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Phylogenetic relationships of *Thlaspi* s.l. (subtribe Thlaspidinae, Lepidieae) and allied genera based on chloroplast DNA restriction-site variation

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Abstract Chloroplast DNA restriction-site variation was analyzed in 30 accessions representing 20 species from the major lineages in *Thlaspi* s.l. (previously described as genera by Meyer 1973, 1979) and allied genera from the subtribe Thlaspidinae (*Peltaria, Teesdalia, Cochlearia, Ionopsidium, Aethionema*). A total of 161 variable restriction sites were detected. Phylogenetic analyses indicated a division of *Thlaspi* s.l. into three groups consistent with Meyer's genera *Thlaspi* s. str., *Microthlaspi* and *Noccaea/Raparia*. The genus *Thlaspi* s.l. as currently described proved to be paraphyletic because one of its major lineages, i.e. *Thlaspi* s. str., appeared to be more closely related to other genera (*Peltaria, Teesdalia*) than to the remaining lineages of *Thlaspi* s.l., i.e. *Noccaea/Raparia* and *Microthlaspi*. Sequence divergence values (100 × p) between the *Thlaspi* s.l. lineages were similar to values between these groups and related genera (*Teesdalia, Peltaria*), respectively. Chloroplast DNA variation was also used to assess subtribal classification of the genera studied. The cpDNA data were inconsistent with the controversial taxonomic classifications based on morphology. The molecular data would suggest that (1) the subtribe Thlaspidinae, as traditionally described, is not monophyletic; (2) the Thlaspidinae should be reduced to a group consisting of *Thlaspi* s. str., *Peltaria, Teesdalia, Microthlaspi, Noccaea/Raparia*, and that *Aethionema* should be excluded from the Thlaspidinae; and (3) *Cochlearia* and *Ionopsidium* represent the subtribe *Cochleariinae*.

Key words *Thlaspi* · Subtribe Thlaspidinae · Brassicaceae · Chloroplast DNA · Restriction-site variation · Molecular systematics

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Introduction

Thlaspi L. s.l. is the largest genus of the subtribe Thlaspidinae (tribe Lepidieae) and comprises approximately 75 species (Al-Shehbaz 1986). This genus is primarily defined by fruit characters (fruit an angustiseptate silicule, the valves keeled and usually winged; locules containing 2–6, rarely 1–10, seeds). Several controversial infrageneric classifications have been proposed, mainly based on fruit characters (reviewed in Mummenhoff and Koch 1994). Meyer (1973, 1979) questioned the naturalness of the genus *Thlaspi* s.l.. By analyzing the anatomy of the seed testa, *Thlaspi* s.l.. was split into 12 segregate genera; the differences between them were considered too great to warrant their subordination, as sections or subgenera, to a single, broadly defined genus (Meyer 1973, 1979). This concept, however, was not followed by other authors (Hedge 1976; Al-Shehbaz 1986; Greuter et al. 1986; Schultze-Motel 1986). Recently we have studied *Thlaspi* s.l. by isoelectric-focusing (IEF) analysis of Rubisco subunits (Mummenhoff and Zunk 1991) and cpDNA restriction-site analysis (Mumenhoff and Koch 1994). The major splits in *Thlaspi* s.l. were strongly confirmed relative to the taxa studied and they correspond to Meyer's segregates *Thlaspi* s.str, *Microthlaspi* und *Noccaea* (including *Raparia*). Chloroplast DNA sequence divergence between these groups was higher than that usually found in intrageneric analyses and comparable to levels of divergence between related genera of other angiosperm families (Mummenhoff and Koch 1994). Nevertheless, critical evaluation of the taxonomic status of these segregates should await the analysis of related genera such as *Aethionema, Peltaria* and *Teesdalia*.

Tribal, subtribal, and even generic, boundaries in the Brassicaceae are often arbitrarily drawn and, therefore, these taxa may often not reflect natural groups (Hedge 1976; Al-Shehbaz 1984). Likewise, there is little agreement among the various morphologically based classifications as to the limits of the Thlaspidinae (Hayek 1911;

Schulz 1936; Janchen 1942; Al-Shehbaz 1986). This subtribe clearly consists of the core genera *Thlaspi* s.l., *Ionopsidium*, *Teesdalia*, *Bivonaea*, and a variable number of mostly monotypic genera distributed by different authors among several subtribes of the tribe Lepidieae and even among other tribes. A more natural classification of the Thlaspidinae would perhaps be achieved by grouping closely related species and genera and working upward, using an alternative approach to the morphologically based taxonomies.

In the Brassicaceae, cpDNA studies have already contributed to a clarification of genetic relationships among *Brassica* and allied genera (Warwick and Black 1991; Warwick et al. 1992), *Arabidopsis* and related genera (Price et al. 1994), and in *Thlaspi* and *Lepidium* (Mummenhoff and Koch 1994; Mummenhoff et al. 1995). A major strength of cpDNA analysis is that it provides numerous, independent molecular characters that can often rigorously define monophyletic lineages (Sytsma 1990). This is important when working with plant groups, e.g. taxa of Brassicaceae that apparently show morphological convergence and parallelism (Dvorak 1971; Meyer 1973; Avetisian 1983). For instance, in *Thlaspi* s.l. fruit shape is particularly convergent (Meyer 1979, 1991), although it has been used previously for infrageneric classification in *Thlaspi* s.l.. Analysis of these non-molecular characters in such groups can easily suggest misleading phylogenetic relationships (Sytsma 1990). The objectives of the present study are:

(1) to test the hypothesis of monophyly in *Thlaspi* s.l. by comparative restriction-site mapping of the cpDNA of *Thlaspi* s.l. and related genera, and in the process (2) to compare the subtribal taxonomies of Hayek (1911), Schulz (1936) and Janchen (1942), relative to the taxa studied.

Materials and methods

Plant material

We have examined cpDNA variation in 20 species (30 accessions) from three major lineages within *Thlaspi* s.l. representing four of Meyer's segregates (Meyer 1973) and from genera assumed to be more or less related to *Thlaspi* s.l. depending on the analysis of different authors. The taxa included in this study are given in Table 1. Information on the origin of plant material can be provided by the authors on request. Voucher specimens are deposited at OSBU.

Molecular methods

Young fresh leaves were usually harvested from plants grown in the greenhouse. In some cases DNA was extracted from field-collected leaves dried and preserved with silica gel (Chase and Hills 1991). Total cellular DNA from individual plants was isolated from 0.5 to 2.0 g of leaf material by a modified CTAB method (Doyle and Doyle 1987), including 2% PVP in the extraction buffer, organic extraction with phenol/chloroform/isoamyl alcohol (25:24:1, v/v/v), and chloroform/isoamyl alcohol (24:1, v/v). DNA-precipitation was carried out with sodium acetate (pH 4.8) and 2-propanol. DNA was digested with ten restriction endonucleases (BRL, Eurogentec, Boehringer) recognizing six-base-pair sequences: *Bgl*I, *Eco*RV, *Hin*dIII, *Kpn*I, *Pst*I, *Pvu*II, *Sca*I, *Sst*I, *Stu*I and *Xho*I. Restriction fragments were separated electrophoretically on 0.4–0.6% agarose gels and transferred to nylon filters (Pharmacia-LKB). Filters were sequentially probed with 19 clones and five subclones representing nearly the entire chloroplast genome of *Brassica juncea* (L.) Coss. Clones were kindly provided by J. Palmer (University of Indiana), R. Price (University of Georgia) and S. Warwick und C. Black (Biosystematic Research Center, Ottawa), respectively. Clone number and size are shown in Warwick and Black (1991, Fig. 1). Preparations of digoxigenin-labelled probe DNAs, filter hybridizations, filter decolorization and probe removal all followed the manufactur's instructions (Boehringer Mannheim) with the modifications described by Mummenhoff and Koch (1994).

Data analysis

Restriction-site maps were constructed for all taxa under study relative to clones of the *Brassica juncea* chloroplast genome. Presence and absence of restriction sites were scored as a 1/0 data matrix. The restriction-site maps and the 1/0 data matrix are available from the authors upon request. To test for non-random structure in the data, the frequency distribution of the lengths of 10000 trees randomly selected by PAUP (version 3.1.1; random trees option, Swofford 1991) from the set of all possible trees was evaluated for left-handed skewness (Hillis and Huelsenbeck 1992). Cladograms were generated from synapomorphic restriction sites using Wagner parsimony in PAUP. Heuristic searches for the most parsimonious trees were conducted by using a simple addition sequence of taxa followed by TBR branch swapping, and employing 100 random addition sequences, followed by the TBR branch-swapping algorithm.

Trees were rooted by the outgroup method. Because taxa included in the present study have been classified by some authors within other subtribes of Lepidieae, and even other tribes, we decided to use more remote outgroups than by simply choosing taxa from other subtribes of the Lepidieae. The morphological and *rbc*L analyses of Rodman (1991) and Rodman et al. (1993), respectively, all indicate that the Brassicaceae is well nested within the core group of capparalean families, and that the Brassicaceae is most closely related to the clemoid subfamily of the Capparaceae, from which it may have been derived (Rodman et al. 1993; Price et al. 1994). The New World tribe Thelypodieae was considered by some to be the most primitive tribe in the Brassicaceae and many authors have postulated that the subfamily Cleomideae (Capparaceae) is the direct progenitor of the Brassicaceae though the intermediate link of the Thelypodieae (for review see Hayek 1911; Hedge 1976; Hauser and Crovello 1982; Al-Shehbaz 1985). This provides justification for the use of members of the Capparaceae and of the tribe Thelypodieae as outgroups in phylogenetic studies of the Lepidieae.

In addition to equal weighting of character states in Wagner parsimony, the data were also analyzed by weighted parsimony (Albert et al. 1992). Character-state weighting was implemented using the step-matrix options of PAUP. Implementation followed recommendations in Albert et al. (1992); costs of site gains over site losses were 1.5:1, 1.3:1, or 1.1:1. Restriction-site data for all mapped enzymes were also used to calculate $100 \times p$ values (Nei and Li 1979, equation 9 and 10), an estimate of nucleotide-sequence divergence between species. The neighbor joining (NJ) method (PHYLIP, version 3.4) was used to construct distance dendrograms from the resulting sequence-divergence matrices (Table 1). Bootstrap analysis (Felsenstein 1985) was performed to determine statistical support for the groups inferred to be monophyletic. One hundred bootstrap replications were run in PAUP with heuristic search options.

Results and discussion

Comparative cpDNA restriction-site mapping allowed us to recognize 259 restriction sites in the 24 taxa surveyed. We detected 161 variable sites (62.2%) of which 103 were synapomorphic and 58 were autapomorphic. No differences in cpDNA restriction-site patterns were found among different accessions of

the species studies, with the exception of *Microthlaspi perfoliatum*. Our recent analysis (Mummenhoff and Koch 1994) of eight populations suggested that "northern" accessions from Germany (*M. perfoliatum*N) have a common plastome type different from the plastomes of populations south of this region (*M. perfoliatum*S; Switzerland, France, Italy). In the current study only two accessions each from the "northern" and "southern" plastome types were included (Fig. 1).

Phylogenetic information content in the data set was estimated by the skewness coefficient (g_1; Hillis and Huelsenbeck 1992). The random search of 10000 of all possible trees produced a highly left-handed skewed ($g_1 = -0.713$) frequency distribution of tree lenghts, indicating significant ($P < 0.01$) non-random structure in the data (Hillis and Huelsenbeck 1992). Initially, phylogenetic analyses were performed using outgroup species from the tribe Thelypodieae (*Streptanthus glandulosus*, *Caulanthus cooperi*) and from the related family Capparaceae (*Capparis spinosa*). However, the resulting trees (data not shown) did not provide support for the monophyly of the Thaspidinae as traditionally described. This was due to the occurrence of many shared-site mutations between the Thelypodieae and some members of the Thaspidinae. Therefore we omitted members of the Thelypodieae as outgroup taxa from subsequent analyses.

Wagner parsimony analysis (heuristic searches) identified 168 equally parsimonious trees with lengths of 189, a consistency index (ci) of 0.55 (without autapomorphies), and a retention index (ri) of 0.77. Variable topologies at the base of the trees, within *Thlaspi* s. str., *Noccaea*, *Ionopsidium*, and the variable position of *Teesdalia* were responsible for the large number of most parsimonious trees. Differentially weighted parsimony analysis has been proposed as a method for analyzing restriction-site data that is preferable to Wagner and Dollo parsimony (Albert et al. 1992; Holsinger and Jansen 1993; Olmstead and Palmer 1994). Justification for weighted parsimony analysis is based on the unrealistic assumption regarding restriction-site evolution required by Wagner parsimony (all changes equally likely) and Dollo parsimony (a site can be gained only once) (Olmstead and Palmer 1994). The three different character-state weights used in the present study gave results that differed only in the relative placement of *Teesdalia*, *Ionopsidium acaule* and variable topologies within *Noccaea*. However, in contrast to the Wagner trees, basal branching was consistent in all weighted parsimony topologies. Weighting at a gain : loss cost ratio of 1.1 : 1

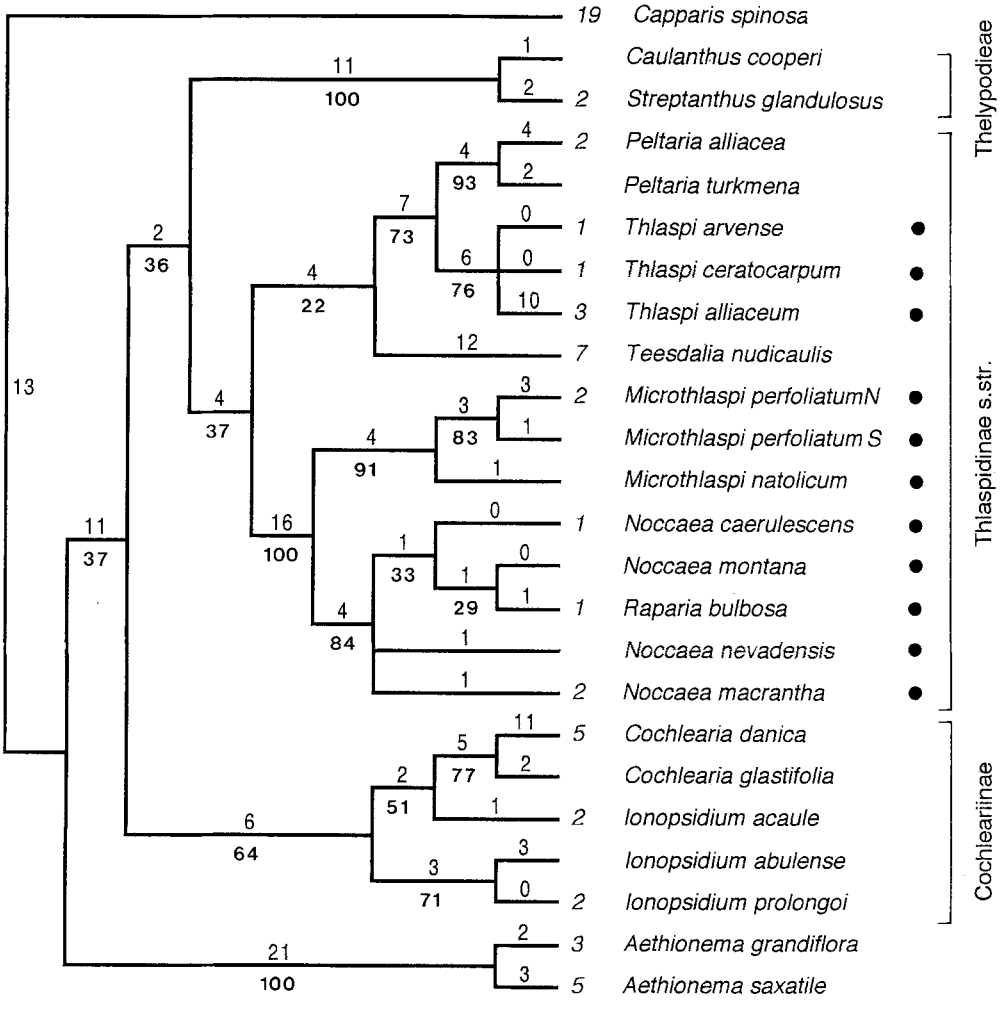

Fig. 1 One of 168 equally parsimonious Wagner tree for *Thlaspi* s.l. and related genera based on cpDNA data. This topology was also identified as among the four shortest trees using character state weighting at a gain loss cost ratio of 1.3 : 1 and 1.5 : 1. Tree length is 189, consistency index 0.55 (without autapomorphies). *Numbers above* the branches refer to the numbers of mutational steps, *numbers below* the branches are percent probability values obtained with bootstrap analysis. Autapomorphies (mutations unique to a given taxon) are indicated at the end of terminal branches. Taxa traditionally described as *Thlaspi* s.l. are marked with a *black dot*. *Capparis spinosa* (Capparaceae) served as the outgroup. For further explanation refer to Results and discussion

identified a single most-parsimonious tree (also among the shortest Wagner trees) with *Teesdalia* as a sister to the *Microthlaspi* and *Noccaea/Raparia* clade, and *Ionopsidium acaule* as a sister to *Cochlearia*. A cost ratio of 1.3:1 and 1.5:1 identified the same four trees (also among the most-parsimonious Wagner trees) only differing in variable topologies within *Noccaea* and the relative position of *Ionopsidium acaule*. In two of these, *I. acaule* is placed as a sister to *Cochlearia* (Fig. 1) whereas the other two trees placed it as next basal to *I. abulense* and *I. prolongoi*. The 1.1 weight topology was very similar to 1.3 and 1.5 weight trees, but it differed from all these in removing the *Teesdalia* plastome from its position as a sister taxon to the *Peltaria/Thlaspi* s. str. clade and placing it as the next basal plastome to the *Microthlaspi, Noccaea/Raparia* clade. The NJ tree (data not shown) constructed from distance matrices included many of the same groups identified as clades by parsimony analyses. The NJ tree was nearly identical in topology with two of the four shortest 1.3 and 1.5 weight trees just mentioned, placing *I. acaule* as a sister to the remainder of *Ionopsidium*; differences among these two parsimony trees and the NJ topology involved the placement of *Raparia bulbosa* and *Noccaea* taxa relative to one another.

Bootstrap analysis (Felsentein 1985) was used to estimate the confidence to be placed in groups inferred to be monophyletic. Recently it was suggested that the narrow application of the bootstrap to accept or reject hypotheses of monophyly on the basis of 95% confidence levels (Felsenstein 1985) is too strict (Hillis and Bull 1993). Using computer simulations and a laboratory generated phylogeny, Hillis and Bull (1993) demonstrated that, for a variety of conditions, clades represented by bootstrap values of > 70% have a high probability of being real. Many clades of our phylogenetic trees are supported by bootstrap values > 70% and, therefore, appear to be genuinely monophyletic (Fig. 1). Some basal clades are characterized by bootstrap proportions < 50% (Fig. 1) apparently due to the lack of characters and/or high rates of homoplasy. In this context it may be noted that, at higher taxonomic levels, few cpDNA characters were found to support basal clades and increased levels of homoplasy were observed (Jansen et al. 1990; Bruneau and Doyle 1993). Furthermore these "uncertain" basal clades with bootstrap proportions < 50% (Fig. 1) were observed in our analyses with > 80% consistency in the rival Wagner trees and with 100% consistency in the weighted parsimony trees and in the NJ tree.

Thlaspi s. str. versus *Thlaspi* s.l.

As outlined in the introduction section, the classification of the genus *Thlaspi* s.l. is difficult and controversial (Al-Shehbaz 1986; Mummenhoff and Koch 1994). Our recent phylogenetic restriction-site analysis of cpDNA from 22 representatives from all sections within *Thlaspi* s. l., as defined by Schulz (1936) and Ball et al. (1993), indicated three distinct lineages that are congruent with the respective genera of Meyer (1973, 1979), i.e. *Thlaspi* s. str., *Microthlaspi*, and *Noccaea* with *Raparia* included (Mummenhoff and Koch 1994, Fig. 1) These three genera embrace the bulk of species formerly classified in *Thlaspi* s.l.. Although cpDNA sequence divergence (100 × p values) between these lineages was higher than usually found in intrageneric analyses the evaluation of the taxonomic status of the segregates was not possible mainly because none of the related genera of *Thlaspi* s. l. were studied (Mummenhoff and Koch 1994). The present analysis includes representatives from four of Meyer's segregates and related genera such as *Aethionema, Teesdalia* and *Peltaria*. Chloroplast DNA divergence values between these major lineages of *Thlaspi* s.l. were similar to values between these groups and related genera such as *Teesdalia* and *Peltaria*, respectively (Table 1). The results of our phylogenetic cpDNA analysis clearly indicate that *Thlaspi* s.l. as traditionally delimited is paraphyletic because one of its major lineages, *Thlaspi* s. str., is more closely related to *Peltaria* and *Teesdalia* than to the other lineages, i.e. *Microthlaspi* and *Noccaea* (Fig. 1). This conclusion is not without parallels in the Brassicaceae. The cpDNA studies of Warwick and Black (1990), Warwick et al. (1992) and Price et al. (1994) apparently suggest that *Brassica, Diplotaxis* and *Arabidopsis*, respectively, are also highly unnatural groups in their current delimitation. The distinctiveness of *Thlaspi* s. str. versus the other *Thlaspi* s.l. lineages in respect of seed testa characters has already been commented on by Meyer (1973, 1979). We also recognized the greatest differences between *Thlaspi* s. str. and the other *Thlaspi* s.l. lineages; *Thlaspi* s. str. differs by at least 37 cpDNA mutations from *Microthlaspi, Noccaea* and *Raparia* (Fig. 1). All species of *Thlaspi* s. str. show no abnormal tendency to take up nickel or zinc, whereas abnormal metal-accumulation potential was reported from other lineages, e.g. *Noccaea* and *Raparia* (Reeves 1988).

The results of our cpDNA studies provide compelling evidence for recognizing *Thlaspi* s. str. as a distinct genus, with *Peltaria* as its closest relative. The cpDNA data would also appear to favour the inclusion of *Raparia* within the genus *Noccaea*, and *Microthlaspi* could easily be treated at the subgeneric level. The genera mentioned above all share common ancestry, and *Teesdalia* also appears to belong in this lineage. Taxonomic evaluation of the remaining segregates of Meyer (1973) was not performed because (1) these taxa are distributed in the Middle East (Turkey, Kurdistan, Caucasus, Azerbaidjan, Armenia, Iran) where sampling is not possible at the moment, and (2) these taxa were not available from botanical gardens or collections.

Subtribal classification

The clades common to all cpDNA parsimony analyses suggest several conclusions about generic groupings.

Table 1 Estimated divergence among chloroplast DNAs of Brassicaceae taxa under study. Sequence divergence values are given as 100 × p (Nei and Li 1979). Abbreviations for the taxa are given below[a]

	CAP	CCO	STG	AEG	AES	COD	COG	IOA	IOB	IOP	PEA	PET	THV	THC	THA	TEN	MPN	MPS	MNA	NOC	NOM	NOV	NOA	RAB
CAP	0																							
CCO	2.93	0																						
STG	3.10	0.28	0																					
AEG	3.67	3.20	3.23	0																				
AES	3.56	3.37	3.40	0.77	0																			
COD	3.18	2.50	2.67	3.05	3.36	0																		
COG	2.48	1.48	1.65	2.91	3.08	1.07	0																	
IOA	2.77	1.42	1.59	2.93	2.97	1.45	0.59	0																
IOB	2.55	1.13	1.37	2.86	2.95	1.75	0.73	0.54	0															
IOP	2.77	1.30	1.59	3.07	3.24	1.71	0.71	0.59	0.30	0														
PEA	3.08	1.82	1.99	3.32	3.50	3.09	1.86	2.00	1.64	2.00	0													
PET	2.98	1.61	1.78	2.85	3.02	2.63	1.45	1.59	1.24	1.59	0.48	0												
THV	2.98	1.30	1.59	3.24	3.36	2.75	1.64	1.58	1.35	1.58	0.96	0.77	0											
THC	2.95	1.29	1.58	3.30	3.33	2.81	1.63	1.57	1.34	1.57	0.95	0.76	0.11	0										
THA	3.35	1.94	2.24	3.50	3.67	2.99	2.17	2.11	1.88	2.11	1.60	1.52	0.83	0.83	0									
TEN	3.41	2.12	2.29	3.56	3.73	2.77	2.23	2.17	2.09	2.17	2.05	1.97	2.02	2.01	2.04	0								
MPN	3.90	2.34	2.63	3.96	4.12	3.10	2.45	2.39	2.37	2.39	2.69	2.33	2.13	2.12	2.67	2.59	0							
MPS	3.67	2.12	2.41	3.59	3.76	2.88	2.22	2.17	2.14	2.17	2.33	1.98	1.91	1.90	2.44	2.63	0.33	0						
MNA	3.50	1.95	2.25	3.42	3.59	2.71	2.05	1.99	1.97	1.99	2.16	1.81	1.74	1.73	2.40	2.59	0.50	0.28	0					
NOC	3.50	2.04	2.21	3.44	3.48	2.94	2.27	2.21	2.19	2.21	2.36	2.01	1.94	1.92	2.61	2.67	1.03	0.69	0.63	0				
NOM	3.44	1.98	2.15	3.39	3.42	2.89	2.21	2.16	2.13	2.16	2.30	1.95	1.88	1.87	2.56	2.61	0.97	0.63	0.58	0.06	0			
NOV	3.38	2.05	2.22	3.33	3.36	2.83	2.16	2.10	2.07	2.10	2.25	1.89	1.82	1.81	2.50	2.56	0.91	0.57	0.52	0.11	0.06	0		
NOA	3.58	2.24	2.41	3.53	3.56	3.02	2.35	2.29	2.26	2.29	2.44	2.08	2.01	1.99	2.69	2.75	1.09	0.75	0.69	0.29	0.23	0.17	0	
RAB	3.53	2.05	2.22	3.47	3.50	2.83	2.16	2.10	2.07	2.10	2.38	2.02	1.95	1.94	2.63	2.69	1.15	0.81	0.76	0.23	0.17	0.23	0.41	0

[a] CAP = *Capparis spinosa* L.; CCO = *Caulanthus cooperi* (S. Wats.) Payson; STG = *Streptanthus glandulosus* Hook.; AEG = *Aethionema grandiflorum* Boiss. & Hohenack; AES = *A. saxatile* (L.) R. Br.; COD = *Cochlearia danica* L.; COG = *C. glastifolia* L.; IOA = *Ionopsidium abulense* (Pau) Rothm.; IOB = *I. acaule* (Desf.) Reichenbach; IOP = *I. prolongoi* (Boiss.) Batt.; PEA = *Peltaria alliacea* Jacq.; PET = *P. turkmena* Lipsky; THV = *Thlaspi alliaceum* L.; THC = *T. ceratocarpum* (Pall.) Murr.; THA = *T. arvense* L.; TEN = *Teesdalia nudicaulis* (L.) R. Br.; MPN = *Microthlaspi perfoliatum* (L.) F.K. Meyer - northern plastome type; MPS = *M. perfoliatum* (L.) F.K. Meyer - southern plastome type; MNA = *M. natolicum* (Boiss.) F.K. Meyer; NOC = *Noccaea caerulescens* (J. & C. Presl) F.K. Meyer; NOM = *N. macranthum* (Lipsky) F.K. Meyer; NOV = *N. nevadensis* (Boiss. & Reut.) F.K. Meyer; NOA = *N. montana* (L.) F.K. Meyer; RAP = *Raparia bulbosa* (Spruner) F.K. Meyer

There was good agreement between one of our plastome clades (Fig. 1) and genera (*Peltaria*, *Teesdalia*, *Thlaspi* s. str., *Microthlaspi*, and *Noccaea/Raparia*) that have been recognized by Hayek (1911) as being related. However, Hayek (1911) included *Cochlearia* in the Thlaspidinae, which is placed on the cpDNA tree along with *Ionopsidium* in a separate clade (Fig. 1). *Cochlearia* was treated by Schulz (1936) and Janchen (1942) as core group of the subtribe Cochleariinae whereas *Ionopsidium* was included by both authors and Hayek (1911) in the Thlaspidinae. The cpDNA data demonstrate close relationships among both genera.

Only taking into account the taxa under study, the cpDNA data would suggest that the subtribe Thlaspidinae should perhaps be reduced to the core genera *Thlaspi* s. str., *Peltaria*, *Teesdalia*, *Microthlaspi* and *Noccaea/Raparia*, whereas *Cochlearia* and *Ionopsidium* seem to represent the subtribe Cochleariinae. Chemosystematic data also give support to this grouping. Genera of the subtribe Thlaspidinae, as delimited here, are characterized by their main seed oil components, i.e. monosaturated fatty acids longer than C_{18}. *Teesdalia* produces high amounts of eicosenoic acid (20:1) and the remaining taxa accumulate erucic acid (22:1); *Microthlaspi* additionally shows remarkable amounts of nervonic acid (24:1) (Kumar and Tsunoda 1980; Avetisian and Fursa 1990). These fatty acids are very uncommon in the tribe Lepidieae (otherwise accumulating linoleic acid $C_{18:3}$) including *Aethionema* and *Cochlearia* (Kumar and Tsunoda 1980; Avetisian and Fursa 1990).

Our cladistic analysis of cpDNA variation suggests that the subtribe Thlaspidinae, as currently or traditionally recognized, is not monophyletic. The plastomes of two members (*Caulanthus*, *Streptanthus*) of the tribe Thelypodieae surveyed were placed as a sister clade to the core genera of Thlaspidinae, recognized here as Thlaspidinae s. str. This result was stable in both Wagner and weighted parsimony analyses. The non-basal position of Thelypodieae is an unexpected result as relationships among the Capparaceae and Thelypodieae have often been suggested on the basis of similarities in floral morphology (Hayek 1911; Hauser and Crovello 1982; Al-Shehbaz 1984). Instead, *Aethionema* turned out as a sister group to all the other taxa studied and, therefore, should be excluded from the Thlaspidinae (Fig. 1). These results are confirmed by recent analyses. Our preliminary sequence studies of the ITS region of the nuclear ribosomal DNA (unpublished data), as well as *rbc*L sequence studies (Price et al. 1994), also demonstrate *Aethionema* as a sister group to other Brassicaceae, using the Capparaceae as an outgroup. Except for Hayek (1911), who assumed *Aethionema* to be a member of the subtribe Iberidinae, later authors (Schulz 1936; Janchen 1942) placed *Aethionema* in the subtribe Thlaspidinae; Al-Shehbaz (1986) even advocated that *Aethionema* is most closely related to *Thlaspi* s.l.. But the problem really is where does *Aethionema*, a genus of about 60 species, belong. One could speculate that *Aethionema*, and perhaps other genera so far not included in our studies, represents an evolutionary lineage in the Brassicaceae, that may have diverged from other lineages very early in the evolution of the family. However, hypotheses on the origin of and evolution within the Brassicaceae are beyond the scope of the present study and certainly should await broad morphological and molecular-based cladistic studies.

Plant breeding and phylogenetic analysis

In the search for renewable oils to replace mineral oils, plant breeders have attempted to develop Brassicaceae oilcrops with a high content of long-chain fatty acids (i.e. nervonic acid, $C_{24:1}$, typical of *Microthlaspi perfoliatum*) by somatic hybridization techniques (Fahleson et al. 1994). However, evaluating the possibilities and limitations of somatic hybridization within the Brassicaceae requires a knowledge of the phylogenetic relatedness between the species utilized (Fahleson et al. 1994). The cpDNA analysis of Warwick and Black (1991) in *Brassica* and allied genera and the present cpDNA study in the Thlaspidinae provided new insights into phylogenetic relationships. From such investigations it should be possible to estimate the degree to which genetic divergence limits the production of somatic hybrids (Fahleson et al. 1993).

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