

Family relationships of the enigmatic rosid genera *Barbeya* and *Dirachma* from the Horn of Africa region

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Abstract: *Barbeya* is a monotypic genus in the Horn of Africa and adjacent parts of Arabia. It is usually treated as the family *Barbeyaceae* and regarded as an aberrant member of *Urticales*. *Dirachma*, with one species on Socotra and one in Somalia, is usually treated as the family *Dirachmaceae*, in *Geraniales*, but a position in *Malvales* has also been suggested. Analyses of molecular data, from both *rbcL* and *trnL-F*, indicate that *Barbeya* and *Dirachma* are closely related inter se as well as to *Rhamnaceae* and *Elaeagnaceae*. In an analysis based on morphology *Barbeya* groups with *Elaeagnaceae*, and *Dirachma* with *Rhamnaceae* and *Ulmaceae*. In a combined molecular and morphological analysis *Barbeya* is the sister group of *Elaeagnaceae* and *Dirachma* is the sister group of the whole *Barbeya-Elaeagnaceae-Rhamnaceae* clade. However, the support for these arrangements is weak and, rather than merging *Barbeyaceae* with *Dirachmaceae* as suggested by the molecular analysis or with *Elaeagnaceae* as suggested by the morphological and combined analyses, it seems best to retain both *Barbeyaceae* and *Dirachmaceae* in their present circumscriptions, but in both cases in completely new positions in the angiosperm system. The results are compatible with a new circumscription of *Rhamnales* comprising *Rhamnaceae*, *Elaeagnaceae*, *Dirachmaceae* and *Barbeyaceae*.

Among major groups of flowering plants the rosids (CHASE & al. 1993) are one of the least understood with respect to familial interrelationships, and many rosid families are still not unequivocally classified to order. The Horn of Africa region houses several peculiar and isolated genera and families of flowering plants. Here we discuss two such rosid genera, *Barbeya* SCHWEINF. and *Dirachma* SCHWEINF. ex BALF. f., both with uncertain positions in the angiosperm system.

Barbeya, with its single arborescent species *B. oleoides* SCHWEINF. in the Horn of Africa and adjacent parts of tropical Arabia, was first described (SCHWEINFURTH 1891) as a member of *Urticaceae* s. l. (sensu BENTHAM & HOOKER 1880). It has also

been included in *Ulmaceae* subfam. *Barbeyoideae* (e.g. ENGLER 1897, ENGLER & DIELS 1936, MELCHIOR 1964) but has during the last 80 years or so generally been treated as a family of its own, *Barbeyaceae* (e.g. RENDLE 1916; HUTCHINSON 1959, 1969, 1973; CRONQUIST 1981, 1988; DAHLGREN 1983; THORNE 1983; GOLDBERG 1986; TAKHTAJAN 1987). Most recent authors have regarded the family as an aberrant member of *Urticales* (e.g. DICKISON & SWEITZER 1970; CRONQUIST 1981, 1988; DAHLGREN 1983, 1989; KUBITZKI 1993; ZAVADA & KIM 1996), whereas BERG (1989) excluded it from this order. Others have treated it as an order of its own, *Barbeyales* (e.g. TAKHTAJAN 1966, 1980, 1987; TOBE & TAKAHASHI 1990). Other alternatives are provided by AIRY SHAW in WILLIS (1966), who suggested a connection with *Simmondsiaceae*, and BARABE & al. (1982), who placed *Barbeyaceae* close to *Leitneriaceae* in their subclass *Hamamelididae*. Finally, THORNE (1973, 1976, 1983, 1989, 1992) placed it among the “*Incertae sedis*” at the end of his system.

Dirachma, a woody genus with one species on Socotra and one in Somalia, was originally placed in *Geraniaceae* (BALFOUR 1884, 1888), in which it has been placed also by many subsequent authors, for example CRONQUIST (1981, 1988) and THORNE (1992). However, BALFOUR (1888) also pointed out similarities between *Dirachma* and *Tiliaceae*, *Sterculiaceae* and *Samydaceae*, and AIRY SHAW in WILLIS (1966) suggested a distant connection with *Greyiaceae*. Currently the genus is often treated as a family of its own, *Dirachmaceae*, associated with *Geraniaceae* in *Geraniales* (DAHLGREN 1983, 1989; TAKHTAJAN 1987) or with *Tiliaceae* in *Malvales* or *Tiliales* (HUTCHINSON 1959, 1969; LINK 1991, 1993, 1994; YAKOVLEVA 1994).

In the present study we analyzed DNA sequences of the plastid regions *rbcL* (protein-coding) and *trnL-F* (composed of an intron, a short exon, and an intergene spacer; TABERLET & al. 1991), morphological data, and a combination of molecular and morphological data to (1) determine the positions of *Barbeya* and *Dirachma* in the angiosperm system, (2) identify the major lineage(s) that encompass and are related to these genera, and (3) suggest a family classification for these lineages.

Materials and methods

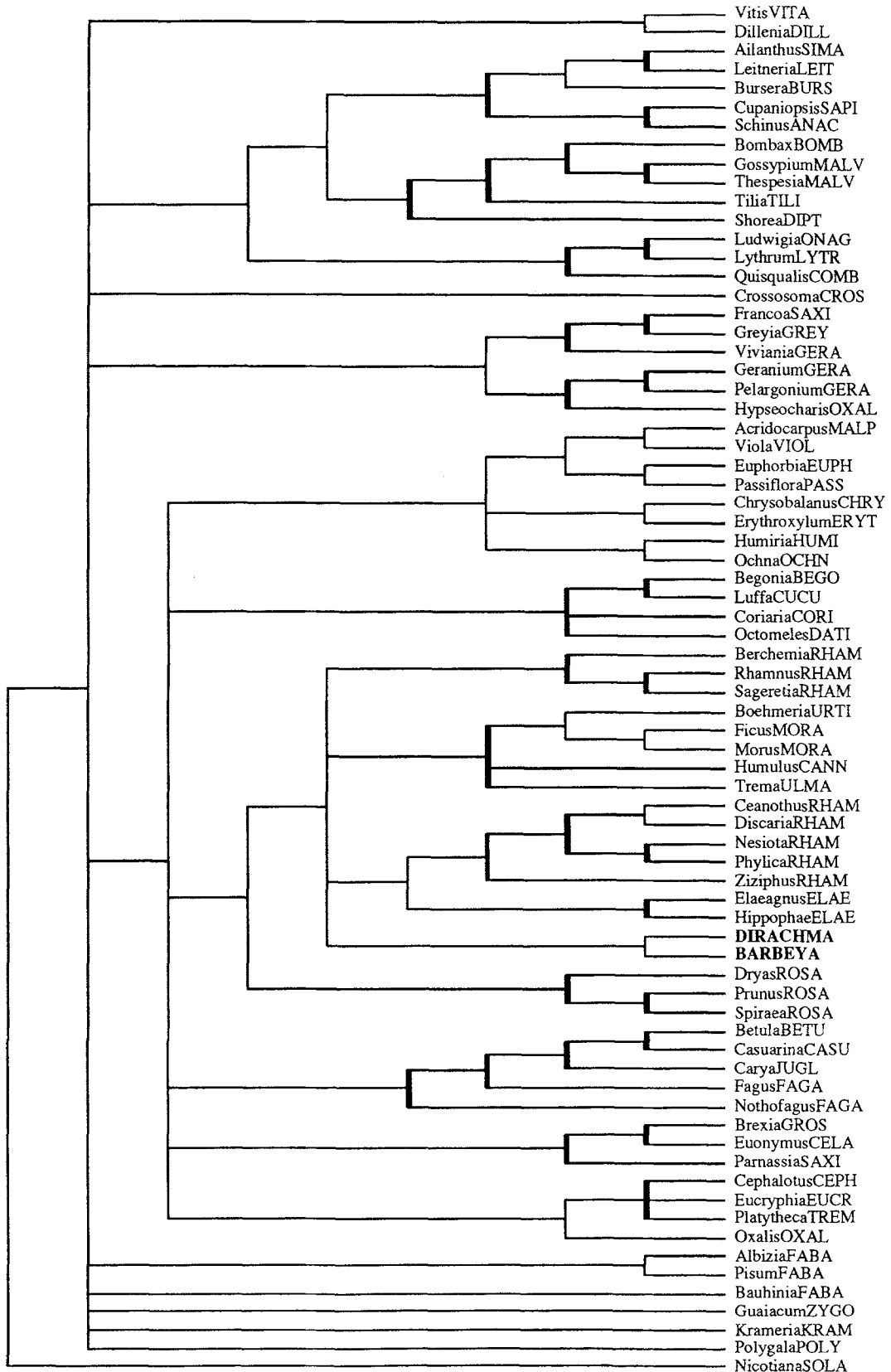
The DNA was extracted, amplified and sequenced following the protocols in BREMER & al. (1995). The *rbcL* data matrices in the phylogenetic analyses comprise characters corresponding to each nucleotide position (27 to 1428, positions 1–26 are excluded as they are identical to one of the primers) of the *rbcL* sequence. The aligned *trnL-F* matrix comprises 1257 nucleotide positions and is available on request.

Vouchers for new sequences and their respective EBI/GenBank accession numbers are listed in Table 1. All *trnL-F* sequences were made at Kew, whereas the *rbcL* sequence of *Dirachma* and part of the *Barbeya* sequence were made in Uppsala. The sequence of *Barbeya oleoides* in EBI/GenBank (from Saudi Arabian material) has a gap of about 100 base pairs. This gap was filled by a new sequence from Somali material (see Table 1).

As a first step in our strategy, *rbcL* sequences of *Barbeya* and *Dirachma* were analysed along with a large sample of sequences of rosids of various families, including members of *Geraniaceae*, *Greyiaceae*, *Leitneriaceae*, *Tiliaceae*, *Ulmaceae* and *Urticaceae*. The sequences in this sample were all taken from EBI/GenBank (CHASE & al. 1993). This initial analysis (see Fig. 1 and under Results) indicates (1) that *Barbeya* and *Dirachma* are sister groups, and (2) that these two genera belong to a large clade including also all

Table 1. New DNA sequences analysed in this paper

Family	Species	DNA	Source/voucher information	New accession EBI/GenBank
<i>Barbeyaceae</i>	<i>Barbeya oleoides</i> SCHWEINF.	<i>rbcL</i> (positions 865–962)	Somalia, THULIN & al. 9178 (K, UPS)	AJ224820
	<i>Barbeya oleoides</i> SCHWEINF.	<i>trnL-F</i>	Saudi Arabia, COLLENETTE 1/93 (K)	AJ225795
<i>Dirachmaceae</i>	<i>Dirachma socotrana</i> SCHWEINF. ex BALF. f.	<i>rbcL</i>	Yemen, Socotra, THULIN & GIFFRI 8812 (E, K, UPS)	AJ225789
	<i>Dirachma socotrana</i> SCHWEINF. ex BALF. f.	<i>trnL-F</i>	Yemen, Socotra, THULIN & GIFFRI 8812 (E, K, UPS)	AJ225796
<i>Elaeagnaceae</i>	<i>Elaeagnus</i> sp.	<i>trnL-F</i>	China, CHASE 2414 (K)	AJ225800
	<i>Hippophae salicifolia</i> D. DON	<i>trnL-F</i>	China, CHASE 856 (K)	AJ225801
<i>Rhamnaceae</i>	<i>Ceanothus caeruleus</i> LAG.	<i>trnL-F</i>	USA, CHASE 2413 (K)	AJ225798
	<i>Nesiotia elliptica</i> HOOK. f.	<i>rbcL</i>	St. Helena, CHASE 500 (K)	AJ225783
	<i>Nesiotia elliptica</i> HOOK. f.	<i>trnL-F</i>	St. Helena, CHASE 500 (K)	AJ225803
	<i>Discaria chacaye</i> (G. DON) TORTOSA	<i>trnL-F</i>	Chile, CHASE 914 (K)	AJ225797
	<i>Ziziphus glabrata</i> HEYNE	<i>trnL-F</i>	Saudi Arabia, COLLENETTE 5/93 (K)	AJ225799
	<i>Sageretia thea</i> (OSBECK) M. C. JOHNST.	<i>rbcL</i>	Saudi Arabia, COLLENETTE 9/93 (K)	AJ225785
	<i>Sageretia thea</i> (OSBECK) M. C. JOHNST.	<i>trnL-F</i>	Saudi Arabia, COLLENETTE 9/93 (K)	AJ225792
	<i>Berchemia discolor</i> (KLOTZSCH) HEMSL.	<i>rbcL</i>	Saudi Arabia, COLLENETTE 14/93 (K)	AJ225786
	<i>Berchemia discolor</i> (KLOTZSCH) HEMSL.	<i>trnL-F</i>	Saudi Arabia, COLLENETTE 14/93 (K)	AJ225793
	<i>Phylica pubescens</i> AITON	<i>rbcL</i>	S Africa, CHASE 859 (K)	Y16769 & Y16770
	<i>Phylica pubescens</i> AITON	<i>trnL-F</i>	S Africa, CHASE 859 (K)	Y16771
<i>Rosaceae</i>	<i>Dryas drummondii</i> RICHARDS.	<i>trnL-F</i>	Canada, CHASE 917 (K)	AJ225802
<i>Ulmaceae</i>	<i>Trema micrantha</i> BLUME	<i>rbcL</i>	Puerto Rico, CHASE 335 (NCU)	TMU03844
	<i>Gironniera subaequalis</i> PLANCH.	<i>trnL-F</i>	Java, CHASE 1384 (K)	Y16772



members of *Elaeagnaceae* and *Rhamnaceae*, as well as all included members of *Urticales* (from the families *Cannabaceae*, *Moraceae*, *Ulmaceae* and *Urticaceae*), and (3) that this large clade is sister to a clade with the included members of *Rosaceae*.

As a second step, to analyse the clade with *Barbeya* and *Dirachma* in more detail, a sample comprising these two genera plus *Elaeagnus*, *Hippophae* and *Shepherdia* (*Elaeagnaceae*), *Berchemia*, *Ceanothus*, *Discaria*, *Nesiota*, *Phyllica*, *Rhamnus*, *Sageretia* and *Ziziphus* (*Rhamnaceae*), *Gironniera* and *Trema* (*Ulmaceae*), and *Dryas* and *Neillia* (*Rosaceae*), was selected.

All these genera were analysed for both *rbcL* and *trnL-F* except *Gironniera* (*rbcL* missing), and *Trema*, *Neillia*, *Rhamnus* and *Shepherdia* (*trnL-F* missing). Also, a data matrix comprising 22 characters from morphology, anatomy and chemistry for all these taxa except *Gironniera* was compiled.

For the analyses of the second step, the molecular data (*rbcL* and *trnL-F*) and the morphological data were analysed both separately and in combination. In both the molecular and combined analyses *Shepherdia* and *Neillia* were excluded, while the *trnL-F* data for *Gironniera* were used with the *rbcL* data for *Trema* in the molecular analysis, and in combination with both the *rbcL* and morphological data for *Trema* in the combined analysis. For *Rhamnus* only the *rbcL* sequence was included in the analyses.

All analyses were performed using the computer program PAUP 3.1.1 (SWOFFORD 1993) on a PowerMac 8100/80, with all character changes weighted equally. All characters were treated as unordered. The methods for the searches were heuristic, with random stepwise addition of sequences and 100 replications, and TBR branch swapping. To estimate the support for each clade bootstrap values (with 10 000 replicates; FELSENSTEIN 1985) were calculated.

Morphological characters

The 22 morphological characters (including also anatomy and chemistry) used in the phylogenetic analyses are listed below. For most of them additional comments as regards definitions, codings and references are also given. The numbers of the characters are the same as in the data matrix in Table 2.

1. Leaves opposite (0), alternate (1).
2. Stipules present (0), absent (1).
3. Indumentum with hairs simple (0), stellate and peltate (1).

Stellate and peltate hairs are, among the studied genera, only found in *Elaeagnaceae* (*Elaeagnus*, *Hippophae* and *Shepherdia*). The dense silvery indumentum of *Barbeya* superficially resembles that of *Elaeagnaceae*, but consists of simple, unicellular hairs only. TOBE & TAKAHASHI (1990: 565) compared the trichomes of *Barbeya* with those in *Urticales* and found that "the curly, non-glandular trichomes of *Barbeya* are much thinner than the attenuate, micropapillate trichomes of *Urticales*, and the two are assigned to different trichome types. Thus, trichome morphology suggests that *Barbeya* does not fit in *Urticales*".

4. Number of sepals four (0), three (1), five (2), six (3), two (4), eight (5).

Fig. 1. Strict consensus tree of four equally parsimonious trees obtained by phylogenetic analysis of *rbcL* sequences from *Barbeya* and *Dirachma* (in bold type), and a large sample of rosid genera of various families (family names abbreviated, in capital letters). Thick bars indicate bootstrap values above 50%

Table 2. Data matrix for the 16 genera (with names) and 22 characters (numbered as in the text) used in the morphological analysis. Polymorphic states are represented by letters as follows: P=(01), Q=(02), S=(0123), K=(12), L=(012), V=(023), B=(235). Unknown and inapplicable states are coded “?”

Taxon	0 0 0 0 0	0 0 0 0 1	1 1 1 1 1	1 1 1 1 2	2 2
	1 2 3 4 5	6 7 8 9 0	1 2 3 4 5	6 7 8 9 0	1 2
<i>Dryas</i>	1 0 0 5 0	0 1 0 5 ?	0 3 0 2 1	0 0 1 0 0	1 1
<i>Neillia</i>	1 0 0 2 0	0 1 0 5 ?	0 S 0 1 1	0 0 1 0 0	1 0
<i>Trema</i>	1 0 0 QP	0 0 ? P 0	0 Q 1 0 ?	0 0 0 ? 1	1 0
<i>Barbeya</i>	0 1 0 PP	0 0 ? 4 ?	0 L 0 0 ?	0 1 1 ? ?	? 1
<i>Hippophae</i>	1 1 1 4 1	0 0 ? 1 P	1 1 0 2 0	1 1 1 1 1	0 1
<i>Shepherdia</i>	0 1 1 0 1	0 0 ? 3 P	1 1 0 2 0	1 1 1 1 1	0 1
<i>Elaeagnus</i>	1 1 1 0 1	0 0 ? 1 1	1 1 0 2 0	1 1 1 1 1	0 1
<i>Dirachma</i>	1 0 0 B 1	0 1 0 V 1	0 3 1 2 0	1 ? ? ? ?	? ?
<i>Ziziphus</i>	P 0 0 2 1	1 P 1 0 1	1 Q 1 2 0	1 0 0 0 1	0 0
<i>Sageretia</i>	0 0 0 2 1	1 1 1 0 1	1 Q 1 2 0	1 0 0 0 1	0 0
<i>Nesiota</i>	0 0 0 Q 1	1 1 ? P 1	1 Q 1 2 0	1 0 0 0 1	0 0
<i>Phylica</i>	1 0 0 2 1	1 P 1 0 1	1 Q 1 2 0	1 0 0 0 1	0 0
<i>Rhamnus</i>	P 0 0 Q 1	1 P P P 1	1 Q 1 2 0	1 0 0 0 1	0 0
<i>Discaria</i>	0 0 0 Q 1	1 P 1 P 1	1 Q 1 2 0	1 0 0 0 1	0 0
<i>Ceanothus</i>	P 0 0 V 1	1 1 1 L 1	1 Q 1 2 0	1 0 0 0 1	0 0
<i>Berchemia</i>	1 0 0 2 1	1 1 1 0 1	1 Q 1 2 0	1 0 0 0 1	0 0

Within *Dirachma*, *D. somalensis* has 5- or 6-merous flowers throughout, whereas *D. socotrana* has 8-merous flowers (LINK 1994). In *Barbeya* the sepals are three or four in number (CRONQUIST 1981). The genera of *Elaeagnaceae* either have four (*Shepherdia*, *Elaeagnus*) or two sepals (*Hippophae*).

5. Aestivation of sepals imbricate (0), valvate (1).

In *Rosaceae* the sepals are imbricate, while in *Elaeagnaceae* and *Rhamnaceae* they are valvate throughout (CRONQUIST 1981). In *Ulmaceae* the sepals (perianthlobes) are generally imbricate, but for example in *Trema* the aestivation of female buds is imbricate and in male buds induplicate-valvate (POLHILL 1966). In *Barbeya* the aestivation of female buds is somewhat imbricate and in male buds it is valvate (FRIIS 1993). Both *Trema* and *Barbeya* and here coded as polymorphic.

6. Sepals not keeled (0), keeled (1).

Sepals which are, to various degrees, keeled are characteristic of all the genera of *Rhamnaceae*.

7. Petals absent (0), present (1).

Petals are absent in *Trema*, *Barbeya*, and the genera of *Elaeagnaceae*, while they are present in *Dryas* and *Neillia*. Within *Rhamnaceae* petals are generally present but, among the included genera, varying numbers of species without petals are found in *Ziziphus*, *Phylica*, *Rhamnus* and *Discaria*.

8. Petals not hooded (0), hooded (1).

The petals are not hooded in *Dryas*, *Neillia* and *Dirachma*, while they are generally hooded in the genera of *Rhamnaceae*, where they seem to protect the

anthers, which fit into the concavity of the petals (SUESSENGUTH 1953, CRONQUIST 1981).

9. Stamens five (0), four (1), six (2), eight (3), 9–12 (4), 15–30 (5).

Barbeya is in the literature said to have 6–12 stamens (e.g. CRONQUIST 1981). However, it seems that there are only occasional flowers with fewer than nine stamens and they occur in inflorescences where the number is otherwise 9–12. In such flowers it is also often possible to see rudiments of “missing” stamens. For this reason *Barbeya* is here coded as having 9–12 stamens. *Dirachma* has five, six or eight stamens (LINK 1994). *Elaeagnus* and *Hippophae* are here coded as having four stamens, although very occasionally other numbers may occur.

10. Stamen position opposite sepals (0), alternate with sepals (1).

This character has been coded with a query in *Neillia*, *Dryas* and *Barbeya*, where the number of stamens is higher than the number of sepals, and no clear positions relative to the sepals can be seen. *Trema* has stamens opposite the sepals, while all *Rhamnaceae*, as well as *Elaeagnus* in *Elaeagnaceae* have stamens alternating with the sepals. *Shepherdia* has four stamens opposite the sepals and four stamens alternating with the sepals, while *Hippophae* has two stamens opposite the sepals and two alternating.

11. Nectary-disk absent (0), present (1).

Various modified intrastaminal nectary-disks are found in all members of *Rhamnaceae* and *Elaeagnaceae*, but are absent in *Rosaceae* and *Barbeya*. The nectaries found at the petal-bases in the flowers of *Dirachma* (LINK 1994) are not regarded as homologous with the nectary-disks of *Rhamnaceae* and *Elaeagnaceae*.

12. Carpels two (0), one (1), three (2), more than three (3).

Barbeya has one (rarely up to three) carpels, *Dirachma* five, six or eight, *Dryas* many, *Neillia* one or rarely up to five, *Elaeagnaceae* one, and *Trema* and the genera of *Rhamnaceae* two or three carpels.

13. Gynoecium apocarpous (0), syncarpous (1).

Syncarpous gynoecia are found in all *Rhamnaceae* and in *Dirachma* and *Trema*. *Dryas* and *Neillia* are apocarpous but other members of *Rosaceae* (subfam. *Maloideae*) may be syncarpous. In *Elaeagnaceae* the gynoecia are apocarpous, always consisting of a single carpel only. The gynoecium of *Barbeya* is composed of one or rarely two or three carpels. If two or three, the carpels are more or less fused laterally (DICKISON & SWEITZER 1970) but have separate styles and are here coded as apocarpous.

14. Ovules pendulous (0), marginal (1), ascending (2).

Pendulous ovules are found in *Barbeya* as well as in *Trema* (CRONQUIST 1981), whereas ascending ovules are characteristic of *Elaeagnaceae* and *Rhamnaceae* (CRONQUIST 1981), and also are found in *Dirachma* (LINK 1994) and *Dryas* (HUTCHINSON 1964). In *Neillia* the ovules have a marginal position (VIDAL 1963).

CRONQUIST (1981) and FRIIS (1993) stated the ovule of *Barbeya* to be “apparently unitegmic”. However, according to studies of BOUMAN & BOESEWINKEL (1997) the ovules of *Barbeya* are bitegmic as in all other taxa included in this study.

15. Seeds exotestal (0), mesotestal (1).

In exotestal seeds the outer epidermis of the testa forms, typically, a rigid palisade with thick-walled cells (CORNER 1976). This is characteristic of all members of *Rhamnaceae* and *Elaeagnaceae* and is the main reason why CORNER

(1976) postulated a relationship between these two families. Exotestal seeds similar to those of *Rhamnaceae* are also found in *Dirachma* (BOESEWINKEL & BOUMAN 1997). *Rosaceae* generally have mesotestal seeds (CORNER 1976), in which the mesotesta becomes more or less sclerotic, and the rest of the seed-coat is unspecialized.

Barbeya (BOUMAN & BOESEWINKEL 1997) and *Trema* have unspecialized seed-coats and are coded with a query for this character.

Vitaceae and *Leeaceae*, families previously often regarded as closely related to *Rhamnaceae* (see above), have endotestal seeds (CORNER 1976).

16. Seed-coat non-multiplicative (0), multiplicative (1).

The characterisation of the seed-coat as multiplicative or non-multiplicative (CORNER 1976) refers to whether the cells of both integuments divide after fertilization and form more cells by periclinal division (i.e. adding cell layers, multiplicative) or by anticlinal division (i.e. adding cells within the same layer of cells, non-multiplicative). The seed-coat of *Barbeya* is non-multiplicative (BOUMAN & BOESEWINKEL 1997), and this also the case in *Ulmaceae* and *Rosaceae* (CORNER 1976).

17. Nodes trilacunar (0), unilacunar (1).

Trilacunar nodes are found in *Rosaceae*, *Ulmaceae* and *Rhamnaceae*, whereas unilacunar nodes are found in *Elaeagnaceae* and *Barbeya* (CRONQUIST 1981). The condition in *Dirachma* is unknown.

18. Fiber pits simple (0), bordered (1).

In simple pits the secondary wall of the fiber ends abruptly at the pit cavity with the result that the cavity retains approximately the same diameter through the depth of the secondary wall, whereas the bordered pit has an overarching secondary wall thus forming a border (ESAU 1977: 51). The fibers of *Barbeya* have bordered pits (DICKISON & SWEITZER 1970). This is also the case in *Rosaceae* and *Elaeagnaceae* (METCALFE & CHALK 1950), whereas the fibers of *Trema* and *Rhamnaceae* have simple pits (METCALFE & CHALK 1950). *Dirachma* has not been studied in this respect.

19. Quebrachitol absent (0), present (1).

The cyclitol quebrachitol is generally present in *Elaeagnaceae* (HEGNAUER 1966), but has not been found in *Rhamnaceae* and *Rosaceae*. In *Ulmaceae* it has been reported from some genera (DARNLEY GIBBS 1974), but *Trema* has apparently not been studied. *Barbeya* and *Dirachma* apparently have not been studied in this respect either.

20. Alkaloids absent (0), present (1).

Alkaloids of various kinds are generally produced in *Elaeagnaceae* and *Rhamnaceae* (HEGNAUER 1966, 1973) and have also been recorded in *Trema* (HEGNAUER 1973), but are absent in *Rosaceae*. *Barbeya* and *Dirachma* have not been investigated in this respect, but *Barbeya* has a strong reputation in both Somalia (M. THULIN) and Eritrea (GHEBREHIWET MEDHANIE, pers. comm.) to be poisonous to goats, which might indicate presence of alkaloids.

21. Cyanogenic pathway absent (0), present (1).

Trema (HEGNAUER 1973), like many *Rosaceae*, including *Dryas* and *Neillia* (DARNLEY GIBBS 1974), produces cyanogenic compounds, but in this respect it is exceptional in *Ulmaceae* and in *Urticales* in general. In *Elaeagnaceae*, and

also in *Rhamnaceae*, cyanogenic compounds seem to be lacking (HEGNAUER 1966, 1973).

22. Ellagic acid absent (0), present (1).

Ellagic acid is produced in *Barbeya* (DICKISON & SWEITZER 1970, GIANNASI 1978, 1986) and *Elaeagnaceae* (HEGNAUER 1966), but not in *Rhamnaceae* and in *Trema* or other *Ulmaceae* (HEGNAUER 1973, GIANNASI 1978). *Rosaceae* are variable in this respect with *Dryas* in subfam. *Rosoideae* producing ellagic acid, but not *Neillia* in subfam. *Spiraeoideae* (HEGNAUER 1973).

Results

The initial analysis, of a large sample of rosids of various families as mentioned under Materials and methods, resulted in four trees with $ci = 0.265$ and $ri = 0.456$, and the consensus tree is shown in Fig. 1.

The morphological analysis resulted in 248 equally parsimonious trees with $ci = 0.875$ and $ri = 0.839$. *Dryas* and *Neillia* (*Rosaceae*), were designated as outgroups as *Rosaceae* is the sister group to the *Dirachma-Barbeya-Elaeagnaceae-Urticales-Rhamnaceae* clade in Fig. 1. The strict consensus tree from the morphological analysis is shown in Fig. 2. This shows two major sister clades, one with *Barbeya* and *Elaeagnaceae*, and one with *Trema* (representing *Urticales*), *Dirachma*, and *Rhamnaceae*.

The analysis of the molecular data (*rbcL* plus *trnL-F*) resulted in four equally parsimonious trees with $ci = 0.636$ and $ri = 0.669$. *Dryas* was designated as the outgroup and the strict consensus tree is shown in Fig. 3. Here *Barbeya* and *Dirachma* form a clade of their own, and this clade is sister to a clade with all the genera of *Rhamnaceae*. Further down on the tree are first *Urticales* (here represented by *Gironniera* and *Trema*), and then a clade with *Hippophae* and *Elaeagnus* representing *Elaeagnaceae*.

The combined analysis (morphological plus molecular data) resulted in two equally parsimonious trees with $ci = 0.636$ and $ri = 0.667$. Also here the trees were rooted by *Dryas*. The consensus tree is shown in Fig. 4. This tree agrees with the morphological tree in that *Barbeya* is forming a clade with *Elaeagnaceae*, and with the molecular tree as regards the general relationships between the genera within *Rhamnaceae*. However, it differs from both the morphological and the molecular tree in that *Urticales* now is sister to all the rest of the ingroup and in that *Dirachma* is sister to a *Barbeya-Elaeagnaceae-Rhamnaceae* clade.

Discussion

The results of the analysis of the large sample of *rbcL* sequences in Fig. 1 can be compared with the results of a recent analysis of another large sample of such sequences, which also includes *Barbeya*, but not *Dirachma* (SWENSEN 1996: fig. 2). The results disagree in many details, probably mainly owing to sampling differences, but agree in that *Barbeya* in both cases belongs to a large clade with various members of *Elaeagnaceae*, *Rhamnaceae* and *Urticales*, and in that this large clade is sister to a clade with various members of *Rosaceae*. The *Rhamnaceae-Elaeagnaceae* complex is one of four major clades among the rosids

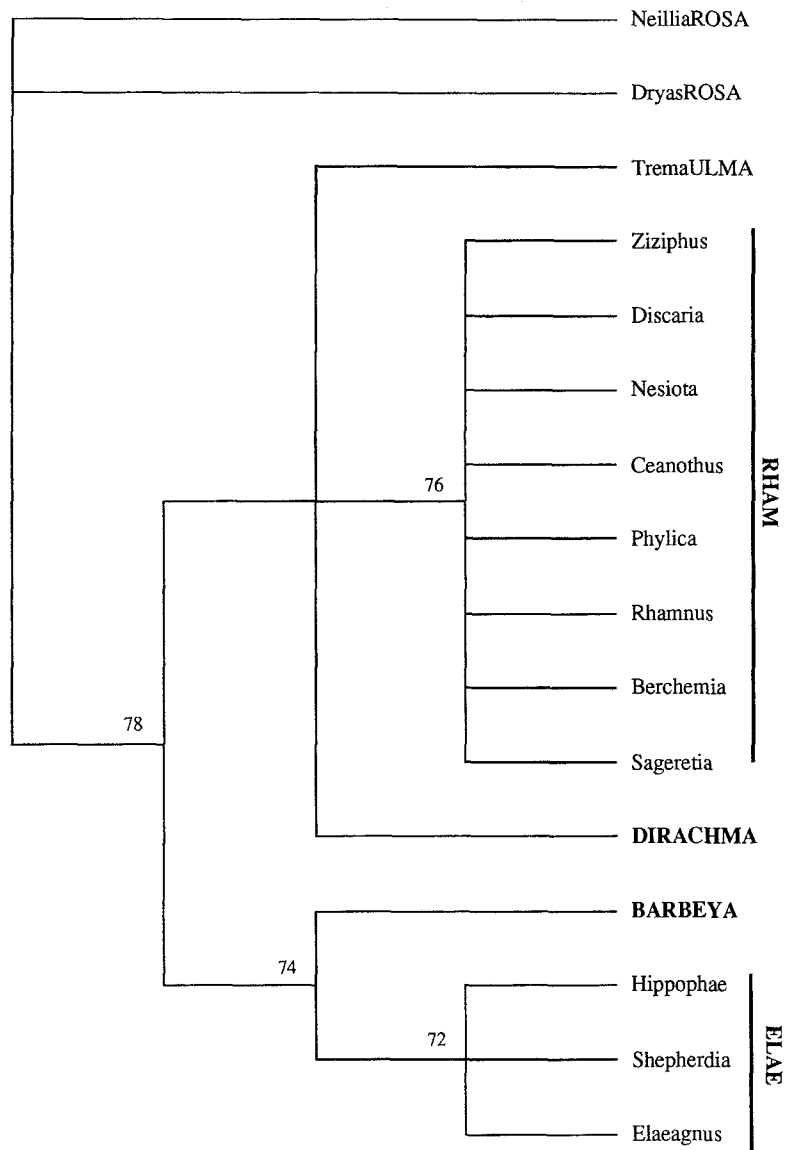


Fig. 2. Strict consensus tree of 248 equally parsimonious trees obtained by phylogenetic analysis of morphological data from *Barbeya* and *Dirachma* (in bold type), and a sample of genera from *Elaeagnaceae*, *Rhamnaceae* and *Ulmaceae*. Vertical bars and corresponding letters represent RHAM = *Rhamnaceae* and ELAE = *Elaeagnaceae*. Numbers above the branches indicate bootstrap values above 50%

that contain actinorhizal plants engaged in nitrogen-fixing symbioses with *Frankia* bacteria (SWENSEN 1996).

The results of the three subsequent analyses are partly in conflict. The morphological analysis (Fig. 2) shows *Barbeya* as sister group to *Elaeagnaceae*, whereas *Dirachma* is close to the *Rhamnaceae*. The bootstrap support value for the *Barbeya-Elaeagnaceae* clade is fairly high, whereas the position of *Dirachma* is poorly supported. In the molecular analysis (Fig. 3), however, there is strong

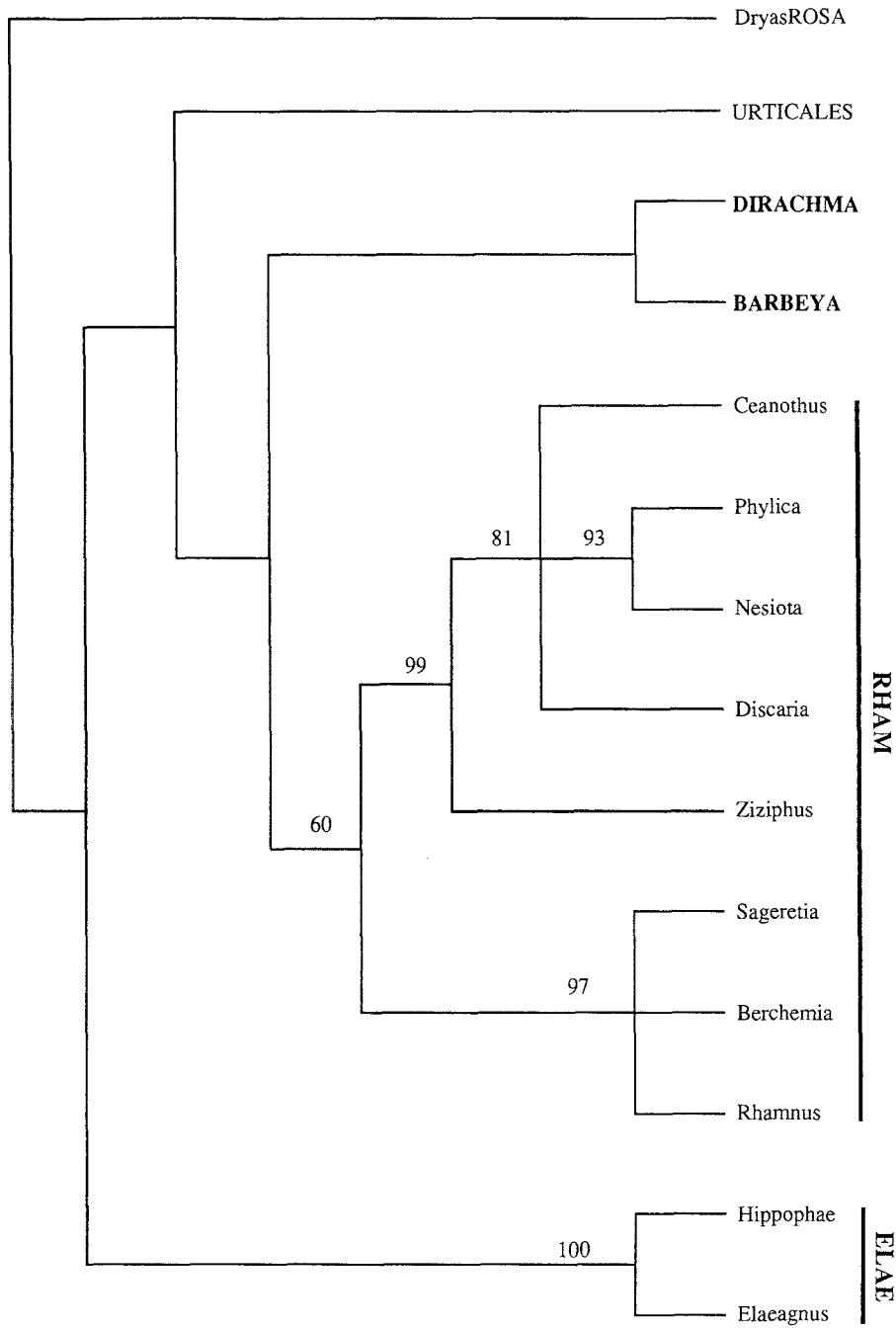


Fig. 3. Strict consensus tree of four equally parsimonious trees obtained by phylogenetic analysis of molecular data (*rbcL* and *trnL-F* sequences) from *Barbeya* and *Dirachma* (in bold type), and a sample of genera from *Elaeagnaceae*, *Rhamnaceae* and *Urticales*. For *Urticales* the *rbcL* data for *Trema* are used in combination with the *trnL-F* data for *Gironniera*. Vertical bars and corresponding letters represent RHAM = *Rhamnaceae* and ELAE = *Elaeagnaceae*. Numbers above the branches indicate bootstrap values above 50%

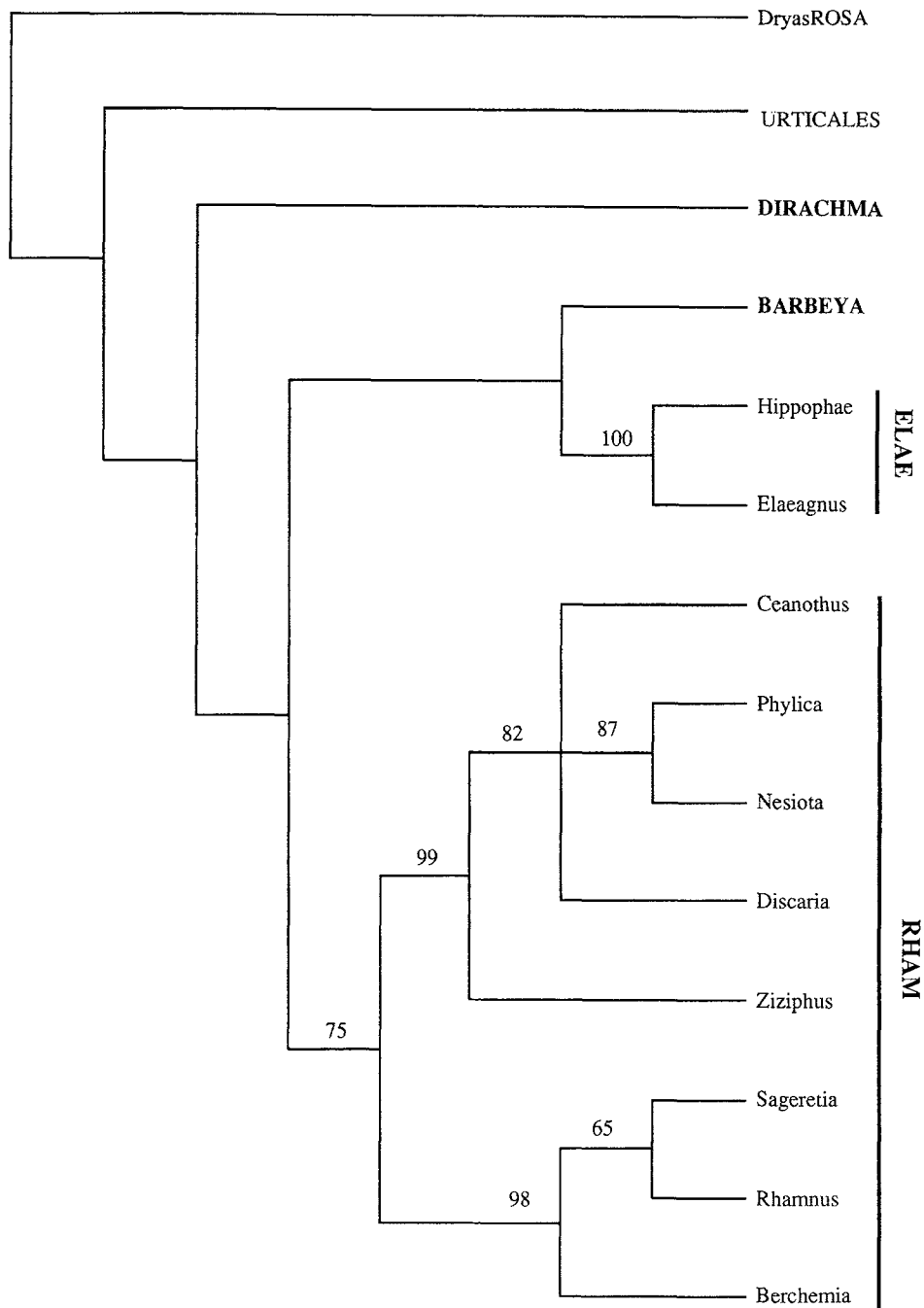


Fig. 4. Strict consensus tree of two equally parsimonious trees obtained by phylogenetic analysis of molecular data (*rbcL* and *trnL-F* sequences) plus morphological data from *Barbeya* and *Dirachma* (in bold type), and a sample of genera from *Elaeagnaceae*, *Rhamnaceae* and *Urticales*. For *Urticales* the *rbcL* and morphological data for *Trema* are used in combination with the *trnL-F* data for *Gironniera*. Vertical bars and corresponding letters represent RHAM = *Rhamnaceae* and ELAE = *Elaeagnaceae*. Numbers above the branches indicate bootstrap values above 50%

support for the clade with the two genera of *Elaeagnaceae* and for the two clades found within *Rhamnaceae*, one with *Ceanothus*, *Discaria*, *Nesiota*, *Phyllica* and *Ziziphus*, and one with *Berchemia*, *Rhamnus* and *Sageretia*. *Rhamnaceae* itself as a monophyletic group is only weakly supported and, actually, if *rbcL* is analysed separately, *Rhamnaceae* becomes biphyletic. The clade with *Barbeya* plus *Dirachma* in the molecular analysis is weakly supported. In the combined analysis (Fig. 4) there is weak support for the *Barbeya-Elaeagnaceae* clade but a relatively strong support for a monophyletic *Rhamnaceae*.

These results indicate that the geographically associated genera *Barbeya* and *Dirachma*, despite their profound morphological differences, are in some way also phylogenetically associated, even if the exact relationships between them and the likewise associated *Rhamnaceae*, *Elaeagnaceae* and *Urticales* are still uncertain.

Obviously both *Barbeya* and *Dirachma* are isolated taxa. In none of the analyses *Barbeya* groups with the representative from the *Urticales*, and the genus should obviously be excluded from this order. *Barbeya* differs from all members of *Urticales* notably by having unilacunar (not tri- or pentalacunar) nodes, more or less apocarpous (not syncarpous) gynoecia, and by producing ellagic acid.

On the other hand, *Barbeya* is associated with *Elaeagnaceae* in both the morphological and the combined analysis. *Barbeya* is strikingly similar to various members of *Elaeagnaceae* in habit and its silvery indumentum, and *Barbeya* agrees with *Elaeagnaceae* also for example in lacking stipules, by having unilacunar nodes and apocarpous gynoecia, and by producing ellagic acid. However, the differences are also substantial (indumentum of simple hairs in *Barbeya* versus stellate and peltate hairs in *Elaeagnaceae*; nectary-disk absent in *Barbeya* but present in *Elaeagnaceae*, ovules pendulous in *Barbeya* versus ascending in *Elaeagnaceae*, and seed coat unspecialized and non-multiplicative in *Barbeya* versus exotestal and multiplicative in *Elaeagnaceae*). According to the bootstrap values the association between *Barbeya* and *Elaeagnaceae* is weakly supported in the combined analysis, and cannot motivate an inclusion of the genus in this family. Instead, *Barbeya* seems best retained in a family of its own.

Dirachma is close to *Rhamnaceae* in the morphological analysis, and characters in common are, for example, presence of stipules and petals, syncarpous gynoecia, and exotestal and multiplicative seed coats. On the other hand, *Dirachma* differs from *Rhamnaceae* for example by having non-keeled sepals, non-hooded petals, and by lacking a nectary-disk. Furthermore, there is no support for a close relationship between *Dirachma* and *Rhamnaceae* in the combined analysis. Therefore, *Dirachma* seems also best retained in a family of its own.

The marked subdivision of *Rhamnaceae* into two clades in the molecular and the combined analysis is curious. This subdivision has no support in the morphological data available to us and is the subject of further study (RICHARDSON, FAY & CHASE, unpubl.).

BACKLUND & BREMER (1998) discuss principles of classification in relation to monotypic plant families and argue that, as far as possible, monotypic families should be reduced to increase the phylogenetic information content of the system. However, following the primary principle of monophyly, this can only be done if the monotypic family is the sister group of a single family, and BACKLUND &

BREMER also give a number of secondary principles to be considered. In the case of *Barbeyaceae* and *Dirachmaceae* one could argue, for example, from the combined analysis (Fig. 4) that *Barbeyaceae* should be included in *Elaeagnaceae* as it is the sister group of a single family, whereas *Dirachmaceae* should be retained as it is the sister group of a clade comprising both *Barbeya* plus *Elaeagnaceae* and *Rhamnaceae*. However, as the monophyly of the *Barbeya-Elaeagnaceae* clade is only weakly supported and as the inclusion of *Barbeya* in *Elaeagnaceae* would create a heterogenous and poorly identifiable family the recognition of *Barbeyaceae* clearly would be the preferable alternative.

At the suprafamilial level the first broad analysis (Fig. 1), as well as all the three subsequent analyses (Figs. 2–4) would be largely congruent with a *Rhamnales* sensu THORNE (1983, 1992), with *Rhamnaceae* and *Elaeagnaceae*, or with the superorder *Rhamnanae* sensu TAKHTAJAN (1987), with the two monotypic orders *Rhamnales* and *Elaeagnales*, if only *Barbeyaceae* and *Dirachmaceae* are added. At the same time the first analysis strongly contradicts MELCHIOR's (1964) or CRONQUIST's (1981, 1988) circumscription of *Rhamnales* (with *Rhamnaceae*, *Leeaceae* and *Vitaceae*). This analysis also contradicts the placement of *Elaeagnaceae* in *Thymelaeales* by MELCHIOR (1964) or its placement in the *Proteales* by CRONQUIST (1981, 1988).

At the order level the following classification would be compatible with our results: *Rhamnales* with *Rhamnaceae*, *Elaeagnaceae*, *Dirachmaceae* and *Barbeyaceae*, *Urticales* with the same circumscription as usual except for the exclusion of *Barbeyaceae*, and a monotypic *Rosales*. However, we refrain from making any formal suprafamilial groupings. To get a balanced classification the circumscription of orders should be seen in a broader perspective than is possible here.

Within the *Rosales-Urticales-Rhamnales* clade actinorrhizal symbiosis (SWENSEN 1996) is known from *Rosaceae* (*Cercocarpus*, *Cowania*, *Dryas* and *Purshia*), *Elaeagnaceae* (all genera), and *Rhamnaceae* (*Ceanothus*, *Colletia*, *Discaria* and *Trevoa*). Whereas the nodules of *Elaeagnaceae* and *Rhamnaceae* are characterized by spherical, usually septate vesicles, the ones of *Rosaceae* are ellipsoid and non-septate. This, according to SWENSEN (1996), supports the view that the actinorrhizal symbiosis of *Rosaceae* and that of *Elaeagnaceae-Rhamnaceae* have separate origins, although she, as well as SOLTIS & al. (1995) postulate a genetic predisposition for actinorrhizal symbiosis in all nodulating species and their close relatives.

No cases of actinorrhizal symbiosis are known from any member of *Urticales*. This indicates either that this lineage has lost its symbiotic capacity or that symbiotic taxa exist but have remained undetected. *Barbeya* and *Dirachma*, both being part of the actinorrhizal *Elaeagnaceae-Rhamnaceae* clade, surely would be interesting objects for an investigation in this respect.

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