Redefined generic limits of Carum (Umbelliferae, Apioideae) and new systematic placement of some of its taxa

Authors: Ekaterina A. Zakharova, Galina V. Degtjareva, and Michael G. Pimenov

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Redefined generic limits of *Carum* (*Umbelliferae, Apioideae*) and new systematic placement of some of its taxa

Abstract


A phylogenetic analysis of nuclear ribosomal DNA internal transcribed spacer (nrDNA ITS) sequences of the species of *Carum* (*Umbelliferae, Apioideae*) in its traditional circumscription is provided. The sampling includes also a wide spectrum of taxa across subfamily *Apioideae*, to identify the relationships of the various disparate elements so far united in *Carum*. The molecular analysis has been supplemented by an analysis of fruit and petal morphology of several critical species of *Carum* and beyond. *Carum* in its traditional interpretation is confirmed to be polyphyletic; it includes at least seven independent evolutionary lines. *C. carvi*, which provides the type of the generic name, and only four Caucasian and Near Eastern species, *C. caucasicum, C. meifolium, C. grossheimii, C. porphyrocoleon*, make up *Carum* s.str., while their relationship to *Grammosciadium, Chamaesciadium* and *Fuernrohria* in the same clade needs further studies. The rare Caucasian *Carum komarovii* as well as the W Asian *Pimpinella antherscoides* and the Japanese *Chamaele decumbens* are closely related to and united here with *Aegopodium*. *Carum verticilatum*, for which a sister group relationship to the *Oenantheae* clade is revealed, is restored as the monotypic genus *Trocdaris*. The previous transfer of the S Siberian and Middle Asian *Carum atrosanguineum* to *Vicatia* is confirmed.

Additional key words: *Apiaceae, Aegopodium, Pimpinella, Tamamschjanella, Trocdaris*, taxonomy, systematics, molecular phylogenetics, nrITS, fruit anatomy, petal morphology

Introduction

At present, generic classification and relationships within the tribe *Apiieae* in its traditional circumscription (*Amineae* in Drude’s classification of *Umbelliferae* subfam. *Apioideae*, Drude 1897–98) have remained complicated and largely unresolved. According to molecular phylogenetic analyses in the *Apiieae*, the larger genera appear nearly all non-monophyletic (Downie & al. 2001, 2010). In the corresponding classifications of the *Apiieae*, the larger genera appear members of the so-called “apioid superclade”, which is analogous to the *Apiae* of the Drude and post-Drude morphological classifications. *Carum* L. is one of these larger, traditional genera and was established at an early stage of scientific plant systematics, primarily on the basis of common European species. The nomenclatural type of *Carum* is *C. carvi* L. (caraway), the only Linnean species of the genus. Later, numerous species from different countries and continents were described within or attributed to *Carum*, because of similarities in the general habit and a rather simple fruit structure, mainly characterised by a glabrous pericarp with almost equal ribs and solitary or coupled vascular vittae. Today, about 200 validly published species names are known in *Carum*, according to the International Plant Names Index (IPNI 2012). However, further
morphological investigations showed the genus to be largely polyphyletic and many species already have been transferred from *Carum* to other, sometimes new genera, after more detailed morphological analysis (Boissier 1872; Drude 1897–98; Korovin 1927; Wolff 1927; Handel-Mazzetti 1933; Kljuykov 1985, 1986). *Carum*, in its narrower sense includes 22–25 (Drude 1897–98; Wolff 1927) to 30 (Mabberley 1987; Pimenov & Leonov 1993) species, which are distributed in Europe, Africa, Asia and North America; *C. carvi* is widely cultivated. A broader concept of *Carum*, with dozens of genera merged (Koso-Poljansky 1916; Hiroe 1979) does not seem to be popular in current *Umbelliferae* systematics. The accepted number of *Carum* species varies in different Floras: USSR (Schischkin 1950: 10 species), Europe (Tutin 1968: 5 species), Caucasus (Tamamschjan 1967: 8 species), Turkey (Hedge & Lamond 1972: 5 species).

Comparative carpological studies (Zakharova 2010) are available for the majority of the currently accepted species (except for some N African ones). According to these, several European and E Asian species (*C. heldreichii* Boiss., *C. appuanum* (Viv.) Grande, *C. verticillatum* (L.) W. D. J. Koch, *C. diversifolium* (DC.) C. B. Clarke) had been demonstrated to be morphologically alien to *Carum*; of these *C. diversifolium* was shown to be member of *Trachyspermum* Link.

Also molecular phylogenetic studies by Papini (2006), Papini & al. (2007) and Degtjareva & al. (2009) indicated that *Carum* is polyphyletic, although their samplings usually included only few species of the genus. Downie & al. (2010) in their classification based on the phylogenetic analyses of nrDNA sequences, classified the diverse elements of *Carum* in their two, newly circumscribed tribes *Careae* (including the generitype) and *Pyramidoptereae*.

The objective of the present study has been (1) to assess through a molecular phylogenetic analysis of nrITS sequences the extent of polyphyly of *Carum* in its current circumscription; (2) to identify, supplemented by an analysis of the fruit and petal morphology of several critical taxa, the relationships of the various elements of current *Carum*; (3) to provide a revised generic classification of the elements to be excluded from *Carum*. The species included in our sampling represent current *Carum* in its entire geographical range except N Africa.

**Materials and methods**

**Taxon sampling for the molecular phylogenetic analyses** — A total of 41 new sequences of the nuclear ribosomal DNA internal transcribed spacer (nrDNA ITS) were generated for this study, including 30 sequences for 16 taxa currently classified in or not unanimously excluded from *Carum*, and 6 sequences for *Aegopodium* species, one sequence for *Chamaele*, three sequences for species of *Pimpinella*, and one for *Chamaesciadium*. In the light of the apparent polyphyly of *Carum*, the initial set of samples was increased, based on previous studies (Papini 2006; Papini & al. 2007; Spalik & Downie 2007; Degtjareva & al. 2009; Downie & al. 2008; Downie & al. 2010; Magee & al. 2010; Zhou & al. 2008) and a BLAST search in GenBank, to include representatives of all genera to which closer affinities of species currently classified in *Carum* could be detected. In this way, representatives of the tribes *Careae*, *Pimpinelleae*, *Pyramidoptereae*, *Oenantheae*, *Selinaceae* and of the Physospermopsid clade according to the classification by Downie & al. (2010), were added. A member of the genus *Physospermum*, *P. cornubiense*, was selected as outgroup based on previous higher-level studies (e.g., Valiejo-Roman & al. 1998, Downie & al. 2010). The final sampling included 146 accessions of 134 species from 49 genera (see Appendix).

**DNA isolation, amplification and sequencing** — Total genomic DNA was isolated from fruits and leaf tissue using the NucleoSpin Plant isolation kit ( Macherey-Nagel, Düren, Germany) following the manufacturer’s instructions. PCR amplification and sequencing strategies (including primer locations and characteristics) followed the protocols given previously (Valiejo-Roman & al. 2002). PCR products were purified using the DNA cleaning kit (Evrogen, Moscow, Russia) following the manufacturer’s instructions. Direct sequencing was performed on the ABI PRISM 3100 Genetic Analyzer (Applied Biosystems), with the use of the ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit for cycle sequencing reactions following the manufacturer’s instructions. Forward and reverse strands of all ITS samples were sequenced. The resulting sequences were checked with the CHROMAS 1.45 software (www technelysium com au/chromas.html). The ITS sequences were edited and manually aligned using the BioEdit program, version 5.0.9 (Hall 1999). Sequences of the 5.8S rRNA gene were also obtained but excluded from the analysis because in many published sequences this region is not represented. Gap-rich columns of the alignment were excluded from the analysis in order to avoid tree topology deformation due to misinterpretation of missing characters (Lemmon & al. 2009). The aligned data matrices are available upon request from the corresponding author.

**Molecular phylogenetic analyses** — Maximum Parsimony (MP) analysis was carried out with PAUP® (version 4.0b8, Swoford 2000) using the heuristic search with TBR branch swapping, all character states specified as equally weighted and all gaps treated as missing data. One hundred replicates with random addition of sequences were performed and all shortest trees were saved. Bootstrap analysis (Felsenstein 1985) was performed to assess the degree of support for particular branches of the tree. Bootstrap support (BS) values were calculated from 100 replicate analyses with TBR branch swapping and random addition sequence of taxa. One
Fig. 1. Maximum Parsimony (MP) analysis of the ITS dataset; strict consensus tree of 100,000 shortest trees. Major clades are denoted by vertical bars to the right. Numbers above the branches are the bootstrap support values > 50% from MP bootstrapping. Carum species are shown in bold.
Fig. 2. Phylogenetic Maximum likelihood (ML) tree inferred from the ITS dataset with RAxML under a GTR+GAMMA nucleotide substitution model approximation. Major clades are denoted by vertical bars to the right. Branch lengths are proportional to the number of the expected nucleotide substitutions. Numbers above the branches are the bootstrap support values > 50% from ML bootstrapping. *Carum* species are shown in bold.
thousand most parsimonious trees from each replicate 
were saved. 

Maximum Likelihood (ML) analysis was carried out 
with RAXML (version 7.2.6, Stamatakis 2006) using the 
GTRGAMMA model. The model was selected by the 
Akaike Information Criterion in the Modelftest program 
(Posada & Crandall 1998). Bootstrapping was made us-
ing the rapid bootstrapping algorithm implemented in 
RAXML with 100 replicates. Bootstrap support (BS) 
values were then drawn onto the best-known likelihood 
tree.

Morphological analysis — The material for morphologi-
cal studies had been obtained from the herbaria B, IRK, 
LD, LE, MHA, MW and WU (abbreviations according to 
Thiers 2008+) and is given in the figure captions. Mor-
phological characters significant in Umbelliferae system-
atics (Kljuykov & al. 2004) were studied and compared 
using a reflected-light microscope. Fruit anatomy was 
examined under a light microscope, the sections being 
made in the middle of the mericarps, with a hand razor. 
Before sectioning, the fruits had been kept for three days 
in a mixture of equal parts of glycerine, ethyl alcohol and 
water. The sections were treated with phloroglycine and 
hydrochloric acid and then mounted in glycerine.

Results

ITS data set — To check the infraspecific variability of ITS 
sequences, for some species of the genera Carum L., 
Chamaele Miq., Pimpinella L. and Vicatia DC., samples 
from several populations were sequenced. No infras-
pecific ITS variation has been found in Carum buriati-
cum Turcz., C. carvi, C. graecum subsp. serpentinicum 
Hartvig, C. komarovi Karjag., C. porphyrocoleon (Freyn 
& Sint.) Woronow ex Schischk., Pimpinella anthers-
coides Boiss., P. cruciata Bornm. & H. Wolff, Carum 
atrosguineum Kar. & Kir. (= Vicatia atrosanguinea 
(Kar. & Kir.) P. K. Mukh. & Pimenov) and Carum ver-
ticillatum. Consequently, only a single accession was 
included in the phylogenetic analyses. Different accessions 
of Carum caucasicum (M. Bieb.) Boiss., C. grossheimii 
Schischk., C. heldreichii, C. mefolium (M. Bieb.) Boiss., 
Chamaele decumbens (Thunb.) Makino and Pimpinella 
anthrrhiscoides Boiss., in contrast, possessed slightly dif-
ferent ITS sequences and were therefore subject to the 
phylogenetic analyses.

Among the species investigated, the length of ITS1 
ranges from 209 bp to 221 bp and that of ITS2 from 
209 bp to 225 bp. Seventy three gap-rich columns of the 
alignment were excluded from the analysis. The align-
ment (ITS1+ITS2) comprised 72.8 % parsimony infor-
mative characters.

MP analyses yielded 100 000 shortest trees, with a 
length of 2108 steps (CI = 0.342, RI = 0.808). The strict 
consensus tree is shown in Fig. 1. The ML tree (Fig. 2) is 
remarkably similar to the MP strict consensus tree (Fig. 
l) in recovering the same major groups. Within the ma-

Phylogenetic relationships — Seven major clades have 
been resolved. Our largely representative sampling of the 
species of Carum is found dispersed over five of these 
seven major clades and represents at least seven inde-
pendent evolutionary lines.

Clade A (100 % MP BS; 100 % ML BS) comprises 
the generitype Carum carvi plus C. porphyrocoleon, C. 
caucasicum, C. mefolium and C. grossheimii, as well as 
species of the genera Cha.maesciadium C. A. Mey., Gram-
mosciadium DC. and Fuernrohria K. Koch. All taxa are 
distributed in the Caucasus and adjacent countries.

Clade B (100 % MP BS; 100 % ML BS) comprises 
the genera Aegopodium L. and Chamaele, two species 
of Pimpinella and the rare Transcaucasian Carum koma-
rovii.

Clade C (88 % MP BS; 96 %ML BS) comprises eight 
Carum species scattered among three well supported 
subclades.

Clade D (100 % MP BS; 100 % ML BS) comprises 
27 species of Pimpinella and Arafoe aromatica Pimenov 
& Lavrova, which is sister to the Pimpinella group.

Clade E (100 % MP BS; 100 % ML BS) comprises 
Carum alpinum (M. Bieb.) Benth. and C. saxicolum Al-
bov as well as C. atrosanguineum, which recently has 
been included in Vicatia.

Clade F (84 % MP BS; 99 % ML BS) comprises Ca-
rum verticillatum, being sister to the remainder of this 
clade.

Clade G (96 % MP BS; 100 % ML BS) comprises three 
Pimpinella species, Physospermopsis cuneata 
H. Wolff, Sinolimprichtia alpina H. Wolff and 
Haplo-
phaera phaea Hand.-Mazz.

Morphology and anatomy of fruits — Below we provide 
a detailed description of fruit morphology and anatomy 
as analysed for some critical species:

Carum carvi (Fig. 3A). Fruit of ovoid or ellip-
soid mericarps, with the carpophore bifid to the base; 
mericarps homomorphic, glabrous, 3–3.5 mm long, 
0.8–1.2 mm broad, terete, primary ribs equal, keeled, 
straight, with entire margin; calyx teeth very short or 
obsolete; stylodors low-conical, styles recurved; meri-
carps in transverse section slightly compressed laterally; 
exocarp cells small; commissure narrow, exocarp almost 
reaching carpophore; mesocarp parenchyma not ligni-
fied; vascular bundles compact, situated in primary rib 
bases; vallecular vittae solitary, two on the commissural 
side; rib secretory ducts solitary, small; endocarp not ligni-
fied, its cells tubular; endosperm flat on the commis-
sural side.
Carum verticillatum (Fig. 3B). Fruit of ellipsoid mericarps, with the carpophore bifid in the upper part; mericarps homomorphic, glabrous, 2.5–2.7 mm long, 0.5–0.7 mm broad, primary ribs approximately equal, keeled, straight, with entire margin; calyx teeth very short or obsolete; stylopods low-conical, styles recurved; mericarps in transverse section slightly compressed laterally; commissure narrow, exocarp almost reaching carpophore; mesocarp parenchyma not lignified; vascular bundles compact, situated in primary rib bases; vallecular vittae solitary, two on the commissural side; rib secretory ducts solitary, small; endosperm on the commissural side flat.

Aegopodium podagraria (Fig. 3C). Fruit of ovoid or ellipsoid mericarps, with the carpophore bifid to the base; mericarps homomorphic, glabrous, 3–6 mm long, 1–2 mm broad, terete, primary ribs equal, filiform, straight; calyx teeth obsolete; stylopodia conical, styles recurved; mericarps in transverse section almost rounded; exocarp cells small; commissure narrow, exocarp almost reaching carpophore; mesocarp parenchyma not lignified, with dark yellow content; vascular bundles compact, situated in primary rib bases; vallecular and commissural vittae small and sometimes invisible, 3–5 in each furrow, 4–7 on the commissural side, rib secretory ducts solitary, small; endocarp slightly lignified, its cells rectangular, big; endosperm flat on the commissural side.

Carum komarovii (Fig. 3D). Fruit of ovoid or ellipsoid mericarps, with the carpophore bifid to the base; mericarps homomorphic, glabrous, 4–6 mm long, 1–1.2 mm broad, terete, primary ribs equal, keeled (or short-winged), straight (or sometimes slightly wavy), with entire margin; calyx teeth obsolete; stylopodia conical, styles recurved; mericarps in transverse section almost rounded; exocarp cells small; commissure narrow, exocarp almost reaching carpophore; mesocarp parenchyma not lignified, with dark yellow content; vascular bundles compact, situated in primary rib bases; vittae vallecular and commissural, small and sometimes invisible, 3–5 in each furrow, 4–6 on the commissural side, rib secretory ducts solitary, small; endocarp slightly lignified, its cells rectangular, big; endosperm flat on the commissural side.

Pimpinella anthriscoides (Fig. 3E). Fruit of ovoid or ellipsoid mericarps, with the carpophore bifid to the base; mericarps homomorphic, glabrous, 3–4 mm long, 1–1.5 mm broad, terete, primary ribs equal, filiform, straight, sometimes slightly wavy, with entire margin; calyx teeth obsolete; stylopodia conical, styles recurved; mericarps in transverse section almost rounded; exocarp cells small; commissure narrow, exocarp almost reaching carpophore; mesocarp parenchyma not lignified, with dark yellow content; vascular bundles compact, situated in primary rib bases; vittae vallecular and commissural, small and sometimes invisible, 5–7 in each furrow, 4 on the commissural side, rib secretory ducts solitary, small; endocarp slightly lignified, its cells rectangular, big; endosperm flat on the commissural side.

Chamaele decumbens (Fig. 3F). Fruit of ellipsoid mericarps, with the carpophore bifid to the middle or the base; mericarps homomorphic, glabrous, 2.4–2.6 mm long, 0.7–0.8 mm broad, terete, primary ribs equal, filiform, straight (or sometimes slightly wavy), with entire margin; calyx teeth obsolete; stylopodia low conical, styles recurved; mericarps in transverse section almost rounded; exocarp cells small; commissure narrow, exocarp almost reaching carpophore; mesocarp parenchyma not lignified, with dark yellow content, vittae vallecular and commissural, obsolete in mature fruit; rib secretory ducts obsolete; endocarp slightly lignified, its cells rectangular, extending in radial direction, big; endosperm flat on the commissural side.

Chamaesciadium acaule. Fruit of ovoid mericarps, with the carpophore bifid to the middle; mericarps homomorphic, glabrous, 3.5–4 mm long, 0.9–1.1 mm broad, terete, primary ribs equal, keeled, straight, with entire margin; calyx teeth obsolete; stylopodia flat or
low conical, styles recurved; mericarps in transverse section slightly compressed laterally; exocarp cells small; commissure narrow, almost reaching carpophore; mesocarp parenchyma not lignified; vascular bundles compact, situated in primary rib bases; vittae vallecular and commissural, 2–4 in each furrow, 4 on the commissural side; rib secretory ducts solitary, small, present in all ribs; endocarp not lignified; endosperm flat on the commissural side.

** Morphology of petals —** The number of petal secretory ducts varies in the taxa studied (Fig. 4). Within *Carum* it ranges from 1 to 7 (*C. carvi* has 1 secretory duct, *C. buriaticum* 7, *C. komarovii* 4–5), within *Aegopodium* from 1 to 7 too (*Aegopodium kashmiricum* and *A. tadshikorum* have 6–7 secretory ducts; *A. alpestre* and *A. latifolium* 1 as was noted by Vinogradova (1991), *A. podagraria* has 4–5). *Pimpinella anthriscoides* has 5–7 secretory ducts.

**Discussion**

When compared with the major clades recognised as tribes within *Apioidae* by Downie & al. (2010), our Clade A + Clade B correspond to *Caraeae*, Clade C to the deeply reformed *Pyramidoteraceae*, Clade D to the expanded *Pimpinelleae*, Clade E to the *Selineae*, Clade F to the *Oenantheae*, and the species of Clade G partly to the E Asian *Physospermopsis* clade.

*Caraeae* in its current wide sense is heavily polyphyletic and represents at least seven independent evolutionary lines spread over five of these major *Apioidae* clades. This confirms in a much more detailed way the preliminary findings in previous molecular phylogenetic analyses including members of *Carum* (Papini 2006, Papini & al. 2007, Degtjareva & al. 2009).

**Carum carvi and its allies —** Clade A, containing *Carum carvi*, which corresponds to *Carum* sensu strictissimo, is taxonomically heterogeneous. Besides *C. carvi*, it includes *C. porphyrocoleon*, *C. caucasicum*, *C. meifolium* and *C. grossheimii*, but also representatives of the genera *Chamaesciadium*, *Grammosciadium* and *Fuernrohria*.

As far as the four *Carum* species clustering with *C. carvi* in this clade are concerned, their position and affinity to each other is not surprising. All are distributed in the Caucasus and adjacent territories of the Near East, only *C. carvi* exceeds this range very considerably. In the ML tree, *C. caucasicum*, *C. meifolium*, *C. grossheimii* and *Chamaesciadium acaule* (M. Bieb.) Boiss. form two subclasses. *C. meifolium* clusters with *C. grossheimii*, while *C. caucasicum* with *Chamaesciadium acaule*. Not only are the members of each subclade closely related, but they are also almost indistinguishable in their ITS sequences. The accessions of *C. caucasicum* and *Chamaesciadium acaule* show moderate infraspecific variation. Specific independence and correct morphology-based identification of *C. meifolium* and *C. grossheimii* are beyond question.

*Chamaesciadium* is regarded by some botanists (Drude 1897–98; Koso-Poljansky 1914, 1916; Rechinger 1987) as a close relative or even a part of *Carum*, although Wolff (1927), Schischkin (1950) and Peşmen (1972) placed it near *Pimpinella*, and Heywood (1971) in *Apiaeae* subtribe *Seselinae*. The fruits of *Chamaescia-
dium acaule (see Pimenov & Kljuykov 1995) are rather similar to those of Carum carvi (Fig. 3A), the main difference being the number of vallecular and commissural vittae.

Based on morphology, the two other genera, Grammosciadium and Fuernrohria, clustered in Clade A, were usually attributed to other tribes, Grammosciadium to Scandiceae and Fuernrohria to Coriandreae. Hedge & Lamond (1987) and Vinogradova (1995), however, doubted the placement of Fuernrohria in Coriandreae. Previous molecular analyses (Katz-Downie & al. 1999; Downie & al. 2000a, b, 2001; Valiejo-Roman & al. 2006b; Terentieva & al. 2008) all supported the affinity of Fuernrohria and Grammosciadium to each other. The fruits of E. setifolia K. Koch are similar to those of G. pterocarpum Boiss. in style, stylopods and calyx teeth structure, and to G. conferum Hub.-Mor. & Lamond in some characters of mechanical and secreteory systems (Vinogradova 1995).

Morphologically, the inferred affinity of the two genera with Carum is surprising and they could by no means be united with the latter. Unlike C. carvi, Grammosciadium daucoides DC. (the nomenclatural type of that genus) is a polycarpic perennial with leaves with filiform terminal segments having acute apices, numerous pinnate bracts and 5–7 pinnate, or ternate, or entire bracteoles, linear to oblong fruits with filiform calyx teeth and broad commissure. Other species of Grammosciadium have winged marginal ribs and a mesocarp with sclerenchyma cell groups. Fuernrohria setifolia differs from C. carvi in its life form (polycarpic perennial), filiform terminal leaf segments having acute apices, numerous ternate bracts and entire filiform or ternate bracteoles, not prominent fruit ribs, a continuous sclerenchyma layer in mesocarp, cyclic vittae, broad commissure. Further studies are needed to assess the relationship between the genera of this clade.

The rare Transcaucasian Carum komarovii — The most interesting result obtained in our molecular phylogenetic appraisal of Carum concerns the rare C. komarovii. The species is only known from a few localised Transcaucasian occurrences, in Armenia and Azerbaijan (Karjagin 1944; Tamanshschan 1967). The ITS sequences of its two accessions were identical. Distant from Carum s.str., C. komarovii is nested in Clade B together with six species of Aegopodium, a species of the monotypic genus Chamaele and Pimpinella anthisricoides, whose four accessions (kept in herbaria under the names of P. anthisricoides, P. cervarifolia Freyn & Sint. and Aegopodium trbracteolatum Schmal.) form a separate subclade in both the MP and ML trees, despite their low infraspecific variability. C. komarovii is sister to the latter subclade and both are in turn sister to the subclade containing the six Aegopodium and the Chamaele species.

Aegopodium is a genus of seven species, widely distributed in the Paleartic (Europe, temperate Asia, N Africa, adventive in North America). The distinguishing features of Aegopodium are glabrous fruits without prominent ribs, inconspicuous vallecular and commissural vittae sometimes invisible in mature fruits (3–5 in each furrow and 4–9 on the commissural side), big, slightly lignified endocarp cells and broad ultimate leaf blade segments; some of the species have long horizontal rhizomes. A. tadhshikorum Schischk. is an Aegopodium species with short rhizomes. The fruit structure of A. podagarria L. (Fig. 3C), the generitype, well represents carpological peculiarities of the genus.

Chamaele decumbens is an endemic genus and species of the Japanese flora, spread on all main islands of Japan, with best known localities in the mountains of Honshu (Hiroe & Constance 1958). Chamaele was regarded as a subgenus of Aegopodium by Yabe (1902), as a part of Carum by Koso-Poljansky (1916) and as an independent genus by most other researchers (Wolff 1927; Ohwi 1953; Hara 1954; Hiroe & Constance 1958; Kitagawa 1960, 1982; Hiroe 1979; Ohba 1999). The fruits of C. decumbens (Fig. 3F) resemble those of Aegopodium. The species has short rhizomes and adventive roots; it differs from A. podagarria by the absence of horizontal rhizomes and is similar in that feature to A. tadhshikorum. The morphological data are fully consistent with the molecular data, so that Chamaele decumbens can safely be transferred to Aegopodium.

Pimpinella anthisricoides appears in our ITS tree far from the Clade D, where the bulk of the species of Pimpinella and also its generitype P. saxifraga L. is placed. In an earlier study by Tabanca & al. (2005), P. anthisricoides appeared as sister taxon to all other Pimpinella species. This is, however, clearly due to their sampling strategy, which a priori assumed monophyly of Pimpinella by including only species of that genus and a single remote outgroup taxon. Its position in our ITS tree, forming with Carum komarovii the sister group to the Aegopodium clade, is in good agreement with the morphological data. P. anthisricoides is similar to Aegopodium in fruit morphology (Fig. 3E) and rhizome structure (thick elongated horizontal black rhizomes), and Zakharova (2010) already noted the morphological similarity between C. komarovii (Fig. 3D) and P. anthisricoides. Fruit structure of C. komarovii, in contrast, differs considerably from the core of Carum. Moreover, C. komarovii and P. anthisricoides, like A. podagarria and most other Aegopodium species but in contrast to C. carvi (Fig. 4), have several petal secretory ducts.

Hence, both molecular analysis and morphological data provide support for a unification of Carum komarovii, Pimpinella anthisricoides and Chamaele with Aegopodium.

According to current taxonomy, Pimpinella anthisricoides is represented by two varieties, i.e. the type variety and var. cruciata (Bormm. & H. Wolff) Matthews. Matthews (1972: 363) supposed that the two differ from each other in the number of rays (6–10 in var. cruciata, 12–20
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in var. anthriscoides) and flowers in umbelets (8–15 in var. cruciata, 15–25 in var. anthriscoides). Var. cruciata was initially described as an independent species, *P. cruciata* Bornm. & H. Wolff (Bornmüller & Wolff 1921), which was together with *P. anthriscoides* attributed to *P.* subsect. Saxifragae H. Wolff (i.e. *P.* subsect. Pimpinellina according to the Code) and the series Anthriscoidae H. Wolff. Wolff (1927: 289, 306) distinguished the two species by the presence (*P. anthriscoides*: “involucr phylla 4–5 linear-lanceolata, radiis pluries breviora”) versus absence (*P. cruciata*: “involucrum nullum”) of involuclar bracts. Absence of involuclar bracts is a usual feature of Pimpinella, whereas their presence is not. Wolff (1927) qualified *P. cerasifolia* Freyn & Sint. as a synonym of *P. anthriscoides*. In our ITS tree, *P. cruciata* appears as sister to Tamamschjannella in Clade C, which represents the Pyramidotereae by Downie & al. (2010), distant from *P. anthriscoides* s.str., whereas *P. cerasifolia* is situated in the same terminal clade as the latter. Two conclusions can be drawn: (1) *P. anthriscoides* and *P. cruciata* are two “good” independent species and can by no means be regarded as varieties of one species. (2) *P. anthriscoides* and *P. cruciata* have to be excluded from Pimpinella and belong to different genera: *P. anthriscoides* to Aegopodium, whereas *P. cruciata* to Tamamschjannella (including the former Ligusticum rhizomaticum Hartvig).

**Carum atrosanguineum and Vicatia** — *Carum atrosanguineum* Kar. & Kir., described from S Siberian mountains and also distributed in Middle Asia and the W Himalaya, was recognised as a species closely related to *Vicatia* by Pimenov (1977) and later included in that genus as *V. atrosanguinea* (Kar. & Kir.) P. K. Mukh. & Pimenov (Pimenov & al. 1991). With the inclusion of *V. atrosanguinea*, the small genus *Vicatia*, formerly regarded as an E Asian (Sino-Himalayan) taxon, became a S Siberian-Himalayan genus (Pimenov & al. 1991).

We tested this relationship hypothesis by including *Carum atrosanguineum* (the two accessions showed identical ITS sequences) and *Vicatia coniifolia* DC., the generitype of *Vicatia*, in our molecular analysis. The analysis revealed a sister group relationship of both species far from *Carum* s.str. in Clade E, which corresponds to the clade identified by Downie & al. (2010) as tribe Selineae. Our analysis thus provides full support for the classification of *Carum atrosanguineum* as a member of the genus *Vicatia*.

**Carum alpinum and C. saxicolum** — Two Caucasian species, *Carum alpinum* (M. Bieb.) Benth. and *C. saxicolum* (Albow) Pimenov, are placed remotely from *Carum* s.str. in Clade E. The ITS sequences of *C. alpinum* and *C. saxicolum* are identical. This increases the doubt on the specific independence of *C. saxicolum*, because the two species are morphologically very similar. Pimenov (1978) revised their generic position and returned (*C. alpinum*) or transferred (*C. saxicolum*) them to Seseli as *S. alpinum* M. Bieb. and *S. saxicolus* (Albow) Pimenov, respectively.

To test the conflicting hypotheses on the taxonomic affinity of these species (*Carum versus Seseli*), we included in our analysis also *S. tortuosum* L., the nomenclatural type of *Seseli*, and *S. libanotis*, the nomenclatural type of *S. sect. Libanotis*. The ITS tree clearly supports the exclusion of the two species from *Carum* s.str. They are nested in a statistically well supported major clade with *Seseli* and representatives of the genera *Cnidioarpa* (*C. alaica* Pimenov), *Gasparrinia* (*G. peucedanoides* (M. Bieb.) Thell. ≡ *Seseli peucedanoides* M. Bieb.), *Katapushis* (*K. silaifolia* (Jacq.) Reduron & al. ≡ *Cnidium silaifolium* (Jacq.) Simonk.), *Saposnhiokovia* (*S. divaricata* (Turcz.) Schischk.), *Selimum* (*S. carvifolia* (L.) L., *S. broteroi* Hoffmanns & Link), *Trinia* (*T. hispida* Hoffm.) and *Vicatia* (see above). Our ITS tree, however, neither supports their placement in *Seseli*. Within Clade E, the two species appear as sister group to *Cnidioarpa alaica*, but further investigations with a denser sampling and, perhaps more markers, appear necessary.

For the time being, *Carum alpinum* might best be treated in the tradition of Fischer & Meyer (1840) in a genus of its own, *Lomatocarum* Fisch. & C. A. Mey., as *L. alpinum* (M. Bieb.) Fisch. & C. A. Mey.

**Carum buriaticum and C. furcatum** — The first species has a large distribution area, including S Siberia (from Altai Mts to Transbaicalia), Mongolia and China (the N, NE and central Asian parts to the Tibetan Plateau), the second was described from China and is now treated as a synonym of the first (Pu FaTing 1985). Molecular data have never been published before. The three accessions from different parts of the area show no variation in their sequences and the species is nested in Clade C of the ITS tree, which corresponds to the *Pyramidoptereae* of Downie & al. (2010). Within Clade C, *C. buriaticum* appears as sister to (MP tree, Fig. 1) or part of (ML tree, Fig. 2) a statistically well supported subclade including *Bunium persicum* and some other geophilic taxa, mainly of Mediterranean (s.l.) origin and distribution, namely *Elaeosticta*, *Galagania*, *Hyalolaena*, *Mogoltavia*, *Oedibasis*, as well as the non-geophilic *Pyramidoptereta* and *Schultzia*. According to the ITS trees, *C. buriaticum* is nearest to *Schultzia*, in the ML tree (Fig. 2) it is even nested among the samples of that genus. The taxa of this subclade are morphologically rather dissimilar, making this placement surprising.

*Carum buriaticum* differs from *C. carvi* in its life form as a polycarpic perennial, in the presence of numerous bracts and bracteoles, in the number of secretory ducts in the petals (Fig. 4G, H) and in a medium-sized commissure. However, compared with the Caucasian species closely related to *C. carvi* according to our ITS tree, the commissure width is the only character separating *C. buriaticum* from them. Hence, the distant position in the ITS tree of *C. buriaticum* from *Carum* s.str. does

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not correspond to any apparent morphological difference. Further investigations appear necessary prior to a revised classification of *Carum buriaticum*.

**Eastern Mediterranean *Carum* species** — This group includes in our view *Carum heldreichii* Boiss., *C. rupestre* Boiss. & Heldr., *C. appu num* (Viv.) Grande, *C. graecum* Boiss. & Heldr. and *C. ru picola* Hartvig & Strid.

The taxonomy of the group is rather controversial. Tutin (1968) regarded *Carum graecum* as a synonym of *C. appu num* and *C. rupestre* as a synonym of *C. heldreichii*. Hartvig (1986), in contrast, accepted *C. graecum*, *C. appu num*, *C. heldreichii* and *C. ru picola* as independent species. *C. graecum* differs from *C. appu num*, according to Hartvig (1986), in leaf lobes being 2–5(–8) mm long, bracts up to 8 and petals with 3–7 secretory ducts, while *C. ru picola* differs from *C. heldreichii* in yellowish petals and leaf lobes 5–20 mm long.

All E Mediterranean species are nested in Clade C of the ITS tree (Fig. 1–2) like *C. buriaticum*, but in two other subclades. The first, composed of *Carum graecum* subsp. *serpentinicum* Hartvig and *C. appu num* together with *Scaliger a napiformis* and *S. moreana*, is statistically well supported and sister to all other subclades of Clade C. The second subclade with badly resolved internal relationships and lacking sufficient statistical support, includes the SE European *C. heldreichii*, *C. ru picola* and the S Anatolian *C. ru picola* in a well supported terminal clade, together with taxa of *Bunium*, *Hellenocarum*, *Postiella*, *Tamanschjanella* and *Trachyspermum*.

The data clearly support (1) the exclusion of the E Mediterranean members of *Carum* from that genus, and (2) indicate that they represent two different evolutionary lines, confirming the preliminary results of analyses by Papini & al. (2007) and Degtjareva & al. (2009).

**Carum verticillatum** — The W European *Carum verticillatum*, morphologically being considerably different from *C. carvi*, never before has been included in a molecular phylogeny (so far only cp *matK* and *rbcL* sequences published). In our ITS trees (Fig. 1–2) it is the only species of *Carum* placed in Clade F. *C. verticillatum* branches at the very base of Clade F, being sister to all its other members. Clade F includes taxa with different morphology but rather similar ecology, all being hydrophilous or hygrophilous plants. The long branch leading to *Carum verticillatum* in the ML tree corresponds to the higher rates of nucleotide substitution in the ITS region.

*Carum verticillatum* is characterised by unusual leaf structure with transversal orientation of the subdivisions of paired first-order pinnae (Rutishauser & Isler 2001). The pinnae along the main rachis are arranged as if they were verticillate leaves along the stem. The attribution of the species to *Carum* (Koch 1824 and the majority of later workers) is based mainly on considerable carpological similarity with *C. carvi*. Like the latter, *C. verticillatum* (Fig. 3B) has glabrous fruits, divided into two homomorphic mericarps, with approximately equal, keeled dorsal and marginal primary ribs, narrow commissure, non-lignified parenchyma, commissural a solitary vallecular vitta and two commissural vittae, and the endosperm flat on the commissural side. However, from the cladistic viewpoint, such a fruit structure must be regarded as pleiomorphic, thus not informative for relationship clarification. It is represented widely in subfamily Apioideae, being found in *Bunium*, *Bupleurum* L., *Cnidio carpa* Pimenov, *Falcaria* Fabr., *Gas sparrinia* Bertol., *Geocar yum* Coss., *Paulita* Sojak, *Physospermopsis*, *Rupiphe la* Pimenov & Lavrova, *Seseli*, *Visnaga* P. Gaertn. and some other Eurasian genera. The very distant positions in the ITS tree of this species and *C. carvi* correlates with morphological dissimilarity in the life form and the root, leaf, involucrum, involucel and pollen morphology. The roots of *C. verticillatum* are fusiform, thickened downwards; its leaves are narrowly oblong in outline, pinnate, with numerous pairs of deeply palmatisect verticillately arranged filiform segments, markedly diminishing towards the leaf base; bracts and bracteoles are 5 to 10, linear to lanceolate, acute, deflexed. The palynological differences between *C. verticillatum* and *C. carvi* were shown by Cerceau-Larrivé (1959) and El Alaoûi-Faris (2005) and include pollen grain shape (*P/E* ≥ 2–2.5, pollen constricted in the equatorial area in *C. carvi* versus *P/E* = 2, pollen not constricted in the equatorial area in *C. verticillatum*), endoaperture structure and size (prominent, 4 × 3 μm in *C. carvi* and plane, 6 × 2.4 μm in *C. verticillatum*), exine thickness (in the equatorial area 2.4 μm, in the polar area 1.6 μm in *C. carvi*, equal in the equatorial and polar areas, 2.5 μm in *C. verticillatum*) and longer columnellae in *C. carvi* as compared with *C. verticillatum*.

The taxonomic history of *C. verticillatum* reflects its critical taxonomic position; it was classified in *Sis on* L. (basionym by Linnaeus 1753), *Seseli* (Crantz 1767; independently Desfontaines 1798), *Sium* (Lamarck 1779), *Troc daris* (Rafinesque 1840), *Bunium* (Godron 1848), *Pimpinella* (Jessen 1879), *Apium* (Carel 1886) and *Selinum* (Krause 1904).

**Taxonomic conclusions**

(1) *Carum s.str.* — Among the species here investigated only four are truly closely related to *C. carvi*, the holocultural type of *Carum*. Therefore we conclude that *Carum s.str.* is composed only of *C. carvi*, *C. meifolium*, *C. caucasicum*, *C. grossheimii* and *C. porphyrocoleon*. All these species are distributed in Caucasus and adjacent countries of the Near East.

(2) *Carum alpinum* — *C. alpinum* (M. Bieb.) Benth. (incl. *C. saxicolum* (Albov) Pimenov) is to be excluded
from *Carum*. The species is nearer to *Seseli*, but has some differences from the species of that genus. For the time being, the best solution is probably to treat it as member of the separate, monotypic genus *Lomatocarum* Fisch. & C. A. Mey. as *L. alpinum* (M. Bieb.) Fisch. & C. A. Mey. in Index Seminum [St. Petersburg]: vi. 59. 1840.

(3) *Carum atrosanguineanum* — The molecular analysis, in accord with the morphological data (Pimenov & al. (1991), provide evidence for the placement the species in *Vicatia* DC. (Pimenov & al. 1991).

(4) *Carum verticillatum* — This species deserves the status of an independent genus, whose name was proposed by Rafinesque in 1840. According to molecular and morphological data, *C. verticillatum* differs considerably from other *Carum* species. After its attribution to *Carum* (Koch 1824), which is currently generally accepted, it was regarded as a member of seven other *Umbelliferae* genera. However, nowadays these generic attributions are solely of historical interest. The only exception is *Trocdaris* Raf., Good Book: 50. 1840, being a validly published generic name, if *C. verticillatum* is regarded as a separate monotypic genus:

*Trocdaris verticillatum* (L.) Raf., Good Book: 50. 1840

Ref. (all sub *Carum verticillatum*) — Reichenbach 1863: pl. 31; Coste 1902: 209; Thellung 1926: 1187; Tutin 1968: 354; Tutin 1980: 150, fig. 57; Cook 1983: 511, fig. 8; Valdés & al.: 1987: 319; Arenas & Garcia 1993: 151, fig. 65(2), 66(3); Villar & al. 1997: 573; Gómez 2003: 300; Reduron 2007: 742

Distribution. — Morocco, Portugal, Spain, France, Belgium, Netherlands, Germany (extreme W, probably extinct), W Great Britain (northwards to Scotland), Ireland. See map in Cook (1983: fig. 8).

(5) *Carum segregates nested in the Pyramidoptereae clade* — Three separate evolutionary lines to be excluded from *Carum* s.str. (a) *Carum heldreichii* Boiss., *C. rupestre* Boiss. & Heldr. and *C. rupeicola* Hartvig & Strid, (b) *Carum burtaticum* (incl. *C. furcatum*), and (c) *C. appuana* (Viv.) Grande and *C. graecum* Boiss. & Heldr. have each still uncertain positions in the *Pyramidoptereae* sensu Downie & al. (2010).

(6) *Carum komarovii* and recircumscription of *Aegopodium* — *C. komarovii* has to be excluded from *Carum* s.str. and to be united with *Aegopodium*. The genus has also to be enlarged by two species, currently treated as *Chamaele decumbens* and *Pimpinella an thriscoides*, see clade B. After these transfers, *Aegopodium* contains 10 species.


Distribution. — Japan (Hokkaido; Honshu; Shikoku; Kyushu).


Ref. (all sub Carum komarovii) — Schischkin 1950: 352; Akhundov 1955: 444; Tamamschjan 1967: 76, map. 82; Mandenova 1973: 353.

Distribution. — Armenia, Azerbaidzhan.


Note. — When Pimpinella annthisroides is transferred to Aegopodium, the epithet annthisroides cannot be used, as the resulting combination is already in use for a different, Chinese species (Boissie 1909). The second oldest available name is A. trichrocteolatum Schmalh.


Distribution. — Iran (W: W Azarbayjan, Kordestan, Hamadan, Lorestan; N: Mazandaran; C: Tehran, Chaharmahal va Bakhteyari), Azerbaidzhan, Georgia, Armenia, Turkey (N (Pontic) Anatolia: Kastamonu, Ordu, Giresun, Çoruh; C Anatolia: Ankara, Çankiri, Sivas, Bilecik, Eskişehir, Konya, Kayseri; S Anatolia: Antalya; E Anatolia: Gümüşhane, Erzincan, Malatya, Tunceli, Bitlis, Van, Hakkari), Iraq, Lebanon, Syria.

(7) Pimpinella cruciata — This species is to be excluded from Pimpinella and transferred to Tamamschjanella; T. rubella (based on Eleutherospermum rubellum) appears to be conspecific.


Note. — Pimpinella cruciata cannot be regarded as a variety of P. anthriscoides. Moreover, it belongs to an­other genus, not closely related to Aegopodium, where P. anthriscoides has been transferred in the present revi­sion. P. cruciata has true affinity with the Balkan - Near Eastern - Caucasian genus Tamamschjanella. Both pre­viously known species of Tamamschjanella (T. rubella and T. rhizomatica) have, like Aegopodium, horizontal rhizomes, differing, however, in the presence of bracts. According to initial descriptions (Bormmüller & Wolff 1921; Busch 1931), P. cruciata differs from Eleuth­erospermum rubellum (basionym of T. rubella) in petal colour (albida = somewhat white in P. cruciata versus red in T. rubella), all other features in the two species are the same. However, our investigation of type material in both Lund (LD) and St-Petersburg (LE) herbaria revealed that the petals of P. cruciata are whitish (pale) only after drying, whereas initially they seem to be red, what can be confirmed by examining better preserved plants. So, the two species are regarded here as conspecific.

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Appendix

Voucher information and GeneBank accession numbers for plants used in the present study.

AY524741 (Lee & Downie 2006). *C. virosa* l.: U78407 (Downie & al. 1998). — *Cnidiocarpa... Wolff]: (1) turkey, turkish-armenien, koesoedagh, 17.7.1889, Sintenis 1311 (lD), jQ792232; (2) turkey, turkish-ar-

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