Annals of Botany **98**: 141–155, 2006
doi:10.1093/aob/mcl079, available online at www.aob.oxfordjournals.org

Phylogenetics of *Papaver* and Related Genera Based on DNA Sequences from ITS Nuclear Ribosomal DNA and Plastid *trnL* Intron and *trnL–F* Intergenic Spacers

JAMES C. CAROLAN[1,*], INGRID L. I. HOOK[2], MARK W. CHASE[3], JOACHIM W. KADEREIT[4] and TREVOR R. HODKINSON[1]

[1]*Department of Botany, School of Natural Sciences, and* [2]*Department of Pharmacognosy, School of Pharmacy, University of Dublin, Trinity College, Dublin 2, Ireland,* [3]*Jodrell Laboratory, Royal Botanic Gardens, Kew, Richmond, Surrey TW9 3DS, UK and* [4]*Institut für Spezielle Botanik, Johannes Gutenberg-Universität, D-55099 Mainz, Germany*

Received: 23 October 2005 Returned for revision: 6 January 2006 Accepted: 26 February 2006 Published electronically: 4 May 2006

• *Background and Aims* Representatives from *Papaver*, *Roemeria*, *Stylomecon* and *Meconopsis* were studied to elucidate phylogenetic relationships between *Papaver* and these closely allied genera.
• *Methods* Two molecular data sets were used individually and combined and included sequences from the internally transcribed spacer region (ITS) of 18S–26S nuclear ribosomal DNA and the *trnL* intron and the *trnL–trnF* intergenic spacer region of plastid DNA.
• *Key Results* Parsimony analysis demonstrated that the genus is not monophyletic unless the closely related *Roemeria*, *Stylomecon* and *Meconopsis cambrica* are included in a revised circumscription of *Papaver*. Three distinct clades are resolved in a combined ITS and *trnL–F* analysis. Clade 1 consists of *Papaver* sect. *Meconella* and Asian *Meconopsis*. Clade 2 contains a group here identified as *Papaver s.s.*, comprising sections *Carinatae*, *Meconidium*, *Oxytona*, *Papaver*, *Pilosa*, *Pseudopilosa* and *Rhoeadium.* Clade 3 consists of *Papaver* sect. *Argemonidium* and *Roemeria refracta.* A number of diagnostic indels support these groupings. Within clade 2, sects. *Papaver* and *Rhoeadium* are either not monophyletic or lack evidence supporting their monophyly.
• *Conclusions* The results of this molecular analysis indicate that a number of morphological characters such as valvate capsule dehiscence, dark or light filaments and sessile stigmatic discs have arisen in parallel. The phylogenetic trees are incongruent with the existing taxonomy of *Papaver*, and a revised classification is suggested.

Key words: DNA, *Meconopsis*, nrITS, *Papaver*, phylogenetics, *Roemeria*, *Stylomecon*, *trnL–F*.

INTRODUCTION

Papaver L. is the largest genus of subfamily Papaveroideae of Papaveraceae *sensu* Kadereit (1993*a*). Based on morphological considerations, subfamily Papaveroideae, including subfamily Platystemonoideae, can be divided into two major clades (Kadereit *et al*., 1997; Schwarzbach and Kadereit 1999). These are a New World clade containing *Arctomecon* Torr. et Frem., *Argemone* L., *Romneya* Harv., *Canbya* Parry, *Platystemon* Benth., *Meconella* Nutt. and *Hesperomecon* Greene, and a largely Old World clade with *Papaver*, *Meconopsis* Vig., *Stylomecon* Benth. and *Roemeria* Medic. The latter group shares characters such as semicampyltropous ovules and a seed coat with a fine layer of crystals. Additionally, meconic acid is found only in species of these four genera (Cordell, 1981). Determining relationships among these four genera of Papaveroideae is the primary focus of this paper.

Papaver consists of approximately 80 annual, biennial and perennial herbs distributed in central and south-western Asia, central and southern Europe and northern Africa (Kadereit, 1988*a*). *Papaver* sect. *Meconella* has a panarctic–alpine distribution that includes north-eastern North America. *Papaver aculeatum* Thunb. (sect. *Horrida*) is indigenous to South Africa, and *P. californicum* A. Gray (sect. *Californicum*) is indigenous to western North America. *Papaver* is characterized by the absence of a style and the possession of stigmatic tissue arranged radially on a sessile stigmatic disc crowning the ovary. The latest taxonomic revision of *Papaver* (Kadereit, 1988*a*) recognized 11 sections (*Argemonidium* Spach.; *Carinatae* Fedde; *Californicum* Kadereit; *Horrida* Elk.; *Oxytona* Bernh.; *Meconidium* Bernh.; *Meconella* Spach; *Papaver* L.; *Pilosa* Prantl; *Pseudopilosa* Gunther; *Rhoeadium* Bernh.). Detailed taxonomic accounts of many of the sections have been published (Goldblatt, 1974; Kadereit, 1986*a*, *b*, 1987, 1988*b*, *c*, 1989, 1993*b*, 1996). The separation of species into sections is based on a combination of characters, including mode of capsule dehiscence (through valves or pores), colour of anthers and filaments (pale or dark), and general capsule characteristics such as size, shape and indumentum. Based on these characters, Kadereit (1988*a*) recognized four groups of sections within *Papaver.* The first group consists of sects. *Californicum*, *Meconella* and *Meconidium* and is characterized by pale filiform filaments and anthers, and valvate capsule dehiscence. The second group consists of sect. *Argemonidium* alone and is characterized by dark clavate filaments and anthers and poricidal capsule dehiscence. The third group comprises sects. *Horrida*, *Pilosa* and *Pseudopilosa* and is characterized by pale filiform filaments and anthers and poricidal capsule dehiscence. Finally, group four comprises sects. *Carinatae*, *Oxytona*, *Papaver* and *Rhoeadium* and is

* For correspondence. Present address: School of Biological and Environmental Science, University College Dublin, Dublin 4, Ireland. E-mail james.carolan@ucd.ie

characterized by dark (sometimes pale) filiform (sometimes clavate) filaments and always dark anthers and poricidal capsule dehiscence.

Meconopsis comprises approximately 50 perennial monocarpic or polycarpic herbs, distributed primarily in southern central Asia. *Meconopsis cambrica* (L.) Vig. is the only European representative of the genus. *Meconopsis* is considered to be distinct from *Papaver* based on the possession of stigmatic tissue borne on top of a style (although species without styles do exist). *Roemeria* comprises three annual species distributed mainly in southwestern and central Asia and Europe. It has long, linear, bristly capsules with sessile stigmas borne directly on top of the ovary. *Stylomecon* is a monotypic genus comprising the annual *S. heterophylla* (Benth.) G. Taylor native to western North America and is characterized by the possession of stigmatic tissue borne on top of a style. Although it is similar to *Meconopsis* in capsule characteristics, it is recognized as a distinct genus primarily based on its annual habit and geographical distribution (Taylor, 1930; Kadereit *et al*., 1997).

Delimitation of taxa into their respective genera (*Papaver*, *Meconopsis*, *Stylomecon*, *Roemeria*) seems straightforward based on the distinction of capsule characteristics. Previous molecular phylogenetic analyses of these genera (Kadereit and Sytsma, 1992; Kadereit *et al*., 1997), however, demonstrated that they form a monophyletic group within Papaveroideae (Kadereit, 1993*a*) and provided evidence that *Papaver sensu* Kadereit (1988*a*) is not monophyletic. These molecular analyses included a restriction site analysis of plastid DNA (Kadereit and Sytsma, 1992) and an RFLP analysis of the plastid *trnK* region (Kadereit *et al*., 1997). It was demonstrated that *Roemeria* was sister to *P*. sect. *Argemonidium* and *Stylomecon* was sister to *P*. sect. *Californicum*, indicating that *Papaver* was monophyletic only if these genera were included in *Papaver*. In addition, the European *Meconopsis cambrica* did not group with the Asian species of this genus. *Meconopsis cambrica* resolved as sister to a group of sections of *Papaver* including *Carinatae*, *Meconidium*, *Oxytona*, *Papaver*, *Pilosa*, *Pseudopilosa* and *Rhoeadium*, leading Kadereit *et al*. (1997) to view these sections as *Papaver s.s.* Determining the interrelationships of these sections was limited by the small number of species sampled in their study. Generally, only a single species was used to represent sections, and single individuals were used to represent species. The nonmonophyly of *Papaver s.l.* indicates that the stigmatic disc typical for the genus may have arisen several times independently. To define *Papaver* based on a single character that has multiple origins would be taxonomically and phylogenetically unsound. The results of these molecular analyses also demonstrated that some of the infrageneric taxonomic groupings suggested by Kadereit (1988*a*) were artificial.

The objective of this paper is to examine phylogenetic relationships within *Papaver* and allied genera by comparing nucleotide sequences obtained from plastid and nuclear ribosomal sequences. The two molecular regions used were the internally transcribed spacer region (ITS) of 18S–26S nuclear ribosomal DNA (Sun *et al*., 1994; Baldwin *et al*., 1995) and the *trnL* intron and the *trnF* intergenic spacer region of plastid DNA (Taberlet *et al*., 1991). All regions are relatively small in size (i.e. *trnL–trnF* ~500–900 bp; and ITS ~700 bp), which facilitates successful amplification and sequencing (Taberlet *et al*., 1991; Baldwin *et al*., 1995; Kelchner, 2000).

Combining sequences from different genomes (nuclear, plastid and mitochondrial) is common in molecular phylogenetics, as long as they produce congruent results, and has resulted in greater understanding of relationships within a wide range of plant groups (see Savolainen and Chase, 2003). Both DNA regions used here have proven useful at similar taxonomic levels in other plant groups (e.g. Gielly *et al*., 1994; Sun *et al*., 1994; Baldwin *et al*., 1995; Wendel *et al*., 1995; Gielly and Taberlet, 1996; Wendel and Doyle, 1998; Kelchner, 2000; Hodkinson *et al*., 2002). However, these DNA regions and their subsequent combination have not been applied to *Papaver* phylogenetics. The topology of the trees obtained here from the comparative analysis of the ITS and *trnL–F* regions is interpreted in terms of morphological, chemotaxonomic and geographical similarities.

MATERIALS AND METHODS

Specimens

Material was obtained from various botanical gardens and commercial sources and grown to maturity either at the National Botanic Garden, Glasnevin, Ireland, or in the glasshouse of the Department of Pharmacognosy, University of Dublin, Trinity College, Ireland. DNA obtained from herbarium material was also used. Voucher specimens were kept for each accession and stored in the Herbarium of the Department of Botany, Trinity College Dublin, Ireland (TCD). DNA was stored at the Department of Botany, TCD, DNA Bank. Voucher specimens for each accession and sequences obtained from GenBank are listed in Table 1.

Outgroup selection

Outgroup taxa were selected on the basis of the plastid DNA restriction site analysis of Kadereit and Sytsma (1992) and the morphological work of Kadereit (1993*a*). *Eomecon chionantha* Hance (Papaveraceae subfamily Chelidonioideae) was chosen as an outgroup taxon owing to its position as given in previous studies. *Argemone mexicana* L. (Papaveraceae subfamily Papaveroideae) and *Chelidonium majus* L. (Papaveraceae subfamily Chelidonioideae) were also included as outgroups.

DNA extraction

DNA was extracted from 0·5–1·0 g of fresh leaf material using a modified 2 % CTAB procedure of Doyle and Doyle (1987), precipitated using 100 % ethanol or isopropanol for at least 48 h at −20 °C, pelleted and washed with 70 % ethanol and purified via the Concert™ Rapid PCR Purification System (Life Technologies, Gaithersburg, MD, USA).

TABLE 1. *Species and associated voucher specimens used in the study*

Taxon	GenBank number ITS: *trnL–F*	ID number*	Voucher or reference
Argemone mexicana L.	AY328303·1; AY328248·1		Y. M. Yuan *et al*., unpubl.
Chelidonium majus L.	AY328251·1; AY328308·1		Y. M. Yuan *et al*., unpubl.
Eomecon chionantha Hance	AY328254·1; AY328306·1		Y. M. Yuan *et al*., unpubl.
Meconopsis aculeata Royle	AY328263·1; AY328227·1		Y. M. Yuan *et al*., unpubl.
Meconopsis betonicifolia Franch.	DQ250323; DQ251174	032	1998·0451
Meconopsis betonicifolia Franch	AY328236·1; AY328292·1		Y. M. Yuan *et al*., unpubl.
Meconopsis cambrica L.	DQ250277; DQ251128	001	2000·0001
Meconopsis cambrica L.	DQ250278; DQ251129	048	1992·0611
Meconopsis delavayi Franch. ex Prain	AY328211·1; AY328285·1		Y. M. Yuan *et al*., unpubl.
Meconopsis lancifolia Franch. ex Prain	AY328212·1; AY328282·1		Y. M. Yuan *et al*., unpubl.
Papaver aculeatum Thunb.	DQ250317; DQ251168	131	2000·0131
Papaver aculeatum Thunb	DQ250316; DQ251167	151	2000·0659
Papaver alpinum spp. *rhaeticum* Mgf.	DQ250261; DQ251112	150	2000·0150
Papaver alpinum spp. *alpinum* L.	DQ250268; DQ251119	102	2000·0568
Papaver anomalum Fedde	DQ250263; DQ251116	106	2000·1321
Papaver anomalum 'album' Fedde	DQ250264; DQ251115	078	2000·1806
Papaver apulum Ten.	DQ250300; DQ251151	084	2000·0601
Papaver argemone L.	DQ250298; DQ251149	153	2000·0153
Papaver armeniacum ssp. *armeniacum* L.	DQ250302; DQ251153	154	2000·1717
Papaver armeniacum ssp. *armeniacum* L.	DQ250297; DQ251148	095	2000·0604
Papaver armeniacum ssp. *armeniacum* L.	DQ250311; DQ251162	087	2000·0651
Papaver armeniacum ssp. *armeniacum* L.	DQ250312; DQ251163	082	2000·0795
Papaver armeniacum ssp. *armeniacum* L.	DQ250259; DQ251110	103	2000·0793
Papaver armeniacum ssp. *armeniacum* L.	DQ250294; DQ251145	107	2000·0107
Papaver atlanticum Ball et Cross.	DQ250307; DQ251158	092	2000·0603
Papaver atlanticum Ball et Cross.	DQ250315; DQ251166	099	2000·0615
Papaver atlanticum Ball et Cross.	DQ250293; DQ251144	077	2000·0472
Papaver atlanticum Ball et Cross.	DQ250303; DQ251154	156	2000·0156
Papaver bracteatum Lindl.	DQ250286; DQ251137	028	2000·0028
Papaver bracteatum Lindl.	DQ250287; DQ251138	031	2000·0031
Papaver californicum A.Gray	DQ250318; DQ251169	170	2000·0170
Papaver croceum Ledeb.	DQ250258; DQ251109	015	1999·00340
Papaver croceum Ledeb.	DQ250257; DQ251108	104	2000·0104
Papaver croceum Ledeb.	DQ250266; DQ251117	105	2000·0653
Papaver croceum Ledeb.	DQ250284; DQ251135	148	AS 95/23 Kadereit *et al*., 1996
Papaver commutatum Fisch et Mey.	DQ250313; DQ251164	102	2000·0605
Papaver dubium ssp. *dubium* L.	DQ250270; DQ251121	024	2000·0024
Papaver dubium ssp. *dubium* L.	DQ250319; DQ251170	162	2000·1706
Papaver dubium L. ssp. *erosum* (Litv.) Kadereit	DQ250271; DQ251122	168	2000·0610
Papaver dubium ssp. *lecoquii* Syme	DQ250322; DQ251173	174	2000·0606
Papaver dubium L. ssp. *lecoquii* (Lamotte) Syme var. *albiflorum* Besser	DQ250267; DQ251118	100	2000·0600
Papaver glaucum Boiss. et Hausskn	DQ250310; DQ251161	089	2000·0609
Papaver glaucum Boiss. et Hausskn	DQ250309; DQ251160	177	2000·0177
Papaver glaucum Boiss. et Hausskn	DQ250308; DQ251159	189	2000·0189
Papaver hybridum L.	DQ250301; DQ251152	167	1999·1723
Papaver miyabeanum Tatew.	DQ250276; DQ251127	019	1999·0339
Papaver miyabeanum Tatew.	DQ250265; DQ251116	186	2000·0186
Papaver macrostomum Boiss. et Huet	DQ250275; DQ251126	160	RBGE 34139
Papaver nudicaule ssp. *nudicaule* L.	DQ250260; DQ251111	086	2000·0598
Papaver orientale L.	DQ250292; DQ251143	011	2000·0011
Papaver orientale L.	DQ250289; DQ251140	035	2000·0035
Papaver orientale L.	DQ250290; DQ251141	135	2000·0135
Papaver orientale L.	DQ250291; DQ251142	179	RBGE 19880542A
Papaver pavonium Fischer & Meyer ssp. *pavonium*	DQ250283; DQ251134	138	2000·0138
Papaver pilosum ssp. *strictum* Wendt ex Kadereit	DQ250321; DQ251172	081	2000·0768
Papaver pilosum Sibth. & Sm. ssp. *pilosum* Wendt	DQ250320; DQ251171	182	2000·0182
Papaver pseudo-orientale (Fedde) Medv.	DQ250269; DQ251120	014	2000·0014
Papaver pseudo-orientale (Fedde) Medv.	DQ250288; DQ251139	039	2000·0039
Papaver pseudo-orientale (Fedde) Medv.	DQ250296; DQ251147	093	2000·0632
Papaver pseudo-orientale (Fedde) Medv.	DQ250285; DQ251136	139	2000·0794
Papaver radicatum Rottb.	DQ250262; DQ251113	094	2000·0769
Papaver rhoeas L.	DQ250272; DQ251123	090	2000·0090
Papaver rhoeas L.	DQ250273; DQ251124	147	KJ93/7 Kadereit *et al*., 1996
Papaver rupifragum Boiss et Reut.	DQ250314; DQ251165	017	1999·0342
Papaver somniferum ssp. *setigerum* (DC.) L.Corb	DQ250279; DQ251130	016	2000·0016
Papaver somniferum L.	DQ250281; DQ251132	052	2000·0052
Papaver somniferum L.	DQ250305; DQ251156	063	2000·0063

TABLE 1. *Continued*

Taxon	GenBank number ITS: *trnL–F*	ID number*	Voucher or reference
Papaver somniferum L.	DQ250280; DQ251131	130	2000·0130
Papaver somniferum L.	DQ250304; DQ251155	166	2000·0166
Papaver somniferum ssp. *somniferum* L.	DQ250282; DQ251133	068	2000·0068
Papaver somniferum ssp. *somniferum* L.	DQ250306; DQ251157	142	2000·0142
Roemeria refracta DC.	DQ250299; DQ251150	171	2000·0171
Stylomecon heterophylla Taylor	DQ250295; DQ251146	183	2000·0183

Vouchers are deposited in the Herbarium of Trinity College Dublin (TCD).
* The ID number represents the identification number used in this study and to differentiate taxa with the same name.

DNA was then stored in TE buffer (10 mM Tris/HCl, 1 mM EDTA, pH 8·0) at −80 °C until required.

DNA sequencing

For amplification and sequencing of the ITS region the forward and reverse primers of Sun *et al*. (1994) were used. The *trnL* intron and the *trnL–trnF* spacer (hereafter the *trnL–F* region) were amplified and sequenced as one segment using primers 'c' and 'f' of Taberlet *et al*. (1991). Difficulties were encountered when attempting to amplify certain sequence regions from herbarium specimens. Their successful amplification and sequencing was achieved using the internal primers (2 and 3) for ITS of Baldwin *et al*. (1995) and the internal primers (d and e) for the *trnL* intron and the *trnL–F* region (Taberlet *et al*., 1991). PCRs for both regions were carried out in 50-μL reactions using 1 % PCR buffer (Promega, Madison, WI, USA), 2·5 mM $MgCl_2$, 0·2 μM of each primer, 0·2 mM of each dNTP, 1 U *Taq* polymerase (Promega) and approximately 50 ng template DNA. Reaction conditions for the *trnL–F* region were: denaturation at 94 °C for 3 min followed by 30 cycles of 1 min at 94 °C, 1 min at 51 °C, 1 min at 72 °C and a final extension at 72 °C for 7 min in a Peltier thermal cycler (PTC 200; MJ Research). PCR amplification of the ITS region was achieved using a touchdown PCR strategy involving denaturation at 94 °C for 3 min followed by 30 cycles of 1 min at 94 °C, 1 min at 60–52 °C (over the first eight cycles with the remaining cycles at 52 °C), 1 min at 72 °C and a final extension at 72 °C for 7 min. Successfully amplified DNA fragments were purified using the Concert™ Rapid PCR Purification System (Life Technologies) and sequenced using Big Dye Terminator Cycle Sequencing Kits v1.1 (Applied Biosystems, Foster City, CA, USA) on an Applied Biosystems 310 or 377 automated DNA sequencer, all according to the manufacturer's protocols and with the same primers as used for the initial amplification.

Sequence analysis and phylogenetic reconstruction

Forward and reverse sequence reads were assembled using Sequencher™ version 3.1 (Gene Codes Corporation, 1998) to obtain a contiguous sequence for the target DNA region. Consensus sequences for all accessions were imported into SE-Al v2.0 (sequence-alignment, Rambaut, 2001) in which sequences were aligned by inserting gaps manually within the data matrix following the guidelines of Kelchner (2000). The aligned matrix was imported into PAUP v4.0b for phylogenetic analysis (Swofford, 2003). Gaps were treated as missing data. Regions of the sequence alignment that contained a substantial number of alignment gaps were omitted from the analyses because the positional homology within these regions is uncertain (Swofford *et al*., 1996). Omitted regions included 12-, 53- and 38-bp hypervariable regions of the ITS aligned matrix (corresponding to positions 138–150, 240–293 and 478–516, respectively) and the initial 14 and final 65 nucleotides of the *trnL–F* aligned matrix. Independent phylogenetic analysis of the *trnL* intron and the *trnL–trnF* spacer regions yielded broadly congruent trees (results not shown). If incongruence was found it was not supported by bootstrap analysis (soft incongruence; Seelanen *et al*., 1997). For the purposes of this study both regions were combined for parsimony analysis. These are part of the non-recombining plastid genome and are frequently combined for phylogenetic reconstruction (e.g. Hopper *et al*., 1999; Chase *et al*., 2000; Hodkinson *et al*., 2002) because they should have the same phylogenetic history.

Maximum parsimony (MP) trees were obtained from the resulting matrices using heuristic search options. Searches included 1000 replicates of random addition sequence (saving no more than 30 trees per replicate to reduce time spent swapping large islands of trees) with the tree bisection reconnection (TBR) branch-swapping algorithm and MulTrees on (keeping multiple equally most-parsimonious trees). Internal support was assessed using 1000 bootstrap replicates (Felsenstein, 1985), simple addition sequence, TBR swapping and MulTrees on (holding 30 trees per replicate; see Salamin *et al*., 2003). Groups with bootstrap percentages (BP) of 90–100 were considered to be strongly supported, 80–89 moderately supported and 50–79 weakly supported. Only groups with BP >50 that are consistent with the strict consensus tree are shown.

No major conflicts (hard incongruence) between the separate trees were identified between single-region analyses. Accordingly, the *trnL–F* data were joined with the ITS data in a combined analysis. The incongruence length difference (ILD) test (or similar congruence tests) was not applied as this can be ineffective in identifying combinability of data and in some cases has been shown to be misleading (Yoder *et al*., 2001). Our decision to combine was based on the pattern of major clades and their respective bootstrap

percentages. The combined analysis of *trnL–F* and ITS data was also performed using the same parameters as stated above for the single gene region analyses.

RESULTS

Analysis of ITS

The lengths of ITS1, ITS2 and 5·8S were confirmed using a comparative alignment of *Papaver rhoeas* ITS1, ITS2 and 5·8S obtained from GenBank (Schwarzbach and Kadereit, 1999; accession no. AF098920). The 5·8S region ranged from 157 to 169 bp across all accessions used in this study. Relatively little variation was encountered within the 5·8S region, with only 10 of the 171 nucleotides (5·8 % of the final aligned matrix) being variable, but all were potentially parsimony-informative. The ITS1 and ITS2 spacers ranged in length from 218 to 260 bp and from 216 to 257 bp, respectively. A considerable proportion of both regions were variable. Of the 189 variable sites within the aligned ITS1 (66·5 % of the aligned ITS1 region), 152 were potentially parsimony-informative. Of the 171 variable sites found within the aligned ITS2 (50·4 % of the ITS2 region) 128 were potentially parsimony-informative. The G+C content of both ITS1 and ITS2 ranged from 50·5 to 60·2 % and 55·3 to 63·8 %, respectively. Independent analysis of the ITS1 and ITS2 spacers yielded broadly congruent trees (results not shown; Carolan, 2004). For the purposes of this study both regions plus the 5·8S gene were combined for parsimony analysis. The entire aligned ITS matrix (ITS1, 5·8S and ITS2) was 705 bp long; 324 sites were variable, and 235 of these were potentially parsimony-informative. Figure 1 shows one of 151 equally most-parsimonious trees from the ITS analysis. It has 826 steps, with a consistency index (CI) of 0·57 and a retention index (RI) of 0·83.

Two distinct clades were found within the ITS tree (Fig. 1). Clade 1 comprises sects. *Argemonidium*, *Meconella* and the Asian representatives of *Meconopsis.* This clade is sister to all other sections of *Papaver* in all equally most-parsimonious trees but is itself weakly supported with only 56 % bootstrap support (bootstrap percentage; BP). *Roemeria refracta* groups with the species of sect. *Argemonidium* (97 BP). Within clade 1, sect. *Meconella* forms a well-supported group (98 BP). The Asian representatives of *Meconopsis* are resolved as sister to sect. *Meconella* in all equally most-parsimonious trees (83 BP).

Clade 2 (53 BP) comprises the remaining sections of *Papaver*, including *Meconopsis cambrica* and *Stylomecon heterophylla*. *Papaver aculeatum* (sect. *Horrida*) and a group comprising sect. *Californicum* and *Stylomecon heterophylla* resolve independently but sister to the remaining sections of clade 2 (53 and 69 BP, respectively). The positioning of *Stylomecon heterophylla* as sister to *P. californicum* is well supported (98 BP). *Meconopsis cambrica* and the remaining sections of *Papaver* form a well-supported group (97 BP). Sections *Papaver* and *Rhoeadium* are not monophyletic in this tree, as indicated by the grouping of *Papaver glaucum* (sect. *Papaver*) with representatives of sect. *Rhoeadium* (including sect. *Carinatae*; <50 BP) and the grouping of *P. dubium* ssp. *erosum* (sect. *Rhoeadium*) with *Papaver somniferum* (sect. *Papaver*; 88 BP). However, there is also little evidence contradicting their monophyly. Within clade 2, sect. *Pseudopilosa* is characterized by the possession of a number of unique indels. These include a 4-bp indel at positions 75–78 (A, Fig. 1; Table 2) and a 4-bp indel at positions 216–219 (B, Fig. 1; Table 2) of the aligned ITS matrix. Omitting these indels (gapped sites) from the analysis did not affect the sister group position of *Pseudopilosa* (with respect to the majority but not all of clade 2).

Analysis of trnL–F

The total lengths of the *trnL* intron and the *trnL–F* spacer were confirmed using a comparative alignment of the *Meconopsis betonicifolia trnL* intron, *trnL–F* spacer and the 3′ *trnL* exon sequence obtained from GenBank (Y. M. Yuan *et al*., unpubl. data, Zhongshan University, P. R. China; accession AY328263). Little variation was encountered within the 3′ *trnL* exon region (50 bp long, including one parsimony informative character). The unaligned *trnL* intron and *trnL–F* spacer regions ranged in length from 467 to 505 bp and 384 to 422 bp, respectively. The final aligned matrix had a total length of 951 characters (539, 50 and 362 sites for the *trnL* intron, the 3′ *trnL* exon and the *trnL–F* spacer, respectively). The 128 variable sites found within the aligned *trnL* intron (representing 23·7 % of the *trnL* intron) consisted of 74 potentially parsimony-informative characters, and the *trnL–F* spacer contained 165 variable characters (representing 45·5 % of the spacer), of which 103 were potentially parsimony informative. The G+C content of both the *trnL* intron and the *trnL–F* spacer ranged from 32 to 36·5 % and 33·6 to 41·4 %, respectively. In total the aligned *trnL–F* matrix was 951 bp long; 294 sites were variable, and 176 of these were potentially parsimony informative. Phylogenetic analysis of the *trnL–F* matrix produced eight equally most-parsimonious trees (468 steps, CI = 0·77, RI = 0·91; Fig. 2).

Three main clades are present in the *trnL–F* trees, which (Fig. 2) are broadly congruent with the ITS analysis. The separation of *P.* sect. *Argemonidium* and *Roemeria refracta* (clade 3, 74 BP) and *Meconella* (clade 1, 100 BP) from the main group containing the remaining sections of *Papaver* (clade 2, 69 BP) is evident. Section *Argemonidium* is well supported (100 BP). The Asian representatives of *Meconopsis* (excluding *M. aculeata*) form a well-supported group (90 BP) and are sister to representatives of sect. *Meconella* (55 BP). Members of sect. *Meconella* possessed two characteristic 4-bp indels at positions 162 and 261 (D and F, Fig. 2; Table 2), which are also present in the outlying sections of clade 2, such as *P.* sects. *Californicum*, *Horrida*, *Stylomecon heterophylla* and Asian *Meconopsis*. *Papaver argemone*, *P. apulum* and *P. hybridum* share a 10-bp deletion at positions 644–653 (G, Fig. 2; Table 2), which is not found in *P. pavonium*.

The remainder of the sections form a weakly supported group (clade 2; 69 BP). *Papaver* sects. *Horrida*, *Californicum* and *Meconopsis cambrica* are resolved independently

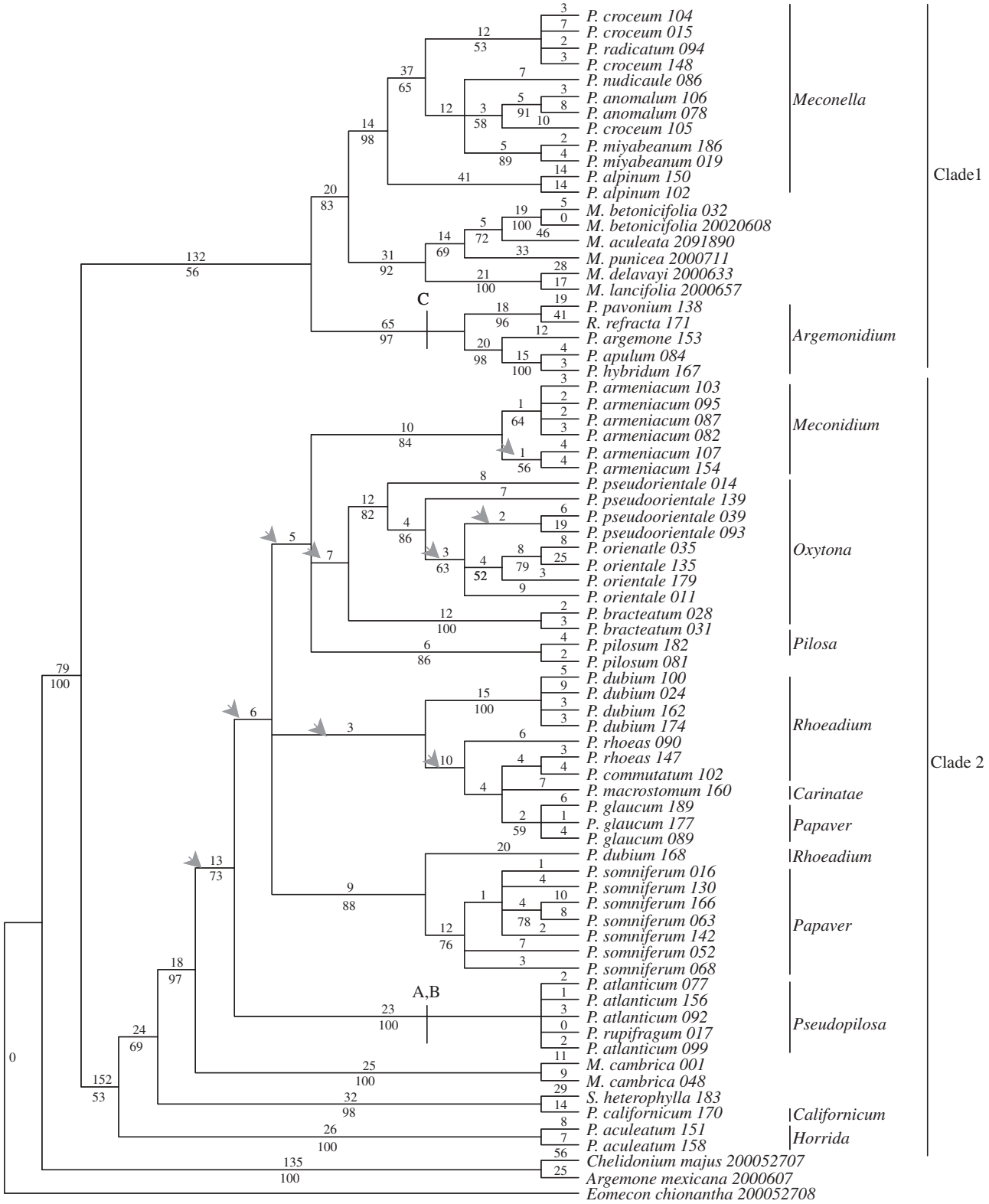

FIG. 1. One of 151 equally most-parsimonious trees generated from the ITS sequences. Support for each node is represented by bootstrap percentages (BP) below the branch (shown only when >50 % and consistent with the strict consensus tree). An arrow indicates clades that did not appear in the strict consensus tree. Numbers above each branch indicate the numbers of character changes along each lineage (accelerated transformation, ACCTRAN, optimization). Groups that possess characteristic indels are indicated using letters (refer to Table 2).

TABLE 2. *Insertions/deletions in the ITS and* trnL–F *regions for representatives used in this study*

Region and start points	Diagnostic indel or sequence	Tree annotation	Taxon
ITS 75	TATA indel	A	Sect. *Pseudo-pilosa*
ITS 216	TCTC indel	B	Sect. *Pseudo-pilosa*
ITS 695	T indel	C	Sect. *Argemonidium*; *Roemeria refracta*
trnL–F 162	TATA indel	D	Sects. *Californicum; Horrida; Meconella; Asian Meconopsis; Stylomecon heterophylla*
trnL–F 186	TAGAG intel	E	*Papaver commutatum*; *P. dubium* ssp. *erosum*; *P. glaucum*; *P. macrostomum*; *P. rhoeas*
trnL–F 261	GCCC indel	F	Sects. *Californicum*; *Horrida*; *Meconella*; Asian *Meconopsis*; *Stylomecon heterophylla*
trnL–F 643	10-bp deletion	G	Sect. *Argemonidium* (excluding *P. pavonium*)

Start points of the indel are based on the aligned matrix for that given region. Characters mapped onto phylogenetic trees are given as letters.

but sister to the main group in clade 2 with similar topologies to the ITS trees. *Papaver californicum* and *Stylomecon heterophylla* form a well-supported group (100 BP). Within clade 2, a group comprising sects. *Carinatae*, *Meconidium*, *Oxytona*, *Papaver*, *Pilosa*, *Pseudopilosa* and *Rhoeadium* was resolved (97 BP). Section *Oxytona* groups with sect. *Meconidium* (58 BP), with sect. *Pilosa* as sister to these (92 BP). The sampled species of section *Pseudopilosa* are well supported (99 BP). The members of sect. *Rhoeadium* do not form a monophyletic group; *P. dubium* (excluding *P. dubium* ssp. *erosum*) groups more closely with *Papaver somniferum* (sect. *Papaver*) than other members of sect. *Rhoeadium* (83 bp). In addition, *P. dubium* (excluding *P. dubium* ssp. *erosum*) does not possess a 5-bp indel at positions 186–190 (E, Fig. 2; Table 2) that the other members of sect. *Rhoeadium* share. *Papaver glaucum* groups within the main *P. rhoeas* clade (83 BP) and shares the indel (E, Fig. 2) with these *Rhoeadium* species.

Analysis of combined ITS and trnL–F

The combined *trnL–F* and ITS matrix was 1659 bp long. Parsimony analysis of the matrix generated eight equally most-parsimonious trees of 1332 steps with a CI of 0·63 and an RI of 0·84 (Fig. 3). The combination of the ITS and *trnL–F* data sets showed increased bootstrap support for the majority of groupings compared with those found in the individual analyses. Three clades are resolved. Clade 1 (90 BP) comprises *P.* sect. *Meconella* (100 BP) and Asian *Meconopsis* (99 BP). Clade 2 (81 BP) comprises the remaining sections of *Papaver*, *Meconopsis cambrica* and *Stylomecon heterophylla*. Section *Horrida* (100 BP) is sister to the rest of clade 2. The single representative of sect. *Californicum* (*P. californicum*) shares a close affinity with *Stylomecon heterophylla* (100 BP). Within clade 2, the main group of sections (*Carinatae*, *Meconidium*, *Oxytona*, *Papaver*, *Pilosa*, *Pseudopilosa* and *Rhoeadium*) is evident and well supported (99 BP). Of these, sect. *Pseudopilosa* is most divergent and monophyletic within *Papaver* (100 BP). Support for the positioning of *Meconopsis cambrica* as sister to the core sections of clade 2 and its separation from the other representatives of *Meconopsis* increased to 99 BP in comparison with 97 BP in the ITS tree and 58 BP in the *trnL–F* tree.

Sections *Meconidium* (99 BP), *Oxytona* (98 BP) and *Pilosa* (94 BP) form a well-supported clade (84 BP). Sections *Papaver* and *Rhoeadium* are not monophyletic. *Papaver glaucum* (sect. *Papaver*) groups with species of sects. *Rhoeadium* and *Carinatae* (56 BP; *P. commutatum*, *P. dubium* ssp. *erosum*, *P. macrostomum* and *P. rhoeas*) and not with the other representatives of sect. *Papaver*. Finally, clade 3 comprises sect. *Argemonidium* plus *Roemeria refracta* (100 BP).

DISCUSSION

Phylogenetics of Papaver *and related genera*

The combination of nuclear ribosomal ITS and plastid *trnL–F* nucleotide sequences in a phylogenetic analysis resulted in well-resolved and well-supported trees. Three main lineages can be identified (clades 1, 2 and 3; Fig. 3). The results also show that *Papaver* is only monophyletic if *Roemeria*, *Stylomecon heterophylla* and *Meconopsis cambrica* are included in this genus. This is consistent with the molecular studies of Kadereit and Sytsma (1992) and Kadereit *et al*. (1997). Evidently, the topologies and major groupings of the phylogenetic trees produced in this analysis are incongruent with the generally accepted definitions of these closely interrelated genera. The major groupings found in this analysis are discussed below and interpreted in light of their morphology and biogeography.

Papaver *sect.* Argemonidium *and* Roemeria. Kadereit (1986*a*) revised *Papaver* sect. *Argemonidium* and concluded that it contains four annual, half-rosette species, *P. apulum*, *P. argemone*, *P. hybridum* and *P. pavonium*. *Papaver apulum*, *P. argemone* and *P. pavonium* are closely related and occur allopatrically from around the Adriatic Sea through Turkey–Iran to the Himalayas. The fourth species, *P. hybridum*, occupies a wide range from the Macaronesian Islands towards the Himalayas (Kadereit, 1986*a*, 1988*a*). The four species of this section are well differentiated in capsule and petal characters (Kadereit, 1986*a*) but are clearly closely related to each other as demonstrated by the groupings within the molecular phylogenetic trees (clade 3; 97 BP; Fig. 3). Within sect. *Argemonidium*, *P. apulum* and *P. hybridum* are sister species in both the ITS and the *trnL–F* analyses. In all analyses sect. *Argemonidium* is distinct from the other sections of *Papaver* and has characteristic indels (Table 2). The molecular distinctness of sect. *Argemonidium* is also supported by morphological differences (Fedde, 1909; Ernst, 1962; Cullen, 1965; Kadereit, 1986*a*; Markgraf, 1958), which

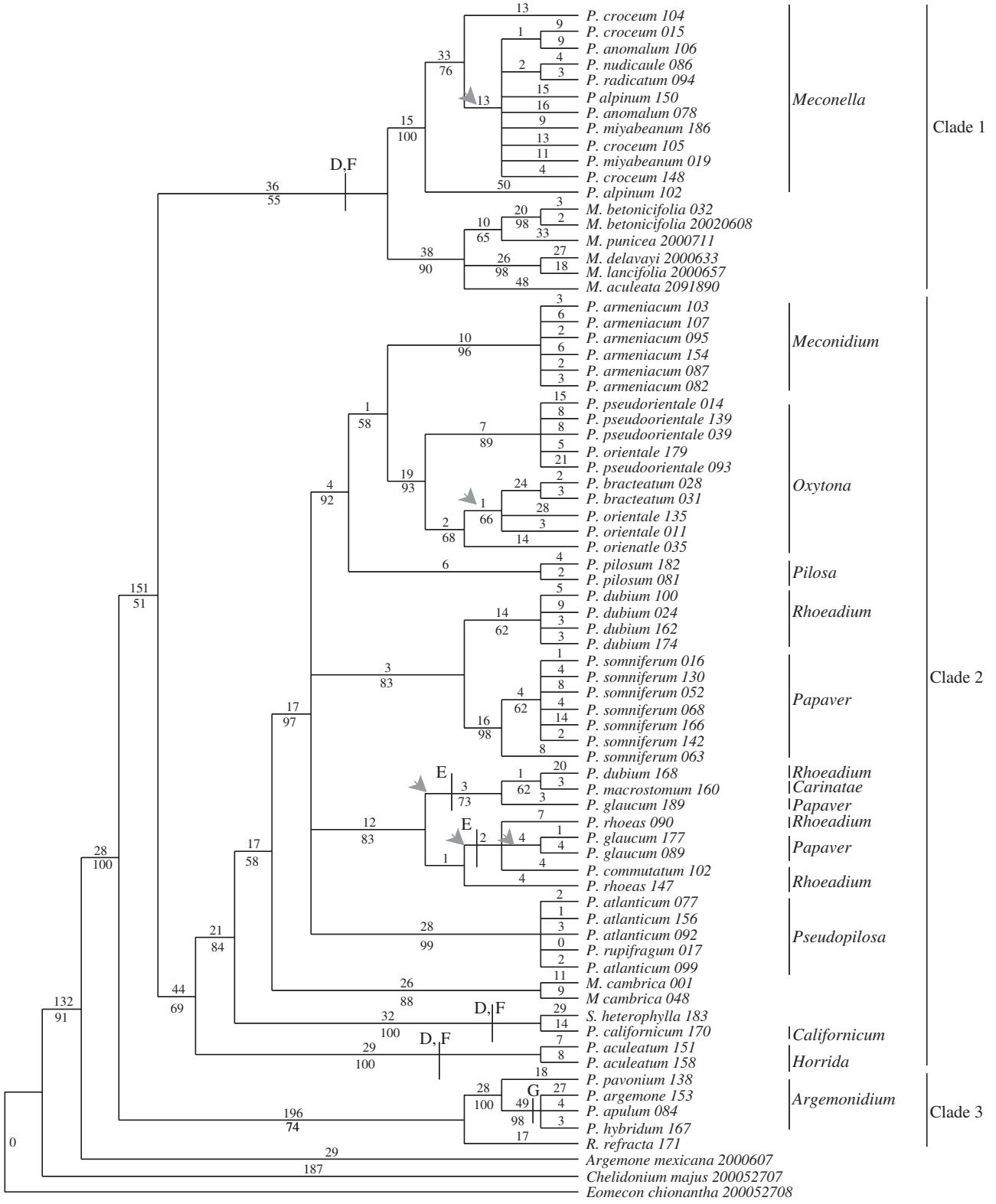

FIG. 2. One of eight equally most-parsimonious trees generated from the *trnL–F* sequences using maximum parsimony. Support for each node is represented by bootstrap percentages (BP) below the branch (shown only when >50 % and consistent with the strict consensus tree). Numbers above each branch indicate the numbers of character changes along each lineage (ACCTRAN optimization). An arrow indicates branches that did not appear in the strict consensus tree. Groups that possess characteristic indels are indicated using letters (refer to Table 2).

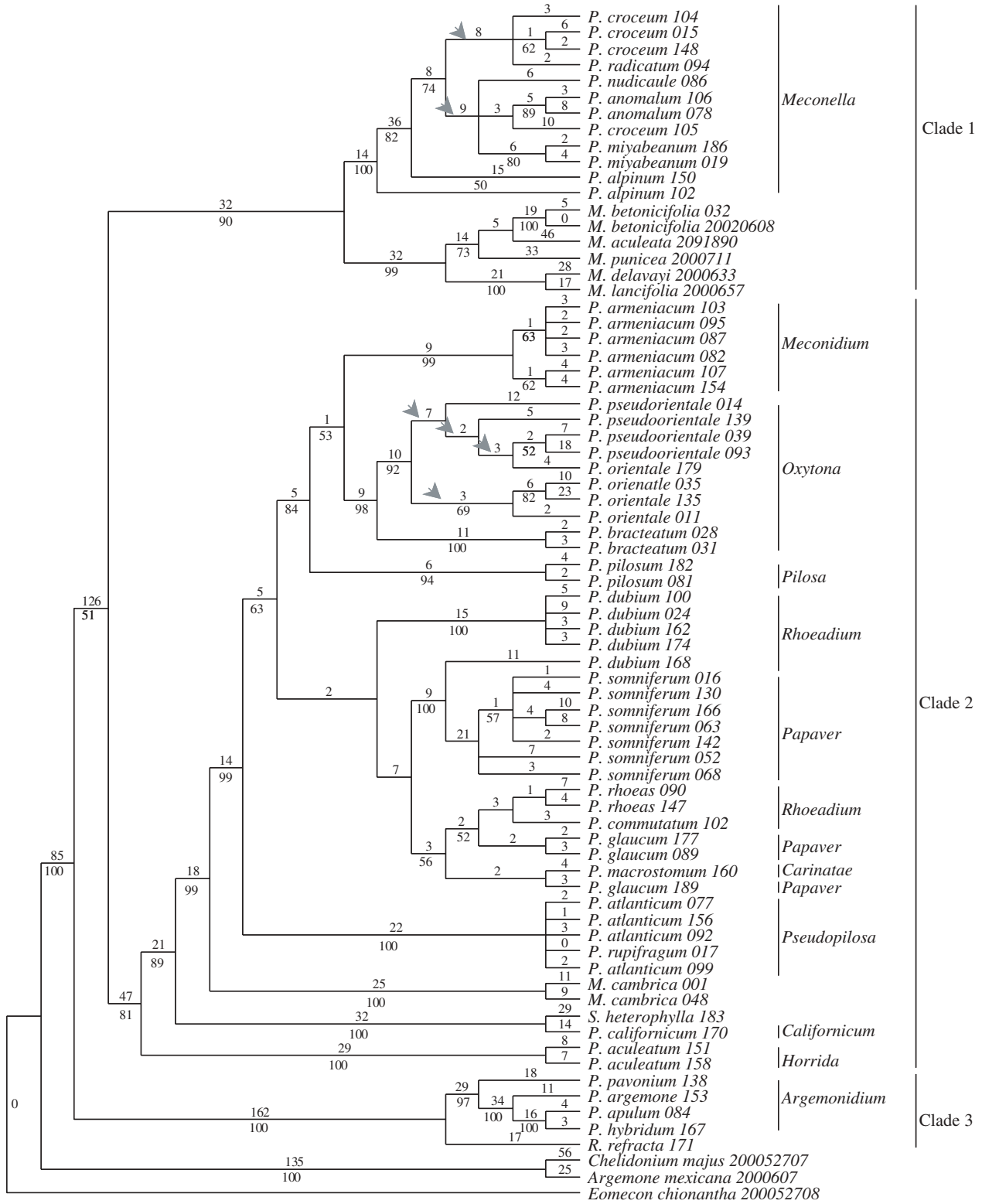

FIG. 3. One of eight equally most-parsimonious trees generated from the combined ITS and *trnL–F* data sets using maximum parsimony. Support for each node is represented by bootstrap percentages (BP) below the branch (shown only when >50 % and consistent with the strict consensus tree). Numbers above each branch indicate the numbers of character changes along each lineage (ACCTRAN optimization). An arrow indicates branches that did not appear in the strict consensus tree.

include presence of an apical plug in the capsules, long internodes above the basal leaf rosette, polyporate pollen grains, bristly capsules and sepal morphology.

From a taxonomic point of view the most significant relationship involving sect. *Argemonidium* is the close grouping of its members with the genus *Roemeria.* In all analyses sect. *Argemonidium* and *Roemeria* are sister to each other. This affinity has been suggested by previous authors based on morphological observations (Günther, 1975; Morales Torres *et al*., 1988) and has been supported by previous molecular analyses (Kadereit and Sytsma, 1992; Kadereit *et al*., 1997). Some of the morphological characters that separate sect. *Argemonidium* from *Papaver* are shared with *Roemeria*, including polyporate pollen grains, sepal morphology and long internodes above the basal leaf rosette (shared with *R. hybrida*). Relationships within the *Argemonidium–Roemeria* group are unclear owing to incongruence between the ITS and *trnL–F* phylogenetic trees and also between these results and previous molecular analyses by Kadereit and Sytsma (1992) and Kadereit *et al*. (1997). The trees resulting from ITS sequences clearly show *P. pavonium* ssp. *pavonium* and *Roemeria refracta* as sister species (99 BP). This placement was also demonstrated by Kadereit and Sytsma (1992) and Kadereit *et al*. (1997). Based on the molecular similarity between *P. pavonium* and *R. refracta* and the fact that *P. pavonium* has a similar geographical distribution to *R. refracta* and *R. hybrida*, Kadereit and Sytsma (1992) and Kadereit *et al*. (1997) postulated that *Roemeria* had arisen from within sect. *Argemonidium* and most probably directly from *Papaver pavonium* or an ancestor of this species. However, in the analyses of the maternally inherited *trnL–F* region (Fig. 2), sect. *Argemonidium* and *Roemeria* are sister groups, indicating that *Roemeria* can be considered distinct from sect. *Argemonidium* but not distinct from *Papaver*. Incongruence of trees generated from these differently inherited DNA regions is sometimes attributed to hybridization. Given that the two species in question are both diploid (Podlech and Dieterle, 1969; Kadereit, 1986*a*), allopolyploidy cannot explain the different topologies of the ITS and *trnL–F* trees. However, hybridization or introgression could explain these differences. Divergence of ITS repeat types could also have occurred before the divergence of the *Argemonidium–Roemeria* group. Paralogy could therefore also explain the pattern, with one ITS repeat type retained in the *P. pavonium–Roemeria* group and an alternative type in the others.

The current taxonomy of *Papaver* and relatives does not take account of the distinctiveness of sect. *Argemonidium* and its close relationship to *Roemeria*. We here suggest a reclassification accommodating our molecular results. Elevation of sect. *Argemonidium* to genus level or a combination of sect. *Argemonidium* as a subgenus of *Roemeria* would be appropriate taxonomic treatments of these groups. We favour the former option because of the substantial morphological differences between sect. *Argemonidium* and *Roemeria*.

Papaver *sect.* Meconella *and* Meconopsis (*excluding* M. cambrica). The scapose, perennial species of *Papaver* sect. *Meconella* (represented in this study by *Papaver alpinum*, *P. anomalum*, *P. croceum*, *P. miyabeanum* and *P. radicatum*) form a monophyletic group (100 BP in the *trnL–F* and combined analyses and 98 BP in the ITS analysis; Figs 1–3). Section *Meconella* is widely distributed across central, inner and eastern Asia, Siberia, Scandinavia through Greenland and northern Canada, with representatives found also in mountainous regions of Europe and the Rocky Mountains in North America (Rändel, 1974; Kadereit, 1988*a*). The species included in this study represent a limited sample from this distribution (five of 30 species; Rändel, 1974; Kadereit, 1988*a*).

The distinctness of this section from *Papaver* (excluding sect. *Argemonidium*) is also supported, as is its placement with *Meconopsis* excluding *M. cambrica* (Fig. 3; clade 1; 90 BP). A number of morphological characters have been used to define sect. *Meconella* (Hanelt, 1969; Rändel, 1974; Kadereit, 1988*a*). These include bristly, valvate capsules, simple or dissected pinnatisect leaves, pale anthers and filaments, and yellow, orange or white petals.

The species of sect. *Meconella* can be divided into two groups based on the degree of leaf dissection (finely dissected leaves: *Papaver alpinum*, *P. miyabeanum* and *P. radicatum*; broad leaf lobes: *Papaver anomalum* and *P. croceum*), but such a grouping is not supported by our molecular analysis. This morphological character has been discussed previously by Kadereit (1990) and Kadereit and Sytsma (1992) with reference to *P. alpinum*. The authors of these studies regarded finely dissected leaves to be a primitive character in sect. *Meconella* and suggested that this character may support a relationship to sect. *Argemonidium* with species of similar leaf morphology. Although *P. alpinum* is sister to the remaining *Meconella* species in the combined analysis, the other representatives of sect. *Meconella* with finely dissected leaves group more closely with species possessing broad leaf lobes. In addition, a molecular analysis of *P. alpinum s.l.* by Bittkau and Kadereit (2002) found that within this species broad leaf lobes are ancestral.

The position of sect. *Meconella* is not fully congruent with topologies obtained from earlier molecular analyses (Kadereit and Sytsma, 1992; Kadereit *et al*., 1997). The results from those analyses indicated that *Meconella* is sister to all sections of *Papaver* (Kadereit and Sytsma, 1992) or that sections *Meconella* and *Argemonidium* were resolved as sister to each other (Kadereit *et al*., 1997). However, bootstrap support for the sister-group relationship of these two sections (in the latter study) was low (<50 BP). Based on the topology of the major clades in our molecular trees, it can be concluded that sect. *Meconella* (and probably *Meconopsis*) is derived from a lineage that separated earlier from that giving rise to most other sections of *Papaver* (excluding *Argemonidium*).

The Asian representatives of *Meconopsis* were resolved as sister to sect. *Meconella* and share the diagnostic indels of sect. *Meconella* (D and F; Table 2). This grouping is incongruent with results of previous molecular analyses (Jork and Kadereit, 1995; Kadereit *et al*., 1997). The results of those analyses demonstrated that within Asian *Meconopsis* two distinct clades existed (based on an RFLP analysis of plastid

DNA fragments). The first clade comprised species such as *Meconopsis chelidonifolia* and *M. villosa* that are sister to the other representatives of Asian *Meconopsis* (clade 2) plus the remaining Old World Papaveroideae (*Meconopsis cambrica*, *Papaver*, *Roemeria*, *Stylomecon*) used in that analysis. Only representatives of this second clade were included in the analyses reported here.

A significant amount of morphological difference exists between sect. *Meconella* and *Papaver s.s.* (clade 2). Although species of sect. *Meconella* possess a sessile stigmatic disc similar to the stigmatic discs typical of *Papaver*, it has been noted (Rändel, 1977; Kadereit *et al*., 1997) that the stigmatic discs of sect. *Meconella* may not be homologous to those found in other sections of *Papaver* (excluding *Argemonidium*). The stigmatic discs of *Meconella* consist in some cases of stigmatic tissue only, or there are deep incisions between the stigmatic rays. In addition, certain species of *Meconella* have polyporate instead of tricolpate pollen grains, a characteristic also found in some species of *Meconopsis* and *Papaver* sect. *Argemonidium*. No species of *Meconella* with polyporate pollen were included in this study.

If the current circumscription of *Papaver* is followed and sect. *Meconella* is retained within *Papaver*, a strict interpretation of the trees produced in this analysis would imply that *Meconopsis* should also be considered to be a member of *Papaver*. To retain *Meconopsis* as a genus would require a separation of sect. *Meconella* from *Papaver*. For example, it could either be raised to genus rank or included in *Meconopsis*. *Meconella* is therefore treated as a subgenus of *Papaver*, recognizing the distinction between *Meconella* and other *Papaver* subgenera but also recognizing that evidence exists for the amalgamation of *Papaver* and *Meconopsis.*

Papaver *sects.* Californicum *and* Horrida. *Papaver* sects. *Californicum* and *Horrida* are distributed outside the main geographical range of *Papaver*. *Papaver aculeatum* (sect. *Horrida*) is native to South Africa and is characterized by an indumentum of relatively long bristles, poricidal capsules, and pale filiform filaments and anthers. All green parts of the plant are covered with patent bristles (Kadereit, 1988*c*). *Papaver californicum* (sect. *Californicum*) is native to the west coast of North America and has a slender, ribbed, glabrous capsule, a many-flowered racemose inflorescence, pale anthers and filaments, and valvate capsule dehiscence (Kadereit, 1988*b*). Both species are annuals. In the ITS, *trnL–F* and combined trees both sections are attached to basal nodes within the main clade of *Papaver* (clade 2; Figs 1 –3), and sect. *Californicum* is sister to the 'core' group of *Papaver* (sects. *Carinatae*, *Meconidium*, *Oxytona*, *Papaver*, *Pilosa*, *Pseudopilosa* and *Rhoeadium*) and *Meconopsis cambrica*. *Papaver aculeatum* shares morphological and cytological characteristics with sects. *Pilosa* and *Papaver.* Similarities between *P. aculeatum* and *P. somniferum* (sect. *Papaver*) include auriculate–amplexicaulous leaves and a chromosome base number of $n = 11$, both characteristics found only in these two species. However, both these characters appear to have evolved in parallel (Figs 1–3). Similarities between sects. *Horrida* and *Pilosa* include racemose inflorescences, pale filiform filaments and the possession of long capsules with flat stigmatic discs (Kadereit, 1988*c*). However, these two sections do not associate in the molecular analysis presented here, and convergence is therefore also implied to explain the similarity in morphology. *Papaver californicum* shares characteristics with sect. *Meconidium*, including valvate capsule dehiscence and pale filiform filaments, but species of these two groups are geographically widely separated and do not associate in the molecular trees.

The results from the molecular analysis support the view of Kadereit *et al*. (1997) that *Stylomecon heterophylla* arose from within *Papaver* and should not be considered a separate genus. *Stylomecon heterophylla* and *P. californicum* are both native to California and grow in similar habitats (Kadereit, 1988*b*). Morphological similarities between these species include leaf shape, glabrous/globose buds, orange petals, and pale anthers and filiform filaments (Ernst, 1962; Kadereit, 1988*b*). The two species are differentiated by capsular morphology, with *S. heterophylla* possessing a distinct style that is similar to those found in many representatives of *Meconopsis.* In the ITS, *trnL–F* and combined analysis, *S. heterophylla* and *P. californicum* form a well-supported group (100 BP in the *trnL–F* and combined trees, Figs 2 and 3; 96 BP in the ITS trees, Fig. 1). The two species appear to have diverged relatively recently. *Stylomecon heterophylla* possesses the 4-bp indel diagnostic for sects. *Meconella*, *Californicum* and *Horrida* (incl. Asian *Meconopsis*) at positions 261–265 in the *trnL–F* region (F, Fig. 2; Table 2). The separation of *S. heterophylla* from *Papaver* therefore is not justified based solely on differences in capsule characteristics.

The results of the molecular analysis can be interpreted in a number of ways for taxonomic conclusions concerning sects. *Californicum* and *Horrida*. Both sections are successively sister to the highly supported (99 BP; Fig. 3) core group of *Papaver* comprising sects. *Carinatae*, *Meconidium*, *Oxytona*, *Papaver*, *Pilosa*, *Pseudopilosa* and *Rhoeadium*. However, sects. *Californicum* and *Horrida* possess the characteristic 4-bp indel at positions 248–252 in the *trnL–F* region shared with Asian *Meconopsis* and representatives of sect. *Meconella*. The disjunct geographical distributions of sect. *Californicum* (North America) and sect. *Horrida* (South Africa) might indicate a wider distribution of *Papaver* at some point during its evolutionary history, with extinction occurring in North America and Africa leaving these two sections geographically isolated (Randel, 1974; Kadereit *et al*., 1997), or indicate long-distance dispersal. Taking into account the outlying positions of sects. *Californicum* and *Horrida* in the molecular trees, they seem to derive from a relatively ancient lineage of *Papaver*. The positions of *Californicum* and *Horrida* within the core *Papaver* clade in the analyses here are congruent with previous molecular analyses (Kadereit and Sytsma, 1992; Kadereit *et al*., 1997).

It is recommended that sects. *Californicum* and *Horrida* be elevated to the rank of subgenera within *Papaver*, i.e. subgen. *Californicum* and subgen. *Horrida*. The separation of *Stylomecon heterophylla* from *Papaver* is rejected. The clear relationship of this species to *P. californicum*, as

indicated by similarities in morphology, geographical distribution, and nucleotide sequences within the ITS and *trnL–F* gene regions, favours its inclusion in subg. *Californicum*. Considering these differences in capsule morphology, subg. *Californicum* should contain two species, *Papaver californicum* and *P. heterophylla* (=*Stylomecon heterophylla*).

Meconopsis cambrica. The only European species of *Meconopsis*, *M. cambrica*, is well separated from the representatives of Asian *Meconopsis* in the molecular analysis here. *Meconopsis cambrica* occupies a well-supported (99 BP; Fig. 3) sister-group position to the remaining sections of *Papaver* (excluding *Argemonidium*, *Californicum*, *Horrida* and *Meconella*). This supports the view (Kadereit *et al*. 1997) that two distinct lineages within *Meconopsis s.l.* exist and that *Meconopsis* in its current circumscription is neither monophyletic nor distinct from *Papaver*. *Meconopsis cambrica* shares diagnostic *trnL–F* indels with the majority of *Papaver* (excluding *Argemonidium*, *Californicum*, *Horrida* and *Meconella*). *Meconopsis cambrica* could have arisen either in parallel with the Asian representatives of *Meconopsis*, clade 2, i.e. core *Papaver* (sects. *Carinatae*, *Papaver*, *Pilosa*, *Pseudopilosa*, *Oxytona*, *Meconidium* and *Rhoeadium*), or from within a lineage best recognized as members of an expanded *Meconopsis*. Both these views were proposed by Kadereit *et al*. (1997), who favoured the latter view based on geographical, phytochemical and morphological considerations. Topological considerations alone favour parallel evolution as *M. cambrica* is embedded in clade 2 in our ITS/*trnL–F* trees.

It is evident from the results of this analysis that incongruence exists with previous taxonomic classifications regarding the positioning of *M. cambrica*. If *M. cambrica* is recognized as *Meconopsis*, *Papaver s.s.* (i.e. after the exclusion of the groups discussed above) is not monophyletic. It is suggested to include *M. cambrica* (as *Papaver cambrica* L.) in *Papaver*. However, an appropriate treatment of this species is difficult owing to the lack of apparent morphological similarities with extant *Papaver* species. There is no obvious section or group of species with which to place *Papaver cambrica.* Although unsatisfactory from a taxonomic perspective it may be necessary to describe a new monotypic section for this species within *Papaver.* The alternative is to leave it as *incertae sedis* until further evidence is found regarding its placement.

Inter-sectional relationships in Papaver s.s.

Clade 2 contains a well-supported (99 BP; Fig. 3) group of sections including *Carinatae*, *Meconidium*, *Oxytona*, *Papaver*, *Pilosa*, *Pseudopilosa* and *Rhoeadium* (hereafter described as *Papaver s.s.*). This is the largest inclusive group of *Papaver s.l.* This group was described by Kadereit *et al*. (1997) as representing the typical species of *Papaver*. Within this group, inter-sectional relations are not fully resolved, but sections are generally well supported (Figs 1–3).

Section *Pseudopilosa* (represented in the combined analysis by *P. atlanticum* and *P. rupifragum*) forms a well-supported group in the combined analysis (100 BP; Fig. 3) and is sister to the remaining sections of *Papaver s.s.* Representatives of sect. *Pseudopilosa* are characterized by having unique 5- and 4-bp indels at positions 75–79 (A, Fig. 1) and 216–219 (B, Fig. 1) of the ITS region, respectively (Table 2). The species of this section are of subscapose to scapose habit and are found in south-western Asia, northern Africa and southern Spain.

Section *Pilosa* comprises a single perennial subscapose species with a number of subspecies found predominantly in western Turkey (Kadereit, 1996). The species is characterized by convolute leaf vernation, poricidal capsule dehiscence and pale filiform filaments. The separation of sect. *Pilosa* from sect. *Pseudopilosa* based on morphological and phytochemical differences (Popov, 1937; Günther, 1975; Kadereit, 1996) is supported by the results of the combined analysis here (Fig. 3). *Papaver pilosum* is sister to sects. *Oxytona* and *Meconidium* (86 BP). Section *Oxytona* comprises a polyploid series including diploid *P. bracteatum* ($2n = 14$), tetraploid *P. orientale* Fedde ($2n = 28$) and allohexaploid *P. pseudo-orientale* Fedde ($2n = 42$) and is found predominantly in the Caucasus Mountains, eastern Turkey and north-western Iran (Goldblatt, 1974). The group is characterized by their perennial habit, poricidal capsule dehiscence, and dark filaments and anthers. Section *Meconidium*, comprising four biennial species (represented in the analysis here by two subspecies of *P. armeniacum*), occupies a continuous geographical range in southern and eastern Turkey, the Caucasus Mountains, northern Iraq and north-western Iran and possesses glabrous or bristly capsules, valvate capsule dehiscence, and pale filaments and anthers. Sections *Meconidium*, *Oxytona* and *Pilosa* are heterogeneous morphologically, and identification of synapomorphies for this group is difficult. The three species of sect. *Oxytona* are clearly monophyletic (98 BP; Fig. 3). Genomic and fluorescence *in situ* hybridization studies (Carolan, 2004) have indicated that the diploid *P. bracteatum* was a parent of the hexaploid *P. pseudo-orientale.* The clear inter-relationship between these species has been demonstrated previously using AFLP fingerprinting (Carolan *et al*., 2002).

The remaining sections of *Papaver s.s.* are sects. *Carinatae*, *Papaver* and *Rhoeadium*. The results of the molecular analyses question whether these sections are monophyletic. *Papaver* sect. *Rhoeadium* consists of 17 predominantly annual species (Günther, 1975; Kadereit, 1989) and is represented in this study by *Papaver commutatum*, *P. dubium* and *P. rhoeas.* The centre of diversity of sect. *Rhoeadium* is south-western Asia and the Aegean area with some species found in the central or western Mediterranean, the Balkans and the western Himalayas (Kadereit, 1989). Characteristic morphological traits include poricidal capsules and dark (sometimes light) filaments. However, the section is extremely diverse in morphological characteristics. Kadereit (1989) recognized three species groups within sect. *Rhoeadium* based on geographical and morphological traits. The first group contains species with longer than broader capsules, such as *P. dubium*, and only tetraploid ($2n = 28$) and hexaploid ($2n = 42$) species. The

second group contains diploid species ($2n = 14$), including *P. arenarium* and *P. commutatum*, and is diverse morphologically. The third group is morphologically more uniform than the *P. arenarium* group and consists of diploid species, including *P. rhoeas.* The similarity of the *P. rhoeas* and *P. arenarium* groups (the latter represented by *P. commutatum*) suspected by Kadereit (1989) is weakly supported (56 BP; Fig. 3) here. In addition, the representatives of these two groups possess a diagnostic 5-bp indel at positions 186–191 of the *trnL–F* region (E, Table 2). A separation exists in some analyses (Figs 2 and 3) between the *P. rhoeas*/*P. arenarium* groups and the *P. dubium* group. In the *trnL–F* trees, the *P. dubium* group is clearly allied to *P. somniferum* (83 BP; Fig.2). However, in the ITS trees obtained (Fig. 1) the *P. dubium* group is weakly allied to the *P. rhoeas* group (BP < 50 %). *Papaver dubium* also lacks a characteristic 5-bp *trnL–F* indel (E, Table 2) unique to the other representatives of sect. *Rhoeadium* (including *P. glaucum*). In addition, some incongruence between the ITS and *trnL–F* topologies exists with respect to *P. dubium* ssp. *erosum.* In the ITS analysis *P. dubium* ssp. *erosum* groups with *P. somniferum* (sect. *Papaver*; 88 BP; Fig. 1), and in the *trnL–F* tree it groups within a subclade comprising *P. commutatum*, *P. glaucum*, *P. macrostomum* and *P. rhoeas* (83 BP; Fig. 2).

The single representative of sect. *Carinatae* (*P. macrostomum*) consistently fell within the *P. rhoeas* group and shares its diagnostic *trnL–F* indel (Figs 1–3; E, Table 2). *Papaver macrostomum*, distributed in Iran, Iraq and Turkey, possesses all the morphological characteristics of sect. *Rhoeadium* but has been separated into a separate section based on the possession of a deciduous stigmatic disc (Fedde, 1909; Kadereit, 1987). No support for the separation of *P. macrostomum* from sect. *Rhoeadium* is found in the ITS and *trnL–F* trees.

The four annual representatives of sect. *Papaver* (represented in this study by *P. glaucum* and *P. somniferum*) from the western Mediterranean and south-western Turkey to Cyprus, Iran, Afghanistan and Pakistan do not form a monophyletic group in our analyses of ITS and *trnL–F*. Species of this section are characterized by the possession of more or less strongly auriculate–amplexicaulous leaves, poricidal capsule dehiscence and dark (sometimes pale) filaments. *Papaver glaucum* shows more sequence similarity to sect. *Rhoeadium*. This division within sect. *Papaver* has previously been demonstrated (Kadereit and Sytsma, 1992). The study by these authors also demonstrated that *P. glaucum* and *P. gracile* (members of this section) are more closely related to *P. rhoeas* and *P. dubium* of sect. *Rhoeadium.* Many morphological and geographical similarities exist between the two sections (see Kadereit, 1988*a*). Phytochemically, *P. glaucum* differs from *P. somniferum* in not accumulating morphinane alkaloids but rather has some alkaloids similar to those found in *P. rhoeas* (Preininger *et al*., 1981; Preininger, 1986). *Papaver gracile*, *P. glaucum* and *P. decaisnii*, like the majority of *Papaver*, have a base chromosome number of $n = 7$. *Papaver somniferum* has a base chromosome number of $n = 11$ (Hammer and Fritsch, 1977). These differences in chromosome number and alkaloid spectra led Novak and Preininger (1980) and Preininger *et al*. (1981) to separate these three species into their new sect. *Glauca.* Reckin (1973) transferred these species to sect. *Rhoeadium.* The presence in *P. glaucum* of the diagnostic 5-bp indel at positions 186–191 of the *trnL–F* region (E, Table 2), characteristic for the *Papaver rhoeas* group, further questions the classification of *P. glaucum* in sect. *Papaver*.

Although our study could demonstrate the non-monophyly of sects. *Papaver* and *Rhoeadium*, limited sampling of species and limited support for some groups do not allow us to reclassify *Papaver s.s.* confidently into sections apart from the inclusion of *M. cambrica* just discussed. However, *Papaver s.s.* should be treated as *Papaver* subg. *Papaver*. It seems likely from the molecular results that subg. *Papaver* will contain sects. *Meconidium*, *Oxytona*, *Papaver* (including *Rhoeadium* and *Carinatae*), *Pilosa* and *Pseudopilosa*.

Evaluation of morphological characters previously viewed as diagnostic for Papaver

Papaver has been defined primarily by the possession of a capsule with a sessile stigmatic disc. The results of the molecular analyses presented here clearly demonstrate that a number of species with sessile stigmatic discs are close relatives of taxa that possess a style. This is demonstrated by *S. heterophylla* and *P. californicum* and *P.* sect. *Meconella* and Asian *Meconopsis*. Furthermore, the structure of the stigmatic disc in sect. *Argemonidium* is different from all other stigmatic discs due to the formation of a plug-like structure in the interior of the capsule. This can be regarded as evidence for its independent evolution from other species with a typical stigmatic disc.

Papaver has generally been considered to represent the most derived lineage of Papaveroideae, and hence the sessile stigmatic disc was deemed to be an advanced character. The results here are congruent with this view with respect to *Papaver s.s.* only. In light of the groupings generated in our phylogenetic analysis it is not inconceivable that the sessile stigmatic disc has arisen on a number of occasions from ancestors with a style. Independent origins of the stigmatic disc in *Papaver* have been suggested previously (Kadereit and Sytsma, 1992).

Two morphological characters were considered of primary significance for the evaluation of relationships within *Papaver*, particularly at the inter-sectional level. These are the mode of capsule dehiscence and the degree of pigmentation of filaments and anthers. The possession of pale filaments and anthers by the majority of genera of Papaveroideae and of dark filaments in part of *Papaver s.s.* indicates that pale filaments might be ancestral. Dark filaments seem to have evolved more than once, or there have been reversals to pale filaments in some sections (e.g. *Meconidium* and *Pilosa*). Molecular and morphological data separate sect. *Argemonidium* from the other sections with dark filaments (*Carinatae*, *Oxytona*, *Papaver* and *Rhoeadium*).

Sections *Meconella* and *Californicum* have valvate capsule dehiscence and an outlying position with respect to the

other sections of *Papaver*. This indicates that valvate capsule dehiscence may be primitive. However, this character is also found in sect. *Meconidium*, which falls within *Papaver s.s*. Its presence here suggests that this character is a synapomorphy for the species of sect. *Meconidium*. Thus, the results of this analysis indicate that valvate capsule dehiscence has evolved independently at least three times within *Papaver s.l.*

The combination of morphological, biogeographical and molecular characters has made possible a novel interpretation of relationships in *Papaver* and allies, and allows for more useful taxonomies to be generated. A formal taxonomic revision of *Papaver* infrageneric groupings is in preparation.

ACKNOWLEDGEMENTS

We thank Matthew Jebb of the National Botanical Garden, Glasnevin, Ireland, for his assistance in the acquisition and cultivation of a considerable portion of the material used in this study. In addition, thanks are due to Brian S. Luther (Seattle, USA), Suzanne Cubey (Herbarium of the Royal Botanic Garden, Edinburgh, UK), Hans Hansen (University of Copenhagen, Botanic Gardens) and Rainer Greissl (Mainz, Germany) for the provision of material, and to Joe Reilly, Mary Deegan and Maureen Brunt for assistance in cultivating and maintaining the living collection at Trinity College, Dublin, Ireland.

LITERATURE CITED

Baldwin BG, Sanderson MJ, Porter JM, Wojciechowski MF, Cambell CS, Donoghue MJ. 1995. The ITS region of nuclear ribosomal DNA: a valuable source of evidence on angiosperm phylogeny. *Annals of the Missouri Botanical Garden* **82**: 247–277.

Bittkau C, Kadereit JW. 2002. Phylogenetic and geographical relationships in *Papaver alpinum* L. (Papaveraceae) based on RAPD data. *Botanische Jahrbücher für Systematik, Pflanzengeschichte und Pflanzengeographie* **123**: 463–479.

Carolan JC. 2004. *Phylogenetic analysis of* Papaver *L.* PhD thesis, University of Dublin, Trinity College, Ireland.

Carolan JC, Hook ILI, Walsh JJ, Hodkinson TR. 2002. Using AFLP markers for species differentiation and assessment of genetic variability of *in vitro* cultured *Papaver bracteatum* (section *Oxytona*). *In vitro* cellular and developmental biology. *Plant* **38**: 300–307.

Chase MW, de Bruijn AY, Reeves G, Cox AV, Rudall PJ, Johnson MAT, Eguiarte LE. 2000. Phylogenetics of Asphodelaceae (Asparagales): an analysis of plastid *rbcL* and *trnL–F* DNA sequences. *Annals of Botany* **86**: 935–956.

Cordell GA. 1981. Alkaloids derived from phenylalanine and tyrosine. In: Cordell GA, ed. *Introduction to alkaloids: a biogenetic approach*. New York: John Wiley and Sons, 490–512.

Cullen J. 1965. Papaveraceae. In: Davis PH, ed. *Flora of Turkey and the east Aegean Islands*, Vol. I. Edinburgh: University Press, 213–247.

Doyle JJ, Doyle JL. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletins* **19**: 11–15.

Ernst WR. 1962. *A comparative morphology of the Papaveraceae*. PhD thesis, Stanford University, Stanford, California.

Fedde F. 1909. Papaveraceae–Hypecoideae et Papaveraceae–Papaveroideae. In: Engler A, ed. *Das Pflanzenreich*. Leipzig: Englemann, 228.

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

Gielly LP, Taberlet P. 1996. A phylogeny of the European gentians inferred from chloroplast *trnL* (UAA) intron sequences. *Botanical Journal of the Linnaean Society* **120**: 57–75.

Gielly LP, Yuan YM, Kupfer P, Taberlet P. 1994. Phylogenetic use of noncoding regions in the genus *Gentiana* L.: chloroplast *trnL* (UAA) intron versus nuclear ribosomal internal transcribed spacer sequences. *Molecular Phylogenetics and Evolution* **5**: 460–466.

Goldblatt P. 1974. Biosystematic studies in *Papaver* section *Oxytona*. *Annals of the Missouri Botanic Garden* **61**: 264–296.

Günther KF. 1975. Beitrage zur Morphologie und verbreitung der *Papaveraceae* 2. Teil: Die Wuchsformen der *Papaverae*, *Escoscholzieae* und *Platystemonoideae*. *Flora* **164**: 393–436.

Hammer K, Fritsch R. 1977. Zur Frage nach der Ursprungsart des Kulturmohns (*Papaver somniferum* L.). *Kulturpflanze* **25**: 113–124.

Hodkinson TR, Chase MW, Takahashi C, Leitch IJ, Bennett MD, Renvoize SA. 2002. The use of DNA sequencing (ITS and *trnL–F*), AFLP and fluorescent *in-situ* hybridisation to study allopolyploid *Miscanthus* (Poaceae). *American Journal of Botany* **89**: 279–286.

Hopper SD, Fay MF, Rossetto M, Chase MW. 1999 A molecular phylogenetic analysis of the bloodroot and kangaroo paw family, Haemodoraceae: taxonomic, biogeographic and conservation implications. *Botanical Journal of the Linnean Society* **131**: 285–299.

Jork KB, Kadereit JW. 1995. Molecular phylogeny of the Old World representatives of Papaveraceae subf. Papaveroideae with special emphasis on the genus *Meconopsis* Vig. In: Jensen U, Kadereit JW, eds. *Systematics and evolution of the Ranunculiflorae*. *Plant Systematics and Evolution* **9** (Suppl.): 171–180.

Kadereit JW. 1986*a*. A revision of *Papaver* section *Argemonidium*. *Notes of the Royal Botanic Garden Edinburgh* **44**: 25–43.

Kadereit JW. 1986*b*. A revision of *Papaver* L. sect *Papaver* (Papaveraceae). *Botanische Jahrbücher für Systematik, Pflanzengeschichte und Pflanzengeographie* **108**: 1–16.

Kadereit JW. 1987. A revision of *Papaver* sect *Carinatae* (Papaveraceae). *Nordic Journal of Botany* **7**: 501–504.

Kadereit JW. 1988*a*. Sectional affinities and geographical distribution in the genus *Papaver* L. (Papaveraceae). *Beiträge zur Biologie der Pflanzen* **63**: 139–156.

Kadereit JW. 1988*b*. *Papaver* L. sect *Californicum* Kadereit, a new section of the genus *Papaver*. *Rodora* **90**: 7–13.

Kadereit JW. 1988*c*. The affinities of the south-hemispherical *Papaver aculeatum* Thunb. (Papaveraceae). *Botanische Jahrbücher für Systematik, Pflanzengeschichte und Pflanzengeographie* **109**: 335–341.

Kadereit JW. 1989. A revision of *Papaver* section *Rhoeadium* Spach. *Notes of the Royal Botanic Garden Edinburgh* **45**: 225–286.

Kadereit JW. 1990. Some suggestions on the geographical origin of the central, west and north European synantropic species of *Papaver* L. *Botanical Journal of the Linnean Society* **103**: 221–231.

Kadereit JW. 1993*a*. *Papaveraceae*. In: Kubitzki K, Rohweer JG, Bittrich V, eds. *The families and genera of vascular plants*, Vol. II. Berlin: Springer-Verlag, 20–33.

Kadereit JW. 1993*b*. A revision of *Papaver* sect. Meconidium. *Edinburgh Journal of Botany* **50**: 125–148.

Kadereit JW. 1996. A revision of *Papaver* L. sects *Pilosa* Prantl and *Pseudopilosa* M. Popov ex Gunther (Papaveraceae). *Edinburgh Journal of Botany* **53**: 285–309.

Kadereit JW, Sytsma KJ. 1992. Disassembling *Papaver*: a restriction site analysis of chloroplast DNA. *Nordic Journal of Botany* **12**: 205–217.

Kadereit JW, Schwarzbach AE, Jork KB. 1997. The phylogeny of *Papaver* s.l. (*Papaveraceae*): polyphyly or monophyly? *Plant Systematics and Evolution* **204**: 75–98.

Kelchner SA. 2000. The evolution of non-coding chloroplast DNA and its application in plant systematics. *Annals of the Missouri Botanical Garden* **87**: 182–198.

Markgraf F. 1958. Familie *Papaveraceae*. In: Hegi P, ed. *Illustrietrte Flora von Mittelurropaa Band IV*, 2nd edn. Teil 1. Munich: Hanser,15–49.

Morales Torres C, Mendoza Castellon R, Romero Garcia AT. 1988. La posicion sistematica de *Papaver argemone* L.: interes evolutivo del orden *Papaverales*: 1. *Lagascalia* **15**: 181–189.

Novak J, Preininger V. 1980. Sect., Glauca-nova sekce rodu *Papaver*. *Presila* **52**: 97–101.

Podlech D, Dieterle A. 1969. Chromosomenstudien an Afghanischen Pflanzen. *Candollea* **24**: 185–243.

Popov MG. 1937. Papaveraceae. In: Komarov VL, ed. *Flora of the USSR*. Moscow-Leningrad: Nauka, 470–474.

Preininger V. 1986. Chemotaxonomy of Papaveraceae and Fumariaceae. In: *The alkaloids*. London: Academic Press, 2–98.

Preininger V, Novak J, Santavy F. 1981. Isolierung und Chemie der Alkaloide aus Pflanzen der Papaveraceae LXXXXI. Glauca, eine neue Sektion der Gattung *Papaver*. *Planta Medica* **41**: 119–123.

Rändel U. 1974. Beitrage zur Kenntnis der Sippenstruktuir der Gattung *Papaver* L. Sectio *Scapiflora* Reihenb. Im vergleich mit *P. alpinum* L. (*Papaveraceae*). *Feddes Repert* **86**: 19–37.

Rändel U. 1977. Uber die grondlandischen Vertreter der section *Lasiotrachyphylla* Bernh. (Papaveraceae). *Feddes Repert* **88**: 421–450.

Reckin J. 1973. A contribution to the cytology of *Papaver gracile* Auch. Including proposals for a revision of the section *Mecones*. *Caryologia* **26**: 245–251.

Salamin N, Chase MW, Hodkinson TR, Savolainen V. 2003. Assessing internal support with large phylogenetic DNA matrices. *Molecular Phylogenetics and Evolution* **27**: 528–539.

Seelanan T, Schnabel A, Wendel J F. 1997. Congruence and consensus in the cotton tribe (Malvaceae). *Systematic Botany* **22**: 259–290.

Savolainen V, Chase M W. 2003. A decade of progress in plant molecular phylogenetics. *Trends in Genetics* **12**: 717–724.

Schwarzbach AE, Kadereit JW. 1999. Phylogeny of prickly poppies, *Argemone* (Papaveraceae), and the evolution of morphological and alkaloid characters based on ITS nrDNA sequence variation. *Plant Systematics and Evolution* **218**: 257–279.

Sun Y, Skinner DZ, Liang GH, Hulbert SH. 1994. Phylogenetic analysis of *Sorghum* and related taxa using internal transcribed spacers of nuclear ribosomal DNA. *Theoretical and Applied Genetics* **89**: 26–32.

Swofford DL. 2003. *PAUP*: Phylogenetic analysis using parsimony (* and other methods)*, version 4.0b 10. Sunderland, MA: Sinauer Associates.

Swofford DL, Olsen GJ, Waddell PJ, Hillis DM. 1996. Phylogenetic inference. In: Hillis DM, Moritz C, Mable BK, eds. *Molecular systematics*, 2nd edn. Sunderland, MA: Sinauer Associates, 407–514.

Taberlet PL, Gielly L, Pautou G, Bouvet J. 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Moleular Biology* **17**: 1105–1109.

Taylor G. 1930. *Stylomecon*: a new genus of Papaveraceae. *Journal of Botany* **68**: 138–140.

Wendel JF, Doyle JJ. 1998. Phylogenetic incongruence: window into genome history and molecular evolution. In: Soltis DE, Soltis PS, Doyle JJ, eds. *Molecular systematics of plants II*. London: Kluwer Academic Publishers, 265–296.

Wendel JF, Schnabel A, Seelanan T. 1995. An unusual ribosomal DNA sequence from *Gossypium gossypioides* reveals ancient, cryptic, intergenomic introgression. *Molecular Phylogenetics and Evolution* **4**: 298–313.

Yoder AD, Irwin JA, Payseur, BA. 2001. Failure of the ILD to determine data combinability for slow Loris phylogeny. *Systematic Biology* **50**: 108–121.