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Evolutionary origin of the Asteraceae capitulum: Insights from Calyceraceae[1]

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- *Premise of the study:* Phylogenies based on molecular data are revealing that generalizations about complex morphological structures often obscure variation and developmental patterns important for understanding the evolution of forms, as is the case for inflorescence morphology within the well-supported MGCA clade (Menyanthaceae + Goodeniaceae + Calyceraceae + Asteraceae). While the basal families share a basic thyrsic/thyrsoid structure of their inflorescences, Asteraceae possesses a capitulum that is widely interpreted as a racemose, condensed inflorescence. Elucidating the poorly known inflorescence structure of Calyceraceae, sister to Asteraceae, should help clarify how the Asteraceae capitulum evolved from thyrsic/thyrsoid inflorescences.
- *Methods:* The early development and structure of the inflorescence of eight species (five genera) of Calyceraceae were studied by SEM, and patterns of evolutionary change were interpreted via phylogenetic character mapping.
- *Key results:* The basic inflorescence structure of Calyceraceae is a cephalioid (a very condensed botryoid/thyrsoid). Optimization of inflorescence characters on a DNA sequence-derived tree suggests that the Asteraceae capitulum derives from a simple cephalioid through two morphological changes: loss of the terminal flower and suppression of the cymose branching pattern in the peripheral branches.
- *Conclusions:* Widely understood as a condensed raceme, the Asteraceae capitulum is the evolutionary result of a very reduced, condensed thyrsoid. Starting from that point, evolution worked separately only on the racemose developmental control/pattern within Asteraceae and mainly on the cymose developmental control/pattern within Calyceraceae, producing head-like inflorescences in both groups but with very different diversification potential. We also discuss possible remnants of the ancestral cephalioid structure in some Asteraceae.

Key words: Asteraceae; Calyceraceae; capitulum; evolution; inflorescence; MGCA clade.

The interpretation of complex plant structures has been historically based on generalizations of comparative morphology, anatomy, and development. But the relatively recent development of phylogenies based on molecular data are revealing that such morphological generalizations often obscure variation and developmental patterns important for understanding the evolution of diversity of forms, key innovations, and evolutionary constraints or potential. Among angiosperms, the inflorescences of Asteraceae and its near relatives well illustrate the problem that generalizations pose to understanding the evolution of complex structures. Within the flowering plant order Asterales, the Menyanthaceae, Goodeniaceae, Calyceraceae, and Asteraceae form a clade (the MGCA clade) that is well supported by both molecular and morphological data (Lundberg and Bremer, 2003; Lundberg, 2009; Tank and Donoghue, 2010). Within this clade, inflorescence morphology is varied and evolutionarily poorly understood.

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The basic inflorescence of the basal families of the MGCA clade is the thyrse and thyrsoid (Fig. 1). Both thyrses and thyrsoids combine a racemose branching pattern of the main axis with a cymose branching pattern at the proximal/basal lateral first-order branches (Endress, 2010). The apical meristem of the main axis of thyrses remains open, but produces a terminal flower in thyrsoids. When the proximal/basal lateral branches of a thyrsoid are much reduced or suppressed, it is named a botryoid to be distinguished from a true raceme or bostryx; in botryoids, the terminal flower still remains. In a more detailed approach, the most frequent inflorescence in Menyanthaceae, sister to the remaining families of the MGCA clade, is a thyrsoid/botryoid (e.g., *Menyanthes trifoliata* L., Troll, 1964; Troll and Weberling, 1989), sometimes reduced to a solitary flower (*Liparophyllum gunii* Hook. f., Troll and Weberling, 1989). Although Goodeniaceae includes several types of inflorescences, the basic structure in this family is the thyrse or the thyrsoid with different development of the first-order branches. These branches can be all one-flowered, all cymes, or have both in the same inflorescence (cymes at the base of the main axis, one-flowered branches toward the apex). Some Goodeniaceae also have condensed forms of their inflorescences (see Carolin, 1967; Rajput and Carolin, 1988; Carolin et al., 1992). Thus, while the two basalmost families of the MGCA clade share a basic thyrsic/thyrsoid structure of their inflorescences, Asteraceae possesses a capitulum (Fig. 1; primary capitulum sensu Harris, 1999) that is widely interpreted as a racemose, condensed inflorescence with centripetal maturation of flowers. As sister to the sunflower family, Calyceraceae may have the inflorescence

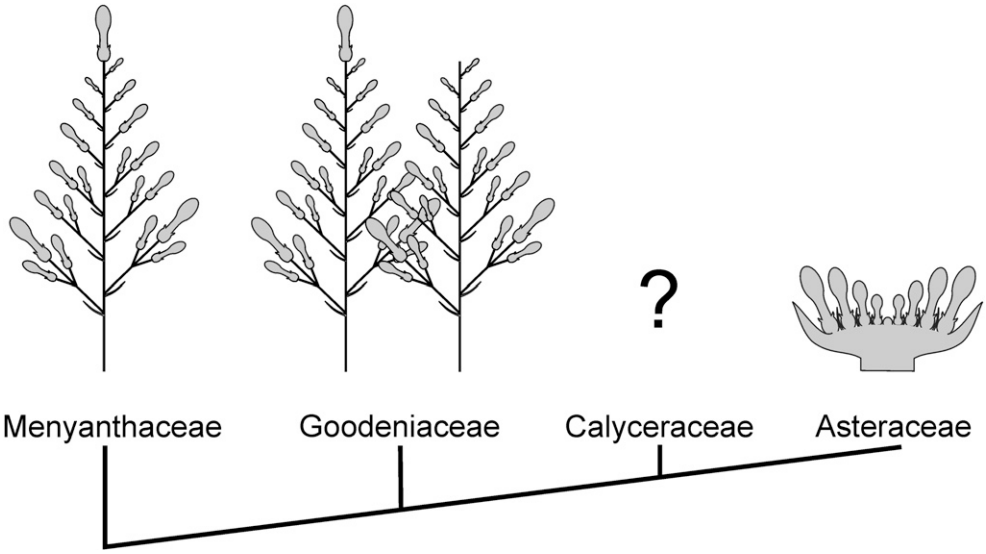

Fig. 1. Basic inflorescence diversity within the MGCA clade. Menyanthaceae based on *Menyanthes trifoliata* L. according to Troll and Weberling (1989: 424). Goodeniaceae based on *Goodenia* (Troll, 1964; Carolin, 1967), *Dampiera* (Rajput and Carolin, 1988; Carolin et al., 1992), *Pentaptilon* and *Verreauxia* (Troll, 1964; Carolin et al., 1992).

structure and diversity that illuminate how the Asteraceae capitulum evolved from thyrsic/thyrsoid inflorescences.

The structure of Calyceraceae inflorescences has been interpreted differently (Fig. 2) since the foundation of the family by Brown (1817). Most taxonomic works describe these inflorescences as capitula or heads (Pontiroli, 1963; Chiapella, 1999; and literature therein). The racemose head structure was also supported by Troll (1964; as "polytelic"), although he recognized basipetal flower maturation in *Boopis*. However, other authors have suggested two additional, very different structures for Calyceraceae inflorescences: compound capitula (Brown, 1825/1834; Harris, 1999) and heads of aggregated cincinni (monochasia also known as scorpioid cymes, Reiche, 1900). DeVore (1994) described the inflorescence of *Acicarpha* as a racemose head (capitulum) and inflorescences of the remaining genera (*Calycera*, *Boopis*, *Moschopsis*, *Nastanthus*, and *Gamocarpha*) as heads composed of cymose subunits. But neither of these interpretations of the Calyceraceae inflorescence has been supported with a published photograph, drawing, or interpretative scheme. Only Harris (1999) published scanning electron microscopy (SEM) photographs illustrating the early inflorescence development of *Calycera leucanthema* Kuntze and some later stages of *C. herbacea* Cav., both cases interpreted as tertiarily condensed capitula (Harris, 1999, p. 361). These incongruent interpretations of the inflorescence structure and lack of adequate documentation are likely attributed, in part, to difficulties in obtaining material of this family. Calyceraceae is a small family with only four to six genera and ca. 54 species endemic to southern South America (Pontiroli, 1963; Chiapella, 1999; Hellwig, 2007; Zanotti and Pozner, 2008). Except for *Acicarpha* and a few species of *Boopis* and *Calycera*, most Calyceraceae grow along the Andes of Chile and Argentina, mainly in Patagonia. Populations are small and infrequent; herbarium material is scarce and usually not well preserved.

To clarify the inflorescence structures in Calyceraceae as a step toward a better interpretation of the origins of the capitulum in Asteraceae, we studied by SEM the early development and structure of the inflorescence of *Boopis anthemoides* and *Nastanthus patagonicus*, analyzed the structure of young inflorescences of *Boopis gracilis*, *Gamocarpha selliana*, *Gamocarpha alpina*, *Calycera crassifolia*, *Acicarpha tribuloides*, and *Acicarpha procumbens*, and interpreted patterns of evolutionary change via phylogenetic character mapping.

MATERIALS AND METHODS

***Structure and development*—**Fresh young inflorescences and buds were collected in the field and fixed in formalin-acetic acid-alcohol (FAA, Ruzin, 1999) during several fieldtrips through Argentinean Patagonia in the summers of 2005–2006 and 2006–2007. Young inflorescences of *Acicarpha* were taken from herbarium material and rehydrated with 1% photographic humectant in water at 60°C for 24 h and then fixed with Craft III (Ruzin, 1999) for a week to

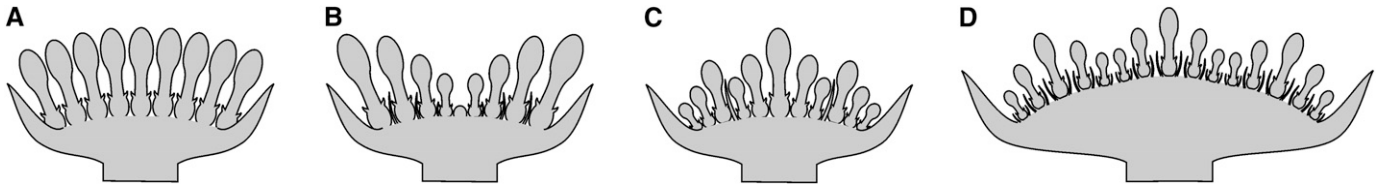

Fig. 2. Inflorescence structures suggested for Calyceraceae. (A) Head (Pontiroli, 1963; Chiapella, 1999). (B) Capitulum (Pontiroli, 1963; Troll, 1964; De Vore, 1994 only for *Acicarpha*; Chiapella, 1999). (C) aggregate of cymose units (Reiche, 1900 for *Boopis*; De Vore, 1994 for *Boopis*, *Calycera*, *Gamocarpha*, *Moschopsis*, and *Nastanthus*). (D) Tertiarily condensed capitulum (Harris, 1999; only *Calycera*).

harden the tissues. All dissected, fixed material (Appendix 1) was dehydrated with a graded ethanol series up to absolute ethanol, transferred to pure acetone, and critical point dried with CO_2 as an intermediate fluid. Photographs were taken with a Philips XL30 microscope of the scanning microcospy service from the Museo Argentino de Ciencias Naturales "Bernardino Rivadavia" (Buenos Aires).

***Phylogenetic reconstruction*—**Relationships among families in the MGCA clade are largely undisputed, and the phylogenetic structure (Menyanthaceae, (Goodeniaceae, (Calyceraceae, Asteraceae))) was used as the basis of interpreting character change in this study. Relationships within Calyceraceae are much less certain, however, and comparative DNA sequencing of the nuclear internal transcribed spacer regions 1 and 2 (ITS; including the 5.8S ribosomal gene; White et al., 1990) combined with three chloroplast intergenic spacers (*trnH–psbA*, Shaw et al., 2005; *ycf6–psbM*, Shaw et al., 2005; and *trnS–trnG*, Hamilton, 1999) was used to resolve relationships among seven of the eight species also examined structurally (quality DNA was not available for the eighth species). DNA was isolated from silica-dried tissue, specific DNA regions amplified via PCR, and sequences obtained following standard protocols reported elsewhere (i.e., Johnson et al., 2008 using primers outlined therein or in the references for each region listed above).

Sequences were aligned by hand using the program Se-Al (Rambaut, 1996) and analyzed separately by region for visual inspection of incongruence and placement of the root within Calyceraceae (varying levels of resolution, but not taxonomic incongruence, was observed), before ultimately combining all regions into a single matrix. Sequence alignment within Calyceraceae was straightforward, and even outgroup sequences were aligned with only a few regions of questionable homology; when encountered, such ambiguous regions were excluded from the final analysis. The final matrix consisted of 14 operational taxononomic units: seven members of Calyceraceae sequenced fully for all regions and seven composite taxa constructed from sequences mostly obtained from GenBank and used to represent each of the other families of the MGCA clade (Appendix 2). Though not presented here, each species of Calyceraceae was sequenced from 2–5 populations with negligible between population variation, and an additional seven species were also sequenced, providing confidence in the resulting phylogenetic structure presented here. Phylogenetic reconstruction employed parsimony with 1000 replications of random addition and 100 000 replications of fast bootstrap analyses as implemented in the program PAUP* 4.0b10 (Swofford, 2003).

***Character mapping*—**Descriptions of inflorescence morphology follow the review of Endress (2010). Inflorescence structure was divided into five binary morphological characters (presence/absence): (1) terminal flower, (2) 1-flowered branches, (3) cymose branches/units, (4) internode elongation of the main axis and pedicels, and (5) involucre differentiation. Menyanthaceae and Goodeniaceae were coded following two criteria: with the plesiomorphic structure, and with all inflorescence diversity considered particularly for Goodeniaceae. Plesiomorphic states were based on Tippery et al. (2008) for Menyanthaceae and on Carolin et al. (1992), Jabaily et al. (2010), and R. S. Jabaily (University of Wisconsin, personal communication) for Goodeniaceae. Inflorescence diversity of Menyanthaceae was taken from Troll (1964) and Troll and Weberling (1989), but we excluded the unusual one-flowered inflorescence of *Liparophyllum gunii*. Inflorescence diversity of Goodeniaceae was based on Carolin (1967), Rajput and Carolin (1988), and Carolin et al. (1992). We optimized characters on the phylogenetic tree recovered from the parsimony analyses described above using the program TNT (Goloboff et al., 2008).

RESULTS

***Structure and development*—**In *Boopis anthemoides*, flower and bract primordia arise acropetally (Fig. 3B, C). The apical meristem (Fig. 3B) finally forms a flower primordium surrounded by 1–3 bracts (Fig. 3C), which is not only as large as or even larger (Fig. 3D–F) than the most basal/peripheral ones, but its development is also more advanced than the neighboring, smaller flower primordia (Fig. 3G). Inflorescences also develop a few cymose units, two flowers each with a monochasial arrangement, at the periphery (Fig. 3D, E) subtended by the outermost (involucral) bracts. The inflorescence of *B. gracilis* follows the same structure. Anthesis begins in the basal/peripheral flowers together with the terminal flower (Fig. 3A), followed by the remaining flowers in acropetal order. Within each two-flowered cymose unit, anthesis also follows the degree of relative size and development of flower buds.

Acicarpha procumbens and *A. tribuloides* have the same structure as *Boopis* (Fig. 4D–F), but without the peripheral cymose units. In the particular case of *A. tribuloides*, the terminal flower may be slightly larger and more advanced in development than the closest surrounding ones (Fig. 4E), or it may have a similar size and stage as those of most neighboring flowers (Fig. 4F).

Inflorescences of *Calycera crassifolia* share the same structure as *Boopis,* but with a greater number of peripheral cymose units (Fig. 4C).

In *Gamocarpha alpina* and *G. selliana*, the zone of individual flowers surrounding the terminal flower is very much reduced (Fig. 4A, B). Cymose units, up to five flowers each, are easily distinguished because bracts are wide, covering and bounding every cymose unit with their sheathing base (Figs. 4A, 4B, 6A–C). Cymose units in *Gamocarpha* may develop up to three flowers with a dichasial arrangement (Figs. 4A, 4B, 6A, 6C) and no prophylls subtending the flowers of the secondary branches.

The complexity of the inflorescences of *N. patagonicus* varies (Fig. 5A–D). The same individual may produce *Boopis*-like inflorescences with a few peripheral, two-flowered cymose units (Fig. 5A) and a total of ca. 30 flowers per inflorescence, to larger inflorescences with up to hundreds of flowers (Fig. 5C, D), where the zone of individual flowers around the terminal flower is very much reduced, and most of the inflorescence area is covered by 2–7-flowered cymose units subtended by a wide, flat bract (Fig. 5C, D). Early stages of large inflorescences show that flower primordia of the cymose units are arranged with a dichasial to monochasial pattern (Figs. 5B, 6E–G), which, when observed at very early stages, seem to repeat the structure of the apical zone of the inflorescence (Fig. 5B). However, the central zone of individual flowers and the boundaries of every cymose group become clearer later, when subtending bracts are more developed (Fig. 5C, D).

A detailed interpretation of the cymose units found in Calyceraceae follows (Fig. 6). The cymose branching pattern (monochasial or dichasial) cannot be seen (even in dissected inflorescences) because of the nature of the flat inflorescence meristem and lack of internode differentiation; hence, the monochasial and dichasial structure is inferred from flower and bract position and relative flower size. Cymose units of *Gamocarpha selliana* are easy to discern because subtending bracts are wide and surround each cymose unit (Fig. 6A–C). Wide outer bracts of the larger inflorescences in *Nastanthus patagonicus* (Figs. 5C, D, 6E) and outermost bracts (involucral bracts) in *Boopis anthemoides* (Fig. 6D), *B. gracilis*, and *Calycera crassifolia* also help to understand the boundaries of every cymose unit. But when bracts are narrow (as it happens with most inner and central bracts), cymose units are not easily discernable from each other (Figs. 5B, 6F, 6G), particularly in *Nastanthus patagonicus* where cymose units may develop up to seven flowers (up to third-order branching; Fig. 6E) with one prophyll subtending a flower of second-order branching (Fig. 6F).

***Phylogenetic reconstruction*—**The combined DNA sequence data set contained 320 parsimony-informative characters, and analyses recovered a single most-parsimonious tree of 1004 steps (introduced later; CI = 0.82, RI = 0.76). All internal branches within Calyceraceae were supported with bootstrap percentages equal to or greater than 85%.

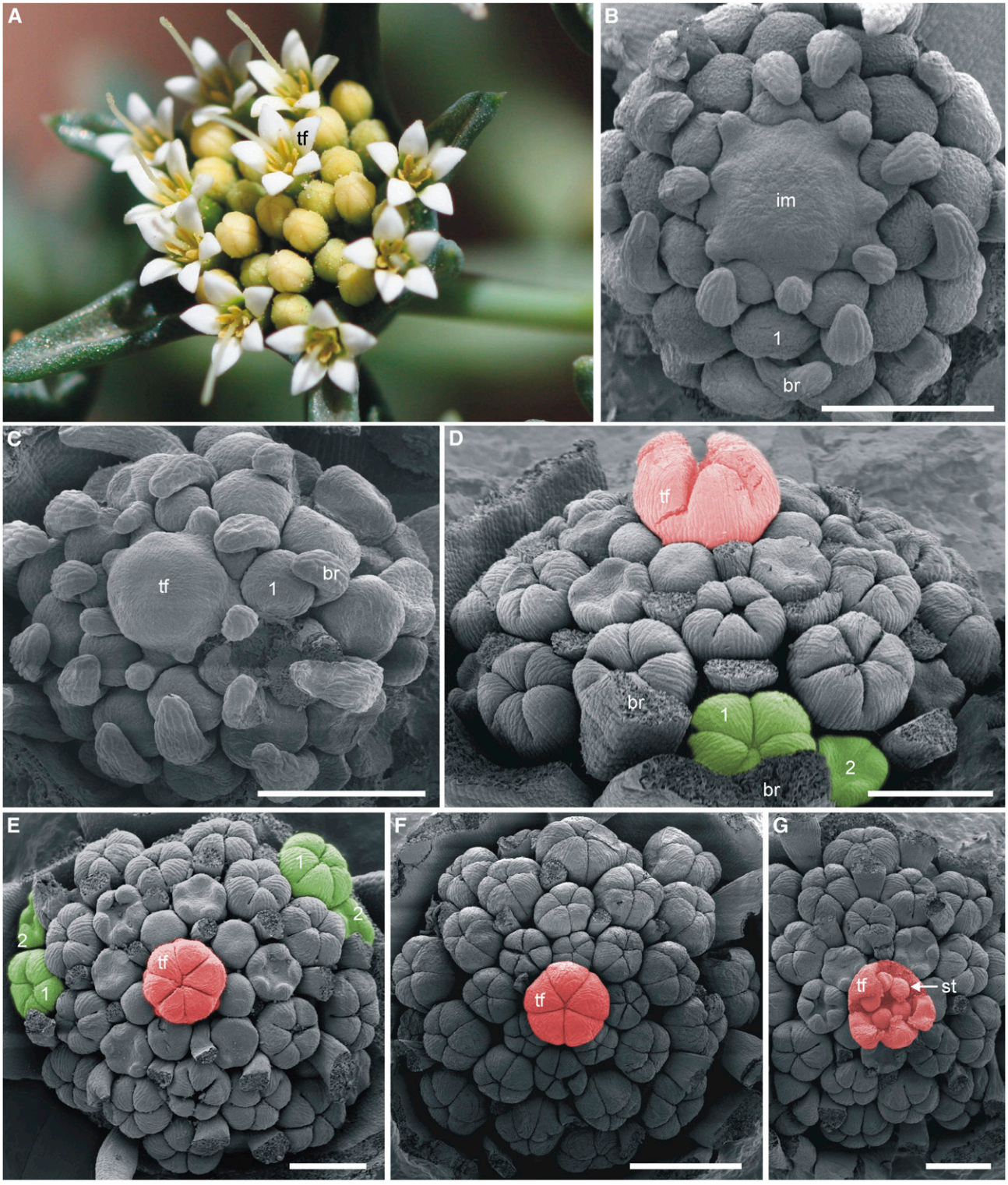

Fig. 3. Inflorescence structure and development in *Boopis*. (A) Photograph of *Boopis gracilis*. (B–G) SEM images of *B. anthemoides*. (A) Inflorescence at the beginning of anthesis; the terminal flower opens together with the peripheral flowers. (B) Early stage of inflorescence development, the inflorescence meristem produces first-order lateral branches meristems with substending bracts. (C) The inflorescence meristem produced the last bracts and begins the differentiation of the terminal flower. (D) Terminal flower, centripetal first-order branching flowers, and peripheral cymose units with second-order branching flower. (E) Upper view of young inflorescence showing terminal flower, centripetal first-order branching flowers, and cymose units. (F) More advanced stage of inflorescence development, in this case with no cymose units. (G) Partial dissection of terminal flower showing that its larger size also corresponds to a more advanced differentiation than that of the closest flowers. *Abbreviations:* 1, 2, 3, branching order of flowers/meristems; br, bract; im, inflorescence meristem; st, stamen primordium; tf, terminal flower; red halftones, terminal flower; some cymose units are colored in green halftones to help their identification within the inflorescence.

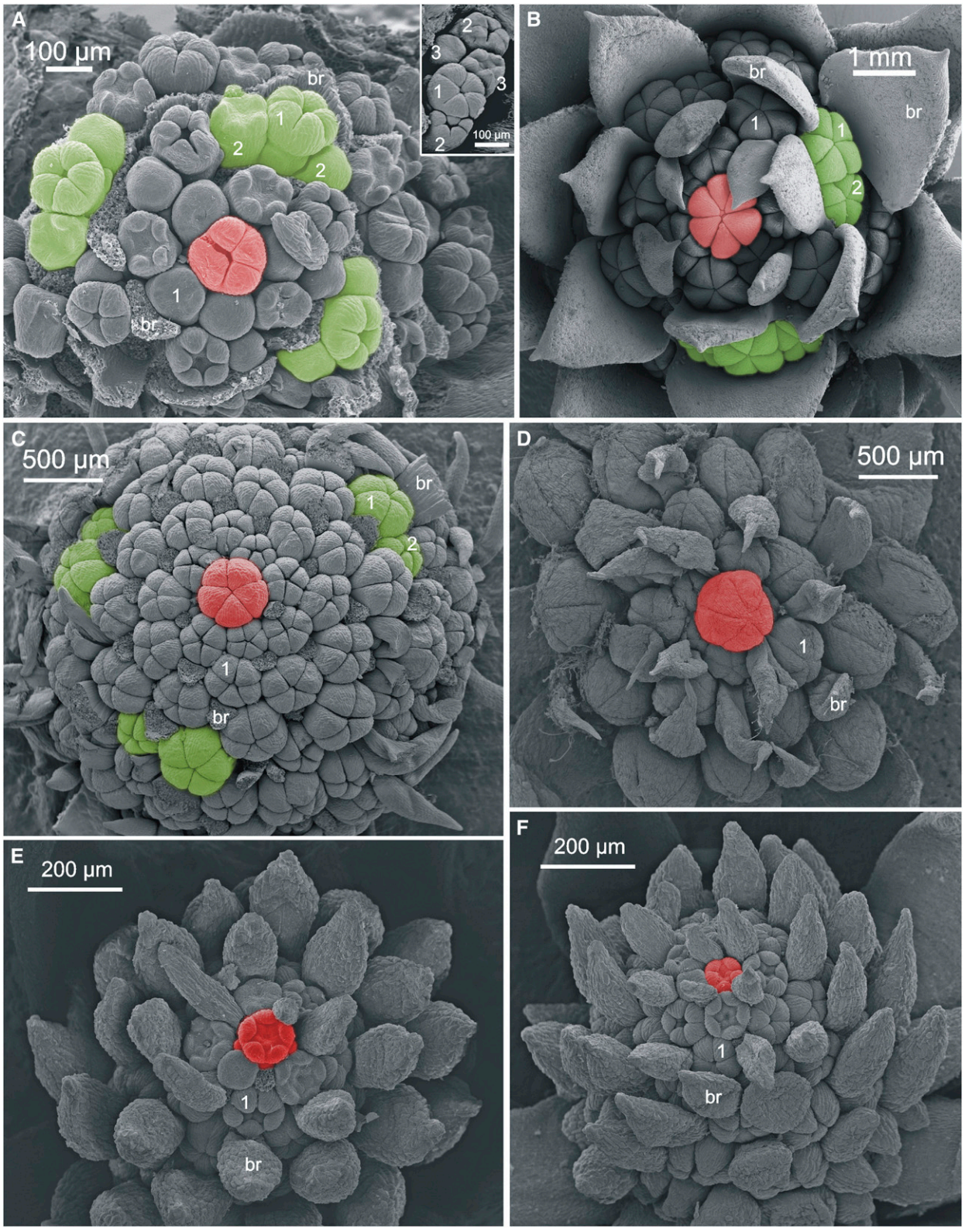

Fig. 4. SEM images of inflorescence structure in *Gamocarpha*, *Calycera*, and *Acicarpha*. (A–B) *Gamocarpha selliana*. (A) Early stage of inflorescence development, bracts partially removed. (B) Later stage, cymose units clearly outlined by the bracts. (C) *Calycera crassifolia*, upper view of young inflorescence showing the terminal flower, one-flowered lateral branches, and cymose, peripheral units. (D) *Acicarpha procumbens*, inflorescence with terminal flower, one-flowered lateral branches and no cymose units. (D, E) *Acicarpha tribuloides*, inflorescence without cymose units and a very reduced terminal flower. *Abbreviations:* 1, 2, 3, branching order of flowers/meristems; br, bract; red halftones, terminal flower; a few cymose units are colored in green halftones to aid identification within the inflorescence.

DISCUSSION

Structure of Calyceraceae inflorescence—The basic Calyceraceae inflorescence is a condensed thyrsoid (formally named a cephalioid) with three main zones or parts: a distal terminal flower, a surrounding group of individual flowers with one subtending bract each, and a peripheral ring of cymose units of 2–7 flowers forming monochasia or dichasia, each unit subtended by a bract. The terminal flower of Calyceraceae inflorescences corresponds to the terminal flower found in Menyanthaceae and some Goodeniaceae thyrsoids/botryoids (Fig. 7), and it is always present in Calyceraceae, although it may be much reduced as in *Acicapha tribuloides*. The solitary flowers surrounding the terminal flower in Calyceraceae correspond to the one-flowered branches of the thyrsoids/botryoids found in Menyanthaceae and Goodeniaceae (Fig. 7). Cymose groups of 2–7 flowers each correspond to the cymose branches of Menyanthaceae and Goodeniaceae inflorescences (Fig. 7). The solitary flowers (one-flowered branches) may form the entire cephalioid, as in *Acicarpha tribuloides* and *A. procumbens*, or most of it, as in *Boopis anthemoides* and *B. gracilis*. In other cases, the individual flowers contribute very little to the inflorescence, as in the cephalioids of *Nastanthus patagonicus*, where the cymose units form most of the head. Therefore, the capituliform inflorescence of Calyceraceae is a very condensed thyrsoid/botryoid with the main axis, branches, and flower pedicels not elongated. Outermost bracts are usually differentiated from the vegetative leaves as involucral bracts and sometimes partially fused (as in *Boopis* and *Nastanthus*); however, some species of *Moschopsis* lack that differentiation (Chiapella, 1999). Inner bracts are usually slender and morphologically different from the outer bracts, and thus they have been called paleae. But they also may be wide, as in *Nastanthus patagonicus*, even sheathing the subtended flowers as in *Gamocarpha selliana*, clearly bounding the cymose units/branches. Basic structural changes needed to transform the Menyanthaceae and Goodeniaceae thyrsoids/botryoids to the cephalioids of Calyceraceae are the lack of elongation of the inflorescence branch system and the differentiation of the outermost bracts to form the involucre (Figs. 8, 9).

Among the previous interpretations of the structure of Calyceraceae inflorescences, only DeVore (1994) and Harris (1999) have published sufficient detail to warrant discussion here. DeVore's (1994) interpretation of the *Acicarpha* inflorescence as a racemose head (capitulum) is not completely accurate because the terminal flower still develops, although it may be

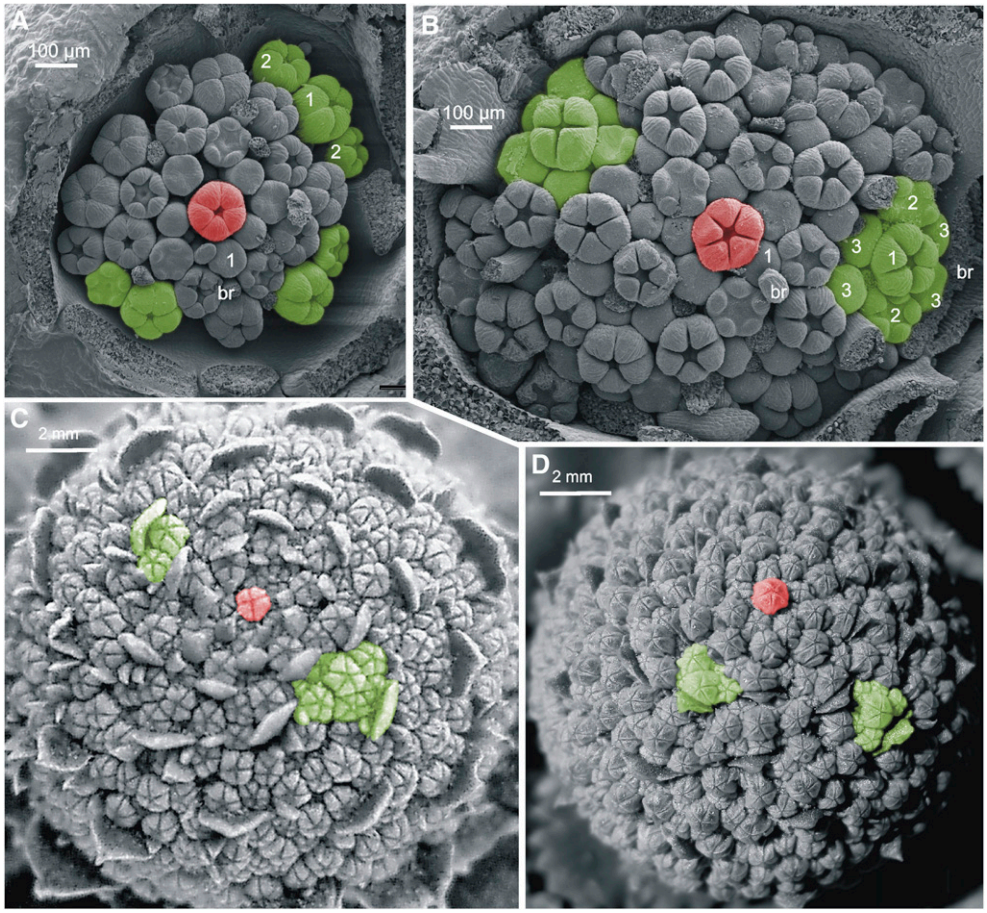

Fig. 5. Inflorescence structure in *Nastanthus patagonicus*. (A) Early stage of development of small inflorescence (SEM), very much alike *Boopis*, cymose units with mono- and dichasial pattern. (B) A larger inflorescence (SEM) where the cymose units produce up to third-order branches following a dichasial pattern. (C, D) Young inflorescences showing high number of flowers, mainly contributed by the cymose units (fresh material). *Abbreviations:* 1, 2, 3, branching order of flowers/meristems; br, bract; red halftones, terminal flower; a few cymose units are colored in green halftones to help their identification within the inflorescence.

similar in size and hardly discernable from the closest surrounding flowers. DeVore's (1994) recognition of the cymose subunits in the inflorescences of the remaining genera (*Calycera*, *Boopis*, *Moschopsis*, *Nastanthus*, and *Gamocarpha*) agrees with our observations. On the other hand, Harris (1999, p. 361) interpreted the inflorescence of *Calycera leucanthema* (Poepp. ex Less.)

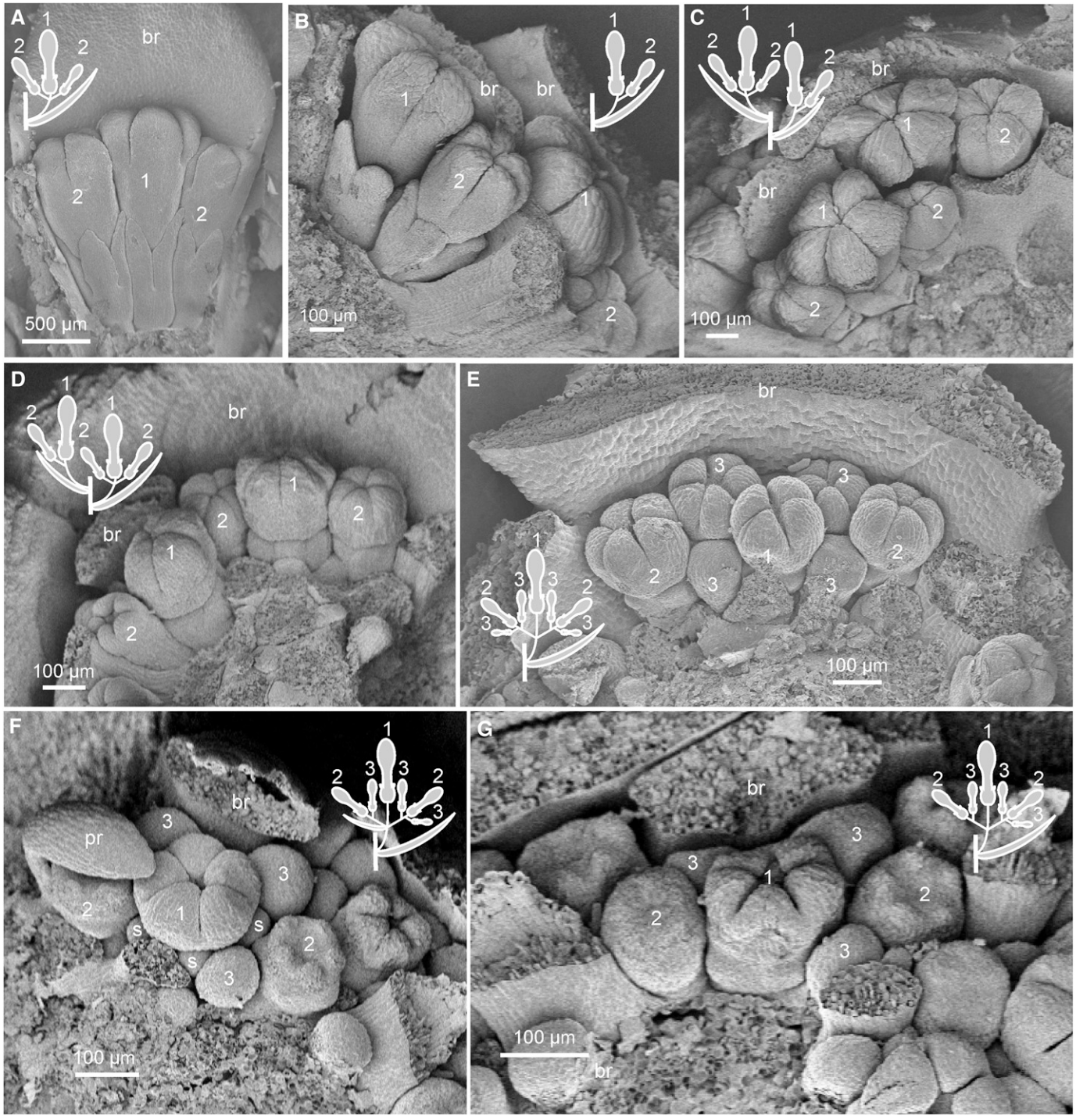

Fig. 6. SEM images of structure of cymose units in Calyceraceae. (A–C) *Gamocarpha selliana*. (A) Dichasium with subtending bract (front of the sheathing base removed to show the flowers). (B) Two monochasia (earlier stage), bract apex and front of the sheathing base were removed to show the flowers. (C) Dichasium (inner) and monochasium (outer). (D) *Boopis anthemoides*, monochasium (inner) and dichasium (outer). (E–G) *Nastanthus patagonicus*, more complex cymose units with 6–7 flowers and up to third-branching order; sometimes one prophyll may develop subtending a flower of the second-order branches (F). Each line drawing shows the interpretation of the cymose braching pattern that cannot be seen because the flat inflorescence meristem lacks internode differentiation; the monochasial and dichasial structure is inferred on flower and bract position, and relative flower size. *Abbreviations:* 1, 2, 3, branching order; br, bract; pr, prophyll.

Kuntze and *C. herbacea* Cav. as tertiarily condsensed capitula, by understanding the cymose units as secondarily condensed capitula made of very reduced, one-flowered capitula. She supported this view with the fact that tertiarily condensed capitula are also found in Goodeniaceae and Brunoniaceae, (apparently based on Carolin, 1978; Erbar and Leins, 1988; specifically in *Brunonia australis* Sm. ex R. Br.; note that *Brunonia* is now included in Goodeniaceae). However, we could not find any evidence in Carolin (1978) and Erbar and Leins (1988) to support the existence of tertiarily condensed capitula in *Brunonia australis*. In fact, Erbar and Leins (1988, p. 264) wrote: "The flowers of *Brunonia* are arranged in condensed partial inflorescences, which are difficult to analyze and have been interpreted as cymose (Abb. 1), gathered in great number into an umbelliform head (about that compare Carolin, 1967)." Philipson (1953) provided a similar interpretation of *Brunonia australis* inflorescences as a condensation of many dichasia, and Carolin (1967) supposed that the individual flowers of each dichasium are in fact reduced partial inflorescences because of some extra sterile bracts around the flowers. In spite of the extra bracts mentioned by Carolin (1967), the cymose nature of *Brunonia australis* partial inflorescences does not change, and they should not be understood as tertiarily condensed capitula. When mapped on the MGCA clade (not shown), the interpretation of Harris (1999), about the inflorescence of *Brunonia australis*, *Calycera leucanthema*, and *C. herbacea* as tertiarily condensed capitula, implies that simple capitula have arisen at least twice (once within Goodeniaceae, and once in the common ancestor of Calyceraceae + Asteraceae), with capitulum condensation (secondarily and tertiarily condensed capitula) occurring independently three times (Goodeniaceae, Calyceraceae and Asteraceae) and with no living taxon bearing simple capitula in the Goodeniaceae and Calyceraceae (*Acicarpha* still develops a terminal flower). As we show next, a simpler hypothesis exists.

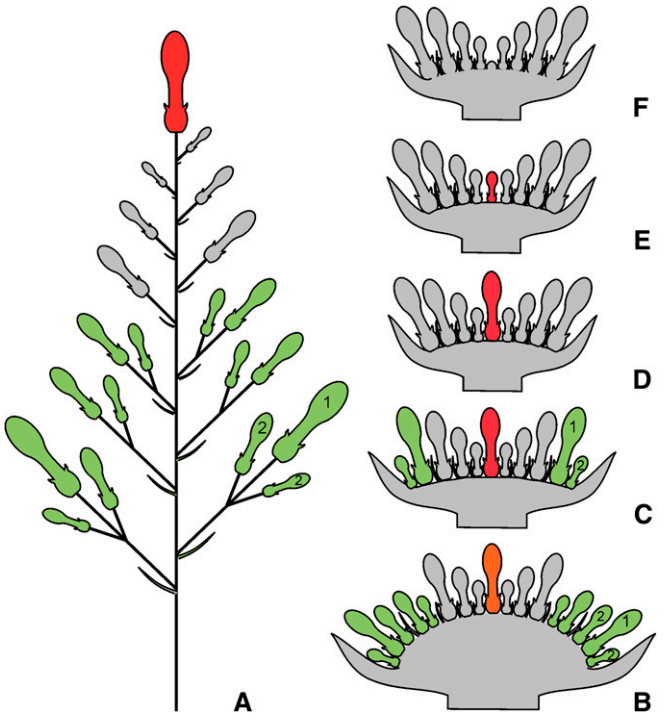

Fig. 7. Homologies among inflorescences of Menyanthaceae, Goodeniaceae, Calyceraceae, and Asteraceae. (A) Basic thyrse of Goodeniaceae and Menyanthaceae. (B–E) Cephalioids of Calyceraceae. (B) *Nastanthus* and *Gamocarpha*. (C) *Calycera* and *Boopis*. (D) *Boopis* and *Acicarpha*. (E) *Acicarpha*. (F) Capitulum of Asteraceae. Terminal flower (red); one-flowered lateral branches (gray); cymose lateral branches/units (green); involucral bracts, inner bracts, receptacle and apical meristem of the capitulum (F) also in gray; 1, 2, branching order of flowers.

The evolutionary origin of the Asteraceae capitulum—Cronquist (1955, 1977) suggested a cymose origin for the capitulum of Asteraceae based on the cymose branching and centrifugal flowering sequence of the capitulescences (the branch system bearing capitula). He proposed that the ancestor of the Asteraceae capitulum had a cymose inflorescence that was condensed into a head and turned into a racemose pattern. But Cronquist based his view on an obsolete use of "cymose" for a racemose inflorescence with a terminal flower (cf. Endress, 2010), with subsequent condensation and loss of the terminal flower. Harris (1999) rejected Cronquist's hypothesis, dismissing the evidence used by Cronquist and arguing instead that early stages of development of *Lobelia* racemes are almost identical to those of simple capitula (Harris, 1999, fig. 7, pp. 364–365). However, Erbar and Leins (2000) re-introduced the possibility that the ancestor of the Asteraceae may have had a complex inflorescence based on the loose arrangement of the last, central-most flower primordia of *Arnaldoa macbrideana* Ferreyra (Barnadesioideae).

Given that phylogenetic analyses of the Asterales based on molecular and morphological data (Lundberg and Bremer 2003, and literature therein) strongly support the MGCA clade, a thyrsic/thyrsoid origin of the Asteraceae capitulum is better supported by available data. Given this phylogenetic context, the central questions are (1) what structural changes are required to transform the thyrsic/thyrsoid basic inflorescence of Menyanthaceae and Goodeniaceae to the capitulum of the Asteraceae, and (2) what morphological evidence is available to support any hypothesis.

Our investigation of the inflorescence structure and development of representative members of Calyceraceae answers both questions. The basic cephalioid structure of Calyceraceae inflorescences is structurally homologous to noncondensed thyrsoids/botryoids of Menyanthaceae (like *Menyanthes trifoliata*, see Troll, 1964; Troll and Weberling, 1989) and to the thyrsoids of some Goodeniaceae, such as some species of *Dampiera* (Rajput and Carolin, 1988; Carolin et al., 1992), and *Pentaptilon* and *Verreauxia* (Troll, 1964; Carolin et al., 1992). According to Endress (2010), thyrses and thyrsoids have a primary racemose structure due to their many (more than two) primary branches, and a cymose structure in their second and following orders of branching. Thus, in the evolutionary origin of the Asteraceae capitulum, the inflorescence kept the racemose pattern of the primary branching of the ancestral thyrsoid, suppressing any further level of branching, internode elongation, and the terminal flower (Figs. 8, 9).

Supporting this hypothesis morphologically, the optimization of inflorescence characters upon the MGCA clade (including all Calyceraceae species studied here), reveals a general pattern of inflorescence evolution within the MGCA clade that enables the reconstruction of the ancestral inflorescence of Calyceraceae + Asteraceae. Inflorescence diversity in Menyanthaceae includes thysoids and botryoids (Troll, 1964; Troll and Weberling, 1989, not considering the unusual one-flowered inflorescence of *Liparophyllum gunii*), and both structures may

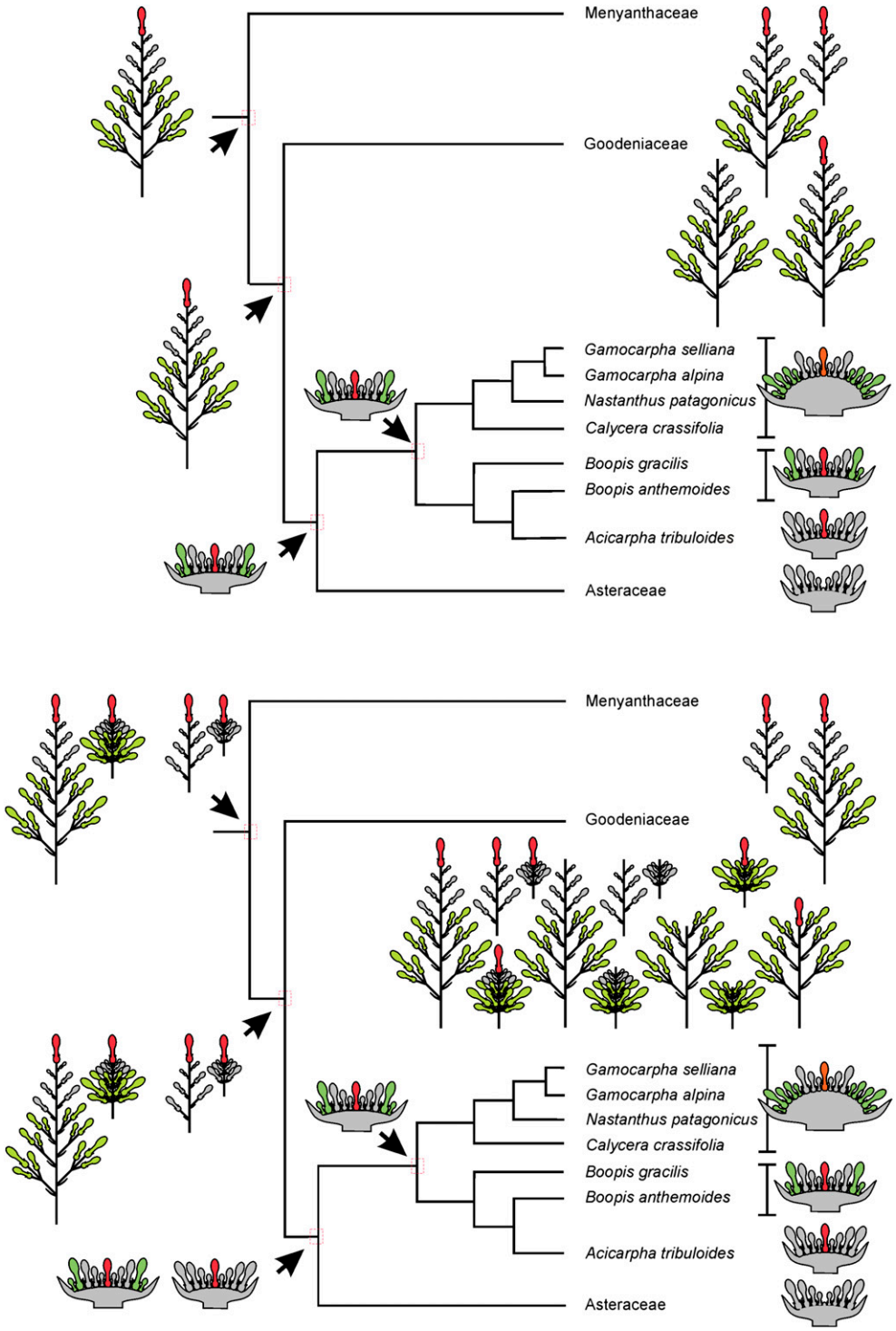

Fig. 8. Optimization of inflorescence characters on a molecular tree of the MGCA clade with some resolution within Calyceraceae, based on the species here studied. Basal families (Menyanthaceae and Goodeniaceae) were coded with the plesiomorphic states (upper tree) or with all possible states (particularly Goodeniaceae, lower tree). In both cases, the ancestor of the Calyceraceae, and the ancestor of Calyceraceae + Asteraceae show a simple cephalioid (as *Acicarpha* or *Boopis*). Cephalioids within Calyceraceae seem to have evolved toward richer forms by the development of more and larger cymose units, while the Asteraceae capitulum arose by the truncation of simple cephalioids and loss of cymose units. Terminal flower (red); one-flowered lateral branches (gray); cymose lateral branches/units (green); involucral bracts, inner bracts, receptacle and apical meristem of the capitulum also in gray.

be considered plesiomorphic following Tippery et al. (2008). The Goodeniaceae show a wide diversity of inflorescences, with different modifications of the basic thyrsoid (*Pentaptilon*, *Verreauxia*) or thyrsic (*Goodenia*) structure, sometimes condensed (*Anthotium*, *Brunonia*, *Dampiera wellsiana*, and *D. eriocephala*) or not, with or without mono/dichasial lateral

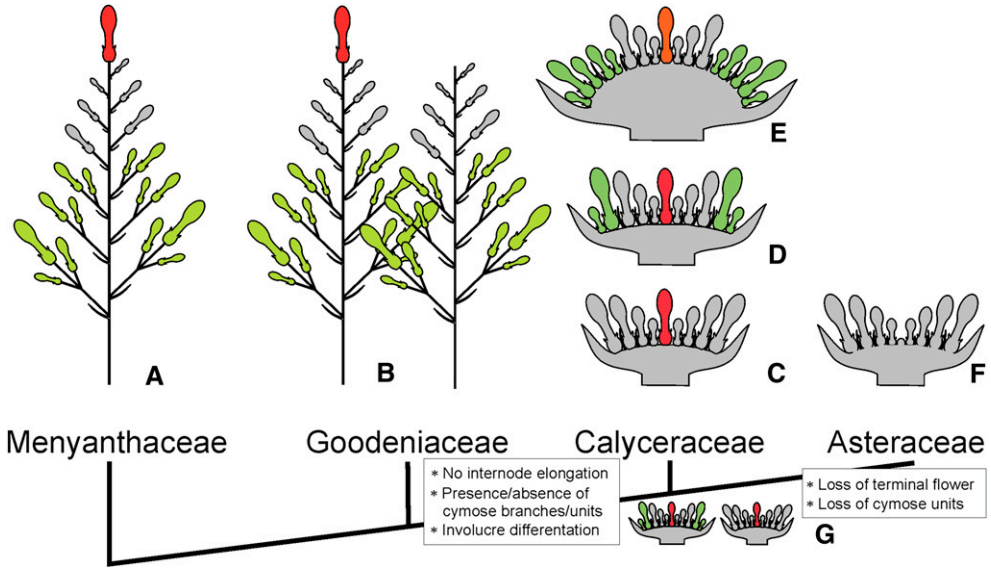

Fig. 9. Proposal for the evolutionary origin of the Asteraceae capitulum. (A) *Menyanthes trifoliata*. (B) *Goodenia*, *Dampiera*, *Pentaptilon*, and *Verreauxia*. (C) *Acicarpha* and *Boopis*. (D) *Boopis* and *Calycera*. (E) *Gamocarpha* and *Nastanthus*. (F) Typical capitulum of Asteraceae. (G) Hypothetical ancestor inflorescence of the clade Asteraceae + Calyceraceae. Terminal flower (red); one-flowered lateral branches (gray); cymose lateral branches/units (green); involucral bracts, inner bracts, receptacle, and apical meristem of the capitulum also in gray.

first order branches (in the latter case, the inflorescence is a raceme as in *Dampiera wellsiana*, or a botryoid if there is a terminal flower), with or without one-flowered lateral branches (i.e., a cyme, as in *Velleia*). A preliminary molecular phylogeny of the Goodeniaceae (Jabaily et al., 2010) proposes two main clades: the *Dampiera/Lechenaultia/Anthotium* group and the *Scaevola*/*Goodenia* (including *Verrauxia* and *Velleia*)/*Coopernookia*/*Brunonia* (plus other monotypic genera) group. Although it is not possible to choose a basalmost genus in the Goodeniaceae phylogeny (R. S. Jabaily, personal communication), we took the thyrse and the thyrsoid as the plesiomorphic structures for Goodeniaceae based on Rajput and Carolin (1988) and Carolin et al. (1992). Following optimizations of inflorescence characters (Fig. 8), the thyrsoid would be the ancestral inflorescence structure within the MGCA clade, present in most Menyanthaceae and some Goodeniaceae. When Menyanthaceae and Goodeniacae are coded as polymorphic, with truncate, nontruncate, condensed, not condensed, and cymose lateral first-order branches present or not (Fig. 8, lower half), the ancestor of the MGCA clade also includes impoverished and condensed thyrsoids, and the ancestor of the Calyceraceae + Asteraceae clade exhibits simple cephalioids, with (e.g., *Boopis*) or without (e.g., *Acicarpha*) peripheral cymose units. When Menyanthaceae and Goodeniacae are coded with the plesiomorphic inflorescence structures (Fig. 8, upper half) the ancestor of Calyceraceae + Asteraceae shows simple *Boopis*-like cephalioids with peripheral cymose units. In both cases, the simple capitulum appears only in Asteraceae. Although our phylogeny of Calyceraceae does not include all taxa, the optimization of the inflorescence characters suggests that simple cephalioids, as in *Acicarpha* and *Boopis*, seem to be most primitive in the family, and more complex cephalioids as in *Calycera*, *Nastanthus*, *Boopis*, and *Gamocarpha* represent a secondary enrichment of the inflorescence within Calyceraceae due to the proliferation of cymose peripheral branches. Thus, Asteraceae and Calyceraceae, although phylogenetically very close, based their inflorescence diversification on two different developmental controls: the cymose pattern (Calyceraceae) and the racemose pattern (Asteraceae). While the number of flowers per inflorescence in Calyceraceae mainly varies by changing the developmental control of the first order branch meristems to a cymose pattern, Asteraceae modifies the number of flowers per capitulum through the activity of the apical (central) inflorescence meristem with a racemose pattern. Both developmental controls are present in the thyrsoids of Menyanthaceae and Goodeniaceae and presumably present in the ancestor of Calyceraceae and Asteraceae. Thus, starting from a condensed structure with both developmental controls (the cephalioid), evolution worked separately on the racemose developmental control/pattern within Asteraceae and mainly on the cymose developmental control/pattern within Calyceraceae, producing head-like inflorescences in both groups but with very different diversification potential. Asteraceae heads evolved central (disc) and peripheral (ray) floral forms and extreme flower polymorphism with different functions, transforming the whole inflorescence into a new reproductive unit (a pseudanthium). In contrast, flowers in Calyceraceae inflorescences remained poorly differentiated, although there is some floral differentiation. *Acicarpha*, for instance, is a well-known example of cephalioids with perfect peripheral flowers and staminate central-apical flowers. The calyx dimorphism observed in the fruits of *Calycera* species is already established in the flower, and cephalioids produce polymorphic flowers in terms of hypanthium length (L. M. Zavala-Gallo, S. Denham, and R. Pozner, Instituto de Botánica Darwinion, unpublished manuscript). There may be more cases of flower polymorphism that still need confirmation in Calyceraceae, like *Nastanthus* (Zavala-Gallo et al., 2010), but compared to the pseudanthia of Asteraceae, inflorescences of Calyceraceae remain just a bunch of little flowers.

It is worth considering if any remnant of the cephalioid ancestral structure (i.e., a terminal central flower and cymose peripheral units) can be found in Asteraceae capitula. Developmental studies of Asteraceae capitula have typically paid little attention to the apical/central meristem in late developmental

stages (usually, SEM photographs of capitulum development were not taken to see what happens to the apical meristem at the end of its growing activity). However, the analysis of taxonomic literature and published images of the last stages of the apical meristem of the capitulum suggests that there is still the possibility of finding remnants of the terminal flower in Asteraceae. In *Gnaphalium purpureum* L. (Harris, 1995), *Arnaldoa macbrideana* (Erbar and Leins, 2000), and *Gerbera hybrida* (Teeri et al., 2006), for instance, the apical meristem does not remain "open", and eventually produces some flower primordia with a similar degree of development; however, these do not follow the spiral arrangement of the outer flowers so that it is difficult to choose a flower as the terminal one. Some *Barnadesia* species have a central flower and eight surrounding flowers per capitulum (Urtubey, 1999), and *Huarpea andina* A. L. Cabrera, produces one central flower and five surrounding flowers per capitulum (Cabrera, 1951). Considering the basal condition of the Barnadesioideae within Asteraceae, it would be interesting to determine whether the central flower of those *Barnadesia* and *Huarpea* is the terminal flower of the inflorescence. In addition, very reduced one-flowered capitula, as those of *Fulcaldea*, could produce only one terminal flower each.

In searching for cymose peripheral units in Asteraceae, attention was paid to the development of peripheral, centrifugal (basipetal) flowers in some Asteraceae such as *Erigeron philadelphicus* (Harris et al., 1991), *Centaurea melitensis* (Porras and Muñoz, 2000), *Cosmos bipinnatus* (Molder and Owens, 1973), *Layia glandulosa* (Gottlieb and Ford, 1987), and *Bellis perennis* L. (Leins and Erbar, 1987; see Harris, 1995 for a complete list of Asteraceae with exceptions to the acropetal initiation of flowers). After the initiation of the flat inflorescence apex, in these species disc flowers are initiated acropetally, and peripheral ray flowers are initiated basipetally. The later basipetal inception and initially delayed development of peripheral ray flowers were interpreted as being related to (1) the ligulate morphology and carpellate or neutral function or (2) the possibility of capitulum condensation (Harris, 1995), so that the ray flowers are not "true flowers" but very reduced primary capitula gathered with the disc flowers in a secondarily condensed capitulum. However, considering the morphological evidence and the evolutionary hypothesis presented here, we suggest that those marginal, later ray flowers produced in basipetal order may be understood as remnants of the cymose peripheral units still present in Calyceraceae. Furthermore, because flower dimorphism of the ray flowers is related to the position and relative inception of flower meristems, the evolution of the genetic control of the ray flowers might be, in some way, related to the cymose genetic control of the peripheral lateral branches of the Calyceraceae.

***Conclusions*—**The MGCA clade is an interesting model for inflorescence evolution because it allows one to test historical interpretations of complex structures with a well-supported phylogenetic hypothesis (Figs. 8, 9). The basalmost family, Menyanthaceae, produces thyrsoids, a structure that combines both racemose and cymose patterns/developmental controls. The next basal family, Goodeniaceae, shows a wide variety of inflorescence structure also combining both patterns/developmental controls, with or without a terminal flower. Calyceraceae has intermediate thyrsic, condensed forms that connect the expanded thyrsoid inflorescences to the inflorescence of Asteraceae, which shows an extremely diverse radiation of the condensed, racemose pattern/developmental control. The production of a terminal flower, a fundamental part of Troll's typology (Troll, 1964), appears less important than the combination of branching patterns and developmental controls (cf. Endress, 2010). The Asteraceae capitulum comes from an ancestral complex inflorescence with both racemose and cymose developmental controls. This mixed developmental origin may be related in some way to the evolution of floral dimorphism in Asteraceae, with the peripheral (ray) flowers developing on meristems under a different developmental control (cymose) than the central (disc) flowers (racemose). This complex evolutionary origin is likely the reason that the capitula of Asteraceae have diversified extensively in form beyond other capituliform, racemose inflorescences in other angiosperm families.

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APPENDIX 1. Vouchers of the species studied by SEM; all are housed at SI (Instituto de Botánica Darwinion). Those marked with an asterisk (*) are the reference for the photographs of fresh material.

Taxon — **Collection site**: *Vouchers*.

Acicarpha procumbens Less. — **Argentina**: *Burkart, A. & Bacigalupo, N. M. 21436.*

Acicarpha tribuloides Juss. — **Argentina**: *J. Hurrell, E. Ulibarri, F. Mallard & D. Bazzano 5300, J. Hurrell, E. Ulibarri & D. Bazzano 5940.*

Boopis anthemoides Juss. var. ***anthemoides*** — **Argentina**: *Winter, J. 1.; Zanotti, C. A. 60.*

Boopis gracilis Phil. **Argentina**: *Pozner, R. et al. 543(*).*

Calycera crassifolia (Miers) Hicken — **Argentina**: *Zanotti, C. A. 61.*

Gamocarpha alpina (Poepp. ex Less.) H. V. Hansen — **Argentina**: *Winter, J. 2.*

Gamocarpha selliana Reiche — **Argentina**: *Picca, P. 324.*

Nastanthus patagonicus Speg. — **Argentina**: *Zanotti, C. A. 26 (*); Zanotti, C. A. 57(*).*

APPENDIX 2. Taxa sampled for DNA sequence analyses. Outgroup operational taxonomic units (OTUs) were constructed at the family level by combining sequences, mostly from GenBank, from sometimes different species or genera to represent as many of the sequenced regions as possible (outgroup sequences marked with an asterisk (*) were sequenced for this study, and the voucher is deposited at BRY for Goodeniaceae or SI for Asteraceae. All ingroup sequences were generated for this study, and vouchers are deposited at SI. Missing sequences (outgroup only) are indicated by a long dash. Published GenBank sequences used to construct the outgroup composite OTUs come from Tippery et al. (2008), Howarth et al. (2003), Gruenstaeudl et al. (2009), Abbott et al. (2009), Wahrmund et al. (2010), Beltrame (2007), and Kim et al. (2005).

Family: ***OTU***, collector and number (for sequences generated for this study only), GenBank no.: ITS; *trnH–psbA*; *ycf6–psbM*; *trnS–trnG*.

Outgroup

Menyanthaceae: ***Menyanthes trifoliata*** L., EF173025; ***Nymphoides cristata*** Kuntze, GU135291; —; —. Goodeniaceae1: ***Goodenia scapigera*** R. Br., AY102793; ***Scaevola taccada*** (Gaertn.) Roxb., GU135368; ***Scaevola gaudichaudiana*** Cham.*, *D. Cann et al. 88*, JN874720; ***Scaevola gaudichaudiana****, *D. Cann et al. 88*, JN874712. Goodeniaceae2: ***Goodenia glabra*** R. Br., AY102792; —; ***Scaevola chamissoniana*** Gaudich.*, *McKinnon et al. 572*, JN874721; —. Goodeniaceae3: ***Velleia spathulata*** R. Br., AY102794; —; —; —. Asteraceae1: ***Nassauvia glomerulosa*** D. Don*, *Nicola 17*, JN874697; ***Fulcaldea laurifolia*** (Bonpl.) Poir, EU841298; ***Atractylis carduus*** C. Chr. EU571351; —. Asteraceae2: ***Barnadesia lehmanni*** Hieron., EU841142; ***Barnadesia lehmannii***, EU841271; ***Centaurea maculosa*** Lam., DQ846397; —. Asteraceae3: —; ***Dasyphyllum brevispinum*** Sagást. & M. O. Dillon EU841290; —; ***Dasyphyllum argenteum*** Kunth AY865177.

Ingroup

Acicarpha tribuloides Juss., *J. M. Bonifacino et al. 1925,* JN874690, JN874698, JN874713, JN874705. ***Boopis anthemoides*** Juss., C. *Zanotti 60,* JN874691, JN874699, JN874714, JN874706. ***Calycera crassifolia*** (Miers) Hicken, C. *Zanotti 61*, JN874692, JN874700, JN874715, JN874707. ***Gamocarpha alpina*** (Poepp. ex Less.) H.V.Hansen, C. *Zanotti 4*, JN874693, JN874701, JN874716, JN874708. ***Gamocarpha selliana*** Reiche, C. *Zanotti 92*, JN874694, JN874702, JN874717, JN874709. ***Nastanthus patagonicus*** Speg., C. *Zanotti 26*, JN874695, JN874703, JN874718, JN874710. ***Boopis gracilis*** Phil., *R. Pozner 543*, JN874696, JN874704, JN874719, JN874711.