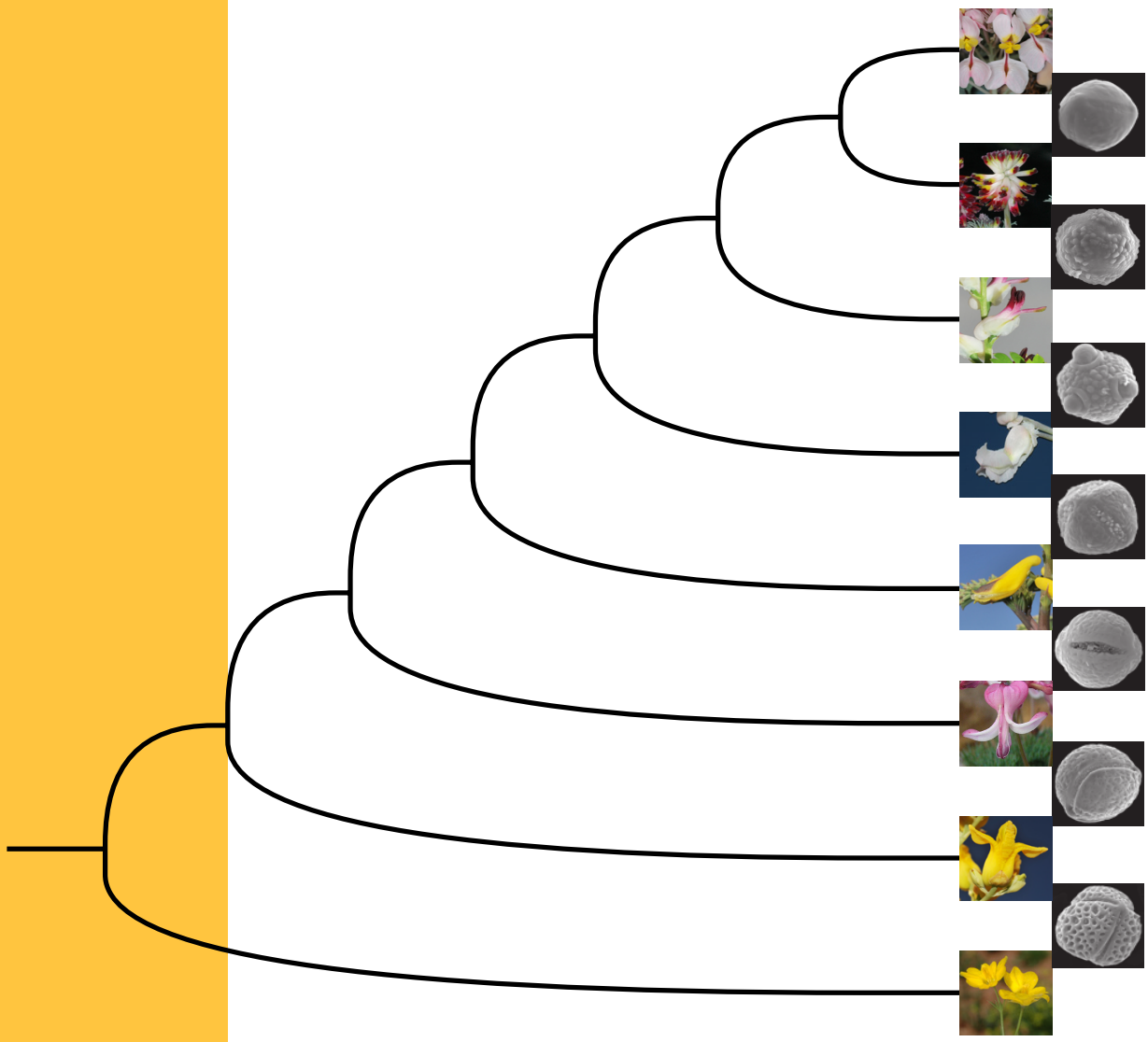
Universidad de Granada

Programa de Doctorado
Biología Fundamenal y de Sistemas

Tesis Doctoral

Sistemática y Evolución en la subfamilia Fumarioideae



Miguel Ángel Pérez Gutiérrez
Octubre, 2015

Editor: Universidad de Granada. Tesis Doctorales
Autor: Miguel Ángel Pérez Gutiérrez
ISBN: 978-84-9125-006-7
URI: http://hdl.handle.net/10481/39522

Tesis Doctoral *Sistemática y evolución en la subfamilia Fumarioideae*

Doctoral Thesis *Systematics and evolution of the subfamily Fumarioideae*

Miguel Ángel Pérez Gutiérrez

Directores: Ana Teresa Romero García
Víctor N. Suárez Santiago

Universidad de Granada
Programa de Doctorado Biología Fundamental y de Sistemas

Granada, Octubre 2015

El doctorando Miguel Ángel Pérez Gutiérrez y los directores de la tesis Dª. Ana Teresa Romero García y D. Víctor N. Suárez Santiago garantizamos, al firmar esta tesis doctoral, que el trabajo ha sido realizado por el doctorando bajo la dirección de los directores de la tesis y hasta donde nuestro conocimiento alcanza, en la realización del trabajo, se han respetado los derechos de otros autores a ser citados, cuando se han utilizado sus resultados o publicaciones.

En **Granada**, a **29** de **Octubre** de **2014**

Director/es de la Tesis

Fdo.: **Ana Teresa Romero García** Fdo. : **Víctor N. Suárez Santiago**

Doctorando

Fdo.: **Miguel Ángel Pérez Gutiérrez**

Prólogo

Esta memoria de tesis contiene los resultados obtenidos durante mi periodo de formación doctoral desarrollado en el Departamento de Botánica de la Universidad de Granada (UGR). Este periodo de formación ha estado supervisado por los doctores Dª Ana Teresa Romero García y de D. Víctor N. Suárez Santiago, también del Departamento de Botánica de la UGR. Todo el trabajo de investigación ha sido realizado entre Septiembre de 2009 y Septiembre de 2014, en el marco del proyecto de investigación CGL-2008-01554 y bajo el soporte del programa de formación de personal investigador a través de la ayuda BES-2009-024836. Los principales resultados obtenidos son plasmados en cuatro manuscritos que bien han sido publicados, están bajo revisión o bajo preparación, en revistas científicas internacionales de prestigio en el ámbito de la investigación en plantas. En la elaboración de dichos trabajos han trabajado, además del doctorando y los directores de tesis, el Dr. Gabriel Blanca López del Departamento de Botánica de la UGR, la Dra. M. Carmen Fernández del Departamento de Biología Celular de la UGR y la Dra. María J. Salinas Bonillo del Departamento de Biología Vegetal y Ecología de la Universidad de Almería. La contribución en cada uno de los manuscritos ha sido la siguiente:

Capítulo 3. 'Phylogeny of the tribe Fumarieae (Papaveraceae s.l.) based on chloroplast and nuclear DNA sequences: evolutionary and biogeographic implications' Miguel A. Pérez-Gutiérrez, Ana T. Romero-García, M. Carmen Fernández, G. Blanca, María J. Salinas-Bonillo, Víctor N. Suárez-Santiago. Víctor Suárez realizó el diseño de la investigación. Víctor Suárez, Gabriel Blanca, M. Carmen Fernández, Ana Teresa Romero y yo recolectamos el material vegetal. V. Suárez-Santiago y yo producimos y analizamos los datos e interpretamos los resultados. V. Suárez elaboró el manuscrito y todos los autores participaron en la revisión del mismo.

Capítulo 4. 'Evolutionary history of fumitories (subfamily Fumarioideae, Papaveraceae): an old story shaped by the main geological climatic events in the Northern Hemisphere' Miguel A. Pérez-Gutiérrez, Ana T. Romero-García, M. Carmen Fernández, G. Blanca, María J. Salinas-Bonillo, Víctor N. Suárez-Santiago. V. Suárez y yo diseñamos el trabajo, realizamos los análisis e interpretamos los resultados. Yo generé los datos. V. Suárez, G. Blanca, M. Carmen Fernández, A.T. Romero y yo recolectamos el material vegetal. Yo elaboré el manuscrito y todos los autores participaron en su revisión.

Capítulo 5. 'Pollen morphology and ontogeny in the subfamily Fumarioideae: the tribe Fumarieae' Miguel A. Pérez-Gutiérrez, Víctor N. Suárez-Santiago, M. Carmen Fernández, María J. Salinas-Bonillo, Ana T. Romero-García. A.T. Romero y yo diseñamos la investigación. M. Carmen Fernández, A.T. Romero, M.J. Salinas y yo producimos los datos. A.T. Romero y yo

interpretamos los resultados. Yo elaboré el manuscrito. Todos los autores participaron en la recolección de material vegetal y en la revisión del manuscrito.

Capítulo 6. Evolutionary trends in the pollen grain of the subfamily Fumarioideae: evidence from morphology and ontogenetic characters of the pollen wall and apertures' Miguel A. Pérez-Gutiérrez, Ana T. Romero-García, M. Carmen Fernández, María J. Salinas-Bonillo, Gabriel Blanca, Víctor N. Suárez-Santiago. Yo diseñé el trabajo. A.T. Romero y yo produjimos los datos. V. Suárez y yo realizamos los análisis e interpretamos los resultados. Yo elaboré el manuscrito. Todos los autores participaron en la recolección del material vegetal y en la revisión del manuscrito.

Agradecimientos (Acknowledgements)

Well, there are many people that I have to thank for helping me in some moments during my PhD job, but it could be a really long list. Probably I forget some of them but I want to thank specifically to Ernst Viteck (Wien), Henrik Zetterlund (Göteborg), Alpheus Mothapo (SANBI), Nahid Heidari (Uppsala), Omar Fiz Palacios (Uppsala), Yu Yan (RASP), Leonidas Brikiatis (Athens), Ron Blackey (Palaeomaps), Isabel Sanmartín (Real Jardín Botánico) and Magnus Lidén (Uppsala). Their contributions allow me to get this thesis document. Also I want to thank Sven Buerki (Kew) and Diego Nieto Lugilde (University of Maryland) for assessing the final thesis document.

Si mostrara mi agradecimiento a todas las personas que de uno u otro modo me han ayudado, animado, orientado o simplemente se han preocupado sobre mi trabajo de tesis doctoral y todo lo relacionado con él, creo que alargaría este documento en demasía. Por ello me restrinjo a aquellos más estrictamente relacionados con mi trabajo y conmigo.

Quiero agradecer en primer lugar a mis directores de tesis, Ana Teresa Romero y Víctor Suárez, ya que nada de lo que se ha realizado y se resume en esta memoria de tesis doctoral se hubiera llevado a cabo sin su ayuda, su asesoramiento y su proximidad. Hemos dedicado muchos momentos para sacar adelante este proyecto y creo que los frutos son proporcionales al esfuerzo dedicado. Les estoy enormemente agradecido por su ayuda y enseñanzas, no sólo a nivel científico o académico, sino que también a nivel humano.

También agradezco su ánimo y consejo a todos los compañeros del departamento de Botánica de la UGR, tanto profesores, personal administrativo, becarios y alumnos internos. En especial a Gabriel Blanca, por su disponibilidad, enseñanzas y recomendaciones a lo largo de estos años, y también a Concha Morales, quien me acompañó a muestrear y que en todo momento que lo he necesitado me ha orientado en la identificación y taxonomía de diferentes especies de esta subfamilia que tanto nos deleita. Destaco también a mis compañeros del laboratorio de sistemática y evolución molecular: Marta, Samira, Laura, Ana e Ismael que también han compartido conmigo muchos momentos de mi trabajo y hemos aprendido juntos.

Mari Carmen Fernández del departamento de Biología Celular de la UGR, María J. Salinas del departamento de Biología y Geología de la UAL y Conchi Hernández del CIC de la UGR también han estado muy integradas en el proyecto de mi tesis, de modo que os agradezco vuestro trabajo y contribución.

A muchos compañeros del departamento de Genética también les agradezco enormemente su ayuda y consejo, en especial a Manolo Garrido, Ester Quesada e Inma López, quienes me han enseñado mucho sobre el trabajo en laboratorio.

A todo el personal del Herbario de la UGR les doy las gracias por su colaboración con mi trabajo y su constante disponibilidad y cercanía, su labor es fundamental para que proyectos como el de esta tesis puedan realizarse.

A mis compañeros del máster en Genética y Evolución: Anja, Elena, Paco y Rubén, por haber compartido formación y haber intercambiado consejos sobre todos los trámites relacionados con el trabajo de tesis, muchas gracias.

A todos mis amigos de la Licenciatura en Ciencias Ambientales y aquellos amigos eternos de mi pueblo también les agradezco el apoyo que siempre me muestran.

Pero en especial quiero agradecer a toda mi familia, a mis padres por haberme educado, dármelo todo y enseñarme a ser persona, y a mi hermana por ser un apoyo y un referente constante. También agradezco a la familia de Silvia el apoyo y ayuda que me han dado en estos últimos años pero sobre todo a Silvia, que me aconseja y ayuda incansablemente y que le da sentido a todo lo que hago.

A todos, gracias!

Table of contents

Resumen introductorio

Uno de los principales objetivos de la sistemática es completar la reconstrucción de las relaciones evolutivas entre todos los organismos. El desarrollo de filogenias moleculares durante las últimas décadas ha permitido avanzar sustancialmente en la consecución de dicho objetivo. Sin embargo aún existe un gran número de grupos para los que no se ha realizado una revisión filogenética completa, como es el caso de la subfamilia Fumarioideae, el grupo más diversificado de la familia Papaveraceae pero que hasta la fecha es el que menos atención ha recibido con respecto al estudio de las relaciones entre sus táxones. Este trabajo de tesis doctoral se desarrolla con dos propósitos generales, el primero, solventar dicha carencia existente en la sistemática de Fumarioideae llevando a cabo una filogenia molecular exhaustiva de la subfamilia; y el segundo, estudiar la morfología de la pared de polen y aperturas de toda Fumarioideae describiendo al mismo tiempo las características que definen dicha morfología durante el proceso ontogénico, para posteriormente identificar las tendencias evolutivas de los caracteres polínicos entre los linajes de la subfamilia. Este segundo objetivo de la tesis introduce las herramientas derivadas de la palinología en el estudio de la sistemática de Fumarioideae. Tradicionalmente el estudio del polen ha sido considerado para abordar cuestiones taxonómicas o para aportar información a la sistemática en plantas. En este trabajo de tesis doctoral se han integrado las posibilidades que ofrece la filogenia molecular con el conocimiento de la diversidad polínica y ontogénica existente en la subfamilia. Por ello, los resultados de esta tesis doctoral se estructuran en dos grandes bloques, estando cada uno de ellos dividido a su vez en dos capítulos.

En el primer bloque se desarrolla el estudio de las relaciones filogenéticas en Fumarioideae. El primero de los capítulos que lo conforman lleva a cabo la reconstrucción de la filogenia de la subtribu Fumariinae (tribu Fumarieae sensu Lidén, 1986). Este es el grupo con mayor riqueza genérica de la subfamilia y que contiene a la mayoría de linajes con distribución mediterránea. Su naturaleza monofilética ha sido previamente confirmada, pero el conocimiento de las relaciones entre sus géneros se basa exclusivamente en información morfológica, no habiendo sido contrastado con datos moleculares. El segundo capítulo desarrolla el análisis filogenético exhaustivo de la subfamilia Fumarioideae, incluyendo representantes de todos sus géneros. En ambos capítulos, además de llevar a cabo la reconstrucción filogenética empleando secuencias de ADN nuclear y cloroplastidial, se desarrollan otras técnicas derivadas de la inferencia filogenética. En el primer capítulo se reconstruyen los estados de carácter para algunos rasgos morfológicos en los linajes analizados, así como se lleva a cabo un análisis de reconstrucción de áreas ancestrales en los mismos. En el capítulo dedicado a toda la subfamilia se realiza una datación mediante métodos Bayesianos e incluyendo información de fósiles para estimar los tiempos de divergencia de las diversificaciones existentes. Con dicha información y mediante el uso de métodos de análisis biogeográfico que implementan modelos

paleogeográficos, la historia evolutiva de la subfamilia fue reconstruida proponiendo los principales eventos de dispersión o vicarianza que la han configurado.

El segundo bloque desarrolla el objetivo de descripción polínica y análisis de su evolución en Fumarioideae. El primer capítulo de este bloque lleva a cabo la descripción de los rasgos morfológicos del grano de polen para todos los géneros de la tribu Fumarieae (información ya disponible para la tribu Hypecoeae) así como de los rasgos ultraestructurales de la pared y aperturas polínicas para buena parte de los mismos. Este capítulo se centra especialmente en el proceso ontogénico de maduración de la pared del polen, describiendo los eventos mediante los que tiene lugar y la diversidad morfológica existente. Los rasgos observados son comparados con el modelo general de ontogenia en angiospermas y se discute sobre su significado biológico y evolutivo. En el segundo capítulo de este bloque se recopila la información morfológica producida para Fumarieae y la información disponible sobre ontogenia polínica de la tribu Hypecoeae, de *Pteridophyllum* y *Euptelea*, para optimizar los principales rasgos morfológicos y ultraestructurales sobre la filogenia de la subfamilia elaborada en el bloque anterior. Siete rasgos morfológicos del grano de polen y diecisiete caracteres ultraestructurales durante los cuatro estadios finales del proceso de maduración polínica (microspora, polen bicelular joven, polen bicelular medio y polen maduro) son evaluados. El ajuste de cada uno de los caracteres a la topología es utilizado como índice del grado de información filogenética que presenta. La presencia de sinapomorfías y las tendencias evolutivas de los caracteres polínicos son descritas, del mismo modo que se identifican los cambios entre los diferentes estadios ontogénicos analizados.

Summary

One of the main goals in systematics is to reconstruct the evolutionary relationships among all the existing organisms. The development of molecular phylogenies during the last decades has facilitated progress in reaching this aim. Nevertheless, many groups of organisms still lack a complete phylogenetic revision as is the case of the subfamily Fumarioideae. This plant subfamily is the most diversified group of the family Papaveraceae but it is also the Papaveraceae group to which less attention has been paid to the study of the relations among its taxa. This doctoral thesis develops two main objectives: the first is to solve this lack of knowledge of the systematics of Fumarioideae by means of a comprehensive molecular phylogeny of the subfamily. The second objective is to study the pollen-wall and aperture system characteristics from the whole subfamily and to describe the features and processes that distinguish the pollen ontogeny in the group. Through this second aim and the use of the phylogenetic hypothesis previously obtained, the pollen evolutionary trends among the lineages of Fumarioideae are identified. This second goal introduces the use of pollen grain, pollen-wall and aperture systems in the study of the systematics of Fumarioideae. Traditionally, research on pollen morphology has been used in taxonomical issues or for providing information about the systematics of plant groups. In this thesis we integrate the possibilities of molecular phylogenetics with the knowledge of the pollen diversity in Fumarioideae. Therefore this doctoral thesis presents the results structured in two main sections, each with two chapters.

The first section contains the study of the phylogenetic relationships in Fumarioideae. In the first of its chapters the phylogeny of the subtribe Fumariinae is reconstructed (tribe Fumarieae sensu Lidén, 1986). This subtribe presents the highest number of genera in the subfamily and also harbors most of the Mediterranean Fumarioideae lineages. The monophyletic nature of Fumariinae has been previously reported but the knowledge about the generic relations is based exclusively on morphological information, without having been contrasted with molecular data. The second chapter delves into the complete phylogenetic analysis of the subfamily Fumarioideae, including representatives from all its genera. In both chapters, besides carrying out the phylogenetic reconstruction using sequences from plastidial and nuclear DNA, other techniques derived from the phylogenetic inference are employed. The reconstructions of the ancestral states for some morphological features of the subtribe Fumariinae and of the ancestral area distribution for its lineages are developed in the first chapter. In the chapter focused on the whole subfamily, we perform a divergence time estimation using Bayesian methods and fossil information. With this data and the use of biogeographical analyses that implement palaeogeographical information, we reconstruct the evolutionary history of Fumarioideae and identify the main dispersal and vicariance events that have shaped it.

The second section accomplishes the goal of pollen description and analysis of its evolution in the subfamily Fumarioideae. Its first chapter provides the description of the pollen grain morphology of all the representatives of the tribe Fumarieae -the tribe Hypecoeae information is already available- and of the ultrastructural characteristics from the pollen-wall and aperture systems of most of the Fumarieae genera. This third chapter of the thesis is especially focused on the pollen maturation ontogenetic process, depicting the events in which this process takes place and showing the extant morphologic diversity. The described traits are compared with the ontogeny model of the angiosperm and their biologic and evolutionary implications are discussed. The fourth chapter collects together all the pollen morphological information produced for the tribe Fumarieae and that available both from pollen morphology and ontogeny of the tribe Hypecoeae, the genus *Pteridophyllum* and the genus *Euptelea*. All this information is optimized on the phylogenetic hypothesis obtained in the former block using the molecular data. Seven traits from the pollen grain morphology and seventeen ultrastructural characters from the four last stages of pollen development –microspore, young bicellular, medium bicellular and mature pollen- are analyzed. The adjustment indexes from each of the studied characters to the topology are used to estimate the level of phylogenetic signal that they include. The presence of synapomorphies and the evolutionary trends from the pollen characteristics are described, at the same time as the changes observed amongst the four ontogenetic stages.

1. Introducción

1. Introducción general

1.1 La familia Papaveraceae

La familia Papaveraceae Juss. está constituida por 44 géneros y en torno a 790 especies, principalmente distribuidas en el Hemisferio Norte pero alcanzado también las regiones del Centro y Sur de América, y Sudáfrica (Stern, 1997; Zhang et al., 2008). Respecto a su encuadre sistemático, la familia forma parte del orden Ranunculales Dumort. (Kubitzki et al., 1993; Chase et al., 1993), primer clado divergente de las eudicotiledóneas basales (Soltis et al., 1997; Hoot et al., 1999). La circunscripción de Ranunculales era conflictiva debido a la escasez de rasgos sinapormórficos que sustentaran su agrupación (Hoot et al., 1999; Wang et al., 2009), pero tras el empleo de filogenias moleculares para esclarecer su sistemática, actualmente su composición está totalmente clarificada (Hoot and Crane, 1995; Soltis et al., 1997; Hoot et al., 1999; Kim et al., 2004; Worberg et al., 2007; Wang et al., 2009). Las distintas hipótesis filogenéticas obtenidas mediante esas reconstrucciones moleculares muestran que las familias Eupteleaceae K. Wihl. y Papaveraceae presentan una posición basal respecto al resto de Ranunculales, aunque cuál de ellas dos ocupa la posición más basal no está totalmente aclarado. Si bien es cierto que Eupteleaceae siempre ocupa esta posición en las reconstrucciones filogenéticas moleculares, también lo es que siempre lo hace con escaso o moderado apoyo estadístico. Cuando los datos moleculares se combinan con datos morfológicos, la posición basal de Eupteleaceae sí recibe fuerte apoyo estadístico (Wang et al., 2009). Por todo ello, actualmente se reconoce Eupteleaceae como el linaje más basal de Ranunculales aunque con ciertas reservas, y Papaveraceae como el segundo linaje en divergir en el orden. En relación con la sistemática de Papaveraceae, su condición monofilética era ya ampliamente aceptada en sistemas de clasificación antiguos (Cronquist, 1981; 1988), siendo descrita como un grupo natural. Los principales rasgos morfológicos que soportan dicha agrupación están relacionados con caracteres florales, ya que todos sus táxones presentan dos sépalos y cuatro o más pétalos, así como gineceo paracárpico (Hoot et al., 1999). La presencia de placentación parietal (Wang et al. 2009) y de laticíferos o de idioblastos secretores en la epidermis interna del tegumento externo también caracterizan a la familia, aunque este último carácter está ausente en el género *Pteridophyllum* Siebold & Zucc. (Friedel, 1938). Los trabajos que han evaluado la naturaleza monofilética de Papaveraceae usando herramientas moleculares han sido pocos. Algunos de estos en un contexto filogenético más amplio, en el conjunto del orden Ranunculales, y sin incluir representantes de todos los linajes de Papaveraceae (Hoot and Crane, 1995; Hoot et al., 1999; Kim et al., 2004; Worberg et al., 2007). El primer trabajo molecular centrado en Papaveraceae fue el realizado por Hoot et al. (1997), donde se resolvió su naturaleza monofilética aunque con bajo apoyo estadístico. La reconstrucción filogenética realizada por Wang et al. (2009) para todas las familias de

Ranunculales combinando marcadores cloroplastidiales y nuclerares, sí resolvió con un elevado apoyo la monofilia de Papaveraceae.

Dentro de Papaveraceae existen cuatro linajes principales (el género *Pteridophyllum*, el género *Hypecoum* L., Papaveroideae Eaton y Fumarioideae Eaton) cuyas relaciones filogenéticas no han sido totalmente resueltas (Kadereit et al., 1994; Hoot et al., 1997). Es por ello que algunos autores han considerado la existencia de cuatro familias independientes (Kadereit et al., 1994; Takhtajan, 2009), mientras que otros consideran Papaveraceae en sentido amplio compuesta por cuatro subfamilias (Thorne, 1992; Mabberley 2008), o por tres subfamilias de las que Fumarioideae incluye el género *Hypecoum* (Zhang et al., 2008).

Pteridophyllum es un género monotípico, endémico de la región de Honshu, Japón, y presenta unas características morfológicas bastante diferentes al resto de la familia (Lidén, 1993a), como la ya mencionada ausencia de laticíferos o idioblastos secretores, a la que se suma la ausencia de cristales de oxalato cálcico en la epidermis interna del tegumento más externo (Brückner, 1985). Este taxon constituye el linaje más problemático de la familia en cuanto a su afinidad filogenética se refiere, ya que ha sido propuesto como linaje hermano a *Hypecoum* formando la familia Hypecoaceae (Prantl & Kundig) Barkley (Fedde, 1936), junto a *Hypecoum* dentro de la familia Fumariaceae DC. (Cronquist, 1981), como próximo a Papaveraceae s.str. (Brückner, 1985), o incluso considerado como una familia independiente (Pteridophyllaceae (Murb) Sigiura ex Nak., Lidén 1993a). En la revisión de la familia realizada por Kadereit et al. (1994), mediante la que reconstruyeron la relaciones filogenéticas del grupo empleando información morfológica, *Pteridophyllum* fue considerado el linaje más basal de Papaveraceae y por ello utilizado como outgroup. Posteriormente, en un análisis cladístico basado tanto en rasgos morfológicos como marcadores moleculares, Hoot et al. (1997) confirmaron la posición basal del género en la familia aunque con un apoyo moderado. Recientemente, Wang et al. (2009) obtuvieron que *Pteridophyllum* es un clado hermano a *Hypecoum* y, ambos al resto de Fumarioideae, por lo que incluyen estos tres linajes en la subfamilia. Sin embargo, esta afinidad de *Pteridophyllum* e *Hypecoum* sólo es obtenida al combinar la información morfológica con la molecular, mientras que el análisis molecular independiente no resolvió la posición de *Pteridophyllum*.

Respecto a la afinidad filogenética del género *Hypecoum*, su posición como grupo hermano de Fumariaceae está ampliamente aceptada (Kadereit et al., 1994; Hoot et al., 1997; 1999). Sin embargo, su tratamiento taxonómico no ha sido siempre homogéneo puesto que en distintos trabajos se le ha considerado como familia independiente (Hypecoaceae; Kadereit et al. 1994), mientras que en otros como subfamilia Hypecoideae Prantl & Kundig dentro de Fumariaceae (Lidén, 1993b; Hoot et al., 1997). En las clasificaciones más recientes se incluye dentro de Fumarioideae (APG II, 2003; APG III, 2009) a nivel de tribu (Stevens, 2001 onwards).

Por su parte, la subfamilia Papaveroideae conforma un grupo monofilético, confirmado tanto en base a rasgos morfológicos (sépalos que envuelven la corola, estambres desordenados generalmente en número elevado y parénquima de la semilla que se extiende por encima de la

Pteridophyllum

Hypecoum

Fumarioideae

Papaveroideae

Figura 1. Fotografías de flores de cada uno de los cuatro linajes principales de Papaveraceae. Fotos de *Pteridophyllum racemosum*; *Hypecoum imberbe*; *Dicentra eximea alba* como representante de Fumarioideae y *Meconopsis dhwojii* por Papaveroideae.

calaza y el rafe; Kadereit et al., 1994) como con información molecular (Hoot et al., 1997). Las relaciones entre este grupo y el resto de Papaveraceae fueron ampliamente discutidas por los discordantes resultados obtenidos por Kadereit et al. (1994) y Loconte et al. (1995). Mientras que los primeros autores obtienen que Papaveroideae es hermano a Fumarioideae tras la posición basal de *Pteridophyllum*, Loconte et al. (1995) proponen que Papaveroideae es parafilético con respecto al resto de los linajes de la familia. Esta disparidad es explicada por Hoot et al. (1997) por la diferente elección de outgroup empleada en cada uno de los análisis, y confirma la hipótesis de Kadereit et al. (1994) con los resultados obtenidos en su análisis filogenético. Dada la carencia de reconstrucciones filogenéticas exhaustivas para Papaveraceae, hasta la fecha esta hipótesis no ha vuelto a ser contrastada.

1.2 La subfamilia Fumarioideae

En este trabajo de tesis doctoral abordamos la sistemática de la subfamilia Fumarioideae siguiendo el tratamiento propuesto para el grupo en la clasificación APG III (2009), sin

considerar la inclusión de *Pteridophyllum* en la subfamilia. De este modo, Fumarioideae es el grupo más diversificado de Papaveraceae incluyendo 20 géneros con alrededor de 590 especies, distribuidas principalmente en el Hemisferio Norte y estando también representadas en la región de Sudáfrica (Lidén, 1993a; Zhang et al., 2008). Los datos de riqueza específica y distribución, así como algunos rasgos diagnóstico de todos sus géneros son resumidos en la Tabla 1.

Tabla 1. Distribución y principales rasgos de los géneros de Fumarioideae

Taxon	Principales características	Nº especies	Distribución
Tribu Hypecoeae			
Hypecoum L.	Inflorescencias cimosas, flores bisimétricas con los pétalos externos trilobados y los internos tripartidos con la parte central espatulada y fimbriada, frutos en lomento o cápsula.	18	Asia/ Mediterráneo
Tribu Fumarieae			
Subtribu Corydalinae	Corolo bisimétrica, frutos en cápsula, estilo clorofílico		
Adlumia DC.	Inflorescencias axilares, nectario ausente.	2	Norteamérica / Siberia
Capnoides (L.)Borckh.	Corola zigomórfica, estambres completamente soldados.	1	Norteamérica
Corydalis DC.	Corola zigomórfica, nectario basalmente fusionado al espolón.	465	Hemisferio Norte
Dactylicapnos Wall.	Pétalos casi totalmente fusionados, gran nectario con horquilla central.	16	Himalaya / Indochina
Dicentra Bernhardi	Nectario pequeño y redondeado, estigma con cuatro papilas pequeñas y simples.	7	Norteamérica / Siberia / Japón
Ehrendorferia T. Fukuhara & Lidén	Flores erectas, estambres dorsalmente aladas y semillas sin eliosomas.	2	Norteamérica
Ichtyoselmis Lidén & Fukuhara	Estambres libres, gran estigma con forma de violín.	1	China / Birmania
Lamprocapnos Fukuhara	Pétalos externos con forma de bolsa, estambres petaloideos, estigma estrecho y alargado.	1	China / Korea
Subtribu Fumariinae	Corola zigomorfa, frutos con bajo número de semillas, estilo no clorofílico.		
Ceratocapnos Dur.	Presencia de zarcillos, flores pequeñas ampliamente aladas, fruto nuez monosperma o cápsula con 2-5 semillas.	3	Mediterráneo
Cryptocapnos Rech.F.	Racimos corimbosos cortamente pedunculados, fruto elipsoidal con pico excavado, monospermo.	1	Afganistán
Cysticapnos Miller	Plantas trepadoras, últimas divisiones foliares zarcillosas, flores cortamente espolonadas, fruto en cápsula.	3	Sudáfrica
Discocapnos Cham & Schldl.	Estilo persistente, fruto con forma de disco alado, monospermo.	1	Sudáfrica
Fumaria L.	Inflorescencias largamente pedunculadas, pedícelos florales cortos, fruto con dos hendiduras apicales, monospermo.	50	Mediterráneo/ India / Macaronesia
Fumariola Korsh.	Racimos corimbosos, fruto subcilíndrico con zona apical aplanada, monospermo.	1	Kazajistán
Platycapnos (DC.)Bernhardi	Racimos de flores en espiga densa, fruto aplanado, monospermo.	3	Mediterráneo / Macaronesia
Pseudofumaria Medikus	Frutos en cápsulas lineares elíptico-oblongas, células suspensoras fuertemente alargadas.	3	Alpes / Balcanes
Sarcocapnos DC.	Hojas largamente pecioladas, fruto elíptico y aplastado, fruto nuez con 1-2 semillas.	7	Península Ibérica / Marruecos
Rupicapnos Pomel	Racimos corimbosos, fruto con un pico corto y agudo, monospermo.	7	Norte de África / Sur de España
Trigonocapnos Schlechter	Racimos con gran número de flores, pedicelo floral largo, fruto trígono, alado, monospermo.	1	Sudáfrica

La subfamilia está constituida por el género *Hypecoum* por un lado, y el resto de táxones por otro, ambos tratados a nivel de tribu en Stevens (2001, onwards): tribu Hypecoeae Dumort. y tribu Fumarieae Dumort. La subfamilia se caracteriza por presentar: flores bisimétricas o zigomórficas, pétalos dimórficos, sépalos que no encierran la corola, estambres con presencia de nectario, filamento del estambre hialino y amplio, óvulo campilótropo y número cromosómico básico igual a 8 (Lidén, 1986, 1993b; Kadereit et al., 1994; Zhang et al., 2008).

La tribu Hypecoeae está definida por la presencia de hojas en roseta basal, inflorescencias cimosas en dicasio largamente pedunculadas, sépalos clorofílicos, pétalos externos ligeramente trilobados, pétalos internos fuertemente divididos en tres lóbulos, con los laterales enteros mientras que el central espatulado o fimbriado para la presentación secundaria de polen (Lidén, 1986; Dahl, 1990; Zhang et al. 2008). El androceo de la tribu Hypecoeae lo conforman cuatro estambres diteca, dos internos de mayor tamaño y con dos haces vasculares cada uno y dos externos con sólo un haz vascular (Lidén, 1986; Dahl, 1990; Kadereit et al., 1995). Sus frutos son fragmentables en tipo lomento o cápsula con muchas semillas en una fila (Dahl, 1990; Zhang et al. 1998). Esta tribu monotípica contiene 18 especies, la mayoría de ellas distribuidas en la región Mediterránea aunque algunas son endémicas del Centro de Asia y China (Zhang et al., 2008).

La tribu Fumarieae se caracteriza por presentar inflorescencias racemosas, flores espolonadas con simetría bilateral o zigomorfa, sépalos petaloideos y el androceo formado por seis estambres organizados en dos haces (con tres haces vasculares respectivamente) albergando cada uno de ellos dos anteras monoteca en posición lateral y uno diteca en posición central (Lidén, 1986; Zhang et al., 2008). Aunque la morfología del androceo distingue las dos tribus, el estudio ontogénico de su desarrollo muestra que ambas tribus comparten el mismo desarrollo temprano con seis primordios independientes de los cuales en *Hypecoum* cuatro se agrupan en parejas formando los estambres internos durante el desarrollo floral (Ronse Decraene and Smets, 1992). Fumarieae también se caracteriza por frutos en cápsula o en nuez con una o dos semillas (Lidén, 1986). Esta tribu incluye 19 géneros y en torno a 570 especies distribuidas ampliamente en el Hemisferio Norte, presentando un pequeño grupo de 3 géneros endémico de la región de Sudáfrica (Lidén, 1986) (Tabla 1).

Algunas características generales de toda la subfamilia Fumarioideae son que todos sus táxones presentan porte herbáceo, aunque existe una amplia variedad de estilos de vida y ecologías. Hay geófitos efímeros, plantas rizomatosas, zarcillosas tepradoras y casmófitos estrictos, existiendo también en la subfamilia una rica gama de ecologías que van desde zonas de sotobosque, alta montaña, suelos arenosos, regiones desérticas hasta campos abiertos de ecosistemas ruderales (Lidén, 1993b). La presencia de néctar en buena parte de las especies (Lidén, 1993b) indica la importancia de la polinización mediada por insectos en Fumarioideae, sin embargo muchas de las especies han sido reconocidas como autocompatibles y con fuertes barreras de esterilidad interespecífica (Lidén, 1986). Respecto a la dispersión de frutos y semillas, en la tribu Hypecoeae aquellas especies con cápsula lomentácea dispersan sus semillas mendiante la fragmentación en artejos (Dahl, 1989). En el caso de la tribu Fumarieae,

la mirmecocoria es uno de los principales mecanismos desarrollados para la dispersión ya que en buena parte de los táxones el fruto es una cápsula dehiscente con elaiosomas bien desarrollados en las semillas (Lidén, 1993b), así como en otros géneros de frutos indehiscentes es común la presencia de un apéndice en el ápice del fruto para la dispersión por hormigas.

Filogenia y sistemática infrasubfamiliar

La sistemática del género *Hypecoum* fue realizada por Dahl (1989; 1990), quien lo subdividió en dos subgéneros (subg. *Chiazospermun* y subg. *Hypecoum*) y agrupó sus especies en cinco secciones (*Chiazospermum* y *Leptocarpae* en el primero de los subgéneros, e *Hypecoum*, *Pendulae* y *Mnemosilla* en el segundo). En la presente tesis doctoral las relaciones intragenéricas en *Hypecoum* no fueron objeto de estudio, de modo que solamente algunos representantes del género han sido incluidos en los análisis para evaluar su relación con el resto de géneros de la subfamilia. Respecto a las relaciones filogenéticas entre las distintas especies de *Hypecoum*, hasta la fecha no se ha realizado ningún análisis molecular, así que la única hipótesis filogenética disponible es la realizada por Dahl (1990) a nivel de secciones en base a algunos de los caracteres morfológicos clave en su taxonomía.

En cuanto a la tribu Fumarieae, su sistemática se ha tratado en más detalle que la de Hypecoeae dada la profunda revisión del grupo realizada por Lidén (1986). Fumarieae está conformada por dos grupos principales (considerados a nivel de tribu por Lidén (1986), que trató el conjunto de Fumarieae a nivel de subfamilia) la tribu Corydaleae Dumort y la tribu Fumarieae. El primero de ellos incluye todos los géneros con flores bisimétricas además de *Corydalis* DC. y *Capnoides* (L.) Borckh. (con flores zigomorfas), y Fumarieae incluye el resto de géneros con flores zigomorfas. Dentro de Fumarieae, Lidén (1986) identificó tres clados principales que describió a nivel de subtribu (Discocapninae Lidén, Fumariinae y Sarcocapninae Lidén). El género *Cysticapnos* Mill. fue calificado como 'incertae sedis' debido a la existencia de caracteres morfológicos ambiguos para incluirlo en uno u otro grupo. Las relaciones entre los géneros de la tribu Corydaleae no fueron evaluadas.

Loconte et al. (1995), en su estudio filogenético sobre el orden Ranunculales proponen una hipótesis filogenética completa para toda la familia Papaveraceae basada también en un análisis cladístico con caracteres morfológicos. En dicha filogenia la tribu Corydaleae es reconocida como grupo parafilético, mientras que para la tribu Fumarieae (sensu Lidén, 1986) se confirma la monofilia de las tres subtribus descritas por Lidén (1986), así como la posición incierta del género *Cysticapnos*. Poco después, se realizó el primer análisis filogenético empleando información molecular en Fumarioideae (Lidén et al. 1997), aunque principalmente centrado en táxones de la tribu Corydaleae y con muy poca representación de la tribu Fumarieae (sensu Lidén, 1986). En dicho trabajo, donde se utilizó el intrón cloroplastidial *rps*16 como marcador molecular, se corroboró la naturaleza parafilética de Corydaleae y la pertenencia del género *Cysticapnos* a la tribu Fumarieae (sensu Lidén, 1986). Uno de los

principales resultados obtenidos en dicho estudio fue la escisión del género *Dicentra* Bernh. en cuatro géneros; *Dicentra* s.str., el rescatado género *Lamprocapnos* Endl. y dos nuevos géneros: *Ehrendorferia* T. Fukuhara & Lidén e *Ichtyoselmis* Lidén & T. Fukuhara.

Desde entonces no se han llevado a cabo nuevos estudios sobre las relaciones filogenéticas en Fumarioideae, por lo que las propuestas realizadas en base a morfología no han sido contrastadas completamente con información molecular. Sí se han elaborado reconstrucciones filogenéticas con herramientas moleculares a nivel genérico, como son el estudio sobre el género *Corydalis* realizado por Lidén et al. (1995) o el trabajo sobre la filogenia de *Sarcocapnos* llevado a cabo por Salinas et al. (2003), que en ambos casos emplean el marcador nuclear de ADN ribosómico ITS ('internal transcribed spacer'). Sin embargo, la necesidad de una hipótesis filogenética completa para toda la subfamilia Fumarioideae se hace cada vez más evidente.

En este sentido, Fukuhara (1999), en su estudio sobre la evolución del funículo y semilla de once géneros de Fumarioideae, se vio obligado a diseñar una topología combinando los resultados de filogenia molecular disponibles para algunos táxones y los de filogenia morfológica para otros, con el fin de polarizar los cambios de estado en los caracteres observados. Recientemente, Fumarioideae se ha convertido en un grupo modelo para el estudio de la evolución de los órganos florales, como demuestra el considerable número de trabajos publicados en los últimos años sobre la morfología floral tanto de Papaveraceae (Kölsch and Gleissberg, 2006; Damerval and Nadot, 2007) como de Fumarioideae (Hidalgo et al., 2012; Damerval et al., 2013). No obstante, la carencia de hipótesis filogenéticas imposibilita una reconstrucción completa de los procesos identificados sobre el patrón evolutivo de la subfamilia. Por todo ello, el desarrollo de un análisis filogenético exhaustivo en este trabajo de tesis doctoral permitirá eliminar las carencias que actualmente existen en la sistemática y filogenia de la subfamilia.

1.3 Biogeografía de Fumarioideae

Como se ha mencionado, la subfamilia Fumarioideae está distribuida en el Hemisferio Norte, alcanzando también la región de Sudáfrica (Tabla 1). La distribución actual de sus géneros y especies presenta un conjunto de disyunciones biogeográficas que convierten a la subfamilia en un modelo interesante para el estudio de patrones de distribución. Dichas disyunciones presentes en la subfamilia pueden resumirse en tres grupos: a) Hemisferio Norte/Sudáfrica; b) Este de Asia/Norte América; y c) disyunciones de la región Mediterránea. El primer grupo es el caso de la subtribu Discocapninae sensu Lidén (1986), único grupo endémico en esa región de toda la subfamilia. Dada la excepcionalidad de la flora Sudafricana, los patrones biogeográficos de táxones Sudafricanos son cuestiones intensamente estudiadas (Linder, 2005; van der Niet and Johnson, 2009; Britton et al., 2014). Sin embargo, el caso particular de Discocapninae (sensu Lidén, 1986) no ha sido evaluado. Respecto al segundo grupo, existen varios géneros y especies con distribución disyunta entre el Este de Asia y Oeste-Este de Norte América (Este

de Asia: *Ichtyoselmis*, *Lamprocapnos*, *Adlumia asiática* Ohwi, *Corydalis*, *Dicentra peregrina* (Rudolph) Makino / Norte América: *Dicentra*, *Ehrendorferia*, *Adlumia fungosa* (Ait.) Britton, Sterns & Poggenb., *Corydalis* secciones *Archaeocapnos* Popov ex Michailova, *Dactylotuber* (Ruprecht) Popov in Schischkin y *Sophorocapnos* (Turcz.) Popov in Schischkin, resto especies *Dicentra*). Un amplio número de trabajos ha abordado el estudio de este tipo de disyunción en el Hemisferio Norte, tanto desde un enfoque general con la finalidad de describir los principales patrones de distribución o identificar los procesos implicados (Xiang et al., 1998; Wen 1999; Xiang et al., 2000; Milne, 2006; Wen et al., 2010; Kadereit and Baldwin 2012; Brikiatis, 2014), como desde un enfoque específico mediante estudios centrados en táxones presentes en ambas regiones (Bastida et al., 2010; Xie et al., 2010; Fior et al., 2013; Huang et al., 2013; Chin et al., 2014). La reconstrucción de la historia biogeográfica de la subfamilia Fumarioideae puede ayudar a resolver algunas de las cuestiones relacionadas con dichos procesos. El tercer grupo incluye a los representantes de la subfamilia presentes en la región mediterránea, de donde son endémicos 6 géneros con alrededor de 65 especies, mostrando disyunciones dispares (Sur/Norte del Mediterráneo; Oeste/Este del Mediterráneo; etc). La diversificación de la flora mediterránea también es una temática que despierta mucho interés en el ámbito de la biogeografía, como así demuestra el amplio número de trabajos que se han dedicado a su estudio (Mansion et al., 2008; 2009; Mao et al., 2010; Salvo et al., 2010; Ali et al., 2012; Barres et al., 2013; Fiz-Palacios and Valcárcel. 2013; Inda et al., 2014; Manafzadeh et al., 2014). El estudio de los patrones reflejados por los táxones mediterráneos de la subfamilia puede aportar nueva información sobre los factores y eventos que han ocasionado la elevada biodiversidad presente en el Mediterráneo.

Hasta la fecha, los trabajos que han estudiado aspectos sobre la biogeografía de la subfamilia son escasos (Lidén, 1986; Zhuang et al., 1993; Kadereit et al., 1995). Uno de ellos aborda ligeramente la biogeografía de la tribu Fumarieae (sensu Lidén, 1986), dedicando un pequeño apartado sobre fitogeografía en una revisión general de la sistemática de dicha tribu (Lidén, 1986). En el resto de trabajos se analizan los patrones de distribución de la subfamilia de forma conjunta al resto de la familia Papaveraceae, pero sin emplear ninguna herramienta específica destinada al análisis de cuestiones biogeográficas (Zhuang et al., 1993; Kadereit et al., 1995). En este sentido, el desarrollo de una filogenia completa para la subfamilia Fumarioideae facilitará el desarrollo de trabajos que estudien los patrones de distribución seguidos por sus linajes durante la evolución del grupo.

1.4 Estudio del grano de polen y su aplicación en sistemática

La palinología es una de las ramas más desarrolladas dentro de la botánica debido a la amplia gama de disciplinas que ha generado (Blackmore, 2007; Hesse et al., 2009). Una de las aplicaciones más antigua y extendida de la palinología es la utilización de los caracteres morfológicos presentes en el grano de polen para el tratamiento de cuestiones sistemáticas (Le Thomas, 1980; 1981; Linder and Ferguson, 1985; Zavada and Dilcher, 1986). Los rasgos polínicos han resultados muy útiles para la actual clasificación de las angiospermas, ya que el número y posición de aperturas permitió definir el grupo de las tricolpadas o eudicotiledóneas (Donoghue and Doyle, 1989; Doyle and Hotton, 1991). Poco después, diferentes filogenias moleculares confirmaban la naturaleza monofilética de dicho grupo (Chase et al., 1993; Qiu et al., 1999; Soltis et al., 2000; Hilu et al., 2003). La integración o contraste de la información polínica con los resultados de análisis cladísticos es una práctica extendida en muchos trabajos orientados a la clarificación de la sistemática en distintos grupos de plantas (Urtubey and Tellería, 1998; Hoot et al., 2009; van der Weide and van der Ham, 2011). Las características más empleadas para esa finalidad son aquellas relacionadas con la morfología externa del grano de polen, especialmente el sistema apertural y la ornamentación de la exina (Blackmore et al., 1995; Kosenko, 1999; Heo et al., 2011; Xie and Li, 2012). Aunque con menor frecuencia, los caracteres relacionados con la información ultraestructural de las distintas capas que constituyen la pared del polen también son incluidos en los estudios sobre sistemática (Cooper et al., 2000; Simpson et al., 2003; Remizowa et al., 2008; Chen and Xia, 2011).

A pesar del alto nivel de desarrollo alcanzado en palinología, del amplio número de trabajos destinados a la descripción de la diversidad polínica y de la gran cantidad de información disponible, el proceso de formación y desarrollo del grano de polen es un fenómeno que todavía presenta lagunas y requiere más investigación (Hesse et al., 2009). En este sentido, el estudio de las distintas capas de la pared de polen y de las aperturas durante su ontogenia permite avanzar en el conocimiento de los procesos implicados en el desarrollo polínico. La observación de los caracteres ultraestructurales durante la maduración del grano de polen ha permitido, además de reflejar la existencia de diversidad ultraestructural dentro de una misma familia (Nashri-Ayachi and Nabli, 2006; Xu and RonsedeCraene, 2013), identificar mecanismos básicos implicados en el desarrollo de la pared y aperturas polínicas (Furness and Rudall, 2004; Gavarayeva and Hemsley, 2006).

1.5 Ultrastructura de la pared del polen

La pared del grano de polen tiene una estructura multiestratificada con dos capas principales, la exina y la intina (Figura 2; Punt et al., 2007; Hesse et al., 2009). La intina es una capa homogénea compuesta principalmente por celulosa y pectina, e interviene en el proceso de germinación al envolver el tubo polínico durante su desarrollo (Hesse et al., 2009). La

presencia y grosor de la intina pueden ofrecer información sobre el grado de madurez del grano de polen. En algunas ocasiones, en la intina pueden diferenciarse diferentes estratificaciones o capas, distinguibles por su tonalidad. Cuando esto ocurre, lo más habitual es la presencia de dos estratos claramente discernibles: la endintina y la exintina (Kress and Stone, 1982). En otras ocasiones una mayor estratificación es posible, como la división en tres capas (intina 1, 2 y 3, o intina externa, media o interna) que ha sido descrita en las zonas aperturales de diferentes táxones (Marquez et al., 1997; Vega-Maray et al., 2003).

A diferencia de la intina, la exina es una capa heterogénea con mayor diversidad de estructuras; en ella se diferencian dos estratos principales: la endexina, capa más interna, y la ectexina, capa más externa (Punt et al., 2007; Hesse et al., 2009). El nivel de desarrollo de la endexina varía durante la ontogenia del grano de polen, mostrando su máximo crecimiento durante los estadíos intermedios de maduración y adelgazándose a medida que se alcanza la madurez. Posiblemente por estar implicada en el transporte y migración de sustancias a través de la pared (Rowley, 1995). Respecto a la ectexina, aunque también implicada en la transferencia de sustancias, posee una función protectora tras la liberación del grano de polen (Payne, 1972). Esta parte más externa de la pared polínica se compone de tres capas, la base ('foot layer'), el infratéctum y el téctum (Punt et al., 2007; Hesse et al., 2009). La primera de ellas es la capa más interna y por ello puede estar en contacto con el citoplasma, la endexina o la intina. De hecho, en algunos casos no es fácil diferenciar la 'foot layer' de la endexina, conformando una capa homogénea conocida como nexina (Erdmant, 1952). La segunda, el infratéctum, es la capa intermedia y presenta una morfología discontinua con huecos que tradicionalmente ha sido clasificada en alveolar, granular y columelar, siendo el primer tipo característico de algunos grupos de gimnospermas, el segundo estando en algunas gimnospermas y angiospermas, y el tercero el más abundante en angiospermas (Doyle, 2009). La parte más externa de la ectexina, el téctum, es la capa que confiere el aspecto externo al grano de polen. En muchos casos, el téctum está cubierto por elementos supratectales que conforman la escultura (espinas, etc) y el conjunto (rasgos de la estructura y la escultura), constituye la ornamentación de la exina (Hesse et al., 2009). Esta última característica de la pared del polen, la ornamentación, es el rasgo más fácilmente observable y por ello el más ampliamente estudiado.

Todas las capas de la exina están compuestas de esporopolenina, sustancia con una enorme estabilidad química, y por ello muy resistente, que permite que el grano de polen pueda dispersarse a grandes distancias durante prolongados períodos de tiempo sin degradarse (Hesse et al., 2009). Este biopolímero, además de caracterizar la pared del polen, está presente en la pared de las esporas de hongos, algas, musgos y helechos. La esporopolenina está principalmente constituida por una mezcla de polihidroxilados, de ácidos grasos de larga cadena y de compuestos orgánicos oxigenados, aunque ni su composición exacta ni su síntesis se conocen por completo (Wallace et al., 2011; Kim and Douglas, 2013).

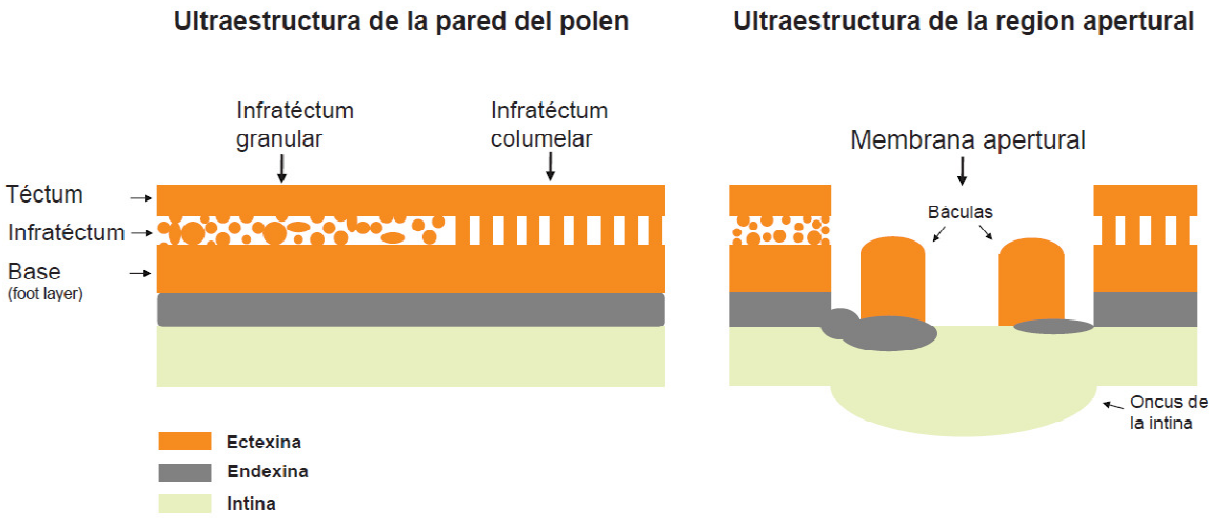


Figura 2. Ilustración de la ultrastructura de la pared de polen y región apertural. A la izquierda se muestran las dos morfologías de la capa de infratéctum más frecuentes en el polen de angiospermas. A la derecha se reproduce los elementos que constituyen las regiones aperturales.

La exina, además de su función protectora frente a agentes externos, también realiza una función de protección sobre el grano de polen a través del proceso conocido como harmomegatia (Payne, 1972). Dicho mecanismo consiste en la contracción y expansión del grano de polen para adaptarse a los ciclos de hidratación y deshidratación que sufrirá tras salir de la antera. Hasta su liberación, el grano de polen se encuentra en condiciones óptimas de humedad en el lóculo de la antera, pero una vez expuesto a las condiciones atmosféricas, es inevitable la desecación del contenido citoplasmático del mismo. La harmomegatia minimiza las afecciones derivadas de dicha desecación y evita daños irreversibles en el gametofito masculino contenido en el grano de polen (Hesse et al., 2009). Las modificaciones de contracción y expansión del grano de polen tienen lugar principalmente gracias a las regiones aperturales, ya que las aperturas se doblan hacia dentro para proteger el citoplasma y reducir la pérdida de agua (Katifori et al., 2010). Aunque en un principio se propuso que la harmomegatia está principalmente condicionada por la forma y disposición aperturales (Payne, 1972), poco después se comprobó que la exina también actúa de forma activa en el proceso (Suárez-Cervera and Seoane-Camba, 1986).

Desde el punto de vista de su aplicación en sistemática es la exina la capa de la pared del polen que proporciona un mayor número de caracteres comparables entre táxones, debido principalmente a la estratificación de la ectexina.

1.6 Aperturas

La morfología presente en un grano de polen también está fuertemente condicionada por la presencia, número y distribución de aperturas. Existen dos tipos de aperturas: los poros, que son aperturas circulares o elípticas de pequeño tamaño; y los colpos, o aperturas alargadas (Punt et al., 2007; Hesse et al., 2009). La coexistencia de ambos tipos de aperturas también es posible, denominándose dicha apertura como colporada. La configuración apertural (número, posición y orientación de las aperturas) es un carácter determinado directamente por el tipo de citocinesis que tiene lugar durante la formación de las microsporas (Furness and Rudall, 2004). Existen dos tipos de microsporegénesis: la sucesiva y la simúltanea; esta última tiene relevancia sistemática ya que está extendida en la mayoría de eudicotiledóneas, resultando en el establecimiento de un eje polar en el grano de polen y en la presencia de tres aperturas con disposición ecuatorial (Furness and Rudall, 2004; Hesse et al., 2009).

Respecto a su ultraestructura la zona apertural es una región muy compleja de la pared del polen ya que suele presentar capa de intina en los estadíos avanzados de maduración, capa de endexina muy desarrollada durante los periodos jóvenes (persistiendo en la madurez en algunos táxones), y restos de la ectexina que constituyen la membrana apertural (Figura 2; Hepslop-Harrison 1963; Simpson, 1985; Fernández and Rodríguez García, 1995).

En todos los casos, la función de la apertura es facilitar la germinación del gametofito masculino durante la fecundación. Sin embargo, desde el punto de vista ontogénico, la apertura del grano de polen también desempeña una función como zona de transferencia de sustancias durante la maduración del mismo (Blackmore and Crane, 1998). Así, las aperturas conjugan las funciones antagónicas de exponer al exterior el contenido de la célula polínica, reducir la pérdida de agua (harmomegatia) y al mismo tiempo facilitar la absorción de la misma una vez el grano ha llegado a medios húmedos.

Como se ha mencionado, el número de aperturas es un importante rasgo para la configuración de la morfología polínica. Junto con el tipo apertural y la ornamentación del téctum, el número y disposición de las aperturas define la apariencia que tendrá el grano de polen una vez maduro, y en base a la cual se definen los tipos polínicos.

1.7 Ontogenia de la pared del polen en angiospermas

La estructuración en capas de la pared de polen descrita en los epígrafes previos sigue la terminología estándar aceptada por la comunidad palinológica internacional (Punt et al., 2007). Del mismo modo existe un modelo general para el tratamiento de los distintos estadíos en los que se produce la maduración del grano de polen. Owen and Makaroff (1995), en una exhaustiva descripción del proceso de desarrollo del polen en *Arabidopsis thaliana* (L) Heynh., establecen un conjunto de doce estadíos de desarrollo reflejando los principales procesos observados durante la maduración del polen, y que resumimos a continuación:

Fase premeiosis I. Esta fase inicial contiene los microesporocitos en el centro de la antera, rodeados por el tapete, la capa intermedia, el endotecio y la epidermis. Todos los microesporocitos presentan un núcleo grande con un gran nucleolo y están conectados por plasmodesmos.

Fase premeiosis II. En este estadío la célula de cada microesporocito aumenta su tamaño y se comienza a depositar la calosa a su alrededor. La deposición de calosa sella los plasmodesmos aunque los microesporocitos siguen conectados por canales citoplasmáticos.

Fase meiosis. El grosor de la capa de calosa aumenta mientras la división meiótica tiene lugar, quedando el microesporocito constituido por cuatro núcleos haploides. Cada uno de los cuatro núcleos se posiciona en la periferia de la célula con una distribución tetraédrica. Pequeñas cantidades de calosa se depositan entre los núcleos del microesporocito.

Fase tétrada. Tras la citocinesis las cuatro microsporas haploides se disponen de forma tetraédrica constituyendo la tétrada y permaneciendo unidas por la calosa del microesporocito. En la pared de cada microspora se acumula la matriz de primexina sobre la que se deposita la primexina, que se constituye por probáculas que ya reflejan la escultura que el grano de polen tendrá en su estadío maduro.

Fase de microspora recién liberada o joven. Cada una de las células de la tétrada se han separado y permanecen independientes. Las capas de la exina empiezan a ser discernibles mediante la deposición de esporopolenina y a partir de este momento de desarrollo la ornamentación del téctum se empieza a definir.

Fase microspora vacuolada. Las células incrementan su tamaño y el contenido citoplasmático se llena de gran cantidad de vacuolas que se fusionan en una única vacuola de gran tamaño que ocupa gran parte del volumen de la célula. En esta fase la estructuración de la exina está completamente definida y es el momento de mayor nivel de desarrollo de la capa de endexina.

Fase de polen bicelular joven. La fase previa finaliza cuando tiene lugar la división mitótica en la que se originan la célula vegetativa y la célula generativa, ocupando esta última un lugar marginal junto a la pared celular y estando rodeada por su propia pared celular. La capa de intina se desarrolla durante este estadío bajo la pared polínica, presentando por ello un grosor delgado.

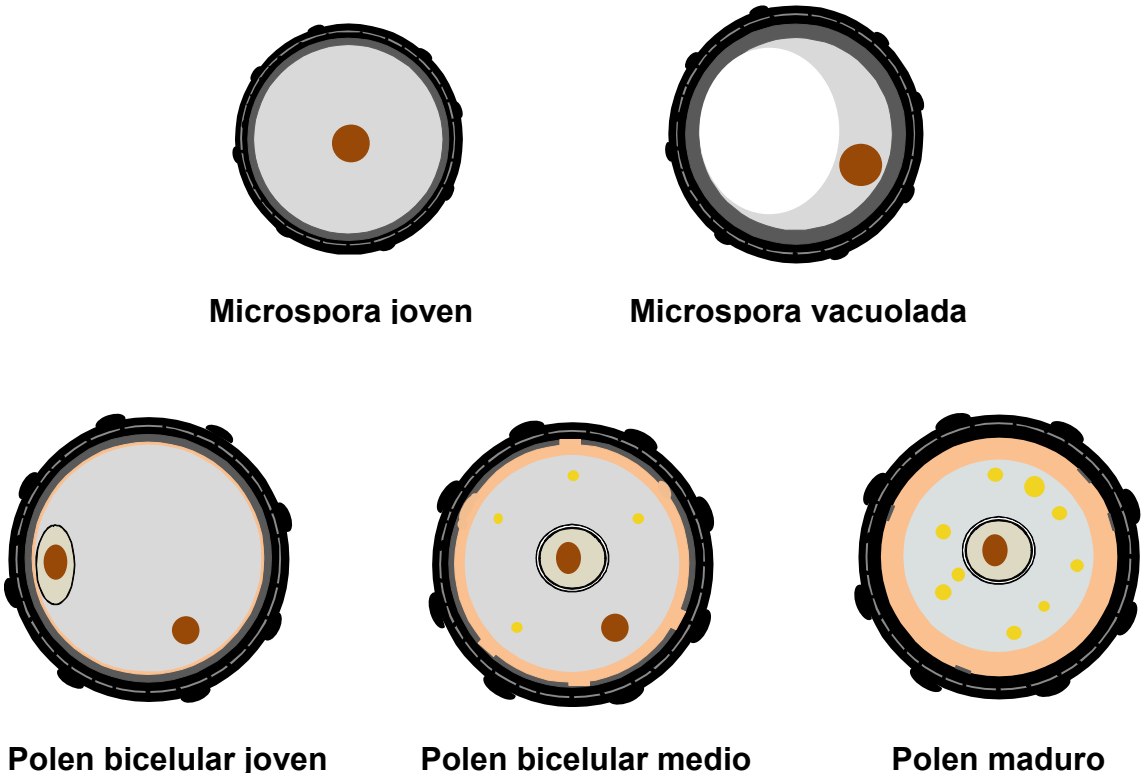


Figura 3. Ilustración de los cinco últimos estadíos en la ontogenia del grano de polen. La ectexina está representada en color negro, la capa de endexina en gris oscuro y la capa de intina en naranja. Durante el estadío de microspora vacuolada se reproduce la presencia de una vacuola de gran tamaño en color blanco, durante los estadíos de polen bicelular medio y polen maduro la presencia de acúmulos de almidón se ilustra en color amarillo. La ornamentación de la exina se reproduce en la parte externa de la pared mediante la ilustración de prolongaciones del téctum.

Fase de polen bicelular medio. Durante este estadío comienza la acumulación de lípidos y almidón en el interior celular, mientras que en la pared la capa de intina aumenta su grosor y la endexina se hace cada vez más delgada llegando a fragmentarse.

Fase de polen maduro. Es la fase final del proceso de desarrollo del grano de polen. En ella el contenido citoplasmático está principalmente formado por gran cantidad de almidón y lípidos, la capa de intina alcanza un tamaño igual o mayor que la capa de exina y en la parte externa de la pared se acumula el 'polenkitt', sustancia que ayuda a la resistencia frente a desecación de la pared del grano de polen y facilita el transporte en especies zoófilas.

En este trabajo de tesis doctoral empleamos los cinco estadíos finales de este resumen sobre el modelo general ontogénico en angiospermas. Dado que la morfología de la exina y sus distintas capas se comienza a desarrollar a partir de los estadíos de microspora recién liberada (Owen and Makaroff, 1995), hemos seleccionado esos cinco estadíos con la finalidad de observar y comparar los caracteres ultrastructurales de la pared del polen (Figura 3).

1.8 Caracteres polínicos en Fumarioideae

El estudio de los caracteres polínicos de Fumarioideae ha sido una tarea a la que se ha dedicado un amplio número de trabajos, la gran mayoría centrados en el análisis de la morfología externa del grano de polen. Uno de los primeros es el trabajo de Stern (1962), centrado en la morfología polínica del antiguo género *Dicentra* (incluyendo *Dactylicapnos*, *Ehrendorferia*, *Ichtyoselmis* y *Lamprocapnos*). Este trabajo, junto a la descripción de morfología polínica para algunas especies andaluzas de Fumariaceae realizada por Candau y Soler (1981), son los únicos que emplean exclusivamente representantes de Fumarioideae ya que el resto analizan el polen de este grupo en trabajos más amplios incluyendo a representantes de Papaveraceae o del orden Ranunculales. Rachele (1974) estudió la morfología polínica de los táxones norte americanos de Papaveraceae, así como poco después Layka (1976) presentó un trabajo más amplio de descripción polínica para Papaveraceae. En la 'Flora polínica del noroeste de Europa' Kalis (1979) presentó sus resultados sobre morfología del polen de representantes de Papaveraceae, incluyendo algunas imágenes a microscopía electrónica de barrido (MEB) de la ultrastructura de la pared del polen. Candau (1987) elaboró la descripción morfológica de la mayoría de taxones de Fumariaceae y Papaveraceae en Andalucía. Por otra parte, Blackmore et al. (1995) realizaron un análisis polínico para el orden Ranunculales e incluyeron también representantes de Papaveraceae; los autores discutieron el valor filogenético de los caracteres analizados así como su evolución para el conjunto de familias que componen dicho orden (Blackmore et al., 1995).

Como resultado del desarrollo de todos estos trabajos, los tipos polínicos y morfología de Fumarioideae son bien conocidos, siendo éste un grupo euripalino con mayor presencia de apertura tipo colpo, pero también con representantes porados. El número general de aperturas es tres o derivado, existiendo algunos casos en los que las aperturas están soldadas entre sí. Respecto a la ornamentación de la exina, Fumarioideae se caracteriza por presentar principalmente superficies de tipo psilado y verrugado. El género *Hypecoum* es una excepción puesto que presenta dos colpos fusionados con disposición ecuatorial en el grano de polen, así como ornamentación puntiforme (Dahl, 1989; 1990; Blackmore et al., 1995).

Aunque el grado de conocimiento de la morfología polínica de Fumarioideae se puede considerar muy amplio, existen varios géneros de Fumarioideae cuyos tipos polínicos no se han descrito, por lo que la información disponible para la subfamilia permanece incompleta.

Por otra parte, los datos sobre información ultraestructural de las distintas capas de la pared del polen en Fumarioidae son muy escasos. Sólo existen dos trabajos que describen la morfología interna de la pared de polen y apertura en la subfamilia, uno de ellos en *Fumaria densiflora* DC. (Romero y Fernández, 2000) y el otro en *Hypecoum imberbe* Sm. (Romero et al., 2003). Ambos trabajos abordaron la información ultrastructural durante varias fases del proceso ontogénico de maduración de la pared del polen. La comparación de los resultados de ambas investigaciones refleja la existencia de grandes diferencias morfológicas en los caracteres ultrastructurales de pared y aperturas, así como en los procesos observados

durante su desarrollo. El polen de *Fumaria densiflora* se caracteriza por un téctum muy desarrollado, infratéctum granular muy delgado y presencia de múltiples lamelaciones en la 'foot layer' y endexina durante los estadíos de microspora joven y vacuolada. Por su parte, *Hypecoum imberbe* presenta téctum equinado, infratéctum con estructura columelar y una 'foot layer' muy delgada con una única lamelación presente sólo durante microspora. Las aperturas de *Fumaria* L. presentan una cobertura protectora compuesta de un material algodonoso denominado 'fluffy material', que está ausente en *Hypecoum*. Completar la información ultraestructural y ontogénica para el conjunto de la subfamilia permitirá conocer la diversidad morfológica de todo el grupo y posibilitará la inclusión de estos rasgos ultraestructurales en el estudio de la sistemática de Fumarioideae.

2. Objetivos

2. Objetivos de la presente tesis doctoral

Como se ha mostrado en la introducción, el eje vertebrador de este trabajo de tesis doctoral es la sistemática de Fumarioideae, subfamilia que se presenta como un grupo muy interesante para el estudio de cuestiones evolutivas tanto por su posición sistemática como por su diversidad morfológica y biogeográfica. La carencia de una hipótesis filogenética exhaustiva para esta subfamilia limita el desarrollo de estudios evolutivos en el conjunto del grupo. El rápido desarrollo de la biología molecular y el perfeccionamiento de las técnicas de inferencia filogenética permiten subsanar dicha carencia. Por su parte, el estudio de la morfología y ultraestructura polínicas también proporciona herramientas con las que evaluar la diversidad existente en Fumarioideae, al mismo tiempo que la incorporación de la ontogenia polínica permite integrar en la compresión de dicha diversidad aquellos procesos implicados en la maduración del polen. Con todo ello, para profundizar en la sistemática y evolución de dicha subfamilia se articula esta tesis doctoral en base a los siguientes objetivos:

1.- Obtener una hipótesis filogenética para toda la subfamilia Fumarioideae incluyendo representantes de todos sus géneros y mediante el empleo de caracteres moleculares del ADN cloroplastidial y del ADN ribosómico. Dicha hipótesis filogenética será empleada para desarrollar diferentes estudios evolutivos en la subfamilia: i) reconstrucción de estados ancestrales para algunos rasgos morfológicos, ii) estimación de tiempos de divergencia entre sus linajes, y iii) reconstrucción de la historia biogeográfica de sus táxones.

2.- Describir la diversidad existente en la morfología polínica en la tribu Fumarieae, haciendo énfasis en los rasgos ultrastructurales presentes en las distintas capas que constituyen la pared del polen y en las aperturas durante su proceso ontogénico. De este modo se completará la información disponible sobre morfología polínica y ultrastructura para toda la subfamilia. Esa información será comparada en conjunto con los conocimientos generales sobre desarrollo del polen en angiospermas.

3.- Investigar la evolución de los caracteres morfológicos y ultrastructurales de la pared y apertura polínicas a lo largo de los linajes de la subfamilia Fumarioideae, evaluando el grado de información filogenética retenida por dichos caracteres y describiendo las principales tendencias evolutivas observadas entre sus estados.

3. Fumarioideae phylogeny 1

3. Phylogeny of the tribe Fumarieae (Papaveraceae s.l.) based on chloroplast and nuclear DNA sequences: evolutionary and biogeographic implications

Miguel A. Pérez-Gutiérrez, Ana T. Romero-García, María J. Salinas-Bonillo, G. Blanca, M. Carmen Fernández and Víctor N. Suárez-Santiago

Manuscript published in *American Journal of Botany*

American Journal of Botany

American Journal of Botany 99(3): 517–528. 2012.

Phylogeny of the tribe Fumarieae (Papaveraceae s.l.) based on chloroplast and nuclear DNA sequences: Evolutionary and biogeographic implications[1]

Miguel A. Pérez-Gutiérrez[2], Ana T. Romero-García[2], María J. Salinas[3], Gabriel Blanca[2], M. Carmen Fernández[4], and Víctor N. Suárez-Santiago[1,5]

[2]Departamento de Botánica, Facultad de Ciencias, Universidad de Granada, c/ Severo Ochoa, s/n 18071 Granada, Spain; [3]Departamento de Biología Vegetal y Ecología, Universidad de Almería, c/ Carretera de Sacramento, s/n 04120 Almería, Spain; and [4]Departamento de Biología Celular, Facultad de Ciencias, Universidad de Granada, c/ Severo Ochoa, s/n 18071 Granada, Spain.

- *Premise of the Study:* Little research has been done at the molecular level on the tribe Fumarieae (Papaveraceae). Papaveraceae is a model plant group for studying evolutionary patterns despite the lack of a reference phylogeny for this tribe. We investigated the phylogenetic relationships within the tribe to complete the molecular data for this family in order to help understand its character evolution and biogeographic pattern.
- *Methods:* We used maximum-parsimony and Bayesian approaches to analyze five DNA regions for 25 species representing 10 of the 11 Fumarieae genera and five outgroups. Evolutionary pathways of four characters (habit, life span, type of fruit, and number of seeds per fruit) were inferred on the phylogeny using parsimony. The ancestral distribution areas were reconstructed using dispersal–vicariance analysis.
- *Key Results:* Fumarieae is monophyletic and includes three groups that agree with the morphology-based subtribes: Discocapninae, Fumariinae, and Sarcocapninae. Within subtribes, the relationships among genera were different from those obtained with morphological data. Annual life span, nonchasmophytic habit, and a several-seeded capsule were the basal character states for the tribe. The ancestor occupied a continuous area between West Eurasia and Africa. Vicariances explain the divergence between lineages Discocapninae (South Africa) and Fumariinae–Sarcocapninae (Mediterranean), and the disjunction of Fumariinae (Mediterranean–Central Asia).
- *Conclusions:* Molecular phylogeny confirms the subtribal classification of Fumarieae based on morphology. However it provides different results regarding the relationships among genera within each subtribe, which affects the inference of the evolutionary pathway followed by the four selected characters. The disjunct distribution of the tribe is explained by different vicariance scenarios.

Key words: Biogeography; molecular phylogeny; morphological evolution; tribe Fumarieae.

In the evolutionary analysis of morphological characters, it is important to have a reference phylogeny on which to establish the evolutionary paths. The family Papaveraceae Juss., at the base of the Eudicotyledoneae, has been taken as a model group in this kind of study. However, the phylogeny of this family is incomplete: there are no detailed analyses of the subfamily Fumarioideae Eaton and, in particular, of the tribe Fumarieae.

Fumarieae accommodates 11 genera and 80 species (Lidén, 1993; Manning et al., 2009) within a distribution area that extends from the Macaronesian region (Azores, Canary Islands, Cape Verde, and Madeira) as far as Afghanistan, with a considerable concentration in the Mediterranean basin (Lidén, 1986). One species, *Fumaria abyssinica* Hammar, can be found in East Africa, and three genera (*Cysticapnos* Mill., *Discocapnos* Cham. & Schltdl., and *Trigonocapnos* Schltr.) are distributed in South Africa. Biogeographically, most Fumarieae genera are narrowly distributed endemics, and there is a strong disjunction (Mediterranean–South Africa–Central Asia).

The systematics of the subfamily Fumarioideae are not clear, and there is an important taxonomic and nomenclatural confusion in the taxonomic categories at the infrasubfamiliar level. Lidén (1986) proposed two tribes, Corydaleae Rchb. and Fumarieae. He considered the genus *Hypecoum* L. at subfamily level within Papaveraceae s.l. Later, when he treated Fumariaceae as an independent family, he maintained *Hypecoum* as a subfamily within Fumariaceae (Lidén, 1993). In further research, Lidén et al. (1997) found Corydaleae to be nonmonophyletic in a phylogenetic analysis using chloroplast DNA (intron of the gene *rps16*), but no systematic restructuring was made at the tribal level. Wang et al. (2009) recently analyzed Ranunculales and found that *Hypecoum* was a sister clade of *Pteridophyllum* Siebold & Zucc. (until then *Pteridophyllum* had been considered the earliest-diverging lineage of Papaveraceae s.l.), and that both genera were related to the Fumarioideae taxa. The latter relationship is doubtful, however, and needs more exhaustive study, because there may have been a sampling bias (Wang et al., 2009) and the relationship appears

[1]Manuscript received 29 July 2011; revision accepted 21 December 2011.

For assistance in location and provision of much of the plant material used in the study, the authors thank D. De Meyere and F. Van Belle, National Botanic Garden of Belgium; R. Wilford, Royal Botanic Gardens, Kew; H. Zetterlund, Gothenburg Botanical Garden; E. Vitek, Naturhistoriches Museum Wien; the staff of the herbarium of the University of Granada; and M. Lidén of the University of Uppsala. We are also grateful to N. Walkington for assistance with English usage in the manuscript. Financial support from the Spanish Ministry of Science and Innovation (Project CGL2008-01554/BOS) is gratefully acknowledged.

[5]Author for correspondence (e-mail: vsuarez@ugr.es)

doi:10.3732/ajb.1100374

only when morphological data are combined with molecular data. Moreover, one of the two synapomorphies supporting the relationship between the group *Pteridophyllum–Hypecoum* and the Fumarioideae, spinose exine sculpturing, is wrong (spinose tectum is characteristic of Papaveroideae; Romero et al., 2003; personal observation). Wang et al. (2009) propose the subfamily composed of two tribes, Hypecoideae (*Hypecoum* and *Pteridophyllum*) and Fumarieae, including all taxa of Fumarioideae *sensu* Lidén (1986) without specifying any infratribe categories. In view of the aforementioned taxonomic and nomenclatural ambiguities, we decided to follow the scheme proposed by Lidén (1986).

According to Lidén (1986, 1993), unlike the other tribe of the subfamily (Corydaleae), Fumarieae is morphologically characterized by a caducous style without conspicuously flattened stigma, fruits that are mostly nuts or few-seeded capsules, and seeds without an elaisome. Species in Fumarieae are grouped on the basis of morphological characters into three subtribes (Lidén, 1986): Fumariinae, Sarcocapninae Lidén, and Discocapninae Lidén. The major differences between the subtribes are to be found in their fruits and stigmas. Thus, Fumariinae includes those genera (*Cryptocapnos* Rech.f., *Fumaria* L., *Fumariola* Korsch., and *Rupicapnos* Pomel) with a hard-walled, one-seeded nutlet, stigma with two large papillae, pantoporate pollen, and a peculiar seed coat anatomy (Lidén, 1986; Fukuhara and Lidén, 1995b); Sarcocapninae comprises genera (*Ceratocapnos* Dur., *Platycapnos* (DC.) Bernh., *Pseudofumaria* Medik., and *Sarcocapnos* DC.) with fruits that have a sterile apex and a papyraceous endocarp (except *Pseudofumaria*), stigma with a dentate ridge or apical appendices and small papillae, and three vesicular suspensor cells (except *Platycapnos*) in the embryo; and Discocapninae includes two of the three South African genera (*Discocapnos* and *Trigonocapnos*) with tendrilliferous leaves, monosperm and not-woody endocarp fruits, stigma with two small papillae, a triangular spur on the upper petal, and a prominent triangular wing on the inner petals. Genus *Cysticapnos* was considered as *incertae sedis* by Lidén (1986, 1993) because of its different morphological affinities with the aforementioned subtribes and even with the tribe Corydaleae. This genus is characterized by tendrilliferous leaves (as in Discocapninae), many-seeded capsules (as in Corydaleae), seeds without elaiosomes (as in Fumarieae), and stigmas that are deeply cleft or have two simple papillae.

Morphological variation within Fumarieae is well documented (e.g., Lidén, 1986, 1993; Loconte et al., 1995; Lidén et al., 1997; Manning et al., 2009). Morphological data have been used as a basis for studying the phylogenetic relationships between the different genera using cladistic methods, and the monophyly of the three subtribes has been asserted (Lidén, 1986; Loconte et al., 1995). The phylogeny by Loconte et al. (1995) showed the genus *Cysticapnos* to be the sister group of the clade formed by the three subtribes. Lidén et al. (1997) included *Cysticapnos* in Fumarieae (without establishing the subtribal affinities) on the basis of their analysis of the chloroplast sequences of the intron of the *rps16* gene. Recently, Manning et al. (2009) included the genus *Cysticapnos* in Discocapninae on the basis of an unpublished preliminary molecular analysis.

Only three phylogenetic studies using DNA sequence data have been performed on the subfamily Fumarioideae. Two of them focused partially on the tribe Corydaleae (Lidén et al., 1995, phylogeny of *Corydalis* DC.; Lidén et al., 1997, phylogeny of *Dicentra* and related genera); only one dealt with the tribe Fumarieae, although it was centered on the genus *Sarcocapnos* (Salinas et al., 2003). There are therefore no molecular studies about the relationships among the genera in Fumarieae and among its three subtribes, and the lack of a reference phylogeny means that researchers cannot examine the evolutionary pattern of morphological characters (e.g., seed and funicle morphology in Fumariaceae; Fukuhara, 1999), floral symmetry in Papaveraceae s.l., and the genes involved (Kölsch and Gleissberg, 2006; Damerval and Nadot, 2007; Damerval et al., 2007).

There are several characters in the tribe Fumarieae that are highly interesting from a biological and a taxonomic point of view because of the distribution of their character states among taxa in Fumarieae (Lidén, 1986, 1993). The fruit is one of the main sources of taxonomic characters in the subfamily Fumarioideae and, especially, in the tribe Fumarieae. As we noted above, the tribe is characterized as having nuts, although several taxa from different, geographically isolated subtribes have capsules (Discocapninae: *Cysticapnos*; Sarcocapninae: *Pseudofumaria*, *Ceratocapnos*), as does the paraphyletic tribe Corydaleae. The number of seeds per fruit also varies and has been used in the classifications at infratribe levels. One of the most striking characters within Fumarieae is the chasmophytic habit, which according to Lidén (1986) is an evolutionary convergence induced by certain climatic conditions, and its acquisition has involved the acquisition of other associated features via the "evolutionary trap" phenomenon (Snogerup, 1971). One of these secondary features associated with the chasmophytic habit is the perennial life span (Lidén, 1986).

Here, we construct a molecular phylogeny of the whole tribe Fumarieae to evaluate the generic circumscriptions and the relationships between genera, as well as to evaluate the monophyly of the subtribes recognized in morphological classifications and the relationships between them. We also use the molecular phylogeny to interpret the distribution of the four aforementioned interesting characters (chasmophytic habit, life span, number of seeds per fruit, and type of fruit) among the genera, as well as the biogeographic pattern shown by the tribe. To this end, we carried out a phylogenetic analysis using four chloroplast DNA (cpDNA) sequences (*rps16* intron, *trnG* intron, *trnL* intron, and *trnL-trnF* intergenic spacer) and the internal transcribed spacer region (ITS-1, 5.8S, ITS-2; hereafter "ITS region") of the nuclear ribosomal DNA.

MATERIALS AND METHODS

***Plant material*—**The study included 25 species from 10 genera of Fumarieae (all the genera except the monotypic *Fumariola*, for which we were unable to obtain material) and five outgroup species (Table 1 and Appendix 1). The outgroup species were selected according to Lidén et al. (1997) and our own data about the phylogeny of the whole family (unpublished data). For all species, fresh leaves were collected in the wild or from botanic gardens or grown at the Department of Botany of the University of Granada. Dry leaves were also taken from herbarium material (see Appendix 1).

***DNA extraction, polymerase chain reaction, and DNA sequencing*—**Total genomic DNA was extracted, using the CTAB method (Doyle and Doyle, 1987). The entire ITS region (ITS1, 5.8S, and ITS2) and the plastid markers (*rps16* intron, *trnG* intron, *trnL* intron, and *trnL-trnF* intergenic spacer) were amplified by polymerase chain reaction (PCR). The amplification primers were N-nc18s10 and C26A (Wen and Zimmer, 1996) for the ITS region, rpS16F and rpS16R (Shaw et al., 2005, modified from Oxelman et al., 1997) for *rps16*, 3′trnG and 5′trnG2G (Shaw et al., 2005) for *trnG* intron, and finally primers C and F (Taberlet et al., 1991) were used to amplify the *trnL* intron and *trnL-F* spacer as a whole (hereafter trnL-F region). The PCR reactions were performed in a volume of 50 μL, under standard conditions (Innis et al., 1990) for ITS, and under the conditions for plastid markers recommended (Taberlet et al., 1991; Shaw et al., 2005).

TABLE 1. Genera of the tribe Fumarieae and outgroups, number of sampled and total species per genus, generalized geographic range, and coded areas for the dispersal–vicariance analysis (DIVA).

Tribe	Genus	Number of species (sampled/total)	Geographic range	DIVA
Fumarieae	*Ceratocapnos*	2/3	W and E Mediterranean/W Europe	E/F
	Cysticapnos	2/3	S Africa	C
	Cryptocapnos	1/1	Afghanistan	D
	Discocapnos	1/1	S Africa	C
	Fumaria	4/50	Mediterranean	E
	Fumariola	0/1	Kyrgyzstan	—
	Platycapnos	3/3	W Mediterranean	E
	Pseudofumaria	2/2	C Mediterranean	E
	Rupicapnos	2/7	W Mediterranean	E
	Sarcocapnos	7/7	W Mediterranean	E
	Trigonocapnos	1/1	S Africa	C
Corydaleae	*Adlumia*	1/2	E Asia/NE America	A/B
	Capnoides	1/1	N America	B
	Corydalis	2/ca. 465	Asia/Europe/N America	A
	Lamprocapnos	1/1	China	A

Notes: A = Sino-Himalayan; B = North America; C = South Africa; D = Irano-Turanian; E = Mediterranean, for taxa distributed around the Mediterranean basin, including those with representatives in other areas but mainly distributed around the Mediterranean; and F = Western Europe for *Ceratocapnos claviculata*, which is distributed in the western Euro-Siberian region. Outgroup taxa were coded according to their main distribution.

Automated sequencing of the purified PCR products was performed in both directions using the amplification primers on a 3100-Avant Genetic Analyzer (Applied Biosystems, Foster City, California, USA).

Thirteen sequences were taken from GenBank (Appendix 1), four of the *rps16* intron (*Adlumia fungosa* [Ait.] Britton, Sterns & Poggenb., *Capnoides sempervirens* [L.] Borckh., *Lamprocapnos spectabilis* [L.] Fukuhara, and *Rupicapnos numidica* [Coss. & Dur.] Pomel), and nine ITS sequences (*Lamprocapnos spectabilis*, *Platycapnos spicata* [L.] Bernh., and *Sarcocapnos* spp.); the remainder were generated as part of this study. The new sequences have been deposited in the EMBL nucleotide sequence database (Appendix 1).

Phylogenetic analysis—Nucleotide sequences were edited and aligned with the SEQMAN II version 3.61 and MEGALIN version 3.18 programs, respectively, from the DNAstar software package (DNASTAR, Madison, Wisconsin, USA) and then adjusted by eye. Two (174 bp), three (41 bp), and one (18 bp) regions of the ITS, trnL-F, and *rps16* aligned matrices, respectively, were ambiguous and excluded from analyses (Table 2). Gaps were treated as missing data. All four plastid data sets were combined in a single matrix for analyses. Separate and combined analyses were conducted on the ITS and plastid data sets. For all cases, congruence between data sets was tested using the incongruence length difference test (IDL: Farris et al., 1995). The ILD was implemented in PAUP* version 4.0b10 as the partition homogeneity test (Swofford, 2003), using 100 replicates with 1000 random-additions sequences each. The combined data matrix is available from TreeBASE (study accession no. S11911).

Phylogenetic analyses were performed using two optimality criteria: maximum parsimony (MP) as implemented in PAUP* and Bayesian inference using MrBayes version 3.1.2 (Ronquist and Huelsenbeck, 2003).

Parsimony analysis used heuristic searches with 1000 replicates of random sequence additions using tree bisection–reconnection (TBR) branch-swapping under the Fitch criterion (unordered states and equal weights). Only 10 trees were maintained at each step, to minimize the time the algorithms spent searching for trees on suboptimal islands. The starting tree was obtained by stepwise addition. Finally, 1000 bootstrap replicates (BS: Felsenstein, 1985) with 10 heuristic searches, as above, were performed to assess internal support for nodes. The amount of phylogenetic signal in the parsimony analyses were given by the consistency index (CI: Kluge and Farris, 1969) and the retention index (RI: Swofford, 1993).

Bayesian analyses were implemented using the best-fit nucleotide substitution model for each data set (GTR+G [nst = 6]; rates = gamma; statefreqpr = dirichlet) for plastid matrix, GTR+I+G [nst = 6; rates = invgamma; statefreqpr = dirichlet] for ITS). These models were selected using MrModeltest version 2.3 (Nylander, 2004) and Akaike's information criterion (Akaike, 1973). A partitioned model was used for the combined analysis, which included the selected models for the independent data sets. The analyses were based on 2 million generations with four simultaneous runs (16 Markov chain Monte Carlo chains) starting from random trees that were sampled every 100 generations. The stationarity of the runs and the convergence between runs were checked with Tracer version 1.5 (Rambaut and Drummond, 2007). The initial 25% of the samples obtained were discarded as burn-in. The remaining trees were used to build 50% majority-rule consensus trees.

Character-state reconstruction—We tested the evolutionary pathways of the four selected characters using parsimony in MacClade version 4.05 (Madison and Madison, 2002). The characters and their character states were (1) life span—annual (including annuals and biennals) or perennial; (2) chasmophytic habit—chasmophyte or nonchasmophyte; (3) number of seeds per fruit—one, two, or more than two; and (4) type of fruit—capsular or nut. Character states were mapped into the strict consensus tree of the three most-parsimonious trees obtained in the combined analysis of plastid and nuclear data sets. The only polytomy in the tree was treated as hard polytomy in McClade. Some genera had few representative species in the analysis and various character states for one character. In the species of these genera the character was coded as polymorphic (regardless of the character state shown for the species included in the analysis). Our main data sources were Lidén (1986, 1993), Manning et al. (2009), and Salinas (2009). The trace option in MacClade was "all most parsimonious states," but we also used the ACCTRAN (accelerate transformation) and DELTRAN (delayed transformation) options.

TABLE 2. Comparative table of the alignment features and most-parsimonious tree statistics for the different data sets.

DNA region	AL	CD	Percent missing	Inf/Var	NT	L	CI	RI
cpDNA matrix (trnL-F, trnG, rpS16)	2755(1152, 733, 870)	59(41, 0, 18)	12.32(18.87, 6.82, 8.52)	198/531(114/245, 53/141, 31/145)	9	702	0.895	0.871
ITS region	681	174	5.08	95/157	24	380	0.645	0.690
ITS/cpDNA matrix	3436	233	11.18	293/688	3	1094	0.805	0.789

Notes: AL = Alignment length; CD = number of ambiguous characters deleted from analyses, percent missing = percentage of data missing in the alignments, Inf/Var = number of parsimony-informative characters in relation to the total variable characters, NT = number of most-parsimonious trees, L = length of the trees, CI = consistence index, and RI = retention index.

***Ancestral-area reconstruction*—**To reconstruct the potential ancestral distribution areas, we conducted a dispersal–vicariance analysis (Ronquist, 1997) as implemented in RASP version 1.107 (Yu et al., 2010, 2011). This method calculates the optimized areas over a set of trees, thus taking into account topological uncertainty. The areas were optimized over the condensed tree of a subsample of 20 000 trees from the Bayesian analysis. Four areas were defined for the ingroup, following Lidén (1986) and a biogeographic criterion and two areas for the outgroup (Table 1).

RESULTS

***Phylogenetic analyses*—**The ILD test failed to reject both the combination of the four plastid markers ($P = 0.299$) and the combination of the plastid and ITS data sets ($P = 0.05$). Although the latter P value may suggest slight incongruence between the data sets, we decided to combine them on the basis of the extremely conservative nature of the ILD test (Sullivan, 1996; Cunningham, 1997). Data sets with slight heterogeneity can be readily combined. Table 2 shows the alignment features and tree statistics for both independent and combined parsimony analyses of the data sets. Combined parsimony analysis yielded only three most-parsimonious trees. The strict consensus tree was the same as the topology obtained via Bayesian inference (Fig. 1). The phylogeny supported the inclusion of the Fumarieae taxa in three clades that agree with the three subtribes established on the basis of morphological characters (Discocapninae [1.00/100], Fumariinae [1.00/99], and Sarcocapninae [0.98/68]). Relationships between subtribes are resolved: Discocapninae is the basal subtribe, and Fumariinae and Sarcocapninae are sister groups (1.00/100). The only unresolved relationship was that involving the placement of *Platycapnos* and *Pseudofumaria* within the Sarcocapninae subtribe. These genera are at the base of the Sarcocapninae clade either as a grade or both forming a clade as sister groups in the most-parsimonious trees. Within each subtribe, the different species are grouped by generic affinity. The only exception is *Cysticapnos*, which appears as paraphyletic. *Discocapnos* and *Trigonocapnos* are sister groups (Fig. 1).

In general, combined analyses resulted in more supported clades and more resolved relationships than independent analyses. Both plastid and ITS data sets yielded trees that were largely congruent where support values were high (Appendices S1, S2; see Supplementary Data with the online version of this article). The only strongly supported incongruence was that involving the grouping of *Fumaria densiflora–F. agraria* in the plastid phylogeny (1.00/96) and *F. densiflora–F. capreolata* in the ITS tree (1.00/94). The trees obtained from the analyses of the plastid matrix differed from those obtained in the combined analyses in the relationships among genera within the Fumariinae subtribe, because all possible combinations appeared (Appendix S1). Most ITS trees reflect the groupings established in the plastid phylogeny (Appendix S2). As occurred in the plastid trees, *Platycapnos* was included in the subtribe Sarcocapninae in most ITS trees (as the basal genus); however, it was related to the subtribe Fumariinae in five most-parsimonious trees. In addition, *Corydalis* was related to the subtribe Discocapninae in six most-parsimonious trees. The ITS analyses did not totally resolve the generic relationships between *Cysticapnos vesicaria* (L.) Fedde, *Discocapnos* and *Trigonocapnos* of the subtribe Discocapninae and between species of *Sarcocapnos* in the subtribe Sarcocapninae. In the latter subtribe, the genus *Ceratocapnos* was nonmonophyletic. Finally, unlike in the plastid trees, the relationships between the genera of the subtribe Fumariinae were well established.

***Character-state reconstruction*—**Reconstructions of the character states for the four explored characters are shown in Fig. 2. All four characters are relatively labile, showing several parallelisms or reversions of their states. Moreover, the heterogeneity of the character states in the subtribe Sarcocapninae causes the ambiguity of the ancestral condition for the internal nodes.

Reconstruction of the life span involved eight steps on the tree. The annual life span is resolved as the ancestral condition for the tribe (Fig. 2). State assignation to the internal nodes was resolved for Discocapninae and Fumariinae, and the perennial life span in *Cysticapnos* and *Rupicapnos* evolved along independent paths. In Sarcocapninae, this character was ambiguous because both conditions (perennial and annual) could be the basal state; a perennial ancestor of the subtribe will involve two reversions (*Ceratocapnos* and *Platycapnos*), whereas, if an annual ancestor is considered, three parallel paths toward perennials should be assumed in *Platycapnos saxicola* Willk., *Pseudofumaria*, and *Sarcocapnos*.

The chasmophytic habit (four steps in length on the tree) was a totally unresolved character for the internal nodes of the clades that included chasmophytes (Fumariinae and Sarcocapninae; Fig. 2). The reconstructions of the character using ACCTRAN on the most-parsimonious trees involve a chasmophytic condition that is ancestral for both subtribes and the reversions to nonchasmophytes in *Ceratocapnos*, *Fumaria*, and *Platycapnos*; while using DELTRAN they yield the independent acquisitions of the chasmophytic habit in *Cryptocapnos*, *Pseudofumaria*, *Rupicapnos*, and *Sarcocapnos*.

The type of fruit (five steps in length on the tree) for the common ancestor of the tribe was a capsule, changing to a nut in the *Discocapnos–Trigonocapnos* ancestor independently of the other nuts in the tribe (Fig. 2). Ambiguities in the internal nodes of Sarcocapninae and the common ancestor of this subtribe and Fumariinae are resolved as nuts when we use the ACCTRAN method, with two reversions to capsule in *Pseudofumaria* and *Ceratocapnos*. An ancestral capsular fruit is selected under the DELTRAN method to resolve the ambiguities and then three more independent changes toward nuts (besides the change in *Discocapnos–Trigonocapnos*) are involved (Fumariinae, *Platycapnos*, and *Sarcocapnos*).

The number-of-seeds character was totally resolved and involved five steps on the tree (Fig. 2). More than two seeds would be the basal condition for the tribe, from which one-seeded fruits evolved independently in *Discocapnos–Trigonocapnos*, Fumariinae, *Platycapnos*, *Sarcocapnos integrifolia* (Boiss.) Cuatrec., and the polymorphic *Ceratocapnos heterocarpa* Dur.

***Ancestral-area reconstruction*—**Table 3 shows the results of the dispersal–vicariance analysis. The analysis established the area for the ancestor of the tribe as being South African and Mediterranean areas with equal probability, explaining as vicariance the divergence between lineages of Discocapninae and Fumariinae–Sarcocapninae. Within Fumarieae, two more vicariance events were detected, one in the Fumariinae ancestor and the other in the ancestor of *Ceratocapnos*. In both cases, vicariance was preceded by a dispersal event from the Mediterranean area (Table 3), which was the ancestral area inferred for the remaining internal nodes.

DISCUSSION

The results clarify the phylogenetic relationships of the tribe Fumarieae and enable us to understand their evolution, thus providing a base for further studies on the evolution and development of the characters in Papaveraceae.

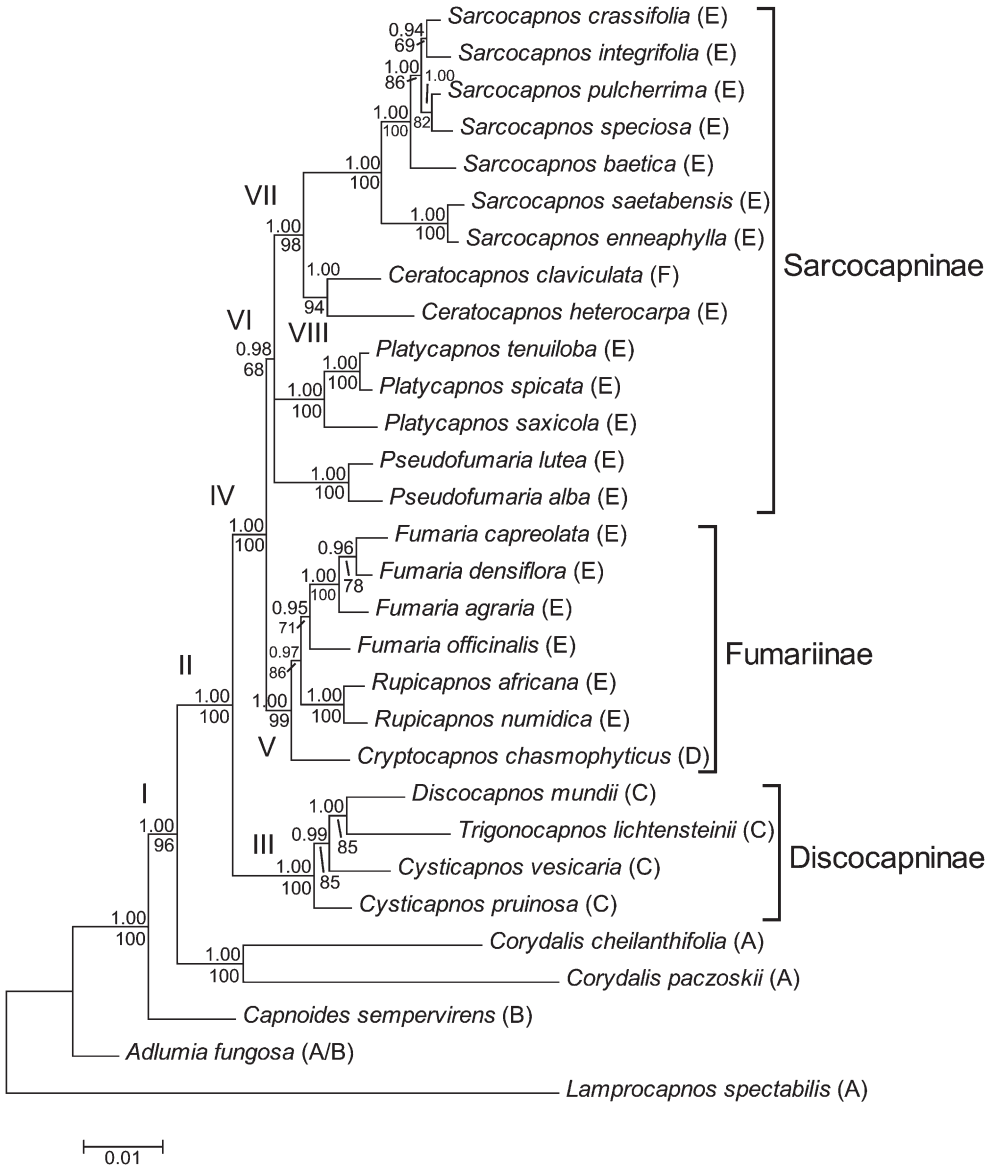


Fig. 1. Bayesian consensus tree of analysis of the combined ITS and cpDNA data set. Numbers above branches are posterior probabilities, and numbers below branches are bootstrap values ≥50% obtained in the parsimony analysis, which generated the identical consensus topology. Subtribes within Fumarieae are indicated on the right. The coded areas for the dispersal–vicariance analysis are shown in brackets after the taxon names. Roman numerals indicate the internal nodes with the reconstruction of the ancestral area shown in Table 3. A = Sino-Himalayan, B = North America, C = South Africa, D = Irano-Turanian, E = Mediterranean, and F = Western Europe.

The different methods applied (parsimony and Bayesian inference) for the phylogenetic analyses produced consistent results. Independent analyses of the nuclear and plastid data sets yielded congruent phylogenies, with few, low-supported topological incongruences. The only strongly supported incongruence was that involving the grouping of *Fumaria densiflora–F. agraria* in the plastid phylogeny and *F. densiflora–F. capreolata* in the ITS tree (Appendices S1, S2). The ILD test failed to reject the convergence between both data sets, and we therefore took the phylogeny estimated from the ITS/cpDNA data set as the "best" phylogeny of Fumarieae.

Monophyly of Fumarieae and subtribal relationships—The most comprehensive taxonomic treatments of the subfamily Fumarioideae were carried out by Lidén (1986, 1993). Two tribes were considered in these studies, Corydaleae and Fumarieae, and the genus *Cysticapnos* remained as *incertae sedis*. The monophyly of Corydaleae was rejected by Lidén et al. (1997) on the basis of *rps16* sequences, whereas the tribe Fumarieae (represented by only three taxa) resulted in a monophyletic assemblage including *Cysticapnos*. As Lidén et al. (1997) stated, the only synapomorphy supporting the tribe Fumarieae including *Cysticapnos* is the presence of small chromosomes. Our results (Fig. 1), based on five molecular markers and including an extensive sampling of the tribe Fumarieae (all genera except *Fumariola*) as well as a representation of the most related genera, strongly support the monophyly of the tribe Fumarieae (containing *Cysticapnos*, 1.00/100).

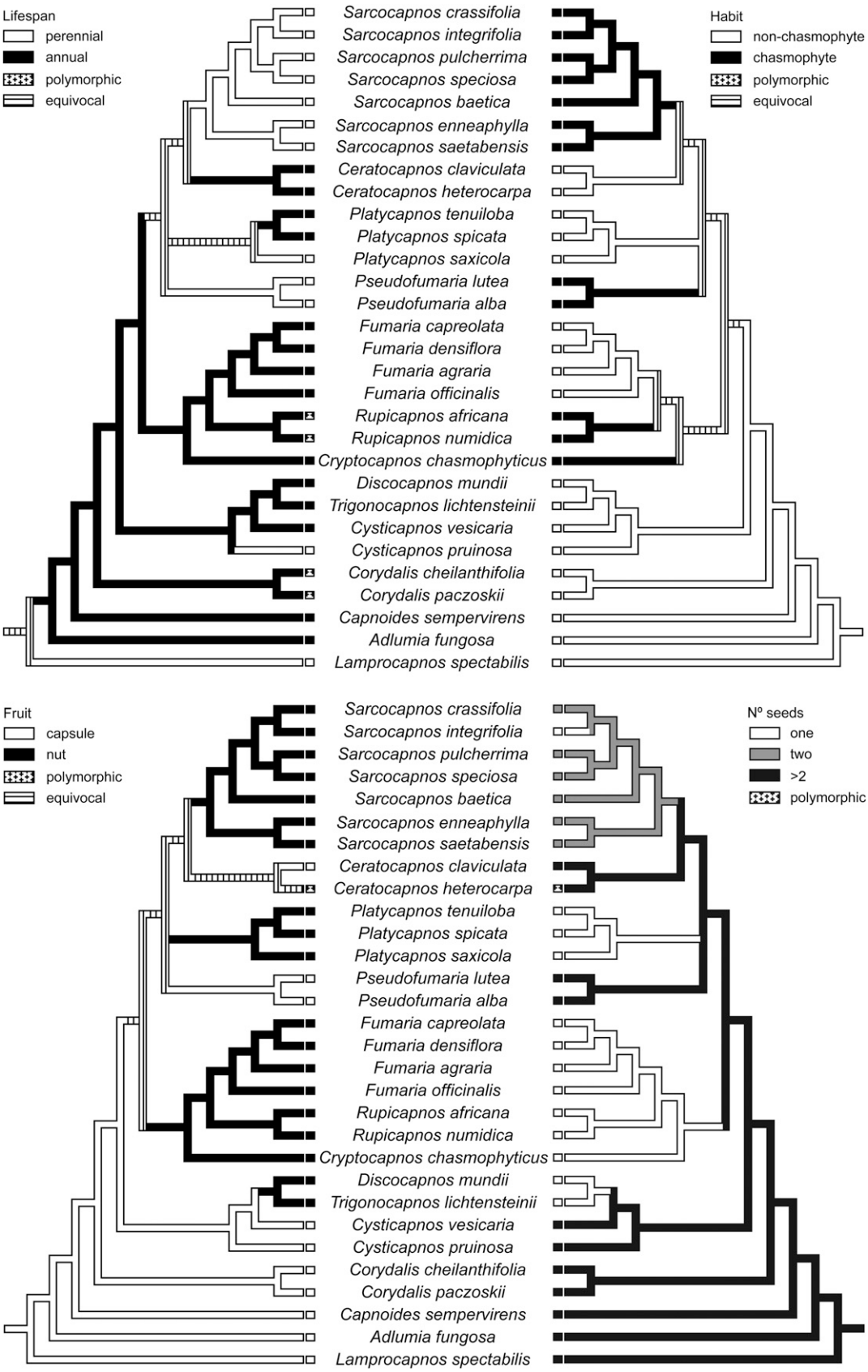


Fig. 2. Parsimony reconstruction of the four morphological characters on the topology shown in Figure 1.

In addition, our phylogenetic reconstruction supports the existence of three big groups within Fumarieae, in line with the subtribal circumscription established by Lidén (1986) on the basis of morphology: Discocapninae, Fumariinae, and Sarcocapninae (Fig. 1). The first two groups were strongly supported, whereas subtribe Sarcocapninae was only moderately supported (0.98/68). Our analysis reflects the same phylogenetic relationships among subtribes shown in the cladistic analysis by Lidén (1986), with the southern African subtribe Discocapninae the most basal subtribe, and Fumariinae and Sarcocapninae forming a sister group. Manning et al. (2009) suggested Discocapninae as a sister group of *Fumaria* on the basis of an unpublished DNA phylogeny, but our analysis strongly rejects this hypothesis.

Subtribe Discocapninae— According to our results, the subtribe Discocapninae includes all three South African endemic genera, which agrees with the emendation of the subtribe done by Manning et al. (2009) on the basis of their unpublished DNA phylogeny. However, in our phylogeny *Cysticapnos* appears as a paraphyletic genus, with *C. pruinosa* as the basal species and *C. vesicaria* as a sister species of the group formed by *Discocapnos* and *Trigonocapnos* (Fig. 1). This result disagrees with the phylogeny suggested by Manning et al. (2009) with *Cysticapnos* as a monophyletic group.

Morphologically, the subtribe is characterized by a combination of characters, none of which are synapomorphic (Lidén, 1986; Manning et al., 2009). The basal position of the subtribe within the tribe could explain this lack of synapomorphies, implying that it may have plesiomorphic characters shared with genera of the paraphyletic Corydaleae. Thus, for example, the tendrilliferous leaves of the genus *Dactylicapnos*, the persistent style (except in *Discocapnos*), and the capsule of *Cysticapnos* are typical characters of Corydaleae *sensu* Lidén (1986). One possible synapomorphy for the subtribe is pollen with six short, broad colpes (personal observation). As part of a wider project, we are studying the pollen features of the Papaveraceae family. This kind of colpe is exclusive for the three genera of the subtribe (suggested by Lidén only for *Discocapnos* and *Trigonocapnos*; Lidén, 1986). The relationship between *Discocapnos* and *Trigonocapnos* is well supported by morphology, in that both species share as main characters an unusual vasculature of the fruit wall that is different from other Fumariaceae (Fukuhara, 1995) and one-seeded indehiscent fruits with a pubescent pericarp (Lidén, 1986) and a marginal wing (Fukuhara, 1995).

Regarding the taxonomy of the subtribe, it is debatable whether all the genera should be included in an enlarged *Cysticapnos* s.l. or should be regarded as independent (cf. Manning et al., 2009). According to Manning et al. (2009), the first option is not reasonable if the taxonomic importance of fruit type in the subfamily is considered, in which case it would be more reasonable to keep the distinction between *Cysticapnos* (with capsules) and *Discocapnos* and *Trigonocapnos* (with nuts). Moreover, these authors refer to a lack of coherence of the lumping proposal with regard to their molecular analysis, given that *Cysticapnos* appears as monophyletic (Manning et al. unpublished data). Therefore, Manning et al. (2009) consider that the best option is to keep all three genera independent. Our results, with a paraphyletic, basal *Cysticapnos*, allow us to reconsider the taxonomic alternatives presented in Manning et al. (2009). Thus, the paraphyly of *Cysticapnos* does not support the recognition of three genera in the subtribe. If we consider only one genus, *Cysticapnos* s.l., we should accept different types of fruit within the genus, with a trend from capsules to nuts (discussed below). However, if different genera are considered, *Cysticapnos* should be split into two genera on the basis of its paraphyly, in which case the subtribe should include four different genera. After the extensive morphological review of *Cysticapnos* by Manning et al. (2009), the genus now includes three species: *Cysticapnos vesicaria*, *C. cracca* (Cham. & Schltdl.) Lidén, and *C. pruinosa*. Originally, *Cysticapnos* was described to include *C. vesicaria* (*Corydalis vesicaria* L.), with a bladder capsule (Miller, 1754). Later, Bernhardi (1838) described the genus *Phacocapnos* Bernh. to include *C. cracca* (*Corydalis cracca* Cham. & Schltdl.) and *C. pruinosa*, based on compressed-capsular fruit. This classification was followed by different authors, including Fedde (1936), and was the basis for the current classification lumping *Phacocapnos* and *Cysticapnos* together (Liden, 1986). The splitting of *Cysticapnos* into two genera is supported not only by the shape of the capsule, but also by other characters. Thus *C. vesicaria* has a geniculate style (Lidén, 1986), a reticulate venation on the fruit valves (Fukuhara and Lidén, 1995a), and seeds with a grooved hilar region (Fukuhara, 1999); whereas *C. cracca* and *C. pruinosa* share a straight style, nonreticulate venation on the fruit valves, and a more or less truncated hilar region.

TABLE 3. Results of the dispersal–vicariance analysis. Nodes refer to Figure 1.

Node	Ancestral Area	Event	Reconstruction	Frequency (%)
I	ACE	Vicariance	ACE → A/CE	100
II	CE	Vicariance	CE → C/E	100
III	C	–	C → C/C	100
IV	E	Dispersal	E → DE/E	100
V	DE	Vicariance	DE → D/E	100
VI	E	–	E → E/E	100
VII	E	Dispersal	E → EF/E	100
VIII	EF	Vicariance	EF → F/E	100

Notes: A = Sino-Himalayan, C = South Africa, D = Irano-Turanian, and E = Mediterranean.

Subtribe Fumariinae— This is a morphologically well-defined subtribe (Lidén, 1986; Fukuhara and Lidén, 1995b). Our results strongly support the monophyly of the group (1.00/99), including the monotypic basal *Cryptocapnos* (Fig. 1). The cladistic analysis of Lidén (1986) yielded a different pattern for the relationships between genera, in which *Fumaria* was the most basal and *Fumariola* was a sister to the *Cryptocapnos*–*Rupicapnos* clade. Although *Fumariola* was missing in our analysis, the relationships shown by the ITS/cpDNA phylogeny have important repercussions on the interpretation of several morphological characters, some of them discussed in the section on character-state reconstruction. For example, according to Lidén (1986), *Rupicapnos* and *Cryptocapnos* are very close genera, sharing many morphological characters. Our results suggest that shared characters among these taxa should be interpreted as plesiomorphies in *Rupicapnos*. An interesting character that must be reinterpreted in light of our phylogeny is the apical beak of the fruit. There seems to be a trend toward diminution of the beak, from the long beak in *Cryptocapnos* to the pits in *Fumaria* (asserted as homologous by Lidén, 1986) with a short apical beak in *Rupicapnos*. The extreme reduction of the beak in *Fumaria* could be related to the change of habit, given that the beak seems to be useful for

dispersion (by geocory) into the cracks in chasmophytic habitats. However, the absence of *Fumariola* (annual chasmophyte with four short mucros) in our analysis means that we cannot confidently assert this possible trend in the beak evolution of the fruit in Fumariinae.

The genus *Fumaria* is the most diversified of the Fumariinae, with 50 species in the treatment by Lidén (1986). Polyploidy seems to play an important role in the diversification of the genus, given that there is an extensive ploidy series (2n = 16, 32, 48, 64, 72, 80, 112; Lidén, 1986). The genus is divided into two sections (sect. *Capreolatae* Hammar and sect. *Fumaria*) on the basis of leaflet shape, size of the flowers, and some corolla characters (cf. Lidén, 1986). In our analysis, we included two representatives of each section (sect. *Capreolatae*: *F. agraria* Lag. and *F. capreolata* L.; sect. *Fumaria*: *F. densiflora* DC. and *F. officinalis* L.), but they did not group according to sectional affinities (Fig. 1). The *Fumaria* clade included one of the topological conflicts between chloroplast and nuclear phylogenies: *F. densiflora* is sister to *F. agraria* (1.00/100) with the chloroplast data and sister to *F. capreolata* (1.00/94) with the ITS data. Neither of these groups reflects the sectional classification, but they suggest the possibility of hybridizations among *Fumaria* species. Lidén (1986) noted reticulations as a factor causing the nonmonophyly of the sections. More studies using molecular markers in combination with morphology are needed to clarify the taxonomy of *Fumaria*.

Subtribe Sarcocapninae— All the genera of this subtribe were strongly supported as monophyletic. However, the monophyly of the subtribe was only moderately supported and the phylogenetic relationships among all four genera were not totally resolved, because of the conflicting positions of *Pseudofumaria* and *Platycapnos* (Fig. 1). The only difference between the three most-parsimonious trees obtained from the ITS/cpDNA data set involved the placement of these two genera, which were either grouped together (in one of the three trees) or formed a grade with regard to the clade *Ceratocapnos–Sarcocapnos*, with *Platycapnos* the basal taxon in one most-parsimonious tree and *Pseudofumaria* in the other. The support values for monophyly of the subtribe increased when either of these two genera were excluded from the analyses (carried out only under the maximum-parsimony criterion; BS: 85% without *Platycapnos*; BS: 88% without *Pseudofumaria*; data not shown).

The topological conflict is not resolved with morphology, given that all three phylogenetic hypotheses are apparently supported by such data. The genus *Platycapnos* was basal in the cladistic analysis carried out by Lidén (1986), because the grouping *Pseudofumaria–Ceratocapnos–Sarcocapnos* was supported by the embryonic suspensor with 3 vesicular cells in one row, the corolla morphology with broadly spatulate outer petals, the inner petals yellow at the apex, and the stigmatic tissue along the base of the stigma. However, this scheme supposes the reversion in *Pseudofumaria* of the papery endocarp that separates from the fruit wall, and of the sterile-beak at the apex of the fruit. These two characters support the grouping of *Platycapnos–Ceratocapnos–Sarcocapnos*. And finally, the hypothesis that *Platycapnos* and *Pseudofumaria* are sister groups is supported by the pollen type: both genera have a synpolypantocolpate pollen (personal observation). This kind of pollen has long been known for *Platycapnos*, but *Pseudofumaria* was reported to have hexacolpate pollen (Lidén, 1986). Our observations of the pollen of both *Pseudofumaria* species show that this genus has variable pollen, as it is mainly synpolypantocolpate, but pollen with much smaller apocolpi also exists. The synpolypantocolpate pollen type is also present in several species of *Corydalis* (personal observation), which suggests that this character has evolved in both groups along separate paths. Others characters shared by *Platycapnos* and *Pseudofumaria* are the lower petal with an even lower margin, the inner petals only unilaterally indented, and the presence of apical appendages in the stigma (Lidén, 1986).

The unresolved positions of these two genera in the molecular tree (Fig. 1) and the absence of morphological characters consistent with a clear relationship pattern suggest a rapid and simultaneous radiation of the three main lineages in Sarcocapninae (*Ceratocapnos–Sarcocapnos*, *Platycapnos*, and *Pseudofumaria*) from the common ancestor. In fact, the lengths of the branches grouping *Pseudofumaria* or *Platycapnos* with the clade *Ceratocapnos–Sarcocapnos* and the branch grouping both genera were always only one change. Molecular markers used in our study have proved effective at resolving relationships even at an infrageneric level (e.g., relationships within *Sarcocapnos*); however, they have failed to resolve the relationships among the three lineages of Sarcocapninae. Many examples exist, at a variety of taxonomic scales, in which a rapid origin of major lineages is suggested by unresolved phylogenetic relationships using molecular markers, which offer excellent support for more recent relationships (Fishbein et al., 2001, and references therein).

The short length of branches leading to subtribes Sarcocapninae and Fumariinae suggests that the radiation of the three main lineages of Sarcocapninae must have occurred not long after the split between the two subtribes. A rapid radiation of the Sarcocapninae lineages after the split of Fumariinae and Sarcocapninae would explain the lack of fixation shown by several morphological characters in these lineages (e.g., the capsular fruit in *Pseudofumaria* and *Ceratocapnos*, and the embryonic suspensor like a bunch of grapes in *Platycapnos* [= Fumariinae]) due to incomplete lineage sorting.

Within Sarcocapninae, the relationships between *Sarcocapnos* and *Ceratocapnos* are strongly supported (1.00/98; Fig. 1), as expected if we consider morphology (fruit with sterile beak and ribbed walls, stigma with an apical crest), pollen characters (angular form, hexacolpate, and a particular ultrastructure of the pollen wall without interapertural endexine and with a very thick foot-layer and intine; personal observation), and phytochemistry (they share an exclusive biosynthetic pathway for the isoquinoline alkaloids, the (s)-crassifoline pathway; whereas the remaining Fumariaceae have the (s)-reticuline route; Valpuesta et al., 1995).

At an infrageneric level, the perennial orophyllous *Platycapnos saxicola* is basal to the clade formed by the annuals of disturbed habitats *P. spicata* and *P. tenuiloba* Pomel (Fig. 1). With regard to *Sarcocapnos*, our phylogenetic hypothesis agrees with the results obtained by Salinas et al. (2003). The only difference is the resolved basal position of *S. baetica* (Boiss. & Reuter) Nyman regarding *S. crassifolia* (Desf.) DC.-*S. integrifolia*-*S. pulcherrima* C. Morales & Romero García–*S.speciosa* Boiss. group, unresolved in the Salinas et al. phylogeny.

Character-state reconstruction—Lidén (1986) determined the evolutionary pathways for the characters he used in his cladistic

analysis of the tribe Fumarieae. The uncertain phylogenetic position of the genus *Cysticapnos* was one of the problems Lidén encountered in exploring character polarity—a problem we do not have, given the well-resolved position of *Cysticapnos* in our phylogeny (Fig. 1).

Fumarieae comprises annual and perennial species. The annual life span was considered basal for the tribe, and the perennial state was classified as a secondary feature probably associated with the chasmophytic habit (Lidén, 1986). Our results agree with this hypothesis, in that annual is the ancestral condition for the tribe and the subfamily (Fig. 2). The perennial state is secondary and acquired independently several times during evolution of the tribe. The equivocal resolution of this character for the internal nodes in Sarcocapninae means that we cannot hypothesize unequivocally about whether the annuals in *Platycapnos* and *Ceratocapnos* are reversals or whether the perennials (*Platycapnos saxicola*, *Pseudofumaria*, and *Sarcocapnos*) are convergences.

The convergence toward perennial in *Cysticapnos* and *Rupicapnos*, and the fact that all perennial species are chasmophytes (*Pseudofumaria*, *Rupicapnos*, and *Sarcocapnos*) or orophyllous plants (*Cysticapnos pruinosa* and *Platycapnos saxicola*), suggests that a basal annual condition is more likely for Sarcocapninae and the adaptative convergence toward perennials related to the chasmophytic or orophyllous habitats (exceptions are the nonperennial chasmophytes *Cryptocapnos* and *Fumariola* from Central Asia and three taxa of *Rupicapnos* restricted to the dry Saharian cliffs: *R. muricaria* Pomel and two subspecies of *R. longipes* [Coss. & Dur.] Pomel; Lidén, 1986).

With regard to the chasmophytic habit, in spite of the fact that the state reconstruction for the common ancestor of Fumariinae and Sarcocapninae was equivocal, a relation between life span and habit would imply that the chasmophytic habit would also be a secondary state acquired independently from an ancestral nonchasmophytic habit (Fig. 2).

One of the most notable changes showed by our study regarding the character polarization carried out by Lidén (1986) refers to the evolutionary pathway of the fruit. Our results show capsular fruit as the ancestral condition for the tribe instead of an indehiscent fruit as Lidén proposed (Fig. 2). Obviously, this change is due to the inclusion of *Cysticapnos* (which has a capsular fruit) in Discocapninae occupying the basal position (from which a nut evolves in *Discocapnos* and *Trigonocapnos*). Once again, the state reconstruction for the internal nodes of Sarcocapninae and Fumariinae–Sarcocapninae clades was equivocal.

The optimization for number of seeds was totally resolved and the basal condition was taken as more than two seeds (Fig. 2), from which the reduction in the number of seeds toward monosperm and disperm fruits happened independently in different lineages (*Discocapnos–Trigonocapnos*, Fumariinae, and *Platycapnos*). The number of seeds is a character that is directly related to the type of fruit, given that the capsular fruits have several seeds (more than two) and the nuts are di- or monosperms. This relation can be seen in the coincident distribution of the character states for the two characters among taxa (Fig. 2). Bearing in mind this relation between the number of seeds and the kind of fruit, a capsular fruit would be the condition for the nonresolved internal nodes whereas the nut would represent a derived state acquired several times during the evolution of the tribe.

Biogeography— Asia is the most speciose region for the subfamily Fumarioideae, where most of the basal lineages of the subfamily are distributed (Lidén, 1986; Dahl, 1990; Kadereit et al., 1995). The tribe Fumarieae shows one of the most striking phytogeographic disjunctions in the family Papaveraceae s.l., in that it is mainly centred around the Mediterranean region but with the subtribe Discocapninae endemic to the Cape region of South Africa, and with two genera of the subtribe Fumariinae endemic in certain parts of Central Asia (*Cryptocapnos* in Afghanistan and *Fumariola* in Turkestan). At the genus level, the geographic distributions are relatively restricted except for *Fumaria*, which is widely distributed (mainly around the Mediterranean but ranging from Macaronesia to northern India and occasionally East Africa).

The results obtained from the reconstruction of ancestral areas suggest that an ancestor of the tribe occupied a continuous area, which after disruption gave rise to a process of differentiation between the lineages, leading to the subtribe Discocapninae on one hand and to the clade Fumariinae–Sarcocapninae on the other. Given the ecological discontinuities in Africa (Quézel, 1978), it seems unlikely that there was a continuous area for this ancestor covering all the current distribution of the taxa. It is however likely that this area covered western Eurasia and North Africa and that a disruption event in the area coupled with a migration toward the south facilitated the beginning of the diversification of the lineages, leading to the subtribe Discocapninae on one hand and to the clade Fumariinae–Sarcocapninae on the other. According to Meusel (1969) and Burtt (1971), disjunctions such as that of the tribe Fumarieae demonstrate the existence of a tertiary arid flora that was widespread in western Eurasia and in Africa, and that the main climatic changes that took place in Africa from the Miocene onward were responsible for the vicariances observed (Quézel, 1978, 1983). Unfortunately, we could not calibrate our phylogenetic hypothesis chronologically, but a possible causal event of the vicariance encountered by the ancestor of the tribe may have been the shift of the Equator toward the south, which occurred in the transition between the Oligocene and the Miocene, causing a drastic aridification of the whole area, which allowed the expansion of the distributional range of a large number of taxa (Quézel, 1978). This hypothesis was supported by Kadereit et al. (1995) for the tribe Fumarieae, which, they proposed, already existed in the mid- or late Tertiary. This hypothesis assumes that the ancestor of the tribe was already adapted to more or less dry, open-area conditions, implying a change in ecological requirements from the basal lineages of the subfamily, all of which have forest floor habitats. Kadereit et al. (1995) and Hoot et al. (1997) proposed this change in habitat as the main stimulus in the morphological evolution and diversification of the different lineages of the family Papaveraceae, in particular the Mediterranean groups.

According to our results, the diversification of the subtribes Fumariinae and Sarcocapninae must have taken place in the Mediterranean area. In the subtribe Fumariinae, the first lineage to diverge was that of the *Cryptocapnos*. The dispersal–vicariance analysis supports a vicariant process for the group, such that the ancestor of the subtribe must have been widespread across the eastern Mediterranean region to the Irano-Turanian region, which it reached by dispersion from the Mediterranean region. In view of the morphological similarities between *Rupicapnos* and *Cryptocapnos*, Lidén (1986) proposed both taxa as an example of the disjunct western Mediterranean–Central Asia and suggested that *Rupicapnos* originated in the Irano-Turanian

region before expanding west through North Africa. Our data support vicariance as a phenomenon that explains the origin of the Central Asian taxa of Fumariinae and, therefore, a later origin of *Rupicapnos* in the Mediterranean area.

With regard to the subtribe Sarcocapninae, the concentration of most of its taxa in the western and central Mediterranean suggests that this area was the center of the origin and radiation of its three main lineages (*Ceratocapnos–Sarcocapnos*, *Platycapnos*, and *Pseudofumaria*). *Ceratocapnos* is the only genus with representation in the eastern Mediterranean (*C. turbinata* in Palestine and Cyprus), and in the west of the Euro-Siberian region (*C. claviculata*). These kinds of disjunction are relatively frequent (Davis and Hedge, 1971) and are associated with the disruption of a continuous ancestral area (probably reached during the late Miocene–early Pliocene) because of later geological and climatic events. The footprint left by this kind of event is also reflected in the distribution of other genera of the tribe (*Fumaria*, *Platycapnos*, *Rupicapnos*, and *Sarcocapnos*), as is the presence of two species of *Sarcocapnos* in North Africa (*S. crassifolia* and *S. enneaphylla*) or the presence of a species of *Rupicapnos* in the south of the Iberian Peninsula (*R. africana*) or of three taxa in the Saharan Mountains (*R. muricaria* and two subspecies of *R. longipes*). Lidén (1986) suggested that the distribution of *Sarcocapnos* in North Africa is attributable to its migration toward the south. Our results suggest that this migration must have happened on at least two separate occasions (the ancestor of *S. integrifolia–S. crassifolia*, and *S. enneaphylla*). This author also suggested that the presence of *Rupicapnos* in the south of the Iberian Peninsula is attributable to the dispersion of the genus northward. Quézel (1978) proposed that the Mediterranean taxa present in the Saharan mountains, as occurs with *Rupicapnos*, are relics of past epochs when the glacial periods forced them to migrate toward the south, after which they became cut off after the return northward during the interglaciar periods. This hypothesis also explains the migrations proposed by Lidén (1986) for *Sarcocapnos* and *Rupicapnos*.

LITERATURE CITED

AKAIKE, H. 1973. Information theory and an extension of the maximum likelihood principle. In: B. N. Petran and F. Csaki [eds.], Second international symposium on information theory, 267–281. Akademiai Kiado, Budapest, Hungary.

BERNHARDI, J. J. 1838. Nachträgliche bemerkungen über Papaveraceen und Fumariaceen. *Linnaea* 12: 651–668.

BURTT, B. L. 1971. From the south: an African view of the floras of Western Asia. In: P. H. Davis, P. C. Harper, and I. C. Hedge [eds.], Plant life of South-West Asia, 135–149. Botanical Society of Edinburgh, Edinburgh, UK.

CUNNINGHAM, C. W. 1997. Can three incongruence tests predict when data should be combined? *Molecular Biology and Evolution* 14: 733–740.

DAHL, A. E. 1990. Infrageneric division of the genus *Hypecoum* L. (Papaveraceae). *Nordic Journal of Botany* 10: 129–140.

DAMERVAL, C., M. LE GUILLOUX, M. JAGER, AND C. CHARON. 2007. Diversity and evolution of CYCLOIDEA-like TCP gene in relation to flower development in Papaveraceae. *Plant Physiology* 143: 759–772.

DAMERVAL, C., AND S. NADOT. 2007. Evolution of perianth and stamen characteristics with respect to floral symmetry in Ranunculales. *Annals of Botany* 100: 631–640.

DAVIS, P. H., AND I. C. HEDGE. 1971. Floristic links between NW Africa and SW Asia. *Annalen des Naturhistorischen Museums in Wien* 75: 43–57.

DOYLE, J. J., AND J. L. DOYLE. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin* 19: 11–15.

FARRIS, J. S., M. KÄLLERSJÖ, A. G. KLUGE, AND C. BULT. 1995. Constructing a significance test for incongruence. *Systematic Biology* 44: 570–572.

FEDDE, F. 1936. Papaveraceae. In: A. Engler and K. Prantl [eds.], Die natürlichen Pflanzenfamilien, ed. 2, 17b, 5-145. Leipzig, Germany.

FELSENSTEIN, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39: 783–791.

FISHBEIN, M., C. HIBSCH-JETTER, D. E. SOLTIS, AND L. HUFFORD. 2001. Phylogeny of Saxifragales (angiomsperms, eudicots): Analysis of a rapid, ancient radiation. *Systematic Biology* 50: 817–847.

FUKUHARA, T. 1995. Vascular pattern in the fruit of *Trigonocapnos* and *Discocapnos* (Papaveraceae; Fumarioideae). *International Journal of Plant Sciences* 156: 547–554.

FUKUHARA, T. 1999. Seed and funicle morphology of Fumariaceae-Fumarioidea: Systematic implications and evolution paterns. *International Journal of Plant Sciences* 160: 151–180.

FUKUHARA, J., AND M. LIDÉN. 1995a. Pericarp anatomy in Fumariaceae-Fumarioideae. *Botanische Jahrbucher fur Systematik, Pflanzengeschichte und Pflanzengeographie* 117: 499–530.

FUKUHARA, J., AND M. LIDÉN. 1995b. Seed-coat anatomy in Fumariaceae-Fumarioideae. *Botanical Journal of the Linnean Society* 119: 323–365.

HOOT, S. B., J. W. KADEREIT, F. R. BLATTNER, K. B. JORK, A. E. SCHWARZBACH, AND P. R. CRANE. 1997. Data congruence and phylogeny of the Papaveraceae s.l. based on four data sets: atpB and rbcL sequences, trnK restriction sites, and morphological characters. *Systematic Botany* 22: 575–590.

INNIS, M. A., D. H. GELFAND, J. J. SNINSKY, AND T. J. WHITE. 1990. *PCR protocols: A guide to methods and applications*. Academic Press, New York, New York, USA.

KADEREIT, J. W., F. R. BLATTNER, K. B. JORK, AND A. SCHWARZBACH. 1995. The phylogeny of the Papaveraceae s.1.: Morphological, geographical and ecological implications. In: U. Jensen, and J.W. Kadereit [eds.], Systematics and evolution of the Ranunculiflorae. *Plant Systematics and Evolution* (Supplement) 9: 133–145.

KLUGE, A. G., AND J. S. FARRIS. 1969. Quantitative phyletics and the evolution of anurans. *Systematic Zoology* 40: 315–328.

KÖLSCH, A., AND S. GLEISSBERG. 2006. Diversification of CYCLOIDEA-like TCP genes in the Basal Eudicots Families Fumariaceae and Papaveraceae s.str. *Plant Biology* 8: 680–687.

LIDÉN, M. 1986. Synopsis of Fumarioideae (Papaveraceae) with a monograph of the tribu Fumarieae. *Opera Botanica* 88: 1–133.

LIDÉN, M. 1993. Fumariaceae. In: K. Kubitzki, J. G. Rohwer, and V. Bittrich [eds.], The families and genera of vascular plants, vol. 2: Florewing plants, dicotyledons: Magnoliid, hamamelid, and caryophyllid families, 310–318. Springer-Verlag, Heidelberg, Germany.

LIDÉN, M., T. FUKUHARA, AND T. AXBERG. 1995. Phylogeny of *Corydalis*, ITS and morphology. In: U. Jensen, and J.W. Kadereit [eds.], Systematics and evolution of the Ranunculiflorae. *Plant Systematics and Evolution* (Supplement) 9: 183–188.

LIDÉN, M., T. FUKUHARA, J. RYLANDER, AND B. OXELMAN. 1997. Phylogeny and classification of Fumariaceae, with emphasis on *Dicentra* s.l., based on the plastid gene *rps16* intron. *Plant Systematics and Evolution* 206: 411–420.

LOCONTE, H., L. M. CAMPBELL, AND D. WM. STEVENSON. 1995. Ordinal and familial relationships of Ranunculid genera. In: U. Jensen, and J.W. Kadereit [eds.], Systematics and evolution of the Ranunculiflorae. *Plant Systematics and Evolution* (Supplement) 9: 99–118.

MADISON, D. R., AND W. P. MADISON. 2002. MacClade 4.05: Analysis of phylogeny and character evolution. Sinauer, Sunderland, Massachusetts, USA.

MANNING, J. C., P. GOLDBLATT, AND F. FOREST. 2009. A revision of Fumariaceae (Fumarioideae) in southern Africa, including naturalized taxa. *Bothalia* 39: 47–65.

MEUSEL, H. 1969. Beziehungen in der Florendifferenzierung von Eurasien und Nordamerika. *Flora* 158: 537–564.

MILLER, P. 1754. The gardeners dictionary (Abr. Ed. 4 Vol. 2.). London, UK.

NYLANDER, J. A. A. 2004. MrModeltest version 2. Computer program distributed by the author, Evolutionary Biology Centre, Uppsala University.

OXELMAN, B., M. LIDÉN, AND D. BERGLUND. 1997. Chloroplast *rps16* intron phylogeny of the tribe Silineae (Caryophyllaceae). *Plant Systematics and Evolution* 206: 393–410.

QUÉZEL, P. 1978. Analysis of the Flora of Mediterranean and Saharan Africa. *Annals of the Missouri Botanical Garden* 65: 479–534.

QUÉZEL, P. 1983. Flore et végétation actuelles de l'Afrique du nord, leur signification en fonction de l'origine, de l'évolution et des migrations des flores et structures de végétation passées. *Bothalia* 14: 411–416.

RAMBAUT, A., AND A. J. DRUMMOND. 2007. Tracer version 1.5. Computer program and documentation distributed by the authors. Website: http://beast.bio.ed.ac.uk/software/tracer.

ROMERO, A. T., M. J. SALINAS, AND M. C. FERNÁNDEZ. 2003. Pollen wall development in *Hypecoum imberbe* Sm. (Fumariaceae). *Grana* 42: 91–101.

RONQUIST, F. 1997. Dispersal-vicariance analysis: A new approach to the quantification of historical biogeography. *Systematic Biology* 46: 195–203.

RONQUIST, F., AND J. P. HUELSENBECK. 2003. MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics (Oxford, England)* 19: 1572–1574.

SALINAS, M. J. 2009. Fumariaceae. In: G. Blanca, B. Cabezudo, M. Cueto, C. Fernández López, and C. Morales Torres [eds.], Flora Vascular de Andalucía Oriental, vol. 2, Ranunculaceae-Polygalaceae, 61–72. Consejería de Medio Ambiente, Junta de Andalucía, Sevilla, Spain.

SALINAS, M. J., A. T. ROMERO, G. BLANCA, R. DE LA HERRÁN, M. GARRIDO-RAMOS, C. RUIZ-REJÓN, C. MORALES, ET AL. 2003. Contribution to the taxonomy and phylogeny of *Sarcocapno*s (DC). (Fumariaceae). *Plant Systematics and Evolution* 237: 153–164.

SHAW, J., E. B. LICKEY, J. T. BECK, S. B. FARMER, W. LIU, M. JERMEY, K. C. SIRIPUN, ET AL. 2005. The tortoise and the hare II: Relative utility of 21 noncoding chloroplast DNA sequences for phylogenetic analysis. *American Journal of Botany* 92: 142–166.

SNOGERUP, S. 1971. Evolutionary and plant geographical aspects on chasmophytic communities. In: P. H. Davis, P. Harper, and I. C. Hedge [eds.], Plant life of Southwest Asia, 157–170. Botanical Society of Edinburgh, Edinburgh, UK.

SULLIVAN, J. 1996. Combining data with different distributions of among-site rate variation. *Systematic Biology* 45: 375–380.

SWOFFORD, D. L. 1993. PAUP: phylogenetic analysis using parsimony, version 3.1.1. Illinois Natural History Survey, Champaign, Illinois, USA.

SWOFFORD, D. L. 2003. PAUP*: Phylogenetic analysis using parsimony (*and other methods), version 4. Sinauer, Sunderland, Massachusetts, USA.

TABERLET, P., L. GIELLY, G. PAUTOU, AND J. BOUVET. 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Molecular Biology* 17: 1105–1109.

VALPUESTA, M., N. POSADAS, I. RUIZ, M. V. SILVA, A. I. GÓMEZ, R. SUAU, B. PÉREZ, ET AL. 1995. Alkaloids from *Ceratocapnos heterocarpa* plants in vitro cultures. *Phytochemistry* 38: 113–118.

WANG, W., A. M. LU, Y. REN, M. E. ENDRESS, AND Z. D. CHEN. 2009. Phylogeny and classification of Ranunculales: Evidence from four molecular loci and morphological data. *Perspectives in Plant Ecology, Evolution and Systematics* 11: 81–110.

WEN, J., AND E. A. ZIMMER. 1996. Phylogeny and biogeography of *Panax* L. (the ginseng genus, *Araliaceae*): Inferences from ITS sequences of nuclear ribosomal DNA. *Molecular Phylogenetics and Evolution* 6: 167–177.

YU, Y., A. J. HARRIS, AND X. HE. 2010. S-DIVA (statistical dispersal-vicariance analysis): A tool for inferring biogeographic histories. *Molecular Phylogenetics and Evolution* 56: 848–850.

YU, Y., A. J. HARRIS, AND X. HE. 2011. RASP (Reconstruct Ancestral State in Phylogenies) version 1.107. Computer program and documentation distributed by the authors. Website: http://mnh.scu.edu.cn/soft/blog/RASP.

APPENDIX 1. Species, voucher information (data for sequences taken from GenBank are not shown), and EMBL accession numbers used in this study. Abbreviations: GBG = Gothenburg Botanical Garden; GDA, GDAC = herbarium of the University of Granada; LHMS = Leslie Hill Molecular Systematics Laboratory of the South African National Biodiversity Institute (SANBI); NBGB = National Botanic Garden of Belgium; W = Herbarium of the Natural History Museum in Vienna.

Species; Voucher or plant register (if from a living collection), EMBL accessions (TrnL-F region, *trnG* intron, *rps16* intron and ITS region). Accessions for sequences taken from GenBank are in italics.

Adlumia fungosa (Ait.) Britton, Sterns & Poggenb.; Living collection: NBGB20001298-92, HE603352, HE603275, *Z82944*, HE603323. ***Capnoides sempervirens*** (L.) Borckh.; Living collection: NBGB19871844, HE603351, HE603274, *Z82956*, HE603322. ***Ceratocapnos claviculata*** (L.) Lidén; Spain: Ourense, Leiro, 6-IV-1989, M. Buján, GDA27019, HE603339, HE603262, HE603291, HE603311. ***C. heterocarpa*** Dur.; Spain: Granada, La Bernardilla, Guájar Faragüit, 19-II-1980, M. Ladero, J. Molero, Mart. Parras & J. Hurtado, GDA9032, HE603338, HE603261, HE603290, HE603310. ***Corydalis cheilanthifolia*** Hemsl.; Living collection: K196919923, HE603349, HE603272, HE603301, HE603320. ***C. paczoskii*** Busch.; Living collection: K19833040, HE603350, HE603273, HE603302, HE603321. ***Cysticapnos pruinosa*** (Bernh.) Lidén; South Africa: Seeds from Silverhill Seeds and Books, 4877, HE603347, HE603270, HE603299, HE603318. ***C. vesicaria*** (L.) Fedde; South Africa: Seeds from Silverhill Seeds and Books, 600, HE603348, HE603271, HE603300, HE603319. ***Cryptocapnos chasmophyticus*** Rech. f.; Afghanistan: Orūzgān, inter Tirin and Orūzgān, 2000m, 1967-05-24, Rechinger, W1969-0013838 holotype, HE603324, HE603247, HE603277, HE603303. ***Discocapnos mundii*** Cham. & Schltdl.; DNA from SANBI DNA Bank, LHMS3135, HE603345, HE603268, HE603297, HE603316. ***Fumaria agraria*** Lag.; Portugal: Algarve, Punta de Sagrés, 27-III-1980, M. Ladero, O. Socorro & J. Hurtado, GDA12614, HE603330, HE603253, HE603282, HE603309. ***F. capreolata*** L.; Spain: Granada, junto Facultad de Farmacia, 20-II-2007, V. N. Suárez-Santiago, GDA52802, HE603328, HE603251, HE603280, HE603307. ***F. densiflora*** DC.; Spain: Granada, junto Facultad de Farmacia, 14-III-2007, V. N. Suárez-Santiago, GDA52804, HE603329, HE603252, HE603281, HE603308. ***F. officinalis*** L.; Spain: Granada, junto parking de la Alhambra, 13/02/2011, M. A. Pérez-Gutiérrez, GDA57985, HE603327, HE603250, HE603279, HE603306. ***Lamprocapnos spectabilis*** (L.) Fukuhara; Cultivated at Derparment of Botany, origin unknown; HE603353, HE603276, *Z82937*, *AJ493444*. ***Platycapnos saxicola*** Willk.; Spain: Jaén, Sierra de Mágina, cerro Cárceles, vert. N., 1850 m, 17-VI-1988, G. Blanca, GDAC43855, HE603341, HE603264, HE603293, HE603313. ***P. spicata*** (L.) Bernh.; Spain: Granada, Albolote, Urbanización Cortijo del Aire, III-1984, C. Morales, GDAC32052, HE603342, HE603265, HE603294, *AJ493448*. ***P. tenuiloba*** Pomel; Spain: Almería, Sierra del Cabo de Gata, cerca de la Playa de Genoveses, 2-III-1984, C. Morales & al., GDAC32047, HE603340, HE603263, HE603292, HE603312. ***Pseudofumaria alba*** (Mill.) Lidén; Living collection, NBGB19763486, HE603344, HE603267, HE603296, HE603315. ***P. lutea*** (L.) Borckh.; Living collection, NBGB19891892, HE603343, HE603266, HE603295, HE603314. ***Rupicapnos africana*** (Lam.) Pomel; Spain, Málaga, Pantano del Chorro, 14-III-1998, M. E. González, GDAC44500, HE603325, HE603248, HE603278, HE603304. ***R. numidica*** (Coss. & Dur.) Pomel; Living collection, GBG19004473, HE603326, HE603249, *Z82954*, HE603305. ***Sarcocapnos baetica*** (Boiss. & Reuter) Nyman; Spain: Jaén, Sierra de Cazorla; 26/05/2010, A. Benamente, V. Suárez Santiago & G. Blanca, GDA56768, HE603331, HE603254, HE603283, *AJ250623*. ***S. crassifolia*** (Desf.) DC.; Morocco: Col du Zad, entre Azrou y Midelt, 03/05/1998, entre Azrou y Midelt, GDAC44364, HE603332, HE603255, HE603284, *AJ429195*. ***S. enneaphylla*** (L.) DC.; Spain: Granada, Vélez de Benaudalla, 7-IV-1997, M. J. Salinas & J. E. Linares, GDAC44351, HE603337, HE603260, HE603289, *AJ493442*. ***S. integrifolia*** (Boiss.) Cuatrec.; Spain: Jaén, La Cerradura, 7-V-1997, G. Blanca, M. J. Salinas & E. Linares, GDAC44352, HE603333, HE603256, HE603285, *AJ250621*. ***S. pulcherrima*** C. Morales & Romero García; Spain: Granada, Moclín, alrededores del castillo, 16-IV-1985, C. Morales, R. Mendoza & A. Ortega, GDAC22850, HE603334, HE603257, HE603286, *AJ250627*. ***S. saetabensis*** Mateo & Figuerola; Spain: Valencia, Castillo de Játiva, 200 m, 14-V-1997, G. Blanca & M. J. Salinas, GDAC44360, HE603336, HE603259, HE603288, *AJ250625*. ***S. speciosa*** Boiss.; Spain: Granada, Puerto la Ragua, 1700 m, 10-VI-1997, M. J. Salinas, C. Morales & L. Baena, GDAC44358, HE603335, HE603258, HE603287, *AJ250626*. ***Trigonocapnos lichtensteinii*** (Cham. & Schltdl.) Lidén; DNA from SANBI DNA Bank, LHMS1875, HE603346, HE603269, HE603298, HE603317.

4. Fumarioideae phylogeny 2

4. Evolutionary history of fumitories (subfamily Fumarioideae, Papaveraceae): an old story shaped by the main geological and climatic events in the Northern Hemisphere

Miguel A. Pérez-Gutiérrez, Ana T. Romero-García, M. Carmen Fernández, G. Blanca, María J. Salinas-Bonillo and Víctor N. Suárez-Santiago

Manuscript in review in *Molecular Phylogenetic and Evolution*

4.1 ABSTRACT

Biogeographical methods based on phylogenies constitute an excellent approach to understanding plant biogeographical models, identifying connections between floristic regions that elucidate the origin of plant diversity that they harbour. Fumitories (subfamily Fumarioideae, Papaveraceae) represent, by their wide mainly northern temperate distribution (also present in South Africa) a suitable plant group to use as a model system for studying biogeographical links between floristic regions of the Northern Hemisphere and also the Southern Hemisphere Cape region. However, the phylogeny of the entire Fumarioideae subfamily remains poorly understood. In this work, we infer a complete molecular phylogeny of Fumarioideae, which we use to interpret the biogeographical patterns in the subfamily and to establish biogeographical links between floristic regions, such as those suggested by its different inter- and intra-continental disjunctions. The phylogenetic analyses resolve the generic relationships in the group except the position of the genus *Pteridophyllum*. The tribe Hypecoeae is the sister group of tribe Fumarieae, this latter holding a basal grade of monotypic or few-species genera with bisymmetric flowers, and a crown group, Crown Fumarieae, of more diversified genera with zygomorphic flowers. The biogeographical analysis shows a subfamily that originated in East Asia at the end of the Early Cretaceous. From here, ancestral range expansions followed three different directions, one at the beginning of the Late Cretaceous by the ancestor of tribe Hypecoeae towards central Asia, and two during the Cretaceous-Palaeogene transition towards western North America and Indochina by the ancestor of the tribe Fumarieae. The ancestor of Crown Fumarieae expanded its range from East Asia into the Himalayas before to the middle Eocene. The uplifts of the Qinghai-Tibetan Plateau together the zonal climate pattern of the Palaeogene are suggested to be responsible both for the accelerated diversification rate resulting in the origin of the basal lineages of Crown Fumarieae as well as for the westward migration of the ancestor of the subtribe Fumariinae into the Irano-Turanian region. From here, Fumariinae reached South Africa during late Eocene and Mediterranean basin during Oligocene. There were two colonization waves of the Mediterranean following two different routes: a northern route during the early Oligocene by the *Sarcocapnos* Clade, probably facilitated by the land bridge resulting of the Mediterranean microplate accretion; and a southern route into North Africa, through the Gomphotherium land bridge, followed by the *Fumaria* Clade between late Oligocene and middle Miocene.

4.2 Introduction

A key aspect of the biogeographical studies is the elucidation of historical connections between different floristic regions to understand the genesis of plant diversity in these floristic regions (Linder, 2005). The establishment of biogeographical links between floristic regions requires the evaluation of the current patterns of plant distribution (MacLaughlin, 1994), and therefore key

taxa need to be studied. In this sense, the subfamily Fumarioideae Eaton (Papaveraceae Juss.) represents a suitable plant group to use as a model system for studying biogeographical links between Northern Hemisphere floristic regions, involving also the Southern Hemisphere Cape region. On the one hand, Fumarioideae shows a wide, mainly northern temperate distribution (also present in South Africa; Fig. 1) and, on the other hand, its species occupy both forest floor and open-dry habitats, being present in floristic regions with different macroclimatic conditions, enabling floristic connections to be tested between contrasting regions.

Molecular dating and ancestral-area reconstruction methods based on phylogenies provide better approaches to understand the plant biogeographical models and enable them to be interpreted in connection to the climatic geological events over the history of the Earth. This requires prior knowledge of the phylogenetic relationships. In this context, the phylogeny of Fumarioideae remains poorly understood, since no complete molecular study has been made, and only some partial phylogenies are available (e.g. Lidén et al., 1995; Lidén et al.1997; Salinas et al., 2003; Pérez-Gutiérrez et al., 2012).

Fumarioideae is one of the two subfamilies (together Papaveroideae Eaton) currently recognized in Papaveraceae (Wang et al., 2009), and includes two genera with controversial phylogenetic affinities in Papaveraceae, *Hypecoum* L. and *Pteridophyllum* Siebold & Zucc. (see Lidén, 1986, 1993a,b; Kadereit et al., 1994; Hoot et al., 1997; Zhang et al., 2008). It includes *c.* 590 species and 21 genera (Lidén, 1986; Lidén et al., 1997; Zhang et al., 2008) grouped into two tribes (Stevens, 2001 onwards): Fumarieae Dumort.(= Fumariaceae s.str.) and Hypecoeae Dumort. (*Hypecoum* + *Pteridophyllum*). Previously *Hypecoum* was placed either in Fumariaceae (as a subfamily or in Papaveraceae subfamily Fumarioideae without suprageneric status, Lidén, 1993a; Zang et al., 2008; respectively) or close to Fumariaceae s.str. (Takhtajan, 1997), and a sister-clade relationships between both groups was found on the basis of morphology and molecular data (Kadereit et al., 1994; Hoot et al., 1997); *Pteridophyllum* was considered the earliest-diverging lineage of Papaveraceae (Kadereit et al., 1994; Hoot et al., 1997). While Wang et al. (2009) seems to solve all these controversies, the classification proposed by them should be taken with caution as noted in Pérez-Gutiérrez et al. (2012). Thus, in Wang et al. (2009) the relationship between *Hypecoum-Pteridophyllum* and the rest of Fumarioideae (Fumariaceae s.str.) is obtained only by combining morphological data with molecular markers, since the latter alone did not resolve the *Hypecoum* and *Pteridophyllum* relationships. Only two morphological characters support that grouping: sepals not enclosing floral bud, and spinose exine (Wang et al., 2009), this latter being true only for *Hypecoum* and *Pteridophyllum*, and wrong for the rest of Fumarioideae representatives, which have non-spinose exines (Kalis, 1979; Blackmore et al., 1995; Pérez-Gutiérrez et al., unpubl. res.).

Classification within the tribe Fumarieae includes two main groups previously recognized at the tribal level by Lidén (1986, 1993a) within its subfamily Fumarioideae, and which should be considered subtribes after the Wang et al. (2009) emendation: Corydalinae Endl. (8 genera) and Fumariinae Endl. (11 genera). However, morphological and molecular analyses show Corydalinae to be a non-monophyletic group (Loconte et al., 1995; Lidén et al., 1997), while the

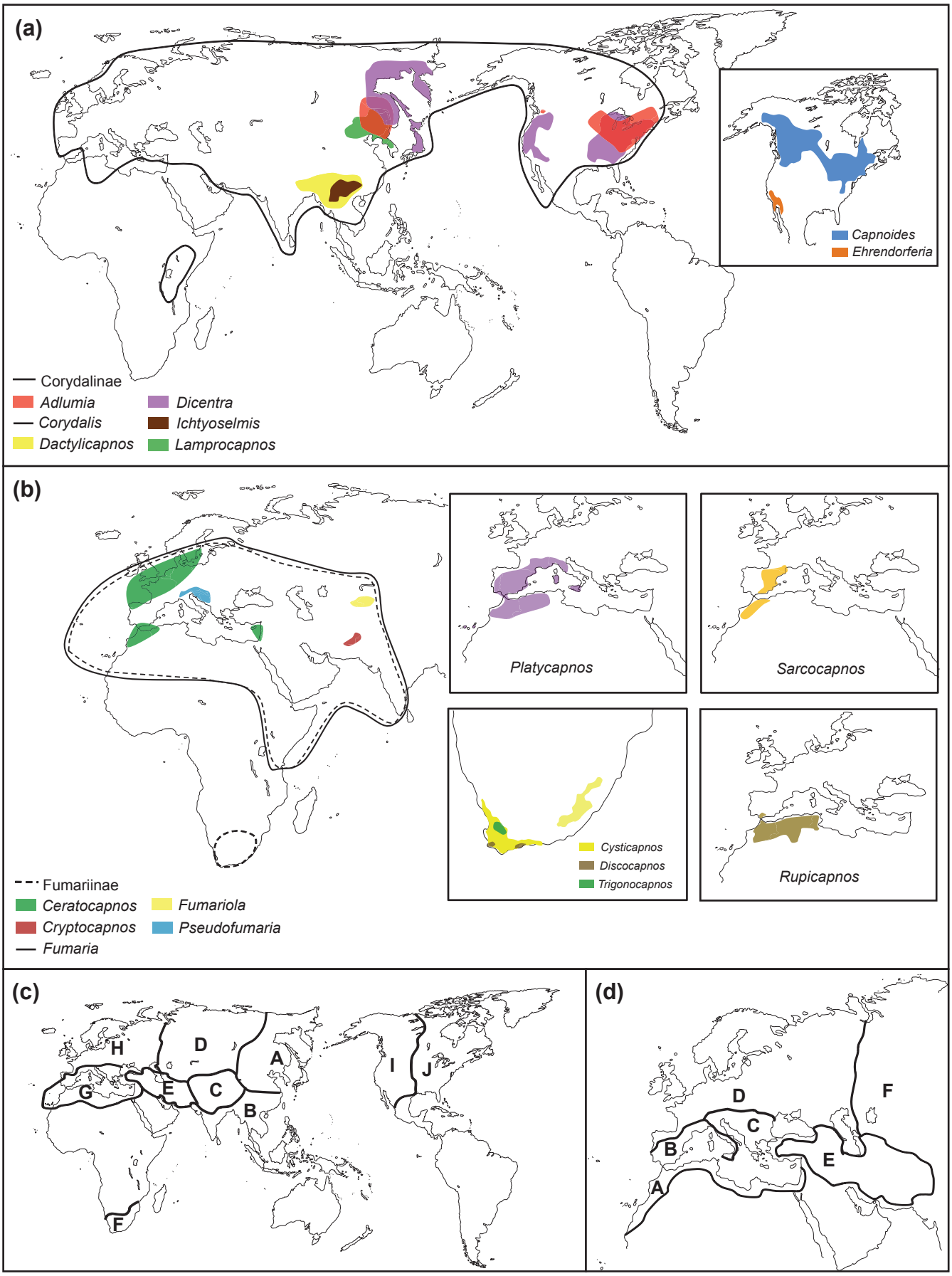


Figure 1. Geographic distribution of tribe Fumarieae and selected areas. (a) Distribution of genera from subtribe Corydalinae. (b) Distribution of genera from subtribe Fumariinae. (c) Areas defined for the ancestral range reconstruction analysis of the subfamily Fumarioideae: A, East Asia; B, Indochina; C, Himalayas; D, Central Asia; E, Irano-Turanian, F, South Africa; G, Mediterranean; H, Europe; I, western North America; J, eastern North America. (d) Areas defined in the ancestral range-reconstruction analysis focusing on the Mediterranean taxa of subtribe Fumariinae: A, southern Mediterranean basin; B, Western Mediterranean; C, Eastern Mediterranean; D, Central and Northern Europe; E, Irano-Turanian; F, Central Asia.

monophyly of Fumariinae has been asserted both with cladistics analysis of morphological characters (Lidén, 1986) and molecular phylogenetic analysis (Pérez-Gutiérrez et al., 2012).

According to Lidén (1986) subtribe Fumariinae (tribe Fumarieae *sensu* Lidén, 1986, 1993a) can be divided into three groups of genera on a morphological basis (this author described them at subtribe level: Discocapninae Lidén, Fumariinae, and Sarcocapninae Lidén). These three groups of genera were confirmed as three monophyletic lineages by Pérez-Gutiérrez et al. (2012) using chloroplast and nuclear DNA phylogenies.

Despite all the controversies surrounding subfamily Fumarioideae, few molecular phylogenetic studies are available on the relationships within Fumarioideae. Some of these works have addressed phylogenies on a particular genus (Lidén et al., 1995; Salinas et al., 2003), and no complete molecular phylogeny has been published. The first molecular approach to the phylogeny of Fumarioideae was that of Lidén et al. (1997) using the intron of the chloroplast *rps*16 gene. These authors focused mainly on the subtribe Corydalinae (tribe Corydaleae *sensu* Lidén, 1986, 1993a), and they not only found Corydalinae to be a non-monophyletic group, but also clarified many relationships among its genera and identified the basal lineages for the subfamily. Taxonomically, this study resulted in the split of *Dicentra* Bernh. in four genera (*Dicentra* s.str., *Ehrendorferia* T. Fukuhara & Lidén, *Ichtyoselmis* Lidén & T. Fukuhara and *Lamprocapnos* Endl.), and the recognition of three subgenera for a monophyletic *Corydalis* DC. [*Chremnocapnos* Wendelbo, *Corydalis* and *Sophorocapnos* (Turcz.) Fukuhara & Lidén]. Within the subtribe Fumariinae (tribe Fumarieae *sensu* Lidén 1986, 1993a), Lidén et al. (1997) found *Cysticapnos* Mill. (hitherto *incertae sedis*) to belong to Fumariinae, but as they included only three Fumariinae species no further conclusions could be drawn. Pérez-Gutiérrez et al. (2012) concentrated their phylogenetic analysis on the subtribe Fumariinae (as tribe Fumarieae *sensu* Lidén 1986, 1993a) using chloroplast and nuclear DNA markers. These authors asserted the monophyly of the subtribe, confirmed the existence of the three lineages inside, and established the generic relationships. Consequently, to date, no complete molecular study including all Fumarioideae genera has been published.

In the present work, we infer a complete molecular phylogeny of Fumarioideae in order to analyse the tribal and generic relationship in the subfamily as a whole. This allows us to interpret the biogeographical patterns in the subfamily and to establish biogeographical links between floristic regions, as those suggested by the various existing disjunctions at different taxonomic levels (Lidén, 1986, 1993a; Kadereit et al., 1994, 1995; Fig. 1).

Roughly, the subtribe Corydalinae (Fig. 1A; Table 1), distributed throughout Asia, North America, and Europe. Three genera show disjunct areas between Asia and North America (*Adlumia* DC., *Corydalis*, and *Dicentra*), since different disjunction patterns can be seen: i) East Asia/North America, between the sister groups *Ichtyoselmis* (*I. macrantha* (Oliv.) Lidén, exclusive from south-central China and Burma) and *Dicentra* (North America) (Lidén et al., 1997), within *Corydalis* subgenus *Sophorocapnos* section *Sophorocapnos* (Turcz.) Popov in Schischkin (widespread in North America), and within *Dicentra* with *D. peregrina* (Rudolph) Makino from East Siberia-Japan and the remainder from North America (within *Dicentra* also a

western North America/eastern North America disjunct pattern exists); ii) East Asia/eastern North America, in *Adlumia* with *A. fungosa* (Ait.) Britton, Sterns & Poggenb. from eastern North America and *A. asiatica* Ohwi restricted to Korea and Manchuria; iii) East Asia/western North America, in *Corydalis* subgenus *Corydalis* section *Archaeocapnos* Popov ex Michajlova; iv) Central to Arctic Asia/NW North America, in *Corydalis* subgenus *Corydalis* section *Dactylotuber* (Ruprecht) Popov in Schischkin. While most *Corydalis* are concentrated in the Sino-Himalayan area and the three above-mentioned sections (*Archaeocapnos*, *Dactylotuber*, *Sophorocapnos* reach North America, many species of different sections reach Europe and south-western Asia, and one species appears in the mountains of East Africa (*C. mildbraedii* Fedde). With regard to rest of the Corydalinae genera, *Lamprocapnos* is exclusive from Asia (NE China, N Korea, and SE Russia), *Ehrendorferia* from western North America, *Dactylicapnos* Wall. is centred in the Hymalayan region to W China, and the monotypic *Capnoides* Mill. is present in northern North America. The subtribe Fumariinae shows a strong Mediterranean-South Africa-Central Asia disjunction (Fig. 1B), since it is centred in the Mediterranean basin but the lineage *Cysticapnos-Discocapnos* Cham. & Schltdl.-*Trigonocapnos* Schltr. (subtribe Discocapninae sensu Lidén 1986, 1993a) is endemic of South Africa, and *Fumariola* Korsh. and *Cryptocapnos* Rech.f. are exclusive from Central Asia (Turkestan and Afghanistan, respectively).

Despite the noteworthy biogeographical pattern of Fumarioideae, very few studies, and only partial ones, have evaluated it in a phylogenetic framework, most of them being based on cladistics analyses of morphological data (focused either on small groups, e.g. *Rupicapnos* Pomel and *Sarcocapnos* DC., Lidén, 1986; or in a wider context on the whole Papaveraceae family, Zhuang, 1993; Kadereit et al., 1994, 1995). Only one study (Pérez-Gutiérrez et al., 2012) has centred on the subtribe Fumariinae (tribe Fumarieae sensu Lidén 1986, 1993a) using a molecular phylogeny to test ancestral ranges. Therefore, no biogeographical analysis including ancestral-area reconstruction and/or dating methods based on phylogenies has been made for the whole subfamily. The poorly understood phylogeny of Fumarioideae and the lack of fossil records have discouraged such studies.

In the present work, we use seven DNA regions [five from chloroplast DNA (*matK* gene, *trnL* intron, *trnL-F* intergenic spacer, *trnG* intron, and *rps16* intron) and two from nuclear ribosomal DNA (partial sequence of *26S* gene and ITS region)] to construct a phylogeny of the entire subfamily Fumarioideae in order to establish the relationships between all its genera. Using this phylogeny we interpret their biogeographical patterns through the analyses of ancestral areas and dating methods.

4.3 Materials and methods

4.3.1 Plant material

This study included 58 species representative of all genera of subfamily Fumarioideae *sensu* Wang et al. (2009). For outgroup species, we used *Eschscholzia californica* Cham. as representatives of the subfamily Papaveroideae and *Euptelea pleiosperma* Hook.f. & Thomson

and *Euptelea polyandra* Siebold & Zucc. as representatives of the family Eupteleaceae K.Wilh. (most basal lineage of Ranunculales; Kim et al., 2004; Worberg et al., 2007; Wang et al., 2009). Plant material was collected in the wild, from botanic gardens, and from herbarium material (Table S1).

4.3.2 DNA extraction, PCR amplification, and DNA sequencing

Total genomic DNA was extracted from fresh or dry leaves, and seeds following the CTAB method (Doyle and Doyle, 1987), and, using the NucleoSpin® Plant Kit (Macherey-Nagel GmbH & Co. KG, Düren, Germany), from difficult, old material.

The plastid markers (*matK* gene, *trnL* intron, *trnL-F* intergenic spacer, *trnG* intron, and *rps16* intron) and nuclear ribosomal regions (partial *26S* gene and ITS region) were amplified by polymerase chain reaction (PCR). The PCR amplifications were performed with following primer pairs: trnK2R (Johnson and Soltis, 1995), matK1166 (5'>GGCTTACTAATGGGAT<3') and matK192 (5'>CGGGTTGCAAMAATAAAGGA<3') for *matK* gene, primers C and F (Taberlet et al., 1991) were used to amplify the *trnL* intron and *trnL-F* spacer as a whole (hereafter trnL-F region), primers 3'trnG and 5'trnG2G (Shaw et al., 2005) for *trnG* intron, rpS16F and rpS16R (Shaw et al., 2005, modified from Oxelman et al., 1997) for the *rps16* intron, the set of primers N-nc26s10, 1229rev, 1839rev (Kuzoff et al., 1998) for *26S* gene, and finally the primers N-nc18s10 and C26A (Wen and Zimmer, 1996) were used for the ITS region. PCR reactions were made in a volume of 50 µL, under standard conditions (Innis et al., 1990) for the nuclear markers and under the recommended conditions for plastid markers (Taberlet et al., 1991; Johnson and Soltis, 1995; Shaw et al., 2005). Automated sequencing of the purified PCR products was performed in both directions using the amplification primers on a 3100-Avant Genetic Analyzer (Applied Biosystems, Foster City, California, USA) in the Centro de Instrumentación Científica of the University of Granada (Spain).

All sequences used in Pérez-Gutiérrez et al. (2012) plus 19 sequences of Fumarioideae and outgroup species (four of *matK*, one of trnL-F region, seven of *rps16* intron, three of *26S* gene and four of ITS region) were taken from Genebank (Table S1). All sequences generated as part of this study were deposited in the Genebank database (Table S1).

4.3.3 Phylogenetic analyses

Nucleotide sequences were edited and aligned with SEQMAN II version 3.61 and MEGALIN version 3.18 programs, respectively, from the DNAstar software package (DNASTAR Madison, Wisconsin, USA) and then adjusted by eye. One (28pb), four (75pb), six (123pb), six (95pb), four (135pb), and two (12pb) regions of the *matK*, *trnG*, trnLF, *rps*16, ITS, and *26S* aligned matrices, respectively, were ambiguous and excluded from analyses. Moreover, due to the high sequence variability shown by the ITS1, we excluded it from the analyses. To test the congruence between data sets, we performed the incongruence-length difference test (ILD; Farris et al., 1994). The ILD was implemented in PAUP* version 4.0b10 as the partition

homogeneity test (Swofford, 2003), using 100 replicates with 1000 random addition sequences each.

Phylogenetic analyses of the whole group included two independent analyses, one with all plastid data combined, and one with *26S* and ITS region (*5.8S*-ITS2) combined. The number of sequences included in each data matrix varied due to the inability to determine some sequences for several taxa (20 sequences in total: four of *matK*, three of *rps16,* 10 of *26S*, and three of ITS; Table S1).

Phylogenetic relationships were inferred using Maximum Parsimony (MP) as implemented in PAUP* 4.0b10 and Bayesian Inference (BI) using MrBayes version 3.1.2 (Ronquist and Huelsenbeck, 2003). Gaps were treated as missing data. The MP analyses used heuristic searches with 1000 replicates of random sequence addition using tree bisection-reconnection (TBR) branch swapping under the Fitch criterion (unordered states and equal weights). Only 10 trees were maintained at each step, to minimize the time the algorithms spent searching for trees on suboptimal islands. The starting tree was constructed by stepwise addition. Finally, 1000 bootstrap replicates (BS: Felsenstein, 1985) with 10 heuristic searches, as above, were performed to assess internal support for nodes. The amount of phylogenetic signal in the analyses was given by consistency index (CI: Kluge and Farris, 1969) and the retention index (RI: Swofford, 1993).

Bayesian analyses were implemented using the best-fit nucleotide substitution model for each data set: GTR+I+G (nst = 6; rates = invgamma; statefreqpr = dirichlet) for plastid, *26S* and ITS2, and K80+I (nst = 2; rates = propinv; statefreqpr = fixed (equal)) for *5.8S*. These models were selected using MrModeltest version 2.3 (Nylander, 2004) and Akaike's information criterion (Akaike, 1973). A partitioned model was used for the combined analysis, which included that selected for the independent data sets. The analyses were based on 2 million generations with four simultaneous runs (16 Markov chain Monte Carlo chains) starting from random trees that were sampled every 100 generations. The stationary of the runs and the convergence between runs were checked with Tracer version 1.5 (Rambaut and Drummond, 2007). The initial 25% of the trees that resulted were discarded as burn-in, and the remaining trees were used to build 50% majority-rule consensus trees.

4.3.4 Divergence-time estimation

To estimate the divergence times of the Fumarioideae lineages, we used a Bayesian relaxed-clock method as implemented in the BEAST 1.7.5 package (Drummond et al., 2012) for the chloroplast-combined datamatrix. For tree calibration, two fossils assigned to Papaveraceae were considered, but the ambiguity in their assignation precludes us from using them as calibration points. One of them, *Palaeoaster* Knowlton (Smith, 2001), an alleged member of Papaveraceae, was recently used by Valtueña et al. (2012) to date Papaveraceae in their population study on *Meconopsis cambrica* Vig.; however, that fossil has been recognized to be close to the order Bennettitales and not to Papaveraceae (Little et al., 2010; Manchester, personal communication). On the other hand, recently Jud and Hickey (2013) have published

leaf fossils from the Lower Cretaceous (from Aptian sediments in Dutch Gap, Virginia, USA), which they assigned to the species *Potomacapnos apeleutheron* Jud & Hickey within the subfamily Fumarioideae. In the cladistic analysis performed by these authors, *P. apeleutheron* includes in the tribe Fumarieae. However, the authors on the basis of the age of the fossils and their fragmentary nature could not rule out the possibility of a phylogenetic position of *P. apeleutheron* as a ranunculalean-or eudicot-stem lineage instead of a Papaveraceae, preventing its use as phylogeny calibration point. Therefore, due to the lack of fossils for Fumarioideae, we followed a two-step strategy for tree calibration.

An initial analysis included sequences of external taxa belonging to all families of Ranunculales order and two species of Ceratophyllales. For this analysis the genes *matK* and *rbc*L were used due to its higher availability in the nucleotide database (Table S1). The inclusion of Ceratophyllales allows us to introduce as calibration point the split of Eudicots, while the Ranunculaceae Juss. and Menispermaceae Juss. representatives allow us to include the fossils both of Ranunculaceae stem lineage from the Early Cretaceous, *Leefructus mirus* Sun, Dilcher, Wang et Chen (Sun et al., 2011), and of the Menispermaceae stem lineage *Prototinomiscium vangerowii* Knobloch & Mai from the Late Cretaceous (Knobloch and Mai, 1986). Therefore three calibration points were used, the first one by constricting Ranunculales origin to the range 131-147 Ma (Wikström et al., 2001), and the other two through a minimum age for Ranunculaceae and Menispermaceae stems of 122.6-125.6 Ma and 91 Ma, respectively.

We used the Wikström et al. (2001) date, based in tricolpate pollen fossil, and no other dating for eudicots (Anderson et al., 2005; Bell et al., 2010) because these other dates are not consistent with the data of the recently discovered Ranunculaceae stem lineage fossil. The partitioned .xml file was made up in BEAUty v1.7.5 (Drummond et al., 2012) by means the selection a GTR model and a four-categories-gamma-shape distribution with invariant sites for the datamatrix, an uncorrelated lognormal relaxed-clock model (Drummond et al., 2006) and a Yule speciation process as the tree priors. BEAST v1.7.5 (Drummond et al., 2012) was launched with 50 million generations sampling one tree and parameters every 1000 generations. Tracer v1.5 (Rambaut and Drummond, 2007) was employed to check chain convergence and effective sampling size of the parameters. The maximum clade credibility tree summarizing the estimated mean age and the 95% confidence intervals from post-burn-in (10%) trees was calculated with TreeAnnotator v1.7.5 (Drummond et al., 2012).

The second analysis was focused on the subfamily Fumarioideae using the chloroplast-combined matrix. For phylogeny calibration, we used the dates from the first analysis as calibration points; the split between Eupteleaceae and rest of Ranunculales, and crown Papaveraceae were used according a normal distribution covering the standard deviation of the highest posterior densities previously found (HPD; 138.01-125.99 Ma and 116.14-99.27 Ma, respectively). BEAST package was used following the same procedure described above.

4.3.5 Biogeographical analysis

To reconstruct the biogeographical history of Fumarioideae, we used the dispersal-extinction-cladogenesis method (DEC) implemented in Lagrange v 2.0.1 (Ree et al., 2005; Ree and Smith, 2008). This method develops parametric likelihood analyses, and it allows data to be included both from dated phylogenies and from palaeogeographic models reflecting the history of the Earth in different time frames. Lagrange takes into account this information, estimating the dispersal and extinction indexes for the whole group and then it computing relative probabilities of inherited areas for each node on the topology. We used the maximum credibility tree derived from the BEAST analysis and performed two independent analyses: i) one on the whole Fumarioideae subfamily, and ii) other focused only on the Mediterranean lineages of the subtribe Fumariinae.

To delimit the biogeographical areas, we defined regions in the framework of the current continents (Buerki et al., 2011), and subdivided these regions according to the current distribution of Fumariodeae taxa and its specific richness. For the biogeographical analysis of the entire subfamily Fumarioideae, we used 10 areas (Fig.1c): (A) East Asia, including eastern Siberia, Manchuria, Korea, Japan and central and east of China, reaching the Verkhoyanks Range in the west, the Lake Baikal in the north, and the Gobi Desert in the south; (B) Indochina, including south and east of China, India and Indochina; (C) Himalayas, including the Qinghai-Tibetan Plateau (QTP) and all mountain areas that delimit it (Qilian and Kunlun in the north, Hymalayan in the south, Karakorum in the west, and Hengduan in the east); (D) Central Asia, covering the region from Ural mountains in the west, to the contact zone of the Iranian Plateau and the Pamir Mountains in the south, and to the limits of regions A and C in the south-east and east, respectively; (E) Irano-Turanian, from the Anatolia Peninsula to the western Himalayas, covering the Caucasus, the Levant region, Iran and Iraq; (F) South Africa, from southern Namibia to the Lesotho region southwards; (G) the Mediterranean, including the Mediterranean basin and Canary Islands; (H) Central and Northern Europe, from the Cantabrian region of the Iberian Peninsula northwards and eastwards to the Ural mountains; (I) western North America, including the western half of North America and north-western Mexico; and (J) eastern North America, covering the eastern half of North America. Using these areas, we tested two different models in the DEC analysis: the first one (M0) without dispersal constraint between areas over time (with equal rates of dispersal among areas), and the second one (M1) for which a time stratification was defined along Fumarioideae history. Thus for M1 model, four time intervals were established (before 80 Ma, 80-40 Ma, 40-20 Ma, 20-0 Ma) following Buerki et al. (2011), and a specific dispersal-rate matrix was defined for each in order to reflect the main palaeogeographical connections between landmasses (Fig. S2). The dispersal-rate values among areas were inversely proportional to the number of areas and/or physical barriers (i.e. water masses) in between. The maximum number of permitted ancestral areas for the analysis was three, because most of the sampled species currently occur in no more than three areas. The Phyton scripts were completed in the online Lagrange configurator (http://www.reelab.net/lagrange). Taxa distribution was assigned following Lidén (1986), Stern

(1997) and Zhang et al. (2008). Due to the high number of species in *Corydalis* (*c*. 465; Zhang et al., 2008), and the low number included in our analysis, we repeated the analyses condensing the included species by subgenera in order to provide a better generic-distribution representation. The subgenus distribution areas were established according to their specific richness (ABCH for *Corydalis* subgenus *Corydalis*, CDE for *Corydalis* subgenus *Chremnocapnos* and ABC for *Corydalis* subgenus *Sophorocapnos*; the initial tree for launching Lagrange was accordingly modified). In this way, we could test whether the insufficient sampling of this genus could bias the result of the ancestral area reconstruction. With the original analyses, i.e. considering the distribution for each *Corydalis* species sampled, we were able to evaluate the North America/Asia connection shown by the subgenus *Corydalis* section *Archaeocapnos*.

With regard to the second biogeographical analysis focused on the Mediterranean region and adjacent territories, we defined the following operational areas on the basis of patterns of endemicity among current taxa and maximizing congruence with the Mediterranean biogeographical pattern and palaeogeographic history (Fig. 1d): (A) the southern Mediterranean basin, including North Africa, the northern portion of the Sahara region and the Canary Islands; (B) Western Mediterranean, covering Mediterranean region of Iberian Peninsula, southern France, west and southern Italy and islands of the central Mediterranean; (C) Eastern Mediterranean, including the Balkans Peninsula, the Alps, and northern Italy; (D) Central and Northern Europe, from northern Spain; (E) Irano-Turanian, as previously defined; and (F) Central Asia, as previously defined. Two different models were also tested, an unconstrained model (MM0) and a second one with constrained dispersal rates stratified into three time intervals (MM1). For the latter the time intervals (35-25 Ma, 25-14 Ma and 14-0 Ma) were defined considering both the dates of the main diversifications of the Mediterranean representatives of subtribe Fumariinae, and the geological events described for this region (Rögl, 1999; Meulenkamp and Sissingh, 2003; Ree and Sanmartín, 2009). The exchange rates for each period were based on those in Ree and Sanmartín (2009) adjusted to our Mediterranean subdivision (see Fig. S3). Lagrange analysis was conducted as described above.

4.4 Results

4.4.1 Phylogenetic analyses

The only ILD test rejecting the data-set combination was the one performed on the combination of nuclear and plastid markers (P= 0.002); all remainder ILD test failed to reject the combinations tested. This result was consistent with the strong incongruences detected between the trees resulting from the nuclear and plastid independent analyses. Therefore, we did not combine the two data sets. The alignment features and tree statistics for parsimony analyses are shown in Table 1.

Bayesian and maximum-parsimony analyses of the chloroplast data set yielded almost the same topologies and same level of node support. Figure 2 shows the Bayesian tree including the posterior probabilities (PP) and the bootstrap (BS) values. Almost all relationships were strongly supported (>70% BS; >0.90 PP). Only three differences were found between BI and MP analyses: i) in the parsimony analysis *Pteridophyllum racemosum* Siebold & Zucc. form a weakly supported group (BS: 57) with the genus *Hypecoum* (taxa not grouped in the BI), ii) *Rupicapnos* grouped, with low PP (0.61), with the clade *Cryptocapnos-Fumariola* in the Bayesian analysis; while in the parsimony analysis the relationships between *Cryptocapnos-Fumariola*, *Fumaria* L., and *Rupicapnos* were not resolved (all three possible combinations resulted in the most-parsimonious trees); and iii) *Sarcocapnos pulcherrima* C. Morales & Romero García and *S. speciosa* Boiss. were sister groups, and *S. crassifolia* DC. was sister to the group formed by the two of them in the Bayesian analysis; while in the parsimony analysis this relationship appeared in half of the six most parsimonious trees, with *S. crassifolia* and *S. integrifolia* (Boiss.) Cuatrec. being sister groups in the other three trees. All species were grouped by generic affinity, except those of *Cysticapnos* which appeared as paraphyletic. Relationship of *Pteridophyllum racemosum* to the remainder Fumarioideae subfamily was weakly supported, as also occurs with its relationship to *Hypecoum* (only supported by the parsimony analysis; BS: 57). Fumarioideae (excluding *Pteridophyllum*) appeared as a strongly supported monophyletic group (PP: 1; BS: 100 when analysis excluded *Pteridophyllum*, data not shown). The tree confirmed the paraphylia of the subtribe Corydalinae, since most of its genera appeared, forming a grade of basal Fumarieae lineages (Fig. 2). The remaining genera formed a strongly supported clade (Crown Fumarieae), with well-resolved relationships inside, including *Capnoides*, *Dactylicapnos*, *Corydalis*, and all genera of subribe Fumariinae grouped in three clades (*Cysticapnos*, *Fumaria*, and *Sarcocapnos*). All relationships were strongly supported except that of the *Sarcocapnos* Clade, which was weakly supported in the parsimony analysis (BS: 51%) but strongly supported in the Bayesian analysis (PP: 0.93). Generic relationships within subtribe Fumariinae were all well resolved except those for the *Fumaria* Clade (Fig. 2).

Table 1. Comparative table of the alignment features, most parsimonious tree statistics for the different data sets, and nucleotide evolution model selected. Al, alignment length; CD, number of ambiguous characters deleted from analyses; PI, number of parsimony-informative characters; Var, number of variable characters; NT, number of most parsimonious trees; L, length of the most parsimonious trees; CI, consistency index; RI, retention index.

	Al	CD	PI/Var	NT	L	CI	RI	Model Selected
cpDNAmatrix	4867	321	1236/1908	6	3700	0.6981	0.8148	GTR+I+G
26S	1762	12	139/251	107	521	0.5643	0.6856	GTR+I+G
5.8S/ITS2	451	115	90/133	10,000	427	0.5633	0.6574	K80+I/GTR+I+G
rDNAmatrix	2213	127	229/384	10,000	974	0.537	0.6531	GTR+I+G/K80+I

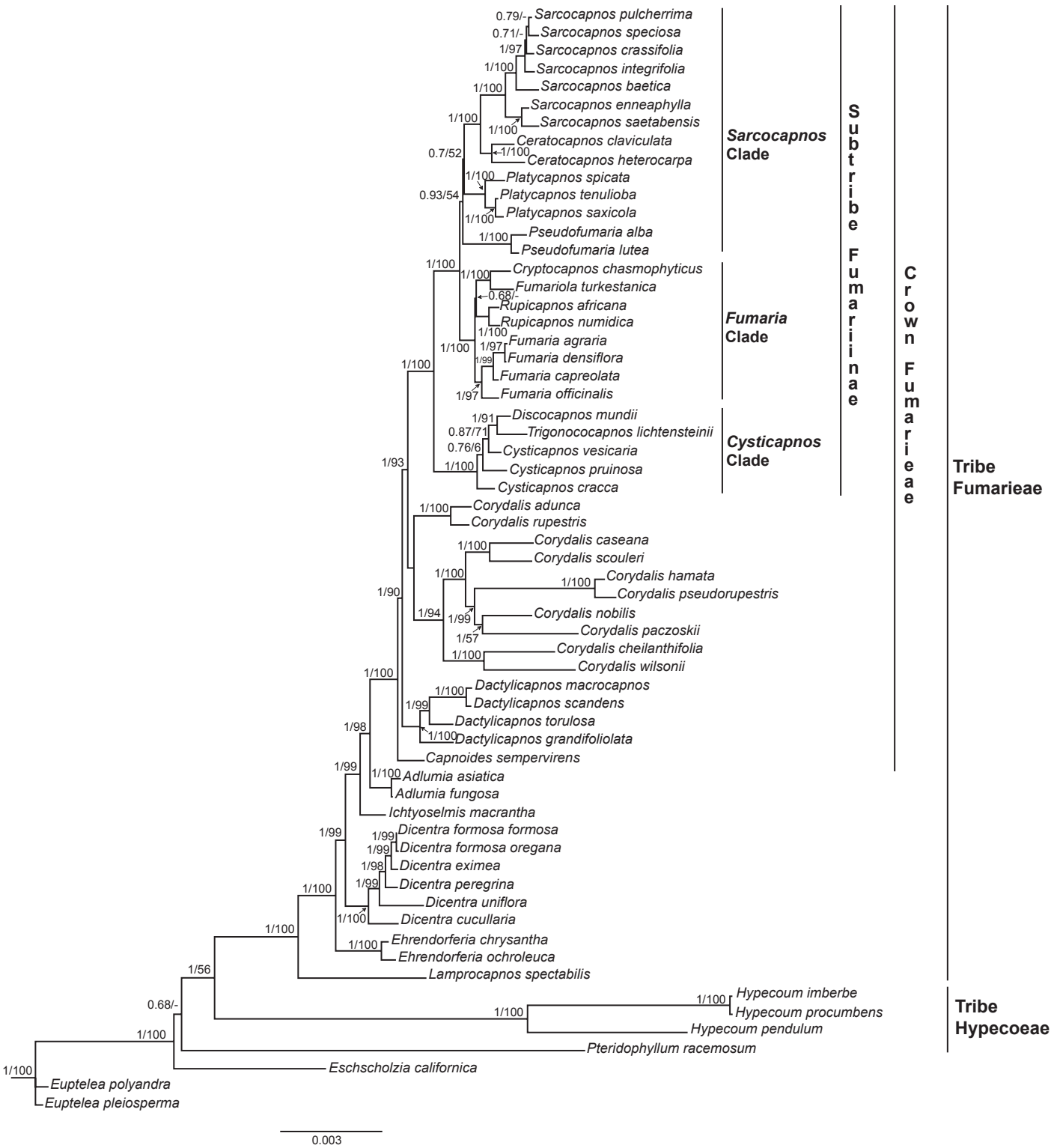


Figure 2. Bayesian 50% majority-rule consensus tree inferred from the combined plastid data matrix. Posterior probabilities and bootstrap values are shown above branches (PP/BS). Hyphens show the branches collapsed in the strict consensus tree from the parsimony analysis. Classification and groupings are indicated on the right.

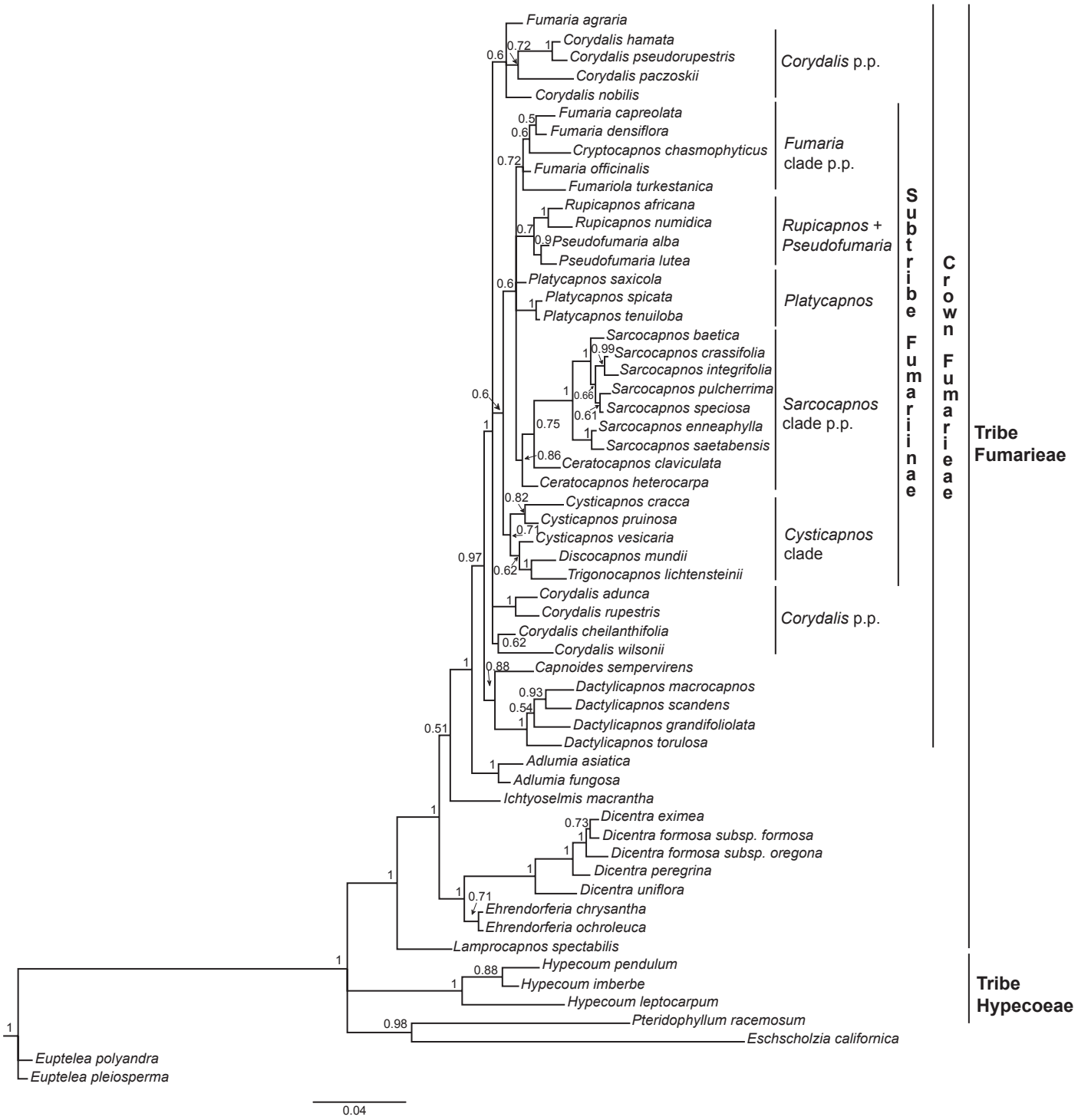


Figure 3. Bayesian 50% majority-rule consensus tree inferred from the combined nuclear ribosomal datamatrix. Posterior probabilities are shown above branches. Classification and groupings are indicated on the right.

Phylogenies found from the parsimony and Bayesian analyses of the combined nuclear ribosomal markers were highly congruent, with only some occasional weakly-supported conflictive relationships inside the Crown Fumarieae clade (Fig. 3; Fig. S4). However, they differed in the support level of the internal nodes; parsimony analysis did not support any internal node, while Bayesian analysis strongly supported almost all internal nodes (Fig. 3; Fig. S4). In general, ribosomal phylogeny resolved much less than did the chloroplast one, especially within the Crown Fumarieae clade; here, several species were not grouped by generic affinity (*Ceratocapnos* Dur., *Corydalis*, *Cysticapnos*, *Fumaria*, *Platycapnos* (DC.) Bernh.), and the generic relationships were not well defined. From the three clades defined within the subtribe Fumariinae in the chloroplast tree only the *Cysticapnos* Clade (BS: 58, PP: 0.71) appears in the ribosomal phylogeny, while the *Sarcocapnos* Clade and *Fumaria* Clade were only partially defined, excluding *Platycapnos* and *Pseudofumaria* Medik., and *Rupicapnos* respectively. *Dactylicapnos* was a monophyletic genus and sister to *Capnoides* (BS: 61; PP: 0.88). Outside the Crown Fumarieae, the ribosomal tree showed the same basal lineages of Fumarioideae as the chloroplast tree with few differences in the relationships between lineages (*Dicentra* and *Ehrendorferia* were sister groups, and *Pteridophyllum* was related to the representative of subfamily Papaveroideae; Figs. 2, 3).

4.4.2 Divergence date estimates and biogeographical analyses

The first step in the divergence-time estimates resulted in the divergence of the family Eupteleaceae from the rest of Ranunculales lineages 130 Ma in the Hauterivian from the Early Cretaceous, with the split of the family Papaveraceae also in the Early Cretaceous (Barremian, 129 Ma) and its crown group originating 107 Ma in the Albian from the end of the Early Cretaceous (Fig. S5). Using the dates for the split of Eupteleaceae and for the crown Papaveraceae as secondary calibration points, and our chloroplast phylogeny, we dated all Fumarioideae nodes (Fig. 4a; Fig. S6).

Unconstrained and constrained biogeographical analyses were congruent across all nodes, resulting in the same ancestral area reconstructions [Table S7 and Table S8 (analysis collapsing the *Corydalis* species by subgenera), in on-line attachments]. The areas and their relative probabilities estimated in the constrained analysis are presented below and shown in Fig. 4a. The detailed biogeographical analysis of the *Sarcocapnos* and *Fumaria* clades is shown in Fig. 4b and Table S9.

Our results show a complex biogeographical history as the result of multiple dispersal events both intercontinental and intracontinental in different directions and time periods (Figs. 4a,b, S6; Table 2, TablesS7-S9). Four dispersal trends between East Asia and North America were detected (two from East Asia to North America and two in the opposite direction), four from western North America into eastern North America, at least eight dispersions between different regions of Asia (mainly from East Asia into other regions; without considering *Corydalis*), one from the Irano-Turanian region into South Africa, four dispersions between Mediterranean basin

and other regions (Central Asia, Irano-Turanian, and North Europe), and multiple intra-Mediterranean dispersals.

4.4.3 Origin and initial diversification of Fumarioideae

Crown Fumarioideae (*Pteridophyllum* excluded) was dated during the transition between the Early and Late Cretaceous (96 Ma; Figs. 4a, S6, node 116), with East Asia being the geographical range of the ancestor (Fig. 4a). The origin of both Fumarioideae tribes was estimated in East Asia for Fumarieae and Central Asia for Hypecoeae (Fig. 4a, node 116). The latter implies the dispersion of the Fumarioideae ancestor from eastern to Central Asia followed by a vicariance in the second half of the Early Cretaceous (Fig. 4a). Before the diversification of *Hypecoum* during the Eocene a range expansion into the Irano-Turanian and Mediterranean regions occurred in the stem lineage of the genus in the Late Cretaceous-Eocene (96-41 Ma; Fig. 4a; Table 2). The crown group of the tribe Fumarieae dates back to 76 Ma in the Late Cretaceous (Fig. 4a, node 115). Our results show that during the diversification of tribe Fumarieae the lineages have originated at different velocities.

4.4.4. Basal lineages of Fumarieae

Origins of the basal lineages occurred in a progressive way within a time window of around 26 Ma (76-50 Ma; Figs. 4a, S6, nodes 115-96). After the origin of the *Lamprocapnos* lineage, the ancestor of the remainder of Fumarieae expanded its range in two ways between 76-64 Ma (Fig. 4a, node 114; Table 2): to Indochina, and into western North America. It was in western North America where the ancestors of *Ehrendorferia* and *Dicentra* originated. The origin of *Ichtyoselmis* lineage was in Indochina 54 Ma after a vicariance event between this region and the East Asia/western North America area (Fig. 4a, node 98). The stem lineage of *Adlumia* was dated back 50 Ma in western North America (Figs. 4a, S6, node 96). *Dicentra* diversified in western North America at the beginning of the Miocene (22 Ma). Two intra-continental (from western into eastern North America) and one inter-continental (from western North America into East Asia between 22-14 Ma) dispersal events were detected in *Dicentra* (Fig. 4a; Table 2). Two range expansions toward eastern North America and East Asia were also detected in the stem lineage of *Adlumia* from western North America (Fig. 4a; Table 2). A vicariance was responsible of the origin of *A. asiatica* in East Asia around 3 Ma (Fig. 4a).

4.4.5 Crown Fumarieae

The ancestor of Crown Fumarieae should occupy a wide area including East Asia and western North America (Fig. 4a, node 95). Diversification began in the Eocene (44 Ma; Figs. 4a, S6), resulting in the split by vicariance of *Capnoides* lineage in western North America (after that the stem lineage of this species dispersed into eastern North America; Fig. 4a; Table 2) and the ancestor of the remainder lineages occupying East Asia and Himalayas. Presence in the Himalayas of this latter implies a previous range expansion of the ancestor of Fumarieae from East Asia (Fig. 4a, node 95; Table 2). After this initial split, the remaining main Fumarieae

Table 2. Biogeographical links between different regions as shown by the dispersal events that occurred during Fumarioideae evolution. Node labels correspond to Fig. 4; when superscript they refer to Fig. 4b. Direction reflects the path of the dispersal. Temporal range considers the mean age value between nodes for each stem lineage, as shown in Fig. S6. Biogeographical areas: CA, Central Asia; EA, East Asia; EM, eastern Mediterranean; ENA, eastern North America; Hi, Himalayas; IC, Indochina; IT, Irano-Turanian region; M, Mediterranean basin; NAf, North Africa; NE, Central and North Europa; SAf, South Africa; WM, western Mediterranean; WNA, western North America.

Biogeographical connection	Node label	Stem lineage	Direction	Time range
East Asia / North America	115-114	*Ehrendorferia* + rest Tribe Fumarieae	EA>WNA	Late Cretaceous-Palaeocene (76.06-63.55 Ma)
	96-12	*Adlumia*	EA>WNA	Cenozoic (49.84-2.34 Ma)
	34-30	*Corydalis* sect. *Archaeocapnos*	WNA>EA	Eocene-Oligocene (31.91-26 Ma)
	109-108	*Dicentra uniflora* + rest *Dicentra*	WNA>EA	Miocene (22.23-13.67 Ma)
Western North America / Eastern North America	96-12	*Adlumia*	WNA>ENA	Cenozoic (50.12-2.74 Ma)
	95	*Capnoides*	WNA>ENA	Cenozoic (44.57 Ma onwards)
	109-108	*Dicentra uniflora* + rest *Dicentra*	WNA>ENA	Miocene (22.23-13.67 Ma)
	109	*Dicentra cucullaria*	WNA>ENA	Neogene (22.23 Ma onwards)
Intra-Asiatic	117-116	*Hypecoum* and Fumarieae	EA>CA	middle Cretaceous (106.48-96.15 Ma)
	116-8	*Hypecoum*	CA>IT	Cretaceous-Palaeogene (96.15-41.34 Ma)
	115-114	Tribe Fumarieae	EA>IC	Late Cretaceous-Palaeocene (76.06-63.55 Ma)
	96-95	Crown Fumarieae	EA>Hi	middle Eocene (44.42-42.4 Ma)
	93-92	*Corydalis* + Fumariinae	Hi>IT	middle Eocene (42.4-40.39 Ma)
	17-16	*Dactylicapnos macrocapnos* + *D. scandens*	Hi>IC	Miocene (16.6-2.34 Ma)
	17	*Dactylicapnos torulosa*	Hi>IC	Neogene (16.6 Ma onwards)
	37-36[a]	*Cryptocapnos-Fumariola* group	IT>CA	Miocene (14.98-8.58 Ma)
South African	92-91	Fumariinae	IT>SAf	Eocene (40.39-33.33 Ma)
Mediterranean	116-8	*Hypecoum*	CA>M	Cretaceous-Palaeogene (96.15-41.34 Ma)
	91-90[a]	*Fumaria* Clade + *Sarcocapnos* Clade	IT>EM	Oligocene (33.33-26.25 Ma)
	90-74[a]	*Sarcocapnos* Clade	EM>WM	Oligocene (26.25-24.51 Ma)
	90-89[a]	*Fumaria* Clade	IT>NAf	Oligocene-Miocene (26.25-16.3 Ma)
	72-71	*Ceratocapnos*	M>NE	Miocene (19.01-12.08 Ma)

lineages appeared in a very short time window, around 4 million years during the second half of the Eocene (44-40 Ma; Figs. 4a, S6, nodes 93-92). Close to this period *Corydalis* also diversified (38 Ma; Figs. 4a, S6, node 38). The genus *Dactylicapnos* originated in the Himalayas 24 Ma ago (Fig. 4a, node 19); within this genus two independent range expansions into Indochina were detected (Fig. 4a; Table 2). The reconstructed ancestral areas for both the stem lineage of *Corydalis* (East Asia/Himalayas/Irano-Turanian) and the subtribe Fumariinae (Irano-

Turanian region; Fig. 4a, node 92) show the westward range expansion into the Irano-Turanian region of their most recent common ancestor (MRCA) (Fig. 4a, node 92; Table 2). After the diversification of *Corydalis* subgenus *Chremnocapnos*, the split between subgenera *Sophorocapnos* and *Corydalis* occurred 32 Ma ago; the crown group of subgenus *Corydalis* was dated back to 26 Ma. Within the genus *Corydalis*, different dispersal events have been detected, from East Asia into Indochina and into Central Asia, and from Central Asia into Northern Europe and into Himalayas, but highlight a dispersion of the ancestor of subgenus *Corydalis* from East Asia into western North America during the Oligocene (Figs. 4a, S6, nodes 34-30; Table 2), explaining the presence there of the section *Archaeocapnos*.

The ancestor of subtribe Fumariinae expanded its area from the Irano-Turanian region towards South Africa and also towards the Mediterranean basin (Fig. 4a, nodes 91 and 90). Split between the Irano-Turanian/Mediterranean and the South African lineages was dated at 33 Ma (Eocene-Oligocene transition; Figs. 4a, S6, node 91); while the diversification of the South African group (crown *Cysticapnos* Clade) took place approximately 17 million years afterwards, in the middle Miocene (Figs. 4a, S6, node 47).

4.4.6 Mediterranean Fumarieae

Ancestor of Mediterranean Fumarieae was dispersed in the Irano-Turanian region during Oligocene (Fig. 4b). Split resulting in the *Sarcocapnos* Clade and *Fumaria* Clade lineages was dated to 26 Ma ago (Figs. 4b, S6, node 90). While the *Fumaria* Clade lineage inherited the area of the ancestor, the *Sarcocapnos* Clade ancestor originated in the eastern Mediterranean, involving a previous westward expansion of the Mediterranean taxa ancestor from the Irano-Turanian region through the north of the Mediterranean basin between 33-26 Ma (Fig. 4b, nodes 91-90; Table 2). From here, this latter ancestor reached the western Mediterranean basin in less than 2 million years (Figs. 4b, S6, nodes 90-74; Table 2); afterwards, the western Mediterranean lineages and the eastern Mediterranean lineage (stem lineage of *Pseudofumaria*) split by vicariance (Fig. 4b, node 74). Conversely to *Sarcocapnos* ancestor, that of *Fumaria* Clade colonized the Mediterranean basin from Irano-Turanian region by expansion westward through North Africa (26-16 Ma, Oligocene-Miocene; Figs. 4b, S6, nodes 90-89; Table 2). *Fumaria* diversified in North Africa 11 Ma ago, from where it expanded its range towards other Mediterranean areas (Fig. 4b, node 88). Lineage of chasmophytes originated in North Africa and Irano-Turanian region, and diversified 15 Ma by vicariance, resulting in the ancestor of *Rupicapnos* in North Africa and the ancestor of *Cryptocapnos* and *Fumariola* in the Irano-Turanian region (Fig. 4b, node 81). This latter expanded its range by dispersal into Central Asia (between 15 and 9 Ma) and originated the ancestors of *Cryptocapnos* and *Fumariola* by vicariance (Fig. 4b, node 77).

4.5 Discussion

Here, we present a phylogenetic analysis of the subfamily Fumarioideae *sensu* Wang et al. (2009), including all its genera; until now the most complete molecular phylogeny made for this plant group. Results found with chloroplast and nuclear markers were highly incongruent with regard to the relationships within Crown Fumarieae, while they were almost congruent in the Fumarioideae basal lineages. Incongruence between chloroplast and nuclear Papaveraceae trees was also detected in Hoot et al. (1997). These cases of incongruence may come from both the low resolution of the *26S* gene [few informative characters, 139 from 1762, and high homoplasy (CI: 0.56, RI: 0.69, RC: 0.39 for independent analysis)], and from the high saturation degree of the ITS region [133 characters variable from 336 used (115 excluded as ambiguous); CI: 0.56, RI: 0.66, RC: 0.37 for independent analysis] (Table 1). Therefore, the discussion presented below focuses mainly on the chloroplast analysis.

Our results enable us to evaluate the generic boundaries and to clarify the relationships among Fumarioideae genera. The phylogeny has provided a basis for a detailed biogeographical analysis of this widely distributed subfamily with noteworthy intercontinental disjunctions.

4.5.1 Relationships of Pteridophyllum with the rest of Fumarioideae

Pteridophyllum is a monotypic genus endemic from Japan and morphologically well differentiated, but with uncertain phylogenetic affinities within Papaveraceae. It has been considered (using morphological and molecular markers) to be an independent lineage occupying the most basal position within Papaveraceae (Kadereit et al., 1994, 1995; Hoot and Crane, 1995; Hoot et al., 1997). However, when only molecular markers are used, this position is always poorly supported and thus unresolved (Hoot et al., 1997). The molecular phylogeny of Ranunculales also resulted in an inconclusive position of *Pteridophyllum* within Papaveraceae (Wang et al., 2009). Nevertheless, combining molecular data with morphology, these authors found a different position of *Pteridophyllum* with respect to that reported by Hoot et al. (1997), with *Pteridophyllum* as sister group to *Hypecoum* and related to Fumarioideae (Wang et al., 2009). Accordingly, Wang et al. (2009) proposed the inclusion of *Pteridophyllum* in the subfamily Fumarioideae.

Our molecular analysis was also inconclusive with regard to *Pteridophyllum* position. Chloroplast phylogeny relate *Pteridophyllum* to Fumarioideae (as a sister group of *Hypecoum* in the parsimony analysis), but without statistical support; while nuclear phylogeny relate it to Papaveroideae (as sister group of *Eschscholzia californica*) in the Bayesian analysis (Pp: 0.98), and to Fumarioideae (without BS support) in the parsimony analysis (Fig. S4). Both the *Pteridophyllum* and *Hypecoum* sequences showed a large number of changes with regard to the rest of taxa, resulting in long branch lengths on the trees; especially for chloroplast and ITS markers, much more variable than gene *26S* (e.g. nº of changes for external branch of the chloroplast tree: *Pteridophyllum* = 302, *Hypecoum* = 249, *Lamprocapnos* = 241, *Ehrendorferia* = 97, *Dicentra* = 44, *Ichtyoselmis* = 43, *Adlumia* = 13, Crown Fumarieae = 44). Long-branch

attraction is a phenomenon affecting the topologies from phylogenetic inferences (Sanderson et al., 2000), especially when the parsimony criterion is used (Swofford et al., 2001). This may be responsible of the ambiguity in the *Pteridophyllum* position.

According to our findings, *Pteridophyllum* should be not considered as belonging to Fumarioideae until its position is resolved. We base this on the inconclusive position of *Pteridophyllum* in the molecular phylogenies, and that the inclusion of *Pteridophyllum* in Fumarioideae by Wang et al. (2009) is based only on two morphological characters, the interpretation of one of them (exine sculpturing of pollen grain) being wrong for Fumarioideae representatives [spinose exine according to Wang et al. (2009), when it is actually a non-spinose exine (Kalis, 1979; Blackmore et al., 1995; Pérez-Gutiérrez et al., unpubl. res.)]. More work is needed, including a much broader sampling of Papaveroideae and more definitive markers at this level, to elucidate the relationship of *Pteridophyllum* with the rest of Papaveraceae lineages. From here on, we will refer to the subfamily Fumarioideae excluding *Pteridophyllum*.

4.5.2 Phylogenetic relationships within subfamily Fumarioideae

Fumarioideae shown as a strongly supported natural group with two well-defined lineages, *Hypecoum* and the rest of Fumarioideae, (Figs. 1, 2). Lidén (1993a) considered both lineages at the subfamily level. Given that the APG III classification (APG, 2009) considers Fumariaceae as a subfamily of Papaveraceae, the most appropriate taxonomic level for the two Fumarioideae lineages is the tribe: tribe Hypecoeae and tribe Fumarieae.

Within Fumarieae, we have identified a grade of basal lineages with all taxa having bisymmetric and two-spurred flowers, and a group of genera with asymmetric and one-spurred flowers (except *Dactylicapnos*, bisymmetric and two-spurred flowers) that we term Crown Fumarieae.The order of diversification of basal lineages largely coincides with that reported by Lidén et al. (1997) using the chloroplast gene *rps16* intron; differences involve the position of the genus *Dicentra*. In Lidén et al. (1997) *Dicentra* was a weakly supported clade, sister to the monotypic *Ichtyoselmis*. In our analyses it is in all cases well-supported, diverging after *Ehrendorferia* divergence in the chloroplast tree or as a sister group of *Ehrendorferia* in the nuclear tree (Figs. 2, 3). However, the latter relationship occurs only when the combined *26S*-ITS data matrix is used (clade not recovered in the independent analyses; data not shown), and it is not supported in the parsimony analyses (Fig. S4). Our results agree with the Lidén et al. (1997) observations since they found no morphological support for the sister relationship among *Dicentra* and *Ichtyoselmis*. Moreover, our results support the split of *Dicentra* (in its traditional concept) in *Lamprocapnos*, *Ehrendorferia*, *Dicentra* s.str., and *Ichtyoselmis* as proposed by Lidén et al. (1997).

One of the major controversies regarding intergeneric relationships within the Crown Fumarieae is the position of the North American *Capnoides sempervirens* (L.) Borckh. (zygomorphic and one-spurred flowers, unlike other species of tribe Corydaleae *sensu* Lidén, 1986, 1993a). Its position was not resolved in Lidén et al. (1997). Moreover, in the latter study the position of

Dactylicapnos (bisymmetric and two-spurred flowers) was weakly supported as sister clade of *Corydalis*. All of the above has prevented the unambiguous reconstruction of the flower symmetry (and evolution of the genes involved) in the subfamily and of the other morphological characters (e.g. Lidén et al., 1997; Fukuhara, 1999; Damerval and Nadot, 2007; Damerval et al., 2007). Our results show a totally resolved phylogeny, offering a basis for morphological character reconstructions, with *Capnoides* as the basal lineage within Crown Fumarieae, followed by *Dactylicapnos*, and *Corydalis* as the sister group to subtribe Fumariinae.

At infrageneric level, the only taxonomic inconsistency is found within *Corydalis*. This is the wider genus of Fumarioideae (*c*. 465 species) and currently is divided into three subgenera (Lidén et al., 1997; Zhang et al., 2008). Our analysis included 10 species representing the three subgenera and covering its biogeographical area (Fig. 2). Our results (using different species) reflect the subgeneric classification of Lidén et al. (1997) based on the gene *rps16* intron sequences and agree with the clades determined in an ITS-tree within *Corydalis* (Lidén et al., 1995). The only inconsistency between our analysis and that of Lidén et al. (1997) concerns the relationships of *C. pseudorupestris* Lidén & Z.Y. Su [subgenus *Sophorocapnos*] with *C. hamata* Franch. (*Corydalis* subgenus *Corydalis* section *Hamatae* C.Y. Wu & Z.Y. Su). The section *Hamatae* includes three species, of which two of them (*C. hamata* and *C. muliensis* C.Y. Wu & Z.Y. Su) share a distribution with *C. pseudorupestris*, which might have favoured contact and hybridization among them. Due to the high number of species, much more molecular work is needed to test the most recent sectional classification of *Corydalis* (Zhang et al., 2008).

Within Crown Fumarieae, the subtribe Fumariinae forms a strongly supported clade, in which the same groupings as in Pérez-Gutiérrez et al. (2012) were recovered [see this study for a detailed discussion of this subtribe (considered as tribe Fumarieae)], the South African clade being the early divergent lineage. Unlike that of Pérez-Gutiérrez et al. (2012), our study includes the monotypic genus *Fumariola* (from Turkestan), which was included in the *Fumaria* clade and sister to the monotypic *Cryptocapnos* (from south-central Afghanistan); however, as in the aforementioned study the relationships between *Fumaria-Rupicapnos-Cryptocapnos+Fumariola* were poorly resolved.

4.5.3 The origin of Fumarioideae and ancestral connections East Asia-North America-Indochina

Fumarioideae (excluding *Pteridophyllum*) originated at the end of the Early Cretaceous in East Asia (Figs. 4a, S6), and the crown group began to diversify 96 Ma at the boundary between Early and Late Cretaceous. Our results almost coincide with those of previous dating studies including the subfamily (Wikström et al., 2001; Anderson et al., 2005; Wang et al., 2012). The estimated ancestral geographical distribution agrees with the Asiatic origin of Fumarioideae proposed by Lidén (1986) and Dahl (1990). Climatic conditions proposed for East Asia during the Cretaceous involves a stable humid and warm climate from the Albian onwards (Hsü, 1983; Clarke and Jenkyns, 1999; Hasegawa, 2003; Morley, 2011), which favoured the development of megathermal vegetation (Golozoubov et al., 1999; Morley, 2011) and suggests the subfamily

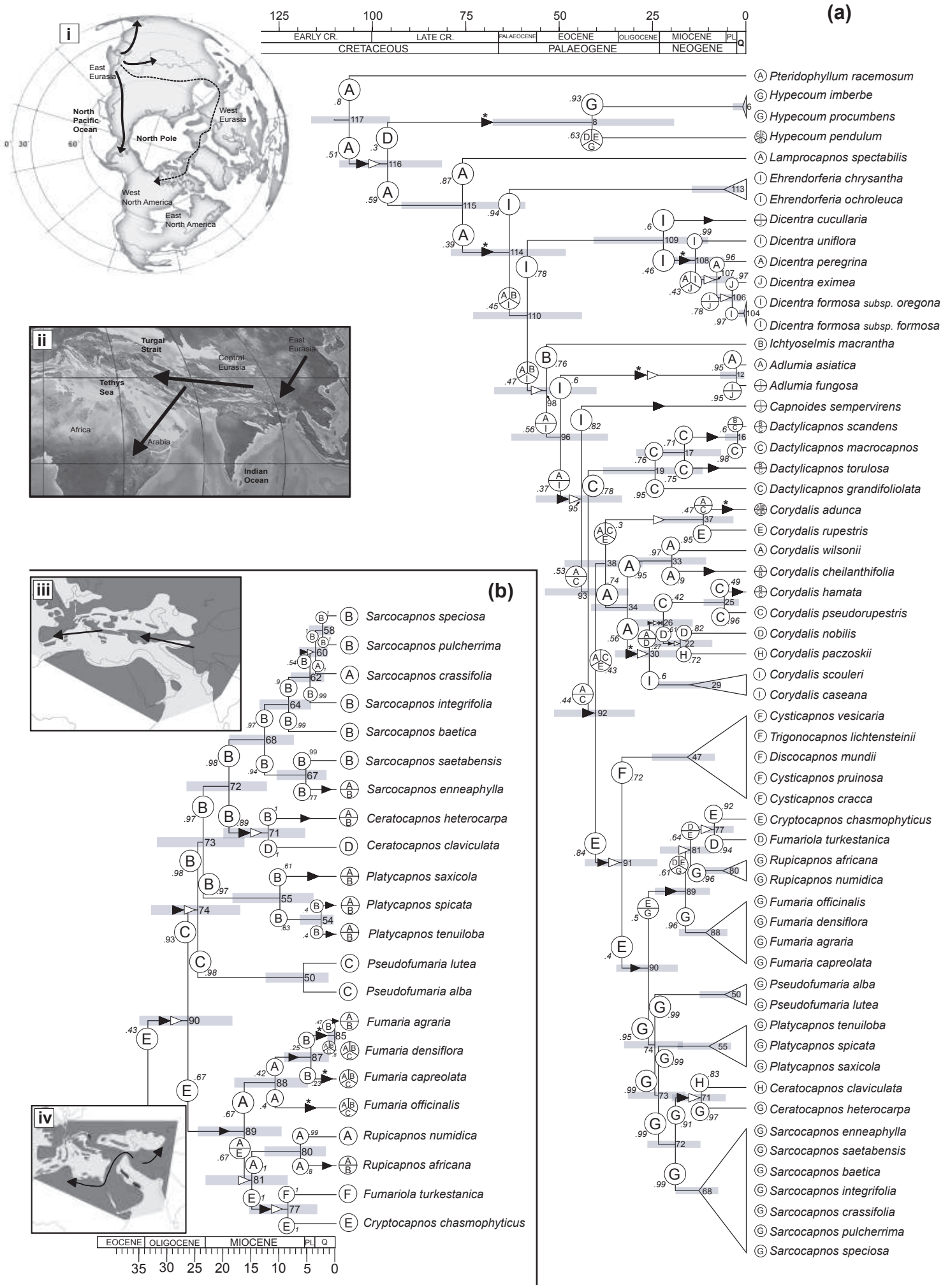


Figure 4. Caption on next page

Figure 4. (On previous page) Spatio-temporal reconstruction of subfamily Fumarioideae. Figure shows the maximum clade credibility tree (MCCT) from the BEAST analysis with the results of the ancestral-area reconstruction from the Lagrange analyses constrained for dispersal between areas over time for the whole subfamily (a), and for the partial analysis focused on the Mediterranean taxa of the subtribe Fumariinae (b). The outgroup species were pruned from the MCCT. Grey bars indicate the 95% highest posterior density of the divergence-time estimation. The current distribution areas of the species are shown beside each one, while the reconstructed areas are shown on the vertical branches linking the stem lineages; both appear within circles. Clades with equally distributed species were condensed in the tree. The nodes are labelled with Roman numerals. Values in italics represent the relative probability values for each reconstructed area. Each black arrowhead illustrates a dispersal event; a black arrowhead with an asterisk represents two or more dispersal events by the same lineage. White arrowheads illustrate vicariance events. Extinction events are represented by a black cross. The main dispersion events are illustrated on palaeo-maps to the left of the MCCT. Maps reflect: i) the main Northern Hemisphere land connections during Late Cretaceous and Palaeocene, ii) Eurasia and Africa continents during Eocene, iii) Mediterranean during late Oligocene, and iv) Mediterranean during Miocene. Maps were partially modified from Brikiatis (2014) and Rögl (1999) with permission from Wiley Online Library editorial and from Geologica Carpathica journal, respectively, and from the Global Palaeogeography Library (cpgeosystems.com/paleomaps.html).

ancestor could occupy a forest-floor habitat. All basal lineages (except the tribe Hypecoeae and *Ehrendorferia*, both growing in arid and open habitats) and *Capnoides*, *Dactylicapnos* and many *Corydalis* show forest-floor habitat (almost all taxa from subtribe Fumariinae occupy arid and open habitat). Our results support the proposal by Kadereit et al. (1995; based on a phylogenetic analysis of morphological characters) that forest-floor habitat is basal for the whole Papaveraceae family, and that transition from forest floor into open arid habitats took place a few times within Fumarioideae.

The diversification of the basal lineages of the subfamily occurs progressively within a large time frame (46 million years between the split of *Hypecoum*, node 116, and that of *Adlumia*, node 96; 26 million years for the tribe Fumarieae, node 115; Fig. 4a). Similar origin patterns with slow initial diversification in the Late Cretaceous were identified also for sapindaceous lineages in South-east Asia (Buerki et al., 2013); and Mao et al. (2010) found a slow diversification within *Juniperus* L. during the climate-stable Oligocene. The latter authors propounded that diversification in *Juniperus* was suppressed by long periods of stable climate, as described for other Tertiary flora (Milne and Abbott, 2002). The Late Cretaceous is the best-known period of warm and equable climate during the Phanerozoic (e.g. Frakes et al., 1992), which could have discouraged a rapid early diversification in Fumarioideae. However, more studies are showing that Late Cretaceous climate was not as stable, having documented even short-term glaciation events (e.g. Barrera and Savin, 1999; Miller et al., 1999). This climatic variability was more pronounced in northern latitudes such as in the area of the Arctic Ocean (*cf.* Brikiatis, 2014), where the tribe Fumarieae ancestor arrived on its way to North America. Together with the possible absence of climate stability, it also highlights the existence of long branch lengths for all subfamily basal lineages, leading to monotypic or few-species genera with crown groups dating very late (Miocene or Pliocene), suggesting an important role for extinction in the evolutionary history of the basal lineages of Fumarioideae.

Three main dispersive events occurred in the basal lineages of the subfamily (Fig. 4a). Firstly, the ancestor of the subfamily expanded its range towards Central Asia during the Early Cretaceous-Late Cretaceous transition, before the vicariance caused the split of the lineage of the tribe Hypecoeae. Secondly, the MRCA of *Ehrendorferia* and remainder tribe Fumarieae expanded its range northwards into western North America and also southwards into Indochina.

These two latter dispersals would have occurred between the end of the Campanian (Late Cretaceous) and the end of the Danian (early Palaeocene) (Fig. 4a).

Range expansions of the Fumarioideae ancestor could have been promoted by the global warming trends during Late Cretaceous, from the Albian to the Turonian, and the Palaeocene, from the end of the Maastrichtian (or beginning of the Palaeocene) to the Palaeocene-Eocene boundary or early Eocene. The westward and northward dispersal routes we suggested for Fumarioideae coincide with those proposed for the warm temperate Tsagayan flora that developed on the continental boundary of the western Pacific (Amur region and northern China). Akhmetiev and Beniamovski (2009), and Akhmetiev (2010) proposed that the global regression at the Maastrichtian-Danian transition provoked the desiccation of the Northern Central Eurasian epicontinental seas and straits, favouring the migration of this flora in a westward way during the Late Cretaceous-Palaeocene boundary, both along mid-latitude from the Amur River to south-eastern Kazakhstan, and at high latitudes along the northern boundary of the West Siberian plate to reach in the Denian the northern and middle Urals as well as the Arctic region. Moiseeva et al. (2009) showed the relationship between the Late Sagwon Flora of the northern slope of Alaska and the Tsagayan Flora, and suggested a northward migration of the latter to high latitudes of the Arctic Pacific via the Bering Land Bridge due to the progressively warming climate of the Palaeocene.

Given that the lineage of the tribe Hypecoeae originated in the early Late Cretaceous in Central Asia (Fig. 4a), its westward migration should have been affected by the Late Cretaceous warming trend. Crown *Hypecoum* was dated at around 55 million years after the split of its stem lineage. *Hypecoum* grows in arid and open habitats, so the transition from forest floor to the *Hypecoum* habitat had to occur during its long stem lineage. Ecology of *Hypecoum* agrees with the Palaeogene climatic environment in Central Asia, with a zonal climate pattern dominated by desert and steppe conditions, since a large expanse of the arid/semi-arid region was distributed between 20ºN and 40ºN palaeolatitude from west to east (Guo et al., 2008; Zhang et al., 2012). To the north of this region, a Mediterranean-like climate was situated in Central Asia (Zhang et al., 2012). Therefore, before the diversification of *Hypecoum* (41 Ma), Mediterranean and arid/semi-arid climates existed in Central Asia, fostering the adaption of *Hypecoum* ancestor to these conditions. Our date for the *Hypecoum* ancestor coincides with the Bartonian (Late Eocene), when sclerophyllous flora distributed from northern Ukraine to eastern Kazakhstan (Akhmetiev, 2010). Recent biogeographical analyses of *Ruta* (Salvo et al., 2010) and *Haplophyllum* (Manafzadeh et al., 2014) proposed a Central Asia origin and initial diversification for these genera during the Eocene, and subsequent colonization of the Mediterranean during the Miocene. Our results show the Mediterranean arrival of *Hypecoum* in the late Eocene but in view of the Mediterranean palaeogeography (Rögl, 1999), this arrival was probably delayed until Oligocene-Miocene boundary, as described for the Rutaceae genera.

With regard to the North America dispersion of the Fumarioideae ancestor, three high-latitude land bridges connected Eurasia and North America during Late Cretaceous and Palaeocene (*cf*. Brikiatis, 2014): Beringia, connecting East Asia and western North America intermittently (100-*c*.

75 Ma, *c*. 65.5 Ma, 58 Ma); De Geer, connecting north-eastern North America-Greenland and Fennoscandia (71-63 Ma); and Thulean, connecting North America and Europe via Greenland (*c*. 56.8 Ma). If the dispersal is dated to between the end of Late Cretaceous and the early Palaeocene (76-63.5 Ma; Figs. 4a, S6, nodes 115-114; Table 2), both the De Geer and Beringia routes could be possible. During this time period, climatic conditions favoured the biotic exchange between Eurasia and North America in two time windows, *c*. 69 Ma and 65.5 Ma (*cf*. Brikiatis, 2014). While the De Geer route functioned in both time windows, Beringia did so only during the 65.5 Ma window. Connection between East Asia and North America through De Geer route was facilitated by the global regression at the Maastrichtian-Danian transition and the interruption of the Turgai strait (epicontinental sea from the Arctic Ocean to the Tethys Seaway, in existence from the Middle Jurassic until the early Oligocene; Tiffney, 1985; *cf.* Brikiatis, 2014). However, De Geer route connected East Asia and north-eastern North America, in disagreement with our results, since the North American ancestral area for Fumarioideae was western North America, supporting a Beringia route (Fig. 4a, nodes 115-114). During Late Cretaceous the Western Interior Sea divided North America into western and eastern regions (extending from the Gulf of Mexico through the western interior lowlands to the Arctic Ocean; Kauffman, 1984), preventing dispersal between the two (*cf*. Graham, 1993). The regression of the Western Interior Sea began at the end of the Cretaceous (*cf*. Graham, 1993), and then a passage from north-eastern to western North America could have existed during the early Palaeocene; and therefore De Geer route cannot be ruled out for the arrival of Fumarioideae to North America.

Western North America was a diversification and dispersal centre of the basal lineages of tribe Fumarieae (Fig. 4a). All North American genera diversified in the West, from where secondary dispersal into eastern North America and into East Asia took place (Table 2). It is noteworthy that diversification of these genera occurred long after the split of their stem lineages (Fig. 4a). These lineages were affected by climatic factors from the Eocene onwards (*cf.* Graham, 1993), as the Palaeocene-Eocene Thermal Maximum, the abrupt cooling near the Eocene-Oligocene boundary (*c*. 33.7 Ma), the onset of a drier climate from the Miocene, or the Pleistocene glaciations, which could have led to extinction events along these lineages. The *Dicentra* crown group was dated to the beginning of the Miocene, when drier climates and colder winters were established. As a consequence, North American megathermal vegetation shifted southwards and began its decline, while mesothermal broad-leaved temperate deciduous vegetation expanded at mid-latitudes (*cf.* Graham, 1993). The area of the southern Rocky Mountains and the Sierra Madre Occidental has been considered an important centre for the evolution of the Madro-Tertiary geoflora (Axelrod, 1958), and it could be the place where *Dicentra* diversified. Two independent eastward dispersals occurred in *Dicentra*, one of the stem lineage of *D. cucullaria* (L.) Bernh., and other of the ancestor of the rest of *Dicentra* during the first part of the Miocene; this latter also expanded its range into East Asia by the Bering land bridge, the only active land bridge at that time (*cf*. Brikiatis, 2014). Crown *Ehrendorferia* was dated in the Miocene-Pliocene boundary. This genus is endemic to California (USA), reaching *E. chrysantha*

(Hook. & Arn.) Rylander Baja California (Mexico). It grows in open and arid habitats from 15 to 2200 m (Stern, 1997), in agreement with the general cooling that occurred from the late Miocene and the drier conditions, especially at the southern end of the Rocky Mountains, where a sclerophyllous vegetation occurred. Finally, *Adlumia* is a classical example of East Asia/eastern North America disjunction (Kadereit et al., 1995). Our results show western North America as the ancestral area for the stem lineage, which expanded its range into East Asia and into eastern North America. The split between *Adlumia* species was dated to the Pliocene-Pleistocene transition (2.7 Ma), somewhat less than a million years after Bering land-bridge disruption (3.5 Ma; *cf*. Sanmartín et al., 2001). Currently *Adlumia fungosa* shows a very narrow distribution in western North America suggesting a past wider distribution which could have shrunk during the Pleistocene climatic oscillations; the effect of this climatic oscillations were milder in the eastern North America (Soltis et al., 2006), since *A. fungosa* is widely distributed.
In the same time period of the initial dispersion into western North America (second half of the Late Cretaceous and beginning of Palaeocene; Fig. 4a, nodes 115-114; Table 2) the Fumarieae lineage arrived to Indochina. South-East Asia has played important roles in the angiosperm evolution, representing a dispersal route between Northern and Southern Hemispheres during periods of climate change, and a refugium for tropical lineages (Buerki et al., 2014). Thus the climatic instability at the end of the Cretaceous, when the Northern Hemisphere humid environments suffered a significant decline, could promote the southward migration of the Fumarioideae ancestor. Between 59-54 Ma (late Palaeocene-early Eocene) the continuous area occupied by the ancestor of the MRCA of *Dicentra* and the rest of Fumarieae is fragmented (western North America + East Asia / Indochina) leading to a vicariance event, which resulted in the origin of *Ichtyoselmis* lineage 54 Ma (Figs. 4a, S6). Split of *Ichtyoselmis* lineage coincides with the instauration of the zonal arid/semi-arid climate from western to eastern China in the Early Eocene epoch (palaeolatitude 20ºN-40ºN), caused by the subtropical high-pressure belt in the Northern Hemisphere (Zhang et al., 2012). *Ichtyoselmis* inhabit the mountainous area in northern Myanmar and west part of south-eastern China, under humid conditions during the Palaeogene (Guo et al., 2008), and considered as refuge for several plants lineages (*cf*. Wen et al., 2014). It grows at middle and high elevations (1500-2000 m) under woods on humid well-drained soils (Zhang et al., 2008). Its distribution and ecology, together it is a monotypic genus, suggest that this plant as a palaeoendemism.

4.5.4 Crown Fumarieae: Eocene Himalayas diversification and westward expansion

The ancestor of Crown Fumarieae expanded from East Asia into the Himalayas before the middle Eocene (Fig. 4a, nodes 96-95). Crown Fumarieae diversification began 44 Ma with the origin of the *Capnoides* lineage in western North America by vicariance (Fig. 4a, node 95). From this moment onwards acceleration occurred in the diversification rate, resulting in the origin of its basal lineages (*Capnoides*, *Dactylicapnos*, *Corydalis*) and the ancestor of subtribe Fumariinae in only four million years (44-40 Ma; Figs. 4a, S6). Moreover, crown *Corydalis* was dated to only two million years after the split of its stem lineage from the subtribe Fumariinae

lineage; however, this latter did not begin diversification until seven million years afterwards in the Irano-Turanian region. The geography and time frame for these fast diversification events suggest that they might be related to the geological events that occurred in the Himalayan region during the late Eocene. The uplifts of the Qinghai-Tibetan Plateau (QTP) together the Palaeogene climate pattern dominated by desert and steppe climates have triggered and facilitated plant speciation and diversification (*cf.* Wen et al., 2014). According to our results these events also must have governed the diversification of the basal lineages of the Crown Fumarieae, and its westward range expansion.

During the Eocene, a strong fluctuation of climate conditions in North America and Asia has been documented (Woodburne et al., 2009; Zhang et al., 2012). Climatic deterioration (Bridgerian Crash) after the Early Eocene Climatic Optimum (EECO) from 50 to 47 Ma, resulted in a strong retreat from tropical climates to the increased seasonality and aridity. This could be a stimulus for southward migration of the Crown Fumarieae ancestor, responsible for the vicariance into Asian and western North American lineages and the arrival of the Asian lineage to the Himalayan region (Fig. 4a). A similar vicariance pattern across Beringia as result of the global cooling during Eocene was proposed by Chin et al. (2014) to explain the diversification of the North American and eastern Asian lineages of *Prunus* subgenus *Prunus*. North-South plant migrations into the Himalayan region through East Asia have been shown, since close biogeographical relationships can be found between the Hengduan-Himalayan forest regions and the Arcto-Tertiary floristic elements and relict taxa in eastern Asia and North America (*cf*. Wen et al., 2014).

The fast diversification of *Dactylicapnos* and *Corydalis* lineages (between the Himalayas and East Asia, 42 and 40 Ma), and the split of the basal subgenera of *Corydalis* (38 and 32 Ma) closely follow the end of the Indian-Asian collision in the easternmost part (41 Ma, Rowley, 1996) and then the first phase of the QTP uplift (Gangdese motion, 45-38 Ma). Palaeogeographic interpretations by Ding et al. (2014) of the Eocene Tibet suggest that relatively low elevation basin was sandwiched by two mountains in excess of 4500 m of Qiangtang to the north and Gangdese to the south.

Dactylicapnos originated and diversified in the Himalayas 24 Ma (Figs. 4a, S6, node 19), coinciding with the second uplift phase of Tibet, which occurred in the early Miocene (the Himalayan motion, 25-17 Ma; Shi et al., 1999); from where it expanded into Indochina. Diversification of many plant groups have been proposed as responses to the early Miocene uplift (*cf*. Zhang and Fritsch, 2010; *cf*. Wen et al., 2014); and several lineages originated on the QTP have been shown to have migrated into other regions (the out-of-QTP hypothesis), as in the case of Eurasia, Central Asia, and northern China (*cf.* Wen et al., 2014). *Dactylicapnos* represents a genus that originated on the QTP and that have migrated eastwards into South-east Asia. Our results show that this dispersal route has been followed independently by different *Dactylicapnos* lineages from the mid-Miocene onwards (Fig. 4a). Most migrations of the QTP lineages have been related with the climatic oscillations that occurred from Pliocene (*cf*.

Wen et al., 2014). This could also be the case of *Dactylicapnos*, which must have also undergone the possible influence of subsequent QTP uplift events.

Corydalis is a numerous (*c.* 465 species) and widely distributed genus, mainly through Northern Hemisphere, but especially well represented in China (357 species, 262 endemic; Zhang et al., 2008). Despite the bias in our biogeographical analysis with regard to *Corydalis* (see Materials an Methods), our results (considering the two analyses made to test the sampling bias; Fig. 4a; Table S8) suggest the Himalayas and a close part of East Asia as the ancestral area for diversification, which is coherent with that area being the most species-rich region; and also suggest that the section *Archaeocapnos* arrived to western North America during Oligocene (Figs. 4a, S6, nodes 34-30; Table 2), when Beringia was the only land bridge between Asia and North America. At least two more dispersion events into North America had to happen in *Corydalis*, one in subgenus *Corydalis* section *Dactylotuber*, and another in subgenus *Sophorocapnos*, section *Sophorocapnos*; these dispersals could not be evaluated in our analysis for lack of plant material. *Corydalis* is the only Fumarieae genus for which the crown group is dated to the Palaeogene (Eocene). In terms of the subfamily basal lineages, only crown *Hypecoum* was dated to the Eocene, as other genera diversified during Neogene, and therefore all (including *Hypecoum*) showed very long branches for their stem lineages, implying a major role of extinction during their evolution. The high number of *Corydalis* species and its short stem lineage (2 million years) suggest less impact of extinction on its evolution. In this sense, the high capacity of *Corydalis* species to adapt to very different habitats (forest, high mountain habitats, desert, rocky places, etc.; Zhang et al., 2008) may have prevented a high rate of extinction and favoured the great diversification and distribution of the genus. The extensive uplifts of the QTP have triggered rapid radiations in several plant groups (*cf*. Wen et al., 2014) and, according to our dating results, this seems also to be the case of *Corydalis*.

The MRCA of *Corydalis* and subtribe Fumariinae arrived to the Irano-Turanian region during the late Eocene (42-40 Ma; Figs. 4a, S6, nodes 93-92; Table 2). Biogeographical connections between the floras of QTP and Central Asia, Minor Asia, or Mediterranean Eurasia have been reported for many plant lineages (*cf*. Wen et al., 2014); however, most of these connections are proposed to be post-Oligocene connections. Barres et al. (2013) proposed a late Eocene-early Oligocene expansion of the subtribe Carlininae ancestor (Asteraceae) from western Asia to Central Asia. Since in the early Eocene the Tethys Sea covered the western Asian portion of the Irano-Turanian region, the westward migration of the *Corydalis*-Fumariinae ancestor should have occurred, coinciding with the vanishing of the Tethys Sea. By the end of the Eocene, the Tethys Sea had nearly disappeared as consequence of the collision of India with Eurasia, the Indian Ocean was born, and the western Tethys was reduced to the Mediterranean Sea (Rögl, 1999). When migration was dated (42-40 Ma) an elongated deep basin (from the Mediterranean eastwards) divided the Irano-Turanian region into two portions (western Asia and Central Asia), defining two possible migration routes from Himalayas, one to the north (from the North Caspian Sea) and one to the south (through the Iranian connection). Barres et al. (2013) proposed the northern route for the eastward migration of Carlininae. Considering the sea basin as a barrier

for southward plant migrations (e.g. *Haplophyllum*; Manafzadeh et al., 2014), which was present at least until early Oligocene (Rögl, 1999), and that the South African dispersal from the Irano-Turanian region of the Fumariinae ancestor had already happened in the early Oligocene (lineage of *Cysticapnos* Clade originated in South Africa 33 Ma; Figs. 4a, S6), the most probable migration route from the Himalayas is the southern route. Therefore, the origin of the Fumariinae lineage was probably the Iranian area. This result agrees with Pérez-Gutiérrez et al. (2012), which estimated the area for the Fumariinae ancestor (tribe Fumarieae in Pérez-Gutiérrez et al., 2012) to be a continuous area between western Eurasia and Africa.

During the late Eocene a vegetation belt extended along the southern areas of Eurasia and North America with sclerophyllous species adapted to warm temperate semi-arid habitats, the Madrean-Tethyan vegetation (Axelrod, 1975; Wen and Ickert-Bond, 2009). Kadereit et al. (1995) suggested that in Fumarioideae the transition from forest floor into arid and open habitats happened in the Fumariinae subtribe. This transition could have occurred before the westward migration and may be related to the QTP uplift, since the high elevation of Gangdese Mountains may have contributed to the aridification of the inner plateau (Ding et al., 2014), probably promoting the adaptation of Fumariinae ancestor to open dry habitats.

4.5.5 The South African dispersal of the subtribe Fumariinae

The beginning of the Fumariinae diversification was 33 Ma, with the split between Mediterranean groups (*Fumaria–Sarcocapnos* clades) and South African ones (*Cysticapnos* Clade; Fig. 4a, node 91). The origin of the latter was stated in South Africa by a vicariance event, involving the previous dispersal of the Fumariinae ancestor from the Irano-Turanian region. Pérez-Gutiérrez et al. (2012) also explained by vicariance the divergence between the two lineages (South African and Mediterranean), suggesting (without dating data and considering the suggestions of other authors, e.g. Kadereit et al., 1995) climatic changes in Africa from the Miocene onward as responsible for the migration and vicariance observed.

The dating methods in our study show that the time framework estimated does not coincide with the hypothesis proposed by those authors to explain the South Africa/Mediterranean disjunction in Fumariinae. Thus our reconstruction involves the idea that the subtribe ancestor expanded its range from the Irano-Turanian region into South Africa during late Eocene, and then a vicariance resulted in the split of the South Africa and Mediterranean (still in the Irano-Turanian region) lineages in the early Oligocene (Fig. 4a, node 91; Table 2). If we consider the Pérez-Gutiérrez et al. (2012) hypothesis and our dating results, then the ancestor of *Cysticapnos* Clade must have diversified in the Irano-Turanian region before its Miocene dispersal; however, our results show the presence in South Africa of that ancestor from the early Oligocene. These results open the door to another possible scenario about South African dispersal; that is, the late Eocene dispersal of the Fumariinae ancestor from West Asia into Africa, across north-eastern Africa, via stepping-stones along the Tethyan coast; and migration along the African mountains to South Africa before the split of lineages in the early Oligocene. This dispersal route during late Eocene has been described for other plant groups such as for Tribe Cardueae

(Barres et al., 2013). South African early Oligocene climate might have been similar to the modern climate (*cf.* Linder, 2005), which could allow the establishment of the arid-habitat-adapted ancestor of Fumariinae. Long-distance dispersal of lineages adapted to the Oligocene climates (temperate climates, low-nutrient soils, and seasonal drought) has been documented for the Cape flora, which after climatic changes gave rise to additional Cape lineages (*cf.* Linder, 2005). Crown *Cysticapnos* clade was dated in the mid-Miocene (16 Ma), coinciding with a more mesic South African climate (established from early Miocene) and before the beginning of the trend towards the modern seasonally arid conditions (14 Ma; Zachos et al., 2001). Climatic change from Oligocene to Miocene climates could have been responsible for the initial diversification of *Cysticapnos* by isolation of the ancestor populations in open and dry disjunct areas.

4.5.6 The Mediterranean colonization by the subtribe Fumariinae

According to our results the arrival to the Mediterranean of Fumariinae from the Irano-Turanian region occurred in two separate dispersion events following two different routes (Fig. 4b). One dispersal event was that of the *Sarcocapnos* Clade following a northern route during the early Oligocene (33-26 Ma); the other was that of the *Fumaria* Clade through northern Africa between late Oligocene and middle Miocene (26-16 Ma). The Irano-Turanian region has been proposed as a geographical source for temperate Mediterranean flora (e.g., Quézel, 1985; Thompson, 2005); and this hypothesis has been shown by biogeographical studies of several plant groups (e.g. Araceae, Mansion et al., 2008; *Ruta*, Salvo et al., 2010; *Consolida* s.l., Jabbour and Renner, 2011; *Haplophyllum*, Manafzadeh et al., 2014), to which the Mediterranean Fumariinae genera need to be included.

Two important tectonic events occurred between the late Eocene and the early Miocene, which could be involved in the expansion of Fumariinae into the Mediterranean during the estimated dates. On one hand, the origin of the Paratethys Sea in the Eocene-Oligocene boundary (Rögl, 1999) and its isolation from a proto-Mediterranean Sea during the early Oligocene by the progressive accretion of the microplates located between them (Rögl, 1999; Meulenkamp and Sissingh, 2003). This microplate accretion resulted in an elongated and more-or-less continuous landmass connecting the proto-Mediterranean basin with Asia Minor, and both two Mediterranean domains (western and eastern Mediterranean); and it allowed floristic exchanges between these areas (Salvo et al., 2010; Manafzadeh et al., 2014). According our results, this land bridge would have allowed the invasion of the Mediterranean by the ancestor of *Sarcocapnos* Clade from the Irano-Turanian region and its westward expansion towards the western Mediterranean (Fig. 4b). This eastern-western dispersal through the Mediterranean was very fast (2 million years, 26-24 Ma; Figs. 4b, S6). Relationships between the three main lineages of the *Sarcocapnos* Clade were not well supported (Fig. 2), and a rapid and simultaneous radiation from the common ancestor was proposed to explain this phylogenetic conflict and the incomplete lineage sorting of morphological characters (Pérez-Gutiérrez et al., 2012). Our results of dating and ancestral area reconstruction support this hypothesis, since the

vicariance explaining the split of the eastern-Mediterranean (*Pseudofumaria*) and western-Mediterranean (*Platycapnos* and *Sarcocapnos-Ceratocapnos*) lineages in the Oligocene-Miocene boundary is compatible with the geological instability shown for the migratory route followed during this time (Rögl, 1999; Meulenkamp and Sissingh, 2003). Thus, the land bridge connecting the western and eastern Mediterranean was repeatedly disrupted by cycles of marine transgression-regression between the Tethys and Paratethys seas (Rögl, 1999; Meulenkamp and Sissingh, 2003), promoting east-west Mediterranean disjunctions, as that in *Sarcocapnos* Clade (Quézel, 1985; Oosterbroek & Arntzen, 1992; Sanmartín, 2003; Mansion et al., 2008; Manafzadeh et al., 2014).

On the other hand, the interruption of the Tethys Sea by the collision of the Arabian plate with the Anatolian microplate around 20 Ma resulted in the formation of a land corridor between western Irano-Turanian region and Africa across Arabia, the Gomphotherium landbridge (Rögl, 1999). The time of the appearance of this land corridor is consistent with our dating of the southern Mediterranean invasion by the *Fumaria* Clade (26-16 Ma), and then it likely facilitated the arrival of the *Fumaria* Clade ancestor from Irano-Turanian region, through the Arabian plate, to North Africa (Fig. 4b). This migration route in the same time period was also proposed for the ancestor of *Ruta* (Salvo et al., 2010). The *Fumaria* Clade ancestor occupied a wide area from western Irano-Turanian region to western North Africa. Progress of the diversification of the *Fumaria* Clade began with the split of *Fumaria* and quickly continued with a vicariance between *Rupicapnos* and *Cryptocapnos-Fumariola* lineages (Fig. 4b). However, relationships between these lineages were not well resolved in our phylogenies, since another hypothesis on this relationship involves *Rupicapnos* as sister group of *Fumaria*; and thus the first diversification event would have been a vicariance between North African lineages and the Irano-Turanian ancestor of *Cryptocapnos-Fumariola* (data not shown). The onset of diversification of *Fumaria* Clade coincided not only with the intense orogenic activity in western Asia during middle Miocene as consequence of the collision between Arabian and Eurasian plates, which has been proposed as responsible for the isolation of plant lineages and for promoting allopatric speciation (Sanmartín, 2003), but also with the emergence of intermittent seaways interrupting the Gomphotherium landbridge (Rögl, 1999). All these factors could have promoted the rapid radiation of the three lineages in the clade (*Fumaria*/*Fumariola-Cryptocapnos* /*Rupicapnos*), explaining the low resolution of the molecular markers to establish the relationships between lineages in the phylogenies. Finally, *Fumaria* Clade reached Central Asia by the dispersion of the ancestor of *Cryptocapnos* and *Fumariola* from the Irano-Turanian region during the late Miocene (Fig. 4b, Table 2).

The current North African-western Mediterranean distribution for several species of Fumariinae genera can be explained by range expansion in both directions from the late Miocene, with a concentration of dispersal events from 6 Ma (Figs. 4b, S6). The coincidence of such dispersion with the closure of the western Mediterranean Sea from the Atlantic Ocean in the late Tortonian, increasing the aridity of western Mediterranean, and with the desiccation of the Mediterranean Sea (*c*. 6 Ma; Duggen et al., 2003) suggests the influence of these geological events on the

exchange of Fumariinae species between western Mediterranean and North Africa across the Strait of Gibraltar.

The Mediterranean lineages of the subtribe Fumariinae appeared and evolved *in situ* in the Mediterranean basin. The structure and composition of Mediterranean flora is believed to have been influenced both by the trend towards increasing aridification starting 9-8 Ma and the onset of the Mediterranean climate (3-2 Ma) (Suc, 1984; Thompson, 2005); however, in Fumariinae, neither the migration events (33-26 Ma and 26-16 Ma) nor the origin of major lineages (all between 24-15 Ma) were affected by such climatic regimes. On the contrary, all generic diversification was triggered during the climatic trend to aridity starting 9 Ma, but before the onset of the Mediterranean climate (9-6 Ma; split of the basal lineage of *Sarcocapnos* occurred 13 Ma, but the core *Sarcocapnos* diversified 9 Ma; Figs. 4b, S6). An important question arising from the above concerns one of the most characteristic and striking adaptations of Fumariinae subtribe, i.e. its perennial-chasmophytic habit. According to Pérez-Gutiérrez et al. (2012) the ancestor of Fumariinae was annual and non-chasmophyte, and the perennial-chasmophytic habit was acquired independently in the *Fumaria* Clade (by *Rupicapnos* and *Cryptocapnos-Fumariola*) and in the *Sarcocapnos* Clade (independently by *Pseudofumaria* and by *Sarcocapnos*). Annual lifespan (considered as an adaptation to open and dry habitats; Raunkiaer, 1918) was acquired by the Fumariinae ancestor (in the Irano-Turanian region or even in the Hymalayas before its range expansion), while the perennial-chasmophytic habit was acquired in the stem lineages of those taxa, and therefore prior to the beginning of the trend towards aridity. Before 16 Ma the Mediterranean basin underwent subtropical conditions, with little seasonal change in temperature and relatively high levels of summer rainfall (Thompson, 2005). Therefore, acquisition of perennial-chasmophytic habit under these climate conditions suggests it was an adaptation to occupy the less-competitive open, dry niches of the cracks of the cliff.

4.6 References

Akaike, H., 1973 . Information theory and an extension of the maximum likelihood principle. In: B. N. Petran and F. Csaki [eds.], Second international symposium on information theory, 267-281. Akademiai Kiado, Budapest, Hungary.

Akhmetiev, M.A., 2010. Paleocene and Eocene floristic and climatic change in Russia and Northern Kazakhstan. Bull Geosci. 85, 77-94.

Akhmetiev, M.A., Beniamovski, V.N., 2009. Palaogene floral assemblages around epicontinental seas and straits in Northern Central Eurasia: proxies for climatic and paleogeographic evolution. Geol. Acta. 7, 297-309.

Anderson, C.L., Bremer, K., Friis, E.M., 2005. Dating phylogenetically basal eudicots using *rbcL* sequences and mutiple fossil reference points. Am. Jor. Bot. 92, 1737-1748.

Axelrod, D.I., 1958. Evolution of the madro-tertiary geoflora. Ann. Missouri Bot. Gard. 24, 280-334.

Axelrod, D.I., 1975. Evolution and biogeography of Madrean-Tethyan sclerophyll vegetation. Ann. Missouri Bot. Gard. 62, 280-334.

Barrera, E., Savin, S.M., 1999. Evolution of late Campanian-Maastrichtian marine climates and oceans. In: Barrera, E., Johnson, C. (Eds.), Evolution of the Cretaceous Ocean-Climate system. Geol. Soc. Am. Spec. Pap. 332, 245-282.

Barres, L., Sanmartín, I., Anderson C.L., Susanna, A., Buerki, S., Casals-Galbany, M., Vilatersana, R., 2013. Reconstructing the evolution and biogeographic history of tribe Cardueae (Compositae). Am. J. Bot. 100, 867-882.

Bell, C.D., Soltis, D.E., Soltis, P.S., 2010. The age and diversification of the angiosperms re-revisited. Am. J. Bot. 97, 1296-1303.

Blackmore, S., Stafford, P., Persson, V., 1995.Palynology and systematics of Ranunculiflorae. Pl. Syst. Evol. (Supplement). 9,71-82.

Bremer, B., Bremer, K., Chase, M.W., Fay, M.F., Reveal, J.L., Soltis, D.E., Soltis, P.S., Stevens, P.F., Anderberg, A.A., Moore, M.J., Olmstead, R.G., Rudall, P.J., Sytsma, K.J., Tank, D.C., Wurdack, K., Xiang, J.Q.Y., Zmarzty, S. 2009. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG III. Bot. J. Linn. Soc. 161, 105-121.

Brikiatis, L., 2014. The De Geer, Thulean and Beringia routes: key concepts for understanding early Cenozoic biogeography. J. Biogeogr. 6, 1036-1054.

Buerki, S., Forest, F., Alvárez, N., 2014. Proto-South-East Asia as a trigger for early angiosperm diversification. Bot. J. Lin. Soc. 174, 326-333.

Buerki, S., Forest, F., Alvarez, N., Nylander, A.A.J., Arrigo, N., Sanmartín, I., 2011. An evaluation of new parsimony-based versus parametric inference methods in biogeography: a case study using the globally distributed plant family Sapindaceae. J. Biogeogr. 38, 531-550.

Buerki, S., Forest, F., Stadler, T., Alvarez, N., 2013. The abrupt climate change at the Eocene-Oligocene boundary and the emergence of South-East Asia triggered the spread of sapindaceous lineages. Ann. Bot. 112, 151-160.

Clarke, L.J., Jenkyns, H.C., 1999. New oxygen isotope evidence for long-term Cretaceous climate change in the Southern Hemisphere. Geology 27, 699-702.

Chin, S.W., Shaw, J., Haberle, R., Wen, J., Potter, D. 2014. Diversification of almonds, peaches, plums and cherries – Molecular systematics and biogeographic history of *Prunus* (Rosaceae). Mol. Phylogenet. Evol. 76, 34-48.

Dahl, Å.E., 1990. Infrageneric division of the genus *Hypecoum* (Papaveraceae). Nord. J. Bot. 10, 129-140.

Damerval, C., Le Guilloux, M., Jager, M., Charon, C., 2007. Diversity and Evolution of *CYCLOIDEA*-Like TCP Genes in Relation to Flower Development in Papaveraceae. Plant Physiol. 143, 759-772.

Damerval, C., Nadot, S., 2007. Evolution of Perianth and Stamen Characteristics with Respect to Floral Symmetry in Ranunculales. Ann. Bot. 100, 631-640.

Ding, L., Qiang, X., Yahui, Y., Houqi, W., Fulong, C., Shun, L., 2014. The Andean-type Gangdese Mountains: Paleoelevation record from the Paleocene-Eocene Linzhou Basin. Earth Planet. Sci. Let. 392, 250-264.

Doyle , J.J., Doyle, J.L., 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem. Bull. 19,11-15.

Drummond, A.J., Ho, S.Y.W., Phillips, M.J., Rambaut, A., 2006. Relaxed phylogenetics and dating with confidence. PLoS Biol. 4, 699-710.

Drummond, A.J., Suchard, M.A., Xie, E. and Rambaut, A., 2012. Bayesian phylogenetic with BEAUty and the BEAST 1.7. Mol. Biol. Evol. 29, 1969-19733.

Duggen, S., Hoernle, K., van den Bogaard, P., Rupke, R., Morgan, J.P., 2003. Deep roots of the messinian salinity crisis. Nature 422, 602-606.

Farris, J.S., Kallersjo, M., Kluge, A.G., Bult, C., 1994. Testing significance of incongruence. Cladistics 10, 315-319.

Felsenstein, J., 1985. Confidence-limits on phylogenies – an approach using the bootstrap. Evolution 39, 783-791.

Frakes, L.A., Francis, J.E., Syktus, J.I., 1992. Climate Modes of the Phanerozoic. Cambridge University Press, Cambridge, UK. 274 pp.

Fukuhara, T., 1999. Seed and funicle morphology of Fumariaceae-Fumarioideae: Systematic implications and evolution patterns. Int. J. Plant. Sci.160, 151-180.

Golozoubov, V.V., Markevich, V.S., Bugdaeva, E.V., 1999. Early Creaceous changes of vegetation and environment in East Asia. Palaeogeogr. Palaeoclimatol. Palaeocl. 153, 139-146.

Graham, A., 1993. History of the vegetation: Cretaceous (Maastrichtian)- Tertiary. In: Flora of North America Editorial Committee (Ed.), Flora of North America,Oxford Univ. Press, New York, pp. 57–70.

Guo, Z.T., Sun, B., Zhang, Z.S., Peng, S.Z., Xiao, G.Q., Gem J.Y., Hao, Q.Z., Qiao, Y.S, Liang, M.Y., Liu, J.F., Yin, Q.Z., Wei, J.J. 2008. A major reorganization of Asian climate by the early Miocene. Clim. Past 4, 153-174.

Hasegawa, T., 2003. Cretaceous terrestrial paleoenvironments of northeastern Asia suggested from carbon isotope stratigraphy: Increased atmospheric pCo_2-induced climate. J. Asian Earth Sci. 8, 849-859.

Hoot, S.B., Crane, P.R., 1995. Inter-familiar relationships in the *Ranunculidae* based on molecular systematic. Pl. Syst. Evol. (Supplement). 9, 119-131.

Hoot, S.B., Kadereit, J.W., Blattner, F.R., Jork, K.B., Schwarzbach, A.E., Crane, P.R., 1997. Data congruence and phylogeny of the Papaveraceae s.l. Based on four data sets: *atpB* and *rbcL* sequences, *trnK* restriction sites, and morphological characters. Syst. Bot. 22, 575-590.

Hsü, J., 1983. Late Cretaceous and Cenozoic vegetation in China, emphasizing their connections with North America. Ann. Missouri Bot. Gard.70, 490–508.

Innis, M. A., Gelfand, D.H., Sninsky, J.J., White, T.J., 1990. PCR protocols: A guide to methods and applications. Academic Press, New York, New York, USA.

Jabbour, F., Renner, S.S., 2011. *Consolida* and *Aconitella* are an annual clade of *Delphinium* (Ranunculaceae) that diversified in the Mediterranean basin and the Irano-Turanian region. Taxon 60, 1029-1040.

Johnson, L.A., Soltis, D.E., 1995. Phylogenetic inference in Saxifragaceae sensu stricto and *Gilia* (Polemoniaceae) using *mat*K sequences. Ann. Mo. Bot. Gard. 86, 1-32.

Jud, N.A., Hickey, L.J., 2013. *Potomacapnos apeleutheron* gen. et sp. nov., a new Early Cretaceous angiosperm from the Potomac group and its implications for the evolution of eudicot leaf architecture.Am. J. Bot. 100, 2437-2449.

Kadereit W.J., Blattner R.F., Jork K.B., Schwarzbach A., 1994. Phylogenetic analysis of the Papaveraceae s.l. (incl. Fumariaceae, Hypecoceae, and Pteridophyllum) based on morphological characters. Bot. Jahrb. Syst. 116(3), 361-390.

Kadereit WJ, Blattner FR, Jork KB, Schwarzbach A., 1995. The phylogeny of the Papaveraceae s.1.: Morphological, geographical and ecological implications. In: Jensen U, and Kadereit JW eds. Systematics and evolution of the Ranunculiflorae. Pl. Syst. Evol.(Supplement) 9, 133-145.

Kalis, A.J., 1979. Papaveraceae. The northwest European pollen flora, 20. Rev. Palaeobot. and Palyno. 28, 209-260.

Kauffman, E.G., 1984. Paleobiograaography and evolutionary response dynamic in the Cretaceous western interior seaway of North America. In: G.E.G., Westermann, (Ed.), Jurassic Cretaceous Biochronology and Paleogeography of North America, Geol. Assoc. Can. Spec. Pap. 27, 273-306.

Kim, S., Soltis, D.E., Soltis, P.S., Zanis, M.J., Suh, Y., 2004. Phylogenetic relationship among early-diverging eudicots based on four genes: were the eudicots ancestrally woody? Mol. Phyl. Evol. 31, 16-30.

Kluge, A.G., Farris., J.S., 1969. Quantitative phyletics and the evolution of anurans. Syst. Zool. 40, 315-328.

Knobloch, E., Mai, D.H., 1986. Monographie der Früchte und Samen in der Kreide von Mitteleuropa. Rozpr. Ustred. Ustavu. Geol. 47, 1-29.

Kuzoff, R.K., Sweere, J.A., Soltis, D.E., Soltis, P.S., Zimmer, E.A., 1998. The phylogenetic potential of entire 26S rDNA sequences in plants. Mol. Biol. Evol. 15, 251-263.

Lidén, M., 1986. Synopsis of Fumarioideae with a monograph of the tribe Fumarieae. Opera Bot. 88. 136pp.

Lidén, M., 1993a. Fumariaceae. In: K. Kubitzki, J. G. Rohwer, and V. Bittrich [eds.], The families and genera of vascular plants, vol. 2:Florewing plants, dicotyledons: Magnoliid, hamamelid, and caryophyllid families. Springer-Verlag, Heidelberg, Germany.

Lidén, M., 1993b. Pteridophyllaceae. In: K. Kubitzki, J. G. Rohwer, and V. Bittrich [eds.], The families and genera of vascular plants, vol. 2:Florewing plants, dicotyledons: Magnoliid, hamamelid, and caryophyllid families. Springer-Verlag, Heidelberg, Germany.

Lidén, M., 2007. New species, combinations and records of *Hypecoum*, *Dactylicapnos* and *Corydalis* (Fumariaceae) in China. Nord. J. Bot. 25, 33-37, 2007.

Lidén, M., 2010. Three new species of *Dactylicapnos* (Fumariaceae) and a synopsis of the *D. macrocapnos* complex. Nord. J. Bot. 28, 656-660.

Lidén, M., Fukuhara, T. and Axberg, T., 1995. Phylogeny of *Corydalis*, ITS and morphology. Pl. Syst. Evol. Suppl. 9, 183-188.

Lidén, M., Fukuhara, T., Rylander, J., Oxelman, B., 1997. Phylogeny and classification of Fumariaceae, with emphasis on *Dicentra* s.l., based on the plastid gene rps16 intron. Pl. Syst. Evol. 206, 411-420.

Linder, H.P., 2005. Evolution of diversity: the Cape Flora. Trends Plant Sci. 10, 536-541.

Little, S.A., Manchester, S.R., Smith, U.R., 2010. Should *Palaeoaster* should expeled from the angiosperm? *In*: Botany 2010. Programm and Abstract Book. Providence, Rhode Island. http://2010.botanyconference.org/engine/search/index.php?func=detail&aid=789

Loconte, H., Campbell, L.M., Stevenson W.M., 1995. Ordinal and familial relationships of Ranunculid genera. In: Jensen, U., and Kadereit, J.W. [eds.], Pl. Syst. Evol. Suppl. 9, 99-118.

MacLaughlin, S.P., 1994. Floristic plant geography: the classification of floristic areas and floristic elements. Prog. Phys. Geogr. 18, 185-208.

Manafzadeh, S., Salvo, G., Conti, E., 2014. A tale of migrations from east to west: the Irano-Turanian floristic region as a source of Mediterranean xerophytes. J. Biogeogr. 41, 366-379.

Mansion, G., Rosenbaum, G., Schoenenberger, N., Bacchetta, G., Rossello, J.A. & Conti, E., 2008. Phylogenetic analysis informed by geological history supports multiple, sequential invasions of the mediterranean basin by the angiosperm family Araceae. Syst. Biol.57, 269-285.

Mao, K.S., Hao, G., Liu, J.Q., Adams, R.P., Milne, R.I., 2010. Diversification and biogeography of *Juniperus* (Cupressaceae): variable diversification rates and multiple intercontinental dispersals. New Phytol. 188, 254-272.

Meulenkamp, J.E., Sissingh, W., 2003. Tertiary paleogeographic and tectonostratigraphic evolution of the northern and southern Peri-Tethys platforms and the intermediate domains of the Africa-Eurasian convergent plate boundary zone. Palaeogeogr. Palaeoclimatol. Palaeoecol. 196, 209-228.

Miller, K.G., Barrera, E., Olsson, R.K., Sugarman, P.J., Savin, S.M., 1999. Does ice drive early Maastrichtian eustasy? Global δ^{18}O and New Jersey sequences. Geology 27, 783-786.

Milne, R.I., Abbot, R.J., 2002. The origin and evolution of Tertiart relict floras. Adv. Bot. Res. 38, 281-314.

Moiseeva, M.G., Herman, A.B., Spicer, R.A., 2009. Late Paleocene Flora of the Northern Alaska Peninsula: The Role of Transberingian Plant Migrations and Climate Change. Paleontol. J. 43, 1298-1308.

Morley, R.J., 2011.Cretaceous and Tertiary climate change and the past distribution of megathermal rainforests.In: Bush, M.B., Flenley, J.R., Gosling, W.D. (Eds.),*Tropical rainforest responses to climatic changes*.Berlin, Germany, Springer, pp. 1-34.

Nylander, J.A.A., 2004. MrModeltest version 2. Computer program distributed by the author, Evolutionary Biology Centre, Uppsala University.

Oosterbroek, J.W., Arntzen, J.W. 1992. Area-cladograms of Circum-Mediterranean taxa in relation to Mediterranean palaeogeography. J. Biogeogr. 19, 3-20.

Oxelman, B., Lidén, M., Berglund, D., 1997. Chloroplast *rps16* intron phylogeny of the tribe Silineae (Caryophyllaceae). Pl. Syst. Evol. 206, 393-410.

Pérez-Gutiérrez, M.A., Romero-García, A.T., Salinas, M.J., Blanca, G., Fernández, M.C., Suárez-Santiago, V.N., 2012. Phylogeny of the tribe Fumarieae (Papaveraceae s.l.) based on chloroplast and nuclear DNA sequences: evolutionary and biogeographic implications. Am. J. Bot. 99, 517-528.

Quézel, P., 1985. Definition of the Mediterranean region and origin of its flora. In: Gómez-Campo, C. (Ed.), Plant Conservation in the Mediterranean Area. W. Junk, Dordrecht, pp. 197-218.

Rambaut, A., Drummond, A.J., 2007. Tracer version 1.5. Computer program and documentation distributed by the authors. Website: http://beast.bio.ed.ac.uk/software/tracer.

Raunkiaer, C., 1918. Récherches statistiques sur les formations végétales. Biol. Meddr. 1, 1-80.

Ree, R.H., Moore, B.R., Webb, C.O., Donoghue, M.J., 2005. A likelihood framework for inferring the evolution of geographic range on phylogenetic trees. Evol. 59, 2299-2311.

Ree, R.H., Sanmartín, I., 2009. Prospects and challenges for parametric models in historical biogeographical inference. J. Biogeogr. 36, 1211-1220.

Ree, R.H., Smith, S.A., 2008. Maximum Likelihood of Geographic Range Evolution by Dispersal, Local Extinction, and Cladogenesis. Syst. Biol. 57, 4-14.

Rögl, F., 1999. Mediterranean and Paratethys. Facts and hypotheses of an Oligocene to Miocene paleogeography (short overview). Geol. Carpath. 50, 339-349.

Ronquist, F., Huelsenbeckn, J.P., 2003. MrBayes 3: Bayesian phylogenetic inferenceunder mixed models. Bioinformatics. 19, 1572-1574.

Rowley, D.B., 1996. Age of initiation of collision between India and Asia: a review of stratigraphic data. Earth Planet. Sci. Let. 145, 1-13.

Salinas, M.J., Romero, A.T., Blanca, G., De la Herrán, R., Garrido-Ramos, M., Ruíz-Rejón, Morales, C., Ruíz-Rejón, M., Suárez, V., 2003. Contribution to the taxonomy and phylogeny of *Sarcocapnos* (DC.) (Fumariaceae). Pl. Syst. Evol. 237, 153-164.

Salvo, G., Ho, S.Y.W., Rosenbau, G., Ree, R., Conti, E., 2010. Tracing the Temporal and Spatial Origins of Islands Endemics in the Mediterranean Region: A Case Study from the Citrus Family (*Ruta* L., Rutaceae). Syst. Biol. 59, 705-722.

Sanderson, M.J., Wojciechowski, M.F., Hu, J.M., Sher Khan, T., Brady, S.G., 2000. Error, bias, and long-branch attraction in data for two chloroplast photosystem genes in seed plants. Mol. Biol. Evol. 17, 782-797.

Sanmartín, I., 2003. Dispersal vs. vicariance in the Mediterranean: historical biogeography of the Palearctic Pachydeminae (Coleoptera, Scarabaeoidea). J. Biogeogr. 30, 1883-1897.

Sanmartín, I., Enghoff, H., Ronquist, F., 2001. Patterns of animal dispersal, vicariance and diversification in the Holartic. Biol. J. Lin. Soc. 73, 345-390.

Shaw, J., Lickey, E.B., Beck, J.T., Farmer, S.B., Liu, W.S., Miller, J., Siripun, K.C., Winder, C.T., Schilling, E.E., Small, R.L., 2005. The tortoise and the hare II: relative utility of 21 noncoding chloroplast DNA sequences for phylogenetic analysis. Am. J. Bot. 92, 142-166.

Shi, Y.F., Li, J.J., Li, B.Y., Yao, T.D., Wang, S.M., Li, S.J., Cui, Z.J., Wang, F.B., Pan, B.T., Fang, X.M., Zhang, Q.S. 1999. Uplift of the Qinghai-Xizang (Tibetan) Plateau and east Asian environmental change during late Cenozoic. Acta Geogr. Sin. 54, 10-21.

Smith, U.R. 2001. Revision of the Cretaceous Fossil Genus Palaeoaster (Papaveraceae) and Clarification of Pertinent Species of *Eriocaulon*, *Palaeoaster*, and *Sterculiocarpus*. Novon 11, 258-260.

Soltis, D.E., Morris, A.B., McLachlan J.S., Manos, P.S., Soltis, P.S., 2006. Comparative phylogeography of unglaciated eastern North America. Mol. Ecol. 15, 4261-4293.

Stern, K.R. 1997. *Dicentra*. In: Flora of North America Editorial Comittee (Eds.), 1993+. Flora of North America North of Mexico. 16+ vols. New York and Oxford. Vol. 3, pp. 341-347.

Stevens, P.F., 2001 onwards. Angiosperm Phylogeny Website. Version 12, July 2012 [and more or less continuously updated since]. http://www.mobot.org/MOBOT/research/APweb/.

Suc, J.P., 1984. Origin and evolution of the Mediterranean vegetation and climate in Europe. Nature 307, 429-432.

Sun, G., Dilcher, D.L., Whang, H., Chen, Z., 2011. A eudicot from the early cretaceous of China. Nature471, 625-628.

Swofford , D.L., 1993. PAUP: phylogenetic analysis using parsimony, version 3.1.1. Illinois Natural History Survey, Champaign, Illinois, USA.

Swofford , D.L. 2003. PAUP*: Phylogenetic analysis using parsimony (*and other methods), version 4. Sinauer, Sunderland, Massachusetts, USA.

Swofford, D.L., Waddel, P.J., Huelsenbeck, J.P., Foster, P.G., Lewis, P.O., Rogers, J.S., 2001. Bias in phylogenetic estimation and its relevance to the choice between parsimony and likelihood methods. Syst. Biol. 50, 525-539.

Taberlet, P., Gielly, L., Pautou, G., Boutev, J., 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. Pl. Mol. Biol.17, 1105-1109.

Takhtajan, A.,1997. Diversity and classification of flowering plants. Columbia University Press, New York.

Thompson, J.D., 2005. Plant Evolution in the Mediterranean. Oxford University Press, Oxford.

Tiffney, B.H., 1985. Perspectives on the origin of the floristic similarity between eastern Asia and eastern north America. J. Arn. Arb. 66, 73-94.

Valtueña, F.J., Preston, C.D., Kadereit, J.W., 2012. Phylogeography of a Tertiary relict plant, *Meconopsis cambrica* (Papaveraceae), implies the existence of northern refugia for a temperate herb. Mol. Ecol. 21, 1426-1437.

Wang, W., Lu, A.-M., Ren, Y., Endress, M.E., Chen, Z.-D., 2009. Phylogeny and classification of Ranunculales: evidence from four molecular loci and morphological data. Persp. Plant. Ecol. Evol. Syst. 11, 81-110.

Wang, W., Ortiz, R.C., Jacques, F.M.B., Xiang, X-G., Li, H-L., Lin, L., Li, R-Q., Liu, Y., Soltis, P.S., Soltis, D.E., Chen, Z-D., 2012. Menispermaceae and the diversification of tropical rainforest near the Cretaceous-Paleogene boundary. New Phytol. 195, 470-478.

Wen, J., Ickert-Bond, S.M. 2009. Evolution of the Madrean-Tethyan disjunctions and the North and South American amphitropical disjunctions in plants. J. Syst. Evol. 47, 331-348.

Wen, J., Zhang, J-Q., Nie, Z.-L., Zhong, Y., Sun, H., 2014. Evolutionary diversifications of plants on the Qinghai-Tibetan Plateau. Front. Genet. 5, 4. *doi:* 10.3389/fgene.2014.00004.

Wen J, Zimmer E.A., 1996. Phylogeny and biogreography of *Panax* L. (the ginseng genus, Araliaceae): Inferences from ITS sequences of nuclear ribosomal DNA. Mol.Phylogenet. Evol. 6, 167-177.

Wikström, N., Savolainen, V., Chase, M.K., 2001. Evolution of the angiosperms: calibrating the family tree. Proc. R. Soc. Lond. 268, 2210-2220.

Woodburne, M.O., Gunnell, G.F., Stucky, R.K., 2009. Climate directly influences Eocene mammal faunal dynamics in North America. Proc. Natl. Acad. Sci. U. S. A., 106: 13399-13403.

Worberg, A., Quandt, D., Barniske, A.M., Löhne, C., Hilu, K.W., Borsch, T. 2007., Phylogeny of basal eudicots: insights from non-coding and rapidly evolving DNA. Org. Divers. Evol. 7, 55-77.

Zachos, J., Pagani, M., Sloan, L., Thomas, E., Billups, K., 2001. Trends, rhythms, andaberrations in global climate 65 Ma to present. Science 292, 686-693.

Zhang, M.L., Fritsch, P.W., 2010. Evolutionary response of *Caragana* (Fabaceae) to Qinghai-Tibetan Plateau uplift and Asian interior aridification. Plant. Syst. Evol. 288, 191-199.

Zhang, M.-L., Su, Z.-Y., Lidén, M., Grey-Wilson, C., 2008. Papaveraceae. In: Wu, Z.-Y., Raven, P.H., & Hong, D.-Y. (Eds.). Flora of China, Vol. 7 (Menispermaceae through Capparaceae). Science Press, Beijing & Missouri Botanical Garden Press, St. Louis, USA.

Zhang, Z. S., Flatoy, F., Wang, H.J., Bethke, I., Bentsen, M., Guo, Z.T., 2012. Early Eocene Asian climate dominated by desert and steppe with limited monsoons. J. Asian Earth Sci. 44, 24-35.

Zhuang, X., 1993. The taxonomic and evolution and distribution of Papaveraceae. Acta Bot. Yunnanica. 15, 137-148.

Supporting information: Table S1

Taxon	Living collection code/Voucher/ Seed collection code/References	*matK*	trnL-F	*trnG*	*rps16*	ITS	*26S*	*rbcL*
Ceratophyllaceae Gray								
Ceratophyllum dermersum L.	[1] Hilu et al. unpubl., [2]Schaefer et al. (2011).	[1]AF543732						[2]HM849883
Ceratophyllum submersum L.	[1]Qiu et al. unpubl., [2]Qiu et al. (1999).	[1]DQ401361						[2]AF197599
Circaeasteraceae Hutch.								
Circaeaster agrestis Maxim.	[1]Wefferling et al. (2013), [2]Wang et al. (2009).	[1]KC494017						[2]FJ626607
Kingdonia uniflora Balf. f. & W. W. Sm.	[1]Wefferling et al. (2013), [2]Wang et al. (2009).	[1]KC494019						[2]FJ626608
Eupteleaceae K.Wilh.								
Euptelea pleiosperma Hook f. & Thomson	NBGB 19831369, [1]Worberg et al. (2006), [2]Feng et al. unpubl., [3]Wang et al. (2009), [4]Wang et al. unpubl.	[1]AM396510	LN610907	LN610811		[2]AF162214	[3]FJ626482	[4]AY048174
Euptelea polyandra Siebold & Zucc.	NBGB 19723330, [1]Qiu et al. unpubl., [2] Kim et al. (2004), [3]Qiu et al. (1993).	[1]DQ401348	LN610908	LN610812			[2]AF389249	[3]L12645
Lardizabalaceae R.Br.								
Akebia quinata (Houtt.) Decne.	[1]Adachi et al. unpubl., [2]Qiu et al. (1993).	[1]AB069851						[2]L12627
Decaisnea fargesii Franch.	[1]Wefferling et al. (2013), [2]Hoot et al. (1995).	[1]KC494018						[2]L37916
Menispermaceae Juss.								
Menispermum canadense L.	[1]Wefferling et al. (2013), [2]Hoot et al. (1999).	[1]KC494041						[2]AF093726
Menispermum dauricum DC.	[1]Wefferling et al. (2013), [2]Xiang et al. unpubl.	[1]KC494042						[2]AF190436
Stephania japonica (Thunb.) Miers	[1]Wang et al. (2012), [2]Hoot et al. (2009).	[1]JN051855						[2]FJ026507
Papaveraceae Juss.								
Adlumia asiatica Ohwi	UPS 20041051	LN610915	LN610885	LN610788	LN610819	LN610767	LN610840	
Adlumia fungosa (Ait.) Britton, Stern & Poggenb.	NBGB 20001298-92, [1]Pérez-Gutiérrez et al. (2012), [2]Lidén et al. (1997).	LN610916	[1]HE603352	[1]HE603275	[2]Z82944	[1]HE603323	LN610841	
Capnoides sempervirens (L.) Borckh.	NBGB 19871844, [1]Pérez-Gutiérrez et al. (2012), [2]Lidén et al. (1997).	LN610917	[1]HE603351	[1]HE603274	[2]Z82956	[1]HE603322	LN610842	
Ceratocapnos claviculata (L.) Lidén	GDA 27019, [1]Pérez-Gutiérrez et al. (2012).	LN610918	[1]HE603339	[1]HE603262	[1]HE603291	[1]HE603311		
Ceratocapnos heterocarpa Dur.	GDA 9032, [1]Pérez-Gutiérrez et al. (2012).	LN610919	[1]HE603338	[1]HE603261	[1]HE603290	[1]HE603310	LN610843	
Corydalis adunca Maxim.	GBH SQE#168	LN610920	LN610886	LN610789	LN610820	LN610768	LN610844	
Corydalis caseana Gray	RBGE 19940005	LN610921	LN610887	LN610790	LN610821			
Corydalis cheilanthifolia Hemsl.	K 196919923, [1]Pérez-Gutiérrez et al. (2012).	LN610922	[1]HE603349	[1]HE603272	[1]HE603301	[1]HE603320	LN610845	

Corydalis hamata Franch.	GBG 19950591		LN610888	LN610791	LN610822	LN610769	LN610846	
Corydalis nobilis (L.) Pers.	GBG 19850489, [1]Hoot et al. (1999).	LN610923	LN610889	LN610792	LN610823	LN610770	LN610847	[1]AF093722
Corydalis paczoskii Busch.	K 19833040, [1]Pérez-Gutiérrez et al. (2012).	LN610924	[1]HE603350	[1]HE603273	[1]HE603302	[1]HE603321	LN610848	
Corydalis pseudorupestris Lidén & Z.Y. Sun	GBG 2007162	LN610925	LN610890	LN610793	LN610824	LN610771		
Corydalis rupestris Kotschy ex Boiss	GBG 2005414, [1]Lidén et al. (1997), [2]Lidén et al. (1995).	LN610926	LN610891	LN610794	[1]Z82942	[2]X85488	LN610849	
Corydalis scouleri Hook.	RBGE 19702476	LN610927	LN610892	LN610795	LN610825			
Corydalis wilsonii N.E. Br.	GBG 19660071	LN610928	LN610893	LN610796	LN610826	LN610772	LN610850	
Cryptocapnos chasmophyticus Rech.f.	W 35122, [1]Pérez-Gutiérrez et al. (2012).	LN610929	[1]HE603324	[1]HE603247	[1]HE603277	[1]HE603303		
Cysticapnos cracca (Cham. & Schltdl.) Lidén	STE 762340		LN610894	LN610797	LN610827	LN610773		
Cysticapnos pruinosa (Bernh.) Lidén	SSS 4877, [1]Pérez-Gutiérrez et al. (2012).	LN610930	[1]HE603347	[1]HE603270	[1]HE603299	[1]HE603318	LN610851	
Cysticapnos vesicaria (L.) Fedde	SSS 600, [1]Pérez-Gutiérrez et al. (2012), [2]Forest et al (2007).	LN610931	[1]HE603348	[1]HE603271	[1]HE603300	[1]HE603319	LN610852	[2]AM234980
Dactylicapnos grandifoliolata Merrill.	UPS 20029376	LN610932	LN610895	LN610798	LN610828	LN610774	LN610853	
Dactylicapnos macrocapnos (Prain) Hutch.	UPS 20061163, [1]Lidén et al. (1997).	LN610933	LN610896	LN610799	[1]Z82947	LN610775	LN610854	
Dactylicapnos scandens (D.Don) Hutch.	UPS 20090029	LN610934	LN610897	LN610800	LN610829	LN610776	LN610855	
Dacylicapnos torulosa (Hook.f. & Thomson) Hutch.	UPS 20080011	LN610935	LN610898	LN610801	LN610830		LN610856	
Dicentra cucullaria (L.) Bernh.	K 195974519	LN610936	LN610899	LN610802	LN610831			
Dicentra eximea (Ker Gawl.) Torr.	K 19862854, [1]Muller et al. (2006), [2]Borsch et al. (2003), [3]Kim et al. (2004), [4]Hoot et al. (2005).	[1]DQ182345	[2]AY145361	LN610803	LN610832	LN610777	[3]AF389262	[4]L37917
Dicentra formosa subsp. formosa (Haw.) Wal.	GBG 19000198	LN610937	LN610900	LN610804	LN610833	LN610778	LN610857	
Dicentra formosa subsp. oregana (Eastw.) Munz	K 19991128, [1]Lidén et al. (1997).	LN610938	LN610901	LN610805	[1]Z82939	LN610779	LN610858	
Dicentra peregrina (Rudolph) Makino	GBG 19952827, [1]Lidén et al. (1997), [2]Dohmoto unpubl.	LN610939	LN610902	LN610806	[1]Z82940	[2]AB571149	LN610859	
Dicentra uniflora Kellogg	RSABG 1042 16763, [1]Lidén et al. (1997).	LN610940	LN610903	LN610807	[1]Z82938	LN610780	LN610860	
Discocapnos mundii Cham. & Schltdl.	LHMS 3135, [1]Pérez-Gutiérrez et al. (2012).	LN610941	[1]HE603345	[1]HE603268	[1]HE603297	[1]HE603316	LN610861	
Ehrendorferia chrysantha (Hook. & Arn.) Rylander	RSABG 353122096, [1]Lidén et al. (1997).	LN610942	LN610904	LN610808	[1]Z82941	LN610781	LN610862	
Ehrendorferia ochroleuca (Engelm.) Fukuhara	RSABG 363721935	LN610943	LN610905	LN610809	LN610834	LN610782	LN610863	
Eschscholzia californica Cham.	RSABG 393122737, [1]Hoot et al. (1997).	LN610944	LN610906	LN610810	LN610835	LN610783	LN610864	[1]U86625
Fumaria agraria Lag.	GDA 12614, [1]Pérez-Gutiérrez et al. (2012).		[1]HE603330	[1]HE603253	[1]HE603282	[1]HE603309		
Fumaria capreolata L.	GDA 52802, [1]Pérez-Gutiérrez et al. (2012), [2]Schaefer et al. (2011).	LN610945	[1]HE603328	[1]HE603251	[1]HE603280	[1]HE603307	LN610865	[2]HM850014
Fumaria densiflora DC.	GDA 52804, [1]Pérez-Gutiérrez et al. (2012).	LN610946	[1]HE603329	[1]HE603252	[1]HE603281	[1]HE603308	LN610866	

Fumaria officinalis L.	GDA 57985, [1]Pérez-Gutiérrez et al. (2012), [2]de Vere et al. unpubl.	LN610947	[1]HE603327	[1]HE603250	[1]HE603279	[1]HE603306	LN610867	[2]JN893098
Fumariola turkestanica Korsch	Field collection	LN610948	LN610909	LN610813	LN610836	LN610784	LN610868	
Hypecoum imberbe Sm.	GDA 52828, [1]Hilu et al. (2008), [2]Kim et al. (2004), [3]Hoot et al. (1997).	[1]GU266596	LN610910	LN610814	LN610837		[2]AF389263	[3]U86628
Hypecoum leptocarpum Hook f. & Thomson	Blattner & Kadereit (1999).					AJ001968		
Hypecoum pendulum L.	GDA 54592, [1]Lidén et al. (1997).	LN610949	LN610911	LN610815	[1]Z82936	LN610785	LN610869	
Hypecoum procumbens L.	GDAC 43575		LN610912	LN610816				
Ichtyoselmis macrantha (Oliv.) Lidén	GBG 19952279	LN610950	LN610913	LN610817	LN610838	LN610786	LN610870	
Lamprocapnos spectabilis (L.) Fukuhara	GBG 19980323, [1]Pérez-Gutiérrez et al. (2012), [2]Lidén et al. (1997), [3]Salinas et al. (2003), [4]Chase et al. (1993).	LN610951	[1]HE603353	[1]HE603276	[2]Z82937	[3]AJ493444	LN610871	[4]L08761
Platycapnos saxicola Willk.	GDAC 43855, [1]Pérez-Gutiérrez et al. (2012).	LN610952	[1]HE603341	[1]HE603264	[1]HE603293	[1]HE603313	LN610872	
Platycapnos spicata (L.) Bernh.	GDAC 32052, [1]Pérez-Gutiérrez et al. (2012), [2]Salinas et al. (2003).	LN610953	[1]HE603342	[1]HE603265	[1]HE603294	[2]AJ493448	LN610873	
Platycapnos tenuiloba Pomel	GDAC 32047, [1]Pérez-Gutiérrez et al. (2012).	LN610954	[1]HE603340	[1]HE603263	[1]HE603292	[1]HE603312		
Pseudofumaria alba (Mill.) Lidén	NBGB 19763486, [1]Pérez-Gutiérrez et al. (2012).	LN610955	[1]HE603344	[1]HE603267	[1]HE603296	[1]HE603315	LN610874	
Pseudofumaria lutea (L.) Borckh.	NBGB 19891892, [1]Pérez-Gutiérrez et al. (2012).	LN610956	[1]HE603343	[1]HE603266	[1]HE603295	[1]HE603314	LN610875	
Pteridophyllum racemosum Siebold & Zucc.	GBG 19820246, [1]Hoot et al. (1997).	LN610957	LN610914	LN610818	LN610839	LN610787	LN610876	[1]U86631
Rupicapnos africana (Lam.) Pomel	GDAC 44500, [1]Pérez-Gutiérrez et al. (2012).	LN610958	[1]HE603325	[1]HE603248	[1]HE603278	[1]HE603304	LN610877	
Rupicapnos numidica (Coss. & Dur.) Pomel	GBG 19004473, [1]Pérez-Gutiérrez et al. (2012), [2]Lidén et al. (1997).	LN610959	[1]HE603326	[1]HE603249	[2]Z82954	[1]HE603305	LN610878	
Sarcocapnos baetica (Boiss. & Reuter) Nyman	GDAC 56768, [1]Pérez-Gutiérrez et al. (2012), [2]Salinas et al. (2003).	LN610960	[1]HE603331	[1]HE603254	[1]HE603283	[2]AJ250623	LN610879	
Sarcocapnos crassifolia (Desf.) DC.	GDAC 44364, [1]Pérez-Gutiérrez et al. (2012), [2]Salinas et al. (2003).	LN610961	[1]HE603332	[1]HE603255	[1]HE603284	[2]AJ429195		
Sarcocapnos enneaphylla (L.) DC.	GDAC 44351, [1]Pérez-Gutiérrez et al. (2012), [2]Salinas et al. (2003).	LN610962	[1]HE603337	[1]HE603260	[1]HE603289	[2]AJ493442	LN610880	
Sarcocapnos integrifolia (Boiss.) Cuatrec.	GDAC 44352, [1]Pérez-Gutiérrez et al. (2012), [2]Salinas et al. (2003).	LN610963	[1]HE603333	[1]HE603256	[1]HE603285	[2]AJ250621		
Sarcocapnos pulcherrima C. Morales & Romero García	GDAC 22850, [1]Pérez-Gutiérrez et al. (2012), [2]Salinas et al. (2003).	LN610964	[1]HE603334	[1]HE603257	[1]HE603286	[2]AJ250627	LN610881	
Sarcocapnos saetabensis Matero & Figuerola	GDAC 44360, [1]Pérez-Gutiérrez et al. (2012), [2]Salinas et al. (2003).	LN610965	[1]HE603336	[1]HE603259	[1]HE603288	[2]AJ250625	LN610882	
Sarcocapnos speciosa Boiss.	GDAC 44358, [1]Pérez-Gutiérrez et al. (2012),	LN610966	[1]HE603335	[1]HE603258	[1]HE603287	[2]AJ250626	LN610883	

	[2]Salinas et al. (2003).							
Trigonocapnos lichtensteinii (Cham. &Schltdl.) Lidén	LHMS 1875, [1]Pérez-Gutiérrez et al. (2012), [2]Forest et al. (2007)	LN610967	[1]HE603346	[1]HE603269	[1]HE603298	[1]HE603317	LN610884	[2]AM234981
Argemone mexicana L.	MSB 0090423, [1]Hoot et al. (1997).	LN614543						[1]U86621
Glaucium flavum Crantz.	GDAC 28365, [1]Hoot et al. (1997).	LN614542						[1]U86626
Hunnemannia fumarifolia Sweet	MSB 0009128, [1]Hoot et al. (1997).	LN614544						[1]U86627
Macleaya cordata (Willd.) R.Br.	[1]Wang et al. (2009), [2]Hoot et al. (1997).	[1]FJ626523						[2]U86629
Meconopsis cambrica Vig.	[1]Xiao unpubl.	[1]JX087883						[1]JX087689
Epimedium koreanum Nakai	[1]Adachi, et al. unpubl., [2]Kim & Jansen (1996).	[1]AB069837						[2]L75869
Nandina domestica Thunb.	[1]Adachi, et al. unpubl., [2]Kim & Jansen (1996).	[1]AB069830						[2]L75843
Ranunculaceae Juss.								
Glaucidium palmatum Siebold & Zucc.	[1]Adachi et al. unpubl., [2]Hoot et al. (1999).	[1]AB069850						[2]AF093723
Ranunculus macranthus Scheele	[1]Leebens-Mack et al. (2005).	[1]DQ069586						[1]DQ069502
Ranunculus muricatus L.	[1]Paun et al. (2005), [2]Schaefer et al. (2011).	[1]AY954191						[2]HM850296

(a) Period Before 80 Ma

	A	B	C	D	E	F	G	H	I	J
A	1.0	1.0	0.01	1.0	0.5	0.01	0.01	0.5	1.0	0.3
B	1.0	1.0	0.01	0.5	1.0	0.01	0.01	0.3	0.5	0.2
C	0.01	0.01	1.0	0.01	0.01	0.01	0.01	0.01	0.01	0.01
D	1.0	0.5	0.01	1.0	0.3	0.01	0.01	1.0	0.5	0.2
E	0.5	1.0	0.01	0.3	1.0	0.01	0.01	0.3	0.3	0.1
F	0.01	0.01	0.01	0.01	0.01	1.0	0.01	0.01	0.01	0.01
G	0.01	0.01	0.01	0.01	0.01	0.01	1.0	0.01	0.01	0.01
H	0.5	0.3	0.01	1.0	0.3	0.01	0.01	1.0	0.3	0.1
I	1.0	0.5	0.01	0.5	0.3	0.01	0.01	0.3	1.0	0.5
J	0.3	0.2	0.01	0.2	0.1	0.01	0.01	0.1	0.5	1.0

(b) Period 80-40 Ma

	A	B	C	D	E	F	G	H	I	J
A	1.0	1.0	1.0	1.0	0.5	0.01	0.2	0.3	1.0	0.5
B	1.0	1.0	1.0	0.5	0.5	0.01	0.2	0.2	0.5	0.3
C	1.0	1.0	1.0	0.5	1.0	0.01	0.3	0.2	0.5	0.3
D	1.0	0.5	0.5	1.0	0.3	0.01	0.2	0.5	0.5	0.3
E	0.5	0.5	1.0	0.3	1.0	0.01	0.5	0.2	0.3	0.2
F	0.01	0.01	0.01	0.01	0.01	1.0	0.01	0.01	0.01	0.01
G	0.2	0.2	0.3	0.2	0.5	0.01	1.0	0.5	0.2	0.3
H	0.3	0.2	0.2	0.5	0.2	0.01	0.5	1.0	0.5	1.0
I	1.0	0.5	0.5	0.5	0.3	0.01	0.2	0.5	1.0	1.0
J	0.5	0.3	0.3	0.3	0.2	0.01	0.3	1.0	1.0	1.0

(c) Period 40-20 Ma

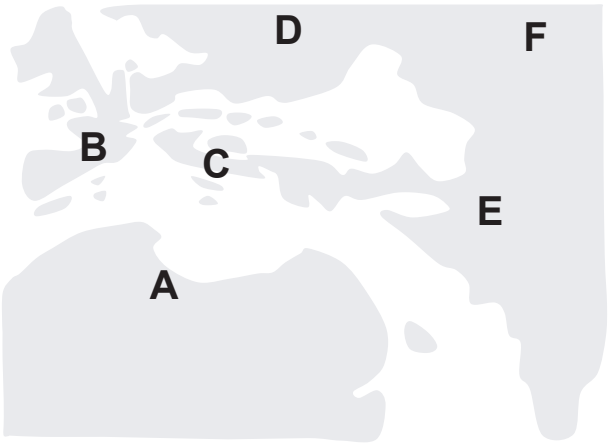
	A	B	C	D	E	F	G	H	I	J
A	1.0	1.0	1.0	1.0	0.5	0.2	0.3	0.5	1.0	0.5
B	1.0	1.0	1.0	0.5	1.0	0.3	0.5	0.5	0.5	0.3
C	1.0	1.0	1.0	1.0	1.0	0.3	0.5	0.5	0.5	0.3
D	1.0	0.5	1.0	1.0	1.0	0.3	0.5	1.0	0.5	0.3
E	0.5	1.0	1.0	1.0	1.0	0.5	1.0	1.0	0.3	0.2
F	0.2	0.3	0.3	0.3	0.5	1.0	1.0	0.3	0.1	0.05
G	0.3	0.5	0.5	0.5	1.0	1.0	1.0	1.0	0.2	0.1
H	0.5	0.5	0.5	1.0	1.0	0.3	1.0	1.0	0.3	0.2
I	1.0	0.5	0.5	0.5	0.3	0.1	0.2	0.3	1.0	1.0
J	0.5	0.3	0.3	0.3	0.2	0.05	0.1	0.2	1.0	1.0

(d) Period 20-0 Ma

	A	B	C	D	E	F	G	H	I	J
A	1.0	1.0	1.0	1.0	0.5	0.3	0.3	0.5	1.0	0.5
B	1.0	1.0	1.0	0.5	1.0	0.5	0.5	0.5	0.5	0.3
C	1.0	1.0	1.0	1.0	1.0	0.5	0.5	0.5	0.5	0.3
D	1.0	0.5	1.0	1.0	1.0	0.5	0.5	1.0	0.5	0.3
E	0.5	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.3	0.2
F	0.3	0.5	0.5	0.5	1.0	1.0	1.0	0.5	0.2	0.1
G	0.3	0.5	0.5	0.5	1.0	1.0	1.0	1.0	0.2	0.1
H	0.5	0.5	0.5	1.0	1.0	0.5	1.0	1.0	0.3	0.2
I	1.0	0.5	0.5	0.5	0.3	0.2	0.2	0.3	1.0	1.0
J	0.5	0.3	0.3	0.3	0.2	0.1	0.1	0.2	1.0	1.0

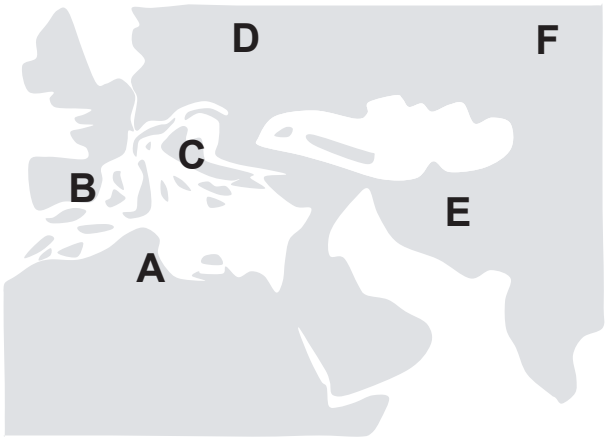
Fig. S2. Scheme of the model constrained for dispersal between areas over time designed for the dispersal-extinction-cladogenesis (DEC) analysis of Fumarioideae (model M1). Dispersal rates and palaeogeographic reconstructions reflecting the main connections among landmasses in: a) Before 80 Ma, highlighting remoteness of South Africa and the absence of the Himalayas and Mediterranean regions; Bering land bridge connecting North America and East Asia and the Western Interior Sea dividing North America were considered. b) Lapse 80-40 Ma, where the Indian-Eurasian collision and the Mediterranean region are already considered; all three possible North Hemisphere land bridges (Beringia, De Geer, and Thulean) and the Turgai strait were considered. c) Lapse 40-20 Ma, allowing the plant interchange with South Africa, and considering Mediterranean as a continuous region; Beringia was the only land bridge connecting North America with Eurasia. d) From 20 Ma to present, where Beringia continued as the only land bridge, and where Arabia connected with Eurasia. Maps are reinterpreted from Library of Palaeogeography (cpgeosystems.com/paleomaps.html).

(a) Period 35-25 Ma



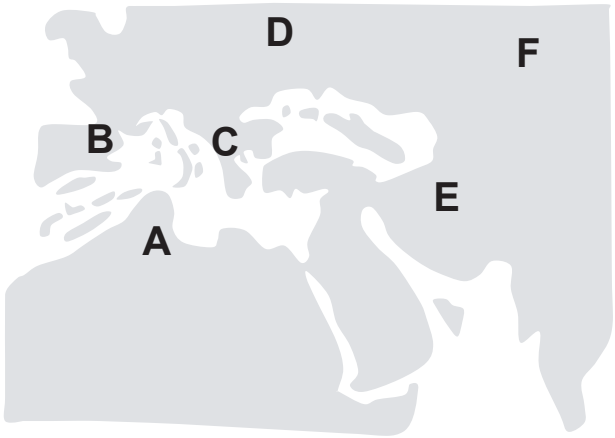
	A	B	C	D	E	F
A	1.0	0.1	0.1	0.01	0.1	0.01
B	0.1	1.0	1.0	0.7	0.1	0.01
C	0.1	1.0	1.0	0.5	1.0	0.1
D	0.01	0.7	0.5	1.0	1.0	1.0
E	0.1	0.1	1.0	1.0	1.0	1.0
F	0.01	0.01	0.1	1.0	1.0	1.0

(b) Period 25-14 Ma



	A	B	C	D	E	F
A	1.0	0.5	0.5	0.01	1.0	0.01
B	0.5	1.0	0.7	0.7	0.1	0.01
C	0.5	0.7	1.0	1.0	1.0	0.1
D	0.01	0.7	1.0	1.0	1.0	1.0
E	1.0	0.1	1.0	1.0	1.0	1.0
F	0.01	0.01	0.1	1.0	1.0	1.0

(c) Period 14-0 Ma



	A	B	C	D	E	F
A	1.0	0.5	0.1	0.01	0.7	0.01
B	0.5	1.0	1.0	1.0	0.1	0.01
C	0.1	1.0	1.0	1.0	1.0	0.1
D	0.01	1.0	1.0	1.0	1.0	1.0
E	0.7	0.1	1.0	1.0	1.0	1.0
F	0.01	0.01	0.1	1.0	1.0	1.0

Fig. S3. Scheme of the model constrained for dispersal between areas over time designed for the dispersal-extinction-cladogenesis (DEC) analysis of Mediterranean taxa of subtribe Fumariinae (model MM1). Dispersal rates and palaeogeographic reconstructions reflecting the main land connections in the Mediterranean during: a) Period 35-25 Ma, promoting connections between Irano-Turanian and East Mediterranean on the one hand, and between East Mediterranean and West Mediterranean on the other. Dispersals towards North Africa are limited because the Arabian plate was separated from Eurasia. b) Lapse 25-14 Ma, connection between Eurasia and North of Africa is fully permitted, while plant interchanges between West Mediterranean and other regions are slightly restricted. c) Period 14 Ma to present, dispersal between West and East Mediterranean is enabled, while the connection of North of Africa with other regions is limited. Connections between Europe, Central Asia and Irano-Turanian are always allowed. Maps are reinterpreted from Rögl (1999), and Ree and Sanmartín (2009).

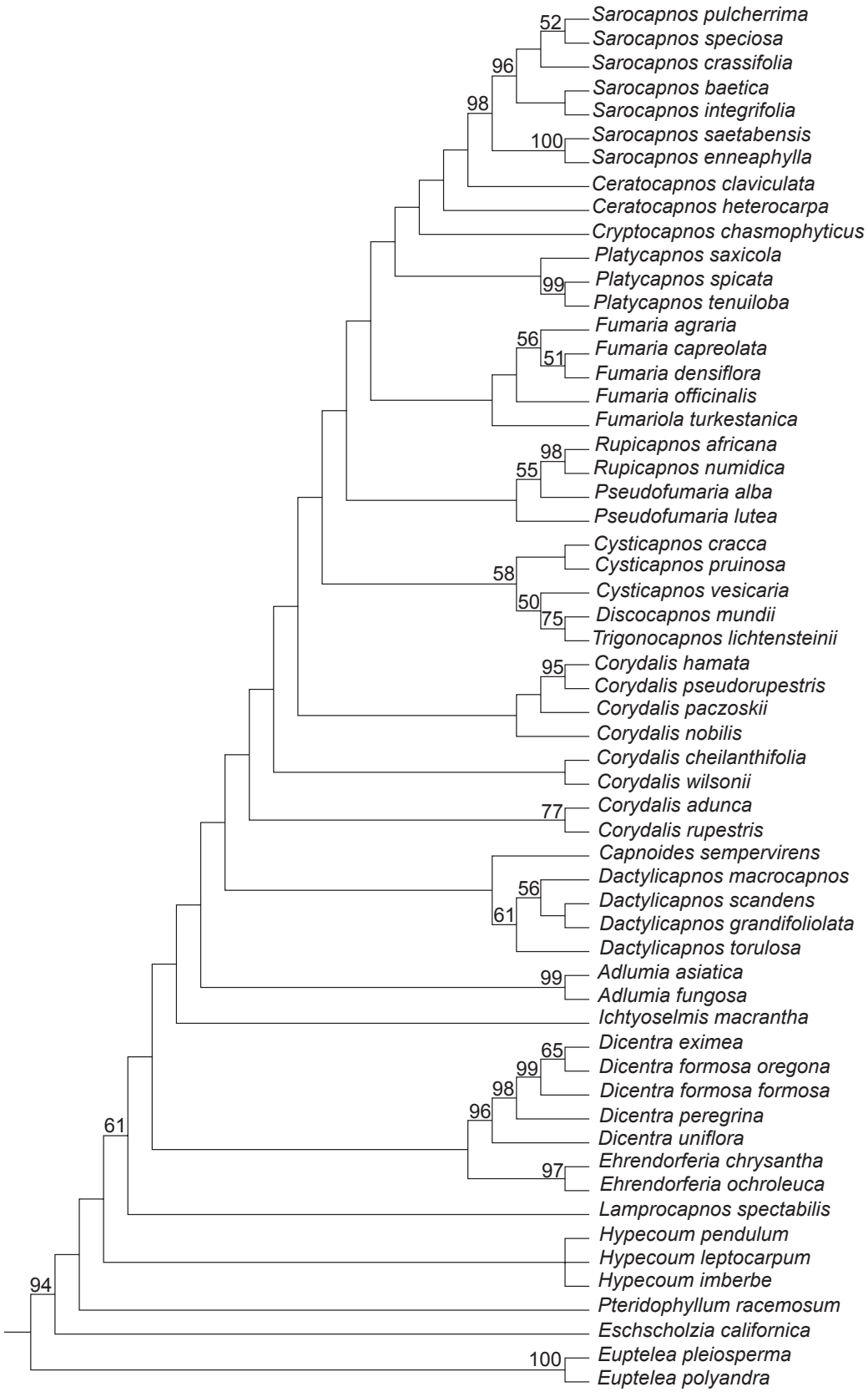


Fig. S4. 50% percentage majority-rule consensus tree from the parsimony analysis of the combined nuclear ribosomal datamatrix for Fumarioideae. Bootstrap support values are shown above branches.

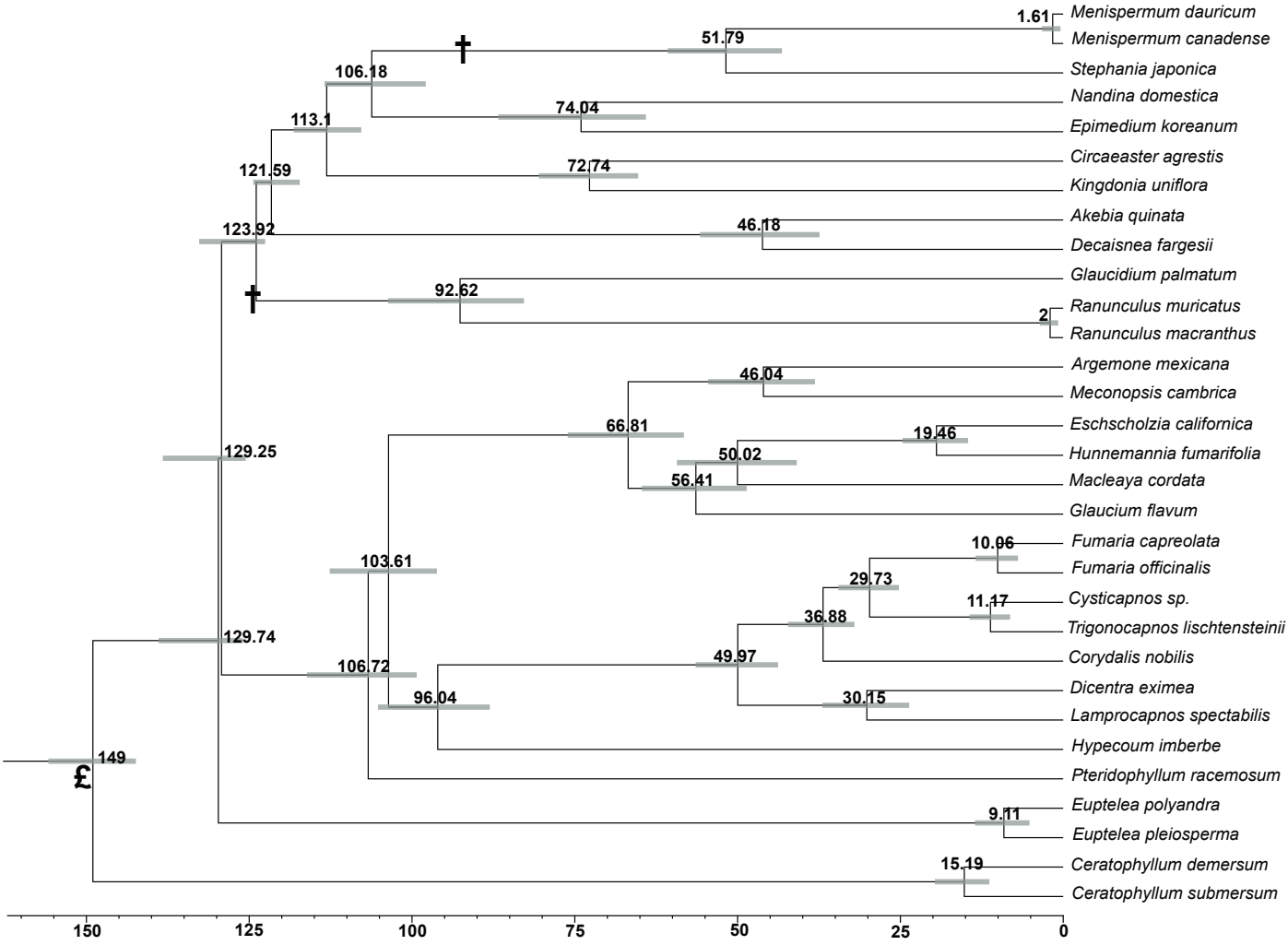


Fig. S5. Chronogram, based on the maximum clade credibility tree, for Ranunculales in the first step of the divergence time estimation analysis. Calibration constraints are indicated with cross where a fossil datum was used, and with £ where a secondary calibration data was used.

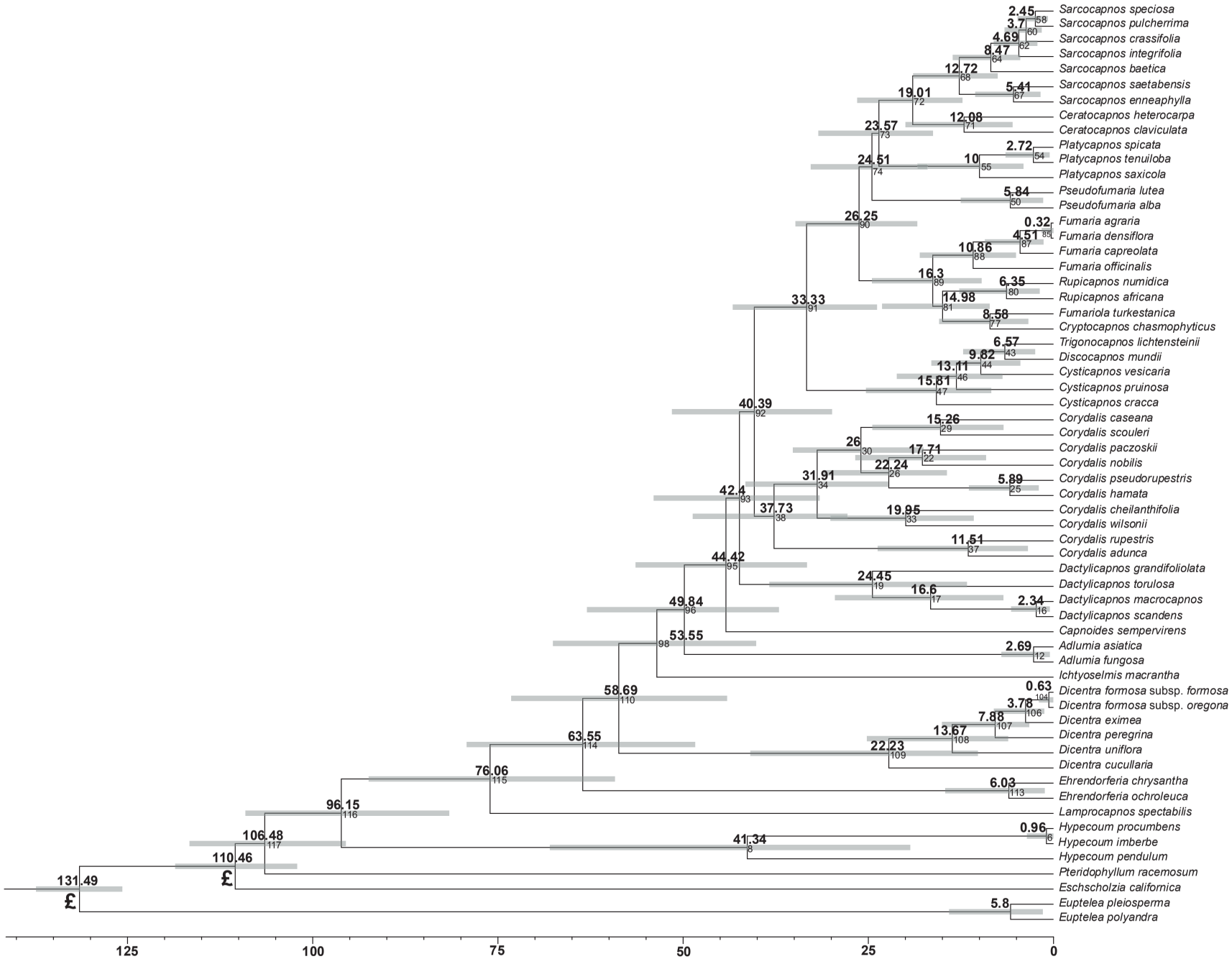


Fig. S6. Chronogram, based on the maximum clade credibility tree, for the subfamily Fumarioideae in the second step of the divergence time estimation analysis.

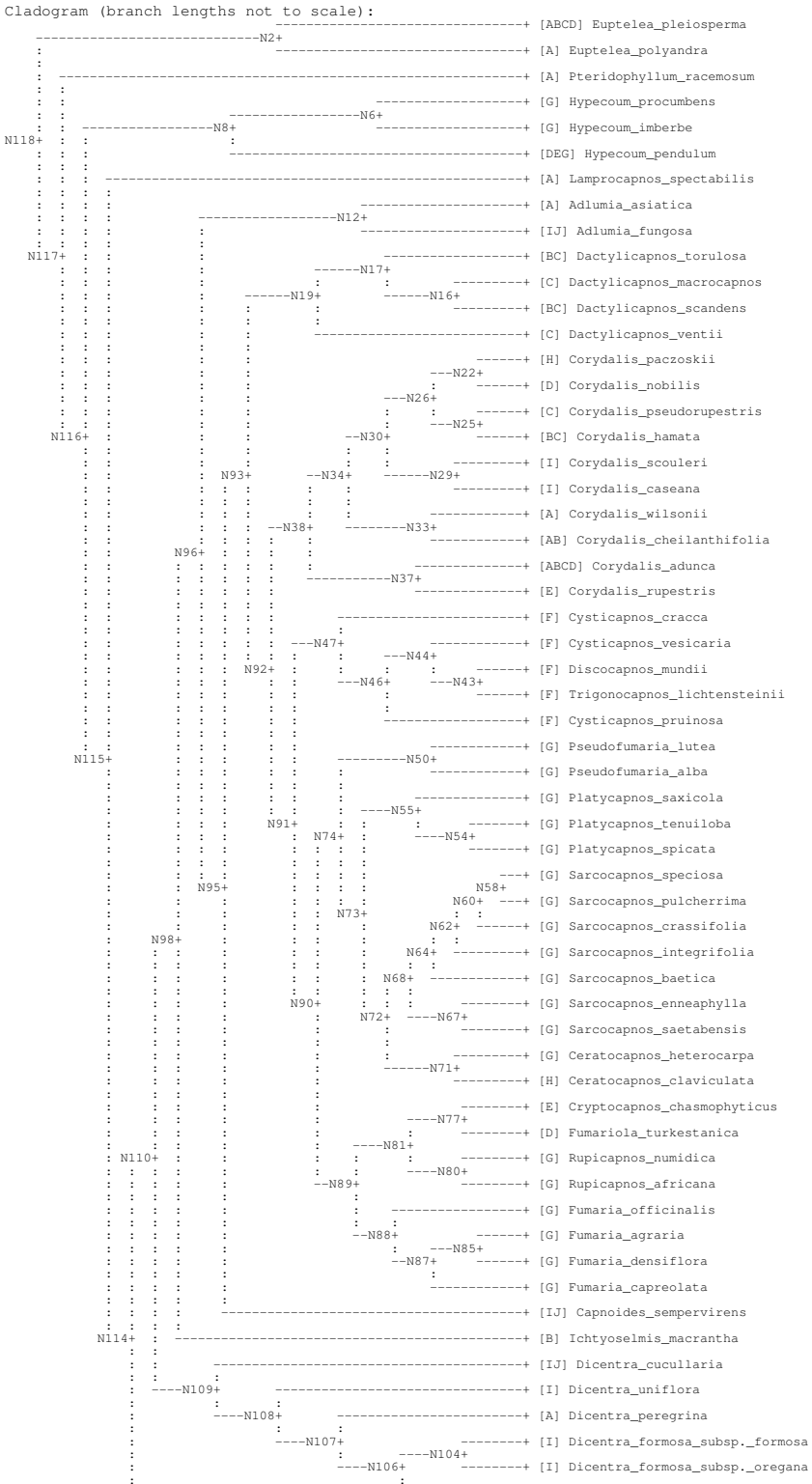
Table S7. Outputs resulting after the Lagrange analyses for the biogeographical reconstruction of the whole subfamily Fumarioideae. a) Result with constraints for dispersal between areas over time (model M1); b) result without constraints for dispersal between areas over time (model M0).

Analysis M1

```
Lagrange: likelihood analysis of geographic range evolution
Version: 20130526
Author: Richard Ree <rree@fieldmuseum.org>
https://github.com/rhr/lagrange-python

Newick tree with interior nodes labeled:
((Euptelea_pleiosperma:5.85443152956,Euptelea_polyandra:5.85443152956)N2:126.399613348,(Pteridophyllum_racemosum:123.8
90142918,(((Hypecoum_procumbens:0.995567103468,Hypecoum_imberbe:0.995567103468)N6:46.1639672934,Hypecoum_pendulum:47.1
595343969)N8:62.6681650943,(Lamprocapnos_spectabilis:83.1710757502,(((((Adlumia_asiatica:2.77654149275,Adlumia_fungosa
:2.77654149275)N12:49.6099815998,((((Dactylicapnos_torulosa:18.0729444509,(Dactylicapnos_macrocapnos:2.41479564896,Dac
tylicapnos_scandens:2.41479564896)N16:15.658148802)N17:8.12504615721,Dactylicapnos_ventii:26.1979906082)N19:18.6347554
205,((((((Corydalis_paczoskii:18.6414526681,Corydalis_nobilis:18.6414526681)N22:4.64722932813,(Corydalis_pseudorupestr
is:6.12158331292,Corydalis_hamata:6.12158331292)N25:17.1670986833)N26:3.98080292051,(Corydalis_scouleri:15.9956444151,
Corydalis_caseana:15.9956444151)N29:11.2738405016)N30:6.25967905281,(Corydalis_wilsonii:20.8197969655,Corydalis_cheila
nthifolia:20.8197969655)N33:12.7093670041)N34:6.41822489843,(Corydalis_adunca:11.81912875,Corydalis_rupestris:11.81912
875)N37:28.128260118)N38:2.75869217446,((Cysticapnos_cracca:16.2866439126,((Cysticapnos_vesicaria:10.077470913,(Discoc
apnos_mundii:6.84073504312,Trigonocapnos_lichtensteinii:6.84073504312)N43:3.23673586983)N44:3.32048502714,Cysticapnos_
pruinosa:13.3979559401)N46:2.88868797254)N47:18.7300885899,(((Pseudofumaria_lutea:6.21759268222,Pseudofumaria_alba:6.2
1759268222)N50:19.18980656,((Platycapnos_saxicola:10.113611122,(Platycapnos_tenuiloba:2.80082327123,Platycapnos_spicat
a:2.80082327123)N54:7.31278785074)N55:14.3090799582,((((((Sarcocapnos_speciosa:2.57793831388,Sarcocapnos_pulcherrima:2
.57793831388)N58:1.26884843825,Sarcocapnos_crassifolia:3.84678675213)N60:0.987678764842,Sarcocapnos_integrifolia:4.834
46551698)N62:3.89224337662,Sarcocapnos_baetica:8.72670889359)N64:4.3306437601,(Sarcocapnos_enneaphylla:5.42541617703,S
arcocapnos_saetabensis:5.42541617703)N67:7.63193647665)N68:6.46508024486,(Ceratocapnos_heterocarpa:12.4425344241,Cerat
ocapnos_claviculata:12.4425344241)N71:7.0798984745)N72:4.90025818158)N73:0.984708162042)N74:1.84315773489,(((Cryptocap
nos_chasmophyticus:9.04818651015,Fumariola_turkestanica:9.04818651015)N77:6.41010120197,(Rupicapnos_numidica:6.3678082
2199,Rupicapnos_africana:6.36780822199)N80:9.09047949013)N81:1.31211406943,(Fumaria_officinalis:11.1794179716,((Fumari
a_agraria:0.335719744366,Fumaria_densiflora:0.335719744366)N85:4.36413849555,Fumaria_capreolata:4.69985823992)N87:6.47
955973167)N88:5.59098380995)N89:10.4801551955)N90:7.7661755254)N91:7.68934853998)N92:2.12666498615)N93:1.96553395509,C
apnoides_sempervirens:46.7982799837)N95:5.58824310892)N96:4.02459869778,Ichtyoselmis_macrantha:56.4111217903)N98:5.952
48575978,(Dicentra_cucullaria:21.5769142442,(Dicentra_uniflora:13.2253771866,(Dicentra_peregrina:7.7722805443,((Dicent
ra_formosa_subsp._formosa:0.650533916236,Dicentra_formosa_subsp._oregana:0.650533916236)N104:3.17160232307,Dicentra_ex
imia:3.82213623931)N106:3.950144305)N107:5.45309664233)N108:8.35153705758)N109:40.786693306)N110:5.36692710134,(Ehrend
orferia_chrysantha:6.33057983639,Ehrendorferia_ochroleuca:6.33057983639)N113:61.3999548151)N114:15.4405410987)N115:26.
6566237411)N116:14.0624434269)N117:8.36390195956)N118:0.0;
```

Cladogram (branch lengths not to scale):



```
Global ML at root node:
  -lnL = 137.8
  dispersal = 0.006908
  extinction = 0.002528

Ancestral range subdivision/inheritance scenarios ('splits') at
internal nodes.

* Split format: [left|right], where 'left' and 'right' are the ranges
  inherited by each descendant branch (on the printed tree, 'left' is
  the upper branch, and 'right' the lower branch).

* Only splits within 2 log-likelihood units of the maximum for each
  node are shown.  'Rel.Prob' is the relative probability (fraction of
  the global likelihood) of a split.

At node N118:
   split     lnL     Rel.Prob
   [A|A]     -139.9  0.1183
   [ABD|A]   -140.8  0.04644
   [AC|A]    -140.9  0.04195
   [ACD|A]   -141    0.03948
   [ABC|A]   -141    0.03905
   [ABCD|A]  -141    0.03765
   [BCD|A]   -141.2  0.03326
   [A|ADE]   -141.2  0.03303
   [AB|A]    -141.2  0.03164
   [AD|A]    -141.4  0.02722
   [BC|A]    -141.6  0.02153
   [A|AD]    -141.6  0.02113
   [CD|A]    -141.6  0.02061
   [D|ADE]   -141.7  0.02015
   [A|ABE]   -141.7  0.01951
   [B|A]     -141.9  0.01568
   [B|ABE]   -141.9  0.01551
   [C|A]     -142    0.0147
   [A|AB]    -142    0.01466
   [D|AD]    -142.1  0.01289
   [B|B]     -142.1  0.01288
   [D|A]     -142.2  0.01203
   [B|AB]    -142.2  0.01166
   [B|AD]    -142.4  0.01008
   [A|ADI]   -142.4  0.01001
   [D|D]     -142.4  0.009633
   [C|AD]    -142.6  0.007875
   [BCD|B]   -142.7  0.007287
   [BCD|D]   -142.7  0.0071
   [A|AI]    -142.7  0.006813
   [A|ACE]   -142.8  0.006391
   [ABD|B]   -142.8  0.006359
   [ABD|D]   -142.8  0.006196
   [D|ADI]   -142.9  0.006105
   [BC|B]    -142.9  0.005897
   [A|ABD]   -142.9  0.00558
   [CD|D]    -143    0.0055
   [C|AB]    -143    0.005465
   [D|AB]    -143    0.005368
   [ABC|B]   -143    0.005347
   [ACD|D]   -143    0.005268
   [ABCD|B]  -143    0.005156
   [AI|A]    -143    0.005137
   [ABCD|D]  -143.1  0.005024
   [ACI|A]   -143.1  0.004906
   [ACE|A]   -143.1  0.004865
   [C|ACE]   -143.1  0.004764
   [ABI|A]   -143.1  0.004625
   [ABE|A]   -143.2  0.004539
   [B|ABD]   -143.2  0.004436
   [A|ABI]   -143.2  0.004356
   [AB|B]    -143.2  0.004332
   [ADI|A]   -143.3  0.004083
   [ACD|B]   -143.3  0.004055
   [ADH|A]   -143.3  0.003961
   [ADE|A]   -143.3  0.003934
   [ABC|D]   -143.3  0.003908
   [A|AC]    -143.3  0.003755
   [AD|D]    -143.4  0.003632
   [B|BE]    -143.4  0.003498
   [D|DE]    -143.4  0.00349
   [B|ABI]   -143.4  0.003463
   [BC|D]    -143.4  0.003448
   [A|DE]    -143.4  0.003432
   [D|ABD]   -143.4  0.003404
   [CD|B]    -143.4  0.003387
   [B|AI]    -143.5  0.003249
   [C|AI]    -143.6  0.003047
   [AC|B]    -143.6  0.002872
   [C|AC]    -143.6  0.002799
   [AC|D]    -143.6  0.002798
   [CE|A]    -143.6  0.00277
   [B|DE]    -143.7  0.002728
   [A|B]     -143.7  0.002701
   [A|BE]    -143.7  0.00264
   [A|D]     -143.7  0.002631
   [C|ABD]   -143.7  0.0026
   [AB|D]    -143.7  0.002533
   [D|AI]    -143.8  0.002494
   [A|ADH]   -143.8  0.002376
   [BE|A]    -143.9  0.002242
   [AD|B]    -143.9  0.002237
   [C|DE]    -143.9  0.002132
   [B|BEG]   -143.9  0.002105
   [DH|A]    -143.9  0.002072
   [C|B]     -144    0.002013
   [C|D]     -144    0.001962
   [E|ADE]   -144    0.001903
   [A|ACD]   -144.1  0.001788
   [C|BE]    -144.2  0.00164
   [D|BE]    -144.2  0.001611
   [DE|A]    -144.2  0.001589
   [B|BDE]   -144.3  0.001493
   [B|AC]    -144.3  0.001492

At node N2:
   split     lnL     Rel.Prob
   [ABCD|A]  -138    0.7929
   [BCD|A]   -140.1  0.09179
   [ABD|A]   -140.9  0.04438
   [ABC|A]   -141.2  0.03209

At node N117:
   split    lnL     Rel.Prob
   [A|A]    -138.5  0.4885
   [A|AD]   -140.4  0.07372
   [A|ADE]  -140.6  0.05731
   [B|B]    -140.9  0.04426
   [A|AB]   -141    0.03876
   [A|ABE]  -141.1  0.0365
   [D|D]    -141.1  0.03391
   [A|ADI]  -141.9  0.01653
   [A|AI]   -142.2  0.01232
   [A|B]    -142.2  0.01191
   [A|BE]   -142.2  0.01172
   [A|ABD]  -142.3  0.01073
   [A|D]    -142.3  0.01059
   [A|ACE]  -142.3  0.01047
   [A|DE]   -142.5  0.008838
   [D|AD]   -142.6  0.007889
   [A|ABI]  -142.8  0.006205
   [D|ADE]  -142.9  0.006133
   [B|BE]   -143    0.005391
   [B|AB]   -143.1  0.004836
   [B|ABE]  -143.2  0.004555
   [A|AC]   -143.2  0.004334
   [AB|A]   -143.4  0.003647
   [A|ADH]  -143.4  0.00355
   [AI|A]   -143.5  0.003307
   [AD|A]   -143.5  0.00306
   [B|AD]   -143.6  0.002952
   [AB|B]   -143.7  0.002749
   [D|DE]   -143.7  0.002607
   [I|ADI]  -143.7  0.0026
   [I|AD]   -143.8  0.00246
   [A|ACD]  -143.9  0.002203
   [AD|D]   -143.9  0.002149
   [B|BEG]  -143.9  0.002107
   [B|A]    -144    0.001971
   [I|AI]   -144    0.001938
   [I|A]    -144    0.001909
   [AC|A]   -144.1  0.00178
   [D|ADI]  -144.1  0.001769
   [D|A]    -144.2  0.001614
   [B|ABD]  -144.4  0.001338

At node N116:
   split    lnL     Rel.Prob
   [A|A]    -139.4  0.1975
   [D|A]    -139.5  0.1709
   [DE|A]   -140    0.1051
   [E|AB]   -140.2  0.0865
   [D|AI]   -140.6  0.05718
   [AD|A]   -141.1  0.03412
   [D|AB]   -141.3  0.02917
   [B|A]    -141.3  0.02872
   [B|B]    -141.3  0.02812
   [B|AB]   -141.5  0.02404
   [E|B]    -141.5  0.02269
   [ADE|A]  -141.6  0.02174
   [D|AD]   -141.6  0.02062
   [BE|A]   -141.7  0.0189
   [D|D]    -141.8  0.01678
   [B|ABI]  -142    0.01395
   [D|ADI]  -142.3  0.01033
   [B|AI]   -142.4  0.009378
   [A|AI]   -142.6  0.00815
   [E|AD]   -142.6  0.007527
   [DH|A]   -142.7  0.007449
   [E|AC]   -142.8  0.006752
   [E|ABE]  -142.8  0.006215
   [CE|A]   -142.9  0.005971
   [BE|B]   -143.2  0.004149
   [A|AB]   -143.3  0.003775
   [DE|B]   -143.3  0.003732
   [AD|I]   -143.4  0.003664

At node N8:
   split    lnL     Rel.Prob
   [G|DEG]  -138.2  0.6248
   [G|EG]   -139.8  0.1248
   [G|DE]   -140.1  0.09346
   [G|BEG]  -141.1  0.03373
   [G|E]    -141.3  0.02875
   [E|E]    -142.2  0.01218
   [E|DE]   -142.5  0.008888
   [G|BE]   -142.5  0.008567
   [G|G]    -142.7  0.007111
   [G|CEG]  -142.9  0.005646
   [E|DEG]  -143    0.005056

At node N6:
   split  lnL     Rel.Prob
   [G|G]  -137.8  0.9988

At node N115:
   split    lnL     Rel.Prob
   [A|A]    -138.7  0.3743
   [A|ABI]  -139.5  0.1799
   [A|AI]   -139.9  0.1231
   [A|AB]   -140.1  0.098
   [A|I]    -140.3  0.07534
   [I|ABI]  -142.2  0.01179
   [AI|I]   -142.2  0.01147
   [B|ABI]  -142.4  0.01012
   [AD|I]   -142.5  0.008585
   [I|AI]   -142.6  0.00807
   [A|ADI]  -142.7  0.007195
   [AD|A]   -142.8  0.006632
   [AB|I]   -143    0.005521
   [B|AB]   -143    0.005514
   [D|AI]   -143    0.005393
   [A|B]    -143    0.005317
   [AB|A]   -143.1  0.004596
   [I|I]    -143.2  0.004439
   [ADI|I]  -143.2  0.004232
   [A|AIJ]  -143.3  0.004112

At node N114:
   split    lnL     Rel.Prob
   [ABI|I]  -138.6  0.4495
   [AI|I]   -139    0.2754
   [I|I]    -140.1  0.09902
   [AB|I]   -141.1  0.03499
   [A|I]    -141.1  0.03439
   [ACI|I]  -141.4  0.02735
   [A|A]    -141.5  0.02413
   [AIJ|I]  -141.7  0.01879

At node N110:
   split    lnL     Rel.Prob
   [ABI|I]  -138.6  0.4191
   [AI|I]   -139.7  0.1417
   [AB|I]   -140.2  0.08449
   [I|I]    -140.4  0.0726
   [ABI|A]  -140.7  0.05031
   [A|A]    -141    0.04102
   [A|I]    -141.1  0.03493
   [ACI|I]  -141.3  0.03046
   [A|AI]   -141.3  0.02763
   [AI|A]   -141.8  0.01702
   [AB|A]   -142    0.01428
   [A|AIJ]  -142.3  0.01101
   [AI|J]   -142.6  0.00828

At node N98:
   split    lnL     Rel.Prob
   [AI|B]   -138.5  0.4997
   [ABI|B]  -139.6  0.1518
   [A|B]    -140.3  0.07535
   [A|A]    -140.5  0.06166
   [AI|A]   -140.6  0.05985
   [ACI|C]  -141.2  0.03088
   [ACI|A]  -141.4  0.02635
   [AC|B]   -141.8  0.01832
   [AB|B]   -141.8  0.01771
   [I|A]    -142.8  0.006414
   [A|AB]   -142.9  0.005972

At node N96:
   split    lnL     Rel.Prob
   [I|AI]   -139.1  0.2749
   [I|ACI]  -139.7  0.1457
   [I|ABI]  -139.9  0.1131
   [A|A]    -140.1  0.09703
   [A|AI]   -140.1  0.09285
   [A|ACI]  -140.8  0.04921
   [AI|A]   -141    0.04052
   [A|ABI]  -141    0.03819
   [I|A]    -141.8  0.01833
   [A|AC]   -141.8  0.01723
   [AI|I]   -141.9  0.01601
   [I|I]    -142    0.01373
   [I|AC]   -142.2  0.01201
   [I|AB]   -142.4  0.009588
   [A|AB]   -142.6  0.00757
   [I|ADI]  -142.8  0.006484

At node N12:
   split   lnL     Rel.Prob
   [A|IJ]  -137.8  0.9493
   [A|I]   -140.9  0.04257

At node N95:
   split    lnL     Rel.Prob
   [AC|I]   -138.4  0.5053
   [AB|I]   -139.6  0.1663
   [A|I]    -140    0.1097
   [A|A]    -141.1  0.03613
   [C|AI]   -141.4  0.02544
   [A|IJ]   -141.6  0.0222
   [AC|A]   -141.6  0.02205
   [AD|I]   -141.7  0.02019
   [ACI|I]  -141.9  0.01614
   [A|AI]   -142.2  0.01204
   [C|A]    -142.4  0.01012
   [ACE|A]  -142.4  0.009944
```

At node N93:

split	lnL	Rel.Prob
[C|AC]	-138.6	0.4366
[C|ACE]	-139.3	0.2214
[B|AB]	-140.2	0.08496
[B|ABE]	-140.8	0.04802
[C|C]	-141.2	0.03269
[C|AB]	-141.7	0.01866
[C|AD]	-141.8	0.01768
[C|A]	-141.8	0.01743
[C|ACI]	-142.2	0.01124
[A|A]	-142.3	0.01104
[C|AI]	-142.5	0.008746
[C|ACD]	-142.6	0.008186
[B|A]	-142.8	0.006575
[C|CE]	-142.8	0.006323
[A|AB]	-142.9	0.005842
[B|AD]	-143	0.00533
[A|AD]	-143.1	0.004939
[B|B]	-143.3	0.003909
[D|AD]	-143.4	0.003646

At node N19:

split	lnL	Rel.Prob
[C|C]	-138	0.7629
[BC|C]	-139.6	0.1529
[B|C]	-141.1	0.03705

At node N17:

split	lnL	Rel.Prob
[C|C]	-138.2	0.6559
[BC|C]	-140.1	0.0983
[B|BC]	-140.6	0.06107
[C|BC]	-140.6	0.05735
[BC|B]	-140.7	0.05161
[B|B]	-141	0.03876

At node N16:

split	lnL	Rel.Prob
[C|BC]	-138.3	0.5958
[C|C]	-138.7	0.3918

At node N92:

split	lnL	Rel.Prob
[ACE|E]	-138.6	0.4297
[AC|E]	-139.3	0.2099
[ABE|E]	-139.9	0.1137
[AB|E]	-140.9	0.04424
[AD|D]	-141.4	0.02602
[CE|E]	-141.9	0.01624
[A|D]	-141.9	0.01548
[ADI|D]	-142	0.01422
[AC|C]	-142	0.01411
[C|E]	-142.2	0.01156
[AC|D]	-142.5	0.008684
[ACI|C]	-142.5	0.008607
[ACD|D]	-142.5	0.008563
[A|C]	-142.5	0.008414
[ADE|E]	-142.6	0.00774
[A|CE]	-142.9	0.005714
[C|C]	-143	0.005492
[AD|E]	-143.3	0.004057

At node N38:

split	lnL	Rel.Prob
[A|ACE]	-139	0.2785
[A|CE]	-139.9	0.1225
[A|ABE]	-140.2	0.08509
[A|AC]	-140.3	0.08027
[AC|C]	-140.7	0.05263
[A|C]	-140.9	0.04464
[A|A]	-141	0.0379
[AC|E]	-141.2	0.03291
[A|AB]	-141.4	0.02725
[A|BE]	-141.4	0.02703
[AC|A]	-141.5	0.02431
[C|C]	-142	0.01376
[C|ACE]	-142.1	0.01359
[A|B]	-142.3	0.01111
[A|AD]	-142.4	0.009454
[ACI|C]	-142.5	0.008581
[AD|D]	-142.6	0.008295
[ADI|D]	-142.6	0.008212
[C|CE]	-142.7	0.006864
[AD|A]	-142.8	0.006189
[ADI|A]	-142.9	0.006127
[A|ADE]	-143	0.005498
[AI|C]	-143	0.005068
[AB|B]	-143.1	0.004707
[AI|A]	-143.2	0.004471
[ACI|A]	-143.3	0.003964
[C|AC]	-143.3	0.003918
[A|D]	-143.3	0.0038
[AB|E]	-143.3	0.003792
[AD|C]	-143.4	0.003625
[A|BC]	-143.5	0.003162
[A|CD]	-143.5	0.003065

At node N34:

split	lnL	Rel.Prob
[A|A]	-138.3	0.555
[AC|A]	-139.9	0.1163
[AI|A]	-140.4	0.07446
[AD|A]	-140.6	0.05993
[ACI|A]	-140.6	0.05724
[ADI|A]	-140.8	0.04823
[ACD|A]	-141.3	0.02774
[AB|A]	-142.4	0.01011
[A|AB]	-142.7	0.007396

At node N30:

split	lnL	Rel.Prob
[AD|I]	-139.1	0.2633
[AC|I]	-139.6	0.1609
[A|I]	-139.8	0.1318
[CD|A]	-140	0.1079
[C|AI]	-140.7	0.05377
[D|AI]	-140.8	0.04932
[D|A]	-140.9	0.04235
[C|A]	-140.9	0.04223
[AB|I]	-141	0.04054
[A|A]	-141.4	0.02668
[BCD|A]	-141.8	0.01742
[DH|A]	-142.2	0.01182
[AD|A]	-142.7	0.007326

At node N26:

split	lnL	Rel.Prob
[D|C]	-139.4	0.1928
[D|A]	-139.7	0.1485
[D|AC]	-140	0.11
[DH|C]	-140.2	0.08912
[AD|C]	-140.2	0.08691
[DH|A]	-140.4	0.06823
[D|AB]	-141	0.03975
[A|C]	-141.1	0.03386
[D|BC]	-141.2	0.0336
[D|D]	-141.2	0.03098
[D|ABC]	-141.5	0.02294
[AD|B]	-141.6	0.02145
[D|AD]	-142	0.01444
[C|C]	-142.2	0.01204
[D|CD]	-142.5	0.0091
[A|B]	-142.5	0.008716
[A|A]	-142.5	0.008668
[CD|C]	-142.6	0.008267
[D|ACD]	-142.7	0.007187
[AD|A]	-142.9	0.006033

At node N22:

split	lnL	Rel.Prob
[H|D]	-138.3	0.5876
[D|D]	-139.3	0.209
[H|AD]	-140.1	0.09739
[H|DH]	-141.5	0.02332
[DH|D]	-141.6	0.02196
[H|CD]	-141.9	0.01607

At node N25:

split	lnL	Rel.Prob
[C|C]	-138.5	0.487
[C|BC]	-138.7	0.3774
[C|ABC]	-140.6	0.05715
[C|AC]	-141.7	0.02002
[C|B]	-141.8	0.01695

At node N29:

split	lnL	Rel.Prob
[I|I]	-137.9	0.848
[AI|I]	-140.4	0.06978
[I|AI]	-140.4	0.06978

At node N33:

split	lnL	Rel.Prob
[A|A]	-137.9	0.8962
[A|AB]	-140.3	0.07815

At node N37:

split	lnL	Rel.Prob
[AC|E]	-138.5	0.4672
[AB|E]	-139.6	0.1649
[BC|E]	-139.8	0.1244
[CD|E]	-140	0.1082
[AD|E]	-140.6	0.05838
[C|E]	-141.6	0.02147
[B|E]	-142.9	0.006053

At node N91:

split	lnL	Rel.Prob
[F|EG]	-139	0.2837
[F|E]	-139.1	0.2646
[E|E]	-139.8	0.1285
[F|DE]	-139.9	0.1142
[G|EG]	-140.9	0.04197
[F|CE]	-141.5	0.02315
[F|EF]	-141.8	0.0176
[E|EG]	-141.9	0.01579
[G|DEG]	-142	0.01429
[E|DE]	-142.1	0.01285
[F|EFG]	-142.1	0.01272
[E|G]	-142.2	0.01127
[EF|E]	-142.4	0.009568

At node N47:

split	lnL	Rel.Prob
[F|F]	-137.8	0.9352
[EF|F]	-141.3	0.02938

At node N46:

split	lnL	Rel.Prob
[F|F]	-137.8	0.9908

At node N44:

split	lnL	Rel.Prob
[F|F]	-137.8	0.9986

At node N43:

split	lnL	Rel.Prob
[F|F]	-137.8	0.9996

At node N90:

split	lnL	Rel.Prob
[G|EG]	-138.4	0.541
[G|DEG]	-138.9	0.3109
[G|E]	-141	0.04039
[G|G]	-141.2	0.03322
[G|DE]	-141.5	0.02458

At node N74:

split	lnL	Rel.Prob
[G|G]	-137.8	0.9963

At node N50:

split	lnL	Rel.Prob
[G|G]	-137.8	0.9982

At node N73:

split	lnL	Rel.Prob
[G|G]	-137.8	0.9951

At node N55:

split	lnL	Rel.Prob
[G|G]	-137.8	0.998

At node N54:

split	lnL	Rel.Prob
[G|G]	-137.8	0.9996

At node N72:

split	lnL	Rel.Prob
[G|G]	-137.9	0.9069
[G|GH]	-140.2	0.08624

At node N68:

split	lnL	Rel.Prob
[G|G]	-137.8	0.999

At node N64:

split	lnL	Rel.Prob
[G|G]	-137.8	0.9994

At node N62:

split	lnL	Rel.Prob
[G|G]	-137.8	0.9997

At node N60:

split	lnL	Rel.Prob
[G|G]	-137.8	0.9999

At node N58:

split	lnL	Rel.Prob
[G|G]	-137.8	0.9999

At node N67:

split	lnL	Rel.Prob
[G|G]	-137.8	0.9993

At node N71:

split	lnL	Rel.Prob
[G|H]	-138	0.825
[G|G]	-139.9	0.1215
[G|GH]	-141.5	0.02353

At node N89:

split	lnL	Rel.Prob
[DEG|G]	-138.2	0.6121
[EG|G]	-138.9	0.3315
[G|G]	-141.8	0.01714

At node N81:

split	lnL	Rel.Prob
[DE|G]	-138.2	0.6402
[E|G]	-139	0.2836
[DEG|G]	-141.4	0.02533
[EG|G]	-142.2	0.01173

At node N77:

split	lnL	Rel.Prob
[E|D]	-137.9	0.8982
[E|E]	-141.5	0.02429
[EG|D]	-141.6	0.02145
[DE|D]	-141.8	0.01802

At node N80:

split	lnL	Rel.Prob
[G|G]	-137.8	0.9966

At node N88:

split	lnL	Rel.Prob
[G|G]	-137.8	0.9988

At node N87:

split	lnL	Rel.Prob
[G|G]	-137.8	0.9994

At node N85:

split	lnL	Rel.Prob
[G|G]	-137.8	1

At node N109:

split	lnL	Rel.Prob
[I|I]	-138.9	0.3312
[J|AIJ]	-139.7	0.1451
[I|AIJ]	-139.8	0.135
[IJ|I]	-139.9	0.1135
[I|AI]	-140.4	0.07179
[J|IJ]	-140.6	0.06079
[I|IJ]	-140.6	0.05657
[J|AI]	-141.4	0.02631
[J|I]	-141.8	0.01788

At node N108:

split	lnL	Rel.Prob
[I|AIJ]	-138.6	0.4266
[I|I]	-138.9	0.3136
[I|AI]	-139.8	0.1311
[I|IJ]	-139.9	0.1193

At node N107:

split	lnL	Rel.Prob
[A|IJ]	-138	0.7802
[A|I]	-139.5	0.1839

At node N106:

split	lnL	Rel.Prob
[I|J]	-137.8	0.9716

At node N104:

```
split  lnL     Rel.Prob
[I|I]  -137.8  0.9991
```

```
At node N113:
   split  lnL     Rel.Prob
   [I|I]  -137.8  0.9945
```

Analysis M0

```
Lagrange: likelihood analysis of geographic range evolution
Version: 20130526
Author: Richard Ree <rree@fieldmuseum.org>
https://github.com/rhr/lagrange-python

Newick tree with interior nodes labeled:
((Euptelea_pleiosperma:5.85443152956,Euptelea_polyandra:5.85443152956)N2:126.399613348,(Pteridophyllum_racemosum:123.8
90142918,(((Hypecoum_procumbens:0.995567103468,Hypecoum_imberbe:0.995567103468)N6:46.1639672934,Hypecoum_pendulum:47.1
595343969)N8:62.6681650943,(Lamprocapnos_spectabilis:83.1710757502,(((((Adlumia_asiatica:2.77654149275,Adlumia_fungosa
:2.77654149275)N12:49.6099815998,((((Dactylicapnos_torulosa:18.0729444509,(Dactylicapnos_macrocapnos:2.41479564896,Dac
tylicapnos_scandens:2.41479564896)N16:15.658148802)N17:8.12504615721,Dactylicapnos_ventii:26.1979906082)N19:18.6347554
205,((((((Corydalis_paczoskii:18.6414526681,Corydalis_nobilis:18.6414526681)N22:4.64722932813,(Corydalis_pseudorupestr
is:6.12158331292,Corydalis_hamata:6.12158331292)N25:17.1670986833)N26:3.98080292051,(Corydalis_scouleri:15.9956444151,
Corydalis_caseana:15.9956444151)N29:11.2738405016)N30:6.25967905281,(Corydalis_wilsonii:20.8197969655,Corydalis_cheila
nthifolia:20.8197969655)N33:12.7093670041)N34:6.41822489843,(Corydalis_adunca:11.81912875,Corydalis_rupestris:11.81912
875)N37:28.128260118)N38:2.75869217446,((Cysticapnos_cracca:16.2866439126,((Cysticapnos_vesicaria:10.077470913,(Discoc
apnos_mundii:6.84073504312,Trigonocapnos_lichtensteinii:6.84073504312)N43:3.23673586983)N44:3.32048502714,Cysticapnos_
pruinosa:13.3979559401)N46:2.88868797254)N47:18.7300885899,(((Pseudofumaria_lutea:6.21759268222,Pseudofumaria_alba:6.2
1759268222)N50:19.18980656,((Platycapnos_saxicola:10.113611122,(Platycapnos_tenuiloba:2.80082327123,Platycapnos_spicat
a:2.80082327123)N54:7.31278785074)N55:14.3090799582,((((((Sarcocapnos_speciosa:2.57793831388,Sarcocapnos_pulcherrima:2
.57793831388)N58:1.26884843825,Sarcocapnos_crassifolia:3.84678675213)N60:0.987678764842,Sarcocapnos_integrifolia:4.834
46551698)N62:3.89224337662,Sarcocapnos_baetica:8.72670889359)N64:4.3306437601,(Sarcocapnos_enneaphylla:5.42541617703,S
arcocapnos_saetabensis:5.42541617703)N67:7.63193647665)N68:6.46508024486,(Ceratocapnos_heterocarpa:12.4425344241,Cerat
ocapnos_claviculata:12.4425344241)N71:7.0798984745)N72:4.90025818158)N73:0.984708162042)N74:1.84315773489,(((Cryptocap
nos_chasmophyticus:9.04818651015,Fumariola_turkestanica:9.04818651015)N77:6.41010120197,(Rupicapnos_numidica:6.3678082
2199,Rupicapnos_africana:6.36780822199)N80:9.09047949013)N81:1.31211406943,(Fumaria_officinalis:11.1794179716,((Fumari
a_agraria:0.335719744366,Fumaria_densiflora:0.335719744366)N85:4.36413849555,Fumaria_capreolata:4.69985823992)N87:6.47
955973167)N88:5.59098380995)N89:10.4801551955)N90:7.7661755254)N91:7.68934853998)N92:2.12666498615)N93:1.96553395509,C
apnoides_sempervirens:46.7982799837)N95:5.58824310892)N96:4.02459869778,Ichtyoselmis_macrantha:56.4111217903)N98:5.952
48575978,(Dicentra_cucullaria:21.5769142442,(Dicentra_uniflora:13.2253771866,(Dicentra_peregrina:7.7722805443,((Dicent
ra_formosa_subsp._formosa:0.650533916236,Dicentra_formosa_subsp._oregana:0.650533916236)N104:3.17160232307,Dicentra_ex
imia:3.82213623931)N106:3.950144305)N107:5.45309664233)N108:8.35153705758)N109:40.786693306)N110:5.36692710134,(Ehrend
orferia_chrysantha:6.33057983639,Ehrendorferia_ochroleuca:6.33057983639)N113:61.3999548151)N114:15.4405410987)N115:26.
6566237411)N116:14.0624434269)N117:8.36390195956)N118:0.0;
```

Cladogram (branch lengths not to scale):

```
                                 ------------------------------+ [ABCD] Euptelea_pleiosperma
    ----------------------------N2+
    :                            ------------------------------+ [A] Euptelea_polyandra
    :
    :  ------------------------------------------------------+ [A] Pteridophyllum_racemosum
    :  :
    :  :                                   -------------------+ [G] Hypecoum_procumbens
    :  :                    ----------------N6+
    :  :  -----------------N8+                -------------------+ [G] Hypecoum_imberbe
N118+  :  :                  :
    :  :  :                  ------------------------------------+ [DEG] Hypecoum_pendulum
    :  :  :
    :  :  :  ---------------------------------------------------+ [A] Lamprocapnos_spectabilis
    :  :  :  :
    :  :  :  :                             ---------------------+ [A] Adlumia_asiatica
    :  :  :  :           ------------------N12+
    :  :  :  :           :                 ---------------------+ [IJ] Adlumia_fungosa
    :  :  :  :           :
   N117+  :  :           :                   -------------------+ [BC] Dactylicapnos_torulosa
       :  :  :           :             ------N17+
       :  :  :           :             :        :        ---------+ [C] Dactylicapnos_macrocapnos
       :  :  :           :     ------N19+        ------N16+
       :  :  :           :     :        :                 ---------+ [BC] Dactylicapnos_scandens
       :  :  :           :     :        :
       :  :  :           :     :        ---------------------------+ [C] Dactylicapnos_ventii
       :  :  :           :     :
       :  :  :           :     :                           ------+ [H] Corydalis_paczoskii
       :  :  :           :     :                     ---N22+
       :  :  :           :     :                     :     ------+ [D] Corydalis_nobilis
       :  :  :           :     :                 ---N26+
       :  :  :           :     :                 :     :     ------+ [C] Corydalis_pseudorupestris
       :  :  :           :     :                 :     ---N25+
     N116+  :           :     :            --N30+           ------+ [BC] Corydalis_hamata
         :  :           :     :            :    :
         :  :           :     :            :    :        ---------+ [I] Corydalis_scouleri
         :  :           :  N93+       --N34+    ------N29+
         :  :           :  :  :       :    :             ---------+ [I] Corydalis_caseana
         :  :           :  :  :       :    :
         :  :           :  :  :       :    :          ------------+ [A] Corydalis_wilsonii
         :  :           :  :  :  --N38+    --------N33+
         :  :           :  :  :  :    :               ------------+ [AB] Corydalis_cheilanthifolia
         :  :        N96+  :  :  :    :
         :  :        :  :  :  :  :    :             --------------+ [ABCD] Corydalis_adunca
         :  :        :  :  :  :  :    -----------N37+
         :  :        :  :  :  :  :                  --------------+ [E] Corydalis_rupestris
         :  :        :  :  :  :  :
         :  :        :  :  :  :  :        ------------------------+ [F] Cysticapnos_cracca
         :  :        :  :  :  :  :        :
         :  :        :  :  :  :  :  ---N47+           ------------+ [F] Cysticapnos_vesicaria
         :  :        :  :  :  :  :  :     :     ---N44+
         :  :        :  :  :  N92+  :     :     :     :     ------+ [F] Discocapnos_mundii
         :  :        :  :  :     :  :     ---N46+     ---N43+
         :  :        :  :  :     :  :           :           ------+ [F] Trigonocapnos_lichtensteinii
         :  :        :  :  :     :  :           :
         :  :        :  :  :     :  :           ------------------+ [F] Cysticapnos_pruinosa
         :  :        :  :  :     :  :
         :  :        :  :  :     :  :                 ------------+ [G] Pseudofumaria_lutea
        N115+        :  :  :     :  :     ---------N50+
            :        :  :  :     :  :     :           ------------+ [G] Pseudofumaria_alba
            :        :  :  :     :  :     :
            :        :  :  :     :  :     :              --------------+ [G] Platycapnos_saxicola
            :        :  :  :     :  :     :  ----N55+
            :        :  :  :     N91+     :  :      :       -------+ [G] Platycapnos_tenuiloba
            :        :  :  :        :  N74+  :      ----N54+
            :        :  :  :        :  :  :  :             -------+ [G] Platycapnos_spicata
            :        :  :  :        :  :  :  :
            :        :  :  :        :  :  :  :                 ---+ [G] Sarcocapnos_speciosa
            :        :  N95+        :  :  :  :             N58+
            :        :     :        :  :  :  :           N60+  ---+ [G] Sarcocapnos_pulcherrima
            :        :     :        :  :  N73+           :  :
            :        :     :        :  :     :        N62+  ------+ [G] Sarcocapnos_crassifolia
            :     N98+     :        :  :     :        :  :
            :     :  :     :        :  :     :     N64+  ---------+ [G] Sarcocapnos_integrifolia
            :     :  :     :        :  :     :     :  :
            :     :  :     :        :  :     :  N68+  ------------+ [G] Sarcocapnos_baetica
            :     :  :     :        :  :     :  :  :
            :     :  :     :        N90+     :  :  :       -------+ [G] Sarcocapnos_enneaphylla
            :     :  :     :           :     N72+  ----N67+
            :     :  :     :           :        :        -------+ [G] Sarcocapnos_saetabensis
            :     :  :     :           :        :
            :     :  :     :           :        :        ---------+ [G] Ceratocapnos_heterocarpa
            :     :  :     :           :        ------N71+
            :     :  :     :           :                 ---------+ [H] Ceratocapnos_claviculata
            :     :  :     :           :
            :     :  :     :           :                  --------+ [E] Cryptocapnos_chasmophyticus
            :     :  :     :           :           ----N77+
            :     :  :     :           :           :      --------+ [D] Fumariola_turkestanica
            :     :  :     :           :    ----N81+
            : N110+  :     :           :    :      :      --------+ [G] Rupicapnos_numidica
            :  :  :  :     :           :    :      ----N80+
            :  :  :  :     :           --N89+             --------+ [G] Rupicapnos_africana
            :  :  :  :     :                :
            :  :  :  :     :                :    -----------------+ [G] Fumaria_officinalis
            :  :  :  :     :                :    :
            :  :  :  :     :                --N88+          ------+ [G] Fumaria_agraria
            :  :  :  :     :                     :    ---N85+
            :  :  :  :     :                     --N87+      ------+ [G] Fumaria_densiflora
            :  :  :  :     :                          :
            :  :  :  :     :                          ------------+ [G] Fumaria_capreolata
            :  :  :  :     :
            :  :  :  :     ----------------------------------------+ [IJ] Capnoides_sempervirens
            :  :  :  :
           N114+  :  ---------------------------------------------+ [B] Ichtyoselmis_macrantha
                :  :
                :  :       ----------------------------------------+ [IJ] Dicentra_cucullaria
                :  :       :
                :  ----N109+       ------------------------------+ [I] Dicentra_uniflora
                :          :       :
                :          ----N108+       ------------------------+ [A] Dicentra_peregrina
                :                  :       :
                :                  ----N107+               --------+ [I] Dicentra_formosa_subsp._formosa
                :                          :       ----N104+
                :                          ----N106+       --------+ [I] Dicentra_formosa_subsp._oregana
                :                                  :
                :                                  -----------------+ [J] Dicentra_eximia
                :
                :                          -------------------------+ [I] Ehrendorferia_chrysantha
               ---------------------N113+
                                           -------------------------+ [I] Ehrendorferia_ochroleuca
```

```
Global ML at root node:
  -lnL = 135.8
  dispersal = 0.005889
  extinction = 0.001826

Ancestral range subdivision/inheritance scenarios ('splits') at
internal nodes.

* Split format: [left|right], where 'left' and 'right' are the ranges
  inherited by each descendant branch (on the printed tree, 'left' is
  the upper branch, and 'right' the lower branch).

* Only splits within 2 log-likelihood units of the maximum for each
  node are shown.  'Rel.Prob' is the relative probability (fraction of
  the global likelihood) of a split.

At node N118:
   split     lnL     Rel.Prob
   [A|A]     -137.9  0.1289
   [ABD|A]   -138.8  0.0511
   [AB|A]    -139    0.04116
   [ABC|A]   -139    0.04078
   [ACD|A]   -139    0.04057
   [A|ADE]   -139.1  0.04037
   [ABCD|A]  -139.2  0.036
   [AD|A]    -139.2  0.03408
   [AC|A]    -139.2  0.0335
   [BCD|A]   -139.3  0.03152
   [A|AD]    -139.4  0.02938
   [D|ADE]   -139.4  0.02765
   [BC|A]    -139.7  0.0202
   [D|AD]    -139.7  0.02013
   [CD|A]    -139.8  0.02008
   [C|A]     -139.8  0.0192
   [B|A]     -139.8  0.01871
   [A|ABE]   -139.8  0.01856
   [B|ABE]   -140    0.01616
   [B|AD]    -140    0.01535
   [D|A]     -140.1  0.01472
   [A|ADI]   -140.1  0.01437
   [C|AD]    -140.2  0.01313
   [A|AB]    -140.3  0.01174
   [B|AB]    -140.4  0.01023
   [D|ADI]   -140.5  0.00984
   [D|D]     -140.5  0.009196
   [A|AI]    -140.8  0.007014
   [B|B]     -140.9  0.006183
   [A|ABD]   -141    0.005808
   [ABD|D]   -141.1  0.005322
   [BCD|D]   -141.1  0.005252
   [C|AB]    -141.1  0.005247
   [B|ABD]   -141.1  0.005058
   [D|AB]    -141.2  0.004826
   [A|ACE]   -141.3  0.004321
   [ACD|D]   -141.3  0.004225
   [CD|D]    -141.3  0.004182
   [D|ABD]   -141.4  0.003978
   [C|ACE]   -141.4  0.003861
   [D|DE]    -141.4  0.003771
   [C|AI]    -141.4  0.003761
   [ABCD|D]  -141.4  0.003749
   [B|AI]    -141.5  0.003665
   [AD|D]    -141.5  0.003549
   [AI|A]    -141.5  0.00348
   [A|AC]    -141.5  0.003434
   [ABI|A]   -141.5  0.003338
   [A|DE]    -141.6  0.003303
   [ABE|A]   -141.6  0.003268
   [C|ABD]   -141.6  0.003244
   [ABC|D]   -141.6  0.003185
   [C|AC]    -141.6  0.003069
   [D|AI]    -141.7  0.002882
   [B|DE]    -141.7  0.002877
   [ADI|A]   -141.7  0.002854
   [ABD|B]   -141.7  0.002814
   [ACI|A]   -141.7  0.002801
   [ADH|A]   -141.7  0.002799
   [BCD|B]   -141.7  0.002777
   [ADE|A]   -141.7  0.002776
   [ACH|A]   -141.7  0.002772
   [ACE|A]   -141.7  0.002757
   [A|ABI]   -141.8  0.002636
   [AB|D]    -141.8  0.002572
   [B|BE]    -141.8  0.002549
   [BC|D]    -141.8  0.002524
   [C|DE]    -141.8  0.00246
   [B|ABI]   -141.9  0.002295
   [AB|B]    -141.9  0.002266
   [ABC|B]   -141.9  0.002246
   [A|D]     -141.9  0.002238
   [BC|B]    -142    0.002224
   [C|D]     -142.1  0.002
   [ABCD|B]  -142.1  0.001982
   [A|ADH]   -142.2  0.001821
   [A|BE]    -142.2  0.001756
   [AC|D]    -142.2  0.001744
   [CH|A]    -142.2  0.001727
   [ACD|B]   -142.2  0.001675
   [A|ACD]   -142.3  0.001606
   [B|AC]    -142.3  0.001496
   [CE|A]    -142.4  0.001485
   [C|ACD]   -142.4  0.001435
   [DH|A]    -142.4  0.001379
   [C|C]     -142.4  0.001365

At node N2:
   split     lnL     Rel.Prob
   [ABCD|A]  -136    0.8457
   [BCD|A]   -138.3  0.0839
   [ABD|A]   -139.6  0.0238

At node N117:
   split    lnL     Rel.Prob
   [A|A]    -136.5  0.5061
   [A|AD]   -138.1  0.1012
   [A|ADE]  -138.3  0.08944
   [A|ABE]  -139.1  0.03924
   [A|AB]   -139.3  0.03198
   [A|ADI]  -139.3  0.03045
   [A|ABD]  -139.9  0.01762
   [D|D]    -140    0.01522
   [A|AI]   -140.1  0.01418
   [B|B]    -140.1  0.01351
   [A|D]    -140.3  0.01184
   [A|DE]   -140.3  0.01164
   [A|BE]   -140.4  0.01056
   [A|ACE]  -140.5  0.009663
   [A|AC]   -140.6  0.008809
   [A|B]    -140.8  0.006845
   [A|ABI]  -141.2  0.004696
   [D|AD]   -141.4  0.003872
   [A|ACD]  -141.4  0.003757
   [D|ADE]  -141.5  0.003422
   [A|ADH]  -141.6  0.003015
   [I|ADI]  -141.8  0.002529
   [I|AD]   -141.9  0.002312
   [B|ABE]  -141.9  0.002311
   [B|AD]   -141.9  0.002279
   [A|ACI]  -142.1  0.001922
   [B|AB]   -142.1  0.001883

At node N116:
   split    lnL     Rel.Prob
   [D|A]    -137.4  0.2123
   [DE|A]   -137.5  0.1923
   [A|A]    -137.8  0.1365
   [D|AI]   -138.3  0.0885
   [E|AB]   -138.4  0.07971
   [D|AB]   -138.9  0.04916
   [AD|A]   -139.8  0.01974
   [ADE|A]  -139.9  0.01666
   [D|AD]   -140    0.01513
   [BE|A]   -140.3  0.0118
   [D|ADI]  -140.3  0.01169
   [CE|A]   -140.4  0.01087
   [B|A]    -140.4  0.01058
   [B|AB]   -140.4  0.01057
   [E|AC]   -140.4  0.01008
   [C|A]    -140.5  0.009858
   [B|ABI]  -140.5  0.00912
   [B|B]    -140.6  0.008277
   [E|B]    -140.7  0.007445
   [D|D]    -140.9  0.00667
   [D|AC]   -141.1  0.005413
   [E|AD]   -141.1  0.005073
   [DH|A]   -141.2  0.004832
   [DE|B]   -141.2  0.004753
   [E|ABE]  -141.3  0.004475
   [CD|A]   -141.3  0.004218
   [C|AI]   -141.3  0.004214
   [B|AI]   -141.3  0.004136

At node N8:
   split    lnL     Rel.Prob
   [G|DEG]  -136.1  0.766
   [G|DE]   -137.9  0.1272
   [G|EG]   -139.1  0.03926
   [G|BEG]  -139.9  0.01645
   [G|CEG]  -140.6  0.008951

At node N6:
   split  lnL     Rel.Prob
   [G|G]  -135.8  0.9995

At node N115:
   split    lnL     Rel.Prob
   [A|A]    -136.7  0.4066
   [A|ABI]  -137.4  0.2191
   [A|AI]   -138    0.1139
   [A|AB]   -138.3  0.08316
   [A|I]    -138.6  0.06091
   [A|ACI]  -139.7  0.02008
   [A|AIJ]  -140.6  0.008271
   [A|ADI]  -140.7  0.007728
   [I|ABI]  -140.8  0.007196
   [A|AC]   -140.9  0.006217
   [B|ABI]  -141    0.005876
   [AD|I]   -141.2  0.00489
   [AI|I]   -141.2  0.004879
   [A|B]    -141.4  0.003905

At node N114:
   split    lnL     Rel.Prob
   [ABI|I]  -136.5  0.498
   [AI|I]   -137.2  0.2645
   [I|I]    -138.5  0.07049
   [ACI|I]  -139    0.04125
   [AB|I]   -139.1  0.03933
   [A|I]    -139.6  0.02436
   [AIJ|I]  -139.7  0.02219

At node N110:
   split    lnL     Rel.Prob
   [ABI|I]  -136.6  0.479
   [AI|I]   -137.8  0.135
   [AB|I]   -138.3  0.08432
   [ABI|A]  -138.7  0.05618
   [I|I]    -138.8  0.05077
   [ACI|I]  -139.2  0.03541
   [A|A]    -139.6  0.02352
   [A|I]    -139.7  0.02114
   [A|AI]   -139.9  0.018
   [AI|A]   -140    0.01583
   [AB|A]   -140.2  0.0124
   [AI|J]   -140.3  0.01122
   [AC|I]   -140.3  0.01108

At node N98:
   split    lnL     Rel.Prob
   [AI|B]   -136.4  0.5507
   [ABI|B]  -137.5  0.196
   [A|B]    -138.7  0.05946
   [AI|A]   -139.3  0.03302
   [A|A]    -139.3  0.03241
   [ACI|A]  -139.5  0.02704
   [ACI|C]  -139.6  0.02447
   [AB|B]   -139.7  0.02041
   [AC|B]   -139.8  0.0186

At node N96:
   split    lnL     Rel.Prob
   [I|AI]   -137.1  0.2794
   [I|ACI]  -137.7  0.1512
   [I|ABI]  -137.7  0.1501
   [A|AI]   -138.2  0.09273
   [A|A]    -138.6  0.06158
   [A|ACI]  -138.8  0.05018
   [A|ABI]  -138.8  0.04981
   [AI|A]   -139.3  0.03307
   [AI|I]   -140.1  0.01449
   [I|A]    -140.1  0.01387
   [A|AC]   -140.2  0.01279
   [I|AB]   -140.3  0.01217
   [I|AC]   -140.3  0.01179
   [I|ADI]  -140.6  0.008774
   [A|AB]   -140.6  0.008169

At node N12:
   split   lnL     Rel.Prob
   [A|IJ]  -135.9  0.9638

At node N95:
   split    lnL     Rel.Prob
   [AC|I]   -136.5  0.5185
   [AB|I]   -137.3  0.2233
   [A|I]    -138.3  0.0826
   [AD|I]   -139.4  0.02891
   [A|IJ]   -139.7  0.02142
   [A|A]    -139.7  0.02071
   [C|AI]   -139.8  0.01822
   [ACI|I]  -139.9  0.01653
   [AC|A]   -140.2  0.0122
   [A|AI]   -140.8  0.00735
   [AI|I]   -140.8  0.006839

At node N93:
   split    lnL     Rel.Prob
```

```
   [C|AC]   -136.8  0.3946
   [C|ACE]  -137.4  0.2108
   [B|AB]   -138    0.1145
   [B|ABE]  -138.5  0.06684
   [C|AD]   -139.4  0.02985
   [C|AB]   -139.6  0.02423
   [C|C]    -139.7  0.02018
   [C|A]    -139.8  0.01879
   [C|AI]   -140.2  0.01333
   [C|ACD]  -140.3  0.01159
   [B|AD]   -140.6  0.008491
   [C|ACI]  -140.7  0.007735
   [B|A]    -140.8  0.006969
   [A|A]    -140.8  0.00673
   [A|AB]   -141.1  0.005434
   [B|AI]   -141.2  0.004587
   [D|AD]   -141.3  0.004296
   [A|AD]   -141.3  0.004235

At node N19:
   split   lnL     Rel.Prob
   [C|C]   -136.2  0.726
   [BC|C]  -137.5  0.1858
   [B|C]   -138.9  0.04584

At node N17:
   split   lnL     Rel.Prob
   [C|C]   -136.3  0.6304
   [BC|C]  -138.1  0.1044
   [B|BC]  -138.6  0.0665
   [C|BC]  -138.7  0.05955
   [BC|B]  -138.7  0.05536
   [B|B]   -138.9  0.04707

At node N16:
   split   lnL     Rel.Prob
   [C|BC]  -136.4  0.5961
   [C|C]   -136.8  0.3929

At node N92:
   split    lnL     Rel.Prob
   [ACE|E]  -136.8  0.3892
   [AC|E]   -137.5  0.1981
   [ABE|E]  -137.7  0.1523
   [AB|E]   -138.7  0.05967
   [AD|D]   -139.4  0.02827
   [ADI|D]  -139.8  0.01822
   [A|D]    -139.9  0.01706
   [AC|D]   -140.1  0.01378
   [ADE|E]  -140.3  0.01165
   [ACD|D]  -140.4  0.01066
   [A|DE]   -140.4  0.009989
   [CE|E]   -140.5  0.009448
   [AC|C]   -140.8  0.007234
   [ACI|C]  -141    0.006
   [C|E]    -141    0.005994
   [AD|E]   -141    0.005791
   [AB|D]   -141.1  0.004997
   [A|CE]   -141.2  0.004733

At node N38:
   split    lnL     Rel.Prob
   [A|ACE]  -137.2  0.2471
   [A|ABE]  -138    0.1196
   [A|CE]   -138.1  0.101
   [A|AC]   -138.6  0.06631
   [AC|C]   -138.7  0.05641
   [AC|E]   -139.1  0.04008
   [A|A]    -139.1  0.03837
   [A|AB]   -139.1  0.03773
   [A|BE]   -139.2  0.03317
   [AC|A]   -139.3  0.03282
   [A|C]    -139.4  0.02948
   [C|ACE]  -139.9  0.0166
   [A|AD]   -140.3  0.01174
   [A|B]    -140.3  0.01142
   [ADI|D]  -140.5  0.009664
   [A|ADE]  -140.5  0.009641
   [ACI|C]  -140.6  0.008713
   [C|C]    -140.6  0.008444
   [AD|D]   -140.7  0.007828
   [ADI|A]  -140.7  0.00781
   [AD|A]   -140.9  0.006327
   [AB|B]   -141.1  0.005281
   [ACI|A]  -141.1  0.00507
   [AI|A]   -141.1  0.004963
   [AB|E]   -141.2  0.004502
   [C|AC]   -141.3  0.004453
   [C|CE]   -141.3  0.004341
   [AB|A]   -141.4  0.003848
   [A|D]    -141.4  0.003762
   [AI|C]   -141.5  0.003668
   [A|CD]   -141.5  0.003587
   [AD|C]   -141.5  0.00338
   [AI|D]   -141.6  0.003034

At node N34:
   split    lnL     Rel.Prob
   [A|A]    -136.5  0.5335
   [AC|A]   -137.9  0.1322
   [ACI|A]  -138.4  0.07438
   [AI|A]   -138.5  0.07174
   [AD|A]   -138.8  0.04997
   [ADI|A]  -138.9  0.04896
   [ACD|A]  -139.9  0.01708
   [ACH|A]  -139.9  0.01706
   [AB|A]   -140.3  0.01149

At node N30:
   split   lnL     Rel.Prob
   [AD|I]  -137.2  0.2461
   [AC|I]  -137.4  0.2097
   [A|I]   -138.2  0.09925
   [C|AI]  -138.2  0.09085
   [CD|A]  -138.6  0.06268
   [C|A]   -138.8  0.05422
   [CH|A]  -138.8  0.05192
   [AB|I]  -138.9  0.04537
   [D|AI]  -139.1  0.03967
   [D|A]   -139.5  0.02554
   [A|A]   -140.1  0.01438
   [DH|A]  -140.3  0.01139

At node N26:
   split    lnL     Rel.Prob
   [D|C]    -138    0.1173
   [D|A]    -138.1  0.1061
   [DH|C]   -138.2  0.09692
   [H|C]    -138.4  0.07485
   [AD|C]   -138.4  0.07443
   [D|AC]   -138.5  0.0722
   [DH|A]   -138.5  0.07211
   [H|AC]   -138.8  0.05179
   [C|C]    -138.9  0.04672
   [D|AB]   -139.1  0.03972
   [AD|B]   -139.5  0.02631
   [A|C]    -139.5  0.02629
   [D|BC]   -139.6  0.02239
   [D|ABC]  -139.8  0.01984
   [D|D]    -139.8  0.01834
   [AC|C]   -140.1  0.01477
   [CD|C]   -140.4  0.01066
   [H|BC]   -140.5  0.009361
   [D|AD]   -140.6  0.008558
   [A|B]    -140.7  0.007584
   [C|AC]   -140.8  0.00733
   [CH|C]   -140.8  0.006715
   [D|CD]   -141.2  0.0046
   [ACH|C]  -141.3  0.004453
   [A|A]    -141.3  0.004386
   [D|ACD]  -141.3  0.004068
   [ACD|C]  -141.4  0.003999

At node N22:
   split   lnL     Rel.Prob
   [H|D]   -136.4  0.5964
   [D|D]   -138    0.1191
   [H|AD]  -138.2  0.09224
   [H|H]   -139.1  0.03773
   [C|D]   -139.7  0.02068
   [H|C]   -139.8  0.01927
   [H|DH]  -140    0.01628
   [DH|D]  -140    0.01628
   [H|CD]  -140.1  0.01439
   [CH|D]  -140.2  0.01305
   [H|AC]  -140.8  0.00694

At node N25:
   split    lnL     Rel.Prob
   [C|C]    -136.5  0.511
   [C|BC]   -136.8  0.3688
   [C|ABC]  -138.8  0.05396
   [C|AC]   -139.9  0.01705

At node N29:
   split   lnL     Rel.Prob
   [I|I]   -136    0.8607
   [AI|I]  -138.6  0.06473
   [I|AI]  -138.6  0.06473

At node N33:
   split   lnL     Rel.Prob
   [A|A]   -135.9  0.9094
   [A|AB]  -138.5  0.07186

At node N37:
   split   lnL     Rel.Prob
   [AC|E]  -136.7  0.4367
   [AB|E]  -137.3  0.2291
   [BC|E]  -138.1  0.1044
   [CD|E]  -138.2  0.09618
   [AD|E]  -138.4  0.07782
   [C|E]   -140    0.01643

At node N91:
   split    lnL     Rel.Prob
   [F|EG]   -137    0.3076
   [F|E]    -137.1  0.2958
   [F|DE]   -137.4  0.2079
   [E|E]    -138.6  0.06551
   [F|CE]   -139.9  0.01718
   [G|EG]   -140.1  0.01386
   [F|EF]   -140.2  0.01338
   [F|EFG]  -140.5  0.009672
   [E|EG]   -140.5  0.009313
   [E|DE]   -140.7  0.007568
   [EF|E]   -140.8  0.007306

At node N47:
   split  lnL     Rel.Prob
   [F|F]  -135.9  0.9723

At node N46:
   split  lnL     Rel.Prob
   [F|F]  -135.8  0.9961

At node N44:
   split  lnL     Rel.Prob
   [F|F]  -135.8  0.9993

At node N43:
   split  lnL     Rel.Prob
   [F|F]  -135.8  0.9998

At node N90:
   split    lnL     Rel.Prob
   [G|EG]   -136.6  0.487
   [G|DEG]  -136.8  0.3924
   [G|DE]   -139.2  0.03425
   [G|E]    -139.3  0.03134
   [G|G]    -139.8  0.01912

At node N74:
   split  lnL     Rel.Prob
   [G|G]  -135.8  0.9971

At node N50:
   split  lnL     Rel.Prob
   [G|G]  -135.8  0.999

At node N73:
   split  lnL     Rel.Prob
   [G|G]  -135.8  0.9955

At node N55:
   split  lnL     Rel.Prob
   [G|G]  -135.8  0.9989

At node N54:
   split  lnL     Rel.Prob
   [G|G]  -135.8  0.9998

At node N72:
   split   lnL     Rel.Prob
   [G|G]   -135.9  0.9126
   [G|GH]  -138.3  0.08207

At node N68:
   split  lnL     Rel.Prob
   [G|G]  -135.8  0.9994

At node N64:
   split  lnL     Rel.Prob
   [G|G]  -135.8  0.9996

At node N62:
   split  lnL     Rel.Prob
   [G|G]  -135.8  0.9998

At node N60:
   split  lnL     Rel.Prob
   [G|G]  -135.8  0.9999

At node N58:
   split  lnL     Rel.Prob
   [G|G]  -135.8  1

At node N67:
   split  lnL     Rel.Prob
   [G|G]  -135.8  0.9996

At node N71:
   split  lnL     Rel.Prob
   [G|H]  -136    0.8649
   [G|G]  -138.2  0.09674

At node N89:
   split    lnL     Rel.Prob
   [DEG|G]  -136.2  0.7032
   [EG|G]   -137.2  0.2561

At node N81:
   split    lnL     Rel.Prob
   [DE|G]   -136.1  0.7372
   [E|G]    -137.4  0.2097
   [DEG|G]  -139.7  0.02105

At node N77:
   split   lnL     Rel.Prob
   [E|D]   -135.9  0.9307
   [EG|D]  -140    0.01631
   [E|E]   -140.2  0.01334

At node N80:
   split  lnL     Rel.Prob
   [G|G]  -135.8  0.9981

At node N88:
   split  lnL     Rel.Prob
```

```
   [G|G]  -135.8  0.9993

At node N87:
   split  lnL     Rel.Prob
   [G|G]  -135.8  0.9997

At node N85:
   split  lnL     Rel.Prob
   [G|G]  -135.8  1

At node N109:
   split    lnL     Rel.Prob
   [I|I]    -136.9  0.3417
   [J|AIJ]  -137.8  0.1459
   [I|AIJ]  -137.8  0.137
   [IJ|I]   -138.1  0.1079
   [J|IJ]   -138.6  0.06474
   [I|AI]   -138.6  0.06128
   [I|IJ]   -138.6  0.06083
   [J|AI]   -139.5  0.02667
   [IJ|J]   -139.9  0.01788

At node N108:
   split    lnL     Rel.Prob
   [I|AIJ]  -136.7  0.4394
   [I|I]    -137    0.3075
   [I|IJ]   -137.9  0.1283
   [I|AI]   -138    0.1179

At node N107:
   split   lnL     Rel.Prob
   [A|IJ]  -136    0.8271
   [A|I]   -137.7  0.1491

At node N106:
   split  lnL     Rel.Prob
   [I|J]  -135.9  0.9799

At node N104:
   split  lnL     Rel.Prob
   [I|I]  -135.8  0.9993

At node N113:
   split  lnL     Rel.Prob
   [I|I]  -135.8  0.997
```

Table S8. Outputs resulting after the Lagrange analyses for the biogeographical reconstruction of the whole subfamily Fumarioideae considering *Corydalis* as represented at subgeneric level. a) Result with constraints for dispersal between areas over time (model M1); b) result without constraints for dispersal between areas over time (model M0).

Analysis M1

```
Lagrange: likelihood analysis of geographic range evolution
Version: 20130526
Author: Richard Ree <rree@fieldmuseum.org>
https://github.com/rhr/lagrange-python

Newick tree with interior nodes labeled:
((((Lamprocapnos_spectabilis:76.062519187,(((Dicentra_cucullaria:22.2346087608,(Dicentra_uniflora:13.6685037579,(Dicen
tra_peregrina:7.88023522507,((Dicentra_formosa_subsp._oregana:0.631625031978,Dicentra_formosa_subsp._formosa:0.6316250
31978)N6:3.14454570673,Dicentra_eximia:3.77617073871)N8:4.10406448636)N9:5.78826853287)N10:8.56610500284)N11:36.451320
5716,(((((Dactylicapnos_ventii:24.4459143759,((Dactylicapnos_macrocapnos:2.33731625505,Dactylicapnos_scandens:2.337316
25505)N15:14.2591035145,Dactylicapnos_torulosa:16.5964197696)N17:7.84949460628)N18:17.9517218384,((Corydalis_subgChrem
nocapnos:37.7311989283,(Corydalis_subgCorydalis:31.9080001978,Corydalis_subgSophorocapnos:31.9080001978)N22:5.82319859
222)N23:2.66152856675,(((((Fumaria_capreolata:4.51389425594,(Fumaria_densiflora:0.32100983992,Fumaria_agraria:0.321009
83992)N27:4.19288441602)N28:6.34144540368,Fumaria_officinalis:10.8553396596)N30:5.4495094079,((Rupicapnos_africana:6.3
491470616,Rupicapnos_numidica:6.3491470616)N33:8.62594944738,(Fumariola_turkestanica:8.57594885624,Cryptocapnos_chasmo
phyticus:8.57594885624)N36:6.39914765274)N37:1.32975255854)N38:9.94766354182,((((Ceratocapnos_claviculata:12.078267137
9,Ceratocapnos_heterocarpa:12.0782671379)N41:6.93012950056,(((((Sarcocapnos_speciosa:2.45428190554,Sarcocapnos_pulcher
rima:2.45428190554)N44:1.25003389389,Sarcocapnos_crassifolia:3.70431579943)N46:0.981646340801,Sarcocapnos_integrifolia
:4.68596214023)N48:3.78065587642,Sarcocapnos_baetica:8.46661801665)N50:4.25792805922,(Sarcocapnos_enneaphylla:5.407567
83341,Sarcocapnos_saetabensis:5.40756783341)N53:7.31697824246)N54:6.28385056259)N55:4.56474240122,((Platycapnos_tenuil
oba:2.72122599764,Platycapnos_spicata:2.72122599764)N58:7.27928390688,Platycapnos_saxicola:10.0005099045)N60:13.572629
1351)N61:0.940185527765,(Pseudofumaria_lutea:5.83564312446,Pseudofumaria_alba:5.83564312446)N64:18.677681443)N65:1.739
1880419)N66:7.08121794989,(Cysticapnos_cracca:15.8131700049,(Cysticapnos_pruinosa:13.1131865307,((Trigonocapnos_lichte
nsteinii:6.57454166181,Discocapnos_mundii:6.57454166181)N71:3.24495108904,Cysticapnos_vesicaria:9.81949275085)N73:3.29
369377982)N74:2.69998347424)N75:17.5205605543)N76:7.05899679759)N77:2.0049088574)N78:1.82342728655,Capnoides_sempervir
ens:44.2210635008)N80:5.61738906726,(Adlumia_fungosa:2.69237597074,Adlumia_asiatica:2.69237597074)N83:47.1460765973)N8
4:3.71087564991,Ichtyoselmis_macrantha:53.5493282179)N86:5.13660111442)N87:4.86701581521,(Ehrendorferia_chrysantha:6.0
3371329199,Ehrendorferia_ochroleuca:6.03371329199)N90:57.5192318556)N91:12.5095740395)N92:20.0834932119,((Hypecoum_pro
cumbens:0.961391095155,Hypecoum_imberbe:0.961391095155)N95:40.3785366501,Hypecoum_pendulum:41.3399277453)N97:54.806084
6536)N98:10.3291565741,Pteridophyllum_racemosum:106.475168973)N100:25.0100021641,(Euptelea_pleiosperma:5.79950808047,E
uptelea_polyandra:5.79950808047)N103:125.685663057)N104:0.0;
```

Cladogram (branch lengths not to scale):

```
            -----------------------------------------------------+ [A] Lamprocapnos_spectabilis
            :
            :             ---------------------------------------+ [IJ] Dicentra_cucullaria
            :            :
            :     -----N11+       -------------------------------+ [I] Dicentra_uniflora
            :     :       :       :
            :     :       -----N10+       ------------------------+ [A] Dicentra_peregrina
            :     :               :       :
            :     :               ------N9+               --------+ [I] Dicentra_formosa_subsp._oregana
            :     :                       :       ------N6+
            :     :                       ------N8+       --------+ [I] Dicentra_formosa_subsp._formosa
            :     :                               :
            :     :                               ----------------+ [J] Dicentra_eximia
            :     :
            :     :                   --------------------------+ [C] Dactylicapnos_ventii
            :     :                   :
            :     :           ------N18+                 ---------+ [C] Dactylicapnos_macrocapnos
            :     :           :       :        ------N15+
            :     :           :       ------N17+        ---------+ [BC] Dactylicapnos_scandens
            :     :           :               :
            :     :           :               ------------------+ [BC] Dactylicapnos_torulosa
            :     :           :
            :     :           :                      -----------------------+ [CDE] Corydalis_subgChremnocapnos
            :     :           :  --------N23+
            :     :           :  :          :           -----------+ [ABCH] Corydalis_subgCorydalis
            :     :           :  :          --------N22+
            :     :           :  :                      -----------+ [ABC] Corydalis_subgSophorocapnos
            :     :           :  :
            :     :           :  :                     ------------+ [G] Fumaria_capreolata
            :     :        N78+  :               --N28+
            :     :        :  :  :               :    :     ------+ [G] Fumaria_densiflora
            :     :        :  :  :          --N30+    ---N27+
            :     :        :  :  :          :    :          ------+ [G] Fumaria_agraria
          N92+    :        :  :  :          :    :
          :  :    :        :  :  :          :    -----------------+ [G] Fumaria_officinalis
          :  :    :        :  :  :     --N38+
          :  :    :        :  :  :     :    :              --------+ [G] Rupicapnos_africana
          :  :    :        :  :  :     :    :      ----N33+
          :  :    :        :  :  :     :    :      :       --------+ [G] Rupicapnos_numidica
          :  :    :        :  :  :     :    ----N37+
          :  :    :        :  :  :     :           :       --------+ [D] Fumariola_turkestanica
          :  :  N87+        :  :  :     :           ----N36+
          :  :  :  :        :  N77+     :                   --------+ [E] Cryptocapnos_chasmophyticus
          :  :  :  :        :    :     :
          :  :  :  :        :    :     :                 ---------+ [H] Ceratocapnos_claviculata
          :  :  :  :        :    :     :        ------N41+
          :  :  :  :        :    :     :        :        ---------+ [G] Ceratocapnos_heterocarpa
          :  :  :  :        :    :     :        :
          :  :  :  :        :    :     :        :             ---+ [G] Sarcocapnos_speciosa
          :  :  :  :        :    :  N66+        :           N44+
          :  :  :  :        :    :  :  :     N55+        N46+  ---+ [G] Sarcocapnos_pulcherrima
          :  :  :  :        :    :  :  :     :  :        :  :
          :  :  :  :        :    :  :  :     :  :     N48+  ------+ [G] Sarcocapnos_crassifolia
          :  :  :  :        :    :  :  :     :  :     :  :
          :  :  :  :        :    :  :  :     :  :  N50+  ---------+ [G] Sarcocapnos_integrifolia
          :  :  :  :     N80+    :  :  :     :  :  :  :
          :  :  :  :     :  :    :  :  :     :  N54+  ------------+ [G] Sarcocapnos_baetica
          :  :  :  :     :  :    :  :  :  N61+     :
          :  :  :  :     :  :    :  :  :  :  :     :       --------+ [G] Sarcocapnos_enneaphylla
          :  :  :  :     :  :    :  :  :  :  :     ----N53+
          :  :  :  :     :  :    :  :  :  :  :             --------+ [G] Sarcocapnos_saetabensis
          :  :  :  :     :  :    N76+  :  :  :
          :  :  :  :     :  :        :  :  :  :              -------+ [G] Platycapnos_tenuiloba
          :  :  :  :     :  :        :  N65+  :      ----N58+
       N98+  :  :  :     :  :        :     :  ----N60+      -------+ [G] Platycapnos_spicata
       :  :  :  :  :     :  :        :     :        :
       :  :  N91+  :     :  :        :     :         -------------+ [G] Platycapnos_saxicola
       :  :     :  :     :  :        :     :
       :  :     :  :     :  :        :     :           ------------+ [G] Pseudofumaria_lutea
       :  :     :  :     :  :        :     ---------N64+
       :  :     :  :  N84+  :        :                 ------------+ [G] Pseudofumaria_alba
       :  :     :  :  :  :  :        :
       :  :     :  :  :  :  :        :     ------------------------+ [F] Cysticapnos_cracca
       :  :     :  :  :  :  :        :     :
       :  :     :  :  :  :  :        ---N75+     ------------------+ [F] Cysticapnos_pruinosa
       :  :     :  :  :  :  :              :     :
       :  :     :  :  :  :  :              ---N74+           ------+ [F] Trigonocapnos_lichtensteinii
       :  :     :  :  :  :  :                    :     ---N71+
       :  :     :  :  :  :  :                    ---N73+     ------+ [F] Discocapnos_mundii
       :  :     :  N86+  :  :                          :
       :  :     :     :  :  :                          ------------+ [F] Cysticapnos_vesicaria
       :  :     :     :  :  :
  N100+  :     :     :  :  ---------------------------------------+ [IJ] Capnoides_sempervirens
    :  :  :     :     :  :
    :  :  :     :     :  :                     ---------------------+ [IJ] Adlumia_fungosa
    :  :  :     :     :  ------------------N83+
    :  :  :     :     :                      ---------------------+ [A] Adlumia_asiatica
    :  :  :     :     :
    :  :  :     :     ---------------------------------------------+ [B] Ichtyoselmis_macrantha
    :  :  :     :
    :  :  :     :                         --------------------------+ [I] Ehrendorferia_chrysantha
    :  :  :     ----------------------N90+
N104+  :  :                               --------------------------+ [I] Ehrendorferia_ochroleuca
    :  :  :
    :  :  :                                   -------------------+ [G] Hypecoum_procumbens
    :  :  :                  ----------------N95+
    :  :  ----------------N97+                  -------------------+ [G] Hypecoum_imberbe
    :  :                     :
    :  :                     --------------------------------------+ [DEG] Hypecoum_pendulum
    :  :
    :  ------------------------------------------------------------+ [A] Pteridophyllum_racemosum
    :
    :                             --------------------------------+ [ABCD] Euptelea_pleiosperma
    --------------------------N103+
                                  --------------------------------+ [A] Euptelea_polyandra
```

```
Global ML at root node:
  -lnL = 111.5
  dispersal = 0.006836
  extinction = 5.681e-09

Ancestral range subdivision/inheritance scenarios ('splits') at
internal nodes.

* Split format: [left|right], where 'left' and 'right' are the ranges
  inherited by each descendant branch (on the printed tree, 'left' is
  the upper branch, and 'right' the lower branch).

* Only splits within 2 log-likelihood units of the maximum for each
  node are shown.  'Rel.Prob' is the relative probability (fraction of
  the global likelihood) of a split.

At node N104:
   split     lnL     Rel.Prob
   [A|A]     -114    0.08436
   [ADE|A]   -114.2  0.06839
   [ADE|D]   -114.7  0.04219
   [A|ABD]   -114.7  0.0395
   [ABE|A]   -114.8  0.03695
   [A|ACD]   -114.9  0.03551
   [A|AC]    -115    0.03127
   [A|ABCD]  -115    0.03116
   [ABE|B]   -115.1  0.02866
   [A|BCD]   -115.1  0.02781
   [AD|A]    -115.1  0.02775
   [A|ABC]   -115.2  0.02593
   [A|AB]    -115.3  0.02302
   [A|AD]    -115.4  0.01987
   [AB|A]    -115.6  0.01772
   [A|CD]    -115.6  0.01731
   [AD|D]    -115.6  0.01712
   [D|D]     -115.6  0.01712
   [A|BC]    -115.7  0.01566
   [B|B]     -115.7  0.01471
   [D|BCD]   -115.7  0.01464
   [AB|B]    -115.8  0.01374
   [D|ABD]   -115.9  0.013
   [AD|B]    -115.9  0.01291
   [DE|D]    -115.9  0.01271
   [DE|A]    -115.9  0.01236
   [D|ACD]   -116    0.01168
   [ADI|A]   -116    0.01148
   [D|CD]    -116    0.01139
   [A|B]     -116    0.0109
   [A|C]     -116    0.01079
   [AD|C]    -116.1  0.01065
   [D|ABCD]  -116.1  0.01025
   [B|BCD]   -116.1  0.01
   [BE|B]    -116.2  0.009612
   [DE|B]    -116.2  0.009588
   [B|ABD]   -116.2  0.008881
   [A|D]     -116.3  0.008674
   [DE|C]    -116.4  0.007906
   [BE|A]    -116.4  0.007436
   [ADI|D]   -116.5  0.007085
   [B|BC]    -116.5  0.00704
   [B|ABCD]  -116.5  0.007006
   [AB|C]    -116.5  0.006799
   [AB|D]    -116.5  0.006559
   [D|AD]    -116.5  0.006535
   [D|ABC]   -116.6  0.006398
   [ABD|A]   -116.6  0.006264
   [D|BC]    -116.6  0.006181
   [B|ACD]   -116.6  0.005988
   [B|ABC]   -116.7  0.00583
   [B|AB]    -116.8  0.005176
   [D|AC]    -116.8  0.005143
   [AI|A]    -116.8  0.004911
   [ABD|B]   -116.8  0.004858
   [BE|C]    -116.9  0.004755
   [B|CD]    -116.9  0.00467
   [D|A]     -116.9  0.004625
   [BE|D]    -116.9  0.004587

At node N100:
   split    lnL     Rel.Prob
   [ADE|A]  -113    0.2178
   [A|A]    -113.1  0.2073
   [ABE|A]  -113.3  0.1618
   [AD|A]   -113.3  0.1612
   [ADI|A]  -114.4  0.0574
   [AB|A]   -114.4  0.05545
   [ABD|A]  -114.7  0.043
   [BE|A]   -114.7  0.0406
   [DE|A]   -115.3  0.02235

At node N98:
   split   lnL     Rel.Prob
   [A|DE]  -112.7  0.3125
   [AB|E]  -112.9  0.2513
   [A|D]   -113    0.2241
   [AI|D]  -113.8  0.1049
   [AB|D]  -114.1  0.07255

At node N92:
   split    lnL     Rel.Prob
   [A|A]    -112.6  0.3333
   [A|ABI]  -112.7  0.3078
   [A|AB]   -113.3  0.1688
   [A|AI]   -113.6  0.1252
   [A|I]    -114.5  0.05252

At node N91:
   split    lnL     Rel.Prob
   [ABI|I]  -112    0.6432
   [AI|I]   -112.9  0.2404
   [I|I]    -114.7  0.04274
   [AB|I]   -114.7  0.04124

At node N87:
   split    lnL     Rel.Prob
   [I|ABI]  -112    0.6129
   [I|AI]   -113.7  0.1185
   [I|AB]   -113.9  0.09283
   [A|ABI]  -114.3  0.0641
   [I|I]    -115.1  0.02779
   [I|A]    -115.8  0.0135
   [A|AI]   -115.9  0.01239
   [A|AB]   -115.9  0.01226

At node N11:
   split    lnL     Rel.Prob
   [I|I]    -112.5  0.3817
   [J|AIJ]  -113.6  0.122
   [IJ|I]   -113.7  0.1179
   [I|AIJ]  -113.7  0.1131
   [I|AI]   -114.2  0.0682
   [J|IJ]   -114.3  0.06441
   [I|IJ]   -114.3  0.0597
   [J|AI]   -115.3  0.0222
   [J|I]    -115.5  0.01862

At node N10:
   split    lnL     Rel.Prob
   [I|AIJ]  -112.5  0.3888
   [I|I]    -112.6  0.3439
   [I|AI]   -113.5  0.131
   [I|IJ]   -113.6  0.13

At node N9:
   split   lnL     Rel.Prob
   [A|IJ]  -111.7  0.8002
   [A|I]   -113.1  0.1998

At node N8:
   split  lnL     Rel.Prob
   [I|J]  -111.5  1

At node N6:
   split  lnL     Rel.Prob
   [I|I]  -111.5  1

At node N86:
   split    lnL     Rel.Prob
   [AI|B]   -111.7  0.7999
   [ABI|B]  -113.9  0.09093
   [A|B]    -114.1  0.0786

At node N84:
   split    lnL     Rel.Prob
   [AI|I]   -112.4  0.3952
   [ACI|I]  -113.4  0.1516
   [AI|A]   -113.5  0.132
   [ABI|I]  -114.2  0.0715
   [ACI|A]  -114.5  0.05066
   [A|A]    -114.6  0.04475
   [A|AI]   -114.9  0.03511
   [ABI|A]  -115.3  0.02389
   [I|AI]   -115.3  0.0228
   [A|I]    -115.7  0.01488
   [AC|A]   -116    0.011

At node N80:
   split   lnL     Rel.Prob
   [AC|I]  -111.9  0.7105
   [A|I]   -113.5  0.1388
   [AB|I]  -113.7  0.1103

At node N78:
   split    lnL     Rel.Prob
   [C|AC]   -112.1  0.5629
   [C|ACE]  -112.8  0.2854
   [B|AB]   -114.4  0.0533
   [B|ABE]  -115.1  0.0282
   [C|ACD]  -115.5  0.01863
   [C|AB]   -116    0.01104

At node N18:
   split   lnL     Rel.Prob
   [C|C]   -111.7  0.8455
   [C|BC]  -113.6  0.1269

At node N17:
   split   lnL     Rel.Prob
   [C|C]   -111.9  0.7042
   [C|BC]  -113.9  0.09537
   [BC|B]  -114.4  0.05806
   [BC|C]  -114.4  0.05474
   [B|BC]  -114.5  0.04886

At node N15:
   split   lnL     Rel.Prob
   [C|BC]  -112.1  0.5742
   [C|C]   -112.4  0.4165

At node N77:
   split    lnL     Rel.Prob
   [AC|E]   -112.3  0.458
   [ACE|E]  -112.5  0.3781
   [ABE|E]  -114.2  0.07015
   [AC|D]   -115.4  0.02089
   [ACD|D]  -115.5  0.01884
   [AB|E]   -115.6  0.01765

At node N23:
   split    lnL     Rel.Prob
   [C|AC]   -112.6  0.3315
   [CE|A]   -113    0.2178
   [E|AC]   -113.3  0.1705
   [C|A]    -113.9  0.08833
   [E|AB]   -114.1  0.072
   [C|ABC]  -114.7  0.04316
   [CD|A]   -115.5  0.01823
   [D|AC]   -115.7  0.01452

At node N22:
   split     lnL     Rel.Prob
   [A|A]     -112.9  0.2437
   [AC|C]    -113.6  0.1186
   [AC|A]    -113.7  0.1145
   [C|AC]    -114.2  0.06773
   [A|AC]    -114.2  0.06606
   [ABC|B]   -114.5  0.05059
   [ABC|C]   -114.6  0.04613
   [ABC|A]   -114.6  0.04455
   [AB|B]    -114.9  0.03333
   [AB|A]    -115    0.02935
   [B|AB]    -115.5  0.01887
   [A|AB]    -115.6  0.01724
   [A|C]     -115.6  0.01681
   [C|A]     -115.6  0.01665
   [B|ABC]   -115.8  0.01356
   [C|ABC]   -115.9  0.0127
   [A|ABC]   -115.9  0.01239
   [ABCH|B]  -116    0.01155
   [ABCH|C]  -116.1  0.01053
   [ABCH|A]  -116.1  0.01017

At node N76:
   split   lnL     Rel.Prob
   [EG|F]  -112.4  0.4289
   [E|F]   -112.4  0.4177
   [DE|F]  -113.4  0.1529

At node N66:
   split    lnL     Rel.Prob
   [EG|G]   -112    0.5973
   [DEG|G]  -112.6  0.3357
   [E|G]    -114.7  0.04132

At node N38:
   split    lnL     Rel.Prob
   [G|DEG]  -111.9  0.6631
   [G|EG]   -112.6  0.3251

At node N30:
   split  lnL     Rel.Prob
   [G|G]  -111.5  1

At node N28:
   split  lnL     Rel.Prob
   [G|G]  -111.5  1
```

```
At node N27:
   split  lnL     Rel.Prob
   [G|G]  -111.5  1

At node N37:
   split   lnL     Rel.Prob
   [G|DE]  -111.8  0.7213
   [G|E]   -112.8  0.2787

At node N33:
   split  lnL     Rel.Prob
   [G|G]  -111.5  1

At node N36:
   split  lnL     Rel.Prob
   [D|E]  -111.5  1

At node N65:
   split  lnL     Rel.Prob
   [G|G]  -111.5  0.9982

At node N61:
   split  lnL     Rel.Prob
   [G|G]  -111.5  0.9949

At node N55:
   split   lnL     Rel.Prob
   [G|G]   -111.6  0.899
   [GH|G]  -113.9  0.09622

At node N41:
   split  lnL     Rel.Prob
   [H|G]  -111.5  1

At node N54:
   split  lnL     Rel.Prob
   [G|G]  -111.5  1

At node N50:
   split  lnL     Rel.Prob
   [G|G]  -111.5  1

At node N48:
   split  lnL     Rel.Prob
   [G|G]  -111.5  1

At node N46:
   split  lnL     Rel.Prob
   [G|G]  -111.5  1

At node N44:
   split  lnL     Rel.Prob
   [G|G]  -111.5  1

At node N53:
   split  lnL     Rel.Prob
   [G|G]  -111.5  1

At node N60:
   split  lnL     Rel.Prob
   [G|G]  -111.5  1

At node N58:
   split  lnL     Rel.Prob
   [G|G]  -111.5  1

At node N64:
   split  lnL     Rel.Prob
   [G|G]  -111.5  1

At node N75:
   split  lnL     Rel.Prob
   [F|F]  -111.5  1

At node N74:
   split  lnL     Rel.Prob
   [F|F]  -111.5  1

At node N73:
   split  lnL     Rel.Prob
   [F|F]  -111.5  1

At node N71:
   split  lnL     Rel.Prob
   [F|F]  -111.5  1

At node N83:
   split   lnL     Rel.Prob
   [IJ|A]  -111.6  0.9608

At node N90:
   split  lnL     Rel.Prob
   [I|I]  -111.5  1

At node N97:
   split    lnL     Rel.Prob
   [G|DEG]  -111.8  0.7308
   [G|EG]   -113.5  0.1375
   [G|DE]   -113.8  0.1035

At node N95:
   split  lnL     Rel.Prob
   [G|G]  -111.5  1

At node N103:
   split     lnL     Rel.Prob
   [ABCD|A]  -111.7  0.8407
   [BCD|A]   -113.9  0.09427
   [ABD|A]   -115    0.03047
```

Analysis M0

```
Lagrange: likelihood analysis of geographic range evolution
Version: 20130526
Author: Richard Ree <rree@fieldmuseum.org>
https://github.com/rhr/lagrange-python

Newick tree with interior nodes labeled:
((((Lamprocapnos_spectabilis:76.062519187,(((Dicentra_cucullaria:22.2346087608,(Dicentra_uniflora:13.6685037579,(Dicen
tra_peregrina:7.88023522507,((Dicentra_formosa_subsp._oregana:0.631625031978,Dicentra_formosa_subsp._formosa:0.6316250
31978)N6:3.14454570673,Dicentra_eximia:3.77617073871)N8:4.10406448636)N9:5.78826853287)N10:8.56610500284)N11:36.451320
5716,(((((Dactylicapnos_ventii:24.4459143759,((Dactylicapnos_macrocapnos:2.33731625505,Dactylicapnos_scandens:2.337316
25505)N15:14.2591035145,Dactylicapnos_torulosa:16.5964197696)N17:7.84949460628)N18:17.9517218384,((Corydalis_subgChrem
nocapnos:37.7311989283,(Corydalis_subgCorydalis:31.9080001978,Corydalis_subgSophorocapnos:31.9080001978)N22:5.82319859
222)N23:2.66152856675,(((((Fumaria_capreolata:4.51389425594,(Fumaria_densiflora:0.32100983992,Fumaria_agraria:0.321009
83992)N27:4.19288441602)N28:6.34144540368,Fumaria_officinalis:10.8553396596)N30:5.4495094079,((Rupicapnos_africana:6.3
491470616,Rupicapnos_numidica:6.3491470616)N33:8.62594944738,(Fumariola_turkestanica:8.57594885624,Cryptocapnos_chasmo
phyticus:8.57594885624)N36:6.39914765274)N37:1.32975255854)N38:9.94766354182,((((Ceratocapnos_claviculata:12.078267137
9,Ceratocapnos_heterocarpa:12.0782671379)N41:6.93012950056,(((((Sarcocapnos_speciosa:2.45428190554,Sarcocapnos_pulcher
rima:2.45428190554)N44:1.25003389389,Sarcocapnos_crassifolia:3.70431579943)N46:0.981646340801,Sarcocapnos_integrifolia
:4.68596214023)N48:3.78065587642,Sarcocapnos_baetica:8.46661801665)N50:4.25792805922,(Sarcocapnos_enneaphylla:5.407567
83341,Sarcocapnos_saetabensis:5.40756783341)N53:7.31697824246)N54:6.28385056259)N55:4.56474240122,((Platycapnos_tenuil
oba:2.72122599764,Platycapnos_spicata:2.72122599764)N58:7.27928390688,Platycapnos_saxicola:10.0005099045)N60:13.572629
1351)N61:0.940185527765,(Pseudofumaria_lutea:5.83564312446,Pseudofumaria_alba:5.83564312446)N64:18.677681443)N65:1.739
1880419)N66:7.08121794989,(Cysticapnos_cracca:15.8131700049,(Cysticapnos_pruinosa:13.1131865307,((Trigonocapnos_lichte
nsteinii:6.57454166181,Discocapnos_mundii:6.57454166181)N71:3.24495108904,Cysticapnos_vesicaria:9.81949275085)N73:3.29
369377982)N74:2.69998347424)N75:17.5205605543)N76:7.05899679759)N77:2.0049088574)N78:1.82342728655,Capnoides_sempervir
ens:44.2210635008)N80:5.61738906726,(Adlumia_fungosa:2.69237597074,Adlumia_asiatica:2.69237597074)N83:47.1460765973)N8
4:3.71087564991,Ichtyoselmis_macrantha:53.5493282179)N86:5.13660111442)N87:4.86701581521,(Ehrendorferia_chrysantha:6.0
3371329199,Ehrendorferia_ochroleuca:6.03371329199)N90:57.5192318556)N91:12.5095740395)N92:20.0834932119,((Hypecoum_pro
cumbens:0.961391095155,Hypecoum_imberbe:0.961391095155)N95:40.3785366501,Hypecoum_pendulum:41.3399277453)N97:54.806084
6536)N98:10.3291565741,Pteridophyllum_racemosum:106.475168973)N100:25.0100021641,(Euptelea_pleiosperma:5.79950808047,E
uptelea_polyandra:5.79950808047)N103:125.685663057)N104:0.0;
```

Cladogram (branch lengths not to scale):

```
            -----------------------------------------------------+ [A] Lamprocapnos_spectabilis
            :
            :             ---------------------------------------+ [IJ] Dicentra_cucullaria
            :             :
            :     -----N11+       -------------------------------+ [I] Dicentra_uniflora
            :     :       :       :
            :     :       -----N10+       ------------------------+ [A] Dicentra_peregrina
            :     :               :       :
            :     :               ------N9+               --------+ [I] Dicentra_formosa_subsp._oregana
            :     :                       :       ------N6+
            :     :                       ------N8+       --------+ [I] Dicentra_formosa_subsp._formosa
            :     :                               :
            :     :                               ----------------+ [J] Dicentra_eximia
            :     :
            :     :                   --------------------------+ [C] Dactylicapnos_ventii
            :     :                   :
            :     :           ------N18+                 ---------+ [C] Dactylicapnos_macrocapnos
            :     :           :       :        ------N15+
            :     :           :       ------N17+        ---------+ [BC] Dactylicapnos_scandens
            :     :           :               :
            :     :           :               ------------------+ [BC] Dactylicapnos_torulosa
            :     :           :
            :     :           :                      -----------------------+ [CDE] Corydalis_subgChremnocapnos
            :     :           :  --------N23+
            :     :           :  :          :           -----------+ [ABCH] Corydalis_subgCorydalis
            :     :           :  :          --------N22+
            :     :           :  :                      -----------+ [ABC] Corydalis_subgSophorocapnos
            :     :           :  :
            :     :           :  :                     ------------+ [G] Fumaria_capreolata
            :     :        N78+  :               --N28+
            :     :        :  :  :               :    :     ------+ [G] Fumaria_densiflora
            :     :        :  :  :          --N30+    ---N27+
            :     :        :  :  :          :    :          ------+ [G] Fumaria_agraria
          N92+    :        :  :  :          :    :
          :  :    :        :  :  :          :    -----------------+ [G] Fumaria_officinalis
          :  :    :        :  :  :     --N38+
          :  :    :        :  :  :     :    :             --------+ [G] Rupicapnos_africana
          :  :    :        :  :  :     :    :      ----N33+
          :  :    :        :  :  :     :    :      :      --------+ [G] Rupicapnos_numidica
          :  :    :        :  :  :     :    ----N37+
          :  :    :        :  :  :     :           :      --------+ [D] Fumariola_turkestanica
          :  :  N87+        :  :  :     :           ----N36+
          :  :  :  :        :  N77+     :                  --------+ [E] Cryptocapnos_chasmophyticus
          :  :  :  :        :    :     :
          :  :  :  :        :    :     :                 ---------+ [H] Ceratocapnos_claviculata
          :  :  :  :        :    :     :        ------N41+
          :  :  :  :        :    :     :        :        ---------+ [G] Ceratocapnos_heterocarpa
          :  :  :  :        :    :     :        :
          :  :  :  :        :    :     :        :             ---+ [G] Sarcocapnos_speciosa
          :  :  :  :        :    :  N66+        :           N44+
          :  :  :  :        :    :  :  :     N55+        N46+  ---+ [G] Sarcocapnos_pulcherrima
          :  :  :  :        :    :  :  :     :  :        :  :
          :  :  :  :        :    :  :  :     :  :     N48+  ------+ [G] Sarcocapnos_crassifolia
          :  :  :  :        :    :  :  :     :  :     :  :
          :  :  :  :        :    :  :  :     :  :  N50+  ---------+ [G] Sarcocapnos_integrifolia
          :  :  :  :     N80+    :  :  :     :  :  :  :
          :  :  :  :     :  :    :  :  :     :  N54+  ------------+ [G] Sarcocapnos_baetica
          :  :  :  :     :  :    :  :  :  N61+     :
          :  :  :  :     :  :    :  :  :  :  :     :       --------+ [G] Sarcocapnos_enneaphylla
          :  :  :  :     :  :    :  :  :  :  :     ----N53+
          :  :  :  :     :  :    :  :  :  :  :             --------+ [G] Sarcocapnos_saetabensis
          :  :  :  :     :  :    N76+  :  :  :
          :  :  :  :     :  :        :  :  :  :              -------+ [G] Platycapnos_tenuiloba
          :  :  :  :     :  :        :  N65+  :      ----N58+
       N98+  :  :  :     :  :        :     :  ----N60+      -------+ [G] Platycapnos_spicata
       :  :  :  :  :     :  :        :     :        :
       :  :  N91+  :     :  :        :     :         --------------+ [G] Platycapnos_saxicola
       :  :     :  :     :  :        :     :
       :  :     :  :     :  :        :     :           ------------+ [G] Pseudofumaria_lutea
       :  :     :  :     :  :        :     ---------N64+
       :  :     :  :  N84+  :        :                 ------------+ [G] Pseudofumaria_alba
       :  :     :  :  :  :  :        :
       :  :     :  :  :  :  :        :     ------------------------+ [F] Cysticapnos_cracca
       :  :     :  :  :  :  :        :     :
       :  :     :  :  :  :  :        ---N75+     ------------------+ [F] Cysticapnos_pruinosa
       :  :     :  :  :  :  :              :     :
       :  :     :  :  :  :  :              ---N74+           ------+ [F] Trigonocapnos_lichtensteinii
       :  :     :  :  :  :  :                    :     ---N71+
       :  :     :  :  :  :  :                    ---N73+     ------+ [F] Discocapnos_mundii
       :  :     :  N86+  :  :                          :
       :  :     :     :  :  :                          ------------+ [F] Cysticapnos_vesicaria
       :  :     :     :  :  :
  N100+  :     :     :  :  ---------------------------------------+ [IJ] Capnoides_sempervirens
    :  :  :     :     :  :
    :  :  :     :     :  :                     ---------------------+ [IJ] Adlumia_fungosa
    :  :  :     :     :  ------------------N83+
    :  :  :     :     :                      ---------------------+ [A] Adlumia_asiatica
    :  :  :     :     :
    :  :  :     :     ---------------------------------------------+ [B] Ichtyoselmis_macrantha
    :  :  :     :
    :  :  :     :                         --------------------------+ [I] Ehrendorferia_chrysantha
    :  :  :     ----------------------N90+
N104+  :  :                               --------------------------+ [I] Ehrendorferia_ochroleuca
    :  :  :
    :  :  :                                   -------------------+ [G] Hypecoum_procumbens
    :  :  :                  ----------------N95+
    :  :  ----------------N97+                  -------------------+ [G] Hypecoum_imberbe
    :  :                     :
    :  :                     --------------------------------------+ [DEG] Hypecoum_pendulum
    :  :
    :  ------------------------------------------------------------+ [A] Pteridophyllum_racemosum
    :
    :                              --------------------------------+ [ABCD] Euptelea_pleiosperma
    --------------------------N103+
                                   --------------------------------+ [A] Euptelea_polyandra
```

```
Global ML at root node:
  -lnL = 108.1
  dispersal = 0.006641
  extinction = 1.759e-09

Ancestral range subdivision/inheritance scenarios ('splits') at
internal nodes.

* Split format: [left|right], where 'left' and 'right' are the ranges
  inherited by each descendant branch (on the printed tree, 'left' is
  the upper branch, and 'right' the lower branch).

* Only splits within 2 log-likelihood units of the maximum for each
  node are shown.  'Rel.Prob' is the relative probability (fraction of
  the global likelihood) of a split.

At node N104:
   split     lnL     Rel.Prob
   [A|A]     -110.4  0.09546
   [ADE|A]   -110.9  0.0607
   [ADE|D]   -111.1  0.04787
   [A|ABD]   -111.2  0.04306
   [A|ACD]   -111.4  0.03583
   [AD|A]    -111.5  0.03137
   [A|ABCD]  -111.6  0.0289
   [A|AD]    -111.6  0.02776
   [ABE|A]   -111.7  0.02744
   [D|D]     -111.7  0.02719
   [A|AB]    -111.7  0.02662
   [A|BCD]   -111.7  0.02654
   [AD|D]    -111.8  0.02474
   [ABE|B]   -111.8  0.02297
   [A|AC]    -111.9  0.02148
   [A|ABC]   -112    0.01917
   [A|CD]    -112.1  0.01694
   [ADI|A]   -112.1  0.01685
   [AD|B]    -112.2  0.01576
   [D|ABD]   -112.2  0.01555
   [D|BCD]   -112.2  0.01534
   [A|C]     -112.3  0.01418
   [AD|C]    -112.3  0.01398
   [A|B]     -112.4  0.01332
   [ADI|D]   -112.4  0.01329
   [A|BC]    -112.4  0.01311
   [AB|A]    -112.4  0.01306
   [D|ACD]   -112.4  0.01294
   [DE|D]    -112.4  0.01293
   [A|D]     -112.4  0.01255
   [D|CD]    -112.5  0.01223
   [B|B]     -112.5  0.0113
   [AB|B]    -112.6  0.01093
   [D|ABCD]  -112.6  0.01044
   [D|AD]    -112.7  0.01003
   [DE|A]    -112.7  0.009834
   [DE|B]    -112.9  0.008232
   [DE|C]    -113    0.007302
   [AI|A]    -113.1  0.006517
   [BE|B]    -113.1  0.006201
   [AB|D]    -113.2  0.006181
   [B|ABD]   -113.2  0.006089
   [B|BCD]   -113.2  0.006004
   [AB|C]    -113.2  0.005819
   [D|AB]    -113.2  0.005769
   [D|A]     -113.2  0.005746
   [D|BC]    -113.2  0.005684
   [D|ABC]   -113.3  0.005193
   [ABD|A]   -113.3  0.005145
   [D|C]     -113.3  0.005119
   [BE|A]    -113.5  0.004445
   [ABD|B]   -113.5  0.004307
   [B|ABCD]  -113.6  0.004087
   [ABD|D]   -113.6  0.004058
   [D|AC]    -113.6  0.003879
   [B|ACD]   -113.6  0.0038
   [B|AB]    -113.6  0.003765
   [B|BC]    -113.7  0.003709
   [BE|D]    -113.7  0.003505

At node N100:
   split    lnL     Rel.Prob
   [ADE|A]  -109.4  0.2683
   [A|A]    -109.5  0.2306
   [AD|A]   -110.1  0.1343
   [ABE|A]  -110.2  0.1139
   [ADI|A]  -110.5  0.08585
   [ABD|A]  -111.2  0.04243
   [AB|A]   -111.4  0.03538
   [DE|A]   -111.6  0.02999
   [BE|A]   -111.6  0.02956

At node N98:
   split   lnL     Rel.Prob
   [A|DE]  -109    0.3856
   [A|D]   -109.7  0.2036
   [AB|E]  -109.8  0.1684
   [AI|D]  -110    0.139
   [AB|D]  -110.6  0.07625

At node N92:
   split    lnL     Rel.Prob
   [A|A]    -109    0.3788
   [A|ABI]  -109.3  0.3028
   [A|AI]   -110.1  0.1247
   [A|AB]   -110.2  0.1209
   [A|I]    -110.9  0.05641

At node N91:
   split    lnL     Rel.Prob
   [ABI|I]  -108.5  0.6192
   [AI|I]   -109.5  0.2494
   [I|I]    -111.1  0.0466
   [AB|I]   -111.1  0.04585

At node N87:
   split    lnL     Rel.Prob
   [I|ABI]  -108.6  0.6074
   [I|AI]   -110.2  0.1226
   [I|AB]   -110.4  0.0947
   [A|ABI]  -110.9  0.05973
   [I|I]    -111.5  0.03087
   [A|AI]   -112.5  0.01206
   [I|A]    -112.5  0.01134
   [A|AB]   -112.6  0.01069
   [J|AI]   -112.7  0.01021

At node N11:
   split    lnL     Rel.Prob
   [I|I]    -109    0.3877
   [J|AIJ]  -110.1  0.126
   [I|AIJ]  -110.2  0.1169
   [IJ|I]   -110.3  0.1094
   [J|IJ]   -110.8  0.06774
   [I|IJ]   -110.8  0.06286
   [I|AI]   -111    0.05352
   [J|AI]   -111.7  0.02514
   [IJ|J]   -112    0.01865

At node N10:
   split    lnL     Rel.Prob
   [I|AIJ]  -109    0.4049
   [I|I]    -109.2  0.3377
   [I|IJ]   -110    0.138
   [I|AI]   -110.2  0.1137

At node N9:
   split   lnL     Rel.Prob
   [A|IJ]  -108.2  0.8428
   [A|I]   -109.9  0.1572

At node N8:
   split  lnL     Rel.Prob
   [I|J]  -108.1  1

At node N6:
   split  lnL     Rel.Prob
   [I|I]  -108.1  1

At node N86:
   split    lnL     Rel.Prob
   [AI|B]   -108.3  0.8284
   [ABI|B]  -110.7  0.07197
   [A|B]    -110.7  0.06888

At node N84:
   split    lnL     Rel.Prob
   [AI|I]   -109    0.402
   [ACI|I]  -109.8  0.175
   [AI|A]   -110.1  0.1256
   [ABI|I]  -110.9  0.05775
   [ACI|A]  -111    0.05471
   [A|A]    -111.3  0.03864
   [A|AI]   -111.5  0.03215
   [I|AI]   -112    0.02028
   [ABI|A]  -112.1  0.01805
   [A|I]    -112.3  0.01481
   [AC|I]   -112.6  0.0109
   [AC|A]   -112.6  0.01027

At node N80:
   split   lnL     Rel.Prob
   [AC|I]  -108.3  0.7718
   [A|I]   -110.3  0.1074
   [AB|I]  -110.5  0.08954

At node N78:
   split    lnL     Rel.Prob
   [C|AC]   -108.6  0.5732
   [C|ACE]  -109.2  0.3225
   [B|AB]   -111.1  0.04714
   [B|ABE]  -111.6  0.02809

At node N18:
   split   lnL     Rel.Prob
   [C|C]   -108.2  0.8679
   [C|BC]  -110.3  0.1046

At node N17:
   split   lnL     Rel.Prob
   [C|C]   -108.4  0.7357
   [C|BC]  -110.6  0.0833
   [BC|B]  -111    0.0523
   [BC|C]  -111.1  0.04647
   [B|BC]  -111.2  0.04309

At node N15:
   split   lnL     Rel.Prob
   [C|BC]  -108.7  0.5487
   [C|C]   -108.9  0.4425

At node N77:
   split    lnL     Rel.Prob
   [AC|E]   -108.8  0.4731
   [ACE|E]  -108.9  0.4315
   [ABE|E]  -110.7  0.07008

At node N23:
   split    lnL     Rel.Prob
   [C|AC]   -109.2  0.3301
   [CE|A]   -109.5  0.2339
   [E|AC]   -109.6  0.2117
   [C|A]    -110.7  0.075
   [E|AB]   -110.7  0.07239
   [C|ABC]  -111.7  0.02653
   [C|ACH]  -112.2  0.01606

At node N22:
   split     lnL     Rel.Prob
   [A|A]     -109.5  0.2378
   [AC|A]    -110.1  0.1262
   [AC|C]    -110.2  0.1167
   [C|AC]    -110.5  0.08959
   [A|AC]    -111.2  0.04477
   [ACH|A]   -111.5  0.03328
   [ACH|C]   -111.5  0.03078
   [ABC|B]   -111.6  0.0288
   [C|A]     -111.7  0.02693
   [ABC|A]   -111.8  0.02506
   [ABC|C]   -111.8  0.02318
   [AB|B]    -111.8  0.02278
   [AB|A]    -112    0.01983
   [B|AB]    -112    0.01897
   [C|ABC]   -112.1  0.01851
   [A|AB]    -112.1  0.01696
   [A|C]     -112.5  0.01245
   [B|ABC]   -112.6  0.01035
   [ABCH|B]  -112.7  0.009677
   [A|ABC]   -112.7  0.009247
   [AC|B]    -112.8  0.008557
   [ABCH|A]  -112.8  0.008421
   [CH|A]    -112.9  0.008348
   [BC|A]    -112.9  0.008312

At node N76:
   split  lnL     Rel.Prob
   [E|F]  -108.1  1

At node N66:
   split    lnL     Rel.Prob
   [EG|G]   -108.4  0.701
   [DEG|G]  -109.5  0.2304
   [E|G]    -111.1  0.04609

At node N38:
   split    lnL     Rel.Prob
   [G|DEG]  -108.5  0.6452
   [G|EG]   -109.1  0.3409

At node N30:
   split  lnL     Rel.Prob
   [G|G]  -108.1  1

At node N28:
   split  lnL     Rel.Prob
   [G|G]  -108.1  1

At node N27:
   split  lnL     Rel.Prob
```

```
   [G|G]  -108.1  1

At node N37:
   split   lnL     Rel.Prob
   [G|DE]  -108.4  0.722
   [G|E]   -109.3  0.278

At node N33:
   split  lnL     Rel.Prob
   [G|G]  -108.1  1

At node N36:
   split  lnL     Rel.Prob
   [D|E]  -108.1  1

At node N65:
   split  lnL     Rel.Prob
   [G|G]  -108.1  0.9988

At node N61:
   split  lnL     Rel.Prob
   [G|G]  -108.1  0.9959

At node N55:
   split   lnL     Rel.Prob
   [G|G]   -108.2  0.9064
   [GH|G]  -110.5  0.08926

At node N41:
   split  lnL     Rel.Prob
   [H|G]  -108.1  1

At node N54:
   split  lnL     Rel.Prob
   [G|G]  -108.1  1

At node N50:
   split  lnL     Rel.Prob
   [G|G]  -108.1  1

At node N48:
   split  lnL     Rel.Prob
   [G|G]  -108.1  1

At node N46:
   split  lnL     Rel.Prob
   [G|G]  -108.1  1

At node N44:
   split  lnL     Rel.Prob
   [G|G]  -108.1  1

At node N53:
   split  lnL     Rel.Prob
   [G|G]  -108.1  1

At node N60:
   split  lnL     Rel.Prob
   [G|G]  -108.1  1

At node N58:
   split  lnL     Rel.Prob
   [G|G]  -108.1  1

At node N64:
   split  lnL     Rel.Prob
   [G|G]  -108.1  1

At node N75:
   split  lnL     Rel.Prob
   [F|F]  -108.1  1

At node N74:
   split  lnL     Rel.Prob
   [F|F]  -108.1  1

At node N73:
   split  lnL     Rel.Prob
   [F|F]  -108.1  1

At node N71:
   split  lnL     Rel.Prob
   [F|F]  -108.1  1

At node N83:
   split   lnL     Rel.Prob
   [IJ|A]  -108.1  0.9737

At node N90:
   split  lnL     Rel.Prob
   [I|I]  -108.1  1

At node N97:
   split    lnL     Rel.Prob
   [G|DEG]  -108.3  0.8166
   [G|DE]   -109.9  0.1528

At node N95:
   split  lnL     Rel.Prob
   [G|G]  -108.1  1

At node N103:
   split     lnL     Rel.Prob
   [ABCD|A]  -108.2  0.8777
   [BCD|A]   -110.4  0.09576
```

Table S9. Outputs obtained after the Lagrange analyses for the biogeographical reconstruction focused on the Mediterranean taxa of subtribe Fumariinae. a) Result with constraints for dispersal between areas over time (model MM1); b) result without constraints for dispersal between areas over time (model MM0).

Analysis MM1

```
Lagrange: likelihood analysis of geographic range evolution
Version: 20130526
Author: Richard Ree <rree@fieldmuseum.org>
https://github.com/rhr/lagrange-python

Newick tree with interior nodes labeled:
((Euptelea_pleiosperma:5.85443152956,Euptelea_polyandra:5.85443152956)N2:126.399613348,(Pteridophyllum_racemosum:123.8
90142918,(((Hypecoum_procumbens:0.995567103468,Hypecoum_imberbe:0.995567103468)N6:46.1639672934,Hypecoum_pendulum:47.1
595343969)N8:62.6681650943,(Lamprocapnos_spectabilis:83.1710757502,(((((Adlumia_asiatica:2.77654149275,Adlumia_fungosa
:2.77654149275)N12:49.6099815998,((((Dactylicapnos_torulosa:18.0729444509,(Dactylicapnos_macrocapnos:2.41479564896,Dac
tylicapnos_scandens:2.41479564896)N16:15.658148802)N17:8.12504615721,Dactylicapnos_ventii:26.1979906082)N19:18.6347554
205,((((((Corydalis_paczoskii:18.6414526681,Corydalis_nobilis:18.6414526681)N22:4.64722932813,(Corydalis_pseudorupestr
is:6.12158331292,Corydalis_hamata:6.12158331292)N25:17.1670986833)N26:3.98080292051,(Corydalis_scouleri:15.9956444151,
Corydalis_caseana:15.9956444151)N29:11.2738405016)N30:6.25967905281,(Corydalis_wilsonii:20.8197969655,Corydalis_cheila
nthifolia:20.8197969655)N33:12.7093670041)N34:6.41822489843,(Corydalis_adunca:11.81912875,Corydalis_rupestris:11.81912
875)N37:28.128260118)N38:2.75869217446,((Cysticapnos_cracca:16.2866439126,((Cysticapnos_vesicaria:10.077470913,(Discoc
apnos_mundii:6.84073504312,Trigonocapnos_lichtensteinii:6.84073504312)N43:3.23673586983)N44:3.32048502714,Cysticapnos_
pruinosa:13.3979559401)N46:2.88868797254)N47:18.7300885899,(((Pseudofumaria_lutea:6.21759268222,Pseudofumaria_alba:6.2
1759268222)N50:19.18980656,((Platycapnos_saxicola:10.113611122,(Platycapnos_tenuiloba:2.80082327123,Platycapnos_spicat
a:2.80082327123)N54:7.31278785074)N55:14.3090799582,((((((Sarcocapnos_speciosa:2.57793831388,Sarcocapnos_pulcherrima:2
.57793831388)N58:1.26884843825,Sarcocapnos_crassifolia:3.84678675213)N60:0.987678764842,Sarcocapnos_integrifolia:4.834
46551698)N62:3.89224337662,Sarcocapnos_baetica:8.72670889359)N64:4.3306437601,(Sarcocapnos_enneaphylla:5.42541617703,S
arcocapnos_saetabensis:5.42541617703)N67:7.63193647665)N68:6.46508024486,(Ceratocapnos_heterocarpa:12.4425344241,Cerat
ocapnos_claviculata:12.4425344241)N71:7.0798984745)N72:4.90025818158)N73:0.984708162042)N74:1.84315773489,(((Cryptocap
nos_chasmophyticus:9.04818651015,Fumariola_turkestanica:9.04818651015)N77:6.41010120197,(Rupicapnos_numidica:6.3678082
2199,Rupicapnos_africana:6.36780822199)N80:9.09047949013)N81:1.31211406943,(Fumaria_officinalis:11.1794179716,((Fumari
a_agraria:0.335719744366,Fumaria_densiflora:0.335719744366)N85:4.36413849555,Fumaria_capreolata:4.69985823992)N87:6.47
955973167)N88:5.59098380995)N89:10.4801551955)N90:7.7661755254)N91:7.68934853998)N92:2.12666498615)N93:1.96553395509,C
apnoides_sempervirens:46.7982799837)N95:5.58824310892)N96:4.02459869778,Ichtyoselmis_macrantha:56.4111217903)N98:5.952
48575978,(Dicentra_cucullaria:21.5769142442,(Dicentra_uniflora:13.2253771866,(Dicentra_peregrina:7.7722805443,((Dicent
ra_formosa_subsp._formosa:0.650533916236,Dicentra_formosa_subsp._oregana:0.650533916236)N104:3.17160232307,Dicentra_ex
imia:3.82213623931)N106:3.950144305)N107:5.45309664233)N108:8.35153705758)N109:40.786693306)N110:5.36692710134,(Ehrend
orferia_chrysantha:6.33057983639,Ehrendorferia_ochroleuca:6.33057983639)N113:61.3999548151)N114:15.4405410987)N115:26.
6566237411)N116:14.0624434269)N117:8.36390195956)N118:0.0;
```

Cladogram (branch lengths not to scale):

```
                                 ------------------------------+ [E] Euptelea_pleiosperma
    ----------------------------N2+
    :                            ------------------------------+ [E] Euptelea_polyandra
    :
    :  -------------------------------------------------------+ [E] Pteridophyllum_racemosum
    :  :
    :  :                                   -------------------+ [E] Hypecoum_procumbens
    :  :                    -----------------N6+
    :  :  -----------------N8+                -------------------+ [E] Hypecoum_imberbe
N118+  :  :                  :
    :  :  :                  ------------------------------------+ [E] Hypecoum_pendulum
    :  :  :
    :  :  :  ---------------------------------------------------+ [E] Lamprocapnos_spectabilis
    :  :  :  :
    :  :  :  :                                 ---------------------+ [E] Adlumia_asiatica
    :  :  :  :           ------------------N12+
    :  :  :  :           :                     ---------------------+ [E] Adlumia_fungosa
    :  :  :  :           :
   N117+  :  :           :                       ------------------+ [E] Dactylicapnos_torulosa
       :  :  :           :              ------N17+
       :  :  :           :                :        :        ---------+ [E] Dactylicapnos_macrocapnos
       :  :  :           :     ------N19+        ------N16+
       :  :  :           :     :        :                  ---------+ [E] Dactylicapnos_scandens
       :  :  :           :     :        :
       :  :  :           :     :        ---------------------------+ [E] Dactylicapnos_ventii
       :  :  :           :     :
       :  :  :           :     :                             ------+ [E] Corydalis_paczoskii
       :  :  :           :     :                       ---N22+
       :  :  :           :     :                       :     ------+ [E] Corydalis_nobilis
       :  :  :           :     :                 ---N26+
       :  :  :           :     :                 :     :     ------+ [E] Corydalis_pseudorupestris
       :  :  :           :     :                 :     ---N25+
     N116+  :           :     :            --N30+           ------+ [E] Corydalis_hamata
         :  :           :     :            :    :
         :  :           :     :            :    :        ---------+ [E] Corydalis_scouleri
         :  :           :  N93+       --N34+    ------N29+
         :  :           :  :  :       :    :             ---------+ [E] Corydalis_caseana
         :  :           :  :  :       :    :
         :  :           :  :  :       :    :          ------------+ [E] Corydalis_wilsonii
         :  :           :  :  :  --N38+    --------N33+
         :  :           :  :  :  :    :               ------------+ [E] Corydalis_cheilanthifolia
         :  :        N96+  :  :  :    :
         :  :        :  :  :  :  :    :             --------------+ [E] Corydalis_adunca
         :  :        :  :  :  :  :    -----------N37+
         :  :        :  :  :  :  :                  --------------+ [E] Corydalis_rupestris
         :  :        :  :  :  :  :
         :  :        :  :  :  :  :        ------------------------+ [E] Cysticapnos_cracca
         :  :        :  :  :  :  :        :
         :  :        :  :  :  :  :  ---N47+           ------------+ [E] Cysticapnos_vesicaria
         :  :        :  :  :  :  :  :     :     ---N44+
         :  :        :  :  :  N92+  :     :     :     :     ------+ [E] Discocapnos_mundii
         :  :        :  :  :     :  :     ---N46+     ---N43+
         :  :        :  :  :     :  :           :           ------+ [E] Trigonocapnos_lichtensteinii
         :  :        :  :  :     :  :           :
         :  :        :  :  :     :  :           ------------------+ [E] Cysticapnos_pruinosa
         :  :        :  :  :     :  :
         :  :        :  :  :     :  :                 ------------+ [C] Pseudofumaria_lutea
       N115+         :  :  :     :  :     ---------N50+
           :         :  :  :     :  :     :           ------------+ [C] Pseudofumaria_alba
           :         :  :  :     :  :     :
           :         :  :  :     :  :     :          --------------+ [AB] Platycapnos_saxicola
           :         :  :  :     :  :     :  ----N55+
           :         :  :  :     N91+     :  :      :      -------+ [AB] Platycapnos_tenuiloba
           :         :  :  :        :  N74+  :      ----N54+
           :         :  :  :        :  :  :  :             -------+ [AB] Platycapnos_spicata
           :         :  :  :        :  :  :  :
           :         :  :  :        :  :  :  :                 ---+ [B] Sarcocapnos_speciosa
           :         :  N95+        :  :  :  :             N58+
           :         :     :        :  :  :  :           N60+  ---+ [B] Sarcocapnos_pulcherrima
           :         :     :        :  :  N73+           :  :
           :         :     :        :  :     :        N62+  ------+ [A] Sarcocapnos_crassifolia
           :     N98+      :        :  :     :        :  :
           :     :  :      :        :  :     :     N64+  ---------+ [B] Sarcocapnos_integrifolia
           :     :  :      :        :  :     :     :  :
           :     :  :      :        :  :     :  N68+  ------------+ [B] Sarcocapnos_baetica
           :     :  :      :        :  :     :  :  :
           :     :  :      :        N90+     :  :  :      -------+ [AB] Sarcocapnos_enneaphylla
           :     :  :      :           :     N72+  ----N67+
           :     :  :      :           :        :        -------+ [B] Sarcocapnos_saetabensis
           :     :  :      :           :        :
           :     :  :      :           :        :        ---------+ [AB] Ceratocapnos_heterocarpa
           :     :  :      :           :        ------N71+
           :     :  :      :           :                 ---------+ [D] Ceratocapnos_claviculata
           :     :  :      :           :
           :     :  :      :           :                  -------+ [E] Cryptocapnos_chasmophyticus
           :     :  :      :           :           ----N77+
           :     :  :      :           :           :      -------+ [F] Fumariola_turkestanica
           :     :  :      :           :    ----N81+
           : N110+  :      :           :    :      :      -------+ [A] Rupicapnos_numidica
           :  :  :  :      :           :    :      ----N80+
           :  :  :  :      :           --N89+             -------+ [AB] Rupicapnos_africana
           :  :  :  :      :                :
           :  :  :  :      :                :    -----------------+ [ABC] Fumaria_officinalis
           :  :  :  :      :                :    :
           :  :  :  :      :                --N88+          ------+ [AB] Fumaria_agraria
           :  :  :  :      :                     :    ---N85+
           :  :  :  :      :                     --N87+      ------+ [ABC] Fumaria_densiflora
           :  :  :  :      :                          :
           :  :  :  :      :                          ------------+ [ABC] Fumaria_capreolata
           :  :  :  :      :
           :  :  :  :      ---------------------------------------+ [E] Capnoides_sempervirens
           :  :  :  :
         N114+  :  :  ---------------------------------------------+ [E] Ichtyoselmis_macrantha
             :  :
             :  :       ---------------------------------------+ [E] Dicentra_cucullaria
             :  :       :
             :  ----N109+       ------------------------------+ [E] Dicentra_uniflora
             :          :       :
             :          ----N108+       ------------------------+ [E] Dicentra_peregrina
             :                  :       :
             :                  ----N107+               --------+ [E] Dicentra_formosa_subsp._formosa
             :                          :       ----N104+
             :                          ----N106+       --------+ [E] Dicentra_formosa_subsp._oregana
             :                                  :
             :                                  -----------------+ [E] Dicentra_eximia
             :
             :                          -------------------------+ [E] Ehrendorferia_chrysantha
             ---------------------N113+
                                        -------------------------+ [E] Ehrendorferia_ochroleuca
```

```
Global ML at root node:
  -lnL = 114
  dispersal = 0.003672
  extinction = 5.787e-10

Ancestral range subdivision/inheritance scenarios ('splits') at
internal nodes.

* Split format: [left|right], where 'left' and 'right' are the ranges
  inherited by each descendant branch (on the printed tree, 'left' is
  the upper branch, and 'right' the lower branch).

* Only splits within 2 log-likelihood units of the maximum for each
  node are shown.  'Rel.Prob' is the relative probability (fraction of
  the global likelihood) of a split.

At node N90:
   split   lnL     Rel.Prob
   [C|E]   -114.4  0.6669
   [C|CE]  -116.3  0.1057
   [C|C]   -116.6  0.08022
   [BC|B]  -116.9  0.05949
   [BC|C]  -116.9  0.05865

At node N74:
   split  lnL     Rel.Prob
   [C|B]  -114.1  0.9811

At node N50:
   split  lnL   Rel.Prob
   [C|C]  -114  1

At node N73:
   split  lnL     Rel.Prob
   [B|B]  -114.1  0.9735

At node N55:
   split   lnL     Rel.Prob
   [B|B]   -114.7  0.501
   [AB|A]  -116.1  0.1326
   [AB|B]  -116.1  0.1291
   [A|AB]  -116.2  0.1165
   [B|AB]  -116.2  0.1139

At node N54:
   split   lnL     Rel.Prob
   [B|B]   -115.5  0.2262
   [AB|A]  -115.7  0.1811
   [A|AB]  -115.7  0.1811
   [AB|B]  -115.7  0.18
   [B|AB]  -115.7  0.18
   [A|A]   -117    0.04993

At node N72:
   split   lnL     Rel.Prob
   [B|B]   -114.2  0.8849
   [B|BD]  -116.3  0.1035

At node N68:
   split   lnL     Rel.Prob
   [B|B]   -114.1  0.9407
   [B|AB]  -117.5  0.03213

At node N64:
   split   lnL     Rel.Prob
   [B|B]   -114.1  0.9043
   [AB|B]  -116.4  0.09506

At node N62:
   split   lnL     Rel.Prob
   [B|B]   -114.6  0.5427
   [AB|B]  -114.8  0.4565

At node N60:
   split  lnL   Rel.Prob
   [B|A]  -114  1

At node N58:
   split  lnL   Rel.Prob
   [B|B]  -114  1

At node N67:
   split   lnL     Rel.Prob
   [B|B]   -114.3  0.772
   [AB|B]  -115.5  0.2258

At node N71:
   split  lnL   Rel.Prob
   [B|D]  -114  1

At node N89:
   split    lnL     Rel.Prob
   [AE|A]   -114.4  0.6714
   [E|C]    -116.2  0.1154
   [A|AC]   -116.3  0.1003
   [A|ABC]  -117    0.05365
   [A|AB]   -117.2  0.04425

At node N81:
   split  lnL   Rel.Prob
   [E|A]  -114  1

At node N77:
   split  lnL   Rel.Prob
   [E|F]  -114  1

At node N80:
   split   lnL     Rel.Prob
   [A|A]   -114.2  0.8051
   [A|AB]  -115.7  0.1927

At node N88:
   split    lnL     Rel.Prob
   [A|A]    -115.3  0.2946
   [AB|B]   -116.5  0.08295
   [B|AB]   -116.8  0.06598
   [AC|A]   -116.9  0.05689
   [AC|C]   -116.9  0.05443
   [C|C]    -117    0.0521
   [ABC|B]  -117.1  0.04842
   [AB|A]   -117.1  0.04553
   [C|AC]   -117.1  0.04469
   [A|AC]   -117.2  0.0425
   [A|AB]   -117.2  0.04062
   [B|ABC]  -117.5  0.03115
   [ABC|A]  -117.7  0.02658
   [ABC|C]  -117.7  0.02543
   [C|ABC]  -117.9  0.02017
   [A|ABC]  -118    0.01918

At node N87:
   split    lnL     Rel.Prob
   [A|A]    -116.3  0.1028
   [B|ABC]  -116.5  0.08505
   [B|AB]   -116.5  0.08388
   [AB|B]   -116.5  0.08364
   [ABC|B]  -116.7  0.0713
   [A|ABC]  -116.9  0.05681
   [A|AB]   -116.9  0.05603
   [AB|A]   -117    0.05116
   [B|B]    -117.1  0.04723
   [ABC|C]  -117.1  0.04636
   [C|ABC]  -117.1  0.04612
   [ABC|A]  -117.2  0.04361
   [B|BC]   -117.4  0.03538
   [BC|B]   -117.6  0.02966
   [C|C]    -117.6  0.02932
   [A|AC]   -117.7  0.02486
   [AC|C]   -117.9  0.02029
   [C|AC]   -117.9  0.02018
   [BC|C]   -118    0.01928

At node N85:
   split    lnL     Rel.Prob
   [A|ABC]  -115    0.3978
   [B|ABC]  -115    0.3975
   [AB|B]   -117.2  0.04248
   [A|AB]   -117.2  0.04066
   [B|AB]   -117.2  0.04063
   [B|BC]   -117.5  0.03041
   [AB|A]   -117.7  0.02587
```

Analysis MM0

```
Lagrange: likelihood analysis of geographic range evolution
Version: 20130526
Author: Richard Ree <rree@fieldmuseum.org>
https://github.com/rhr/lagrange-python

Newick tree with interior nodes labeled:
((Euptelea_pleiosperma:5.85443152956,Euptelea_polyandra:5.85443152956)N2:126.399613348,(Pteridophyllum_racemosum:123.8
90142918,(((Hypecoum_procumbens:0.995567103468,Hypecoum_imberbe:0.995567103468)N6:46.1639672934,Hypecoum_pendulum:47.1
595343969)N8:62.6681650943,(Lamprocapnos_spectabilis:83.1710757502,(((((Adlumia_asiatica:2.77654149275,Adlumia_fungosa
:2.77654149275)N12:49.6099815998,((((Dactylicapnos_torulosa:18.0729444509,(Dactylicapnos_macrocapnos:2.41479564896,Dac
tylicapnos_scandens:2.41479564896)N16:15.658148802)N17:8.12504615721,Dactylicapnos_ventii:26.1979906082)N19:18.6347554
205,((((((Corydalis_paczoskii:18.6414526681,Corydalis_nobilis:18.6414526681)N22:4.64722932813,(Corydalis_pseudorupestr
is:6.12158331292,Corydalis_hamata:6.12158331292)N25:17.1670986833)N26:3.98080292051,(Corydalis_scouleri:15.9956444151,
Corydalis_caseana:15.9956444151)N29:11.2738405016)N30:6.25967905281,(Corydalis_wilsonii:20.8197969655,Corydalis_cheila
nthifolia:20.8197969655)N33:12.7093670041)N34:6.41822489843,(Corydalis_adunca:11.81912875,Corydalis_rupestris:11.81912
875)N37:28.128260118)N38:2.75869217446,((Cysticapnos_cracca:16.2866439126,((Cysticapnos_vesicaria:10.077470913,(Discoc
apnos_mundii:6.84073504312,Trigonocapnos_lichtensteinii:6.84073504312)N43:3.23673586983)N44:3.32048502714,Cysticapnos_
pruinosa:13.3979559401)N46:2.88868797254)N47:18.7300885899,(((Pseudofumaria_lutea:6.21759268222,Pseudofumaria_alba:6.2
1759268222)N50:19.18980656,((Platycapnos_saxicola:10.113611122,(Platycapnos_tenuiloba:2.80082327123,Platycapnos_spicat
a:2.80082327123)N54:7.31278785074)N55:14.3090799582,((((((Sarcocapnos_speciosa:2.57793831388,Sarcocapnos_pulcherrima:2
.57793831388)N58:1.26884843825,Sarcocapnos_crassifolia:3.84678675213)N60:0.987678764842,Sarcocapnos_integrifolia:4.834
46551698)N62:3.89224337662,Sarcocapnos_baetica:8.72670889359)N64:4.3306437601,(Sarcocapnos_enneaphylla:5.42541617703,S
arcocapnos_saetabensis:5.42541617703)N67:7.63193647665)N68:6.46508024486,(Ceratocapnos_heterocarpa:12.4425344241,Cerat
ocapnos_claviculata:12.4425344241)N71:7.0798984745)N72:4.90025818158)N73:0.984708162042)N74:1.84315773489,(((Cryptocap
nos_chasmophyticus:9.04818651015,Fumariola_turkestanica:9.04818651015)N77:6.41010120197,(Rupicapnos_numidica:6.3678082
2199,Rupicapnos_africana:6.36780822199)N80:9.09047949013)N81:1.31211406943,(Fumaria_officinalis:11.1794179716,((Fumari
a_agraria:0.335719744366,Fumaria_densiflora:0.335719744366)N85:4.36413849555,Fumaria_capreolata:4.69985823992)N87:6.47
955973167)N88:5.59098380995)N89:10.4801551955)N90:7.7661755254)N91:7.68934853998)N92:2.12666498615)N93:1.96553395509,C
apnoides_sempervirens:46.7982799837)N95:5.58824310892)N96:4.02459869778,Ichtyoselmis_macrantha:56.4111217903)N98:5.952
48575978,(Dicentra_cucullaria:21.5769142442,(Dicentra_uniflora:13.2253771866,(Dicentra_peregrina:7.7722805443,((Dicent
ra_formosa_subsp._formosa:0.650533916236,Dicentra_formosa_subsp._oregana:0.650533916236)N104:3.17160232307,Dicentra_ex
imia:3.82213623931)N106:3.950144305)N107:5.45309664233)N108:8.35153705758)N109:40.786693306)N110:5.36692710134,(Ehrend
orferia_chrysantha:6.33057983639,Ehrendorferia_ochroleuca:6.33057983639)N113:61.3999548151)N114:15.4405410987)N115:26.
6566237411)N116:14.0624434269)N117:8.36390195956)N118:0.0;
```

```
Cladogram (branch lengths not to scale):
                                 -------------------------------+ [E] Euptelea_pleiosperma
    ----------------------------N2+
    :                            -------------------------------+ [E] Euptelea_polyandra
    :
    :  ------------------------------------------------------------+ [E] Pteridophyllum_racemosum
    :  :
    :  :                                   -------------------+ [E] Hypecoum_procumbens
    :  :                    -----------------N6+
    :  :  -----------------N8+                -------------------+ [E] Hypecoum_imberbe
N118+  :  :                  :
    :  :  :                  ------------------------------------+ [E] Hypecoum_pendulum
    :  :  :
    :  :  :  ---------------------------------------------------+ [E] Lamprocapnos_spectabilis
    :  :  :  :
    :  :  :  :                             ---------------------+ [E] Adlumia_asiatica
    :  :  :  :           ------------------N12+
    :  :  :  :           :                 ---------------------+ [E] Adlumia_fungosa
    :  :  :  :           :
   N117+  :  :           :                   -------------------+ [E] Dactylicapnos_torulosa
       :  :  :           :             ------N17+
       :  :  :           :             :        :        ---------+ [E] Dactylicapnos_macrocapnos
       :  :  :           :     ------N19+        ------N16+
       :  :  :           :     :        :                 ---------+ [E] Dactylicapnos_scandens
       :  :  :           :     :        :
       :  :  :           :     :        ---------------------------+ [E] Dactylicapnos_ventii
       :  :  :           :     :
       :  :  :           :     :                            ------+ [E] Corydalis_paczoskii
       :  :  :           :     :                      ---N22+
       :  :  :           :     :                      :     ------+ [E] Corydalis_nobilis
       :  :  :           :     :                 ---N26+
       :  :  :           :     :                 :     :     ------+ [E] Corydalis_pseudorupestris
       :  :  :           :     :                 :     ---N25+
     N116+  :           :     :            --N30+           ------+ [E] Corydalis_hamata
         :  :           :     :            :    :
         :  :           :     :            :    :        ---------+ [E] Corydalis_scouleri
         :  :           :  N93+       --N34+    ------N29+
         :  :           :  :  :       :    :             ---------+ [E] Corydalis_caseana
         :  :           :  :  :       :    :
         :  :           :  :  :       :    :          ------------+ [E] Corydalis_wilsonii
         :  :           :  :  :  --N38+    --------N33+
         :  :           :  :  :  :    :               ------------+ [E] Corydalis_cheilanthifolia
         :  :       N96+  :  :  :    :
         :  :       :  :  :  :  :    :             --------------+ [E] Corydalis_adunca
         :  :       :  :  :  :  :    -----------N37+
         :  :       :  :  :  :  :                  --------------+ [E] Corydalis_rupestris
         :  :       :  :  :  :  :
         :  :       :  :  :  :  :        ------------------------+ [E] Cysticapnos_cracca
         :  :       :  :  :  :  :        :
         :  :       :  :  :  :  :  ---N47+           ------------+ [E] Cysticapnos_vesicaria
         :  :       :  :  :  :  :  :     :     ---N44+
         :  :       :  :  :  N92+  :     :     :     :     ------+ [E] Discocapnos_mundii
         :  :       :  :  :     :  :     ---N46+     ---N43+
         :  :       :  :  :     :  :           :           ------+ [E] Trigonocapnos_lichtensteinii
         :  :       :  :  :     :  :           :
         :  :       :  :  :     :  :           ------------------+ [E] Cysticapnos_pruinosa
         :  :       :  :  :     :  :
         :  :       :  :  :     :  :                ------------+ [C] Pseudofumaria_lutea
       N115+        :  :  :     :  :     ---------N50+
           :        :  :  :     :  :     :           ------------+ [C] Pseudofumaria_alba
           :        :  :  :     :  :     :
           :        :  :  :     :  :     :          --------------+ [AB] Platycapnos_saxicola
           :        :  :  :     :  :     :  ----N55+
           :        :  :  :     N91+     :  :      :      -------+ [AB] Platycapnos_tenuiloba
           :        :  :  :        :  N74+  :      ----N54+
           :        :  :  :        :  :  :  :             -------+ [AB] Platycapnos_spicata
           :        :  :  :        :  :  :  :
           :        :  :  :        :  :  :  :                 ---+ [B] Sarcocapnos_speciosa
           :        :  N95+        :  :  :  :             N58+
           :        :     :        :  :  :  :           N60+  ---+ [B] Sarcocapnos_pulcherrima
           :        :     :        :  :  N73+           :  :
           :        :     :        :  :     :        N62+  ------+ [A] Sarcocapnos_crassifolia
           :     N98+     :        :  :     :        :  :
           :     :  :     :        :  :     :     N64+  ---------+ [B] Sarcocapnos_integrifolia
           :     :  :     :        :  :     :     :  :
           :     :  :     :        :  :     :  N68+  ------------+ [B] Sarcocapnos_baetica
           :     :  :     :        :  :     :  :  :
           :     :  :     :        N90+     :  :  :      --------+ [AB] Sarcocapnos_enneaphylla
           :     :  :     :           :     N72+  ----N67+
           :     :  :     :           :        :        --------+ [B] Sarcocapnos_saetabensis
           :     :  :     :           :        :
           :     :  :     :           :        :        ---------+ [AB] Ceratocapnos_heterocarpa
           :     :  :     :           :        ------N71+
           :     :  :     :           :                 ---------+ [D] Ceratocapnos_claviculata
           :     :  :     :           :
           :     :  :     :           :                  --------+ [E] Cryptocapnos_chasmophyticus
           :     :  :     :           :           ----N77+
           :     :  :     :           :           :      --------+ [F] Fumariola_turkestanica
           :     :  :     :           :    ----N81+
           : N110+  :     :           :    :      :      --------+ [A] Rupicapnos_numidica
           :  :  :  :     :           :    :      ----N80+
           :  :  :  :     :           --N89+             --------+ [AB] Rupicapnos_africana
           :  :  :  :     :                :
           :  :  :  :     :                :    -----------------+ [ABC] Fumaria_officinalis
           :  :  :  :     :                :    :
           :  :  :  :     :                --N88+          ------+ [AB] Fumaria_agraria
           :  :  :  :     :                     :    ---N85+
           :  :  :  :     :                     --N87+      ------+ [ABC] Fumaria_densiflora
           :  :  :  :     :                          :
           :  :  :  :     :                          ------------+ [ABC] Fumaria_capreolata
           :  :  :  :     :
           :  :  :  :     ----------------------------------------+ [E] Capnoides_sempervirens
           :  :  :  :
         N114+  :  ---------------------------------------------+ [E] Ichtyoselmis_macrantha
             :  :
             :  :       ----------------------------------------+ [E] Dicentra_cucullaria
             :  :       :
             :  ----N109+       ------------------------------+ [E] Dicentra_uniflora
             :          :       :
             :          ----N108+       ------------------------+ [E] Dicentra_peregrina
             :                  :       :
             :                  ----N107+               --------+ [E] Dicentra_formosa_subsp._formosa
             :                          :       ----N104+
             :                          ----N106+       --------+ [E] Dicentra_formosa_subsp._oregana
             :                                  :
             :                                  -----------------+ [E] Dicentra_eximia
             :
             :                          -------------------------+ [E] Ehrendorferia_chrysantha
             ---------------------N113+
                                        -------------------------+ [E] Ehrendorferia_ochroleuca
```

```
Global ML at root node:
  -lnL = 105.9
  dispersal = 0.003071
  extinction = 3.949e-10

Ancestral range subdivision/inheritance scenarios ('splits') at
internal nodes.

* Split format: [left|right], where 'left' and 'right' are the ranges
  inherited by each descendant branch (on the printed tree, 'left' is
  the upper branch, and 'right' the lower branch).

* Only splits within 2 log-likelihood units of the maximum for each
  node are shown.  'Rel.Prob' is the relative probability (fraction of
  the global likelihood) of a split.

At node N90:
   split    lnL     Rel.Prob
   [C|E]    -106.8  0.4157
   [A|AE]   -107    0.3291
   [C|CE]   -108.9  0.04997
   [C|C]    -109.5  0.02911
   [ABC|A]  -109.6  0.02579
   [BC|A]   -109.7  0.02383
   [AC|A]   -109.7  0.02377
   [BC|B]   -109.8  0.02083
   [BC|C]   -109.8  0.02072
   [A|A]    -110    0.01677

At node N74:
   split   lnL     Rel.Prob
   [C|B]   -106.5  0.5681
   [C|AB]  -107.4  0.232
   [C|A]   -107.6  0.1962

At node N50:
   split  lnL     Rel.Prob
   [C|C]  -105.9  1

At node N73:
   split   lnL     Rel.Prob
   [B|B]   -106.5  0.5623
   [AB|B]  -107.2  0.2922
   [A|A]   -108.3  0.09173
   [A|B]   -109.6  0.02598

At node N55:
   split   lnL     Rel.Prob
   [B|B]   -107.1  0.2987
   [AB|B]  -107.7  0.1662
   [AB|A]  -107.7  0.1662
   [B|AB]  -107.8  0.1464
   [A|AB]  -107.8  0.1464
   [A|A]   -108.6  0.06602

At node N54:
   split   lnL     Rel.Prob
   [AB|B]  -107.6  0.1855
   [B|AB]  -107.6  0.1855
   [AB|A]  -107.6  0.1855
   [A|AB]  -107.6  0.1855
   [B|B]   -107.7  0.1693
   [A|A]   -108.4  0.08549

At node N72:
   split   lnL     Rel.Prob
   [B|B]   -106.2  0.7785
   [AB|B]  -108.1  0.116
   [B|BD]  -108.2  0.1005

At node N68:
   split   lnL     Rel.Prob
   [B|B]   -106.1  0.8373
   [B|AB]  -108.4  0.08731
   [AB|B]  -108.8  0.05515

At node N64:
   split   lnL     Rel.Prob
   [B|B]   -106.1  0.8613
   [AB|B]  -107.9  0.1371

At node N62:
   split   lnL     Rel.Prob
   [B|B]   -106.6  0.5177
   [AB|B]  -106.7  0.4809

At node N60:
   split  lnL     Rel.Prob
   [B|A]  -105.9  1

At node N58:
   split  lnL     Rel.Prob
   [B|B]  -105.9  1

At node N67:
   split   lnL     Rel.Prob
   [B|B]   -106.3  0.7184
   [AB|B]  -107.2  0.277

At node N71:
   split  lnL     Rel.Prob
   [B|D]  -105.9  1

At node N89:
   split   lnL     Rel.Prob
   [AE|A]  -106.1  0.8081
   [E|C]   -108.8  0.0539
   [A|AC]  -109    0.04487
   [A|A]   -109.1  0.0401
   [A|AB]  -109.7  0.02259

At node N81:
   split  lnL     Rel.Prob
   [E|A]  -105.9  1

At node N77:
   split  lnL     Rel.Prob
   [E|F]  -105.9  1

At node N80:
   split   lnL     Rel.Prob
   [A|A]   -106.1  0.801
   [A|AB]  -107.6  0.1952

At node N88:
   split    lnL     Rel.Prob
   [A|A]    -106.6  0.4855
   [AC|A]   -108.8  0.05378
   [AC|C]   -109    0.04852
   [AB|A]   -109    0.04617
   [AB|B]   -109    0.04617
   [A|AC]   -109.1  0.04307
   [C|AC]   -109.1  0.04259
   [B|AB]   -109.2  0.0397
   [A|AB]   -109.2  0.0397
   [C|C]    -109.4  0.03101
   [ABC|A]  -109.9  0.0192
   [ABC|B]  -109.9  0.0192
   [ABC|C]  -110    0.01732
   [B|ABC]  -110.2  0.01378
   [A|ABC]  -110.2  0.01378

At node N87:
   split    lnL     Rel.Prob
   [A|A]    -107.7  0.1666
   [B|ABC]  -108.7  0.06022
   [A|ABC]  -108.7  0.06022
   [A|AC]   -108.8  0.05811
   [A|AB]   -108.8  0.05393
   [B|AB]   -108.8  0.05393
   [AB|A]   -108.9  0.05347
   [AB|B]   -108.9  0.05347
   [ABC|B]  -108.9  0.04932
   [ABC|A]  -108.9  0.04932
   [ABC|C]  -108.9  0.04908
   [C|ABC]  -108.9  0.04883
   [AC|A]   -109    0.04758
   [AC|C]   -109    0.04735
   [C|AC]   -109    0.04711
   [C|C]    -109.6  0.02469
   [B|B]    -109.8  0.0199
   [B|BC]   -110.3  0.01282

At node N85:
   split    lnL     Rel.Prob
   [B|ABC]  -106.8  0.4013
   [A|ABC]  -106.8  0.4013
   [A|AC]   -109.3  0.03386
   [B|AB]   -109.3  0.03266
   [A|AB]   -109.3  0.03266
   [AB|B]   -109.3  0.03265
   [AB|A]   -109.3  0.03265
```

5. Pollen description

Pollen morphology and ontogeny in the subfamily Fumarioideae (Papaveraceae): the tribe Fumarieae

Miguel A. Pérez-Gutiérrez, Víctor N. Suárez-Santiago, M. Carmen Fernández, María J. Salinas Bonillo and Ana T. Romero-García

Manuscript in review in *Botanical Journal of the Linnean Society*

5.1 ABSTRACT

The tribe Fumarieae are the most diversified group of the Papaveraceae family, an eurypalinous group whose pollen information has classically been used for studying systematic issues. In this paper we present a study of pollen morphology and ultrastructural evolution during the ontogeny process of the pollen wall in most of the Fumarieae representatives. We describe the high variability in pollen grain ornamentation and in aperture shape and number among genera of the tribe, even at intraspecific level. Remarkable features of the pollen-wall layer development in this group are the lamellated foot layer and granular infratectum. The development of the aperture shows a change in layer stratification from two layers in the early stages to three layers in advanced periods (aperture membrane, exinous oncus and the internal fibrillar material), and in most genera apertures are covered by a fluffy plug from microspore vacuolate. The changes in pollen-wall layer ultrastructure within the tribe Fumarieae and significant differences with the sister group tribe Hypecoeae in pollen features, such as microechinate exine and columellate infratectum, are described. We consider the presence of atypical pollen features in Fumarieae within the framework of current knowledge for pollen wall development and their implications for a better understanding of pollen evolution.

5.2 INTRODUCTION

The subfamily Fumarioideae are included within the family Papaveraceae, which are located in the order Ranunculales at the base of the eudicots (Wang *et al*., 2009). In turn, Fumarioideae are subdivided into two main groups, the tribe Hypecoeae and the tribe Fumarieae (Stevens, 2001 onwards). The former include the monotypic genera *Hypecoum* L., whilst the latter correspond to the largest group of the subfamily which previously were termed Fumarioideae (Lidén, 1993) and also Fumariaceae s.str. (Kadereit *et al*., 1994). In Wang *et al*. (2009) the genus *Pteridophyllum* Siebold & Zucc. is also included in the tribe Hypecoeae mainly based on the kind of exine sculpturing of the pollen (spinose), while the molecular data were not resolutive. Due to the conflictive placement of *Pteridophyllum* (Hoot *et al*., 1997) and following also our recent molecular results of Fumarioideae phylogeny (Pérez-Gutiérrez *et al*., unpubl. data), we do not follow the treatment suggested by Wang *et al*. (2009). The tribe Fumarieae (sensu Stevens, 2001 onwards), are the topic of this research and have been classically well defined mainly by floral characters (Kadereit *et al*., 1994). Later research based on molecular markers has also confirmed its identity showing a monophyletic nature (Lidén *et al*., 1997; Pérez-Gutiérrez *et al.*, unpubl. data). The group consists of 19 genera including about 570 species (Lidén, 1986; Zhang *et al*., 2008), with mainly north temperate distribution (the exception is the *Cysticapnos* Clade, subtribe Discocapninae sensu Lidén, 1986, endemic to South Africa).

Pollen morphology in Fumarioideae has been addressed in different studies (Layka, 1976; Candau, 1987; Morales Torres, Romero & Mendoza, 1988; Blackmore, Stafford & Persson,

1995) but always within dissertations on broader plant groups. Fumarioideae pollen wall morphology is known in broad terms and is defined as a euripalynous group with high variation both in type and number of apertures as well as in the tectum ornamentation. Nevertheless, to the best of our knowledge, there are no published studies concerning the ontogeny of the pollen and the pollen-wall layer morphology. The only exception is a study on pollen wall development in *Fumaria densiflora* DC. (Romero & Fernández, 2000) in which both the ontogeny of the wall as well as the ontogeny of the aperture are described. Another noteworthy reference is a paper on pollen wall development of *Hypecoum imberbe* Sm. (Romero, Salinas & Fernández, 2003), a species from the other tribe of the subfamily, Hypecoeae. In this paper a detailed display of the varied transformation of the pollen wall layers is shown, enabling subsequent comparison with the pollen morphology of close groups. The description of pollen features for the rest of the Fumarioideae could be useful for the study of their taxonomical importance among the infrasubfamiliar groups.

Pollen grain maturation is a complex process in which differentiation in pollen wall layers depicts a great deal of morphological information (Blackmore *et al*., 2007). A number of studies of both morphological and ultrastructure description from ontogenetic pollen wall analysis in angiosperm have been developed for a better understanding of the pollen maturation process (Gabarayeva, Grigorjeva & Rowley, 2003; Saad-Limam, Nabli & Kirchoff, 2005; Furness, 2008) as well as to increase the systematic knowledge from the studied groups (Moon *et al*., 2008; Xu & Kirchoff, 2008).

This study submits pollen morphology features for all the current Fumarieae genera observed by scanning electron microscopy (SEM) and describes the development of apertures and wall layers in fifteen Fumarieae genera through transmission electron microscopy (TEM) observations. With these tools, we have been able to complete the subfamily Fumarioideae pollen information. We detail the main changes of pollen wall formation in the tribe Fumarieae and their general characteristics are discussed within the current knowledge of pollen wall development, assessing evolutionary changes and comparing with their close relatives.

5.3 MATERIALS AND METHODS

5.3.1 PLANT MATERIAL

Material from thirty six representatives of the nineteen genera of Fumarieae was collected from various botanical garden visits and from the herbarium collection loans. Fresh pollen and anthers at different development stages from twenty-two species of nine genera were acquired from the living collection of the Royal Botanical Gardens of Kew, the Royal Botanic Garden of Edinburgh, the National Botanic Garden of Belgium, the Gothenburg Botanical Garden and the Uppsala Botanical Garden. For some rare specimens pollen material from the herbarium collection was taken, all the information about each species and provenance are resumed in the Supporting Information Table S1. For the pollen morphology study twenty-five species

representing all Fumarieae genera were analysed, whereas for the ontogenetic and ultrastructural analysis twenty-nine species representing fifteen genera were used.

5.3.2 METHODS

Scanning analyses

Mature pollen destined for SEM was acetolysed following the Erdtman method (Erdtman, 1960), dehydrated with ethanol series and the pollen passed to a series of alcohol:liquid CO_2 to pure CO_2 submitted to the critical point and then mounted onto SEM stubs using double-sided sticky tapes. All preparations were spatter coated with gold and viewed in a FEI *Quanta* 400 environmental scanning electron microscope in the Centro de Instrumentación Científica (CIC) of the University of Granada. For some samples, especially for those obtained from herbarium loans, only a small amount of material was available. In order not to waste pollen grains with an aggressive treatment, the acetolysis step was replaced by a washing step with Xylene before visualization. Measurements of pollen grain size and aperture number were taken from 20 pollen grains in each sample using light microscopy in pollen samples mounted in glycerine. The information from samples where the amount of pollen was limited was obtained only from SEM measurements. Descriptive palynological terminology was used according to Punt *et al*. (2007).

Transmission analyses

For TEM, anthers were prefixed in 3% glutaraldehyde with 0.025 M cacodylate sodium buffer (pH=7,2) for 24h at 4ºC and then washed three times in a cacodylate buffer. Subsequently, a post-fixed step with a mixture of 1% of OsO_4 and 2% $C_6N_6FeK_4$ in the same buffer was performed for 2 hrs. Then they were dehydrated in a graded series of ethanol and embedded in *Epon* resin. Ultra-thin sections were cut on a Reichter Ultracut E (Leica Microsystems, Wetzlar, Germany) and stained with uranyl acetate and lead citrate (Reynolds, 1963). Observations were performed with a Carl Zeiss (Jena Germany) *LIBRA 120 Plus* transmission electron microscope in the CIC of the University of Granada.

The ontogenetic process was studied at five maturation stages: the young microspore stage from the end of the tetrad period after the callose wall disappears and before cytoplasm vacuolization; the vacuolate microspore stage from the cytoplasm filled with vacuoles to the big vacuole which moves the cytoplasm to the periphery of the cell; young bicellular pollen stage, during the first mitosis when the generative cell is close to the pollen wall; the medium bicellular pollen stage, during the displacement of the generative cell inside of the vegetative cell cytoplasm; and the mature pollen stage when the anther dehiscence is completed. The ultrastructural features studied from pollen wall layers were: size proportion for tectum, infratectum, foot layer, endexine and ectexine, referring to the total ectexine size in all cases except for intine that is refers to the total exine size; white lines presence; infratectum shape; stratification of apertures and fluffy plug presence in apertures (see Tables 2-6 for detailed descriptions).

Cytochemical analyses

In the samples of *Sarcocapnos pulcherrima* C. Morales & Romero García and *Fumaria densiflora* a phosphotungstic acid (PTA) acetone analysis for protein detection was carried out through fixation in a mixture of 1% glutaraldehyde and 2% paraformaldehyde in order to check the presence of endexine layer. These samples were dehydrated with ethanol series and treated as previously described for TEM.

5.4 RESULTS

5.4.1 POLLEN MORPHOLOGY

Pollen grain characteristics studied with SEM are summarized in Table 1. Mean pollen size ranged from 19.5 µm grain diameter of polar axis in *Fumariola turkestanica* Korsh. to 52.6 µm in *Pseudofumaria lutea* (L.) Borkh. (Table 1), showing mainly homogeneous records in most genera. Type and aperture number were tricolpate (Fig. 1A-C) or derived tricolpate in most of the representatives (Table 1, Fig. 1 D-T). Hexapantocolpate pollens predominate in Fumarieae (Fig. 1 D-L), but in taxa such as *Dicentra formosa* subsp. *formosa* (Eastw.) Munz or *Dactylicapnos macrocapnos* (Prain) Hutch. twelve colpi were found in some samples (Table 1; Fig. 1M, N), and in *Pseudofumaria* Medik. representatives and *Platycapnos tenuiloba* Pomel sinpolipantocolpate pollen grains with twelve fused colpi were identified (Table 1; Fig. 1 O, P). *Adlumia fungosa* (Aiton) Britton, Sterns & Poggenb. samples had colpi with margo, a thickening on the colpi edges (Fig. 1 E). In *Cryptocapnos chasmophyticus* Rech. f., *Fumaria densiflora*, *Fumariola turkestanica* and *Rupicapnos numidica* Pomel pores were the aperture type (pantoporate) with aspis (Table 1; Fig. 1 Q-T). Pollen shape was spheroidal in the entire group, with most representatives showing small deviations to the oblate or prolate axis (Table 1; Fig. 1 A-T). Exine ornamentation was generally verrucate (Fig. 1 F, I, P, R, S), psilate (Fig. 1 L, Q, T), rugulate (Fig. 1 E, K), perforate (Fig. 1 B) and reticulate (Fig. 1 A) (see Table 1). The concept pseudorugulate was employed to described a rugulate tectate exine as the observed in *Ehrendorferia chrysantha* (Hook. & Arn.) Rylander (Fig. 1 D). It was necessary to name several samples with a combination of ornamentation terms in order to describe their intermediate morphologies. In this way, psilate perforate was identified in *Dactylicapnos macrocapnos, Dicentra formosa* (Haw.) Walp. subspecies, *D. eximea* (Ker Gawl.) Torr and *Ichtyoselmis macrantha* (Oliv.) Lidén specimens (Fig. 1 N, M, C; Table 1), describing a psilate exine surface with very small perforations (much smaller than 1 µm in diameter). The psilate pseudorugulate type was described for *Cysticapnos cracca* (Cham. & Schltdl.) Lidén and *Trigonocapnos lichtensteinii* (Cham. & Schltdl.) Lidén, since an intermediate type between a psilate surface and a light rugulate tectate pattern was found (Fig. 1 G, H). Pseudorugulate perforate was employed to describe the ornamentation in *Pseudofumaria alba* (Mill.) Lidén and *Pseudofumaria lutea*, where a rugulate tectate exine with irregular perforations was found. Psilate microfoveolate was

used in *Corydalis rupestris* Kotschy, describing a psilate ornamentation with depressions smaller than 1 µm (Fig. 1 J).

Table 1. Pollen grain morphological of Fumarieae genera. PA length of pollar axis (µm), ED equatorial diameter (µm) and P/E ratio for the pollen shape measure. Lengths are presented with minimum-(mean ± standar deviation)-maximum values. Pollen grain shape: Sph spheroidal, Pro prolate, Obl oblate, and exine ornamentation following Punt et al., (2007) terminology. Number and type of apertures: C colpus, P pore and sC sincolpate.

Taxa	PA	ED	P/E	Shape	Exine ornamentation	Apertures
Adlumia fungosa	27.6-(34.4±3.6)-40.4	26.1-(33.2±3.8)-38.4	1.04	Pro-sph	Rugulate	C 3-6 Marged colpi
Capnoides sempervirens	22.7-(29.9±3.7)-35.3	22.5-(28.8±3.1)-33.7	1.04	Pro-sph	Verrucate	C 6
Ceratocapnos heterocarpa	25.1-(27.2±2.8)-34.9	24.3-(26.7±1.9)-29.8	1.01	Pro-sph	Psilate	C 6
Corydalis cheilanthifolia	27.5-(35.8±4.3)-43.1	27.2-(33.1±3.7)-38.7	1.08	Pro-ph	Rugulate	C 3-6
Corydalis temulifolia	36.7-(44.3±4.1)-50.1	36.9-(42.2±4)-47.2	1.05	Pro-sph	Verrucate	C 6
Corydalis rupestris	36.1-(41.4±3.8)-49.7	33.5-(40±3.6)-46.5	1.04	Pro-sph	Psilate microfoveolate	C 6
Cryptocapnos chasmophyticus	21.2-(24.7±2.5)-28.2	24.5-(25.8±0.8)-26.6	0.96	Obl-sph	Psilate	P 6
Cysticapnos cracca	18.3-(20±1.1)-21.2	18.9-(19.8±0.7)-20.3	1	Sph	Psilate pseudorugulate	C 6
Dactylicapnos macrocapnos	27.1-(33.3±4.3)-45.6	25-(31.9±4.7)-44.1	1.03	Pro-sph	Psilate perforate	C 9-12
Dicentra eximea	28.6-(31.6±2.4)-36.3	24.5-(29.5±2.5)-34.7	1.07	Obl-sph	Psilate perforate	C 3-6
Dicentra formosa subsp. *formosa*	25.8-(30.8±3.2)-38.6	25.8-(30.5±3.2)-39.9	1	Sph	Psilate perforate	C 6-12
Dicentra formosa subsp. *oregona*	22.5-(32.6±3.9)-38	24-(32.5±3.7)-39.5	1	Sph	Psilate perforate	C 6-12
Dicentra peregrina	28,4-(37.1±4.1)-42.5	24.6-(34.5±5.2)-40.7	1.07	Pro-sph	Perforate	C 6
Discocapnos mundii	22.7-(23.2±1.1)-25.3	21.5-(22.8±0.9)-23.8	1.02	Pro-sph	Verrucate	C 6-9
Ehrendorferia chrysantha	22.3-(30.5±3.6)-33.7	23.5-(29.7±2.9)-32.8	1.02	Pro-sph	Pseudorugulate	C 6
Fumaria densiflora	31.4-(33.5±2.4)-36	29.6-(32.9±3.06)-37.2	1.01	Pro-sph	Verrucate	P 6-8
Fumariola turkestanica	17.6-(19.5±1.2)-21.2	17.9-(20.5±2.2)-24.4	0.95	Obl-sph	Verrucate	P 6
Ichtyoselmis macrantha	32.1-(37.9±3.2)-42.5	31.8-(37.4±3.1)-43.2	1.01	Pro-sph	Psilate perforate	C 3
Lamprocapnos spectabilis	20.1-(32.4±5.3)40.9	20.5-(30.4±4.8)-40.2	1.06	Pro-sph	Reticulate	C 3
Platycapnos tenuiloba	42.4-(49.3±3.1)-58.6	45-(50.7±2.2)-55.6	0.97	Obl-sph	Verrucate	sC 12
Pseudofumaria alba	44.2-(51.6±4.1)-57.5	42.5-(49.5±3.7)-55.3	1.04	Pro-sph	Pseudorugulate perforate	sC 6-12
Pseudofumaria lutea	42.1-(52.6±4.3)-58.8	44.6-(50.9±3.9)-56.7	1.03	Pro-sph	Pseudorugulate perforate	sC 12
Rupicapnos numidica	17.8-(25±3.9)-29.9	16.6-(24.2±3.5)-29.4	1.03	Pro-sph	Psilate	P 6
Sarcocapnos pulcherrima	31.7-(35.6±2.7)-39.8	30.9-(35.4±2.3)-43	1	Sph	Psilate	C 6
Trigonocapnos lichtensteinii	19.2-(20.9±1.3)-23.6	19.6-(20.7±1.1)-23.3	1	Sph	Psilate pseudorugulate	C 6

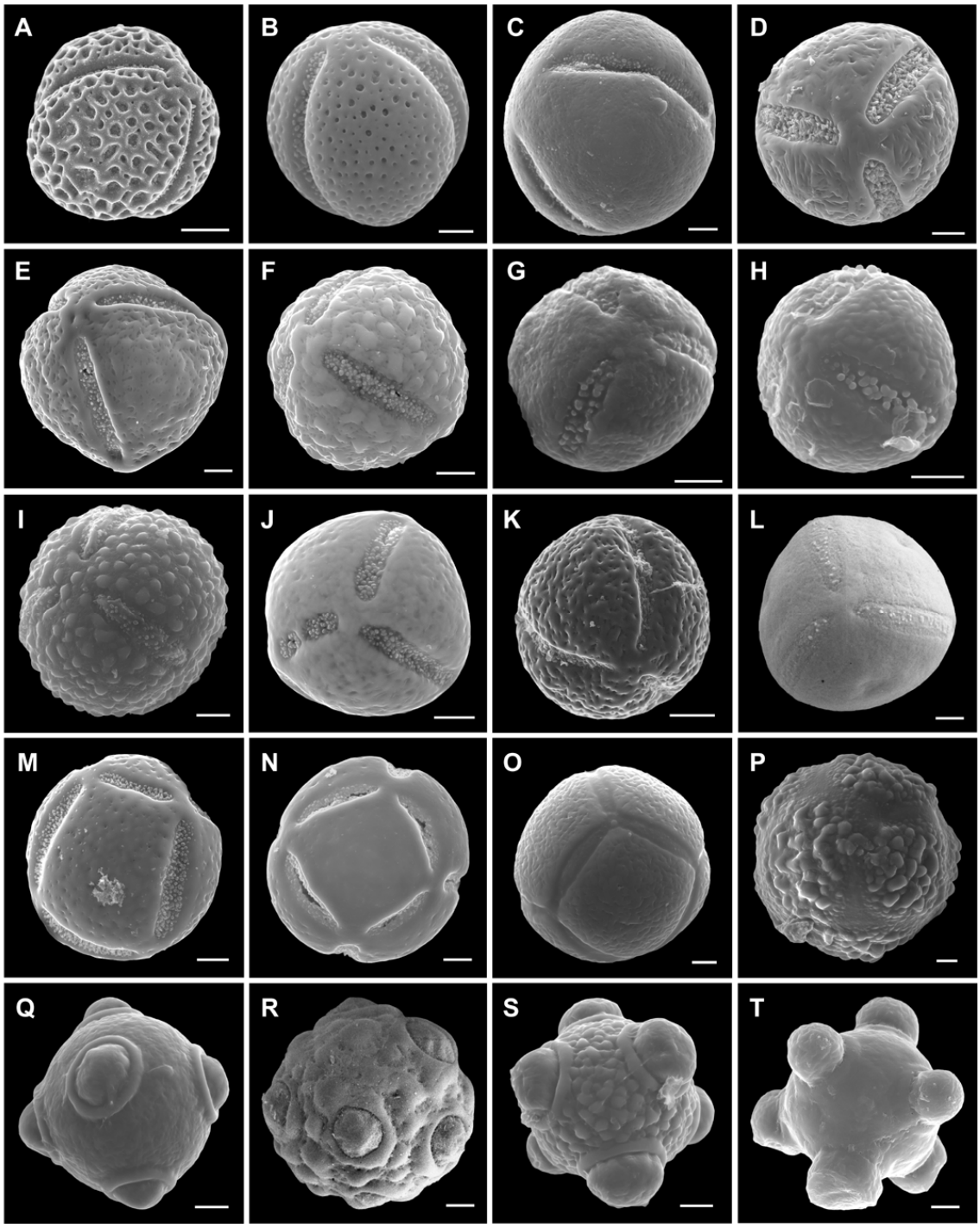


Figure 1. Pollen grain morphology of Fumarieae representatives. A, *Lamprocapnos spectabilis*. B, *Dicentra peregrina*. C, *Ichtyoselmis macrantha*. D, *Ehrendorferia chrysantha*. E, *Adlumia fungosa*. F, *Capnoides sempervirens*. G, *Cysticapnos cracca*. H, *Trigonocapnos lichtensteinii*. I, *Corydalis temulifolia*. J, *Corydalis rupestris*. K, *Corydalis cheilanthifolia*. L, *Sarcocapnos pulcherrima*. M, *Dicentra formosa* subsp. *formosa*. N, *Dactylicapnos macrocapnos*. O, *Pseudofumaria lutea*. P,*Platycapnos tenuiloba*. Q, *Cryptocapnos chasmophyticus*. R, *Fumaria densiflora*. S, *Fumariola turkestanica*. T, *Rupicapnos numidica*. Scale bars = 5 µm in all cases.

5.4.2 ULTRASTRUCTURE AND ONTOGENY OF THE POLLEN WALL

All observations of pollen wall maturation obtained from these Fumarieae representatives reflected variations within the general ontogeny model established in angiosperms. The main findings by pollen stages are summarized below.

Young microspore stage

In the young free microspore stage, ectexine layers were completely differentiated with the tectum and the infratectum clearly formed and the foot layer including white lines (Fig. 2 A-C; Table 2). White lines were not present in *Ceratocapnos heterocarpa* Durieu and *Sarcocapnos pulcherrima* (Fig. 2 D; Table 2). The tectum formed the greater proportion of exine size, excluding *Ceratocapnos heterocarpa* and *Sarcocapnos pulcherrima* (Table 2). Infratectum shape was found to be granular in most cases, with differences in size and grain density (Table 2), with the exception of *Lamprocapnos spectabilis* (L.) Fukuhara and *Ehrendorferia chrysantha,* where an intermediate shape of infratectum between granulate and columellate (Fig. 2 A, B; Table 2) was shown. The foot layer had a regular size proportion among genera (Table 2), although showed very different ways of fragmentation such as perforations or invaginations (Fig. 2 A-D). Endexine layer presence was a generality in most of the Fumarieae genera, although its size proportion compared to the whole exine varied from a third to a tenth (Table 2). Meanwhile, endexine was a thin layer with the same electrodensity or a little more electrodense than the foot layer, therefore it was not easy to make out (Fig. 2 A-C, F-J) although its presence was identified easily in most taxa with the exception of *Sarcocapnos pulcherrima* (Fig. 2 D) and *Ceratocapnos heterocarpa* (not shown). A PTA treatment revealed the presence of endexine in *Sarcocapnos pulcherrima* forming a very thin inner layer along the pollen wall (Fig. 2 E).

Apertures in Fumarieae consisted of three parts, firstly an external one that contains aperture membrane elements as seen in *Lamprocapnos spectabilis* and *Ehrendorferia chrysantha* (Fig. 2 G, H), or only a fibrillar layer membrane as observed in *Fumaria densiflora* (Fig. 2 F) and *Platycapnos spicata* (L.) Bernh. (Table 2). There was an exception for the external part structure, *Cysticapnos vesicaria* (L.) Fedde sample showed both types of shield (Fig. 2 I; Table 2). Secondly, an intermediate layer formed by thickening endexine or exinous oncus (Fig. 2 F, J; Table 2) consisting of foot layer remains (sometimes lamellated) and endexine, as found in all the sampled genera. The structure of the endexine varied in shape from islets in *Lamprocapnos spectabilis*, *Ehrendorferia chrysantha* and *Cysticapnos vesicaria* (Fig. 2 G-I; Table 2), small granules in *Fumaria densiflora* (Fig. 2 F, Table 2) to granule association forming glomeruli in *Sarcocapnos pulcherrima* (Fig. 2 J; Table 2). And the third internal one, an inner fibrillar material layer (IFM) which was developed at this stage (Fig. 2 G, H inappreciable and F, I, J well developed).

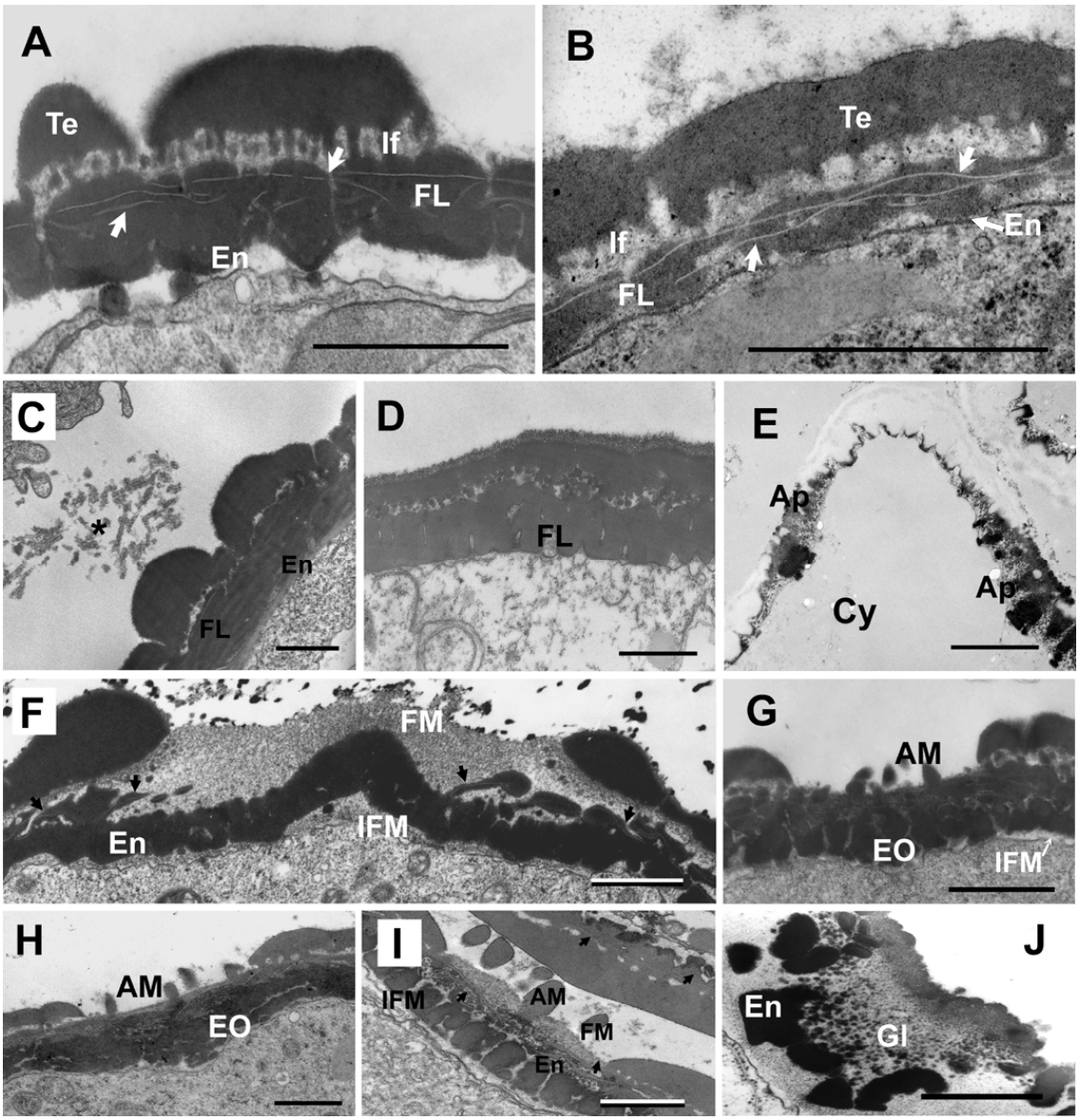


Figure 2. Sections of pollen wall and apertures in the young microspore period of Fumarieae representatives. A *Lamprocapnos spectabilis*, B *Ehrendorferia chrysanta*, C *Platycapnos spicata*, D-E *Sarcocapnos pulcherrima*, F *Fumaria densiflora*, G *Lamprocapnos spectabilis*, H *Ehrendorferia chrysantha*, I *Cysticapnos vesicaria* and J *Sarcocapnos pulcherrima*. Pollen-wall cross section (A-E) and detail of aperture (F-J). In all images tectum (Te), infratectum (If), foot layer (FL), endexine (En), cytoplasm (Cy), apertures (AP), aperture membrane (AM), outer fibrillar membrane (FM), endexinous oncus (EO), inner layer of fibrillar material (IFM) and glomeruli arrangement of endexine (Gl). In images A,B,I, arrowheads highlight the presence of whitelines in foot layer. Image C shows tapetum activity with remains (*) expelled towards pollen wall, and images E and J show PTA results and endexine detection in *Sarcocapnos pulcherrima*. Scale bars =1 µm in all images.

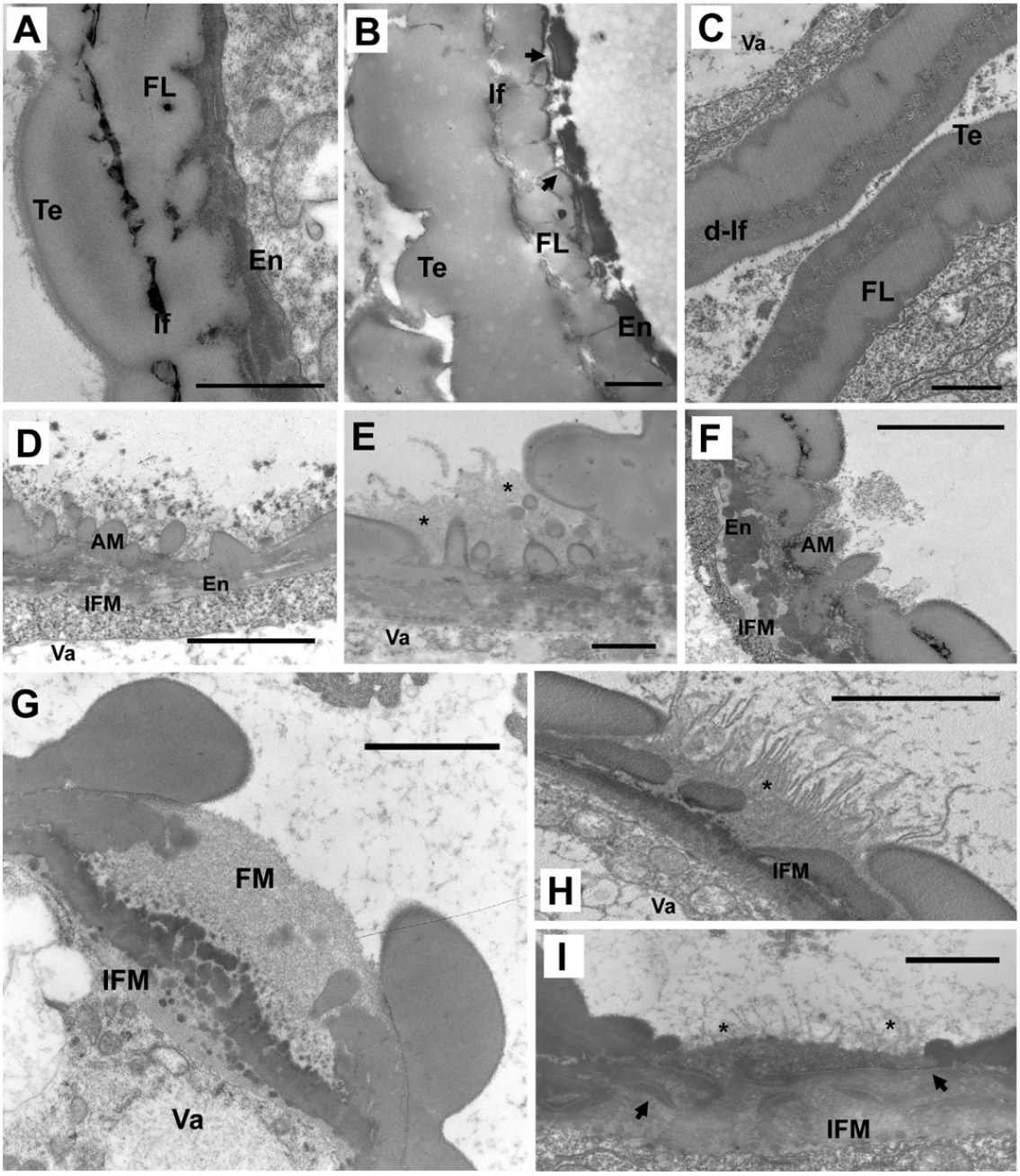


Figure 3. Sections of vacuolated microspore representatives of Fumarieae. A *Corydalis scouleri*, B *Fumaria densiflora* and C *Ceratocapnos heterocarpa*, D *Dicentra formosa* subsp. *formosa*, E *Adlumia fungosa*, F *Corydalis scouleri*, G *Rupicapnos africana*, H *Rupicapnos numidica* and I *Platycapnos spicata*. Pollen-wall cross section (A-C) and detail of aperture (D-I). In all images tectum (Te), infratectum (If), dendroid infratectum (d-If), foot layer (FL), endexine (En), intine (In), vacuole presence (Va), fluffy plug (*), apetural membrane (AM), outer fibrillar membrane (FM) and inner layer of fibrillar material (IFM). In images A,B,I arrowheads highlight the presence of white lines, and image B shows PTA results in *Fumaria densiflora*. Scale bars = 1 µm in A-F pictures and 2 µm in G-I.

Vacuolate microspore stage

In this stage the cell had the cytoplasm mainly filled by a large vacuole (Fig. 3 C-E, G-H). Ectexine layers maintained ultrastructure proportions as in the previous stage (Tables 2, 3) though electrodensity between endexine and foot layer changed and both layers were easily distinguishable (Fig. 3 A, B). The location of the white lines had also changed, disappearing from the foot layer in all genera and located only in the endexine contact face with the foot layer (Fig. 3 A, B; Table 3), except *Ceratocapnos heterocarpa*, where the endexine was not observable in the interapertural areas (Fig. 3 C). The presence of lamellated endexine was also common in the apertures (Fig. 3 I).

In the apertural areas the third layer (IFM) increased under the endexine, being found in all taxa with a higher development than in the previous stage (Table 3; Fig. 3 D-I). The exinous oncus had reduced its size and the structure of the endexine continued to show the presence of islets (Fig. 3 D, F), granules (Fig. 3 E, G) and glomeruli (Table 3). It was during this period when the deposit of a fluffy fibrous material started to appear on the opening areas of most samples. Thus, *Dicentra formosa*, *Adlumia fungosa* and *Corydalis* DC. species (Fig. 3 D-F) presented different and amorphous electrodense deposits, but in *Platycapnos spicata* and *Rupicapnos numidica* the typical fluffly material was complete (Fig. 3 H, I), whilst in the sample of *Rupicapnos africana* (Lam.) Pomel (Fig. 3G), the apertural area at the early vacuolate microspore stage had not yet developed the fluffy plug.

Young bicellular pollen stage

Electrodensity of pollen wall decreased compared with that in the cytoplasm (Fig. 4). All ectexine layers were completely formed, endexine was noticeably differentiated and the intine began to grow. This phase was also characterized by the thin size of the intine layer, the plasmalemma with extremely abundant protrusions (Fig. 4 B, C, F) and by an infratectum filled with a lot of eletrondense material. The tectum layer was the main part of ectexine in most samples, being close to one half of this structure in most genera (Table 4). *Fumaria densiflora* presented the widest tectum (Table 4), close to three quarters of total ectexine and *Ceratocapnos heterocarpa* had the thinnest one (Table 4; Fig. 4 N). Regarding the infratectum, most genera presented a thin infratectum area (around 1/10 of ectexine size), although *Ehrendorferia chrysantha* (Fig. 4 A), *Lamprocapnos spectabilis*, and *Pseudofumaria lutea* (Table 4) had an intermediate thickness. *Ceratocapnos heterocarpa* showed the widest infratectum in the group with an atypical shape of granulation, a net of small granules that formed branches that we have called ‘dendroid granules’ (Fig. 4 N). This kind of infratectum shape was also found in *Sarcocapnos pulcherrima* samples. The large amount of filling electrodense material (Fig. 4 A, D, E) reflected the high accumulation of remains of secretory tapetum. The foot layer showed a similar ectexine proportion to the previous stage (Tables 3, 4) with the exception of *Fumaria densiflora*, which was greatly reduced. Foot layer irregularities were more developed than in the previous stage (Fig. 4 A, C, F). Endexine thickness became

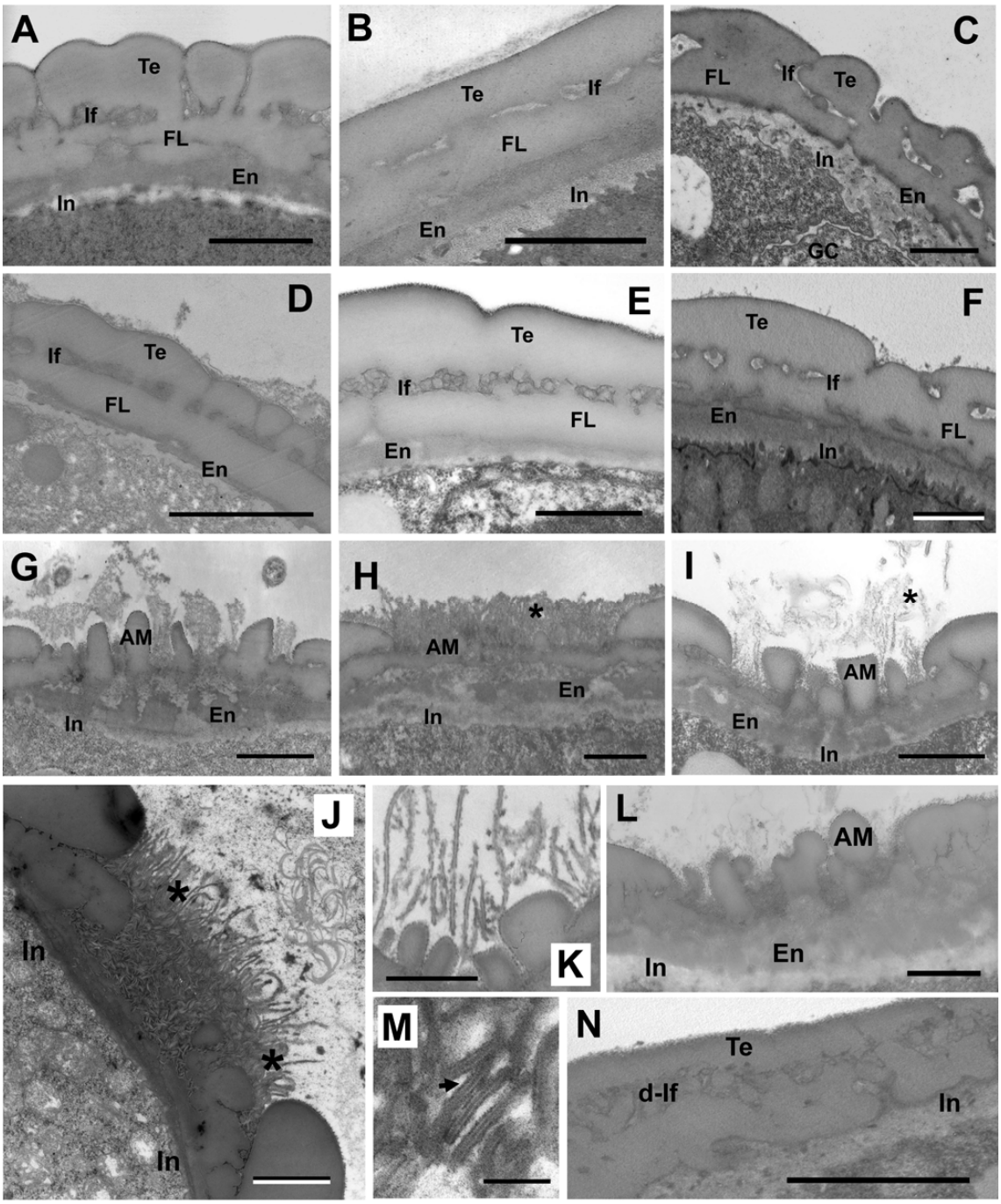


Figure 4. Sections of pollen wall representatives of Fumarieae during the young bicellular stage. A *Ehrendorferia chrysantha*, B *Dicentra formosa* subsp. *formosa*, C *Adlumia fungosa*, D *Dactylicapnos torulosa*, E *Corydalis cheilanthifolia*, F *Cysticapnos vesicaria*, G *Capnoides sempervirens*, H *Dactylicapnos macrocapnos*, I *Corydalis cheilanthifolia*, J *Fumaria densiflora*, K *Corydalis popovii*, L *Cysticapnos vesicaria*, M *Fumaria densiflora* and N *Ceratocapnos heterocarpa.* Pollen-wall cross section (A-F,N), detail of aperture (G-L) and magnification of fluffy plug (M). In all images tectum (Te), infratectum (If), dendroid infratectum (d-If), foot layer (FL), endexine (En), intine (In), fluffy plug (*) and aperture membrane (AM). In image M arrow head highlights pentalamellated nature of fluffy elements. Scale bars = 1 µm in all images, except 0,5 µm in K and 0,1µm in M.

thinner (Fig. 4 A-F; Table 4) due to the grain growth and began to fragment. The newly created intine layer formed a thin sheet in most of the sampled genera (Fig. 4 A, D-F, J) ranging to a fifth in *Ehrendorferia chrysantha* to 1/10 in *Dactylicapnos* Wall., *Corydalis* and *Fumaria densiflora* (Table 4).

The apertural region still presented the structure in three parts (Fig. 4 G-L), the outer formed by aperture membrane elements or fibrillar layer membrane, the intermediate consisting of an endexinous layer, whose structure was homogenized in this stage amongst all the samples showing appearance of islets; and the inner one where fibrillar elements of the previous stage were replaced by intine deposition. In most genera, fluffy depositions in the outer of apertures clearly shaped a net of organised lamellated structures (Fig. 4 H, J, K). In some samples these structures were composed of pentallamelated elements (Fig. 4 M) and in other samples by trilamellated elements (Figs. 4 K; 5 J). In the *Fumaria densiflora* apertures, a great deal of this material was found (Fig. 4 J) whilst small or medium levels of fluffy material were present in most of Fumarieae genera (Fig. 4H, I; Table 4). In all genera the exinous oncus was reduced and the intine size did not shape the oncus, in spite of the thickening (Fig. 4G-L).

Medium bicellular pollen stage

The reduction and fragmentation of the endexine layer by the general growth of the pollen grain as well as the increase of intine layer distinguished this stage (Fig. 5 A-H). Morphologically, in this stage the tectum layer reached its biggest size proportion so far (Fig. 5 A-H; Table 5). The infratectum layer was thin as far as the total ectexine proportion was concerned, ranging from a fifth to thinner than a tenth (Fig. 5 B, C, E, G; Table 5). The foot layer maintained the same size as in the previous stages, with an intermediate proportion from ectexine thickness (Table 5). In *Fumaria densiflora* and *Rupicapnos africana* (Fig. 5 E), the foot layer stood out for being thinner than in the rest of the samples (Table 5). Irregularities in this layer were well developed although they had a variable shape and there were even samples with a continuous foot layer as in *Dactylicapnos macrocapnos* and *Pseudofumaria alba* (Mill.) Lidén (Fig. 5 C, F). Meanwhile, endexine size was greatly decreased and at this stage was formed by islets or by a very fragmented layer (Fig. 5 A, B, D, F). Intine size was a main part of pollen wall (Fig. 5 A-F; Table 5) with an intermediate thickness of exine layer in all genera.

The apertural areas were wider (Fig. 5 I-M), showing the same structuring as in the previous phase, although a reduction in the endexinous part and an increase in the intine was observed (Fig. 5 I, K-M). The external face of the aperture continued to accumulate fluffy material which replaced the fibrillar layer membrane present in the previous stages in taxa as in *Platycapnos spicata* (not shown) and *Fumaria capreolata* L. (Fig. 5L), or that was accumulated in the aperture membrane as in *Ichtyoselmis macrantha*, *Dactylicapnos torulosa* (Hook. & Thomson) and *Corydalis integra* Barbey & Fors.-Major. (Fig. 5 I-K). The presence of intinous oncus was common in apertures in many of the samples observed, but usually had a small size (Fig. 5 I, L).

However, in a sample of *Cysticapnos vesicaria* (Fig. 5 M), a highly developed oncus was observed in the apertural intine.

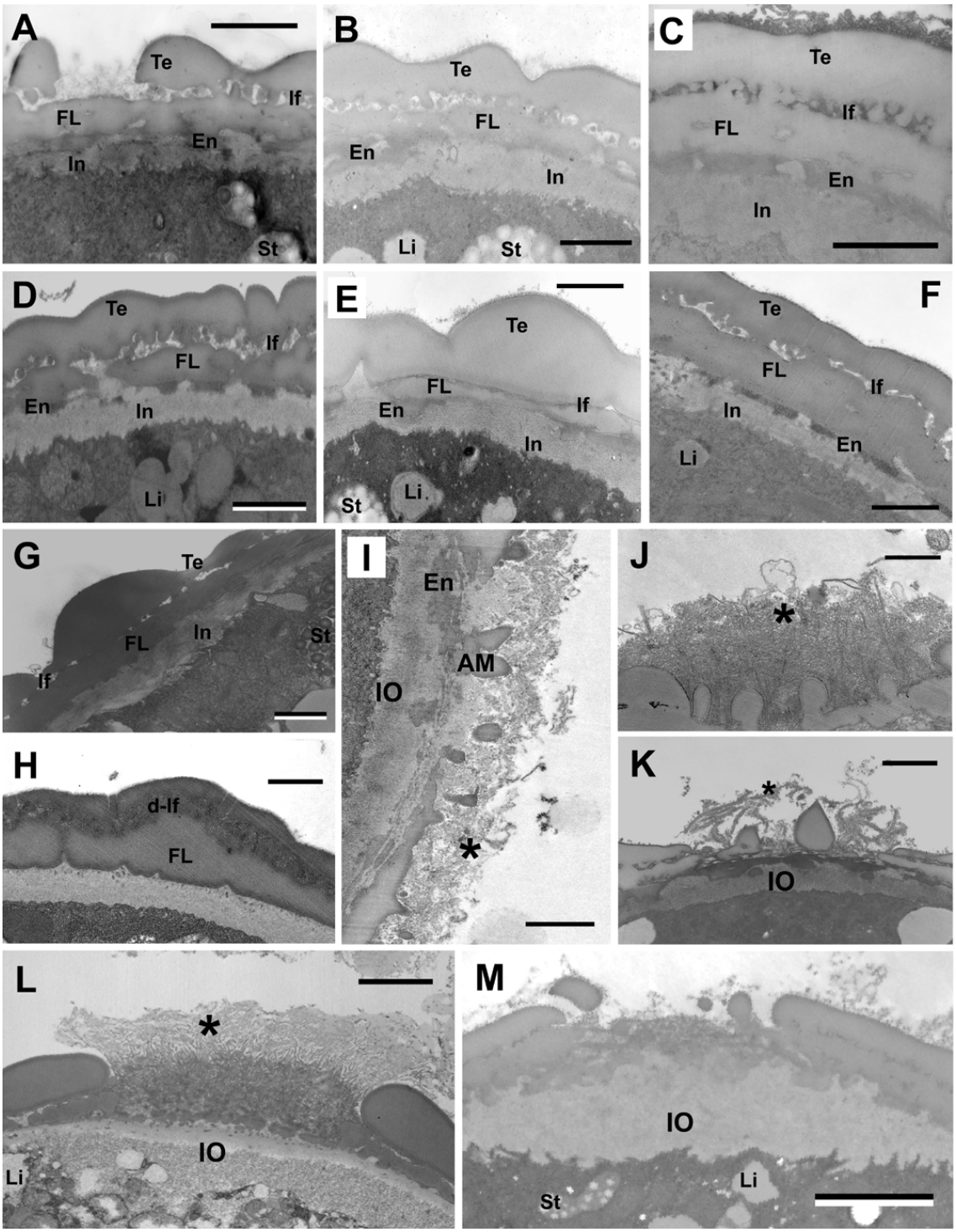


Figure 5. Sections of Fumarieae representatives from the medium bicellular stage. A, *Lamprocapnos spectabilis*, B *Dicentra formosa* subsp. *formosa*, C *Dactylicapnos macrocapnos*, D *Corydalis popovii*, E *Rupicapnos africana*, F *Pseudofumaria alba* and G, *Platycapnos spicata*, detail of pollen wall. H *Ceratocapnos heterocarpa*, I *Ichtyoselmis macrantha*, J *Dactylicapnos torulosa*, K *Corydalis integra*, L *Fumaria capreolata* and M *Cysticapnos vesicaria.* Pollen-wall cross section (A-H) and detail of aperture (I-M). In all images: tectum (Te), infratectum (If), dendroid infratectum (d-If), foot layer (FL), endexine (En), intine (In), lipids (Li), starch (St), fluffy plug (*), aperture membrane (AM) and intinous oncus (IO). Scale bars = 1 µm images A-F, H-K; 2 µm images G,L,M.

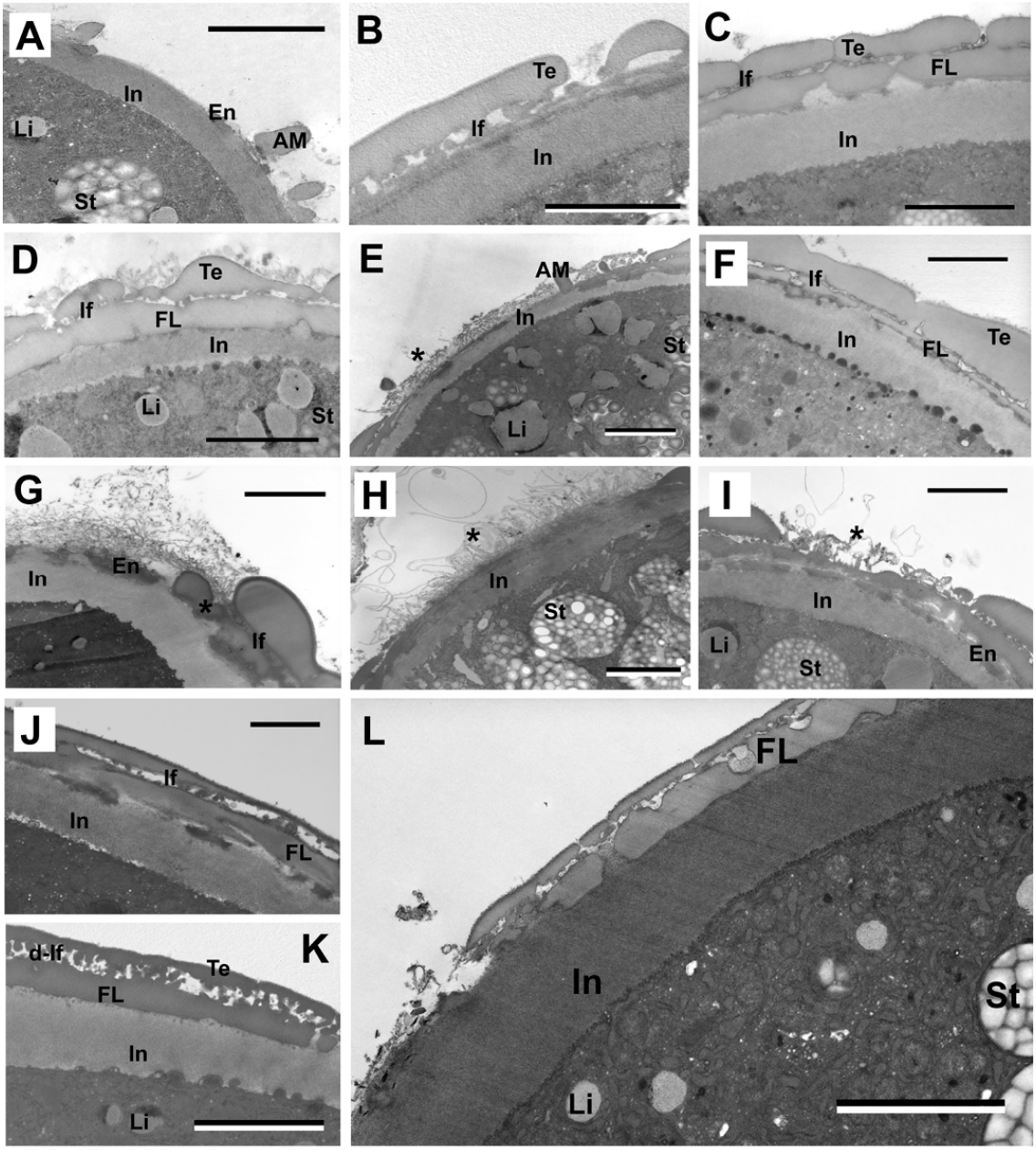


Figure 6. Sections of pollen grains of Fumarieae representatives from the mature stage. A *Dicentra oregona* x *peregrina* 'gothoburgensis' (sensu H. Zetterlund), B *Dicentra peregrina*, C *Dactylicapnos torulosa*, D *Corydalis temulifolia*, E *Corydalis tauricola,* F *Fumaria capreolata*, G *Fumaria densiflora*, H *Platycapnos spicata*, I-J *Pseudofumaria lutea*, K *Ceratocapnos heterocarpa* and L *Sarcocapnos pulcherrima*. Pollen-wall cross section (B-D,F,J-I) and detail of aperture (A,E,G-I). In all images tectum (Te), infratectum (If), dendroid infratectum (d-If), foot layer (FL), endexine (En), intine (In), lipids (Li), starch (St), fluffy plug (*) and aperture membrane (AM). Scale bars = 2 µm all images, except for A where = 1 µm.

Mature pollen stage

During the last stage of pollen grain maturation, the ectexine layers development was similar to the previous stage (Fig. 6 B-D, F, J-L; Table 6), whereas a few remains of the endexine layer were found on the inner face of the ectexine (e.g. *Corydalis temulifolia* Franch., *Fumaria capreolata* and *Pseudofumaria lutea*, Fig. 6 D, F, J). The main proportion of the intine layer as far as the exine size is concerned, also characterized the mature stage together with the halt in the segregation of the intine, which could be observed by the disappearance of the plasmalemma protrusions (Fig. 6 A-L). In this stage, the exine size seemed have reduced due to the greater intine thickness (Fig. 6 B-D, F, J, K), however, the exine components displayed similar size and proportion to the previous stage (Tables 5; 6). The external tectum made up the broader ectexine layer in most of the samples, excluding *Ceratocapnos* Durieu and *Sarcocapnos* DC. samples (Table 6). In the *Corydalis temulifolia* sample (Fig. 6 D), the tectum proportion oscillated reflecting the verrucate tectum shape, as well as in *Dicentra peregrina* the perforation structures were discernable (Fig. 6 B; Table 6). The infratectum layer maintained a very thin size in relation with the total ectexine, even though *Ichtyoselmis macrantha*, *Pseudofumaria lutea*, *Platycapnos spicata*, *Ceratocapnos heterocarpa* and *Sarcocapnos pulcherrima* differed from the rest, having an intermediate infratectum size (Table 6). The infratectum filler was emptier in this last stage, as shown by the lower electrodensity presented in all the genera (Fig. 6 B, J, K). The foot layer continued to occupy around a third of ectexine bulk in most of the genera (Table 6) with the exception of *Fumaria* L. (Fig. 6 F, G) and *Rupicapnos africana* (Table 6) which showed a very thin foot layer. Endexine remains formed a discontinuous layer and were a very insignificant part of the pollen wall size (Fig. 6).

Aperture membranes were very diffuse on the broad apertural regions (Fig. 6 A, E, G-I), where endexine remains were also diffused and a thick layer of intine was present. Fluffy deposits were still present, although there were accumulations in small amounts in most genera with the exception of *Fumaria densiflora* (Fig. 6 G) and *Rupicapnos africana* (Table 6).

5.5 DISCUSSION

This comprehensive review of pollen morphology in the tribe Fumarieae is the first study to present data from all the genera currently recognized. Previous studies published on Fumarioideae pollen (Stern, 1962; Layka, 1976; Kalis 1979; Candau, 1987; Blackmore *et al.*, 1995) highlighted most of the pollen morphology features extant in Fumarieae. We have now enlarged that morphological analysis and coupled it with an ontogenic description of the apertures and the pollen walls, detailing diversity in pollen morphology and determining the main ontogenetic changes.

5.5.1 POLLEN MORPHOLOGY IN FUMARIEAE

Our results show that pollen morphology of Fumarieae is quite different to the morphological features of the pollen in its sister group, the tribe Hypecoeae (reviewed in Dahl, 1989; 1990); involving the main differences both in the exine ornamentation (Fumarieae, non-microechinate; Hypecoeae, microechinate) and the number of apertures (Fumarieae, tricolpate or derived tricolpate; Hypecoeae, two colpate). As it has been mentioned in the introduction, we do not consider the inclusion of *Pteridophyllum* in the tribe Hypecoeae or in the subfamily Fumarioideae appropiate. Wang *et al.* (2009) make this inclusion based on results in which they assigned the character 'pollen with spinose exine sculpturing' to *Pteridophyllum*, *Hypecoum* and Fumarieae, in opposition to the Papaveroideae group. The results presented in this study, as well as previous studies on pollen of Papaveraceae (Kalis, 1979; Dahl, 1990; Blackmore *et al*., 1995) show that the use of this characteristic is a misinterpretation and as far as Fumarioideae is concerned, the inclusion of *Pteridophyllum* should be ruled out.

The pollen sizes we have recorded from Fumarieae, show that it is a plant group with a small or intermediate pollen grain measurement according to Hesse *et al*. (2009). Data obtained were consistent with previous descriptive works on Fumarieae representatives (Kalis, 1979; Candau & Soler, 1981) on pollen size. Pollen-grain shape agreed with the previous results (Blackmore *et al.*, 1995) where mainly spheroidal and similar shapes are described (Table 1).

The findings about aperture type and number presented in this paper show that three is the basic number of apertures for the tribe Fumarieae. Six apertures are the most common and the increase of the aperture number to 12 or more is possible. This is the case of some *Dactylicapnos*, *Dicentra, Fumaria*, *Platycapnos* (DC.) Bernh. and *Pseudofumaria* representatives. In fact, for some species a great diversity in the number of apertures has been detected (from three to twelve, see Table 1) showing that it can be a polymorphic character. High variability in the same anther has previously been alluded to (Blackmore *et al.*, 1995), and has also been documented in other studies, such as in different genera of the subtribe Nepetineae, Lamiaceae (Moon *et al*., 2008), therefore this might be a more common characteristic than previously documented in angiosperm. The pore type aperture is present in all the genera of the *Fumaria* Clade (subtribe Fumariinae, sensu Lidén, 1986), as already stated by Lidén (1986). *Platycapnos* and *Pseudofumaria* species share the aperture model, having twelve colpi fused in their extreme, this characteristic is only present in both these genera of Fumarieae. We consider it makes sense, given that both genera are phylogenetically close (Lidén, 1986; Pérez-Gutiérrez *et al*., 2012). In relation to the ornamentation of the tectum, our results show a predominance of pollen types with non-perforated tectum (verrucate, psilate and pseudorugulate; Table 1); among the pollen grains with perforate tectum, psilate-perforate is the most common type. *Lamprocapnos* (L.) Fukuhara is the only genus with reticulate tectum ornamentation. It is noteworthy that the non-perforated pollens characterize (with some exceptions) a large phylogenetic clade of Fumarieae, the Crown Fumarieae (Pérez-Gutiérrez *et al*., unpubl. data.), which groups (in alphabetical order): *Capnoides* Mill., *Corydalis*, *Cysticapnos* Clade (*Cysticapnos* Mill., *Discocapnos* Cham.& Schltdl., *Trigonocapnos* Schltdl.),

Dactylicapnos, *Fumaria* Clade (*Fumaria*, *Fumariola* Korsh., *Cryptocapnos* Rech.f., *Rupicapnos* Pomel), and *Sarcocapnos* Clade (*Ceratocapnos*, *Platycapnos*, *Pseudofumaria*, *Sarcocapnos*); whilst basal lineages in the phylogeny share pollens with perforations in the tectum, usually perforate or rugulate with small holes but *Lamprocapnos*, the most-basal lineage in the tribe (Lidén *et al.,* 1997; Pérez-Gutiérrez *et al*., unpubl. data), presents reticulate tectum and large lumina. This fact suggests an evolutionary trend toward the disappearance of perforations in the tectum in Fumarieae, from the basal lineages to the most derived ones. Tectum with perforations seems to be the basal state in Papaveraceae, since it is also present in other Papaveraceae as *Pteridophyllum* (microreticulate; Pérez-Gutiérrez *et al*., unpubl. data) and subfamily Papaveroideae (reticulate in the most-basal tribe Eschscholzieae; Pérez-Gutiérrez *et al*., unpubl. data); this agrees with the early acquisition of this kind of tectum in the angiosperm phylogeny (before Austrobaileyales divergence; Doyle, 2005). The network-like pattern of the perforations of the tectum in *Lamprocapnos*, *Pteridophyllum*, and other lineages of Ranunculales (e.g., Berberidaceae, Blackmore *et al*., 1995; Menispermaceae, Del Rei Teixeira, Amorim & Ribeiro, 2013), including the earliest-divergent lineage (Eupteleaceae; Pérez-Gutiérrez et al., unpubl. data) suggest the reticulate tectum as basal in Ranunculales.

Our results and the comparison with those of other authors (Kalis, 1979; Candau & Soler, 1981) show that in general in Fumarieae the pollen morphology is well conserved among congeneric species. However, we have found infrageneric variability in the tectum ornamentation and/or aperture number (even at infraspecific level) within *Corydalis*, *Dicentra* Berhn., and *Rupicapnos*. Stern (1962), before the split of *Dicentra* in several genera by posterior authors (*Dactylicapnos*, *Dicentra* s.str., *Ehrendorferia* T.Fukuhara & Lidén, *Ichtyoselmis* Lidén & T.Fukuhara and *Lamprocapnos*; Khánh, 1973; Lidén *et al.,* 1997), describes its pollen as highly variable. The observations completed in this study for all these genera broadly agree, with some exceptions and nomenclatural differences (as result of different interpretations of the characters), with Stern's pollen descriptions. Even considering *Dicentra* in the strict sense, this genus continues to prove high pollen variation (Fig. 1; Table 1).

5.5.2 ULTRASTRUCTURE AND ONTOGENY OF FUMARIEAE POLLEN WALL

The ultrastructure of the Fumarieae genera comparison has shown that most of characteristics depicted are very homogeneous. Nonetheless, for some genera very specific changes in morphology from pollen wall layers and apertural regions were found.

In Fumarieae both the size of the endexine and its fragmentation vary widely during pollen grain ontogeny. However, endexine proportion is homogenous among the genera and species, whereas different ways of endexine fragmentation may occur at the same time in the same sample. These phenomena are explained given that the endexine presence and dissipation is a general process in pollen development (Rowley, 1995; Blackmore *et al*., 2007). The foot layer shows a common proportion size in all genera during the early stages and this proportion vary slightly and reaches to fragment in some taxa to facilitate the harmomegathy process.

Ceratocapnos and *Sarcocapnos* are exceptions given that the foot layer increases markedly. Greater differences in foot layer semblance are found amongst tribes of the Euphorbiaceae family (Suárez-Cervera *et al*., 2001). Romero *et al.* (2003), debate that the foot layer in *Fumaria* is easier to identify in the earlier stages than in the *Hypecoum* genus. Our results show that in the rest of the Fumarieae samples the foot layer can be distinguished in a similar way to the *Fumaria* and without resemblances to *Hypecoum*.

The presence of the lamellate foot layer in the first step of development is very unusual in the angiosperm pollen grain. This characteristic is discussed in depth by Romero & Fernández (2000) and we show it as a generalized feature in Fumarieae, except in the genera *Ceratocapnos* and *Sarcocapnos*, where this characteristic is not present. *Ceratocapnos* and *Sarcocapnos* are phylogenetically closely related (they are sister groups; Lidén, 1986; Pérez-Gutiérrez *et al*., 2012), and so we can consider the absence of lamellate foot layer in both genera as a synapomorphy, similar to other pollen characteristics they share: absence of lamellated foot layer, dendroidal infratectum shape (discussed later), inappreciable interapertural endexine, and absence of fluffy plug. This set of synapomorphies indicates that the pollen type of *Ceratocapnos* and *Sarcocapnos* it is a well differentiated pollen grain which experienced many changes before the split of both genera.

The proportion of infratectum is very small in most of the Fumarieae genera, although there is a strong thinning in *Fumaria* and *Rupicapnos*, where in some places it seemed to be without granules (Figs. 5 E; 6 F). Infratectum shape was chiefly granular, with some taxa showing intermediate weave (*Lamprocapnos* and *Ehrendorferia*), following the definition for this state proposed by Doyle & Endress (2000). A particular kind of granular infratectum has been described for *Ceratocapnos* and *Sarcocapnos*, 'dendroid granules' in order to differentiate this particular granular shape compared to the rest of the genera where a less dense set of granules was found (Figs. 4 L; 5 H). Although we found granular shapes for most Fumarieae representatives, it seems to be a trend in the tribe infratectum from intermediate shapes (Figs. 1 A, B, H; 4 A; 5 B) to small dense granules (dendroid granules, Figs. 4 N ; 5 H; 6 K). Transformation series for the infratectum are recognized in Magnoliaceae representative taxa from irregular granules to columellate structures (Xu & Kirchoff, 2008). In our study we present a transformation series that could be considered as the opposite, from intermediate shape (similar to columellate) to irregular granules. In turn, the tribe Hypecoeae presents a well developed columellate infratectum (Romero *et al.*, 2003) showing a great contrast for this layer between the two tribes of Fumarioideae.

Tectum proportion also resulted as a homogeneous trait amongst most genera and changed very little in the different stages. The presence of a size range in the same sample was common due to irregularities derived from the reticulate, rugulate and verrucate tectum shapes. This tectal variation is common in pollen grains and has been reported previously within a single grain in Alyxeae representatives (Van Der Ham *et al*., 2001). Although there was homogeneity in tectum proportion in Fumarieae, we detected that *Fumaria* and *Rupicapnos* samples have a thicker tectum layer, whereas *Ceratocapnos* and *Sarcocapnos* exhibit a thinner one, both

groups being clearly different to rest of the genera in the tribe. Differentiation in pollen wall traits found for these genera in some of ectexine layers makes sense, given that *Fumaria* and *Rupicapnos*, as well as *Ceratocapnos* and *Sarcocapnos*, are phylogenetically closely related genera, according to both morphological (Lidén, 1986) and molecular data (Pérez-Gutiérrez *et al*., 2012).

5.5.3 ULTRASTRUCTURE OF FUMARIEAE APERTURES

As far as aperture ontogeny and ultrastructure are concerned, the development of three layers in Fumarieae is noteworthy. The aperture membrane (external layer) is absent in some genera and replaced by a dense fibrillar layer (*Fumaria*, *Rupicapnos* and *Platycapnos*), although in *Cysticapnos vesicaria* the presence of both structures was found, a fact that hints that they are not mutually exclusive (Figs. 2 I; 3 H). During maturation, the lower fibrillar material identified in microspore stages seemed to be replaced by the intine layer (Figs. 3 D-I; 4 G-K). Considering this process, we have called this layer the inner fibrillar material layer (IFM) which may correspond to the MGL (membranous granular layer) of El-Ghazaly & Huysmann (2001). This coincides in its occurrence after endexine layer formation and disappears in the bicellular pollen stage. However, it differs in structure, since we observed a fibrillar material appearance and not a granular material. Moreover, this layer develops in apertural areas and is absent in interapertural areas, except in those close to the apertures. We have not studied the resistance to acetolysis, but recent papers in *Magnolia* species (Galati *et al*., 2012; Gabarayeva & Grigorjeva 2012) confirm the non resistance to acetolysis and consider this MGL layer as part of the intine layer. We agree with these authors and we think that this fibrillar layer might be the result of the beginning of intine accumulation as Romero & Fernández (2000) describe. In the external part of the apertures, we have found that the fluffy plug (Romero & Fernández, 2000) is a common structure in most of Fumarieae representatives. Our results also show that is during vacuolate microspore stage when the fluffy plug starts to be present. In the sample of *Rupicapnos africana* during the early vacuolate microspore stage there are no fluffy elements (Fig. 2 G), whereas in the sample of *Rupicapnos numidica* it is completely formed in a more advanced vacuolate microspore stage (Fig. 2 H).

The apertural stratification (three layers) in Fumarieae has already been compared to those present in the *Hypecoum* genus and a higher complexity in Fumarieae aperture than in the Hypecoeae tribe is mentioned (Romero *et al.*, 2003). All these characteristics, together with the other differences in pollen wall layers described here, exhibit a strong disparity in pollen morphology and ultrastructure between the two tribes of Fumarioideae.

5.5.4 FUMARIEAE POLLEN TRAITS: MORPHOLOGICAL AND EVOLUTIONARY IMPLICATIONS

Endexine

The knowledge about endexine development and function is scarce (Hesse, 2000), mainly because of the endexine is difficult to identify in samples of some plant groups, as it is remarked

by Doyle (2005), and also because this layer develops during early stages of pollen wall ontogeny and most of studies on pollen wall are focused on the mature pollen stages. Recently, some advances in the understanding of endexine development have been made from the study of the pollen of *Passiflora racemosa* Brot. (Gabarayeva, Grigorjeva & Kosenko, 2013b), although it is not yet known if these advances could be extended to other plant groups.

Hesse *et al.* (2009) define endexine of angiosperm as a layer 'continuous or discontinuous, spongy or compact, is present overall, only in apertures, or even completely absent'. In Fumarieae we have found that the presence of endexine is common to most genera, being drastically reduced in some genera (*Ceratocapnos* and *Sarcocapnos*) and mainly restricted to apertures (close to disappearing in interapertural areas). In our analysis we have defined the endexine both in staining and in development terms, the two criteria classically employed and that sometimes have been in dispute (Doyle, 2005). We have checked that during early stages of microspore the electrodensity of the endexine and foot layer are quite similar, whereas from the stage of vacuolate microspore onwards the distinction is clear. Moreover, we have used the PTA treatment and showed that it is an effective technique, even when endexine is heavily reduced in the interapertural areas, as in *Ceratocapnos* and *Sarcocapnos*. Contrary to most of angiosperm, the white lines in the endexine layer are very scarce and difficult to observe in the tribe Fumarieae and they mainly appear close to the foot layer and in the apertural areas.

Regarding the evolutionary trend of endexine, Doyle (2005) evidences that endexine was an ancestral feature in angiosperm versus the hitherto established concept that endexine absence was ancestral (Brenner, 1996). The presence of this layer in Fumarieae provides another example for a basal angiosperm lineage (basal eudicots) with a clearly developed endexine layer. In this study we have found two genera (*Ceratocapnos* and *Sarcocapnos*) without endexine, which considering the Fumarieae phylogeny (Pérez-Gutiérrez *et al*., 2012; Pérez-Gutiérrez *et al*., unpubl. data) is explained by the loss of this layer in the most recent common ancestor of these two genera. The loss of endexine has been also evidenced in closely related taxa of early divergent angiosperm as Magnoliales (Galati *et al*., 2012; Xu & Ronse de Craene, 2013).

Infratectum morphology

Infratectum morphology is another trait for which an evolutionary trend has been discussed on several occasions (Van Campo & Lugardon, 1973; Doyle & Endress, 2000; Doyle, 2009). There are three main recognized types of infratectum for angiosperm: columellar, granular and intermediate (Doyle & Endress, 2000). Traditionally the granular type is thought to be ancestral in angiosperm due to its presence in some Magnoliales groups, although later, columellate infratectum was discovered to be the ancestral state in angiosperm (Doyle, 2005). The Fumarieae tribe contain representatives from two of these general infratectum shapes: intermediate in *Lamprocapnos* and *Ehrendorferia* genera (basal taxa in the tribe, Pérez-Gutiérrez *et al*., unpubl. data), and granular in the rest. Therefore, the granular state is characteristic of Fumarieae infratectum and this tribe is put forward as a new example of a

granular infratectum group in angiosperm, joining to some monocots, Magnoliales representatives, some Fagales and the Apocynaceae family (Doyle, 2009). Moreover, considering the phylogeny of subfamily Fumarioideae (Pérez-Gutiérrez *et al*. unpubl. data) an evolutionary trend in this subfamily from columellar infratectum (tribe Hypecoeae) to granular through the intermediate infratectum shape in the two most basal lineages of Fumarieae tribe (*Lamprocapnos* and *Ehrendorferia*) exists.

White lines

Lamellated structures are largely described from pollen wall layers of angiosperm (Southworth, 1966; Rowley, 1976), gymnosperm (Kurman, 1989), or even in sporoderm of pteridophyte (Lugardon, 1990). In the pollen wall, they are traditionally considered to be found exclusively in the endexine layer (Faegri & Iversen, 1975) and to set the limit between the endexine and the foot layer (Nabli, 1975; Gabarayeva, Grigorjeva & Rowley, 2010). However, in the plant group studied here, the foot layer is found to be lamellate as Romero & Fernández (2000) describe in *Fumaria densiflora* contrary to the opinion of Guédès (1982), Blackmore & Crane (1988) and Blackmore (1990), who consider that a real foot layer is not lamellated during development and that the taxa with lamellations below the infratectum lack a foot layer as such. In fact, Gabarayeva *et al.* (2013b) discuss the presence of lamellated structures away from the endexine layer and they state that it must be a 'universal mechanism' for white line formation in order to explain the widespread occurrence of these structures in pollen observations. Our results report the presence of white lines, both in the endexine and in the foot layer, but observed at different maturation stages. Whereas a lamellated endexine is found in the microspore vacuolate stage, the foot layer shows a high level of white lines only in the young microspore stage. After these periods, lamellations disappear as Romero & Fernández (2000) have already described in *Fumaria densiflora*. Lamellations in the foot layer are depicted during tetrad stages in *Impatiens parviflora* (Vinckier *et al*., 2012) although are also present in the endexine, whilst they are also found in mature pollen grains of representatives of *Cypripedium*, *Phragmipedium* and *Selenipedium* (Burns-Balogh & Hesse, 1988). In turn, a lamellated foot layer is observed in the young microspore pollen wall of *Lycopersum esculentum* (Polowick & Sawhney, 1993a) but this characteristic is also stored in mature grains (Polowick & Sawhney, 1993b). However, in Fumarieae lamellated structures disappear from the foot layer in the following vacuolate microspore stage, when they were practically absent from the endexine and remained restricted to apertural areas. It is widely recognized that when the foot layer and the endexine are not discernible, the entire region is called nexine (Dahl & Rowley, 1991), especially in young microspores. However, in our samples the endexine layer begins to be recognizable in the young microspore stage (Fig. 2), even the PTA tests only show a positive reaction in the endexine and in many cases we are able to assert that white lines are present in all the foot layer region, not only in the contact region with the endexine. All this hints that the foot layer in Fumarieae performs a similar function during pollen wall maturation in the young microspore stage to the currently recognized role described for lamellated endexine

(Gabarayeva & Hemsley, 2006; Hemsley & Gabarayeva, 2007). According to these authors, the white lines are the result of sporopollenin transport and deposition through dynamic mesophases of cramped micellar systems (Gabarayeva *et al.*, 2009). We propose that this activity, which has been extensively identified by means of lamellation and white line observation in endexine areas (Gabarayeva *et al.*, 2010), might occur in the foot layer before the endexine formation, as observations of Fumarieae representatives suggest (Fig. 2).

The fluffy plug

An extremely particular trait in Fumarieae pollen is the fluffy plug found in the apertures of most of their genera. Romero & Fernández (2000) describe this material in apertures of *Fumaria densiflora* for the first time and suggest a protective function in the pollen wall, especially against desiccation in pore areas. They highlight that the fibrillar material of the fluffy plug consists of a pentalamellated structure in contrast to the fibrillar material described at that date (Romero & Fernández, 2000, see Figures 2 and 3). We have now located fluffy elements homologous to those described in *F. densiflora*, although showing a both pentalamellated and trilamellated nature. The most striking type of fluffy material was found in *Rupicapnos* (Fig. 3 H) and *Fumaria* (Figs. 4 J; 5 L) genera, where a large accumulation of this material covered the pore aperture and was visible from the vacuolate microspore stage. *Platycapnos* and *Dactylicapnos* contained a high amount of fluffy material, although to a lesser degree than for the aforementioned taxa, whereas for rest of genera only a slight amount of apertural fluffy plug was found. Fibrillar structures are mentioned related to the outer covering of tetrads in the young stages of pollen growth (Dickinson & Sheldon, 1986; Rowley & Skvarla, 2004; Gabarayeva *et al.*, 2010; Gabarayeva, Grigorjeva & Kosenko, 2013a), as well as the tectum (see discussion in Romero & Fernandez, 2000), integrating the intine layer (Xu & Kirchoff, 2008; Gabarayeva *et al.*, 2010) or in accumulations under the aperture (Saad-Limam *et al.*, 2005; Kuang *et al.*, 2008), but never on the apertures and besides, none of these structures seem to be similar to the pentalamellated shape described for Fumarieae fluffy plug (Romero & Fernández, 2000). Fluffy textures are used to describe surfaces in the outer material of tubes from germinated pollen grains (Derksen *et al.*, 1999; 2002) and in the internal membrane between vegetative and generative cells of pollen (Hess, 1998), but neither the aspect (non-lamellated versus lamellated) nor the setup (light layer versus mass) are similar to the Fumarieae fluffy plug. Regarding the nature of Fumarieae fluffy material, Romero & Fernández (2000) propose that it might originate from rest of the tapetum, possibly from the endoplasmic reticulum (Fig 2C). The idea that this material might be sporopolenin material should be ruled out because the fluffy plug disappears when the acetolysis method is used.

5.6 CONCLUSIONS

1-Fumarieae pollen morphology presents high variability, especially in ectexine sculpturing, ornamentation and aperture number. Most of the features reported here are consistent with previous morphological descriptions of the group, even though pollen information has been enlarged to include all the currently recognized Fumarieae genera in this study.

2- Pollen-wall layer development in Fumarieae shows changes towards the disappearance of the lamellated foot layer and the fluffy material, the accumulation of dense small granules in the infratectum layer and a reduction of the interapertural endexine which remains confined only to apertural areas. The size proportion of the tectum and the foot layer in the pollen wall exhibits a reversed behaviour between *Fumaria*/*Rupicapnos* and *Ceratocapnos*/*Sarcocapnos* representatives.

3-Three main regions or layers are clearly defined in Fumarieae apertures from microspore vacuolate stage onwards. The external layer is the aperture membrane, generally made up of pollen wall fragments or with a fibrillar substance in the pore type apertures, mixed aperture membrane is also possible. Endexine forms the intermediate layer and generally shapes the oncus during the initial stages. The innermost layer contains a fibrillar material during the initial stage and is later replaced by the intine.

4-Great differences in ornamentation, type and number of apertures, infratectum morphology, proportion of foot layer and tectum, and in aperture stratification are present between the tribe Fumarieae and the sister group Hypecoeae, showing high levels of pollen change between both lineages.

5- Some features of Fumarieae pollen are unusual when compared to the pollen of other angiosperms and especially to the pollen of other eudicots. Endexine, which is considered ancestral in angiosperm, disappears in the interapertural regions of some Fumarieae representatives. For its part, there are three different infratectum shapes in Fumarieae, here we describe the type of dendroid granules defining a granular infratectum that consists in a net of dense small granules that may be ramified. A lamellated foot layer is extended in the early stage during Fumarieae pollen wall ontogeny and suggests a similar performance of the already identified endexine white lines in the foot layer during this period. A well defined assembly of fibrous material on the Fumarieae apertures is described as a fluffy plug, so far, an exclusive apertural element in pollen morphology knowledge.

5.7 REFERENCES

Blackmore S. 1990. Sporoderm homologies and morphogenesis in land plants, with a discussion of *Echinops sphaerocephala* (Compositae). *Plant Systematics and Evolution* **5** (Suppl.)**:** 1-12.

Blackmore S, Crane PR. 1988. Systematic implications of pollen and spore ontogeny. In: Humphries CJ, ed. *Ontogeny and systematics*. Columbia University Press, New York, 83-115.

Blackmore S, Stafford P, Persson V. 1995. Palynology and systematics of Ranunculiflorae. *Plant Systematics and Evolution* (Supplement) **9:** 71-82.

Blackmore S, Wortley H, Skvarla JJ, Rowley JR. 2007. Pollen wall development in flowering plants. *New Phytologist* **174:** 483-498.

Brenner GJ. 1996. Evidence for the earliest stage of angiosperm pollen evolution: a palaeoequatorial section from Israel. In: Taylor DW, Hickey LJ, eds. *Flowering plant origin, evolution and phylogeny*. Chapman & Hall, New York, 99-116.

Burns-Balogh, Hesse M. 1988. Pollen morphology of the cypripedioid ochids*. *Plant Systematics and Evolution* **158:** 165-182.

Candau P. 1987. Familia Papaveraceae y Fumariaceae. In: Valdés B, Díez MJ, Fernández I, eds. *Atlas polínico de Andalucía occidental*. Inst. Desarr. Reg. Univ. Sevilla & Diput. Cádiz, Sevilla, 84-89.

Candau P, Soler A. 1981. Contribución a la palinología de la familia Fumariaceae en la Península Ibérica. *Botanica Macaronesica* **8-9:** 147-161.

Dahl ÅE. 1989. Taxonomic and morphological studies in *Hypecoum* sect. *Hypecoum* (Papaveraceae). *Plant Systematics and Evolution* **163:** 227-280.

Dahl ÅE. 1990. Infrageneric division of the genus *Hypecoum* (Papaveraceae). *Nordic Journal of Botany* **10:** 129-140.

Dahl AO, Rowley JR. 1991. Microspore development in *Calluna* (Ericaceae). Exine formation. Annales des science naturelles, botanique. Paris Serie 13, **11:** 155-176.

Del Rei Teixeira M, Amorim AM, Riberio FA. 2013. Pollen morphology of Menispermaceae in the state of Bahia, Brazil. *Acta Botanica Brasilica* **27:** 436-444.

Derksen J, Li Y-Q, Knuiman B, Geurts H. 1999. The wall of *Pinus sylvestris* L. pollen tubes. *Protoplasma* **208:** 26-36.

Derksen J, Knuiman B, Hoedemaekers K, Guyon A, Bonhomme S, Pierson ES. 2002. Growth and cellular organization of *Arabidopsis* pollen tubes in vitro. *Sexual Plant Reproduction* **15:** 133-139.

Dickinson HG, Sheldon JM. 1986. The generation of patterning at the plasma membrane of the young microspore of *Lilium*. In: Blackmore S, Ferguson IK eds. *Pollen and spores: form and function*. Linnean Society Symposium Series No. 12. Academic Press, London, pp. 1-18.

Doyle JA. 2005. Early evolution of angiosperm pollen as inferred from molecular and morphological phylogenetic analysis. *Grana* **44:** 227-251.

Doyle JA. 2009. Evolutionary significance of granular exine structure in the light of phylogenetic analyses. *Review of Palaeobotany and Palynology* **156:** 198-210.

Doyle JA, Endress PK. 2000. Morphological phylogenetic analysis of basal angiosperms: comparison and combination with molecular data. *International Journal of Plant Sciences* 161 Suppl. **6**: S121-S153.

El-Ghazaly G, Huysmann S. 2001. Re-evaluation of a neglected layer in pollen wall development with comments on its evolution. *Grana* 40: 3-16.

Erdtman G. 1960. The acetolysis method: a revised description. *Svensk Botanisk Tidskrift* **54:** 561-564.

Faegri K, Iversen J. 1975. Textbook of pollen analysis, 3r ed: Faegri, K. Blackwell, Oxford.

Furness CA. 2008. Successive microsporogenesis in Eudicots with a reference to Berberidaceae (Ranunculales). *Plant Systematics and Evolution* **273:** 211-223.

Gabarayeva NI, Hemsley AR. 2006. Merging concepts: the role of self-assembly in the development of pollen wall structure. *Review of Paleobotany and Palynology* **138:** 121-139.

Gabarayeva NI, Grigorjeva VV, Rowley JR, Hemsley AR. 2009. Sporoderm development in *Treversia burckii* (Araliaceae) II. Post tetrad period: further evidence for the participation of self-assembly processes. *Review of Paleobotany and Palynology* **156:** 233-247.

Gabarayeva NI, Grigorjeva VV, Rowley JR. 2010. Sporoderm development in *Acer tataricum* (Aceraceae): an interpretation. *Protoplasma* **247:** 65-81.

Gabarayeva NI, Grigorjeva VV. 2012. Sporoderm development and substructure in *Magnolia sieboldii* and other Magnoliaceae: an interpretation. *Grana* **51:** 119-147.

Gabarayeva NI, Grigorjeva VV, Kosenko Y. 2013a. I. Primexine development in *Passiflora racemosa* Brot.: overlooked aspects of development. *Plant Systematics and Evolution* **299:** 1013-1035.

Gabarayeva NI, Grigorjeva VV, Kosenko Y. 2013b. II. Exine development in *Passiflora racemosa* Brot.: post-tetrad period. Overlooked aspects of development. *Plant Systematics and Evolution* **299:** 1037-1055.

Galati BG, Zarlavsky G, Rosenfeldt S, Gotelli MM. 2012. Pollen ontogeny in *Magnolia liliflora* Desr. *Plant Systematics and Evolution* **298:** 527-534.

Guédès M. 1982. Exine stratification, ectexine structure and angiosperm evolution. *Grana* **21:** 161-170.

Hess MW. 1998. Membrane coatings on the generative cell surface of freeze-substituted monocotyledon pollen. *Protoplasma* **176:** 84-88.

Hesse M. 2000. Pollen wall stratification and pollination. *Plant Systematics and Evolution* **222:** 1-17.

Hesse M, Halbritter H, Zetter R, Weber M, Buchner R, Frosch-Radivo A, Ullrich S. 2009. Pollen terminology. An illustrated handbook. Springer, New York.

Hemsley AR, Gabarayeva NI. 2007. Exine development: the importance of looking through a colloid chemistry “window”. *Plant Systematics and Evolution* **263:** 25-49.

Hoot SB, Kadereit JW, Blattner FR, Jork KM, Schwarzbach AE, Crane PR. 1997. Data congruence and phylogeny of the Papaveraceae s.l. based on four data sets: atpB and rbcL sequences, trnK restriction sites, and morphological characters. *Systematic Botany* **22:** 575-590.

Kadereit WJ, Blattner RF, Jork KB, Schwarzbach A. 1994. Phylogenetic analysis of the Papaveraceae s.l. (incl. Fumariaceae, Hypecoceae, and Pteridophyllum) based on morphological characters. *Botanische Jahrbücher für Systematik* **116:** 361-390.

Kalis AJ. 1979. Papaveraceae. The northwest European pollen flora, 20. *Review of Palaeobotany and Palynology* **28:** 209-260.

Kuang YF, Kirchoff BK, Tang YJ, Liang YH, Liao JP. 2008. Palynological character and their systematic significance in Naucleeae (Cinchonoideae, Rubiaceae). *Review of Paleobotany and Palynology* **151:** 123-135.

Kurman MH. 1989. Pollen wall formation in *Abies concolor* and a discussion on wall layers homologies. *Canadian Journal of Botany* **67:** 2489-2504.

Layka S. 1976. Les méthodes modernes de la palynologie appliqués à l'étude des Papaverales. – Ph.D. diss., l'Université de Montpellier, C.N.R.S.A.O. 12.535.

Lidén M. 1986. Synopsis of Fumarioideae with a monograph of the tribe Fumarieae. *Opera Botanica* 88. 136pp.

Lidén M. 1993. Fumariaceae. In: Kubitzki K, Rohwer JG, and Bittrich V, eds. *The families and genera of vascular plants*, vol. 2:Florewing plants, dicotyledons: Magnoliid, hamamelid, and caryophyllid families. Springer-Verlag, Heidelberg, Germany.

Lidén M, Fukuhara T, Rylander J, Oxelman B. 1997. Phylogeny and classification of Fumariaceae, with emphasis on *Dicentra* s.l., based on the plastid gene *rps16* intron. *Plant Systematics and Evolution* **206:** 411-420.

Lugardon M. 1990. Pteridophyte sporogenesis: a survey of spore wall ontogeny and fine structure in a polyphyletic plant group. In: Blackmore S, Knox RB, eds. *Microspores: Evolution and Ontogeny*. Academic Press, London, 95-120.

Moon HK, Vinckier S, Smets E, Huysmans S. 2008. Comparative pollen morphology and ultrastructure of Mentheae subtribe Nepetinae (Lamiaceae). *Review of Paleobotany and Palynology*. **149:** 174-186.

Morales Torres C, Romero AT, Mendoza R. 1988. La posición sistemática de *Papaver argemone* L.: interés evolutivo del Orden Papaverales. *Lagascalia* (Extra) **15:** 181-191.

Nabli MA. 1975. Mise en évidence de deux lamelles primordiales, ectexinique et endexinique, dans l'exine de quelques Labiatae. Comptes Rendus hebdomadaires des séances de l'Académie des sciences, Paris **281:** 251–254.

Pérez-Gutiérrez MA, Romero-García AT, Salinas MJ, Blanca G, Fernández MC, Suárez-Santiago VN. 2012. Phylogeny of the tribe Fumarieae (Papaveraceae s.l.) based on chloroplast and nuclear DNA sequences: evolutionary and biographic implications. *American Journal of Botany* **99:** 517-528.

Polowick PL, Sawhney VK. 1993a. An ultrastructural study of pollen development in tomato (*Lycopersicon esculentum*). I. Tetrad to early binucleate microspore stage. *Canadian Journal of Botany* **71:** 1039-1047.

Polowick PL, Sawhney VK. 1993b. An ultrastructural study of pollen development in tomato (*Lycopersicon esculentum*). II. Pollen maturation. *Canadian Journal of Botany* **71:** 1048-1055.

Punt W, Hoen PP, Blackmore S, Nilsson S, Le Thomas A. 2007. Glossary of pollen and spore terminology. *Review of Palaeobotany and Palynology*. **143:** 1-81.

Reynolds ES. 1963. The use of lead citrate at high pH as an electron-opaque stain for electron microscopy. *Journal of Cell Biology* **17:** 208-212.

Romero AT, Fernández MC. 2000. Development of exine and apertures in *Fumaria densiflora* DC from the tetrad stage to maturity. In: Harley MM, Morton CM, Blackmore S, eds. *Pollen and Spores: Morphology and Biology*, 45-56. Royal Botanic Gardens, Kew.

Romero AT, Salinas MJ, Fernández MC. 2003. Pollen wall development in *Hypecoum imberbe* (Fumariaceae). *Grana* **42:** 91-101.

Rowley JR. 1976. Dynamic changes in pollen wall morphology. In: Ferguson IK, Muller J, eds. *The Evolutionary Significance of the Exine*. Linnean Society Symposium Series, **1:** 39-66.

Rowley JR. 1995. Are the endexines of pteridophytes, gymnosperm and angiosperm structurally equivalent? *Review of Paleobotanic and Palynology* **85:**13-34.

Rowley JR, Skvarla JJ. 2004. Pollen development in *Epilobium* (Onagraceae): early microspore stages. *Review of Paleobotany and Palynology* **132:** 237-260.

Saad-Limam SB, Nabli MA, Kirchoff BK. 2005. Pollen wall ultrastructure and ontogeny in *Heliotropium europaeum* L. (Boraginaceae). *Review of Paleobotany and Palynology* **133:** 135-149.

Southworth D. 1966. Ultrastructure of *Gerbera jamenosii* pollen. *Grana palynology* **6:** 324-337.

Stern KR. 1962. The use of pollen morphology in the taxonomy of *Dicentra*. *American Journal of Botany* **55:** 626-628.

Stevens PF. 2001 onwards. Angiosperm Phylogeny Website. Version 12, July 2012 [and more or less continuously updated since]. http://www.mobot.org/MOBOT/research/APweb/.

Suárez-Cervera M, Gillespie L, Arcalís E, Le Thomas A, Lobreau-Callen, Seoane-Camba JA. 2001. Taxonomic significance of sporoderm structure in pollen of Euphorbiaceae: Tribes Plukenetieae and Euphorbieae. *Grana* **40:** 78-104.

Van Campo M, Lugardon B. 1973. Structure grenue infratectale de l'ectexine des pollens de quelques Gymnospermes et Angiospermes. *Pollen et Spores* **15:** 171–187.

Van Der Ham R, Zimmermann Y-M, Nilsson S and Igersheim A. 2001. Pollen morphology and phylogeny of the Alyxieae (Apocynaceae). *Grana* **40:** 169-191.

Vinckier SA, Janssens SB, Huysmans S, Vandevenne A, Smets EF. 2012. Pollen ontogeny linked to tapetal cell maturation in *Impatiens parviflora* (Balsaminaceae). *Grana* **51:** 10-24.

Wang W, Lu AM, Ren Y, Endress ME, Chen ZD. 2009. Phylogeny and classification of Ranunculales: evidence from four molecular loci and morphological data. *Perspectives in Plant Ecology, Evolution and Systematics* **11:** 81-110.

Xu FX, Kirchoff BK. 2008. Pollen morphology and ultrastructure of selected species of Magnoliaceae. *Review of Paleobotany and Palynology* **150:** 140-153.

Xu FX, Ronse de Craene LP. 2013. Pollen morphology and ultrastructure of selected species from Annonaceae. *Plant Systematics and Evolution* **299:** 11-24.

Zhang ML, Lidén M, Grey-Wilson C. 2008. Papaveraceae. In: Zhengy W, Raven PH, Deyuan H, eds. *Flora of China, Vol. 7 (Menispermaceae through Capparaceae).* Science Press, Beijing & Missouri Botanical Garden Press, St. Louis, USA.

5.8 TABLES

Table 2. Pollen wall and aperture features of Fumarieae in young microspore stages. Pollen-wall layer proportion refers to ectexine size. Infratectum shape: G granulate, I intermediate. Aperture: ER external region, AM aperture membrane, FM fibrillar membrane, MR, medium region, Gl endexine in glomeruli, Gr granular endexine and Is endexine in islets; IR inner region, IFM inner fibrillar material.

	Endexine	Foot layer		Infratectum		Tectum	Aperture		
		Size	White lines	Size	Shape		ER	MR	IR
Ceratocapnos heterocarpa	Inappreciable	1/2-3/4	Absent	1/10-1/3	G	1/8-1/5	AM	Gl	IFM
Cysticapnos vesicaria	1/6-1/10	1/8-1/3	Present	<1/10-1/6	G	1/3-1/2	AM/FM	Is	IFM
Dicentra peregrina	1/3	1/4-1	Present	1/10-1/6	G	0-1/2	AM	Is	IFM
Ehrendorferia chrysantha	1/3	1/6-1/3	Present	1/8-1/5	I	1/2	AM	Is	IFM
Fumaria densiflora	1/6-1/4	1/5-1/3	Present	1/10-1/6	G	1/2	FM	Gr	IFM
Platycapnos spicata	1/10	1/3-1/2	Present	<1/10	G	1/2	FM	Gr	IFM
Lamprocapnos spectabilis	1/8-1/3	1/2-1	Present	1/10-1/5	I	0-1/2	AM	Is	IFM
Sarcocapnos pulcherrima	Inappreciable	1/2-3/4	Absent	1/8-1/5	G	1/6-1/4	AM	Gl	IFM

Table 3. Pollen wall and aperture features of Fumarieae during vacuolate microspore stages. Layer proportion refers to total ectexine size. Infratectum shape: DG dendroid granules, G granulate. Aperture: ER external region, AM aperture membrane, FM fibrillar membrane, MR medium region, Gl endexine in glomeruli, Gr endexine in granules and Is endexine in islets, IR inner region, IFM inner fibrillar material.

	Endexine		Foot layer	Infratectum		Tectum	Aperture		
	Size	White lines	Size	Size	Shape		ER	MR	IR
Adlumia fungosa	1/10	Present	1/4-1/3	1/8-1/6	G	1/2	AM	Gr	IFM
Ceratocapnos heterocarpa	Inappreciable	Absent	1/2-3/4	1/6-1/4	DG	1/6	AM	Gl	IFM
Corydalis cheilanthifolia	1/10	Not found	1/5-1/3	1/8-1/4	G	1/2	AM	Is	IFM
Corydalis rupestris	1/10	Not found	1/4-1/3	1/10-1/4	G	1/2	AM	Is	IFM
Corydalis scouleri	1/10	Present	1/2-3/4	<1/10-1/6	G	1/4-1/2	AM	Is	IFM
Cysticapnos vesicaria	1/6	Not found	1/5-1/3	1/10-1/6	G	2/3	AM	Is	IFM
Dicentra formosa subsp. *formosa*	1/4-1/3	Not found	1/6-1/3	1/10-1/6	G	1/2	AM	Is	IFM
Fumaria densiflora	1/6-1/4	Present	1/6-1/4	<1/10	G	1/2-3/4	FM	Gr	IFM
Platycapnos spicata	1/4	Present	1/5-1/3	<1/10	G	1/2-2/3	FM	Is	IFM
Rupicapnos africana	1/6	Not found	1/8-1/4	<1/10	G	2/3-3/4	FM	Gr	IFM
Rupicapnos numidica	1/5	Not found	1/10-1/6	<1/10	G	4/3	FM	Gr	IFM
Sarcocapnos pulcherrima	Inappreciable	Absent	1/3-3/4	1/10-1/5	DG	1/6-1/4	AM	Gl	IFM

Table 4. Pollen wall and aperture features of Fumarieae in the young bicellular pollen stage. Intine layer proportion is refers to exine total size, rest of layer to the ectexine size. Infratectum shape: DG dendroid granules, G granulate, I intermediate. Aperture, fluffy plug amount: H high, L low, M medium, NF not found and VH very high.

	Intine	Endexine	Foot layer	Infratectum		Tectum	Aperture
		Thickness	Size	Size	Shape	Size	Fluffy
Adlumia fungosa	1/6-1/5	<1/10	1/3-2/3	1/10-1/6	G	1/4-2/3	L
Capnoides sempervirens	1/10-1/6	1/8-1/6	1/3-1/2	1/10-1/8	G	1/3-1/2	L
Ceratocapnos heterocarpa	1/8-1/6	Inappreciable	1/2-2/3	1/4-1/3	DG	1/6-1/4	NF
Corydalis cheilanthifolia	<1/10-1/8	1/15-1/6	1/4-1/3	1/8-1/6	G	1/3-1/2	L
Corydalis paczoskii	<1/10	1/5-1/3	1/4-1/2	1/10-1/8	G	1/5-1/3	L
Corydalis popovii	1/8-1/6	1/8-1/6	1/5-1/3	1/10-1/5	G	1/2-2/3	M
Corydalis taliensis	1/8-1/6	<1/10	1/5-3/4	<1/10	G	1/4-3/4	L
Cysticapnos vesicaria	1/8-1/6	1/10-1/6	1/5-1/4	1/8-1/6	G	1/2-2/3	L
Dactylicapnos macrocapnos	1/8-1/6	1/8-1/5	1/3-1/2	1/10	G	1/2-2/3	H
Dactylicapnos torulosa	1/10-1/6	1/10-1/6	1/3-1/2	1/10-1/8	G	1/3-2/3	H
Dicentra eximea	1/8-1/6	1/6-1/4	1/3-1/2	1/8-1/6	G	1/2-2/3	L
Dicentra formosa subsp. *formosa*	1/8-1/5	1/8-1/4	1/4-1/3	<1/10	G	1/3-1/2	M
Dicentra peregrina	1/10-1/5	1/6-1/5	1/6-1/4	1/10-1/4	G	1/3-1/2	M
Ehrendorferia chrysantha	<1/10-1/6	1/6-1/3	1/4-1/2	1/8-1/5	I	1/2-3/4	M
Fumaria densiflora	1/10-1/8	1/15-1/10	1/8-1/6	<1/10	G	2/3-3/4	VH
Lamprocapnos spectabilis	1/10-1/6	1/10-1/6	1/3-1	1/8-1/4	I	0-1/2	NF
Platycapnos spicata	1/8-1/6	1/10-1/8	1/3-1/2	1/10-1/6	G	1/3-3/4	M
Pseudofumaria lutea	1/8-1/4	1/10	1/3-1/2	1/8-1/6	G	1/3-1/2	M
Sarcocapnos pulcherrima	1/8-1/6	Inappreciable	2/3-3/4	1/8-1/5	DG	1/8	NF

Table 5. Pollen wall and aperture features of Fumarieae in the medium bicellular pollen stage. Intine proportion refers to exine size and for the rest of the layers only to ectexine size. Infratectum shape: DG dendroid granules, G granulate, I intermediate. Aperture, fluffy plug amount: H high, L low, M medium, NF not found and VH very high.

	Intine	Endexine	Foot layer	Infratectum		Tectum	Aperture
		Thickness	Size	Size	Shape	Size	Fluffy
Capnoides sempervirens	1/4-1/3	<1/10	1/5-1/2	1/10-1/6	G	1/2-2/3	L
Ceratocapnos heterocarpa	1/5-1/4	Inappreciable	1/2-3/4	1/5-1/3	DG	1/6-1/4	NF
Corydalis cheilanthifolia	1/4-1/3	1/10-1/6	1/6-1/4	1/10-1/5	G	1/3-2/3	L
Corydalis popovii	1/4-1/2	<1/10-1/8	1/4-1/3	1/10-1/6	G	1/3-1/2	M
Corydalis rupestris	1/4-1/3	1/10-1/4	1/4-2/3	1/10-1/8	G	1/3-1/2	L
Corydalis temulifolia	1/3-1/2	1/10-1/5	1/3-2/3	<1/10-1/8	G	1/4-1/2	L
Cysticapnos vesicaria	1/3-1/2	1/10-1/8	1/4-1/2	<1/10-1/6	G	1/3-1/2	L
Dactylicapnos macrocapnos	1/5-1/3	1/8-1/6	1/4-1/3	1/10-1/5	G	1/3-1/2	M
Dicentra formosa subsp. *formosa*	1/3-1/2	1/10-1/5	1/6-1/2	1/10-1/5	G	1/4-2/3	L
Fumaria densiflora	1/4-1/2	<1/10	<1/10-1/6	<1/10	G	2/3-3/4	VH
Ichtyoselmis macrantha	1/5-1/3	1/8-1/4	1/4-1/2	1/10-1/5	G	1/3-1/2	L
Lamprocapnos spectabilis	1/6-1/4	1/8/1/3	1/3-1	1/6-1/4	I	0-1/2	NF
Platycapnos spicata	1/6-1/2	<1/10	1/7-1/2	<1/10-1/6	G	1/4-2/3	M
Pseudofumaria alba	1/4-1/3	1/8-1/6	1/2-2/3	1/10-1/5	G	1/4-1/2	M
Rupicapnos africana	1/6-1/2	1/10-1/5	1/10-1/3	<1/10	G	1/2-4/3	VH
Sarcocapnos pulcherrima	1/3-1/2	Inappreciable	1/2-3/4	1/10-1/5	DG	1/6-1/4	NF

Table 6. Pollen wall and aperture features of Fumarieae in the mature pollen stage. Intine proportion refers to total exine size, the rest of the layers to ectexine size. Infratectum shape. DG dendroid granules, G granulate. Aperture, fluffy plug amount: H high, L low, M medium, NF not found, VH very high.**Dicentra oregona* x *peregrina* 'gothoburgensis' sensu H. Zetterlund.

	Intine	Endexine	Foot layer	Infratectum		Tectum	Aperture
		Thickness	Size	Size	Shape	Size	Fluffy
Capnoides sempervirens	1/2-3/4	<1/10-1/8	1/6-1/3	1/10-1/4	G	1/3-2/3	L
Ceratocapnos heterocarpa	1:1	Inappreciable	1/2-3/4	1/4-1/3	DG	1/8-1/5	NF
Corydalis cheilanthifolia	2/3-1:1	<1/10-1/8	1/4-1/2	1/8-1/5	G	1/2-2/3	L
Corydalis paczoskii	2/3-1:1	1/10-1/8	1/6-1/2	<1/10-1/3	G	1/4-2/3	L
Corydalis rupestris	2/3-3/4	<1/10	1/6-1/3	1/10-1/6	G	1/5-1/2	L
Corydalis tauricola	1/2-2/3	<1/10	1/8-1/2	1/10-1/4	G	1/2-2/3	M
Corydalis temulifolia	1/3-3/4	<1/10	1/4-2/3	<1/10-1/6	G	1/5-1/2	L
Cysticapnos vesicaria	2/3-1:1	<1/10	1/3-1/2	1/10-1/5	G	1/3-1/2	L
Dactylicapnos torulosa	1:1	<1/10	1/5-1/2	1/10-1/6	G	1/4-1/2	M
Dicentra formosa subsp. *formosa*	1/2-3/4	1/10-1/6	1/6-1/3	<1/10-1/5	G	1/4-2/3	L
Dicentra 'gothoburgensis'*	1/2-2/3	<1/10-1/6	1/6-1/3	1/10-1/5	G	1/2-2/3	L
Dicentra peregrina	2/3-1:1	<1/10	1/8-1	1/8-1/4	G	0-2/3	M
Fumaria capreolata	1/2-2/3	<1/10	<1/10-1/5	<1/10	G	2/3-5/6	VH
Fumaria densiflora	4/3-1:1	1/10-1/8	1/10-1/6	<1/10	G	2/3-4/5	VH
Ichtyoselmis macrantha	1:1	1/10-1/6	1/6-1/3	1/5-1/3	G	1/4-1/2	L
Platycapnos spicata	1/2-1:1	1/10	1/5-1/3	<1/10-1/4	G	1/8-2/3	H
Pseudofumaria lutea	1:1	1/10-1/8	1/2-2/3	1/6-1/3	G	1/8-1/2	M
Rupicapnos africana	1/2-3/4	1/8	1/10-1/5	<1/10	G	1/3-4/5	H
Sarcocapnos pulcherrima	1:1-2:1	Inappreciable	2/3-4/5	1/8-1/2	DG	1/8-1/4	NF

Supporting Information. M.A., Pérez-Gutiérrez, V.N. Suárez-Santiago, M. C., Fernández, M.J. Salinas Bonillo, A.T. Romero-García. "Pollen morphology and ontogeny in the subfamily Fumarioideae (Papaveraceae): the tribe Fumarieae"

Table S1. Taxa included, provenance and analyses completed for them

Taxa	Origin	ID	SEM	TEM
Adlumia fungosa (Ait.) Britton, Stern & Poggenb.	LC National Botanical Garden of Belgium	NBGB 20001239-92	x	x
Capnoides sempervirens (L.) Borkh.	LC National Botanical Garden of Belgium	NBGB 19871844	x	x
Ceratocapnos heterocarpa Dur.	H Herbarium of the University of Granada	GDA 9032	x	x
Corydalis cheilanthifolia Hemsl.	LC Royal Botanic Gardens of Kew	K 196919923	x	x
Corydalis paczoskii N. Busch	LC Royal Botanic Gardens of Kew	K 19833040		x
Corydalis popovii Nevski ex Popov	LC Royal Botanic Gardens of Kew	K 19952322		x
Corydalis rupestris Kotschy	LC Gothenburg Botanical Gardens	GBG 2005414	x	x
Corydalis scouleri Hook.	LC Royal Botanic Garden Edinburgh	RBGE 19702476		x
Corydalis taliensis Franch.	LC Royal Botanic Garden Edinburgh	RBGE 19973257		x
Corydalis tauricola (Cullen & P.H. Davis) Lidén	LC Royal Botanic Garden Edinburgh	RBGE 19952472		x
Corydalis temulifolia Franch.	LC Gothenburg Botanical Gardens	GBG AY328202??	x	x
Cryptocapnos chasmophyticus Rech. f.	H Natural Museum History Vienna	W 35122	x	
Cysticapnos cracca (Cham. & Schltdl.) Lidén	H Compton Herbarium	STE 762340	x	
Cysticapnos vesicaria (L.) Fedde	LC Material grow from seeds of SSS	SSS 600		x
Dactylicapnos macrocapnos (Prain) Hutch.	LC Uppsala Botanical Gardens	UPS 20061163	x	x
Dactylicapnos torulosa (Hook.f. & Thomson) Hutch.	LC Uppsala Botanical Gardens	UPS 20080011		x
Dicentra eximea (Ker Gawl.) Torr.	LC Royal Botanic Gardens of Kew	K 19862854	x	x
Dicentra formosa subsp. *formosa*	LC Gothenburg Botanical Gardens	GBG 19000198	x	x
Dicentra formosa subsp. *oregona* (Eastw.) Munz	LC Royal Botanic Garden of Kew	K 19991128	x	
Dicentra gonthoburgensis (*D. formosa oregana* x *peregrina*)	LC Gothenburg Botanical Gardens	GBG 20032244		x
Dicentra peregrina (Rudolph)Makino	LC GBG	GBG 19952827	x	x
Discocapnos mundii Cham. & Schltdl.	H Pretoria National Herbarium	PRE 3612	x	
Ehrendorferia chrysantha (Hook. & Arn.) Rylander	LC Material grow from seed of RSABG	RSABG	x	x
Fumaria capreolata L.	LC Collected from wild	GDA 52802		x
Fumaria densiflora DC.	LC Collected from wild	GDA 52804	x	x
Fumariola turkestanica Korsch	Field collection	----	x	
Ichtyoselmis macrantha (Oliv.) Lidén	LC Gothenburg Botanical Gardens	GBG 19952279	x	x
Lamprocapnos spectabilis (L.) Fukuhara	LC Gothenburg Botanical Gardens	GBG 19980323	x	x
Platycapnos spicata (L.) Bernh.	LC Collected from wild	GDAC 32052		x
Platycapnos tenuiloba Pomel	LC Collected from wild	GDAC 32047	x	
Pseudorumaria alba (Mill.) Lidén	LC National Botanic Garden of Belgium	NBGB 19763486	x	x
Pseudofumaria lutea (L.) Borkh.	LC National Botanic Garden of Belgium	NBGB 19891892	x	x
Rupicapnos africana (Lam.) Pomel	LC Collected from wild	GDAC 44500		x
Rupicapnos numidica Pomel	LC Gothenburg Botanical Gardens	GBG 19004473	x	x
Sarcocapnos pulcherrima	LC Collected from wild	GDA C 22850	x	x
Trigonocapnos lichtensteinii	H Pretoria National Herbarium	PRE 592	x	

6. Pollen evolution

Evolutionary trends in the pollen grain of the subfamily Fumarioideae: evidence from morphology and ontogenetic characters of the pollen wall and apertures

Miguel A. Pérez-Gutiérrez, Ana T. Romero-García, M. Carmen Fernández, María J. Salinas Bonillo, Gabriel Blanca and Víctor N. Suárez-Santiago

Manuscript in preparation

6.1 Abstract

The pollen wall and apertures are classic features for taxonomy and systematics in plants. The survey of trends in the evolution of pollen features is an interesting field of study that reflects the importance of pollen fitness in plant diversification and the mechanisms by which evolution occurs in the pollen grain. In this paper, the evolution of the pollen wall and apertures is studied through the phylogeny of the subfamily Fumarioideae. The optimization of ultrastructural characters on the Fumarioideae topology shows that the proportional size of the ectexine layers (tectum, infratectum and foot layer) is a feature that fits the phylogeny of the subfamily, showing clear evolutionary trends. Four pollen models are described reflecting these trends in ectexine development. The organization of the aperture endexine and the type of aperture membrane are also informative characters, but with different development during the ontogeny process. Trends toward the increase in the number of apertures and toward the reduction of the exine perforations are found for the evolution of the pollen morphology in the subfamily. The acquisition of rugulate and verrucate exine surfaces in most of the Fumarioideae lineages is also an extended trend in the evolution of the pollen wall of this group.

6.2 Introduction

Fumarioideae Eaton is the most diversified group of the family Papaveraceae Juss., containing 20 genera and around 590 species (Liden 1986; Zhang et al. 2008). The group has a complex biogeographical history that has resulted in a wide distribution throughout the northern temperate regions and even, reaching South Africa (Pérez-Gutiérrez et al. unpubl. data a). From an evolutionary point of view, Papaveraceae is interesting for being one of the early divergent plant groups inside the order Ranunculales Dumort. (Kim et al. 2004; Wang et al. 2009), the earliest-branched eudicot order (APG III 2009). Evolutionary studies in Ranunculales are important for understanding core eudicot character diversification (Soltis et al. 2002). The interest of the evolutionary studies in Papaveraceae, and Fumarioideae in particular, has increased notably in the last decade; especially the studies on the floral characters such as floral symmetry and the genes involved (Köhl and Gleissberg 2006; Damerval and Nadot 2007; Damerval et al. 2007, 2013).

Pollen grain morphology as well as pollen wall ultrastructure and aperture data are also plant features that arouse great interest in plant evolution (Doyle 2005; Furness 2007); however, little research has been done on the evolutionary trends in the basal groups of eudicots. Fumitories are a euripalynous plant group, since almost each genus shows an exclusive pollen type (Pérez-Gutiérrez et al. unpunbl. data b), making this subfamily a good basal-eudicot group to study the evolution of the pollen characters. At a morphological level the main differences between Fumarioideae pollen concern the aperture system (number and type of aperture) and exine ornamentation, however ultrastructural studies at different pollen development stages

also show important differences between taxa in the pollen wall and aperture developments (Romero and Fernández 2000; Romero et al. 2003; Pérez-Gutiérrez et al. unpubl. data b).

Pollen morphological studies on Fumarioideae have been carried out on different occasions although generally in wider research focused on the whole Papaveraceae family. The studies carried out by Layka (1975, 1976) and Kalis (1979) are some of the most remarkable due to the high number of representatives included. Pantoaperturate distribution of apertures, the higher number of apertures or absence of columellae structures in the inner layers are clearly different pollen features between most Fumarioidae and the rest of the Papaveraceae. Similarly, in a wider study on Ranunculiflorae, Blackmore et al. (1995) published a morphological description for Fumarioideae pollen and described systematic implications at interfamiliar level. They concluded that aperture features are not suitable for relationship inference at family level but highlighted the higher systematic potential from exine ornamentation and stratification. As examples of studies focused on the subfamily Fumarioideae, Stern (1962) makes a detailed description of pollen morphology from 19 representatives of *Dicentra* Bernh. (now segregated in four genera: *Dactylicapnos* Wall., *Dicentra*, *Ehrendorferia* T. Fukuhara & Lidén, I*chtyoselmis* Lidén & T. Fukuhara and *Lamprocapnos* (L.) Fukuhara) and an interpretation of the phylogenetic trends in ornamentation and number of apertures throughout the genus. Pollen morphology in *Hypecoum* L. is depicted in the studies by Dahl (1989, 1990), as well as measurements from pollen grain of the genus *Sarcocapnos* presented by Salinas et al. (2003). Thereby, diversity in Fumarioideae pollen grain morphology is well known thanks to the aforementioned research and other regional pollen descriptive publications, both general (Candau 1987) and specific approximations (Rachele 1974; Candau and Soler 1981).

As far as ultrastructural information is concerned, the only surveys that delve into pollen wall layer features are those made by Romero and Fernández (2000) and Romero et al. (2003) dedicated to *Fumaria densiflora* DC and *Hypecoum imberbe* Sm. respectively. Recently, we have performed an exhaustive description of features from ontogeny and also from external pollen grain morphology for representatives of all the genera of the tribe Fumarieae (Pérez-Gutiérrez et al. unpubl. data b). This study has increased the morphological information available from Fumarioideae pollen and established the main ultrastructural changes during the pollen grain ontogeny of the group. In this paper, we analyze all this information in the phylogenetic context of the subfamily in order to elucidate evolutionary trends that have taken place in pollen wall layers and aperture systems during the evolution of this subfamily.

From a phylogenetic point of view, Fumarioideae consists of two distinct groups recognized at tribal level (Steven 2001 onwards), the tribe Hypecoeae Dumort. (1 genus, *Hypecoum*) and the tribe Fumarieae Dumort. (19 genera) (Pérez-Gutiérrez et al. unpubl data a; Fig. 1). Within the tribe Fumarieae, a group of genera conform a basal grade (denominated as basal Fumarieae in Pérez-Gutiérrez et al. in press a) and the rest of the genera are the core of the tribe (Crown Fumarieae in Pérez-Gutiérrez et al. unpubl. data a). The basal Fumarieae group contains *Adlumia* DC. *Dicentra*, *Ehrendorferia*, *Ichtyoselmis* and *Lamprocapnos*; whilst the Crown Fumarieae harbours *Capnoides* Mill., *Corydalis* DC., *Dactylicapnos* and the subtribe Fumariinae

(formed by three monophyletic groups of genera, the *Cysticapnos* Clade, the *Fumaria* Clade and the *Sarcocapnos* Clade; Pérez-Gutiérrez et al. unpubl. a).

In this paper, we optimize a set of morphological and ultrastructural characters from the pollen wall and the aperture system of Fumarioideae on the plastidial phylogeny of the subfamily (Fig. 1). The ontogeny of the ultrastructural characters is checked by analysis during four different pollen grain maturation stages. In this way, (1) we study the evolution of the morphological and ultrastructural features in the subfamily by means of the identification of the ancestral states and (2) we evaluate the phylogenetic information that these pollen characters contain by the use of the statistics fit to the molecular tree. This second objective allows us to contrast the information that morphological characters offer compared to the ultrastructural ones. We also evaluate whether the evolution of the ultrastructural features and their phylogenetic signal are homogeneous amongst the four developmental stages.

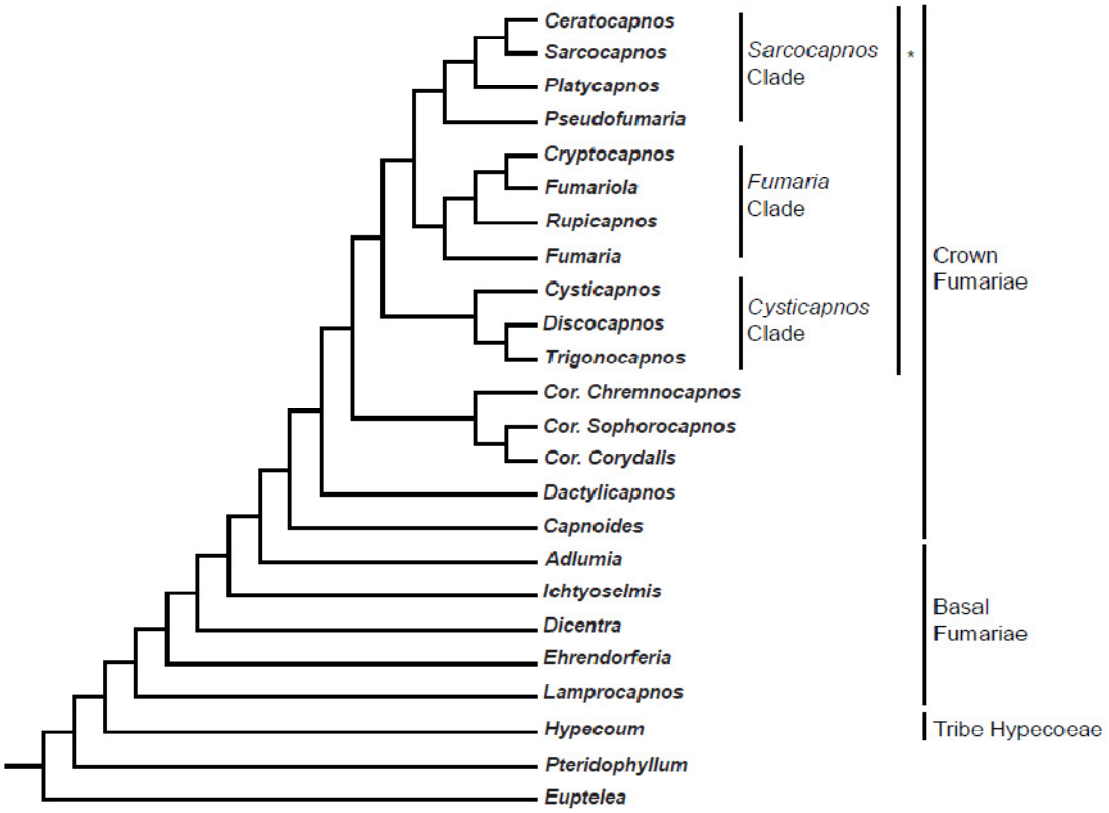


Fig 1. Phylogeny of subfamily Fumarioideae based on molecular data (Pérez-Gutiérrez et al., unpubl data). The main groups recognized for the subfamily are shown on the right (* subtribe Fumariinae).

6.3 Materials and Methods

Material

The pollen information collected for this study was obtained from Pérez-Gutiérrez et al. (unpubl. data b) for the tribe Fumarieae, and from Pérez-Gutiérrez et al. (unpubl. data c) for *Pteridophyllum* and *Euptelea* genera. Pollen morphology and ultrastructure of *Hypecoum* (tribe Hypecoeae) were collected from Dahl (1990) and Romero et al. (2003) respectively. Some information about aperture number in *Euptelea* was also obtained from Praglowski (1974). All the data were summarized at generic level, with the exception of the genus *Corydalis* for which we used the pollen features at subgeneric level (*Chremnocapnos* Wendelbo, *Corydalis* and *Sophorocapnos* Turcz.). Seventeen characters from the ultrastructure of the pollen-wall layers and apertures were obtained from images of transmission electron microscopy (TEM), using between three and ten images per genera and stage. This set of characters was analyzed during four stages of the ontogenetic process of pollen wall maturation, microspore cell, young bicellular pollen, medium bicellular pollen and mature pollen grain (see Pérez-Gutiérrez et al. unpubl. data b, for the description of each stage). To analyze the size of the ectexine layers, we used the ratio referring to the total size of the ectexine in order to avoid the bias among different angles in the ultrastructural cuts. Regarding pollen morphology, seven characters describing the main information observed on the outside of the pollen grain (structure and sculpture) were obtained from images of scanning electron microscopy (SEM), using between three and ten images per genera. Features were coded in most cases as multistate character or as presence-absence, all the characters and their states are presented in the Table 1. The pollen terminology has been used according to Punt et al. (2007) and Hesse et al. (2009).

Methods

In order to reconstruct the ancestral states of the extent groups of Fumarioideae all of the analyzed characters were optimized on the chloroplast topology of the subfamily (Fig. 1) using Mesquite v3.00 (Maddison and Maddison, 2014), and parsimony as reconstruction method. The genus *Pteridophyllum* Sieblod. & Zucc., as representative of the basal lineage of Papaveraceae (Hoot et al. 1997), and the genus *Euptelea* Siebold. & Zucc. as representative of the earliest divergent family of Ranunculales order, are used as outgroup taxa (Kim et al. 2004; Wang et al. 2009). The goodness-of-fit of the pollen characters to the molecular tree was established by the consistency (CI: Kluge and Farris, 1969) and retention (RI: Swoford, 1993) indices, also calculated in Mesquite v3.00. Changes of the ultrastructural features during the ontogeny process were evaluated by observation and comparison of each character among the four pollen development stages. A one-way ANOVA for the rescaled consistency index (RC) values was used, using IBM SPSS Statistics for Windows, version 20 (IBM Corp., Armonk, NY) to test for significant differences in the tree-fit-goodness of the pollen characters among pollen-developmental stages and also between ultrastructure and morphological characters. Heterocedasticity was confirmed by the Bartlett test.

6.4 Results

Pollen feature adjustment to the topology

Measurements of CI and RI indices are presented in Table 1. Most of the pollen ultrastructural features exhibit high levels of homoplasy and low levels of apparent-synapomorphy retention (Table 1a). This is the case for the infratectum content character, the foot layer morphology, most of the endexine layer features, the intine size and most of the characters related to the aperture. The rest of characters show medium or high levels of consistency and apparent-synapomorphy retention. The ratio of tectum/ectexine, the infratectum setup and the presence of endexine in the interapertural areas stand out as being informative characters in most of the development stages, whilst the tectum perforations, the ratio of infratectum/ectexine, the ratio of foot layer/ectexine, the inner foot layer and the amount of fluffy material are informative in some of the stages. Regarding the character-adjustment level among the different stages, the ANOVA test does not show significant differences in the RC values. The tree-fit statistics reflect a high level of homoplasy and low apparent-synapomorphy retention for the characters related to pollen morphology (Table 1b). All the features observed from the structure and sculpture of the pollen grain present low levels of CI and RI, with the exception of apertural type, which is a character highly informative (Table 1b). However, the ANOVA test did not find significant differences for RC from ultrastructure and morphological characters. The character states for all the ontogenetic stages in the Fumarioideae taxa are presented in supplementary material Table S1 and the states for the pollen morphology characters in Table S2.

Evolution of ultrastructural pollen characters

The optimization of all the characters on the Fumarioideae phylogeny allows us to identify the ancestral states for the pollen features as well as their evolutionary changes. The reconstruction during the mature stage of development shows that the ancestor of Fumarioideae presents: the proportions of the ectexine layers with a thick tectum, a thick infratectum and a very thin foot later (Figs. S3, S6, S8). The ancestor also has tectum with perforations, a columellate infratectum, and a discontinuous foot layer with an irregular inner face (Figs. S4, S5, S9, S10). The interapertural endexine is present, being very thin and slightly fragmented (Figs. S11-S14), whilst the intine is a thick layer (Figs. S15). The ancestor apertures have the endexine arranged in islets, an echinate apertural membrane and without fluffy material (Figs. S17-S19). Ancestral states for the tribe Fumarieae show that the characters changed toward a medium proportion of tectum layer, thin for the infratectum and medium for the foot layer (Figs. S3, S6, S8). This ancestor presents a granular infratectum and a baculate aperture membrane (Figs. S5, S18). The fluffy material is acquired for the common ancestor of *Ehrendorferia* and the rest of the tribe

Table 1. Values of statistical adjustment to the topology obtained for a: ultrastructural pollen features during the four development stages analyzed ([a] microspore, [b] young bicellular, [c] medium bicellular and [d] mature pollen) and b: pollen morphology features. CI, consistency index; RI retention index and RC, rescaled retention index. In bold are highlighted those characters with CI>0.75. na means not applicable due to be invariable.

	Characters and character states	CI[a]	RI[a]	RC[a]	CI[b]	RI[b]	RC[b]	CI[c]	RI[c]	RC[c]	CI[d]	RI[d]	RC[d]
a) Ultrastructure													
1	**Ratio tectum/ectexine:** Very thin [0], Thin [1], Medium [2], Thick [3], Very thick [4]	**0.80**	**0.50**	**0.40**	**0.75**	**0.50**	**0.38**	**0.75**	**0.80**	**0.60**	**0.75**	**0.80**	**0.60**
2	**Tectum perforations:** Absence [0], Perforations [1], Wider perforations [2], Narrow perforations [3], Mixed [4]	0.60	0.67	0.40	**0.80**	**0.80**	**0.64**	0.67	0.71	0.48	**0.80**	**0.75**	**0.60**
3	**Infratectum setup:** Few granules [0], Granules [1], Intermediate [2], Dendroids [3], Columellate [4]	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**
4	**Ratio infratectum/ectexine:** Very thin [0], Thin [1], Medium [2], Thick [3]	0.60	0.50	0.30	0.60	0.50	0.30	0.60	0.60	0.36	**0.75**	**0.83**	**0.63**
5	**Infratectum content:** Empty [0], Electrodense material [1], Gray material [2]	0.67	0.67	0.44	0.40	0.00	0.00	0.40	0.00	0.00	0.20	0.00	0.00
6	**Ratio foot layer/ectexine:** Very thin [0], Thin [1], Medium [2], Thick [3], Very thick [4]	**0.75**	**0.75**	**0.56**	**0.75**	**0.67**	**0.50**	0.38	0.29	0.11	0.57	0.50	0.29
7	**Foot layer morphology:** Continuous [0], Discontinuous [1]	0.20	0.43	0.09	0.14	0.00	0.00	0.20	0.00	0.00	0.33	0.00	0.00
8	**Inner foot layer:** Regular [0], Irregular [1], Very irregular [2]	**1.00**	**1.00**	**1.00**	0.40	0.40	0.16	0.50	0.50	0.25	0.33	0.50	0.17
9	**Presence of endexine:** Inappreciable [0], Presence [1]	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**
10	**Ratio endexine/ectexine:** Very thin [0], Thin [1], Medium [2]	0.50	0.50	0.25	0.25	0.00	0.00	na	na	na	na	na	na
11	**Fragmentation of endexine:** Unfragmented [0], Slightly fragmented [1], Very fragmented [2]	0.20	0.33	0.07	0.33	0.00	0.00	0.33	0.20	0.07	0.67	0.75	0.50
12	**Shape endexine fragmentation:** Almost continuous layer [0], Islets [1], Mixed [2]	0.67	0.50	0.34	0.50	0.00	0.00	0.33	0.20	0.07	**1.00**	**1.00**	**1.00**
13	**Ratio intine/exine:** Very thin [0], Thin [1], Medium[2]	na	na	na	0.50	0.00	0.00	0.50	0.00	0.00	0.40	0.25	0.10
14	**Apertural oncus:** Absence [0], Presence [1]	na	na	na	0.25	0.00	0.00	0.17	0.29	0.05	0.20	0.00	0.00
15	**Apertural endexine setup:** Islets [0], Granules [1], Layered [2], Glomeruli [3], Small granules [4], Homogeneus [5]	**0.71**	**0.67**	**0.48**	**1.00**	-	-	**1.00**	-	-	**1.00**	-	-
16	**Apertural membrane:** Baculate [0], Fibrillar [1], Mixed [2], Echinate [3]	**0.75**	**0.75**	**0.30**	0.67	0.75	0.50	0.67	0.80	0.53	0.67	0.80	0.53
17	**Fluffy material:** Absence [0], Low [1], Middle [2], High [3], Very high [4]	0.50	0.83	0.42	0.67	0.72	0.48	0.43	0.55	0.24	0.57	0.57	0.33
b) Pollen morphology													
18	**Pollen grain size:** Small [0], Medium [1], Big [2], Very big [3]	-	-	-	-	-	-	-	-	-	0.40	0.50	0.2
19	**Pollen grain shape:** Oblate spheroidal [0], Prolate spheroidal [1], Spheroidal [2]	-	-	-	-	-	-	-	-	-	0.44	0.17	0.08
20	**Tectum continuity:** Discontinuous [0], Foveolate [1], Continuous [2]	-	-	-	-	-	-	-	-	-	0.38	0.29	0.11
21	**Muri orientation:** Unoriented [0], Reticulate [1], Rugulate [2]	-	-	-	-	-	-	-	-	-	0.33	0.14	0.05
22	**Tectum sculpturing:** Echinate [0], Verrucate [1], Smooth [2]	-	-	-	-	-	-	-	-	-	0.50	0.33	0.17
23	**Apertural type:** Colpus [0], Pore [1]	-	-	-	-	-	-	-	-	-	**1.00**	**1.00**	**1.00**
24	**Number apertures:** 3 [0],2[1],6 [2],9 [3],12 [4]	-	-	-	-	-	-	-	-	-	0.87	0.00	0.00

(Fig. S19). The interapertural endexine fragmentation in islets develops during the evolution of the ancestor of *Ichtyoselmis* and the rest of Fumarieae (Fig. S14), whilst a very fragmented interapertural endexine appears in the Crown Fumarieae ancestor (Fig. S13). A reduction in the tectum perforations with an increase of the presence of narrow perforations is present for the ancestor of the subtribe Fumariinae (Fig. S4). The reduction in the infratectum ectexine ratio and the presence of a fibrillar aperture membrane is acquired by the common ancestor of the *Fumaria* and *Sarcocapnos* Clades (Figs. S6, S18). The ancestor of *Ceratocapnos* and *Sarcocapnos* genera shows a great deal of synapomorphies: a thin ratio of tectum/ectexine, a thick ratio of infratectum and a thick or very thick ratio of foot layer; the infratectum in dendroid granules, the interapertural endexine inappreciable, a very thick proportion of intine layer and an absence of fluffy material (Figs. S3, S6, S8, S5, S11, S15). These taxa present a reversal toward a baculate aperture membrane (Fig. S18). The *Fumaria* Clade also presents a set of synapomorphies such as a very thick tectum/ectexine ratio, a very thin infratectum/ectexine ratio, a very thin ratio of foot layer, an infratectum with few granules and the presence of high or very high levels of fluffy material (Figs. S3, S6, S8, S5, S19).

From an ontogenetic point of view, some of the characters are invariable among the four development stages. This is the case of the infratectum setup, the ratio of foot layer/endexine and the presence of interapertural endexine (Figs. S5, S8, S11). The character perforation of the tectum is also invariable but the presence of a mixed state in some taxa is observed (Fig. S4), as well as the aperture membrane that is mainly homogeneous with the exception of a multistate between fibrillar and baculate in the *Cysticapnos* genus (Fig. S18). The rest of studied characters present variations among the development stages. The ratio of tectum/ectexine is medium in the ancestor of Fumarioidae during the microspore and young bicellular stages and becomes thick from medium bicellular stage. The representatives of the *Fumaria* Clade present a thickening in this proportion from the young bicellular stage (Fig. S3). The character ratio of infratectum/ectexine has the internal nodes of the subtribe Fumariinae resolved as thin in the bicellular stages but as very thin at the mature pollen stage, the genus *Ceratocapnos* reaches its thickest infratectum at the medium bicellular stage, whilst *Sarcocapnos* does so at the mature stage (Fig. S6). A gray material fills the infratectum in the ancestor of Fumarioideae during the microspore and medium bicellular stages but changes to electrodense at the young bicellular stage and becomes empty in mature pollen (Fig. S7). The morphology of the foot layer is continuous in the majority of the lineages of Fumarieae during the microspore stage but discontinuous in the ancestor of Fumarioideae and in most lineages during all the stages (Fig. S9). The inner face character of the foot layer is mainly homogeneous during development, but the ancestral stage for the subfamily and outgroups becomes regular during the bicellular stages with a reversal to irregular in the mature pollen (Fig. S10). The ratio of endexine/ectexine becomes thinner from the microspore stage (Fig. S12) whilst the fragmentation of this layer and the formation of islets increase with maturity (Fig. S13, S14). The ratio of intine/exine is thin, medium and thick for all the nodes of Fumarioideae at the young bicellular, medium bicellular and mature pollen stages, respectively (Fig. S15). The apertures

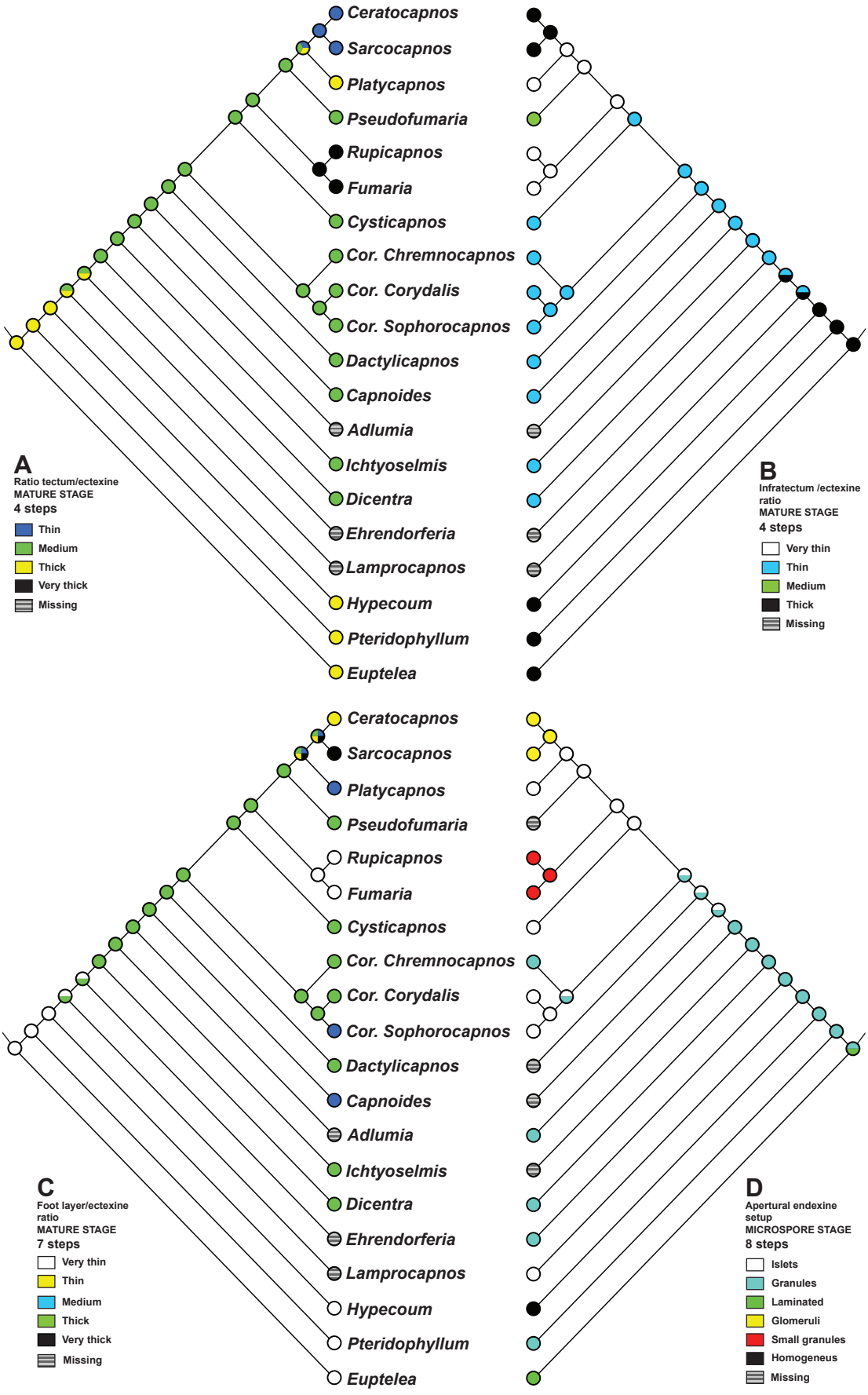


Fig 2. Optimization of some ultrastructural features on the phylogeny of Fumarioideae: A, ratio of tectum/ectexine at the mature pollen stage; B, ratio of infratectum/ectexine at the mature pollen stage; C, ratio of foot layer/ectexine at the mature pollen stage; D, endexine setup of apertural endexine during the microspore stage.

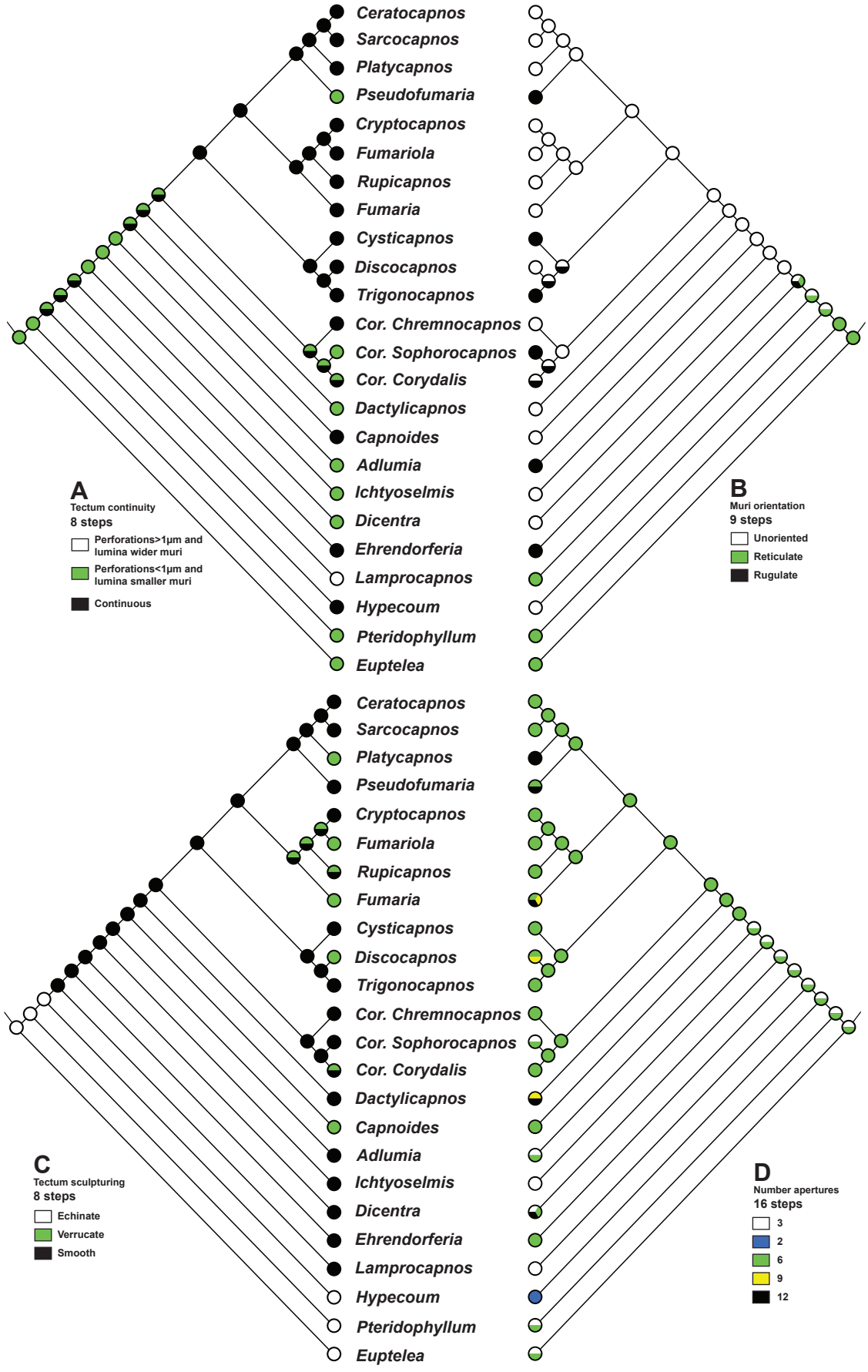


Fig 3. Optimization of some pollen morphology features on the phylogeny of Fumarioideae: A, tectum continuity; B, muri orientation; C, tectum sculpturing; D, number of apertures.

have an endexinous oncus during the microspore stage and this is replaced by intinous oncus at the bicellular stages, but during the mature stage the oncus is rarely observed (Fig. S16). The apertural endexine setup in granules is ancestral for Fumarioideae at the microspore stage and changes to islets in the subtribe Fumariinae while the states mixed and glomeruli are synapomorphies of the *Fumaria* Clade and the group *Ceratocapnos*-*Sarcocapnos* respectively. From the young bicellular stage an aperture endexine in islets is ancestral for the subfamily and all its lineages, contrary to the *Euptelea* genus that presents a laminated aperture endexine (Fig. S17). The presence of fluffy material in the apertures shows its highest levels during the young bicellular stage for the majority of the Fumarieae lineages, with the exception of the *Fumaria* Clade representatives which always present high amounts of this material (Fig. S19).

Evolution of pollen morphology features

With regard to the morphological features of pollen grains, a medium pollen size is ancestral in the subfamily, with changes to small in the *Cysticapnos* and *Fumaria* Clades and very big in the genera *Platycapnos* and *Pseudofumaria* Medik. (Fig. S20A). The pollen grain shape is not resolved for the subfamily ancestor, but the prolate spheroidal is the most frequent in the tribe Fumarieae and is the ancestral state for this group (Fig. S20B). Changes to spheroidal or oblate spheroidal are found in different lineages of the subfamily. The continuity of the tectum is not resolved for the ancestor of Fumarioideae but perforate (mainly holes smaller than 1 µm) is the most usual state in the basal taxa of Fumarieae and outgroups (Fig. 3A). A continuous tectum is acquired in the ancestor of the subtribe Fumariinae. The orientation of the tectum ornamentation is unresolved for Fumarioideae ancestor, but most of the internal nodes in Fumarieae are unoriented (Fig. 3B). The presence of rugulate orientation has appeared in independent occasions during the evolution of Fumarieae lineages. An echinate tectum sculpture is proposed as ancestral for the subfamily, whilst a smooth tectum is ancestral in the tribe Fumarieae (Fig. 3C). The presence of verruca has occurred on different occasions among the Fumarieae groups. The aperture pore type is a synapomorphy for the *Fumaria* Clade (Fig. S20C). The ancestral state for the number of apertures in the subfamily is three or six, and an increase in this character occurs in several independent lineages (Fig. 3D). In basal representatives of the tribe Fumarieae the presence of three apertures is usual. The tribe Hypecoeae presents only two apertures fused in an equatorial position.

6.5 Discussion

In this study we combine the information from pollen morphology and ultrastructure of Fumarioideae (Kalis, 1979; Dahl, 1990; Blackmore et al. 1995; Romero et al. 2003; Pérez-Gutiérrez et al, unpubl. data b) with the current phylogeny for the group (Pérez-Gutiérrez et al. unpubl. data a) enabling us to assess the evolutionary trends of pollen features in the subfamily and the phylogenetic information that they contains. The results present a large comparative survey of sculpture and structure of Fumarioideae mature pollen grain and of the ultrastructural

characteristics during pollen wall (including the apertural region) development from the microspore stage onwards. To the best of our knowledge, this is the first study that analyzes the evolution of pollen wall ultrastructure data together with the ontogeny process.

The ultrastructural evolution of the Fumarioideae pollen wall layer

For many of the ultrastructural features analyzed, a low level of consistency and phylogenetic signal has been found (Table 1a). Some of these low-informative characters are related to well defined and conserved pollen processes in the angiosperm, such as the infratectum content, the intine growth and the apertural oncus presence (Blackmore et al. 2007; Hesse et al. 2009; Kuang et al. 2012). But others are labile features that change quickly among taxa and stages (foot layer morphology and the inner foot layer).

In spite of the high levels of homoplasy found in many of the ultrastructural characters, some evolutionary trends have been observed, reflecting the existence of different pollen models in some groups of Fumarioideae. This is the case of the evolution in the thickness of the ectexine layers, which presents different proportions in the tribe Hypecoeae and the tribe Fumarieae, and within Fumarieae, a distinct proportion of the ectexine layers is found for the representatives of the *Fumaria* Clade and for the *Ceratocapnos* and *Sarcocapnos* genera.

In the light of the proportion of the ectexine layers throughout the phylogeny of Fumarioideae, we propose the existence of four models of ectexine development: a) the columellate model, with a bigger infratectum and a very thin foot layer, present in the tribe Hypecoeae and outgroups; b) the granulate model, with a medium tectum and foot layer but a thin infratectum, which is found in most of the Fumarieae genera; c) the *Fumaria* model, with a big tectum and very small infratectum and foot layer, identified in *Fumaria* and *Rupicapnos* genera; and d) the *Sarcocapnos* model, with a very thin tectum, a medium or big infratectum and a thick foot layer, observed in the genera *Ceratocapnos* and *Sarcocapnos* (Fig. 4). If we focus on the infratectum organization of each of the models, it can be stated that this feature is the trait that most influences the final ultrastructure of the ectexine, given that columellate infratectum contains a wide infratectum size with the columellae well developed (columellate model, Fig. 4A), the granulate type presents a thin or medium infratectum with granules (granulate model, Fig. 4B), the ‘few granules’ type (defined in Pérez-Gutiérrez et al., unpubl. data b, as a thin infratectum with few or no granules) has a very thin infratectum (*Fumaria* model, Fig. 4C), and the particular granulate infratectum ‘dendroid granules’ type (defined in Pérez-Gutiérrez et al., unpubl. data b, as a infratectum with branched and irregular granules) contains a medium or thick infratectum (*Sarcocapnos* model, Fig. 4D). Traditionally, when the evolution of pollen exine structure is discussed in the literature only the infratectum ultrastructure is mentioned (Doyle, 2005; Doyle 2009), without giving importance to the tectum or the foot layer. In this study we have observed that all the ectexine layers are correlated, as when one of them is very developed, the other one is reduced (Figs. S3, S6, S8). We have proved that considering all the ectexine layers allows us to better explain the evolutionary trends in the pollen wall of Fumarioideae. For this reason we

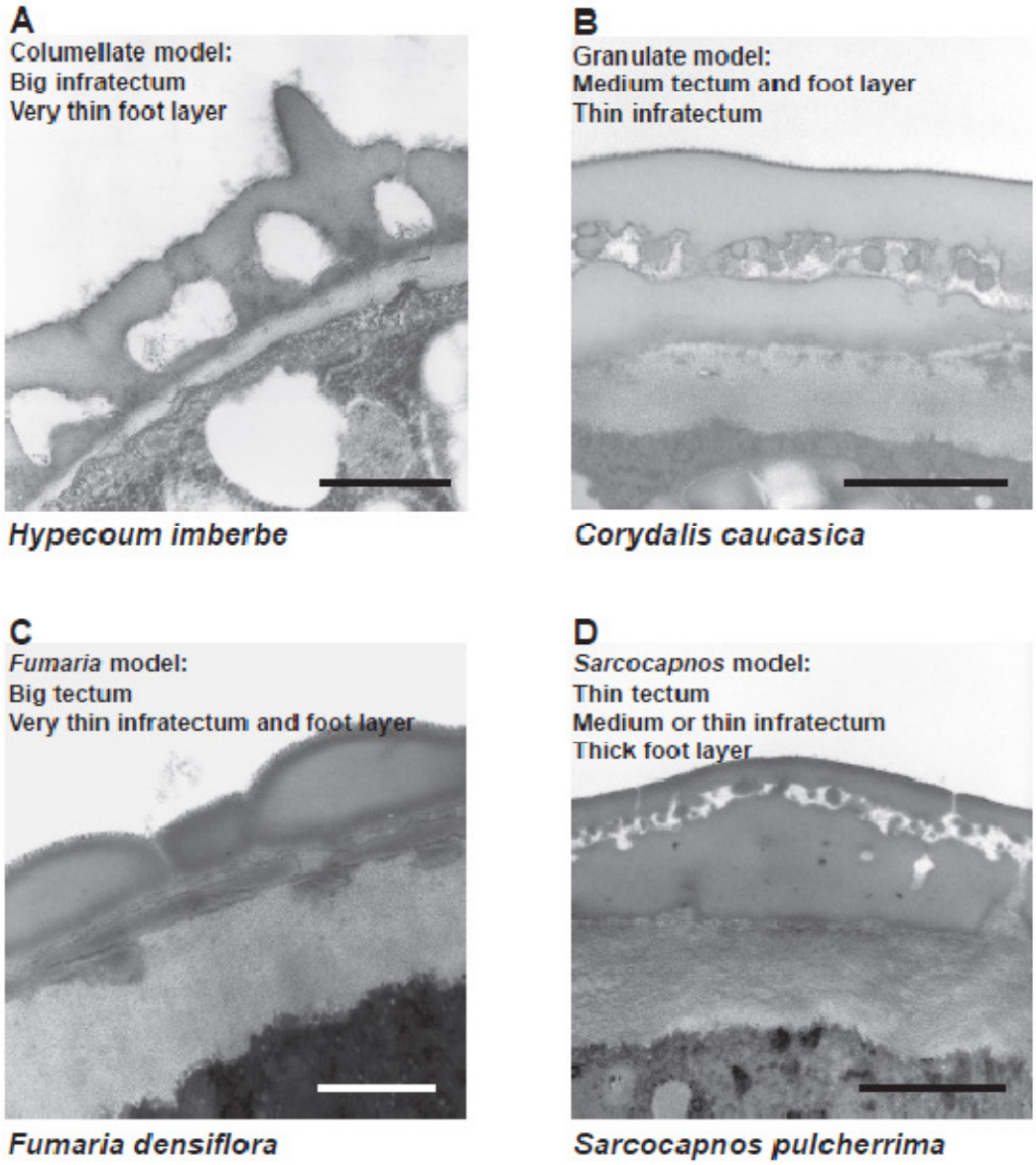


Figure 4. Images of examples for the ectexine model identified in Fumarioideae representatives: A, collumelate model; B, granulate model; C, Fumaria model; and D, Sarcocapnos model. All scale bars= 1µm.

consider that the study of the ectexine as a whole could improve the understanding of the evolution of the pollen wall structure.

The ancestral pollen model existing in Fumarioideae is columellate and, for the tribe Fumarieae, a change took place toward a middle size of tectum and foot layer as well as a thin infratectum: the granulate model. That the columellate model is ancestral in the subfamily is an interesting fact since the columellate infratectum is the most extended in eudicots (Doyle, 2009), but in the tribe Fumarieae the infratectum shape has evolved in a different way to the majority of the eudicots. At the same time, the tribe reaches quite different models of ectexine morphology, given that during its evolution two independent new changes occurred. One was a thickening in

the tectum accompanied of a reduction of the infratectum and foot layer size: the *Fumaria* model. The other occurred in the *Ceratocapnos-Sarcocapnos* lineage, for which a thickening of the foot layer and infratectum, as well a strong thinning in the tectum layer took place (*Sarcocapnos* model). Differences in the morphology of the ectexine layers are observed in members of the same subtribe in the tribe Naucleae (Kuang et al. 2008), but keeping the same infratectum shape. At family level, differences among ectexine layers and changes between granulate and columellate infratectum are present in the pollen ultrastructure of Annonaceae, Magnoliaceae and Myristicaceae (Sauquet and Le Thomas, 2003; Xu and Kirchoff 2008; Doyle and Le Thomas 2012; Xu and Ronse de Craene 2013). Our study could represent the basis for developing future research related to ectexine evolution in the angiosperm pollen wall, especially in eudicots.

The ultrastructural evolution of the Fumarioideae aperture

The ultrastructural features of apertures used in our study have also shown low levels of phylogenetic signal for the subfamily Fumarioideae, since most characters showed low values for the tree-fit statistics. Despite this, the apertural endexine setup and the aperture membrane contain high and medium levels of apparent-synapomorphy retention in the earlier stages (Table 1a). Especially noteworthy is the deposition of the endexine layer character during the microspore stage. In this stage there are different states in Fumarioideae that characterize certain groups (Fig. 2D) however, during the rest of the development stages the apertural endexine in Fumarioideae is homogeneized among all its representatives with the islets morphology in most of them (also in *Pteridophyllum*). In a comparative study of extant and fossil *Platanus* L. representatives, Denk and Tekleva (2006) use features of the apertural endexine - comparing the size between the apertural and interapertural endexine, and considering the endexine fragmentation in the apertural regions- to recognize two different groups in their studied material. In Fumarioideae, in spite of the differences observed at the beginning of the endexine deposition process, we have been unable to establish different groups within due to the homogenization of this layer during the pollen maturation. However, apertural endexine distinguishes Fumarioideae (also *Pteridophyllum*) from the outgroup *Euptelea*, with a laminated apertural endexine. The existence of different ways of endexine organization during the microspore stage and its change during pollen development is a little studied issue. Recently, Gabarayeva et al. (2013) observed a clearly different arrangement of the apertural endexine as far as the interapertural one in *Passiflora racemosa* Brot. is concerned, which changes during the different pollen development stages. More comparative studies are needed to increase the knowledge of type and evolution of the endexine deposition process in angiosperms.

The ancestral state for the aperture membrane in Fumarioideae is echinate, whilst a synapomorphy for the tribe Fumarieae is baculate (Fig. S18). For the representatives of the *Fumaria* Clade, and for the *Platycapnos* and *Pseudofumaria* genera (*Sarcocapnos* Clade), the aperture membrane (usually composed of tectum fragments) is absent, being replaced by a fibrillar membrane (Pérez-Gutiérrez et al. unpubl. data b). This replacement occurred in the

ancestor of the *Fumaria* and *Sarcocapnos* Clades and reversed in the *Ceratocapnos-Sarcocapnos* ancestor (where the baculate apertural membrane appears and the fibrillar one is lost; Fig. S18). The lack of congruence between this character and the phylogeny of Fumarieae agrees with those found for other non-pollen characters by Pérez-Gutiérrez et al. (2012). *Platycapnos* and *Pseudofumaria* shows a set of non-fixed characters (shared with species of the *Fumaria* Clade) which are explained by an incomplete lineage sorting as a consequence of a rapid and simultaneous radiation of the *Sarcocapnos* Clade (1,7 Mya after the split of the most recent common ancestor with *Fumaria* Clade; Pérez-Gutiérrez et al. unpubl. data a). Here, we have observed that the aperture membrane morphology and the infratectum ectexine ratio are other examples of this phenomenon. Regarding the occurrence of the fibrillar aperture membrane for some Fumarieae members, the presence of both a fibrillar and a baculate aperture membrane at the microspore stage of *Cysticapnos* genus (a mixed structure that disappears in the rest of development stages) suggests that it develops during the evolution of the Fumariinae ancestor (Fig. S18).

A very characteristic element of Fumarioideae apertures is the fluffy material that covers the apertural region. This fibrillar structure, defined firstly in the apertures of *Fumaria densiflora* (Romero and Fernández 2000), has been found in most of the representatives of the tribe Fumarieae, and it represents an acquisition in the first lineages of the tribe. The lack of this material in the lineage of *Ceratocapnos-Sarcocapnos* is explained by a loss during evolution. The amount of this fibrillar material varies greatly among genera, but its level is high in all the pore type representatives. So far, little is known about the nature of fluffy material and its function, only that it has a lamellar structure and that it is exclusive to the tribe Fumarieae (Romero and Fernández 2000; Pérez-Gutiérrez et al. unpubl. data b).

The evolution of structure and sculpture in Fumarioideae

The tree-fit statistics related to pollen morphology features show that the majority are highly homoplasic characters, with the exception of the apertural type (Table 1 b). The evolution of these features shows that there is a high diversity with a big deal of reversals and convergences. Similar patterns of exine evolution with an abundance of reversals and convergences have been identified in other plant groups (subtribe Nepetinae, Moon et al. 2008; Annonaceae, Doyle and Le Thomas 2012; Aquifoliales, Schori and Furness 2014). We have identified an increase in the aperture number from three and six in the ancestor of the subfamily to nine, twelve or more in independent lineages of the tribe Fumarieae (Fig. 3D). This increase of the aperture number in Fumarioideae is a common trend in pollen evolution of angiosperms given that it may facilitate the germination process and harmomegathy mechanism (Furness and Rudall 2004). We have observed that the higher pollen grain sizes correspond to the species with sinpolipantocolpate pollen (*Platycapnos* and *Pseudofumaria*, with twelve fused colpi) and for this reason we consider this kind of aperture system is related to a large pollen size. In other studies with species of sincolpate pollen (Lippi and Rossi 1999; Wang et al. 2003)

there was no relation between a large pollen size and fused apertures, but in these cases, the number of apertures was not so high.

The ancestral tectum in Fumarioideae is discontinuous and a trend towards continuous tectum occurs in the ancestor of the Crown Fumarieae. This discontinuous exine is congruent with the semitectate-reticulate pollen morphology that was proposed as basal in eudicots by Donoghue and Doyle (1989). The reduction of holes and perforations during Fumarioideae pollen evolution could be a consequence of the adaptation of most of the Crown Fumarieae lineages to open, arid habitats and to the exposure of pollen to hostile environments. In a recent paper on *Lillium* Hill pollen evolution in China (Du et al. 2014), the authors recognized the importance of adaptation to environmental constraints for pollen evolution, as well as referring to the value of the pollination system. Although they cannot relate any pollen ornamentation to a specific environment, they show the existence of connections between pollen size and shape with high mountain habit and annual precipitation. In our group, most of the basal Fumarieae taxa are present in forest-floor and humid conditions, whilst most of the Crown Fumarieae lineages are adapted to semi-desert and Mediterranean type environments from central Asia, the Mediterranean basin and South Africa (Pérez-Gutiérrez et al. in press a). In spite of the fact that we cannot relate a pollen feature to a particular environmental condition, the high diversity of pollen features in Fumarioideae leads us to believe it is an ideal group for developing studies to test the effect of habit adaptation and pollination system in pollen wall morphology evolution.

One of the mechanisms that mainly determine the effectiveness of the pollen grain is harmomegathy (Payne 1972; Hesse et al. 2009; Katifori et al. 2010), a process that allows the pollen grain to adapt to cycles of hydration and dehydration without damages. The presence of discontinuous exine lets the pollen grain adapt to these changes of volume in an easy way (Payne 1972; Blackmore and Barnes 1986), but when the pollen grain is exposed to very dry conditions the dehydration can be too great and affect the male gametophyte. The presence of smooth surfaces in pollen grains might decrease water loss during the harmomegathy process and for this reason could be the most widespread exine type in Fumarioideae. The role of rugulate and psilate surfaces in the harmomegathy process has been highlighted by Volkova et al. (2013), reviewing the knowledge of harmomegathy and analyzing the exine of some Boraginaceae representatives. In their study they show the importance of the apertures in harmomegathy and the additional flexibility provided by the elements from the surface and ultrastructure of the pollen wall. We have observed the preponderance of rugulate and verrucate ornamentation in Fumarioideae pollen as well as the presence of microperforations in the psilate surfaces. According to Volkova et al. (2013), the perforations in psilate exines provide flexibility to the wall at the same time that the presence of rugulate or verrucate morphologies supplies elasticity to the interapertural areas, pollen wall regions that also take part in the harmomegathy process. The contribution of these exine morphologies to this very important mechanism in pollen survival might explain the multiple independent acquisitions of rugulate and verrugate surfaces in Fumarioideae evolution.

6.6 Conclusions

In this study we combine the available information about the pollen wall and aperture system for the Fumarioideae subfamily with its phylogeny. Most of the features analyzed are labile and present a great deal of reversal and convergences, but we have identified synapomorphies for some characters at different levels of the systematics of Fumarioideae. We have defined the existence of four different ultrastructural pollen models in the subfamily Fumarioideae, based on information from the proportional size of the ectexine layers. The columellate model, observed in the tribe Hypecoeae, in *Pteridophyllum* and in *Euptelea*; the granulate model, widespread in most of the tribe Fumarieae; the *Fumaria* model, present in representatives from the *Fumaria* Clade; and the *Sarcocapnos* model, which is only present in the genera *Ceratocapnos* and *Sarcocapnos*. Each of these models is related to one type of infratectum organization: columellate, granulate, few granules and dendroid granules. The definition of these models considers the entire ectexine layer as a whole in order to describe the morphology of the most external layer of the pollen exine in a more accurate way and reflect the existence of evolutionary trends in their sizes. During the evolution of Fumarioideae lineages an increase in the number of apertures has also been reported, and in relation to the ornamentation of the pollen wall a trend in the reduction of perforations as well as the acquisition of rugulate and verrucate surfaces has been identified. This latter trend in exine morphology might be related to a better effectiveness of the pollen wall during the harmomegathy process as a consequence of the adaptation to open, arid habitats.

6.7 References

APG III Bremer B, Bremer K, Chase MW, Fay MF, Reveal JL, Soltis DE, Soltis PS, Stevens PF, et al. (2009) An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG III. Bot J Linn Soc 161:105-121

Blackmore S, Barnes SH (1986) Harmomegathic mechanisms in pollen grains. In: Blackmore S, Ferguson IK (eds) Pollen and spores: form and function. Academic Press, London, pp 137-149

Blackmore S, Stafford P, Persson V (1995) Palynology and systematics of Ranunculiflorae. Plant Syst Evol 9(Supp):71-82

Blackmore S, Wortley H, Skvarla JJ, Rowley JR (2007) Pollen wall development in flowering plants. New Phytol 174:483-498

Candau P (1987) Familia Papaveraceae y Fumariaceae. In: Valdés B, Díez MJ, Fernández I (eds) Atlas polínico de Andalucía occidental. Inst Desarr Reg Univ Sevilla & Diput Cádiz, Sevilla, pp 84-89

Candau P, Soler A (1981) Contribución a la palinología de la familia Fumariaceae en la Península Ibérica. Bot Macaron 8-9:147-161

Dahl ÅE (1989) Taxonomic and morphological studies in *Hypecoum* sect. *Hypecoum* (Papaveraceae). Plant Syst Evol 163:227-280

Dahl ÅE (1990) Infrageneric division of the genus *Hypecoum* (Papaveraceae). Nord J Bot 10:129-140

Damerval C, Nadot S (2007) Evolution of perianth and stamen characteristics with respect to floral symmetry in Ranunculales. Ann Bot 100:631-640

Damerval C, Le Guilloux M, Jager M, Charon C (2007) Diversity and Evolution of *CYCLOIDEA*-Like TCP Genes in Relation to Flower Development in Papaveraceae. Plant Physiol 143:759-772

Damerval C, Citerne H, Le Guilloux M, Domenichini S, Dutheil J, Ronse de Craene L, and Nadot S (2013) Asymmetric morphogenetic cues along the transverse plane: shift from disimmetry to zygomorphy in the flower of Fumarioideae. Amer J Bot 100:391-402

Denk T, Tekleva MV (2006) Comparative pollen morphology and ultrastructure of *Platanus*: Implications for phylogeny and evaluation of the fossil record. Grana 43:195-221

Donoghue MJ, Doyle JA (1989) Phylogenetic analysis of angiosperms and the relationships of Hammamelidae. In: Crane PR, Blackmore S (eds) Evolution, systematic, and fossil history of the Hammamelidae, vol. 1. Clarendon Press, Oxford pp 17-45

Doyle JA (2005) Early evolution of angiosperm pollen as inferred from molecular and morphological phylogenetic analysis. Grana 44:227-251

Doyle JA (2009) Evolutionary significance of granular exine structure in the light of phylogenetics analyses. Rev Paleobot Palynol 156:198-210

Doyle JA, Le Thomas A (2012) Evolution and phylogenetic significance of pollen in Annonaceae. Bot J Linn Soc 169:190-221

Du Y-P, Wei C, Wan Z-X, Li S, He H-B, Jia G-X (2014) *Lillium* spp. pollen in China (Liliaceae): Taxonomic and phylogenetic implications and pollen evolution related to environmental condition. PLoS ONE 9: e87841

Furness CA (2007) Why does some pollen lack apertures? A review of inaperturate pollen in eudicots. Bot J Linn Soc 155:29-48

Furness CA, Rudall PJ (2004) Pollen aperture evolution - a crucial factor for eudicot success? Trends Plant Sci 9:154-158

Gabarayeva NI, Grigorjeva VV, Kosenko Y (2013) II. Exine development in *Passiflora racemosa* Brot.: post-tetrad period. Overlooked aspects of development. Plant Syst Evol 299:1037-1055

Hesse M, Halbritter H, Zetter R, Weber M, Buchner R, Frosch-Radivo A, Ullrich S (2009) Pollen terminology. An illustrated handbook. Springer, New York

Hoot SB, Kadereit JW, Blattner FR, Jork KM, Schwarzbach AE, Crane PR (1997) Data congruence and phylogeny of the Papaveraceae s.l. based on four data sets: atpB and rbcL sequences, trnK restriction sites, and morphological characters. Syst Bot 22:575-590

Kalis AJ (1979) Papaveraceae. The northwest European pollen flora, 20. Rev Palaeobot Palynol 28:209-260

Katifori E, Alben S, Cerda E, Nelson DR, Dumais J (2010). Foldable structures and the natural design of pollen grain. Proc Nat Acad Sci 107:7635-7639

Kim S, Soltis DE, Soltis PS, Zanis MJ, Suh Y (2004) Phylogenetic relationship among early-diverging eudicots based on four genes: were the eudicots ancestrally woody? Mol Phyl Evol 31:16-30

Kluge AG, Farris JS (1969). Quantitative phyletics and the evolution of anurans. Syst Zool 40:315-328

Kölsch A, Gleissberg S (2006) Diversification of CYCLOIDEAlikeTCP genes in the Basal Eudicots Families Fumariaceae and Papaveraceae s.str. Plant Biol 8:680-687

Kuang YF, Kirchoff BK, Tang YJ, Liang YH, Liao JP (2008) Palynological characters and their systematic significance in Naucleeae (Cinchonoideae, Rubiaceae). Rev Paleobot Palynol 151:123-135

Kuang Y-F, Kirchoff BK, Liao JP (2012) Presence of the protruding oncus is affected by anther dehiscence and acetolysis technique. Grana 51:253-262

Layka S (1975) Les caractères de l'endexine chez les Papavéracées. Soc Bot Fr, Coll Palynol 122:103-107

Layka S (1976) Les méthodes modernes de la palynologie appliqués à l'étude des Papaverales. – Ph.D. diss., l'Université de Montpellier, CNRSAO 12.535

Lidén M (1986) Synopsis of Fumarioideae with a monograph of the tribe Fumarieae. Opera Bot 88 136pp

Lippi MM, Rossi W (1999) Pollen morphology of some Acanthaceae from West Africa. Plant Biosyst 133:73-82

Maddison WP, Maddison DR (2014) Mesquite: A modular system for evolutionary analysis. Version 3.0. http://mesquiteproject.org

Moon HK, Vinckier S, Smets E, Huysmans S (2008) Comparative pollen morphology and ultrastructure of Mentheae subtribe Nepetinae (Lamiaceae). Rev Paleobot Palynol 149:174-186

Payne WW (1972) Observations of harmomegathy in pollen of anthophyta. Grana 12:93–98

Pérez-Gutiérrez MA, Romero-García AT, Salinas MJ, Blanca G, Fernández MC, Suárez-Santiago VN (2012) Phylogeny of the tribe Fumarieae (Papaveraceae s.l.) based on chloroplast and nuclear DNA sequences: evolutionary and biographic implications. Amer J Bot 99:517-528

Praglowski J (1974) The pollen morphology of the Trochodendraceae, Tetracentraceae, Cercidiphyllaceae, and Eupteleaceae with reference to taxonomy. Pollen Spores 16:449-467

Punt W, Hoen PP, Blackmore S, Nilsson S, Le Thomas A (2007) Glossary of pollen and spore terminology. Rev Palaeobot Palynol 143:1-81

Rachele LD (1974) Pollen morphology of the Papaveraceae of the Northeastern United States and Canada. Bull Torrey Bot Club 101:152-159

Romero AT, Fernández MC (2000) Development of exine and apertures in *Fumaria densiflora* DC from the tetrad stage to maturity. In: Harley MM, Morton CM, Blackmore S, (eds) Pollen and Spores: Morphology and Biology. Royal Botanic Gardens, Kew, pp 45-56

Romero AT, Salinas MJ, Fernández MC (2003) Pollen wall development in *Hypecoum imberbe* (Fumariaceae). Grana 42:91-101

Salinas MJ, Romero AT, Blanca G, De la Herrán R, Garrido-Ramos M, Ruiz-Rejón C, Morales C, Ruiz-Rejón M, Suárez V. (2003) Contribution to the taxonomy and phylogeny of *Sarcocapno*s (DC). (Fumariaceae). Plant Syst Evol 237:153-164

Sauquet H, Le Thomas A (2003). Pollen diversity and evolution in Myristicaceae. Int J Plant Sci 164:613-628

Schori M, Furness CA (2014). Pollen diversity in Aquifoliales. Bot J Linn Soc 175:169-190

Soltis DE, Soltis PS, Albert VA, Oppenheimer DG, dePamphilis CW, Ma H, Frohlich MW, Theiβen G (2002). Missing links: the genetic architecture of flower and floral diversification. Trends Plant Sci 7:22-30

Stern KR (1962) The use of pollen morphology in the taxonomy of *Dicentra*. Amer J Bot 55:626-628

Stevens PF (2001 onwards) Angiosperm Phylogeny Website. Version 12, July 2012 [and more or less continuously updated since]. http://www.mobot.org/MOBOT/research/APweb/

Swofford DL (1993) PAUP: phylogenetic analysis using parsimony, version 3.1.1. Illinois Natural History Survey, Champaign, Illinois, USA

Volkova OA, Severova EE, Polevova SV (2013) Structural basis of harmomegathy: evidence from Boraginaceae pollen. Plant Syst Evol 299:1769-1779

Wang H, Mill RR, Blackmore S (2003) Pollen morphology and infrageneric evolutionary relationships in some Chinese species of *Pedicularis* (Scrophylariaceae). Plant Syst Evol 237:1-17

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. Persp Plant Ecol Evol Syst 11:81-110

Xu F-X, Kirchoff BK (2008) Pollen morphology and ultrastructure of selected species of Magnoliaceae. Rev Paleobot Palynol 150:140-153

Xu FX, Ronse de Craene LP (2013) Pollen morphology and ultrastructure of selected species of Annonaecae. Plant Syst Evol 299:11-24

Zhang M-L, Su Z-Y, Lidén M, Grey-Wilson C (2008) Papaveraceae. In: Wu, Z.-Y., Raven, P.H., & Hong, D.-Y. (eds) Flora of China, Vol. 7 (Menispermaceae through Capparaceae). Science Press, Beijing & Missouri Botanical Garden Press, St. Louis, USA.

Supplementary material, M.A. Pérez-Gutiérrez, A.T. Romero-García, M. Carmen Fernández, M.J. Salinas-Bonillo, V.N. Suárez-Santiago. "Evolutionary trends in the pollen grain of the subfamily Fumarioideae: evidence from morphology and ontogenetic characters of the pollen wall and apertures".

Table S1. State of characters table of the ultrastructural features analyzed in Fumarioideae pollen wall ontogeny (stages: microspore/young bicellular/medium bicellular/mature stages)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Adlumia	2/2/?/?	1/1/?/?	1/1/?/?	1/1/?/?	1/2/?/?	2/2/?/?	0/1/?/?	1/1/?/?	1/1/?/?	0/0/?/?	0/1/?/?	?/1/?/?	-/1/?/?	1/1/?/?	1/0/?/?	0/0/?/?	2/1/?/?
Capnoides	?/2/2/2	?/1/1/1	?/1/1/1	?/1/1/1	?/2/2/0	?/2/2/1	?/1/0/0	?/1/1/1	?/1/1/1	?/1/0/0	?/1/2/2	?/0/1/1	-/1/2/3	?/1/0/0	?/0/0/0	?/0/0/0	?/1/1/1
Ceratocapnos	1/1/1/1	3/3/3/3	3/3/3/3	2/2/3/3	1/1/1/0	3/3/3/3	1/1/1/1	2/2/2/2	0/0/0/0	0/?/?/?	?/?/?/?	?/?/?/?	-/1/1/4	1/1/1/0	3/0/0/0	0/0/0/0	0/0/0/0
Cor. Corydalis	2/2/2/2	1/1/1/1	1/1/1/1	1/1/1/1	1/1/2/0	2/2/2/2	0/1/1/1	1/1/1/1	1/1/1/1	0/1/0/0	0/1/2/2	?/1/1/1	-/1/2/3	1/1/0/0	0/0/0/0	0/0/0/0	1/1/2/1
Cor. Chremnocapnos	2/?/2/2	1/?/0/1	1/?/1/1	1/?/1/1	1/?/2/2	2/?/2/2	0/?/1/1	1/?/1/1	1/?/1/1	0/?/0/0	0/?/1/2	?/?/1/1	-/?/2/3	1/?/0/0	1/?/0/0	0/?/0/0	1/?/1/1
Cor. Sophorocapnos	2/2/2/2	1/1/1/1	1/1/1/1	1/1/1/1	1/1/2/0	2/2/1/1	1/1/0/1	1/1/1/1	1/1/1/1	0/1/0/0	0/1/1/2	?/0/1/1	-/1/2/3	1/1/0/1	0/0/0/0	0/0/0/0	1/1/1/1
Cysticapnos	3/2/2/2	0/0/0/0	1/1/1/1	1/1/1/1	1/1/2/0	2/2/2/2	0/0/0/0	1/1/1/1	1/1/1/1	1/1/0/0	1/1/1/1	2/2/0/1	-/1/2/4	1/1/1/0	0/0/0/0	0,1/0/0/0	1/1/1/2
Dactylicapnos	?/2/2/2	?/1/0/1	?/1/1/1	?/1/1/1	?/1/1/2	?/2/2/2	?/0/0/1	?/0/1/1	?/1/1/1	?/1/0/0	?/1/1/2	?/0/0/1	-/1/2/4	?/0/1/0	?/0/0/0	?/0/0/0	?/3/2/2
Dicentra	2/2/2/2	1/1/1/1	1/1/1/1	1/1/1/1	1/1/2/0	2/2/2/2	0/0/1/1	1/1/1/1	1/1/1/1	2/1/0/0	1/1/1/1	0/0/2/2	-/1/2/3	1/1/1/1	1/0/0/0	0/0/0/0	1/2/1/1
Ehrendorferia	2/3/?/?	1/4/?/?	2/2/?/?	1/1/?/?	2/1/?/?	2/2/?/?	1/1/?/?	1/2/?/?	1/1/?/?	2/1/?/?	1/0/?/?	0/?/?/?	-/0/?/?	1/1/?/?	1/0/?/?	0/0/?/?	0/2/?/?
Euptelea	2/2/3/3	2/2/2/2	4/4/4/4	3/3/3/3	0/0/0/0	0/0/0/0	1/0/1/1	1/0/0/1	1/1/1/1	0/0/0/0	0/0/0/0	?/?/?/?	-/1/2/3	1/1/1/0	2/2/2/2	2/2/2/2	0/0/0/0
Fumaria	3/4/4/4	3/3/3/3	0/0/0/0	0/0/0/0	1/1/2/0	1/1/0/0	0/1/1/1	1/1/1/2	1/1/1/1	1/0/0/0	1/1/2/2	1/0/2/1	-/1/2/4	1/0/1/1	4/0/0/0	1/1/1/1	3/4/4/4
Hypecoum	2/2/3/3	3/1/1/1	4/4/4/4	3/3/3/3	0/1/0/0	0/0/0/0	1/1/1/1	0/0/0/1	1/1/1/1	0/1/0/0	0/0/0/1	?/?/?/0	-/0/2/3	1/1/0/0	5/0/0/0	2/2/2/2	0/0/0/0
Ichtyoselmis	?/?/2/2	?/?/1/1	?/?/1/1	?/?/1/1	?/?/1/2	?/?/2/2	?/?/1/1	?/?/2/2	?/?/1/1	?/?/0/0	?/?/1/1	?/?/0/1	-/?/2/3	?/?/1/0	?/?/0/0	?/?/0/0	?/?/2/1
Lamprocapnos	2/2/2/?	2/2/2/?	2/2/2/?	1/2/2/?	2/2/2/?	2/2/3/?	0/0/1/?	1/1/1/?	1/1/1/?	2/0/0/?	1/1/1/?	1/0/0/?	-/1/2/?	1/0/0/?	0/0/0/?	0/0/0/?	0/0/0/?
Platycapnos	2/2/3/3	3/3/4/4	1/1/1/1	0/0/0/0	1/2/2/0	2/2/1/1	0/0/0/0	1/1/1/1	1/1/1/1	0/1/0/0	0/1/1/2	?/0/1/1	-/1/2/3	1/1/1/1	0/0/0/0	1/1/1/1	1/2/2/3
Pseudofumaria	?/2/2/2	?/3/3/4	?/1/1/1	?/1/1/2	?/1/2/2	?/2/2/2	?/0/1/1	?/1/1/1	?/1/1/1	?/1/0/0	?/1/2/2	?/0/1/1	-/1/2/3	?/1/1/1	?/0/0/0	?/1/1/1	?/2/2/2
Pteridophyllum	4/3/3/3	1/1/1/1	4/4/4/4	2/3/3/3	2/1/2/2	1/1/1/0	1/1/1/1	1/2/2/1	1/1/1/1	0/1/0/0	1/1/1/1	1/1/2/2	-/1/1/2	1/1/1/0	1/0/0/0	2/2/2/2	0/0/0/0
Rupicapnos	3/?/4/4	3/?/3/3	0/?/0/0	0/?/0/0	1/?/2/0	1/?/1/0	1/?/1/1	1/?/1/2	1/?/1/1	1/?/0/0	1/?/2/2	0/?/1/1	-/?/2/3	1/?/0/0	4/?/0/0	0/?/1/1	3/?/4/3
Sarcocapnos	0/1/1/1	3/3/3/3	3/3/3/3	1/1/1/3	1/1/2/0	3/3/3/4	1/1/1/1	2/2/2/2	0/0/0/0	0/?/?/?	?/?/?/?	?/?/?/?	-/1/2/4	1/0/0/0	3/0/0/0	0/0/0/0	0/0/0/0

1 Ratio tectum/ectexine: Very thin [0], Thin [1], Medium [2], Thick [3], Very thick [4]; **2 Tectum perforations:** Absence [0], Perforations [1], Wider perforations [2], Narrow perforations [3], Mixed [4]; **3 Infratectum setup:** Few granules [0], Granules [1], Intermediate [2], Dendroids [3], Columellate [4]; **4 Ratio infratectum/ectexine:** Very thin [0], Thin [1], Medium [2], Thick [3]; **5 Infratectum content:** Empty [0], Electrodense material [1], Gray material [2]; **6 Ratio foot layer/ectexine:** Very thin [0], Thin [1], Medium [2], Thick [3], Very thick [4]; **7 Foot layer morphology:** Continuous [0], Discontinuous [1]; **8 Inner foot layer:** Regular [0], Irregular [1], Very irregular [2]; **9 Presence of endexine:** Inappreciable [0], Presence [1]; **10 Ratio endexine/ectexine:** Very thin [0], Thin [1], Medium [2]; **11 Fragmentation of endexine:** Unfragmented [0], Slightly fragmented [1], Very fragmented [2]; **12 Shape endexine fragmentation:** Almost continuous layer [0], Islets [1], Mixed [2]; **13 Ratio intine/exine:** Very thin [0], Thin [1], Medium[2]; **14 Apertural oncus:** Absence [0], Presence [1]; **15 Apertural endexine setup:** Islets [0], Granules [1], Layered [2], Glomeruli [3], Small granules [4], Homogeneus [5]; **16 Aperture membrane:** Baculate [0], Fibrillar [1], Echinate[2]; **17 Fluffy material:** Absence [0], Low [1], Middle [2], High [3], Very high [4].

Table S2. State of characters table for pollen morphological features (structure and sculpture) of Fumarioideae

	21	22	23	24	25	26	27
Adlumia	1	1	1	2	2	0	0,2
Capnoides	1	1	2	0	1	0	2
Ceratocapnos	1	1	2	0	2	0	2
Cor. Chremnocapnos	2	1	1,2	0,2	1,2	0	2
Cor. Corydalis	2	1	2	0	2	0	2
Cor. Sophorocapnos	2	1	1	2	2	0	0,2
Cryptocapnos	0	0	2	0	2	1	2
Cysticapnos	0	2	2	2	2	0	2
Dactylicapnos	1	1	1	0	2	0	3,4
Dicentra	1,2	0,1,2	1	0	2	0	0,2,4
Discocapnos	0	1	2	0	1	0	2,3
Ehrendorferia	1	1	2	2	2	0	2
Euptelea	2	2	1	1	0	0	0,2
Fumaria	1	1	2	0	1	1	2,3,4
Fumariola	0	0	2	0	1	1	2
Hypecoum	0	2	2	0	0	0	1
Ichtyoselmis	2	1	1	0	2	0	0
Lamprocapnos	1	1	0	1	2	0	0
Platycapnos	3	0	2	0	1	0	4
Pseudofumaria	3	1	1	2	2	0	2,4
Pteridophyllum	1	1	1	1	0	0	0,2
Rupicapnos	0	1	2	0	1,2	1	2
Sarcocapnos	2	2	2	0	2	0	2
Trigonocapnos	0	2	2	2	2	0	2

21 Pollen grain size: Small [0], Medium [1], Big [2], Very big [3]; **22 Pollen grain shape:** Oblate spheroidal [0], Prolate spheroidal [1], Spheroidal [2]; **23 Tectum continuity:** Perforations >1µm and lumina > muri [0], Perforations <1 µm and lumina < muri [1], Continuous [2]; **24 Muri orientation:** Unoriented [0], Reticulate [1], Rugulate [2]; **25 Tectum sculpturing:** Echinate [0], Verrucate [1], Smooth [2]; **26 Apertural type:** Colpus [0], Pore [1]; **27 Number apertures:** 3 [0], 2 [1], 6 [2], 9 [3], 12 [4].

Fig. S3. Ratio tectum/ectexine optimization on the Fumarioideae topology in the four different ontogenetic stages

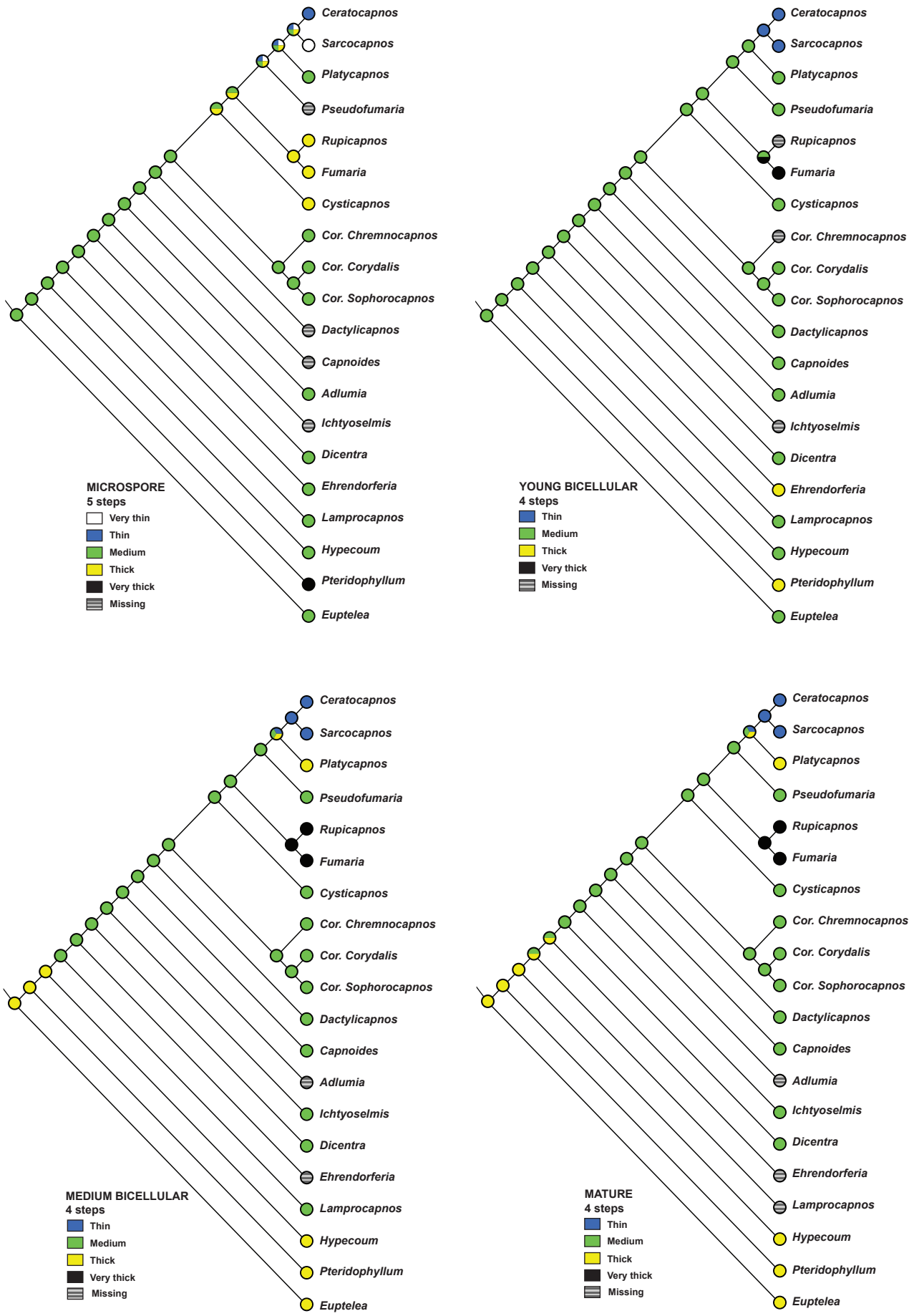


Fig. S4. Tectum perforations optimization on the Fumarioideae topology in the four different ontogenetic stages

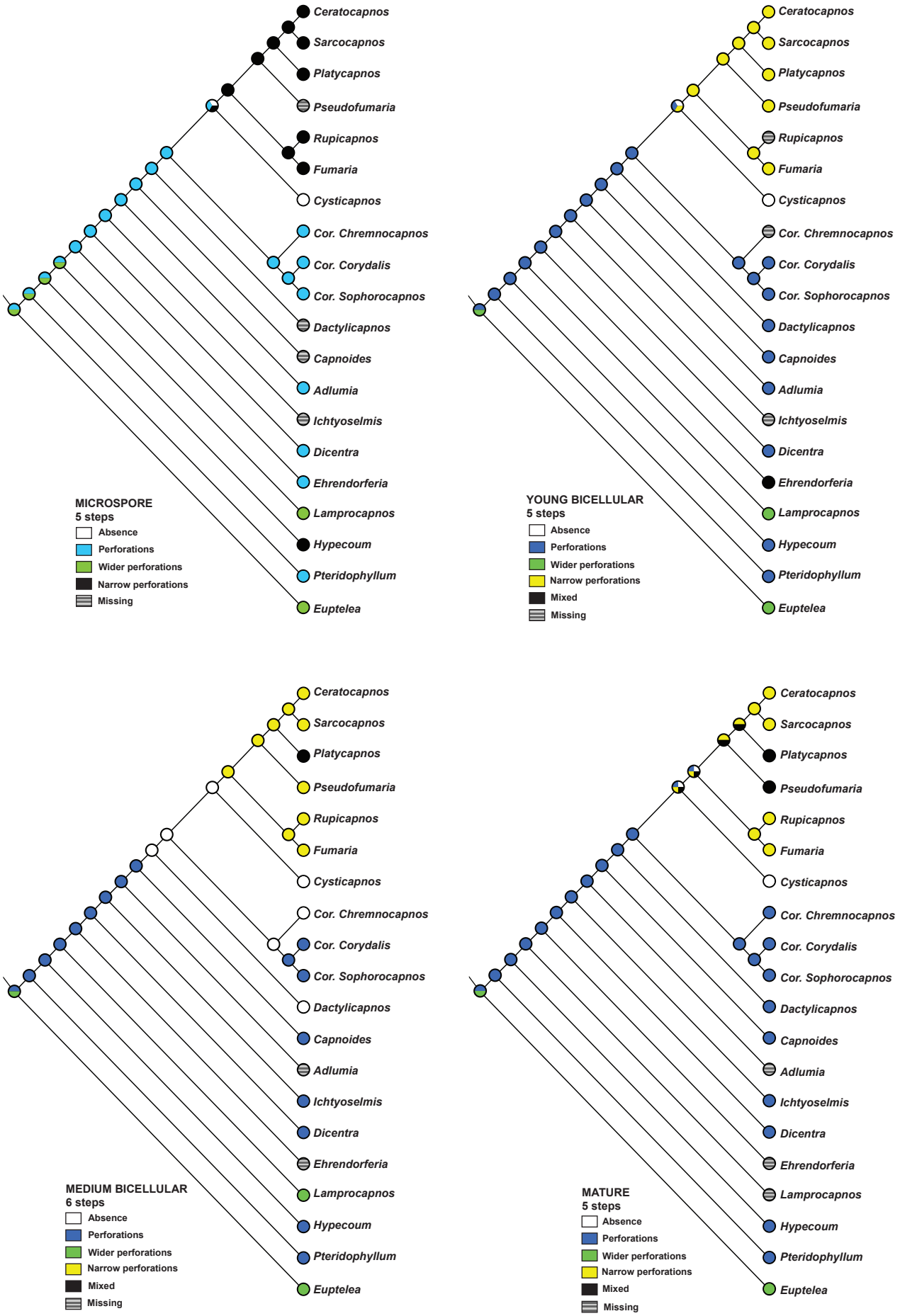


Fig. S5. Infratectum setup optimization on the Fumarioideae topology in the four different ontogenetic stages

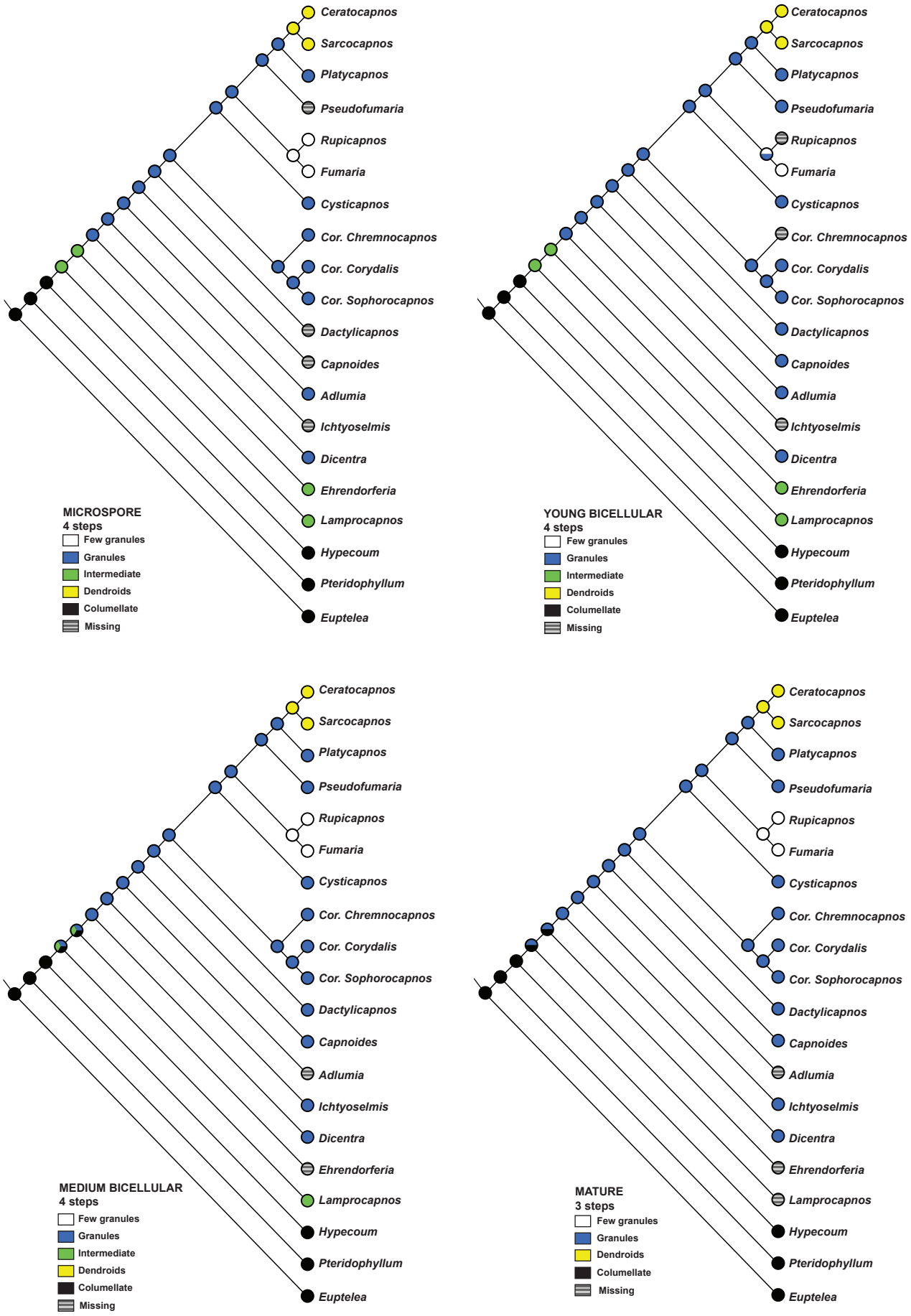


Fig. S6. Ratio infratectum/ectexine optimization on the Fumarioideae topology in the four different ontogenetic stages

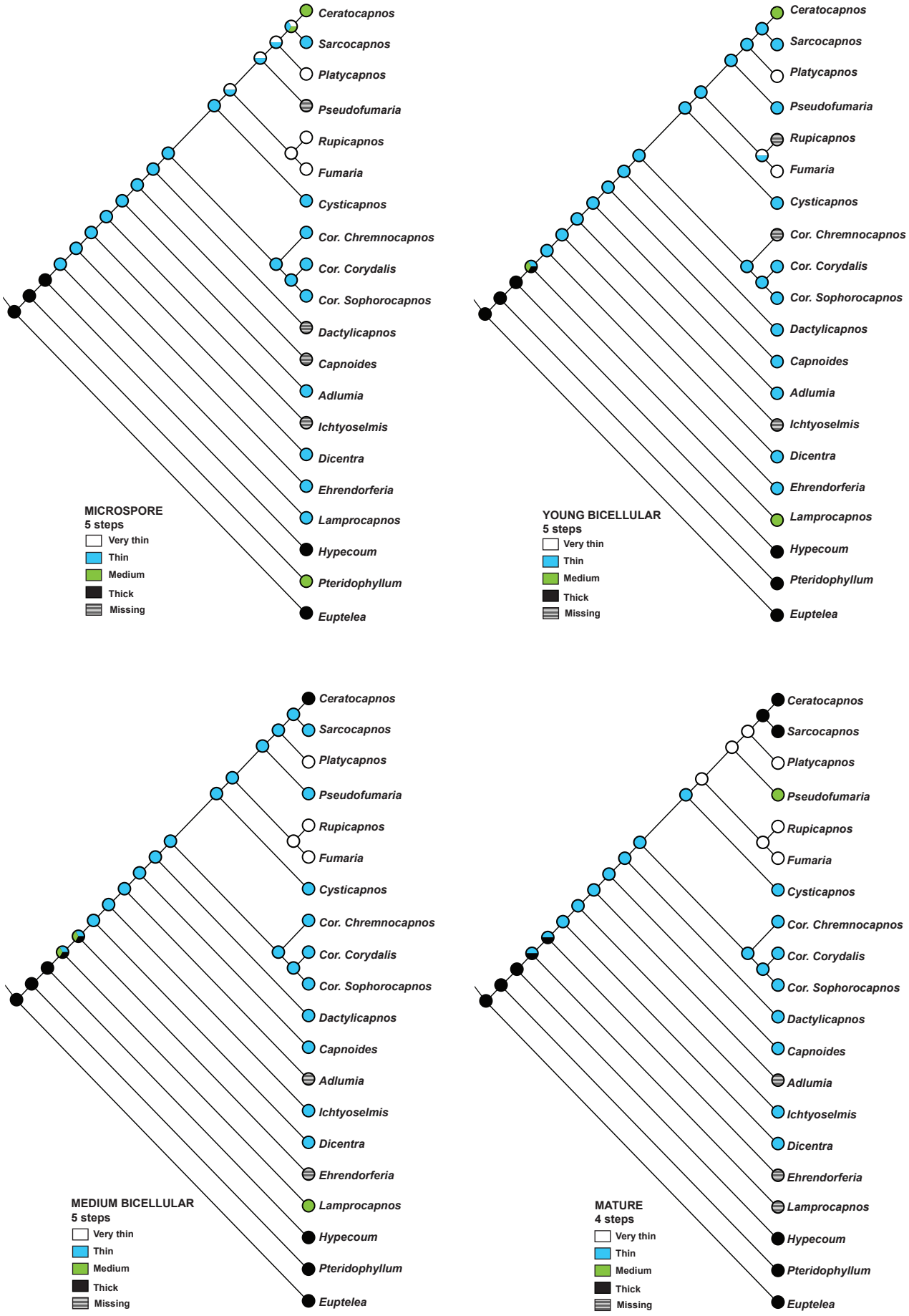


Fig. S7. Infratectum content optimization on the Fumarioideae topology in the four different ontogenetic stages

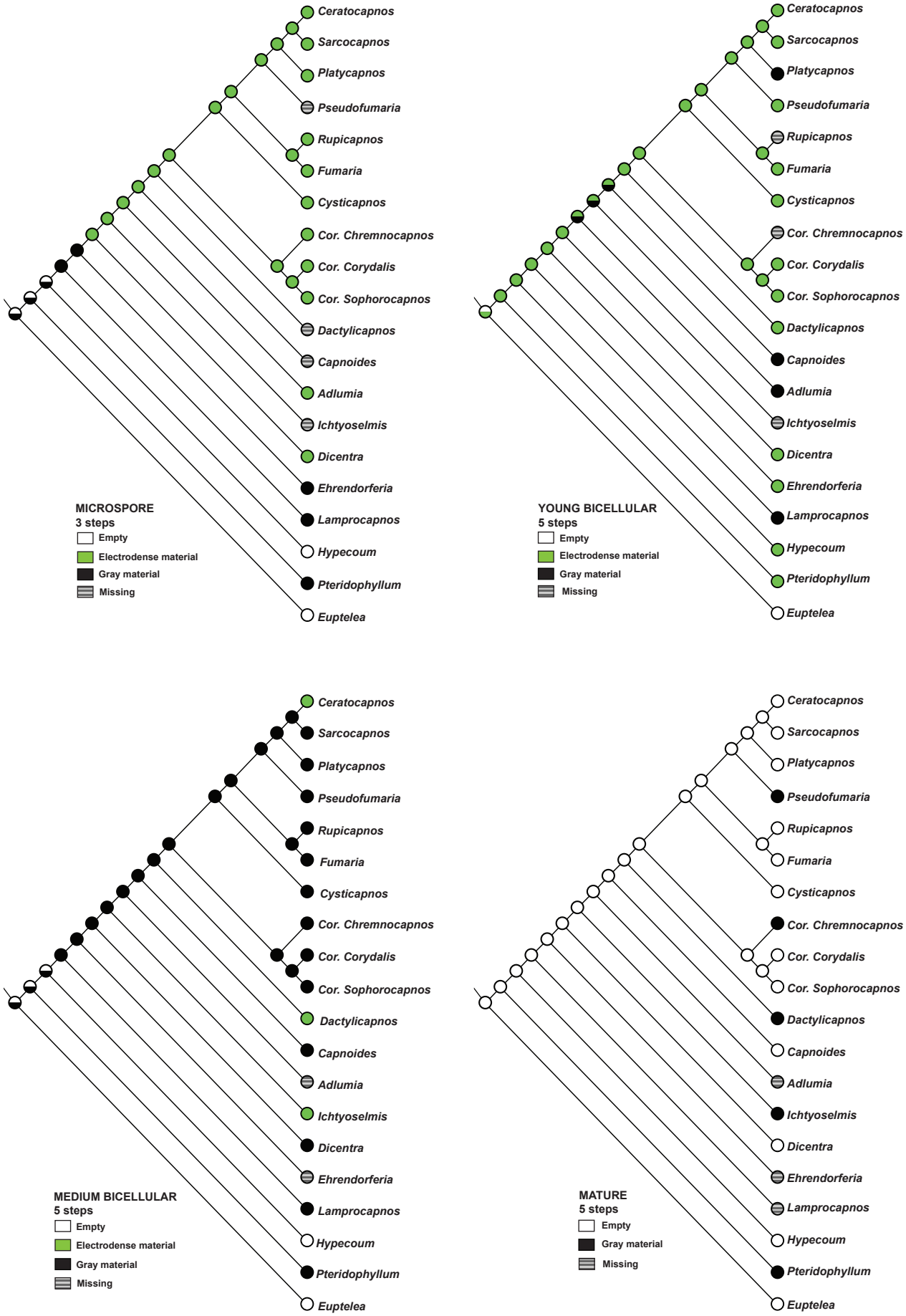


Fig. S8. Ratio foot layer/ectexine optimization on the Fumarioideae topology in the four different ontogenetic stages

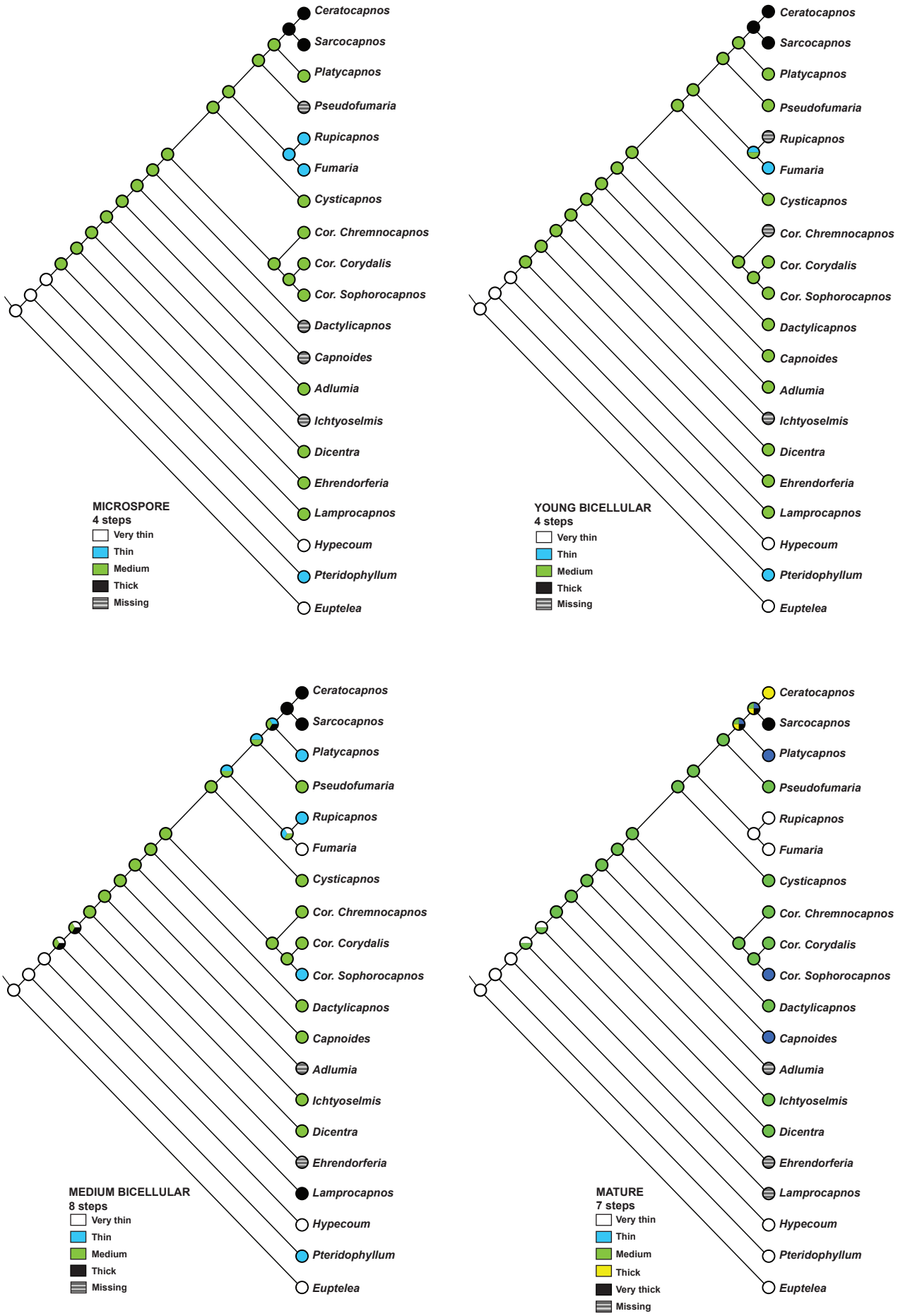


Fig. S9. Foot layer morphology optimization on the Fumarioideae topology in the four different ontogenetic stages

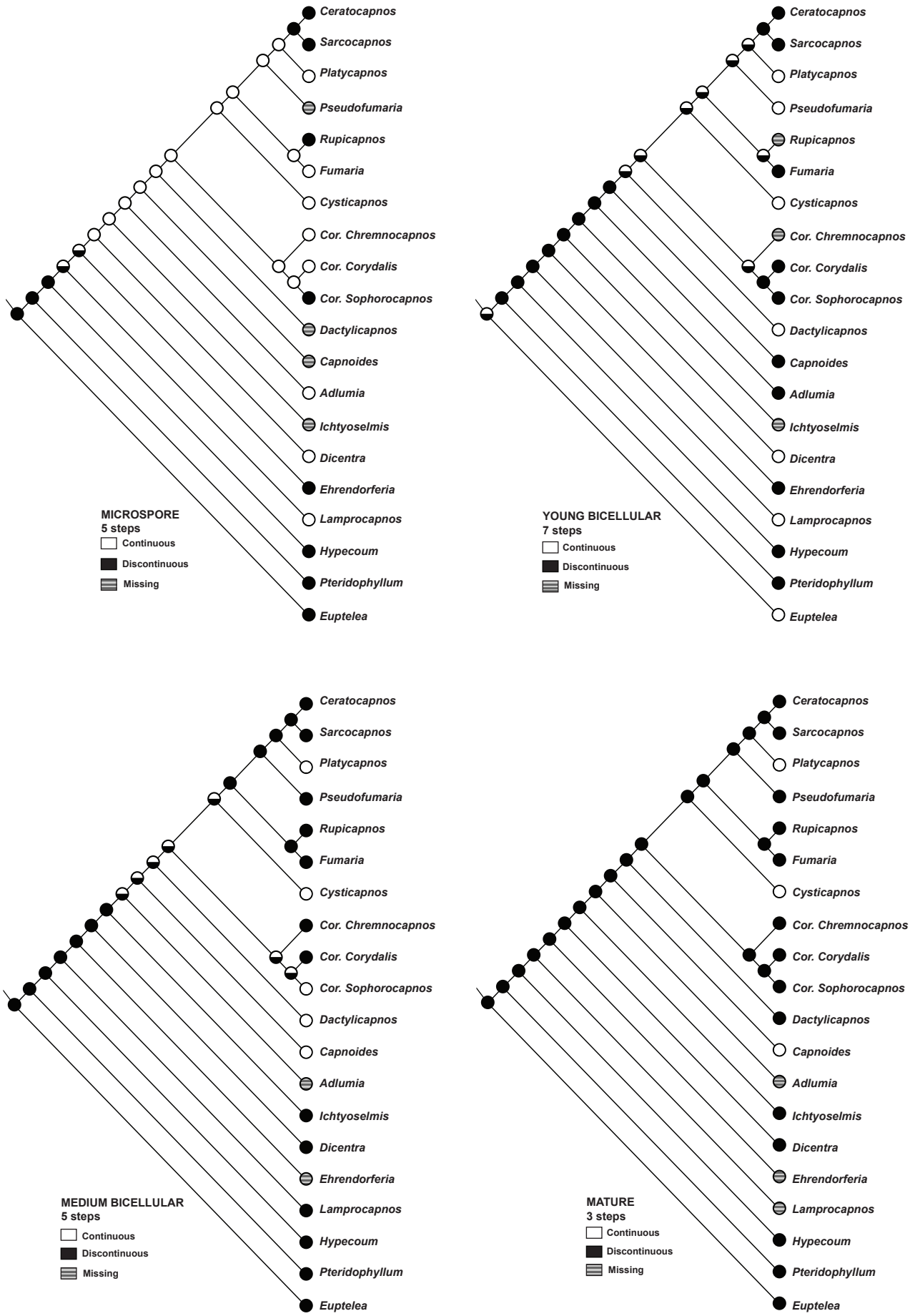


Fig. S10. Morphology of the foot layer inner face optimization on the Fumarioideae topology in the four different ontogenetic stages

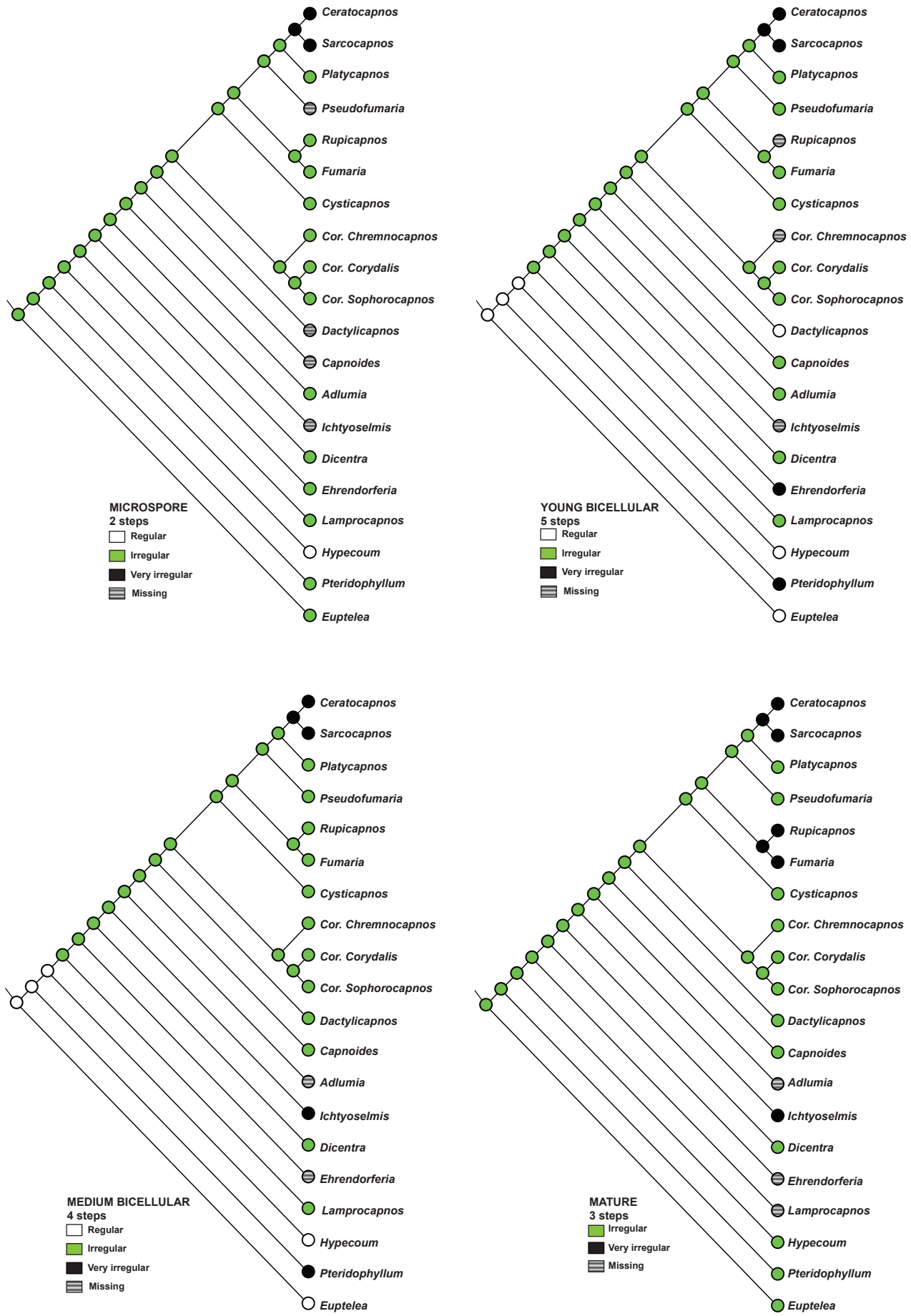


Fig. S11. Presence of interapertural endexine optimization on the Fumarioideae topology in the four different ontogenetic stages

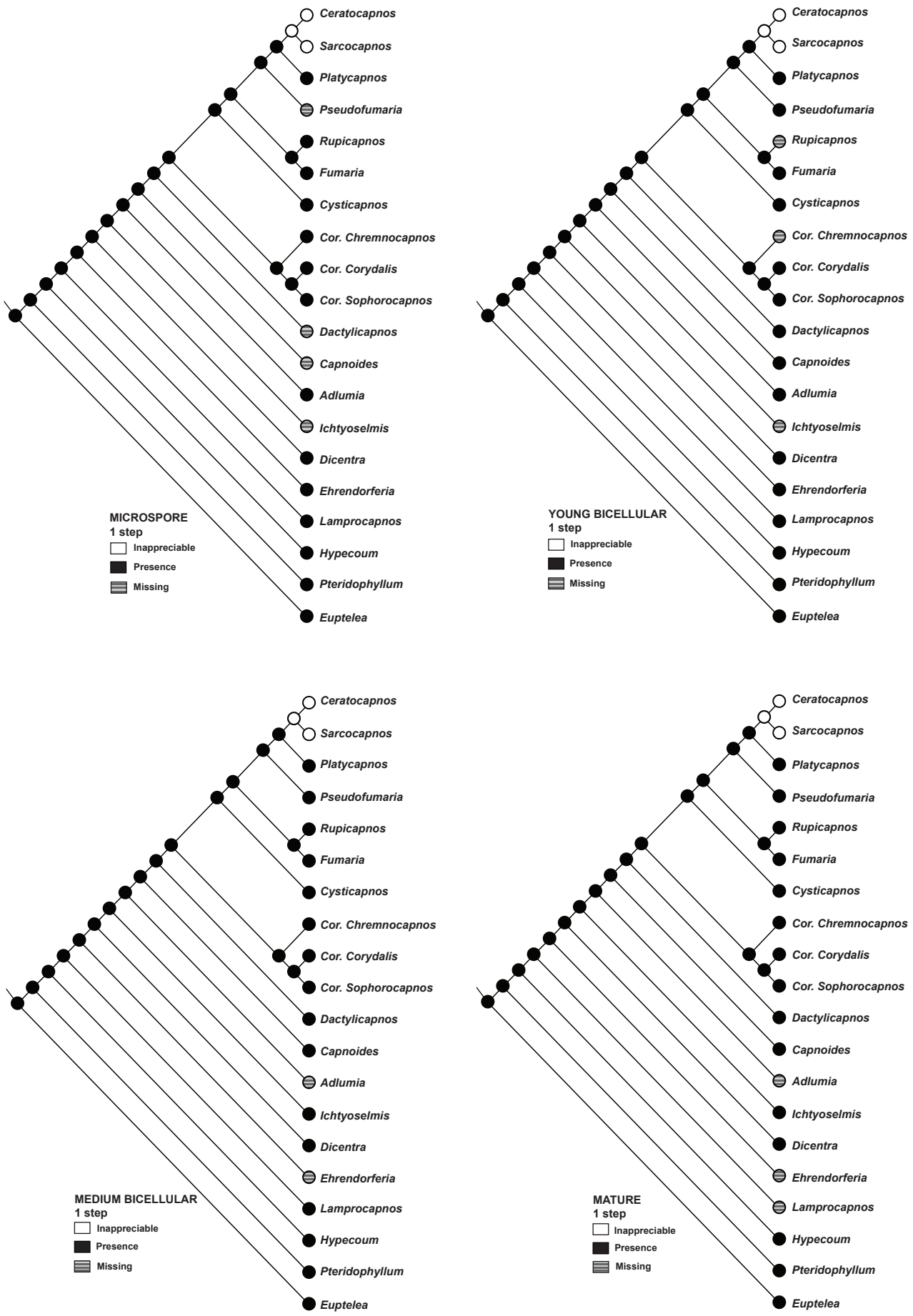


Fig. S12. Ratio endexine/ectexine optimization on the Fumarioideae topology in the four different ontogenetic stages

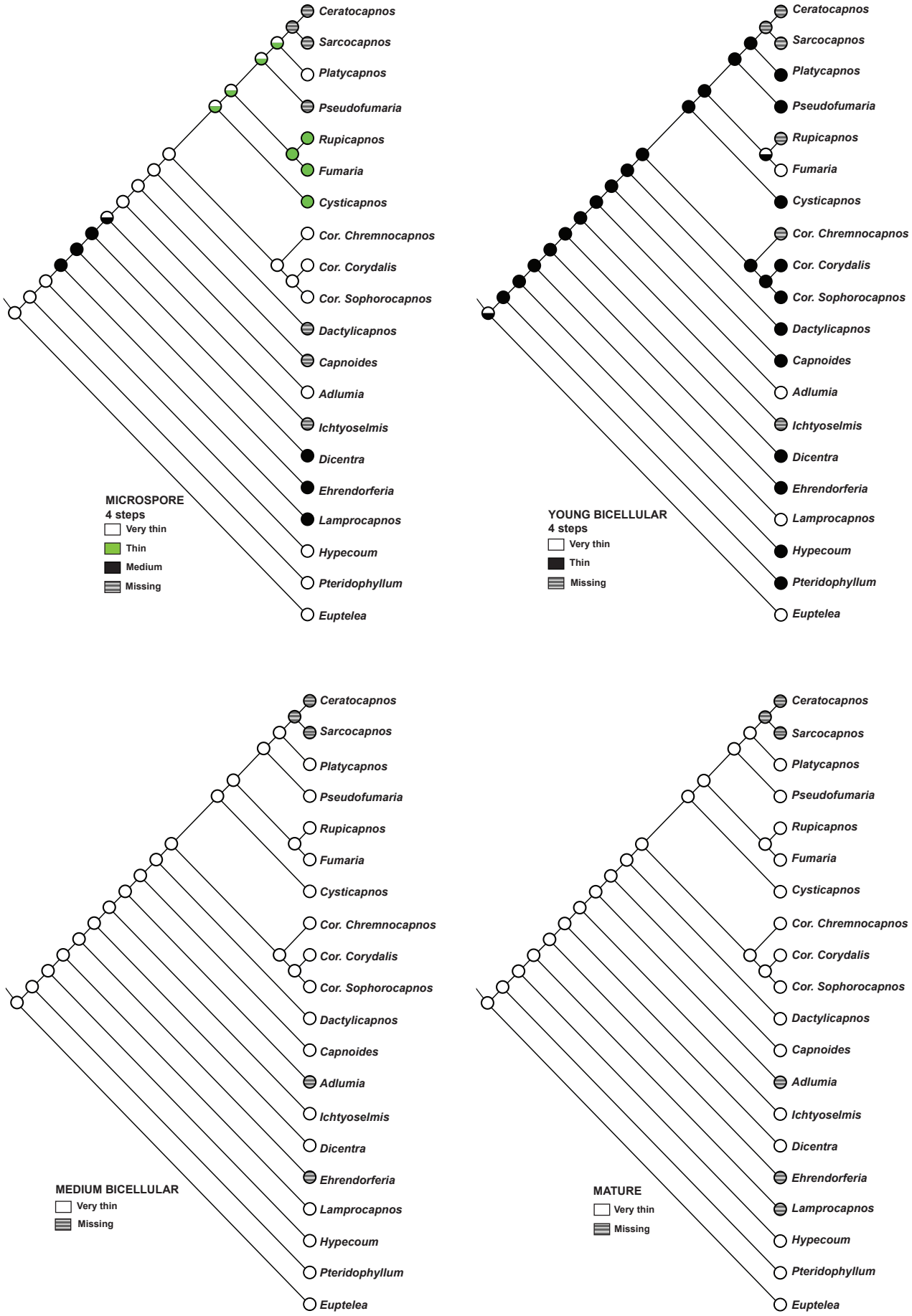


Fig. S13. Fragmentation of endexine optimization on the Fumarioideae topology in the four different ontogenetic stages

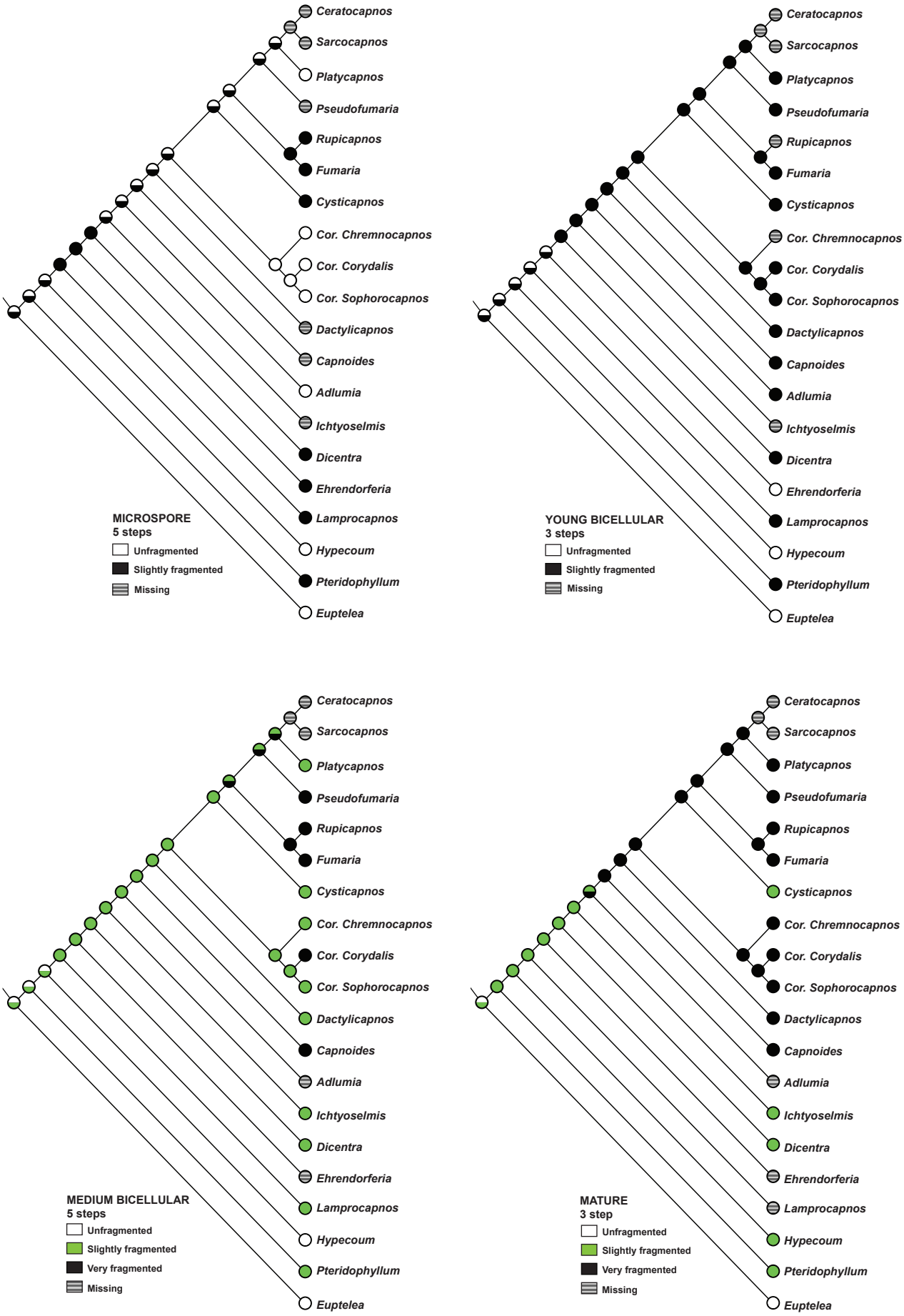


Fig. S14. Shape endexine fragmentation optimization on the Fumarioideae topology in the four different ontogenetic stages

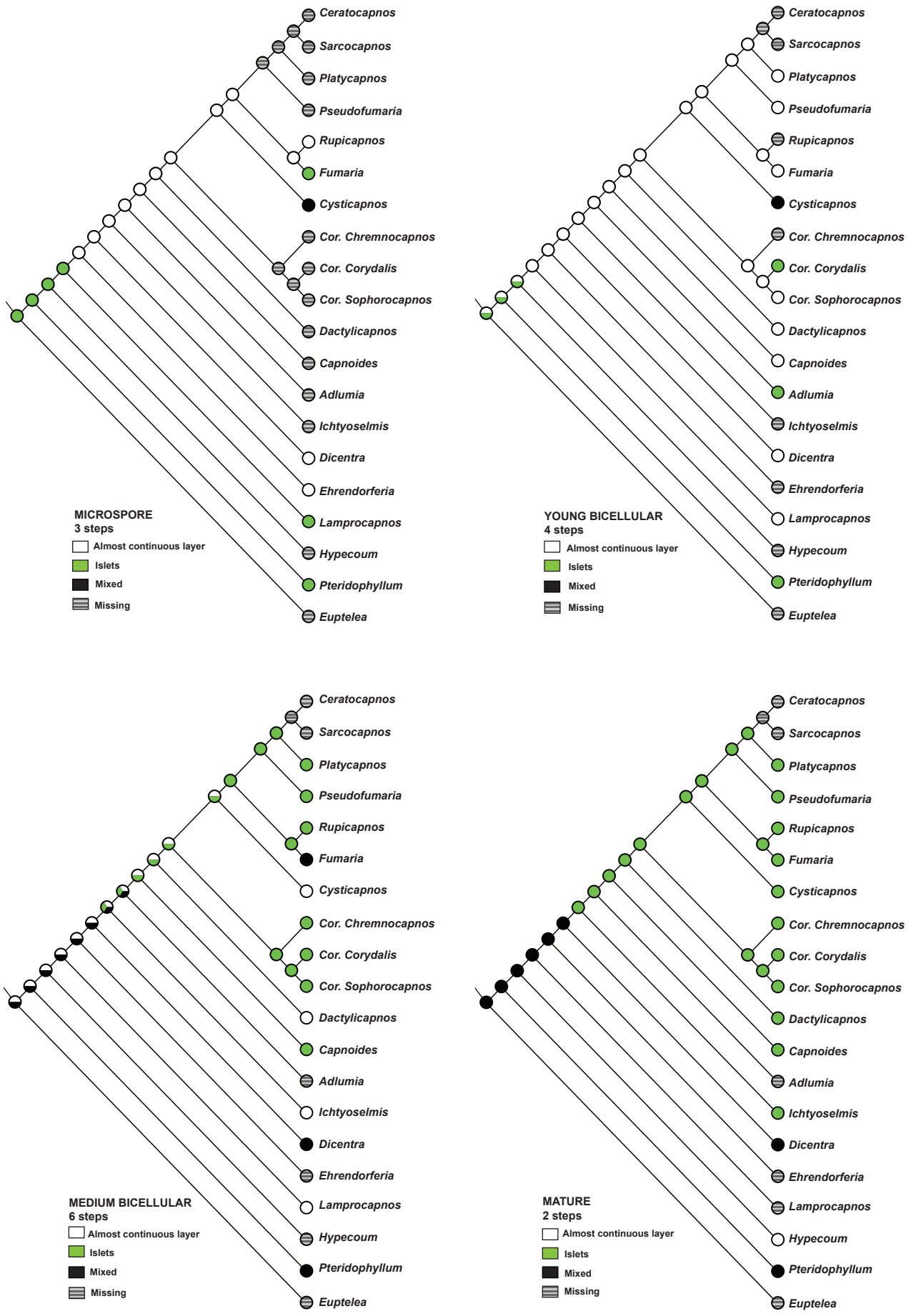


Fig. S15. Ratio intine/exine optimization on the Fumarioideae topology in the four different ontogenetic stages

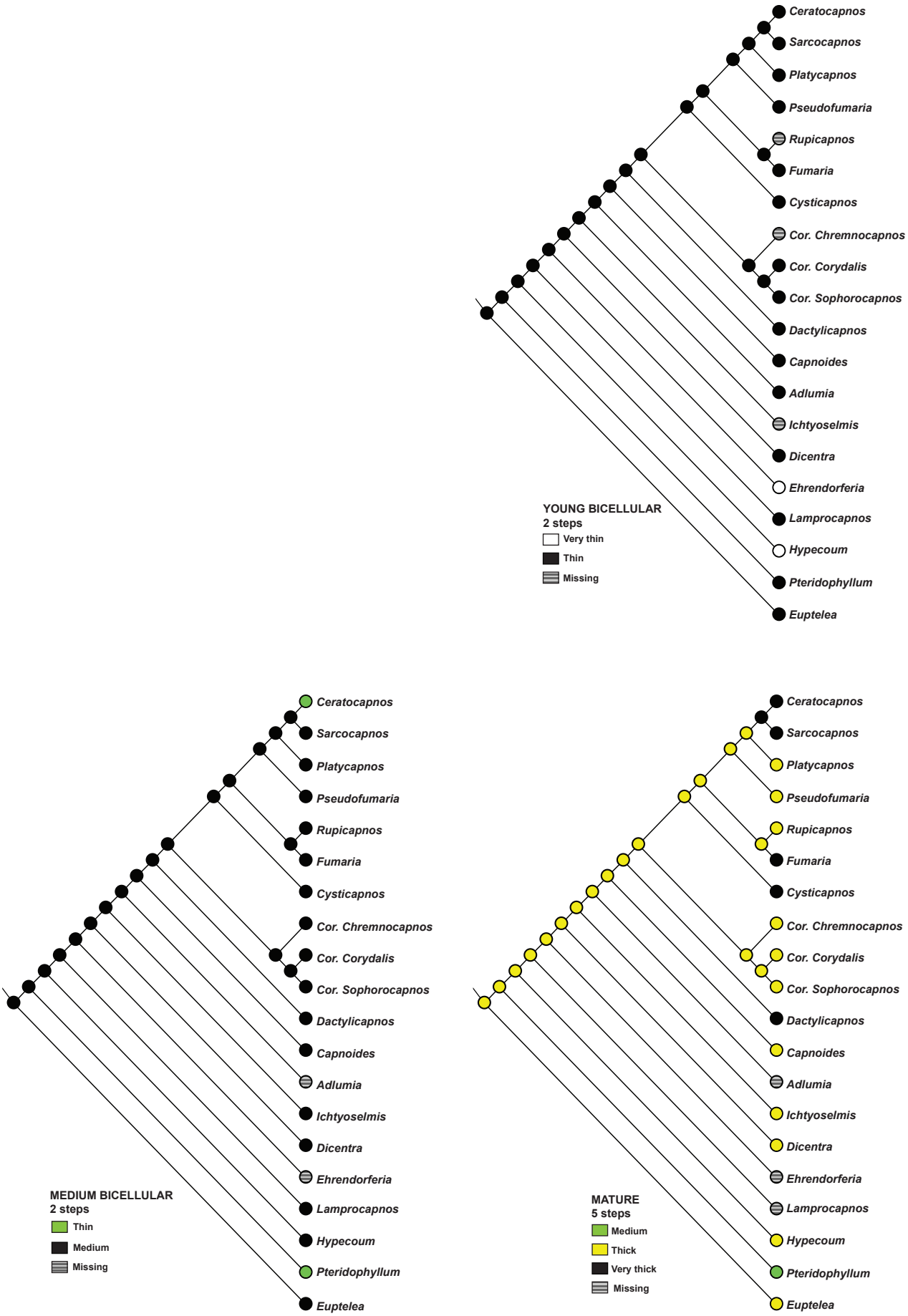


Fig. S16. Aperture oncus optimization on the Fumarioideae topology in the four different ontogenetic stages

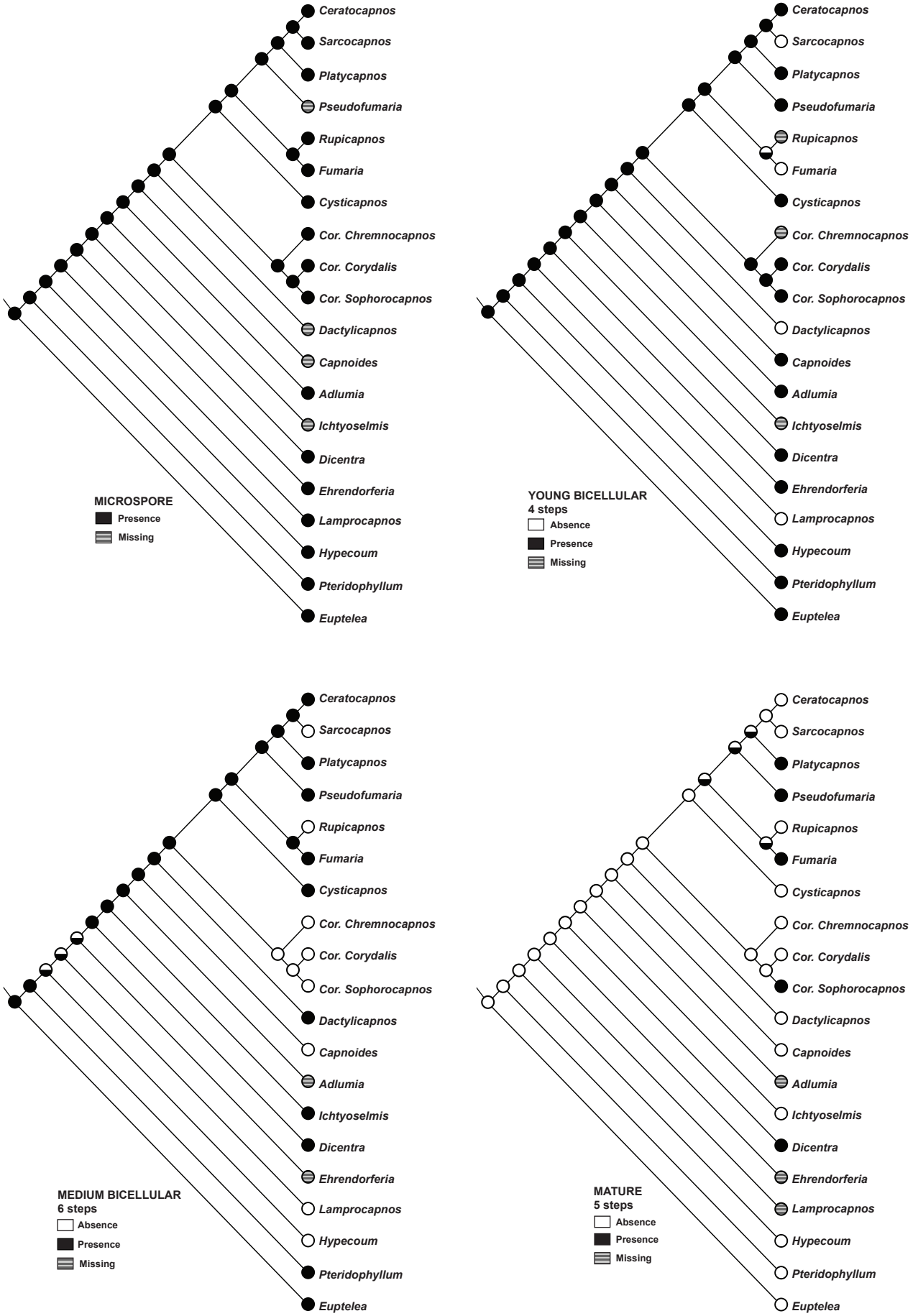


Fig. S17. Apertural endexine setup optimization on the Fumarioideae topology in the four different ontogenetic stages

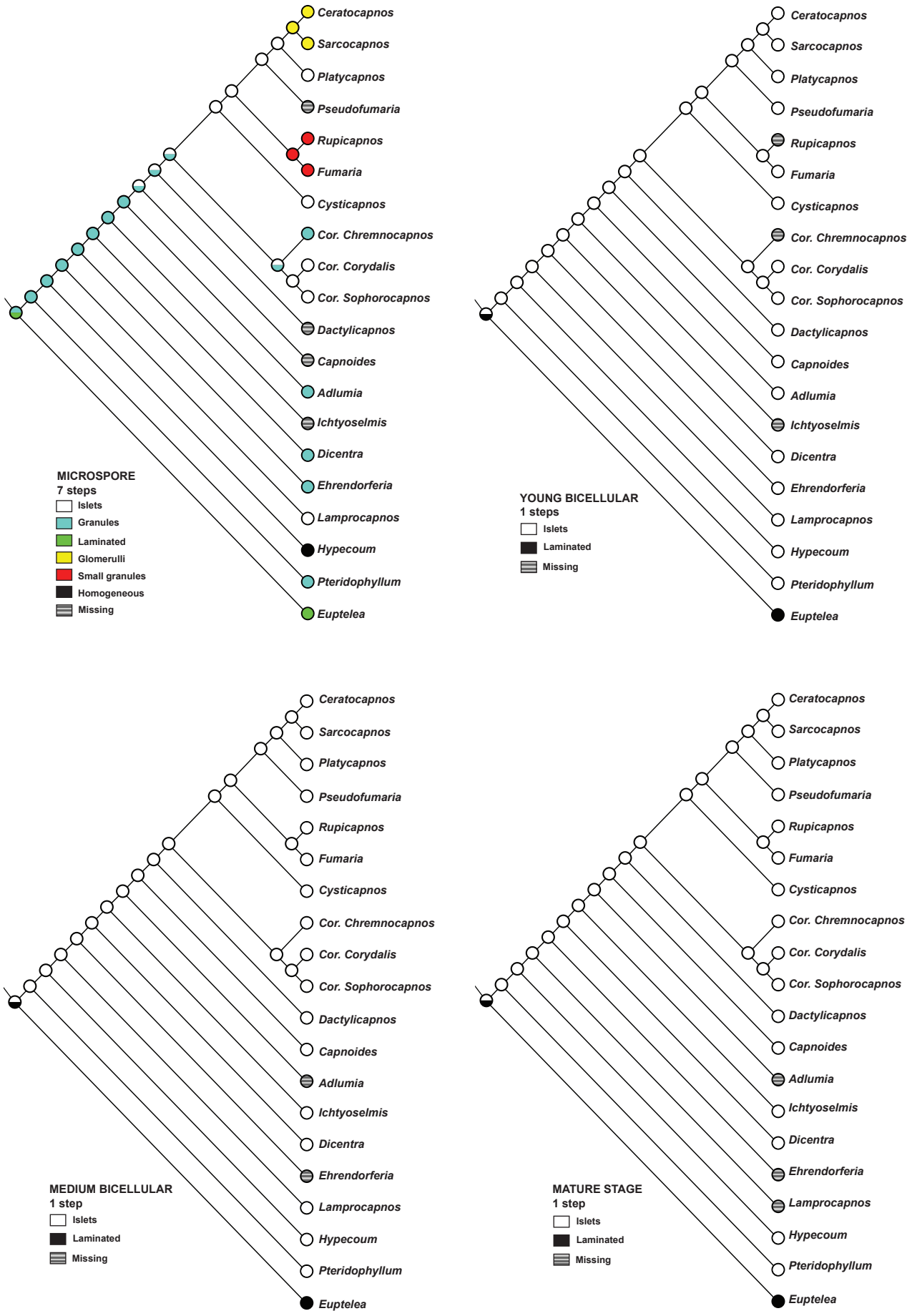


Fig. S18. Aperture membrane optimization on the Fumarioideae topology in the four different ontogenetic stages

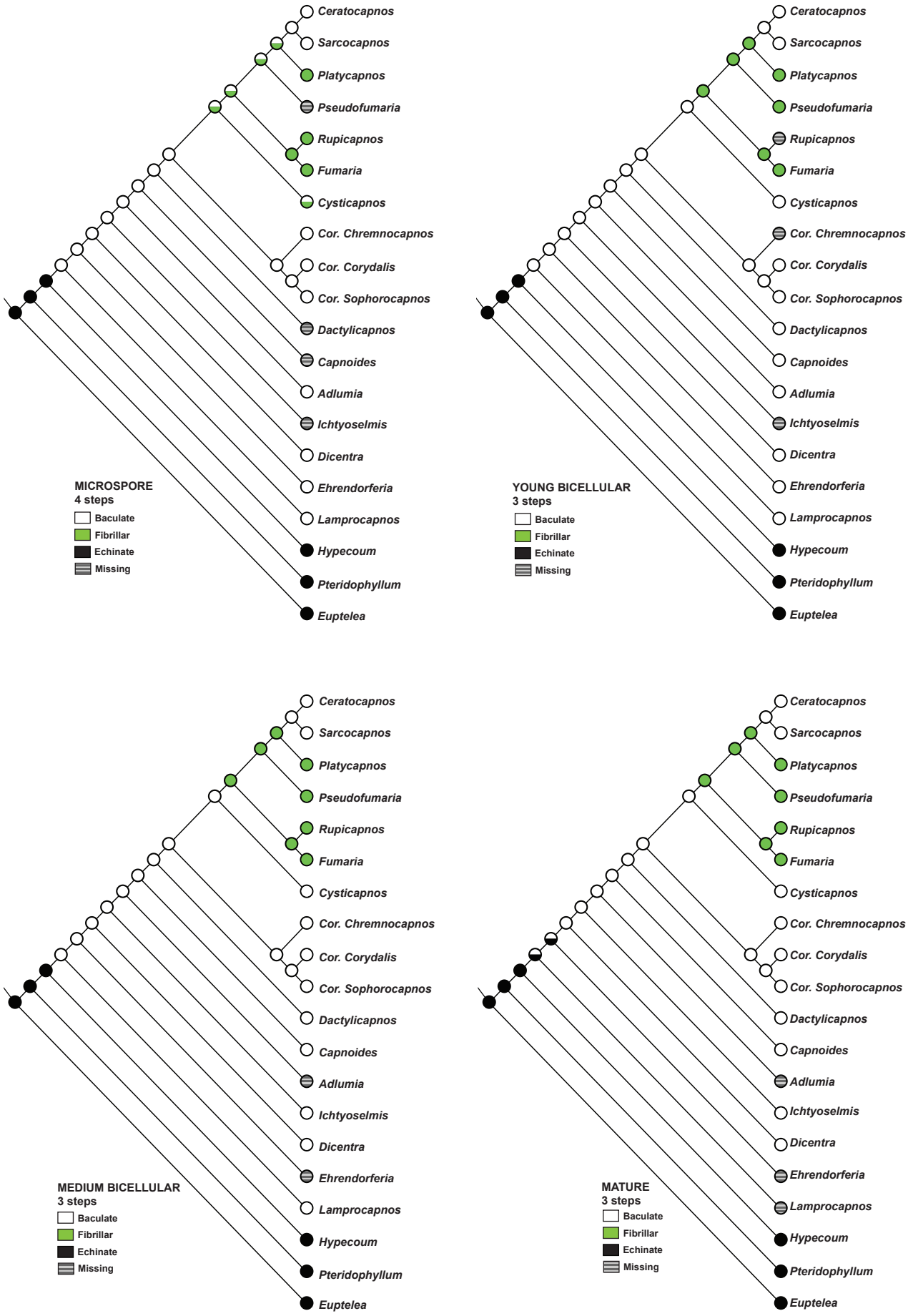


Fig. S19. Fluffy material in aperture optimization on the Fumarioideae topology in the four different ontogenetic stages

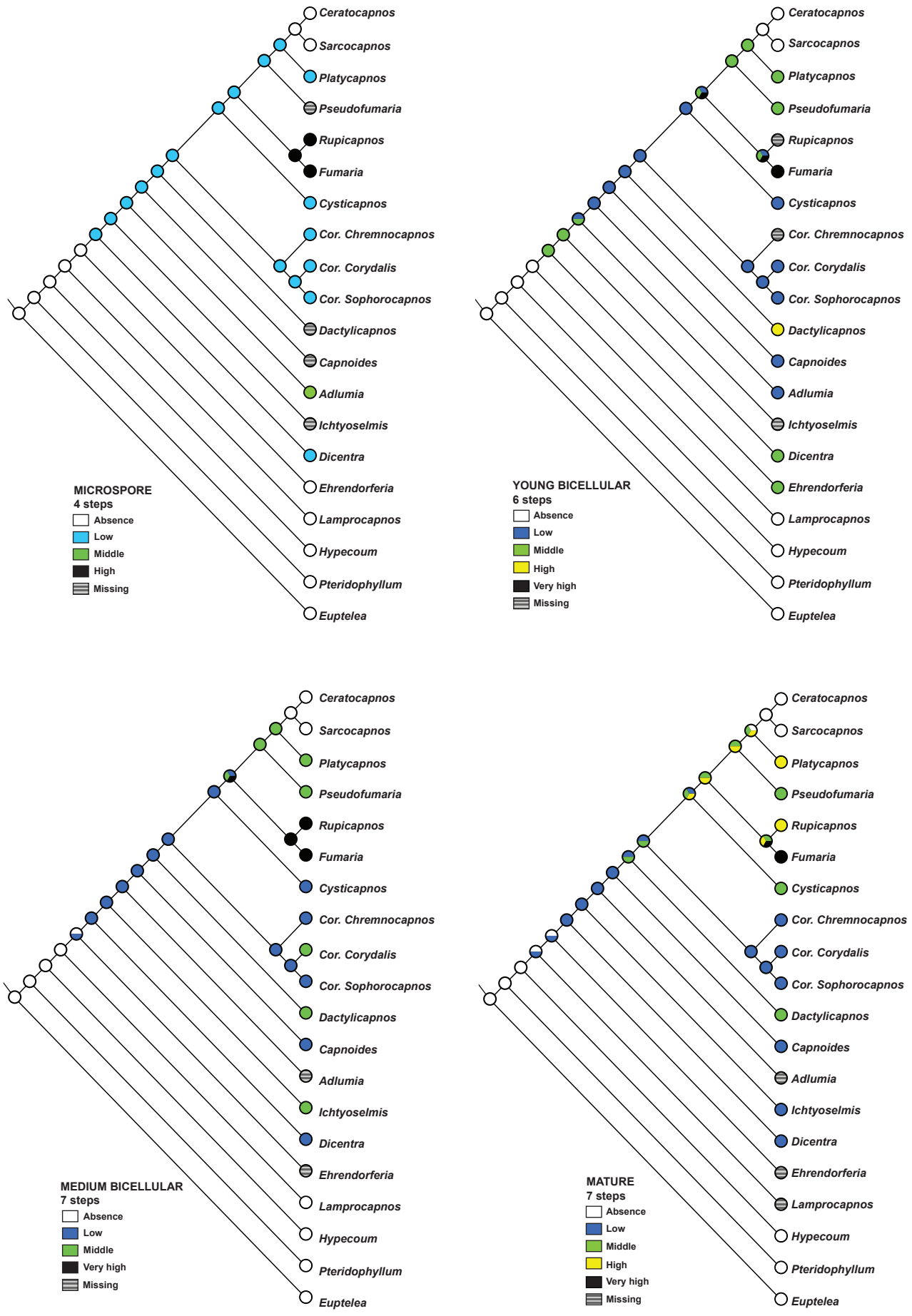


Fig. S20. Pollen morphology features optimization on the Fumarioideae topology in the four different ontogenetic stages

7. Conclusiones generales

7. Conclusiones generales

Filogenia de la subfamilia Fumarioideae

1. Los representantes de la subfamilia Fumarioideae se agrupan en dos clados principales congruentes con las tribus Hypecoeae y Fumarieae consideradas tradicionalmente en base a datos morfológicos. A expensas de un mayor número de evidencias, *Pteridophyllum* debería excluirse de Hypecoeae y mantenerse como una subfamilia independiente. En la tribu Fumarieae existe un grado de linajes basales, constituido por la mayoría de géneros con corola bisimétrica y biespolonada, al que nos referimos como Fumarieae Basales. Empleamos el término 'Crown' Fumarieae para aludir al grupo monofilético conformado por el resto de táxones, caracterizado por corola zigomorfa y presencia de un único espolón (a excepción de *Dactylicapnos*). En este grupo se incluyen los linajes de *Capnoides*, *Dactylicapnos*, *Corydalis* y la subtribu Fumariinae.

2. Las relaciones filogenéticas para la subtribu Fumariinae confirman la existencia de tres grupos monofiléticos que denominamos Clado *Cysticapnos*, Clado *Fumaria* y Clado *Sarcocapnos*, y que coinciden con las agrupaciones descritas por Lidén (1986) a nivel de subtribu en base a datos morfológicos. Las relaciones intergenéricas para el Clado *Fumaria* no se resuelven con los marcadores empleados, mientras que las relaciones entre los géneros del Clado *Sarcocapnos* están débilmente apoyadas. Se propone un fenómeno de 'incomplete lineage sorting' para explicar la baja capacidad resolutiva de los marcadores empleados en este último grupo, como consecuencia de la rápida separación de sus linajes por la inestabilidad geológica en el Mediterráneo durante el Oligoceno.Todas las secuencias empleadas se agruparon según su afinidad genérica a excepción de aquellas pertenecientes al género *Cysticapnos*, que en todos los análisis fue resuelto como parafilético.

3. La reconstrucción de los estados de carácter para algunos rasgos morfológicos de interés sistemático y evolutivo en la subtribu Fumariinae muestra que el estilo de vida anual, el hábito no casmofítico y el fruto en cápsula polisperma son los estados ancestrales para el grupo.

Historia biogeográfica de la subfamilia Fumarioideae

1. La subfamilia Fumarioideae se originó en el Este de Asia al final del Cretácico Inferior. Tres eventos dispersivos tuvieron lugar durante la diversificación de los linajes basales de la subfamilia: 1) una migración hacia el Centro de Asia durante el Cretácico Superior resultó en el linaje de la tribu Hypecoeae; 2) una expansión de rango hacia el Oeste de Norte América a través del puente terrestre de Beringia permitió la llegada a Norte América de los linajes basales de Fumarieae; y 3) una expansión hacia Indochina seguida de una vicarianza originó

el linaje de *Icthyoselmis*. Estos dos últimos procesos dispersivos ocurrieron durante la transición Cretácico-Paleógeno.

2. En la tribu Fumarieae hemos identificado la existencia de cuatro conexiones biogeográficas por expansión de área entre el Este de Asia y el Oeste de Norte América en diferentes periodos. Dos siguiendo el sentido Este de Asia-Oeste de Norte América, una de ellas ocurrió durante la transición Cretácico-Paleógeno e implicó al ancestro común de *Ehrendorferia* y el resto de la tribu; y otra durante la transición Eoceno-Oligoceno realizada por el género *Corydalis*. Las otras dos conexiones siguieron un sentido Norte América-Este de Asia, la primera de ellas durante el Mioceno inferior realizada por el género *Dicentra*; mientras que la segunda ocurrió durante el Cenozoico e implicó al género *Adlumia*.

3. Tras la llegada del ancestro del Crown Fumarieae a la región del Himalaya se produjo un aumento en la tasa de diversificación que resultó en la aparición de los linajes de *Dactylicapnos*, *Corydalis* y la subtribu Fumariinae en tan sólo cuatro millones de años. Este incremento en la tasa de diversificación coincidió con el inicio de las elevaciones de la cordillera Tibetana y el establecimiento en la región de un patrón climático dominado por condiciones desérticas y esteparias.

4. El ancestro de la subtribu Fumariinae alcanzó la región Irano-Turánica durante el Eoceno medio. La diversificación de la subtribu comenzó hace 33 millones de años, durante el Eoceno inferior, con la separación de los grupos mediterráneo (Clados *Fumaria* y *Sarcocapnos*) y sudafricano (Clado *Cysticapnos*). Este último se originó en la región de Sudáfrica mediante un evento de vicarianza que tuvo lugar tras la dispersión previa del ancestro de Fumariinae desde Irano-Turania.

5. La colonización de la cuenca Mediterránea por la subtribu Fumariinae ocurrió mediante dos dispersiones independientes. La primera por el linaje del Clado *Sarcocapnos* siguiendo una ruta norte durante el Oligoceno inferior, probablemente a través de la masa de tierra emergida que conectó Asia Menor con el Mediterráneo durante el origen y aislamiento del Mar Paratetis en ese periodo. El linaje del Clado *Fumaria* llegó a la región Mediterránea entre el Oligoceno superior y el Mioceno siguiendo una ruta sur, a través del puente terrestre de Gomphotherium que conectó la región Irano-Turánica con el Norte de África.

Descripción del polen de la tribu Fumarieae

1. La morfología polínica de la tribu Fumarieae presenta una alta diversidad de tipos de ornamentación y número de aperturas. Los pólenes con exina psilada y verrugada son los más frecuentes, mientras que el número de aperturas más extendido es seis. Respecto a la ultraestructura, la pared del polen de la tribu Fumarieae se distingue por presencia de 'foot layer' lamelada durante el estadío de microspora joven y por infratéctum con morfología granular.

2. En la tribu Fumarieae, la ultraestructura de las regiones aperturales presenta tres capas que se desarrollan durante el estadío de microspora joven. La capa más externa alberga la membrana apertural o, en su ausencia, una cobertura de material fibrilar. La capa intermedia se constituye de endexina, que puede estructurarse en islotes, en gránulos pequeños o en glomérulos. La capa más interna está formada por material fibrilar durante los estadíos iniciales que posteriormente es reemplazado por la intina. La presencia de material algodonoso o 'fluffy material' en la región exterior de las aperturas está extendida en la mayoría de géneros de la tribu.

3. Los caracteres morfológicos y ultraestructurales de la pared del polen en la subfamilia Fumarioideae muestran la existencia de dos tipos polínicos muy diferentes que se corresponden con cada una de las tribus de la subfamilia. El polen de la tribu Hypecoeae, caracterizado por infratéctum columelar y ornamentación de la exina equinada, se asemeja más al polen de *Pteridophyllum* o al de la subfamilia Papaveroideae que al de la tribu Fumarieae, caracterizada por infratéctum granular y exina no espinosa.

Evolución de caracteres polínicos en la subfamilia Fumarioideae

1. Los caracteres polínicos analizados en la subfamilia Fumarioideae presentan bajo nivel de señal filogenética, mostrando la mayoría de ellos altos niveles de homoplasia y bajos niveles de retención de sinapomorfía aparente. Los caracteres relacionados con el tamaño de las capas de la ectexina, la morfología del infratéctum, la disposición de la endexina apertural y el tipo de apertura destacan como los más informativos filogenéticamente.

2. La morfología de la endexina bajo las aperturas se muestra como un carácter útil para estudios en sistemática. Durante el estadío de microspora la disposición de la endexina apertural es un carácter filogenéticamente informativo para la tribu Fumarieae. En polen maduro, la endexina bajo las aperturas presenta una morfología claramente distinta entre la subfamilia Fumarioideae y el grupo basal externo, *Euptelea*.

3. En la subfamilia Fumarioideae hemos descrito cuatro modelos polínicos en función del tamaño de las capas de la ectexina y cuya distribución entre los táxones es coherente con la filogenia de la subfamilia. 1) El modelo columelar presenta infratéctum grueso y capa basal muy delgada, caracteriza a la tribu Hypecoeae y es ancestral para la subfamilia; 2) el modelo granular contiene téctum y base con tamaño medio e infratéctum delgado, este modelo está presente en la mayoría de géneros de la tribu Fumarieae y es ancestral para el conjunto de la tribu; 3) el modelo *Fumaria* se define por téctum grueso e infratéctum y base muy delgados, y caracteriza a los taxones del Clado *Fumaria*; y 4) el modelo *Sarcocapnos* presenta téctum muy delgado y base muy gruesa, siendo exclusivo de los géneros *Ceratocapnos* y *Sarcocapnos*.

4. Para la subfamilia Fumaroideae se han identificado tendencias evolutivas en los caracteres número de aperturas y tipo de ornamentación de la exina. El número de aperturas en el grano de polen tiende a incrementarse a lo largo de la evolución de la subfamilia, mientras que la ornamentación de la exina tiende hacia la reducción en la presencia de perforaciones y la adquisición de superficies verrugadas y ruguladas.

7. General conclusions

Phylogeny of the subfamily Fumarioideae

1. The representatives of the subfamily Fumarioideae are grouped in two main clades congruent with the tribes Hypecoeae and Fumarieae traditionally considered based on morphological data. Until further evidence comes to light, *Pteridophyllum* should be excluded from Hypecoeae and kept as an independent subfamily. The tribe Fumarieae harbours a grade of basal lineages with all the taxa having bisymmetric and two-spurred flowers that we term Basal Fumarieae. We use Crown Fumarieae to refer to the monophyletic group formed by the rest of the taxa and defined by asymmetric and one-spurred flowers (except *Dactylicapnos* genus). The lineages of *Capnoides*, *Dactylicapnos*, *Corydalis* and the subtribe Fumariinae are included in this group.

2. The phylogenetic relationships for the subtribe Fumariinae confirm the existence of three monophyletic groups that we have named *Cysticapnos* Clade, *Fumaria* Clade and *Sarcocapnos* Clade, and that concur with the groups described by Lidén (1986) at a subtribal level based on morphology. The intergeneric relationships for the *Fumaria* Clade are not resolved with the markers used, whilst the relationships among the genera of *Sarcocapnos* Clade are weakly supported. We propose an incomplete lineage sorting phenomenon to explain the low resolution of the molecular markers in this latter group, as a consequence of the rapid split of its lineages due to the geological instability in the Mediterranean during the Oligocene. All the sequences used are grouped by generic affinity with the exception of those belonging to the *Cysticapnos* genus, which in all analyses is resolved as paraphyletic.

3. The reconstruction of the ancestral state for some morphological features with systematic and evolutionary interest in the subtribe Fumariinae shows that annual life span, nonchasmophytic habit, and a several-seeded capsule were the basal character state for the group.

Biogeographic history of the subfamily Fumarioideae

1. The subfamily Fumarioideae originated at the end of the Early Cretaceous in East Asia. Three dispersive events occurred during the diversification of the basal lineages of the subfamily: 1) a migration toward Central Asia during the Late Cretaceous resulted in the tribe Hypecoeae lineage; 2) a range expansion toward the west of North America across the Beringia land bridge allowed the arrival of the basal lineages of Fumarieae to North America; and 3) an expansion toward Indochina followed by a vicariance, originated the lineage of *Ichtyoselmis*. These last two dispersals took place during the Cretaceous-Palaeogene transition.

2. In the tribe Fumarieae we have identified four biogeographic connections by area expansion between East Asia and the west of North America in different periods. Two of them following East Asia-west North America direction, one of them during the Cretaceous-Palaeogene transition and that involved the common ancestor of *Ehrendorferia* and the rest of the tribe; and the other during the Eocene-Oligocene transition made by the genus *Corydalis*. The other two connections followed a North America-East Asia direction, one of them during the early Miocene and made by the genus *Dicentra*; whilst the second took place during the Cenozoic and involved the genus *Adlumia*.

3. After the arrival of the Crown Fumarieae ancestor to the region of the Himalayas an acceleration occurred in the diversification rate that resulted in the origin of the lineages of *Dactylicapnos*, *Corydalis* and subtribe Fumariinae in only four million years. This increase in the diversification rate coincided with the beginning of the Qinghai-Tibetan Plateau uplifts and with the establishment in the region of a climate pattern dominated by desert and steppe conditions.

4. The ancestor of the subtribe Fumariinae reached the Irano-Turanian region during the middle Eocene. The beginning of the subtribe diversification was 33 Ma, during the early Eocene, with the split between the Mediterranean group (*Fumaria* and *Sarcocapnos* Clades) and the South African one (*Cysticapnos* Clade). The origin of the latter was resolved in South Africa by a vicariance event, involving the previous dispersal of the Fumariinae ancestor from the Irano-Turanian region.

5. The colonization of the Mediterranean basin by the subtribe Fumariinae took place by mean of two independent dispersals. The first of them made by the lineage of the *Sarcocapnos* Clade following a northern route during the early Oligocene, probably through the emerged land-mass that connected Asia Minor with the Mediterranean during the origin and isolation of the Paratetis Sea in this period. The *Fumaria* Clade lineage arrived in the Mediterranean region during the Oligocene-Miocene transition following a southern route, across the Gomphoterium land bridge that connected the Irano-Turanian region with North Africa.

Pollen description of the tribe Fumarieae

1. The pollen morphology of the tribe Fumarieae shows a high diversity of ornamentation and aperture number. Pollen grains having psilate or verrucate exine are the most common, whilst the most common number of apertures is six. In relation to the ultrastructure, the pollen walls of the tribe Fumarieae are defined by the presence of a lamellated foot layer during the young microspore stage and by an infratectum layer with granulate morphology.

2. In the tribe Fumarieae, the ultrastructure of the apertural regions has three layers that develop at the young microspore stage. The outer layer contains the aperture membrane or, in its absence, a cover of fibrillar material. The intermediate layer is formed of endexine and may be structured in islets, small granules or glomeruli. The inner layer is made of fibrillar material during the microspore stages and is later replaced by the intine. The presence of fluffy material on the outside of the apertures is common in most genera of the tribe.

3. Morphological and ultrastructural characteristics of the pollen wall of the subfamily Fumarioideae show the existence of two very different pollen types that concur with each of tribe of the subfamily. The pollen of the tribe Hypecoeae, defined by columellate infratectum and echinate ornamentation of the exine, is more similar to the pollen of *Pteridophyllum* or to the pollen of Papaveroideae than to the pollen of Fumarieae, that is defined by granulate infratectum and non-spinose exine.

Evolution of pollen characters in the subfamily Fumarioideae

1. The pollen characters analyzed for the subfamily Fumarioideae have low levels of phylogenetic signal, most of them showing high levels of homoplasy and low levels of apparent-synapomorphy retention. The characters related to the size of the ectexine layers, the infratectum morphology, the apertural endexine setup and the apertural type stand out as being the most phylogenetically informative characters.

2. The morphology of the endexine below the apertures appears as a useful character in systematics studies. The endexine setup at the microspore stage is a phylogenetically informative character for the tribe Fumarieae. At the mature pollen stage, the apertural endexine shows a clearly different morphology between Fumarioideae and the outgroup, *Euptelea*.

3. We have described four pollen models in Fumarioideae in relation to the size of the ectexine layers and whose distribution among the taxa is consistent with the phylogeny of the subfamily. 1) The columellate model presents a thick infratectum and a very thin foot layer, which characterizes the tribe Hypecoeae and is ancestral for the subfamily; 2) the granulate model has a medium size tectum and foot layer and a thin infratectum, this model is common in most genera of Fumarieae and is ancestral for the tribe; 3) the *Fumaria* model is defined by a thick tectum and a very thin infratectum and foot layer and characterizes the taxa of the *Fumaria* Clade; and 4) the *Sarcocapnos* model that presents a very thin tectum and a very thick foot layer, which is exclusive to the genera *Ceratocapnos* and *Sarcocapnos*.

4. In the subfamily Fumarioideae we have identified evolutionary trends for the aperture number and exine ornamentation characters. The aperture number in the pollen grain trends to increase during the evolution of the subfamily, whilst the exine ornamentation trends to reduce the presence of perforations and to acquire verrucate and rugulate surfaces.

8. Bibliografía general

8. Bibliografía general

Ali, S.S., Yu, Y., Pfosser, M. and W. Wetschnig. 2012. Inferences of biogeographical histories within subfamily Hyacinthoideae using S-DIVA and Bayesian binary MCMC analysis implemented in RASP (Reconstruct Ancestral State in Phylogenies). *Annals of Botany* 109:95-107.

APG II: Bremer, B., Bremer, K., Chase, M.W., Reveal, J.L., Soltis, D.E., Soltis, P.S., Stevens, P.F., Anderberg, A.A., Fay, M.F., Goldblatt, P., Judd, W.S., Källersjö, M., Kårehed, J., Kron, K.A., Lundberg, J., Nickrent, D.L., Olmstead, R.G., Oxelman, B., Pires, J.C., Rodman, J.E., Rudall, P.J., Savolainen, V., Sytsma, K.J., van der Bank, M., Wurdack, K., Xiang, J.Q.-Y. and S. Zmarzty. 2003. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG II. *Botanical Journal of the Linnean Society* 141:399-436.

APG III: Bremer, B., Bremer, K., Chase, M.W., Fay, M.F., Reveal, .J.L, Soltis, D.E., Soltis, P.S., Stevens, P.F., Anderberg, A.A., Moore, M.J., Olmstead, R.G., Rudall, P.J., Sytsma, K.J., Tank, D.C., Wurdack, K., Yiang, J.K.Y. and S. Zmarzty. 2009. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG III. *Botanical Journal of the Linnean Society* 161:105-121.

Barres, L., Sanmartín, I., Anderson, C.L., Susanna, A., Buerki, S., Galbany-Casals, M. and R. Vilatersana. 2013. Reconstructing the evolution and the biogeographic history of tribe Cardueae (Compositae). *American Journal of Botany* 100:867-882.

Bastida, J.M., Alcántara, J.M., Rey, P.J., Vargas, P. and C.M. Herrera. 2010. Extended phylogeny of *Aquilegia*: the biogeographical and ecological patterns of two simultaneous but contrasting radiations. *Plant Systematics and Evolution* 284:171-185.

Blackmore, S., Stafford, P. and V. Persson. 1995. Palynology and systematics of Ranunculiflorae. *Plant Systematics and Evolution* 9(Supp):71-82.

Blackmore, S. and P.R. Crane. 1998. The evolution of apertures in the spores and pollen grains of the embryophytes. In: Owen, S.J. and P.J. Rudall (eds.), *Reproductive biology*. London, UK: Royal Botanic Gardens, Kew, 159-182.

Blackmore, S. 1997. Polle and spores: microscopic keys to understanding the earth's biodiversity. *Plant Systematics and Evolution* 263:3-12.

Blackmore, S., Wortley, H., Skvarla, J.J. and J.R. Rowley. 2007. Pollen wall development in flowering plants. *New Phytologist* 174:483-498.

Brikiatis, L. 2014. The De Geer, Thulean and Beringia routes: key concepts for understanding early Cenozoic biogeography. *Journal of Biogeography* 6:1036-1054.

Brückner, C. 1985. Frucht und Samenanatomie von Pteridophyllum racemosum Sieb. et. Zucc un der Position der monotypischen Gattung in den Papaverales. Feddes Repertorium 96:199-213.

Candau, P. 1987. Familia Papaveraceae y Fumariaceae. In: Valdés, B., Díez, M.J. and I. Fernández (eds.), *Atlas polínico de Andalucía occidental*. Instituto de Desarrollo Regional Universidad de Sevilla & Diputación de Cádiz, Sevilla, 84-89.

Candau, P. y A. Soler. 1981. Contribución a la palinología de la familia Fumariaceae en la Península Ibérica. *Botanica Macaronesica* 8-9: 147-161.

Chase, M.W., Soltis, D.E., Olmstead, R.G., Morgan, D., Les, D.H., Mishler, B.D., Duvall, M.R., Price, R.A., Hills, H.G., Qiu, Y.-L., Kron, K.A., Rettig, J.H., Conti, E., Palmer, J.D., Manhart, J.R., Sytsma, K.J., Michaels, H.J., Kress, W.J., Karol, K.G., Clark, W.D., Hedren, M., Gaut, B.S., Jansen, R.K., Kim, K.-J., Wimpee, C.F., Smith, J.F., Furnier, G.R., Strauss, S.H., Xiang, Q.-Y., Plunkett, G.M., Soltis, P.S., Swensen, S.M., Williams, S.E., Gadek, P.A., Quinn, C.J., Eguiarte, L.E., Golenberg, E., Learn, G.H., Graham Jr., S.W., Barrett, S.C.H., Dayanandan, S. and V.A. Albert. 1993. Phylogenetics of seed plants: an analysis of nucleotide sequences from the plastid gene *rbc*L. *Annals of the Missouri Botanical Garden* 80:528-580.

Chen, J. and N-H. Xia. 2011. Pollen morphology of Chinese *Curcuma* L. and *Boesenbergia* Kuntz (Zingiberaceae): taxonomic implications. Flora 206:458-467.

Chin, S.-W., Shaw, J., Haberle, R., Wen, J. and D. Potter. 2014. Diversification of almonds, peaches, plums and cherries – Molecular systematics and biogeographic history of *Prunus* (Rosaceae). *Molecular Phylogenetics and Evolution* 76:34-48.

Cooper, R.L., Osborn, J.M. and C.T. Philbrick. 2000. Comparative pollen morphology and ultrastructure of the Callithricaceae. *American Journal of Botany*. 87:161-175.

Dahl, Å.E. 1989. Taxonomic and morphological studies in *Hypecoum* sect. *Hypecoum* (Papaveraceae). *Plant Systematics and Evolution* 163:227-280.

Dahl, Å.E. 1990. Infrageneric division of the genus *Hypecoum* (Papaveraceae). *Nordic Journal of Botany* 10:129-140.

Damerval, C. and S. Nadot. 2007. Evolution of perianth and stamen characteristics with respect to floral symmetry in Ranunculales. *Annals of Botany* 100:631-640.

Damerval, C., H. Citerne, M. Le Guilloux, S. Domenichini, J. Dutheil, L. Ronse de Craene, and S. Nadot. 2013. Asymmetric morphogenetic cues along the transverse plane: shift from disimmetry to zygomorphy in the flower of Fumarioideae. *American Journal of Botany* 100:391-402.

Denk, T. and M.V. Tekleva. 2006. Comparative pollen morphology and ultrastructure of *Platanus*: Implications for phylogeny and evaluation of the fossil record. *Grana* 43:195-221.

Donoghue, M. J. & Doyle, J. A. 1989. Phylogenetic analysis of angiosperms and the relationships of Hamamelidae. In: Crane, P.R. and S. Blackmore (eds.), *Evolution, systematics, and fossil history of the Hamamelidae*, vol. 1. Clarendon Press, Oxford, pp. 17-45.

Doyle, J.A. 2005. Early evolution of angiosperm pollen as inferred from molecular and morphological phylogenetic analysis. *Grana* 44:227-251.

Doyle, J.A. 2009. Evolutionary significance of granular exine structure in the light of phylogenetics analyses. *Review of Paleobotany and Palynology* 156:198-210.

Doyle, J. A. and C. L. Hotton. 1991. Diversification of early angiosperm pollen in a cladistic context. In: Blackmore, S. and S.H. Barnes, (eds.) *Pollen and spores: patterns of diversification*. Clarendon Press, Oxford, pp. 169-195.

Erdmant, G. 1952. Pollen morphology and plant taxonomy: Angiosperms. Almqvist and Wiksell, Stockholm, 539 pp.

Fedde,, F. 1936. Papaveraceae. In: Engler, A. and H. Harms (eds.), Die natürlichen Planzenfamilien, 17b, 2nd ed, Leipzig, Engelman, pp 5-145.

Fernández, M.C. and M.I. Rodríguez García. 1995. Pollen grain apertures in *Olea europaea* L. *Review of Paleobotany and Palynology* 72:41-48.

Fior, S., Li, M., Oxelman, B., Viola, R., Hodges, S.A., Ometto, L. and C. Varotto. 2013. Spatiotemporal reconstruction of the Aquilegia rapid radiation throught next-generation sequencing of rapidly evolving cpDNA regions. *New Phytologist* 198:579-592.

Fiz-Palacios, O. and V. Valcárcel. 2013. From Messinian crisis to Mediterranean climate: A temporal gap of diversification recovered from multiple plant phylogenies. *Perspectives in Plant Ecology, Evolution and Systematics* 15:130-137.

Friedel, J. 1938. Note sur la structure anatomique du Pteridophyllum racemosum Sieb. et. Zucc. Bulletin de la Société Botanique de France 85:406-408.

Fukuhara, T. 1999. Seed and funicle morphology of Fumariaceae-Fumarioideae: Systematic implications and evolution patterns. *International Journal of Plant Sciences* 160:151-180.

Furness, C.A. and P.J. Rudall. 2004. Pollen aperture evolution - a crucial factor for eudicot success? *Trends in Plant Sciences* 9:154-158.

Gabarayeva, N.I., Grigorjeva, V.V. and J.R. Rowley. 2003. Sporoderm ontogeny in *Cabomba aquatica* (Cabombaceae). *Review of Paleobotany and Palynology* 127:147-173.

Gabarayeva, N.I. and A.R. Hemsley. 2006. Merging concepts: the role of self-assembly in the development of pollen wall structure. *Review of Paleobotany and Palynology* 138:121-139.

Gabarayeva, N.I., Grigorjeva, V.V. and J.R. Rowley. 2010. Sporoderm development in *Acer tataricum* (Aceraceae): an interpretation. *Plant Systematics and Evolution* 247:65-81.

Heo, X-I., Lee, S., Lee, C. and S-C. Kim. 2011. Generic delimitation and infrageneric classification of *Stewartia* and *Hartia* (Theaceae; Stewartieae): insight from pollen morphology. *Plant Systematic and Evolution* 297:33-50.

Heslop-Harrison, J. 1963. An ultrastructural study of pollen wall ontogeny in *Silene pendula*. *Grana palynologica* 4:7-24.

Hesse, M., Halbritter, H., Zetter, R., Weber, M., Buchner, R., Frosch-Radivo, A. and S. Ullrich. 2009. Pollen terminology. An illustrated handbook. Springer, New York.

Hidalgo, O., Bartholmes, C. and S. Gleissberg. 2013. Virus-induced gene silencing (VIGS) in *Cysticapnos vesicaria*, a zygomorphic-flowered Papaveraceae (Ranunculales, basals eudicots). *Annals of Botanny* 109:911-920.

Hilu, K.W., Borsch, T., Müller, K., Soltis, D.E., Soltis, P.S., Savolainen, V., Chase, M.W., Powell, M.P., Alice, L.A., Evans, R., Sauquet, H., Neinhuis, C., Slotta, T.A.B., Rohwer, J.G., Campbell, C.S. and L.W. Chatrou. 2003. *American Journal of Botany* 90:1759-1776.

Hoot, S.B., Kadereit, J.W., Blattner, F.R., Jork, K.B., Schwarzbach, A.E. and P.R. Crane. 1997. Data congruence and phylogeny of the Papaveraceae s.l. Based on four data sets: *atpB* and *rbcL* sequences, *trnK* restriction sites, and morphological characters. *Systematic Botany* 22:575-590.

Hoot, S.B., Zautke, H., Harris, D.J., Crane, P.R. and S.S. Neves. 2009. Phylogenetic patterns in Menispermaceae based on multiple chloroplast sequence data. *Systematic Botany* 34:44-56.

Huang, W.-P., Sun, H., Deng, T., Razafimandimbison, S.G., Nie, Z.-L. and J. Wen. 2013. Molecular phyogenetics and biogeography of the eastern Asian-eastern North American disjunct *Mitchella* and its close relative *Damnacanthus* (Rubiaceae, Mitchelleae). *Botanical Journal of the Linnean Society* 171:395-412.

Inda, L.A., Sanmartin, I., Buerki, S. and P. Catalán. 2014. Mediterranean origin and Eocene-Holocene Old World diversification of meadow fescues and ryegrasses (*Festuca* subgenus *Schenodorus* and *Lolium*) *Journal of Biogeography* 41:600-614.

Kadereit, W.J., Blattner, R.F., Jork, K.B. and A. Schwarzbach. 1994. Phylogenetic analysis of the Papaveraceae s.l. (incl. Fumariaceae, Hypecoceae, and Pteridophyllum) based on morphological characters. *Botanische Jahrbücher für Systematik* 116:361-390.

Kadereit, W.J., Blattner, F.R., Jork, K.B. and A. Schwarzbach. 1995. The phylogeny of the Papaveraceae s.1.: Morphological, geographical and ecological implications. In: Jensen, U. and J.W. Kadereit (eds.), Systematics and evolution of the Ranunculiflorae. *Plant Systematics and Evolution* Suppl. 9:133-145.

Kadereit, W.J. and B.G. Baldwin. 2012. Western Eurasian – western North America disjunct plant taxa: The dry-adapted ends of formerly widespread north temperate mesic lineages- and examples of long-distance dispersal. *Taxon* 61:3-17.

Kalis, A.J. 1979. Papaveraceae. The northwest European pollen flora, 20. *Review of Palaeobotany and Palynology* 28:209-260.

Katifori, E., Alben, S., Cerda, E., Nelson, D.R. and J. Dumais. 2010. Foldable structures and the natural design of pollen grain. *Proceedings of the National Academy of Science* 107:7635-7639

Kim, S.S. and C.J. Douglas. 2013. Sporopollenin monomers biosynthesis in *Arabidopsis*. Journal of Plant Biology 56:1-6.

Kosenko, V.N. 1999. Contributions to the pollen morphology and taxonomy of the Liliaceae. Grana 38:20-30.

Kölsch, A. and S. Gleissberg. 2006. Diversification of CYCLOIDEAlikeTCP genes in the Basal Eudicots Families Fumariaceae and Papaveraceae s.str. *Plant Biology* 8:680-687.

Kress, W.J. and D.E. Stone. 1982. Nature of the sporoderm in monocotyledons, with special reference to the pollen grains of Canna and Heliconia. *Grana* 21:129-148.

Layka, S. 1976. Les méthodes modernes de la palynologie appliqués à l'étude des Papaverales. – Ph.D. diss., l'Université de Montpellier, CNRSAO 12.535.

Le Thomas, A. 1980. Ultrastructural characters of the pollen grains of African Annonaceae and their significance for the phylogeny of primitive angiosperms. Pollen Spores 22:267-342.

Le Thomas, A. 1981. Ultrastructural characters of the pollen grains of African Annonaceae and their significance for the phylogeny of primitive angiosperms. *Pollen Spores* 23:5-36.

Lidén, M. 1986. Synopsis of Fumarioideae with a monograph of the tribe Fumarieae. *Opera Botanica* 88. 136pp.

Lidén, M. 1993a. Pteridophyllaceae. In: Kubitzki, K., Rohwer, J.G., and V. Bittrich, (eds.), *The families and genera of vascular plants*, vol. 2: Florewing plants, dicotyledons: Magnoliid, hamamelid, and caryophyllid families. Springer-Verlag, Heidelberg, Germany.

Lidén, M. 1993b. Fumariaceae. In: Kubitzki, K., Rohwer, J.G., and V. Bittrich (eds.), *The families and genera of vascular plants*, vol. 2: Florewing plants, dicotyledons: Magnoliid, hamamelid, and caryophyllid families. Springer-Verlag, Heidelberg, Germany.

Lidén, M., Fukuhara, T. and T. Axberg. 1995. Phylogeny of *Corydalis*, ITS and morphology. *Plant Systematics and Evolution* Suppl. 9:183-188.

Lidén, M., Fukuhara, T., Rylander, J. and B. Oxelman. 1997. Phylogeny and classification of Fumariaceae, with emphasis on *Dicentra* s.l., based on the plastid gene *rps16* intron. *Plant Systematics and Evolution* 206:411-420.

Linder, H.P. and I.K. Ferguson. 1985. Notes on the pollen morphology and phylogeny of the Restionales and Poales. *Grana* 24:65-76.

Loconte, H., Campbell, L.M. and W.M. Stevenson. 1995. Ordinal and familial relationships of Ranunculid genera. In: Jensen, U. and J.W. Kadereit, (eds.), *Plant Systematic and Evolution* Suppl. 9:99-118.

Mabberley, D.J. 2008. Mabberley's plant-book. A protable dictionary of plants, their classification and uses. Cambridge, Cambridge University Press.

Manafzadeh, S., Salvo, G. and E. Conti. 2014. A tale of migrations from east to west: the Irano-Turanian floristic region as a source of Mediterranean xerophytes. *Journal of Biogeography* 41:366-379.

Mansion, G., Rosenbaum, G., Schoenenberger, N., Bacchetta, G., Rossello, J.A. and E. Conti. 2008. Phylogenetic analysis informed by geological history supports multiple, sequential invasions of the mediterranean basin by the angiosperm family Araceae. *Systematic Biology* 57:269-285.

Mansion, G., Selvi, F., Guggisberg, A. and E. Conti. 2009. Origin of Mediterranean insular endemics in the Boraginales: integrative evidence from molecular dating and ancestral area reconstruction. *Journal of Biogeography* 36:1282-1296.

Mao, K., Hao, G., Liu, J., Adams, R.P. and R.I. Milne. 2010. Diversification and biogeography of *Juniperus* (Cupressaceae): variable diversification rates and multiple intercontinental dispersals. *New Phytologist* 188:254-272.

Marquez, J., Seaone-Camba, J.A. and M. Suárez-Cervera. 1997. The role of the intine and cytoplasm in the activation and germination processes of Poaceae pollen grains. *Grana* 36:328-342.

Milne, R.I. 2006. Northern hemisphere plant disjunctions: a window on tertiary land bridges and climate changes? *Annals of Botany* 98:465-472.

Nasri-Ayachi, M.B. and M.A. Nabli. 2006. Comparative exine ontogeny in some members of the family Zygophyllaceae sensu lato. *Protoplasma* 228:49-53.

Owen, H.A. and C.A. Makaroff. 1995. Ultrastructure of microsporogenesis and microgametogenesis in *Arabidopsis thaliana* (L.) Haynh. Ecotype Wassilewskija (Brassicaceae). *Protoplasma* 185: 7-21.

Payne, W.W. 1972. Observations of harmomegathy in pollen of anthophyta. *Grana* 12:93-98.

Qiu, Y.-L., Lee, J., Bernasconi-Quadroni, F., Soltis, D.E., Soltis, P.S., Zanis, M., Zimmer, E.A., Chen, Z., Savolainen, V. and M.W. Chase. 1999. The earliest angiosperms: evidence from mitochondrial, plastid and nuclear genomes. *Nature* 402:404-407.

Rachele, L.D. 1974. Pollen morphology of the Papaveraceae of the Northeastern United States and Canada. *Bulletin of the Torrey Botanical Club* 101:152-159.

Remizowa, M.V., Sofoloff, D.D., Macfarlane, T.D., Yadav, S.R., Prychid, C.J. and P.J. Rudall. 2008. Comparative pollen morphology in the early-divergent angiosperm family Hydatellaceae reveals variation at infraspecific level. *Grana* 47:81-100.

Romero, A.T. and M.C. Fernández. 2000. Development of exine and apertures in *Fumaria densiflora* DC from the tetrad stage to maturity. In: Harley, M.M., Morton, C.M. and S. Blackmore (eds.), *Pollen and Spores: Morphology and Biology*. Royal Botanic Gardens, Kew, pp 45-56.

Romero, A.T., Salinas, M.J. and M.C. Fernández. 2003. Pollen wall development in *Hypecoum imberbe* (Fumariaceae). *Grana* 42:91-101.

Rowley, J.R. 1995. Are the endexines of pteridophytes, gymnosperms and angiosperms structurally equivalent? *Review of Paleobotany and Palynology* 85:13-34.

Salinas, M.J., Romero, A.T., Blanca, G., De la Herrán, R., Garrido-Ramos, M., Ruíz-Rejón, Morales, C., Ruíz-Rejón, M. and V. Suárez. 2003. Contribution to the taxonomy and phylogeny of *Sarcocapnos* (DC.) (Fumariaceae). *Plant Systematics and Evolution* 237:153-164.

Salvo, G., Ho, S.Y.W., Rosenbaum, G., Ree, R. and E. Conti. 2010. Tracing the temporal and spatial origins of island endemics in the Mediterranean region: a case study from the Citrus family (*Ruta* L., Rutaceae). *Systematic Biology* 59:705-722.

Simpson, D.A., Furness, C.A., Hodkinson, T.R., Muasya, A.M. and M. W. Chase. 2003. Phylogenetic relationship in Cyperaceae subfamily Mapanioideae inferred from pollen and plastid DNA sequence data. *American Journal of Botany*. 90:1071-1086.

Simpson, M.G. 1985. Pollen ultrastructure of the Tecophilaceae. *Grana* 24:77-92.

Soltis, D.E., Soltis, P.S., Chase, M.W., Mort, M.E., Albach, D.C., Zanis, M., Savolainen, V., Hahn, W.H., Hoot, S.B., Fay, M.F., Axtell, M., Swensen, S.M., Prince, L.M., Kress, W.J., Nixon, K.C., and J.S. Farris. Angiosperm phylogeny inferred from 18S rDNA, rbcL, and atpB sequences. Botanical Journal of the Linnean Society 133:381-461.

Stern, K.R. 1962. The use of pollen morphology in the taxonomy of *Dicentra*. *American Journal of Botany* 55:626-628.

Stevens, P.F. 2001 onwards. Angiosperm Phylogeny Website. Version 12, July 2012 [and more or less continuously updated since]. http://www.mobot.org/MOBOT/research/APweb/.

Suárez-Cervera, M. and J.A. Seoane-Camba. 1986. On the exine elasticity in the *Lavandula dentata* L. pollen grains. In: Blackmore, S. and I.K. Ferguson, (eds.), *Pollen and Spores: Form and function*. Linnean Society Symposium Series nº 12. Academic Press, London, pp 409-411.

Thorne, R.F. 1992. Classification and geography of the flowering plants. Botanical review 58:225-348

Urtubey, E. and M.C. Tellería. 1998. Pollen morphology of the subfamily Barnadesioideae (Asteraceae) and its phylogenetic and taxonomic significance. *Review of Palaeobotany and Palynology* 104:19-37.

Van der Ham, R., Zimmerman, Y.-M., Nilsson, S. and A. Igershem. 2001. Pollen morphology and phylogeny of the Alyxieae (Apocynaceae). *Grana* 40: 169-191.

Van der Weide, J.C. and R.W.J.M. van der Ham. 2011. Pollen morphology and phylogeny of the tribe Tabernaemontanae (Apocynaceae, subfamily Rauvolfioideae). *Taxon* 61:131-145.

Vega-Maray, A.M., Fernández-González, D., Valencia-Barrera, R., Seoane-Camba, J.A. and M. Suárez-Cervera. 2003. Ultrastructural modification in the apertural intine of *Parietaria judaica* L. (Urticaceae) pollen during the early stages of hydration. *Grana* 42:220-226.

Wallace, S., Fleming, A., Wellman, C.H. and D.J. Beerling. 2011. Evolutionary development of the plant spore and pollen wall. *AoB Plants* plr027.

Wang, W., Lu, A.M., Ren, Y., Endress, M.E. and Z.D. Chen. 2009. Phylogeny and classification of Ranunculales: evidence from four molecular loci and morphological data. *Perspectives in Plant Ecology, Evolution and Systematics* 11:81-110.

Wen, J. 1999. Evolution of eastern Asia and eastern North American disjunct distributions in flowering plants. *Annuals Review of Ecology and Systematics* 30:421-455.

Wen, J., Ickert-Bond, S.M., Nie, Z.L. and R. Li. 2010. Timing and modes of evolution of eastern Asian-North American biogeographic disjunctions in seed plants. Darwin's Heritage Today: Proceedings of the Darwin 200 Beijing International Conference, Higher Education Press, Beijing. pp.252-269.

Xiang, Q.-Y., Soltis, D.E. and P.S. Soltis. 1998. The Eastern Asian and Eastern and Western North American Floristic Disjunction: Congruent Phylogenetic Patterns in Seven Diverse Genera. *Molecular Phylogenetics and Evolution* 10:178-190.

Xiang, Q.-Y., Soltis, D.E., Soltis, P.S., Manchester, S.R. and D.J. Crawford. 2000. Timing the eastern Asian-eastern North American floristic disjunction: molecular clock corroborates paleontological estimates. *Molecular Phylogenetics and Evolution* 15:462-472.

Xie, L., Yi, T.-S., Li, R., Li, D.-Z. and J. Wen. 2010. Evolution and biogeographic diversification of the witch-hazel genus (*Hamamelis* L., Hamamelidaceae) in the Northern Hemisphere. *Molecular Phylogenetics and Evolution* 56:675-689.

Xie, L. and L-Q. Li. 2012. Variation of pollen morphology, and its implications in the phylogeny of Clematis (Ranunculaceae). *Molecular Phylogenetics and Evolution* 298:1437-1453.

Xu, F.X. and L.P. Ronse de Craene. 2013. Pollen morphology and ultrastructure of selected species of Annonaecae. *Plant Systematics and Evolution* 299:11-24.

Zavada, M.S. and D.L. Dilcher. 1986. Comparative pollen morphology and its relationship to phylogeny of pollen in the Hamamelidae. *Annals of the Missouri Botanical Garden* 73:348-381.

Zhang, M.L, Lidén, M. and C. Grey-Wilson–. 2008. Papaveraceae. In: Zhengy W, Raven PH, Deyuan H, eds. *Flora of China, Vol. 7 (Menispermaceae through Capparaceae).* Science Press, Beijing & Missouri Botanical Garden Press, St. Louis, USA.

Zhuang, X. 1993. The taxonomic and evolution and distribution of Papaveraceae. *Acta Botanica Yunnanica* 15:137-148.

Universidad de Granada