

Molecular systematics and biogeography of Resedaceae based on ITS and *trnL-F* sequences

Santiago Martín-Bravo ^{a,*}, Harald Meimberg ^{c,d}, Modesto Luceño ^a, Wolfgang Märkl ^c, Virginia Valcárcel ^a, Christian Bräuchler ^c, Pablo Vargas ^b, Günther Heubl ^c

^a Pablo de Olavide University, Ctra. Utrera km 1, 41013 Sevilla, Spain

^b Royal Botanical Garden of Madrid, CSIC, Plaza de Murillo 2, 28014 Madrid, Spain

^c Department of Biology I, Section: Biodiversity Research, Ludwig-Maximilians-University Munich, Menzinger Str. 67, 80638 Munich, Germany

^d Department of Bioagricultural Sciences and Pest Management, Colorado State University, Plant Sciences Building, University Ave. 307, Fort Collins, CO 80523, USA

Received 9 October 2006; revised 11 December 2006; accepted 18 December 2006

Available online 31 December 2006

Abstract

The Resedaceae, containing 6 genera and ca. 85 species, are widely distributed in the Old World, with a major center of species diversity in the Mediterranean basin. Phylogenetic analyses of ITS and plastid *trnL-trnF* sequences of 66 species from all genera of the Resedaceae reveal (1) monophyly of the family, in congruence with preliminary phylogenetic studies; (2) molecular support for the traditional morphological subdivision of the Resedaceae into three tribes according to ovary and placentation types, and carpel number; (3) two monophyletic genera (*Caylusea*, *Sesamoides*), and one natural group (core *Reseda*), which includes the remaining four genera of the family (*Ochradenus*, *Oligomeris*, *Randonia*, *Reseda*); (4) a monophyletic origin for four of the six taxonomic sections recognized within *Reseda* (*Leucoreseda*, *Luteola*, *Glaucoreседа*, *Phyteuma*). Our results lead us to interpret an increment of the basic chromosome number in the family from $x = 5$ to $x = 6$ in at least two independent instances, and a broad representation of polyploids in multiple lineages across phylogenies, including association between octoploids and alien invasion in many parts of the world. Species diversity, endemism number, phylogenetic relationships and sequence divergence in Resedaceae suggest two major centers of differentiation, one in the western Mediterranean, and the other in the eastern Mediterranean and SW Asia. Two independent colonization events to the Canary Islands from Africa are indicated for the two Canarian *Reseda* endemics.

© 2007 Elsevier Inc. All rights reserved.

Keywords: Biogeography; Brassicales; Canary Islands; Character evolution; Chromosome evolution; Endemics; Mediterranean; Phylogenetics; *Reseda*

1. Introduction

Resedaceae are included in the order Brassicales (Judd et al., 1994), and have been traditionally considered closely related to Capparaceae and Brassicaceae (Abdallah and de Wit, 1978; Cronquist, 1988; Norris, 1941; Takhtajan, 1969; Thorne, 1976). However, recent studies based on embryological, morphological and molecular data, revealed unexpected relationships for Resedaceae (Gadek et al., 1992; Hall et al., 2002, 2004; Hufford, 1996; Karol et al., 1999; Rodman,

1991a,b; Rodman et al., 1993, 1994, 1996a,b, 1998; Tobe and Raven, 1991), which together with the Australian endemic Gyrostemonaceae and two Capparaceae genera (*Forchhammeria* Liebm. and *Tirania* Pierre; Pax and Hoffmann, 1936) formed the GRFT-clade (Hall et al., 2004). Unfortunately, none of these studies could unambiguously determine the sister-group of the Resedaceae. Although several molecular studies have been conducted in the order Brassicales, little attention has been paid to the origin and internal phylogenetic relationships of the Resedaceae. Hall et al. (2002, 2004) performed an extensive survey of the phylogenetic relationships within core Brassicales, based on plastid sequence data from various markers (*matK*, *ndhF*, *rbcL*). They suggested for

* Corresponding author. Fax: +95 4349151.

E-mail address: smarbra@upo.es (S. Martín-Bravo).

the first time monophyly of Resedaceae, although based on a very restricted sampling (only one accession each of *Reseda* and *Oligomeris*).

Taxonomy of the Resedaceae has been traditionally based on morphological data (e.g. ovary structure, petal shape, seed ornamentation, floral merosity). The most comprehensive taxonomic accounts of the Resedaceae were published by Müller Argoviensis (1857, 1864), and by Abdallah and de Wit (1978). Both treatments are mostly in agreement with respect to infrafamilial subdivisions, although differing in species number (Supplementary Table S1). The family was divided in three tribes (Astrocarpeae, Cayluseae, Resedeae), circumscribed by ovary and placentation types (Fig. 1). The tribe Resedeae was also divided into two subtribes on the basis of the relative position of sepals, petals and stamens: Randoninae, with *Randonia*, as characterized by its perigynous flowers; and Resedinae, with the remaining genera (*Ochradenus*, *Oligomeris*, *Reseda*) typically showing hypogynous flowers. However, the infrageneric subdivision of genus *Reseda* has been controversial. The circumscription of subgenera and sections depends on authorship (Supplementary Table S1). More recent taxonomic novelties include rearrangements of *Ochradenus* (Miller, 1984) and *Sesamoides* (López González, 1986, 1990), in addition to the description of some new spe-

cies of *Reseda* (Miller and Nyberg, 1994; Thulin, 1990; Valdés Bermejo and Kaercher, 1984), and *Ochradenus* (Miller, 1984; Miller and Morris, 2004; Thulin, 1994a), mainly from NE Africa and Arabian Peninsula.

The approximately 85 species of the Resedaceae primarily occur on limestone soils of arid environments (steppes and deserts). Some species are widespread weeds favoured by human activities, and a few are confined to high mountains. Four of the six genera (*Caylusea*, *Ochradenus*, *Oligomeris*, *Randonia*) occur in desert regions, while the remaining two (*Reseda*, *Sesamoides*) are mainly Mediterranean genera. The three *Caylusea* species are distributed in desert areas from Cape Verde Islands to southwestern Asia, and in the highlands of E Tropical Africa. *Oligomeris* occupies similar desert habitats and comprises two species in SW Africa, and a widespread species with a disjunct area, distributed in the Old World (from the Canary Islands to N India), and in the New World (SW North America). *Ochradenus* (9 species) and the monotypic genus *Randonia* are desert shrubs, the former occurring from N Africa to SW Asia, the latter restricted to gypsum soils of W and C Sahara. *Reseda* is by far the largest genus (c. 65 species) in the family. Many species of *Reseda* are restricted to the Mediterranean basin, while four species are worldwide weeds (*Reseda alba*, *R. lutea*, *R. luteola*, *R. phyteuma*).

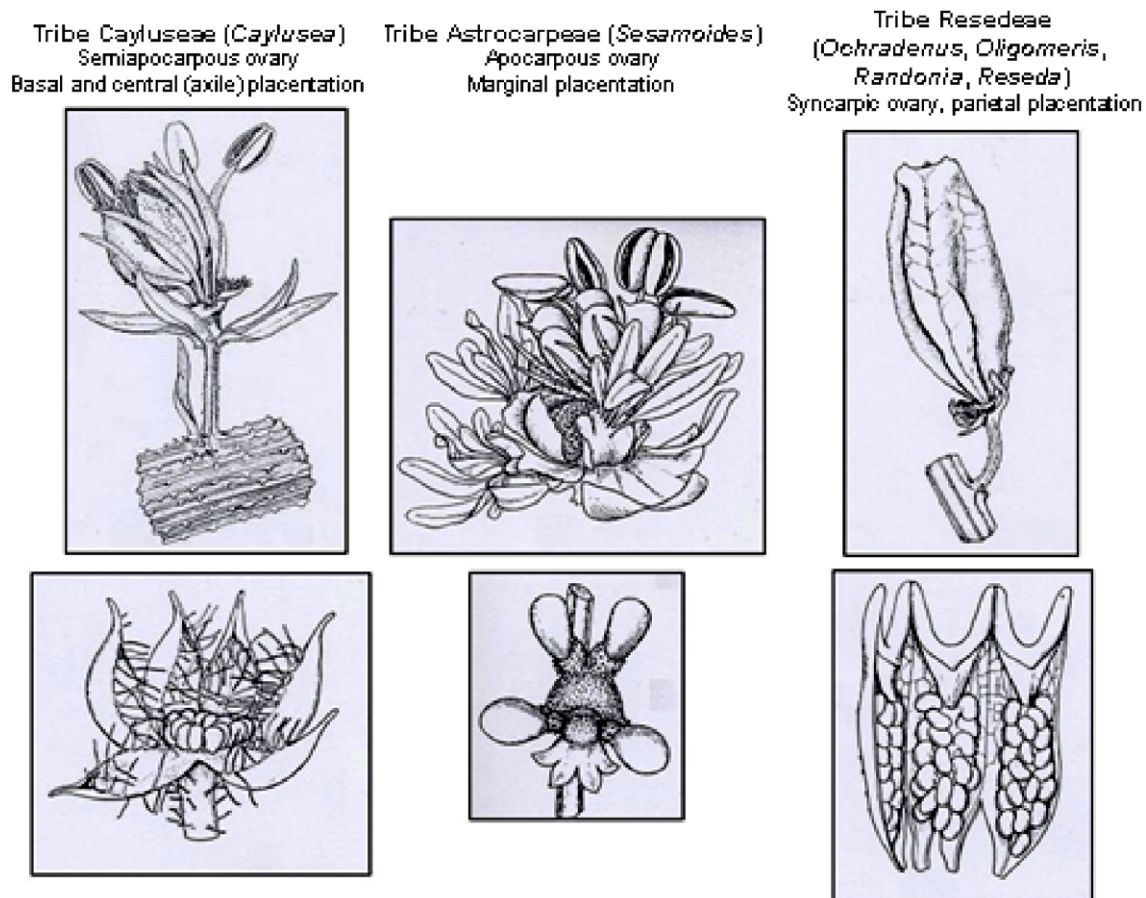


Fig. 1. Diagnostic characters of tribal classification of Resedaceae based on type of ovary and placentation. Illustrations taken from Abdallah and de Wit (1978).

Finally, the genus *Sesamoides*, with 1–6 species depending on the taxonomic treatment (Abdallah and de Wit, 1978; López González, 1993; Müller Argoviensis, 1857, 1864), occurs exclusively in the W Mediterranean region.

Cytogenetic studies have proven to be of major importance in Resedaceae, and could contribute to understand the evolutionary patterns inferred from phylogenetic reconstructions. A congruent pattern of different basic chromosome numbers and the infrageneric taxonomic classification of *Reseda* based on morphology (Table 1) was first detected by Eigsti (1936). These results were later confirmed by a series of cytogenetic studies of Iberian species of *Reseda* and *Sesamoides* (Fernández Peralta and González Aguilera, 1982; González Aguilera et al., 1980a,b; González Aguilera and Fernández Peralta, 1981, 1983, 1984). In these studies, the authors interpreted a basic chromosome number of $x=5$ for *Sesamoides* and three different ones within *Reseda* ($x=5$, $x=6$, $x=7$), which characterized different sections (Table 1). They also suggested $x=5$ as the primitive basic number for the whole family. Both dysploidy and polyploidy have been invoked as the primary driving forces in chromosome evolution of Resedaceae (review in González Aguilera and Fernández Peralta, 1984). Dysploidy may have been involved in the early occurrence of the different basic chromosome numbers of the Resedaceae, and also in the differentiation of certain sections of genus *Reseda* (sects. *Phyteuma*, *Reseda*). Polyploidy is associated to speciation of particular groups (*Sesamoides*, *Reseda* sects. *Glaucoseda*, *Leucoseda*, *Reseda*). As a result, number of complements in Resedaceae varies from two in *Reseda* sect. *Phyteuma* to 16 in *Sesamoides* (Table 1). Frequent anomalies were observed in chromosome pairing during meiosis, such as multivalent configurations, chromosome bridges, and early or lagged segregation of chromosomes (Fernández Peralta and González Aguilera, 1982; González Aguilera et al., 1980a,b; González Aguilera and Fernández Peralta, 1981, 1983). Despite a considerable number of cytogenetic studies, further investigations are needed to obtain new cytogenetic data for some species, particularly for those of *Caylusea*, *Ochradenus*, *Oligomeris* and *Randonia*.

In this paper we present the first molecular phylogenetic hypothesis of the Resedaceae with special emphasis on the largest genus (*Reseda*), using sequence data from the Internal Transcribed Spacer region (ITS) of the nuclear ribosomal DNA and the plastid *trnL-trnF* region. Particular issues addressed in this paper are to (i) test the monophyly of the six genera of the family and to analyze phylogenetic relationships between them; (ii) evaluate previous taxonomic classifications of the whole family and particularly the internal subdivision of genus *Reseda*; (iii) elucidate possible patterns of morphological and chromosome evolution inferred from phylogenetic relationships; and (iv) identify biogeographic patterns of diversity and endemism.

2. Materials and methods

2.1. Plant material and sampling strategy

Species delimitation of generic and infrageneric subdivisions within Resedaceae was established following two taxonomical treatments (Abdallah and de Wit, 1978; Müller Argoviensis, 1857, 1864), with some modifications (Supplementary Table S1). One hundred and fifty-six populations of 66 species were sampled (Supplementary Table S2 and Table 2), as follows: *Caylusea* (3 species), *Ochradenus* (6), *Oligomeris* (3), *Randonia* (1), *Reseda* (48), and *Sesamoides* (5). Up to 6 populations (*Caylusea hexagyna*) were sampled to represent the geographical and morphological variation within species. Special emphasis was placed on *Reseda*, including representatives of all infrageneric groups defined by Müller Argoviensis (1864) and Abdallah and de Wit (1978), and at least two populations per species, when possible.

Outgroup taxa were selected on the basis of recent molecular studies conducted across Brassicales using plastid markers (Hall et al., 2002, 2004; Rodman et al., 1993, 1996a,b, 1998). Four populations of Gyrostemonaceae (genera *Gyrostemon*, *Codonocarpus*, *Tersonia*), two of Tovariaceae (*Tovaria*), two of Capparaceae (*Forchhammeria*) and one of Pentadiplandraceae (*Pentadiplandra*) were included (Supplementary Table S2).

Table 1
Summary of the cytogenetic data available for Resedaceae

Taxon	Inferred basic chromosome number (x)	Selected references	Ploidy level
<i>Caylusea</i>	?	—	—
<i>Oligomeris</i>	5?	Dalgaard (1986)	6?
<i>Ochradenus</i>	7?	Spellenberg and Ward (1988), Mohamed (1997)	8?
<i>Randonia</i>	5?	Reese (1957)	6?
<i>Sesamoides</i>	5	González Aguilera and Fernández Peralta (1981)	4,8,12,16
<i>Reseda</i>			
<i>Glaucoseda</i>	7	González Aguilera et al. (1980a)	4
<i>Leucoseda</i>	5	Kaercher and Valdés Bermejo (1975), González Aguilera and Fernández Peralta (1983)	4,8
<i>Luteola</i>	6	Eigsti (1936), Fernández Peralta and González Aguilera (1982)	4
<i>Neoreseda</i>	?	—	—
<i>Phyteuma</i>	6	Eigsti (1936), González Aguilera et al. (1980b)	2
<i>Reseda</i>	6	Eigsti (1936), González Aguilera et al. (1980b)	4,8

Note. Specific haploid chromosome numbers are shown in Supplementary Table S2.

2.2. DNA extractions, PCR amplification and sequencing

A total of 154 accessions (66 species, 11 subspecies) of the Resedaceae were sequenced as the ingroup for the ITS analysis, and 95 accessions (59 species, 7 subspecies) for the *trnL-F* study. Combined analysis was performed for 93 samples of 59 species and 7 subspecies, from which we obtained both ITS and *trnL-F* sequences (Table 2).

Total genomic DNA was extracted from silica-dried material, fresh tissue from cultivated plants and herbarium specimens (BRNM, C, DBN, FT, GB, GH, HBG, HUI, LD, M, MA, MSB, NY, O, OXF, PRE, RNG, UPOS, UPS, WU), using the DNeasy Plant Mini Kit (Qiagen, California, USA) or NucleoSpin Plant-Kit (Macherey-Nagel). Standard polymerase chain reaction (PCR) was used for amplification of double-stranded DNA on a Perkin-Elmer PCR system 9700 (California, USA). Standard primers were used for amplification and cycle sequencing of the ITS region (Blattner, 1999 for ITS A; White et al., 1990 for ITS 4; Meimberg, 2002 for aITS1 and aITS4) and the *trnL(UAA)–trnF(GAA)* spacer (*trnC* and *trnF*, Taberlet et al., 1991). After 1–5 min pre-treatment at 94 °C, PCR conditions were: 24–35 cycles of 1 min at 94 °C, 30 s–1 min at 50–52 °C, 1–2 min at 72 °C, and a final stage of 15 min at 72 °C. Amplified products were cleaned using spin filter columns (PCR Clean-up kit, MoBio Laboratories, California, USA) and MicroconYM-100 Filter Tubes (Amicon Bioseparations), following the manufacturer's protocols. Cleaned products were directly sequenced using dye terminators (Big Dye Terminator v. 2.0, Applied Biosystems, Little Chalfont, UK) following the manufacturer's protocols, and samples were run on an Applied Biosystems Prism Model 3700 automated sequencer. Sequenced data were assembled and edited using the program SeqEd v. 1.0.3 (Applied Biosystems, California, USA). IUPAC symbols were used to represent nucleotide ambiguities in ITS sequences.

2.3. Phylogenetic analyses

Three different matrices were analyzed: ITS (157 sequences), *trnL-F* (104 sequences), and the ITS-*trnL-F* combined (96 accessions). Alignment was performed using

Table 2
Number of species of the six genera for ITS and *trnL-F* sequence analyses

Taxon	Marker			Percentage sampled (%)
	ITS	<i>trnL-F</i>	Combined	
<i>Caylusea</i>	3	3	3	100
<i>Ochrademus</i>	6	4	4	66
<i>Oligomeris</i>	3	3	3	100
<i>Randonia</i>	1	1	1	100
<i>Reseda</i>	48 (59)	45 (52)	45 (52)	77
<i>Sesamoides</i>	5	3	3	83
Total (family)	66	59	59	80

Note: Number of infraspecific taxa are specified in brackets for *Reseda*. Percentage of sampled species are given with respect to total number of species recognized (see supplementary Table S1).

the windows interface Clustal X v. 1.62b (Thompson et al., 1997), with manual adjustments. Maximum parsimony (MP) and Bayesian Inference (BI) analyses were performed on the complete ITS and *trnL-F* matrices as well as on reduced matrices resulting from the exclusion of multiple sequences per taxon. In MP analyses, all characters and transitions/transversions were equally weighted (Fitch, 1971), as implemented in PAUP* version 4.0b10 (Swofford, 2002). In order to avoid overweighting characters, gaps were treated as missing data. Heuristic searches were replicated 100 times with random taxon-addition sequences, tree-bisection-reconnection (TBR) branch swapping, and the options Multrees and Steepest Descent in effect. For cases in which the running was interrupted due to a memory fault, a second heuristic search was performed retaining only 500 trees per replicate with a number of steps equal to the one found in the previous heuristic search (Schultheis, 2001). In addition to standard measure of fit of characters to the trees produced (Consistency Index (CI) (Kluge and Farris, 1969); Retention Index (RI) (Farris, 1989)), the strength of support for individual branches was estimated by fast bootstrapping (Felsenstein, 1985) with 100,000 re-sampling. Congruence of ITS and *trnL-F* datasets was tested using the Hompart test (1000 replicates with a max-trees setting of 10) and the Templeton (1983) and Kishino and Hasegawa (1989) tests as implemented in PAUP.

In order to test whether interruptions of heuristic searches caused by memory faults could affect the results of analyses, we performed complete parsimony searches using the improved algorithms (Goloboff, 1999; Nixon, 1999) implemented in the program TNT v. 1.0 (Goloboff et al., 2003). These analyses were performed by using the new technologies sectorial search, ratchet and tree fusing, in a 1000 random addition sequence replicate analysis, with default parameters in effect and gaps treated as missing data. Trees retained after completion of each search were submitted to a “traditional” search with TBR branch swapping.

Insertions/deletions mutations needed for the alignment of the ITS and *trnL-F* matrices were coded with the program IndelCoder (Müller, 2006) using the Modified Complex Indel Coding (MCIC) algorithm. In order to evaluate the relevance of considering indels as additional coded characters for phylogenetic inference, two different MP analyses were carried out for each matrix, one including all indels as exactly coded by IndelCoder as additional characters, and one without coded indels. In order to test the accuracy of this new program, IndelCoder codification was manually revised and a third matrix was obtained and analyzed. This third matrix was compiled by excluding those additional characters which codified indels that only affected outgroup, were ambiguous (hypervariable ends) or autoapomorphic.

The complete ITS matrix was split into three different matrices, including the ITS-1, ITS-2 spacers, and the 5.8S region, respectively. These three matrices and the complete *trnL-F* matrix were analyzed to determine the simplest

model of sequence evolution, both under the Hierarchical Likelihood Ratio Test (hLRT) and the Akaike Information Criterion (AIC) as implemented in MrModeltest 1.1b (Nylander, 2002; Posada and Crandall, 1998). When each criterion selected a different evolutionary model, Bayesian analyses were performed under both models by using MrBayes 3.0b4 (Ronquist and Huelsenbeck, 2003). Four Markov Chain Monte Carlo were run simultaneously in each Bayesian analysis for 5,000,000 generations with an interval of 1000 generations. Burn-in was evaluated over generations. After discarding trees yielded before the Likelihood stationary, the remaining trees were summarised in a majority rule consensus tree, using posterior probability (pp) as a measure of clade support. Tree topology depicted by different evolutionary models selected by each criterion was similar and differing slightly in clade support. Therefore pairwise differences, tree topology and posterior probabilities herein shown, were those obtained when applying the simplest model selected by the hLRT criterion. The evolutionary models that best fit the ITS-1 and ITS-2 spacers were different from the one that best fits the 5.8S region (see Section 3.2). Accordingly, character partition was performed on the complete ITS matrix for the Bayesian analysis.

3. Results

3.1. Characteristics of ITS and *trnL-F* sequences

The characteristics of ITS and *trnL-F* sequences are summarised in Table 3. According to phylogenetic results, these characteristics are given for (1) the family Resedaceae, which comprises 154 ITS and 95 *trnL-F* sequences, and (2) the core *Reseda*, with 136 ITS and 85 *trnL-F* accessions of *Ochradenus*, *Oligomeris*, *Randonia*, and *Reseda*.

Visual inspection of ITS chromatograms of the core *Reseda* revealed clear nucleotide additivities (positions containing double nucleotide peaks) in 131 positions. These additivities were found in 62 accessions, representing 39 species of the four genera of the core. Forty five of these 131 additive positions were present in 4 species of *Reseda* sect. *Leucoreseada* (*R. alba*, *R. attenuata*, *R. suffruticosa*, *R. valentina*). Although it was not possible to determine whether double-peaks were due to sequencing artifacts or to different ITS copies, equimolar proportions of alternative nucleotide peaks in many accessions suggested the presence of more than one ITS copy as the most probable explanation. This view is also supported by the fact that 86 of the 131 additive positions were variable sites for the remaining accessions with no additivities. Nonetheless, further studies which include cloning of those species showing ITS double-peaks are necessary to check if they are caused by multiple ITS copies due to hybridization processes. Corrected GTR pairwise distances of ITS sequences within the Resedaceae vary between 0% (80 pairs of sequences) and 28.56% (*Caylusea latifolia* and the two populations of *C. abyssinica* vs. *Reseda alphonsi*). Within the core *Reseda*, the minimum pairwise distance (0%) was found between 58 pairs of sequences, and the maximum (23.34%) between *Reseda undata* ssp. *undata* and *R. alphonsi*. The highest sequence divergence within the same species in core *Reseda* (3.57%) was found when comparing *Reseda valentina* ssp. *valentina* pop.2 and *R. valentina* ssp. *almijarensis*.

Corrected GTR pairwise distances of *trnL-F* sequences within the Resedaceae vary between 0% (59 pairs of sequences) and 7.46% (*Sesamoides interrupta* vs. *Randonia africana* pop.2). Within the core *Reseda*, the minimum pairwise distance (0%) was found between 55 pairs of sequences, and the maximum one (4.35%) between *Reseda luteola* pop.3 and *R. urnigera*. The highest sequence

Table 3

Summary of phylogenetic results obtained from the analyses of ITS and *trnL-F* sequences of Resedaceae and the core *Reseda*, once outgroup sequences were excluded

	ITS region	ITS			<i>trnL-trnF</i>
		ITS-1	5.8S	ITS-2	
<i>Resedaceae</i>					
Length range (bp)	624–639	251–266	160–162	208–213	697–787
Aligned length (bp)	681	281	162	238	1002
Number of variable vs. informative characters	298/258	176/156	11/6	111/96	139/99
Maximum sequence divergence (GTR)	28.56%	43.35%	2.71%	25.68%	7.46%
Informative indels (no. bp)	15 (1–18)	9 (1–18)	0	6 (1–2)	10 (1–93)
Number of nucleotide additivities	151	98	5	48	0
Number of accessions with nucleotide additivities	67	50	5	37	0
CI' (CI)	0.55 (0.57)	—	—	—	0.71 (0.76)
RI	0.92	—	—	—	0.93
Mean G + C content	57.80%	57.56%	55.69%	59.59%	33.22%
<i>Core Reseda</i>					
Number of variable vs. informative characters	266/220	154/133	9/4	103/83	113/76
Maximum sequence divergence (GTR)	23.34%	25.81%	1.29%	25.68%	4.35%
Informative indels (no. bp)	8 (1–2)	4 (1)	0	4 (1–2)	6 (1–93)
Number of nucleotide additivities	131	86	5	40	0
Number of accessions with nucleotide additivities	65	47	6	34	0

divergence within the same species in core *Reseda* (1.07%) was found when comparing the two populations of *Randonia africana*.

3.2. Phylogenetic analyses

Phylogenetic reconstructions of the ITS, *trnL-F* and combined matrices performing BI and MP, using algorithms implemented in PAUP (specified below) and TNT (results not shown), yielded similar topologies. Both plastid and nuclear data sets were congruent when testing topological congruence with Templeton and Kishino–Hasegawa tests as implemented in PAUP (results not shown). However, the Hompart test showed that both data sets were incongruent. By partitioning the taxon sampling and testing independently, the incongruence could be traced back to six accessions (*Reseda attenuata* pop.2, *R. barrelieri*, *R. ellenbeckii* pop.2, *R. gayana* pop.2, *R. sessilifolia* and *R. valentina* ssp. *valentina* pop.2). Of these, four taxa belong to *Reseda* sect. *Leucoreseda*, where topological incongruences affect terminal branches. *Reseda sessilifolia* and *R. ellenbeckii* pop.2 were positioned as polytomies to most of the other accessions of the ingroup with *trnL-F*, but were positioned within well-resolved clades with ITS. After excluding these six accessions and the outgroup, the Hompart test revealed congruence between both data sets (p -value = 0.13). Despite the lack of congruence for the whole data matrix using the Hompart, the combined analyses were conducted, as topological tests revealed congruence and the few incongruences detected did not affect deep nodes of phylogenies. Additionally, only minor differences were detected in the consensus topologies of the single matrix analyses, and combined results increased branch support for nearly all clades. BI analyses of the complete ITS, *trnL-F*, and combined matrices are shown in Figs. 2–4, respectively. Resolution and clade support were considerably lower in the individual *trnL-F* analysis compared to the ITS and combined, irrespective of the method used, due to a reduced amount of informative positions of the data set (Table 3). Topologies obtained from phylogenetic analyses of the complete ITS, *trnL-F* and combined matrices were identical and the measure of fit similar to those inferred from the reduced matrices (one accession per species). The MP analyses of the complete ITS matrix resulted in 47,000 shortest trees of 1085 steps (CI' = 0.551; RI = 0.922); 49,501 shortest trees of 391 steps (CI' = 0.713; RI = 0.934) for the *trnL-F* matrix; 47,503 shortest trees of 1397 steps (CI' = 0.579; RI = 0.880) for the combined matrix. Similar bootstrap supports were obtained for each of the three different MP analyses (without indel coding, only coding informative indels, and including all indels coded), as conducted to evaluate the accuracy of the program IndelCoder. MrModeltest retrieved SYM + I + G as the most likely evolutionary model for the ITS-1 spacer, SYM + G for the ITS-2 spacer, K80 + I for the 5.8S region, and GTR + G for *trnL-F*.

Resedaceae form a strongly-supported monophyletic group (100% bs; 100% pp) irrespective of the sequences and analyses performed. The ITS (Fig. 2) and ITS-*trnL-F* (Fig. 4) trees revealed three well-supported clades (all $\geq 92\%$ bs; 100% pp). Two of them clustered all accessions of *Caylusea* and *Sesamoides* together, and the third one includes all accessions of *Ochradenus*, *Oligomeris*, *Randonia*, and *Reseda*. *Caylusea* is sister group to the rest of the Resedaceae (100% bs; 100% pp) and then *Sesamoides* is sister ($\geq 83\%$ bs; 100% pp) to the third major clade (core *Reseda*). Two lineages of moderate to high support contain accessions of *Reseda* and *Oligomeris* (lineage A, $\geq 69\%$ bs; 100% pp) and accessions of *Reseda*, *Ochradenus* and *Randonia* (lineage B, $\geq 93\%$ bs; 100% pp). Lineage A is formed by four sublineages. Sublineage A1 has accessions exclusively of *Reseda* sect. *Leucoreseda* ($\geq 99\%$ bs; 100% pp) and is sister to the other three. The sublineage A2 contains accessions of *Reseda* sect. *Luteola* (100% bs; 100% pp) and is, in turn, sister to the sublineage A3, with accessions of *Reseda* sect. *Glaucoseseda* ($\geq 98\%$ bs; 100% pp), and the sublineage A4, with those of *Oligomeris* ($\geq 71\%$ bs; $\geq 90\%$ pp). Lineage B displays limited resolution of sublineages containing the rest of accessions of core *Reseda*. Three basal, well-supported sublineages (B1, B2, B4) and one with weak support (B3) are recognized and further discussed (Figs. 2 and 4).

4. Discussion

4.1. Character evolution and systematic implications

Analyses of nuclear ITS and plastid *trnL-F* sequences of nearly all species of the Resedaceae strongly support its monophyly, as already proposed in previous molecular studies based on plastid markers (*rbcL*, *ndhF*, *matK*; Hall et al., 2004). Within Resedaceae, *Caylusea* and *Sesamoides* constitute well-supported monophyletic genera, while the large genus *Reseda* is not monophyletic, since the genera *Oligomeris*, *Ochradenus*, and *Randonia* are embedded within *Reseda* (core *Reseda*; Figs. 2–4). The three tribes (Cayluseae, Astrocarpeae, and Resedeae) traditionally recognized in the Resedaceae share a most recent common ancestor, as retrieved in all phylogenies ($\geq 73\%$ bs; 100% pp; Figs. 2–4). The use of two diagnostic morphological characters (ovary and placentation type; Abdallah and de Wit, 1978; Bolle, 1936; Müller Argoviensis, 1857, 1864) is congruent with our result of three lineages including all accessions of the three tribes. Placentation appears to have evolved from the plesiomorphic central-axial condition found in the tribe Cayluseae to the apomorphic parietal in the tribe Resedeae, as it has been traditionally proposed (Puri, 1945, 1950; Ronse de Craene, 2002; Stebbins, 1974). Our analysis however reveals marginal placentation (tribe Astrocarpeae) as the intermediate state (Fig. 1). On the other hand, an unidirectional increment in carpel fusion in the course of evolution is not supported by our data. Cayluseae, the earliest divergent tribe, has a semiapocarpic ovary,

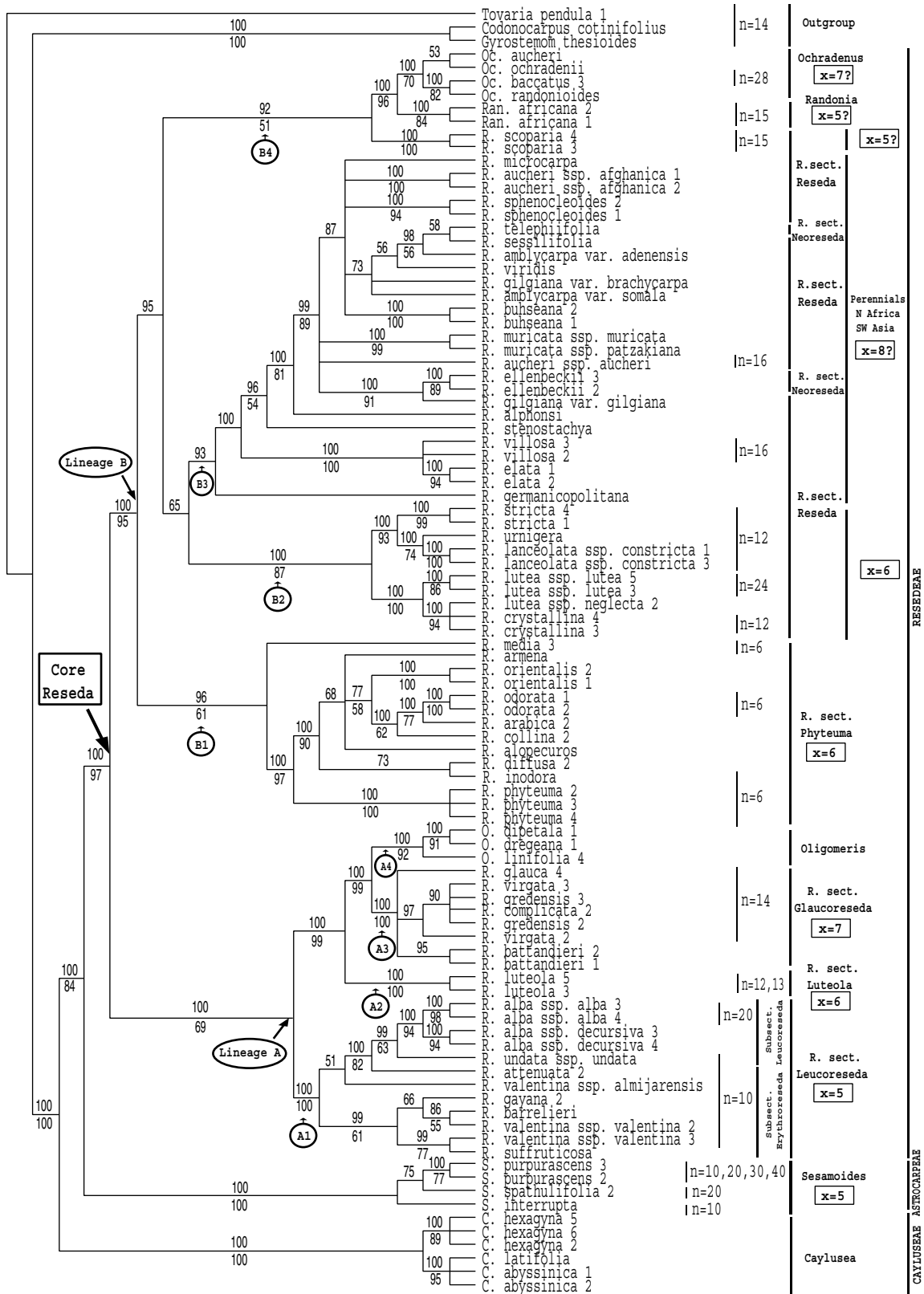


Fig. 4. Majority rule consensus tree of the 49,601 trees retained in the Bayesian Inference of the 93 combined *trnL-F/ITS* sequences of the same samples of Resedaceae plus three outgroup sequences. Posterior probabilities and bootstrap values are given above and below branches, respectively. Vertical bars indicate supraspecific taxa from the same taxonomic group. Haploid (*n*) and inferred basic chromosome numbers (*x*) also shown.

indicating that apocarpic ovaries (tribe Astocarpeae) originated later, and then syncarpic unilocular ovaries (tribe Resedaceae). Accordingly, our nuclear and plastid phylogenies disagree with acceptance of apocarpy as the primitive condition, as historically admitted by most taxonomists (i.e. Armbruster et al., 2002; Eames, 1961; Endress, 1982, 2001; Soltis et al., 2005; Stebbins, 1974). Syncarpy is a key innovation and has a broad adaptive advantage over apocarpy, so conditions favouring apocarpy over syncarpy are uncommon (Armbruster et al., 2002; Endress, 2001), and reversals to apocarpy in angiosperms rare (Endress et al., 1983; Fallen, 1986; Jenny, 1988; Ramp, 1988). However, this reversal event affecting *Sesamoides* was suggested by systematists (Cronquist, 1981; Hufford, 1996; Kubitzki, 2003; Sobick, 1983; Takhtajan, 1997). A general pattern of reduction in carpel number in Resedaceae is also deduced from our phylogenetic hypothesis, in agreement with a main trend in angiosperms (Soltis et al., 2005). Carpel number is somehow variable in the early divergent lineages (5–6 in *Caylusea* and 4–8 in *Sesamoides*), and then decreases in core *Reseda* (usually 3–4), followed by acquisition of two carpels in *Randonia africana* and *Reseda* sect. *Neoreseda*.

Our molecular phylogeny is not conclusive to resolve the taxonomic complexity of *Sesamoides*, in which some authors (Abdallah and de Wit, 1978; Müller Argoviensis, 1864) circumscribed a single species, while others recognized up to six different species (López González, 1993). The low morphological variability found among *Sesamoides* taxa is also supported by their limited ITS and *trnL-F* sequence divergence, and give evidence for limited differentiation in this genus.

The phylogenetic placement of *Oligomeris*, *Ochradenus* and *Randonia* within genus *Reseda* is surprising if we consider the diagnostic, clear-cut characters of these genera. However, it could be a good example of how ecological adaptation to extremely xerophytic conditions may induce homoplastic morphological variation in *Reseda*, which leads to taxonomic decisions that hide true systematic relationships. Thus, the acquisition of woodiness and deciduous leaves in *Ochradenus* and *Randonia*, and the polygamous flowers and reduction or loss of petals in *Oligomeris* and *Ochradenus* are interpreted as morphological adaptations to desert conditions. In fact, simplification of floral structure, i.e. reduction in size or loss of petals and a tendency towards dioecy (polygamous flowers), has been considered as a transitional state in the evolutionary pathway from hermaphroditism to dioecy (Barrett, 2002; Charlesworth and Charlesworth, 1978). Convergent evolution for these characters has been reported in desert habitats as those where Resedaceae genera occur (Hall et al., 2004; Hufford, 1996). Acquisition of woodiness in *Ochradenus* and *Randonia* is intriguing since the annual habit, considered to be an adaptation to dryness, appears to be predominant in the Sahara flora (Braun-Blanquet, 1964).

Despite lack of evidence for monophyly of *Reseda*, the general evolutionary pattern within the core *Reseda* is mainly in agreement with previous sectional classification

of *Reseda*. Thus, four of the six sections recognized within *Reseda* are monophyletic (*Leucoreseada* (lineage A1), *Luteola* (A2), *Glaucoreseada* (A3), *Phyteuma* (B1); Figs. 2, 4). Two different major lineages are reported within the core *Reseda*, clade A and clade B. Lineage A comprises mostly 4-carpelled species (except for *R. luteola*), whereas members of lineage B usually bear 3 carpels, with rare exceptions of 2 carpels (*Randonia*, *Reseda* sect. *Neoreseda*). Tree topology of ITS and *trnL-F* sequence analyses indicates not only a general pattern of reduction in carpel number in the Resedaceae but also within the core *Reseda* in a homoplastic fashion.

Section *Leucoreseada* (lineage A1) is formed by the Mediterranean widespread *R. alba* and six endemics to Iberian Peninsula and NW Africa. This section has been divided in two subsections considering habit and floral structure (Aránega, 1992, 1994; Supplementary Table S1), which are partly supported by our ITS and combined analyses. Accessions of both subsections are segregated in two major sublineages (Figs. 2–4). However, incongruences in the phylogenetic placement of several accessions (*R. attenuata*, *R. barrelieri*, *R. valentina*) were found when comparing nuclear (Fig. 2) and plastid (Fig. 3) phylogenies. These incongruencies concerned the internal resolution of sect. *Leucoreseada*, and may also be responsible for the lack of congruence depicted when applying the Hompart test. Species identity is clarified by our analysis in some cases but not in others. Our results support identity of *R. gayana*, traditionally subsumed under *R. undata* (Abdallah and de Wit, 1978; Müller Argoviensis, 1864; Valdés Bermejo, 1993; Yeo, 1964, 1996), and its placement in subsect. *Erythroreseda*, as already proposed on the basis of morphology (Aránega, 1992, 2005). Populations of the Mediterranean widespread and highly variable *R. alba* (subsect. *Leucoreseada*) do not form a monophyletic group according to our ITS and *trnL-F* trees (Figs. 2 and 3). We hypothesize, as already suggested (Abdallah and de Wit, 1980; Zohary, 1966), that hybridization between taxa of *R. alba* complex plays an important role and is responsible for numerous admixtures (23) found in six ITS accessions of this group.

Reseda sect. *Glaucoreseada* (lineage A3) consists of restricted endemics to the high mountain ranges and plateaus of the Iberian Peninsula and Morocco. Low internal resolution in this section (Figs. 2–4) is due to low level of sequence divergence rather than nucleotide incongruences among informative characters (results not shown). This result is congruent with recent, ongoing processes of allopatric speciation. The striking sister-relationship between *Reseda* sect. *Glaucoreseada* and *Oligomeris* has never been proposed in taxonomic accounts, although both taxa share several morphological features (4 carpels, entire leaves, basal leaf teeth, persistent sepals and stamens).

The monotypic *Reseda* sect. *Luteola* is the only member of lineage A which consistently bears 3 carpels. However, it has traditionally been considered close to sect. *Glaucoreseada*, because both taxa are the only ones in the family showing a forked placenta.

The remaining species of *Reseda* (sections *Reseda*, *Phyteuma*, *Neoreseda*), together with those of *Ochradenus* and *Randonia*, form lineage B (Figs. 2–4). The large, basal polytomy displayed in this lineage B, prevents us from establishing major clades and determining phylogenetic relationships among them.

Monophyly of section *Phyteuma* is not clearly retrieved in our analyses. All the species of sect. *Phyteuma* cluster together (B1) in the *trnL-F* (Fig. 3) and combined (Fig. 4) analyses, but not in the ITS tree (Fig. 2). Our results support the identity of the NW African endemic *R. collina* (Müller Argoviensis, 1857, 1864), as well as its independence from *R. phyteuma*, in contrast with the view of several authors (Abdallah and de Wit, 1978; Ibn Tattou, 1999; Valdés, 2002). *Reseda phyteuma* has been proposed as one of the putative ancestors of the cultivated *R. odorata*, as well as *R. arabica* and *R. orientalis* (Abdallah and de Wit, 1978). Nuclear and plastid discordance suggests a hybrid origin, in which *R. arabica* and *R. orientalis* may have been involved (Figs. 2 and 3).

All species of section *Reseda* are included in sublineages B2, B3, and B4 (Figs. 2 and 4) together with those of sect. *Neoreseda* (B3) and *Ochradenus* and *Randonia* (B4). Sublineage B2 (*Reseda* sect. *Reseda sensu stricto*) shows high congruence between taxonomic and monophyletic groups, with the exception of the NW African *R. lutea* ssp. *neglecta*, which is linked with the Canarian endemic *R. crystallina* and not with its conspecific ssp. *lutea* (see discussion in biogeography section).

Mostly perennial species from N Africa and SW Asia included in section *Reseda* form the weakly supported sublineage B3. However, phylogenetic relationships within this group are hindered by the poor internal resolution in all analyses. In this particular case, low resolution may be due to active reticulation processes, as 26 additivities have been detected in 14 ITS accessions representing 12 species of sublineage B3. In addition to this, some conspecific accessions do not cluster together (*Reseda amblycarpa*, *R. aucherii*, *R. ellenbeckii*, *R. gilgiana*; Figs. 2–4). The atypic two-carpelled shrublets *R. ellenbeckii* and *R. telephiifolia* also fall within this clade. Both species were treated in the separate subgenus or section *Neoreseda* (Abdallah and de Wit, 1978; Perkins, 1909, respectively). However, in light of our results and in order to seek a natural classification, there is no evidence for support of an independent group for these species.

Accessions of *Ochradenus* and *Randonia* form a monophyletic group in the same sublineage (B4) in all analyses. Occurrence of the two genera in lineage B coincides with a relatively reduced number of carpels (3 and 2, respectively). Both genera were recognized by Müller Argoviensis (1864) and Abdallah and de Wit (1978) based on floral differences, while Miller (1984) considered no morphological evidence to differentiate them. The two genera display biological affinities, i.e. desert shrubs very similar in habit, with spinescent branches and deciduous leaves. However, occurrence of three-carpelled flowers without corolla in *Ochradenus*,

while two-carpelled with 8 petals in *Randonia*, coupled with grouping of ITS species accessions into monophyletic groups (Figs. 2, 4; but see Fig. 3), lead us to recognize the taxonomic validity of both genera.

4.2. Cytogenetic evolution

There exists a strong relationship between cytogenetic evolution, as inferred from the ITS and *trnL-F* phylogenies, and taxonomic classification of the family (Fig. 4). Our results suggest an evolutionary increment of haploid number from the proposed basic chromosome number $x=5$ (González Aguilera and Fernández Peralta, 1984). Dysploid processes may have been involved in early acquisition of the two secondary basic numbers ($x=6$, $x=7$), and therefore would have acted as the driving force in the cytogenetic evolution of the family. It could not be tested whether $x=5$ constitutes the ancestral state, because no chromosome number is available for the basal-most genus *Caylusea*. *Sesamoides*, sister to the remaining genera, displays haploid chromosome numbers of 10, 20, 30, and 40 (González Aguilera and Fernández Peralta, 1981). It has been stated that the basic chromosome number of *Sesamoides* is $x=5$, in spite of the series of haploid numbers ranging from 10 to 40, because meiotic tetravalents have been observed in species with $2n=20$ (González Aguilera and Fernández Peralta, 1981, 1984). Our phylogenetic reconstructions suggest that the derived basic number $x=6$ may have been acquired twice or three times in the course of the evolution of the family, as it is the inferred basic number in three independent sublineages (A2, B1, B2). Similar arguments of those given for *Sesamoides* led to accept $x=7$ as the basic chromosome number of *Reseda* sect. *Glaucoseda* (sublineage A3; González Aguilera et al., 1980a). The position of sect. *Glaucoseda* in all phylogenetic reconstructions indicates a more recent acquisition of the basic number $x=7$ in *Reseda*.

In addition to dysploidy, polyploidy seems to have acted as an important cytogenetic mechanism in the evolution of the Resedaceae. *Sesamoides purpurascens* contains several ploidy levels (from $n=10$ to $n=40$), which is a strong pattern of ploidy increment also found at a lower extent in *Reseda* sects. *Leucoseda* and *Reseda s.s.* (Table 1, Supplementary Table S2). Our phylogenetic results help to interpret evolution of chromosome number in the course of polyploidization in two cases. A pectinate topology of lineage A and sister-group relationships indicate that acquisition of $n=20$ in *Reseda alba* (sect. *Leucoseda*) is the result of polyploidy from $n=10$. In lineage B2, species of sect. *Reseda s. s.* ($n=12$), form a well-supported sister-group to *Reseda lutea* ($n=24$), leading us to suggest a second case of polyploidization in the Mediterranean region. It is interesting to notice that these two unique known octoploids in *Reseda* (*R. alba*, *R. lutea*) form part of the two main lineages (A, B; Figs. 2 and 4) and are the most morphologically variable and widely distributed species within their sections (*Leucoseda* and *Reseda*, respectively). In fact, *R. alba* and

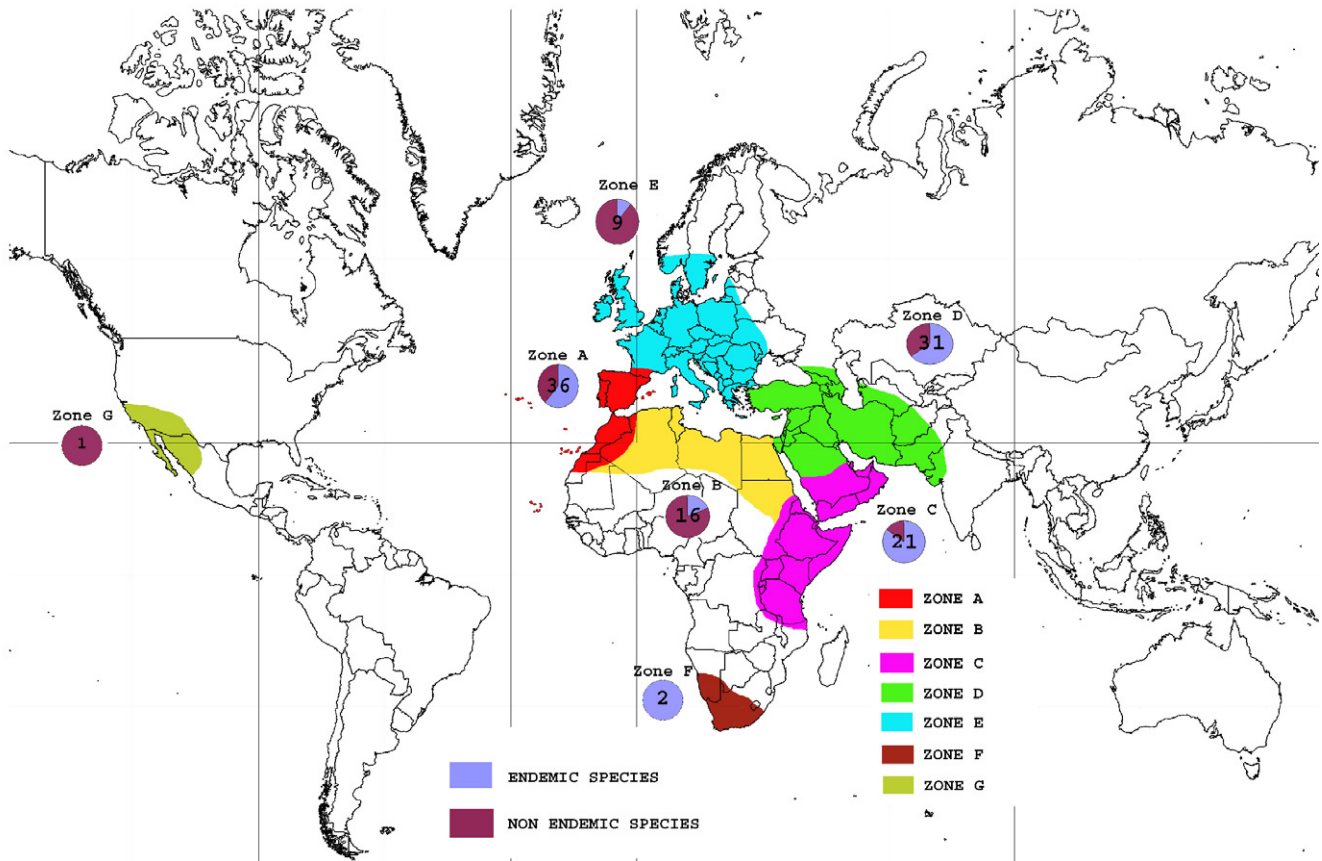


Fig. 5. Distribution map of the Resedaceae displaying defined zones used in the biogeography discussion as indicated by different area colours. Numbers encircled and pie diagrams include total number of species in each zone and proportion of endemics and total number.

R. lutea are becoming invasive aliens in many parts of the world (i.e. Daniel, 1993; Davis et al., 1993; Harris et al., 1995; Heap, 1997; Heap et al., 1993; Leistner, 1970; Pearce, 1982), whereas the species with lowest chromosome numbers in sects. *Leucoreseda* and *Reseda* show a more limited distribution (Fig. 4; Supplementary Table S2). This fact agrees with the presumed improvement of colonization ability attributed to polyploids and their predominance in the checklists of the more extended weeds (Ehrendorfer, 1980; Soltis and Soltis, 2000; Stebbins, 1972).

4.3. Biogeographic implications

Resedaceae are primarily distributed in the Old World, with the Mediterranean basin as the primary center of diversity in terms of not only species but also genera number. Species distribution of the Resedaceae is summarised in seven different zones (Fig. 5), characterized by vegetation, habitat, climatic, and geographic characteristics. The region including the Iberian Peninsula, NW Africa and Macaronesia (zone A), is one of the two main hotspots of the Resedaceae, with 36 species and five genera. High level of endemism with 22 endemic species, the endemic *Reseda* sect. *Glaucoreseda* and the subendemic sect. *Leucoreseda* (all endemic species except for the widespread *R. alba*) lead us to interpret an active, long-term diversification process

in zone A. The diversity of habitats (steppes, savannas, garigues, semiarid and arid deserts), the presence of high mountain ranges and long-term isolated archipelagos, as well as the influence of past climatic and geological events, help to understand the number of taxa and endemics' abundance. In the case of the mountain sect. *Glaucoreseda*, our ITS phylogeny support the monophyly of the Moroccan *R. battandieri* and *R. glauca* from Pyrenees (Fig. 2). The remaining species appear unresolved in a polytomy due to the low number of informative characters, that may be caused by recent diversification as a result of post-glacial isolation. At least four independent dispersal events may have been involved in the colonization of Resedaceae to the Canary Islands. The Canary flora contains the widespread *Oligomeris linifolia*, the Mediterranean *Reseda luteola*, and two endemic species (*R. crystallina*, *R. scoparia*), which are placed in four independent lineages of the ITS, *trnL-F* and combined phylogenies (Figs. 2–4). Concerning continental sources of dispersals, no general pattern can be established with confidence. Assuming current species distributions as ancestral areas, our results place the origin of the endemic *R. crystallina* in NW Africa from an ancestor related to the endemic *R. lutea* ssp. *neglecta* (Figs. 2–4), as already proposed by Abdallah and de Wit (1978) on the basis of morphology. The case of the endemic *R. scoparia* is difficult to explain, given that its phylogenetic placement remains still

unclear. This taxon is sister to the *Ochradenus/Randonia* clade if plastid (Fig. 3) and combined analyses (Fig. 4) are considered, while unresolved in the basal polytomy of lineage B of the ITS tree (Fig. 2).

Zone B, which includes desert countries of N and NE Africa (from Algeria to Egypt), harbours representatives of five of the six genera of the Resedaceae. In contrast to zone A, this zone is not significantly rich in species number and local endemics. This homogeneous pattern of diversity may be due to relatively similar habitats and uniform climates over time.

Tropical E African countries (Kenya, Uganda, Tanzania, Ethiopia, Somalia, Eritrea, Djibouti) and S Arabian Peninsula (zone C) are also rich in species (21) and genera (4). This zone has an extraordinary percentage of endemic species of the Resedaceae (80%), since 17 out of the 21 species are exclusive to this area. Particularly, two of the three species of genus *Caylusea* and all the nine *Reseda* species occurring in zone C are endemics. These nine *Reseda* species are characterized by shrubby habit, and belong to the same lineage (B3) in our phylogenetic reconstructions (Figs. 2 and 4). New arid habitats generated since aridification in the Pleistocene (Cane and Molnar, 2001; Chiarugi, 1933; deMenocal, 1995; Quézel, 1978) were suitable for *Reseda*. An earlier isolation time by Pleistocene aridification processes, together with a diverse topography and geology (some edaphic endemics), may have been responsible for high levels of speciation rates in zone C. Recurrent aridification processes may have also affected *Ochradenus*, which also displays the greatest diversification of the genus in E Africa and S Arabia (Miller, 1984), with eight of the nine species (6 endemics) in zone C. These two territories are separated by the Red Sea and Gulf of Aden, whose formation is a fairly old geological event (~20–10 million years ago, Miocene) for plant speciation (Meulenkaamp and Sissingh, 2003; Roberts, 1969; Wickens, 1976). A strong disjunct pattern is observed in this zone, where *Reseda* differentiated preferentially in the horn of Africa (6 endemics), whereas *Ochradenus* displays a high number of endemics (4) in the southern-most stripe of the Arabian Peninsula. Additionally, an ancient part of the African continent (the island of Socotra) displays a similar pattern of allopatric speciation. Our phylogenetic results agree with two independent speciation processes (lineages B3, B4) in Socotra to form two unrelated Resedaceae endemics (*Reseda viridis*, *Ochradenus socotranus*; Fig. 2).

Zone D primarily comprises SW part of Asia, from E Mediterranean basin to W India (Fig. 5). This broad area is the second main center of diversity of the Resedaceae (31 species, 4 genera). All the zone D endemics are circumscribed in *Reseda* sects. *Phyteuma* and *Reseda* (except for one species of *Ochradenus*), reflecting a great diversification pattern within two sublineages (B1, B3; Figs. 2 and 4). At least 15 endemic species of sect. *Reseda* are present in zone D, accounting for nearly 60% of the total number of species within the largest section of the genus.

Zone E comprises the C Mediterranean basin (from Sardinia and Corsica to Greece) and C Europe. The low number of species and genera (9/2) in this large territory, coupled with occurrence of multiple sublineages of recent origin (*Reseda* sects. *Leucoreseda*, *Luteola*, *Phyteuma*, *Reseda*), reveals a relatively new colonization of most European countries. In fact, there is only one endemic species in this area (*Reseda inodora*).

Zone F consists of the remote area of SW Africa where two endemic species of *Oligomeris* can be found (*O. dregeana*, *O. dipetala*). Several cases of disjunct distribution between the arid regions of N and S Africa have been studied (de Winter, 1971; Goldblatt, 1978; Thulin, 1994b). This pattern of disjunction has been traditionally explained by the existence of an arid corridor facilitating N-S connection through E Africa during dry phases of the Pleistocene (Jürgens, 1997; Verdcourt, 1969; Werger, 1978). This corridor may have been operating in alternate arid-humid phases in pre-Pleistocene periods to account for long isolation processes (Besnard et al., 2006). Alternatively, the possibility of long-distance dispersal has also been proposed (Thulin, 1994b). The small zone G (SW North America), which only harbours the widespread species *Oligomeris linifolia*, represents the most remarkable disjunction of the Resedaceae. Further work is needed to investigate whether long-distance dispersal is responsible for recent colonization of *Oligomeris* in SW North America, as suggested by sequence similarity of populations from Yemen, Morocco, Tunisia and the isolated Canary Islands.

Acknowledgments

The authors thank to M. Míguez, F.J. Fernández, and T. Ernst for technical support; M. Escudero for advice on some analyses; the curators of BRNM, C, DBN, FT, GB, GH, HBG, HUI, LD, M, MA, MSB, NY, O, OXF, PRE, RNG, UPOS, UPS, and WU herbaria for the loan of specimens and granting permissions for DNA extractions. The following contributors provided plant material: P. Escobar, P. Jiménez, J.M. Marín, and J. Martínez. This research was supported by the Spanish Ministry of Education and Science through the project REN2002-04354-C02-01 and through a Ph.D scholarship to S. Martín-Bravo.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ympev.2006.12.016.

References

- Abdallah, M.S., de Wit, H.C.D., 1978. The Resedaceae: a taxonomical revision of the family (final instalment). Meded. Landbouwhoogeschool Wageningen, 78.
- Abdallah, M.S., de Wit, H.C.D., 1980. Resedaceae. In: Townsend, C.C., Guest, E. (Eds.), Flora of Iraq, vol. 4. Ministry of Agriculture and Agrarian Reform, Baghdad, pp. 1085–1094.

- Aránega, R., 1992. Estudio Biosistemático de *Reseda* L. sect. *Leucoseda* DC. (Resedaceae) en el Mediterráneo Occidental. Ph. D. Thesis. Universidad Complutense de Madrid.
- Aránega, R., 1994. Notas sobre *Reseda* sect. *Leucoseda* DC. en la Península Ibérica. *Anales Jard. Bot. Madrid* 52, 216–221.
- Aránega, R., 2005. Aclaraciones taxonómicas y nomenclaturales sobre *Reseda decursiva* Forssk. y *Reseda gayana* Boiss. en Andalucía. *Acta Bot. Malacit.* 30, 189–197.
- Armbruster, W., Debevec, E.M., Willson, M.F., 2002. Evolution of syncarpy in angiosperms: theoretical and phylogenetic analyses of the effects of carpel fusion on offspring quantity and quality. *J. Evol. Biol.* 15, 652–657.
- Barrett, S.C.H., 2002. The evolution of plant sexual diversity. *Nat. Rev. Genet.* 3, 274–284.
- Besnard, G., Rubio de Casas, R., Vargas, P., in press. Plastid and nuclear DNA polymorphism reveals historical processes of isolation and reticulation in the olive tree complex (*Olea europaea* L.). *J. Biogeogr.*
- Blattner, F.R., 1999. Direct amplification of the entire ITS region from poorly preserved plant material using recombinant PCR. *Biotechniques* 27, 1180–1186.
- Bolle, F., 1936. Resedaceae. In: Engler, A. (Ed.), *Die Natürlichen Pflanzenfamilien*, vol. 17b. W. Engelmann, Leipzig, pp. 659–693.
- Braun-Blanquet, J., 1964. *Pflanzensoziologie. Grundzüge der Vegetationskunde*. Springer, Vienna.
- Cane, M.A., Molnar, P., 2001. Closing of the Indonesian seaway as a precursor to east African aridification around 3 million years ago. *Nature* 411, 157–162.
- Charlesworth, B., Charlesworth, D., 1978. A model for the evolution of dioecy and gynodioecy. *Am. Nat.* 112, 975–977.
- Chiarugi, A., 1933. Paleoxilologia della Somalia Italiana. *Giorn. Bot. Ital.* 40, 306–307.
- Crespo, M.B., 1993. *Reseda valentina* (Resedaceae), a legitimate name. *Willdenowia* 23, 103–106.
- Cronquist, A., 1981. *An Integrated System of Classification of Flowering Plants*. Columbia University Press, New York.
- Cronquist, A., 1988. *The Evolution and Classification of Flowering Plants*, second ed. New York Botanical Garden, New York.
- Dalgaard, V., 1986. Chromosome studies in flowering plants from Macaronesia. *Anales Jard. Bot. Madrid* 43, 83–111.
- Daniel, T.F., 1993. Resedaceae. In: Hickman, J.C. (Ed.), *The Jepson Manual. Higher Plants of California*. University of California Press, Berkeley, pp. 930–931.
- Davis, E.S., Wichman, D.M., Harris, J.D., 1993. Yellow mignonette biology and control. *Proc. West. Soc. Weed Sci.* 46, 89–90.
- deMenocal, P.B., 1995. Plio-Pleistocene African climate. *Science* 270, 53–59.
- de Winter, B., 1971. Floristic relationship between the northern and southern arid areas in Africa. *Mitt. Bot. Staatssamml. München* 10, 424–437.
- Díaz Lifante, Z., Luque, T., Santa-Bárbara, C., 1992. Chromosome numbers of plants collected during Iter Mediterraneum II in Israel. *Bocconea* 3, 229–250.
- Eames, A.J., 1961. *Morphology of the Angiosperms*. McGraw-Hill, New York.
- Ehrendorfer, F., 1980. Polyploidy and distribution. In: Lewis, W.H. (Ed.), *Polyploidy, Biological Relevance*. Plenum press, New York and London, pp. 45–60.
- Eigsti, O.J., 1936. Cytological studies in the Resedaceae. *Bot. Gaz.* 98, 363–369.
- Endress, P.K., 1982. Syncarpy and alternative modes of escaping disadvantages of apocarpy in primitive angiosperms. *Taxon* 31, 48–52.
- Endress, P.K., 2001. Origins of flower morphology. *Mol. Dev. Evol.* 29, 105–115.
- Endress, P.K., Jenny, M., Fallen, M.E., 1983. Convergent elaboration of apocarpous gynoecia in higher advanced dicotyledons (Sapindales, Malvales, Gentianales). *Nord. J. Bot.* 3, 293–300.
- Fallen, M.E., 1986. Floral structure in the Apocynaceae: morphological, functional, and evolutionary aspects. *Bot. Jahrb. Syst.* 106, 245–286.
- Farris, J.S., 1989. The retention index and the rescaled consistency index. *Cladistics* 5, 417–419.
- Fedorov, A.A., Bolkhovskikh, Z.V., 1974. Chromosome Numbers of Flowering Plants. O. Koeltz Science Publish, Koenigstein.
- Felsenstein, J., 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39, 783–791.
- Fernández Peralta, A.M., González Aguilera, J.J., 1982. Cytogenetic and evolutionary studies on the Spanish species of *Reseda* L.: section *Luteola* Dumort. (Resedaceae). *Taxon* 31, 1–8.
- Fitch, W.M., 1971. Towards defining the course of evolution: minimum change for a specific tree topology. *Syst. Zool.* 20, 406–416.
- Gadek, P.A., Quinn, C.J., Rodman, J.E., Karol, K.G., Conti, E., Price, R.A., Fernando, E.S., 1992. Affinities of the Australian endemic Akaniaceae: new evidence from *rbcL* sequences. *Aust. Syst. Bot.* 5, 717–724.
- Ghaffari, S.M., 1988. Chromosome number reports XCIX. *Taxon* 37, 396–399.
- Goldblatt, P., 1978. An analysis of the flora of southern Africa: its characteristics, relationships, and origins. *Ann. Missouri Bot. Gard.* 65, 369–436.
- Goloboff, P.A., 1999. Analyzing large datasets in reasonable times: solutions for composite optima. *Cladistics* 15, 415–428.
- Goloboff, P.A., Farris, S., Nixon, K., 2003. TNT: tree analysis using new technology. Program and documentation, available at <<<http://www.zmuc.dk/public/phylogeny/tnt/>>>.
- López González, G., 1993. *Sesamoides* All. In: Castroviejo, S., et al. (Eds.), *Flora Ibérica*, vol. 4. Consejo Superior de Investigaciones Científicas, Madrid, pp. 475–483.
- González Aguilera, J.J., Fernández Peralta, A.M., 1981. Caryology and evolution in *Sesamoides* (Resedaceae). *Plant Syst. Evol.* 139, 147–154.
- González Aguilera, J.J., Fernández Peralta, A.M., 1983. The Nature of Polyploidy in *Reseda* sect. *Leucoseda* (Resedaceae). *Plant Syst. Evol.* 142, 223–237.
- González Aguilera, J.J., Fernández Peralta, A.M., 1984. Phylogenetic relationships in the family Resedaceae. *Genetica* 64, 185–197.
- González Aguilera, J.J., Fernández Peralta, A.M., Sañudo, A., 1980a. Estudios citogenéticos y evolutivos en especies españolas de la familia Resedaceae L. sección *Glaucoreseda* DC. *Anales Inst. Bot. Cavanilles* 36, 311–320.
- González Aguilera, J.J., Fernández Peralta, A.M., Sañudo, A., 1980b. Cytogenetic and evolutive studies on the Spanish species of the family Resedaceae L.: sections *Phyteuma* L. and *Resedastrum* Duby. *Bol. Soc. Brot.* 53, 519–536.
- Hall, J.C., Sytsma, K.J., Iltis, H.H., 2002. Phylogeny of Capparaceae and Brassicaceae based on chloroplast sequence data. *Am. J. Bot.* 89, 1826–1842.
- Hall, J.C., Iltis, H.H., Sytsma, K.J., 2004. Molecular phylogenetics of core Brassicales, placement of orphan genera *Emblingia*, *Forchhammeria*, *Tirania* and character evolution. *Syst. Bot.* 29, 654–669.
- Harris, J.D., Davis, E.S., Wichman, D.M., 1995. Yellow mignonette (*Reseda lutea*) in the United States. *Weed Technol.* 19, 196–198.
- Heap, J.W., 1997. Biology and control of *Reseda lutea* L. I. Seed biology and seedling growth. *Aust. J. Agr. Res.* 48, 511–515.
- Heap, J.W., Willcocks, M.C., Kloot, P.M., 1993. The biology of Australian weeds. 17. *Reseda lutea* L. *Plant Prot. Quar.* 2, 178–185.
- Hufford, L., 1996. Developmental morphology of female flowers of *Gyrostemon* and *Tersonia* and floral evolution among Gyrostemonaceae. *Am. J. Bot.* 83, 1471–1487.
- Ibn Tattou, M., 1999. Resedaceae. In: Fennane, M., Ibn Tattou, M., Mathez, J., Ouyahya, A., El Oualidi, J. (Eds.), *Flora pratique du Maroc*, vol. 1. Institute Scientifique, Université Mohammed V, Rabat, pp. 440–448.
- Jenny, M., 1988. Different gynoecium types in Sterculiaceae: ontogeny and functional aspects. In: Leins, P., Tucker, S.C., Endress, P.K. (Eds.), *Aspects of Floral Development*. J. Cramer, Berlin, pp. 225–236.
- Judd, W.S., Sanders, R.W., Donoghue, M.J., 1994. Angiosperm family pairs: preliminary phylogenetic analyses. *Harvard Pap. Bot.* 5, 1–51.
- Jürgens, N., 1997. Floristic biodiversity and history of African arid regions. *Biodivers. Conserv.* 6, 495–514.
- Kaercher, W., Valdés Bermejo, E., 1975. Contribución al estudio cariológico del género *Reseda* L. en España. Nota I. Sección *Leucoseda* DC. *Anales Inst. Bot. Cavanilles* 32, 165–174.

- Karol, K.G., Rodman, J.E., Conti, E., Sytsma, K.J., 1999. Nucleotide sequence of *rbcl* and phylogenetic relationships of *Setchellanthus caeruleus* (Setchellanthaceae). *Taxon* 48, 303–315.
- Keighery, G.J., 1975. Chromosome numbers in the Gyrostemonaceae Endl. and the Phytolaccaceae Lindl.: a comparison. *Aust. J. Bot.* 23, 335–338.
- Kishino, H., Hasegawa, M., 1989. Evaluation of the maximum likelihood estimates of the evolutionary tree topologies from sequence data, and the branching order in Hominoidea. *J. Mol. Evol.* 29, 170–179.
- Kluge, A.G., Farris, J.S., 1969. Quantitative phyletics and the evolutions of the anurans. *Syst. Zool.* 18, 1–32.
- Kubitzki, K., 2003. Resedaceae. In: Kubitzki, K. (Ed.), *The Families and Genera of Vascular Plants. Malvales, Capparales and non-betalain Caryophyllales*, vol. 5. Springer, Berlin, pp. 334–338.
- Leistner, O.A., 1970. Resedaceae. In: Codd, L.E., de Winter, B., Killick, D.J.B., Rycroft, H.B. (Eds.), *Flora of Southern Africa*, vol. 13. Botanical Research Institute and National Botanical Gardens, Kirstenbosch, pp. 177–184.
- López González, G., 1986. De Linnaei Plantis Hispanicis Novitates Nonnullae. II. *Anales Jard. Bot. Madrid* 42, 319–324.
- López González, G., 1990. Notas referentes al género *Sesamoides* Gómez Ortega (Resedaceae). *Anales Jard. Bot. Madrid* 48, 97–100.
- Mabberley, D.J., 1997. *The Plant Book: A Portable Dictionary of the Vascular Plants*, second ed. Cambridge University Press, Cambridge.
- Martín Ciudad, A., 1991. Números cromosómicos de plantas vasculares ibéricas, I. In: Castroviejo, S., Valdés Bermejo, E. (Eds.), *Archivos de Flora Ibérica*, vol. 1. Consejo Superior de Investigaciones Científicas, Madrid, pp. 142–143.
- Meimberg, H., 2002. Molekular-Systematische Untersuchungen an den Familien Nepenthaceae und Ancistrocladaceae sowie verwandter Taxa aus der Unterklasse Caryophyllidae s.l. Dissertation Universität München.
- Meulenkamp, J.E., Sissingh, W., 2003. Tertiary palaeogeography and tectonostratigraphic evolution of the Northern and Southern Peri-Tethys platforms and the intermediate domains of the African-Eurasian convergent plate boundary zone. *Palaeogeogr. Palaeoclimatol.* 196, 209–228.
- Miller, A.G., 1984. A revision of *Ochradenus*. *Notes Roy. Bot. Gard. Edinburgh* 41, 491–594.
- Miller, A.G., Morris, M., 2004. *Ethnoflora of the Socotra Archipelago*. Royal Botanic Garden, Edinburgh.
- Miller, A.G., Nyberg, J.A., 1994. Studies in the flora of Arabia: XXVII. Some new taxa from the Arabian Peninsula. *Edinburgh J. Bot.* 51, 33–47.
- Mohamed, M.K., 1997. Chromosome counts in some flowering plants from Egypt. *Egypt. J. Bot.* 37, 129–156.
- Müller, K., 2006. Incorporating information from length-mutational events into phylogenetic analysis. *Mol. Phylogenet. Evol.* 38, 667–676.
- Müller Argoviensis, J., 1857. *Monographie de la famille des Résédacées*. Zürcher and Furrer, Zürich.
- Müller Argoviensis, J., 1864. Resedaceae. In: De Candolle, A.P. (Ed.), *Prodromus Systematis Naturalis Regni Vegetabilis*, vol. 16(2). Victor Masson, Paris, pp. 548–589.
- Nixon, K.C., 1999. The parsimony ratchet, a new method for rapid parsimony analysis. *Cladistics* 15, 407–414.
- Norris, T., 1941. Torus anatomy and nectary characteristics as phylogenetic criteria in the Rhoeadales. *Am. J. Bot.* 28, 101–113.
- Nylander, J.A.A., 2002. MrModeltest v. 1.0b. Department of Systematic Zoology, Uppsala University (program distributed by the author).
- Pax, F., Hoffmann, K., 1936. Capparaceae. In: Engler, A. (Ed.), *Die Natürlichen Pflanzenfamilien*, vol. 17b. W. Engelmann, Leipzig, pp. 146–224.
- Pearce, R.D., 1982. Resedaceae. In: George, A.S. (Ed.), *Flora of Australia*, vol. 8. Australian Government Publishing Service, Canberra, pp. 359–361.
- Perkins, J., 1909. Resedaceae Africa tropicae. In: Engler, A. (Ed.), *Beiträge zur Flora von Afrika*, XXXV. Bot. Jahrb. Syst. 43, pp. 415–418.
- Posada, D., Crandall, K.A., 1998. Modeltest: testing the model of DNA substitution. *Bioinformatics* 14, 817–818.
- Puri, V., 1945. Studies in floral anatomy. III. On the origin and orientation of placentar strands. *Proc. Natl. Acad. Sci. India B* 15, 74–91.
- Puri, V., 1950. Studies in floral anatomy. VI. Vascular anatomy of the flower of *Crateva religiosa* Forst., with special reference to the nature of the carpels in the Capparaceae. *Am. J. Bot.* 37, 363–370.
- Quézel, P., 1978. Analysis of the flora of Mediterranean and Saharan Africa. *Ann. Missouri Bot. Gard.* 65, 479–534.
- Ramp, E., 1988. Struktur, Funktion und systematische Bedeutung des Gynoeciums bei Rutaceae und Simaroubaceae. Ph.D. Dissertation, University of Zürich, Zürich.
- Reese, G., 1957. Über die Polyploidiespektren in den Nord-Saharanischen Wüstenpflanzen. *Flora* 144, 534–548.
- Roberts, D.G., 1969. Structural evolution of the rift zones in the Middle East. *Nature* 223, 55–57.
- Rodman, J.E., 1991a. A taxonomic analysis of glucosinolate-producing plants. Part I: Phenetics. *Syst. Bot.* 16, 598–618.
- Rodman, J.E., 1991b. A taxonomic analysis of glucosinolate-producing plants. Part II: Cladistics. *Syst. Bot.* 16, 619–629.
- Rodman, J.E., Price, R.A., Karol, K., Sytsma, K.J., Palmer, J.D., 1993. Nucleotide sequences of the *rbcl* gene indicate monophyly of mustard oil plants. *Ann. Missouri Bot. Gard.* 80, 686–699.
- Rodman, J.E., Karol, K.G., Price, R.A., Conti, E., Sytsma, K.J., 1994. Nucleotide sequences of *rbcl* confirm the Capparalean affinity of the Australian endemic Gyrostemonaceae. *Aust. Syst. Bot.* 7, 57–69.
- Rodman, J.E., Karol, K.G., Price, R.A., Sytsma, K.J., 1996a. Molecules, morphology and Dahlgren's expanded order Capparales. *Syst. Bot.* 21, 289–307.
- Rodman, J.E., Soltis, P.S., Soltis, D.E., Sytsma, K.J., 1996b. Dual origin of mustard oil biosynthesis inferred from congruent nuclear 18S ribosomal RNA and plastid *rbcl* gene phylogenies. *Am. J. Bot.* 83 (Suppl.), 188.
- Rodman, J.E., Soltis, P.S., Soltis, D.E., Sytsma, K.J., Karol, K.G., 1998. Parallel evolution of glucosinolate biosynthesis inferred from congruent nuclear and plastid gene phylogenies. *Am. J. Bot.* 85, 997–1006.
- Ronquist, F., Huelsenbeck, J.P., 2003. MrBayes 3: Bayesian phylogenetic inference under mix models. *Bioinformatics* 19, 1572–1574.
- Ronse de Craene, L.P., 2002. Floral development and anatomy of *Pentadiplandra* (Pentadiplandraceae): a key genus in the identification of floral morphological trends in the core Brassicales. *Can. J. Bot.* 80, 443–459.
- Schultheis, L.M., 2001. Systematic of *Downingia* (Campanulaceae) based on molecular sequence data: implications for floral and chromosome evolution. *Syst. Bot.* 26, 603–621.
- Sobick, U., 1983. Ontogenetic studies of flowers in the Resedaceae with special regards to androecium and gynoecium. *Bot. Jahrb. Syst.* 104, 203–248.
- Soltis, P.S., Soltis, D.E., 2000. The role of genetic and genomic attributes in the success of polyploids. In: Ayala, F.J., Fitch, W.M., Clegg, M.T. (Eds.), *Variation and Evolution in Plants and Microorganisms: Towards a New Synthesis 50 years After Stebbins*. National Academy Press, Irvine, California, pp. 310–328.
- Soltis, D.E., Soltis, P.S., Endress, P.K., Chase, M.W., 2005. *Phylogeny and Evolution of Angiosperms*. Sinauer Associates, Sunderland, Massachusetts.
- Spellenberg, R., Ward, D., 1988. Chromosome number reports XCIX. *Taxon* 37, 398.
- Stebbins, G.L., 1972. The evolution of the grass family. In: Younger, V.B., McKell, C.B. (Eds.), *The Biology and Utilization of Grasses*. Academic Press Inc., New York and London, pp. 1–17.
- Stebbins, G.L., 1974. *Flowering Plants: Evolution Above the Species Level*. Harvard University Press, Cambridge, Massachusetts.
- Swofford, D.L., 2002. PAUP*: Phylogenetic Analysis Using Parsimony (* and other methods). Version 4.0b10. Sinauer Associates, Sunderland, Massachusetts.
- Taberlet, P., Gielly, L., Pautou, G., Bouvet, J., 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Mol. Biol.* 17, 1105–1109.
- Takhtajan, A., 1969. *Flowering Plants: Origins and Dispersal*. Smithsonian Institution Press, Washington, DC.
- Takhtajan, A., 1997. *Diversity and Classification of Flowering Plants*. Columbia University Press, New York.

- Templeton, A.R., 1983. Phylogenetic inference from restriction endonuclease cleavage site maps with particular reference to the evolution of humans and the apes. *Evolution* 37, 221–244.
- Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F., Higgins, D.G., 1997. The Clustal_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res.* 25, 4876–4882.
- Thorne, R.F., 1976. A phylogenetic classification of the Angiopermaeae. *Evol. Biol.* 9, 35–106.
- Thulin, M., 1990. A new species of *Reseda* from Somalia. *Kew Bull.* 45, 667–669.
- Thulin, M., 1994a. A new species of *Ochradenus* (Resedaceae) from southern Arabia. *Nord. J. Bot.* 14, 383–384.
- Thulin, M., 1994b. Aspects of disjunct distributions and endemism in the arid parts of the horn of Africa, particularly Somalia. In: Seyani, J.H., Chikuni, A.C. (Eds.), *Proceedings of the XIIIth Plenary Meeting AET-FAT 2, Malawi*, pp. 1105–1119.
- Tobe, H., Raven, P.H., 1991. The embryology and relationships of Gyrostemonaceae. *Aust. Syst. Bot.* 4, 407–420.
- Valdés, B., 2002. Resedaceae. In: Valdés, B., Rejdali, M., Achhal, A., Jury, S.L., Montserrat, J.M. (Eds.), *Checklist of vascular plants of N Morocco with identification keys, vol. 1. Consejo Superior de Investigaciones Científicas, Madrid*, pp. 265–269.
- Valdés Bermejo, E., 1993. *Reseda* L. In: Castroviejo, S. (Ed.), *Flora Ibérica, vol. 4. Consejo Superior de Investigaciones Científicas, Madrid*, pp. 440–475.
- Valdés Bermejo, E., Kaercher, W., 1984. Dos nuevos táxones ibéricos del género *Reseda* L., sect. *Leucoreseda* DC. *Anales Jard. Bot. Madrid* 41, 198–201.
- Verdcourt, B., 1969. The arid corridor between the north-east and south-west areas of Africa. *Palaeoecol. Afr.* 4, 140–144.
- Werger, M.J.A., 1978. The Karoo–Namib region. In: Werger, M.J.A. (Ed.), *Biogeography and Ecology of Southern Africa*. W. Junk, The Hague, pp. 233–299.
- White, T.J., Bruns, T., Lee, S., Taylor, J., 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis, M., Gelfand, D., Sninsky, J., White, T.J. (Eds.), *PCR Protocols: A Guide to Methods and Amplifications*. Academic Press, San Diego, California, pp. 315–322.
- Wickens, G.E., 1976. The flora of Jbel Marra (Sudan Republic) and its geographical affinities. *Kew Bull. Addit. Ser.* 5, 1–368.
- Yeo, P.F., 1964. *Reseda* L. In: Tutin, T.G., Burges, N.A., Valentine, D.H., Walters, S.M., Webb, D.A. (Eds.), *Flora Europaea, first ed., vol. 1. Cambridge University Press, Cambridge*, pp. 346–349.
- Yeo, P.F., 1996. *Reseda* L. In: Tutin, T.G., Burges, N.A., Charteret, A.O., Heywood, V.H., Moore, D.M., Valentine, D.H., Walters, S.M., Webb, D.A. (Eds.), *Flora Europaea, second ed., vol. 1. Cambridge University Press, Cambridge*, pp. 417–420.
- Zohary, M., 1966. *Flora Palaestina. The Israel Academy of Sciences and Humanities, Jerusalem*.