

Phylogeny of the tribe Antirrhineae (Scrophulariaceae) based on morphological and *ndhF* sequence data

Medhanie Ghebrehiwet¹, Birgitta Bremer², and Mats Thulin²

¹Department of Biology, University of Asmara, Asmara, Eritrea

²Department of Systematic Botany, Uppsala University, Uppsala, Sweden

Received November 5, 1998

Accepted May 18, 1999

Abstract. Phylogenetic relationships within the tribe Antirrhineae (Scrophulariaceae) are analysed and discussed on the basis of parsimony analyses of morphological and *ndhF* gene sequence data. The results indicate that the tribe Antirrhineae consists of four major groups of genera, the *Anarrhinum* clade, the *Gambelia* clade, the *Maurandya* clade, and the *Antirrhinum* clade. The *Anarrhinum* clade, consisting of the Old World bee-pollinated genera *Anarrhinum* and *Kickxia*, is sister to the rest of the tribe. The *Gambelia* clade consists of the New World genera *Gambelia* and *Galvezia*, which are very closely related and pollinated by hummingbirds. The *Maurandya* clade consists of one subclade including *Maurandya* and a number of related bee- or hummingbird-pollinated New World genera and another subclade with the Old World bee-pollinated genera *Asarina* and *Cymbalaria*. The *Antirrhinum* clade consists mainly of bee-pollinated Old World genera, such as *Antirrhinum*, *Linaria*, *Chaenorhinum*, and their segregates, but also includes the New World genera *Mohavea* and *Howelliella*, of which the latter is known to be partly pollinated by hummingbirds. It is concluded that hummingbird-pollination has evolved independently within Antirrhineae at least three times from bee-pollinated ancestors.

Key words: Scrophulariaceae, Antirrhineae, morphology, *ndhF*, phylogeny, pollination.

The tribe Antirrhineae as revised by Sutton (1988) consists of 27 genera representing 328 species. Familiar members include various garden ornamentals, such as snapdragons (*Antirrhinum*), toadflax (*Linaria*), and kenilworth (*Cymbalaria*). The group is a clearly circumscribed tribe in the family Scrophulariaceae and is characterised, above all, by its poricidal capsule dehiscence, unique in the family, and by the presence of apparently unique iridoid glycosides, such as antirrhinoside (Kooiman 1970). Preliminary studies of morphological and *rps2* sequence data by Depamphilis et al. (1994) indicate strong support for the monophyly of the Antirrhineae. The relatively stable taxonomic history of the Antirrhineae is a reflection of the marked internal continuity and external discontinuity of its morphology. Its circumscription has remained more or less the same whether it was treated as a single genus (Linnaeus 1753), as a tribe (Chavannes 1833), or as a subfamily (Betsche 1984).

The supra-generic relationships within the Antirrhineae have been discussed by, e.g., Rouy (1909), Rothmaler (1943), and Speta (1982). Mainly on the basis of the palate structure (the basal convexity of the abaxial lip) Rouy (1909) classified the Antirrhineae into three subtribes, Linariinae (as “Linariae”), Anarrhininae (as “Anarrhineae”), and Rhodochitoninae (as “Rhodochitoneae”). Plants with a prominent palate were put in the Linariinae (*Linaria*, *Antirrhinum* and *Schweinfurthia*), and those without a clearly defined palate into Anarrhininae (most genera including *Anarrhinum* and *Maurandya*). *Rhodochiton* was treated as a separate subtribe on the basis of its unique membranaceous calyx.

Rothmaler (1943) divided the tribe into five groups: “*Maurandya*-Gruppe” (five genera), “*Gambelia*-Gruppe” (three genera), “*Linaria*-Gruppe” (11 genera), “*Mohavea*-Gruppe” (*Mohavea* only), and “*Anarrhinum*-Gruppe” (*Anarrhinum* only). These groups have been treated later on by Rothmaler himself (Rothmaler 1954) and subsequent authors as the subtribes Maurandyinae, Gambeliinae, Linariinae, Mohaveinae and Anarrhininae (as “Simbuletinae” by Rothmaler, 1954). Rothmaler (1943) further attempted to show phylogenetic relationships between the genera within the tribe. He assumed that palmate venation was a relatively primitive character state, while development of palate and spur were amongst the derived states. He placed the “*Maurandya*-Gruppe” in a basal position on one of the main branches on the tree with *Rhodochiton* nearest the root. The “*Anarrhinum*-Gruppe” was placed as a sister to all other genera of Antirrhineae.

Rothmaler’s classification and evolutionary interpretation has been changed and criticised by several subsequent workers. Speta (1982) confined the Linariinae to *Linaria* only, whereas the remaining genera were placed in the new subtribe Antirrhininae. Rothmaler’s evolutionary interpretation on the status of the Maurandyinae was rejected by Elisens (1985a), who suggested that the

Maurandyinae have several derived characters that will place its members in a relatively advanced position in the tribe, though his view was not supported by others (Sutton 1988, Thompson 1988). In his revision of the tribe Antirrhineae Sutton (1988) made some comments and remarks of disagreement with Elisens’ work, suggesting the need for a detailed phylogenetic study of the whole tribe.

Recent studies based on molecular data have begun to unravel relationships within the family Scrophulariaceae that will also give some clues about the position of the Antirrhineae in the family. These include the studies by Depamphilis et al. (1994) and Olmstead and Reeves (1995), who have demonstrated the polyphyly of the family. The results obtained by Olmstead and Reeves (1995) by using *rbcL* and *ndhF* gene sequences indicate that the Scrophulariaceae are composed of two distinct clades designated by the authors as “Scroph I” and “Scroph II”. The Antirrhineae represented by *Antirrhinum* is shown to fall within “Scroph II”. This clade further includes *Digitalis* and *Veronica* from Scrophulariaceae as well as representatives of Plantaginaceae, Callitrichaceae, and Hippuridaceae. Recently, it has been shown that also *Globularia* belongs to this clade (Oxelmann et al. 1999).

The floral diversity within Antirrhineae is considerable and includes for example corollas closed by a palate, narrow tubular corollas, wide flaring corollas, and corollas with a spur. Flowers of the Old World species of the tribe (including *Anarrhinum*, *Antirrhinum*, *Asarina*, *Chaenorhinum*, *Cymbalaria*, *Kickxia*, *Linaria*, and *Misopates*) are normally zygomorphic, but many of the New World Antirrhineae (particularly *Lophospermum*, *Maurandya*, and *Rhodochiton*) have peloric flowers with corollas that approach the actinomorphic state (Sutton 1987). In the Antirrhineae most of the species are bee-pollinated, but pollination by hummingbirds is known from several New World members of the tribe (Pennell 1935, Elisens 1986, Sutton 1988).

The aim of the present work is to present a hypothesis for the phylogenetic relationships within the tribe based on morphological data for all the genera recognised by Sutton (1988) and complemented by molecular data for a selected sample of genera, and to discuss trends in floral evolution and pollination systems within the tribe, as well as previous subtribal classifications, in the light of this phylogenetic hypothesis.

Materials and methods

Outgroup. In all analyses *Digitalis* and *Chelone* were used as outgroups. *Chelone* was selected on the basis of ongoing research suggesting it to be close to the Antirrhineae (Eberhardt Fischer, pers. comm.). A common origin of Cheloneae and Antirrhineae is also suggested by Raman (1990) on the basis of similarity in the trichomes present on the corolla. *Digitalis* was selected as a member of the "Scroph II" clade of Olmstead and Reeves (1995) along with *Antirrhinum*.

Morphological analysis. A data matrix was prepared comprising 30 taxa and 30 characters. All genera recognised by Sutton (1988) were included in the analyses. The genera were used as terminals in all cases except *Kickxia*, where the two sections *K. sect. Kickxia* and *K. sect. Valvatae* were used to be able to test the monophyly of the genus. These two sections show marked differences and a subdivision into distinct genera has been proposed by Betsche (1984). The information on the characters is based on literature (Sutton 1988, Elisens 1985a, Thompson 1988) supplemented by studies of herbarium material of all genera and cultivated material of many taxa. The various morphological characters used are listed in Table 1 and the data matrix is presented in Table 2.

The data matrix was analysed using the computer program PAUP version 3.1.1 (Swofford 1993). The parsimony analysis was carried out using heuristic methods and with all character changes weighted equally. The options used were TBR branch swapping with MULPARS on, steepest descent off and random addition sequences with 100 entry replicate searches. All multistate characters were treated as unordered. To assess the relative support for clades bootstrap (Felsenstein 1985) and Bremer support (Bremer

1994) analyses were performed. Bootstrap was calculated from 1000 replicates, each with a single, random additional sequence of the taxa and nearest-neighbour interchanges (NNI) branch swapping saving a single tree. Bremer support is defined as the number of extra steps necessary to lose a group in the consensus. Character evolution was traced using the computer program MacClade (Maddison and Maddison 1992).

Morphological characters. Several of the characters and codings require some additional comments. Polymorphisms are coded by all observed states but, the widely common state is used when there is an absolute majority for one of the states (see Weins 1995). Unknown character states are coded with a question mark. In order to minimise unknown states in characters inapplicable for taxa lacking particular organs, absence of organs was included as a state in a multistate character (Maddison 1993, Swenson and Bremer 1997). Coding of quantitative characters was done following Thiele (1993), who in contrast to Stevens (1991) supports the applicability of overlapping morphometric data in phylogenetic analyses.

Habit. In their habit the Antirrhineae are typically herbs, but some are subshrubs or small shrubs. The stems may be erect, procumbent or climbing. Approximately half of the genera of the Antirrhineae includes only perennial species.

Leaves. The shape of the leaves is quite variable in the Antirrhineae and often diagnostic at generic level (Sutton 1988). The range of shape is coded here in three states as isodiametric leaves, elongate leaves, and linear to filiform leaves. Isodiametric leaves include such with deltate, hastate, sagittate, orbiculate, or reniform shape, while elongate leaves may be elliptic, ovate, obovate, oblanceolate, or oblong. Leaf shape in the Antirrhineae may vary considerably in different parts of the plant. Basal leaves are in some genera often markedly different in size and/or shape from the leaves higher up on the stem, i.e. the leaves are heteromorphic. The homomorphic condition is coded as one state and the heteromorphic condition as two states, different in size and different in shape.

Rothmaler (1943) sometimes used the venation pattern of the leaves in his delimitation of genera in the tribe. Palmate venation is prevalent in the genera with more or less isodiametric leaves. Most genera in the Antirrhineae have entire or lobed

Table 1. Characters and character states used in the morphological analysis of Antirrhineae

1.	Habit herbaceous (0), suffrutescent (1), shrubby (2)
2.	Duration perennial (0), annual/biennial (1)
3.	Stem erect (0), procumbent or climbing (1)
4.	Upper and lower leaves homomorphic (0), different in size (1), different in shape (2)
5.	Leaf shape isodiametric (0), elongate (1), linear to filiform (2)
6.	Leaf margin dentate (0), lobed or entire (1)
7.	Petiole not cirrhose (0), cirrhose (1) absent (2)
8.	Venation pinnate (0), palmate (1)
9.	Pedicel not cirrhose (0), cirrhose (1)
10.	Calyx lobes equal (0), adaxial lobe longest (1), adaxial lobe shortest (2)
11.	Calyx shorter than corolla tube (0), longer than corolla tube (1)
12.	Corolla up to 20 mm long (0), more than 20 mm long (1)
13.	Corolla tube up to 5 mm wide (0), more than 5 mm wide (1)
14.	Corolla tube length $< 2x$ width (0), $\geq 2x$ width (1)
15.	Corolla tube not spurred (0), spurred (1)
16.	Corolla lips equal (0), adaxial lip shorter (1), adaxial lip longer (2)
17.	Abaxial lip without palate (0), with palate (1)
18.	Anthers free (0), marginally coherent (1), forming ring (2)
19.	Staminode with rudimentary anther (0), without rudimentary anther (1)
20.	Shape of capsule ovoid to subglobose (0), globose (1), oblong (2)
21.	Loculus size of capsule equal (0), unequal (1)
22.	Capsule wall coriaceous (0), papery (1)
23.	Dehiscence by single split (0), valvate (1), irregular rupture (2)
24.	Seed symmetry radial (0), bilateral (1), dorsiventral (2)
25.	Position of hilum basal or subbasal (0), median (1)
26.	Seed size > 2 mm (0), $1 - 2$ mm (1), < 1 mm (2)
27.	Wing of seed of <i>Epixiphium</i> type absent (0), present (1)
28.	Tubercles absent (0), present (1)
29.	Periclinal wall of testa cell flat (0), convex (1), concave (2)
30.	Epicuticular wax absent (0), present (1)

leaves, but in some the margin is dentate or crenate.

The presence or absence of a petiole is an important character in the delimitation of Antirrhineae genera. The petiole can be cirrhose or not. All species of *Linaria* have sessile leaves.

Pedicel. The flowers in Antirrhineae are pedicellate, and the pedicel can be cirrhose or not. Only in few species the pedicel is virtually absent.

Calyx. The calyx is normally deeply divided into five lobes. A few taxa, *Rhodochiton* and one species of *Anarrhinum*, have a shallowly lobed calyx. The relation between the length of the calyx to that of the corolla-tube has been used as a diagnostic character at generic level by Rothmaler

(1943). The character is here coded as calyx lobes shorter or longer than corolla-tube.

Corolla. The corolla of most Antirrhineae is gamopetalous, 5-lobed, tubular and bilabiate, and the abaxial lip has a basal convexity, the palate, which occludes the mouth of the tube (Sutton 1988). The floral tube is variable in length, width, and shape. The abaxial base of the tube is known to exhibit different shapes variably known as entire, saccate, gibbous, or spurred (Sutton 1988). There is no clear-cut separation between these shapes as one type continues to the other. Only two states are used here, spurred and not spurred. In *Antirrhinum* the corolla-tube is generally gibbous, but one species, *Antirrhinum braun-blانquetii*, is spurred. In *Kickxia*, on the other hand, all species except

Table 2. Data matrix for the terminal taxa (with names) and the characters (numbered as in Table 1). Polymorphic states are represented by letters as follows: a = 0/1, b = 0/2, c = 0/1/2, d = 1/2. Unknown and inapplicable states are coded “?”

Taxa	1									1 1 1 1 1 1 1 1 1 2									2 2 2 2 2 2 2 2 2 3												
	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	
<i>Digitalis</i>	0	0	0	1	1	0	0	0	0	0	0	1	1	0	0	0	0	0	0	?	0	0	0	?	1	0	1	0	0	?	0
<i>Chelone</i>	0	0	0	0	1	0	0	0	0	0	0	1	1	0	0	a	0	0	0	0	0	0	0	?	0	0	0	1	0	0	0
<i>Acanthorrhinum</i>	2	0	0	0	2	1	2	0	0	0	0	0	0	0	0	2	1	1	1	1	1	1	0	1	1	0	1	0	1	a	1
<i>Albraunia</i>	0	1	0	0	1	1	0	0	0	2	1	0	0	0	1	0	1	1	1	2	1	0	0	0	0	0	1	0	1	a	1
<i>Anarrhinum</i>	0	0	0	2	1	0	0	0	0	0	0	0	0	0	1	1	1	2	1	0	1	0	a	1	1	1	2	0	1	1	0
<i>Antirrhinum</i>	c	0	0	0	b	1	0	0	0	0	0	a	1	1	0	2	1	1	1	0	1	1	0	1	0	0	2	0	0	1	1
<i>Asarina</i>	1	0	1	0	0	0	0	1	0	0	0	1	1	1	0	0	1	1	1	0	1	0	1	1	0	0	1	0	0	0	0
<i>Chaenorhinum</i>	a	a	0	0	1	1	0	0	0	1	0	0	0	0	1	0	1	1	1	c	1	1	a	1	0	0	2	0	1	1	1
<i>Cymbalaria</i>	0	a	1	0	0	1	0	1	0	1	0	0	0	0	1	0	1	1	1	1	1	0	1	1	0	0	c	0	1	a	1
<i>Epixiphium</i>	0	0	1	0	0	1	1	1	0	0	0	1	1	1	0	0	0	1	0	0	1	1	0	1	2	0	0	1	1	0	1
<i>Galvezia</i>	2	0	0	0	1	1	0	0	0	0	0	a	0	1	0	0	1	0	0	0	0	0	1	2	0	0	2	0	1	0	0
<i>Gambelia</i>	2	0	0	0	1	1	0	0	0	0	0	1	a	1	0	1	1	0	1	0	1	1	0	0	1	0	d	0	a	1	0
<i>Holmgrenanthe</i>	1	0	0	0	0	0	0	1	0	1	0	1	1	1	0	0	1	0	0	1	0	1	1	2	0	0	0	0	1	0	0
<i>Holzneria</i>	0	1	0	0	1	1	0	0	0	2	1	0	0	0	1	0	1	1	1	0	1	1	0	1	0	0	2	0	0	1	a
<i>Howelliella</i>	0	1	0	0	1	1	0	0	0	1	0	a	0	1	1	1	1	1	0	0	1	1	1	2	0	0	2	0	0	1	0
<i>Kickxia</i> sect. <i>K.</i>	c	a	1	2	1	1	0	1	0	0	0	0	0	0	1	1	1	2	1	1	1	0	a	0	1	1	2	0	a	1	0
<i>Kickxia</i> sect. <i>V.</i>	c	a	1	2	c	1	0	1	0	0	0	0	a	0	1	1	1	2	1	0	1	0	a	1	1	1	2	0	1	1	0
<i>Linaria</i>	0	a	a	a	2	1	2	0	0	c	0	0	a	0	1	0	1	1	1	c	1	a	1	1	b	0	a	?	a	c	0
<i>Lophospermum</i>	1	0	1	1	0	0	1	1	1	0	0	1	1	1	0	1	0	0	0	1	0	0	1	2	2	0	0	1	1	2	0
<i>Mabrya</i>	1	0	0	0	0	0	0	1	0	0	0	1	1	1	0	1	0	0	0	0	0	0	1	2	0	0	1	0	1	2	0
<i>Maurandella</i>	1	0	1	1	0	1	1	1	0	0	0	1	1	1	0	1	1	1	0	b	1	1	1	2	1	0	1	0	1	0	1
<i>Maurandya</i>	1	0	1	1	0	1	1	1	1	1	0	1	1	1	0	1	0	1	0	2	1	0	1	2	0	0	1	0	1	?	0
<i>Misopates</i>	0	1	0	0	1	1	0	0	0	1	0	0	0	1	0	1	1	1	1	0	1	1	0	1	1	0	2	1	1	a	1
<i>Mohavea</i>	0	1	0	0	1	1	0	0	0	1	0	1	0	1	0	0	1	1	0	0	1	1	1	1	1	0	1	1	0	0	0
<i>Neogaerrhinum</i>	0	1	0	2	1	1	0	0	1	0	0	0	a	0	0	1	1	1	1	1	1	a	0	2	0	0	2	0	1	0	0
<i>Nuttallanthus</i>	0	1	0	0	1	1	2	0	0	0	0	0	0	1	1	1	1	1	1	2	1	0	1	1	0	0	2	0	1	?	0
<i>Pseudorontium</i>	0	1	0	0	1	1	0	0	0	0	0	0	0	1	0	1	1	1	0	1	0	0	1	1	1	0	0	1	1	0	1
<i>Rhodochiton</i>	1	0	1	0	0	0	1	1	0	0	0	1	1	1	0	0	0	0	0	2	1	0	1	2	2	0	0	1	1	0	0
<i>Sairocarpus</i>	0	1	0	0	1	1	0	0	0	1	0	0	0	1	0	1	1	1	1	b	1	1	1	1	0	0	1	0	1	?	?
<i>Schweinfurthia</i>	1	a	0	0	a	1	0	0	0	1	0	0	0	0	0	0	1	1	1	1	1	1	1	2	0	0	1	0	1	1	1

K. saccata are spurred. *Antirrhinum* is coded here as not spurred, while *Kickxia* is coded as spurred.

Stamens. Normally the Antirrhineae have four fertile stamens with the fifth (adaxial) stamen reduced to an inconspicuous staminode with or without a rudimentary anther. In the majority of the genera the stamens are conspicuously didynamous. Commonly the adjacent stamens are coherent by the margins of the anthers, but in *Kickxia* and *Anarrhinum* all four anthers are connate, forming a ring-like structure.

Capsule. The capsules of Antirrhineae vary markedly in size and shape. The most common

shapes are globose, ovoid, and oblong. The capsule is bilocular and in most genera the loculi are equal, but a few have conspicuously unequal loculi with the abaxial loculus usually the largest. The loculus wall can either be thin and papery or thick and coriaceous. Although all of the Antirrhineae basically have a porose pattern of dehiscence, the mode of dehiscence can be by a single split, valvate, or by irregular rupture.

Seeds. The seeds of Antirrhineae show a great variation in size, shape, and external ornamentation. This variation has been used by several authors (Munz 1926, Rothmaler 1943, Elisens

Table 3. Sources of plant material for taxa included in this study and previously published sequences with voucher specimens and EMBL/GenBank accession numbers

Taxon	Source/Voucher information	EMBL/GenBank acc.
<i>Anarrhinum corsicum</i> Jordan and Fourr.	Cult., Uppsala Bot. Gard., Ghebrehwet 372 (UPS)	AJ245815
<i>Antirrhinum majus</i> L.	Olmstead and Reeves, 1995	L36392
<i>Asarina procumbens</i> Mill.	Cult., Uppsala Bot. Gard., Ghebrehwet 461 (UPS)	AJ250380
<i>Chaenorhinum minus</i> (L.) Lange	Cult., Uppsala Bot. Gard., Ghebrehwet 377 (UPS)	AJ250381
<i>Chelone obliqua</i> L.	Cult., Uppsala Bot. Gard., Thulin 9500 (UPS)	AJ245814
<i>Cymbalaria muralis</i> P. Gaertner	Cult., Uppsala Bot. Gard., Ghebrehwet 378 (UPS)	AJ250382
<i>Digitalis grandiflora</i> Mill.	Olmstead and Reeves, 1995	L363999
<i>Galvezia fruticosa</i> J.F. Gmel.	Ecuador, Herb. material, Asplund 15951 (UPS)	AJ250383
<i>Gambelia speciosa</i> Nutt.	Cult., Uppsala Bot. Gard., Ghebrehwet 369 (UPS)	AJ250384
<i>Howelliella ovata</i> (Eastw.) Rothm.	U. S. A., California, Herb. material, Storer 453 (UPS)	AJ250385
<i>Kickxia elatine</i> (L.) Dumort.	Cult., Uppsala Bot. Gard., seeds from Ryding 1210 (UPS)	AJ245816
<i>K. gracilis</i> (Benth.) D.A. Sutton	Cult., Uppsala Bot. Gard., seeds from Ghebrehwet 205(UPS)	AJ245817
<i>Linaria amethystea</i> (Vent.) Hoffmanns	Cult., Uppsala Bot. Gard., Ghebrehwet 370 (UPS)	AJ250386
<i>Maurandella antirrhiniflora</i> (Willd.) Rothm.	Cult., Uppsala Bot. Gard., Ghebrehwet 462 (UPS)	AJ250387
<i>Maurandya scandens</i> (Cav.) Pers.	Cult., Uppsala Bot. Gard., Ghebrehwet 367 (UPS)	AJ245818
<i>Misopates orontium</i> (L.) Rafin.	Cult., Uppsala Bot. Gard., Ghebrehwet 376 (UPS)	AJ250388
<i>Mohavea confertiflora</i> (A. DC.) A.A. Heller	U. S. A., Arizona, Herb. material, Nelson 1348 (UPS)	AJ250389
<i>Rhodochiton atrosanguineum</i> (Zucc.) Rothm.	Cult., Uppsala Bot. Gard., Ghebrehwet 375 (UPS)	AJ250390
<i>Schweinfurthia pterosperma</i> (A. Rich.) A. Braun	Yemen, Herb. material, Thulin 8205 (UPS)	AJ250391

1985b, Sutton 1988) for generic and infrageneric delimitation within the tribe. The size of the seeds in the tribe varies from c. 4 mm in genera such as *Holmgrenanthe*, *Lophospermum*, and *Rhodochiton* to c. 0.3 mm in some sections of *Linaria* and in *Nuttallanthus*. An average size, as exemplified by species of *Maurandya*, is between 1 and 2 mm.

In the majority of the genera the position of the hilum is basal or sub basal, but also a median position is sometimes found. Sutton (1988) has indicated that the wings of the seeds of *Linaria*, when compared in relation to the position of hilum, are not homologous to those of *Epixiphium*, *Lophospermum*, *Rhodochiton*, *Mohavea*, and *Pseudorontium*. The symmetry of the seeds in Antirrhineae is variable, and three types have been distinguished by Sutton (1988), radial, bilateral, and dorsiventral.

The seeds of Antirrhineae possess an intact outer periclinal wall to the testa-cells at maturity. The curvature of the outer periclinal wall is responsible for the often microscopically visible roughness of the seed surface. Individual testa-cells may have the periclinal wall flat, convex, or concave (Sutton 1988). Epicuticular secretions on seeds usually occur as irregular to very regular striations with a high micromorphological diversity (Barthlott 1981). In the Antirrhineae several genera have seeds with an overlaid fine texturing of granules or filaments of epicuticular waxes (Sutton 1988). In the analysis this character is coded as present or absent.

Molecular analysis. Sequences from 19 terminal taxa, including outgroups and the two sections of *Kickxia*, were used for the cladistic analysis of molecular data. The species used in the analysis are listed in Table 3, with details of their name, voucher specimens and EMBL accession numbers. Most of the plant material used for extracting DNA was grown from seeds, but in a few cases herbarium material was used. DNA was extracted, amplified, and sequenced following the protocols in Kim and Jansen (1995) or Backlund et al. (in press). Alignments were made manually to the reading frames of the corresponding protein sequence, gaps were treated as missing data. In the data analysis 15 sequences of species representing genera in the Antirrhineae and the one of *Chelone* are new, while the sequences for *Antirrhinum* and *Digitalis* were already published. The

data matrix in the phylogenetic analyses comprise 2133 aligned nucleotide positions.

The parsimony analysis was carried out using heuristic methods and with all character changes weighted equally. The options used were the same as in the morphological analysis.

Combined analysis. The molecular and morphological data for the same terminal taxa as in the molecular analysis were merged into one data matrix with a total of 2163 characters. The parsimony analysis was carried out using heuristic methods and the options used were the same as in the previous analyses.

Results

Morphological analysis. The cladistic analysis of the morphological data with 30 characters and 30 taxa generated 134 equally most parsimonious trees all with a length of 140 steps. The consistency index is 0.300 and the retention index 0.616. Within Antirrhineae two major clades appear in the strict consensus tree (Fig. 1). The first clade is composed of New World genera that belong to the subtribe Maurandyinae (Rothmaler 1954, Elisens 1985a) with the Old World genus *Asarina* nested within it. The second clade is less resolved, with *Galvezia* coming out as the sole sister to the rest. The subclade containing *Anarrhinum* and the two sections of *Kickxia* is strongly supported.

Molecular analysis. The molecular search resulted in two equally most parsimonious trees, 977 steps long with a consistency and retention index of 0.735 and 0.634 respectively. The results indicate that Antirrhineae can be subdivided into four well supported groups of genera (Fig. 2). The first group, corresponding to the *Anarrhinum* clade including *Anarrhinum* and the two sections of *Kickxia*, is sister to the rest of the Antirrhineae. This clade is highly supported with a bootstrap value of 100 and a Bremer support of 27. The two sections of *Kickxia* are sister groups with *Anarrhinum* as sister to the genus *Kickxia*.

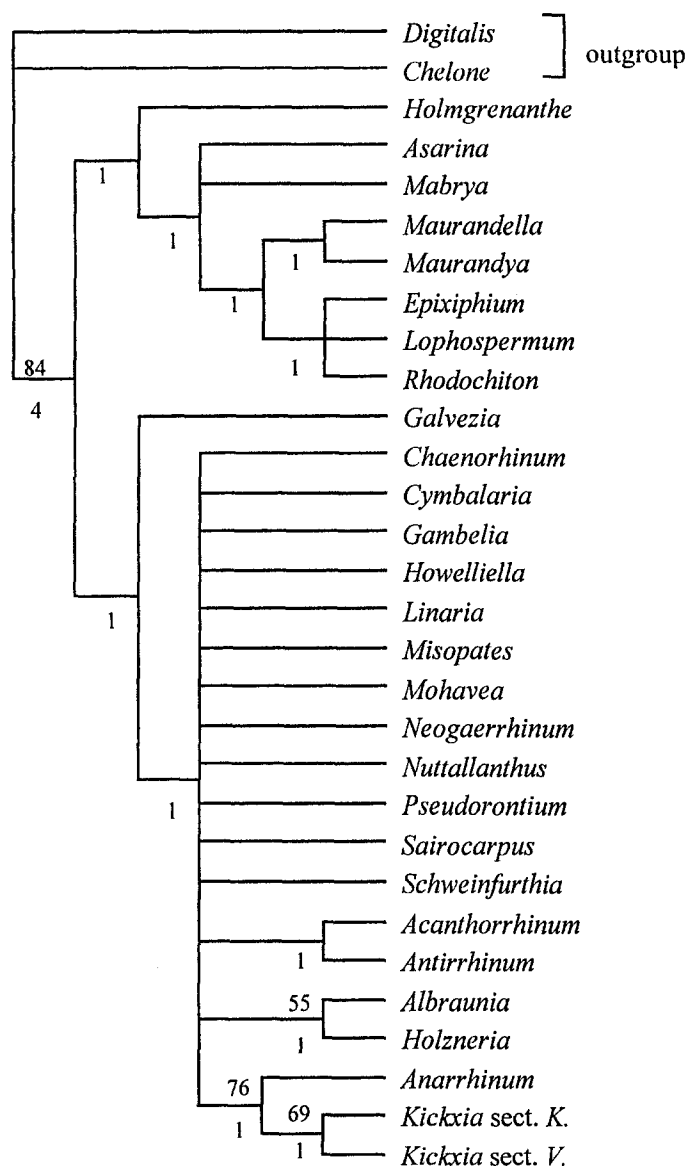


Fig. 1. Strict consensus tree of the 11 equally most parsimonious trees of the Antirrhineae based on morphological characters. Numbers above nodes correspond to bootstrap values above 50%. Numbers below nodes are Bremer support values, i.e. numbers of extra steps required to collapse that particular node

The second group is the *Maurandya* clade that consists of one subclade including the New World Maurandyinae sensu Elisens (1985a) and sister to it another subclade with the Old World *Asarina* and *Cymbalaria*. The clade is well supported with a bootstrap value of 84 and a Bremer support of 6, and all the subclades are also well supported (Fig. 2).

The remaining genera are in a large clade with a bootstrap value of 55 only, but it is possible to divide it into two well supported subclades. One of these, the *Gambelia* clade,

contains the two shrubby genera *Gambelia* and *Galvezia* from Central and South America. This clade has a bootstrap support value of 100 and a Bremer support of 23. The rest of the genera, including *Chaenorhinum*, *Linaria*, *Antirrhinum*, *Misopates*, *Schweinfurthia*, *Howelliella*, and *Mohavea*, make up the *Antirrhinum* clade, which has a bootstrap support of 80 and a Bremer support of 5 (Fig. 2).

Combined analysis. The combined analysis of the morphological and molecular data

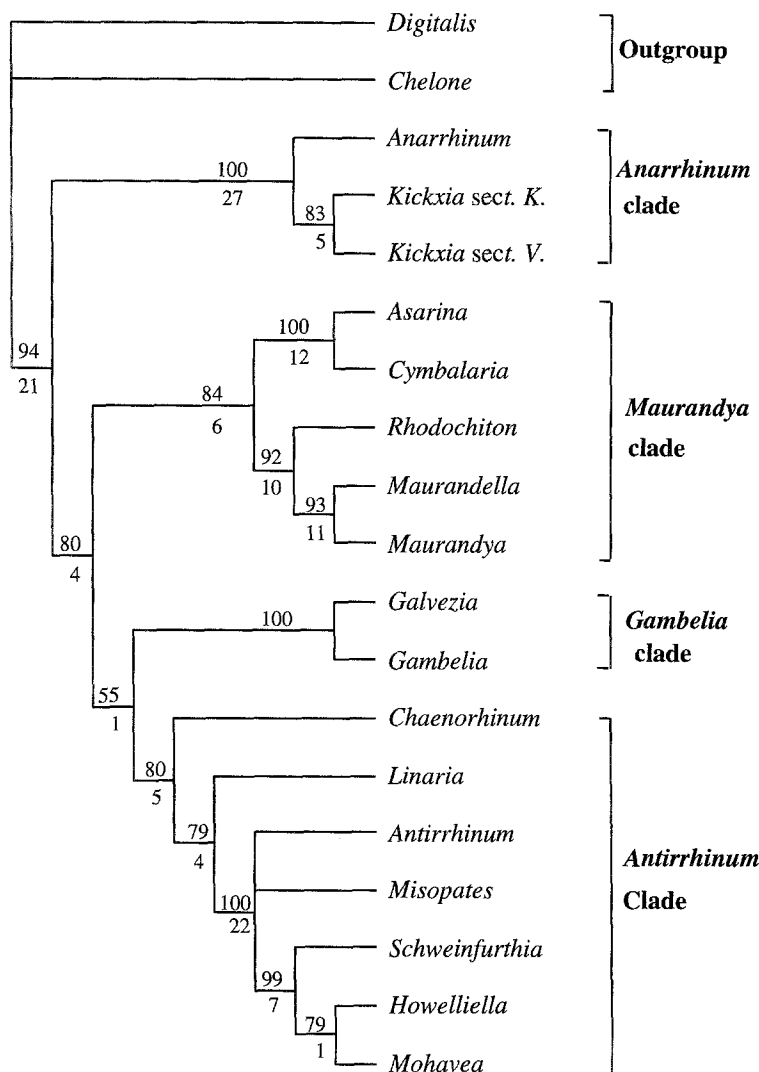


Fig. 2. Strict consensus tree of the two equally most parsimonious trees of the Antirrhineae based on molecular (*ndhF*) characters. Numbers above nodes correspond to bootstrap values above 50%. Numbers below nodes are Bremer support values, i.e. numbers of extra steps required to collapse that particular node

resulted in two most parsimonious trees both 1085 steps long, with consistency and retention indices of 0.698 and 0.613 respectively. The strict consensus tree is shown in Fig. 3. Except for the collapse of the branch uniting the *Gambelia* clade and the *Antirrhinum* clade the topology of the combined tree agrees with that of the molecular tree. All four major clades are well supported with a bootstrap value of 100 for the *Anarrhinum* and the *Gambelia* clades, 90 for the *Maurandya* clade, and 74 for the *Antirrhinum* clade. The corresponding Bremer support values are 29, 21, 8, and 3. Most of the subclades are also well supported. The

morphological characters have been optimised on one of the combined trees in Fig. 4. Presumed pollinators (information from Grant and Grant 1966, Elisens 1986, Sutton 1988, Thompson 1988, Kampany 1995), which were not included as characters in the analyses, are also indicated for each genus on the tree.

Discussion

The results from both the molecular and the combined analyses suggest that the Antirrhineae can be subdivided into four main groups of related genera corresponding to the *Anar-*

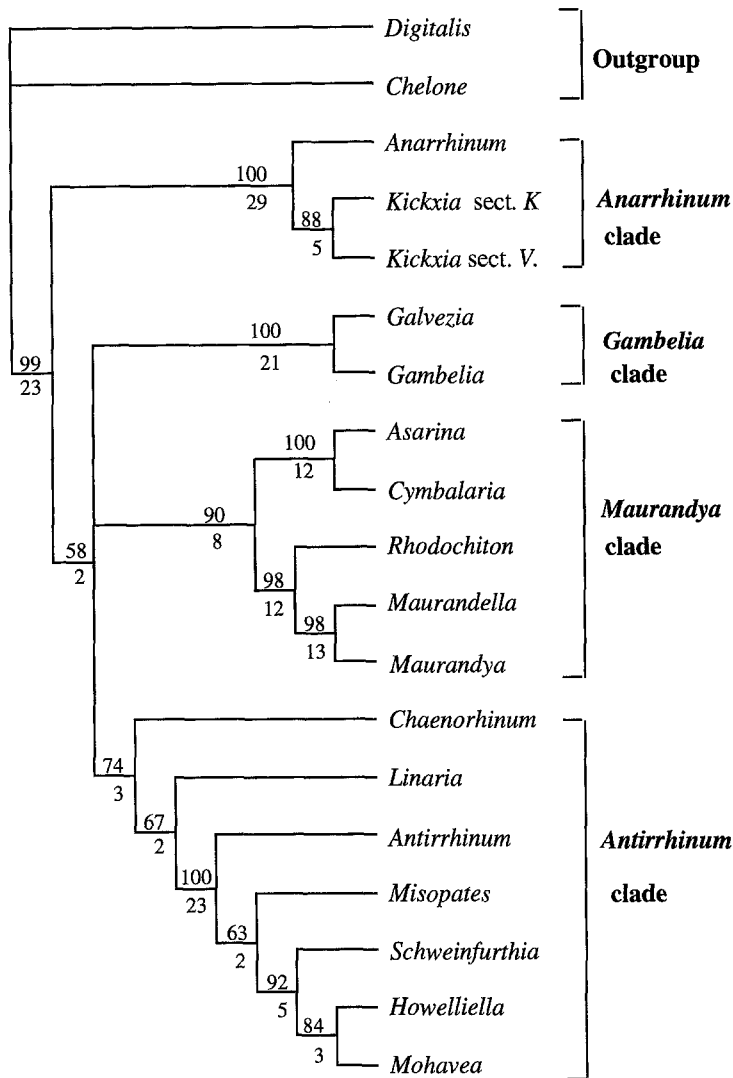


Fig. 3. Strict consensus tree of the two equally most parsimonious trees of the Antirrhineae based on a combined data set of morphological and molecular (*ndhF*) characters. Numbers above nodes correspond to bootstrap values above 50%. Numbers below nodes are Bremer support values, i.e. numbers of extra steps required to collapse that particular node

rhinum clade, the *Gambelia* clade, the *Maurandya* clade, and the *Antirrhinum* clade (Figs. 2, 3). The clades are all well supported with bootstrap values ranging between 74 and 100 and Bremer support values ranging between 3 and 29.

The analysis of the morphological data produced trees with low CI and a relatively poorly resolved consensus tree (Fig. 1). Most of the clades in the morphological tree have low support values. It is therefore scarcely possible to deduce phylogenetic relationships from the morphological results alone. Among

the four groups mentioned above, only the *Anarrhinum* clade appeared as a distinct group with a bootstrap value of 76 and a Bremer support of 1. Although well resolved, the *Maurandya* clade has a low support and, unlike in the molecular analysis, *Cymbalaria* is not included. Nevertheless, in the combined tree the support for several branches, including that of the *Maurandya* clade, was increased (Fig. 3) compared to the molecular analysis (Fig. 2). This indicates that the morphological data to some extent support the results of the molecular data.

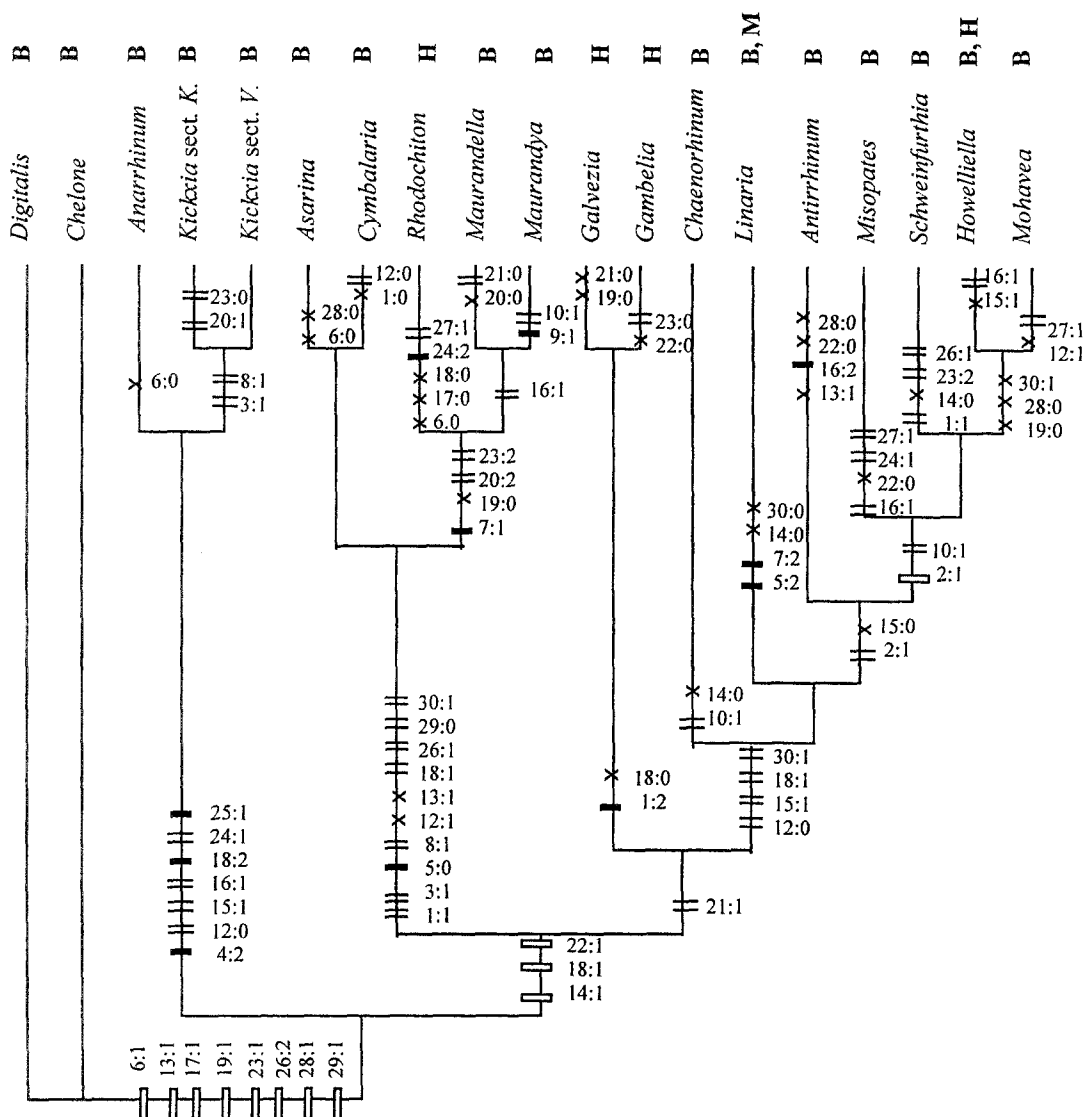


Fig. 4. Morphological characters optimised on one of the most parsimonious trees of the combined analysis. Black bars indicate synapomorphies with $ci = 1$, open bars indicate homoplastic characters with subsequent changes, crosses indicate reversals, and double bars indicate parallelisms. Type of pollinators are indicated in bold after each taxon. Pollinator codes: *B* bee; *H* hummingbird; *M* moth

An interesting result in the molecular and combined analyses is the appearance of the Old World *Cymbalaria* and *Asarina* as sister taxa to the New World *Maurandya* and its relatives. In his phylogenetic tree Rothmaler (1943) placed *Asarina* and *Cymbalaria* close to each other and as an offshoot of the *Maurandya* group. The close relationship between *Asarina* and *Maurandya* was also

pointed to by Pennell (1947), who even treated the two taxa as congeneric. Opposing Pennell's view Elisens (1985a, 1985c) rejected a close relationship between *Asarina* and *Maurandya* and its relatives, and selected the New World *Neogaerrhinum* as an outgroup taxon to detect phylogeny in the Maurandyinae.

In the present molecular and combined analyses the support for the *Maurandya* clade

is strong. Isodiametric leaves is a synapomorphy for this clade. This is a character otherwise found only in certain members of *Kickxia* sect. *Valvatae*. Also, Sutton (1988) stated that a high proportion of radially expanded epidermal cells give rise to the swollen, multicellular tubercles and ridges present in both Maurandyinae, *Cymbalaria* and *Asarina*, but as data for many of the genera were not available, this character was not included in the analysis.

Trends in floral evolution. Most members of the family Scrophulariaceae have flowers with a five-lobed calyx, a campanulate corolla with a slightly gibbous corolla-tube bearing five lobes, four stamens attached to the inside of the corolla-tube, and a bicarpellate pistil (Kampany 1995, Kampany and Dengler 1997). The occurrence of this type of flower in most tribes of the Scrophulariaceae and related families indicate that it is the basic (ancestral) form of flower in the group (Kampany 1995). The two outgroup species *Chelone obliqua* and *Digitalis grandiflora* share all the ancestral floral characteristics. Some close relatives of *Chelone* have the corolla-tube closed by a palate (Kampany 1995).

Studies on evolution of floral morphology in the “Scroph II” clade by Reeves and Olmstead (1998) suggest a common ancestry for *Digitalis* and *Antirrhinum*. It was also suggested that *Chelone* and *Collinsia*, both of which are members of the tribe Cheloneae, are basal members of the “Scroph II” clade. It is likely, therefore, that floral evolution in the “Scroph II” clade has taken two main lines. One of these is the *Digitalis–Plantago–Callitriche* line discussed by Reeves and Olmstead (1998), involving various reductions in floral parts. The second line is the floral evolution in the Antirrhineae as discussed here. In the Antirrhineae, the flower has evolved to form corollas closed by a palate, narrow tubular corollas, wide flaring corollas, and corollas with a spur (Pennell 1935, Kampany 1995).

The members of the *Anarrhinum* clade, which appeared sister to the rest of the

Antirrhineae in the molecular and combined analyses, are all bee-pollinated with basally spurred corolla-tube and with the mouth partially closed by a palate. When compared to the outgroup, these floral characteristics of the *Anarrhinum* clade can be considered as derived. Both groups are bee-pollinated but show differences in the strategy of attracting particular pollinators. The palate functions as a barrier to ineffective visitors, and only strong, heavy, long-tongued bees are capable of reaching the nectar. In *Chelone* and *Digitalis*, on the other hand, the corolla-tube is very wide and allows pollinators to freely move inside. It should be noted also that there are close relatives of *Chelone* and *Digitalis* that are pollinated by hummingbirds, butterflies, or flies (Kampany 1995).

The floral diversity is considerable in the *Maurandya* clade. The flower size ranges from 6 to 15 mm in *Cymbalaria* and from 45 to 55 mm in *Rhodochiton*. The corolla-tube is variable in length, width, as well as in shape. It is abaxially spurred in *Cymbalaria*, gibbous in *Maurandya* and entire in *Rhodochiton*. This variation in floral structure is directly related to the various pollination systems of the genera. Members of *Cymbalaria*, *Asarina*, and *Maurandella*, have snapdragon-like flowers, with a conspicuous palate closing the mouth until depressed by a bee of suitable size and weight. Similar but slightly larger are the flowers of *Maurandya* and *Epixiphium*. Among the large-flowered members are *Rhodochiton* and *Lophospermum*, which are bird-pollinated and have corollas with a gaping throat (Elisens 1986, Sutton 1988). The present results agree with Pennell’s remark on the floral evolution of the Maurandyinae from bee-pollinated ancestry into enlarged and reddened corollas fitted to the visits of hummingbirds (Pennell 1935).

Compared to the other clades, the flowers in the *Gambelia* clade have a uniform structure. In both *Galvezia* and *Gambelia* the flowers are trumpet-shaped with a long, narrow corolla-tube somewhat swollen at the

base. They are red in colour and are reported to be hummingbird-pollinated (Grant and Grant 1966).

The floral diversity in the *Antirrhinum* clade is comparable to that of the *Maurandya* clade. The corollas vary from strongly bilateral with the tube closed by an enlarged palate, as in *Linaria* and *Antirrhinum*, to corollas with a gaping throat, as in *Howelliella* and *Mohavea*. The corolla-tube varies from spurred in *Linaria* to gibbous in *Antirrhinum* and saccate in *Mohavea*. *Linaria* and *Antirrhinum* provide classical examples of flowers specialised for bee-pollination. *Mohavea* was by Sutton (1988) considered to be the most unusual genus in the tribe as the lips of the corolla are greatly enlarged and only the two abaxial stamens are fertile. The orientation of the flower is also different from that of the other genera in the Antirrhineae, as the tube is nearly erect pointing the mouth upwards rather than more or less horizontal, and suggesting a quite different pollination strategy (Sutton 1988). The pollination strategy of *Mohavea* as reported by Little (1983) involves floral food deception mimicry based upon morphological similarities of the flowers to that of the proposed model species *Mentzelia involucrata* (Loasaceae). The only known pollinators for *Mohavea* are solitary bees in the genera *Xeralictus* (Halictidae) and *Hesperapis* (Melittidae), which are the primary pollinators of *Mentzelia* (Little 1983).

The unique floral features and the cyathiform seed structure encouraged several authors (Rothmaler 1943, Speta 1982, Elisens 1985c) to treat *Mohavea* as a subtribe of its own. Despite these unique features, *Mohavea* in the present analyses is nested within the *Antirrhinum* clade (Figs. 2, 3). In the analyses *Mohavea* is placed in a position with *Howelliella* as a sister taxon. *Howelliella* has a poorly developed palate and widely gaping throat with two prominent longitudinal folds in its floor. It is reported to be pollinated by hummingbirds as well as by bees (Thompson 1988).

Characters related to pollination systems are reported to be labile due to selective pressure by pollinators resulting in repeated parallelisms and reversals (Armbruster 1992, 1993; Johnson et al. 1998). In Antirrhineae this is true particularly for the evolution of spur and palate.

In the Antirrhineae nectar is presented to the pollinator in the abaxial base of the tube which is either gibbous, saccate or spurred (Elisens and Freeman 1988). The present results indicate that a spur has evolved several times in the *Anarrhinum*, *Maurandya* and *Antirrhinum* clades as a result of parallelisms and repeated reversals. All members of the *Anarrhinum* clade are spurred, whereas *Cymbalaria* is the only spurred member of the *Maurandya* clade and both *Gambelia* and *Galvezia* are without spur. In the *Antirrhinum* clade both spurred and non-spurred taxa are present. The spur is reported to be sometimes very variable within species or genera. In *Linaria vulgaris*, variants with no spur occur in addition to occasional mutants with five spurs (Sutton 1987). In *Kickxia* sect. *Valvatae* a single species without spur has been described (*K. saccata*), and *Antirrhinum braun-blanquetii* is the only spurred species in the otherwise gibbous or saccate genus *Antirrhinum* (Sutton 1988).

The palate is a structure formed by the arching of the bottom of the corolla-tube upwards to close the entrance. Other than in the Antirrhineae, a palate is known to be present in some members of Cheloneae and Gratioloae (Pennell 1935, Kampany 1995). In certain members of Antirrhineae, e.g. *Linaria*, *Antirrhinum*, *Asarina*, and *Maurandella*, the palate is conspicuous and completely blocks the corolla-tube, whereas in *Anarrhinum*, *Kickxia* and *Chaenorhinum* the arching is low and does not block the mouth of the tube. Several of the New World Antirrhineae, including *Rhodochiton*, *Lophospermum*, and *Maurandya*, have flowers that are relatively large, more or less actinomorphic, and without a palate. Many of them have been reported to

be pollinated by hummingbirds (Elisens 1986). Although bee-pollination is most common and probably ancestral in the Antirrhineae, the present results indicate that hummingbird-pollination has obviously evolved independently at least three times, in the *Maurandya*, *Gambelia*, and *Antirrhinum* clades (Fig. 4).

Comparison with previous subtribal classification. The present results though differing in many ways have some similarities with Rothmaler's classification. His treatment of Mohaveinae and Anarrhinae as monotypic subtribes (Rothmaler 1943) is not supported by the present results. The results are also incongruent with the circumscriptions of the subtribe Maurandyinae made by Rothmaler (1943) and Elisens (1985a), and the subtribes Linariinae and Antirrhinae as circumscribed by Speta (1982). No formal subtribal classification is proposed here, but some further comments on the four clades are given below, including the placing of all genera recognised by Sutton (1988).

The Anarrhinum clade. In his subtribal classification Rothmaler (1943) was unable to group *Anarrhinum* with any other genus and therefore placed it in a subtribe of its own, *Anarrhinae*. The present results show that the Old World genera *Anarrhinum* and *Kickxia* are sister taxa. *Anarrhinum* is similar to *Kickxia* in its unusual arrangement of the anthers, dehiscence of the capsule, position of the hilum, and in its ornamentation of the seeds (Sutton 1988). The close relationship between *Anarrhinum* and *Kickxia* is also supported by the similarity of the protein bodies in the cell nuclei (Speta 1979). The present results show that the *Anarrhinum* clade is strongly supported and is characterised above all by three unique synapomorphies, different shapes of upper and lower leaves, connate anthers forming a ring, and median position of hilum. The present results further indicate that the group is sister to the rest of the Antirrhineae. The two sections of *Kickxia* are sister groups and the genus, as circum-

scribed by Sutton (1988), appears to be monophyletic.

The Gambelia clade. The clade is composed of a small but taxonomically complex group in the New World historically variously treated as the three genera *Galvezia*, *Gambelia*, and *Saccularia* as by Rothmaler (1943), as one genus *Galvezia* as by Munz (1926), or as a section of *Maurandya* or *Antirrhinum* as by Bentham (1876), and Wettstein (1891). In his subtribal classification Rothmaler (1943) treated the group as a separate subtribe *Gambeliinae*. Recently Sutton (1988) recognised two genera, *Galvezia* in South America and *Gambelia* in North America. He also pointed out that *Galvezia* is unique in the tribe in having a totally southern hemispheric distribution, but stressed its close relationship with *Gambelia*. Elisens and Tomb (1983) stated that it is difficult to draw any conclusions regarding the inter- or infrageneric classification of these two taxa using seed morphology. In the present analyses *Galvezia* and *Gambelia* come out as sister taxa with a bootstrap support of 100. The molecular differences detected between the genera are very small, which may support the view that they should be treated as one widely circumscribed genus. All members of the clade have a shrubby habit and are unique in possessing flowers with long narrow corolla-tubes pollinated by hummingbirds (Fig. 4).

The Maurandya clade. The Maurandyinae as delimited by Rothmaler (1943) included only New World Antirrhineae. On the basis of similarities in foliage and floral characters Pennell (1947) concluded that *Asarina* and *Maurandya* are congeneric. Elisens (1985a) rejected Pennell's view and argued that the two taxa are very distinct in many ways, including different chromosome numbers (*Asarina* $x=9$ and *Maurandya* $x=12$) and geographic distribution.

The present results indicate that *Asarina* and *Cymbalaria* are sister taxa to the New World Maurandyinae and suggest that the subtribe Maurandyinae sensu Elisens could

well be expanded further to include both *Asarina* and *Cymbalaria*.

As can be deduced from the morphological tree (Fig. 1) some further genera that probably belong to the *Maurandya* clade, but which were not included in the molecular analysis, are *Holmgrenanthe*, *Mabrya*, *Lophospermum*, and *Epixiphium*. All members of this group are suffrutescent herbs with procumbent or climbing stems and have orbiculate or deltate leaf-blades with palmate venation.

The Antirrhinum clade. This clade largely corresponds to the subtribe Linariinae sensu Rothmaler (1943, 1954), which includes among others *Linaria*, *Antirrhinum*, *Chaenorhinum* and their respective segregate genera. On the basis of chromosome numbers Speta (1982) delimited Linariinae only to *Linaria* with $x=6$ and proposed a new subtribe, Antirrhiniinae, for the remaining genera with $x=7, 8$ and 9 . The present results show that both *Linaria* and *Antirrhinum* belong to a clade that contains both the Old World genera *Chaenorhinum*, *Linaria*, *Antirrhinum*, *Misopates*, and *Schweinfurthia*, and the New World genera *Howelliella* and *Mohavea*. The clade is well supported in the molecular analysis with a bootstrap value of 80, but this is slightly reduced to 74 in the combined analysis. Among the genera that are included in Linariinae by Rothmaler (1943) or in Antirrhiniinae by Speta (1982) are *Asarina*, *Cymbalaria* and *Kickxia*, but this is not compatible with the present results. *Howelliella* and *Mohavea* are sister groups in the analyses.

Howelliella, *Neogaerrhinum*, and *Pseudorontium*, are New World segregate genera of *Antirrhinum* that were erected by Rothmaler (1943, 1954), and a fourth American genus, *Sairocarpus*, was added by Sutton (1988). Thompson (1988) argued for the keeping of all the species included in these genera within *Antirrhinum* sect. *Saerorhinum*. The present analyses support the segregation of *Howelliella* from *Antirrhinum*. Further investigation is required to decide the relationship between the remaining segregate genera and *Antirrhinum*.

A number of other segregate genera of *Antirrhinum*, *Linaria*, and *Chaenorhinum*, and which, like *Neogaerrhinum*, *Pseudorontium*, and *Sairocarpus*, were not sampled for the present molecular analysis are *Acanthorrhinum*, *Albraunia*, and *Holzneria*, in the Old World, and *Nuttallanthus* in the New World. They agree in various characters with either *Antirrhinum*, *Linaria*, or *Chaenorhinum*, and probably all belong to the *Antirrhinum* clade.

We thank Mattias Iwarsson, the Botanical Garden of Uppsala University, and Hans V. Hansen, the Botanical Garden of the University of Copenhagen, for providing seeds, and Roland Moberg, UPS, for access to herbarium material. Christina Grusell is acknowledged for help with the greenhouse work, Nahid Heidari for sequencing 12 of the taxa, and Anders Nilsson for valuable information on literature on pollination. We also thank Kåre Bremer and Bengt Oxelman for constructive comments on the manuscript. The study was supported by a scholarship from Sida/SAREC (Swedish International Development Agency/Swedish Agency for Research Co-operation with Developing Countries), and a grant from the Swedish Natural Science Research Council to Birgitta Bremer.

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Addresses of the authors: Ghebrehiwet Medhanie, Department of Biology, University of Asmara, P.O. Box 1220, Asmara, Eritrea (permanent). Evolutionary Biology Centre, Department of Systematic Botany, Norbyvägen 18D, SE-752 36 Uppsala, Sweden (temporary). Birgitta Bremer, Mats Thulin, Evolutionary Biology Centre, Department of Systematic Botany, Norbyvägen 18D, SE-752 36 Uppsala, Sweden.