

FLOWERING NEWSLETTER REVIEW

A cornucopia of diversity—*Ranunculales* as a model lineage

The RanOmics group, Annette Becker^{1,*}, Julien B. Bachelier², Laetitia Carrive³, Natalia Conde e Silva⁴, Catherine Damerval⁴, Cédric Del Rio⁵, Yves Deveaux⁴, Verónica S. Di Stilio⁶, Yan Gong⁷, Florian Jabbour⁸, Elena M. Kramer⁷, Sophie Nadot⁹, Natalia Pabón-Mora¹⁰, and Wei Wang¹¹

¹ Plant Development Group, Institute of Botany, Justus-Liebig-University, Giessen, Germany

² Institute of Biology/Dahlem Centre of Plant Sciences, Freie Universität Berlin, D-14195 Berlin, Germany

³ Université de Rennes, UMR CNRS 6553, Ecosystèmes-Biodiversité-Evolution, Campus de Beaulieu, 35042 Rennes cedex, France

⁴ Université Paris-Saclay, INRAE, CNRS, AgroParisTech, Génétique Quantitative et Evolution-Le Moulon, 91190 Gif-sur-Yvette, France

⁵ CR2P - Centre de Recherche en Paléontologie - Paris, MNHN - Sorbonne Université - CNRS, 43 Rue Buffon, 75005 Paris, France

⁶ Department of Biology, University of Washington, Seattle, WA 98195-1800, USA

⁷ Department of Organismic and Evolutionary Biology, Harvard University, MA, 02138, USA

⁸ Institut de Systématique, Evolution, Biodiversité (ISYEB), Muséum national d'Histoire naturelle, CNRS, Sorbonne Université, EPHE, Université des Antilles, 57 rue Cuvier, CP39, Paris, 75005, France

⁹ Université Paris-Saclay, CNRS, AgroParisTech, Ecologie, Systématique et Evolution, Gif-sur-Yvette, France

¹⁰ Instituto de Biología, Universidad de Antioquia, Medellín, 050010, Colombia

¹¹ State Key Laboratory of Systematic and Evolutionary Botany, Institute of Botany, Chinese Academy of Sciences, Beijing, 100093 China and University of Chinese Academy of Sciences, Beijing, 100049 China

* Correspondence: Annette.Becker@bot1.bio.uni-giessen.de

Received 15 September 2023; Editorial decision 29 November 2023; Accepted 11 December 2023

Editor: Rainer Melzer, University College Dublin, Ireland

Abstract

The *Ranunculales* are a hyperdiverse lineage in many aspects of their phenotype, including growth habit, floral and leaf morphology, reproductive mode, and specialized metabolism. Many *Ranunculales* species, such as opium poppy and goldenseal, have a high medicinal value. In addition, the order includes a large number of commercially important ornamental plants, such as columbines and larkspurs. The phylogenetic position of the order with respect to monocots and core eudicots and the diversity within this lineage make the *Ranunculales* an excellent group for studying evolutionary processes by comparative studies. Lately, the phylogeny of *Ranunculales* was revised, and genetic and genomic resources were developed for many species, allowing comparative analyses at the molecular scale. Here, we review the literature on the resources for genetic manipulation and genome sequencing, the recent phylogeny reconstruction of this order, and its fossil record. Further, we explain their habitat range and delve into the diversity in their floral morphology, focusing on perianth organ identity, floral symmetry, occurrences of spurs and nectaries, sexual and pollination systems, and fruit and dehiscence types. The *Ranunculales* order offers a wealth of opportunities for scientific exploration across various disciplines and scales, to gain novel insights into plant biology for researchers and plant enthusiasts alike.

Keywords: Ancestral states, carpels, distribution, fossils, fruits, genomic resources, nectaries, phyllotaxy, phylogeny, sexual systems, spurs, symmetry.

Introduction

Ranunculales are the sister order to all other eudicots and have diverged before the core eudicots, which include approximately three-quarters of all angiosperms species (The Angiosperm Phylogeny Group, 2016). Studying *Ranunculales* can thus provide clues to the core eudicot's ancestral states in terms of morphology and genetics. The order *Ranunculales* encompasses >4500 species and is composed of seven families: *Ranunculaceae*, *Berberidaceae*, *Menispermaceae*, *Lardizabalaceae*, *Circaeasteraceae*, *Papaveraceae*, and *Eupteleaceae* (Fig. 1; The Angiosperm Phylogeny Group, 2016). They are remarkably diverse in terms of floral and fruit form, life history traits, leaf shape, growth shape, and their secondary metabolite composition. The flowers of *Ranunculales* are not only unusually diverse in their morphology, they are also unique in concentrating a variety of evolutionary transitions, such as changes in merism (number of floral organs), in phyllotaxy (whorled versus spiral) potentially leading to the emergence of organ fusion (in reproductive organs and perianth), and in the origin of novel organs.

These transitions are only rarely observed in monocot or core eudicot model lineages. Further, *Ranunculales* exhibit a suite of homoplasious characters (shared character states that did not arise from a direct common ancestor, but independently via convergent evolution) such as transitions between sexual systems and pollination modes in closely related taxa, petal loss, spur formation, or transition to zygomorphy (Endress, 1995; Soza *et al.*, 2012; Damerval and Becker, 2017; Becker *et al.*, 2023). Homoplasies and the emergence of novel organs provide premier opportunities to study the molecular and genetic mechanisms involved in the origin of these special traits using species within *Ranunculales* as case studies.

Aside from their morphological diversity, *Ranunculales* produce a multitude of secondary metabolites, many of them of pharmaceutical importance. Consequently, *Ranunculales* species have been used in traditional medicine since at least the early civilizations. Some species, such as *Nigella sativa*, were already mentioned in writing by, for example, Ayurveda, Siddha,

The Ranunculales

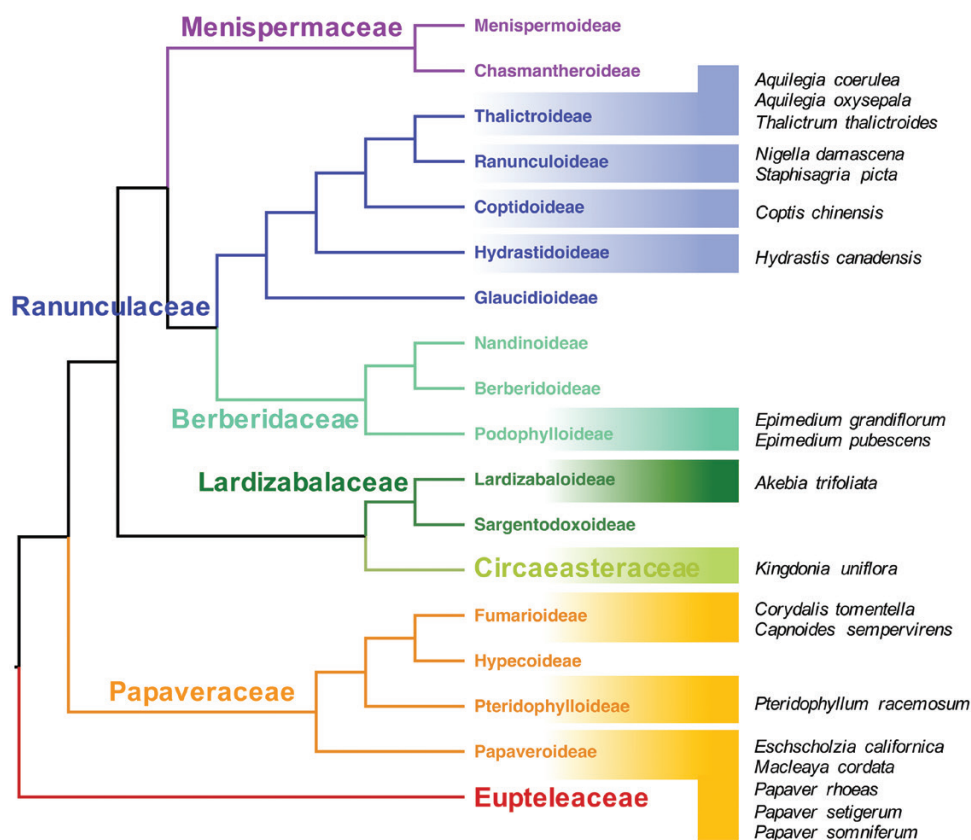


Fig 1. Simplified phylogeny of *Ranunculales* based on Wang *et al.* (2009), Ortiz *et al.* (2016), and Peng *et al.* (2023). Species for which major genomic resources are or will become available in the near future are next to their respective branches. Representative photos of *Ranunculales* flowers: (A) *Aquilegia coerulea*, (B) *Thalictrum thalictroides*, (C) *Nigella damascena*, (D) *Staphisagria picta* (*Ranunculaceae*), (E) *Epimedium grandiflorum* (*Berberidaceae*), (F) *Pteridophyllum racemosum*, (G) *Capnoides sempervirens*, (H) *Eschscholzia californica*, (I) *Macleaya cordata*, (J) *Papaver somniferum* (*Papaveraceae*). (Photo credit: A, D–G, H, J, Becker lab; B, Di Stillo lab; C, F, Jabbour; I, N, Pabón Mora.)

Unani, Greek–Roman, Malay, Tibb–e–Nabwi, and Jewish civilizations (Heiss and Oegg, 2005; Dabeer *et al.*, 2022). The use of opium poppy (*Papaver somniferum*) as a narcotic drug dates even back to the Neolithic (Guerra-Doce, 2015; Yang *et al.*, 2021). New World indigenous cultures used *Ranunculales*; for example, Navajos used *Thalictrum fendleri* tea during ceremonial war dance rites (Elmore, 1943) and Pomo women used *Eschscholzia californica* during infant weaning (Barrett, 1952). *Ranunculales* are a rich source of economically important phytochemicals, such as alkaloids, diterpenes, triterpenes, isoquinoline alkaloids, and cardiac as well as cyanogenic glycosides (Hao *et al.*, 2015). These compounds contribute to a vast array of medicinal uses for different *Ranunculales* species, for example in ulcer treatment, and as antimicrobe and anti-inflammatory agents (Hao *et al.*, 2015). *Papaveraceae* are notoriously known for their secondary metabolite diversity, and many of their compounds are essential pharmaceuticals of high economic value, including morphine, codeine, protopine, isocorydine, or berberine. Benzisoquinoline alkaloids (BIAs) in particular are well known for their analgesic, antitussive, antimicrobial, anticancer, and anti-inflammatory effect (Li *et al.*, 2020; Avci *et al.*, 2021; Becker *et al.*, 2023). Of special pharmaceutical importance are morphine and codeine used as analgesics, the anticancer drug noscapine, and antibacterial compounds such as sanguinarine (Hagel and Facchini, 2013). Members of the other *Ranunculales* families synthesize unique and overlapping subsets of secondary metabolites (Hao *et al.*, 2015). Consequently, a wide array of species is used as herbal extracts, even nowadays, for example in Chinese traditional medicine (Hao *et al.*, 2015, 2017).

An informative phylogenetic position, combined with pharmacological relevance and stunning floral morphological diversity, has led to a strong research interest in the *Ranunculales*, resulting in the development of an array of genetic tools to aid in the investigation of gene function and regulation (Di Stilio, 2011; Becker *et al.*, 2023). The powerful combination of genetic studies, comparative morphology, and secondary metabolite profiling will further enable the reconstruction of ancestral traits before the major core eudicot radiation.

In this review, we present an update on the phylogeny, fossil records, and ecology of *Ranunculales*, before addressing recent findings concerning the genetic origin, diversity, and evolution of floral and fruit traits. We also recapitulate the available omics resources and functional tools, and introduce the RanOmics project, aiming at selecting phylogenetically informative species to unravel the evolution of ecologically and economically important traits.

Genetic resources and functional tools for *Ranunculales*

In the ‘omics’ era, several genetic resources have been established for *Ranunculales*, mostly for mining genes related to secondary metabolite biosynthesis and regulation. The number

of high-quality *Ranunculales* genomes, starting with the first sequenced genome from *Macleaya cordata* (Liu *et al.*, 2017), has increased enormously in the past few years, allowing for comparative genome analysis (Fig. 1; Table 1). However, the suitability criteria for high quality reference genomes are unclear, hence we define them here as follows: the rate of Benchmarking Universal Single-Copy Orthologs (BUSCO; Manni *et al.*, 2021) matches should be >95%. Table 1 shows that only two *Ranunculales* genomes match this criterion, these are *P. somniferum* (opium poppy) and *Corydalis tomentella* (Guo *et al.*, 2018; Xu *et al.*, 2022). Genomes with lower BUSCO values are available for *Thalictrum thalictroides*, *Coptis chinensis*, *Aquilegia coerulea*, and *Aquilegia oxysepala* (*Ranunculaceae*), *Kingdonia uniflora* (*Circaeasteraceae*), *Akebia trifoliata* (*Lardizabalaceae*), *Epimedium pubescens* (*Berberidaceae*), *Eschscholzia californica*, *Corydalis tomentella*, *Papaver somniferum*, *Papaver rhoeas*, *Papaver setigerum*, and *Macleaya chordata* (*Papaveraceae*) (Liu *et al.*, 2017, 2021; Filiault *et al.*, 2018; Hori *et al.*, 2018; Sun *et al.*, 2020; Xie *et al.*, 2020; Arias *et al.*, 2021; Chen *et al.*, 2021; Huang *et al.*, 2021; Yang *et al.*, 2021; Shen *et al.*, 2022).

The available genomes already provide sufficient data for the inference of whole-genome duplications (WGDs) within the *Ranunculales*. When the genomes of *P. somniferum*, *M. cordata*, *A. coerulea*, and *C. chinensis* were analyzed in combination, one WGD was found to have probably occurred in the lineage leading to *C. chinensis* and *A. coerulea*, and another one in the lineage leading to *P. somniferum* and *M. cordata* (Liu *et al.*, 2021). An additional WGD was identified in the lineage leading to *P. somniferum* and *P. setigerum*, which is not shared by *P. rhoeas* and *M. cordata*. Moreover, the *P. setigerum* genome shows an additional WGD (Yang *et al.*, 2021), most probably contributing to its large genome size, which is almost double that of the closely related *P. somniferum*. These recent comparative genome studies suggest that the genome duplication history of *Ranunculales* is most likely to be as complex as those of the core eudicots, considering that the number of sequenced *Ranunculales* genomes is still relatively small.

Recently, the molecular evolution of morphine biosynthesis in the *Papaveraceae* was unraveled by comparative genomics: the final morphine biosynthesis steps, which require the *STORR* gene modules, was found to be <18 million years old (Li *et al.*, 2020). The *STORR* gene, coding for the key enzyme converting morphinans to morphine, originated from a translational fusion of a cytochrome P450 and an oxidoreductase enzyme that occurred after the split of *P. setigerum* and *P. somniferum* from *P. rhoeas* and was then duplicated in the *P. setigerum*-specific WGD (Li *et al.*, 2020; Yang *et al.*, 2021).

The 1KP project (One Thousand Plant Transcriptomes Initiative, 2019) has provided transcriptomic data for a single or a few tissues of these *Ranunculales* species: the *Lardizabalaceae* *A. trifoliata*; the *Menispermaceae* *Cocculus laurifolius*; the *Eupteleaceae* *Euptelea pleiosperma*; the *Berberidaceae* *Nandina domestica* and *Podophyllum peltatum*; the *Ranunculaceae* *Anemone hupehensis*, *Anemone pulsatilla*, *Cimicifuga racemosa*, *Hydrastis canadensis*, and

Table 1. Genomic resources for RanOmics Ranunculales species

Species	Genome available	BUSCO ^a	Cell/callus culture system	Cell culture transformation system	Stable transformation	Virus-induced-gene silencing	Spontaneous mutants
<i>Papaveraceae</i>							
<i>Eschscholzia californica</i>^b	Hori <i>et al.</i> (2018)	Not given	Hauschild <i>et al.</i> (1998)	Fujii <i>et al.</i> (2007)	Park and Facchini, (2000b); Lotz <i>et al.</i> (2022)	Wege <i>et al.</i> (2007)	Lange <i>et al.</i> , 2013; Wakelin <i>et al.</i> (2003); Barrell <i>et al.</i> (2010); Pollock <i>et al.</i> (2019); Conner and Barrell (2014)
<i>Papaver somniferum</i>^b	Guo <i>et al.</i> (2018)	95.3	Eilert <i>et al.</i> (1985)	Belyi <i>et al.</i> (1997)	Park and Facchini, (2000a); Chitty <i>et al.</i> (2003)	Hilemann <i>et al.</i> (2005)	Singh <i>et al.</i> (2017); Pathak <i>et al.</i> (2013); Dhawan <i>et al.</i> (2007); Belyaeva and Nevkrytaya (1979); Prajapati <i>et al.</i> (2001); Singh <i>et al.</i> (2014)
<i>Macleaya cordata</i>	Liu <i>et al.</i> (2017)	90.4	Franke and Böhm (1982)	No	Huang <i>et al.</i> (2017)	No	O'Donnell <i>et al.</i> (1993); Foote <i>et al.</i> , (1994)
<i>Papaver rhoeas</i>	Yang <i>et al.</i> (2021)	92.8	No	No	No	No	
<i>Papaver setigerum</i>	Yang <i>et al.</i> (2021)	94.5	No	No	No	no	
<i>Corydalis tomentella</i>	Xu <i>et al.</i> (2022)	97.67	Holländer-Czytko <i>et al.</i> (1988) (<i>Corydalis sem-pervirens</i>)	No	No	No	
<i>Capnoides sem-pervirens</i>^b							
<i>Pteridophyllum racemosum</i>^b							
<i>Ranunculaceae</i>							
<i>Coptis chinensis</i>	Chen <i>et al.</i> (2021)	91.5	No	No	No	No	
<i>Aquilegia coerulea</i> ^b	Filaut <i>et al.</i> (2018)	Not given	No	No	No	Gould and Kramer (2007)	Cabin <i>et al.</i> (2022)
<i>Aquilegia oxysepala</i>	Xie <i>et al.</i> (2020)	93.2	No	No	No	No	
<i>Thalictrum thalictroides</i>^b	Arias <i>et al.</i> (2021)	84.5	Smolko and Peretti (1994)	Samanani <i>et al.</i> (2002) (<i>Thalictrum flavum</i>)	No	Di Stilio <i>et al.</i> (2010)	Martínez-Gómez <i>et al.</i> (2021); Galimba <i>et al.</i> (2012)
<i>Nigella damascena</i> ^b			Klimek-Chodacka <i>et al.</i> (2020)		No	Wang <i>et al.</i> (2015)	Goncalves <i>et al.</i> (2013); Jabbour <i>et al.</i> (2021); Conde e Silva <i>et al.</i> (2023); Greyson and Raman (1975)
<i>Staphisagria picta</i>^b			No	No	No	No	
<i>Hydrastis canadensis</i>^b			No	No	No	No	
<i>Ciccaeasteraceae</i>							

Table 1. Continued

Species	Genome available	BUSCO ^a	Cell/callus culture system	Cell culture transformation system	Stable transformation	Virus-induced-gene silencing	Spontaneous mutants
<i>Kingdonia uniflora</i>	Sun et al. (2020)	90.6	No	No	No	No	
Lardizabalaceae							
<i>Akebia trifoliata</i>	Huang et al. (2021)	94.0	No	No	No	No	
Barberridaceae							
<i>Epimedium pubescens</i>	Shen et al. (2022)	89.9	No	No	No		Wang et al. (2017)
<i>Epimedium gran-diflorum</i>^b							

^a Benchmarking Universal Single-Copy Orthologs (BUSCO) percentages are provided as a measure for genome completeness.

^b Reference genomes are being sequenced and/or expression atlases are being produced by the PanOmics group or are already available via Phytozome (for *E. californica*).

T. thalictroides; and the *Papaveraceae* *Argemone mexicana*, *Capnoides sempervirens*, *Ceratocapnos vesicaria*, *Chelidonium majus*, *Corydalis linstowiana*, *E. californica*, *Hypecoum procumbens*, *Papaver bracteatum*, *P. rhoeas*, *P. setigerum*, *P. somniferum*, and *Sanguinaria canadensis*.

Additional resources have been developed for species in the *Ranunculales*, with the aim of elucidating gene function and molecular processes, mainly for studies in evolutionary developmental genetics of flowers and secondary metabolite analysis. The latter may be studied by inducing their production in cell culture systems, providing the cultures with standardized substrates and analyzing their products. These cell culture systems were established for the *Ranunculaceae* *T. thalictroides* and *Nigella damascena* (Smolko and Peretti, 1994; Klimek-Chodacka et al., 2020), and the *Papaveraceae* *E. californica*, *M. cordata*, *Corydalis sempervirens*, and *P. somniferum* (Franke and Böhm, 1982; Eilert et al., 1985; Holländer-Czytko et al., 1988; Hauschild et al., 1998). Even more useful are stably transformed cell culture systems, allowing the careful analysis of genes involved in the regulation of biosynthesis. Protocols for these are available for *Thalictrum flavum*, *N. damascena*, *E. californica*, and *P. somniferum* (Belny et al., 1997; Samanani et al., 2002; Fujii et al., 2007; Mohammed and Masyab, 2020).

The analysis of developmental processes can be achieved only in growing plants, ideally using knockout mutants. However, stable transformation of plants and regeneration of the transgenics is a very challenging process, often requiring labor-intensive tissue culture steps, and has thus not been established for many *Ranunculales* species. The notable exceptions here are *E. californica*, *M. cordata*, and *P. somniferum* (Park and Facchini, 2000a, b; Huang et al., 2017; Lotz et al., 2022), but publications of gene function analysis based on regenerated *Ranunculales* transgenics do not exist to date.

An alternative approach to down-regulate gene expression in plants is virus-induced gene silencing (VIGS), which utilizes the plant's immune system to repress viral transcript synthesis. Specific VIGS vector systems using modified plant viruses were developed to efficiently down-regulate target genes (Dinesh-Kumar et al., 2003; Liu et al., 2022). While this is a transient approach requiring careful analysis of the manipulated plants, tissue culture is not necessary, speeding up the process of gene function analysis considerably (Dommes et al., 2019; Rössner et al., 2022). This method is available for *Aquilegia coerulea*, *T. thalictroides*, *T. clavatum* (on dormant tubers), *T. dioicum*, *N. damascena*, *Delphinium ajacis*, *E. californica*, *P. somniferum*, and *Cysticapnos vesicaria* (Hileman et al., 2005; Gould and Kramer, 2007; Wege et al., 2007; Di Stilio et al., 2010; Hidalgo et al., 2012; Wang et al., 2015; Zhao et al., 2023) allowing for the comparative analysis of gene function among species and the assessment of functional conservation (Di Stilio, 2011)

Phylogeny of Ranunculales

Over the past several decades, tremendous progress has been made in delimiting and elucidating phylogenetic relationships

among the infraordinal taxa within *Ranunculales*. As currently circumscribed by molecular data, the order consists of seven monophyletic families: *Berberidaceae*, *Circaeasteraceae*, *Eupteleaceae*, *Lardizabalaceae*, *Menispermaceae*, *Papaveraceae*, and *Ranunculaceae* (Wang *et al.*, 2009; The Angiosperm Phylogeny Group, 2016).

Three major clades are recovered: *Eupteleaceae*, *Papaveraceae*, and the core *Ranunculales* (Kim *et al.*, 2004; Wang *et al.*, 2009). The relationships among these three clades are not well resolved, but the majority of phylogenetic analyses recognize the monogeneric *Eupteleaceae* as the earliest diverging lineage with weak to moderate support (e.g. Kim *et al.*, 2004; Worberg *et al.*, 2007; Wang *et al.*, 2009; Sun *et al.*, 2017; Peng *et al.*, 2023). Within the core *Ranunculales*, *Circaeasteraceae* and *Lardizabalaceae* form a clade, and *Menispermaceae*, *Berberidaceae*, and *Ranunculaceae* form another clade, with *Berberidaceae* as sister to *Ranunculaceae* (Kim *et al.*, 2004; Wang *et al.*, 2009; Sun *et al.*, 2017; Peng *et al.*, 2023).

The *Eupteleaceae* include a single genus with two species only, *Euptelea pleiosperma* and *Euptelea polyandra* (Cao *et al.*, 2016). Genome sequence or other resources are not available for this genus.

Papaveraceae sensu lato contain four subfamilies: *Fumarioideae*, *Hypecoideae*, *Papaveroideae* (including *Chelidonioideae*, *Eschscholziaceae*, and *Papavereae*), and *Pteridophylloideae* (Hoot *et al.*, 2015). The position of *Pteridophylloideae* has been controversial (reviewed by Peng *et al.*, 2023). Recently, a complete genus-level phylogeny was built for *Papaveraceae*, in which *Papaveroideae* form a clade, whereas *Pteridophylloideae*, *Hypecoideae*, and *Fumarioideae* form another clade, with *Hypecoideae* as sister to *Fumarioideae*; the relationships among 91% of all currently recognized genera in the family are well resolved (Peng *et al.*, 2023).

Circaeasteraceae consists of two monotypic genera, *Circaeaster* and *Kingdonia*, and it is the sister group to *Lardizabalaceae* (Wang *et al.*, 2009; Sun *et al.*, 2017). Within *Lardizabalaceae* (the sister family of *Circaeasteraceae*), *Sargentodoxa*, *Decaisnea*, and *Sinofranchetia* are successive sister taxa to the other genera (Wang *et al.*, 2009, 2020).

Within *Menispermaceae*, two subfamilies are recognized: *Chasmantheroideae* and *Menispermoideae* (Ortiz *et al.*, 2016). *Chasmantheroideae* comprises *Coscinieae* and *Burasaieae*, and *Menispermoideae* comprises eight tribes, among which *Menispermeae* is the earliest diverging, followed by *Anomospermeae*, then *Limacidae*. *Cebatheae*, *Cissampelideae*, *Pachygoneae*, *Spirospermeae*, and *Tiliacoreae* form a clade with strong support, but the relationships among these five tribes are not resolved because they might have diversified rapidly over a period of <6 million years (Wang *et al.*, 2017; Lian *et al.*, 2020).

Berberidaceae contain three subfamilies, *Podophylloideae*, *Berberidoideae*, and *Nandinoideae*, corresponding to the chromosome base numbers $x=6$, 7 and 8, or 10, respectively (Wang *et al.*, 2007, 2009; Sun *et al.*, 2018). Recently, Hsieh *et al.* (2021) further updated the classification system for this family at the tribal and generic levels.

Ranunculaceae (Tamura, 1965, 1993) consists of five subfamilies: *Coptidoideae*, *Glaucidoideae*, *Hydrastidoideae*, *Ranunculoideae*, and *Thalictroideae* (Wang *et al.*, 2009). Most studies support *Glaucidoideae* as sister to the remaining taxa of the family, followed by *Hydrastidoideae*, then *Coptidoideae* (e.g. Kim *et al.*, 2004; Wang *et al.*, 2009, 2016; Cossard *et al.*, 2016; Zhai *et al.*, 2019), whereas other studies place *Glaucidoideae* as sister to *Hydrastidoideae* (Hoot *et al.*, 1999; Soltis, 2000). *Thalictroideae* and *Ranunculoideae* are characterized by the T- and R-type chromosomes, respectively (with R-type being metacentric and T-type telocentric, with only one arm; Wang *et al.*, 2009), but the monophyly of *Ranunculoideae* remains controversial. Based on eight DNA loci from three genomes, Cossard *et al.* (2016) placed *Thalictroideae* in *Ranunculoideae*, as sister to *Adonoideae*. That was confirmed by a plastid phylogenomic analysis (Zhai *et al.*, 2019), whereas a phylotranscriptomic analysis strongly supports the monophyletic *Ranunculoideae* (He *et al.*, 2022). He *et al.* (2022) suggest that the different positions of *Adonoideae* in the nuclear and plastid trees could result from ancient hybridization and/or subsequent introgression events. The currently recognized *Ranunculoideae* contains 10 tribes, which together with *Thalictroideae* appear to have diversified rapidly over a period of <14 million years, and perhaps in as little as 1–2 million years (Wang *et al.*, 2016).

Fossil record

The fossil record of the *Ranunculales* includes nearly 800 occurrences (Xing *et al.*, 2016), but most of them should be considered with caution. In particular, few reliable fossils have been described from the Cretaceous period (Friis *et al.*, 2011). Three northern hemisphere fossils could illustrate the early diversification of *Ranunculales* during this period. The flower of *Teixeiraea lusitanica* from the Cretaceous [~113 million years ago (Ma)] of Portugal is considered to be part of the stem or crown of the *Ranunculales* without family assignment von Balthazar *et al.*, 2005). Also, from Portugal and with similar age, the flower *Kajanthus lusitanicus* is the first Cretaceous occurrence of *Ranunculales* assigned to the family *Lardizabalaceae* (Mendes *et al.*, 2014). However, a new study considers this flower as more confidently assigned to the crown group of *Ranunculales*, making it undefined at the family level (Schönenberger *et al.*, 2020). The anatomy of the stem of the liana *Atli mornii* Smith, Little, Cooper, Burnham, and Stockey from the Late Cretaceous (77–74 Ma) of Canada allows for the identification of *Ranunculales* without family affinity, and reinforces the early presence of *Ranunculales* in Laurasia (Smith *et al.*, 2013). However, the recent description of *Santaniella lobata* based on fruits and stems from the Cretaceous (Barremian/Aptian, ~125 Ma) of Brazil related to *Ranunculales* (Gobo *et al.*, 2022) along with the leaf with unknown affinity but close to *Ranunculales* in shape, named *Baderadea pinnatissecta* described from the same region (Pessoa *et al.*, 2021), could indicate a Lower Cretaceous

origin of the *Ranunculales* in Gondwana rather than Laurasia. Nevertheless, additional data from *S. lobata* indicate that this fossil belongs to angiosperms without certainty about the order (Pessoa *et al.*, 2023).

With the exclusion of the monotypic family *Circaeasteraceae*, the other families are represented in the fossil record (Xing *et al.*, 2016). Although the families of *Ranunculales* appear to have diverged early on, no Cretaceous fossil can be confidently assigned to any extant family. The unequivocal fossils assigned to a particular family are mostly fruits, seeds, leaves, wood, and pollen from the Paleogene (Friis *et al.*, 2011).

The family *Berberidaceae* is represented by ~100 fossils from the Oligocene to the Pliocene, mainly from North America and Europe, but also from Asia (Friis *et al.*, 2011; Xing *et al.*, 2016; Chen *et al.*, 2020). Fossils of *Berberidaceae* are represented by only two genera, *Mahonia* and *Berberis*, based on leaves and seeds (Xing *et al.*, 2016). Fossils of the *Eupteleaceae* family are scarce; however, Friis *et al.* (2011) indicate the presence of a few fossils from this family in the Northern Hemisphere from the Paleocene to the Miocene.

The fossil record of *Lardizabalaceae* was recently reviewed (Wang *et al.*, 2020). During the Cenozoic, fossils attributed to this family come from the Eocene to Miocene of Europe and the USA, as well as from the Miocene of Japan and the Cenozoic of South America. Most of the fossils belong to the genus *Sargentodoxa*, with the exception of *Decaisnea* seeds from the Oligocene of Germany and a liana attributed to the family level (Wang *et al.*, 2020).

The very diverse woody family *Menispermaceae* has a very abundant fossil record compared with other *Ranunculales* families, with many fossil fruits, leaves, and wood having been described (Jacques, 2009; Xing *et al.*, 2016). Several Cretaceous fossils may be credible, such as the morphological genus *Menispermites*, but need revision (Jacques, 2009). Characteristic endocarps named ‘moonseed’ are traditionally found in North America and Europe (Jacques, 2009), and were also recently found in South America (Herrera *et al.*, 2011; Jud *et al.*, 2018) and Asia (Han *et al.*, 2018, 2020) as early as the Paleocene. Within this family, a total of 44 genera have been found in the fossil record, of which 17 are extant and 27 are extinct (Jacques, 2009). This fossil record attests to a rapid and universal diversification of *Menispermaceae* during the Paleogene as well as a complex migration of flora during this period.

Reliable fossil record of *Papaveraceae* is meager and is represented, to our knowledge, only by a *Corydalis* from the Pliocene of Italy (Mai, 1995). The fossil record of the *Ranunculaceae* family, mostly based on fruits, was revised by Pigg and Devore (2005). Most of these fossils are distributed in Europe and North America, from the Paleocene to the Pliocene, and some seeds were recently found in the Pliocene of China (Huang *et al.*, 2021).

It is noteworthy that the fossil record of *Ranunculales* is relatively sparse in comparison with the present diversity of the order and knowing its ancient evolutionary history. A large part of the extant diversity is represented by plants with herbaceous

or climbing habitus, which have low fossilization potential (Friis *et al.*, 2011). Moreover, the potential *Ranunculales* fossils from the Cretaceous are also difficult to distinguish from indirectly related early-diverging eudicot lineages (e.g. Sun *et al.*, 2011; Pessoa *et al.*, 2021). The *Ranunculales* fossil record also illustrates a well-known bias in collecting and studies in paleobotany, namely the historical focus on Europe and North America (Xing *et al.*, 2016). Recent discoveries, particularly from South America and Asia, may strengthen the fossil record of the order in the future.

Distribution and ecological niches

The order *Ranunculales* comprises ~4500 species, primarily occupying temperate areas of the world, with few members cosmopolitan or reaching into the tropics. Namely, the two species of *Euptelea* (*Eupteleaceae*) occur in Japan between 400 m and 1500 m (*E. polyandra*) and from India to China between 900 m and 3600 m (*E. 3 pleiosperma*; Endress, 1993). In contrast, the *Papaveraceae* with ~430 species in 42 genera are primarily distributed in the northern hemisphere with few exceptions, including *Papaver aculeatum* in South Africa, and the genus *Bocconia* that reaches central and South America (Kadereit, 1993). The *Fumarioideae* are concentrated in the Sino-Himalayan and Mediterranean regions, with occurrences in South Africa and North America (Lidén, 1993a). Both *Papavereae* and *Chelidoniaceae* (*Papaveroideae*) contain Old and New World genera. Most *Papavereae* in the New World inhabit western North America, while the Old World genera are concentrated in southwest and central Asia, and the Mediterranean. The genus *Papaver* is broadly distributed in the Old and the New World. The *Eschscholzieae* (*Papaveroideae*) are found in the New World and almost exclusively in Pacific North America. *Hunnemanina* is present in the east of Mexico. Most *Papavereae* and *Eschscholzieae* are found in open vegetation arid and warm climates, with a few exceptions that have colonized arctic areas. Conversely, the *Chelidoniaceae* of the New World occupy regions in Northeast America with the exceptions of *Bocconia* (Central and South America) and *Glaucium* and *Dicranostigma* (West and Central Asia). They can inhabit dry open areas (*Glaucium*, *Dicranostigma*, and *Macleaya*) or deciduous forests (*Hylomecon*, *Sanguinaria*, and *Stylophorum*). The only species of *Pteridophyllum*, *Pteridophyllum racemosum*, is a Japanese endemic (Lidén, 1993b).

Species of *Papaveraceae* selected as part of the RanOmics project include: *Corydalis tomentella*, *Capnoides sempervirens*, *Eschscholzia californica*, *Macleaya cordata*, *Papaver rhoeas*, *Papaver setigerum*, *Papaver somniferum*, and *Pteridophyllum racemosum*. *Corydalis tomentella* is a perennial, native to China, that grows in rock crevices, between 700 m and 1000 m. The plant itself reaches 15–20 cm, it has characteristic golden yellow flowers in dense inflorescences, and it can tolerate freezing temperatures (http://www.efloras.org/flora_page.aspx?flora_id=2). *Capnoides sempervirens* (pale corydalis or rock harlequin) is a

biennial plant from the mid-latitudes of North America where it grows on exposed ridges and rocky outcrops (Sprengelmeyer and Rebertus, 2015), and it produces monosymmetric flowers, which are exceptional in that they are in a terminal position (Hidalgo and Gleissberg, 2010). *Eschscholzia californica* is a small herb able to grow as annual or perennial with native ranges from Northern California to Southwestern Mexico, with cymose inflorescences and flowers with deciduous sepals and characteristic yellow petals (Becker et al., 2023). *Macleaya cordata* is a herbaceous, perennial native to China, Japan, and Taiwan, unusual in that it can reach sizes of up to 3 m, it spreads by rhizomes, and it has massive inflorescences of showy but apetalous flowers (Kadereit, 1993; Arango-Ocampo et al., 2016). *Macleaya cordata* is the source of alkaloids with broad uses as detoxifiers, antimicrobials, and insecticides (Liu et al., 2017). All *Papaver* species are herbs with cymes carrying large showy flowers. *Papaver somniferum* is the source of opium, and its center of domestication was the Mediterranean basin (Salavert et al., 2020; Hong et al., 2022). Numerous biochemical accounts with emphasis on the production of BIAs are available for different landraces (Pei et al., 2021), and two features have been linked to domestication, namely changes in capsule dehiscence and seed size (Zohary et al., 2012). Interestingly, morphine, codeine, and thebaine are lacking in capsules of the closely related and geographically overlapping *P. setigerum* (LaValva et al., 1985). Albeit the two species were thought to be part of the same taxonomic unit, *P. somniferum* is 30–150 cm high, self-pollinated, and diploid, while *P. setigerum* is 60 cm high, a field weed occurring in disturbed grounds that can be diploid or tetraploid (Hammer 1977; Jesus et al., 2021). *Papaver rhoeas*, the red poppy, is a remarkable species with exceptional beauty that has reproduced and expanded its native range across the Mediterranean as an agricultural weed (Colledge et al., 2004). *Papaver rhoeas* is a self-incompatible herb, currently pollinated by bees, flies, and beetles (McNaughton and Harper, 1960; Foote, 1994). Finally, the rare *P. racemosum* is a herb with leaves of astonishing shape convergent to those of ferns, is only found in Japan, and it grows between 1000 m and 2000 m in coniferous forests. It shares with the rest of *Papaveraceae* the caducous sepals and the dimerous floral organization, despite the unusual leaf phenotypes (Lidén, 1993a, b).

The Lardizabalaceae (35 species in eight genera) are primarily present in Japan, the Sino Himalayan mountains, Central and East China, and Vietnam. All genera are woody vines in subtropical evergreen forests or warm temperate green forests. Only *Lardizabala* and *Boquila* are endemic to temperate forests of Central and South Chile (Cheng-Yih and Kubitzki, 1993). Their most prominent member is *Akebia trifoliata*, a deciduous to evergreen twining vine, reaching up to 10 m in height with functionally unisexual flowers. Its berries are a rich source of vitamin C and pectin, and the seeds contain a high percentage of unsaturated fatty acids; the species is widely advertised as a new fruit crop. *Akebia trifoliata* consists of three subspecies, all with different but overlapping distributions ranging from

subtropical to temperate regions from 20 m up to 2800 m in elevation in China and Taiwan (Zhang et al., 2021; Zou et al., 2022). Only *A. trifoliata* is a member of the RanOmics project.

Conversely, the *Circaeasteraceae* (two species) are herbs. Two genera are recognized: *Circaeaster* is present in India, Nepal, and China, and grows in moist coniferous forests between 1200 m and 5000 m. *Kingdonia uniflora*, on the other hand, is endemic to China (provinces of Shaanxi, Sichuan, Gansu, and Yunnan) between 2800 m and 3200 m (Cheng-Yih and Kubitzki, 1993) and is selected as the representative species of *Circaeasteraceae* for the RanOmics project.

The *Menispermaceae* consist of ~450 species in 71 genera, including many woody climbers, and rarely trees, shrubs, or herbs. They are of cosmopolitan distribution, mostly confined to the tropical lowlands in the Old and the New World. They are extremely diverse in their habitats and found in Africa and Southeast Asia (*Stephania*), extra tropical North America (*Cocculus* and *Menispermum*), the Mediterranean (*Cocculus*), Japan (*Cocculus* and *Stephania*), and South America (*Abuta* and *Chondrodendron*) (Kessler, 1993; Ortiz et al., 2007).

Species of *Berberidaceae* selected as part of the RanOmics project include *Epimedium grandiflorum* and *Epimedium pubescens*. The *Berberidaceae* include ~650 species organized in 14–17 genera. They are herbs or woody shrubs, often a component of mesophytic forests in East Asia, Northeast America (*Achlys*, *Diphylleia*, *Jeffersonia*, *Podophyllum*, and *Sinopodophyllum*), Andean South America (*Berberis*); even desert xerophytes are found in Southwest Asia. Members of *Berberis* are also found in South America, from Colombia to Chile, Juan Fernandez Islands, and Argentina (Loconte, 1993). In addition, a few species of *Berberis* have become invasive in North America and South Africa (Keet et al., 2016). The pharmaceutically and horticulturally relevant *Epimedium* genus includes only herbaceous species growing mainly in woodlands. Its center of diversity is East Asia, with most species native to China. However, some species grow in the Alps, the Balkan region, Algeria, Caucasia, Japan, east Russia, and Kashmir (Zhang et al., 2022). *Epimedium pubescens* is native to the Chinese provinces Anhui, Jiangxi, and Sichuan (Stearn et al., 2002). *Epimedium grandiflorum*, a species with large flowers comprising curved nectar spurs, grows in Japan, North Korea, and South China, and varies greatly in flower color between white, light yellow, and purple-pink (Stearn et al., 2002).

The *Ranunculaceae* is a cosmopolitan family with ~2500 species in 59 genera. With large preferences for temperate or cool climates, they are a rare element in the tropics (Chartier et al., 2016). The most broadly distributed elements in northern and southern hemispheres include *Anemone*, *Caltha*, *Clematis*, *Myosurus*, *Ranunculus*, and *Thalictrum*. A total of 44 genera are present in East Asia, 24 in Europe, with few genera in temperate North America and in Highlands in South America (Tamura, 1993). Species of *Ranunculaceae* selected as part of the RanOmics project include *Coptis chinensis*, *Aquilegia coerulea*, *Aquilegia oxysepala*, *Thalictrum thalictroides*, *Nigella damascena*, *Staphisagria picta*, and *Hydrastis canadensis*.

In many phylogenies, *H. canadensis* or goldenseal is the sister species to all remaining *Ranunculaceae*. It is native to the eastern deciduous forests of North America. It grows in dense patches resulting from clonal growth via rhizome and lateral root formations (Sanders and McGraw, 2005). The rhizomes of this species are highly prized as a food supplement and as a traditional remedy for diverse conditions, including wound healing, digestive disorders, and cancer, with berberine as the pharmacologically most active ingredient (Mandal et al., 2020). Several *H. canadensis* populations are under serious threat caused by commercial and private harvesting of natural populations (Albrecht and McCarthy, 2006).

Sister to the *Thalictrioideae* and *Ranunculoideae* are the *Coptidoideae*, with *Coptis chinensis* as a RanOmics species representative. The species has an at least 2000 year long history as traditional Chinese medicine, with berberine also as the dominant alkaloid. The rhizomes of *C. chinensis* are harvested, and it is cultivated in several Chinese provinces in shady, moist, and cool mountainous regions between 1200 m and 1800 m (Chen et al., 2021). *Coptis chinensis* is endangered in the wild and its remaining populations are found in the woodlands of central China at altitudes of 500–2000 m. This species, like *H. canadensis*, suffers from harvesting of the rhizomes (He et al., 2007).

Nigella damascena (commonly known as love-in-the-mist) is an annual herbaceous weedy species growing throughout the Mediterranean. As a popular ornamental plant, it was most probably distributed by seeds along ancient trade routes (Heiss and Oeggel, 2005). Interestingly, a mutant that lost petal identity and has numerous petaloid tepals was described as early as in 1601 (Clusius, 1601).

Staphisagria picta is a species endemic to Corsica, Sardinia, and Majorca, growing between 150 m and 600 m in open grasslands (Orellana et al., 2009). *Aquilegia oxysepala* is broadly found throughout Southeastern China and grows in open patches, along roadsides and forest margins at low altitudes (Li et al., 2014). *Aquilegia coerulea* (also described as *Aquilegia caerulea*) also has a large area of distribution, stretching across the Southern and central Rocky Mountains of western North America from 2100 m to 3700 m altitude (Miller, 1981). For genetic studies, mainly the commercially available, fast cycling cultivar ‘Origami’ is used (Sharma and Kramer, 2013). *Thalictrum thalictroides* (*Ranunculaceae*, also known as *Anemone thalictroides*, commonly called rue anemone) is a spring ephemeral growing on streams and open woods in the Eastern USA (Lubbers and Christensen, 1986).

Taken together, the *Ranunculales* species for which genomic resources of various kinds are available occupy diverse habitats that range from dry Mediterranean islands (*S. picta*) over high altitudes (*A. coerulea*), to damp temperate forests (*P. racemosum*). Some species are abundant (*N. damascena*) or even invasive (*E. californica*), but several *Ranunculales* are rare and threatened in the wild (*H. canadensis*, *C. chinensis*, *S. picta*, and *P. racemosum*).

Floral diversity in *Ranunculales*

Floral structure and perianth in families of *Ranunculales*

Like floral phyllotaxis and symmetry, perianth organ identity, development, and function(s) are extremely diverse in *Ranunculales*, and range from absent to undifferentiated tepals, or more or less differentiated and petaloid sepals and modified and nectariferous petals (Fig. 2). For instance, flowers of

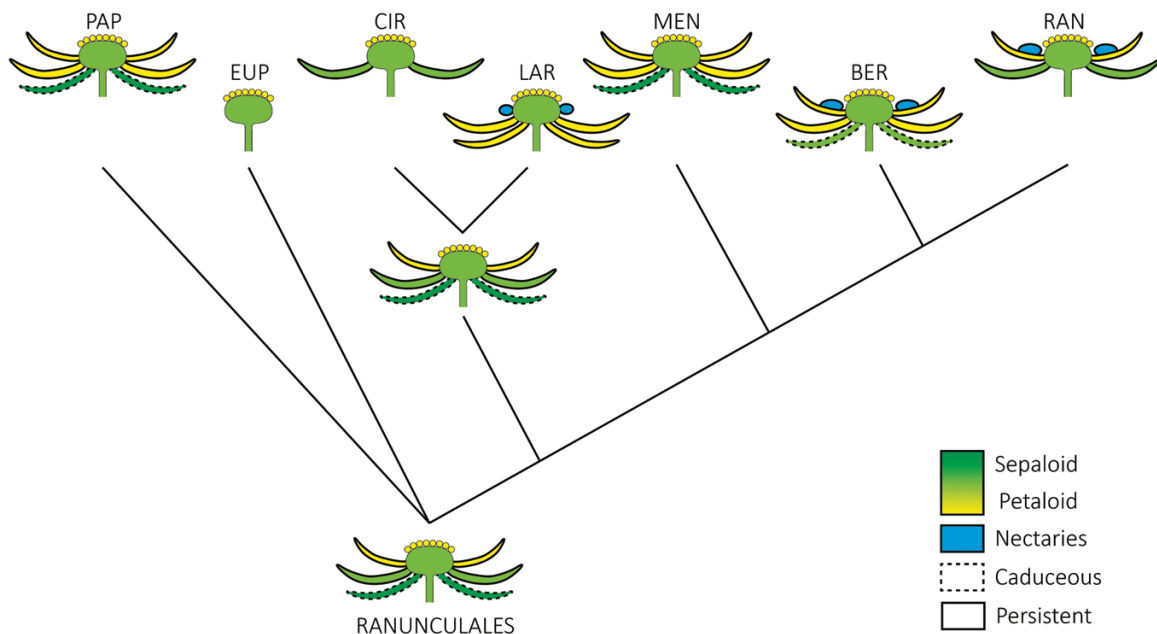


Fig. 2. Simplified phylogeny of *Ranunculales* showing ancestral floral traits of the *Ranunculales* families.

the monotypic *Eupteleaceae* are perianthless (Ren *et al.*, 2007), whereas those in both monotypic genera of *Circaeasteraceae* typically have tepals, which in *Kingdonia* co-occur with modified nectariferous petals interpreted as staminodial in origin (Ren *et al.*, 2004; Tian *et al.*, 2005). In contrast, flowers in *Menispermaceae* and *Lardizabalaceae* typically have persistent and more or less petaloid sepals and, when present, nectariferous petals (Endress, 1995). *Papaveraceae* have caduceous (easily detached) sepals and nectarless petals in the *Papaveroideae*, and more or less persistent petaloid sepals and spurred and nectar-collecting petals in *Fumarioideae* (Sauquet *et al.*, 2015). In *Berberidaceae* and *Ranunculaceae*, flowers can also be perianthless or have a perianth differentiated into more or less caducous or petaloid and persistent sepals, and more or less modified and nectariferous petals traditionally referred to as 'Nektarblätter' (Hiepko, 1965; Terabayashi, 1985; Endress, 1995).

As sister to all other eudicots, *Ranunculales* are thus pivotal to understanding the evolution of perianth and petaloidy in the largest clade of flowering plants. Previous comparative studies have shown that *Ranunculales* petals can be more similar to sepals in position, development, structure, and function(s) (e.g. petaloidy), and thus referred to as petaloid sepals of bracteopetalous origin, or more similar to stamens called Nektarblätter or nectariferous staminodia of andropetalous origin (Hiepko, 1965; Terabayashi, 1985; Endress, 1995). As in other eudicot lineages, the line between bracteo- and andropetals is usually defined by a set of developmental, structural, and functional traits which are thought to have evolved independently several times, including in the *Ranunculales* (Ronse De Craene and Brockington, 2013). However, a comparative study of gene expression patterns and floral organ identity challenged this view by suggesting that petals are deeply homologous and correlate with duplications and subfunctionalizations of B-class MADS box genes (Rasmussen *et al.*, 2009, and see below).

Ancestral floral characters

Based on current ancestral character reconstructions of floral traits, the most recent common ancestor (MRCA) of *Ranunculales* had a differentiated perianth with at least three series (or whorls) of organs, assumed to be petaloid (Fig. 2; Carrive *et al.*, 2020). The question of how different these whorls were remains unanswered, as does the question of which of the two outer whorls was lost in the families with only two whorls of perianth organs. Reconstructions of other perianth characters are consistent with the eudicot ancestor of Sauquet *et al.* (2017); that is, the androecium would have been composed of more than two whorls of stamens, and the gynoecium would have consisted of a few free carpels.

Unfortunately, such ancestral character reconstructions are sometimes hampered by the confusion surrounding the identity of perianth organs, and the definition of petaloidy. In *Ranunculales*, for instance, highly modified nectariferous petals described in previous literature as staminodia may be

misinterpreted as belonging to the androecium, and as a result nectaries would be coded as present on the androecium, whereas these organs are more likely to be homologous to the other, less modified petals of andropetalous origin in other taxa. In addition, the distinction in the perianth between outermost sepals that may be caducous or become gradually petaloid, and the innermost ones persisting as bracteopetals that are regularly associated with modified and nectariferous andropetals, as in *Berberidaceae* or *Ranunculaceae*, has received little attention (e.g. Terabayashi, 1985). Such a re-evaluation of older literature would show that in *Berberidaceae*, for instance, nectaries always differentiate on more or less modified petaloid organs of androecial origin that were likely to have not only been present in their MRCA, but also associated with a distinct series of persistent petaloid organs of bracteo- and an outermost one more or less caducous and/or petaloid. This interpretation is supported by the similarities observed in the recent reconstruction of the *Berberidaceae* and *Ranunculales* MRCA floral Bauplan. It suggests that the occurrence of petals of both bracteo- and androecial origin in flowers of the MRCA of *Berberidaceae*, surrounded by an outermost series of more or less caducous and/or petaloid sepals, may be ancestral for the order as a whole.

Floral organ identity and petaloidy

The ABC homeotic genes define a strict model of organ identity in angiosperms (Coen and Meyerowitz, 1991). The identity of perianth organs is determined based on the expression of the A- and B-class genes, with the B-class giving the petaloid character of the organs. Class A genes are specific to angiosperms, characterized by the presence of sterile organs forming the perianth, surrounding the reproductive organs. Positive self-regulatory loops and antagonistic relationships among members of the ABC-class genes can modulate the timing of accumulation of the products of the different homeotic genes (Schwarz-Sommer *et al.*, 1992; Halfter *et al.*, 1994; Jack *et al.*, 1994; Causier *et al.*, 2010; Conde E Silva *et al.*, 2023). The expression and functional evaluation of the ABC model of flower development genes has provided valuable insight into the evolution of flower patterning in *Ranunculaceae* *Aquilegia* (Kramer *et al.*, 2003, 2004), *Thalictrum* (Di Stilio *et al.*, 2005; Galimba *et al.*, 2012, 2018; Larue *et al.*, 2013; Galimba and Di Stilio, 2015; Soza *et al.*, 2016; Martínez-Gómez *et al.*, 2021), *N. damascena* (Wang *et al.*, 2015), and *Delphinium ajacis* (Zhao *et al.*, 2023), and in the *Papaveraceae* *P. somniferum* (Drea *et al.*, 2007; Pabón-Mora *et al.*, 2012) and *E. californica* (Yellina *et al.*, 2010; Lange *et al.*, 2013).

In core eudicots, an antagonistic relationship between classes A and C restricts their mutual expression (Causier *et al.*, 2010). However, in *Ranunculales*, duplication events and subfunctionalization of members of the different gene classes suggest that the well-characterized Arabidopsis core eudicot model does not strictly apply. For instance, the role of A-class homologs

(*FUL*-like genes) in sepal identity has only been demonstrated in *E. californica* and *P. somniferum* (Pabón-Mora *et al.*, 2012). Knockdown of *FUL* function in *E. californica* or *P. somniferum* by VIGS reveals slight defects in petal shape and color, but petal identity is not lost. In *Aquilegia coerulea*, A-class genes have been recruited primarily in the proper patterning of leaves and have no function in perianth identity (Pabón-Mora *et al.*, 2013). In *N. damascena*, *FUL* homologs have no role in floral organ identity, and an *AGAMOUS-Like* gene (*AGL6*) promotes sepal identity (Wang *et al.*, 2016). All these results support the idea that *FUL* homologs do not have a strict A function in basal eudicots as they do in core eudicot models (Litt, 2007).

B-class genes, particularly *AP3* homologs, have duplicated locally in *Ranunculales*, allowing for subfunctionalization and independent loss of petal identity genes (*AP3-3*) without affecting stamen identity factors (*AP3-1* and *AP3-2*), and resulting in apetalous flowers independently (Zhang *et al.*, 2013; Arango-Ocampo *et al.*, 2016).

In *Thalictrum*, one such genus with apetalous flowers, certain B-class genes are expressed in the sepals only when they are petaloid, as in *T. thalictroides* (Galimba *et al.*, 2018). In this species, E-class genes are also involved in the petaloidy of sepals, and have been suggested to keep the boundaries between either sepal and stamen zones or stamen and carpel zones by interacting with B- and C-class genes (Soza *et al.*, 2016). Perianth organ identity in *T. thalictroides* would therefore be controlled by a sliding boundary model, with a shift towards sepals in the expression of B-class genes (Larue *et al.*, 2013).

Members of the B-class genes are positive regulators of the expression of the C-class genes in *E. californica*, *A. coerulea*, and *N. damascena*, which in turn restrain the expression of the B-class genes (Yellina *et al.*, 2010; Lange *et al.*, 2013; Sharma and Kramer, 2013). The balance between the expression of the different paralogs of each gene class in the transition zones between floral organs is essential to maintain full organ identity and the proper number of each organ type. Flexibility in perianth organ identity may therefore result from the extension or restriction of B- or C-class genes, by modulating the interactions between ABC genes during species evolution. These mechanisms vary among species and individuals, depending on environmental conditions, particularly in flowers with spiral phyllotaxis.

Given the widespread occurrence of petaloidy in sepals or tepals in *Ranunculales*, and the potential that the ancestral flower had a perianth with whorled phyllotaxy (Sauquet *et al.*, 2017; Sokoloff *et al.*, 2018), perianth organ identity in the MRCA of *Ranunculales* may have been controlled by a sliding boundary model of floral organ identity. Either one, two, or even the three whorls (in the perianthless *Eupteleaceae*) would have been lost, and shifts in petaloidy could have occurred in the remaining whorls, depending on taxa, resulting in a strict ABC model of perianth identity evolving independently in some *Papaveraceae* (such as *Eschscholzia*, Chandrabali *et al.*, 2010) and *Berberidaceae*, similar to the ABC model at play in core eudicots.

The petaloid appearance of sepals in different members of the *Ranunculaceae* (Fig. 2) has a different genetic basis. In *Thalictrum*, petaloid sepals express B- and E-class genes, and their targeted silencing or mutation leads to green leafy sepals (Soza *et al.*, 2016; Galimba *et al.*, 2018; Martínez-Gómez *et al.*, 2021), whereas in *Aquilegia* the B gene *AP3-1* controls the novel identity of the staminodium, and contributes to color but not papillate cell types in the sepals (Kramer *et al.*, 2007; Sharma and Kramer, 2017). A 'B' gene paralog product of a *Ranunculales*-specific duplication, *APETALA3-3*, has become subfunctionalized to petal identity in *Aquilegia* (Sharma *et al.*, 2011). This B gene is expressed in petals across other *Ranunculales* (Kramer *et al.*, 2003) and has been secondarily lost in apetalous taxa such as *Thalictrum* (Di Stilio *et al.*, 2005; Zhang *et al.*, 2013).

Loss-of-function mutations in *Thalictrum* B-class genes, as found in natural and horticultural mutants (Martínez-Gómez *et al.*, 2021) or by VIGS, result in female (carpellate) flowers, suggesting a recapitulation of one step in unisexual flower evolution (Larue *et al.*, 2013). This hypothesis has played out in recent findings that B-class MADS box genes are involved in sex determination in other taxa, such as cycads and the rubber tree (Guo *et al.*, 2022; Liu *et al.*, 2022). Ovule identity is induced by the 'D' gene lineage (*STK*-like genes), based on studies in *Petunia* (Angenent *et al.*, 1995). D-class and C-class genes originated from a gene duplication preceding the diversification of angiosperms (Kramer *et al.*, 2004). Studies in *T. thalictroides* led to the finding that of the two *AG* paralogs, one performed the typical C function (in stamen and carpel identity, and floral determinacy) while the other subfunctionalized, taking on a D function role in ovule identity (Di Stilio *et al.*, 2005; Zahn *et al.*, 2006; Galimba *et al.*, 2012; Galimba and Di Stilio, 2015). In *Ranunculaceae* no D-class genes were found, but a family-wide C lineage duplication was recorded (*RanAG1/2*, Kramer *et al.*, 2004). In the *Papaveraceae* *E. californica*, a D lineage gene is found and an independent duplication occurred in the C lineage, resulting in two *AG* paralogs (Zahn *et al.*, 2006; Yellina *et al.*, 2010).

Ranunculales in the evolution of sexual and pollination systems

Most *Ranunculales* species are insect pollinated, some are hummingbird pollinated (*Aquilegia*), and the fly pollination syndrome (small, dull-colored, open flowers with nectariferous petals) is present in at least one genus in each family, whereas wind pollination syndrome (apetalous flowers with drooping stamens and filiform stigmas) is present in *Eupteleaceae*, *Papaveraceae*, and *Ranunculaceae* (Endress, 2010). Among American *Aquilegia* species, there is directionality in the evolution of pollination mode: substantially showier flowers with spurred petals and petaloid sepals are ancestrally pollinated by bees, with spurs getting longer with multiple transitions to hummingbird pollination and then to moth pollination (wind

pollination is not known in this genus) (Whittall and Hodges, 2007).

Petals have been secondarily lost independently in *Thalictrum* and *Enemion*, the latter with flowers that resemble *Thalictrum thalictroides* and that are visited by small pollen-collecting bees. *Thalictrum* flowers are pollinated by small generalist insects, wind pollinated, or both (Kaplan and Mulcahy, 1971; Pellmyr, 1995). Very few systems lend themselves to the study of transitions between insect and wind pollination among closely related taxa at the genus level, and *Thalictrum* is one of them (Timerman and Barrett, 2019). From insect-pollinated, diploid, and hermaphrodite ancestors, *Thalictrum* species have transitioned at least eight times to wind pollination (Wang *et al.*, 2019) in association with polyploidy and unisexual flowers (dioecy, cryptic dioecy, andromonoecy, and gynomonoecy, Soza *et al.*, 2012, 2013). The search for pollination syndromes in *Thalictrum* by multivariate analysis of flower morphology identified four distinct flower morphotypes: ‘petaloid sepal’, ‘showy stamens’, and ‘small unisexual’, associated with insect pollination in the first two and wind pollination in the third. An ‘intermediate’ type that included a known mixed-pollinated (ambophilous) species was also identified, and the pattern held after considering phylogeny (Martínez-Gómez *et al.*, 2023). These data broadly support the existence of detectable flower morphotypes from convergent evolution underlying the pollination mode in *Thalictrum*, presumably via different paths (petaloid sepals or showy stamens) from an ancestral mixed pollination state. Thus, pollination mode in *Thalictrum* is best described as a continuum between insect (the ancestral state) and wind pollination. An interesting research avenue would be to apply a comparable analysis of flower morphotypes to the direct outgroups and to other sister genera and families of Ranunculales. This approach would enable a deeper understanding of the evolutionary trajectory of flower morphologies in relation to pollinators and the sexual system at a broader phylogenetic scale.

Floral phyllotaxis and symmetry

Floral phyllotaxis (the arrangement of organs on the floral receptacle) may be whorled, spiral, and/or irregular (Endress, 2011). In spiral phyllotaxis, there is a delay (plastochron) between the initiation of two subsequent organs, whereas in whorled phyllotaxis, there is a marked plastochron only between whorls of organs belonging to different categories. The ancestral flower of Ranunculales was reconstructed as having a whorled phyllotaxis at anthesis, a condition that is observed today in most families of the order except *Circaeasteraceae* and some *Ranunculaceae* (Carrive *et al.*, 2020). Although many members of this latter family have flowers with an apparently whorled perianth at anthesis, the initiation of perianth organs may follow a spiral pattern (Ren *et al.*, 2011; Zhao *et al.*, 2012). Reproductive organs are usually spirally arranged (Jabbour *et al.*, 2009; Zhao *et al.*, 2012), except in *Aquilegia* (Tucker and

Hodges, 2005). However, together with their regular increase in numbers, especially in *Ranunculaceae*, their phyllotaxis may become more or less irregular with the insertion of incomplete parastichies on the onset of the androecium (Zhao *et al.*, 2012).

With the exception of the perianthless monotypic family *Eupteleaceae*, almost all *Ranunculales* have flowers with at least one series of perianth organs (tepals, sepals, and/or petals), and the vast majority of these species have actinomorphic (i.e. polysymmetric) flowers. Reconstructing the ancestral state for the perianth is somewhat problematic in this order because *Eupteleaceae* are sister to the remaining six families of the order. However, it is very likely that the ancestral flower of all *Ranunculales* had a perianth that was actinomorphic (Damerval and Nadot, 2007; Carrive *et al.*, 2020), as well as the ancestral flower of *Berberidaceae*, *Ranunculaceae*, *Menispermaceae*, *Circaeasteraceae*, and *Lardizabalaceae*. The flowers of *Papaveraceae*, and those of the genus *Epimedium* (*Berberidaceae*) were ancestrally dimerous and therefore dissymmetric, even if the corolla of *Pteridophyllum* (strongly supported as sister to *Hypecoideae*+*Fumarioideae*, Peng *et al.*, 2023) and *Papaveroideae*, but also *Epimedium*, is visually actinomorphic (Sauquet *et al.*, 2015; Carrive *et al.*, 2020; Guo *et al.*, 2022). Zygomorphy (i.e. monosymmetry) evolved once within *Ranunculaceae*, in the ancestral flower of the speciose tribe *Delphinieae*, once in *Menispermaceae*, in the ancestor of *Antizoma*, *Cissampelos*, and *Cyclea* (Ortiz *et al.*, 2016), and probably twice within the subfamily *Fumarioideae* (*Papaveraceae*) (Hoot *et al.*, 2015; Sauquet *et al.*, 2015). Interestingly, zygomorphy evolved from dissymmetry in *Papaveraceae*, in which the dimerous ancestral state itself evolved from an actinomorphic state, a highly uncommon situation in angiosperms. Zygomorphy in *Fumarioideae* is created by the morphological differentiation of the two symmetry planes (e.g. in *Lamprocapnos* and *Dicentra*) followed by the formation of a single spur in the transverse plane during floral development (Damerval *et al.*, 2013). Before anthesis, there is a 90° rotation of the pedicel (resupination) leading to a secondary vertical orientation of the symmetry plane (Endress, 1999; Hidalgo and Gleissberg, 2010). In *Ranunculaceae* and *Menispermaceae*, zygomorphy evolved from actinomorphy as in the vast majority of angiosperms (Reyes *et al.*, 2016).

While zygomorphy has evolved independently in *Papaveraceae* and *Ranunculaceae*, their genetic bases could rely on *CYCLOIDEA-Like* (*CYL*) genes, as in several other angiosperm groups (for a review, see Hileman, 2014). The *CYL* lineage has probably undergone a duplication in the *Ranunculales* after the divergence of the *Eupteleaceae* (Damerval *et al.*, 2022). In *Fumarioideae*, an asymmetric expression has been observed at late developmental stages in the zygomorphic flower of *C. sempervirens* (Damerval *et al.*, 2013). *CYL* silencing by VIGS in the zygomorphic flower of *Cysticapnos vesicaria* reveals a role in sepal and petal identity and a possible involvement in zygomorphy (Zhao *et al.*, 2018). In *Ranunculaceae*, additional duplications took place in both *CYL* lineages in the common ancestor of the zygomorphic tribe *Delphinieae* (Jabbour *et al.*,

2014). Asymmetric expression of some paralogs was observed in the perianth (sepals and/or petals) of several species (Jabbour *et al.*, 2014; Zhao *et al.*, 2023). Silencing of *CYL2* paralogs in *Delphinium ajacis* reveals a role for these genes in the sepal and primordia number, and in the dorsal identity for *CYL2b* or latero-ventral identity for *CYL2a*. It has been suggested that these identity roles were achieved through regulatory interactions with *APETALA3-3* for *CYL2b*, and *AGAMOUS-Like6-1a* and *DIVARICATA1* for *CYL2a* (Zhao *et al.*, 2023).

3D morphogenesis of petals

Petal shape refers to the 3D structure of the organs from the inner whorl of the perianth (the andropetals). As in most angiosperm flowers, the petals of *Ranunculales* were ancestrally leaf shaped, with a flat blade and clawed at the base. This shape, combined with bright or colorful cues, is commonly referred to as ‘petaloid’ (Carrive *et al.*, 2020). Among the six families of *Ranunculales* that have flowers bearing a perianth (*Eupteleaceae* are perianthless), such petaloid petals are observed in *Menispermaceae*, *Circaeasteraceae*, and in the subfamily *Papaveroideae* (*Papaveraceae*). In the other three families, the regular development of nectaries on the petals changes the shape, which becomes three dimensional due to the development of more or less pronounced nectar-storing invaginations ‘Nektarblätter’ (Hiepkko, 1965); they are considered as having an elaborate form compared with flat petals and have evolved several times in *Ranunculales*. The ancestor of *Lardizabalaceae* already had strongly reduced and nectariferous petals (Zhang and Ren, 2011). In the subfamily *Fumarioideae* of *Papaveraceae*, petals are highly elaborate, and fused at the top.

In *Ranunculaceae*, some species have flat and regular petals with only a scale at the base protecting the nectary (e.g. *Ranunculus*), while other species have petals of various and elaborate shapes (tube shaped in *Eranthis* and *Helleborus*, spatula shaped in *Actaea*, including long stalks in *Aconitum*). The development of nectaries on these elaborate petals has been reviewed by Zhao *et al.* (2018) and will be discussed in the next section.

Ancestral state reconstruction of petal shape in *Ranunculaceae* showed that petals were ancestrally flat with a clawed base (Delpeuch *et al.*, 2022). Elaborate, 3D shapes evolved independently from this ancestral petal by differential elongation of organ regions, depending on species. Recently, petal 3D morphogenesis was studied in the genus *Staphisagria*, which belongs to the only zygomorphic clade of *Ranunculaceae* (Zalko *et al.*, 2021). Here, the complex petal shape seems to be the result of synorganization in the whole flower.

Flowers in the *Berberidaceae* family also have nectary-bearing petals ranging from flat to fan or funnel shaped. This morphological diversity results from developmental heterochrony and differential thickening (Su *et al.*, 2021). Interestingly, these elaborate petals were probably ancestral in the family. In *Epimedium* (*Berberidaceae*), the co-occurrence of a spur with

nectary development is responsible for the complex petal shape (Xie *et al.*, 2022). Morphogenesis of simple and elaborate petals in angiosperms in general has been recently reviewed elsewhere (Fu *et al.*, 2022).

Spurs

Spurs are tridimensional structures borne on the perianth, most often on petals, and occur frequently in *Ranunculales*. They are present in *Ranunculaceae*, where they have three independent origins [in *Delphinieae*, *Myosurus* and *Aquilegia* (Carrive *et al.*, 2020), in *Berberidaceae* with a single origin in the common ancestor of *Vancouveria* and *Epimedium* (Sun *et al.*, 2018; Guo *et al.*, 2022), and in *Papaveraceae* with a single origin in the ancestor of all *Fumarioideae*]. In contrast to the rest of the angiosperms, where spurred flowers are most often zygomorphic (Jabbour *et al.*, 2008; Citerne *et al.*, 2010), in *Ranunculales* spurs are observed in flowers with various types of symmetry (Damerval and Nadot, 2007; Damerval and Becker, 2017; Carrive *et al.*, 2020). In actinomorphic flowers, spurs are borne on each of the petals (*Aquilegia*—*Ranunculaceae*, *Vancouveria*—*Berberidaceae*). In dissymmetric flowers, the spurs are also borne on petals but their number varies depending on the degree of differentiation among the two whorls of petals. In *Epimedium* (*Berberidaceae*), spurs are borne on each of the four petals, whereas in dissymmetric flowers of *Fumarioideae* (*Papaveraceae*), only the outermost petals (two in number) are spurred (Endress, 1999). In zygomorphic flowers of *Fumarioideae*, a spur is borne on the outermost petal that is secondarily dorsal after resupination of the floral pedicel (Endress, 1999). The situation is more complex in *Delphinieae* (*Ranunculaceae*) where the number of spurs varies among genera and also among organ categories. All *Delphinieae* flowers have a spur (or hood in *Aconitum*, *Gymnaconitum*, and in some representatives of *Delphinium* subg. *Consolida*) that develops on the dorsalmost sepal, with a single exception (*D. turcicum* with peloric flowers devoid of corolla; Espinosa *et al.*, 2017). Depending on the lineage, one (in the species included in *Delphinium* subg. *Consolida*) or two (in *Staphisagria*, the remaining species of *Delphinium*, *Aconitum*, and *Gymnaconitum*) spurred and nectariferous petals are nested within the dorsal sepal (Jabbour and Renner, 2012; Zalko *et al.*, 2021).

The genetic origin of spurs was investigated in *Aquilegia*. Several transcription factors have been identified in the formation and elongation of the cup of the spur, some of which involved auxin signaling (Yant *et al.*, 2015; Ballerini *et al.*, 2020; Zhang *et al.*, 2020). Whether the same or different genetic mechanisms have been recruited in the several independent evolutionary occurrences of spurs in *Ranunculales* is still unknown.

Nectary development

The production and secretion of nectar is a key innovation in flowering plants that attracts pollinators and facilitates sexual

reproduction. In many taxa of angiosperms, various floral organs develop such as secreting tissues to offer sugary rewards to pollinators in exchange for their service in pollen transfer. These floral nectaries are believed to have evolved many times independently in angiosperms and may be located on various organs of the flower (Erbar, 2014). They may be located on the adaxial side of inner perianth organs or on members of the androecium (stamens or staminodes), as in some basal angiosperms, monocots (*Liliales*), and eudicots (e.g. *Oxalidales* and *Caprifoliaceae*). The monocot orders *Asparagales* and *Zingiberales* are characterized by septal nectaries. Receptacular nectaries often develop between the androecium and gynoecium, in association with the filament bases (Bernardello, 2007); the nectaries may be located on the receptacle (as in many rosids) or on the gynoecium (as in many asterids). The floral nectaries in *Ranunculales* exhibit a great diversity (Fig. 3). All families of *Ranunculales*, except for *Eupteleaceae*, have species that develop

floral nectaries. A recent study focusing on the ancestral traits of *Ranunculales* flowers indicated that these nectaries are likely to have evolved many times independently (Carrive *et al.*, 2020), reflecting the various floral organs that bear the nectaries in different families.

Most *Ranunculaceae* species develop floral nectaries on their petals, associated with nectar-storing invaginations of various shapes, such as spurs (e.g. *Aquilegia* and *Aconitum*), funnels (e.g. *Helleborus* and *Eranthis*), urns (e.g. *Nigella*), or cups (e.g. *Coptis*). However, nectaries can also be found on other floral organs in this family, including stamens and carpels. In *Clematis alpina*, which re-evolved petals after the petal loss in the common ancestor of *Clematis*, the nectary is not present on petals but on carpels instead (Erbar, 2014). In several apetalous genera, including *Caltha* and *Anemone*, the nectary probably re-evolved and is also present on carpels (Peterson *et al.*, 1979; Erbar and Leins, 2013). It is worth noting that in the wind-pollinated

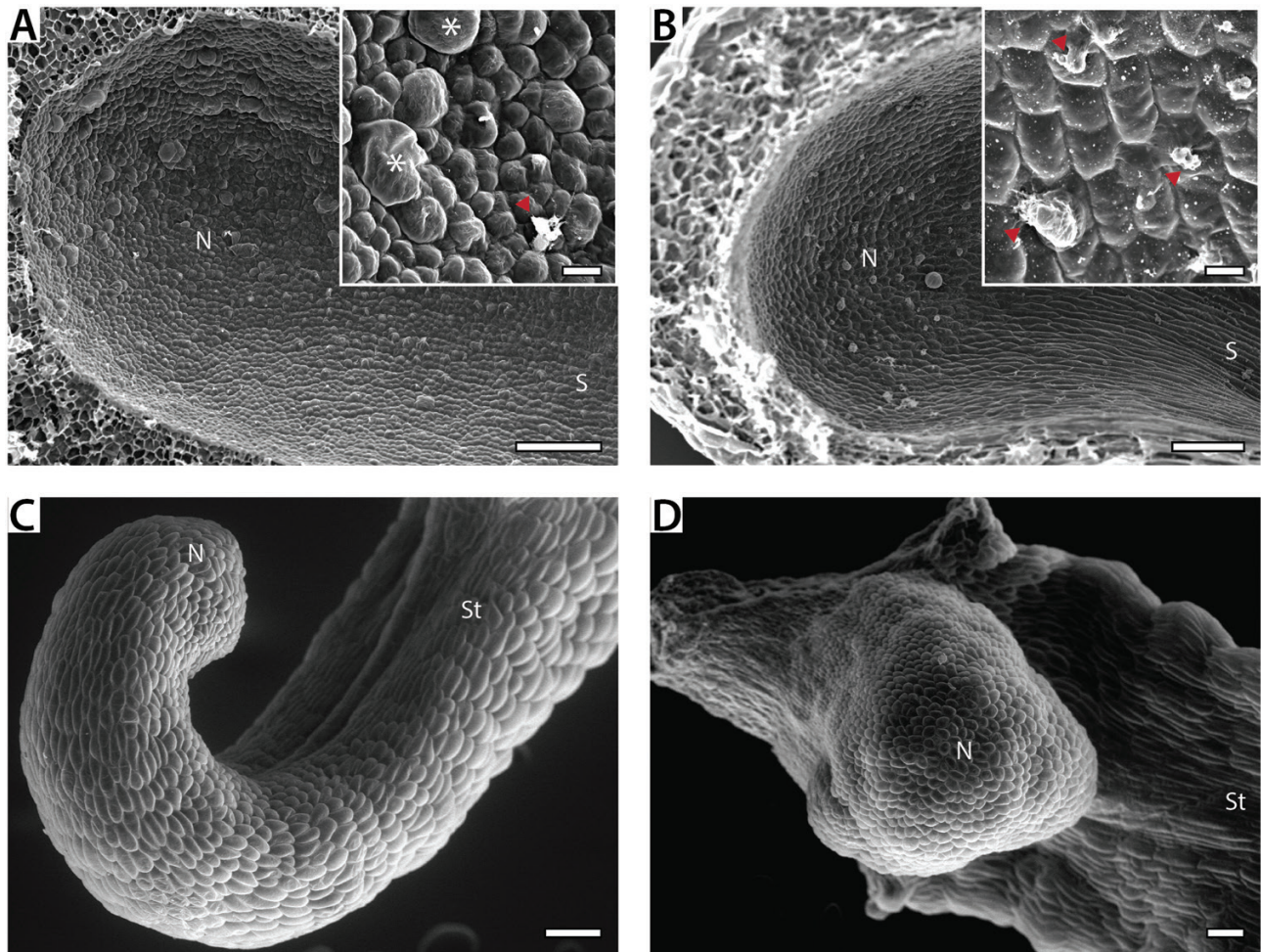


Fig. 3. SEM images of mature nectaries from four *Ranunculales* species: (A) *Aquilegia coerulea* (*Ranunculaceae*), (B) *Epimedium grandiflorum* (*Berberidaceae*), (C) *Corydalis aurea* (*Papaveraceae*), (D) *Lamprocapnos spectabilis* (*Papaveraceae*). Enlarged views of the nectary cells for *Aquilegia* and *Epimedium* are shown in insets. N, nectary; S, spur; St, stamen. Asterisks indicate swelling epidermal cells, and red arrowheads indicate active secreting cells. Scale bars: 100 μm (insets 10 μm).

genera *Thalictrum*, floral nectaries and petals were lost, possibly due to the relaxation of selection pressure to maintain these costly structures for pollinator attraction.

The close association between nectaries and elaborate petaloid organs in *Ranunculaceae* has been hypothesized to facilitate the diversification of pollinator interaction for *Ranunculaceae* species. In *Aquilegia*, a recently established model for nectary development, nectaries develop inside the tips of the spurs (Fig. 3A), and secrete sucrose- and hexose-abundant nectar to be stored in spurs. The amount of secreted nectar and the length/curvature of the spurs are highly diverse in different *Aquilegia* species (Puzey *et al.*, 2012; Edwards *et al.*, 2021). Together, these traits limit nectar access to a specific type of pollinator and can function as reproductive barriers among *Aquilegia* species.

In the closely related family *Berberidaceae*, nectaries are also commonly found on the perianth (Su *et al.*, 2021). Many of these nectariferous organs are historically considered staminodes due to their locations and developmental origins. However, gene expression profiling and phylogenetic analysis showed that these nectariferous organs from several genera, including *Berberis* and *Epimedium*, express the petal identity B-class gene *AP3-3* (Kramer *et al.*, 2003; Rasmussen *et al.*, 2009). In *Berberis*, the inner two perianth whorls bear elliptical, markedly protruding nectaries that embrace the fertile stamens (Erbar, 2014). In *Epimedium*, each petal develops a 3D spur, similar to *Aquilegia*, and bears a nectary at each tip (Xie *et al.*, 2022) (Fig. 3B).

In the *Papaveroideae* subfamily of the *Papaveraceae*, floral nectaries are absent, while they are usually present in the members of the *Fumarioideae* clade (Wang *et al.*, 2023), for example *Dicentra*, *Corydalis*, *Capnoides*, and *Fumaria*, and these nectaries are likely to be homologous (Carrive *et al.*, 2020). Interestingly, the perianths from these genera also develop spurs that hold nectar, but the sites of nectar production and secretion are shifted to the bases of the stamens. In the bisymmetric flower of *Dicentra* (*Lamprocapnos*), six stamens are organized as two triplets, and the filaments of each triplet are basally fused. At the abaxial base of the central filament of each stamen triplet, a ball-shaped nectary develops and is completely enclosed by the petal spur (Zhang and Zhao, 2018) (Fig. 3C). In the zygomorphic flower of *Corydalis*, one out of four petals forms a nectar spur. A 'stalklet' develops from the base of stamen bundles, is inserted into the spur, and bears a nectary at the free end (Erbar, 2014) (Fig. 3D).

In recent years, many comparative studies have surveyed the organization and gene expression profiles of floral nectaries in *Ranunculales* and reported distinct cellular and molecular mechanisms of nectary development and nectar secretion (Vesprini *et al.*, 1999; Damerval *et al.*, 2013; Erbar and Leins, 2013; Erbar, 2014; Antoń and Kamińska, 2015; Zhang and Zhao, 2018; Min *et al.*, 2019; Xie *et al.*, 2022). While most nectaries in core eudicots employ nectary stomata or secretory trichomes to release nectar, such structures are typically absent in *Ranunculales*

nectaries, with a potentially notable exception in *Fumarioideae* (i.e. *A. asiatica*) (Fig. 3A–D; Wang *et al.*, 2023). Instead, nectar secretion by ruptured epidermis or cuticle micro-channels was proposed. At the molecular level, the YABBY family transcription factor gene *CRABS CLAW* (*CRC*) was required for nectary development in several asterid and rosid lineages (Bowman and Smyth, 1999; Lee *et al.*, 2005). However, *CRC* expression was not detected in *Ranunculaceae* nectaries, and nectary development in *Aquilegia* is instead directed by the *STYLISH* (*STY*) family of transcription factor genes (Min *et al.*, 2019). Expression of *STY* genes has also been reported in the nectariferous petals of *Delphinium exaltatum* and *Epimedium* (Min *et al.*, 2019). In contrast, expression of *CRC* orthologs was observed at the nectariferous base of the stamen filaments in the *Papaveraceae* *C. sempervirens* and *Lamprocapnos spectabilis* (Damerval *et al.*, 2013), potentially reflecting the independent evolution of nectaries in *Ranunculaceae* and *Papavaceae*. Future functional studies are required to fully elucidate the cellular and developmental mechanisms of nectar production and nectary formation in *Ranunculales*.

Fruit morphology and dehiscence types

Gynoecium and fruit type vary greatly in *Ranunculales* (Fig. 4). The ancestral condition was identified for the entire order after careful character optimization and found to be a multicarpellate, apocarpous gynoecium. However, different morphologies have become fixed in different families. Whereas *Papaveraceae sensu lato* (including former *Fumariaceae*) have a syncarpous gynoecium, the apocarpous condition is common in *Eupteleaceae* and is a synapomorphy for *Lardizabalaceae* [*Menispermaceae* [*Berberidaceae*+*Ranunculaceae*]]. Members of the *Ranunculaceae* have predominantly an apocarpous gynoecium, but the carpels are frequently described as being connate to different degrees in some genera (such as *Nigella* and *Glaucidium* for instance). *Berberidaceae* are unique in that all members regularly possess a unicarpellate gynoecium, which, as in some *Ranunculaceae*, is probably derived from an ancestral multicarpellate and apocarpous condition, that is also distinctly entirely ascidiate (versus more or less plicate in all other *Ranunculales*, e.g. Endress, 1995) (Fig. 4)

In terms of fruit type, the ancestral condition is the presence of dry dehiscent fruits. Within that category, indehiscent samaras (a winged achene with the wing developing from the ovary wall) are predominant in *Eupteleaceae*. In contrast, longitudinally dehiscent fruits, whether derived from a syncarpous gynoecium (capsules) or from an apocarpous gynoecium (follicles), are plesiomorphic for the rest of the families in the order. Capsules are typical in *Papaveraceae* and *Ranunculaceae* (Fig. 4). Fleshy fruits have been independently acquired in *Lardizabalaceae* (in the genus *Sinofranchetia*) and many *Berberidaceae*, as well as in *Hydrastis* (*Ranunculaceae*). Drupaceous fruits, also indehiscent, are characteristic of the *Menispermaceae*

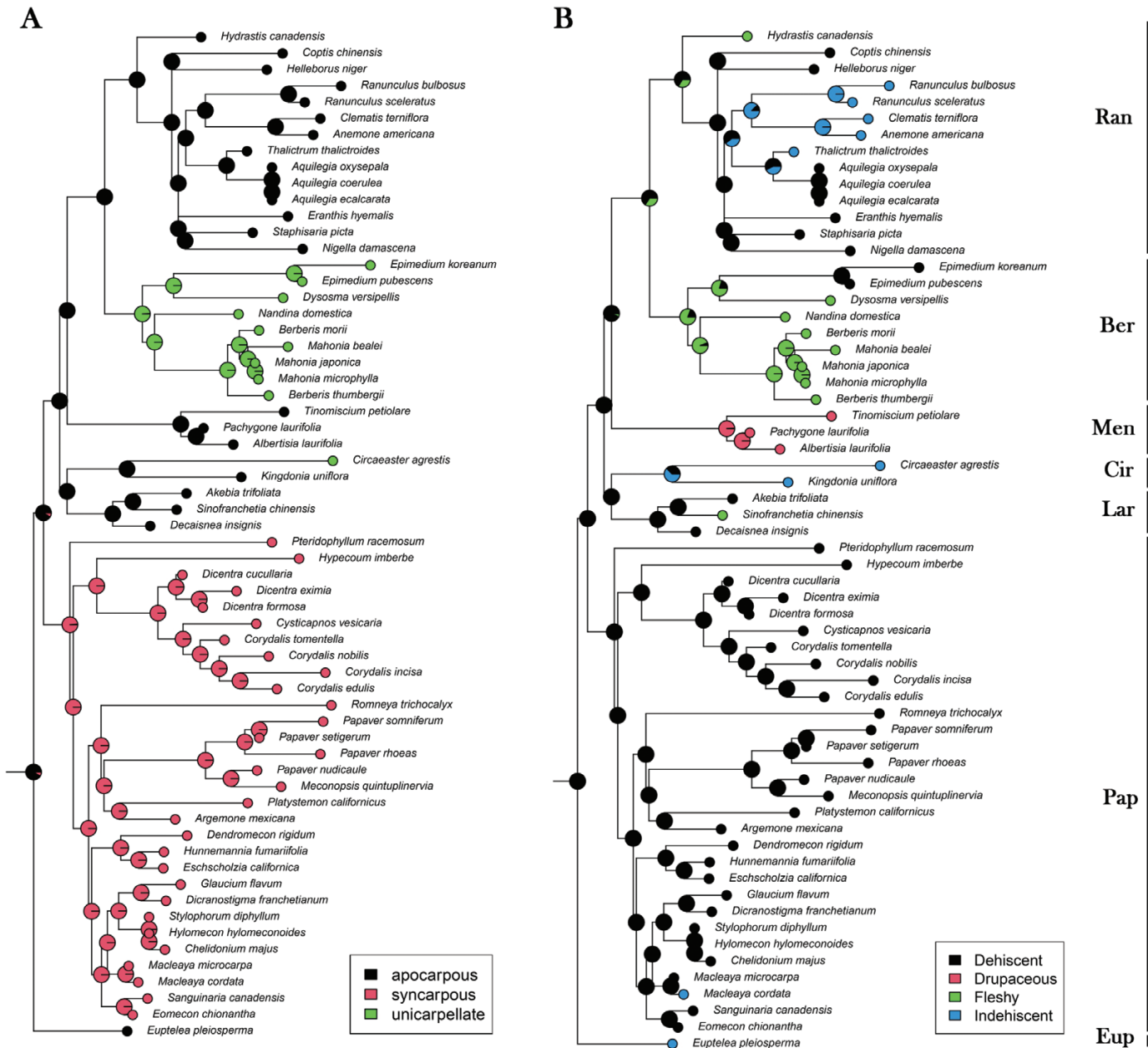


Fig. 4. Ancestral state reconstruction of gynoecium (left) and fruit (right) characters based on a phylogeny using *rbcL* as the marker gene. Trait descriptions are from Cheng-Yih and Kubitzki (1993), Endress (1993), Kaderit (1993), Lidén (1993a, b), Loconte (1993), and Tamura (1993).

(Fig. 4). Achenes, dry indehiscent fruits, were independently acquired in *Circaeasteraceae* and some *Ranunculaceae*.

Regarding the genetic bases for fruit development, there are a number of genes whose function seems to be maintained in both *Papaveraceae* and *Arabidopsis*. They include *FRUITFULL* (*FUL*) genes largely expressed in the fruit wall in *E. californica* and *P. somniferum*. When *FUL* genes are down-regulated, fruit defects include premature rupture of the fruit wall and numerous cell proliferation defects, especially in the endocarp (Pabón-Mora et al., 2012). *APETALA2* (*AP2*) genes are, on the other hand, very different. The two copies show overlapping

expression only in the commissural tissue, and one of the homologs is also expressed in the fruit wall. Very important is the fact that both copies are absent from the dehiscence zone (DZ). These expression patterns suggest a role for *AP2* genes in fruit wall development, most probably acting as repressors of DZ-specific genes (Zumajo-Cardona et al., 2021). Further, the *E. californica* homolog of *CRC* (*EcCRC*) is required for adaxial gynoecium tissue development, and down-regulation leads to a complete abolishment of the DZ (Orashkova et al., 2009).

Genes probably controlling the formation of the DZ in *Papaveraceae* are *SPATULA*/*ALCATRAZ* homologs specifically

restricted to those layers (Zumajo-Cardona *et al.*, 2017), acting together with *REPLUMLESS* genes, which were observed in the DZ not only in *Bocconia*, but also in *Papaver*, suggesting that this is a common putative role for many *Papaveraceae* (Zumajo-Cardona *et al.*, 2018). In *A. thaliana*, *INDEHISCENT* and *SHATTERPROOF1* and *2* are essential for the formation of the DZ. However, as their orthologs do not exist in *Ranunculales* (Zahn *et al.*, 2006; Pabón-Mora *et al.*, 2014), the dry dehiscent fruits predominant in the *Ranunculales* require a gene regulatory network very different from that of *A. thaliana*.

Conclusions

This review has highlighted the *Ranunculales* as an emerging model lineage for comparative analysis of morphological and metabolic traits in angiosperms, pointing out recent developments in the field of genomics and genetic manipulation of several members from diverse families. The amazing morphological diversity of *Ranunculales* raises the question of the underlying genetic bases (particularly concerning convergent traits), still largely unexplored, but also the question of floral integration (whether traits evolve independently from each other or in a correlated manner). Addressing these questions in *Ranunculales*, an order with a key phylogenetic position, may contribute to a better understanding of the drivers of morphological evolution in angiosperms as a whole. Combining a solid phylogeny and fossils for its calibration, molecular tools and genetic resources, together with high morphological diversity, convergent evolution of characters, frequent switching between reproductive systems, and developmental trajectories and functions of perianth organs, the *Ranunculales* order offers new avenues for investigations into plant evolution and adaptation.

Acknowledgements

The authors thank Annalena Kurzweil (Giessen, Germany) for support during the writing process.

Author contributions

AB: conceptualization; CD, FJ, VdS, and AB: review and editing. All authors participated in writing the original draft.

Conflict of interest

The authors declare no conflict of interest.

Funding

Work in AB's group on *E. californica* and on genomic resources of *Ranunculales* was continuously funded by the DFG (German Research Foundation, grants BE2547/3-1; 6-1; 6-2; 7-2; 14-1; 24-1, the RanOmics

project is funded by 27-1). VD was funded by the National Science Foundation (USA), Division of Environmental Biology (Opportunities for Promoting Understanding through Synthesis—Mid-Career Synthesis) grant no. 1911539. YG is supported by National Science Foundation (USA) Postdoctoral Research Fellowships in Biology Program under grant no. 2305493. YG and EMK are supported by National Science Foundation (USA) EDGE Award IOS no. 2128195.

References

- Albrecht MA, McCarthy BC. 2006. Comparative analysis of golden-seal (*Hydrastis canadensis* L) population re-growth following human harvest: implications for conservation. *The American Midland Naturalist* **156**, 229–236.
- Angenent GC, Franken J, Busscher M, van Dijken A, van Went JL, Dons HJ, van Tunen AJ. 1995. A novel class of MADS box genes is involved in ovule development in petunia. *The Plant Cell* **7**, 1569–1582.
- Antoń S, Kamińska M. 2015. Comparative floral spur anatomy and nectar secretion in four representatives of Ranunculaceae. *Protoplasma* **252**, 1587–1601.
- Arango-Ocampo C, González F, Alzate JF, Pabón-Mora N. 2016. The developmental and genetic bases of apetaly in *Bocconia frutescens* (Chelidoniaceae: Papaveraceae). *EvoDevo* **7**, 16.
- Arias T, Riaño-Pachón DM, Di Stilio VS. 2021. Genomic and transcriptomic resources for candidate gene discovery in the Ranunculids. *Applications in Plant Sciences* **9**, e11407.
- Avci FG, Atas B, Gulsoy Toplan G, Gurer C, Sariyar Akbulut B. 2021. Antibacterial and antifungal activities of isoquinoline alkaloids of the Papaveraceae and Fumariaceae families and their implications in structure–activity relationships. *Studies in Natural Products* **70**, 87–118.
- Ballerini ES, Min Y, Edwards MB, Kramer EM, Hodges SA. 2020. POPOVICH, encoding a C2H2 zinc-finger transcription factor, plays a central role in the development of a key innovation, floral nectar spurs, in *Aquilegia*. *Proceedings of the National Academy of Sciences, USA* **117**, 22552–22560.
- Barrell PJ, Wakelin AM, Gatehouse ML, Lister CE, Conner AJ. 2010. Inheritance and epistasis of loci influencing carotenoid content in petal and pollen color variants of California poppy (*Eschscholzia californica* Cham.). *Journal of Heredity* **101**, 750–756.
- Barrett SA. 1952. *Material aspects of Pomo culture*. Milwaukee, WI: Public Museum of the City of Milwaukee.
- Becker A, Yamada Y, Sato F. 2023. California poppy (*Eschscholzia californica*), the Papaveraceae golden girl model organism for evodevo and specialized metabolism. *Frontiers in Plant Science* **14**, 1084358.
- Belny M, Herouart D, Thomasset B, David H, Jacquin-Dubreuil A, David A. 1997. Transformation of *Papaver somniferum* cell suspension cultures with *sam1* from *A. thaliana* results in cell lines of different S-adenosyl-L-methionine synthetase activity. *Physiologia Plantarum* **99**, 233–240.
- Belyaeva RG, Nevkrytaya NV. 1979. A phonological and genetical analysis of mutants with altered flower structure in *Papaver somniferum*. *Plant Breeding Abstracts* **19**, 10360.
- Bernardello G. 2007. A systematic survey of floral nectaries. In: Nicolson SW, Nepi M, Pacini E, eds. *Nectaries and nectar*. Dordrecht: Springer Netherlands, 19–128.
- Bowman JL, Smyth DR. 1999. CRABS CLAW, a gene that regulates carpel and nectary development in Arabidopsis, encodes a novel protein with zinc finger and helix–loop–helix domains. *Development* **126**, 2387–2396.
- Cabin Z, Derieg NJ, Garton A, Ngo T, Quezada A, Gasseholm C, Simon M, Hodges SA. 2022. Non-pollinator selection for a floral homeotic mutant conferring loss of nectar reward in *Aquilegia coerulea*. *Current Biology* **32**, 1332–1341.
- Cao Y-N, Comes HP, Sakaguchi S, Chen L-Y, Qiu Y-X. 2016. Evolution of East Asia's Arcto-Tertiary relict Euptelea (Eupteleaceae) shaped by Late

- Neogene vicariance and Quaternary climate change. *BMC Evolutionary Biology* **16**, 66.
- Carrive L, Domenech B, Sauquet H, Jabbour F, Damerval C, Nadot S.** 2020. Insights into the ancestral flowers of Ranunculales. *Botanical Journal of the Linnean Society* **194**, 23–46.
- Causier B, Schwarz-Sommer Z, Davies B.** 2010. Floral organ identity: 20 years of ABCs. *Seminars in Cell & Developmental Biology* **21**, 73–79.
- Chanderbali AS, Yoo M-J, Zahn LM, et al.** 2010. Conservation and canalization of gene expression during angiosperm diversification accompany the origin and evolution of the flower. *Proceedings of the National Academy of Sciences, USA* **107**, 22570–22575.
- Chartier M, Dressler S, Schönenberger J, Mora AR, Sarthou C, Wang W, Jabbour F.** 2016. The evolution of afro-montane Delphinium (*Ranunculaceae*): morphospecies, phylogenetics and biogeography. *TAXON* **65**, 1313–1327.
- Chen D-X, Pan Y, Wang Y, et al.** 2021. The chromosome-level reference genome of *Coptis chinensis* provides insights into genomic evolution and berberine biosynthesis. *Horticulture Research* **8**, 121.
- Chen X-H, Xiang K-L, Lian L, Peng H-W, Erst AS, Xiang X-G, Chen Z-D, Wang W.** 2020. Biogeographic diversification of Mahonia (*Berberidaceae*): implications for the origin and evolution of East Asian subtropical evergreen broadleaved forests. *Molecular Phylogenetics and Evolution* **151**, 106910.
- Cheng-Yih W, Kubitzki K.** 1993. *Circaeasteraceae*. In: Kubitzki K, Rohwer JG, Bittrich V, eds. *Flowering plants · Dicotyledons*. Berlin, Heidelberg: Springer, 288–289.
- Chitty JA, Allen RS, Fist AJ, Larkin PJ.** 2003. Genetic transformation in commercial Tasmanian cultivars of opium poppy, *Papaver somniferum*, and movement of transgenic pollen in the field. *Functional Plant Biology* **30**, 1045–1058.
- Citerne H, Jabbour F, Nadot S, Damerval C.** 2010. The evolution of floral symmetry. *Advances in Botanical Research* **54**, 85–137.
- Clusius C.** 1601. *Melanthium pleno flore*. In: *Rariorum Plantarum Historia*. Ex officina Plantiniana Apud Ioannem Moretum, 207–208.
- Coen ES, Meyerowitz EM.** 1991. The war of the whorls: genetic interactions controlling flower development. *Nature* **353**, 31–37.
- Colledge S, Conolly A, Shennan S.** 2004. Archaeobotanical evidence for the spread of farming in the eastern Mediterranean. *Current Anthropology* **45**, S35–S58.
- Conde E Silva N, Leguilloux M, Bellec A, Rodde N, Aubert J, Manicacci D, Damerval C, Berges H, Deveaux Y.** 2023. A MITE insertion abolishes the AP3-3 self-maintenance regulatory loop in apetalous flowers of *Nigella damascena*. *Journal of Experimental Botany* **74**, 1448–1459.
- Conner AJ, Barrell PJ.** 2014. Inheritance of pleated petals in California poppy (*Eschscholzia californica*). *New Zealand Natural Sciences* **39**, 25–30.
- Cossard G, Sannier J, Sauquet H, Damerval C, de Craene LR, Jabbour F, Nadot S.** 2016. Subfamilial and tribal relationships of Ranunculaceae: evidence from eight molecular markers. *Plant Systematics and Evolution* **302**, 419–431.
- Dabeer S, Rather MA, Rasool S, et al.** 2022. History and traditional uses of black seeds (*Nigella sativa*). In: Khan A, Rehman M, eds. *Black seeds (Nigella sativa)*. Amsterdam: Elsevier, 1–28.
- Damerval C, Becker A.** 2017. Genetics of flower development in Ranunculales—a new, basal eudicot model order for studying flower evolution. *New Phytologist* **216**, 361–366.
- Damerval C, Citerne H, Le Guilloux M, Domenichini S, Dutheil J, Ronse de Craene L, Nadot S.** 2013. Asymmetric morphogenetic cues along the transverse plane: shift from disymmetry to zygomorphy in the flower of *Fumarioideae*. *American Journal of Botany* **100**, 391–402.
- Damerval C, Claudot C, Le Guilloux M, et al.** 2022. Evolutionary analyses and expression patterns of TCP genes in Ranunculales. *Frontiers in Plant Science* **13**, 1055196.
- Damerval C, Nadot S.** 2007. Evolution of perianth and stamen characteristics with respect to floral symmetry in Ranunculales. *Annals of Botany* **100**, 631–640.
- Delpuech P, Jabbour F, Damerval C, Schönenberger J, Pamperl S, Rome M, Nadot S.** 2022. A flat petal as ancestral state for Ranunculaceae. *Frontiers in Plant Science* **13**, 961906.
- Dhawan OP, Dubey MK, Khanuja SPS.** 2007. Detection of a true breeding homeotic gene mutant Pps-1 with partially petaloid sepals in opium poppy (*Papaver somniferum* L.) and its genetic behavior. *Journal of Heredity* **98**, 373–377.
- Dinesh-Kumar SP, Anandalakshmi R, Marathe R, Schiff M, Liu Y.** 2003. Virus-induced gene silencing. *Methods in Molecular Biology* **236**, 287–294.
- Di Stilio VS.** 2011. Empowering plant evo-devo: virus induced gene silencing validates new and emerging model systems. *BioEssays* **33**, 711–718.
- Di Stilio VS, Kramer EM, Baum DA.** 2005. Floral MADS box genes and homeotic gender dimorphism in *Thalictrum dioicum* (*Ranunculaceae*)—a new model for the study of dioecy. *The Plant Journal* **41**, 755–766.
- Di Stilio VS, Kumar RA, Oddone AM, Tolkin TR, Salles P, McCarty K.** 2010. Virus-induced gene silencing as a tool for comparative functional studies in *Thalictrum*. *PLoS One* **5**, e12064.
- Dommes AB, Gross T, Herbert DB, Kivivirta KI, Becker A.** 2019. Virus-induced gene silencing: empowering genetics in non-model organisms. *Journal of Experimental Botany* **70**, 757–770.
- Drea S, Hileman LC, Martino G de, Irish VF.** 2007. Functional analyses of genetic pathways controlling petal specification in poppy. *Development* **134**, 4157–4166.
- Edwards MB, Choi GPT, Derieg NJ, Min Y, Diana AC, Hodges SA, Mahadevan L, Kramer EM, Ballerini ES.** 2021. Genetic architecture of floral traits in bee- and hummingbird-pollinated sister species of *Aquilegia* (columbine). *Evolution* **75**, 2197–2216.
- Eilert U, Kurz WW, Constabel F.** 1985. Stimulation of Sanguinarine accumulation in *Papaver somniferum* cell cultures by fungal elicitors. *Journal of Plant Physiology* **119**, 65–76.
- Elmore FH.** 1943. *Ethnobotany of the Navajo*. Santa Fe, NM: The School of American Research.
- Endress PK.** 1993. *Eupteleaceae*. In: Kubitzki K, Rohwer JG, Bittrich V, eds. *Flowering plants · Dicotyledons*. Berlin, Heidelberg: Springer, 299–301.
- Endress PK.** 1995. Floral structure and evolution in Ranunculanae. In: Jensen U, Kadereit JW, eds. *Systematics and evolution of the Ranunculiflorae*. *Plant Systematics and Evolution Supplement* 9, vol 9. Vienna: Springer, 47–61.
- Endress PK.** 1999. Symmetry in flowers: diversity and evolution. *International Journal of Plant Sciences* **160**, S3–S23.
- Endress PK.** 2010. Flower structure and trends of evolution in eudicots and their major subclades 1. *Annals of the Missouri Botanical Garden* **97**, 541–583.
- Endress PK.** 2011. Evolutionary diversification of the flowers in angiosperms. *American Journal of Botany* **98**, 370–396.
- Erbar C.** 2014. Nectar secretion and nectaries in basal angiosperms, magnoliids and non-core eudicots and a comparison with core eudicots. *Plant Diversity and Evolution* **131**, 63–143.
- Erbar C, Leins P.** 2013. Nectar production in the pollen flower of *Anemone nemorosa* in comparison with other Ranunculaceae and *Magnolia* (*Magnoliaceae*). *Organisms Diversity & Evolution* **13**, 287–300.
- Espinosa F, Deroin T, Xiang K-L, Wang W, Castro MP, Byng JW, Aytac Z, Nadot S, Jabbour F.** 2017. The Turkish endemic *Pseudodelphinium turcicum* (*Ranunculaceae*): an unusual population of delphinium with peloric flowers that has persisted in the wild for 20 years. *International Journal of Plant Sciences* **178**, 546–555.
- Filiault DL, Ballerini ES, Mandáková T, et al.** 2018. The *Aquilegia* genome provides insight into adaptive radiation and reveals an extraordinarily polymorphic chromosome with a unique history. *eLife* **7**, e36426.
- Footo HC, Ride JP, Franklin-Tong VE, Walker EA, Lawrence MJ, Franklin FC.** 1994. Cloning and expression of a distinctive class of self-incompatibility (S) gene from *Papaver rhoeas* L. *Proceedings of the National Academy of Sciences, USA* **91**, 2265–2269.

- Franke J, Böhm H.** 1982. Accumulation and excretion of alkaloids by *Macleaya microcarpa* cell cultures II. Experiments in liquid medium. *Biochemie und Physiologie der Pflanzen* **177**, 501–507.
- Friis EM, Crane PR, Raunsgaard Pedersen K.** 2011. Early flowers and angiosperm evolution. Cambridge: Cambridge University Press.
- Fu X, Shan H, Yao X, Cheng J, Jiang Y, Yin X, Kong H.** 2022. Petal development and elaboration. *Journal of Experimental Botany* **73**, 3308–3318.
- Fujii N, Inui T, Iwasa K, Morishige T, Sato F.** 2007. Knockdown of berberine bridge enzyme by RNAi accumulates (S)-reticuline and activates a silent pathway in cultured California poppy cells. *Transgenic Research* **16**, 363–375.
- Galimba KD, Di Stilio VS.** 2015. Sub-functionalization of ovule development following duplication of a floral organ identity gene. *Developmental Biology* **405**, 158–172.
- Galimba KD, Martínez-Gómez J, Di Stilio VS.** 2018. Gene duplication and transference of function in the paleoAP3 lineage of floral organ identity genes. *Frontiers in Plant Science* **9**, 334.
- Galimba KD, Tolkin TR, Sullivan AM, Melzer R, Theißen G, Di Stilio VS.** 2012. Loss of deeply conserved C-class floral homeotic gene function and C- and E-class protein interaction in a double-flowered ranunculus mutant. *Proceedings of the National Academy of Sciences, USA* **109**, E2267–E2275.
- Gobo WV, Kunzmann L, Iannuzzi R, Bachelier JB, Coiffard C.** 2022. First evidence of ranunculids in Early Cretaceous tropics. *Scientific Reports* **12**, 5040.
- Gonçalves B, Nougé O, Jabbour F, Ridet C, Morin H, Laufs P, Manicacci D, Damerval C.** 2013. An APETALA 3 homolog controls both petal identity and floral meristem patterning in *Nigella damascena* L. (Ranunculaceae). *The Plant Journal* **76**, 223–235.
- Gould B, Kramer EM.** 2007. Virus-induced gene silencing as a tool for functional analyses in the emerging model plant *Aquilegia* (columbine, Ranunculaceae). *Plant Methods* **3**, 6.
- Greyson RI, Raman K.** 1975. Differential sensitivity of 'double' and 'single' flowers of *Nigella damascena* (Ranunculaceae) to emasculation and to GA3. *American Journal of Botany* **62**, 531–536.
- Guerra-Doce E.** 2015. The origins of inebriation: archaeological evidence of the consumption of fermented beverages and drugs in Prehistoric Eurasia. *Journal of Archaeological Method and Theory* **22**, 751–782.
- Guo L, Winzer T, Yang X, et al.** 2018. The opium poppy genome and morphinan production. *Science* **362**, 343–347.
- Guo M, Pang X, Xu Y, Jiang W, Liao B, Yu J, Xu J, Song J, Chen S.** 2022. Plastid genome data provide new insights into the phylogeny and evolution of the genus *Epimedium*. *Journal of Advanced Research* **36**, 175–185.
- Hagel JM, Facchini PJ.** 2013. Benzylisoquinoline alkaloid metabolism: a century of discovery and a brave new world. *Plant and Cell Physiology* **54**, 647–672.
- Halfter U, Ali N, Stockhaus J, Ren L, Chua NH.** 1994. Ectopic expression of a single homeotic gene, the *Petunia* gene green petal, is sufficient to convert sepals to petaloid organs. *The EMBO Journal* **13**, 1443–1449.
- Hammer K.** 1977. The question of ancestral species of cultivated poppy (*Papaver somniferum* L.). *Kulturpflanze* **25**, 113–124.
- Han M, Manchester SR, Fu Q-Y, Jin J-H, Quan C.** 2018. Paleogene fossil fruits of *Stephania* (Menispermaceae) from North America and East Asia. *Journal of Systematics and Evolution* **56**, 81–91.
- Han M, Wu X-K, Tu M, Kodrul TM, Jin J-H.** 2020. Diversity of Menispermaceae from the Paleocene and Eocene of South China. *Journal of Systematics and Evolution* **58**, 354–366.
- Hao D-C, He C-N, Shen J, Xiao P-G.** 2017. Anticancer chemodiversity of Ranunculaceae medicinal plants: molecular mechanisms and functions. *Current Genomics* **18**, 39–59.
- Hao D-C, Xiao P-G, Ma H-Y, Peng Y, He C-N.** 2015. Mining chemodiversity from biodiversity: pharmacophylogeny of medicinal plants of Ranunculaceae. *Chinese Journal of Natural Medicines* **13**, 507–520.
- Hauschild K, Pauli HH, Kutchan TM.** 1998. Isolation and analysis of a gene *bbe1* encoding the berberine bridge enzyme from the California poppy *Eschscholzia californica*. *Plant Molecular Biology* **36**, 473–478.
- He J, Lyu R, Luo Y, Xiao J, Xie L, Wen J, Li W, Pei L, Cheng J.** 2022. A phylotranscriptome study using silica gel-dried leaf tissues produces an updated robust phylogeny of Ranunculaceae. *Molecular Phylogenetics and Evolution* **174**, 107545.
- He S, Liu C, Saxena PK.** 2007. Plant regeneration of an endangered medicinal plant *Hydrastis canadensis* L. *Scientia Horticulturae* **113**, 82–86.
- Heiss AG, Oeggel K.** 2005. The oldest evidence of *Nigella damascena* L. (Ranunculaceae) and its possible introduction to central Europe. *Vegetation History and Archaeobotany* **14**, 562–570.
- Herrera F, Manchester SR, Hoot SB, Wefferling KM, Carvalho MR, Jaramillo C.** 2011. Phytogeographic implications of fossil endocarps of Menispermaceae from the Paleocene of Colombia. *American Journal of Botany* **98**, 2004–2017.
- Hidalgo O, Bartholmes C, Gleissberg S.** 2012. Virus-induced gene silencing (VIGS) in *Cysticapnos vesicaria*, a zygomorphic-flowered Papaveraceae (Ranunculales, basal eudicots). *Annals of Botany* **109**, 911–920.
- Hidalgo O, Gleissberg S.** 2010. Evolution of reproductive morphology in the Papaveraceae s.l. (Papaveraceae and Fumariaceae, Ranunculales). *International Journal of Plant Developmental Biology* **4**, 76–85.
- Hiepkö P.** 1965. Vergleichend-morphologische und entwicklungsgeschichtliche Untersuchungen über das Perianth bei den Polycaricaceae. *Botanisches Jahrbuch für Systematik, Pflanzengeschichte und Pflanzengeographie* **84**, 359–508.
- Hileman LC.** 2014. Trends in flower symmetry evolution revealed through phylogenetic and developmental genetic advances. *Philosophical Transactions of the Royal Society B: Biological Sciences* **369**, 20130348.
- Hileman LC, Drea S, Martino G, Litt A, Irish VF.** 2005. Virus-induced gene silencing is an effective tool for assaying gene function in the basal eudicot species *Papaver somniferum* (opium poppy). *The Plant Journal* **44**, 334–341.
- Holländer-Czytka H, Johanning D, Meyer HE, Amrhein N.** 1988. Molecular basis for the overproduction of 5-enolpyruvylshikimate 3-phosphate synthase in a glyphosate-tolerant cell suspension culture of *Corydalis sempervirens*. *Plant Molecular Biology* **11**, 215–220.
- Hong UVT, Tamiru-Oli M, Hurgobin B, Okey CR, Abreu AR, Lewsey MG.** 2022. Insights into opium poppy (*Papaver* spp) genetic diversity from genotyping-by-sequencing analysis. *Scientific Reports* **12**, 111.
- Hoot SB, Magallon S, Crane PR.** 1999. Phylogeny of basal eudicots based on three molecular data sets: atpB, rbcL, and 18S nuclear ribosomal DNA sequences. *Annals of the Missouri Botanical Garden* **86**, 1–32.
- Hoot SB, Wefferling KM, Wulff JA.** 2015. Phylogeny and character evolution of Papaveraceae s.l. (Ranunculales). *Systematic Botany* **40**, 474–488.
- Hori K, Yamada Y, Purwanto R, Minakuchi Y, Toyoda A, Hirakawa H, Sato F.** 2018. Mining of the uncharacterized cytochrome P450 genes involved in alkaloid biosynthesis in California poppy using a draft genome sequence. *Plant and Cell Physiology* **59**, 222–233.
- Hsieh C-L, Yu C-C, Huang Y-L, Chung K-F.** 2021. *Mahonia* vs *Berberis* unloaded: generic delimitation and infrafamilial classification of Berberidaceae based on plastid phylogenomics. *Frontiers in Plant Science* **12**, 720171.
- Huang H, Liang J, Tan Q, Ou L, Li X, Zhong C, Huang H, Møller IM, Wu X, Song S.** 2021. Insights into triterpene synthesis and unsaturated fatty-acid accumulation provided by chromosomal-level genome analysis of *Akebia trifoliata* subsp. *australis*. *Horticulture Research* **8**, 33.
- Huang P, Xu M, Xia L, Qing Z, Tang Q, Liu W, Zeng J.** 2017. Establishment of an efficient *Agrobacterium*-mediated genetic transformation method in *Macleaya cordata*. *Scientia Horticulturae* **226**, 302–306.
- Jabbour F, Cossard G, Le Guilloux M, Sannier J, Nadot S, Damerval C.** 2014. Specific duplication and dorsoventrally asymmetric expression patterns of Cycloidea-like genes in zygomorphic species of Ranunculaceae. *PLoS One* **9**, e95727.

- Jabbour F, Damerval C, Nadot S.** 2008. Evolutionary trends in the flowers of Asteridae: is polyandry an alternative to zygomorphy? *Annals of Botany* **102**, 153–165.
- Jabbour F, Du Pasquier PE, Chazalviel L, Le Guilloux M, Conde e Silva NC, Deveaux Y, Galipot P, Heiss AG, Manicacci D, Damerval C.** 2021. Evolution of the distribution area of the Mediterranean *Nigella damascena* and a likely multiple molecular origin of its perianth dimorphism. *Flora* **274**, 151735.
- Jabbour F, Renner SS.** 2012. Spurs in a spur: perianth evolution in the Delphinieae (Ranunculaceae). *International Journal of Plant Sciences* **173**, 1036–1054.
- Jabbour F, Ronse De Craene LP, Nadot S, Damerval C.** 2009. Establishment of zygomorphy on an ontogenic spiral and evolution of perianth in the tribe Delphinieae (Ranunculaceae). *Annals of Botany* **104**, 809–822.
- Jack T, Fox GL, Meyerowitz EM.** 1994. Arabidopsis homeotic gene APETALA3 ectopic expression: transcriptional and posttranscriptional regulation determine floral organ identity. *Cell* **76**, 703–716.
- Jacques FM.** 2009. Fossil history of the Menispermaceae (Ranunculales). *Annales de Paléontologie* **95**, 53–69.
- Jesus A, Bonhomme V, Evin A, Ivorra S, Soteris R, Salavert A, Antolín F, Bouby L.** 2021. A morphometric approach to track opium poppy domestication. *Scientific Reports* **11**, 9778.
- Jud NA, Iglesias A, Wilf P, Gandolfo MA.** 2018. Fossil moonseeds from the Paleogene of West Gondwana (Patagonia, Argentina). *American Journal of Botany* **105**, 927–942.
- Kadereit JW.** 1993. Papaveraceae. In: Kubitzki K, Rohwer JG, Bittrich V, eds. *Flowering plants · Dicotyledons*. Berlin, Heidelberg: Springer, 494–506.
- Kaplan SM, Mulcahy DL.** 1971. Mode of pollination and floral sexuality in *Thalictrum*. *Evolution* **25**, 659–668.
- Keet J-H, Cindi DD, Du Preez PJ.** 2016. Assessing the invasiveness of *Berberis aristata* and *B. julianae* (Berberidaceae) in South Africa: management options and legal recommendations. *South African Journal of Botany* **105**, 288–298.
- Kessler PJA.** 1993. Annonaceae. In: Kubitzki K, Rohwer JG, Bittrich V, eds. *Flowering plants · Dicotyledons*. Berlin, Heidelberg: Springer, 93–129.
- Kim S, Soltis DE, Soltis PS, Zanis MJ, Suh Y.** 2004. Phylogenetic relationships among early-diverging eudicots based on four genes: were the eudicots ancestrally woody? *Molecular Phylogenetics and Evolution* **31**, 16–30.
- Klimek-Chodacka M, Kadluczka D, Lukasiewicz A, Malec-Pala A, Baranski R, Grzebelus E.** 2020. Effective callus induction and plant regeneration in callus and protoplast cultures of *Nigella damascena* L. *Plant Cell, Tissue and Organ Culture* **143**, 693–707.
- Kramer EM, Di Stilio VS, Schlüter PM.** 2003. Complex patterns of gene duplication in the APETALA3 and PISTILLATA lineages of the Ranunculaceae. *International Journal of Plant Sciences* **164**, 1–11.
- Kramer EM, Holappa L, Gould B, Jaramillo MA, Setnikov D, Santiago PM.** 2007. Elaboration of B gene function to include the identity of novel floral organs in the lower eudicot *Aquilegia*. *The Plant Cell* **19**, 750–766.
- Kramer EM, Jaramillo MA, Di Stilio VS.** 2004. Patterns of gene duplication and functional evolution during the diversification of the AGAMOUS subfamily of MADS box genes in angiosperms. *Genetics* **166**, 1011–1023.
- Lange M, Orashakova S, Lange S, Melzer R, Theißen G, Smyth DR, Becker A.** 2013. The seirena B class floral homeotic mutant of California poppy (*Eschscholzia californica*) reveals a function of the enigmatic PI motif in the formation of specific multimeric MADS domain protein complexes. *The Plant Cell* **25**, 438–453.
- Larue NC, Sullivan AM, Di Stilio VS.** 2013. Functional recapitulation of transitions in sexual systems by homeosis during the evolution of dioecy in *Thalictrum*. *Frontiers in Plant Science* **4**, 487.
- La Valva V, Sabato S, Siniscalco Gigliano G.** 1985. Morphology and alkaloid chemistry of *Papaver setigerum* DC (Papaveraceae). *TAXON* **34**, 191–196.
- Lee J-Y, Baum SF, Oh S-H, Jiang C-Z, Chen J-C, Bowman JL.** 2005. Recruitment of CRABS CLAW to promote nectary development within the eudicot clade. *Development* **132**, 5021–5032.
- Li L-F, Wang H-Y, Pang D, Liu Y, Liu B, Xiao H-X.** 2014. Phenotypic and genetic evidence for ecological speciation of *Aquilegia japonica* and *A. oxysepala*. *New Phytologist* **204**, 1028–1040.
- Li Y, Winzer T, He Z, Graham IA.** 2020. Over 100 million years of enzyme evolution underpinning the production of morphine in the Papaveraceae family of flowering plants. *Plant Communications* **1**, 100029.
- Lian L, Del C Ortiz R, Jabbour F, Zhang C-F, Xiang X-G, Erst AS, Gao T-G, Chen Z-D, Wang W.** 2020. Phylogeny and biogeography of Pachygoneae (Menispermaceae), with consideration of the boreotropical flora hypothesis and resurrection of the genera *Cebatha* and *Nephroia*. *Molecular Phylogenetics and Evolution* **148**, 106825.
- Lidén M.** 1993a. Fumariaceae. In: Kubitzki K, Rohwer JG, Bittrich V, eds. *Flowering plants · Dicotyledons*. Berlin, Heidelberg: Springer, 310–318.
- Lidén M.** 1993b. Pteridophyllaceae. In: Kubitzki K, Rohwer JG, Bittrich V, eds. *Flowering plants · Dicotyledons*. Berlin, Heidelberg: Springer, 556–557.
- Litt A.** 2007. An evaluation of A-function: evidence from the APETALA1 and APETALA2 gene lineages. *International Journal of Plant Sciences* **168**, 73–91.
- Liu X, Liu Y, Huang P, et al.** 2017. The genome of medicinal plant *Macleaya cordata* provides new insights into benzyloisoquinoline alkaloids metabolism. *Molecular Plant* **10**, 975–989.
- Liu Y, Wang B, Shu S, et al.** 2021. Analysis of the *Coptis chinensis* genome reveals the diversification of protoberberine-type alkaloids. *Nature Communications* **12**, 3276.
- Liu Y, Wang S, Li L, et al.** 2022. The *Cycas* genome and the early evolution of seed plants. *Nature Plants* **8**, 389–401.
- Loconte H.** 1993. Berberidaceae. In: Kubitzki K, Rohwer JG, Bittrich V, eds. *Flowering plants · Dicotyledons*. Berlin Heidelberg: Springer, 147–152.
- Lotz D, Imani J, Ehlers K, Becker A.** 2022. Towards a genetic model organism: an efficient method for stable genetic transformation of *Eschscholzia californica* (Ranunculales). *Plant Cell, Tissue and Organ Culture* **149**, 823–832.
- Lubbers AE, Christensen LN.** 1986. Intra-seasonal variation in seed production among flowers and plants of *Thalictrum thalictroides* (Ranunculaceae). *American Journal of Botany* **73**, 190–203.
- Mai DH.** 1995. Tertiäre Vegetationsgeschichte Europas. Jena: Gustav Fischer Verlag.
- Mandal SK, Maji AK, Mishra SK, Ishfaq PM, Devkota HP, Silva AS, Das N.** 2020. Goldenseal (*Hydrastis canadensis* L.) and its active constituents: a critical review of their efficacy and toxicological issues. *Pharmacological Research* **160**, 105085.
- Manni M, Berkeley MR, Seppey M, Zdobnov EM.** 2021. BUSCO: assessing genomic data quality and beyond. *Current Protocols* **1**, e323.
- Martínez-Gómez J, Galimba KD, Coté EY, Sullivan AM, Di Stilio VS.** 2021. Spontaneous homeotic mutants and genetic control of floral organ identity in a ranunculid. *Evolution & Development* **23**, 197–214.
- Martínez-Gómez J, Park S, Hartogs SR, Soza VL, Park S, Di Stilio VS.** 2023. Flower morphology as a predictor of pollination mode in a biotic to abiotic pollination continuum. *Annals of Botany* **132**, 61–76.
- McNaughton IH, Harper JL.** 1960. The comparative biology of closely related species living in the same area: I. External breeding-barriers between Papaver species. *New Phytologist* **59**, 15–26.
- Mendes MM, Grimm GW, Pais J, Friis EM.** 2014. Fossil *Kajanthus lusitanicus* gen et sp nov from Portugal: floral evidence for Early Cretaceous Lardizabalaceae (Ranunculales, basal eudicot). *Grana* **53**, 283–301.
- Miller RB.** 1981. Hawkmoths and the geographic patterns of floral variation in *Aquilegia caerulea*. *Evolution* **35**, 763–774.
- Min Y, Bunn JI, Kramer EM.** 2019. Homologs of the STYLISH gene family control nectary development in *Aquilegia*. *New Phytologist* **221**, 1090–1100.
- Mohammed AA-H, Masyad HM.** 2020. Genetic transformation of *Nigella sativa* L. plants with *Agrobacterium rhizogenes* 35S GUS R1000 and

- estimation of thymoquinone level in transformed hairy roots cultures. *Plant Archives* **20**, 3649–3652.
- O'Donnell S, Lane MD, Lawrence MJ.** 1993. The population genetics of the self-incompatibility polymorphism in *Papaver rhoeas*. VI. Estimation of the overlap between the allelic complements of a pair of populations. *Heredity* **71**, 591–595.
- One Thousand Plant Transcriptomes Initiative.** 2019. One thousand plant transcriptomes and the phylogenomics of green plants. *Nature* **574**, 679–685.
- Orashakova S, Lange M, Lange S, Wege S, Becker A.** 2009. The CRABS CLAW ortholog from California poppy (*Eschscholzia californica*, Papaveraceae), EcCRC, is involved in floral meristem termination, gynoecium differentiation and ovule initiation. *The Plant Journal* **58**, 682–693.
- Orellana MR, Blanché C, Simon J, Bosch M.** 2009. Genetic diversity within and among disjunct populations of the Mediterranean island endemic *Delphinium pictum* and *D. requienii* (Ranunculaceae). *Folia Geobotanica* **44**, 47–63.
- Ortiz RDC, Kellog AA, van der Werff H.** 2007. Molecular phylogeny of the moonseed family (Menispermaceae): implications for morphological diversification. *American Journal of Botany* **94**, 1425–1438.
- Ortiz RDC, Wang W, Jacques FM, Chen Z.** 2016. Phylogeny and a revised tribal classification of Menispermaceae (moonseed family) based on molecular and morphological data. *TAXON* **65**, 1288–1312.
- Pabón-Mora N, Ambrose BA, Litt A.** 2012. Poppy APETALA1/FRUITFULL orthologs control flowering time, branching, perianth identity, and fruit development. *Plant Physiology* **158**, 1685–1704.
- Pabón-Mora N, Sharma B, Holappa LD, Kramer EM, Litt A.** 2013. The *Aquilegia* FRUITFULL-like genes play key roles in leaf morphogenesis and inflorescence development. *The Plant Journal* **74**, 197–212.
- Pabón-Mora N, Wong GK-S, Ambrose BA.** 2014. Evolution of fruit development genes in flowering plants. *Frontiers in Plant Science* **5**, 300.
- Park S-U, Facchini PJ.** 2000a. *Agrobacterium*-mediated transformation of opium poppy, *Papaver somniferum*, via shoot organogenesis. *Journal of Plant Physiology* **157**, 207–214.
- Park S-U, Facchini PJ.** 2000b. *Agrobacterium*-mediated genetic transformation of California poppy, *Eschscholzia californica* Cham, via somatic embryogenesis. *Plant Cell Reports* **19**, 1006–1012.
- Pathak S, Lakhwani D, Gupta P, Mishra BK, Shukla S, Asif MH, Trivedi PK.** 2013. Comparative transcriptome analysis using high papaverine mutant of *Papaver somniferum* reveals pathway and uncharacterized steps of papaverine biosynthesis. *PLoS One* **8**, e65622.
- Pei L, Wang B, Ye J, et al.** 2021. Genome and transcriptome of *Papaver somniferum* Chinese landrace CHM indicates that massive genome expansion contributes to high benzylisoquinoline alkaloid biosynthesis. *Horticulture Research* **8**, 5.
- Pellmyr O.** 1995. Pollination biology. In: Hiepko P, ed. *Die Natürlichen Pflanzenfamilien*, Ranunculaceae. Berlin: Duncker & Humblot, 160–184.
- Peng H-W, Xiang K-L, Erst AS, Lian L, Ortiz RDC, Jabbour F, Chen Z-D, Wang W.** 2023. A complete genus-level phylogeny reveals the Cretaceous biogeographic diversification of the poppy family. *Molecular Phylogenetics and Evolution* **181**, 107712.
- Pessoa EM, Ribeiro AC, Christenhusz MJM, Coan AI, Jud NA.** 2023. Is *Santaniella* a ranunculid? Reassessment of this enigmatic fossil angiosperm from the Lower Cretaceous (Aptian, Crato Konservat-Lagerstätte, Brazil) provides a new interpretation. *American Journal of Botany* **110**, e16163.
- Pessoa EM, Ribeiro AC, Jud NA.** 2021. A eudicot leaf from the lower Cretaceous (Aptian, Araripe Basin) Crato Konservat-Lagerstätte. *American Journal of Botany* **108**, 2055–2065.
- Peterson RL, Scott MG, Miller SL.** 1979. Some aspects of carpel structure in *Caltha palustris* L (Ranunculaceae). *American Journal of Botany* **66**, 334.
- Pigg KB, Devore ML.** 2005. *Paleoactaea* gen nov (Ranunculaceae) fruits from the Paleogene of North Dakota and the London Clay. *American Journal of Botany* **92**, 1650–1659.
- Pollack AJ, Gong X, Pollack JR.** 2019. A common phytoene synthase mutation underlies white petal varieties of the California poppy. *Scientific Reports* **9**, 11615.
- Prajapati S, Bajpai S, Gupta MM, Kumarz S.** 2001. The floral androcarpel organ (ACO) mutation permits high alkaloid yields in opium poppy *Papaver somniferum*. *Current Science* **81**, 1109–1112.
- Puzey JR, Gerbode SJ, Hodges SA, Kramer EM, Mahadevan L.** 2012. Evolution of spur-length diversity in *Aquilegia* petals is achieved solely through cell-shape anisotropy. *Proceedings of the Royal Society B: Biological Sciences* **279**, 1640–1645.
- Rasmussen DA, Kramer EM, Zimmer EA.** 2009. One size fits all? Molecular evidence for a commonly inherited petal identity program in Ranunculales. *American Journal of Botany* **96**, 96–109.
- Ren Y, Gu T, Chang H.** 2011. Floral development of *Dichocarpum*, *Thalictrum*, and *Aquilegia* (Thalictrioideae, Ranunculaceae). *Plant Systematics and Evolution* **292**, 203–213.
- Ren Y, Li H-F, Zhao L, Endress PK.** 2007. Floral morphogenesis in *Euptelea* (Eupteleaceae, Ranunculales). *Annals of Botany* **100**, 185–193.
- Ren Y, Li Z, Chang H, Lei Y, Lu A.** 2004. Floral development of *Kingdonia* (Ranunculaceae s. l., Ranunculales). *Plant Systematics and Evolution* **247**, 145–153.
- Reyes E, Sauquet H, Nadot S.** 2016. Perianth symmetry changed at least 199 times in angiosperm evolution. *TAXON* **65**, 945–964.
- Ronse De Craene LP, Brockington SF.** 2013. Origin and evolution of petals in angiosperms. *Plant Ecology and Evolution* **146**, 5–25.
- Rössner C, Lotz D, Becker A.** 2022. VIGS goes viral: how VIGS transforms our understanding of plant science. *Annual Review of Plant Biology* **73**, 703–728.
- Salavert A, Zazzo A, Martin L, et al.** 2020. Direct dating reveals the early history of opium poppy in western Europe. *Scientific Reports* **10**, 20263.
- Samanani N, Park S-U, Facchini PJ.** 2002. In vitro regeneration and genetic transformation of the berberine-producing plant, *Thalictrum flavum* ssp *glaucum*. *Physiologia Plantarum* **116**, 79–86.
- Sanders S, McGraw J.** 2005. *Hydrastis canadensis* L. (Ranunculaceae) distribution does not reflect response to microclimate gradients across a mesophytic forest cove. *Plant Ecology* **181**, 279–288.
- Sauquet H, von Balthazar M, Magallón S, et al.** 2017. The ancestral flower of angiosperms and its early diversification. *Nature Communications* **8**, 16047.
- Sauquet H, Carrive L, Poullain N, Sannier J, Damerval C, Nadot S.** 2015. Zygomorphy evolved from disymmetry in Fumarioideae (Papaveraceae, Ranunculales): new evidence from an expanded molecular phylogenetic framework. *Annals of Botany* **115**, 895–914.
- Schönenberger J, von Balthazar M, López Martínez A, et al.** 2020. Phylogenetic analysis of fossil flowers using an angiosperm-wide data set: proof-of-concept and challenges ahead. *American Journal of Botany* **107**, 1433–1448.
- Schwarz-Sommer Z, Hue I, Huijser P, Flor PJ, Hansen R, Tetens F, Lönning WE, Saedler H, Sommer H.** 1992. Characterization of the *Antirrhinum* floral homeotic MADS-box gene *deficiens*: evidence for DNA binding and autoregulation of its persistent expression throughout flower development. *The EMBO Journal* **11**, 251–263.
- Sharma B, Guo C, Kong H, Kramer EM.** 2011. Petal-specific subfunctionalization of an APETALA3 paralog in the Ranunculales and its implications for petal evolution. *New Phytologist* **191**, 870–883.
- Sharma B, Kramer EM.** 2013. Virus-induced gene silencing in the rapid cycling columbine *Aquilegia coerulea* 'Origami'. *Methods in Molecular Biology* **975**, 71–81.
- Sharma B, Kramer EM.** 2017. *Aquilegia* B gene homologs promote petaloidy of the sepals and maintenance of the C domain boundary. *EvoDevo* **8**, 22.
- Shen G, Luo Y, Yao Y, et al.** 2022. The discovery of a key prenyltransferase gene assisted by a chromosome-level *Epimedium pubescens* genome. *Frontiers in Plant Science* **13**, 1034943.

- Singh SK, Gupta S, Ahmad N, Shukla AK, Shashany AK, Lal RK, Gupta MM, Dhawan OP.** 2017. Variability and heritability studies in floral homeotic mutants of *Papaver somniferum* L. *Industrial Crops and Products* **95**, 276–285.
- Singh SK, Shukla AK, Dhawan OP, Shasany AK.** 2014. Recessive loci Pps-1 and OM differentially regulate PISTILLATA-1 and APETALA3-1 expression for sepal and petal development in *Papaver somniferum*. *PLoS One* **9**, e101272.
- Smith SY, Little SA, Cooper RL, Burnham RJ, Stockey RA.** 2013. A Ranunculalean Liana stem from the cretaceous of British Columbia, Canada: *Atli morinii* gen et sp nov. *International Journal of Plant Sciences* **174**, 818–831.
- Smolko DD, Peretti SW.** 1994. Stimulation of berberine secretion and growth in cell cultures of *Thalictrum minus*. *Plant Cell Reports* **14**, 131–136.
- Sokoloff DD, Remizowa MV, Bateman RM, Rudall PJ.** 2018. Was the ancestral angiosperm flower whorled throughout? *American Journal of Botany* **105**, 5–15.
- Soltis DE.** 2000. Angiosperm phylogeny inferred from 18S rDNA, rbcL, and atpB sequences. *Botanical Journal of the Linnean Society* **133**, 381–461.
- Soza VL, Brunet J, Liston A, Smith PS, Di Stilio VS.** 2012. Phylogenetic insights into the correlates of dioecy in meadow-rues (*Thalictrum*, Ranunculaceae). *Molecular Phylogenetics and Evolution* **63**, 180–192.
- Soza VL, Haworth KL, Di Stilio VS.** 2013. Timing and consequences of recurrent polyploidy in meadow-rues (*Thalictrum*, ranunculaceae). *Molecular Biology and Evolution* **30**, 1940–1954.
- Soza VL, Snelson CD, Hewett Hazelton KD, Di Stilio VS.** 2016. Partial redundancy and functional specialization of E-class SEPALLATA genes in an early-diverging eudicot. *Developmental Biology* **419**, 143–155.
- Sprengelmeyer EE, Rebertus AJ.** 2015. Seed bank dynamics in relation to disturbance and landscape for an ant-dispersed species. *Plant Ecology* **216**, 371–381.
- Stearn WT, Shaw J, Green PS, Mathew B.** 2002. Genus *Epimedium* and other herbaceous Berberidaceae. Portland, OR: Timber Press.
- Su S, Zhao L, Ren Y, Zhang X-H.** 2021. Diversity of petals in Berberidaceae: development, micromorphology, and structure of floral nectaries. *Protoplasma* **258**, 905–922.
- Sun G, Dilcher DL, Wang H, Chen Z.** 2011. A eudicot from the early Cretaceous of China. *Nature* **471**, 625–628.
- Sun Y, Deng T, Zhang A, et al.** 2020. Genome sequencing of the endangered *Kingdonia uniflora* (Circaceasteraceae, Ranunculales) reveals potential mechanisms of evolutionary specialization. *iScience* **23**, 101124.
- Sun Y, Moore MJ, Landis JB, et al.** 2018. Plastome phylogenomics of the early-diverging eudicot family Berberidaceae. *Molecular Phylogenetics and Evolution* **128**, 203–211.
- Sun Y, Moore MJ, Lin N, Adelalu KF, Meng A, Jian S, Yang L, Li J, Wang H.** 2017. Complete plastome sequencing of both living species of Circaceasteraceae (Ranunculales) reveals unusual rearrangements and the loss of the *ndh* gene family. *BMC Genomics* **18**, 592.
- Tamura M.** 1965. Morphology, ecology and phylogeny of the Ranunculaceae IV. *Scientific Reports of Osaka University* **14**, 53–71.
- Tamura M.** 1993. Ranunculaceae. In: Kubitzki K, Rohrer JG, Bittrich V, eds. *Flowering plants · Dicotyledons*. Berlin, Heidelberg: Springer Heidelberg, 563–583.
- Terabayashi S.** 1985. The comparative floral anatomy and systematics of the Berberidaceae: II. Systematic considerations. *Acta Phytotaxonomica et Geobotanica* **36**, 1–13.
- The Angiosperm Phylogeny Group.** 2016. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG IV. *Botanical Journal of the Linnean Society* **181**, 1–20.
- Tian X, Zhang L, Ren Y.** 2005. Development of flowers and inflorescences of *Circaeaster* (Circaceasteraceae, Ranunculales). *Plant Systematics and Evolution* **256**, 89–96.
- Timerman D, Barrett SCH.** 2019. Comparative analysis of pollen release biomechanics in *Thalictrum*: implications for evolutionary transitions between animal and wind pollination. *New Phytologist* **224**, 1121–1132.
- Tucker SC, Hodges SA.** 2005. Floral ontogeny of *Aquilegia*, *Semiaquilegia*, and *Enemion* (Ranunculaceae). *International Journal of Plant Sciences* **166**, 557–574.
- Vesprini JL, Nepi M, Pacini E.** 1999. Nectary structure, nectar secretion patterns and nectar composition in two helleborus species. *Plant Biology* **1**, 560–568.
- von Balthazar M, Pedersen KR, Friis EM.** 2005. *Teixeiria lusitanica*, a new fossil flower from the Early Cretaceous of Portugal with affinities to Ranunculales. *Plant Systematics and Evolution* **255**, 55–75.
- Wakelin AM, Lister CE, Conner AJ.** 2003. Inheritance and biochemistry of pollen pigmentation in California poppy (*Eschscholzia californica* Cham.). *International Journal of Plant Sciences* **164**, 867–875.
- Wang P, Liao H, Zhang W, Yu X, Zhang R, Shan H, Duan X, Yao X, Kong H.** 2015. Flexibility in the structure of spiral flowers and its underlying mechanisms. *Nature Plants* **2**, 15188.
- Wang TN, Clifford MR, Martínez-Gómez J, Johnson JC, Riffell JA, Di Stilio VS.** 2019. Scent matters: differential contribution of scent to insect response in flowers with insect vs wind pollination traits. *Annals of Botany* **123**, 289–301.
- Wang W, Chen Z-D, Liu Y, Li R-Q, Li J-H.** 2007. Phylogenetic and biogeographic diversification of Berberidaceae in the northern hemisphere. *Systematic Botany* **32**, 731–742.
- Wang W, Del Ortiz RC, Jacques FMB, et al.** 2017. New insights into the phylogeny of Burasaieae (Menispermaceae) with the recognition of a new genus and emphasis on the southern Taiwanese and mainland Chinese disjunction. *Molecular Phylogenetics and Evolution* **109**, 11–20.
- Wang W, Lin L, Xiang X-G, Ortiz RDC, Liu Y, Xiang K-L, Yu S-X, Xing Y-W, Chen Z-D.** 2016. The rise of angiosperm-dominated herbaceous floras: insights from Ranunculaceae. *Scientific Reports* **6**, 27259.
- Wang W, Lu A-M, Ren Y, Endress ME, Chen Z-D.** 2009. Phylogeny and classification of Ranunculales: evidence from four molecular loci and morphological data. *Perspectives in Plant Ecology, Evolution and Systematics* **11**, 81–110.
- Wang W, Xiang X-G, Xiang K-L, Del Ortiz RC, Jabbour F, Chen Z-D.** 2020. A dated phylogeny of Lardizabalaceae reveals an unusual long-distance dispersal across the Pacific Ocean and the rapid rise of East Asian subtropical evergreen broadleaved forests in the late Miocene. *Cladistics* **36**, 447–457.
- Wang X-J, Lv X-Q, Zhu Q-Q, Zhang X-H.** 2023. Diversity of staminal nectariferous appendages in disymmetric and zygomorphic flowers of Fumarioideae (Papaveraceae). *Protoplasma* **260**, 1453–1467.
- Wege S, Scholz A, Gleissberg S, Becker A.** 2007. Highly efficient virus-induced gene silencing (VIGS) in California poppy (*Eschscholzia californica*): an evaluation of VIGS as a strategy to obtain functional data from non-model plants. *Annals of Botany* **100**, 641–649.
- Whittall JB, Hodges SA.** 2007. Pollinator shifts drive increasingly long nectar spurs in columbine flowers. *Nature* **447**, 706–709.
- Worberg A, Quandt D, Barniske A-M, Löhne C, Hilu KW, Borsch T.** 2007. Phylogeny of basal eudicots: insights from non-coding and rapidly evolving DNA. *Organisms Diversity & Evolution* **7**, 55–77.
- Xie J, Zhao H, Li K, et al.** 2020. A chromosome-scale reference genome of *Aquilegia oxysepala* var *kansuensis*. *Horticulture Research* **7**, 113.
- Xie S-Y, Hou X-Q, Zhang X-H.** 2022. Are the spurs more complex than other petal types in *Epimedium*? Evidence from development, micromorphology, and nectary structure. *Flora* **293**, 152101.
- Xing Y, Gandolfo MA, Onstein RE, et al.** 2016. Testing the biases in the rich Cenozoic angiosperm macrofossil record. *International Journal of Plant Sciences* **177**, 371–388.
- Xu Z, Li Z, Ren F, et al.** 2022. The genome of *Corydalis* reveals the evolution of benzylisoquinoline alkaloid biosynthesis in Ranunculales. *The Plant Journal* **111**, 217–230.
- Yang X, Gao S, Guo L, et al.** 2021. Three chromosome-scale *Papaver* genomes reveal punctuated patchwork evolution of the morphinan and noscapine biosynthesis pathway. *Nature Communications* **12**, 6030.

- Yant L, Collani S, Puzey J, Levy C, Kramer EM.** 2015. Molecular basis for three-dimensional elaboration of the *Aquilegia* petal spur. *Proceedings of the Royal Society B: Biological Sciences* **282**, 20142778.
- Yellina AL, Orashakova S, Lange S, Erdmann R, Leebens-Mack J, Becker A.** 2010. Floral homeotic C function genes repress specific B function genes in the carpel whorl of the basal eudicot California poppy (*Eschscholzia californica*). *EvoDevo* **1**, 13.
- Zahn LM, Leebens-Mack JH, Arrington JM, Hu Y, Landherr LL, dePamphilis CW, Becker A, Theissen G, Ma H.** 2006. Conservation and divergence in the AGAMOUS subfamily of MADS-box genes: evidence of independent sub- and neofunctionalization events. *Evolution & Development* **8**, 30–45.
- Zalko J, Frachon S, Morel A, et al.** 2021. Floral organogenesis and morphogenesis of *Staphisagria* (Ranunculaceae): implications for the evolution of synorganized floral structures in Delphinieae. *International Journal of Plant Sciences* **182**, 59–70.
- Zhai W, Duan X, Zhang R, Guo C, Li L, Xu G, Shan H, Kong H, Ren Y.** 2019. Chloroplast genomic data provide new and robust insights into the phylogeny and evolution of the Ranunculaceae. *Molecular Phylogenetics and Evolution* **135**, 12–21.
- Zhang J-M, Peng X-Y, Song M-L, Li Z-J, Xu X-Q, Wang W.** 2022. Effects of climate change on the distribution of wild *Akebia trifoliata*. *Ecology and Evolution* **12**, e8714.
- Zhang R, Guo C, Zhang W, et al.** 2013. Disruption of the petal identity gene APETALA3-3 is highly correlated with loss of petals within the buttercup family (Ranunculaceae). *Proceedings of the National Academy of Sciences, USA* **110**, 5074–5079.
- Zhang R, Min Y, Holappa LD, Walcher-Chevillet CL, Duan X, Donaldson E, Kong H, Kramer EM.** 2020. A role for the Auxin Response Factors ARF6 and ARF8 homologs in petal spur elongation and nectary maturation in *Aquilegia*. *New Phytologist* **227**, 1392–1405.
- Zhang X, Zhao L.** 2018. Morphology, structure and ultrastructure of staminal nectary in *Lamprocapnos* (Fumarioideae, Papaveraceae). *Flora* **242**, 128–136.
- Zhang X-H, Ren YI.** 2011. Comparative floral development in Lardizabalaceae (Ranunculales). *Botanical Journal of the Linnean Society* **166**, 171–184.
- Zhang Z, Zhang J, Yang Q, Li B, Zhou W, Wang Z.** 2021. Genome survey sequencing and genetic diversity of cultivated *Akebia trifoliata* assessed via phenotypes and SSR markers. *Molecular Biology Reports* **48**, 241–250.
- Zhao H, Liao H, Li S, et al.** 2023. Delphinieae flowers originated from the rewiring of interactions between duplicated and diversified floral organ identity and symmetry genes. *The Plant Cell* **35**, 994–1012.
- Zhao L, Bachelier JB, Chang H, Tian X, Ren Y.** 2012. Inflorescence and floral development in *Ranunculus* and three allied genera in Ranunculaceae (Ranunculoideae, Ranunculaceae). *Plant Systematics and Evolution* **298**, 1057–1071.
- Zhao Y, Pfannebecker K, Dommies AB, Hidalgo O, Becker A, Elomaa P.** 2018. Evolutionary diversification of CYC/TB1-like TCP homologs and their recruitment for the control of branching and floral morphology in Papaveraceae (basal eudicots). *New Phytologist* **220**, 317–331.
- Zohary D, Hopf M, Weiss E.** 2012. Domestication of plants in the old world: the origin and spread of domesticated plants in Southwest Asia, Europe, and the Mediterranean Basin. Oxford: Oxford University Press.
- Zou S-Y, Feng C, Gao P-X, Li T-J, Jia T-J, Huang H.** 2022. Germplasm resources and genetic improvement of *Akebia*: a new fruit crop in China. *Plant Diversity* **45**, 712–721.
- Zumajo-Cardona C, Ambrose BA, Pabón-Mora N.** 2017. Evolution of the SPATULA/ALCATRAZ gene lineage and expression analyses in the basal eudicot, *Bocconia frutescens* L (Papaveraceae). *EvoDevo* **8**, 5.
- Zumajo-Cardona C, Pabón-Mora N, Ambrose BA.** 2018. Duplication and diversification of REPLUMLESS—a case study in the Papaveraceae. *Frontiers in Plant Science* **9**, 1833.
- Zumajo-Cardona C, Pabón-Mora N, Ambrose BA.** 2021. The evolution of euAPETALA2 genes in vascular plants: from plesiomorphic roles in sporangia to acquired functions in ovules and fruits. *Molecular Biology and Evolution* **38**, 2319–2336.