PHYLOGENETIC PLACEMENT OF *PODOLOTUS* SUGGESTS INDEPENDENT ORIGIN OF LOMENTACEOUS FRUITS IN *CORONILLA* AND *HIPPOCREPIS* (LEGUMINOSAE: LOTEAE)

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Abstract

Tribe Loteae has major diversity centres in the Mediterranean region and in California. However, four monospecific genera are restricted to other parts of Africa and Asia. This paper is focused on the monospecific Asian genus *Podolotus*, which is crucial for understanding evolution of Loteae. Evidence from four DNA markers (nrITS, *psb*A-*trn*H, *pet*B-*pet*D and *rps*16 intron) is used to infer phylogenetic relationships of *Podolotus*. Analysis of the combined data set strongly suggests that *Podolotus* is closest to *Coronilla*. These two genera share several important morphological features but differ in fruit type (dehiscent vs. lomentaceous). Traditionally, *Coronilla* was thought to be closest to two other genera with lomentaceous fruits, *Hippocrepis* and *Scorpiurus*. Our data support the view that lomentaceous fruits evolved in *Coronilla* independently from all other members of Loteae that bear this fruit type. At least five independent origins of lomentaceous fruits took place in evolution of Loteae. Molecular phylogenetic rooting of the tribe Loteae is discussed.

Introduction

The temperate herbaceous tribe Loteae (Leguminosae) has two major diversity centres in the Mediterranean region and in California. However, a number of monospecific genera of Loteae are restricted to non-Mediterranean parts of Africa and Asia. These are *Kebirita* Kramina & Sokoloff in NW Africa, *Antopetitia* A. Rich. in mountains of Tropical Africa, *Podolotus* Royle ex Benth. (mountains of Afghanistan, Iran, Pakistan, India and disjunctively in Oman) and *Pseudolotus* Rech. f. from Oman and Makran desert in Iran and Pakistan (Polhill, 1981; Rechinger, 1984; Ali & Sokoloff, 2001; Kramina & Sokoloff, 2001). Though comprising an insufficient part of species diversity of the tribe, they are highly significant for understanding higher-level relationships in Loteae. It is an open question whether these genera should be viewed as "ousted relics" (see Eskov, 1992, 2002), like the species-poor Australian lineage of *Lotus* (Kramina & Sokoloff, 2004).

Podolotus hosackioides Benth. is one of most enigmatic representatives of the tribe Loteae. The plant was first collected by Royle in Himalayas (Mussoorie). Royle suggested that the plant belongs to a new monospecific genus. The name *Podolotus hosackioides* was adopted by Bentham (1835) in his account of Leguminosae in Royle's illustrated book on Himalayan botany. Bentham (1835) has noted that *Podolotus* resembles the North American genus *Hosackia* Douglas ex Benth. in general habit and leaf morphology and the Old World genus *Lotus* L. in corolla structure but differs from these genera in stalked fruit. Both *Lotus* and *Hosackia* belong to the tribe Loteae.

Bentham & Hooker (1865) proposed a new relationship of *Podolotus*. They accepted *P. hosackioides* as a member of the large genus *Astragalus* (tribe Galegeae). *Podolotus*

shares the character of stalked fruits with many species of *Astragalus*, a feature that is rare in Loteae (though this obvious argument was not explicitly expressed by Bentham & Hooker). The new combination, *Astragalus hosackioides* was proposed by Baker (1876). This view was accepted in other accounts, including the important survey of legumes by Taubert (1894).

Rechinger (1957) re-discovered *Podolotus hosackioides* among new collections from Afghanistan. He had the opinion that the plant should be classified as a member of the tribe Loteae. Since *Podolotus* was accepted as a synonym of *Astragalus*, Rechinger did not consider this name and the work of Bentham (1835). Therefore he described a new genus and a new species, *Kerstania nuristanica* Rech. f. Due to his great experience in *Astragalus*, Ali (1958) was the first who demonstrated that *Kerstania niristanica* was identical to *Podolotus hosackioides*. Ali (1958, 1961, 1977) reduced *K. nuristanica* under the synonymy of *Astragalus hosackioides*.

Polhill (1981), Rechinger (1984), Lassen (1989) and Sokoloff (2003a) provided further morphological evidence in favour of accepting *Podolotus* as a member of Loteae rather than as a species of *Astragalus* in Galegeae. At least four important morphological features distinguish *Podolotus* from *Astragalus*: (1) ovules with micropylae alternantes (see Sokoloff (1999) for illustration and Tikhorimov and Sokoloff (1997) for terminology), similar to many Loteae; (2) occurrence of transversal septa between adjacent seeds in fruits, like in most Loteae with many-seeded fruits; (3) stamen filaments dilated upwards, like in all Loteae; (4) the so-called parchment layer of the pericarp consists of oblique fibres in *Podolotus* (Sokoloff, 1997), while in the few species of *Astragalus* studied so far (e.g., Fahn & Zohary, 1955; Roth, 1977) each fruit valve has two strata of fibres of different orientation.

Polhill (1981) adopted a wide generic concept in Loteae. In particular, he placed many genera, including *Hosackia* and *Podolotus*, in synonymy of *Lotus*. Ali (1994) followed Polhill (1981) and proposed a new combination, *Lotus hosackioides* (Royle ex Benth.) Ali based on *Podolotus hosackioides*.

Though suggesting placement of *Podolotus* in synonymy of *Lotus* Polhill (1981) viewed *Podolotus* as one of key taxa for understanding evolution of Loteae[1]. According to Polhill (1981) the tribe Loteae is derived from Galegeae. He used morphological similarities between *Podolotus* and *Astragalus* to support this hypothesis. Furthermore, Polhill (1981) used evidence from *Podolotus* in support of his view that all genera formerly assigned to the tribe Coronilleae should be placed in Loteae. Traditionally, Loteae and Coronilleae were distinguished on fruit morphology. Fruits of Coronilleae are lomentaceous while fruits of Loteae are not lomentaceous (either dehiscent or indehiscent). Polhill pointed out that *Podolotus* had a fruit of *Lotus* and a calyx of *Coronilla*. Apart from being stalked, the fruit of *Podolotus* is very similar to that of *Lotus* because it is dehiscent by two valves and has the same basic anatomical structure. Besides the similarity of calyx morphology between *Podolotus* and *Coronilla*, both of genera lack a sterile bract on peduncle, a characteristic feature of *Lotus* and many other Loteae (Sokoloff *et al.*, 2007). According to Lassen (1989), the monotypic *Podolotus* belongs to the *Coronilla*-group within the

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[1] Note that Polhill did not intend to reject paraphyletic taxa. The genus *Lotus*, in the wide concept proposed by Polhill (1981), differed from other genera of Loteae in plesiomorphic character states. The most important feature of *Lotus* s.l. is a fruit actively dehiscent into two valves. Based on general assumptions, it was logical to estimate that all other genera of Loteae were derived from *Lotus* sensu Polhill.

expanded tribe Loteae. Sokoloff (2003b) and Sokoloff & Lock (2005) placed *Podolotus* just after *Coronilla* in the linear sequence of genera of the tribe Loteae.

Molecular phylogenetic data supported the broader concept of Loteae (to include Coronilleae) (Allan & Porter, 2000; Allan *et al*., 2003; Degtjareva *et al*., 2003, 2006a, 2006b; Sokoloff *et al*., 2007), but failed to support its origin from Galegeae. Instead, members of Robinieae and Sesbanieae were unexpectedly identified as closest relatives of Loteae (e.g., Hu *et al*., 2000; Wojciechowski *et al*., 2000, 2004).

The first molecular marker used to establish relationships of *Podolotus* was nuclear ribosomal ITS (Degtjareva *et al*., 2006a). The analysis of Degtjareva *et al*., (2006a) was focused on *Lotus* s.str., but included also representatives of all genera of Loteae plus *Robinia* and *Sesbania*. Depending on the method of analysis, the genus *Podolotus* appeared to be sister to a clade comprising *Coronilla*, *Scorpiurus* and *Hippocrepis* (all three genera formerly assigned to Coronilleae) or to *Hippocrepis* plus *Scorpiurus*. Neither of two topologies received significant support in the study of Degtjareva *et al*., (2006a).

Degtjareva *et al*., (submitted) used nrITS plus three plastid markers to analyse phylogenetic relationships in *Anthyllis*. Apart from *Anthyllis*, representatives of all other genera of Loteae plus an outgroup, *Sesbania* were included. They found position of *Podolotus* to be unresolved in analyses of nrITS data set. In Bayesian analysis of plastid data, *Podolotus* was sister to *Coronilla* with posterior probability 0.99, but in maximum parsimony analysis, this grouping did not receive bootstrap support exceeding 50%. Only one species of *Coronilla* was included in this study.

Morphological cladistic analysis of Sokoloff (2006) placed *Podolotus* as a sister to (*Hosackia* ((*Scorpiurus* + *Hippocrepis*) *Coronilla*)) with bootstrap support 63%, though another morphological cladistic analysis based on just slightly different data set failed to resolve relationships of *Podolotus* (Sokoloff, 2003b). The most stable result of all morphological cladistic analyses was appearing a clade comprising *Scorpiurus*, *Hippocrepis* and *Coronilla*. These genera share several important morphological features, including lomentaceous fruits, the absence of a sterile bract on peduncles of partial inflorescences, joint initiation of adaxial sepals in course of flower development, the presence of well-developed stipules (Sokoloff *et al*., 2007). When the morphological data set was analysed together with nrITS sequence data, the *Scorpiurus-Hippocrepis-Coronilla* clade was also revealed (Sokoloff *et al*., 2007).

To summarize, both morphological and molecular phylogenetic analyses show that *Podolotus* is not closely related to *Lotus* s.str. therefore supporting its recognition as a distinct genus. However, neither molecular nor morphological phylogenetic analyses provided robust and unequivocal data on relationships of this interesting genus. The present study is aimed in understanding this issue. We use the four-marker molecular data set produced to investigate phylogeny of *Anthyllis* (Degtjareva *et al*., submitted) and extend sampling of some key genera, especially *Coronilla* to achieve a more representative sampling of taxa potentially related to *Podolotus*. The position of *Coronilla* appears to be crucial. Indeed, some analyses suggest that this genus is closest to *Hippocrepis* and *Scorpiurus* while an alternative possibility implies sister-group relationships between *Coronilla* and *Podolotus*.

Material and Methods

Taxonomic sampling: For molecular phylogenetic analysis, sequences for each of four markers of *Hippocrepis (H. balearica, H. multisiliquosa)*, *Coronilla (C. repanda, C.*

rostrata, C. somalensis, C. vaginalis), *Ornithopus* (*O. micranthus*) and *Lotus* (*L. schimperi*) were generated and added to the already existing alignment produced to investigate phylogeny of *Anthyllis* (Degtjareva *et al.*, submitted). The taxon sampling covered representatives of all genera of the tribe Loteae worldwide plus one of the closest relatives of Loteae (Hu *et al.*, 2000; Wojciechowski *et al.*, 2000), *Sesbania* (Sesbanieae). GenBank accession numbers and voucher information for newly investigated species are listed in Appendix.

DNA extraction, amplification and sequencing: Total DNA was isolated from leaf tissue using NucleoSpin Plant isolation kit (Macherey-Nagel, Düren, Germany) following manufacturer's instructions. The strategies used to obtain ITS, *pet*B-*pet*D, *rps*16 regions are presented elsewhere (ITS: Valiejo-Roman *et al.*, 2002; *pet*B-*pet*D: Löhne & Borsch, 2005; *rps*16: Marazzi *et al.*, 2006). Amplification and sequencing of the *psb*A-*trn*H spacer was conducted as for the ITS region. The *psb*A-*trn*H spacer was amplified using the primers *trn*H (Tate & Simpson, 2003) and *psb* A (Sang *et al.*, 1997). PCR products were purified using the DNA cleaning kit from Cytokine (St.-Petersburg, Russia) following instructions from the manufacturer. Direct sequencing was performed on the ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA), using ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit for cycle sequencing reactions following the manufacturer's instructions. Forward and reverse strands of all samples were sequenced.

Sequence analysis: The sequences were aligned separately using the program MUSCLE (Edgar, 2004) and manually adjusted using the program BioEdit (Hall, 1999), then concatenated.

Pairwise comparison of divergence between *Astragalus* and tribe Loteae representatives was performed using Phylogenetic Analysis using Parsimony and other methods (PAUP), maximum likelihood distances according to the GTR+Γ model of evolution were computed.

Maximum parsimony analysis involved a heuristic search conducted with PAUP (version 4.0b8; Swofford, 2003) using tree bisection–reconnection (TBR) branch swapping with character states specified as equally weighted. 500 replicates with random addition of sequences were performed, 1,000 most parsimonious trees from each replicate were saved. Bootstrap values (Felsenstein, 1985) were calculated from 100 replicate analyses with TBR branch swapping and random addition sequence of taxa, saving 1,000 most parsimonious trees from each replicate.

Bayesian inference of phylogeny was explored using the MrBayes v. 3.1.2 program (Ronquist & Huelsenbeck, 2003). The GTR model of substitution and Gamma rate categories (GTR+Γ) for plastid sequences and the same model with the presence of invariant sites (GTR+Γ+I) for ITS were selected by the Akaike information criterion (AIC) in MrModeltest (Nylander, 2004). Bayesian analysis was performed with four chains in each of the two parallel runs, each chain started with a random tree, and 15,000,000 replicates were generated. The trees obtained were sampled every 100 generations, trees from first 20100 generations were discarded as burn-in. The number of generations to be discarded was determined using the cold-chain log-likelihood examination. Each marker was considered as a separate partition, substitution rates and state frequencies were unlinked across partitions.

Results

1. General features of sequences: Characteristics of ITS and cpDNA sequence data variability are summarized in Table 1. The most informative marker is nuclear ITS spacer (41% informative sharacters), however in the whole data set it represents only 46% of informative variation. Three plastid markers in total contain more informative characters than the ITS.

The most variable in length is *psb*A-*trn*H spacer (from 192 bp to 475 bp). Such variation is associated with some large deletions and amplification of short fragments. Compared to other plastid markers, this spacer is the most variable (23% informative sites). Pairwise comparison of ITS sequences of *Podolotus* with *Astragalus cicer* and *Astragalus glycyphyllos* indicate that *Podolotus* is not a close relative to *Astragalus* : genetic distances are 0.419 and 0.425 accordingly and comparable with distances of *Astragalus* with *Kebirita* or *Antopetitia*, whereas distances from *Podolotus* to other members of tribe do not exceed 0.269.

2. Analyses based on nrITS data

2a. Maximum parsimony analysis: When the tree was rooted using *Sesbania* as the outgroup, all genera of Loteae except *Coronilla* formed a clade with bootstrap support 74%. Within this large clade, sister-group relationships of *Podolotus* were unresolved. When *Sesbania* was removed, *Podolotus* appeared to be closest to *Hippocrepis*, *Coronilla* was close to *Lotus*, *Hammatolobium*, *Coronilla* was close to *Lotus*, *Hammatolobium*, *Cytisposis* and *Tripodion* and *Scorpiurus* grouped with *Anthyllis* (unrooted tree); however, none of these grouping received bootstrap support exceeding 50%.

2b. Bayesian analysis: When the tree was rooted using *Sesbania* as the outgroup, all genera of Loteae except *Coronilla* formed a clade with very low posterior probability (0.56). Within this large clade, *Podolotus* occupied an isolated position (as sister to all genera except *Lotus, Tripodion, Cytisopsis, Hammatolobium*); *Scorpiurus* was sister to *Hippocrepis* with posterior probability 1.00. When *Sesbania* was removed (unrooted tree), relationships between other taxa remained unchanged.

3. Analyses based on plastid data

3a. Maximum parsimony analysis: When the tree was rooted using *Sesbania* as the outgroup, all genera of Loteae except *Podolotus* formed a clade with bootstrap support 57%. Within this large clade, *Coronilla* and *Hippocrepis+Scorpiurus* formed two successive basal subclades. Bootstrap support for grouping all genera of Loteae except *Podolotus, Coronilla, Hippocrepis* and *Scorpiurus* was 90%. When *Sesbania* was removed from analysis (unrooted tree), *Podolotus, Coronilla, Hippocrepis* and *Scorpiurus* formed a cluster with bootstrap support 99%; *Podolotus* clustered with *Coronilla* (bootstrap support 100%) and *Hippocrepis* clustered with *Scorpiurus* (bootstrap support 100%).

3b. Bayesian analysis: When the tree was rooted using *Sesbania* as the outgroup, three major clades of Loteae were revealed, each with posterior probability 1.00: (1) *Scorpiurus* plus *Hippocrepis*; (2) *Coronilla* plus *Podolotus*; (3) all other genera. Relationships between these three clades were unresolved. Removing *Sesbania* did not affect occurrence of three major clades and their posterior probabilities.

Table 1. Characteristics of ITS and cpDNA sequences.

	ITS	*pet*B-*pet*D	*psb*A-*trn*H	*rps*16
Length variation	551-645	847-977	192-475	830-931
Total of aligned characters	770	1172	715	1093
Total of excluded positions	179	249	433	223
Informative sites	243	92	71	119
% of informative characters	41	10	25	14
(across all markers)	(46)	(17,5)	(13,5)	(23)
Constant sites	250	678	152	605

Length variation in both *pet*B-*pet*D and *rps*16 is mostly a result of duplication or insertion/deletion of short fragments (1-10bp), which often are autapomorphic.

Apart from analyzing all three plastid markers taken together, each of individual markers was analyzed separately. When *rps*16 intron was used, the same three major clades were found as in the analysis of all three plastid markers. Posterior probabilities of these groups were relatively low when *Sesbania* was used as the otgroup but increased up to 1.00 when *Sesbania* was removed. When *psb*A-*trn*H region was used, *Hippocrepis* grouped with *Scorpiurus* in an unrooted tree only, and relationships of *Podolotus* and *Coronilla* were unresolved. When *pet*B-*pet*D region was used, *Podolotus* was strongly supported as sister to *Coronilla*, but close relationships between *Hippocrepis* and *Scorpiurus* were not supported.

4. Analyses based on combined nrITS plus plastid data

4a. Maximum parsimony analysis: When *Sesbania* was used as the outgroup (Fig. 1), *Coronilla*, *Podolotus* and *Scorpiurus+Hippocrepis* formed three successive basal clades of Loteae (the sequence of their insertion differed between the tree inferred from bootstrap analysis and the strict consensus of shortest trees). When *Sesbania* was removed (unrooted tree, Fig. 2) a cluster comprising *Coronilla, Podolotus, Scorpiurus* and *Hippocrepis* had bootstrap support 93%, *Coronilla + Podolotus* received 96% and *Scorpiurus + Hippocrepis* 98%. These figures are all greater than bootstrap support values in this region of the rooted tree.

4b. Bayesian analysis: When *Sesbania* was used as the outgroup (Fig. 3), *Coronilla* was sister to *Podolotus* (posterior probability 0.98) and *Hippocrepis* was sister to *Scorpiurus* (posterior probability 1.00). The rest of Loteae formed a clade with posterior probability 1.00, which was sister to *Hippocrepis + Scorpiurus* clade (posterior probability 0.6). When *Sesbania* was removed (Fig. 4), the cluster of ((*Coronilla + Podolotus*) (*Hippocrepis + Scorpiurus*)) and each of its subclusters received posterior probabilities of 1.00.

Discussion

Relationships of *Podolotus* and rooting of Loteae: Position on *Podolotus* in different trees can be summarized as following. Nuclear ribosomal ITS data do not provide robust evidence on relationships of *Podolotus*. Unrooted trees based on plastid data alone and on combined plastid plus nrITS data show the same cluster, ((*Coronilla + Podolotus*) (*Hippocrepis + Scorpiurus*)). This result appears both in Bayesian and maximum parsimony analyses. In Bayesian analyses, the entire cluster and each of its subclusters received posterior probabilities of 1.00. In maximum parsimony analyses, bootstrap support was more than 90%. When *Sesbania* was inserted as the outgroup, it clustered

with *coronilla* (maximum parsimony analysis of combined data set), with *Podolotus* (maximum parsimony analysis of plastid data set), with *Podolotus* plus *Coronilla* (Bayesian analysis of combined data set), or fallen in an unresolved position between *Podolotus+Coronilla*, *Hippocrepis+Scorpiurus* and the rest of Loteae (Bayesian analysis of plastid data set). The unstable rooting with *Sesbania* is not surprising, because of high differences between sequences of *Sesbania* and any member of Loteae. It is clear that long branch attraction does not allow precise rooting of Loteae in our study. Still, higher-level studies of legume phylogeny suggest that *Sesbania* could be the closest outgroup of Loteae (Wojciechowski *et al.*, 2004).
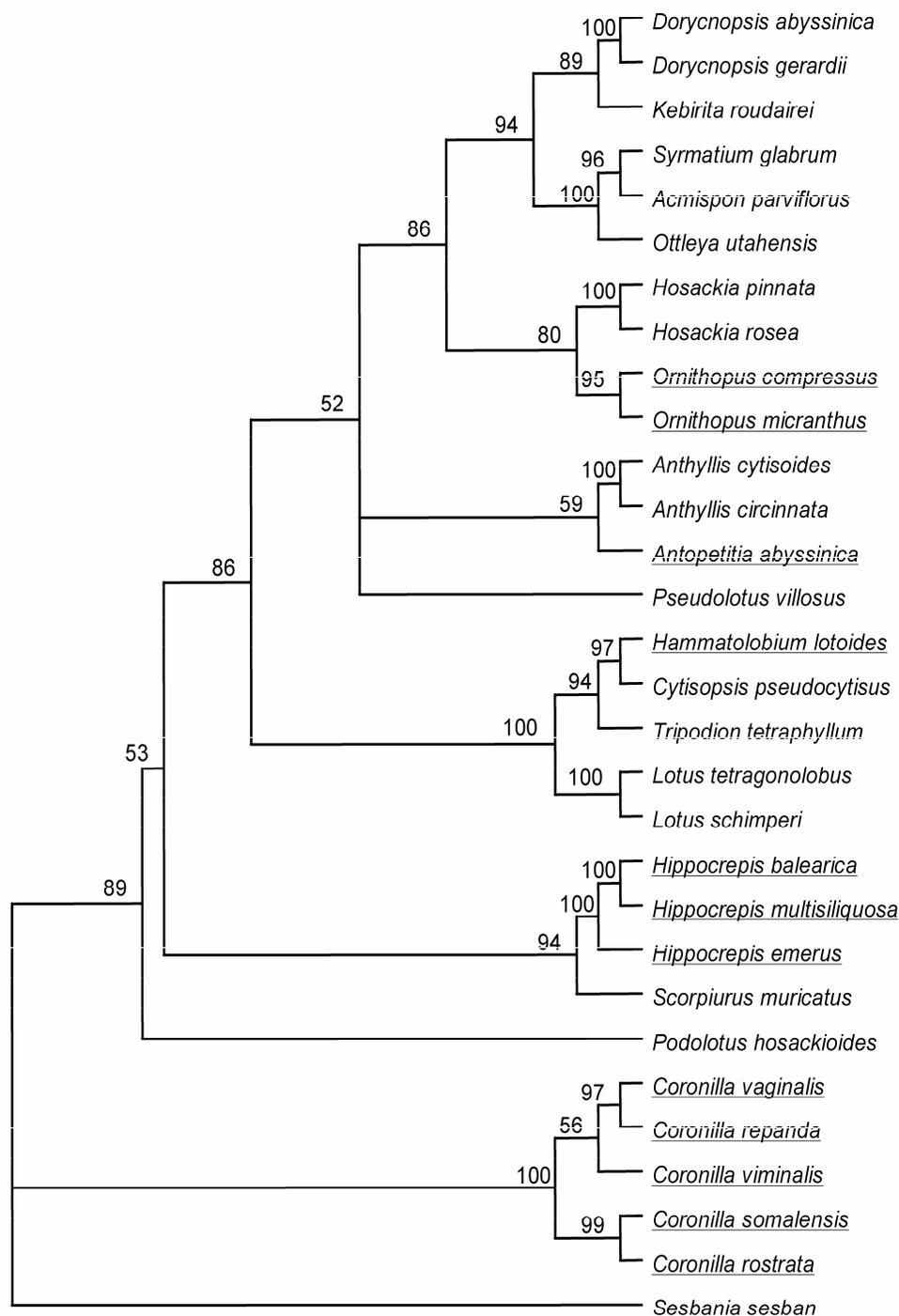
Fig. 1. 50%-majority rule bootstrap consensus tree inferred from maximum parsimony analysis of combined nrITS plus plastid data. Bootstrap support values are indicated. The tree is rooted using *Sesbania* as the outgroup. Names of taxa with lomentaceous fruits are underlined.
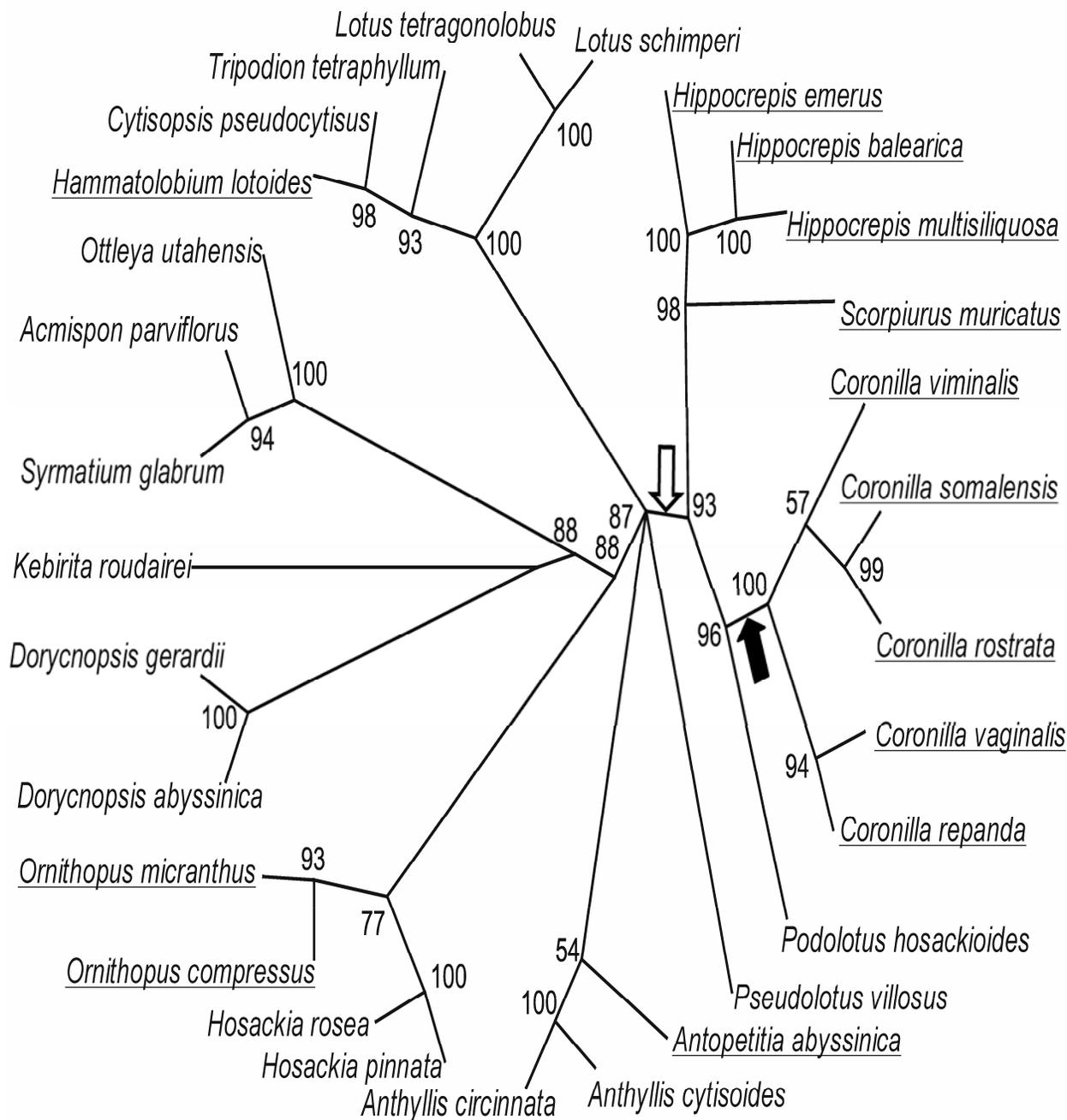
Fig. 2. Unrooted 50%-majority rule bootstrap consensus tree inferred from maximum parsimony analysis of combined nrITS plus plastid data. Bootstrap support values are indicated. Names of taxa with lomentaceous fruits are underlined. Solid arrow = position of rooting through *Sesbania*. Open arrow = position of an alternative rooting that agrees with distribution of some key morphological characters (see the text).

Occurrence of unique indels (a 6 bp duplication and a 2 bp deletion in *pet*B-*pet*D region) strongly suggests that *Coronilla* plus *Podolotus* form a clade rather than a basal grade in phylogenetic tree of Loteae. However, the present molecular phylogenetic data do not allow deciding whether (*Coronilla+Podolotus*) and (*Hippocrepis+Scorpiurus*) represent two successive basal clades of Loteae or group together being sister to the rest of Loteae.
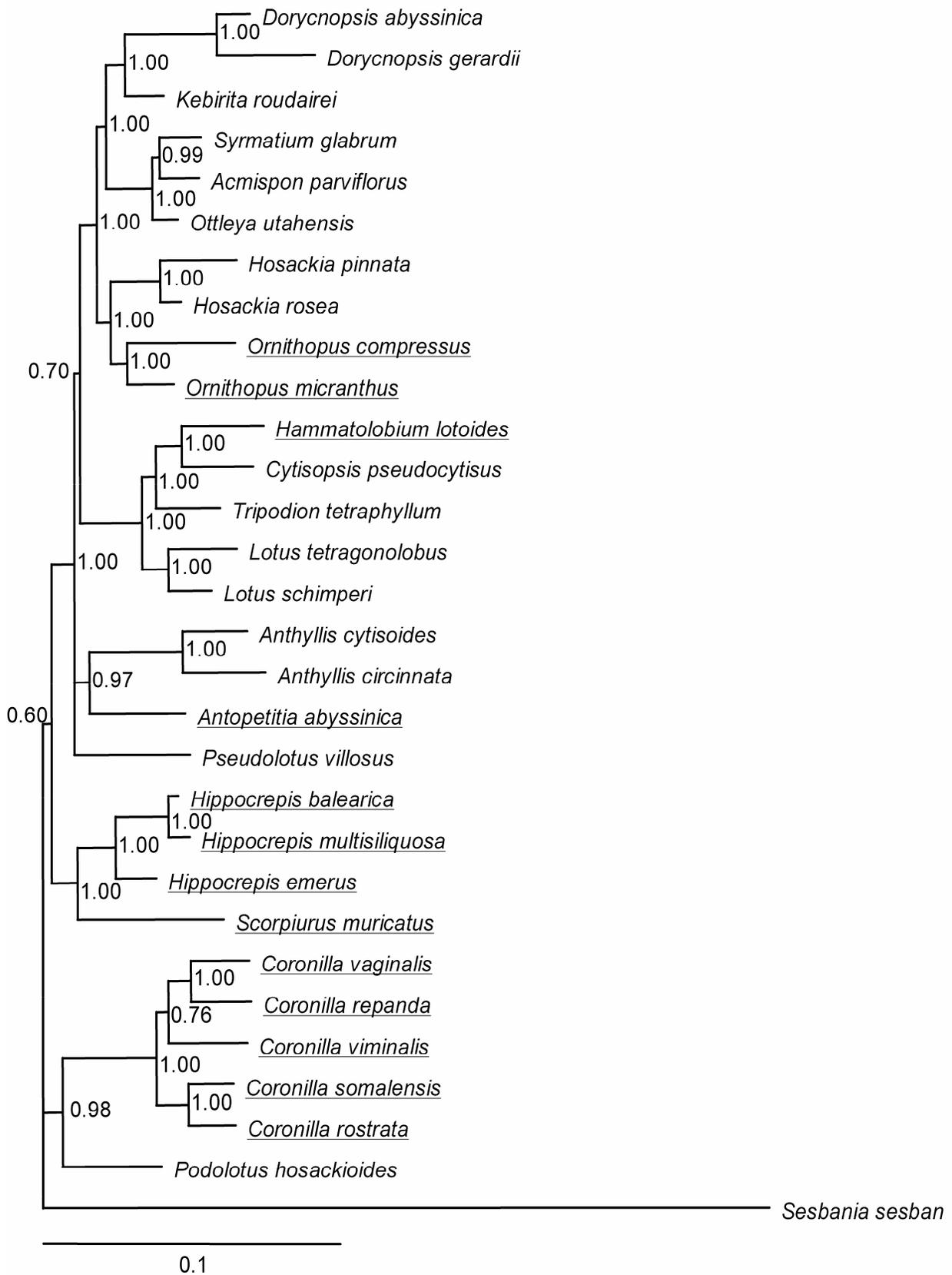
Fig. 3. Tree inferred from Bayesian analysis of combined nrITS plus plastid data. Posterior probabilities of branches are indicated. Scale = 0.1 substitution/site. The tree is rooted using *Sesbania* as the outgroup. Names of taxa with lomentaceous fruits are underlined.
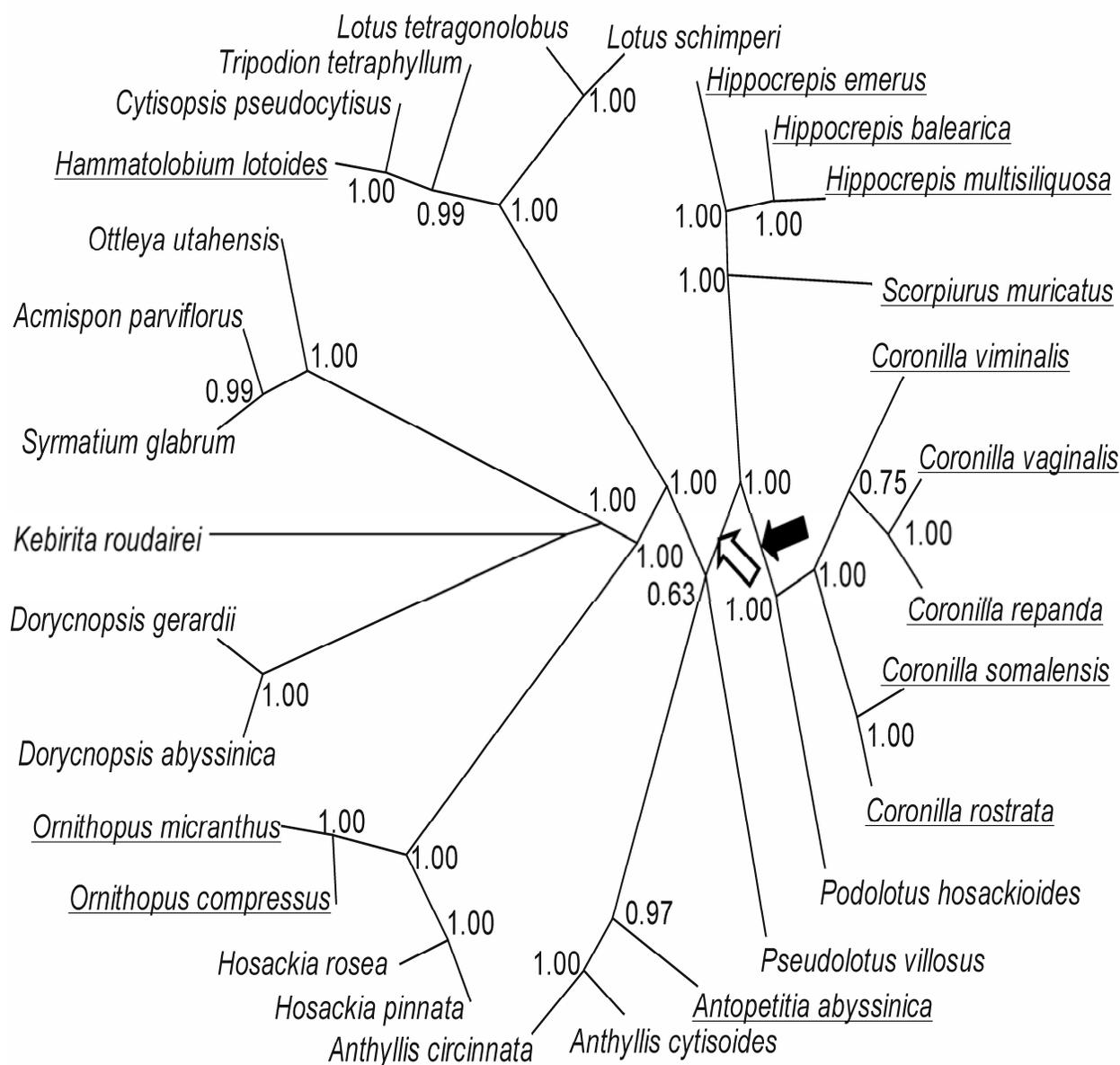
Fig. 4. Unrooted tree inferred from Bayesian analysis of combined nrITS plus plastid data. Posterior probabilities of branches are indicated. Names of taxa with lomentaceous fruits are underlined. Solid arrow = position of rooting through *Sesbania*. Open arrow = position of an alternative rooting that agrees with distribution of some key morphological characters (see the text).

Both combined nrITS/morphology analysis (Sokoloff *et al.*, 2007) and cladistic analyses of morphological characters (Sokoloff, 2003b, 2006) suggest occurrence of a clade comprising *Coronilla, Hippocrepis* and *Scorpiurus*. In all of these analyses *Podolotus* was not placed into this clade. Rather, it occurred in an unresolved position close to the base of the tree of Loteae. *Coronilla, Hippocrepis* and *Scorpiurus* share several morphological features, including lomentaceous fruits. The fruit character is the most conspicuous, but it is homoplastic in Loteae (see below). The most unique common feature of *Coronilla, Hippocrepis* and *Scorpiurus* found by Sokoloff *et al.*, (2007) was the mode of calyx initiation with two adaxial sepals initiating together as a joint primordium. Sokoloff *et al.*, (2007) did not observe this mode of calyx initiation in any other member of Loteae. However, they failed to study this character in some members of the tribe, including *Podolotus*, due to the absence of appropriate fixed material. The

mature calyx of *Podolotus* is morphologically similar to the calyx of *Coronilla* (Polhill, 1981). Therefore we cannot rule out the possibility that *Podolotus* also has joint initiation of adaxial sepals.

When we consider the evidence from morphology together with recent molecular phylogenetic data, it appears to be most logical to estimate a basal split of Loteae between the ((*Coronilla* + *Podolotus*)(*Hippocrepis* + *Scorpiurus*)) clade (the /Coronilla clade) and the rest of Loteae (the /core Loteae clade). If we accept this rooting, several interesting tendencies in morphological evolution can be traced (data from Sokoloff *et al.*, 2007). Two unusual morphological features are recorded only from the /Coronilla clade, namely, the joint initiation of adaxial sepals and flower buds bent backwards. Stipules reduced to dark glands and occurrence of a sterile bract on peduncles of partial inflorescences appear to be synapomorphies of the /core Loteae clade (with a few scattered reversions in species-poor lineages). Both character states are absent from the /Coronilla clade, and the occurrence of a sterile bract appears to be a unique feature of Loteae, at least among higher papilionoid tribes (Sokoloff *et al.*, 2007).

Homoplastic evolution of lomentaceous fruits in Loteae: If *Podolotus* is sister to *Coronilla*, important conclusions can be made on fruit evolution in Loteae. The following genera of Loteae have lomentaceous fruits: *Hippocrepis, Scorpiurus, Coronilla, Hammatolobium, Antopetitia, Ornithopus*. This is clearly a derived character state in Loteae (e.g., Allan *et al.*, 2003; Degtjareva *et al.*, 2003). The ancestral fruit type of Loteae would be the fruit that dehisces by two valves, as in *Lotus*, *Podolotus*, *Hosackia* and other genera that were assembled in the Polhill's expanded *Lotus* s.l. The primitiveness of dehiscent fruits is supported by the fact that this kind of fruits is present in and typical to outgroups of Loteae (Sesbanieae and Robinieae). Molecular phylogenetic data show that *Ornithopus* is sister to *Hosackia*, *Antopetitia* is sister to *Anthyllis*, *Hammatolobium* is sister to *Cytisposis*, *Coronilla* is sister to *Podolotus*. In all these pairs, one of two genera has lomentaceous fruits while another one has non-lomentaceous (either dehiscent or indehiscent) fruit. The only sister pair of genera both having lomentaceous fruits is *Hippocrepis* and *Scorpiurus*. In total, phylogenetic data suggest occurrence of at least five independent origins of lomentaceous fruits in Loteae. It is most interesting that the placement of *Podolotus* suggests independent origins of lomentaceous fruits in *Coronilla* and *Hippocrepis*. These two genera were always considered to be closely related, and the fruit morphology was usually seen as the key feature to unite them. Lassen (1989) however suggested that lomentaceous fruits possibly evolved more than once in course of evolution of this group. Based on comparative morphology, he suggested that *Coronilla, Hippocrepis* and *Scorpiurus* are possibly not closer to each other than to *Podolotus* and *Hosackia*. Lassen (1989) highlights the fact that the moniliform fruit of *Podolotus* is very peculiar; although it is dehiscent, the innerhyaline stratum at maturity separates from the valves and surrounds the individual seeds like plastic bags. Therefore, the fruit of *Podolotus* can be seen as a step towards lomentaceous fruits. Our study largely supports the conclusions of Lassen, though we did not find evidence for close relationships between *Podolotus* and *Hosackia*.

We agree with Lassen (1989) in the hypothesis that the structure of inner pericarp layer in *Podolotus* can be seen as a precondition for the origin of lomentaceous fruits. However, this feature is not restricted to *Podolotus*. Rather, presence of parenhymatous septa between adjacent seeds is characteristic to nearly all members of Loteae with dehiscent fruits. Separation of the inner parenchymatous layer of pericarp in mature fruits

can be seen not only in *Podolotus* (for example, it is quite prominent in *Lotus tetragonolobus*). It is possible that the origin of parenchymatous septa (which was likely a synapomorphy of Loteae plus *Sesbania*) was a precondition to multiple homoplastic origins of lomentaceous fruits in Loteae. It is important that this fruit type is not documented in the close tribe Robinieae where the septa are also absent (see Lavin & Sousa, 1995).

Acknowledgement

Appendix

Alphabetical list of voucher specimens and Genbank accession numbers for species not included in the analyses of Degtjareva *et al.*, (submitted). Genbank accession numbers are in the following order: ITS, *pet*B-*pet*D, *psb*A-*trn*H, *rps*16. Sequences of plastid regions were generated for this study while sequences of the ITS region are taken from our previous studies (Degtjareva *et al.*, 2003; Degtjareva *et al.*, 2006a; Sokoloff *et al.*, 2007).

Coronilla repanda (Poir.) Guss.: Spain, Huelva, 29.v.1976, *Cabezudo et al., s.n.* (MHA), DQ641994, HQ199614, HQ199623, HQ199630.

Coronilla rostrata Boiss. & Sprun. (=*Securigera parviflora* [Desv.] Lassen): Turkey, Antalya, 6.iv.2005, *Majorov s.n.* (MW), DQ641995, HQ199612, HQ199621, HQ199632.

Coronilla somalensis Thulin (=*Securigera somalensis* [Thulin] Lassen): Somalia; *Thulin et al., 8918* (MW), DQ641997, HQ199611, HQ199620, HQ199629.

Coronilla vaginalis Lam.: Germany, 17.vi.2001, *Degtjareva s.n.* (MW), DQ641998, HQ199613, HQ199622, HQ199631.

Hippocrepis balearica Jacq.: Spain, Balearic Islands, *Orell 14184* (MHA), DQ642000, HQ199607, HQ199616, HQ199625.

Hippocrepis multisiliquosa L.: Cyprus, *Seregin & Sokoloff A-292* (MW), DQ642003, HQ199608, HQ199617, HQ199626.

Hosackia rosea Eastw. (=*Lotus aboriginus* Jeps.): California, *Akulova-Barlow 4* (MW), DQ642005, HQ199609, HQ199618, HQ199627.

Lotus schimperi Steud. ex Boiss.: Oman, 11.iii.1994, *McLeish 3458* (E), DQ166218, HQ199606, HQ199615, HQ199624.

Ornithopus micranthus (Benth.) Arechav.: Argentina, Prov. Entre Rios, 14.xi.1988, *Bacigalupo et al., 545* (SI), AY325277, HQ199610, HQ199619, HQ199628.

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