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# On generic rank and phylogenetic relationships of *Dorycnopsis* Boiss. (Leguminosae, Loteae)

by

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## Abstract

Nuclear ribosomal ITS sequence data as well as morphological data show that *Dorycnopsis gerardii* (L.) Boiss. can not be placed in the genus *Anthyllis* L. The genus *Dorycnopsis* Boiss. includes two species, *D. gerardii* and *D. abyssinica* (A. Rich.) V.N. Tikhom. et D.D. Sokoloff (= *Vermifrufrux abyssinica* (A. Rich.) J.B. Gillett). Morphological similarity between *Dorycnopsis gerardii* and *Anthyllis onobrychioides* Cav. might be best explained by evolutionary parallelism. *Anthyllis* (including *Hymenocarpus* Savi but excluding *Dorycnopsis* and the monotypic *Tripodion* Medik.) is well-resolved as a highly supported monophyletic group in analyses of nrITS data set.

**Keywords:** *Anthyllis*, *Dorycnopsis*, nrITS, phylogeny, *Vermifrufrux*.

## Resumen

Datos sobre la secuencia de ITS ribosómico nuclear así como datos morfológicos revelan que *Dorycnopsis gerardii* (L.) Boiss. no puede pertenecer al género *Anthyllis* L. El género *Dorycnopsis* Boiss. incluye dos especies, *D. gerardii* y *D. abyssinica* (A. Rich.) V.N. Tikhom. et D.D. Sokoloff (= *Vermifrufrux abyssinica* (A. Rich.) J.B. Gillett). La similitud morfológica entre *Dorycnopsis gerardii* y *Anthyllis onobrychioides* Cav. encuentra su explicación en un paralelismo evolutivo. *Anthyllis* (incluyendo a *Hymenocarpus* Savi, pero excluyendo a *Dorycnopsis* y al monotípico *Tripodion* Medik.) se considera, a partir del análisis del nrITS, un grupo monofilético con un buen apoyo estadístico.

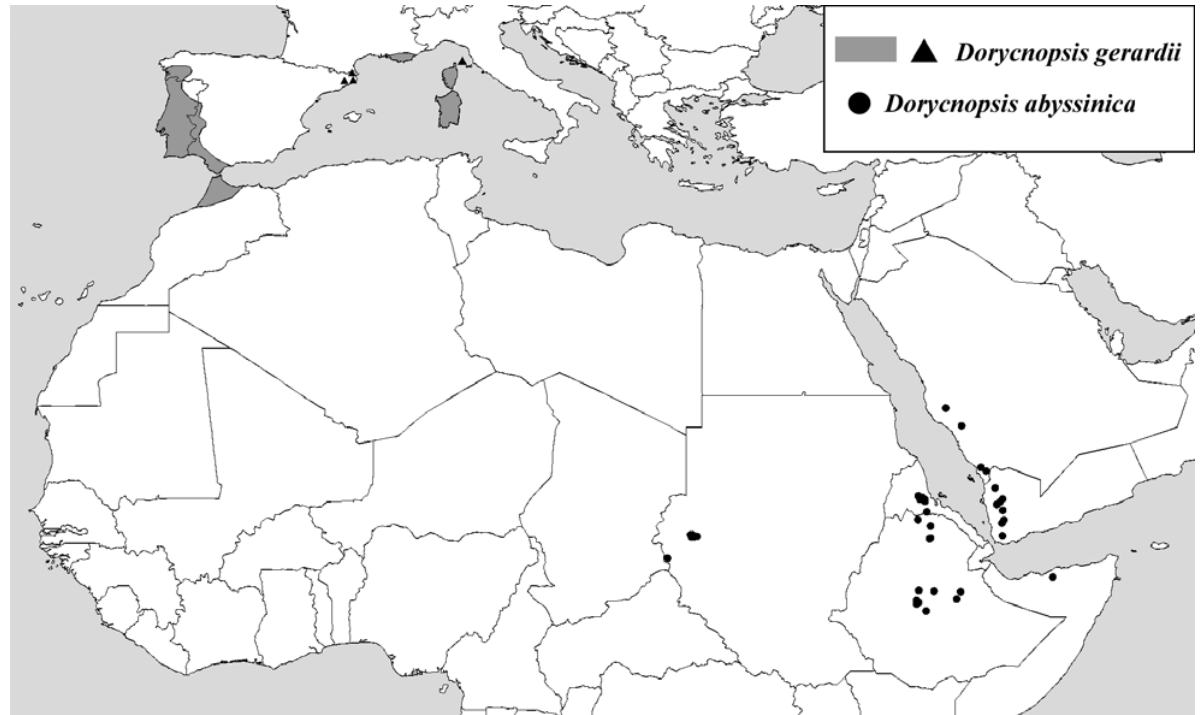
**Palabras clave:** *Anthyllis*, *Dorycnopsis*, nrITS, filogénia, *Vermifrufrux*.

## Introduction

The genus *Dorycnopsis* was described by Boissier (1840). He accepted *Dorycnopsis gerardii* (L.) Boiss. as the only species of the genus. Boissier considered two names as synonyms of *D. gerardii*, namely *Anthyllis gerardii* L. and *Anthyllis onobrychioides* Cav. Most other studies have accepted these two species as distinct. *Anthyllis onobrychioides* was always placed in *Anthyllis* while the other species was placed either in *Anthyllis* (Bentham & Hooker, 1865; Nyman, 1878-1882; Taubert, 1894; Cullen, 1976; Polhill, 1981; Bolòs & Vigo, 1984; Akulova, 1985, 1986) or in the monotypic *Dorycnopsis* (Willkomm, 1880; Hutchinson, 1964; Greuter & al., 1989; Lassen, 1989; Polhill, 1994; Benedí González, 1998; Díaz Lifante, 2000). *Anthyllis* (*Dorycnopsis*) *gerardii* is distributed in the

western and central part of the Mediterranean region (Spain, Portugal, Morocco, S France, islands of Italy – Fig. 1) while *A. onobrychioides* is restricted to Spain.

Tikhomirov & Sokoloff (1997) have accepted two species of *Dorycnopsis*, *D. gerardii* and *D. abyssinica* (A. Rich.) V.N. Tikhom. et D.D. Sokoloff. The latter species has previously been placed in the monotypic genus *Vermifrufrux* J.B. Gillett, as *V. abyssinica* (A. Rich.) J.B. Gillett (Gillett, 1966; Lock, 1989; Thulin, 1989, 1993; Lock & Simpson, 1991; Polhill, 1994). *Dorycnopsis abyssinica* occurs in Sudan, Eritrea, Ethiopia, N Somalia, Yemen and W Saudi Arabia (Fig. 1), i.e., far away from the distribution areas of *D. gerardii*, *A. onobrychioides* and most other *Anthyllis* species. The genus *Anthyllis* in our opinion (Sokoloff, 2003a) includes 23 species in Europe (except for the extreme northeast and southeast), Madeira, northern and east-



**Fig. 1.** Distribution ranges of *Dorycnopsis gerardii* (after Bolòs & Vigo, 1984) and *D. abyssinica* (original, based on herbarium specimens from Kew (K) and localities given by Schweinfurth, 1896).

ern Africa, and western Asia. Most *Anthyllis* species occur in the western Mediterranean area. The only *Anthyllis* species that co-occurs with *D. abyssinica* in Ethiopia and Eritrea is *A. vulneraria* s.l. However, the main part of the distribution area of this latter species lies in Europe, W Asia, and N Africa.

López González (2004) emphasized the substantial morphological similarity between *Anthyllis onobrychioides* and *Dorycnopsis gerardii*. In his opinion, these species differ mainly in flower colour and inflorescence morphology. He considered these differences as insufficient to maintain the genus *Dorycnopsis* as distinct from *Anthyllis* and included *D. gerardii* in *Anthyllis*. Consequently, he concluded that *Vermifruux* should be retained as a separate genus noting that molecular phylogenetic data by Allan & al. (2003) showed no close relationship between *Vermifruux abyssinica* and the genus *Anthyllis*. Allan & al. (2003) presented a phylogenetic analysis of nuclear ribosomal ITS data of more than 80 species of *Loteae*, including *Vermifruux abyssinica* and *Anthyllis onobrychioides*. In a maximum parsimony analysis of nrITS sequences, these species do not group together: *A. onobrychioides* is closely related to other *Anthyllis* species while *Vermifruux* grouped with *Coronilla*, although with only low bootstrap support. In Neighbour-Joining and Bayesian analyses *Vermifruux*

does not group with *Coronilla*; it is also not resolved as a relative of *Anthyllis* (Allan & al., 2003; Degtjareva & al., 2003). These data clearly show that *Vermifruux abyssinica* cannot be placed in *Anthyllis*. However, the type species of *Dorycnopsis*, *D. gerardii*, has not yet been included in molecular phylogenetic analyses of *Loteae* (e.g., Allan & Porter, 2000; Kropf & al., 2002; Allan & al., 2003; Degtjareva & al., 2003; Nanni & al., 2004). Therefore, we have decided to produce and analyse nrITS sequences of *Dorycnopsis gerardii* to resolve its taxonomic position.

## Materials and methods

Complete sequences of ITS1, ITS2 and the 5.8S region were generated for four accessions of *Dorycnopsis gerardii* and one accession of *D. abyssinica*. In addition, nrITS and 5.8S sequences were produced for four species of *Anthyllis*. Voucher information is presented in Table 1. Total genomic DNA was isolated from leaf tissue (in one case from fruit material) using the CTAB method of Doyle and Doyle (1987). PCR reactions were performed with universal primers (White & al. 1990). The sequence data were obtained through direct sequencing of double-stranded templates on an ABI 310 automatic sequencer. Both spacer regions

**Table 1.** GenBank accession numbers of sequences used and voucher information for newly produced sequences.

Species (in brackets, name used in the original publication)	GenBank number	First publication of the sequence
<i>Acmispon americanus</i> (Nutt.) Rydb. [= <i>Lotus unifoliolatus</i> (Hook.) Benth.]	AF450183	Allan & 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 & al. (2003)
<i>Anthyllis aurea</i> Welden	AF450219	Allan & al. (2003)
<i>Anthyllis barba-jovis</i> L.	AF450214	Allan & al. (2003)
<i>Anthyllis circinnata</i> (L.) D.D. Sokoloff [= <i>Hymenocarpus circinnatus</i> (L.) Savij]	AF218504	Allan & Porter (2000)
<i>Anthyllis cornicina</i> L. Voucher: Spain, Guadalupe, 12 Jul. 1977, A. Segura Zubizarreta 15.044 (LE).	DQ340286	This paper
<i>Anthyllis cytisoides</i> L. Voucher: Spain, Alicante, 16 Mai 1980, A. Charpin & al. 16023 (LE).	DQ340285	This paper
<i>Anthyllis hamosa</i> Desf. Voucher: Spain, Estremadura, 13 Apr. 1985, K.U. Kramer 9039 (Z).	DQ340287	This paper
<i>Anthyllis hermanniae</i> L.	AF450213	Allan & al. (2003)
<i>Anthyllis lagascana</i> Benedi Voucher: Algeria, Dept. Médéa, 2 May 1971, U. Hofmann 57 (Z).	DQ340288	This paper
<i>Anthyllis lotoides</i> L.	AF450216	Allan & al. (2003)
<i>Anthyllis montana</i> L.	AF450218	Allan & al. (2003)
<i>Anthyllis onobrychioides</i> Cav.	AF450210	Allan & al. (2003)
<i>Anthyllis ramburei</i> Boiss.	AF450217	Allan & al. (2003)
<i>Anthyllis tejedensis</i> Boiss.	AJ315511	Kropf & al. (2002)
<i>Anthyllis terniflora</i> (Lag.) Pau	AF450212	Allan & al. (2003)
<i>Anthyllis vulneraria</i> L.	AF218499	Allan & Porter (2000)
<i>Coronilla coronata</i> L.	AY325281	Degtjareva & al. (2003)
<i>Coronilla minima</i> L. s.l.	AF450232	Allan & al. (2003)
<i>Coronilla orientalis</i> Mill. [= <i>Securigera orientalis</i> (Mill.) Lassen]	AF450230	Allan & al. (2003)
<i>Coronilla scorpioides</i> (L.) W.D.J. Koch	AY325280	Degtjareva & al. (2003)
<i>Coronilla varia</i> L. [= <i>Securigera varia</i> (L.) Lassen]	AF218537	Allan & Porter (2000)
<i>Cytisopsis pseudocytisus</i> (Boiss.) Fertig	AY325282	Degtjareva & al. (2003)
<i>Dorycnopsis abyssinica</i> (A. Rich.) V.N. Tikhom. & D.D. Sokoloff Voucher: Ethiopia, Prov. Begemdir, 15 Oct. 1968, Nievergell 1032 (Z).	DQ340281	This paper
<i>Dorycnopsis gerardii</i> (L.) Boiss. Vouchers: (1) France, Corsica, 13 June 1972, C. Simon s.n. (H); (2) Spain, Avila, 30 June 1979, A. Segura Zubizarreta 18.110 (H); (3) Spain, Gerona, 25 June 1974, P. Litzler 74/955 (Z); (4) fruit material that was received in early 1990s from Jardim Botânico da Universidade de Lisboa	DQ340283 DQ340284 DQ340282 DQ005954	This paper This paper This paper This paper
<i>Hammatolobium kremerianum</i> (Coss.) C. Muell.	AF450233	Allan & al. (2003)
<i>Hammatolobium lotoides</i> Fenzl	AY325279	Degtjareva & al. (2003)
<i>Hebestigma cubense</i> (Kunth) Urban	AF450157	Allan & al. (2003)
<i>Hippocrepis emerus</i> (L.) Lassen	AF218531	Allan & Porter (2000)
<i>Hippocrepis unisiliquosa</i> L.	AF450238	Allan & 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 & al. (2003)
<i>Lotus angustissimus</i> L.	AF450185	Allan & al. (2003)
<i>Lotus australis</i> Andrews	AF450187	Allan & al. (2003)
<i>Lotus campylocladus</i> Webb & Berth.	AF450196	Allan & al. (2003)
<i>Lotus conimbricensis</i> Brot.	AF450186	Allan & 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 & al. (2003)
<i>Lotus ornithopodioides</i> L.	AF450205	Allan & 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	Degtjareva & al. (2003)
<i>Ornithopus perpusillus</i> L.	AF450226	Allan & al. (2003)
<i>Ornithopus pinnatus</i> (Mill.) Druce	AY325278	Degtjareva & al. (2003)
<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 & 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)

were sequenced in their entirety on both strands. Details of DNA extraction, PCR amplification (including primer locations and characteristics), and DNA purification and sequencing strategies used are the same as described by Valiejo-Roman & al. (2002).

The newly generated nrITS and 5.8S sequences were analysed together with a number of GenBank accessions (see Table 1). In some cases we have changed the nomenclature used in the original sequence publications. We here use generic limits and nomenclature of Loteae as proposed by Sokoloff (2003a). The data set used for the present analysis includes most genera of tribe Loteae and all taxa that were indicated as relatives of *Anthyllis*, *Dorycnopsis* and *Vermifruux* by different authors. Members of Robinieae (*Robinia* and *Hebestigma*) and Sesbanieae (*Sesbania*) were used as outgroups.

DNA sequences were aligned using CLUSTAL W 1.75 (Thompson & al., 1994) and corrected manually using the SED editor of the VOSTORG phylogenetic analysis package (Zharkikh & al., 1990). The alignment is available from the authors on request. Maximum parsimony analysis involved a heuristic search conducted with PAUP\* 4.08b (Swofford, 2000) using TBR branch swapping, options mulpars, steepest descent, collapse, and acctran selected, with character states specified as unordered and equally weighted. 1000 replicates with random addition of sequences were performed and all shortest trees were saved. All gaps were treated as missing data. Bootstrap values were calculated from 100 replicate analyses with random addition sequence of taxa. 1000 most parsimonious trees from each replicate were saved. The GTR+I+ $\Gamma$  model of nucleotide substitution was selected by the AIC in Modeltest (Posada & Crandall, 1998). Maximum likelihood distances were computed using the selected model of molecular evolution. Distance trees were calculated using the Neighbour-Joining method as implemented in PAUP\*. 1000 bootstrap resamplings were performed; insertions and deletions were not taken into account. Bayesian inference of phylogeny was explored using the MrBayes program version 3.1 (Huelsenbeck & Ronquist, 2001). The GTR+I+ $\Gamma$  model of evolution was used.  $5 \times 10^6$  generations were performed; trees from the first  $4 \times 10^6$  generations were discarded. The number of generations to be discarded was determined using a convergence diagnostic.

## Results

All four newly produced nrITS and 5.8S sequences of *D. gerardii* are identical. These sequences are de-

rived from material from different parts of the species range (Portugal, Spain and Corsica). The new sequence of *D. abyssinica* (based on material from Ethiopia) is identical to the sequence produced by Allan & al. (2003) using material from Yemen. Therefore, *D. gerardii* and *D. abyssinica* represent single terminals in our trees.

The length of the ITS1 region ranges from 226 to 233 bp in *Anthyllis*. It is 236 bp long in *D. gerardii* and 226 bp long in *D. abyssinica*. The length of the ITS2 region ranges from 201 to 214 bp in *Anthyllis*. It is 221 bp long in *D. gerardii* and 213 bp long in *D. abyssinica*. The length of the 5.8 S region ranges from 164 to 165 bp in *Anthyllis*. It is 165 bp long in *D. gerardii* and 164 bp long in *D. abyssinica*. Pairwise comparisons of percentage sequence divergence of the entire ITS region among species of *Anthyllis* and *Dorycnopsis* are summarised in Table 2. The sequence divergence between *D. abyssinica* and *D. gerardii* is 11%. Sequence divergence among species of *Anthyllis* ranges between 0% (*A. hermanniae* vs. *A. lagascana*) and 19.1% (*A. vulneraria* vs. *A. circinnata*). Sequence divergence between species of *Anthyllis* and *Dorycnopsis* ranges between 21.6% (*A. hamosa* vs. *D. abyssinica*) and 38% (*A. vulneraria* vs. *D. gerardii*).

Numerous indels were introduced in the sequences during alignment, mainly in regions of ambiguous alignment. Such positions together with adjacent bases were excluded from analysis. The alignment of all 60 ITS sequences after exclusion of 220 ambiguous positions resulted in a matrix of 603 nucleotide positions. Of these, 306 positions are parsimony-informative, 247 invariable, and 50 variable but parsimony-uninformative. In the maximum parsimony analysis, 492 shortest trees (1631 steps) are found, with a consistency index of 0.41 and a retention index of 0.69.

A strict consensus of all shortest trees is shown in Fig. 2. *Anthyllis* (*sensu* Sokoloff 2003a, i.e., including *Hymenocarpos* Savi. but excluding *Dorycnopsis* and *Tripodion*) is well-resolved as a highly supported monophyletic group. *Anthyllis* subgen. *Cornicina* (= gen. *Hymenocarpos*) is not resolved as monophyletic in the strict consensus tree (Fig. 2) but forms a clade with 63% bootstrap support in the majority-rule bootstrap consensus tree (not shown). Subgenus *Terniflora* is monophyletic while subgen. *Barba-Jovis* again is not resolved as monophyletic. A clade combining these two subgenera is sister to *A. vulneraria* (subgen. *Anthyllis*). All these relationships within *Anthyllis* have bootstrap support not exceeding 80% (Fig. 2). *Dorycnopsis gerardii* and *Dorycnopsis* (*Vermifruux*) *abyssinica* are highly supported as a single clade. No close relationship between *Dorycnopsis*

**Table 2.** Pairwise comparisons of percentage sequence divergence of the entire ITS region among species of *Anthyllis* and *Dorycnopsis*.

	D.abys	D.ger	A.ono	A.ram	A.tej	A.aur	A.mon	A.bar	A.her	A.lag	A.ter	A.cyt	A.vul	A.lot	A.cir	A.cor	A.ham
<i>D.abyssinica</i>	0																
<i>D.gerardii</i>	11	0															
<i>A.onobrychioides</i>	23.9	32.5	0														
<i>A.ramburei</i>	23.8	31.8	0.2	0													
<i>A.tejedensis</i>	23.4	32.8	1	1.2	0												
<i>A.aurea</i>	24.3	31.1	3.3	3.5	3	0											
<i>A.montana</i>	24.3	32.9	2.9	3.1	2.6	2	0										
<i>A.barba-jovis</i>	24.1	32.6	2.7	2.9	2.4	1.9	1.5	0									
<i>A.hermanniae</i>	24.5	32.6	3.9	4.1	3.2	2.6	1.2	2.4	0								
<i>A.lagascana</i>	24.4	32.7	3.7	3.9	3	2.4	1	2.2	0	0							
<i>A.terniflora</i>	26.8	34.7	3.4	3.6	3.6	3.9	3.4	3.3	4.4	4.2	0						
<i>A.cytisoides</i>	22.8	31	3.2	3.4	3.4	4	3.4	3.6	4.5	4.3	0.6	0					
<i>A.vulneraria</i>	30.5	38	14.8	15.1	14.9	16.6	16	15.4	16.3	16	18.2	16.6	0				
<i>A.lotoides</i>	23.8	31.6	8.1	8.3	8.3	8.1	7	6.6	8.2	7.8	8.6	8.6	17.7	0			
<i>A.circinnata</i>	25.7	33.9	10.8	11	10.8	11.1	9.9	9.3	11.2	10.7	11.2	10.2	19.1	6.3	0		
<i>A.cornicina</i>	23.3	32.2	7.8	8.1	7.2	7.9	6.8	6.3	7.5	7.1	8.4	8.1	16.3	1.2	5.7	0	
<i>A.hamosa</i>	21.6	32.1	8.5	8.8	8.3	8.5	7.4	7	8.6	8.2	9.1	8.4	16.1	3.1	5.6	2.5	0

(incl. *Vermifruux*) and *Anthyllis* is found (Fig. 2). Maximum parsimony resolves a clade comprising *Dorycnopsis* (incl. *Vermifruux*), *Ornithopus* L., *Kebirita* Kramina & D.D. Sokoloff and the American genera *Hosackia* Benth., *Ottleya* D.D. Sokoloff, *Acmispon* Rafin. and *Syrmatium* Vogel. Relationships among these groups are largely unresolved.

Tree topology and bootstrap values found in the Neighbour-Joining analysis are similar to those found in the maximum parsimony analysis (Fig. 2). However, monophyly of subgen. *Cornicina* received higher bootstrap support in the Neighbour-Joining analysis (92%).

The Bayesian tree (Fig. 3) is generally similar to the trees inferred from the maximum parsimony and Neighbour-Joining analyses. Most important differences include: (1) *Dorycnopsis* (incl. *Vermifruux*) is resolved as sister to a clade comprising the American genera *Ottleya*, *Acmispon* and *Syrmatium* (posterior probability 0.79); (2) *Anthyllis vulneraria* (subgen. *Anthyllis*) is embedded within a clade comprising members of subgenera *Barba-Jovis* and *Terniflora*; (3) monophyly of *Anthyllis* subgen. *Cornicina* (= gen. *Hymenocarpos*) and its sister-group relationship to the rest of *Anthyllis* are well supported (posterior probabilities 1.00).

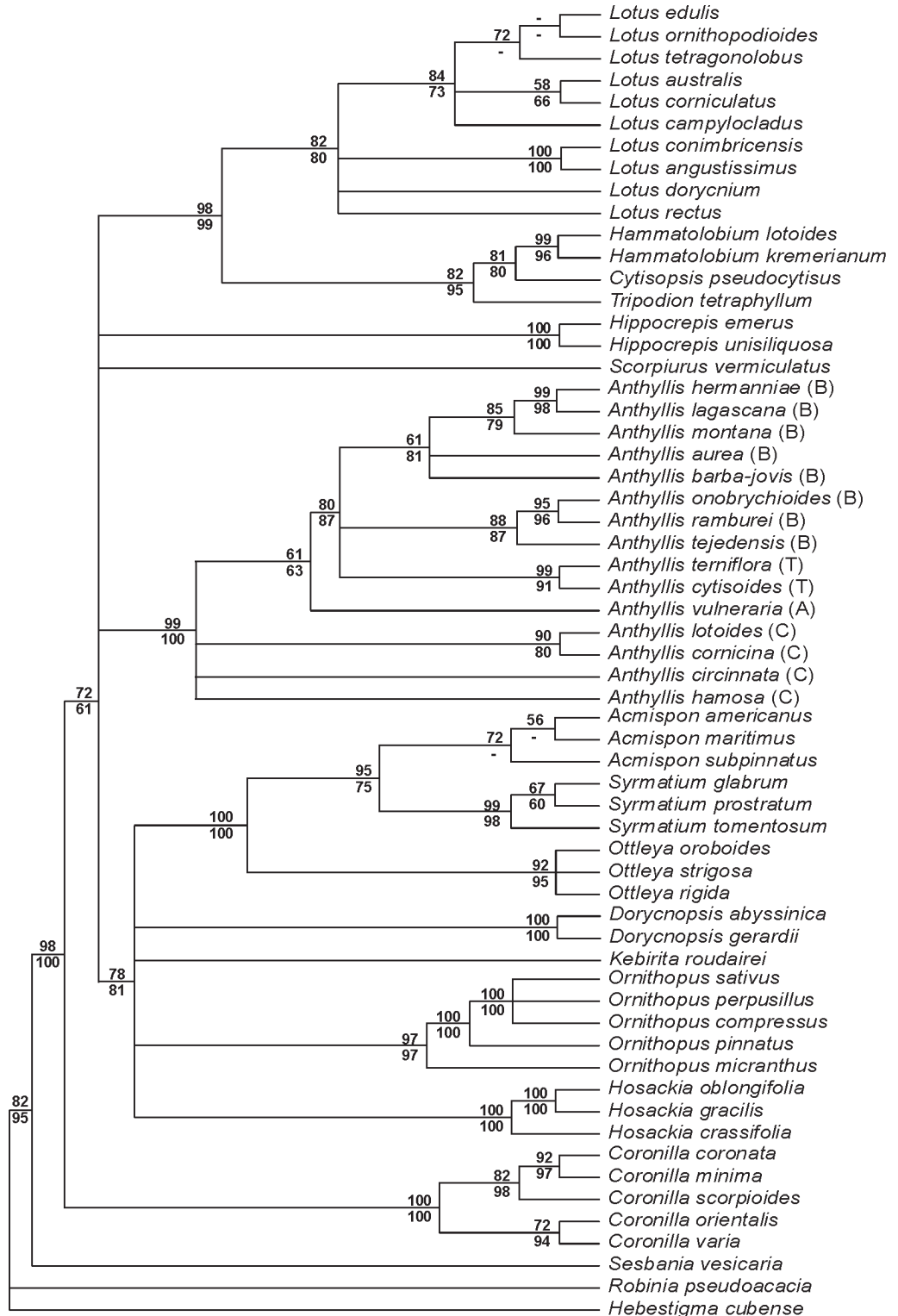
## Discussion

Our molecular phylogenetic analyses show monophyly of both *Anthyllis* (incl. *Hymenocarpos*) and *Dorycnopsis*

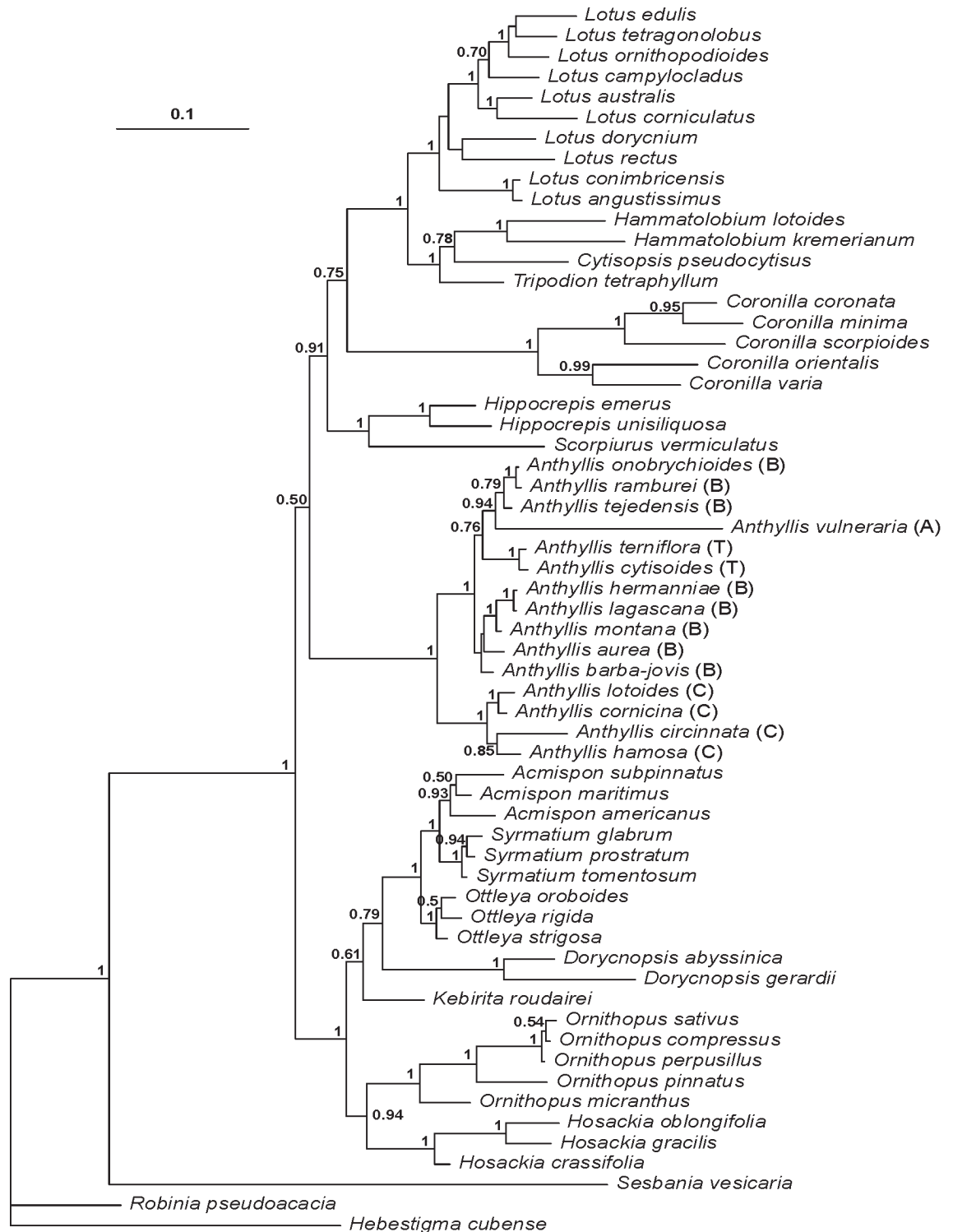
(incl. *Vermifruux*). A morphological cladistic analysis also showed monophyly of these two genera, but the morphological data suggested that *Dorycnopsis* is closely related to *Anthyllis* (Sokoloff, 2003a,b; see also Sokoloff, 2006).

Our nrITS data clearly show that *Dorycnopsis gerardii* cannot be placed in the genus *Anthyllis*. The molecular data are also consistent with the placement of *Vermifruux* into synonymy of *Dorycnopsis*. In our opinion, *D. abyssinica* and *D. gerardii* are morphologically so similar to each other (Table 3) that it is not reasonable to maintain two monotypic genera, *Dorycnopsis* and *Vermifruux*. It is obvious that characters such as leaflet morphology, flower number per umbel, calyx teeth and standard blade length are not significant at the generic level. Basic chromosome numbers of  $2n = 14$  and  $2n = 12$  co-occur within Loteae (e.g., within *Lotus* and *Anthyllis*). There exist some differences in pollen morphology between the two species of *Dorycnopsis*, but this difference does not exceed variation found among species of *Anthyllis* (see Díez & Ferguson, 1990, 1994).

The fruits of *D. abyssinica* are morphologically very different from those of *D. gerardii*. However, fruits of *D. gerardii* and *D. abyssinica* share the same anatomical structure (Sokoloff, 1997; Tikhomirov & Sokoloff, 1997). In details of fibre orientation pattern, fruits of *Dorycnopsis* are very different from those of *Anthyllis*. In tribe Loteae, only members of *Ornithopus* have the same fibre orientation pattern as found in *Dorycnopsis*



**Fig. 2.** Strict consensus of 492 trees (1631 steps) derived from a maximum parsimony analysis of ITS sequence data. Numbers above branches are maximum parsimony bootstrap values, numbers below branches are Neighbour-Joining bootstrap values. Only bootstrap values above 50% are shown. Letters in parentheses indicate subgenera of *Anthyllis* according to Sokoloff (2003a) as follows: (A) = subgen. *Anthyllis*; (B) = subgen. *Barba-Jovis* V.N. Tikhom. & D.D. Sokoloff; (C) = subgen. *Cornicina* (DC.) Akulova ex V.N. Tikhom. & D.D. Sokoloff; (T) = subgen. *Terniflora* V.N. Tikhom. & D.D. Sokoloff.



**Fig. 3.** Relationships among taxa as determined by Bayesian inference. Posterior probabilities above 0.5 are shown. Branch lengths are proportional to the number of expected nucleotide substitutions; scale bar corresponds to 1 substitution per 10 sites. Letters in parentheses indicate subgenera of *Anthyllis* according to Sokoloff (2003a) as follows: (A) = subgen. *Anthyllis*; (B) = subgen. *Barba-Jovis* V.N. Tikhom. & D.D. Sokoloff; (C) = subgen. *Cornicina* (DC.) Akulova ex V.N. Tikhom. & D.D. Sokoloff; (T) = subgen. *Terniflora* V.N. Tikhom. & D.D. Sokoloff.



**Table 3.** Morphological differences between *Dorycnopsis abyssinica* and *Dorycnopsis gerardii*.

Character	<i>Dorycnopsis abyssinica</i>	<i>Dorycnopsis gerardii</i>
Leaflet shape	oblanceolate to obovate	oblanceolate to almost linear
Adaxial leaflet surface	usually pubescent	usually glabrous
Flower number per umbel	(2)4-6	many
Calyx length	about 2 mm	2.5-3 mm
Calyx teeth	shorter than tube	slightly longer than tube
Standard blade	slightly shorter than the claw, with clear basal auricles	slightly longer than the claw, with unclear basal auricles
Fruit diameter	4-5 mm	2-3 mm
Fruit shape	strongly curved	straight
Seeds per fruit	two	one
Pollen ornamentation at mesocolpium (Diez & Ferguson, 1990, 1994)	striate regulate	psilate, perforate and fossulate
Chromosome number (Fernandes & Santos, 1971; Spellenberg & Ward, 1988)	2n = 14	2n = 12

(Degtjareva & al., 2003). According to molecular phylogenetic data, *Dorycnopsis* is more closely related to *Ornithopus* than to *Anthyllis* (Degtjareva & al., 2003; this study). Main differences between fruits of *D. abyssinica* and *D. gerardii* are seed number (two vs. one) and fruit curvature. It is necessary to stress that in both species there are two ovules in the ovary (Tikhomirov & Sokoloff, 1997). Thus the difference in seed number is not relevant for identifying phylogenetic relationships. In some groups of *Loteae* (e.g., the North American *Syrmatium*) one- and few-seeded fruits as well as straight and strongly curved fruits also co-occur. We believe that all this argues for the broad circumscription of *Dorycnopsis* as proposed here.

Sequence divergence data among species of *Anthyllis* and *Dorycnopsis* (Table 2) also support a broad circumscription of *Dorycnopsis*. Sequence divergence between the two species of *Dorycnopsis* is much smaller than the maximum sequence divergence among *Anthyllis* species. Many authors suggest to segregate members of subg. *Cornicina* (*Anthyllis circinnata*, *A. hamosa*, *A. cornicina*, and *A. lotoides*) as the separate genus *Hymenocarpos* (e.g., Lassen, 1986; Greuter & al., 1989; Benedí González, 1998). Maximum ITS sequence divergence among species of *Anthyllis* s.str. (excl. *Hymenocarpos*) is 18.2% (*A. vulneraria* vs. *A. terniflora*), i.e., more than between the two species of *Dorycnopsis*. The nrITS sequence of *A. vulneraria* strongly differs from all other *Anthyllis* species included in the present study. The sequence divergence data suggest that *A. vulneraria* should also be placed in a separate genus if *Hymenocarpos* is segregated from *Anthyllis*. However, this would not be the best

choice from a nomenclatural point of view because *A. vulneraria* is the type species of *Anthyllis*. It is more logical to place *Hymenocarpos* into synonymy of *Anthyllis*. Our data (in contrast to those of Nanni & al., 2004) show close relationships between all species that were segregated in *Hymenocarpos*. However, in a strict consensus of all shortest trees (Fig. 2), these species do not form a clade. This is another argument for placing all these species in *Anthyllis* rather than in a separate genus *Hymenocarpos*.

Both molecular and morphological data clearly show that *Anthyllis onobrychioides* is a member of *Anthyllis*. Its ostensible similarity with *Dorycnopsis gerardii* might be best interpreted as a result of parallel evolution. *Anthyllis onobrychioides* is the type of *Anthyllis* sect. *Dorycnioides* DC. that belongs to subg. *Barba-Jovis* (Tikhomirov & Sokoloff, 1996). Section *Dorycnioides* was often regarded as monotypic (e.g., Willkomm, 1880; Akulova, 1985, 1986). In our opinion (Sokoloff, 2003a), section *Dorycnioides* also includes *A. tejedensis* Boiss., *A. polycephala* Desf., *A. podocephala* Boiss., *A. warnieri* Emb., *A. ramburei* Boiss., and *A. rupestris* Coss. These species were previously often associated with *A. montana* L. and placed in section *Oreanthyllis* Griseb. However, *A. montana* differs from these species in having sessile partial inflorescences, a different bract structure, reduced stipules as well as other characters. We believe that *A. montana* should be placed within a monotypic section *Oreanthyllis* (Sokoloff, 2003a). Although much more work needs to be done to understand the phylogeny of *Anthyllis*, current molecular phylogenetic data tend to support this conclusion. Three of

seven species of section *Onobrychioides* (*sensu* Sokoloff, 2003a) are included in the present study (*A. onobrychioides*, *A. ramburei*, and *A. tejedensis*). They form a clade with 88% bootstrap support (Fig. 2).

In general, we believe that nrITS and morphological data are highly congruent in tribe Loteae. In particular, both morphological (Sokoloff, 2003a,b, 2006; Arambarri & al., 2005; Degtjareva & al., 2006) and molecular (Degtjareva & al., 2003, 2006; present study) cladistic analyses support the monophyly of all genera of Loteae (as defined by Sokoloff, 2003a). Molecular and morphological data for the two species of *Dorycnopsis* also are congruent. The main diagnostic character of *D. gerardii*, one-seeded fruit, is an apomorphic character state. *Dorycnopsis gerardii* also has the basic chromosome number of  $x = 6$ , which is a derived condition in Loteae (Goldblatt, 1981; Degtjareva & al., 2006). Therefore, *D. gerardii* was placed after *D. abyssinica* in the classification system of Loteae (Sokoloff, 2003a,b). Also, *D. gerardii* occupies a longer branch in the molecular phylogram than *D. abyssinica* (Fig. 3).

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