

Taxonomic and phylogenetic relationships between Old World and New World members of the tribe Loteae (Leguminosae): new insights from molecular and morphological data, with special emphasis on *Ornithopus*

Galina V. Degtjareva, Carmen M. Valiejo-Roman, Tatiana E. Kramina,
Evgeny M. Mironov, Tahir H. Samigullin & Dmitry D. Sokoloff

Summary: The tribe Loteae s.l. (incl. Coronilleae) comprises about 275 species distributed in Eurasia, Africa, Australia, North and South America. 47 species of Loteae are endemic to the New World, while all others are restricted to the Old World. Main centres of diversity are Mediterranean region and California. The genus *Ornithopus* has an unusual disjunctive distribution, with one species (*O. micranthus*) in subtropical regions of Eastern South America and five species in Europe, Mediterranean region, Macaronesia and the Caucasus. We have produced sequences of nuclear ribosomal DNA ITS1-2 region of six Loteae species, and have studied fruit anatomy, pollen morphology and other morphological characters in several members of the tribe, with special emphasis on *Ornithopus*. Our data confirm that the genus *Ornithopus*, in its traditional circumscription, represents a natural, monophyletic group. The ITS data strongly suggest sister group relationships between *O. micranthus* and Old World species of *Ornithopus*. We have confirmed results by ALLAN & PORTER (2000) and ALLAN et al. (2003) that *Ornithopus* tend to group with North American genus *Hosackia* on trees inferred from analyses of ITS sequences. There is little morphological support for such a grouping. For example, significant similarities between *Ornithopus micranthus* and *Hosackia* are found in pollen morphology. Our data confirmed that *Ornithopus* is the only genus of Loteae with native distribution on both sides of the Atlantic Ocean. It is highly unlikely that *Ornithopus* was introduced by man and then naturalised in South America.

Zusammenfassung: Die Tribus Loteae s.l. (incl. Coronilleae) umfasst 275 Arten, deren Verbreitungsgebiet sich über Eurasien, Afrika, Australien und Nord- und Südamerika erstreckt. 47 Arten sind Endemiten der Neuen Welt, alle anderen sind altweltlich. Haupt-Diversitätszentren sind die mediterrane Region und Kalifornien. Die Gattung *Ornithopus* zeigt eine ungewöhnliche Disjunktion: eine Art, nämlich *O. micranthus*, ist in den subtropischen Regionen des östlichen Südamerika beheimatet, fünf weitere Arten kommen jedoch in Europa, der mediterranen Region, in Makaronesien und im Kaukasusgebiet vor. Wir erstellten DNA Sequenzdaten der ITS1-2 Region von sechs ausgewählten Arten und untersuchten Fruchtanatomie, Pollenmorphologie sowie zusätzliche morphologische Merkmale an weiteren Vertretern der Tribus. Besonderes Augenmerk legten wir auf die Gattung *Ornithopus*. Unsere Daten bestätigen die Gattung *Ornithopus* in ihrer gängigen Abgrenzung als natürliche, monophyletische Gruppe. ITS-Daten legen eine Beziehung von *O. micranthus* als Schwestergruppe zu den altweltlichen *Ornithopus*-Arten nahe. ALLAN & PORTER (2000) und ALLAN et al. (2003) zeigten, dass *Ornithopus* dazu tendiert, in auf ITS Sequenzdaten basierenden Stammbäumen mit der Nordamerikanischen Gattung *Hosackia* zu gruppieren. Diese Ergebnisse konnten wir bestätigen. Morphologische Unterstützung für eine derartige Gruppierung gibt es jedoch nur wenig. Als Beispiel seien hier signifikante Ähnlichkeiten zwischen *Ornithopus micranthus* und *Hosackia* im Bereich der Pollenmorphologie genannt. Unsere Daten bestätigten, dass *Ornithopus* die einzige Gattung der Tribus Loteae ist, die eine natürliche Verbreitung beiderseits des Atlantischen Ozeans aufweist. Es ist höchst unwahrscheinlich, dass *Ornithopus* durch Menschen in Südamerika eingeschleppt wurde.

Keywords: Leguminosae, Loteae, *Ornithopus*, biogeography, molecular systematics, nuclear ribosomal ITS sequences, morphology, fruit anatomy, palynology

The tribe Loteae s.l. (incl. Coronilleae) is a diverse group of mainly temperate legumes, which comprises about 275 species and ten (cf. POLHILL 1981) to twenty one (SOKOLOFF 1998) genera distributed in Eurasia, Africa, Australia, North and South America. 47 species of

Loteae are endemic to the New World, while all others are restricted to the Old World. No species of the tribe has a native distribution in both Old and New World. In the Old World, the tribe has a major centre of diversity in the Mediterranean and adjacent regions. In the New World, the centre of diversity is California and adjacent regions of USA and Mexico. Only two native species of Loteae occur in South America, both being endemic to this continent.

Taxonomic relationships between North American and Mediterranean members of *Loteae* have been extensively discussed in literature. The majority of authors (e.g. GREENE 1890; OTTLEY 1923, 1944; ISELY 1981, 1993; POLHILL 1981, 1994; BARNEBY 1989; KIRKBRIDE 1999; KARTESZ & MEACHAM 1999) included all North American species into the largest genus of the tribe, *Lotus* L., which has also more than hundred Old World species. Another viewpoint was to accept a separate genus, *Hosackia* Dougl. ex Benth., comprising all species of North American Loteae, and no species from the Old World (e.g. Bentham, 1829; Taubert, 1890; Abrams, 1944; Gillett, 1959). Some authors suggested a need of splitting of *Hosackia* and accepted two or several endemic New World genera (e.g. RYDBERG 1906, 1913; BRAND 1898; SOKOLOFF; 1998–2003a). SOKOLOFF, for example, accepted four genera, *Hosackia* s.str., *Ottleya* D.D. Sokoloff, *Acmispon* Raf. s.str., and *Syrmatium* Vogel. Bentham (1837) included majority of the North American species into *Hosackia* and some species into *Lotus*. LASSEN (1986, 1989, 1998) accepted three endemic New World genera (*Hosackia*, *Syrmatium*, and *Anisolotus* Bernh.) plus a genus *Acmispon* s.l. with one species in Africa (*L. roudairei* Bonnet) and more than a dozen in North America. All above mentioned viewpoints were based on data of comparative morphology.

Recent molecular phylogenetic studies (ALLAN & PORTER 2000; ALLAN 2001; ALLAN et al. 2003), based on sequences of ITS1-2 region of nuclear ribosomal DNA added much of knowledge on relationships between Old World and North American species of Loteae.

ALLAN & PORTER (2000) analysed ITS sequences of 38 Loteae species and revealed that North American taxa are not closely related to Old World species of *Lotus*. Old World species of *Lotus* form a clade sister to Old World genus *Dorycnium*. American species of *Lotus* s.l. included by SOKOLOFF (1998–2003a) in the genera *Acmispon*, *Ottleya* and *Syrmatium* form a strongly supported clade. However, this clade includes also an Old World species *Coronilla valentina* L. (ALLAN & PORTER 2000). Another strongly supported clade comprises three species of North American genus *Hosackia* (according to the classification by SOKOLOFF) plus an Old World species *Coronilla scorpioides* (L.) W.D.J. Koch, which is placed as a sister to *Hosackia crassifolia* Benth. (= *Lotus crassifolius* (Benth.) Greene). The *Hosackia-Coronilla scorpioides*-clade is placed as a sister to Old World species of *Ornithopus*, but the bootstrap support of this grouping is low (ALLAN & PORTER 2000). Although morphological similarity between “peripheral” groups of *Lotus* s.l. and *Coronilla* was mentioned by POLHILL (1981), LASSEN (1989) and SOKOLOFF (1998), surprisingly the two species of Old World *Coronilla* s.str. included in the molecular phylogenetic analysis are nested with different American species (ALLAN & PORTER 2000). The genus *Coronilla* was traditionally allied with another Old World genus, *Securigera* DC. Some authors treated *Securigera* as a synonym of *Coronilla* (SCHMIDT 1979; POLHILL 1981; SOKOLOFF 2003b). However, the ITS data suggested that *Securigera varia* (L.) Lassen (= *Coronilla varia* L.) is close to *Anthyllis* and *Hymenocarpus* rather than to *Coronilla* s.str. (ALLAN & PORTER 2000).

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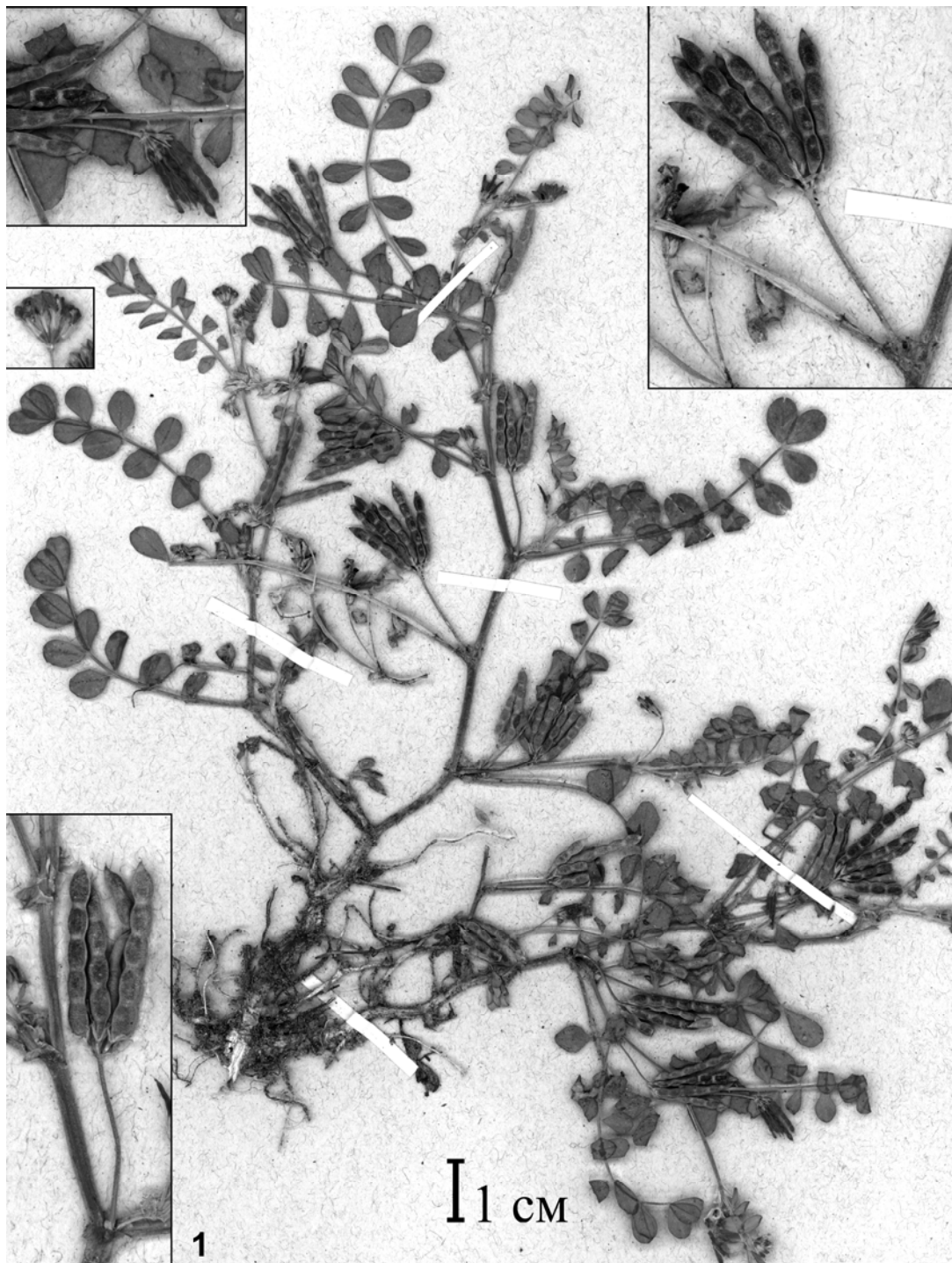


Figure 1: Herbarium specimen of the South American species *Ornithopus micranthus* (Benth.) Arechav.: Brazil, Rio Grande do Sul, 1.XI.1901, G.O.A. Malme 130 (S).

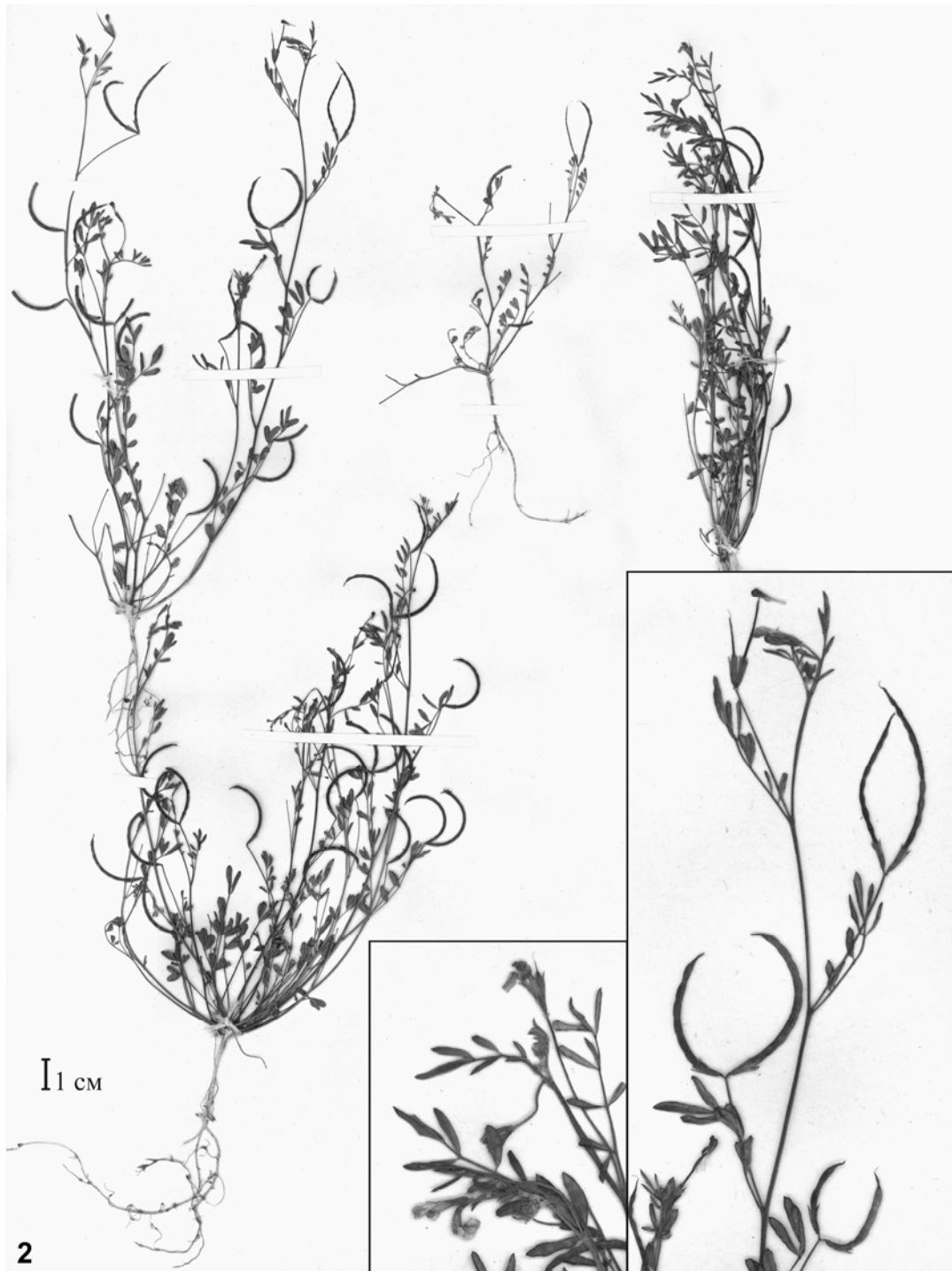


Figure 2: Herbarium specimen of the Old World species *Ornithopus pinnatus* (Mill.) Druce: Portugal, 20.VII.1964, *Matos s.n.* (MHA). Among the Old World species, *O. pinnatus* is morphologically the most similar to *O. micranthus*.

ALLAN et al. (2003) analysed ITS sequences of 80 Loteae species. Sequences of *Coronilla scorpioides* and *C. valentina* were excluded from the analysis. Instead, a sequence of another *Coronilla* species, *C. minima* L. was used. In this analysis, *C. minima* is nested with three species of *Securigera* (ALLAN et al. 2003). No close relationship between *Coronilla* and New World Loteae was revealed (ALLAN et al. 2003). In the analysis by ALLAN et al. (2003), New World Loteae species of *Lotus* s.l. form two well supported clades, each exclusively consisting of American representatives. The first clade includes species of the segregate genus *Hosackia* s.str., while the second clade comprises species of *Ottleya*, *Syrmatium* and *Acmispon* (in the limits proposed by SOKOLOFF 1998–2003a). As in the earlier analysis (ALLAN & PORTER 2000), *Hosackia* is found to be associated with Old World species of *Ornithopus*, but this grouping has low bootstrap support. The *Hosackia-Ornithopus*-clade is found to be a sister group to *Ottleya-Syrmatium-Acmispon*-clade, but also with low bootstrap support (ALLAN et al. 2003).

The idea that *Hosackia*, *Ornithopus*, *Ottleya*, *Syrmatium*, and *Acmispon* are related to each other was new and interesting from biogeographically viewpoint (ALLAN et al. 2003). Indeed, this group of genera comprises all New World species of the tribe Loteae. All species of *Hosackia*, *Ottleya*, *Syrmatium*, and *Acmispon* are restricted to North America, with exception of *Acmispon subpinnatus* (Lag.) D.D. Sokoloff, which is endemic to Chile (SOKOLOFF 2000a, 2003a). The genus *Ornithopus* has six species classified into three sections (SOKOLOFF 2003a). Section *Ornithopus* has four Old World species. Section *Coronilloides* Rouy is monotypic and includes an Old World species, *O. pinnatus* (Mill.) Druce (fig. 2). Finally, section *Austropus* D.D. Sokoloff includes a South American species, *O. micranthus* (Benth.) Arechav. (fig. 1).

The disjunctive distribution of *Ornithopus* is unusual because in the Old World, all species are restricted to the Northern Hemisphere (mainly Mediterranean region and Europe), while in the New World *O. micranthus* is distributed in South America (S. Brazil, Uruguay, Argentina). As far as we know, no detailed comparative study of Old World and New World species of *Ornithopus* has been carried out so far. Only Old World species of section *Ornithopus* were included in the molecular phylogenetic analyses (ALLAN & PORTER 2000; ALLAN et al. 2003). Fruit anatomy was extensively studied in Old World species of *Ornithopus* (e.g. FUCSKÓ 1914; MOELLER & GRIEBEL 1928; FAHN & ZOHARY 1955; KANIEWSKI & WAŻYŃSKA 1968), but not in the South American species *Ornithopus micranthus*. Pollen morphology was studied in all species of *Ornithopus* (e.g. PIRE 1974; ASINA ASER 1984; PRADOS et al. 1985; CROMPTON & GRANT 1993; DÍEZ & FERGUSON 1996) but was not critically compared to pollen morphology of *Hosackia* and other American members of Loteae.

In this paper, we are intending to discuss the following questions:

- 1) Does the genus *Ornithopus*, as traditionally circumscribed, represent a natural, monophyletic group? Or, on the contrary, the New World species *O. micranthus* is not closely related to Old World species of the genus. The question arises from recent morphological (DÍEZ & FERGUSON 1994, 1996; SOKOLOFF 1998, 1999, 2000a, 2000b) and molecular (ALLAN & PORTER 2000; ALLAN et al. 2003) studies which support an exclusion of American species from the other genus of the tribe, *Lotus* s.l.
- 2) If the genus *Ornithopus* is natural, is it possible to explain its unusual disjunctive distribution by man-influenced introduction event from Europe to South America? The question arises from a number of European species of the tribe Loteae which are naturalized in temperate

Eastern South America, while only *O. micranthus* is traditionally treated as a natural one (e.g. BURKART 1987; ARAMBARRI 1997; GÓMEZ SOSA 1997).

- 3) Does any additional evidence exist in support of grouping between all genera of Loteae containing American species and particularly between *Ornithopus* and *Hosackia*? That question is important because such clades were only weakly supported in the published molecular phylogenetic study (ALLAN et al. 2003). The genus *Ornithopus* has never been found as closely related to North American *Loteae* on the basis of morphological studies.
- 4) How comparable are molecular and morphological data in relationships between various genera of Loteae?

To discuss these questions, we have produced new sequences of nrITS1-2 region of six Loteae species, including *Ornithopus micranthus*, and studied fruit anatomy, pollen morphology and other morphological characters in several members of the tribe, with special emphasis on *Ornithopus*.

Materials & Methods

Nomenclature: There is no universally accepted generic system of the tribe Loteae. In this paper, we are using nomenclature published by SOKOLOFF (2003a).

Taxon sampling for molecular analysis: New DNA sequences were produced for six Loteae species (*Ornithopus micranthus*, *O. pinnatus*, *Hammatolobium lotoides*, *Cytisopsis pseudocytisus*, *Coronilla scorpioides*, *C. coronata*). Two species of *Ornithopus* represent two monotypic sections of the genus. Adding GenBank sequences published by ALLAN & PORTER (2000) and ALLAN et al. (2003), five of six species of the genus we can include in the analysis, representing all three sections recognised by SOKOLOFF (2003a). The genus *Hammatolobium* (two species) was sometimes placed in the system near *Ornithopus* (e.g. TAUBERT 1894; SOKOLOFF 1998). However, the ITS data strongly suggest that *Hammatolobium kremerianum* (Coss.) C. Muell. is closely related to Old World *Lotus* (ALLAN et al. 2003). Now we have analysed the second species of *Hammatolobium*. The genus *Cytisopsis* was identified as the basalmost lineage of Loteae in a cladistic analysis based on morphological characters (SOKOLOFF 2003a). ITS sequences of *Cytisopsis* have not been included in the previous analyses. Two new sequences of *Coronilla* were produced because the genus, contrary to the traditional viewpoint, was found to be polyphyletic in the analysis by ALLAN & PORTER (2000).

The six new sequences were analysed together with 43 GenBank sequences, which were first published by ALLAN & PORTER (2000), ALLAN et al. (2003) and LAVIN et al. (2001). These 43 species represent all genera of Loteae, for which ITS sequences are available from the GenBank. The ITS sequences for three monotypic genera (*Antopetitia* A.Rich., *Podolotus* Benth., and *Pseudolotus* Rech. f.) were not obtainable. Members of the tribe *Robinieae* (*Sesbania*, *Robinia*, and *Hebestigma*) are used as an outgroup.

GenBank accession numbers for complete ITS1 and ITS2 nucleotide sequences of all studied species in addition to their source are presented in Table 1. The voucher information for newly produced sequences is given in the Table 2.

Molecular analysis: The sequence data were obtained through direct sequencing of double-stranded templates on ABI 310 automatic sequencer. The templates were derived from the PCR

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Table 1. GenBank accession numbers of sequences used.

Species (names in brackets are used in original publications)	GenBank number	First publication of the sequence
<i>Acmispon americanus</i> (Nutt.) Rydb. [= <i>Lotus unifoliolatus</i> (Hook.) Benth.]	AF450183	ALLAN et al. (2003)
<i>Acmispon maritimus</i> (Nutt.) D.D. Sokoloff [= <i>Lotus salsuginosus</i> Greene]	AF218512	ALLAN & PORTER (2000)
<i>Acmispon subpinnatus</i> (Lag.) D.D. Sokoloff [= <i>Lotus subpinnatus</i> Lag.]	AF450207	ALLAN et al. (2003)
<i>Anthyllis circinnata</i> (L.) D.D. Sokoloff [= <i>Hymenocarpus circinnatus</i> (L.) Savi]	AF218504	ALLAN & PORTER (2000)
<i>Anthyllis lotoides</i> L.	AF450216	ALLAN et al. (2003)
<i>Anthyllis onobrychioides</i> Cav.	AF450210	ALLAN et al. (2003)
<i>Anthyllis terniflora</i> (Lag.) Pau	AF450212	ALLAN et al. (2003)
<i>Anthyllis vulneraria</i> L.	AF218499	ALLAN & PORTER (2000)
<i>Coronilla coronata</i> L.	AY325281	This paper
<i>Coronilla minima</i> L. s.l.	AF450232	ALLAN et al. (2003)
<i>Coronilla orientalis</i> Mill. [= <i>Securigera orientalis</i> (Mill.) Lassen]	AF450230	ALLAN et al. (2003)
<i>Coronilla scorpioides</i> (L.) W.D.J. Koch	AY325280	This paper
<i>Coronilla varia</i> L. [= <i>Securigera varia</i> (L.) Lassen]	AF218537	ALLAN & PORTER (2000)
<i>Cytisopsis pseudocytisus</i> (Boiss.) Fertig	AY325282	This paper
<i>Dorycnopsis abyssinica</i> (A. Rich.) V.N. Tikhom. & D.D. Sokoloff [= <i>Vermifrux abyssinica</i> (A. Rich.) J.B. Gillett]	AF450235	ALLAN et al. (2003)
<i>Hammatolobium kremerianum</i> (Coss.) C. Muell.	AF450233	ALLAN et al. (2003)
<i>Hammatolobium lotoides</i> Fenzl	AY325279	This paper
<i>Hebestigna cubense</i> (Kunth) Urban	AF450157	ALLAN et al. (2003)
<i>Hippocrepis emerus</i> (L.) Lassen	AF218531	ALLAN & PORTER (2000)
<i>Hippocrepis unisiliquosa</i> L.	AF450238	ALLAN et al. (2003)
<i>Hosackia crassifolia</i> Benth. [= <i>Lotus crassifolius</i> (Benth.) Greene]	AF218523	ALLAN & PORTER (2000)
<i>Hosackia gracilis</i> Benth. [= <i>Lotus formosissimus</i> Greene]	AF218524	ALLAN & PORTER (2000)
<i>Hosackia oblongifolia</i> Benth. [= <i>Lotus oblongifolius</i> (Benth.) Greene]	AF218525	ALLAN & PORTER (2000)
<i>Kebirita roudairei</i> (Bonnet) Kramina & D.D. Sokoloff [= <i>Lotus roudairei</i> Bonnet]	AF450200	ALLAN et al. (2003)
<i>Lotus angustissimus</i> L.	AF450185	ALLAN et al. (2003)
<i>Lotus australis</i> Andrews	AF450187	ALLAN et al. (2003)
<i>Lotus campylocladus</i> Webb & Berth.	AF450196	ALLAN et al. (2003)
<i>Lotus conimbricensis</i> Brot.	AF450186	ALLAN et al. (2003)
<i>Lotus corniculatus</i> L.	AF218527	ALLAN & PORTER (2000)
<i>Lotus dorycnium</i> L. s.l. [= <i>Dorycnium herbaceum</i> Vill.]	AF218501	ALLAN & PORTER (2000)
<i>Lotus edulis</i> L.	AF450184	ALLAN et al. (2003)
<i>Lotus ornithopodioides</i> L.	AF450205	ALLAN et al. (2003)
<i>Lotus rectus</i> L. [= <i>Dorycnium rectum</i> (L.) Ser.]	AF218503	ALLAN & PORTER (2000)
<i>Lotus tetragonolobus</i> L. [= <i>Tetragonolobus purpureus</i> Moench]	AF218506	ALLAN & PORTER (2000)
<i>Ornithopus compressus</i> L.	AF218533	ALLAN & PORTER (2000)
<i>Ornithopus micranthus</i> (Benth.) Arechav.	AY325277	This paper
<i>Ornithopus perpusillus</i> L.	AF450226	ALLAN et al. (2003)
<i>Ornithopus pinnatus</i> (Mill.) Druce	AY325278	This paper
<i>Ornithopus sativus</i> Brot. s.l. [= <i>O. isthmocarpus</i> Cosson]	AF218534	ALLAN & PORTER (2000)
<i>Ottleya oroboides</i> (Kunth) D.D. Sokoloff [= <i>Lotus oroboides</i> (Kunth) Ottley]	AF218510	ALLAN & PORTER (2000)
<i>Ottleya rigida</i> (Benth.) D.D. Sokoloff [= <i>Lotus rigidus</i> (Benth.) Greene]	AF218511	ALLAN & PORTER (2000)
<i>Ottleya strigosa</i> (Nutt.) D.D. Sokoloff [= <i>Lotus strigosus</i> (Nutt.) Greene]	AF218513	ALLAN & PORTER (2000)
<i>Robinia pseudoacacia</i> L.	AF218538	ALLAN & PORTER (2000)
<i>Scorpiurus vermiculatus</i> L.	AF218536	ALLAN & PORTER (2000)
<i>Sesbania vesicaria</i> (Jacq.) Elliott	AF398761	LAVIN et al. (2001)
<i>Syrmatium glabrum</i> Vogel [= <i>Lotus scoparius</i> (Nutt.) Ottley]	AF218521	ALLAN & PORTER (2000)
<i>Syrmatium prostratum</i> (Nutt.) Greene [= <i>Lotus nuttallianus</i> Greene]	AF218520	ALLAN & PORTER (2000)
<i>Syrmatium tomentosum</i> (Hook. & Arn.) Vogel [= <i>Lotus heermannii</i> (Durand & Hilg.) Greene]	AF218519	ALLAN & PORTER (2000)
<i>Tripodion tetraphyllum</i> (L.) Fourr. [= <i>Anthyllis tetraphylla</i> L.]	AF218498	ALLAN & PORTER (2000)

Table 2. Information on previously unpublished sequences. For GenBank accession numbers see Table 1.

Species	Voucher
<i>Coronilla coronata</i> L.	Ukraine, Crimea, neighbourhoods of Bakhchisarai, 2 km N of the village Mashino, 12 June 1996, <i>S. Majorov & V. Skvortsov s.n.</i> (MW).
<i>Coronilla scorpioides</i> (L.) W.D.J. Koch	Ukraine, Crimea, neighbourhoods of Bakhchisarai, 3 km N of the village Mashino, 10 June 1996, <i>S. Majorov & V. Skvortsov s.n.</i> (MW).
<i>Hammatolobium lotoides</i> Fenzl	Greece, Pelop., Laconia, M. Taygetos, in <i>Pinus nigra</i> forest, alt. 1500 m, 8 June 1996, <i>E. Emanuelsson 1932</i> (S).
<i>Ornithopus micranthus</i> (Benth.) Arechav.	Argentina, Prov. Entre Rios. Dpto. Gualaguaychu, balneario Ñandubaysal, 14. Nov. 1988, <i>M.N. Bacigalupo et al. 545</i> (SI).
<i>Ornithopus pinnatus</i> (Mill.) Druce	New Zealand, North Island, sandy beach in the North of Auckland, Pacific coast, Nov. 2001, leg. <i>Z.V. Akulova-Barlow s.n.</i> det. <i>D.D. Sokoloff</i> (MW) [an alien plant in New Zealand; native to Europe, Mediterranean region, and Macaronesia].
<i>Cytisopsis pseudocytisus</i> (Boiss.) Fertig	Turkey, C1, Mugla, Datca, Knidos, 29–31 May 1995, <i>A.P. Khokhryakov & M.T. Mazurenko s.n.</i> (MHA)

procedure. Both spacer regions were sequenced in their entirety on both strands. Details of the DNA extractions, the PCR (polymerase chain reaction) amplifications (including primer locations and characteristics), and the DNA purification and sequencing strategies are the same as in VALIEJO-ROMAN et al. (2002).

Only the ITS1 and ITS2 regions were included in the analysis. The DNA sequences were aligned initially using CLUSTAL W version 1.75 (THOMPSON et al. 1994) and corrected manually using the SED editor of the VOSTORG phylogenetic analysis package (ZHARKIKH et al. 1990). The alignment is available from the authors on request.

The resulting data matrix was analysed by programs MrBayes (HUELSENBECK & RONQUIST 2001) and PAUP* version 4.0b8 (SWOFFORD 2000).

Parsimony analysis involved a heuristic search conducted with PAUP* using TBR (tree-bisection-reconnection) branch swapping, options mulpars, steepest descent, collapse, and acctran selected, with character states specified as unordered and equally weighted. All gaps were treated as missing data. Bootstrap analyses (FELSENSTEIN 1985) were performed to assess the degree of support for particular branches on the tree. Bootstrap values were calculated from 100 replicate analyses with random addition sequence of taxa. 1000 most parsimonious trees from each replicate were saved.

Distance trees were calculated using the neighbour-joining method (SAITOU & NEI 1987) as it is implemented in PAUP*. The GTR model of substitutions was used. Rates across sites were assumed to follow gamma distribution. 1000 bootstrap resamplings were performed, insertions and deletions were not taken into account.

Bayesian inference of phylogeny was explored using the MrBayes program. The GTR model of substitutions with presence of invariant sites and 4 Gamma rate categories was used. 1100000 generations were performed, trees from first 100000 generations were discarded.

Morphological data: General morphology was studied using herbarium specimens deposited at Herbaria A, BM, CAS, DS, GH, K, LE, LL, MHA, MW, NSW, NY, P, S, TEX, UPS, US. All species of the genus *Ornithopus* and representatives of all Loteae genera are studied.

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Pollen morphology was studied by means of a scanning electron microscope HITACHI S-405A. The material was sputtered with Pt/Pd. The following specimens were studied:

- Ornithopus micranthus* (Benth.) Arechav.: Uruguay, Nov. 1927, *Herter 974 (82341)* (S).
Ornithopus perpusillus L.: Denmark, 24 June 1965, *B. Øllgaard 189* (MW).
Ornithopus pinnatus (Mill.) Druce: Portugal, 20 July 1964, *Matos s.n.* (MHA).
Ornithopus sativus L. 70% alcohol-fixed material collected in 2002 by T.A. Tsutsupa at the Botanic Garden of Orel State University, Orel, Russia.
Lotus australis Andrews: Australia (NSW).
Lotus corniculatus L. (incl. *L. dvinensis* Min. et Ulle): Russia, Arkhangelsk prov., 1992, *T.E. Kramina s.n.* (MW).

For study of fruit anatomy, dry fruits were softened for two days with a warm ($t = 56\text{ }^{\circ}\text{C}$) mixture of glycerine – ethyl alcohol – water (1:1:1). Longitudinal and cross sections of the pericarp were prepared by use of a sliding microtome. The sections were treated with an alcoholic solution of phloroglucine, then with hydrochloric acid, and mounted in glycerine. Besides, some fruits were treated with boiling water for 20 min. to extract a sclerenchymatous zone (hard layer) of the pericarp. The isolated hard layer was studied *in toto* with a stereo microscope. The following specimens were studied:

- Ornithopus micranthus* (Benth.) Arechav.: Argentina, 14. Nov. 1988, *M.N. Bacigalupo et al. 545* (SI).
Ornithopus pinnatus (Mill.) Druce: Algeria, 25 May 1939, *R. Maire s.n.* (K).
Ornithopus sativus Brot.: several fruit samples from the Carpological collection of Higher Plants Department, Moscow University.
Ornithopus uncinatus Maire et G. Sam.: Morocco, *G. Samuelsson 7237* (K).
Antopetitia abyssinica A. Rich.: Cameroon, 14. Nov. 1974, *B. Satabil 95* (P).

Most parsimonious reconstructions of morphological character state evolution were generated using program WINCLADA version 1.00.08 (NIXON 2002). The reconstructions are based on a tree inferred from Bayesian analysis of the molecular data set. The tree inferred from Bayesian analysis is selected against parsimony and neighbour-joining trees because it is more resolved. Besides, data on inflorescence development (SOKOLOFF 2003c) strongly suggest against basal position of *Coronilla* found in parsimony analysis of molecular data.

Results

Molecular data

Alignment of the 49 complete ITS1 and ITS2 sequences after exclusion of 11 ambiguous positions resulted in a matrix of 827 nucleotide positions. 314 characters were parsimony-informative, 421 characters were constant, and 92 variable characters were parsimony-uninformative.

Trees inferred from various analyses of the same molecular data set have many common features (figs. 3–5). In all trees representatives of Loteae s.l. (incl. Coronilleae) form a highly supported clade. As in previous studies based on nuclear ribosomal internal transcribed spacer data (ALLAN & PORTER 2000; ALLAN et al. 2003), species previously placed in the tribe Coronilleae do not form a single clade. Our data clearly confirm a result by ALLAN et al. (2003) that the genus *Hammatolobium*, which was formerly placed into Coronilleae, is closely

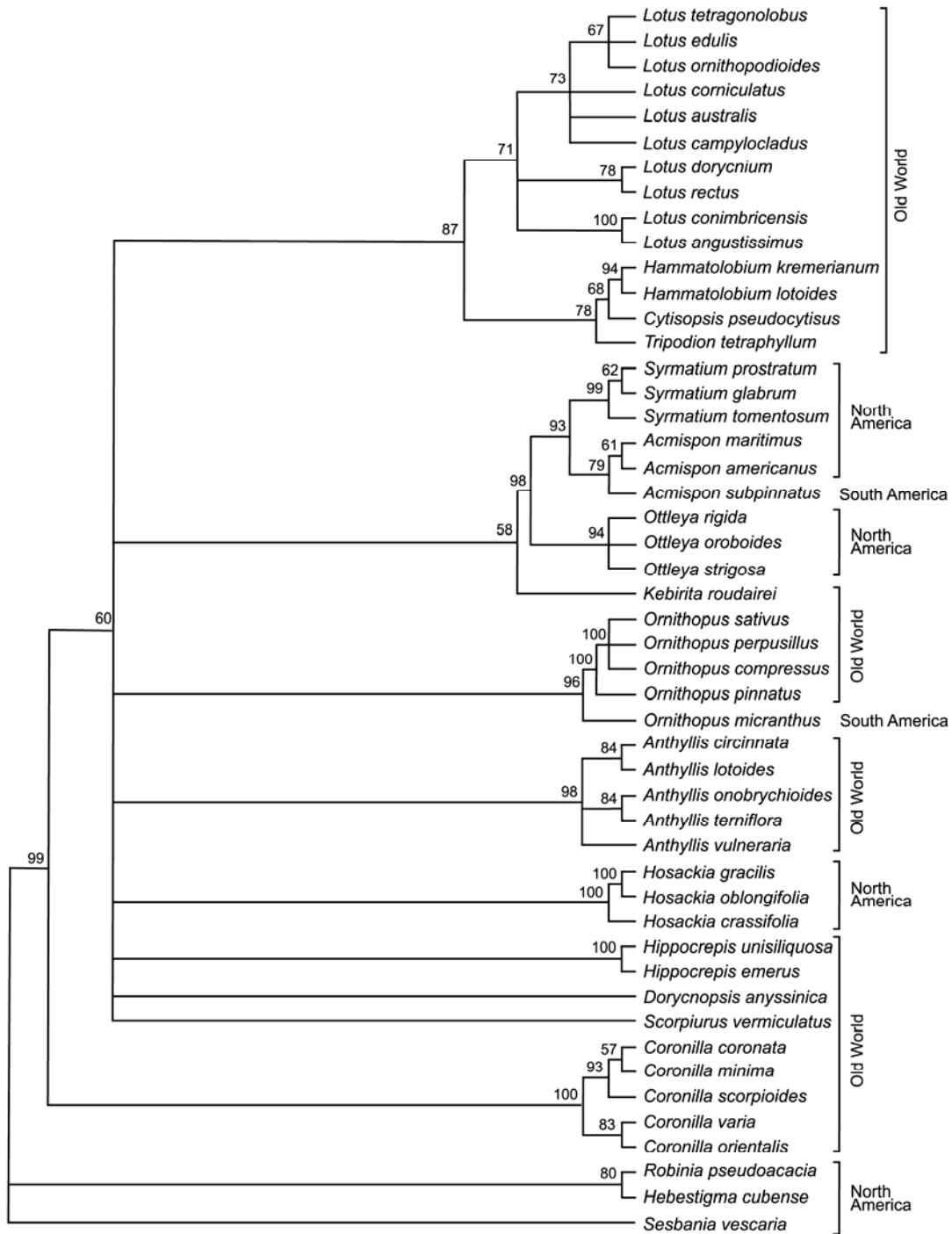


Figure 3: 50% majority rule consensus tree derived from equally weighted parsimony analysis with bootstrap resampling. Numbers at nodes indicate bootstrap estimates (less than 50 are shown as unresolved) for 100 replicate analyses.

related to the genus *Lotus* s.str. Increased taxon sampling allows us to extend conclusions by ALLAN et al. (2003) and to indicate a clade *Hammatolobium*+*Cytisopsis*+*Tripodion* (rather than *Hammatolobium kremerianum* only) as a group sister to *Lotus* s.str.

As in the study by ALLAN et al. (2003), representatives of New World genera *Syrmatium*, *Acmispon*, and *Ottleya* form a highly supported clade in all our analyses. In the tree published by ALLAN et al. (2003), relationships between these three genera are unresolved. In all our analyses, basal position of *Ottleya* and sister group relationships between *Acmispon* and *Syrmatium* are highly supported. This result agrees with morphological data (e.g. SOKOLOFF 2003a). We have not produced new sequences of *Ottleya*, *Syrmatium* and *Acmispon* for this study. Moreover, we have included fewer species of these genera than ALLAN et al. (2003) did. This might be the reason of better resolution in this clade. Besides, we have produced a new alignment, which also may effect in better resolution within the *Ottleya-Acmispon-Syrmatium* clade.

All trees (fig. 3–5) are in agreement with generic limits within Loteae proposed by SOKOLOFF (2003a). Although bootstrap support and posterior probabilities vary between analyses and genera, all genera accepted by SOKOLOFF (2003a) appear as separate clades on all trees. Among genera represented in this study by two or more species, highest support received *Hippocrepis* (100% in all three analyses). High support received also *Syrmatium*, *Ottleya*, *Coronilla* (incl. *Securigera*), *Anthyllis* (incl. *Hymenocarpos*), *Hammatolobium* and *Ornithopus* (80–100%). *Lotus* (incl. *Dorycnium* and *Tetragonolobus*), *Acmispon* and *Hosackia* were only 53–56%-supported in parsimony bootstrap analysis. However, the support indices were higher in neighbour-joining bootstrap analysis (71%, 79%, and 100%, respectively), and in the Bayesian analysis (posterior probabilities 1, 0.84, and 1, respectively).

The species *Coronilla scorpioides* is nested in our analysis together with other species of *Coronilla* s.l. In a previous analysis of the ITS data set (ALLAN & PORTER 2000), *C. scorpioides* was revealed as a close relative of *Hosackia*. The results of the present study, based on a new sequence of *C. scorpioides*, are preferable from the morphological viewpoint (see SOKOLOFF 2003b). Our study revealed a highly supported clade corresponding to *Coronilla* subgen. *Coronilla* (i.e. *C. scorpioides*, *C. minima*, and *C. coronata*).

Within the genus *Ornithopus*, all three analyses strongly suggest basal position of the South American species *O. micranthus*. The rest of studied *Ornithopus* species (all are native to the Old World) forms a 100%-supported clade in all trees. Among Old World species, representatives of the section *Ornithopus* (*O. compressus*, *O. perpusillus*, and *O. sativus*) also form a 100%-supported clade.

Significant differences between results of parsimony analysis, neighbour-joining analysis, and Bayesian analysis occur mainly in basal nodes of Loteae phylogeny.

Parsimony analysis with bootstrap resampling (fig. 3) identified *Coronilla* s.l. as a basal clade within the tribe Loteae. All other representatives of Loteae form a 60%-supported clade. Within this large clade, relationships of *Ornithopus* are not resolved.

The neighbour-joining analysis (fig. 4) resulted in a tree with nine basic clades of Loteae. Relationships between these clades are not clear. In general, the neighbour-joining tree is very similar to the tree inferred from the parsimony analysis, with the difference that the basal position of *Coronilla* is less than 50% supported.

Both parsimony and neighbour-joining analyses identified NW African monotypic genus *Kebirita* as a sister group to New World *Ottleya-Acmispon-Syrmatium* clade (58% and 80% of

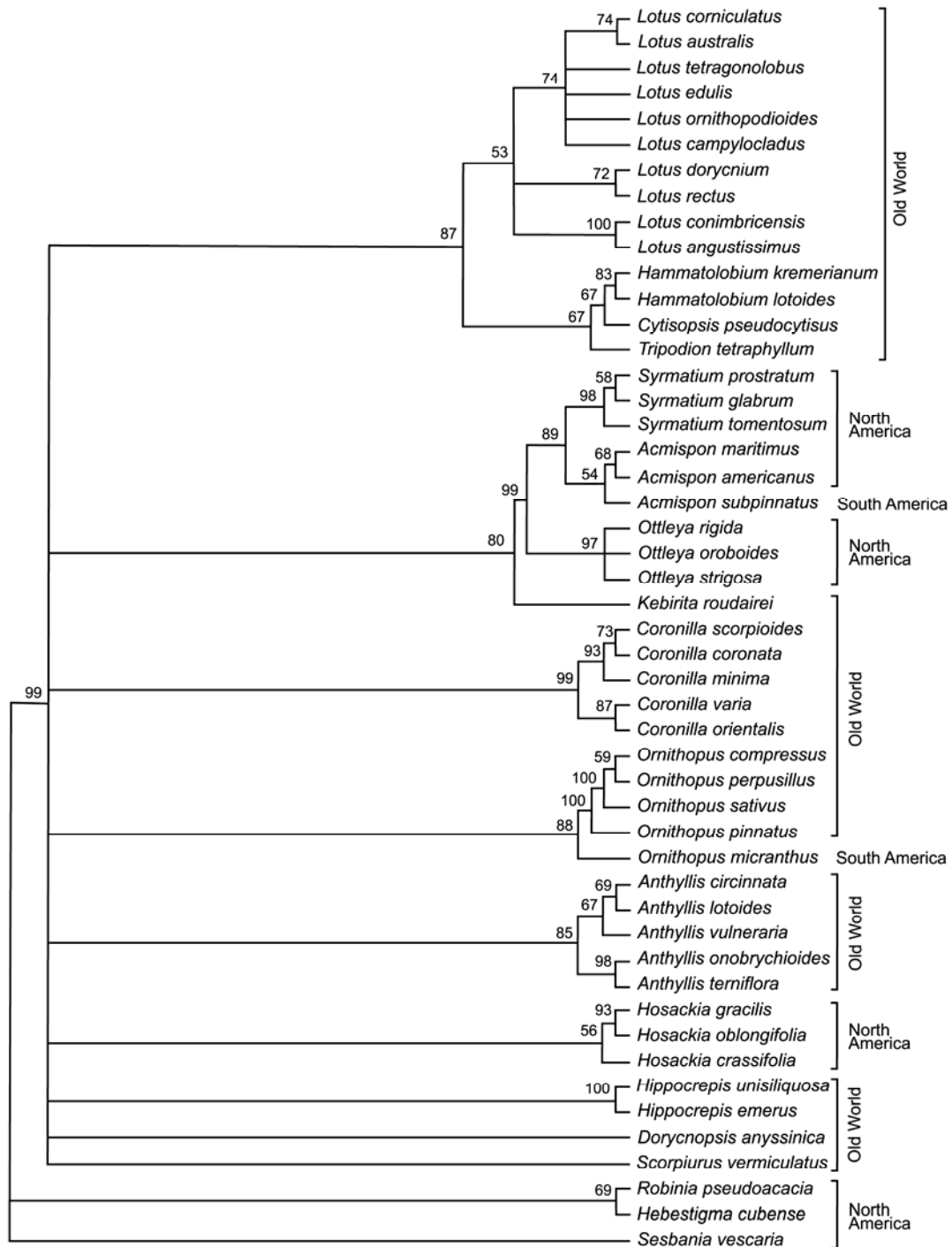


Figure 4: Neighbour-joining tree. Numbers at nodes indicate bootstrap estimates for 1000 replicate analyses.

support, respectively). ALLAN et al. (2003) found the same result in the neighbour-joining analysis of ITS data, but the bootstrap support was less than 50%.

The Bayesian analysis (fig. 5) resulted in a tree with three basic Loteae clades: (1) *Anthyllis*, (2) *Kebirita*, *Dorycnopsis*, *Ottleya*, *Acmispon*, *Syrmatium*, *Hosackia*, *Ornithopus*; (3) *Coronilla*, *Scorpiurus*,

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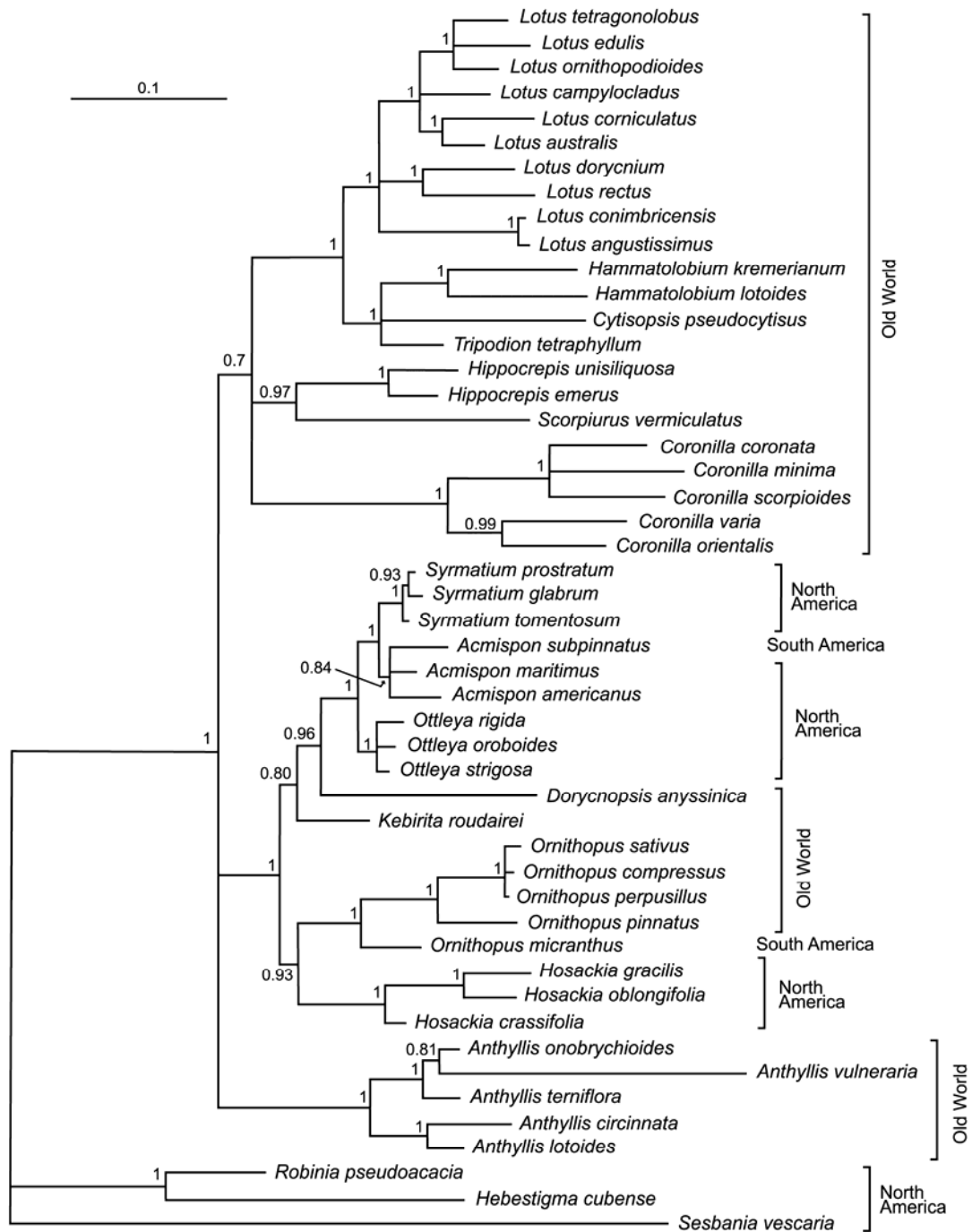


Figure 5: Relationships of studied taxa, determined by Bayesian inference. Nodes with posterior probabilities lower than 0.70 are shown as unresolved. Branch lengths are proportional to the number of expected nucleotide substitutions; scale bar corresponds to 1 substitution per 10 sites.

Hippocrepis, *Tripodion*, *Cytisopsis*, *Hammatolobium*, *Lotus*. The relationships between these three clades are not resolved. It is important that clade 2 comprises all studied New World members of the tribe Loteae. It includes also *Dorycnopsis abyssinica*, *Kebirita roudairei*, and Old World species of *Ornithopus*. Interestingly, African species *D. abyssinica* and *K. roudairei* have been

previously allied with American species on the basis of morphological data (see, e.g. OTTLEY 1944; LASSEN 1986).

Within the clade 2, *Ornithopus* is placed as a sister group to North American genus *Hosackia*, in agreement with the results of the parsimony analyses of ITS data by ALLAN & PORTER (2000) and ALLAN et al. (2003), where, however, *Hosackia*+*Ornithopus* clade received bootstrap support less than 50%. The Bayesian analysis also revealed the following grouping within the clade 2: *Kebirita*(*Dorycnopsis abyssinica*(*Ottleya*(*Acmispon Syrmaticum*))). Thus, position of *D. abyssinica* is different than in trees inferred from parsimony and neighbour-joining analyses. ALLAN et al. (2003) found different position of this species in neighbour-joining and parsimony analyses of the ITS data set. Phylogenetic relationships of *Dorycnopsis* should be subject of future studies. As a matter of fact, there is an importance of studying the second species of the genus, *D. gerardii* (L.) Boiss.

Morphological data

In this section, we will discuss morphology of *Ornithopus* and other Loteae representatives. We have selected characters, which may clarify relationships between *Ornithopus* and *Hosackia* and may cause a question on monophyly and phylogeny of *Ornithopus*.

Life form: *Ornithopus* comprises annuals, whereas *Hosackia* perennials. However, some species of *Hosackia* can produce flowers during the first vegetation season (e.g. *H. repens* G. Don). Many species of *Hosackia* are characterized by presence of adventitious roots on shoots. If compared to other North American species of Loteae, representatives of *Hosackia* usually occupy moister habitats (e.g. ISELY 1981). Some species, e.g. *H. pinnata* (Hook.) Abrams, can grow as (sub-)aquatic plants. Old World species of *Ornithopus* usually grow in dry or moderately humid habitats and would not produce adventitious roots on stems. The South American *O. micranthus* usually grows in humid and moist conditions, often near the water. It was tested as a winter cover crop cultivated in succession with rice (MENEZES et al. 2001). *O. micranthus* can produce adventitious roots. These data appear to be in agreement with basal position of *O. micranthus* in the phylogeny of *Ornithopus*. It should be noted, however, that life form similar to *O. micranthus* has, for example, not closely related species *Lotus benoistii* (Maire) Lassen from Morocco. *L. benoistii* is a (sub-)aquatic annual plant with prostrate stems bearing adventitious roots.

Stipule morphology: Two basic stipule types are represented in the tribe Loteae: (1) membranous or herbaceous and (2) reduced to dark gland. These types are stable within genera accepted by SOKOLOFF (2003a). Outgroup comparison (to Robinieae representatives) clearly shows that type 2 is apomorphic. Fig. 6 shows single most parsimonious reconstruction of this character evolution based on Bayesian ITS tree topology. These data suggest homoplastic nature of the character evolution in the tribe. However, within the clade comprising New World taxa, stipule morphology is in agreement with recognition of two sub-clades: (1) *Hosackia-Ornithopus* (with membranous or herbaceous stipules) and (2) *Kebirita-Dorycnopsis-Ottleya-Acmispon-Syrmaticum* (with stipules reduced to dark glands).

All species of *Hosackia* have free stipules with bases extending to abaxial side of the petiole; stipule margin and tip usually are without dark pigmentation. *Ornithopus micranthus* is similar to *Hosackia* in these features (although stipule base is only slightly extending to abaxial petiole side in *O. micranthus*). In *O. pinnatus*, stipules are also free, but do not cover abaxial side of the

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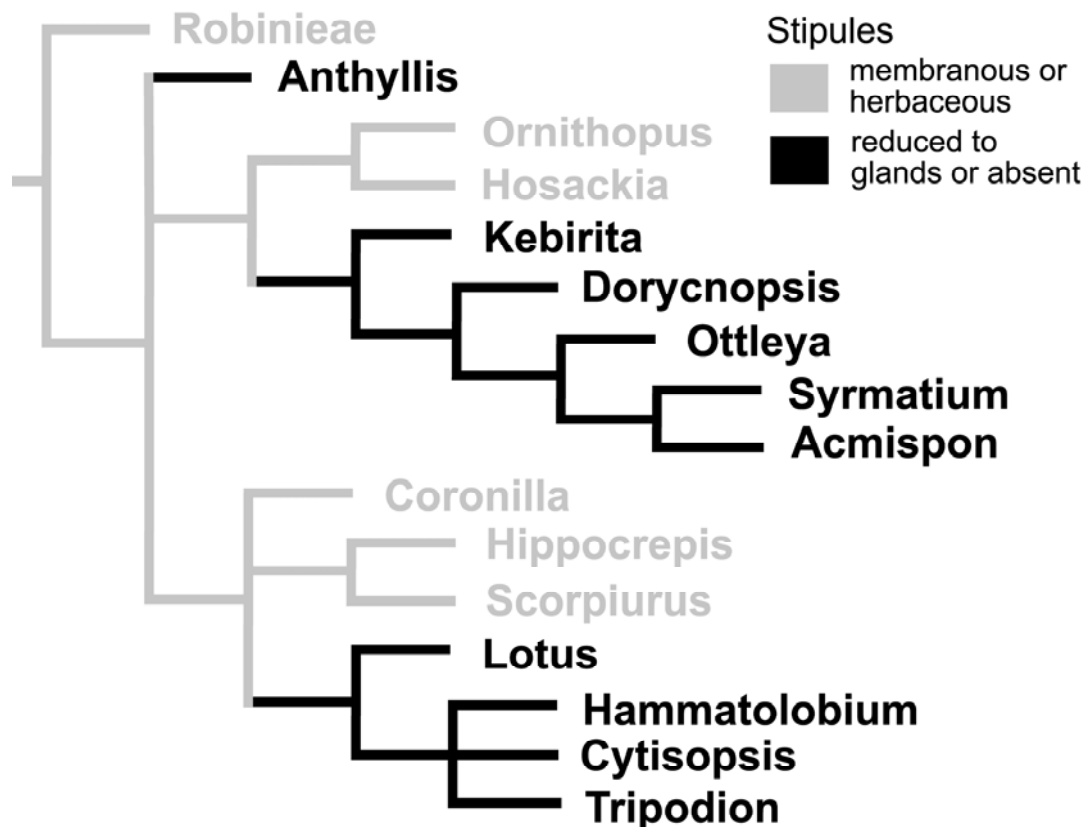


Figure 6: Single most parsimonious reconstruction of character state evolution for stipule morphology in the tribe Loteae. Stipules are absent in few species of *Anthyllis* and *Lotus* as well as in *Cytisopsis*. (Simplified cladogram inferred from Bayesian analysis)

petiole at all; stipule margin is dark. In members of *Ornithopus* sect. *Ornithopus*, stipules of lower leaves are partially fused with petiole and do not cover its abaxial side; dark pigmentation is confined to stipule tip. These data support basal position of *O. micranthus* in phylogeny of the genus.

Inflorescence morphology: The majority of Loteae species, including all members of *Hosackia* and *Ornithopus*, have partial inflorescences in form of axillary umbels and bracts (i.e. subtending leaves of flowers) shaped like small, inconspicuous scales. Many members of Loteae also have a leaf that we have suggested to call *pseudobract* (SOKOLOFF 2003a). The pseudobract is located on axillary peduncles below bracts. Morphologically, this is the first developed leaf on the axillary peduncle. In contrast to bracts, the pseudobract never possesses an axillary flower. The pseudobract usually is represented by a foliage leaf (fig. 6a) being quite different from bracts in morphology and size. Sometimes, however, the pseudobract is scale-like and as small as true bracts (fig. 6a). Many scientists, especially in taxonomic literature, described the pseudobract in various representatives of Loteae as a true bract. Probably they often did not notice small and inconspicuous true bracts. The pseudobract, when present, usually is located immediately below the umbel (i.e. the second internode on the peduncle is shortened). Presence of a pseudobract seems to be a unique (though not universal) feature of the tribe Loteae. Such a structure was not noticed in related papilionoid tribes. A comparison

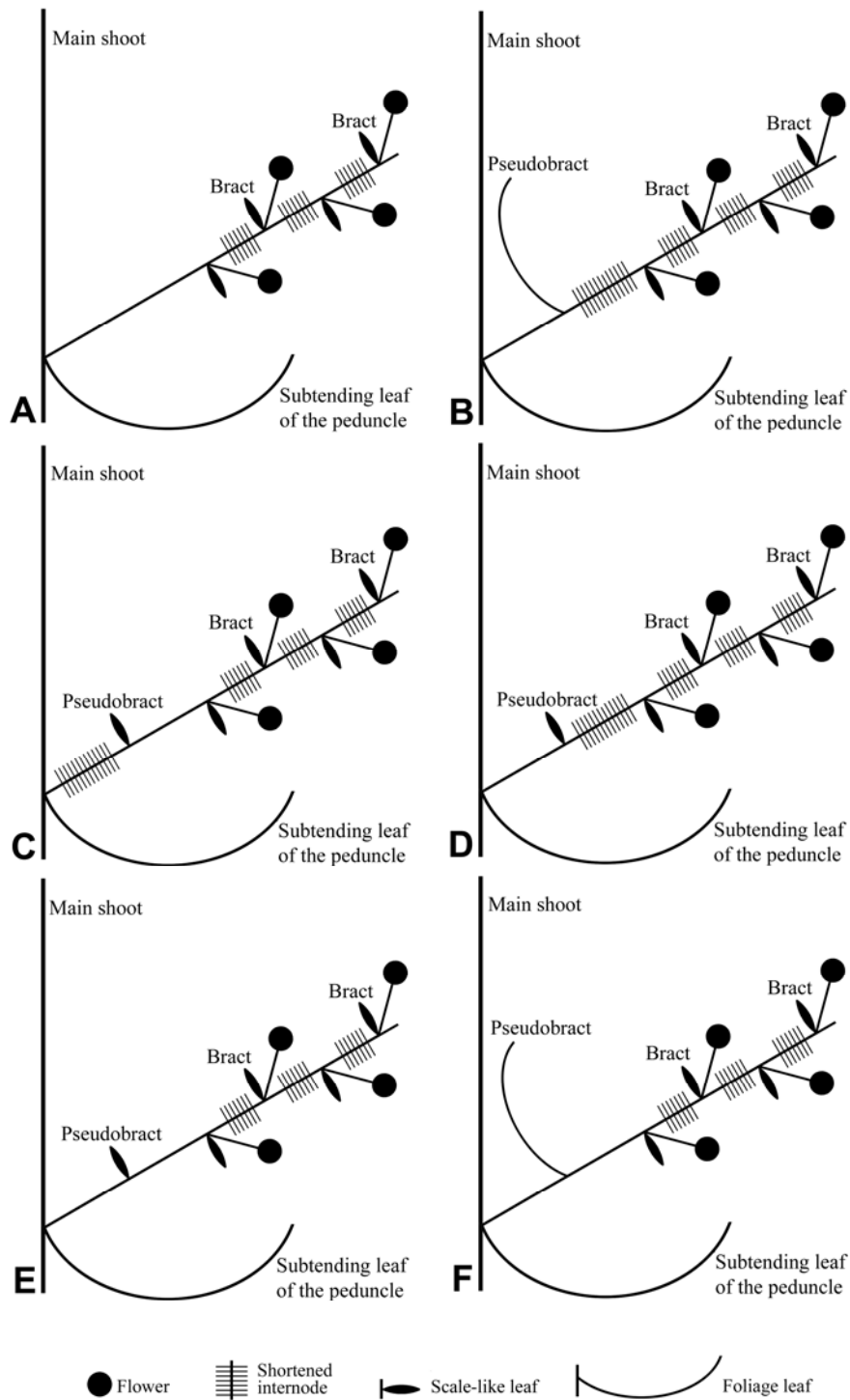


Figure 6a: Simplified schemes of axillary peduncles of selected representatives of the tribe Loteae. Flowers are in fact usually arranged on the peduncle in almost circular manner. A) Pseudobract is absent (*Coronilla*, *Hippocrepis*, *Scorpiurus*, *Dorycnopsis*, *Cytisopsis*, and, seemingly, *Ornithopus pinnatus*). B) Most usual for Loteae peduncle structure, which is found, for example, in *Ornithopus* sect. *Ornithopus*. C) *Ornithopus micranthus*. D) A rare type of peduncle structure found, for example, in some representatives of *Hosackia* sect. *Hosackia*. In this section, a typical structure of the peduncle (as in scheme B) is also found. E & F) Two types of peduncle structure found in *Hosackia* sect. *Protohosackia*.

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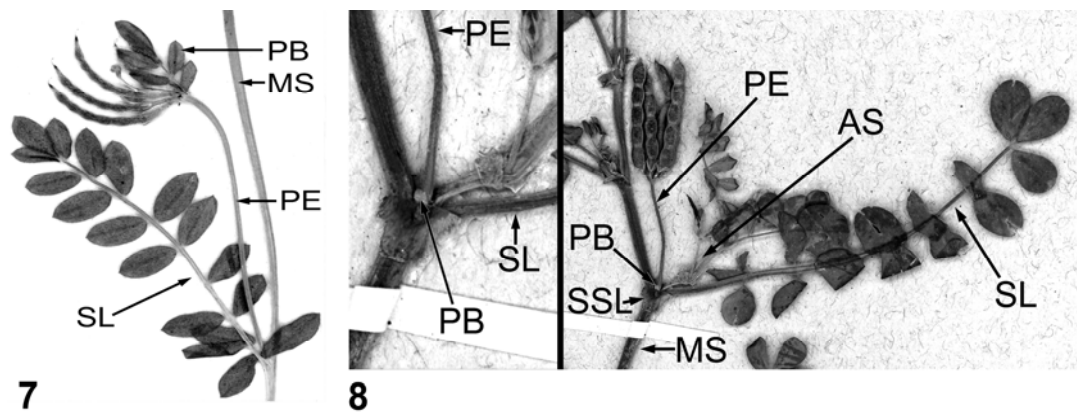


Figure 7: *Ornithopus perpusillus* L. (Denmark, B. Øllgaard 189 – MW). Axillary peduncle (PE) bearing a partial inflorescence (umbel). Stage of young fruits is shown. PB – pseudobract; SL – subtending leaf of the peduncle; MS – main shoot. As in other species of sect. *Ornithopus*, pseudobract is represented by a foliage leaf situated immediately below the umbel.

Figure 8: *Ornithopus micranthus* (Benth) Arechav. (Brazil, G.O.A. Malme 130 – S). Axillary peduncle (PE) bearing a partial inflorescence (umbel). Stage of young fruits is shown. PB – pseudobract; SL – subtending leaf of the peduncle; SSL – stipule of the subtending leaf; MS – main shoot; AS – additional shoot formed from a serial bud in the axis of the subtending leaf of the peduncle. The pseudobract is very small, inconspicuous and situated at the base of the peduncle.

of molecular and morphological data (fig. 9) allows the conclusion that the pseudobract either was evolved several times in course of Loteae evolution or was lost in several lineages (e.g. in *Cytisopsis*, *Dorycnopsis*).

In spite of homoplastic nature of this character at the tribal level, it was concluded that presence or absence of the pseudobract usually is a constant feature within Loteae genera (SOKOLOFF 1998, 2003a). The only important exception is *Ornithopus*. According to SOKOLOFF (2003a), pseudobract is present in *Ornithopus* sect. *Ornithopus* (fig. 7), but absent in sect. *Austropus* (i.e. in *O. micranthus*) and sect. *Coronilloides* (i.e. in *O. pinnatus*). These data might be treated as a reason for splitting *Ornithopus*, especially because the pseudobract is absent in the genus *Dorycnopsis*, which was found to be close to *Ornithopus* according to data of fruit anatomy (SOKOLOFF 1997, 1998) and in some molecular analyses (see ALLAN et al. 2003).

In the present study, we were able to analyse more material of *O. micranthus*. We have concluded, in contrast to previous studies, that this species does have a pseudobract. The pseudobract is very small, scale-like and located at the base of the peduncle (fig. 6a; 8). There is a long internode between the pseudobract and the umbel. Such a peduncle structure has not been previously described for any Loteae species. In the light of these data the presumed absence of the pseudobract in *O. pinnatus* should be confirmed by a study of developmental morphology.

Our present finding of the pseudobract in *O. micranthus* confirms placement of this species in the genus *Ornithopus*. Significant difference between *O. micranthus* and species of the type section in position and structure of the pseudobract confirms recognition of the monotypic section *Austropus*. It is important that in the genus *Hosackia* pseudobract position and structure varies in a manner similar to *Ornithopus* (fig. 6a).

Pollen morphology: DÍEZ & FERGUSON (1996) have published most comprehensive data on pollen morphology in Old World species of *Ornithopus*, which are in consensus with results of

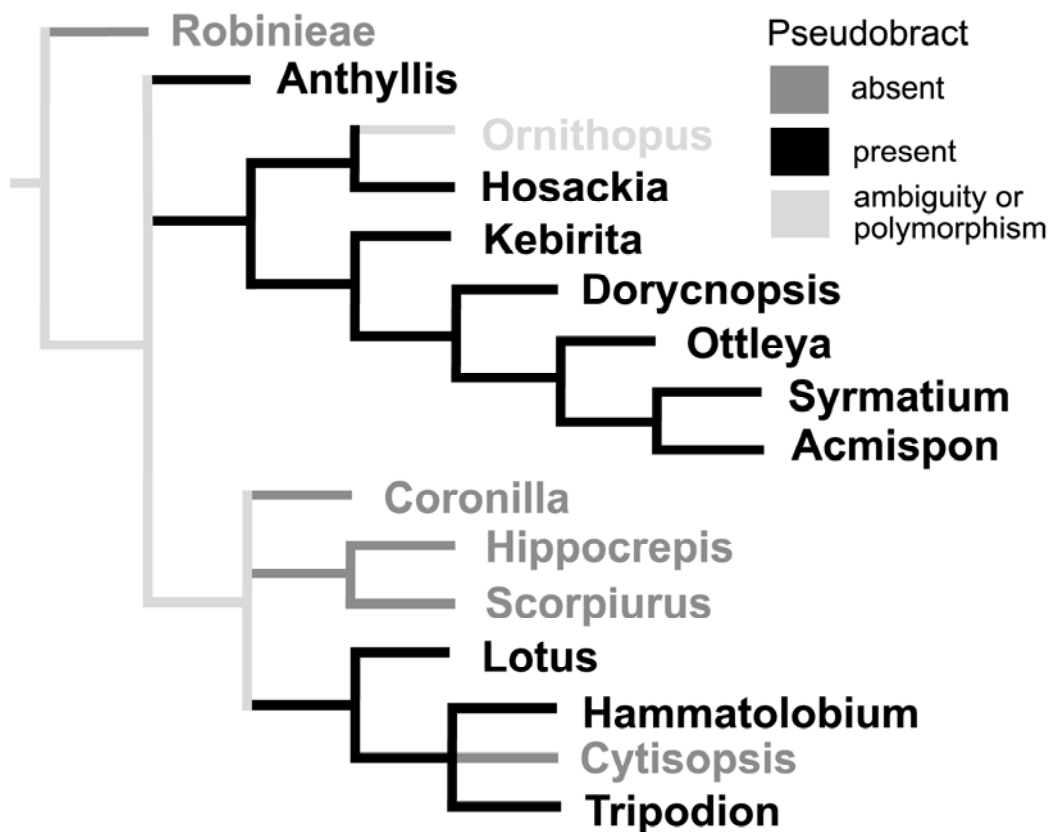


Figure 9: A summary of most parsimonious reconstructions of character state evolution for presence/absence of a pseudobract on peduncle in the tribe Loteae. (Simplified cladogram inferred from Bayesian analysis)

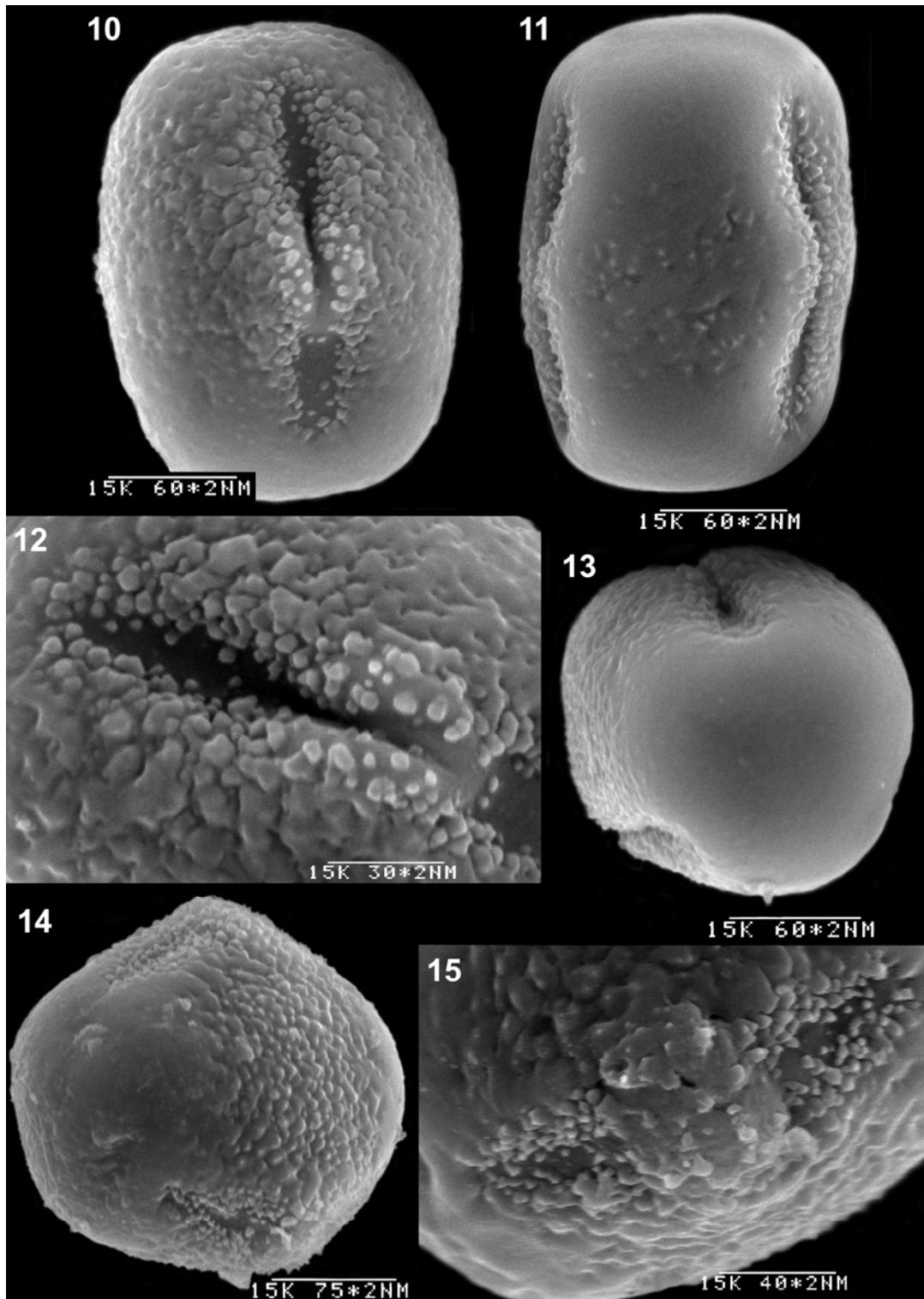
previous studies. They have revealed that pollen grains are 3-zonocolporate, rectangular in equatorial outline and more or less triangular-angulaperturate in polar outline. Ornamentation is granulate-verrucate and perforate at the mesocolpium and more or less psilate at the apocolpia; colpi with granular membrane. The type of pollen ornamentation is distinctive within the tribe Loteae, and Old World species of *Ornithopus* were included into a separate pollen type (DÍEZ & FERGUSON 1996).

Our SEM studies of the Old World species *Ornithopus perpusillus* (figs. 10–13), *O. sativus* (fig. 14–15) and *O. pinnatus* (fig. 16–17) are in good agreement with the description by DÍEZ & FERGUSON (1996), in addition to *O. sativus* pollen is sometimes almost elliptical in equatorial outline. Besides, we found that in *O. perpusillus* ornamentation at mesocolpium is variable. Sometimes granules are almost absent between the apertures.

In the American species *O. micranthus* (figs. 18–21), according to our data, the pollen is 3-zonocolporate, elliptical in equatorial outline and more or less triangular-angulaperturate in polar outline. Ornamentation is psilate-perforate at the mesocolpium, more or less psilate at the apocolpia, and granulate-verrucate around the colpi.

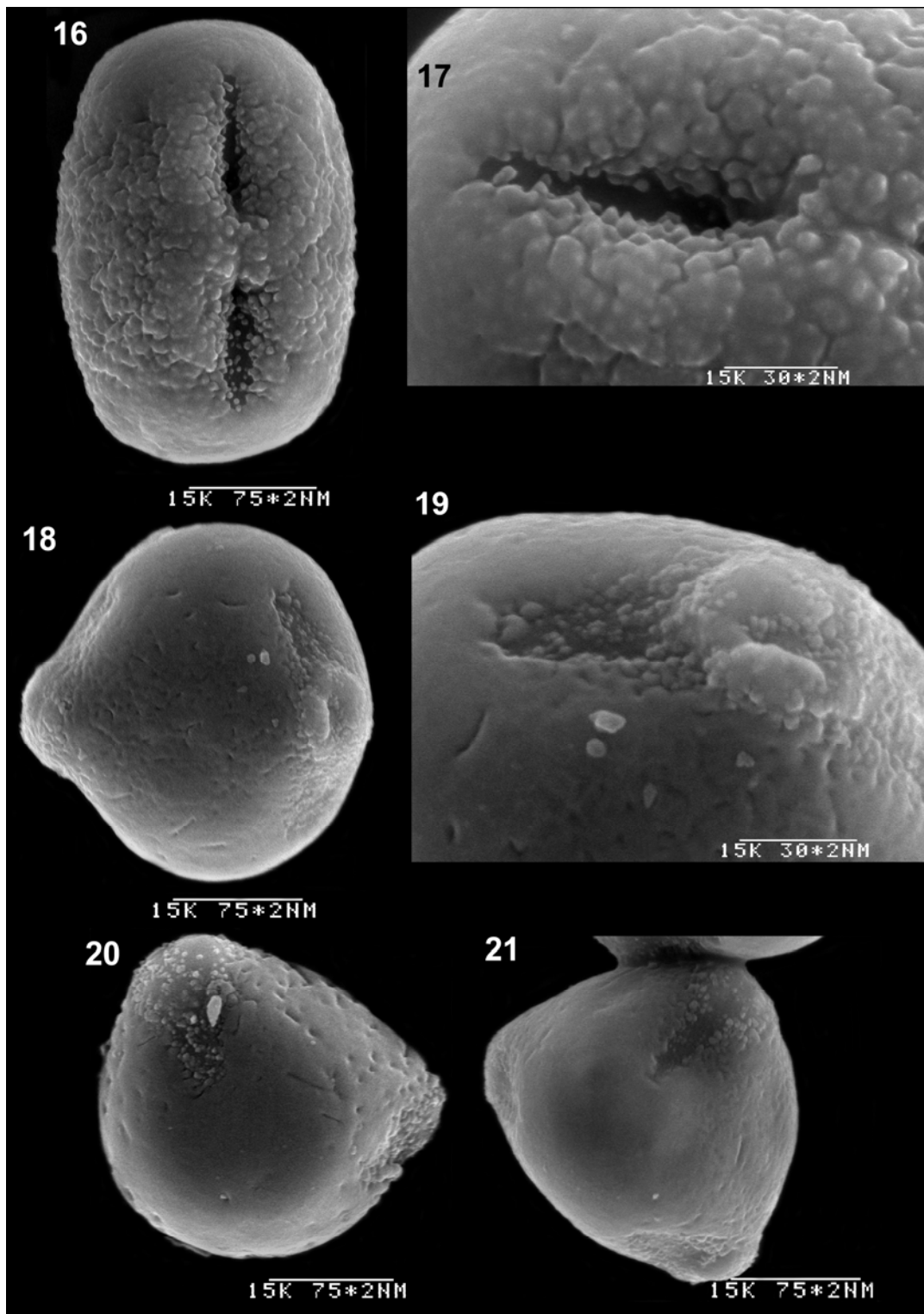
The pollen of *O. micranthus* share many important features with pollen of Old World species of the genus, namely aperture type and number, presence of granules on colpus membrane and around the colpus margin, presence of perforations at the mesocolpium, and psilate apocolpia. The main distinction of *O. micranthus* from Old World species is psilate-perforate ornamentation at

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Figures 10–13: Pollen morphology of *Ornithopus perpusillus* L. (SEM). 10–11) Equatorial view. 12) Aperture. 13) Polar view.

Figures 14–15: Pollen morphology of *Ornithopus sativus* Brot. (SEM). 14) Pollen grain in oblique position showing difference between sculpture on apocolpium (left) and mesocolpium (right). 15) Details of aperture and sculpture.



Figures 16–17: Pollen morphology of *Ornithopus pinnatus* (Mill.) Druce (SEM). 16) Equatorial view. 17) Aperture.

Figures 18–21: Pollen morphology of *Ornithopus micranthus* (Benth) Arechav. (SEM). 18) Equatorial view. 19) Aperture. 20–21) Polar view.

the mesocolpium. PIRE (1974) also noticed that *O. micranthus* differs from Old World species of the genus in exine ornamentation.

Published data on pollen morphology in *Hosackia* (CROMPTON & GRANT 1993; DÍEZ & FERGUSON 1996) allow to conclude that *Hosackia* is very similar palynologically to *Ornithopus*. As far as we know, such a conclusion is new, probably because in previous studies no attempt was made to compare *Hosackia* and *Ornithopus* in detail.

The pollen of the studied species of the American genus *Hosackia* is similar to the pollen of Old World species of *Ornithopus* in many important features. The most important distinctive character of *Hosackia* is psilate-perforate (not granulate-verrucate and perforate) ornamentation between the colpi. However the American species *Ornithopus micranthus* differs from the rest of *Ornithopus* mainly in this same feature, i.e. psilate-perforate mesocolpium. Among members of Loteae described in literature (CROMPTON & GRANT 1993; DÍEZ & FERGUSON 1991, 1994, 1996), *O. micranthus* seems to be most similar palynologically to *Hosackia*. Presence of granules around the colpus margin is probably most important similarity in pollen morphology of these taxa. There are some other members of Loteae with granules around the colpus margin, but they have either more than three apertures (e.g. *Ottleya*) or different exine ornamentation in areas between the colpi (e.g. *Dorycnopsis*).

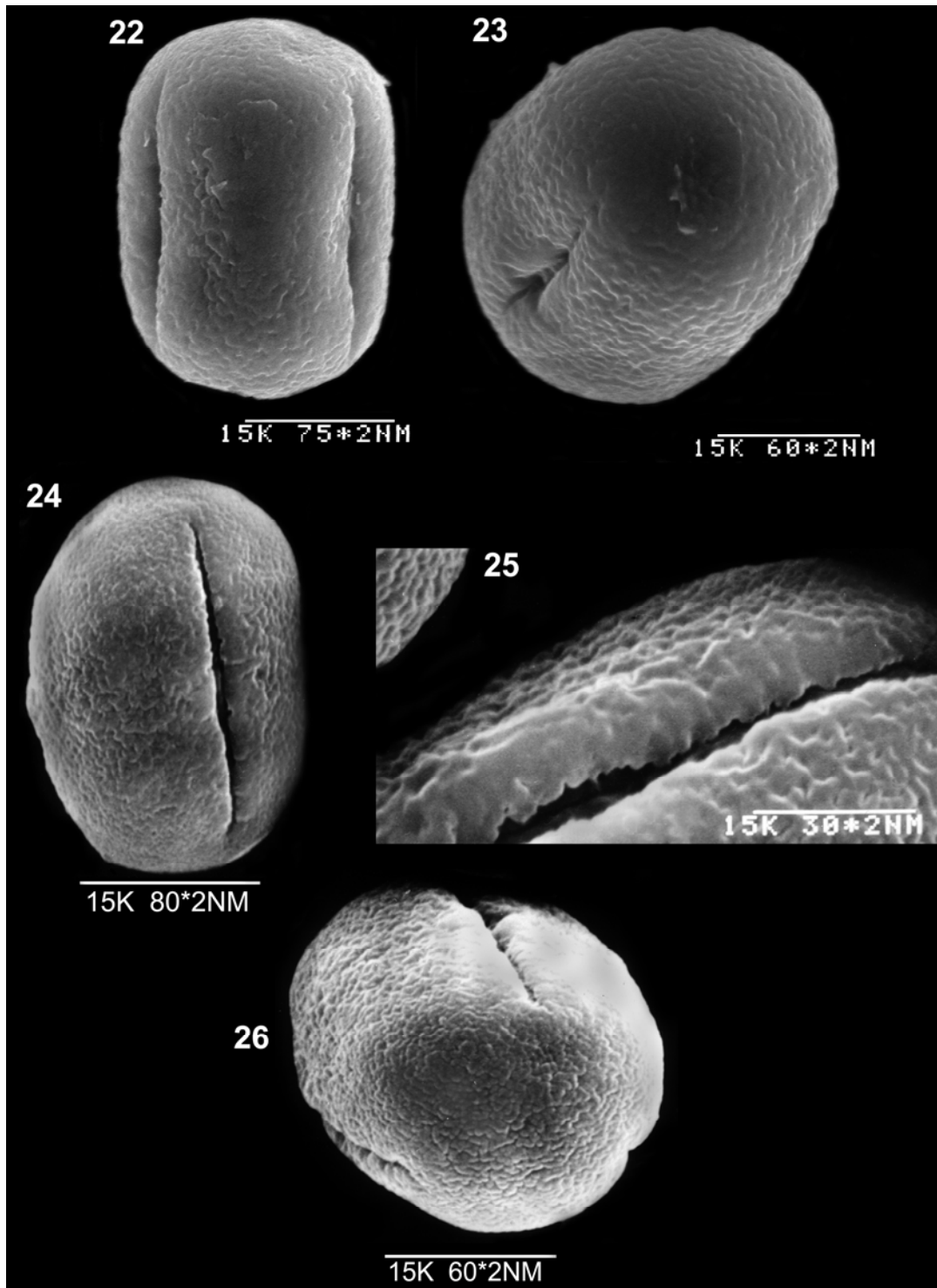
The fact that pollen of *O. micranthus* is especially similar to pollen of *Hosackia* is in good agreement with basal position of *O. micranthus* within the genus *Ornithopus* and with sister group relationships between the two genera on trees inferred from molecular data.

DÍEZ & FERGUSON (1994) noticed that presence of granules around the colpus margin allows distinguishing members of American *Hosackia* from species of Old World *Lotus* s.str. Our data confirm absence of granules in Old World species of *Lotus* (figs. 22–26). An analysis of published data (CROMPTON & GRANT 1993; DÍEZ & FERGUSON 1991, 1994, 1996) allows the conclusion that this character might be even more taxonomically important. It seems that all studied New World representatives of the Loteae tribe have granules on colpus membrane and/or around the colpi. These data, together with clear molecular evidence, prove segregation from *Lotus* s.l. of American genera *Hosackia*, *Acmispon*, *Ottleya*, and *Syrmatium*.

Another area where pollen morphology is in good agreement with molecular phylogeny is aperture number in American Loteae. Only members of *Acmispon*, *Ottleya*, and *Syrmatium* possess more than three apertures (data after DÍEZ & FERGUSON 1994). They form a well-supported clade on trees inferred from molecular data.

Fruit morphology and anatomy: Fruits of Loteae vary in two basic characters, namely (1) presence or absence of longitudinal dehiscence and (2) presence or absence of transversal fruit splitting. Both characters are stable (with very few exceptions) within each genus recognized by SOKOLOFF (2003a). However they are quite homoplastic if analysed at tribal level. Figures 27 and 28 show fruit dehiscence and presence of lomentaceous fruits mapped onto the tree inferred from the molecular data. Multiple gains of lomentaceous and indehiscent fruits in course of Loteae evolution are in agreement with morphological studies by LASSEN (1989) and SOKOLOFF (1998) and with molecular phylogenetic studies by ALLAN & PORTER (2000) and ALLAN et al. (2003).

Ornithopus has lomentaceous indehiscent fruits, which are quite different from dehiscent, not lomentaceous fruits of *Hosackia*. This was main reason why these two genera were not critically compared earlier. However if to take into account a high amount of homoplasy in



Figures 22–23: Pollen morphology of *Lotus australis* Andrews (SEM). 22) Equatorial view. 23) Pollen grain in oblique position showing absence of granules around the colpus margin.

Figures 24–26: Pollen morphology of *Lotus corniculatus* L. (incl. *L. dvinensis* Min. et Ulle) (SEM). 25) Aperture. 26) Polar view.

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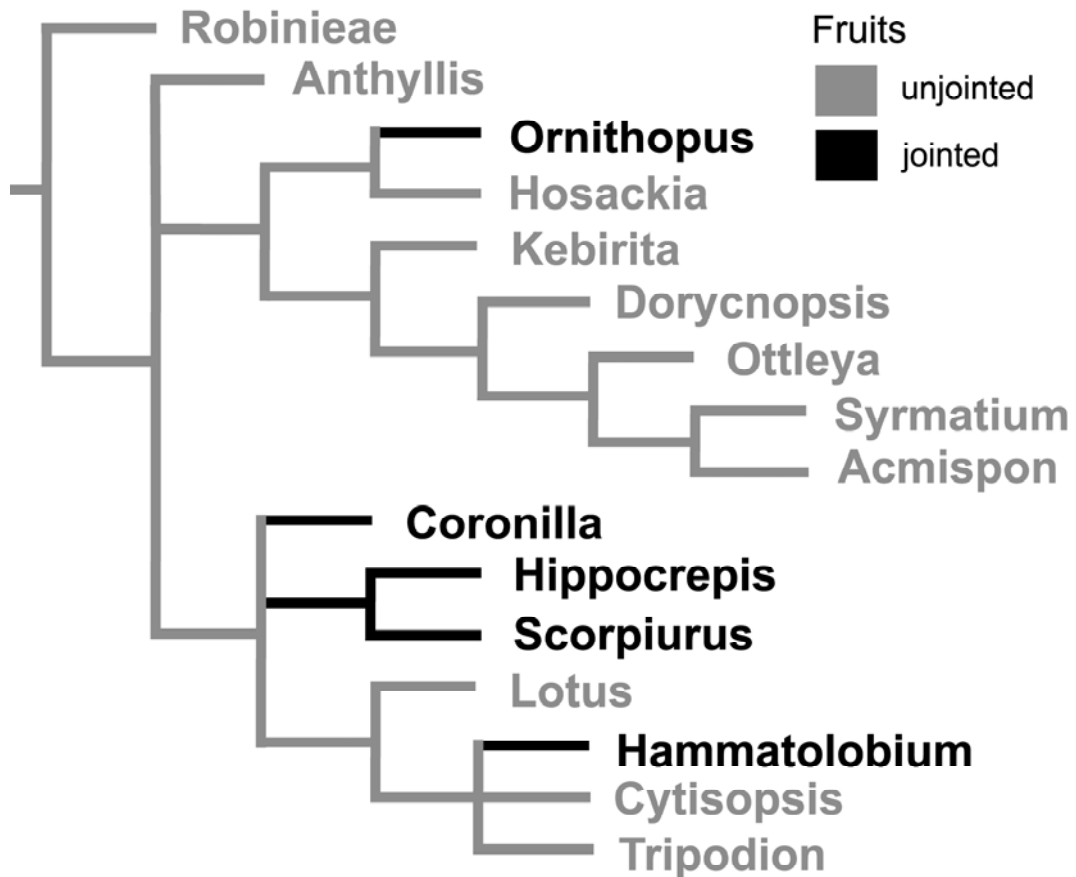


Figure 27: Most parsimonious reconstruction of character state evolution for fruit morphology in the tribe Loteae. Jointed versus unjointed fruits were analysed. DELTRAN optimisation is shown.

evolution of fruit dehiscence within the tribe in general (figs 27, 28), these distinctions, probably, are not so crucial.

Our data on fruit anatomy in Old World species of *Ornithopus* are in good agreement with published descriptions (FUCSKÓ 1914; MOELLER & GRIEBEL 1928; FAHN & ZOHARY 1955; KANIEWSKI & WAŻYŃSKA 1968). We have illustrated previously not studied Old World species *O. uncinatus* (figs. 29–34).

Old World species of *Ornithopus* have relatively large epidermal cells with thickened outer cell walls. The hairs (abundant only in members of sect. *Ornithopus*) are three-celled with first cell situated at the level of normal epidermal cells, second cell very small and third cell very long and thick-walled. Such hair structure is typical for the majority of Loteae species. Three to five layers of thin-walled parenchymatous cells with transparent content are situated below the epidermis. Below them a layer of large thin-walled parenchymatous cells with brown content is located. This layer is uncontinuous. Deeper in the pericarp, a sclerenchyma zone (so-called ‘parchment layer’ or ‘hard layer’) is situated. The sclerenchyma zone consists of lignified fibres. Outer fibres are transversally oriented, inner fibres are oriented along the fruit axis (therefore on pericarp cross section outer fibres are cut lengthways, and internal across – see figs. 29, 30). The outer margin of the sclerenchyma zone is conspicuously undulate on cross sections (fig. 29).

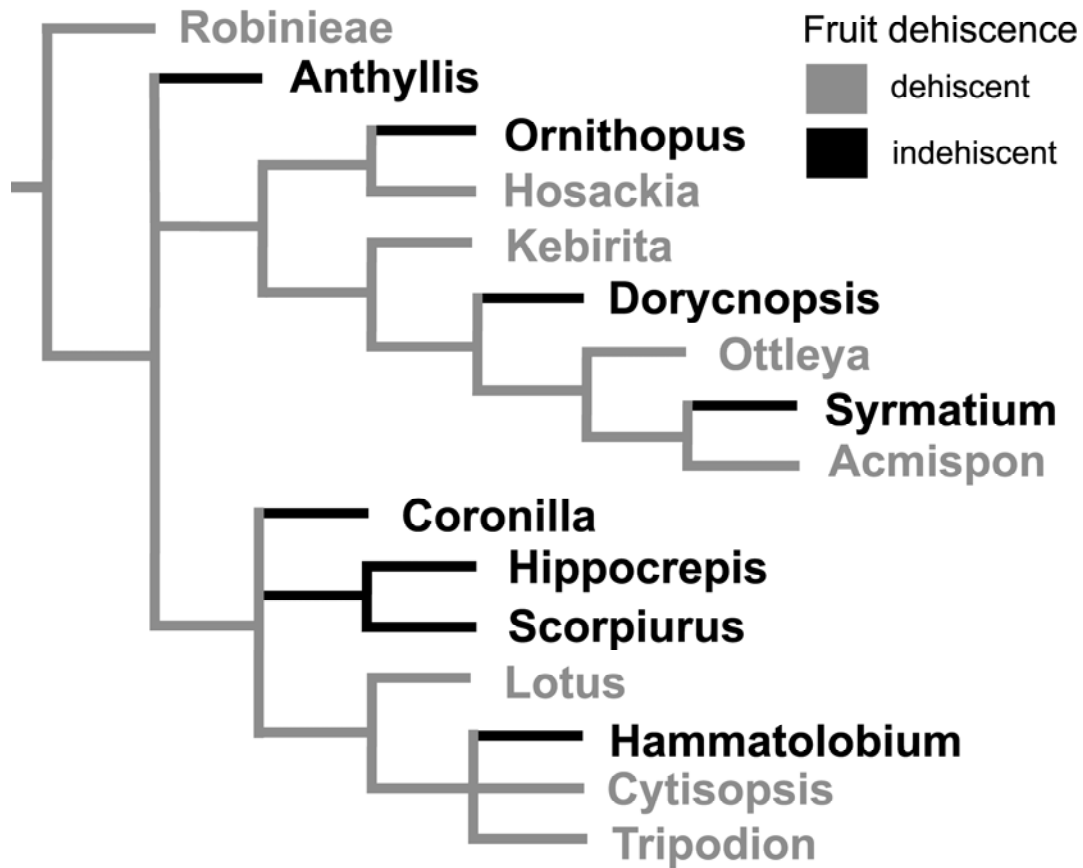


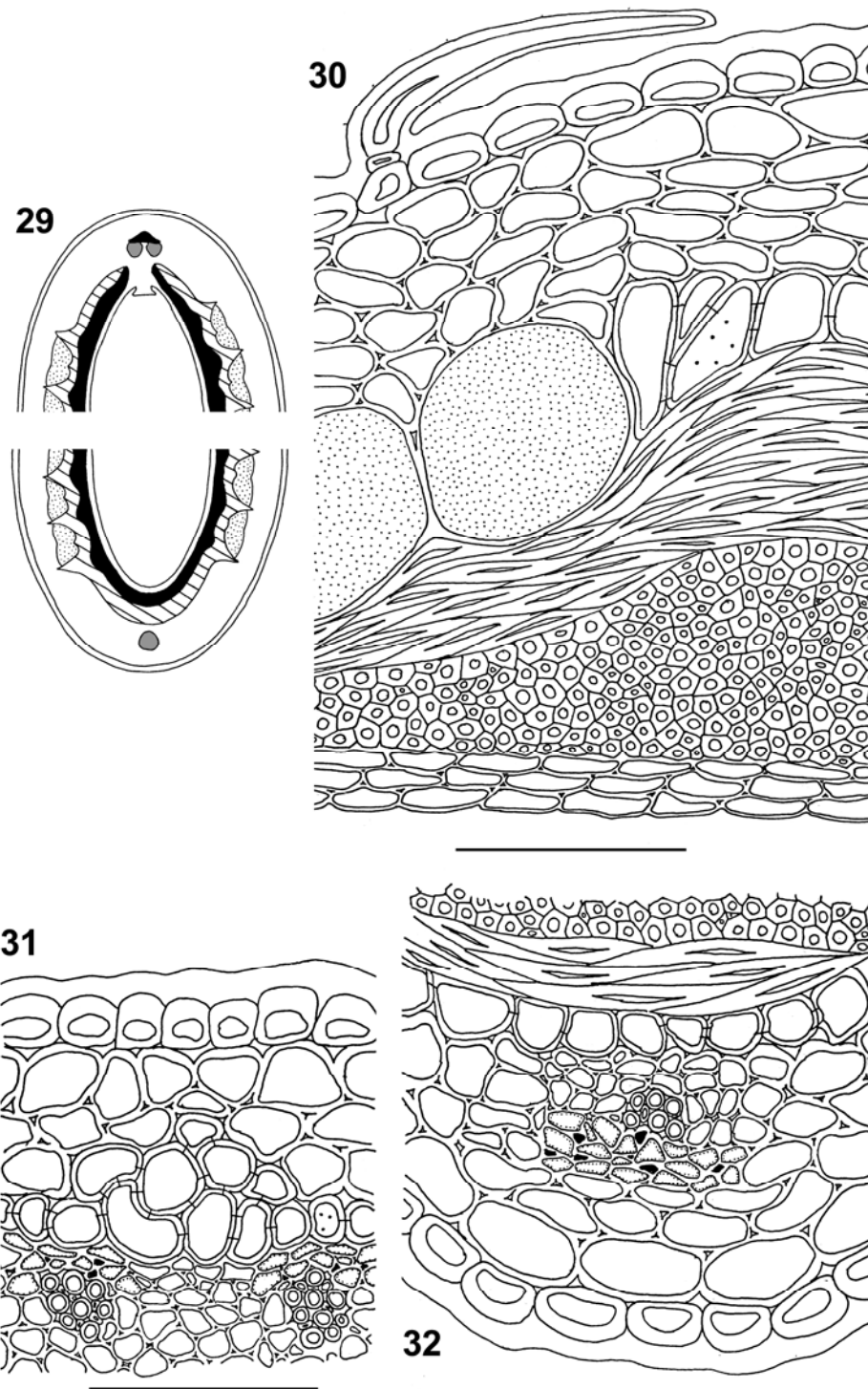
Figure 28: Most parsimonious reconstruction of character state evolution for fruit morphology in the tribe Loteae. Dehiscent versus indehiscent fruits were analysed. DELTRAN optimisation is shown.

A study of isolated sclerenchyma zone with binocular microscope revealed that its outer surface shows regular more or less rounded depressions. The mentioned above brown parenchymatous cells are confined to these depressions (one, two or several cells per a depression). Deeper the sclerenchyma zone, three to four layers of parenchymatous cells with transparent content are located. In the ventral region, the pericarp has two vascular bundles (fig. 31). The sclerenchyma zone is interrupted in the ventral region. In the dorsal region, there is a single vascular bundle (fig. 32). The sclerenchyma zone is continuous in the dorsal region and situated deeper than the bundle (fig. 29). Longitudinal pericarp sections revealed specialized structure of regions where loments are separating from each other (fig. 33, 34). Sclerenchyma zone is interrupted in these regions.

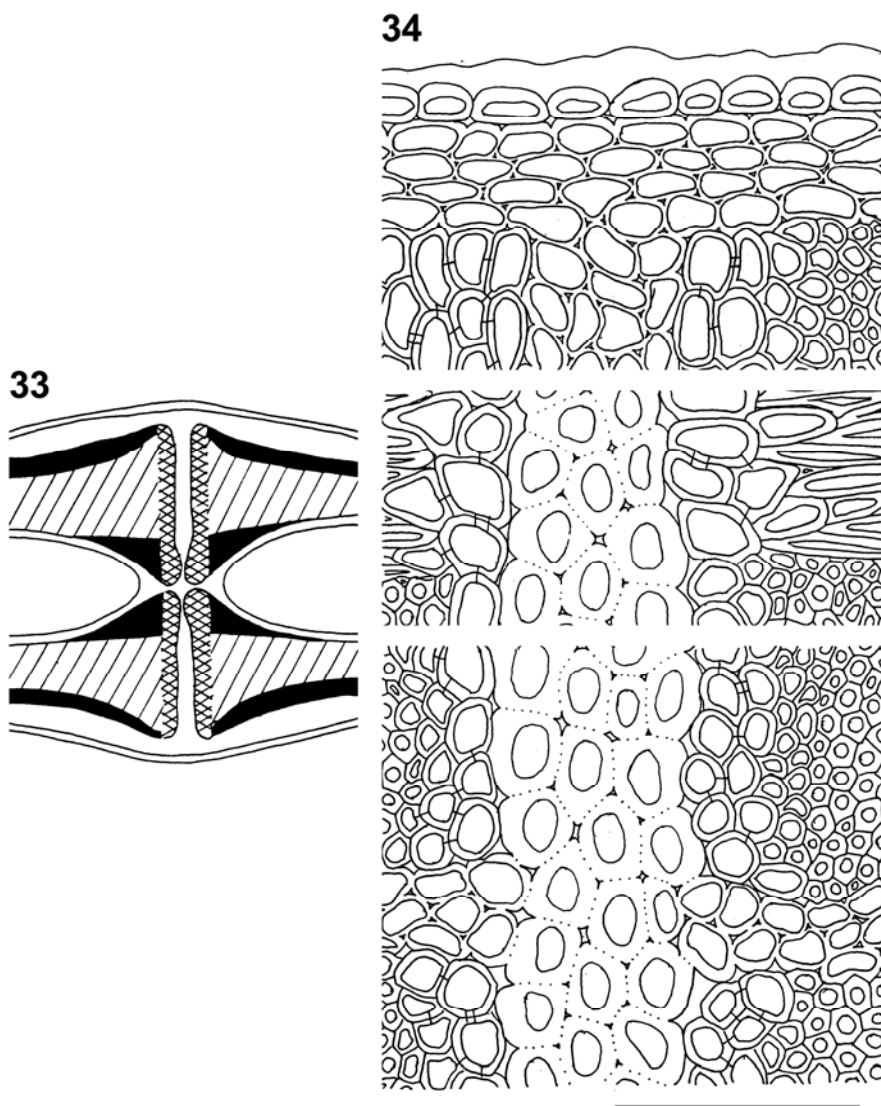
The S. American species *O. micranthus* is very similar to Old World species of the genus in fruit anatomy (figs. 35–40). The description of the Old World species can be almost completely repeated for *O. micranthus*. A peculiar feature of *O. micranthus* is a distribution of brown cells. Each depression of sclerenchyma zone is shadow and always has only one brown cell (fig. 36). In regions where loments are separating from each other the layer of brown cells is present (fig. 39, 40, compare fig. 33, 34).

Our data show that Old World and New World species of the genus *Ornithopus* share the same type of pericarp anatomy. It is important that no one other studied species of the tribe Loteae

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Figures 29–32: Fruit anatomy of *Ornithopus uncinatus* Maire et G. Sam. 29) A scheme of the pericarp structure in cross section. Here and below the following marking is used on the schemes: black areas = sclerenchyma fibres in cross section; oblique shading = sclerenchyma fibres in longitudinal section; dotted areas = cells with a brown content; grey areas = xylem and phloem of vascular bundles. Ventral side is atop. 30) Pericarp anatomy in the middle part of a valve in cross section. Here and below: cells with a brown content are dotted. Here and below: scale = 0.1 mm. 31) Ventral region on cross section. 32) Dorsal region on cross section.



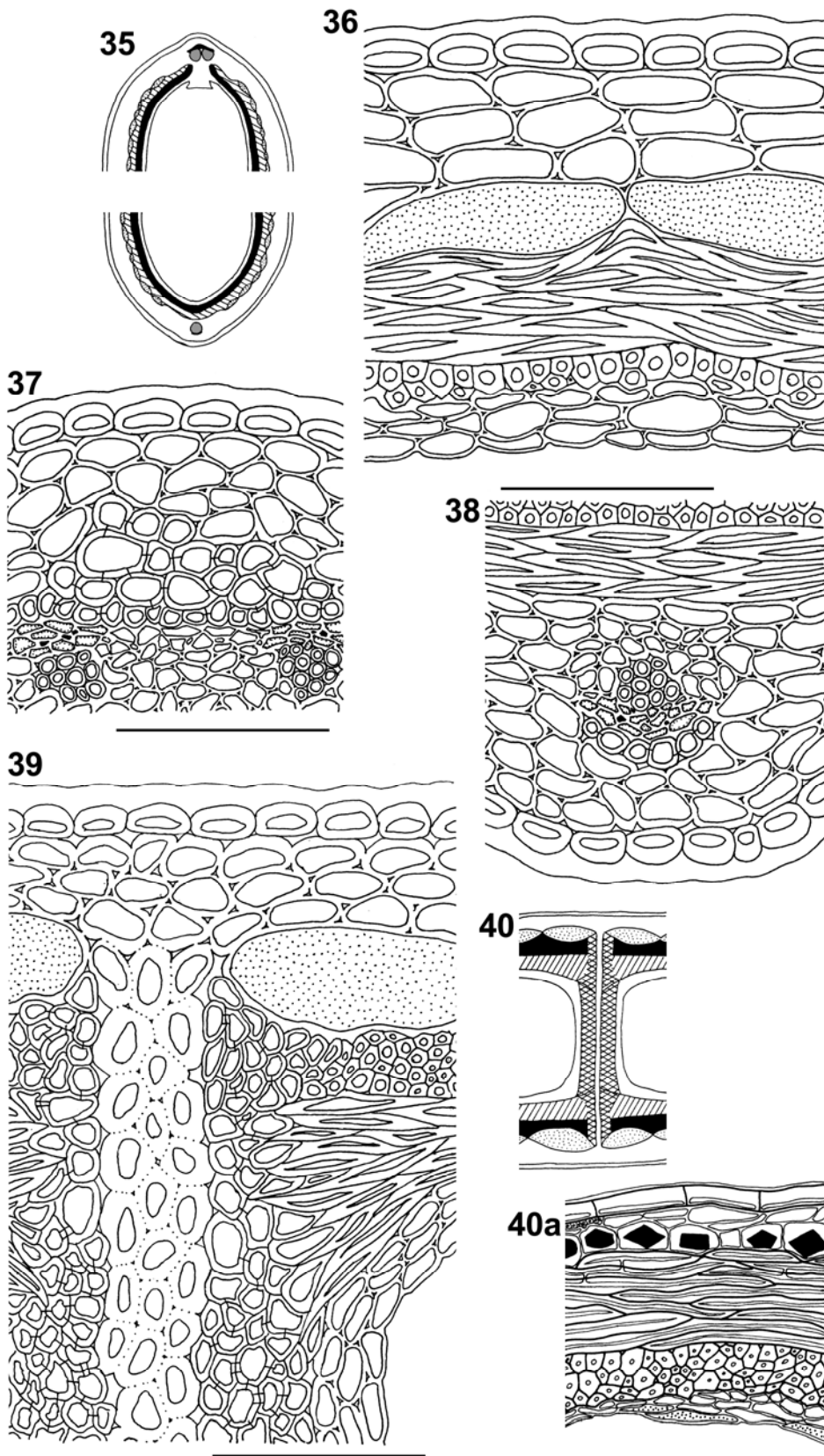
Figures 33–34: Fruit anatomy of *Ornithopus uncinatus* Maire et G. Sam. 33) A scheme of the pericarp in longitudinal section in a region where loment are separating from each other. Here and below: double oblique shading = short lignified cells. 34) Identical but details.

has the same type of pericarp anatomy as *Ornithopus*. The most important diagnostic features of fruit anatomy in *Ornithopus* are sclerenchyma zone with outer fibres oriented transversally and inner fibres oriented longitudinally as well as brown cells located in contact with outer fibres of the sclerenchyma zone. In the tribe Loteae, only *Ornithopus* and *Dorycnopsis* has this

Figures 35–40: Fruit anatomy of *Ornithopus micranthus* (Benth.) Arechav. 35) Scheme of the pericarp in cross section. 36) Pericarp anatomy in the middle part of a valve in cross section. 37) Ventral region in cross section. 38) Dorsal region in cross section. 39) Scheme of the pericarp in longitudinal section in a region where loment are separating from each other. 40) Identical but details.

Figure 40a: *Dorycnopsis gerardii* (L.) Boiss. Pericarp anatomy in the middle part of a valve in cross section. Modified from TIKHOMIROV & SOKOLOFF (1997). Black figures = crystals.

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kind of fibre arrangement in the pericarp (SOKOLOFF 1997). *Dorycnopsis* is similar to *Ornithopus* also in indehiscent fruits and in sclerenchyma zone continuous in the dorsal region of the pericarp. However, the sclerenchyma zone in *Dorycnopsis* has no regular depressions on the outer side, which are characteristic to *Ornithopus*; in *Dorycnopsis*, no brown cells are outside from the sclerenchyma zone, but they are located in the most internal part of the endocarp (fig. 40a) (TIKHOMIROV & SOKOLOFF 1997). Besides, fruits of *Dorycnopsis* are not lomentaceous.

The fruit anatomy data suggest monophyly of *Ornithopus*. The pattern of distribution of brown cells in pericarp appears to be a synapomorphy of *Ornithopus*. Monophyly of *Ornithopus* is also strongly suggested by all molecular analyses. Interestingly, *Dorycnopsis abyssinica* was found to be closely related to *Ornithopus* in some of molecular analyses by ALLAN et al. (2003). In our analyses position of *Dorycnopsis* was unstable. Therefore it is not clear whether similarity in fruit anatomy between *Dorycnopsis* and *Ornithopus* represent homoplasies or synapomorphies.

The monotypic African genus *Antopetitia* A. Rich. is traditionally regarded as closely related to *Ornithopus* (e.g. POLHILL 1981). Taubert (1894) even included *Antopetitia abyssinica* into *Ornithopus*. We were unable to extract DNA of *Antopetitia* for this study. However, we were able to study fruit anatomy of *Antopetitia* and test integrity of the genus *Ornithopus* on the basis of these data.

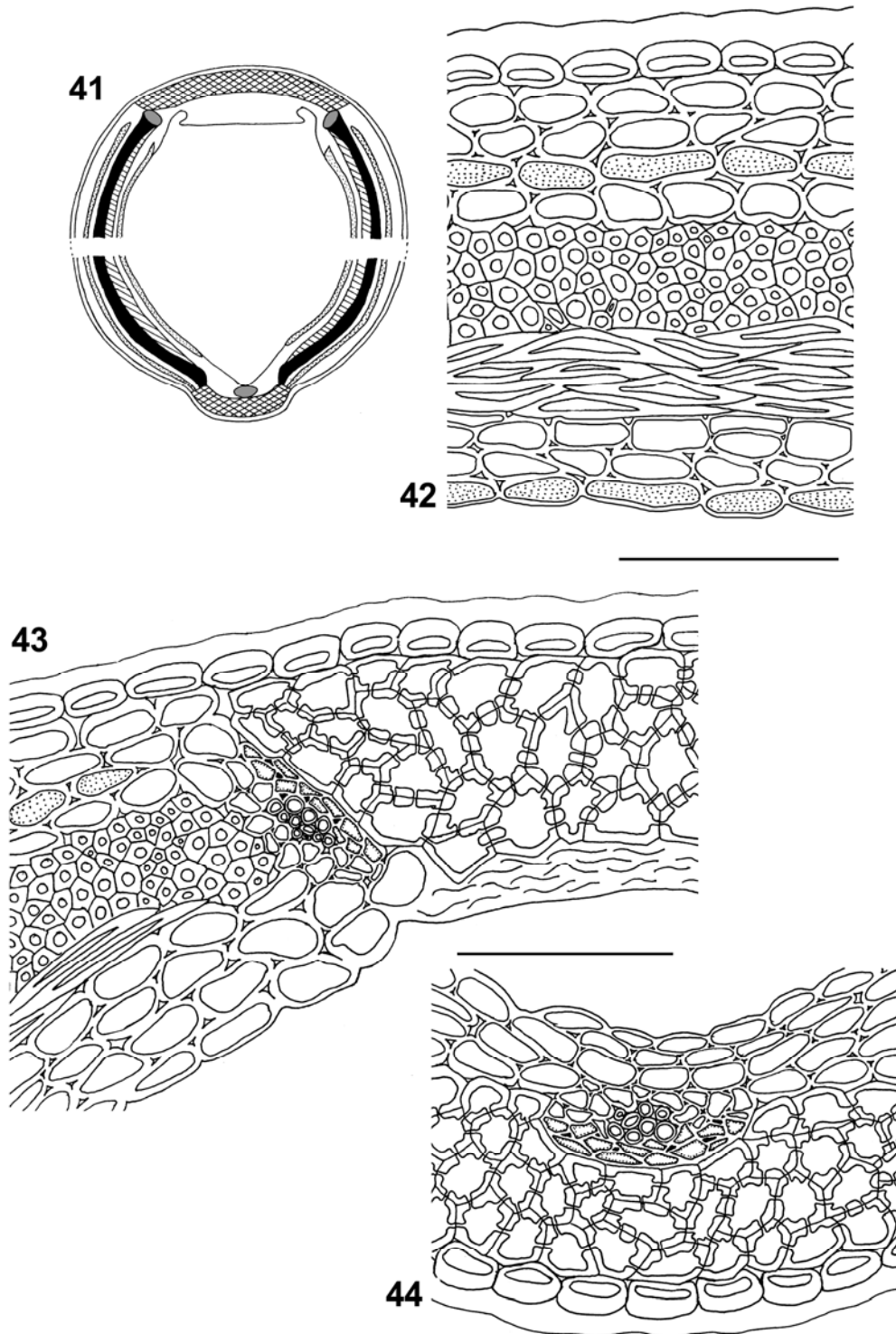
Our data show that *Antopetitia* has the following major differences from *Ornithopus* in fruit anatomy (figs. 41–46). There are two layers of brown cells, of which no one is corresponding to brown cells layer in *Ornithopus* (fig. 42, compare figs. 30, 36). In *Antopetitia*, outer fibres of sclerenchyma zone are oriented along the fruit axis, and inner fibres are transversally oriented (fig. 42). In *Ornithopus*, the reverse pattern of fibres orientation is found (figs. 30, 36). No depressions in sclerenchyma zone is found in *Antopetitia*. In contrast to *Ornithopus*, the sclerenchyma zone is interrupted in dorsal region of the pericarp (fig. 41, compare figs. 29, 35). In the ventral region of the pericarp, the two vascular bundles are situated in close contact to each other in *Ornithopus* (figs. 29, 35) and far away from each other in *Antopetitia* (fig. 41). In *Antopetitia*, lignified cells associated with outer part of vascular bundles are abundant and show wide pores (figs. 41, 43, 44). In *Ornithopus*, these cells are not numerous and have narrow pores (figs. 31, 37, 38). There are also important differences in structure of pericarp in the region where lomenta are separating from each other (compare fig. 45 to figs. 33 and 39).

Fruit anatomy data suggest against inclusion of *Antopetitia abyssinica* into *Ornithopus*.

Discussion

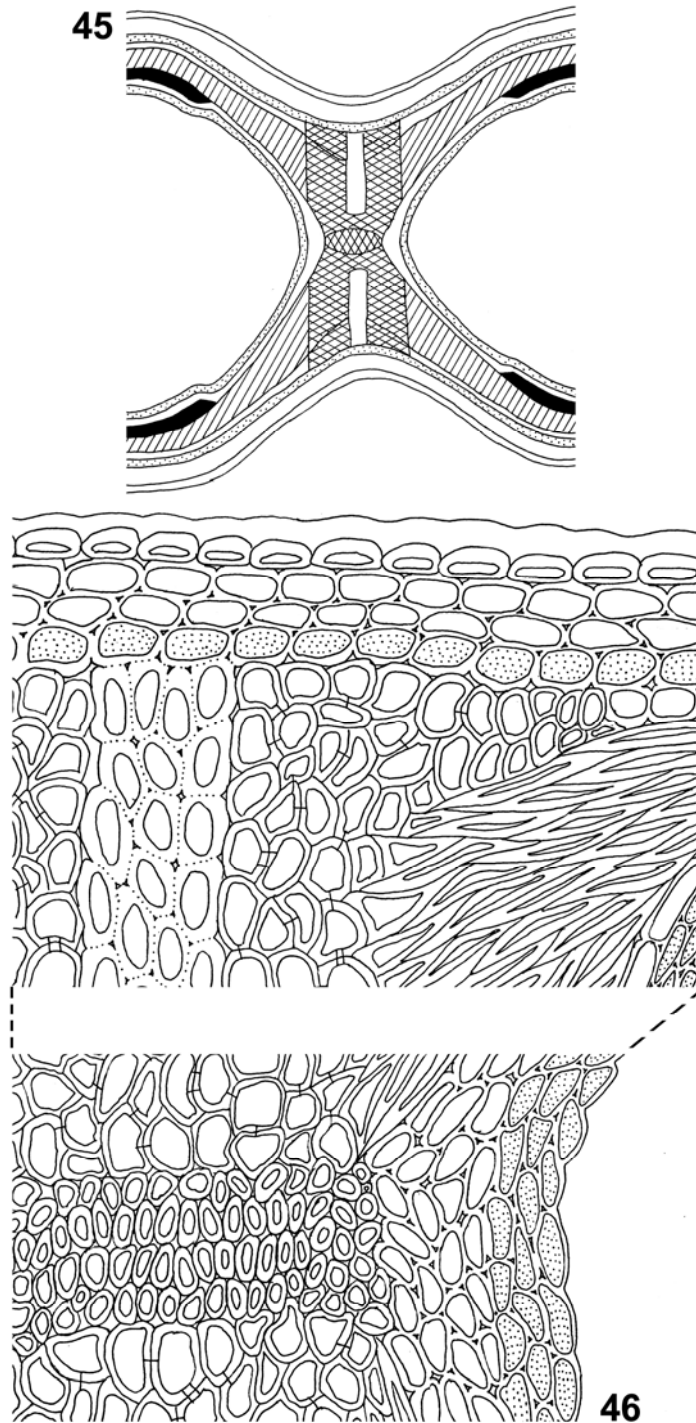
Compatibility of molecular and morphological data: SOKOLOFF (2003a) concluded that, in the tribe Loteae, morphological and molecular data usually are in a consensus at the generic level, and are often incongruent at the level above genera. He also suggested that genera of Loteae (in the limits proposed in SOKOLOFF 2003) represent ‘islands of stability’ in knowledge on Loteae phylogeny. The present study generally confirms this idea. All genera of Loteae appear to be monophyletic groups in all analyses of molecular data set. This is in agreement with morphological data. In contrast, relationships between Loteae genera are quite different in trees inferred from morphological (SOKOLOFF 2003) and molecular data. For example, *Coronilla*, *Hippocrepis*, and *Scorpiurus* form a well-supported clade in analyses of morphological data. All three genera share apparently unique and derived type of partial inflorescence development (SOKOLOFF 2003c). No molecular phylogenetic analyses revealed such a clade.

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Figures 41–44: Fruit anatomy of *Antopetitia abyssinica* A. Rich. 41) Scheme of the pericarp in cross section. 42) Pericarp anatomy in the middle part of a valve on cross section. 43) Ventral region in cross section. 44) Dorsal region in cross section.

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Figures 45–46: Fruit anatomy of *Antopetitia abyssinica* A. Rich. 45) Scheme of the pericarp in longitudinal section in a region where lobes are separating from each other. 46) Identical but details.

The genus *Cytisopsis* has a basal position in trees of Loteae inferred from morphological data. The present molecular study strongly suggests *Cytisopsis* to be closely related to *Hammatolobium* and *Tripodion* and more distantly to *Lotus*.

Monophyly of *Ornithopus*: The genus *Ornithopus* in its traditional circumscription is strongly supported as a monophyletic group in all analyses of molecular data. In addition to diagnostic characters of *Ornithopus* extensively discussed in taxonomic literature, we found new morphological features common to all species of the genus. These are (1) pollen grains with three apertures, (2) different exine sculpture at apocolpium and mecosolpium, (3) presence of granules around the colpus margin, (4) pericarp sclerenchyma zone with outer fibres oriented transversally and inner fibres oriented longitudinally, (5) pericarp sclerenchyma zone with regular depressions at the outer ‘surface’, and (6) brown cells in close contact with sclerenchyma zone of pericarp. A comparison with other Loteae representatives shows that features of pollen morphology (1–3) are likely symplesiomorphies. The features 5 and 6 are likely synapomorphies of *Ornithopus*, which proves monophyly of the genus. It is not clear if feature 4 represents a synapomorphy of *Ornithopus*, because it is found also in another Loteae genus, *Dorycnopsis*.

The South American species *O. micranthus* clearly belongs to the genus *Ornithopus*. However, it has several significant differences from the Old World species of the genus, especially in stipule and pollen morphology. It also has a peculiar type of pseudobract structure and position. Both molecular and non-cladistic morphological data suggest basal position of *O. micranthus* in the phylogeny of *Ornithopus*. These data are in agreement with the placement of *O. micranthus* in a monotypic section, *Austropus* (SOKOLOFF 2003a).

Sister group relationship of *Ornithopus*: Previous molecular phylogenetic studies based on ITS sequences demonstrated sister group relationships between *Ornithopus* and North American genus *Hosackia*, but the bootstrap support of this grouping was less than 50% (ALLAN & PORTER 2000; ALLAN et al. 2003). Our study, including more sequences of *Ornithopus*, also showed sister group relationships of *Ornithopus* with bootstrap support less than 50% in parsimony and neighbour-joining analyses. We have conducted a Bayesian analysis of the molecular data set of the tribe Loteae. This analysis revealed *Ornithopus* and *Hosackia* as sister groups with a posterior probability 0.93. This result disagrees with the traditional view of relationships between the two genera. The genus *Hosackia* was traditionally treated as close to *Lotus*. Often *Hosackia* was even included into *Lotus*. Some authors indicated similarity between *Hosackia* and *Coronilla* (e.g. LASSEN 1989). Nobody suggested close relationships between *Hosackia* and *Ornithopus* on the basis of morphological data. We have concluded, probably for the first time, pollen morphology data are in good agreement with sister group relationships between *Ornithopus* and *Hosackia*. We think it to be important that the apparently basalmost member of *Ornithopus* (*O. micranthus*) is especially similar to *Hosackia* in pollen morphology. We found also some similarities between *Hosackia* and *Ornithopus* in structure of vegetative organs. However, these data are less robust than data of pollen morphology.

Unfortunately we were unable to analyse ITS sequences of *Antopetitia*, which is morphologically close to *Ornithopus* (e.g. POLHILL 1981; SOKOLOFF 2003a). Our data of fruit anatomy strongly suggest that *Antopetitia* at least should not be included in *Ornithopus* (as proposed by TAUBERT 1894).

The ‘Mainly American Clade’ in the tribe Loteae: The Bayesian analysis of the molecular data set revealed a clade containing all New World members of the tribe Loteae (*Hosackia*, *Ornithopus micranthus*, *Ottleya*, *Acmispon*, *Syrmatium*). This clade also contains Old World species of *Ornithopus* as well as African *Kebirita roudairei* and *Dorycnopsis abyssinica*. The posterior

probability for the ‘Mainly American Clade’ is 1 – in contrast to parsimony and neighbour-joining analyses where such a clade received less than 50% of bootstrap support (ALLAN et al. 2003; present study). It is very difficult to indicate morphological evidence for grouping of these genera together. Almost all members of the clade have pollen with granules on or around the colpi (DÍEZ & FERGUSON 1994, 1996). However, similar granules are also found in some other members of Loteae. LASSEN (1986) suggested grouping of *Kebirita* with *Acmispon* and *Ottleya* on the basis of morphological data (although using different nomenclature). Close similarity between *Dorycnopsis* (= *Vermifruix*) *abyssinica* and *Syrmatium* was noticed by OTTLEY (1944) and POLHILL (1981), but criticized by TIKHOMIROV & SOKOLOFF (1997). On the other hand, *Dorycnopsis* share some features of fruit anatomy with *Ornithopus* (TIKHOMIROV & SOKOLOFF 1997). *Dorycnopsis* and *Ornithopus pinnatus* share absence of pseudobract on peduncles. Many authors noticed similarities between representatives of *Hosackia*, *Ottleya*, *Acmispon*, and *Syrmatium*. Sometimes members of all four genera were included in *Hosackia* s.l. (e.g. BENTHAM 1829; TAUBERT 1890; ABRAMS 1944; GILLET 1959). According to DÍEZ & FERGUSON (1994) *Dorycnopsis abyssinica* is similar in exine ornamentation to members of *Acmispon* sect. *Anisolotus* (nomenclature after SOKOLOFF 2003a).

Morphological evidence for the ‘Mainly American Clade’ is very weak. We can recognize a geographical tendency and some morphological tendencies. With a different arrangement of taxa on a molecular tree, we probably could indicate some weak morphological arguments in its advantage too. For example, we have noticed similarity between *Dorycnopsis abyssinica* and *Acmispon* sect. *Anisolotus* in exine sculpturing. However, a similar type of exine ornamentation has also *Coronilla* (Díez & Ferguson, 1996), which appears to be close to *D. abyssinica* in parsimony analysis of molecular data by ALLAN et al. (2003).

Origin of transatlantic disjunction in *Ornithopus*: We think our data show clearly that the transatlantic disjunction in the genus *Ornithopus* is a natural phenomenon rather than a result of man-influenced migration and naturalization of Old World plant in South America. The South American species *O. micranthus* differs from Old World species in several significant morphological features and is supposed to be segregated in a monotypic section. Unlikely such a rapid morphological evolution occurred in post-Columbus time. Some morphological diagnostic features of *O. micranthus* should be regarded as plesiomorphies. Pollen morphology data are important because they indicate relationship of *O. micranthus* to N. American genus *Hosackia*. The basal position of *O. micranthus* on molecular trees also argues against a hypothesis of man-influenced migration from the Old World.

The following most simple scenarios can be proposed to explain transatlantic disjunction in *Ornithopus*. (1) *Ornithopus* was widely distributed in the past, with the area probably covering North America. (2) Long distance dispersal event from S. America to Old World or from Old World to S. America has taken place. It is not possible to select one of the two scenarios without data of fossil *Ornithopus*. As far as we know, no clear data on fossil Loteae has been published.

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Addresses of the authors:

Galina V. Degtjareva,
 Dr. Carmen M. Valiejo-Roman,
 Dr. Tahir H. Samigullin
 Department of Evolutionary Biochemistry
 A.N.Belozersky Institute of Physicochemical Biology
 Moscow State University
 Moscow, 119992
 Russia
 E-mail: degavi@genebee.msu.su
 vallejo@genebee.msu.su
 samigul@belozersky.msu.ru

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Dr. Tatiana E. Kramina,
Dr. Dmitry D. Sokoloff
Higher Plants Department
Biological Faculty
Moscow State University
Moscow, 119992
Russia
E-mail: kramina@herba.msu.ru
sokoloff@dds.srcc.msu.ru

Dr. Evgeny M. Mironov
Department of Botany
Biological Faculty
Lipetsk State Pedagogical University
42, Lenin str.,
398020, Lipetsk
Russia
E-mail: mironovz@mail.ru
(Present address: 26, 13, Lenin str., 398050, Lipetsk, Russia)

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