ANNALS OF BOTANY
Founded 1887

PART OF A SPECIAL ISSUE ON INFLORESCENCES

Floral development and evolution of capitulum structure in *Anacyclus* (Anthemideae, Asteraceae)

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• *Background and Aims* Most of the diversity in the pseudanthia of Asteraceae is based on the differential symmetry and sexuality of its flowers. In *Anacyclus*, where there are (1) homogamous capitula, with bisexual, mainly actinomorphic and pentamerous flowers; and (2) heterogamous capitula, with peripheral zygomorphic, trimerous and long-/short-rayed female flowers, the floral ontogeny was investigated to infer their origin.
• *Methods* Floral morphology and ontogeny were studied using scanning electron microscope and light microscope techniques
• *Key Results* Disc flowers, subtended by paleae, initiate acropetally. Perianth and androecium initiation is unidirectional/simultaneous. Late zygomorphy occurs by enlargement of the adaxial perianth lobes. In contrast, ray flowers, subtended by involucral bracts, initiate after the proximal disc buds, breaking the inflorescence acropetal pattern. Early zygomorphy is manifested through the fusion of the lateral and abaxial perianth lobes and the arrest of the adaxials. We report atypical phenotypes with peripheral 'trumpet' flowers from natural populations. The peripheral 'trumpet' buds initiate after disc flowers, but maintain an actinomorphic perianth. All phenotypes are compared and interpreted in the context of alternative scenarios for the origin of the capitulum and the perianth identity.
• *Conclusions* Homogamous inflorescences display a uniform floral morphology and development, whereas the peripheral buds in heterogamous capitula display remarkable plasticity. Disc and ray flowers follow different floral developmental pathways. Peripheral zygomorphic flowers initiate after the proximal actinomorphic disc flowers, behaving as lateral independent units of the pseudanthial disc from inception. The perianth and the androecium are the most variable whorls across the different types of flowers, but their changes are not correlated. Lack of homology between hypanthial appendages and a calyx, and the perianth double-sided structure are discussed for *Anacyclus* together with potential causes of its ray flower plasticity.

Key words: *Anacyclus*, capitulum, Compositae, disc flowers, evo-devo, inflorescence, pseudanthium, ray flowers.

INTRODUCTION

Historically, recognition of the identity of the Asteraceae has been centred on their capitulum with flowers tightly arranged on a receptacle surrounded by bracts (Troll, 1928; Claßen-Bockhoff, 1990, 1992; Funk *et al.*, 2009). Structurally, the pseudanthium is synchronized to work as an effective reproductive unit (Weberling, 1989). A frequent arrangement of the Asteraceae capitulum consists of peripherally located ray flowers which are highly specialized in pollinator attraction, and disc flowers which assume the reproductive function, thereby improving the chances of reproductive success and facilitating a more flexible basis for breeding system evolution than does a single flower (Lane, 1996; Jeffrey, 2009). A particularly diverse trait in the capitulum is the perianth symmetry exhibited by different flowers. Peripheral flowers can be strongly zygomorphic, whereas the disc flowers are mainly actinomorphic (Bremer, 1994; Jeffrey, 2009). There is often a variation in the expression of the floral sexuality (e.g. the peripheral zygomorphic flowers are usually female while the actinomorphic disc flowers are usually bisexual) associated with the changes in symmetry, which has important consequences for the evolution of the reproductive biology in the family (Burtt, 1977; Torices *et al.*, 2011). For instance, in several species, the presence of peripheral ray flowers has a marked effect on attractiveness to pollinators, rate of outcrossing and fitness (Lack, 1982; Marshall and Abbott, 1984; Stuessy *et al.*, 1986; Sun and Ganders, 1990; Andersson, 2008; Chapman and Abbott, 2009). Despite these evolutionary trends, the presence of peripheral zygomorphic flowers in Asteraceae is not always consistent within tribes, genera or even species (e.g. *Anacyclus* L., *Anthemis* L., *Bidens* L., *Inula* L., *Matricaria* L., *Senecio* L. and *Tanacetum* L.).

The pseudanthial phenomenon represents a shared evolutionary trait in several families of eudicots (Ronse De Craene, 2010). Calyceraceae and Goodeniaceae, which molecular evidence suggests are the closest relatives of Asteraceae (Bremer *et al.*, 2002; Lundberg and Bremer, 2003; Lundberg, 2009), possess inflorescences forming condensed thyrsoids (cephaloids) and thyrses with extended pedicels, respectively (Pozner *et al.*, 2012). In the clade comprising Asteraceae, Calyceraceae, Goodeniaceae and

Menyanthaceae, the evolution of the inflorescence morphology is prominent (Pozner *et al.*, 2012), with characters such as the involucre differentiation and several floral traits supporting the sister relationship of Asteraceae and Calyceraceae (Devore and Stuessy, 1995; Gustafsson, 1995; Lundberg and Bremer, 2003; Soltis *et al.*, 2005). Throughout the whole of the 20th century the pseudanthial phenomenon in Asteraceae has been extensively studied from the morphological point of view (e.g. von Uexküll-Gylleband, 1901; Small, 1917; Koch, 1930*a*, *b*; Leins and Erbar, 1987; Claßen-Bockhoff, 1992; Gustafsson, 1995; Harris, 1999; Erbar and Leins, 2000; Leins and Erbar, 2000). Similarly, differential floral phenotypes in the capitula together with their ecological or molecular/genetic characterization have been discussed in selected Asteraceae lineages (Carr *et al.*, 1996; Andersson, 2001; Chapman *et al.*, 2008; Broholm *et al.*, 2010), inviting a more integrative and reciprocal view about this key trait in Asteraceae evolution.

As part of an ongoing project focused on the evolution of ray and disc flowers of *Anacyclus* L. (Anthemideae) from a developmental genetics and morphological perspective (M.A. Bello *et al.*, Real Jardín Botánico RJB-CSIC, unpubl. res.), we have studied the floral ontogeny in this genus searching for the basis of the differential morphological traits. *Anacyclus* is a Mediterranean genus of mostly annual herbs (Fig. 1) with about 12 species distributed in North Africa, South Europe and the Middle East (Bremer and Humphries, 1993). It is circumscribed in Matricariinae together with *Achillea* L., *Heliocauta* Humphries, *Leucocyclus* Boiss., *Matricaria* L. and *Otanthus* Hoffmanns. & Link. Although according to phylogenetic analyses based on internal transcribed spacer (ITS) data (Oberprieler, 2004; Oberprieler *et al.*, 2009) *Anacyclus* is closely related to *Heliocauta*, the *trn*L/*trn*F intergenic spacer (IGS) region suggests *Anacyclus* to be closest to the *Lepidophorum repandum* + *Lonas annua* clade (Oberprieler, 2004). Alternative cladistic hypotheses based on morphological traits indicate *Leucocyclus* as its closest relative (Humphries, 1979; Bremer and Humphries, 1993).

There are two types of capitula in *Anacyclus*, according to floral sexuality and morphological arrangement within the inflorescence (Figs 1 and 2). The first is the homogamous type, with all flowers bisexual and tubular with pentamerous corollas. This type is present in *A. homogamos* (Maire) Humphries and *A. monanthos* (L.) Thell. (Figs 1A–C and 2G). The second is the heterogamous type, which differs from the homogamous type by the presence of female flowers on the periphery of the capitulum (Fig. 1D–J). Female flowers are zygomorphic and may have an apparently unilobed white, dark or light yellow, sometimes purple underside to the perianth (Figs 1D, E and 2D). The heterogamous capitulum is present in *A. alboranensis* Esteve & Varo, *A. anatolicus* Behçet & Almanar, *A. clavatus* (Desf.) Pers., *A. inconstans* Pomel, *A. latealatus* Hub.-Mor., *A. linearilobus* Boiss. & Reuter, *A. maroccanus* (Ball) Ball, *A. nigellifolius* Boiss., *A. pyrethrum* (L.) Link, *A. radiatus* Loisel. and *A. valentinus* L. In some of these species with heterogamous capitula, the female peripheral flowers may vary in number (Fig. 1H) and be inconspicuously rayed (Figs 1F, G and 2A, B), or even lack a lamina (Fig. 2C), in which case the capitulum has a discoid aspect (Fig. 1F, G). This occurs in *A. alboranensis* Esteve & Varo, *A. inconstans* Pomel and *A. valentinus* L. The presence of hybrids in the genus has been suggested by the comparison of former distributions and morphological continuity between the species (Humphries, 1979, 1981), which reveals an effect of hybridization events on the floral phenotype at the population level. Specifically, the heterogamous capitulum with short/absent perianth lamina of the ray flowers seems to be an intermediate phenotype resulting from the crossing of homogamous and heterogamous individuals with long ray flowers. For example, *A. valentinus* (presenting heterogamous capitula in which ray flowers are underdeveloped or have reduced perianth lamina, Fig. 1G, H) is considered the result of hybridization between *A. homogamos* (with homogamous capitula, Fig. 1A, B) and *A. radiatus* (with heterogamous capitula). Similarly, *A. inconstans* (heterogamous with inconspicuous ray flowers) results from the
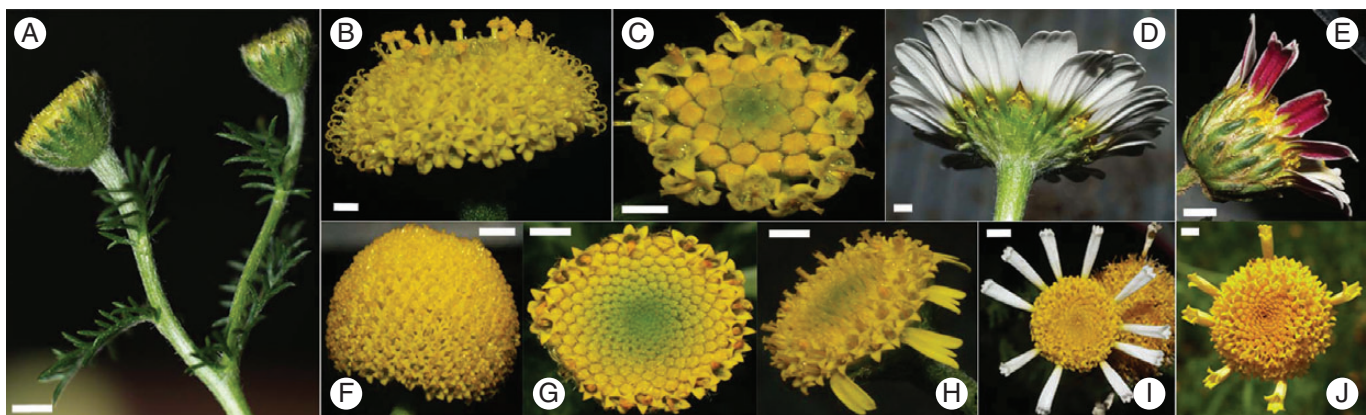

FIG. 1. Inflorescences of *Anacyclus*. *Anacyclus homogamos* (A, B); *A. monanthos* (C); *A. clavatus* (D); *A. pyrethrum* (E); *A. alboranensis* (F); *A. valentinus* (G, H, J); *A. clavatus* 'trumpet' (I). (A) Capitula. (B, C) Homogamous capitula. (D, E) Heterogamous capitula with peripheral ray flowers in anthesis. (F, G) Heterogamous capitula with reduced ray flowers giving a discoid aspect to the inflorescence. (H) Heterogamous capitulum with reduced and scarce ray flowers in anthesis. (I) Heterogamous capitulum with peripheral flowers with a 'trumpet' perianth. Taken from a field population of *A. clavatus*. (J) Heterogamous capitulum with peripheral flowers with a 'trumpet' perianth. Taken from a field population of *A. valentinus*. Scale bars: (A, D, E) = 5 mm; (B) = 1·6 mm; (C) = 2 mm; (F) = 1 cm; (G, H–J) = 2·5 cm.
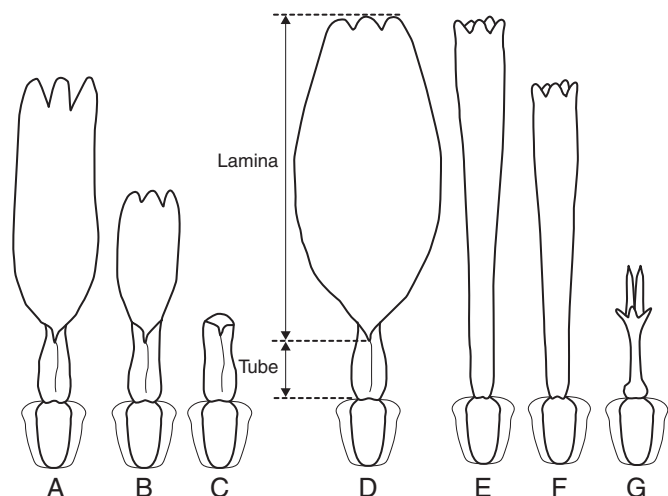
FIG. 2. Schematic drawing of the peripheral (A–F) and disc flowers (G). (A–C) Peripheral flowers found in *A. valentinus*, with relatively narrow (A), short (B) or null (C) perianth lamina. (D) Typical long and wide ray flower observed in heterogamous species such as *A. clavatus*. (E, F) 'Trumpet' flowers present in wild populations of *A. clavatus* (E) and *A. valentinus* (F). (G) Disc flower. The structure surrounding the ovaries corresponds to the wings, flat prolongations of the ovary wall.

crossing between *A. homogamos* and *A. clavatus* (with heterogamous capitula, Fig. 1D).

Apart from the variation in the capitula used in taxonomic species circumscription (Humphries, 1979), we found unusual heterogamous capitula with peripheral 'trumpet' flowers in natural populations of *A. clavatus* and *A. valentinus* in southern Spain (Fig. 1I, J). In these capitula, the 'trumpet' flowers are peripheral like the ray flowers but differ morphologically from them, as well as from the disc flowers. Taking into account the floral morphology diversity exhibited in the capitula of *Anacyclus*, we performed a comparative study of the developmental patterns to (1) describe the different types of capitula in the genus; (2) study the floral development patterns of *Anacyclus*; and (3) discuss the relevance of these observations in the context of the evolution and structure of the inflorescence in Asteraceae.

MATERIALS AND METHODS

Seeds stored in the seed bank collection at the Real Jardín Botánico-CSIC at Madrid were used to grow five species of *Anacyclus* under greenhouse controlled conditions (16 h light, 24 °C) in humidified Petri dishes from September to November 2009. In addition, flowering individuals of *A. pyrethrum*, *A. radiatus* and 'trumpet' forms of *A. clavatus* and *A. valentinus* were collected in the field and transplanted to the greenhouse (Table 1). After germination of the seeds (2–7 d usually; up to 25 d in *A. monanthos*), the seedlings were potted in a bed with clean peat/sand soil and monitored until flowering. Capitula emerged in 5 (*A. alboranensis*, *A. homogamos*, *A. monanthos* and *A. valentinus*) and 7 months (*A. clavatus*) after sowing. Two hundred and sixty-eight live pictures were taken with an Olympus E-500 to document the floral macromorphology.

Floral buds were collected in a solution of FAA, made up of 10 parts of formaldehyde solution, 85 parts of 70 % ethanol and 5 parts of glacial acetic acid, and left for at least 4 d. For the scanning electron microscopy (SEM) study, the buds were embedded in 70 % ethanol, dissected under a Nikon SMZ 1000 stereoscope and dried using a Polaron CPD7501 critical point drier. After mounting the samples on aluminium stubs and covering them with gold in a BALZERS SDC004 sputter coater at 45 mA, SEM microphotographs were taken using a HITACHI S-3000N scanning electron microscope. Nine hundred and eighty microphotographs were taken. For light microscopy (LM), capitula were dehydrated through an ethanol series to 100 %, then taken through an ethanol–Histoclear® series to 100 % Histoclear®. Inflorescences were embedded in MacCormick Paraplast Plus paraffin, sectioned using a Jung RM rotary microtome, (Jung AG) and stained using safranin and alcian blue. Slides were mounted with DePeX mounting medium (Serva). Two hundred and sixty-one microphotographs were taken with an Olympus BX 51 microscope using the QCapture 2·6 software. Digital images were processed using Adobe Photoshop version 8·0·1.

RESULTS

Inflorescence and floral organography

Capitulum. Individuals with heterogamous and homogamous capitula are gynomonoecious and hermaphroditic, respectively. The capitula arranged in a corymbose pattern develop basipetally within the individual plant. In all species, the anthesis occurs acropetally through the capitulum and it does not follow the direction of the parastichies, i.e. flowers at the same distance from the centre of the receptacle open simultaneously (Fig. 1C, G). In the heterogamous capitula, all female flowers start anthesis earlier than the bisexual flowers, making the pseudanthium protogynous. Ray flowers may start senescence before the upper disc flowers are entirely open. Therefore, the same capitulum may present proximal newly formed achenes and distal pre-anthetic floral buds. There are 2–4 rows of free involucral bracts (i). The hairy external involucral bracts have long flat and striate cells at the lower epidermal surface (Fig. 3A). They are glabrous at the upper epidermal surface and sometimes narrowly scarious on the margin and apex. Internal involucral bracts are similar to the external ones, but they tend to be narrower, less hairy and more scarious than the external ones. The receptacle is paleate, almost flat before anthesis of the disc flowers (df) to sub-conical after further elongation. External paleae (pa) may be glabrous or hairy towards the apex, with a scarious and sometimes long ciliated margin and apex, decreasing in size towards the centre of the capitulum (Fig. 3B).

Apart from the usual variation in each of the *Anacyclus* species, we have observed four infrequent capitulum phenotypes in natural populations of *A. clavatus*. First, in one single individual (Carchuna, Málaga, *Agudo 1*), we found the typical heterogamous capitula in lateral branches and a terminal homogamous capitulum (Fig. 4A). In a second case, the peripheral flowers adopt an intermediate morphology between a 'trumpet' and a ray flower (Vélez-Málaga, Málaga, *Álvarez*

TABLE 1. *List of Anacyclus species used in this study and their types of capitula*

Species	Origin (voucher)	Capitulum type and peripheral flower type
A. alboranensis Esteve & Varo	Spain: Almería, Alborán island (Accession no. 090440002 UPM-GEM)	Heterogamous with discoid aspect (Figs 1F, 2C)
A. clavatus (Desf.) Pers.	Spain: Soria, Castilruiz (Álvarez 2072); Madrid, Soto del Real (Álvarez 2074)	Heterogamous with white peripheral ray flowers (Figs 1D, 2D)
A. clavatus 'trumpet'	Spain: Granada, Carchuna (Agudo 1). Capitula collected in the field	Heterogamous with white, actinomorphic and pentamerous peripheral 'trumpet' flowers (Figs 1I, 2F)
A. homogamos (Maire) Humphries	Morocco: Askaun (Quintanar 3505); Marrakech, Imouzzer (Gonzalo 1275)	Homogamous with pentamerous disc flowers (Figs 1B, 2G)
A. monanthos (L.) Thell.	Tunisia: Gabès (Aedo 16194, 16233, 16310).; Medenine (Aedo 16266)	Homogamous with pentamerous disc flowers (Figs 1C, 2G)
A. pyrethrum (L.) Link	Spain: Albacete, Peñascosa (Álvarez 2093). Capitula collected in the field. Morocco: Askaun (Quintanar 3531)	Heterogamous, ray flowers in white adaxially and purple abaxially (Figs 1E, 2D)
A. radiatus Loisel.	Spain: Sevilla, Aznalcázar (Álvarez 2077) Capitula collected in the field	Heterogamous with yellow ray flowers (Fig. 2D)
A. valentinus L.	Spain: Valencia, Altea (Medina 4434)	Heterogamous with discoid aspect (Figs 1G, 2C) or with short yellow to white ray flowers (Figs 1H, 2A, B)
A. valentinus 'trumpet'	Spain: Málaga, Chilches (Álvarez 2133). Capitula collected in the field	Heterogamous with yellow, actinomorphic and pentamerous peripheral 'trumpet' flowers (Figs 1J, 2G)

2144, Fig. 4B). The third one involves one plant bearing a single capitulum in which one peripheral flower possesses 'trumpet' perianth while the remainder are the typical long-rayed flowers (not shown). In the last phenotype, several plants from a population (Villarejo de Salvanés, Madrid, *Álvarez 2274*) exhibit one or several ray flowers growing among the outermost disc flowers (Fig. 4C, D).

Perianth. Bisexual disc flowers are long, tubular and slightly to clearly zygomorphic with two adaxial, two lateral and one abaxial perianth lobe (Figs 2G, 3B and 5A). They are protandric with valvate perianth (p) aestivation. The lower epidermal surface of the perianth (p) consists of flat cells with scattered multicellular hairs (Fig. 3C), while the upper epidermal surface has proximally long flat cells and swollen papillae covering the lobes (Fig. 3D). These papillose cells are bottle-like or rounded, and their external walls are striated (Fig. 3E). The lower epidermis of the perianth lobes tends to extend, covering the tip of each lobe, giving the adaxial perianth lobes their characteristic hooded concavity (Fig. 3D, F). The adaxial perianth lobes can be up to three times longer than the rest of the lobes, with a papillose upper epidermis enclosed by the lower epidermis (Fig. 3F, G). The ray female flowers present a flat, wide and extended zygomorphic perianth (p) (Figs 2D, 3H and 5B), with a lower epidermis formed by flat and striate cells and a dense and papillose upper epidermis (Fig. 3I). The mature perianth displays a green area proximally on the lower epidermis (Fig. 1D) and a perianth tube (p) enclosing the gynoecium up to the distal part of the style (st), leaving the stigmatic branches uncovered during anthesis (Fig. 3H). The perianth consists of three fused lobes, two laterals and one abaxial (Figs 2A, B, D and 5B). The lobes differentiate only at the very distal part (Fig. 1D, E, H). Occasionally the lamina is lacking in the peripheral female flowers, in which case they are still zygomorphic and possess a perianth tube and the gynoecium (Fig. 2C).

Peripheral flowers with 'trumpet' perianth may be white and female (Figs 1I and 5D) or yellow and bisexual (Figs 1J and 5C), but not rayed. Their perianth is usually pentamerous and actinomorphic with five equal lobes (two adaxial, two lateral and one abaxial, Figs 2E, F and 5C, D), although some peripheral 'trumpet' flowers with six lobes and with two adaxial perianth lobes that are slightly longer than the others were also observed (results not shown). Cell morphology is similar to that observed in the ray flowers, with its upper epidermal surface covered by papillate cells. The disc flowers (df) of the capitula with peripheral 'trumpet' flowers are similar to those in the homogamous and heterogamous capitula (Figs 3J, K and 5A).

Androecium. There is a pentamerous androecium in the disc flowers of all species and in the bisexual peripheral 'trumpet' flowers of *A. valentinus* (Fig. 5A, C). The filaments (f) are free, inserted inside the corolla tube. The typical Asteraceae filament collar has rounded cells, whereas the rest of the cells of the filament (f) are long and narrow (Fig. 3L). The anthers (a) are ecaudate, apiculate, introrse and connate.

Gynoecium. A gynoecium is present in all flowers of *Anacyclus* (Fig. 5). As in other Asteraceae, the inferior ovary is bicarpellar and monospermic with basal placentation. Despite the
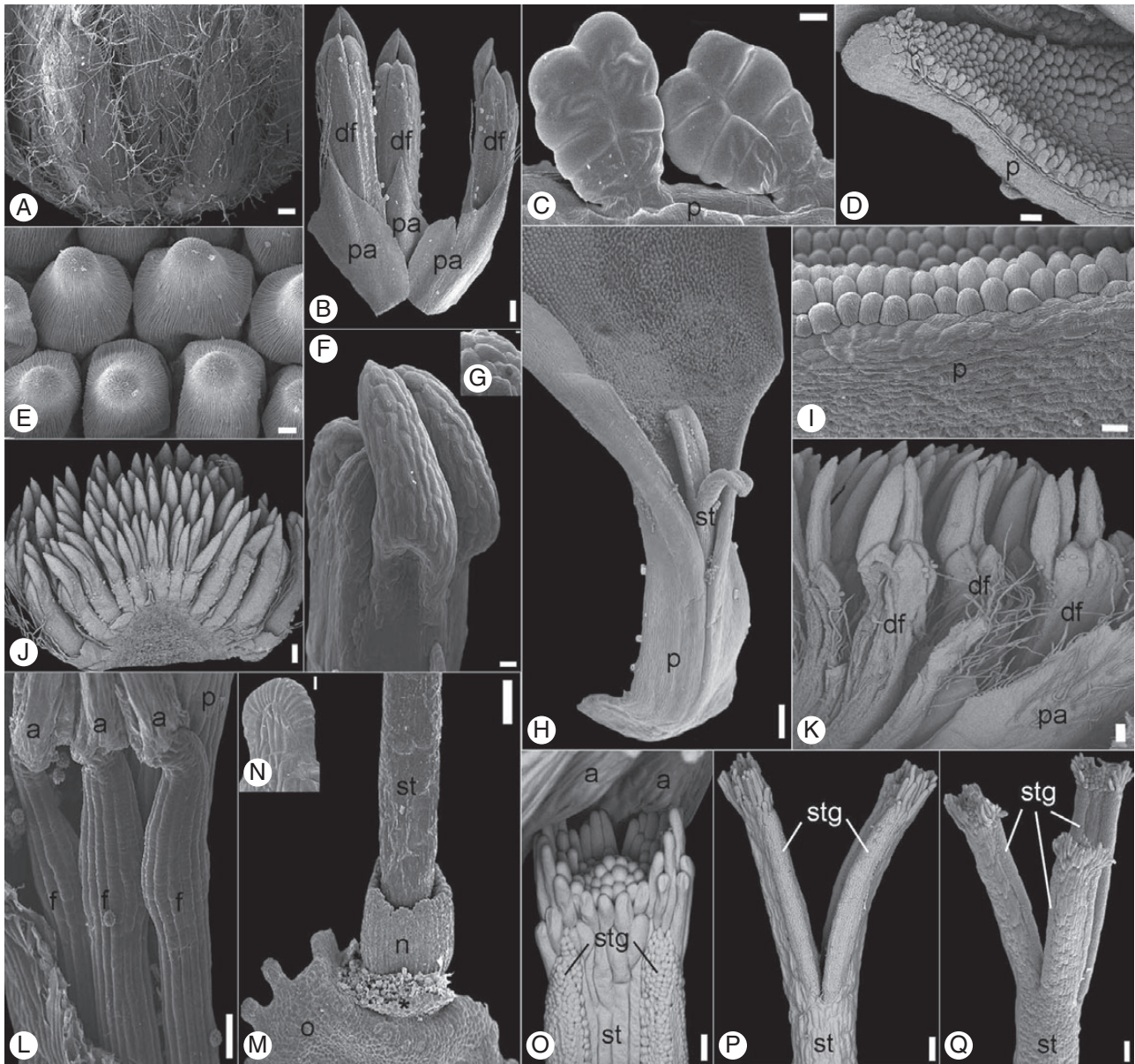
FIG. 3. SEM microphotographs. Adult floral tissues and organs of *Anacyclus. Anacyclus homogamos* (A, B, O, P), *A. monanthos* (C, F, G, L, M, N), *A. valentinus* (D, E, I), *A. clavatus* (H), *A. clavatus* 'trumpet' (J, Q), *A. valentinus* 'trumpet' (K). (A) Side view of the involucre. (B) Front view of disc flowers with subtending paleae. (C) Front view of the perianth hairs in disc flowers. (D) Side view of a lateral perianth lobe of a disc flower. Upper epidermal surface upwards. (E) Top view of the papillose cells on the upper epidermal surface of a disc flower perianth lobe. (F) Side view of the adaxial flank of a disc flower. The adaxial perianth lobes (pointing upwards) are taller than the other lobes. (G) Detail of the surface of an adaxial perianth lobe. (H) Front view of a ray flower with bifid style. (I) Side view of the lower (downwards) and upper perianth surface (upwards) of a ray flower. (J) Side view of a capitulum with 'trumpet' flowers sliced to expose the radial plane. Only disc flowers are detailed. (K) Side view of disc flowers present in a capitulum with 'trumpet' flowers prior to anthesis. (L) Side view of filaments and the proximal part of the anthers in a disc flower. The thick area of the filament closer to the anther is the collar. (M) Side view of a disc flower (removed perianth marked with an asterisk) displaying the stylopodium (nectary) ring surrounding the proximal part of the style. The irregular extensions of the ovary wall correspond to myxogenic cells. (N) Detail of a myxogenic cell from M. (O) Front view of a style branch from a disc flower touching the anthers. (P) Side view of a bifid style and its stigmatic lined surfaces, from a disc flower. (Q) Trifid style from a peripheral 'trumpet' flower. Abbreviations: a, anther; df, disc flower; f, filament; i, involucral bract; n, nectary; o, ovary; p, perianth; pa, palea; st, style; stg, stigmatic tissue. Scale bars: (A, B, H, J, K) = 0·2 mm; (C, N) = 10 μm; (D, F) = 40 μm; (E, G) = 6 μm; (I, O) = 20 μm; (L, M) = 100 μm; (P, Q) = 60 μm.

presence of bicarpellar vascularization, it is pseudomonomer-ous with only one functional carpel (Figs 5 and 7D, E). The ovary is flat and obovate. It has characteristic myxogenic cells (mx) (myxo = slime, mucus; Stearn, 2004) scattered along the external epidermis and an associated nectarial (n)

structure, the stylopodium, surrounding the style (st) at the level of the perianth base (Fig. 3M, N). The stylopodium is present in the disc flowers and in the peripheral 'trumpet' flowers (Fig. 5A, C, D). Although it is usually absent in the female ray flowers (Fig. 5B), it is frequently observed in

F<small>IG</small>. 4. Pseudanthia with unusual phenotypes taken from natural populations of *Anacyclus clavatus*. (A) Individual with both homogamous and heterogamous capitula. (B) Capitulum with peripheral flowers with an intermediate morphology between a 'trumpet' and a ray flower perianth. (C) Capitulum with a unique ray flower (indicated by a black arrowhead) in the middle of the disc flowers. (D) Capitula with several ray flowers towards the periphery of the disc flower area. Scale bars: (A) = 15 mm; (B) = 5 mm; (C) = 7·5 mm; (D) = 6·5 mm.

long and reduced ray flowers of *A. valentinus* (not shown). In bisexual disc flowers and in the 'trumpet' peripheral flowers there is an apically scarious corona on the adaxial part of the gynoecial hypanthium (Fig. 5A, C, D). The corona consists of several lobes and is variable in size and shape depending on the species. It can be reduced or inconspicuous in the 'trumpet' flowers and is lacking in ray flowers. Styles (st) bifurcate forming two stigmatic branches; each branch bears two rows of stigmatic tissue (stg), and exhibits several distal appendices (Fig. 3O, P). Occasionally, in 'trumpet' flowers and in some ray flowers of *A. valentinus*, well-developed styles (st) with three branches were observed (Fig. 3Q). Rarely, styles with just one stigmatic branch were observed in the 'trumpet' flowers of *A. clavatus* (not shown). Achenes are heteromorphic, flattened to sub-cylindrical, obovate and decreasing in size from the periphery to the centre of the receptacle. The external achenes usually present scarious wings that are variable in shape and size depending on the species (Fig. 2). Its wings sometimes are inconspicuous or even reduced to a narrow hyaline margin, and are usually absent in internal achenes.

Anatomy

The description is general for all the studied species because anatomical features did not show significant variation.

Peduncle, receptacle and involucral bracts. The peduncle is cylindrical, has a thin epidermal layer (e) and is occasionally covered by multicellular uniseriate hairs (Fig. 6A, B). Stomatic chambers show the presence of stomata around the peduncle (Fig. 6A). Surrounding the central vascular tissue there is a mesophyll formed by a parenchymatic palisade layer. The vascular bundles have some wide parenchyma cells and duct-like interspersed structures (Fig. 6A). The receptacle is mostly filled with parenchyma, and there is a strip of ducts under the base of the flowers (Fig. 6C). Young involucral bracts have bilayered abaxial epidermis (e) and their mesophyll is parenchymatic (Fig. 6D). Mature involucral bracts have an aerenchymatic mesophyll (not shown). A dense layer of fibres on their lower epidermis occupies most of the area of the bract (Fig. 6E).

Bisexual disc flowers. A short pedicel together with the palea (pa) subtends each floral bud (Fig. 6C, F). The palea has prominent nuclei in its epidermis and a uniform parenchymatic mesophyll. The stylopodium (n) is formed by tight multilayered parenchyma (Fig. 6G). At the level of the stylopodium, the perianth possesses a parenchymatic mesophyll with a rhomboid shape in transversal section. At the level of the anthers, the perianth (p) is reduced to two layers in transverse section (Fig. 6H). At the distal portion of the perianth, the valvate lobes interlock their papillae (Fig. 6I). The filaments (f) have a unilayered epidermis and their vascular bundle is surrounded by parenchyma (Fig. 6J). The anthers are tetrasporangiate (sp). When young, they are made up of the epidermis (e), a unilayered endothecium (en), a narrow middle layer (m) and a distinctive tapetum (t) (Fig. 6K). Around the connective (co) several crystals are interspersed. After meiosis, the sporangia appear full of tetrahedral tetrads. Mature pollen grains are triaperturate (Fig. 6L). The ovary wall consists of an outer epidermis and 3–4 layers of mesophyll. Myxogenic cells (mx) and hairs are frequent elements of its outer epidermis (e) (Fig. 7A, B), and a unilayered endocarp (ec) surrounds the embryo (eb) when formed (Fig. 7C). In front of the filaments, the style is a solid structure displaying the vascular central bundles coming from each carpel and a dense pollen transmitting tissue (tt) (Fig. 7D, E). Each style branch displays a unilayered distinctive epidermis and two groups of stigmatic secretory cells oriented towards the centre of the flower (Fig. 7F).

Female ray flowers. In ray flowers, the marginal veins alternate with the perianth lobes and converge at the tip of the perianth. The upper epidermal surface (e) is covered by a tight layer of papillate cells along the whole surface (Fig. 7G, I). At the proximal portion, the perianth adopts a more typical petal transversal section, showing ribs towards the abaxial side and a mesophyll with a lower zone occupied by collenchyma (c) (Fig. 7G, H), and an upper area filled with parenchyma (pq) (Fig. 7G). Vascular bundles are interspersed and tend to occur in the collenchyma/parenchyma border. Towards their distal half, the perianth becomes narrower in transversal section, keeping a more rounded dorsiventral shape without ribs at the abaxial side, and there is no trace of collenchyma (Fig. 7I). The mesophyll is parenchymatic (pq) with relatively small cells and few schizogenous areas. The ovary wall (io, ow) is similar to that observed in the disc flowers (Fig. 7J). A layer parallel to the inner ovary epidermis (io) is filled with crystals (Fig. 7K). Small groups of cells with prominent
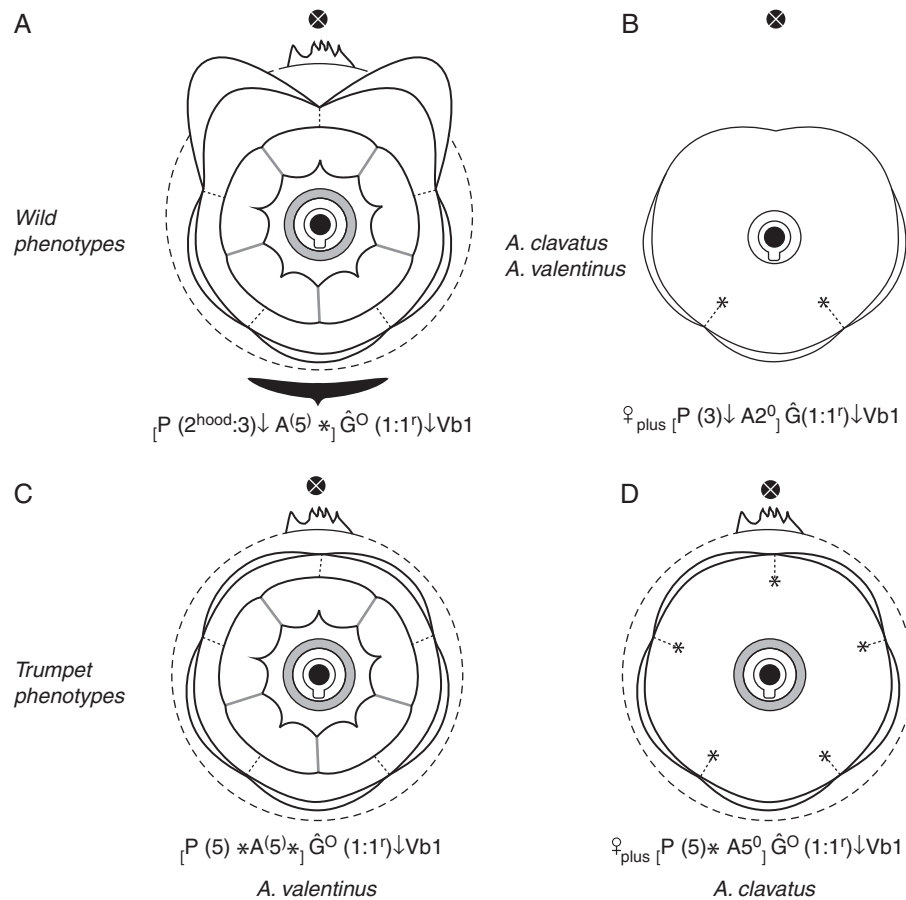
FIG. 5. Diagrams and formulas representing the floral morphologies in *Anacyclus* (for formula symbols, see Prenner *et al.*, 2010). (A) Bisexual disc flower, present in all species. The black structure below the flower corresponds to the subtending palea. (B–D) Different types of peripheral flowers. (B) Female ray flower without adaxial perianth lobes. (C, D) Peripheral flowers with 'trumpet' perianths. (C) Bisexual peripheral 'trumpet' flower with almost equal perianth lobes. Present in populations of *A. valentinus*. (D) Female peripheral 'trumpet' flower with equal perianth lobes. Present in populations of *A. clavatus*. Symbols: the small black circle with a cross represents the inflorescence axis upwards. A dashed circle around the flower diagram in (A), (C) and (D) represents the gynoecial hypanthium. The 'crest-like' structure drawn at the adaxial side of these ovaries corresponds to the corona, which is an extension of the gynoecial hypanthium. Perianth lobes are white. Dashed lines represent the androecium and corolla adnation. Androecium in (A) and (C) displays grey lines between stamens, representing the post-genital stamen connation. Asterisks represent aborted stamens. A grey ring symbolizes the stylopodium. A central circle represents the gynoecium with two carpels: one adaxial with the basal seed (black dot) and the other abaxial reduced, representing a pseudomonomerous condition. Abbreviations: A, androecium; $\hat{G}$, inferior ovary; P, perianth; r, reduced; Vb, basal ovule. Numbers indicate the number of organs per whorl. The superscript zero ('0') in (B) and (C) indicates that the stamens are lost in adult flowers. Symbols: ↓, zygomorphic whorl; *, actinomorphic whorl; ♀plus, female flower' [ ], proximal fusion between organs of different whorls; (), connate organs; ^{O}, distal fusion of organs of the same whorl; o, nectarial gland (stylopodium) surrounding the base of the style.

nuclei form part of the usually large-celled inner ovary (io) epidermis (Fig. 7L).

Floral development

Our observations of the comparative floral development do not suggest differences between the studied species during early stages (i.e. during organ initiation). During mid and late developmental stages (i.e. organ enlargement), a few noteworthy differences have been observed (see below for details).

Capitulum. Capitula initiate as rounded meristems enclosed by lateral compound leaves (cl) (Fig. 8A). The capitula meristem develops the involucral bracts (i) in an acropetal and alternating sequence, with the younger bracts formed at the

largest distance from the last one (Figs 8B and 9A–C). Before the involucral bracts (i) cover the capitula meristem, they become hairy on their abaxial side and the first floral buds initiate (Figs 8B and 9A). While the capitulum adopts a cone shape, well-defined parastichies formed by the disc flower meristems occupy the branch acropetally (Figs 8C, D and 9C, K). Disc flowers belonging to different parastichies and located at the same distance from the floral axis display a similar but not identical degree of development. Soon after the initiation of floral disc buds, the subtending palea differentiates from the common meristem that it shares with these floral buds (Figs 8E and 9C). The paleae differentiation follows an acropetal initiation pattern along the inflorescence. In heterogamous capitula, each peripheral ray flower is opposite to an involucral bract (i) and initiates after at
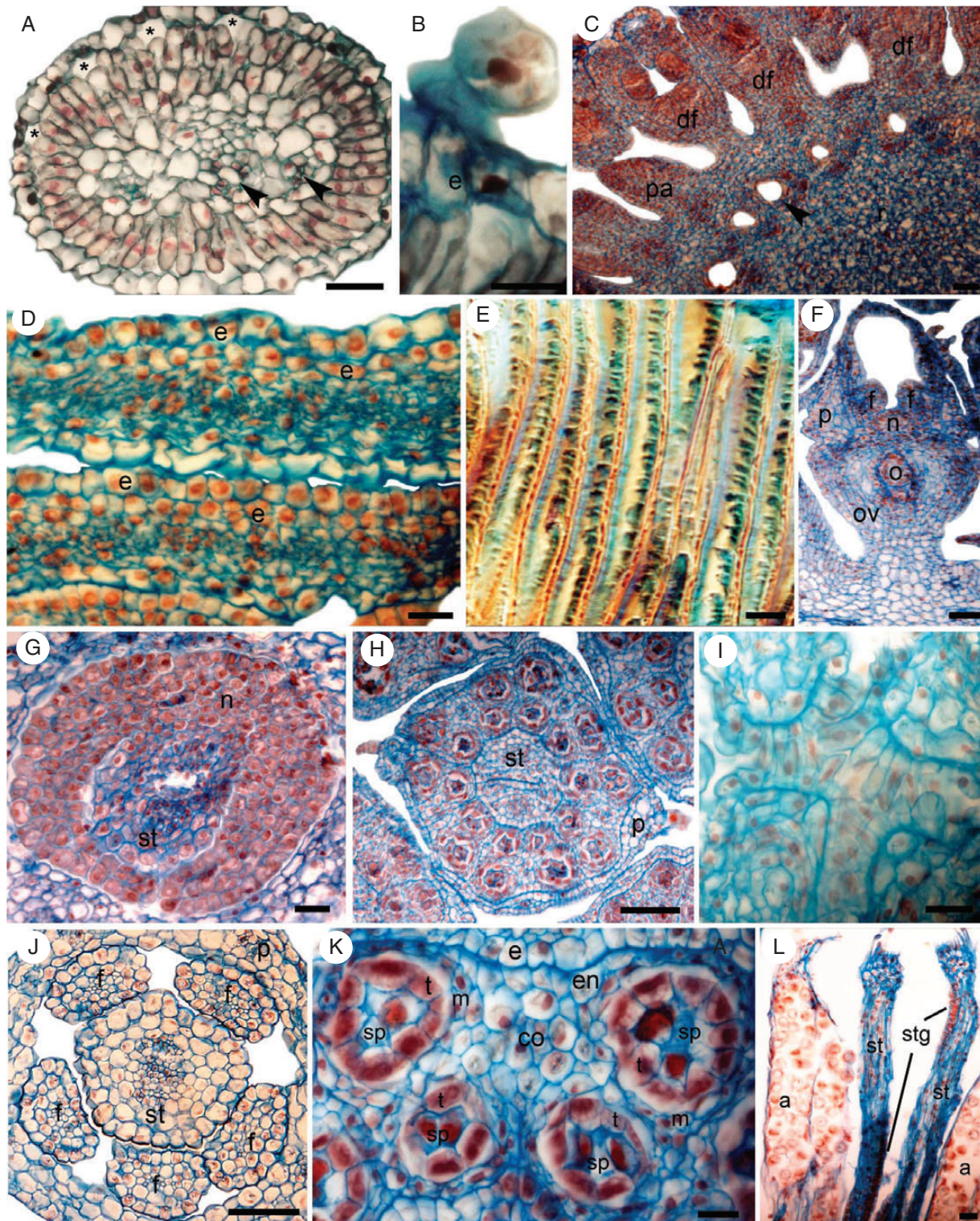
FIG. 6. LM microphotographs. Floral anatomy of *Anacyclus*. *Anacyclus clavatus* (A–D, F, H, I, K); *A. valentinus* (G); and *A. homogamos* (E, J). (A) Transversal section of the penduncle. Asterisks indicate stomatic chambers. Arrowheads point to secretory ducts. (B) Detail of a capitate hair at the peduncle epidermis. (C) Longitudinal section of a young capitulum. The arrowhead points to one of the serial secretory ducts along the inflorescence below the floral buds. (D) Transversal section of two involucral bracts. (E) Transversal section of a mature involucral bract with a thick fibre layer. (F) Longitudinal section of a disc flower. (G) Transversal section of a disc flower at the level of the nectary (stylopodium) near the proximal part of the perianth. (H) Transversal section of a disc flower at the anther level. (I) Transversal section of a disc flower bud at the level of the union of perianth lobes. The lobes interlock their papillate surfaces to seal the top of the perianth. (J) Transversal section of a disc flower with a central style and five surrounding filaments. (K) Detail of the anther anatomy in transverse section. (L) Longitudinal section of two mature anthers full of pollen and the two style branches. The uniserial rows of red cells correspond to the stigmatic area of each branch. Abbreviations: a, anther; co, connective; df, disc flower; e, epidermis; en, endothecium; f, filament; m, middle layers; n, nectary; o, ovule; ov, ovary; p, perianth; pa, palea; r, receptacle; sp, sporangium; st, style; stg, stigmatic tissue; t, tapetum. Scale bars: (A, F) = 50 µm; (B, D, K, L) = 20 µm; (C, G) = 30 µm; (E) = 10 µm; (F) = 50 µm; (H) = 0·2 mm; (I) = 25 µm; (J) = 75 µm.
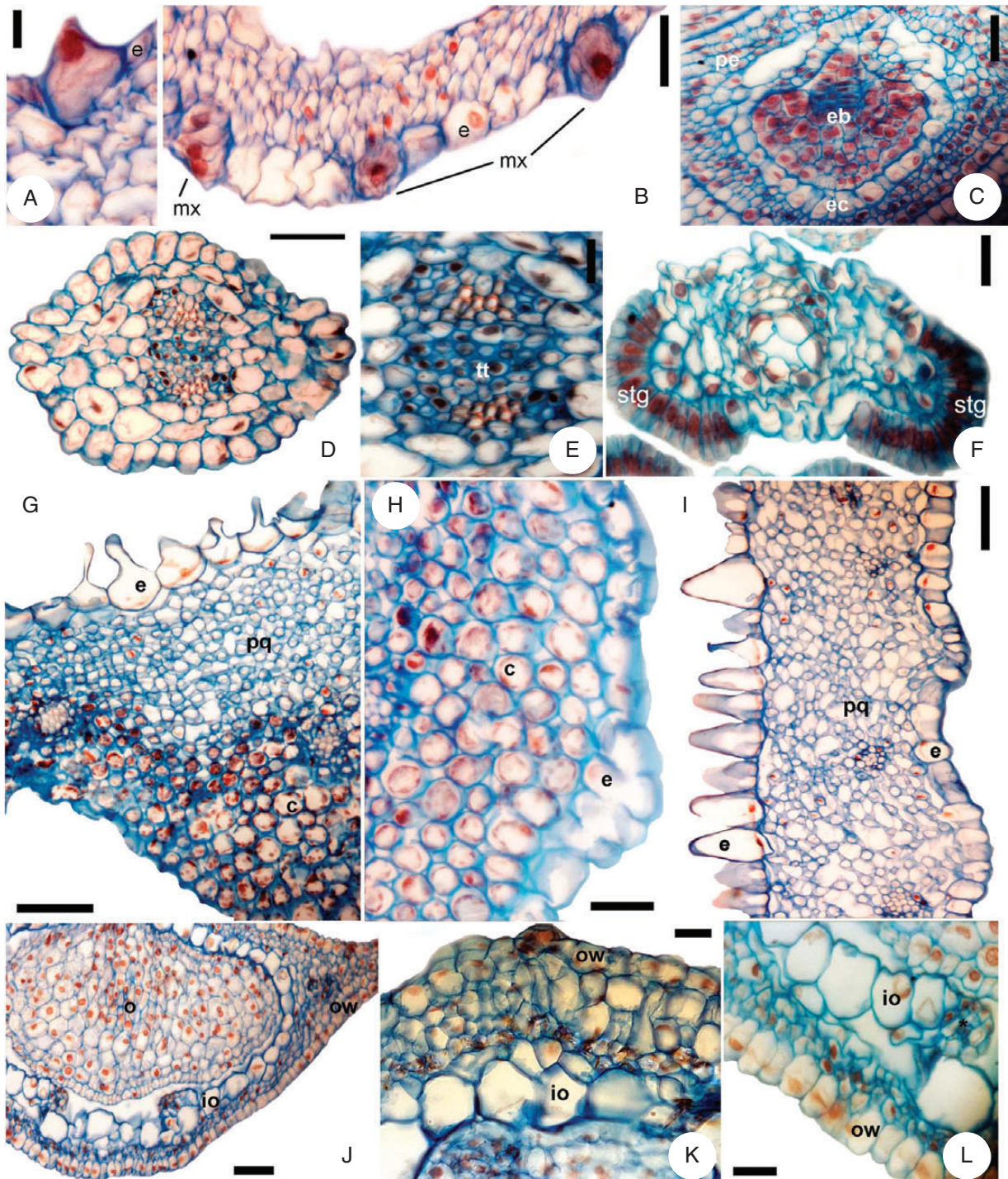
FIG. 7. LM microphotographs. Floral transversal sections of *Anacyclus*. *Anacyclus valentinus* (A, B); and *A. clavatus* (C–O). (A–F) Disc and (G–L) ray flower tissues. (A) Hair at the ovary wall. (B) Ovary wall, with three myxogenic cells at the external epidermis. (C) Ovary of a disc flower with a young embryo. (D) Style at the level below the anthers with two main vascular bundles from each carpel. (E) Detail of (D), with the vascular bundles separated by the pollen transmitting tissue. (F) Style branch with the stigmatic areas towards the side. (G) Proximal part of the perianth. At the abaxial side (at the bottom) is a rib of the lamina filled by several layers of collenchyma. The upper half possesses parenchyma and is limited by a papillate epidermis. (H) Detail of the collenchymatic tissue with chloroplasts. (I) Distal portion of the perianth without traces of collenchyma. The lower epidermal surface is on the right and the upper epidermal surface is on the left. (J) Distal section of the ovary. The ovule tissue is at the centre. (K) Ovary wall with a layer of crystals parallel to the inner ovary epidermis. (L) Ovary wall with the inner ovary epidermis interspersed by small groups of cells similar to vascular bundles (asterisk). Abbreviations: c, collenchyma; e, epidermis; eb, embryo; ec, endocarp; io, inner ovary wall; mx, myxogenic cells; o, ovule; ow, outer ovary wall; pe, pericarp; pq, parenchyma; stg, stigmatic tissue; tt, transmitting pollen tissue. Scale bars: (A, D, F, K) = 25 μm; (B, I, J) = 50 μm; (C) = 0·1 mm; (E, L) = 12·5 μm; (G) = 80 μm; (H) = 35 μm.

least two disc flowers buds have developed in contiguous parastichies (Fig. 9A, C). In contrast, in homogamous capitula (*A. homogamos* and *A. monanthos*), there are no floral buds opposite to any of the involucral bracts (i) (Fig. 8D). When the open inflorescence is entirely crowded up to the top with floral buds (Fig. 8D), the involucral bracts (i) reach their final size and a mosaic of stages of development is displayed simultaneously. There is no trace of a terminal flower or structure (Fig. 8F). Ray and disc flowers remain straight during the mid and late stages of development (Fig. 8G).

Bisexual disc flowers. Soon after the rounded floral meristems initiate together with their subtending palea, they turn slightly concave, forming an ephemeral perianth ring meristem (Fig. 8H). The paleae continue growing more slowly than the floral buds, which are always slightly longer than them. Perianth (p) initiation is usually evident from the differentiation of the adaxial and the lateral lobes, followed afterwards by the abaxial lobe (Fig. 8I), although sometimes all perianth (p) primordia initiate simultaneously (Fig. 8J). After the perianth lobe initiation the floral bud increases its concavity and adopts a funnel-like shape. Once the perianth (p) lobes adopt a slight dorsiventral disposition, five alternating stamen primordia initiate either simultaneously or unidirectionally, when the abaxial stamens initiate slightly earlier than the adaxial stamens (Fig. 8J). Once the stamens initiate, they equalize their sizes and are rapidly covered by the enlarging perianth lobes (p), which adopt a tight valvate disposition (Fig. 8K). During the ovary (o) and perianth (p) differentiation, there is no evidence of pappus/sepal initiation at the junction between these whorls (Fig. 8L, M). Shortly after the closing of the buds, it is remarkable that the perianth (p) edges become sealed with papillate cells proliferating from the adaxial surface of the lobes (Figs 6I and 8K, L). At the same time, the proximal sympetalous part of the perianth enlarges, the differentiation between the ovary (o) and perianth (p) becomes evident and multicellular hairs start to protrude along the epidermis of the whole perianth (Fig. 8L). After the ovary (o) differentiation (Fig. 8M), a rapid enlargement of the adaxial perianth (p) lobes occurs, resulting in these lobes having a cylindrical final shape (Fig. 8N). This enlargement breaks the initial actinomorphic symmetry of the perianth (p) (Fig. 8K, M), turning it secondarily zygomorphic (Fig. 8N). In *A. monanthos*, *A. pyrethrum* and *A. radiatus*, the differentiation of the adaxial petal lobes is delayed after further enlargement of the perianth tube and when the limit between the perianth (p) and the ovary is well established (Fig. 8O). The enlargement of the adaxial perianth lobes follows an acropetal sequence in the capitula, from the older to the younger disc flowers (Fig. 8G, P). The anther–filament differentiation occurs soon after the ovary and the perianth have established their limit. Once the perianth starts to enlarge, the tube bends upwards, leaving the perianth lobes in a straight disposition.

Female ray flowers. The ray flowers initiate after the more proximal disc floral buds and undergo a delayed development during organogenesis (Figs 8C and 9A–C). This is evidence that in young heterogamous capitula the earliest floral buds

(corresponding to disc flowers) alternate with the primordia of involucral bracts (i) (Fig. 9A). The primordia corresponding to ray flowers initiate after the earliest disc flower primordia opposite to the involucral bracts (i) (Fig. 9C). The primordia of the ray flowers are not subtended by paleae originating from a common meristem as in the disc flowers. They are subtended by the leafy organs from the involucrum (i) (Fig. 9C, E). The perianth (p), made up of the congenitally fused lateral and abaxial lobes, is initiated from a defined ring meristem (Fig. 9D). When the ray flowers initiate the perianth (p) primordia differentiation, the nearest disc flowers already have well-defined petals and incipient stamen primordia, and the involucral bracts (i) already cover the young capitulum (Fig. 9E). The adaxial side of the perianth (p) is soon arrested and does not develop a perianth lobe (Fig. 9D, E, G). When the three perianth (p) primordia start their differentiation, ephemeral stamen primordia are formed (Fig. 9D), but they are totally aborted during further perianth development. In species such as *A. radiatus*, incipient style branches (g) can be observed before the floral bud is enclosed (Fig. 9F). Once the perianth (p) enlarges, the lateral sides of the lamina overlap, it rolls (Fig. 9H, I) and several multicellular hairs can be observed (Fig. 9I, J). The perianth initially has a valvate aestivation, but finally becomes imbricate. Although congenitally fused, the three perianth (p) lobes are well distinguished during development (Fig. 9G, H, J). Despite its initial delayed development (Figs 8C and 9C), the ray flowers display extended lamina before the anthesis of the disc flowers (Fig. 1H).

'Trumpet' flowers. The capitulum develops in a similar way to the normal heterogamous type, i.e. the disc floret buds initiate and differentiate acropetally and the peripheral 'trumpet' flowers display initial delayed development (Fig. 9K, L) as in the typical ray flowers. However, this delay is not as long as in the typical ray flowers. Therefore, when the surrounding older disc flowers close their buds, the perianth (p) lobes of the peripheral 'trumpet' flowers are already at the stage of covering the buds (Fig. 9M, N). Although most of the peripheral (p) flowers are pentamerous with two adaxial, two lateral and one abaxial perianth (p) lobe, merosity changes were observed in only a few of them (Fig. 9M, N). The lateral and the adaxial lobes seem to initiate before the abaxial lobe (results not shown), and stamen primordia were identified alternating with them in *A. clavatus* and *A. valentinus* 'trumpet' forms just before the enclosing of the buds by the perianth lobes. These stamen primordia are aborted in *A. clavatus*. During mid and late development stages, the whole set of flowers adopt the characteristic straight and upward position, which is kept until maturity mainly in the disc flowers (df) (Fig. 9O).

DISCUSSION

Interpretations on the pseudanthium structure in Asteraceae

If the emergence of novel body plans or organs often coincides with the rise of a wealth of new species (Becker *et al.*, 2011), the evolution of the capitulum with several
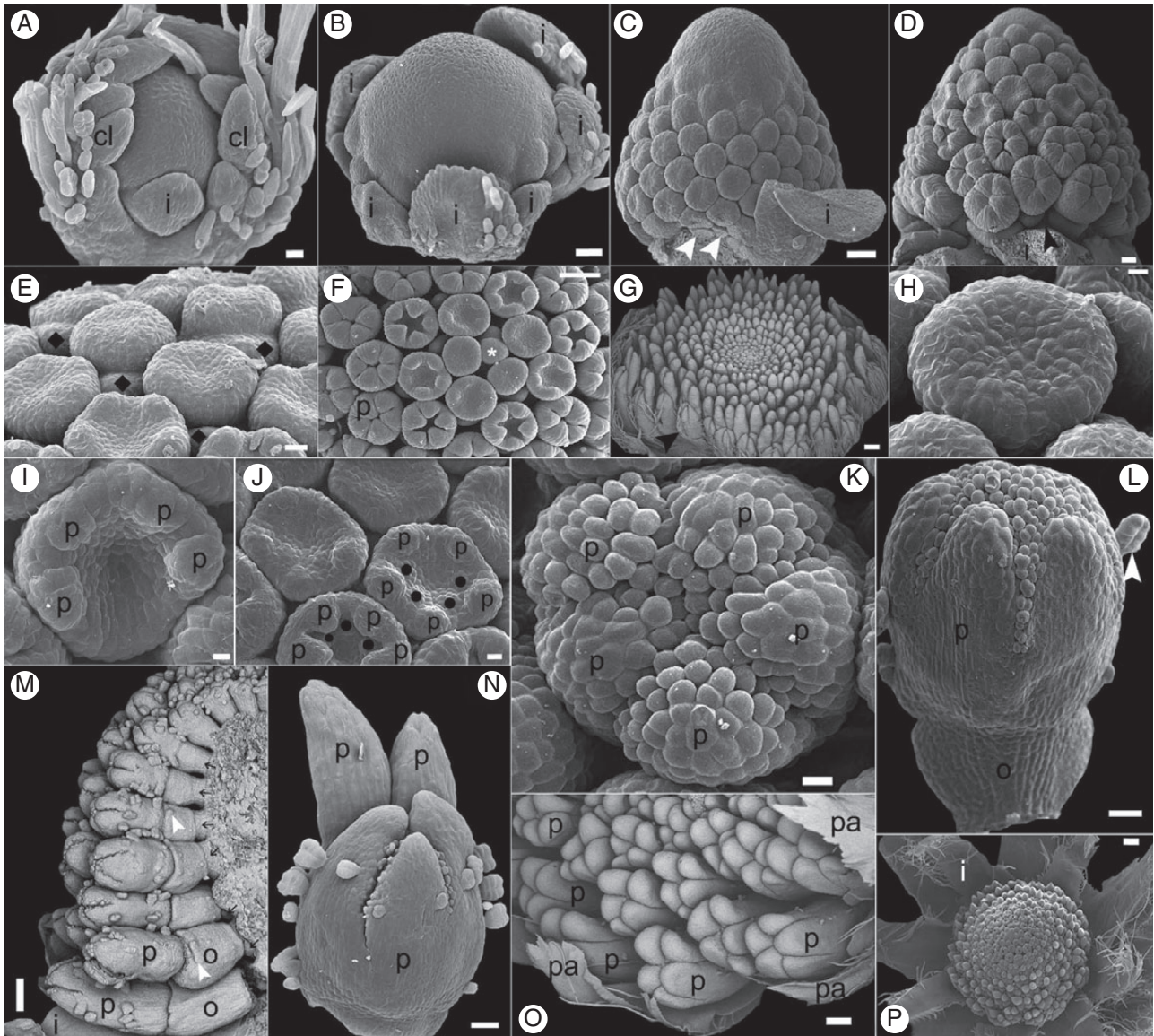
Fɪɢ. 8. SEM microphotographs. Inflorescence (A–D) and disc flower (E–P) development in *Anacyclus*. *Anacyclus homogamos* (A, B, D, I, P), *A. clavatus* (C, F, M, N), *A. alboranensis* (E), *A. valentinus* (G, J), *A. monanthos* (H, K, L) and *A. pyrethrum* (O). (A) Front view of a young reproductive branch. (B) Side view of a young capitulum with involucral bracts initiating helically. Disc flowers buds initiate at the proximal part. (C) Side view of a young heterogamous capitulum with developing parastichies. White arrowheads point to delayed floral meristems of ray flowers. (D) Side view of a homogamous inflorescence. Involucral bract partially removed without the opposite floral bud (black arrowhead). Surrounding disc floral buds are subtended by paleae. (E) Side view of the abaxial flank of developing disc flowers. Black diamonds point to the paleae emerging from a common meristem shared with the floral bud. The inflorescence axis is upwards. (F) Top view of a capitulum. The white asterisk points to the inflorescence meristem. (G) Side view of a developing inflorescence with flat receptacle; pre-anthetic proximal disc flowers flank the undeveloped central buds. The black arrowhead points to a disc flower with subtending paleae. (H) Disc flower bud before organ initiation. (I) Top view of a floral bud with incipient perianth primordia. The adaxial and lateral primordia initiate first, then the abaxial one (adaxial side upwards). (J) Side view of floral buds during stamen primordia initiation (adaxial side upwards). Black dots point to stamen primordia. (K) Top view of valvate perianth of a disc flower. The perianth lobes are thick with a papillose upper surface. Their lower epidermal surface possesses flat cells. (L) Side view of the adaxial flank of a developing disc flower. The two adaxial perianth lobes become taller in comparison with the rest of the lobes. Top of the bud populated by rounded cells and perianth hairs (white arrow). (M) Front view of a radial section of a young inflorescence with developing disc floral buds. Flowers show an actinomorphic perianth, without enlargement of the adaxial perianth lobes. White arrowheads indicate apical ovary appendages. Black arrows point to individual pedicels. (N) Front view of the adaxial side of a disc flower at mid development. Perianth hairs are conspicuous. (O) Elongated disc flowers with delayed adaxial perianth lobe enlargement. (P) Elongation of the adaxial perianth lobes following an acropetal direction. Abbreviations: cl, compound leaves; i, involucral bract; o, ovary; p, perianth; pa, palea. Scale bars: (A, E) = 20 μm; (B, C, N) = 40 μm; (D, M) = 100 μm; (F) = 90 μm; (G) = 0·2 μm; H–K) = 10 μm; (L)= 30 μm; (O, P) = 0·2 mm.

symmetry arrangements along the inflorescence is an important macroevolutionary change associated with the origin of the relatively recent and species-rich Asteraceae. This family is considered one of the largest families of eudicots, with 25 000 species (Heywood *et al.*, 2007). Closer relatives with different inflorescence architecture have fewer species: Goodeniaceae (approx. 420 species) possess elongate short racemes and dichasial, or condensed, head-like inflorescences,
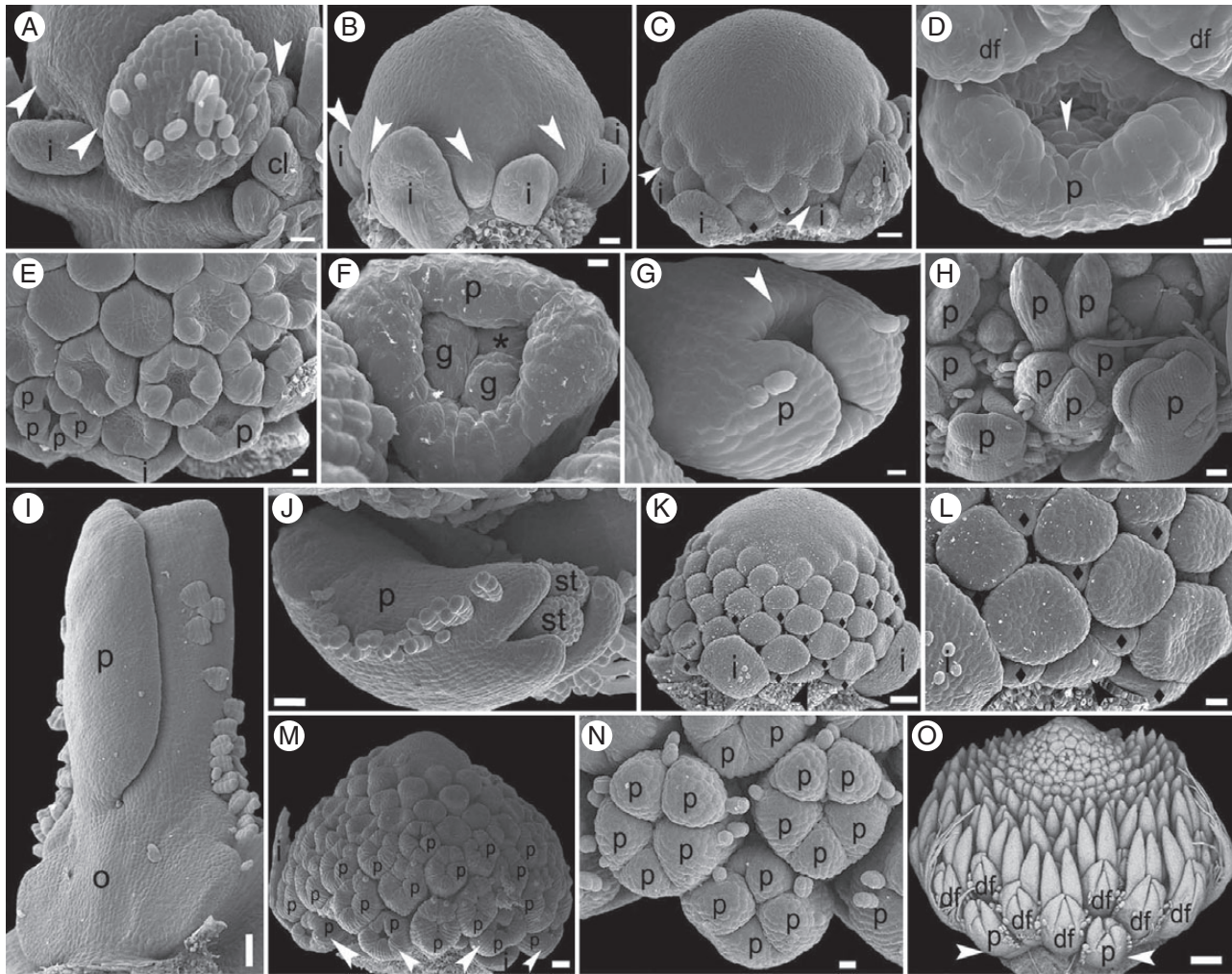
Fɪɢ. 9. SEM microphotographs. Development of heterogamous inflorescences with ray (A–J) and 'trumpet' flowers (K–O). *Anacyclus clavatus* (A, D, E, G, I), *A. pyrethrum* (B), *A. valentinus* (C, J), *A. radiatus* (F), *A. valentinus* with 'trumpet' flowers (K, L, N, O) and *A. clavatus* with 'trumpet' flowers (M). (A) Side view of a young heterogamous capitulum with disc floral buds (white arrowheads) initiating and alternating with young involucral bracts; note the absence of a ray floral bud at this stage. (B) Side view of a heterogamous inflorescence with parastichies initiated by disc flower buds (white arrowsheads). (C) Top/side view of a heterogamous capitulum with young ray flower buds (white arrowhead) and differentiation of a disc floral bud/palea (black diamond). (D) Top view of a ray flower bud with the congenitally united perianth, with the adaxial side repressed and an enlarging abaxial side with a stamen remnant (white arrowhead). (E) Side view of the periphery of a heterogamous capitulum with two ray floral buds: one subtended by an involucral bract (centre, downwards), and the other (at the right) with a congenitally fused perianth. They are surrounded by disc flowers. (F) Top view of a ray flower bud with two carpel primordia. The black asterisk points to a stamen primordium. (G) Side view of a ray floral bud with fused lateral and abaxial perianth lobes. The adaxial perianth side remains repressed (white arrowhead). (H) Side view of the periphery of a heterogamous capitulum with two ray floral buds separated by a disc flower. Note the three fused perianth lobes of the ray flowers and the pentamery of the disc flower with conspicuous adaxial perianth lobes (adaxial side upwards). (I) Side view of the adaxial flank of a ray flower with the perianth rolled and covered by hairs. (J) Side view of a developing ray floral bud displaying enlarging style branches. (K) Side view of a heterogamous capitula with a peripheral 'trumpet' flower bud (black arrow) delayed in comparison with the surrounding disc flowers. Disc flowers have differentiated subtending paleae (black diamonds). (L) Detail of the 'trumpet' floral bud (black arrowhead) and disc floral buds with their paleae (black diamonds) shown in (F). (M) Side view of a heterogamous capitula with peripheral 'trumpet' flowers (white arrowheads). (N) Front view of a tetramerous 'trumpet' flower and two pentamerous disc flowers. (O) Side view of a heterogamous capitulum with 'trumpet' flowers (white arrowheads). Abbreviations: cl, compound leaf; df, disc flower; g, gynoecium; i, involucral bract; o, ovary; p, perianth; st, style. Scale bars: (A, B, E, L, N) = 20 μm; (D, G) = 10 μm; (F) = 6 μm; (I, K, M) = 60 μm; (C, H, J) = 40 μm; (O) = 0·2 mm.

whereas Menyanthaceae (63 species) and Calyceraceae (approx. 60 species) have monotelic thyrses and cephaloids, respectively (Heywood *et al.*, 2007; Lundberg, 2009; Jabaily *et al.*, 2012; Pozner *et al.*, 2012). Pozner *et al.* (2012) suggest that the Asteraceae family differs from its putative sister family Calyceraceae in the developmental control pattern of the inflorescences: the former is mainly racemose and the latter is cymose. It seems that both developmental patterns existed in the ancestor of Asteraceae and closer relatives Calyceraceae, Goodeniaceae and Menyanthaceae.

With this background and the developmental patterns of the capitula (e.g. Harris, 1995), the pseudanthium in Asteraceae can be viewed in two alternative ways apart from the racemose interpretation (e.g. Weberling, 1989). The first hypothesis

suggests that the delayed zygomorphic ray flowers can be interpreted as remnants of the former cymose peripheral units that still prevail in some cephaloids of Calyceraceae (Pozner *et al.*, 2012). Alternatively, the ray flowers can be interpreted as flowers (rather than remnants of cymose units), which develop differently due to their peripheral position. In this second hypothesis, since the Asteraceae capitulum as a whole is an actinomorphic terminal structure, the zygomorphy of the ray floral buds would be a response to its lateral position (Endress, 1999).

Certainly our results show that in heterogamous capitula the peripheral flowers are always subtended by involucral bracts (i) (Fig. 9C, E) rather than by paleae, as consistently occurs in the disc flowers (Fig. 8E) disrupting the pseudanthial acropetal development (Figs 8C and 9A–C). Furthermore in *Anacyclus*, as in other groups of Asteraceae (Leins and Gemmeke, 1979; Harris *et al.*, 1991; Harris, 1995, 1999), natural populations display a remarkable variation in the perianth and the androecium of the peripheral flowers, as opposed to the constant morphology in disc flowers.

With our developmental and structural evidence, a strong case cannot be made for any of the alternative interpretations of the evolution of peripheral floral buds in the Asteraceae heterogamous capitulum. However, it seems clear that in *Anacyclus* peripheral flowers behave as lateral independent units of the pseudanthial disc from inception, in accordance with the first hypothesis presented.

Capitula diversity in Anacyclus *and associated genetic regulation mechanisms*

Capitula with pentamerous, zygomorphic, bilabiate and bisexual flowers are considered ancestral in this family (Jeffrey, 1977, 2009; Torices *et al.*, 2011). The ray corolla has been interpreted as derived from the disc flower (Small, 1918; Koch, 1930a), although in Anthemideae – a derived tribe within the Asteroideae subfamily (Panero and Funk, 2008) – the presence of heterogamous capitula with bisexual disc flowers and female ray flowers is plesiomorphic (Bremer and Humphries, 1993). The reductive trend of the ray flowers, resulting in homogamous capitula, has occurred several times within the tribe (Bremer and Humphries, 1993) and seems to respond to microevolutionary events not only associated with the origin of new species. In well known cases such as *Senecio* (Senecioneae) and the Silversword alliance (Madieae), the transition from rayed (heterogamous) to discoid (homogamous) heads is associated with hybridization events (Stace, 1977; Carr *et al.*, 1996; Andersson, 2001; Kadereit *et al.*, 2006; Chapman and Abbott, 2009). Although the phenotype plasticity of the peripheral flowers in *Anacyclus* (Fig. 2A–F) could be linked to hybridization in natural populations, it would be necessary to test epigenetic factors in the molecular and physical/morphological contexts directly involved in the origin and adaptation of these and novel phenotypes (Hilu, 1983). In *Anacyclus* there is experimental evidence (Humphries, 1981) and complementary field observations of intermediate phenotypes (I. Alvarez, Real Jardín Botánico-CSIC, unpubl. res.) accounting for the occurrence of hybridization between homogamous and heterogamous species.

Looking at this capitula diversity, we observe that the floral symmetry is the most variable morphological trait in *Anacyclus*. Symmetry is a multiform morphological feature, genetically regulated and set up at various stages of floral development (Jabbour *et al.*, 2009). The candidate gene approach is currently implemented in the study of the flower symmetry differentiating expression patterns and genetics of *CYCLOIDEA* (*CYC*) and associated genes such as *DICHOTOMA* (*DICH*) and *RADIALIS* (*RAD*) (for further review of these genes, see, for example, Hileman and Cubas, 2009; Preston and Hileman, 2009; Preston *et al.*, 2011a). Because in eudicots the evolution of *CYC* and associated genes proceeds through gene duplication (Zhang *et al.*, 2010; Howarth *et al.*, 2011) and through heterotopic (Hileman *et al.*, 2003; Gao *et al.*, 2008; Howarth *et al.*, 2011; Preston *et al.*, 2011b) and heterochronic (Busch and Zachgo, 2007; Zhou *et al.*, 2008; Zhang *et al.*, 2010) changes during floral ontogeny, it is worth exploring their activity in different species even within the same family.

Within Asteraceae in *Gerbera hybrida* Hort (Mutisieae), the *CYC*-like homologues *GhCYC2* and *GhCYC3* are expressed in ray flower buds (Broholm *et al.*, 2008; Tähtiharju *et al.*, 2012). However, the overexpression of *GhCYC2* produces longer zygomorphic disc flowers with fused perianth lobes and shorter ray flowers (Broholm *et al.*, 2008). The overexpression of the *CYC* homologue *RAY1* in *Senecio* in non-radiate individuals in a radiate background also produced shorter ray flowers forming discoid-like capitula (Kim *et al.*, 2008), as occurs in the peripheral flowers of some individuals of *A. alboranenesis*, *A. inconstans* and *A. valentinus*, with very short or null lamina (Figs 1F–H and 2A, C). In contrast, in *Senecio vulgaris*, the overexpression of the *CYC2* orthologue (*RAY2*) does not affect the disc morphology like *GhCYC2*. The overexpression of *RAY2* in a radiate background of *S. vulgaris* also produces tubular ventralized 'trumpet'-like ray florets (Kim *et al.*, 2008). The 'trumpet'-like morphology in Asteraceae is one of the possible phenotypes found in hybrid populations from rayed and discoid parents in genera such as *Senecio* (see fig. 1 in Andersson, 2001), *Layia* (see Fig. 1I in Ford and Gottlieb, 1989) and *Dubautia* (Carr *et al.*, 1996). This phenotype in the tubular ray flower of *Helianthus annuus* ('*tub*') is associated in part with transposon mutagenesis (Fambrini *et al.*, 2007; Chapman *et al.*, 2012).

Expression changes of *CYC* genes in time (heterochrony) and space (heterotopy) can be regulating and/or maintaining the observed floral morphological diversity. In the case of *Anacyclus*, hybridization seems to be a common phenomenon (Humphries, 1981; unpubl. res.) among heterogamous (*A. alboranensis*, *A. clavatus* and *A. valentinus*) and homogamous (*A. homogamos*) species, and could be the cause of heterochronic and heterotopic developmental changes affecting the capitula variability.

Floral whorl variation in Anacyclus

Four different flower types were recorded in the inflorescences of *Anacyclus* (Fig. 5). They can be organized in a sequence from the most complex (i.e. the disc bisexual flower) to the simplest (i.e. the ray female flower). When only the perianth whorl is considered, there are three phenotypes:

pentamerous and (late) zygomorphic with the two enlarged adaxial lobes (Fig. 5A), pentamerous and actinomorphic with all perianth lobes equal (Fig. 5C, D) and trimerous and (early) zygomorphic with arrested adaxial lobes (Fig. 5B). When the androecium is the whorl considered, three different phenotypes can also be described: five adult stamens (Fig. 5A, C), five aborted stamen primordia (Fig. 5D) and two abaxial aborted stamen primordia (adaxial or lateral stamen primordia do not initiate, Fig. 5B). Thus, although four flower types can be distinguished in *Anacyclus* (Fig. 5), they consist of three variants of the perianth and three variants of the androecium, which are not correlated. The perianth is affected particularly in the adaxial/abaxial side of the floral bud, whereas the androecium is affected by total or partial (adaxial/lateral) reduction of the stamen primordia. The androecium can be similar in flowers with different perianth (e.g. Fig. 5A, C). Likewise, comparing the 'trumpet' phenotypes (Fig. 5C, D), the perianth is similar but the androecium is different. This observation in *Anacyclus*, where zygomorphic flowers are associated with a reduction in the number of stamens, has been reported as a generalized trend for the Asteridae (Jabbour *et al.*, 2008).

In contrast, the gynoecium basic pseudomonomerous structure does not change; it is a stable whorl and is usually fertile. The stylopodium, a nectary topologically associated with the gynoecium (Ronse De Craene, 2010), is absent in the ray female flowers of *Anacyclus* (Fig. 5B) unlike other Asteraceae where ray flower nectaries are prominent (e.g. *Otopappus*, Heliantheae; Anderson *et al.*, 1979). Even though the presence of the stylopodium is not associated with obvious floral symmetry changes in *Anacyclus* (Fig. 5), it seems that it is the innermost vulnerable organ to be modified in the ray peripheral flower. In the other floral phenotypes. it is as stable as the gynoecium. Therefore, in the *Anacyclus* pseudanthium, the sexual and morphological differentiation of the flowers is restricted to the outermost whorls, the perianth and the androecium.

The pappus-less flowers and a novel perianth interpretation

In *Anacyclus*, the pappus is absent (Bremer and Humphries, 1993), and in disc flowers there are apical adaxial projections (corona) on the achenes continuous with the wings (Humphries, 1979: p. 109, see in Figs 4 and 7M in this study). In *Anacyclus*, organogenesis proceeds from the floral bud stage to initiation of the corolla and the androecium, finishing with emergence of the gynoecium. No traces of lateral 'pappus/calyx-like' primordia were obvious (Fig. 8M). Peripheral flowers of the capitulum do not present corona (Fig. 9I). From the basic types of pappus initiation identified, Harris (1995) distinguishes three patterns: the sequential (when alternating with the corolla lobes, see figs 66 and 87 in Harris, 1995), the random (when it locates in the available space) and the ring-shaped meristem (fig. 55 in Harris, 1995). None of these patterns correspond to the origin of the corona in *Anacyclus*, which results from late developed enations of hypanthial origin, definitely discarding a calycine nature. Alternatively, based on vascularization patterns, it has been considered that in Asteraceae the calyx is fused to the ray corolla somehow keeping its identity as an outer whorl (Koch, 1930*a*, *b*). Asteraceae has a singular petal anatomy –manifested

by, for example, the suppression of the midvein of the petal lobes (Stebbins, 1977) or the fusion of the lateral veins of adjacent petals (Gustafsson and Bremer, 1995; Gustafsson, 1995) – which could be the product of either fusion or reduction processes of perianth vascularization. In *Anacyclus*, there are several bundles associated with the collenchyma tissue in the ray perianth that could be associated with a fused calyx, but in the disc flowers there is nothing similar to this tissue. Still, due the differential behaviour of disc and ray flowers in terms of morphology and development, it is possible that the loss/reduction of the calyx could also be differential.

Evidence such as the lack of homology between the hypanthial appendages on the disc flowers of *Anacyclus* and a calyx structure, the relatively rapid perianth ontogeny and its thick, bilayered structure might back up the hypothesis that the known 'corolla' in this genus could actually be a double-sided perianth with sepal properties on the abaxial side and petal identity on the adaxial side. There are also some differential expression patterns/effects of certain B-class genes in the perianth of *G. hybrida* Hort. (Broholm *et al.*, 2010). However, no compelling evidence is yet available to interpret the perianth as a double-whorled organ.

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