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MOLECULAR PHYLOGENETIC ANALYSES OF TRIBE LOTEAE (LEGUMINOSAE): IMPLICATIONS FOR CLASSIFICATION AND BIOGEOGRAPHY

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Abstract

Molecular phylogenetic analyses have provided new insight into relationships among temperate herbaceous (TH) legumes. In this analysis, we examine molecular phylogenetic relationships among one group of TH legumes, the tribe Loteae. Members of tribe Loteae are largely centred in Old World regions in the Mediterranean, but are also distributed in various regions throughout the world. One genus, *Lotus s.l.*, occurs in both the Old and the New World, with a New World centre of diversity in western North America and Mexico. To examine more closely the monophyly of tribe Loteae and relationships among its component genera, we conduct molecular phylogenetic analyses using sequences of the internal transcribed spacer (ITS) regions and the 5.8S region of nuclear ribosomal DNA. These data are used to evaluate both the biogeography and classification of tribe Loteae, with particular emphasis on the genus *Lotus*. Our results show that tribe Loteae is a monophyletic group, originating most likely in Eurasia and diversifying in temperate regions throughout the Mediterranean and western North America. The genus *Lotus s.l.* does not appear to be monophyletic in that members of Old World *Lotus* form a closer alliance to Old World *Hammatolobium*, than to western North American *Lotus*. Although other genera of Loteae form well-supported monophyletic groups, relationships among these genera are unresolved with respect to one another and to *Lotus*. These results encompass the most comprehensive taxonomic sampling of Loteae genera to date, and bring insight to the taxonomic circumscription of the tribe and its biogeography.

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Introduction

Understanding evolutionary relationships among papilionoid legumes is currently a topic of considerable interest and discussion (Käss and Wink, 1995, 1997; Pennington *et al.*, 2001; Kajita *et al.*, 2001). Facilitated by molecular phylogenetic analyses, this research focus has employed a wide range of molecular markers including chloroplast DNA (e.g., Hu *et al.*, 2000), phytochrome C (Lavin *et al.*, 1998) and the internal transcribed spacer (ITS) regions of nuclear ribosomal DNA (e.g., Wojciechowski *et al.*, 1993). One area of interest has focused exclusively on the evolutionary history of temperate herbaceous (TH) papilionoid legumes. This group has historically included members of tribes Galegeae, Carmichaelieae, Cicereae, Hedysareae, Vicieae, Trifolieae, Loteae and Coronilleae (Polhill, 1981a). Restriction fragment analysis of cpDNA has revealed that tribe Loteae *s.l.* (including Coronilleae) is the only member of the TH group that does not exhibit a rare molecular mutation: the loss of a 25 kb inverted repeat region (Lavin *et al.*, 1990; Liston, 1995), the presence of which is highly conserved among angiosperms (including Loteae) and most gymnosperms (Strauss *et al.*, 1988). The relevance of this mutation is made clear in higher-level phylogenetic analyses (e.g., Doyle *et al.*, 1997; Wojciechowski *et al.*, 2000), which place the Loteae *s.l.* (together with the Robinieae) sister to all other TH legumes. Individual phylogenetic assessments of tribes Galegeae (Sanderson and Liston, 1995), Trifolieae and Vicieae (Steele and Wojciechowski, 2003), Carmichaelieae (Wagstaff *et al.*, 1999), Cicereae (K. Steele, California State University, unpubl.), and Loteae (Allan and Porter, 2000) have provided a comparative basis for evaluating competing classifications and investigating biogeography. Additional taxonomic sampling and the use of independent molecular markers are continually improving our understanding of the taxonomy and biogeography of these groups. This study provides a taxonomically comprehensive assessment of tribe Loteae *s.l.*, with an emphasis on understanding its biogeography and classification.

Tribe Loteae (Polhill, 1981b) consists of approximately 10 genera and nearly 300 species, which have been traditionally segregated into two tribes, Loteae and Coronilleae (Polhill, 1981b). Although the two tribes have been extensively studied from a cytogenetic (Larsen, 1955; Goldblatt, 1981), palynological (Diez and Ferguson, 1990, 1994, 1996) and morphological (Dormer, 1945; Corby, 1981; Sprent, 1981, 2000; Sokoloff, 1997, 1998) perspective, their taxonomic delimitation has remained controversial (Polhill, 1981b). This historical controversy has largely focused on morphological characters relating to fruit type (jointed fruits in Coronilleae, unjointed in Loteae) and to some extent root nodule type (branched in Coronilleae, unbranched in Loteae). Although morphological-based phylogenetic studies are underway (Sokoloff, unpubl. data) recent evidence based on nrDNA (Allan and Porter, 2000) suggests that recognition of a single, expanded tribe, Loteae *s.l.*, is warranted. This recognition is also consistent with Polhill's (1994), non-phylogenetic reassessment of the Loteae and Coronilleae in which he includes Coronilleae within Loteae.

One of the more taxonomically problematic genera with Loteae *s.l.* is the cosmopolitan genus *Lotus*. Owing to its extreme morphological diversity, *Lotus* has been the topic of considerable taxonomic debate (Ottley, 1923, 1944; Isely, 1981; Polhill 1981b). This debate has centred on the Old and New World species, which show considerable overlap in morphological characteristics. Recently, two morphological cladistic studies (Arambarri, 2000a, 2000b) have addressed the question of whether the Old and New World species are monophyletic. Although these studies independently identify a monophyletic New World *Lotus* group (Arambarri, 2000a) and a non-monophyletic Old World *Lotus* group (Arambarri, 2000b), it is not yet clear what the relationship between Old and New World *Lotus* is. Additional non-phylogenetic morphological studies have also been conducted by Sokoloff (1999a, 2000) and Kramina and Sokoloff (1997, 2001). These morphological-based studies have contributed significantly to our overall understanding of Loteae *s.l.*, and *Lotus* in particular.

TABLE 1. List of accessions used in phylogenetic analysis of tribe Loteae *s.l.* Biogeographic designations are based on geographic regions as determined from species distributions given in International Legume Database & Information Service (ILDIS). EUR+ = Europe + Asia + Africa including Macaronesia; NA = western North America (including Mexico and the Caribbean); AUS-SPI = Australia and South Pacific Islands; SA = South America. Actual geographic distribution is shown as follows: EUR = Eurasia including Japan and Taiwan; MED-AFR = mediterranean Africa; SAH-AFR = Sahara Africa; TROP-AFR = Tropical Africa; MAC = Macaronesia; AUS-SPI = Australia and South Pacific Islands; NA = North America (including Mexico and the Caribbean); SA = South America. Numbers in parentheses indicate number of species sampled per genus. Traditional taxonomy of Loteae (e.g., Ball, 1968; Polhill, 1994) is used, with a few nomenclatural corrections.

Taxon	Geographic distribution	Biogeographic designation	GenBank number	Collector number
Anthyllis L. (9/22)				
A. aurea Welden in Host	EUR	EUR+	AF450219	s.n., leg., Anonymous, det., D.D. Sokoloff (MW)
A. barba-jovis L.	EUR/MED-AFR	EUR+	AF450214	s.n., leg. *G. Galasso*; MHA
A. cytisoides L.	EUR/MED-AFR	EUR+	AF450211	*R. Figuerola* YJ-26 (NY)
A. hermanniae L.	EUR	EUR+	AF450213	*R. Figuerola* YJ-17 (NY)
A. lotoides L.	EUR/MED-AFR	EUR+	AF450216	s.n. Det. J. Fernandez Casas (NY)
A. montana L.	EUR/MED-AFR	EUR+	AF450218	*A. Charpin* 22723 (MW)
A. ramburei Boiss.	EUR	EUR+	AF450217	*A. Segura Zubizarreta* 14899 (MHA)
A. terniflora (Lag.) Pau	EUR/MED-AFR	EUR+	AF450212	s.n., 1leg. *F. Sanz*; (MHA)
A. onobrychioides Cav.	EUR	EUR+	AF450210	*A. Ortiz* 504 (NY)
Coronilla L. (4/22)				
C. elegans Pančič = *Securigera elegans* (Pančič) Lassen.	EUR	EUR+	AF450231	s.n. leg. *T Karaeva*; (MW)
C. minima L. *s.l.*	EUR/MED-AFR	EUR+	AF450232	*A. Segura Zubizarreta* 19437 (MHA)
C. orientalis Mill. = *Securigera orientalis* (Mill.) Lassen	EUR	EUR+	AF450230	s.n., leg. *N. Orlova*, I. Rodionov (MW)
C. varia L.=*Securigera varia* (L). Lassen	EUR	EUR+	AF218537	*Doyle* 1559 (BH)
Dorycnium Mill. (4/8)				
D. graecum (L.) Ser.	EUR	EUR+	AF218500	*Korshenevsky* s.n. (RSA)
D. hirsutum (L.) Ser.	EUR/MED-AFR	EUR+	AF218502	*LeDoux* s.n. (RSA)
D. pentaphyllum Scop.	EUR/MED-AFR	EUR+	AF218501	*Elias* 6472 (RSA)
D. rectum (L.) Ser.	EUR/MED-AFR	EUR+	AF218503	*Podlech* 47695 (RSA)
Hammatolobium Fenzl (1/2)				
H. kremerianum (Coss.) Muell.	MED-AFR	EUR+	AF450233	*D. Podlech* 51378 (MHA)
Hippocrepis L. (3/34)				
H. bourgaei (Nyman) Hervier	EUR	EUR+	AF450239	*W. Lippert* 23354(NY)
H. emerus (L.) Lassen	EUR/MED-AFR	EUR+	AF218531	*Raven* 6805 (RSA)

Table 1. continued

H. unisiliquosa L.	EUR/MED-AFR	EUR+	AF450238	s.n. leg. *A.K.Skvortsov*; M.G. Pimenov MW
Hymenocarpos L. (1/1)				
H. circinnatus (L.) Savi	EUR/MED-AFR	EUR+	AF218504	*Sofajy*, s.n. (RSA)
Kebirita Kramina & D. D. Sokoloff (1/1)				
K. roudairei (Bonnet) Kramina & D. D. Sokoloff	SAH-AFR	EUR+	AF450200	*J. Ewald, F. Schuhwerk* 90/639 (NY)
Lotus L. (49/>150)				
subg. **Acmispon** (Raf.) Ottley				
L. cedrosensis Greene	NA	NA	AF218507	*Moran* 350020 (RSA)
L. grandiflorus (Benth.) Greene	NA	NA	AF218508	*Allan* 262 (RSA)
L. oroboides (Kunth) Ottley ex Kearney & Peebles	NA	NA	AF218510	*Steinmann* 1334 (RSA)
L. rigidus (Benth.) Greene	NA	NA	AF218511	*Allan* 290 (RSA)
L. salsuginosus Greene	NA	NA	AF218512	*Allan* 132 (RSA)
L. strigosus (Nutt.) Greene	NA	NA	AF218513	*Allan* 199 (RSA)
L. subpinnatus Lag.	SA	SA	AF450207	cult. #PI368908
L. unifoliolatus (Hook.) Benth.	NA	NA	AF450183	cult. #PI215230
L. wrangelianus Fisch. & Mey.	NA	NA	AF218514	*Allan* 339 (RSA)
subg. **Hosackia** (Benth.) Ottley				
L. chihuahuanus (Watson) Greene	NA	NA	AF450159	*L. Brouillet* 12585
L. crassifolius (Benth.) Greene	NA	NA	AF218523	*Allan* 243 (RSA)
L. formosissimus Greene	NA	NA	AF218524	*Ertter* 6845 (RSA)
L. oblongifolius (Benth.) Greene	NA	NA	AF218525	*Boyd* 2566 (RSA)
subg. **Syrmatium** (Vogel) Ottley				
L. argophyllus (Gray) Greene	NA	NA	AF218516	*Allan* 030 (RSA)
L. benthamii Greene	NA	NA	AF450203	*Allan* 053 (RSA)
L. dendroideus (Greene) Greene	NA	NA	AF218518	*Allan* 012 (RSA)
L. distichus (Greene) Greene	NA	NA	AF450199	*D. Stevenson* 635 (RSA)
L. hamatus Greene	NA	NA	AF450161	*Allan* 198 (RSA)
L. heermannii (Durand & Hilgard) Greene	NA	NA	AF218519	*Allan* 296 (RSA)
L. nevadensis (Watson) Greene	NA	NA		*Allan* 242 (RSA)
L. nuttallianus Greene	NA	NA	AF218520	*Allan* 268 (RSA)
L. procumbens (Greene) Greene	NA	NA	AF450168	*Boyd & Ross* 7855 (RSA)
L. scoparius (Nutt.) Ottley	NA	NA	AF218521	*Allan* 192 (RSA)

Table 1. continued

subg. **Lotus** L.				
L. angustissimus L.	EUR/MED-AFR/MAC	EUR+	AF450185	cult. #PI368894
L. arabicus L.	EUR/SAH-AFR/ TROP-AFR	EUR+	AF450176	cult. #PI214109
L. australis Andrews	AUS-SPI	AUS/SPI	AF450187	*F. J. Badman* 3733 (NY)
L. longesiliquosus Roem. (*L. collinus* (Boiss.) Heldr.)	EUR/MED-AFR	EUR+	AF218526	*Davis* 58797 (RSA)
L. conimbricensis Brot.	EUR/MED-AFR/MAC	EUR+	AF450186	cult. #PI308033
L. corniculatus L.	EUR/MED-AFR/ TROP-AFR	EUR+	AF218527	*Allan* 293 (RSA)
L. creticus L.	EUR/MED-AFR/MAC	EUR+	AF450192	cult. #PI287858-64i
L. cruentus Court	AUS-SPI	AUS/SPI	AF450182	*D. Whibley* 1187 (NY)
L. edulis L.	EUR/MED-AFR	EUR+	AF450184	cult. #PI368897
L. gebelia Vent.	EUR/MED-AFR	EUR+	AF450188	*G. Samuelsson* 6051 (NY)
L. halophilus Boiss. & Spruner	EUR/MED-AFR/ TROP-AFR	EUR+	AF450208	cult. #PI300237
L. krylovii Schischk. & Serg.	EUR	EUR+	AF450209	s.n., leg., *E. Korotkova*, *O. Yemel'yanova* 967 (MW)
L. michauxianus Ser.	EUR	EUR+	AF450206	s.n., leg., *M. Raizi* 6109 (MHA)
L. ornithopodioides L.	EUR/MED-AFR/MAC	EUR+	AF450205	Cultivated, Orel University Botanic Garden, #74
L. palustris Willd.	EUR/MED-AFR	EUR+	AF450195	cult. #PI311427
L. weilleri Maire	MED-AFR	EUR+	AF450180	cult. #PI368910
L. parviflorus Desf.	EUR/MED-AFR/MAC	EUR+	AF450194	cult. #PI283615
L. peczoricus Miniaev & Ulle	EUR	EUR+	AF450191	leg., *S. Ulle* 6383a (MW)
L. peregrinus L.	EUR/MED-AFR	EUR+	AF450177	cult. #PI368905
L. subbiflorus Lag.	EUR/MED-AFR/MAC	EUR+	AF450160	cult. #PI308036
L.glinoides Del. (*L. trigonelloides* Webb. & Berth.)	EUR/SAH-AFR/T ROP-AFR/MAC	EUR+	AF450202	*K. Larsen* LHN-473 (NY)
L. uliginosus Schkuhr	EUR/MED-AFR/MAC	EUR+	AF450197	cult. #PI282156
subg. **Pedrosia** (Lowe) Brand				
L. arenarius Brot.	EUR/MED-AFR	AFR	AF218528	*Podlech* 46130 (RSA)
L. assakensis Coss. ex Brand	MED-AFR	AFR	AF450204	*F. Schuhwerk* #90/320 (NY)
L. campylocladus Webb & Berth.	MAC	MAC	AF450196	*G. P. Lewis* 2180 (NY)
L. maroccanus Ball	MED-AFR	AFR	AF450181	cult. #G31279
Tetragonolobus Scop. (2/5)				
T. maritimus L.	EUR/MED-AFR	EUR+	AF218505	*Skvortsov* s.n. (RSA)

Table 1. continued

T. purpureus Moench	EUR/MED-AFR	EUR+	AF218506	*Vasak* s.n. (RSA)
Ornithopus L. (3/5)				
O. compressus L.	EUR/MED-AFR/MAC	EUR+	AF218533	*Halse* 4904 (RSA)
O. perpusillus L.	EUR/MED-AFR/MAC	EUR+	AF450226	*P. M. Uribe-Echebarria* 270 (RSA)
O. sativus Brot.	EUR/MED-AFR/MAC	EUR+	AF218534	*Podlech* 44720 (RSA)
Scorpiurus L. (2/2)				
S. muricatus L.	EUR/MED-AFR/ TROP-AFR	EUR+	AF218535	*Galiano* 1769 (RSA)
S. vermiculatus L.	EUR/MED-AFR/MAC	EUR+	AF218536	*Galiano* 97/70 (RSA)
Vermifrux Gillett (1/1)				
V. abyssinica (A. Rich.) Gillett	EUR/TROP-AFR	EUR+	AF450235	*Thulin* 9304 (UPS)
Outgroups				
Hebestigma cubense (Kunth) Urban	NA	NA	AF450157	*Lavin* 5611 (MONT)
Hybosema robustum Sousa & Lavin	NA	NA	AF450156	*M. Sousa et al.* 13212 (MEXU)
Lennea melanocarpa (Schltdl.) Harms	NA	NA	AF450155	*Lavin* 5787a (TEX)
Olneya tesota A. Gray	NA	NA	AF450158	*Lavin* 4642 (TEX)
Robinia pseudoacacia L.	NA	NA	AF218538	*Allan* 263 (RSA)
Sesbania vesicaria (Jacq.) Elliott	NA	NA	AF398761	*Lavin* 6194 (MONT)

Most genera of Loteae *s.l.* are centred in Old World regions around the Mediterranean, but a few are distributed more broadly throughout the world (Table 1). *Lotus s.l.* is one such taxon, with approximately 200 species occupying two centres of diversity: the Mediterranean and Macaronesia; and western North America. Additional minor occurrences include Australia and New Caledonia (2 spp.), Chile (1 sp.), tropical Africa (c. 25 spp.) and Asia eastward to China (3–6 spp.) and Japan (2–3 spp.). Four segregate genera, *Dorycnium*, *Tetragonolobus*, *Podolotus* and *Pseudolotus* often included in *Lotus*, are controversial as to their taxonomic status (Gillett, 1959; Ball, 1968; Isely, 1981; Polhill, 1981b, Lassen, 1986; Greuter *et al.*, 1989).

One recently accepted (Kramina and Sokoloff, 2001) monotypic segregate genus is *Kebirita*, which occurs in tropical and North Africa. Other traditionally accepted Old World genera include *Anthyllis* (20–25 species), *Hymenocarpos* (1–4 spp.), *Coronilla* (c. 20 spp.), *Hippocrepis* (30 spp.), *Scorpiurus* (2–4 spp.), *Cytisopsis*, and *Hammatolobium* (2 spp.). One genus, *Ornithopus* (6 spp.), is found in both the Old World and South America [*Ornithopus micranthus* (Benth.) Arechav.], while two monotypic genera, *Antopetitia* and *Vermifrux* are found in tropical Africa, with *Vermifrux* also occurring in extreme southwestern Asia.

Here we expand on previous molecular phylogenetic analyses of tribe Loteae *s.l.*, with an emphasis on sampling Old World genera and additional Old World species of the genus *Lotus*, the largest and most complex taxon within the tribe. Issues we address include: (1) the monophyly of the tribe; (2) the monophyly and relationships among its component genera; (3) the classification of Loteae *s.l.*; and (4) the biogeography of New and Old World Loteae genera.

Materials and methods

Taxonomic sampling

Ingroup representatives from eight of ten traditionally recognised genera of Loteae *s.l.*, (Loteae + Coronilleae as defined by Polhill, 1981b) were included in this study (Table 1). This representation constitutes 87 species, 49 of which belong to the genus *Lotus s.l.* In addition, we included the recently recognised (Kramina and Sokoloff, 2001) African monotypic genus, *Kebirita*, (*K. roudairei* (Bonnet) Kramina & D. D. Sokoloff). Representatives from each of the nine genera cover a broad geographic area, including all major regions of distribution and ecological habitat. Material for the monotypic African genus *Antopetitia*, the very rare Mediterranean genus, *Cytisopsis*, and the South American species, *Ornithopus micranthus,* was not obtainable. Two species of the genus *Coronilla* (*C. scorpioides* (L.) Koch and *C. valentina* L. subsp. *glauca* (L.) Batt.), which were included in a previous molecular phylogeny (Allan and Porter, 2000), were not included in this study. These two species showed peculiar relationships to North American *Lotus* and therefore have been excluded until new accessions can be obtained.

Outgroup representatives included one species from each of six genera of the predominantly tropical New World tribe Robinieae (Table 1). Molecular phylogenetic analysis of the temperate herbaceous tribes using a supertree approach confidently identifies a sister group relationship between Robinieae and Loteae (Wojciechowski *et al.*, 2000). This result was also found in a higher-level phylogenetic analysis of the tropical tribe Millettieae using the chloroplast spacer regions *trn*K/*mat*K (Hu *et al.*, 2000). Members of Robinieae have a pantropical distribution occurring in North America and Mexico, Central America, South America and Africa (Lavin and Sousa, 1995).

DNA extraction and sequence data

Tissue for DNA extraction was obtained from either field-collected, greenhouse-grown leaf material of individual plants, or herbarium samples (see Table 1). DNA isolation, polymerase chain reaction (PCR), cycle sequencing, and automated DNA

sequencing were performed as specified in Allan and Porter (2000), or were slightly modified. Modifications included isolation of DNA from leaf tissue of c. 40 additional species using Qiagen (Qiagen Inc., Valencia, CA) spin columns and the collection of sequence data for these 40 species using an automated 377 DNA sequencer at the Smithsonian's Laboratory of Molecular Systematics. DNA of all taxa was sequenced in both directions to ensure accuracy of base calling.

Sequences were initially aligned using Clustal X (Thompson *et al.*, 1994). This alignment was manually adjusted by sequential pairwise comparisons using the program Se-Al ver. 1.0 (Rambaut, 1996). Inference of numerous single and multiple-base indels (insertion/deletion) was required. Of these, two indels (positions 196 and 662) were found to be absolutely unambiguous. These two indels were coded separately using binary characters (0 = absence; 1 = presence) and included in the phylogenetic analyses (see below). Details regarding sequence length and alignment (e.g., indels), number of informative characters, and pairwise divergence values were determined using PAUP*ver. 4.0b* (Swofford, 2000). Individual sequences from all taxa included in this study have been deposited in Genbank (see Table 1 for accession numbers).

Phylogenetic analysis

Given the large size of the dataset, multiple heuristic searches were conducted using PAUPRat (Sikes and Lewis, 2001), a computer program that implements Parsimony Ratchet (Nixon, 1999) searches in PAUP*. The Parsimony Ratchet operates on the principle of searching for the shortest tree(s) via short, independent searches on many different tree islands. Essentially, this method allows an increase in the number of islands visited in exchange for exhaustively searching each tree island. By perturbing a random set of characters during each search (usually 5–20%) neighbouring tree islands are visited in an attempt to find better tree scores. The best tree from each search is passed along to each subsequent search. A typical search strategy involves conducting an initial unperturbed search, followed by character perturbance. Each search is iterated approximately 200 times and anywhere from 10 – 20 searches (200 iterations each) are performed until tree scores and tree topology remain constant. In this analysis, preliminary Ratchet searches were conducted in order to determine the percent of perturbed characters (5–20%) that yielded optimal tree scores. Following this, 20 (200 iteration) Ratchet searches were performed and the results from all heuristic searches were combined into a single consensus tree.

For comparison to the Ratchet method, single heuristic searches were conducted under a maximum parsimony (MP) criterion, in addition to Neighbour-joining (NJ) distance methods, which employed a Jukes-Cantor distance correction (Jukes and Cantor, 1969). The MP analyses were again limited to multiple heuristic searches (ADDSEQ = SIMPLE, CLOSEST, RANDOM) without branch swapping, or branch swapping on a limited number of trees (e.g., MAXTREES = 400 [maximum number of trees retained in RAM]). In each search, characters were given equal weight and gaps were treated as missing data. Support for clades was evaluated via bootstrapping (Felsenstein, 1985) on trees derived from the Ratchet and MP heuristic searches, and the NJ analysis. The options for bootstrapping were ADDSEQ = SIMPLE, MAXTREES =1, SWAP = TBR, with 500 replicates.

To examine biogeography, we optimised "geographic region" onto one of the MP trees using the TRACE CHARACTER function in MacClade ver. 4.0 (Maddison and Maddison, 2000). All possible paths of character evolution, given the topology and the distribution of characters states, were determined using the MPR option in MacClade. Character states corresponded to the following four biogeographic areas: (1) Europe + Asia + Africa including Macaronesia, (2) North America including Mexico and the Caribbean region, (3) South America, and (4) Australia including the South Pacific Islands. The first of these four areas is clearly the most comprehensive from a biogeographic standpoint. That we consider Europe, Asia

and Africa (including Macaronesia) as a broad-scale biogeographic region is consistent with a Tethyan-Mediterranean distribution as identified in Takhtajan's (1986) categorisation of the floristic regions of the world. For simplicity, we refer to these three geographic regions (Europe + Asia + Africa) as "Old World." The other three biogeographic areas clearly constitute independent areas both geographically and floristically.

Results

ITS sequence variation

Sequence lengths of the entire ITS1&2 and 5.8S region ranged from 590 to 610 bp. Pairwise sequence divergences between outgroup and ingroup taxa were less than 35%. The smallest divergence between any one outgroup and ingroup species was 21% (*Robinia vs. Kebirita roudairei*). The greatest divergence between any two ingroup taxa was 25% (*Lotus creticus* L. and *Coronilla minima* L.). Divergences between ingroup genera typically ranged from as high as 23% to 14%. There were 363 parsimony informative sites (of 746 total sites) in the ITS data set. Indels were particularly common in ITS1, less common in ITS2 and uncommon in the 5.8S region. Using a conservative approach, we included two indels as additional characters, which were appended to the end of the data matrix. One of these indels (position 196 in ITS1) is a single base substitution, which groups together all Loteae genera to the exclusion of *Lotus s.l.* The other, also a single base substitution (beginning at position 662 in ITS2), groups all New World *Lotus* with *Ornithopus*, *Vermifrux* and *Scorpiurus*. Other ambiguous indels (i.e., positional homology uncertain) were not coded separately.

Parsimony Ratchet analyses

Multiple heuristic searches of the full ITS dataset using Parsimony Ratchet/PAUPRat converged on sets of 201 trees each (user-defined limit; 4020 trees total) of 2255 steps (CI = 0.40, RI = 0.7). Perturbation tests in which 5%, 10%, 15% and 20% of the characters were randomly perturbed gave the same tree scores (2255 steps). Excluding uninformative characters from the searches resulted in trees of length 2115 (CI = 0.35, RI = 0.73). Trees from each of the searches were combined in PAUP* and a filter was applied to retain only the shortest length trees. The strict consensus of the remaining 1350 trees is shown in Fig. 1. The strict consensus of each individual set of trees (uninformative characters excluded *vs.* included) is identical. Analyses that excluded indel characters produced a consensus tree that was also identical to those with indel characters included (weighted equally with substitution characters).

The bootstrap consensus tree derived from the individual Ratchet searches reveals four major clades (marked A–D on Fig. 1). Clade A includes all members of tribe Loteae and is well-supported (100%). Clade B contains all members of Old World *Lotus* including the segregate genera *Dorycnium* and *Tetragonolobus*, plus Old World *Hammatolobium*. In this topology, *Hammatolobium* is strongly supported (95%) as sister to the Old World *Lotus* clade, which is supported by a bootstrap value of 63%. Clade C_1 includes three traditionally recognised groups of New World *Lotus,* which are sometimes recognised as separate genera: *Syrmatium*, *Acmispon* and *Ottleya*. Although relationships among these groups are unresolved, the clade containing them receives high bootstrap support (97%). Another equally well supported (100%), albeit minor, New World *Lotus* clade, C_2, contains members of subgenus *Hosackia*. The relationship between these two New World *Lotus* clades, however, is unresolved. Clade D is highly supported (100%), consisting of all members of Old World *Anthyllis* plus *Hymenocarpos*. Several minor, but well-supported clades include *Coronilla s.l.* (100%), *Ornithopus* (100%), *Hippocrepis* (93%) and *Scorpiurus* (100%). Relationships among these Old World genera, however, are unresolved. The monotypic African and SW

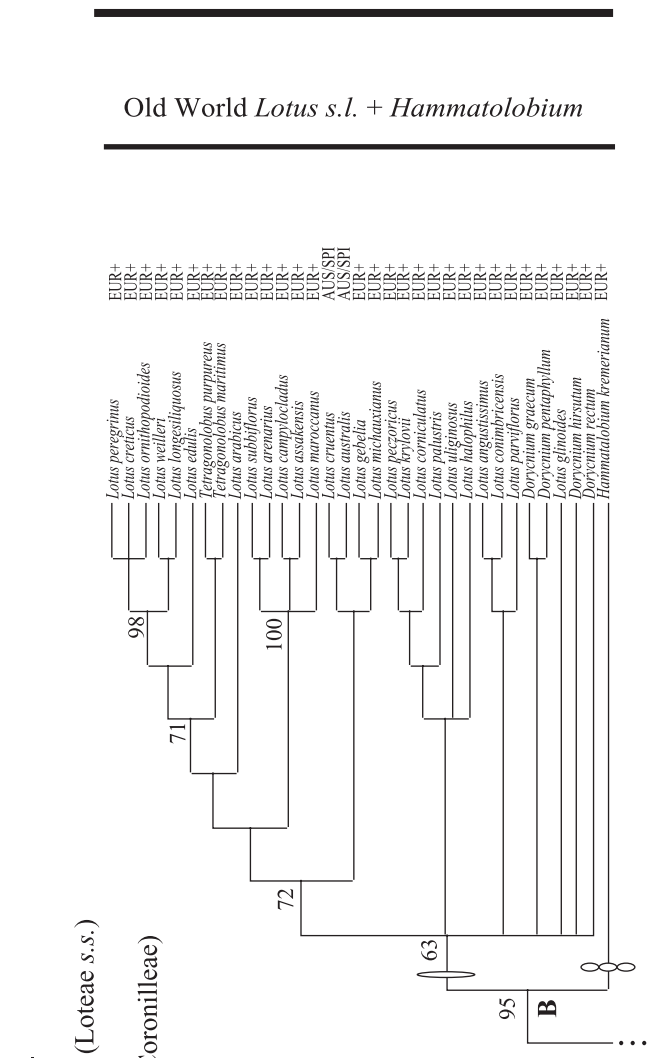
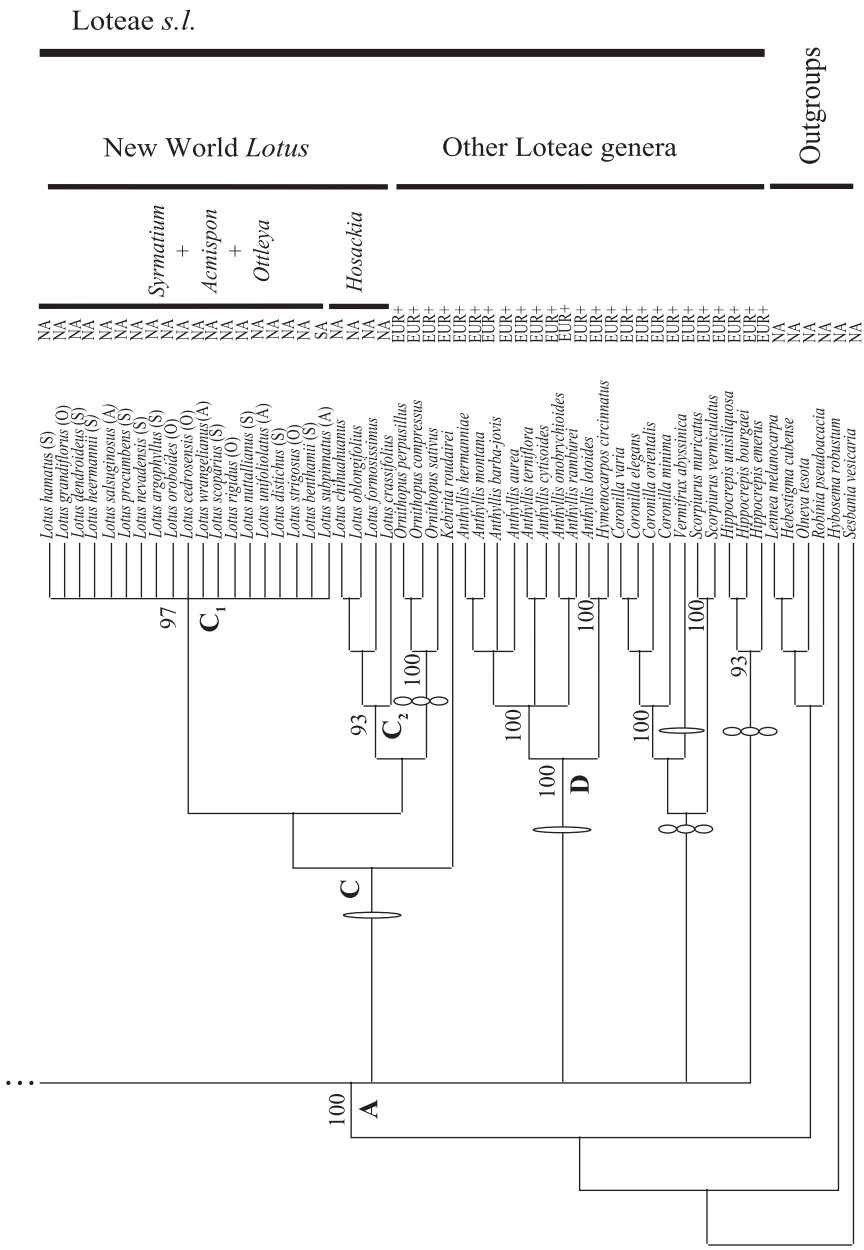


Fig. 1. Strict consensus of 1350 trees (2255 steps) based on ITS sequence data. Parsimony bootstrap values (numbers above branches) are shown only for those clades whose proportions exceeded 50%. Major clades are designated as A–D. Clade A defines a monophyletic Loteae *s.l.* Clade B combines Old World *Lotus* with *Hammatolobium*. Clade C (including C_1 and C_2) corresponds to two minor New World *Lotus* species groups. Clade D includes several members of *Anthyllis* plus *Hymenocarpos*. Letters in parentheses indicate North American Loteae group names as follows: (A) = *Acmispon*, (S) = *Syrmatium*, (O) = *Otilleya*. Fruit type is also mapped onto the phylogeny showing the occurrence of jointed fruits (Coronilleae) and unjointed fruits (Loteae *s.s.*).


Loteae s.l.
New World Lotus
Other Loteae genera
Outgroups
Syrmatium + Acmispon + Ottleya
Hosackia
Lotus hamatus (S)
Lotus grandiflorus (O)
Lotus dendroideus (S)
Lotus heermannii (S)
Lotus salsuginosus (A)
Lotus procumbens (S)
Lotus nevadensis (S)
Lotus argophyllus (S)
Lotus oroboides (O)
Lotus cedrosensis (O)
Lotus wrangelianus (A)
Lotus scoparius (S)
Lotus rigidus (O)
Lotus nuttallianus (S)
Lotus unifoliolatus (A)
Lotus distichus (S)
Lotus strigosus (O)
Lotus benthamii (S)
Lotus subpinnatus (A)
Lotus chihuahuanus
Lotus oblongifolius
Lotus formosissimus
Lotus crassifolius
Ornithopus perpusillus
Ornithopus compressus
Ornithopus sativus
Kebrid roudairei
Anthyllis hermanniae
Anthyllis montana
Anthyllis barba-jovis
Anthyllis aurea
Anthyllis terniflora
Anthyllis cytisoides
Anthyllis onobrychioides
Anthyllis rambirei
Anthyllis lotoides
Hymenocarpos circinnatus
Coronilla varia
Coronilla elegans
Coronilla orientalis
Coronilla minima
Vermifrax abyssinica
Scorpiurus muricatus
Scorpiurus vermiculatus
Hippocrepis unisiliquosa
Hippocrepis bourgaei
Hippocrepis emerus
Lennea melanocarpa
Hebestigma cubense
Oneya tessota
Robinia pseudoacacia
Hybosema robustum
Sesbania vesicaria
NA
SA
EUR+
97
93
100
C1
C2
C
D
A
...

Asian genus *Vermifrux* appears sister to members of *Coronilla*, while the monotypic African genus *Kebirita* is sister to a clade containing members of *Ornithopus* and New World *Lotus*. In both of these latter cases, however, sister group relationships are supported by values less than 50%.

Individual heuristic and Neighbour-joining analyses

In general, the Ratchet strict consensus tree (Fig. 1) agrees well with the strict consensus of the individual heuristic searches (tree not shown). For example, individual heuristic searches also place *Hammatolobium* sister to Old World *Lotus*, but *Hippocrepis* is placed (bootstrap value < 50%) sister to both *Hammatolobium* and Old World *Lotus*. In addition, individual heuristic searches place New World *Lotus* together with *Ornithopus* (sister to subg. *Hosackia*; as in Fig. 1), and *Kebirita* is positioned basal to the entire New World *Lotus/Ornithopus* complex (as in Fig. 1).

The individual heuristic searches also place Old World *Anthyllis* together with *Hymenocarpos*, and *Coronilla s.l.* is placed with *Scorpiurus* and *Vermifrux* in a single clade.

The neighbour-joining tree (not shown) differed from the Ratchet and individual heuristic analyses in the following ways: (1) *Scorpiurus* and *Hippocrepis* are together placed sister to the Old World *Lotus/Hammatolobium* clade, (2) *Kebirita* is sister only to the *Syrmatium* + *Acmispon* + *Ottleya* clade (C_1 in Fig.1), and (3) *Vermifrux* is placed sister to *Ornithopus*. Neighbour-joining bootstrapping, however, shows these contrasting relationships to be weakly supported (< 50%).

Discussion

Monophyly of Loteae

The taxonomic status of tribe Loteae has been debated for some time (Bentham, 1865; Boissier, 1872; Hutchinson, 1964; Polhill, 1981b, 1994; Sokoloff, 1998). Early workers (e.g., Boissier, 1872) routinely assembled the different genera into two tribes, those with unjointed fruits (tribe Loteae), versus those with jointed fruits (tribe Coronilleae). This distinction, however, is blurred by the fact that one species in particular, *Coronilla securidaca* L., has unjointed fruits and thus exhibits characteristics diagnostic of Loteae *s.s.* Corby (1981) found another distinction between Loteae and Coronilleae, namely that Loteae possess unbranched root nodules whereas Coronilleae have branched root nodules. However, Corby did not list the examined species, and thus it is uncertain whether his study includes sufficient generic sampling of the two tribes. Ferguson and Skvarla (1981) suggested that pollen morphology provides evidence in support of maintaining Loteae and Coronilleae as separate tribes. However, a more extensive study, which involved all genera of Loteae and Coronilleae (Díez and Ferguson, 1990, 1994, 1996) led Díez and Ferguson (1996) to conclude that there is little evidence to support the separation of Loteae and Coronilleae as distinct entities.

This analysis identifies a monophyletic Loteae *s.l.* (including all members of the tribe Coronilleae). Especially relevant is the placement of Old World *Hammatolobium*, traditionally recognised within Coronilleae, as sister to Old World *Lotus*. This novel sister-group relationship brings new insight to the monophyly of tribe Loteae, and has important implications for its taxonomic circumscription.

The monophyly of an expanded Loteae was suggested in an earlier study, also based on the ITS and 5.8S regions, but using a considerably smaller taxonomic sampling (Allan and Porter, 2000). In this earlier study, an expanded Loteae was identified based partly on strongly supported sister group relationships of two Old World species, *Coronilla valentina* and *C. scorpioides*, with members of *Lotus s.l.* These two species were identified as more closely related to New World *Lotus* than to any other member of Coronilleae (Allan and Porter, 2000). Although a number of morphological characters support this placement, the result was considered inconclusive for at least

one of these species (*C. valentina*, an Old World waif species in California) because of the possibility that the sampled accession might have been a *Lotus-Coronilla* hybrid. Verification of this possibility awaits the acquisition of new accessions of these two *Coronilla* species. It is equally important to note, however, that the previous ITS analysis (Allan and Porter, 2000) did not include the Old World genus *Hammatolobium*. The strongly supported position of *Hammatolobium* in the phylogeny, independently confirms that, at least one other member of Coronilleae is more closely related to Loteae (i.e., Old World *Lotus*) than to any other member of Coronilleae. This finding, together with previous results based on ITS, supports recognition of a single tribe, Loteae. This conclusion agrees with recent non-taxonomic (Polhill, 1994) and taxonomic (Sokoloff, 1998) assessments based on morphology.

By the monophyletic tribal definition given above, the Loteae *s.l.* includes members with both jointed or unjointed fruits and branched or unbranched root nodules. The occurrence of the fruit type character (jointed vs. unjointed fruits) is shown on Fig. 1. This mapping of fruit type clearly shows the homoplastic nature of this character. Root nodule type is not mapped onto the phylogeny because the occurrence of this character is incompletely known for members of Loteae *s.l.*

Distinguishing characters for Loteae *s.l.* (as contrasted with members of tribe Robinieae) include androecium with filaments dilated upwards, capitate or umbellate partial inflorescences (except *Anthyllis* subg. *Terniflora*, Tikhomirov and Sokoloff (1996a)), wing petals adnate to keel petals by a "ball-and-socket joint" (also in the *Poitea florida*-alliance of Robinieae; Lavin and Sousa, 1995), a base chromosome number of x = 7 or 6 (with some exceptions), epulvinate (except *Hippocrepis emerus s.l.*; García Martín and Talavera (2000)) and distichous (except *Anthyllis* subg. *Terniflora*; Tikhomirov and Sokoloff (1996b)), leaves lacking stipels and a closed vascular system.

Monophyly of *Lotus*

Lotus is a complex genus consisting of nearly 200 species distributed worldwide. There are two centres of species diversity, the Mediterranean region including Macaronesia (c. 135 spp.), and western North America (c. 50 spp.). Despite this apparent geographic disjunction, species of *Lotus* exhibit considerable morphological overlap. The degree of overlap and its significance for *Lotus* taxonomy has been debated for nearly 150 years. When Linnaeus (1753) first described the genus, the New World species of *Lotus* were not yet discovered. Upon discovery of the North American species, however, the question arose as to whether they should be placed within *Lotus*. In fact, the first species identified in North America was placed in *Lotus* (Pursh, 1814). However, Bentham (1829) described a new genus, *Hosackia*, to segregate this species and all other New World plants related to *Lotus*. Some authors (mainly of 19th to mid-20th century) followed Bentham's (1829) original description and placed all the New World species in the genus *Hosackia* (Gray, 1864; Taubert, 1894; Abrams, 1944), while others (mainly twentieth century writers) continued to regard them as *Lotus* (Greene, 1890; Ottley, 1923, 1944; Callen, 1959; Isely, 1981; Polhill, 1981b; Barneby, 1989). Until recently, this debate was largely considered resolved in favour of recognising all the New and Old World species as members of *Lotus* (Isely, 1981; Polhill, 1981b).

Recent studies using morphological comparisons (Kramina and Sokoloff, 1997; Sokoloff, 1999a, 2000), morphology-based cladistics (Arambarri, 2000a, 2000b), and molecular phylogenetic methods (Allan and Porter, 2000) have revisited the issue of the taxonomic circumscription of *Lotus*. Sokoloff (1999a, 2000), for example, advocates the segregation of the New World species into four groups (i.e., genera): *Hosackia*, *Acmispon* (Sokoloff, 2000), *Ottleya* (Sokoloff, 1999a), and *Syrmatium*. Arambarri (2000a, 2000b), based on separate morphological cladistic analyses, suggests that Old World *Lotus* is not monophyletic (Arambarri, 2000a), but that New World *Lotus* is (Arambarri, 2000b). Allan and Porter (2000) suggest that Old and New

World *Lotus* do not form a monophyletic group, but rather two separate groups, each of which is related to other Old World Loteae genera (also see Fig. 1). This study, the most taxonomically comprehensive to date, again finds *Lotus* to be non-monophyletic.

Molecular phylogenetic analyses based on the ITS region robustly place Old World *Hammatolobium kremerianum* (Coss.) Muell. sister to Old World *Lotus*. When this same accession is analysed with the original ITS dataset (Allan and Porter, 2000), the same robust result is found (Allan, unpubl. data). Although a close association between *Hammatolobium* and Old World *Lotus* had not previously been demonstrated, Polhill (1981b) suggested that *Hammatolobium* may be closer to either *Lotus* or Old World *Anthyllis*, than to *Coronilla*. Given that *Hammatolobium* consists of two rather dissimilar species (classified as separate subgenera; Tikhomirov and Sokoloff, 1996b), it remains to be seen whether both are closely allied to Old World *Lotus*. Unlike all Old World and New World *Lotus* species, both species of *Hammatolobium* have jointed fruits. This condition, however, has been shown to be homoplastic within the tribe (Allan and Porter, 2000 and this study, Fig. 1) and as previously suggested based on morphology (Lassen, 1989). Interestingly, however, *Hammatolobium* exhibits flowers with a beaked keel, which is similar to a number of other Old World genera, including *Lotus*. Like Old World *Lotus* and *Dorycnium*, *Hammatolobium* usually has five leaflets, with the basal pair resembling leafy stipules (Polhill, 1981b). Thus, aside from the feature of jointed fruits, *Hammatolobium* shows a strong morphological resemblance to *Lotus* (Sokoloff, 1999b).

Although Old and New World *Lotus* do not form a monophyletic group, Old World *Lotus* is monophyletic if *Tetragonolobus* and *Dorycnium* are included. Bootstrap values supporting the monophyly of Old World *Lotus* are moderate (63%), but this result corroborates that found in an earlier analysis (Allan and Porter, 2000) in which Old and New World *Lotus* formed individual, well supported clades (97% and 100%, respectively). As in this earlier study, we also find the two segregate genera, *Dorycnium* and *Tetragonolobus*, to be nested within Old World *Lotus*. Although *Dorycnium* is not found to be monophyletic within *Lotus*, it seems clear that both genera could be easily accommodated within Old World *Lotus*, a circumscription promoted by Polhill (1981b).

Finally, separate phylogenetic analyses in which Old and New World *Lotus* are constrained to be monophyletic resulted in the addition of 53 steps to the current tree (results not shown). Combined with the newly identified sister-group relationship of *Hammatolobium* and previous ITS analyses, this suggests that New and Old World *Lotus* may not constitute a monophyletic group. This conclusion remains tentative given the low support for clade C (Fig. 1). We anticipate undertaking phylogenetic studies based on other molecular markers as an independent test of this result.

Unlike Old World *Lotus*, it is not yet clear if New World *Lotus* is monophyletic, or what its sister group is. Rather, New World *Lotus* appears to be composed of two clades (C_1 and C_2), each of which is well supported (C_1 = 97%; C_2 = 93%). Again, it is important to note that, given the lack of resolution between these two groups, the current data cannot rule out the possibility of a monophyletic New World group. Nevertheless, two important conclusions emerge. First, clade C_1 includes representatives of three traditionally recognised groups (placed in separate genera by Sokoloff, 1999a, 2000): *Syrmatium*, *Acmispon* and *Ottleya*. Phylogenetic relationships among these taxa are unresolved, however. Morphological features that unite all members of this New World clade are the presence of gland-like stipules and usually 4–7-colporate (not 3-colporate) pollen grains (cf. Díez and Ferguson, 1994, 1996). Second, clade C_2 comprises members of subgenus *Hosackia*, which are diagnosed by the presence of leaf-like stipules. This result, however, contrasts with Arambarri (2000a) who did not find subg. *Hosackia* to be monophyletic. Rather, Arambarri's (2000a) morphological cladistic analysis, based primarily on seed morphology characters, revealed two distinct clades within subg. *Hosackia*. Despite this, independent morphological cladistic analyses (Sokoloff, unpubl. data), which include additional critical taxa and more characters, strongly suggest that subg. *Hosackia* is monophyletic.

It is interesting to note that species of subg. *Hosackia* have three-aperturate pollen, which is similar to that of Old World Loteae, including Old World *Lotus* (Crompton and Grant, 1993; Díez and Ferguson, 1994, 1996). This fact is intriguing in light of the weakly supported association between subg. *Hosackia* and Old World *Ornithopus* (Figs. 1, 2). It may be, however, that this pollen feature (in *Lotus s.s.* and *Hosackia*) is plesiomorphic within the tribe. Indeed, tricolporate pollen is the most common type within the Papilionoideae (Ferguson and Skvarla, 1981).

Given these results two tentative conclusions may be drawn with regard to *Lotus* taxonomy. First, it appears that *Lotus*, as traditionally circumscribed (including both Old and New World taxa), is not a natural group. Thus, Old and New World *Lotus* may be best treated as separate entities. Second, New World *Lotus* appears to comprise at least two groups, one including *Syrmatium*, *Acmispon* and *Ottleya*, and the other, members of subg. *Hosackia*. Thus, two lineages can be unambiguously identified, both having clear morphological synapomorphies.

Relationships and monophyly of other Loteae genera

ITS sequence data provide an important assessment of the monophyly of Old World Loteae genera. Our data identify well-supported monophyletic groups for *Coronilla s.l.* (100%), *Ornithopus* (100%), *Hippocrepis* (93%) and *Scorpiurus* (100%). Relationships among these genera are unresolved, however. One exception is *Anthyllis*, which appears paraphyletic with respect to *Hymenocarpos* (Fig. 1). This issue of paraphyly aside, it is clear that all species of *Anthyllis* except one (*A. lotoides*) form a well-supported (100%) monophyletic group. The sister group relationship between *A. lotoides* L. and *Hymenocarpos circinnatus* (L.) Savi is well supported (100%) and therefore instructive.

It is noteworthy that a similar association between *Anthyllis* and *Hymenocarpos* was identified in an earlier study (Allan and Porter, 2000) using the same accession of *H. circinnatus* and a different species of *Anthyllis* (*A. vulneraria* L.; not included in the present study). That *Anthyllis* is closely allied to *Hymenocarpos* is not surprising: examination of herbarium specimens reveals a high degree of morphological similarity between the two taxa (Allan, unpubl. data). Sokoloff (1998) also places *Hymenocarpos* close to *Anthyllis*, considering it to be one of two satellite genera (along with *Dorycnopsis s.l.*) around *Anthyllis*. The strongly supported (100%) sister group relationship between *Hymenocarpos*, *A. lotoides* and all other *Anthyllis* species, coupled with their shared morphological similarities, suggests that *Anthyllis* should be expanded to include *Hymenocarpos*.

Weakly supported results worth noting are the placements of the monotypic African/SW Asian genus *Vermifrux* and of the African monotypic genus *Kebirita*. In the phylogeny presented here, *Vermifrux* appears sister to *Coronilla* (Fig. 1). In NJ analyses (data not shown), however, *Vermifrux* is, together with *Ornithopus*, sister to members of New World *Lotus*. Ottley (1944) noted that *Vermifrux* is morphologically similar to New World *Lotus* (in particular members of the traditionally recognised subg. *Syrmatium*) in many respects. In fact, she recommended combining the New World species of *Lotus* subg. *Syrmatium* under *Vermifrux*, if in fact segregation of the New World species was warranted (Ottley, 1944). Tikhomirov and Sokoloff (1997), however, regard this taxonomy to be untenable on the basis of detailed morphological comparisons (e.g., features of habit, inflorescence and fruit indumentum): there is a strong similarity (e.g., in fruit anatomy) between *Vermifrux* and *Ornithopus* (Tikhomirov and Sokoloff, 1997; Sokoloff and Mironov, Moscow State University, unpubl. data).

The strict consensus tree (Fig. 1) points to an alliance between New World *Lotus* and African *Kebirita roudairei*. Interestingly, this African species shares morphological features with New World *Lotus* species and appears to be closely related to them. In fact, *K. roudairei* has been the subject of considerable taxonomic revision. Originally considered close to *Lotus*, Cosson (see Maire (1931) for discussion) first named this

species *L. hosackioides* (not validly published), suggesting a close relationship to the New World *Hosackia* group. Bonnet (1893), however, was the first to describe and validly publish this species as *L. roudairei*, and also compared it with New World *Hosackia*. Lassen (1986) considered *L. roudairei* to be quite distinct and transferred it to *Acmispon* sect. *Simpeteria* giving it the name *Acmispon roudairei* (Bonnet) Lassen. While this name is accepted by Lock (1989) and Greuter *et al.* (1989), Kirkbride (1994, 1999) and Lebrun and Stork (1992) place *Acmispon roudairei* within *Lotus*. Finally, Kramina and Sokoloff (2001) have described this species as the monotypic genus *Kebirita* (i.e., *K. roudairei* (Bonnet) Kramina & D. D. Sokoloff), which is the taxonomic scheme used here.

Biogeographic implications

Figure 2 shows a most parsimonious reconstruction (MPR) for the biogeography of Loteae *s.l.* Our choice of this topology for the optimisation of biogeography is based on the following observations: (1) the overall topology is very similar to the topology of individual heuristic searches (data not shown); and (2) it closely matches the individual topologies of nearly all the 1350 trees derived from the Ratchet analysis. Thus, we consider the chosen topology to be a reliable estimate on which to interpret phylogeographic patterns of Loteae *s.l.*

Examination of the most parsimonious optimisation for biogeography (Fig. 2) reveals three general patterns. First, it is clear that all four MPRs unequivocally identify the geographic area "Europe + Asia + Africa (including Macaronesia)" as an important source area for Loteae *s.l.* This biogeographic area coincides with Takhtajan's (1986) designation of a Tethyan-Mediterranean floristic region, and is one that is essentially centred on the Mediterranean Sea, with semi-desert extensions trending eastward and far into central Eurasia. Although it is tempting to postulate a Mediterranean origin for Loteae based on these data, we recognise that the basal-most nodes in our strict consensus tree are in fact unresolved. Thus, given that outgroup members of Robinieae are all North American in distribution, we cannot exclude the possibility of a New World geographic origin for Loteae. It is interesting to note, however, that recent molecular phylogenetic analyses based on *mat*K (M. Lavin and M. F. Wojciechowski, Montana State University and Arizona State University, unpubl. data) identify *Sesbania* as more closely related to Loteae *s.l.* than any other member of Robinieae. This finding could have important implications for ultimately understanding Loteae biogeography because *Sesbania* is the only member of Robinieae with a non-American distribution (i.e., some are more pantropical).

Second, these data indicate that Old and New World members of Loteae are not as closely related to each other as has been previously thought. For example, the North American members of the *Acmispon s.l.* clade (*Syrmatium* + *Acmipson* + *Ottleya*) are closely related to each other and only distantly related to the Old World genus *Vermifrux* as well as to Old World *Lotus s.s.* (see e.g., Ottley, 1944). The monotypic African genus *Kebirita*, on the other hand, appears closely related to members of the North American *Acmispon s.l.* clade, an alliance that has important implications for interpreting the biogeography of New World Loteae.

Third, examination of the four MPRs reveals different scenarios (as implied by the equivocal assignment) for the geographic origin and migration of New World Loteae and Old World species of *Ornithopus*. Two of these reconstructions postulate independent migrations for members of the *Hosackia* and North American *Acmispon s.l.* clades. A third reconstruction suggests a single migration from the Tethyan-Mediterranean region to North America, followed by: (a) dispersal to South America (to account for *Lotus subpinnatus*) and; (b) back dispersal to Eurasia/Africa (to account for Old World *Ornithopus*). A fourth scenario involves two independent migrations from the Tethyan-Mediterranean region to (a) North America (*Hosackia* clade) and (b) South America (*L. subpinnatus*), with subsequent dispersal to North

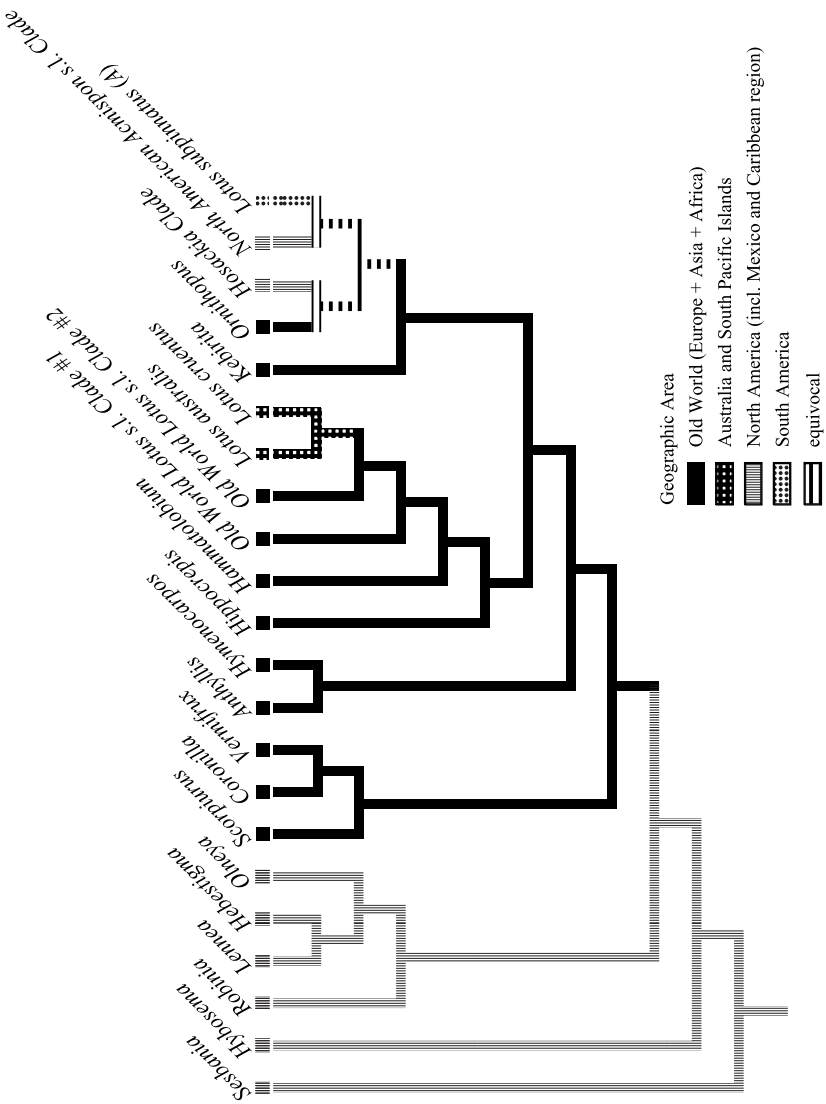


Fig. 2. Maximum parsimony optimisation of biogeography for Loteae *s.l.* Patterns of biogeography were inferred from a simplified cladogram summarising major lineages of Loteae *s.l.* Old World *Lotus s.l.* clade #1 and Old World *Lotus s.l.* clade #2 correspond to the Old World *Lotus* clades with bootstrap values of 63 and 72% in Fig. 1. All most parsimonious reconstructions were derived using equivocal cycling in MacClade 4.0 (Maddison and Maddison, 2000). Note that *L. subpinnatus* (A) is a member of the North American *Acmispon s.l.* group (see Fig. 1), but is shown separately to highlight its South American geographic distribution. Likewise, *Lotus australis* and *L. cruentus* both belong to *Lotus s.l.* (Fig. 1), but are shown separately to emphasise their monophyly and geographic distribution.

America to account for the North American *Acmispon s.l.* clade. In fact, in NJ analyses *L. subpinnatus* is placed basal to the *Acmispon s.l.* clade, albeit with low bootstrap support (data not shown). This migration from Europe/Africa directly to South America, however, would almost certainly imply long distance dispersal, because direct land connections between these two areas have been absent since the early Cretaceous and possibly even since the mid-to-late Jurassic period (Kalandadze and Rautian, 1997).

A final biogeographic pattern concerns the two Australian and South Pacific Island species, *L. australis* and *L. cruentus*. In all topologies, these two species form a well-supported clade and are reconstructed as having a single origin, presumably from Eurasia. It is particularly noteworthy that in this and all other cases cited above migration has occurred over considerable geographic distances. This, together with the fact that members of Loteae (*Lotus* in particular) are believed to be of relatively recent origin (Polhill 1981b), suggests long distance dispersal (rather than vicariance) as a predominant mechanism for producing the biogeographic patterns observed here.

Conclusions

The present study provides evidence for recognising a monophyletic tribe Loteae (including Coronilleae). This is based on the fact that at least one member of the formerly recognised tribe Coronilleae, *Hammatolobium*, is more closely related to the genus *Lotus* (Loteae) than to any other member of the Coronilleae. That Loteae is monophyletic is concordant with previous molecular phylogenetic analyses based on the ITS region (Allan and Porter, 2000). Members of Old World *Lotus* appear to form a monophyletic group distinct from species of *Lotus* in western North America, Mexico, and South America. This finding has important implications for resolving the taxonomic debate over whether to recognise the New World species as members of *Lotus* or not. Since members of New World *Lotus* fall into two separate, albeit unresolved, clades, it is not yet possible to determine whether they are best treated as a single group, two groups, or possibly even several segregate genera (as promoted by Sokoloff, 1999a). Despite this limitation, clear morphological synapomorphies identify two groups, *Hosackia* and *Acmispon s.l.* Although we do not put forward a formal recommendation for revising generic concepts of *Lotus*, we demonstrate justification for revision, based in part on independent molecular and morphological data sets.

In biogeographic analyses of the tribe we conclude that the Old World (Europe + Asia + Africa) has been an important source area related to the geographic origin and diversification of Loteae *s.l.* This is based on the finding that members of Old World Loteae (e.g., *Coronilla*, *Anthyllis*) occupy a basal position in the optimised phylogeny, and thus impose an unequivocal "Old World" assignment to the node leading to the ingroup. Despite this, we cannot exclude the possibility of a North American origin for Loteae given the lack of resolution of individual basal nodes in the phylogeny (Fig. 1). We also identify several different scenarios for explaining the geographic distribution of New World Loteae and Old World *Ornithopus*. These alternative scenarios suggest at most two independent migrations from the Tethyan-Mediterranean (i.e., Old World) region to North America, followed by back dispersal to Eurasia, or independent migrations to North and South America. Finally, we suggest that dispersal, rather than vicariance, is the mostly likely mechanism accounting for the current biogeographic distributions of Loteae. This conclusion is based on the high dispersability of legumes in general (Raven and Polhill, 1981) and of *Lotus* in particular, and on the relatively recent derivation of the tribe from other tropical legumes (i.e., members of Robinieae).

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