

FRUITS AND SEEDS OF THE VALERIANA CLADE (DIPSACALES): DIVERSITY AND EVOLUTION

Bart Jacobs,^{1,*} Charles Bell,[†] and Erik Smets^{*‡}

^{*}Laboratory of Plant Systematics, Katholieke Universiteit Leuven, Kasteelpark Arenberg 31, P.O. Box 2437, BE-3001 Leuven, Belgium;

[†]Department of Biological Sciences, University of New Orleans, 2000 Lakeshore Drive, New Orleans, Louisiana
70148, U.S.A.; and [‡]National Herbarium of the Netherlands, Leiden University Branch,

P.O. Box 9514, NL-2300 RA Leiden, The Netherlands

Recent molecular investigations have greatly improved our understanding of the phylogenetic relationships of the *Valeriana* clade. Our investigation of the fruits and seeds of the *Valeriana* clade provides morphological support for these findings. In particular, seed anatomy, calyx morphology, the presence of sterile locules, and fruit polymorphism provide support for the major lineages in the *Valeriana* clade (i.e., Valerianaceae). Fruit and seed morphology and anatomy of all genera but *Nardostachys* were investigated. Our results indicate that an important shift in seed anatomy took place at the origin of the core valerians (i.e., Valerianeae), including the loss of endosperm and a shift in seed coat anatomy. Furthermore, our data suggest that the evolution toward polymorphic fruits occurred at least twice, once at the origin of the clade holding *Fedia* and *Valerianella* and a second time at the inception of *Plectritis*. Our sampling indicates that the loss of sterile locules in mature achenes took place along the branch leading to *Centranthus*, *Plectritis*, and *Valeriana*. To confirm this, it will be necessary to examine South and Central American *Valeriana* species.

Keywords: Valerianaceae, fruit, seed, evolution, morphology, anatomy.

Online enhancements: appendix tables.

Introduction

The order Dipsacales (campanulids; APG III 2009) accommodates two families, Adoxaceae and Caprifoliaceae (Donoghue et al. 2001, 2003; Winkworth et al. 2008). We chose to assign informal names to the major lineages of Caprifoliaceae (fig. 1) because previous classifications (Backlund and Pyck 1998; Donoghue et al. 2001) have led to considerable confusion. In our study (fig. 1), the *Valeriana* clade corresponds to the family Valerianaceae of Backlund and Pyck (1998) and the Valerianaceae (no rank) of Donoghue et al. (2001).

With 350 species of annuals and perennials, the *Valeriana* clade is the most species-rich lineage in the Caprifoliaceae. The valerians are sister to a clade holding the *Dipsacus* clade and *Triplostegia* Wall. ex DC. (fig. 1; Donoghue et al. 1992, 2001, 2003; Judd et al. 1994; Backlund 1996; Backlund and Bremer 1997; Pyck et al. 1999; Olmstead et al. 2000; Pyck and Smets 2000; Bell et al. 2001; Winkworth et al. 2008). With the exception of Australia and New Zealand, valerians can be found in most parts of the world, with a center of diversity in South America and the Mediterranean Basin.

The *Valeriana* clade accommodates seven genera: *Centranthus* DC., *Fedia* Gaertner, *Patrinia* Juss., *Nardostachys* DC., *Plectritis* (Lindl.) DC., *Valeriana* L., and *Valerianella* Mill. Graebner (1906) divided the *Valeriana* clade into three tribes:

¹ Author for correspondence; e-mail: bart.jacobs@bio.kuleuven.be.

Triplostegieae Höck, Patrinieae Höck, and Valerianeae Höck. Recent studies (Bell et al. 2001; Bell 2004), however, have provided convincing evidence that Graebner's classification should be abandoned for several reasons. First, the only member of Triplostegieae, *Triplostegia*, is more closely related to members of the *Dipsacus* clade (fig. 1) than to any of the valerians (Bell 2004, 2007; Bell and Donoghue 2005b; Winkworth et al. 2008). Therefore, *Triplostegia* is no longer considered a member of the *Valeriana* clade. Second, although molecular data support the basal position of Patrinieae, comprising *Patrinia* and *Nardostachys*, the tribe is paraphyletic (Bell 2004, 2007; Bell and Donoghue 2005b). Third, Graebner (1906) divided Valerianeae into three subtribes: Centranthinae Graebner, Fediinae Graebner, and Valerianinae Graebner. Weberling (1970) redefined Fediinae to include *Plectritis* in addition to *Fedia* and *Valerianella*. Recent molecular investigations, however, link *Plectritis* with *Valeriana*, resulting in the paraphyly of Fediinae sensu Weberling (Bell 2004, 2007; Hidalgo et al. 2004; Bell and Donoghue 2005b). Fourth, Graebner's description of Valerianinae included several other South American genera in addition to *Valeriana*. These genera are now included in *Valeriana* (Larsen 1986; Eriksen 1989; Bell 2004; Bell and Donoghue 2005b).

Valerians have sympetalous, bilaterally symmetric to strongly asymmetric flowers with an inferior, tricarpeolate ovary. The two adaxial locules are sterile, whereas the abaxial locule is fertile and contains one pendulous, anatropous ovule. After fertilization, the ovary matures into a dry, single-

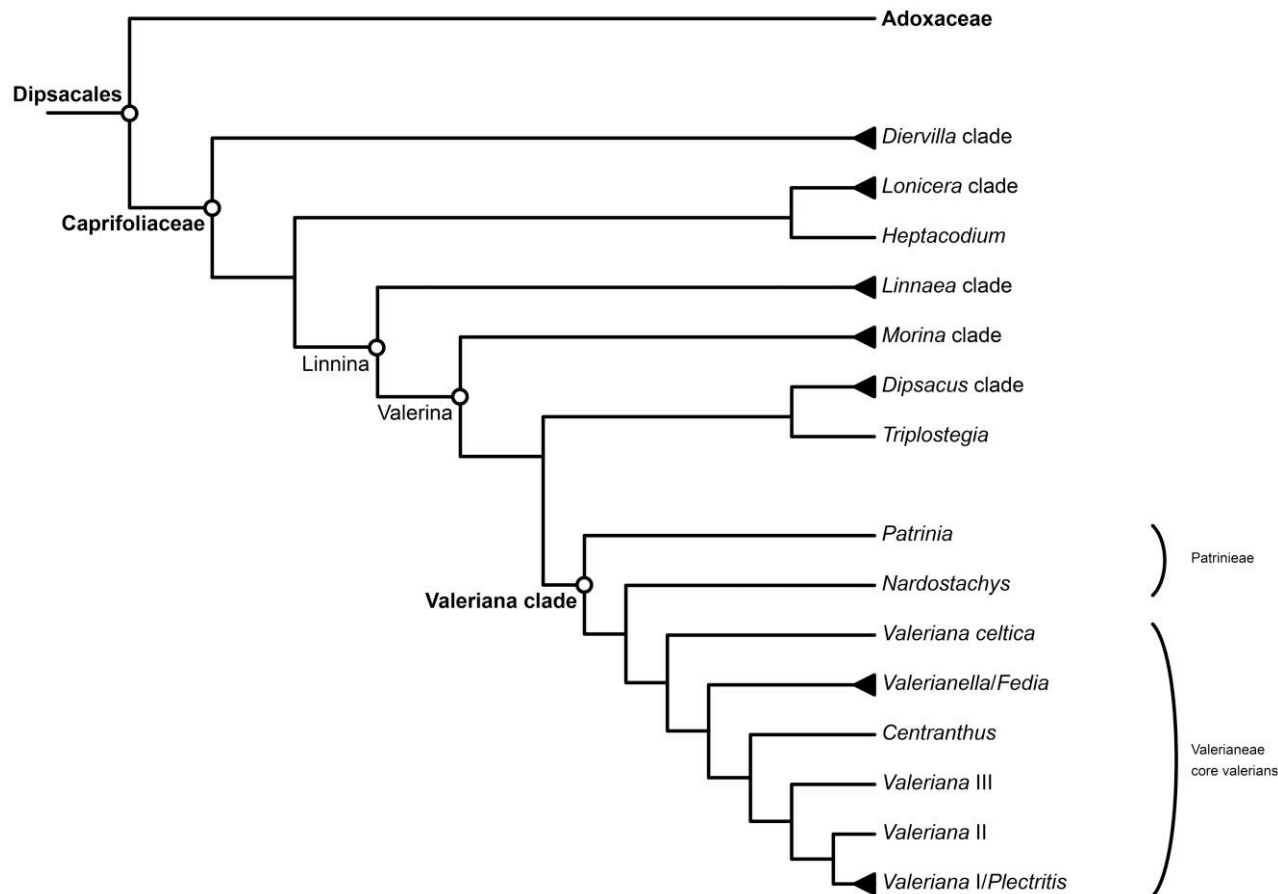


Fig. 1 Phylogeny of the order Dipsacales, with special attention to the intergeneric relationships of the *Valeriana* clade. Dipsacales phylogeny is adopted from Winkworth et al. (2008; their fig. 3A). Intergeneric relationships of the *Valeriana* clade are based on Bell (2007), and tribal classification of the *Valeriana* clade follows Graebner (1906). *Valeriana* clade I contains South and Central American species, *Valeriana* clade II comprises both New World and Old World (North America only) species, and *Valeriana* clade III accommodates only European species.

seeded achene. In the *Valeriana* clade, flower and fruit morphologies, as well as stamen numbers, are highly diverse and play an important role in species delimitation. Iridoids of the valepotriate type are found throughout the *Valeriana* clade, except in *Patrinia* and *Nardostachys*. Surprisingly, valepotriates are also found in *Triplostegia*.

Several recent studies have resulted in an improved understanding of the phylogenetic relationships of the *Valeriana* clade (Pyck et al. 2002; Raymúndez et al. 2002; Bell 2004, 2007; Hidalgo et al. 2004; Bell and Donoghue 2005b). It is clear that *Patrinia* is the first clade to diverge, followed by *Nardostachys*. The rest of the valerians are often referred to as the core valerians. The systematic positions of *Valeriana celtica*, *Valeriana hardwickii*, and *Valeriana longiflora* are peculiar, as those species do not appear to be closely related to the rest of the *Valeriana* species. *Valeriana celtica* and *V. hardwickii* seem to be the basalmost core valerians (Hidalgo et al. 2004; Bell 2007), whereas *V. longiflora* is hypothesized to be sister to *Centranthus* (Hidalgo et al. 2004). In several analyses, *Fedia* was found to be nested inside *Valerianella*, resulting in a paraphyletic *Valerianella* (Bell 2004, 2007). *Fedia* and *Valerianella* are, in turn, sister to a clade in which *Centranthus* is sister to *Valeriana* and *Plectritis* (Bell 2004,

2007; Hidalgo et al. 2004). Relationships among *Centranthus*, *Plectritis*, and *Valeriana* remain uncertain. With the exception of *V. celtica*, *V. hardwickii*, and *V. longiflora*, species of *Valeriana* form three major lineages (Bell 2004, 2007), the first comprising South and Central American taxa (*Valeriana* I; fig. 1), the second comprising both Old and New World (North America only) taxa (*Valeriana* II; fig. 1), and the third comprising only European taxa (*Valeriana* III; fig. 1). Several analyses suggest that *Plectritis* might be nested inside *Valeriana*, closely related to the clade of South and Central American taxa (*Valeriana* I; Bell 2007).

As with most major Dipsacales lineages, the *Valeriana* clade most likely originated in eastern Asia (Bell et al. 2001; Donoghue et al. 2003; Bell 2004, 2007; Bell and Donoghue 2005a, 2005b) and then dispersed into Europe and the New World on several occasions (Bell 2004, 2007; Bell and Donoghue 2005b). In a biogeographical study, Bell and Donoghue (2005b) suggested that members of the *Valeriana* clade migrated from North to Central America and subsequently colonized South America on at least four separate occasions.

With this study, we present an overview of the fruit and seed morphology and anatomy of the valerians. We discuss fruit and seed evolution in the *Valeriana* clade and assess the

usability of fruit and seed characters to support its current phylogeny.

Material and Methods

Taxon Sampling

Fruits and seeds of 24 species were obtained through collaboration with seed banks and herbaria (table A1 in the online edition of the *International Journal of Plant Sciences*). In addition, we sampled 10 species of Caprifoliaceae outside the *Valeriana* clade, corresponding to all the major lineages of the family. Our sampling of *Valeriana* was limited to six species and did not include any South American species (*Valeriana* I; fig. 1). *Nardostachys* was not included in the study because we were unable to obtain material.

Morphological Methods

Before fixation, fruits were cut longitudinally and transversely to improve infiltration of the fixating agent. The material was fixed with glutaraldehyde (2.5%) buffered in sodium cacodylate buffer (0.05 M) for 24 h and subsequently dehydrated in an ethanol series ending with absolute ethanol. For anatomical investigations, fruits were embedded in LR-White and polymerized at 60°C (24 h). Longitudinal and transverse sections were cut with a rotary microtome (HM360, Microm, Walldorf, Germany) and stained with toluidine blue. The combination of a Leitz Dialux light microscope (Leitz, Wetzlar, Germany), a PL-B622CF PixeLINK digital camera,

and Microscopica v1.3 (Orbicule, Leuven, Belgium) was used for observing and photography. For morphological investigations, after dehydration, the material was critical-point dried and sputtered with gold (Spi-Supplies, West Chester, PA). Fruits were mounted on stubs and then observed and photographed with a JSM-6360 scanning electron microscope. Achenes were measured (table 1) with a Leica MZ6 stereomicroscope (Leica Microsystems, Heerbrugg, Switzerland). Mean and standard deviation values of fruit dimensions are based on five specimens per species. In general, between 15 and 20 fruits were investigated per species.

Character State Delimitation

Two character sets with different scopes were created. A first set of eight characters was chosen to study evolution in Caprifoliaceae with special attention to the *Valeriana* clade. A second set of eight characters was used to study evolution at the generic level in the *Valeriana* clade. Character coding is described in table A2, and the character matrices are included as tables A3 and A4; tables A2–A4 are in the online edition of the *International Journal of Plant Sciences*.

Character Evolution

MacClade v4.04 (Maddison and Maddison 2002) and parsimony optimization were used to investigate character evolution. The parsimony optimization analyses are based on previously published phylogenies. The phylogeny of Bell (2007, his fig. 2; our fig. 7) was adopted for the intergeneric

Table 1

Fruit Dimensions of Sampled Species

Taxon	Tribal classification (sensu Graebner 1906)	Length (mm)	Width (mm)	L/W ratio
<i>Centranthus angustifolius</i>	Valerianeae	5.2 ± .1	1.7 ± .2	3.1
<i>Centranthus calcitrapae</i>	Valerianeae	2.9 ± .1	1.2 ± .1	2.3
<i>Centranthus macrosiphon</i>	Valerianeae	3.8 ± .3	1.8 ± .1	2.1
<i>Centranthus ruber</i>	Valerianeae	3.8 ± .1	1.7 ± .1	2.2
<i>Fedia cornucopiae</i>	Valerianeae	4.1 ± .7	2.9 ± .6	1.4
<i>Patrinia gibbosa</i>	Patrinieae	4.5 ± .3	1.9 ± .1	2.4
<i>Patrinia scabiosifolia</i>	Patrinieae	2.1 ± .1	1.4 ± .1	1.4
<i>Patrinia scabra</i>	Patrinieae	3.9 ± .3	2.1 ± .0	1.9
<i>Patrinia triloba</i>	Patrinieae	4.1 ± .3	1.9 ± .1	2.1
<i>Patrinia villosa</i>	Patrinieae	3.5 ± .3	1.7 ± .1	2.1
<i>Plectritis anomala</i>	Valerianeae	2.9 ± .2	2.9 ± .3	1.0
<i>Valeriana montana</i>	Valerianeae	4.2 ± .2	1.7 ± .2	2.4
<i>Valeriana officinalis</i>	Valerianeae	3.9 ± .1	1.8 ± .1	2.1
<i>Valeriana pyrenaica</i>	Valerianeae	4.4 ± .3	.9 ± .1	4.9
<i>Valeriana repens</i>	Valerianeae	4.7 ± .1	1.7 ± .2	2.7
<i>Valeriana sambucifolia</i>	Valerianeae	3.4 ± .3	1.5 ± .1	2.2
<i>Valeriana tripteris</i>	Valerianeae	3.8 ± .3	1.1 ± .1	3.5
<i>Valerianella carinata</i>	Valerianeae	2.0 ± .1	1.5 ± .1	1.4
<i>Valerianella coronata</i>	Valerianeae	2.9 ± .2	1.6 ± .2	1.8
<i>Valerianella dentata</i>	Valerianeae	2.8 ± .2	2.6 ± .2	1.1
<i>Valerianella discoidea</i>	Valerianeae	2.4 ± .1	1.8 ± .1	1.3
<i>Valerianella locusta</i>	Valerianeae	2.2 ± .2	2.1 ± .3	1.0
<i>V. locusta</i>	Valerianeae	3.1 ± .2	2.4 ± .2	1.3
<i>Valerianella rimosa</i>	Valerianeae	2.8 ± .2	2.2 ± .0	1.2
<i>Valerianella turgida</i>	Valerianeae	2.6 ± .1	2.6 ± .1	1.0

relationships of the *Valeriana* clade, whereas the phylogeny of Winkworth et al. (2008, their fig. 3A; our fig. 6) was adopted for the phylogenetic relationships of Caprifoliaceae.

Results

General Fruit and Seed Morphology

Although fruit morphology in the *Valeriana* clade seems diverse at first glance, a basic blueprint is found throughout the clade. Fruits develop from a tricarpeolate ovary with one abaxial (dorsal) fertile and two adaxial (ventral) sterile carpels. Most of the diversity in fruit morphology is the result of two processes: (1) the development of sterile carpels after fertilization and (2) calyx or pericarp outgrowths promoting fruit dispersal.

Of the species we investigated, *Patrinia* (fig. 2), *Fedia* (fig. 3A–3G), and *Valerianella* (fig. 3H–3R) have inflated sterile

locules. The sterile locules have a significant effect on fruit morphology because they generally are several times larger than the fertile locule containing the mature seed. The fruits of *Centranthus* (fig. 4), *Plectritis* (fig. 5H–5N), and *Valeriana* (fig. 5A–5G) examined in our study lack not only inflated sterile locules but also any trace of the sterile locules at maturity. Therefore, it appears that fruit morphology is largely determined by the fertile locule in *Centranthus*, *Plectritis*, and *Valeriana*.

Calyx morphology in the *Valeriana* clade can be divided into five types: (1) large and leafy (e.g., *Patrinia*, fig. 2A, 2D), (2) pappus-like (e.g., *Centranthus*, fig. 4A, and *Valeriana*; fig. 5A), (3) reduced to small teeth (e.g., *Fedia*, *Valerianella*), (4) one to five horns, fused at the base (e.g., *Fedia*; *Valerianella*, fig. 3K), and (5) absent (e.g., *Plectritis*, fig. 5H). *Fedia*, *Valerianella*, and *Plectritis* are known to have polymorphic fruits (Morey 1962; Ernet 1978; Xena de Enrech and Mathez 1990, 1998).

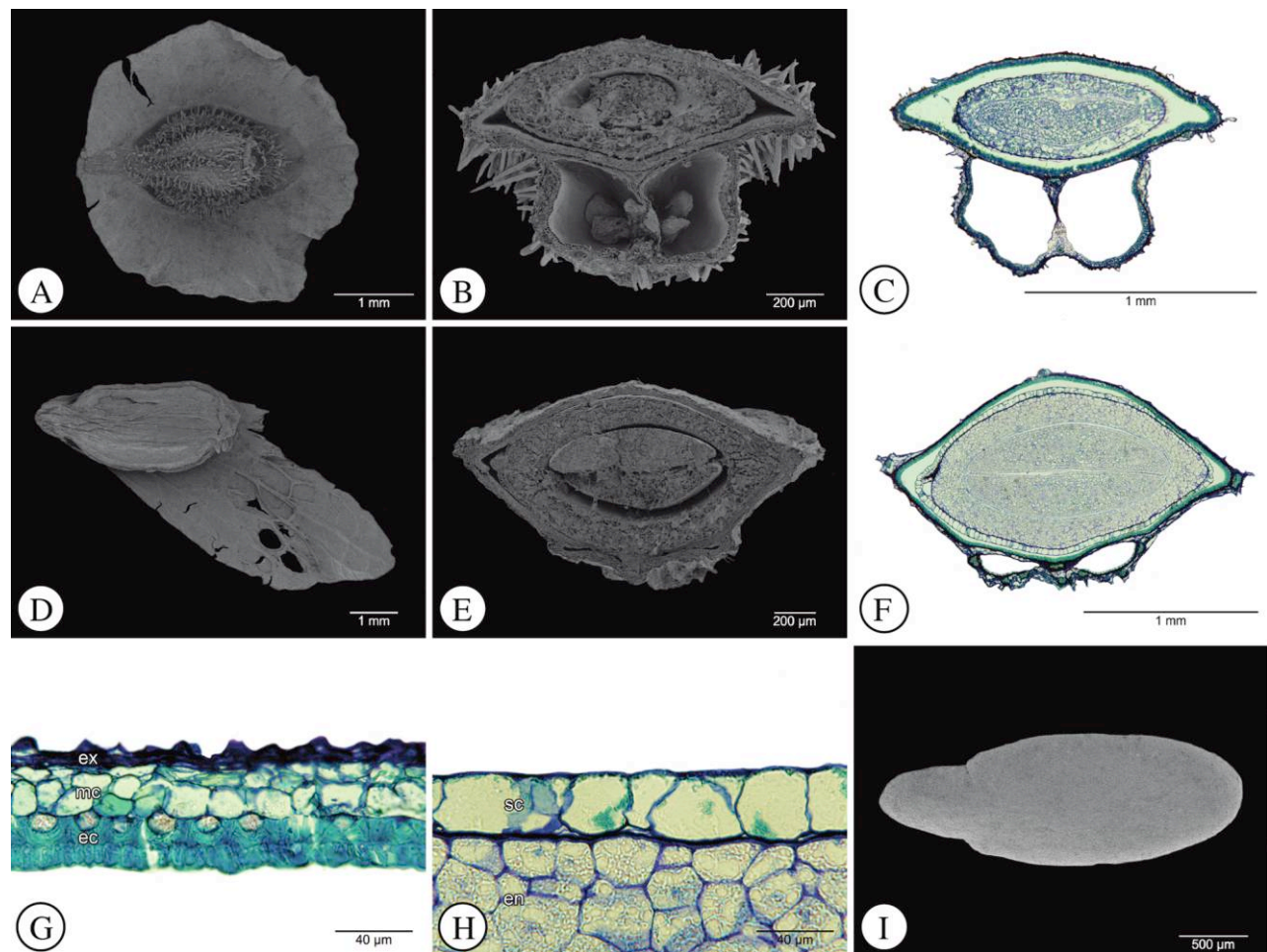


Fig. 2 Fruit and seed morphology and anatomy of *Patrinia*. A, B, D, E, I, SEM; C, F, G, H, LM. A–C, *Patrinia scabiosifolia*. A, Adaxial view of bottle-shaped fruit with large, circular calyx wing. B, Fruit cross section showing sterile locules with unfertilized ovules and seed with endosperm. C, Fruit cross section with parenchymatous septum between sterile locules. D–F, *Patrinia gibbosa*. D, Adaxial view of fruit with large, lanceolate calyx wing. E, Fruit cross section with small sterile locules. F, Fruit cross section with one central and nine peripheral vascular bundles. G, *Patrinia scabra*, pericarp cross section with unilayered exocarp (ex), multilayered, parenchymatous mesocarp (mc), and sclerified endocarp (ec). H, I, *Patrinia gibbosa*. H, Unilayered seed coat (sc) and adjacent endosperm (en). I, Abaxial view of embryo.

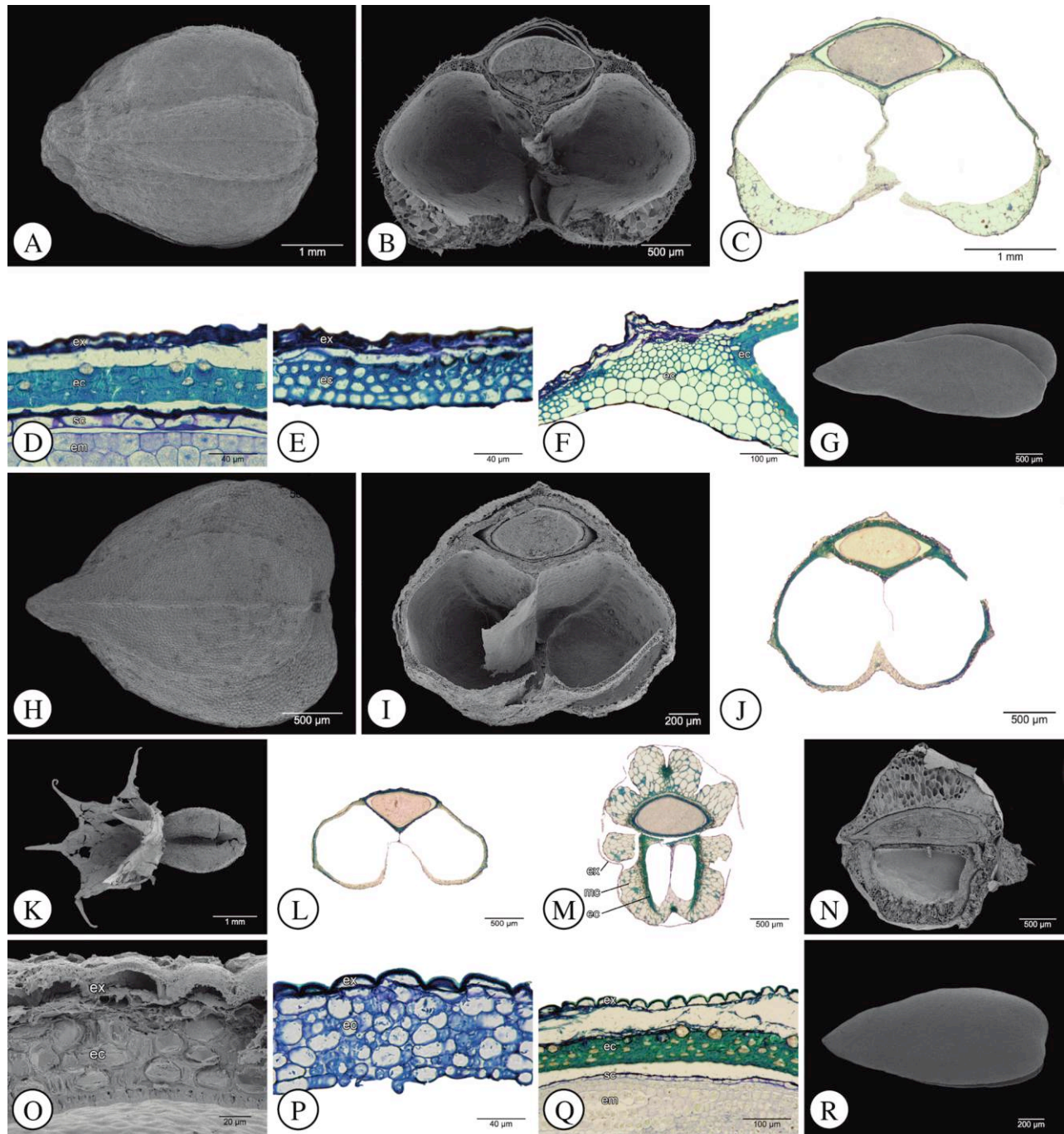


Fig. 3 Fruit and seed morphology and anatomy of *Fedia cornucopiae* and *Valerianella*. A, B, G–I, K, N, O, R, SEM; C–F, J, L, M, P, Q, LM. A–G, *Fedia cornucopiae*. A, Abaxial view of fruit. B, C, Fruit cross section with endocarp inflation in sterile locules (adaxially) and seeds lacking endosperm. D, Fruit cross section with unilayered exocarp (*ex*), sclerified endocarp (*ec*; mesocarp compressed), seed coat (*sc*), and embryo (*em*). E, F, Pericarp cross section of sterile locule with weakly (F) to moderately (E) sclerified endocarp cells (*ec*; *ex*, exocarp). G, Abaxial view of embryo. H–J, *Valerianella rimosa*. H, Abaxial view of fruit. I, J, Fruit cross section with a large, thin, parenchymatous septum between the sterile locules. K, *Valerianella coronata*, adaxial view of fruit with five calyx horns fused at the base. L, *Valerianella dentata*, fruit cross section with a small septum between the sterile locules, resulting in a prominent adaxial groove. M, N, *Valerianella turgida*. M, Fruit cross section with mesocarp (*mc*) inflation (*ex*, exocarp; *ec*, endocarp). N, Fruit longitudinal section. O–Q, *Valerianella rimosa*. O, Pericarp cross section with unilayered exocarp (*ex*), reduced mesocarp (not visible), and multilayered, sclerified endocarp (*ec*) with crystals. P, Cross section of adaxial part of sterile locule with unsclerified endocarp (*ec*; *ex*, exocarp). Q, Fruit cross section with pericarp (*ex*, exocarp; *ec*, endocarp), unilayered seed coat (*sc*), and adjacent embryo (*em*). R, *Valerianella turgida*, abaxial view of embryo.

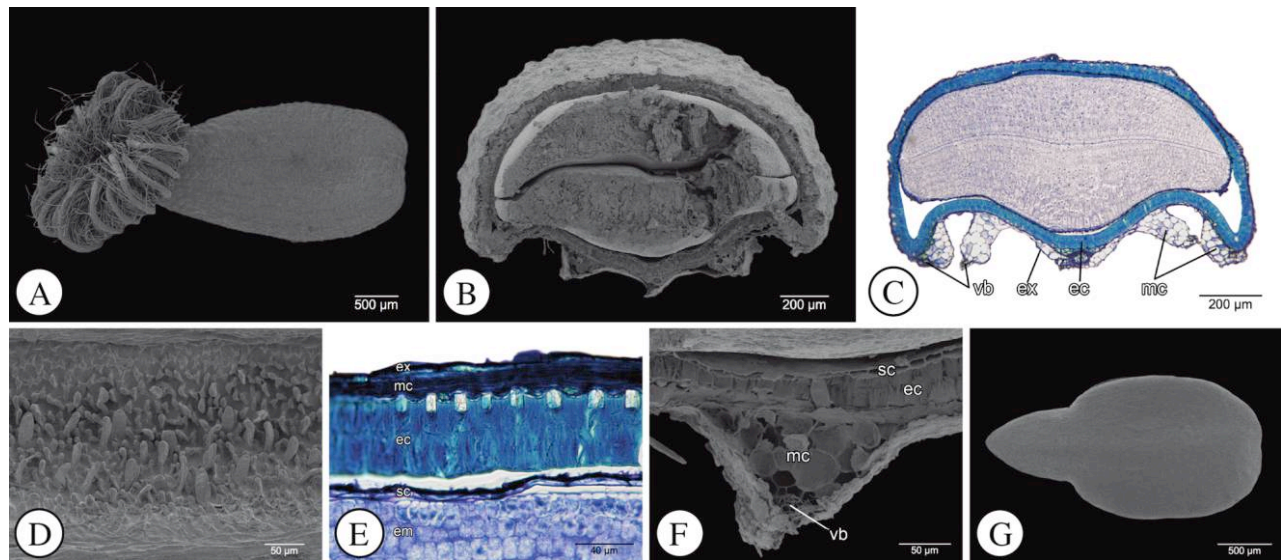


Fig. 4 Fruit and seed morphology and anatomy of *Centranthus*. A, B, D, F, G, SEM; C, E, LM. A, B, *Centranthus ruber*. A, Abaxial view of fruit with pappus-like calyx and abaxial vascular bundle. B, Fruit cross section with four adaxial grooves, mesocarp outgrowths, U-shaped endocarp, and no sterile locules. C, *Centranthus calcitrapae*: four adaxial grooves and U-shaped endocarp (*ec*; *ex*, exocarp; *mc*, mesocarp; *vb*, vascular bundle). D, *Centranthus angustifolius*: trichomes are present in outer pair of adaxial grooves. E, *Centranthus macrosiphon* fruit cross section with unilayered exocarp (*ex*), compressed, amorphous mesocarp (*mc*), sclerified endocarp (*ec*), and unilayered seed coat (*sc*) and embryo (*em*). F, *Centranthus ruber*, pericarp cross section with detail of adaxial mesocarp (*mc*) outgrowth (*ex*, exocarp; *sc*, seed coat; *vb*, vascular bundle). G, *Centranthus macrosiphon*, abaxial view of embryo.

Patrinia

Fruits of *Patrinia* are weakly to strongly bottle shaped (fig. 2A, 2D). Shape in cross section is directly related to the variable size of the sterile locules (fig. 2B, 2C, 2E, 2F). In most species, the sterile locules are large and well developed (fig. 2B, 2C). In *Patrinia gibbosa* (fig. 2D–2F) and *Patrinia triloba*, the sterile locules are smaller, and the endocarp surrounding the sterile locules is weakly sclerified or not sclerified at all. In several species (e.g., *Patrinia scabiosifolia*, fig. 2B), two sterile ovules in each sterile locule were observed in mature fruits. The calyx is reduced to a single, large leaf acting as a fruit wing. The leaf is much larger than the actual fruit and is positioned parallel to the length axis of the fruit. Two calyx subtypes were observed: (1) lanceolate calyx leaves (*P. gibbosa*, fig. 2D; *P. triloba*) and (2) circular to elliptical calyx leaves surrounding the entire fruit (*P. scabiosifolia*, fig. 2A; *Patrinia villosa*). We have no data for the calyx morphology of *Patrinia scabra*. The terminal end of the fruit is often elongated—hence the bottle shape of the fruit—and rotated 90 degrees (fig. 2A, 2D). This rotation causes the parallel orientation of fruit and calyx.

The exocarp is composed of a single layer of small (mostly globoid) cells (fig. 2G) and is often characterized by the emergence of large trichomes (e.g., *P. scabiosifolia*, fig. 2A, 2B). The mesocarp is parenchymatous and well developed in *P. scabra* (fig. 2G) and *P. triloba*. In all other species, however, the mesocarp is difficult to discern as a result of compression during fruit development. The endocarp of the fertile locule is always composed of two cell layers, an outer layer of sclereids containing prismatic crystals and an inner layer of smaller fibers (fig. 2G). Endocarp anatomy of the sterile locules shows

more interspecific variation. The number of cell layers and the degree of cell sclerification, in particular, vary considerably. The frail septum separating the sterile locules consists of a combination of parenchymatous and weakly sclerified cells (fig. 2C, 2F). In some species, the septum cells are all parenchymatous (e.g., *P. scabiosifolia*, fig. 2C). The mesocarp (or its remains) comprises up to 10 vascular bundles: one dorsal, two lateral, up to six ventral, and one located in the center of the fruit (fig. 2F).

Of all genera we investigated, *Patrinia* is the only member of the *Valeriana* clade with seeds in which the embryo is surrounded by multiple cell layers of endosperm (fig. 2B, 2C, 2E, 2F, 2H). The endosperm cells are considerably larger than the cells of the seed coat or the embryo (fig. 2H).

The seed coat is composed of a single layer of well-developed parenchymatous cells of exotestal origin (fig. 2H). The raphe is well developed in most species and is located in what remains of the mesotesta. An antiraphe is absent in all members of the *Valeriana* clade.

The embryo is twice as long as it is wide, with embryo length almost matching seed length (fig. 2I). Embryos are mostly bottle shaped, with large cotyledons and a tapered radicle (fig. 2I). Although general embryo morphology is similar between species, minor differences, such as the relative length of the radicle with respect to embryo length, occur intra- and inter-specifically.

Fedia

Fruits of *Fedia cornucopiae* are ovate to circular in dorsal view (fig. 3A) and elliptical in cross section (fig. 3B, 3C). The inflated sterile locules are large and take up most of the fruit

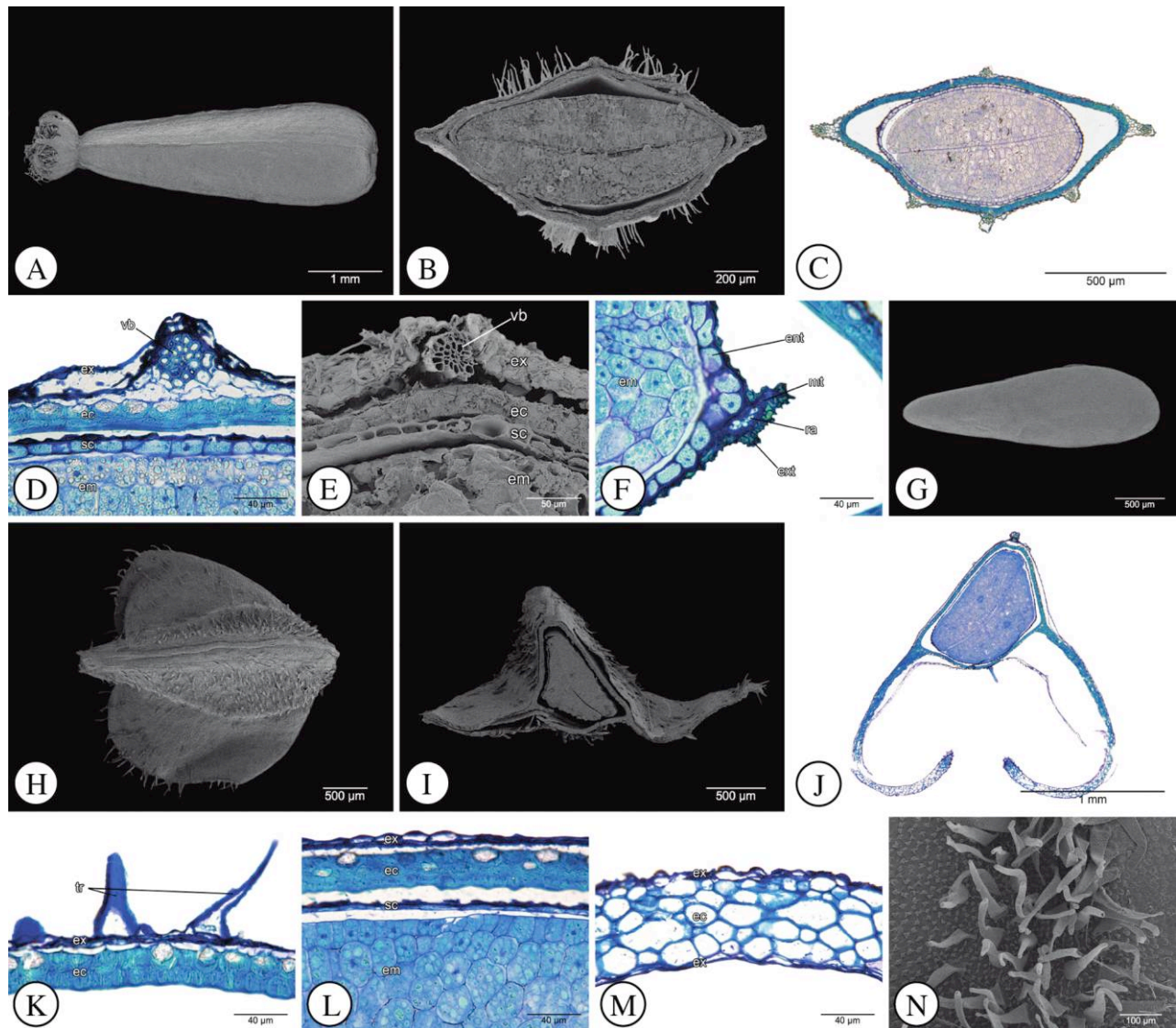


Fig. 5 Fruit and seed morphology and anatomy of *Valeriana* and *Plectritis anomala*. A, B, E, G–I, N, SEM; C, D, F, J–M, LM. A, *Valeriana repens*, abaxial view of fruit with pappus-like calyx and abaxial vascular bundle. B, *Valeriana sambucifolia*, fruit cross section with trichomes on abaxial and adaxial sides. C, *Valeriana officinalis*, fruit cross section with one abaxial, two lateral, and three adaxial vascular bundles. D, *Valeriana montana*, fruit cross section with unilayered exocarp (*ex*), vascular bundle (*vb*) in mesocarp remnants, sclerified endocarp (*ec*), and unilayered seed coat (*sc*) and embryo (*em*). E, *Valeriana officinalis*, fruit cross section with unilayered exocarp (*ex*), vascular bundle (*vb*) in mesocarp remnants, sclerified endocarp (*ec*), and unilayered seed coat (*sc*) and embryo (*em*). F, *Valeriana montana*, seed cross section with thin, outer layer of exotestal tissue (*ext*), raphe (*ra*) located in mesotestal remains (*mt*), and well developed inner endotestal cell layer (*ent*) and adjacent embryo (*em*). G, *Valeriana repens*, abaxial view of embryo. H–N, *Plectritis anomala*. H, Abaxial view of fruit with lateral wings. I, J, Fruit cross section with endocarp wings and rotated seed. K, Pericarp cross section with trichomes (*tr*) emerging from unilayered exocarp (*ex*), mesocarp compressed (not visible), and sclerified endocarp (*ec*). L, Fruit cross section with unilayered seed coat (*sc*) and embryo (*em*; *ex*, exocarp; *ec*, endocarp). M, Cross section of fruit wings with weakly sclerified endocarp (*ec*) cells and adjacent unilayered exocarp (*ex*). N, Adaxial view of fruit surface with two types of trichomes.

(fig. 3B, 3C). Stomata and trichomes are scattered over the entire fruit surface. Fruits of *Fedia* are known to be polymorphic. We encountered only one type (type G sensu Xena de Enrech and Mathez 1998) during our observations, and the following description is based on that type. The calyx is absent or dramatically reduced.

The exocarp is composed of a single layer of cells (fig. 3D–3F). The endocarp of the fertile locule is composed of one or

two cell layers of sclereids and an inner layer of fibers (fig. 3D). The sclereids have heavily thickened cell walls and often contain crystals. The mesocarp of the fertile locule is parenchymatous and difficult to observe because of compression during fruit development (fig. 3D). The ventral sides of the sterile locules are characterized by several layers of large, isodiametric, weakly sclerified endocarp cells (fig. 3B, 3C). Hereafter, we refer to this condition as endocarp inflation.

Cells at the dorsal sides of the sterile locules are weakly sclerified but much smaller (fig. 3F) than those at the ventral side. The thin septum separating the sterile locules is composed of parenchymatous endocarp cells (fig. 3C). Five peripheral vascular bundles are present in the pericarp, one dorsal, two lateral, and two medioventral. The dorsal and medioventral vascular bundles are easily visible on the fruit surface (fig. 3A–3C). Another vascular bundle is present in the fruit center.

Mature seeds of *F. cornucopiae* lack endosperm. The seed coat is composed of a single layer of weakly compressed cells of endotestal origin (fig. 3D). On top of these cells is an additional, amorphous layer of exo- and mesotestal origin (fig. 3D). The raphe is located in what remains of the mesotestal layer.

The embryo is elliptical in cross section, with two large cotyledons and a tapered radicle (fig. 3B, 3C, 3G). The cotyledons are twice as long as the radicle.

Valerianella

Fruits of *Valerianella* have well-developed, inflated sterile locules, resulting in a wide range of fruit morphologies (fig. 3H–3N). Four factors characterize fruit morphology: (1) size of the sterile locules relative to the fertile locule; (2) presence or absence of endocarp and mesocarp inflation; (3) size of the septum separating the sterile locules; and (4) calyx morphology. Sterile-locule size ranges from the same as that of the fertile locule (fig. 3M) to many times that size (fig. 3H–3J, 3L). The sterile locules of *Valerianella turgida* are small (relative to those of other *Valerianella* species), but fruit morphology is largely determined by significant mesocarp (and endocarp) inflation (fig. 3M, 3N). The size of the septum separating the sterile locules can range from equal to the length of the sterile locules (e.g., *Valerianella discoidea*; *Valerianella rimosa*, fig. 3I, 3J) to almost nonexistent (e.g., *Valerianella dentata*, fig. 3L). The smaller the contact area, that is, the size of the septum, the more prominent the ventral groove (fig. 3K, 3L). As in *Fedia*, some *Valerianella* species are known to have polymorphic fruits (Eggers Ware 1983; Martin and Mathez 1991; Devesa et al. 2005). Our sampling included species with a calyx composed of five horns, which are fused at the base (e.g., *Valerianella coronata*, fig. 3K), and species without a calyx (e.g., *V. rimosa*, fig. 3H), the latter much resembling specimens of *F. cornucopiae* (fig. 3A).

We discern two types of pericarp anatomy. All investigated *Valerianella* species but *V. turgida* have a pericarp with a unilayered exocarp, a well-developed to strongly compressed or absent mesocarp, and a well-developed endocarp (fig. 3O–3Q). In *V. turgida* (fig. 3M, 3N), however, the mesocarp is exceptionally well developed and is composed of large, weakly sclerified cells taking up more than half of the fruit volume. The exocarp of *V. turgida* is unilayered as well but does not follow the outline of the mesocarp (fig. 3M, 3N). This results in cavities between exo- and mesocarp at distinct places (fig. 3M, 3N). The endocarp of the fertile locule of *V. turgida* is similar to that of other *Valerianella* species, that is, built up of one or two cell layers of crystal-containing sclereids and one inner cell layer of fibers (fig. 3O, 3Q).

Seeds of *Valerianella* lack endosperm and are elliptical to triangular in cross section (fig. 3I, 3J, 3L–3N). The seed coat

of all studied *Valerianella* species is composed of a single cell layer of moderately compressed parenchyma cells of endotestal origin with an amorphous layer on top (fig. 3Q). The latter layer is what remains of the exo- and mesotesta.

Valerianella embryos are similar to embryos of *Fedia* in that they are dorsoventrally flattened and ovate to drop shaped in dorsal view (fig. 3I, 3J, 3L–3N, 3R). Cotyledon morphology ranges from elliptical (e.g., *Valerianella locusta*; *V. turgida*, fig. 3R) to almost circular (e.g., *V. rimosa*).

Centranthus

Fruits of *Centranthus* are elliptical to ovate in dorsal view (fig. 4A) and elliptical to U-shaped in cross section (fig. 4B, 4C). The calyx consists of several long, thick, and numerous hairlike structures, referred to as a pappus-like calyx (fig. 4A). Fruit morphology and anatomy of *Centranthus* are uniform in all species we investigated. No traces of sterile locules are present at maturity (fig. 4A–4C). Fruits of *Centranthus* are marked by four ventral grooves with several to numerous trichomes (fig. 4B–4D). Only *Centranthus calcitrapae* lacks trichomes. Although few in number, stomata were found on the fruit surface in all investigated species.

The pericarp is composed of three prominent layers: exocarp, mesocarp, and endocarp (fig. 4E, 4F). The exocarp is unilayered and composed of weakly sclerified cells that are often flattened (fig. 4E, 4F). At maturity, the mesocarp is present as two or four ventral outgrowths stretching the entire length of the seed (fig. 4B, 4C, 4F). The outgrowths consist of weakly sclerified cells (fig. 4F). Anatomical sections show the reticulate sclerification pattern, which clearly differs from that of the sclerified endocarp cells. The innermost pair of mesocarp outgrowths was present in all species and visible as two longitudinal, ventral ribs on the ventral seed surface (fig. 4B, 4C). A vascular bundle is present at the terminal end of each outgrowth (fig. 4F). In some species (e.g., *C. calcitrapae*, fig. 4C; *Centranthus macrosiphon*), an additional pair of outgrowths is present at the fruit margins. A vascular bundle also resides at the terminal end of each rib (fig. 4C). The endocarp is composed of two cell layers, an outer layer of sclereids, which comprise prismatic crystals or druses, and an inner layer of smaller fibers (fig. 4E, 4F).

Although seeds of *Centranthus* generally lack endosperm, in one specimen of *Centranthus angustifolius*, a small amount of endosperm was present around the radicle. The seed is elliptical in cross section and therefore differs from the U shape of the surrounding endocarp (fig. 4B, 4C). The seed coat consists of a thin, amorphous layer on top of a well-developed, parenchymatous cell layer (fig. 4E). The embryo is dorsoventrally flattened (fig. 4B, 4C), with two large cotyledons that are twice as long as the radicle (fig. 4G). The radicle is half the width of the cotyledons (fig. 4G).

Valeriana

As in *Centranthus*, mature fruits of *Valeriana* lack any trace of the sterile locules (fig. 5A–5C), and fruit morphology is therefore uniform in comparison with, for example, *Valerianella*. Fruits of *Valeriana* are narrow and elongated (fig.

5A). In dorsal view, fruits are ovate, triangular (fig. 5A) or cordate, and elliptical (fig. 5B, 5C) to circular in cross section. In all investigated species, six prominent ribs are apparent, one dorsal, two lateral, and three ventral (fig. 5B, 5C). Each rib corresponds to a vascular bundle located in what remains of the mesocarp (fig. 5D, 5E). The calyx is papus-like, with several long, thick structures and numerous, hair-like structures (fig. 5A). Trichomes are present in *Valeriana sambucifolia* (fig. 5B), *Valeriana pyrenaica*, and *Valeriana officinalis*. Stomata are present on the fruit surface in all species.

At maturity, the pericarp is composed of exocarp and endocarp (fig. 5D, 5E). Remnants of the mesocarp are mostly absent or visible as a thin, amorphous layer (fig. 5D, 5E). In some species (e.g., *Valeriana repens*), the lateral vascular bundles are embedded in small amounts of mesocarp with large cells that have a reticulate sclerification pattern. The exocarp is composed of a single layer of cells (fig. 5D, 5E), whereas the endocarp is built up of an outer layer of sclereids and an inner layer of fibers (fig. 5D, 5E). The sclereids contain druses or prismatic crystals (fig. 5D, 5E).

Endosperm is absent in all *Valeriana* species studied. The seed coat is composed of a unilayered exotesta, an inner, well-developed, unilayered endotesta, and between them a multilayered mesotesta comprising the raphal bundle (fig. 5D–5F). At maturity, the exo- and mesotesta are reduced to a thin, amorphous layer because of compression during seed development (fig. 5F). At the terminal ends of the seed, remnants of exo- and mesotesta can often be observed (e.g., *Valeriana montana*, fig. 5F).

Like the seed, the embryo is long, narrow (fig. 5G), and usually dorsoventrally flattened (fig. 5B, 5C). The radicle is half the length of the elliptic cotyledons (fig. 5G). In contrast to *Centranthus*, the transition from radicle to cotyledons is more gradual.

Plectritis

Fruits of *Plectritis* are dimorphic. Both winged and wingless fruits have been reported. We encountered only winged fruits, and the following description is based on winged fruits of *Plectritis anomala*. Fruits are characterized by two lateral wings (fig. 5H–5J), which are outgrowths of the lateral parts of the fertile locule (fig. 5I, 5J). Each wing is approximately as wide as the fertile locule. Because of the presence of the wings, the fruit is slightly cordate in dorsal view (fig. 5H). Without wings, the fruit is reminiscent of the fruits of some *Valeriana* species. The dorsal and ventral sides of the fertile locule have numerous long trichomes, as do the margins of the wings (fig. 5H, 5I, 5K, 5N). Sterile locules are absent in mature fruits.

The exocarp is a well-developed single layer of cells, from which numerous trichomes emerge (fig. 5K–5M). The mesocarp is reduced or absent at maturity (fig. 5K–5M). The endocarp of the fertile locule is composed of one or two cell layers of crystal-containing sclereids and an inner layer of fibers (fig. 5K, 5L). The endocarp of the wings differs from the endocarp surrounding the seed (fig. 5J, 5M). The first half to two-thirds of the wing, starting from the fertile locule, consists of three or more moderately to heavily sclerified cell

layers (fig. 5J). The rest of the endocarp cells of the wings are weakly sclerified, with cell walls showing a perforate sclerification pattern (fig. 5J, 5M). The cells at the margins of the wings are more sclerified (fig. 5J). On top of the endocarp of the wings is a unilayered exocarp (fig. 5M).

The seeds of *P. anomala* are triangular in cross section and lack endosperm (fig. 5I). The seed coat consists of one layer of flattened parenchyma cells (fig. 5L). On top of this layer is an amorphous layer composed of exo- and mesotestal remains (fig. 5L).

In contrast to those of all other species investigated, the cotyledons do not lie in a dorsoventral plane but are rotated 45°–90° over their longitudinal axis (fig. 5I, 5J). The embryo is gradually tapered toward the radicle.

Discussion

Phylogenetic Implications

In the past decade, a number of studies (Pyck et al. 2002; Raymúndez et al. 2002; Bell 2004, 2007; Hidalgo et al. 2004; Bell and Donoghue 2005b) have dealt with the phylogenetic relationships of the *Valeriana* clade, resulting in a good understanding of the classification, biogeography, and phylogenetic relationships of this diverse group. These studies, however, have been based exclusively on molecular data. Our study provides morphological support for the findings of these molecular investigations.

The position of *Patrinia* as the basalmost lineage in the *Valeriana* clade is supported by the presence of seeds with both embryo and endosperm. Although the presence of seeds with endosperm is the plesiomorphic condition in the *Valeriana* clade, it has often been assumed that the loss of endosperm is synapomorphic for the clade (Donoghue et al. 2003). In addition, *Patrinia*'s large, persistent, leafy calyx is unique in the *Valeriana* clade and autapomorphic for *Patrinia*. As we did not include *Nardostachys* in our study, we cannot be conclusive about the presence or absence of endosperm in its seeds.

Recent molecular investigations have found *Fedia* nested inside *Valerianella*, thereby rendering *Valerianella* paraphyletic (Bell 2007). On the basis of molecular and morphological evidence, Bell (2007) suggested treating species of *Fedia* as members of *Valerianella*. Both are annuals with similar inflorescences and have polymorphic fruits with persistent sterile locules (Ernet 1978; Xena de Enrech and Mathez 1990, 1998). Molecular evidence suggests that *Valerianella locusta* is the closest relative of *Fedia* (Hidalgo et al. 2004; Bell and Donoghue 2005b; Bell 2007). The fruit morphologies of *Fedia cornucopiae* and *V. locusta* are highly similar, with two large, sterile locules at maturity, a large septum between the sterile locules, and identical fruit wall vascularization. Although calyx morphology is tremendously diverse in the *Fedia-Valerianella* clade, the calyx morphologies of *Fedia* and several *Valerianella* species are strikingly similar, confirming the close relationship between the two genera. In both genera, fruits with well-developed calyxes (types C and M sensu Xena de Enrech and Mathez 1998) and reduced calyxes (types E and G sensu Xena de Enrech and Mathez 1998) are encountered. Differences between *Fedia* and *Valerianella* in-

clude dissimilarities in flower symmetry and stamen number. Fruits of *F. cornucopiae* show clear endocarp inflation in the ventral parts of the sterile locules, which is uncommon in *Valerianella* (although a weak form of endocarp inflation is present in *Valerianella turgida*).

Our sampling suggests that *Centranthus*, *Plectritis*, and *Valeriana* have achenes that lack sterile locules, resulting in a morphology highly different from that of achenes of the *Fedia-Valerianella* clade. However, a number of morphological investigations report that *Valeriana* contains species with and without persistent sterile locules (Asplund 1920; Borsini 1966, 1999; Gütlein and Weberling 1982; Eriksen 1989; Kutschker 2008). A recent fruit morphological study of South Andean *Valeriana* species, for example, describes mature achenes with inflated, persistent sterile locules in several species (e.g., *Valeriana sphaerocarpa* Phil.; Kutschker 2008). It is clear that broad sampling is needed to map the distribution of persistent sterile locules in *Valeriana*. Although Gütlein and Weberling (1982) observed persistent sterile locules in mature fruits of *Valeriana montana*, our results do not confirm these observations. The reticulate sclerification pattern of endocarp cells in several species of *Centranthus* and *Valeriana* was also observed by Vidal (1903; "Netzzellen") and Gütlein and Weberling (1982; "Fensterzellen") and is most likely the result of the uneven sclerification of the cell wall. Additional observations are required to assess the phylogenetic value of this character.

Achenes of *Centranthus* and *Valeriana* have a persistent, pappus-like calyx that promotes wind dispersal. *Plectritis* fruits have no calyx at maturity. Instead, wind dispersal is promoted by the development of two lateral wings. Wingless fruits have also been observed in *Plectritis*, as the genus is known for its dimorphic fruits (Ganders et al. 1977; Xena de Enrech and Mathez 1998).

Recent molecular evidence suggests that *Plectritis* is nested inside *Valeriana* (Hidalgo et al. 2004; Bell and Donoghue 2005b; Bell 2007) and is sister to a clade containing the South and Central American *Valeriana* species (*Valeriana* I, fig. 1; Bell and Donoghue 2005b; Bell 2007). Morphological support for this relationship is scarce. A thorough morphological study of the South and Central American *Valeriana* species, the closest relatives of *Plectritis*, might shed more light on this relationship.

Although the *Valeriana* clade is a highly derived clade characterized by a number of derived features, we were not able to identify any new synapomorphies for it. This, however, is largely due to the presence of both primitive and highly derived taxa in the clade. *Patrinia* (together with *Nardostachys*), on the one hand, is often described as a primitive valerian because of the presence of endosperm, the lack of valepotriates, and its flowers with four stamens, supernumerary bracts subtending the ovary, and pollen with spines mounted on verrucae. The core valerians, on the other hand, are considered highly derived. *Centranthus*, for example, has many derived features, such as seeds without endosperm, the occurrence of valepotriates, asymmetric flowers with one stamen, and the loss of supernumerary bracts. As long as morphological data about *Nardostachys* are lacking, it is unclear which characters are useful for separating the core valerians from *Patrinia* and *Nardostachys*.

Fruit and Seed Evolution

Three evolutionary trends are apparent in Caprifoliaceae and culminate in the *Valeriana* clade, emphasizing its highly derived nature. (1) Seeds in Caprifoliaceae have evolved from seeds with small embryos and copious endosperm in the basal-most clades (*Diervilla* and *Lonicera* clades) to seeds with larger embryos and little endosperm in the *Morina* and *Dipsacus* clades (characters [char.] 5, 6; fig. 6; Jacobs et al. 2009). In the *Valeriana* clade, the seeds of the core valerians have embryos that occupy the entire seed and therefore lack endosperm. *Patrinia*, and possibly *Nardostachys*, retained the ancestral state of having seeds with smaller embryos and a multilayered endosperm. The seed anatomy of *Patrinia* strongly resembles that of members of the *Dipsacus* clade. (2) A second trend is related to transfer of function and embryo protection (char. 7, 8; fig. 6). The first mechanical layer is defined as the outermost layer or structure of the diaspore that offers mechanical protection to the embryo. In the *Diervilla* and *Lonicera* clades, embryo and endosperm are enclosed by a sclerified seed coat or endocarp (Jacobs et al. 2009). In more derived angiosperm clades, the function of the first mechanical layer is often transferred to the outer tissues of the diaspore, and this is also true for Caprifoliaceae. In the *Linnina* clade, the function of the mechanical layer is transferred to the pericarp (*Limnaea*, *Morina*, and *Valeriana* clades) or the epicalyx (*Dipsacus* clade and *Triplostegia*). (3) The third trend involves stamen number. For a detailed discussion of stamen evolution, we refer to Donoghue et al. (2003).

In the *Valeriana* clade, several evolutionary trends are apparent as well. The evolution of the calyx has led to a broad diversity of morphologies in this group (char. 10, 15; fig. 7), starting with large, leafy calyces in *Patrinia* and well-developed, toothed calyces in *Nardostachys*. A shift in calyx morphology occurred at the origin of the *Fedia-Valerianella* clade. Calyx morphology in this clade is diverse, ranging from asymmetric horn-shaped calyces and elaborate five-lobed, cup-shaped calyces to fruits without calyces (see "Fruit Polymorphism"). Ernet (1978) performed a detailed study of fruit morphology in *Valerianella* and *Fedia* and described the high diversity of calyces in these genera. The clade with *Centranthus*, *Plectritis*, and *Valeriana* is characterized by a shift to a pappus-like calyx, which differs dramatically from the other calyx types in the *Valeriana* clade. This calyx shows strong similarities with that of several members of the *Dipsacus* clade, which is also known for its broad diversity in calyx morphology. Our parsimony optimization analyses indicate that a pappus-like calyx is the plesiomorphic condition for the *Valeriana* clade if considered homologous with the pappus-like calyx of, for example, *Dipsacus fullonum* L. Our results indicate that persistent sterile locules, although not synapomorphic for the entire *Valeriana* clade, originated with the first valerians (char. 9; fig. 7). Fruits with sterile locules are present in *Fedia*, *Nardostachys*, *Patrinia*, and *Valerianella*. Our results indicate that a subsequent loss took place along the path leading to the clade with *Centranthus*, *Plectritis*, and *Valeriana*. However, with the results of previous investigations in mind (Asplund 1920; Borsini 1966, 1999; Eriksen 1989; Kutschker 2008), a more complex evolutionary pattern explaining the

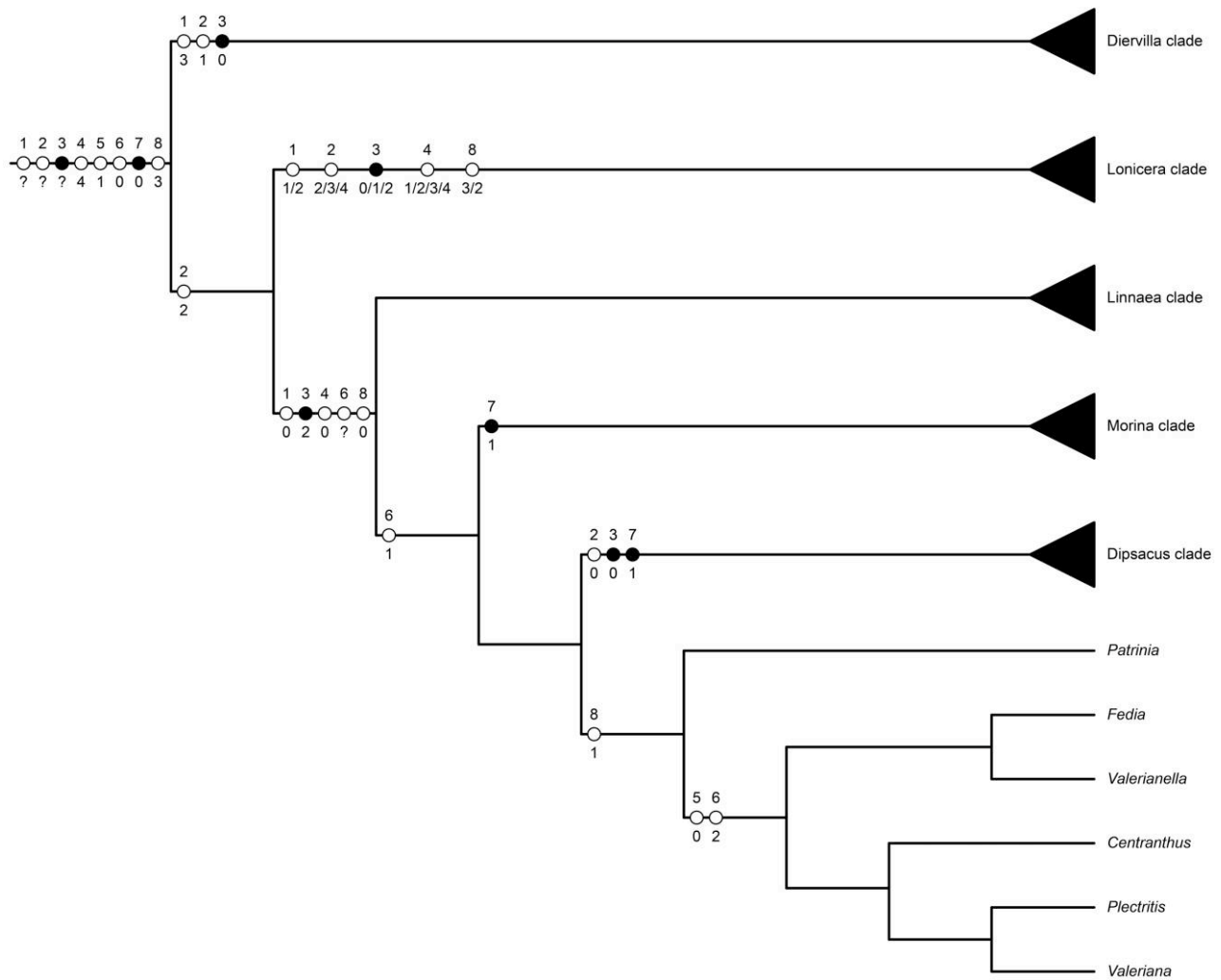


Fig. 6 Evolution of characters 1–8, using parsimony optimization. The top number is the character number (table A2 in the online edition of the *International Journal of Plant Sciences*). The bottom number refers to the character state of the particular character. Characters with filled circles are homoplasious.

occurrence of persistent, sterile locules might be appropriate, as the latter investigations report persistent sterile locules in the genus *Valeriana*. Fruit polymorphism is known to occur in *Fedia*, *Plectritis*, and several *Valerianella* species (Ernet 1978; Xena de Enrech and Mathez 1998). The phylogeny presented by Bell (2007) and the observations of Ernet (1978) indicate that several independent shifts to polymorphic fruits are likely to have taken place. A broader sampling of *Valerianella* and a thorough knowledge of fruit polymorphism in *Valerianella* species are required to confirm this hypothesis.

Our results suggest that fruit polymorphism in *Plectritis* evolved independently from fruit polymorphism in *Fedia* and *Valerianella*. The strongest argument for this hypothesis is the difference in morphology between the fruit types in *Plectritis*. In *Fedia* and *Valerianella*, the differences between the fruit types are related to the development of the calyx and the sterile locules. In *Plectritis*, however, a persistent calyx is absent, as are persistent sterile locules. Differences between

fruit types are instead limited to the presence or absence of fruit wings, which are outgrowths of the fruit wall of the fertile locule (char. 11; fig. 7). In other words, the fruits of *Fedia* and *Valerianella* are morphologically and anatomically quite different from those of *Plectritis*.

Fruit wing morphology is diverse in the *Valeriana* clade. Two main types are considered: calyx wings, as found in most valerians except for *Plectritis* and *Nardostachys*, and pericarp wings, as found in *Plectritis*. Another evolutionary shift in the *Valeriana* clade is the loss of endosperm and the increase in embryo size. When this shift took place is unclear because of the lack of seed anatomical data for *Nardostachys*. It is clear, however, that all core valerians lack endosperm at maturity and that the shift most likely took place at the origin of the clade containing the core valerians. Finally, the seed coat in the *Valeriana* clade is composed of exo-, meso-, and endotesta. In *Patrinia*, meso- and endotesta degenerate during seed development. The prominent layer of the seed coat is therefore of exotestal origin, with the raphe

chory. The mesocarp outgrowths in *Centranthus* achenes and endo- and mesocarp inflation in, for example, *V. turgida* have a similar effect with respect to dispersal. In *Plectritis*, the wings and trichomes aid in anemochory and epizoochory.

As mentioned above, fruits of *Fedia*, *Plectritis*, and several *Valerianella* species are polymorphic. Morey (1962) exemplifies differential dissemination by speculating that winged fruits of *Plectritis* are more likely to get stuck in the soil by rainfall, whereas wingless fruits tend to be washed away. Field experiments to investigate whether differential dissemination does in fact take place in *Plectritis* have not been carried out as of this writing. Studies by Mathez (1991) and Mathez and Xena de Enrech (1985) have shown that each deciduous fruit type in *Fedia* “more or less” corresponds to a mode of dispersal. Fruit types without calyx horns are dispersed by ants (myrmecochory), whereas fruits with two prominent calyx horns are dispersed by adherence to passing animals (epizoochory). A study by Ernet (1978) dealing with fruit dispersal in *Fedia* and *Valerianella* describes all possible dispersal mechanisms for the fruits of the latter genera, ranging from passive dispersal by falling water drops to dispersal by humans. Although it is likely that fruits are dispersed by many means, as described by Ernet (1978), most of these forms of dispersal are rare and contribute little to actual fruit dispersal. On the basis of fruit morphology, we can confidently conclude that the main agents of dispersal are wind, water, and animals (epizoochory).

In several species, small, glandular trichomes were encountered on the fruit surface (e.g., *Centranthus angustifolius*, fig. 4D; *Plectritis anomala*, fig. 5N). It is possible that these trichomes promote myrmecochory; field experiments are required to confirm this hypothesis. Ernet (1978) describes an elaiosome rich in oils in all *Fedia* species and several *Valerianella* species, which could also promote myrmecochory.

Future Prospects

With this study, we successfully identified morphological characters supporting the systematic relationships in the *Valeriana* clade as well as the placement of the clade in the Caprifoliaceae. Our results give a first look at character evolution in the *Valeriana* clade.

Studying *Nardostachys* will further improve our understanding of character evolution in the *Valeriana* clade and will allow us to more accurately pinpoint the shift from seeds with endosperm to seeds without endosperm. Furthermore, the fruit and seed morphology and anatomy of *Valeriana celtica* will help us understand why phylogenetic studies do not link *V. celtica* with any other *Valeriana* species. Finally, an expanded sampling of *Valeriana* (especially South American taxa) is needed to improve our knowledge of evolution in this species-rich genus.

Acknowledgments

We want to show our appreciation to the seed banks of the Botanical Garden of Nantes, the Natural History Museum of Paris, the National Botanical Garden of Belgium, the Botanical Garden of Jena, the Botanical Garden of Utrecht University, the Institute of Ecology and Botany of the Hungarian Academy of Sciences, the Cruickshank Botanical Garden, the Botanical Garden of Regensburg University, the Botanical Garden of St. Andrews, and the Botanical Garden of Ljubljana University. We also would like to thank the National Herbarium of the Netherlands for granting us access to their herbaria and the Linnaeus Garden of Uppsala University for sending us fresh material of *Linnaea borealis* L. Research at the Laboratory of Plant Systematics is supported by a grant of the Research Council of the Katholieke Universiteit Leuven (OT/05/35).

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