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FLOWERING NEWSLETTER REVIEW

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

The RanOmics group, Annette Becker[1,*], Julien B. Bachelier[2], Laetitia Carrive[3], Natalia Conde e Silva[4], Catherine Damerval[4], Cédric Del Rio[5], Yves Deveaux[4], Verónica S. Di Stilio[6], Yan Gong[7], Florian Jabbour[8], Elena M. Kramer[7], Sophie Nadot[9], Natalia Pabón-Mora[10], and Wei Wang[11]

[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

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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*, *Circeasteraceae*, *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,

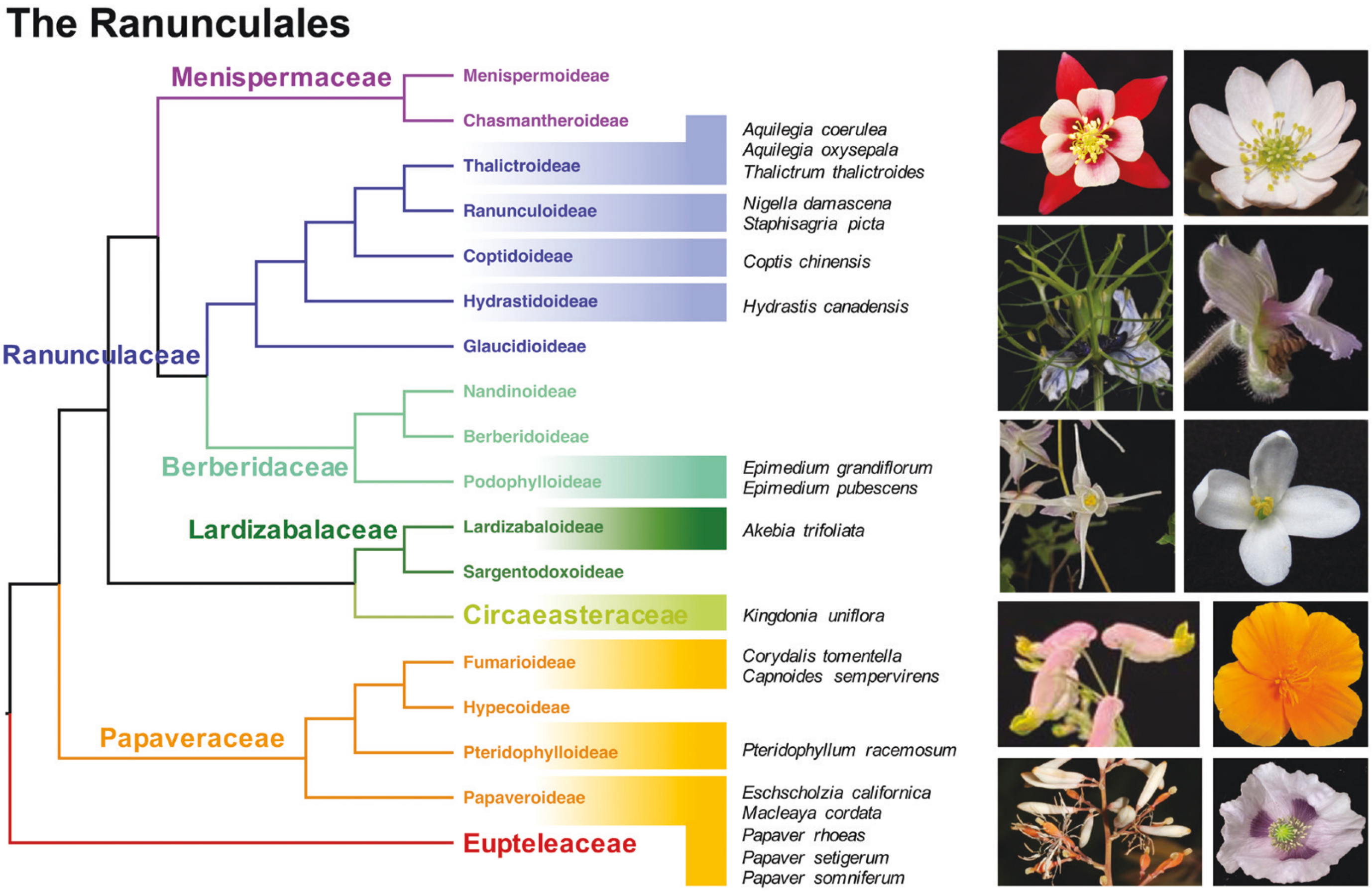

Fig 1. Simplified phylogeny of *Ranunculales* based on Wang *et al.* (2009), Ortiz *et al.* (2016), and Peng *et al.* (2023). Species for which major genomic resources are or will become available in the near future are next to their respective branches. Representative photos of Ranunculales flowers: (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 Stilio lab; C, F, Jabbour; I, N, Pabón Mora.)

Unani, Greek–Roman, Malay, Tibb-e-Nabwi, and Jewish civilizations (Heiss and Oeggl, 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 adressing 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* (*Circeasteraceae*), *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
Papaveraceae							
Eschscholzia californica[b]	Hori *et al.* (2018)	Not given	Hauschild *et al.* (1998)	Fujii *et al.* (2007)	Park and Facchini, (2000b); Lotz *et al.* (2022)	Wege *et al.* (2007)	Lange *et al.*, 2013; Wakelin *et al.* (2003); Barrell *et al.* (2010); Pollack *et al.* (2019); Conner and Barrell (2014)
Papaver somniferum[b]	Guo *et al.* (2018)	95.3	Eilert *et al.* (1985)	Belny *et al.* (1997)	Park and Facchini, (2000a); Chitty *et al.* (2003)	Hilemann *et al.* (2005)	Singh *et al.* (2017); Pathak *et al.* (2013); Dhawan *et al.* (2007); Belyaeva and Nevkrytaya (1979); Prajapati *et al.* (2001); Singh *et al.* (2014)
Macleaya cordata	Liu *et al.* (2017)	90.4	Franke and Böhm (1982)	No	Huang *et al.* (2017)	No	
Papaver rhoeas	Yang *et al.* (2021)	92.8	No	No	No	No	O'Donnell *et al.* (1993); Foote *et al.*, (1994)
Papaver setigerum	Yang *et al.* (2021)	94.5	No	No	No	no	
Corydalis tomentella	Xu *et al.* (2022)	97.67	Holländer-Czytko *et al.* (1988) (*Corydalis sempervirens*)	No	No	No	
Capnoides sempervirens[b]			No	No	No	Hidalgo *et al.* (2012)	
Pteridophyllum racemosum[b]			No	No	No	No	
Ranunculaceae							
Coptis chinensis	Chen *et al.* (2021)	91.5	No	No	No	No	
Aquilegia coerulea[b]	Filiaut *et al.* (2018)	Not given	No	No	No	Gould and Kramer (2007)	Cabin *et al.* (2022)
Aquilegia oxysepala	Xie *et al.* (2020)	93.2	No	No	No	No	
Thalictrum thalictroides[b]	Arias *et al.* (2021)	84.5	Smolko and Peretti (1994)	Samanani *et al.* (2002) (*Thalictrum flavum*)	No	Di Stilio *et al.* (2010)	Martinez-Gómez *et al.* (2021); Galimba *et al.* (2012)
Nigella damascena[b]			Klimek-Chodacka *et al.* (2020)		No	Wang *et al.* (2015)	Goncalvez *et al.* (2013); Jabbour *et al.* (2021); Conde e Silva *et al.* (2023); Greyson and Raman (1975)
Staphisagria picta[b]			No	No	No	No	
Hydrastis canadensis[b]			No	No	No	No	

Cicaeasteraceae

Table 1. Continued

Species	Genome available	BUSCO[a]	Cell/callus culture system	Cell culture transformation system	Stable transformation	Virus-induced gene silencing	Spontaneous mutants
Kingdonia uniflora	Sun *et al.* (2020)	90.6	No	No	No	No	
Lardizabalaceae							
Akebia trifoliata	Huang *et al.* (2021)	94.0	No	No	No	No	
Berberidaceae							
Epimedium pubescens	Shen *et al.* (2022)	89.9	No	No	No		Wang *et al.* (2017)
Epimedium grandiflorum[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 RanOmics 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 latu contain four subfamilies: *Fumarioideae*, *Hypecoideae*, *Papaveroideae* (including *Chelidonieae*, *Eschscholzieae*, 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 *Limacieae*. *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*, *Glaucidioideae*, *Hydrastidoideae*, *Ranunculoideae*, and *Thalictroideae* (Wang *et al.*, 2009). Most studies support *Glaucidioideae* 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 *Glaucidioideae* 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 *Adonideae*. 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 *Adonideae* 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 *Chelidonieae* (*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. *Hunnemania* 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 *Chelidonieae* 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 insecticidals (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* (La Valva *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 speciess 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 *Thalictroideae* 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 Oeggl, 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 *Anemonella 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

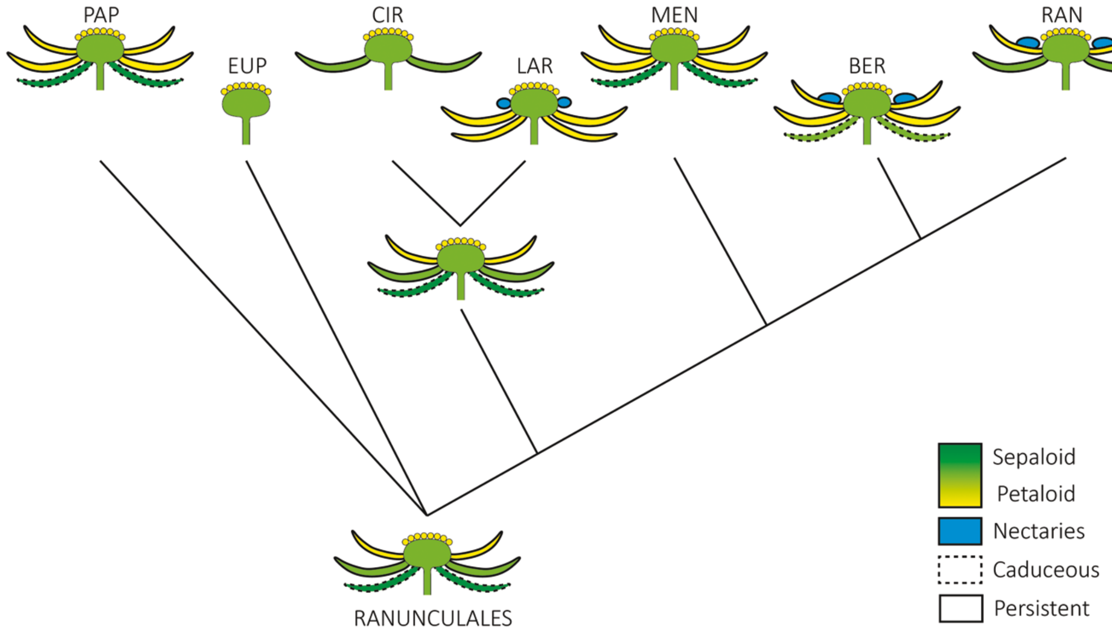

Fig. 2. Simplified phylogeny of *Ranunculales* showing ancestral floral traits of the *Ranunculales* families.

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 caduceus (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 2be 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 bracteal origin 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 bracteal 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*, Chanderbali *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' (Hiepko, 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

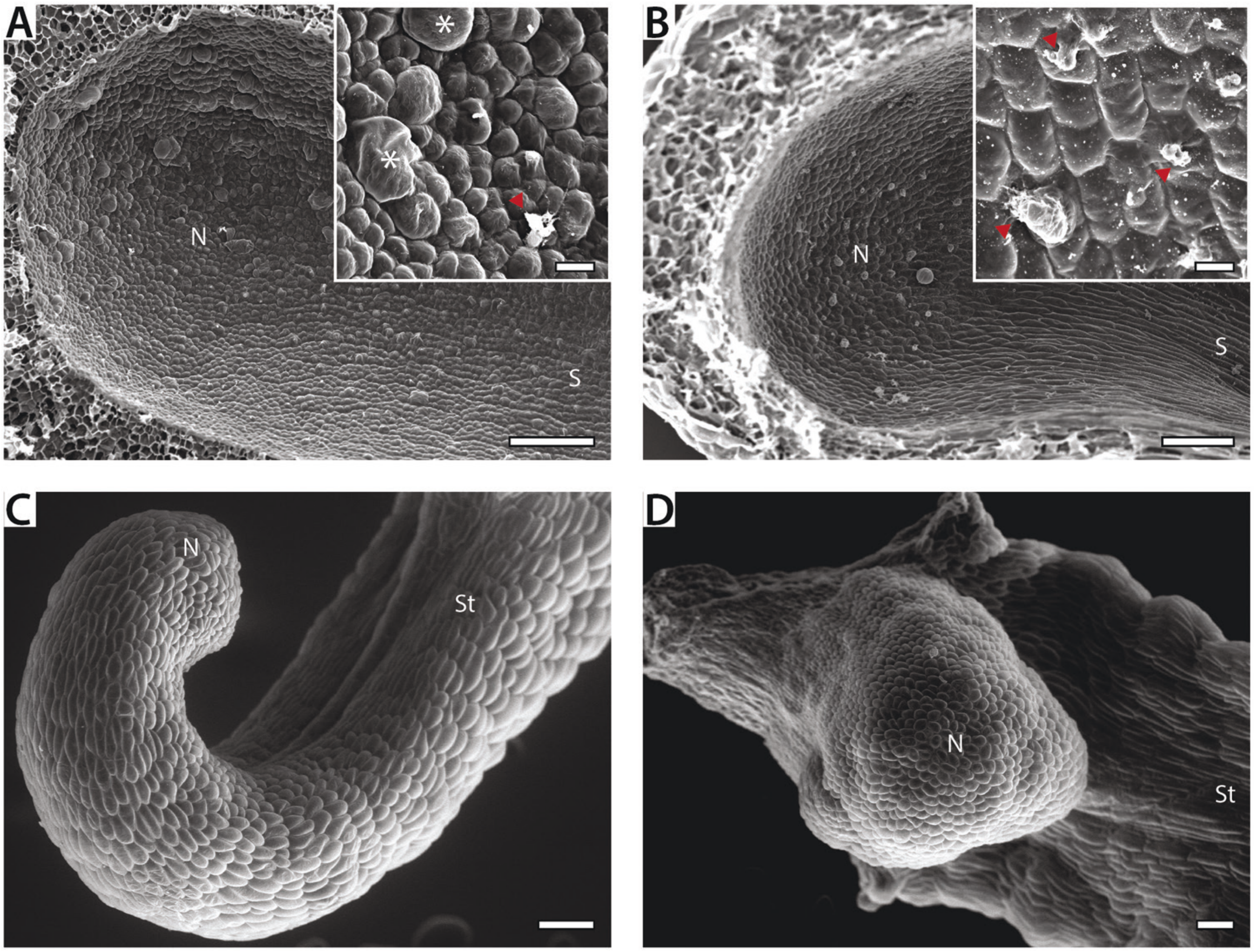

Fig. 3. SEM images of mature nectaries from four Ranunculales species: (A) *Aquilegia coerulea* (*Ranunculaceae*), (B) *Epimedium grandiflorum* (*Berberidaceae*), (C) *Corydalis aurea* (*Papaveraceae*), (D) *Lamprocapnos spectabilis* (*Papaveraceae*). Enlarged views of the nectary cells for *Aquilegia* 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 identify 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. asiatiaca*) (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 *Papaveaceae*. 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 *Lardizabalacaeae* [*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*

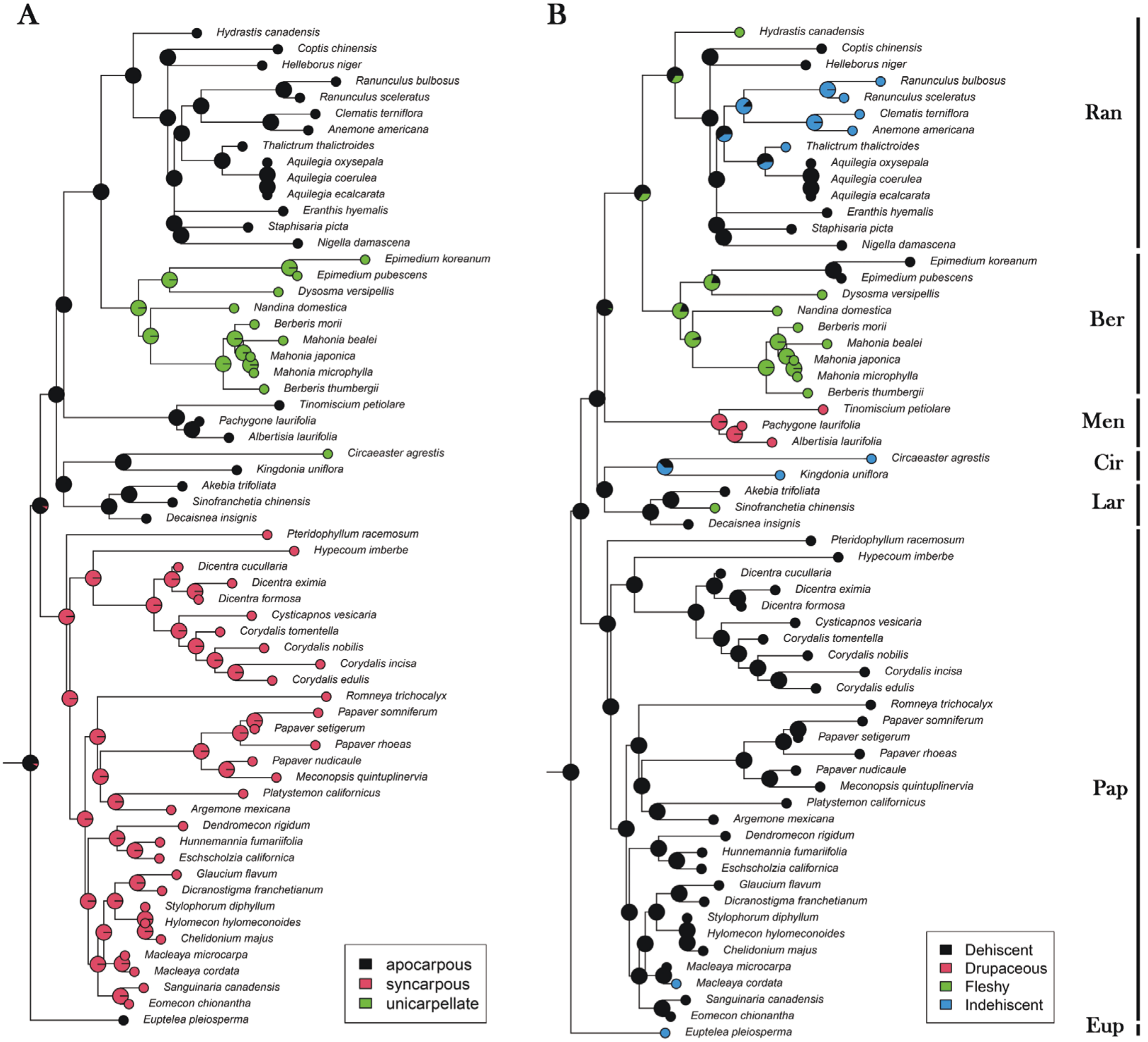

Fig. 4. Ancestral state reconstruction of gynoecium (left) and fruit (right) characters based on a phylogeny using *rbcL* as the marker gene. Trait descriptions are from Cheng-Yih and Kubitzki (1993), Endress (1993), Kadereit (1993), Lidén (1993a, b), Loconte (1993), and Tamura (1993).

(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 (Orashakova *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.

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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.

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