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Subgeneric classification of *Linaria* (Plantaginaceae; Antirrhineae): molecular phylogeny and morphology revisited

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ABSTRACT

Linaria Mill. (Plantaginaceae) with about 160 spp. is the largest genus of the tribe Antirrhineae. We conducted phylogenetic analyses of nuclear ribosomal DNA internal transcribed spacer region (ITS) and chloroplast DNA (*rpl32-trnL*) sequence data to test the monophyly of currently recognized sections in *Linaria*. For this purpose 86 species representing seven sections of *Linaria* and one species of *Nuttallanthus* along with representatives of four outgroup taxa of tribe Antirrhineae were analyzed. Phylogenetic analyses using Maximum Parsimony and Bayesian Inference reveal *Linaria-Nuttallanthus* as a monophyletic group composed of seven supported major clades that match partly with the current subgeneric treatment of the genus. Following sections are recognized here: *Macrocentrum*, *Lectoplectron*, *Pelisserianae*, *Versicolores*, *Supinae*, *Diffusae*, and *Linaria*. Based on our results sect. *Linaria* is expanded to include sect. *Speciosae* and some members of sect. *Diffusae*. A diagnostic key to sections and subsections of *Linaria* according this revised classification is presented. Our results indicate that seed features provide some synapomorphies for the main clades of *Linaria*, but their importance should be cautiously evaluated. In the case of winged and discoid seeds versus oblongoid ones, although the former seems to be the advanced state, it has been evolved independently in several sections/clades, i.e. *Pelisserianae*, *Supinae*, and *Linaria*. We propose major changes in circumscription of sect. *Linaria* which now embraces also some representatives with oblongoid seeds formerly assigned to sects. *Diffusae* and *Speciosae*.

Keywords: Taxonomy, Seed morphology, nrITS sequences, *rpl32-trnL* marker, monophyly.

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Introduction

Linaria Mill. (toadflaxes, including *Nuttallanthus* D.A.Sutton) is the largest genus of the snapdragon lineage (tribe Anthirrhineae Dumort.) with about 160 species (1-3). Based on molecular phylogenetic studies it is placed currently in Plantaginaceae Juss. (4-5). The species are annual or perennial herbs with heteromorphic shoots, bracteate racemes, zygomorphic corolla, tubular and spurred at the base and winged or wingless seeds.

The genus is distributed throughout the northern hemisphere with highest species diversity in the Mediterranean region, Northern Africa, Western South Asia and Eastern Asia (1, 6). Many species (such as *L. vulgaris* Mill.) are widely cultivated ornamental plants favored by the gardeners due to their large and showy flowers (Fig. 1).

Taxonomy of the genus has been very controversial. Being recognized by pre-Linnaean botanists (7-8), the species of *Linaria* were placed in *Antirrhinum* L. by Linnaeus (9). Miller (10) was the first who described the genus validly and accepted it as a distinct genus. Early authors generally considered *Linaria* as a genus including all those species related to *Antirrhinum* that have spurred corolla tube and accepted *Linaria* in a wide sense (11-13) including the members of the currently recognized genera *Chaenorhinum* (DC.) Rchb., *Cymbalaria* Hill, *Kickxia* Dumort. and *Nanorrhinum* Betsche (14). Today, *Linaria* includes only taxa with entire and sessile leaves, terminal, racemose inflorescences as well as cylindrical and mostly curved corolla spur. All recent taxonomic and molecular phylogenetic studies confirmed this view and circumscribe *Linaria* much narrower than before (1, 14-19).

There are also remarkable disagreements about the infrageneric classification of *Linaria* (2). Dumortier (20) divided the species of *Linaria* in four unplaced ranks (indicated by "§") in the Belgian flora, two of which representing the currently recognized genera *Chaenorhinum* and *Cymbalaria*. Chavannes (12) classified *Linaria* in four sections: *Chaenorhinum*, *Cymbalaria*, *Elatinoides* and *Linariastrum*. The three first mentioned sections were later separated as distinct genera, and *Linaria* s.s. was divided into six sections (21). Today the genus *Linaria* embraces only the members of *L.* sect. *Linariastrum* Chav. Bentham (13) divided the members of *L.* sect. *Linariastrum* in five unranked groups. Boissier (22) recognized only two unranked groups under *L.* sect. *Linariastrum* based on the seed type which might be winged (§ *Discoideae*) or oblongoid and wingless (§ *Oblongae*). Viano (23-24) was the first who hypothesized that species with winged and wingless seeds constitute two sister evolutionary lineages. Sutton (1) increased the number of sections in *Linaria* to seven. Today, Sutton's classification is widely accepted (see as e.g., 25). According to this classification, species with encircling wing are distributed among three sections: *Linaria*, *Pelisserianae* Valdés and *Supinae* (Benth.) Wettst., while species with oblongoid seeds lacking the encircling wing are assigned to four sections: *Speciosae* (Benth.) Wettst., *Diffusae* (Benth.) Wettst., *Versicolores* (Benth.) Wettst. and *Macrocentrum* D.A. Sutton (Table 1).

Four toadflax species that are native to the New World have been circumscribed in different genera, such as *Anarrhinum* Desf. (26) and *Linaria*, as part of sect. *Versicolores* (13, 21) or as the distinct sect. *Lectoplectron* Penn. (16, 27). Sutton transferred these species to his new genus *Nuttallanthus*, based on flower and seed characters (1).

Several molecular phylogenetic studies have been conducted on *Linaria* indicating its monophyly if including also *Nuttallanthus* (2). Additional analyses including more species of *Linaria* and its allies as well as multiple nuclear and plastid markers confirmed the monophyly of the genus within Antirrhineae again (2-3). However, it remained uncertain whether the sections constitute natural groups.

Although reliable fossils are missing for this alliance, dating analyses based on *rpl32-trnL* and *trnK-matK* sequences of tribe Antirrhineae estimated the divergence between *Chaenorhinum* and *Linaria* at a mean age of 23 million years ago and the origin of *Linaria* in the early Miocene (28).

Table 1. Overview of *Linaria* in different classification systems

Sutton (1), Sáez and Bernal (17)	Type species	Dumortier (20)	Chavannes (12)	Wettstein (21)	Bentham (13)	Boissier (22)	Podlech and Iranshahr (25)
Sect. *Linaria*	*L. vulgaris* Mill.	§ *Leontorrhinum*	Sect. *Linariastrum* Chav.	Sect. *Diffusae* (Benth.) Wettst. Sect. *Grandes* (Benth.) Wettst. Sect. *Supinae* (Benth.) Wettst.	§ *Diffusae* Benth. § *Grandes* Benth. § *Supinae* Benth.	Sect. *Linariastrum* §*Discoideaei* §*Oblongae*	Sect. *Linaria*
Sect. *Speciosae* (Benth.) Wettst.	*L. genistifolia* (L.) Mill.	§ *Lycorrhinum*	Sect. *Linariastrum*	Sect. *Diffusae* Sect. *Speciosae* (Benth.) Wettst. Sect. *Versicolores* (Benth.) Wettst.	§ *Diffusae* § *Speciosae* Benth. §*Versicolores* Benth.	§Discoideae §*Oblongae*	Sect. *Speciosae*
Sect. *Diffusae* (Benth.) Wettst.	*L. reflexa* (L.) Chaz.	§ *Lycorrhinum*	Sect. *Linariastrum*	Sect. *Speciosae* Sect. *Diffusae* (Benth.) Wettst.	§ *Speciosae* § *Minutiflorae* Benth. § *Diffusae*	§*Oblongae*	Sect. *Diffusae*
Sect. *Supinae* (Benth.) Wettst. Subsect. *Supinae*	*L. supina* (L.) Chaz.	§ *Leontorrhinum*	Sect. *Linariastrum*	Sect. *Arvenses* (Benth.) Wettst. Sect. *Supinae*	§ *Arvenses* Benth. § *Supinae* § *Grandes* § *Diffusae*	§*Discoideae*	Sect. *Supinae*
Subsect. *Saxatile* Valdes				§ *Supinae*	§ *Supinae* §*Versicolores*		
Subsect. *Trimerocalyx* (Murb.) D.A. Sutton				-	-		
Sect. *Pelisserianae* Valdes	*L. pelisseriana* (L.) Mill.	-	Sect. *Linariastrum*	Sect. *Grandes* Sect. *Arvenses*	§ *Grandes* § *Arvenses*	§*Discoideae*	-
Sect. *Versicolores* (Benth.) Wettst. Subsect. *Versicolores*	*L. viscosa* (L.) Chaz.	-	Sect. *Linariastrum*	Sect. *Diffusae* Sect. *Versicolores*	§ *Supinae* § *Diffusae* §*Versicolores*	-	Sect. *Versicolores*
Subsect. *Elegantes* (Viano) D.A. Sutton				Sect. *Versicolores*	§*Versicolores*		
Sect. *Macrocentrum* D.A. Sutton	*L. armeniaca* Chav.	-	Sect. *Linariastrum*	Sect. *Versicolores*	§*Versicolores* -	§*Oblongae*	Sect. *Macrocentrum*

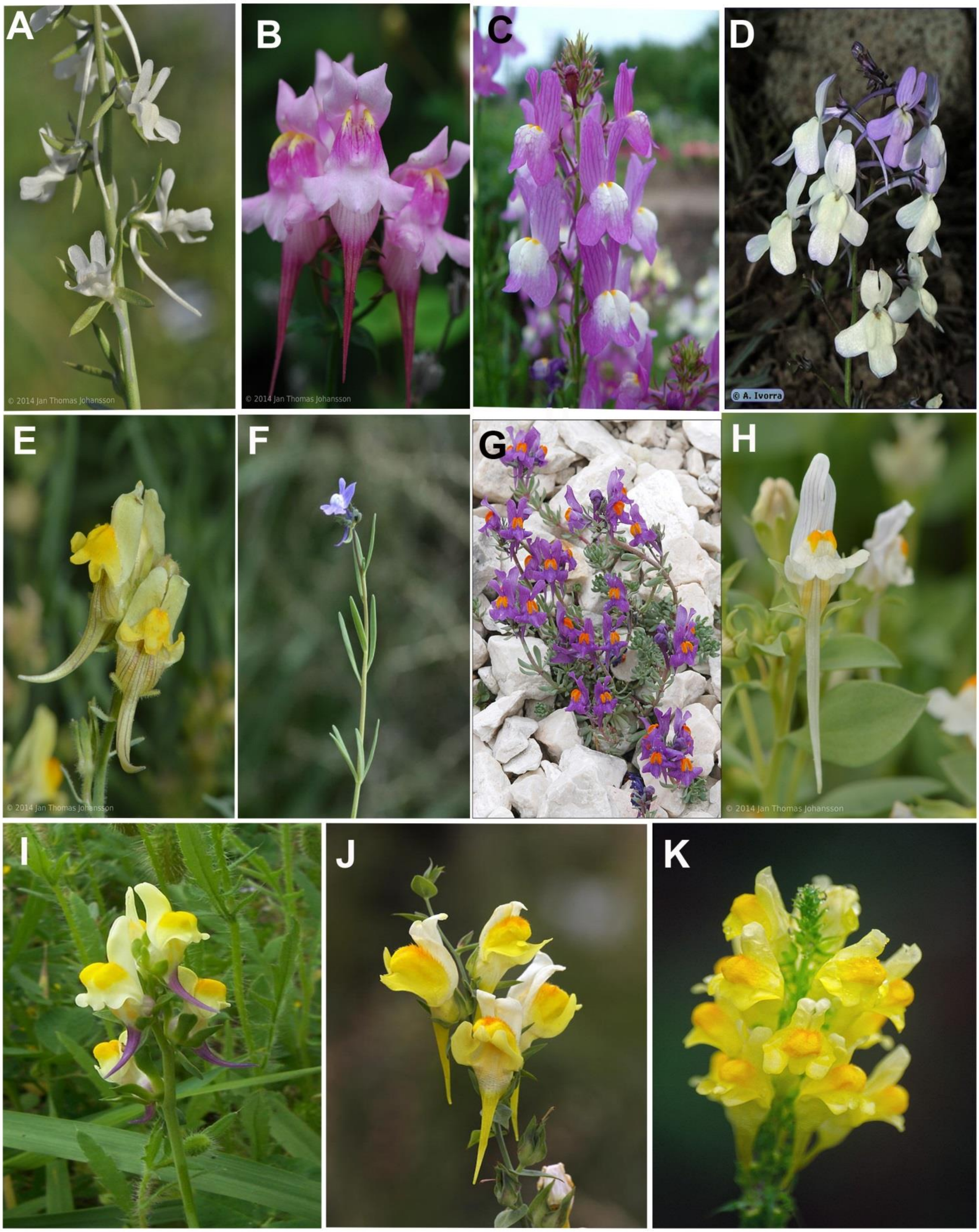

Figure 1. Representatives of *Linaria*. Classification follows Sutton (1). *Linaria* sect. *Macrocentrum*: A, *L. chalepensis*. *Linaria* sect. *Pelisserianae*: B, *L. triornithophora*. *Linaria* sect. *Versicolores*; subsect. *Versicolores*: C, *L. maroccana* Hook.f.; subsect. *Elegantes:* D, *L. nigricans* Lange. *Linaria* sect. *Supinae*; subsect. *Supinae:* E, *L. supina* (L.) Chaz.; subsect. *Arvenses*: F, *L. arvensis* (L.) Desf.; subsect. *Saxatile:* G, *L. alpina* Mill. *Linaria* sect. *Diffusae*: H, *L. reflexa*. *Linaria* sect. *Linaria*: I, *L. triphylla*; J, *L. genistifolia*; K, *L. vulgaris*. Photos by Johansson, T. (A, B, E, H), Landry, LM. (C), Ivorra, A. (D), Blanco-Pastor, J.L. (F), Trnkoczy, A. (G), Rignanese, L. (I), Wolf, R. (J), Kurzenko, V. (K). All photos were used with permission.

These studies also revealed five to six well-supported clades in the genus corresponding partly with the sections recognized by Sutton (1). However, the circumscription of sect. *Diffusae*, sect. *Linaria*, sect. *Speciosae* and sect. *Supinae* remained unclear, due to weak phylogenetic signals provided by the markers used, and probably also because of poor sampling. Blanco-Pastor et al. (29) extended the sampling of *Linaria* sect. *Supinae* as well as the markers used and concluded that this section is also monophyletic and composed of three well-supported subclades corresponding to following subsections: *Supinae*, *Saxatile* Valdés and *Arvenses* (not formally described yet). However, the unbalanced and poor sampling of other sections had probably biased the monophyly of this section, as adding more representatives of sect. *Diffusae* have blurred the borders between these two sections (2). Furthermore, the members of three sections, i.e. *Diffusae*, *Linaria* and *Speciosae* are still intermingled in the obtained phylogenetic trees, so these sections cannot be considered as monophyletic groups (2-3). The molecular phylogenetic studies showed also that the New World genus *Nuttallanthus* is nested within *Linaria* (2).

Despite of intensive molecular phylogenetic studies conducted on *Linaria*, the subgeneric classification of this important genus with several cultivated species have not been improved yet according to the relationships reconstructed. Therefore, the current classification of the genus (1, 25) does not match with phylogenetic reconstructions appropriately. The main goal of the present study is to provide an integrative and linear system addressing both molecular and morphological data that could reflect natural relationships in the genus *Linaria*.

Materials and methods

Taxon sampling

Sequence data in the present study were generated from specimens held at Botanische Staatssammlung München (M), München Systematische Botanik (MSB) and Central Herbarium of the University of Tehran (TUH) or extracted from GenBank. The sampling strategy included multiple individuals and species representing all sections of *Linaria* according to Sutton (1) (Table 1), as well as one species of *Nuttallanthus* plus outgroups. A common origin of tribes Cheloneae Benth. and Antirrhineae was suggested by Raman (30), and *Antirrhinum* (tribe Antirrhineae), *Chelone* L. and *Tetranema* Sweet (tribe Cheloneae) were used as outgroups in our analyses, as suggested also by Vargas et al. (19) and Fernández-Mazuecos et al. (2). ITS and *rpl32-trnL* matrices comprised 140 sequences each representing 86 *Linaria* spp., one *Nuttlallanthus* spp. and four outgroups: 21 newly generated here and 119 obtained from GenBank for the nrDNA ITS, and 22 newly generated here and 118 obtained from GenBank for the plastid marker (Appendix S1).

DNA extraction and amplification

Total DNA was extracted from dried leaf material using the NucleoSpin Plant Kit (Macherey-Nagel, Düren, Germany). Protocols followed those provided by the manufacturer. The DNA was dissolved in 30 µl elution buffer (10 mM Tris- HCl) and checked for quality on a 1% agarose gel. The extracted DNA was resuspended in 50 µl elution buffer (10 mM Tris-HCl), 1 µl of which was used for amplification (higher amounts up to 3 µl in cases where PCR yielded insufficient amounts of product). The internal transcribed spacer (ITS) region (ITS1, 5.8S rDNA, ITS2) of nuclear DNA and partial *rpl32-trnL* from plastid DNA were analyzed. The markers were amplified from total DNA using *Taq*-polymerase (AGS, Heidelberg, Germany). Amplification of the ITS region was conducted using the primers Leu1 (31) and ITS4 (32). In some difficult cases ITS2 and ITS3 were used as described by White et al. (32).

PCR reactions were performed in volumes of 50 µl containing a dNTP solution of 2.5 mM, *Taq*-polymerase with 1 U/µl, primer solutions with a concentration of 100 pmol/µl, and differing amounts of unquantified genomic DNA. When necessary, an alternative preparation containing 0.05% bovine serum albumin (BSA) and 100% dimethyl sulfoxide (DMSO) was used for ITS. Amplification programs for ITS started with a 5 min initial denaturation step at

94°C; followed by 40 cycles of 30 s denaturation (94°C), 30 s annealing (54°C), and 1 min 15 s extension (72°C); ending with a final extension step of 10 min (72°C).

For amplification of *rpl32-trnL* as one fragment we used the primers rpl32F and trnL (UAG) (33) under following parameters: 80°C, 5 min; 35 cycles (94°C, 30 s; 50-55°C, 30 s; 72°C, 1 min); 72°C, 5 min, which were modified from Oxelman et al. (34).

All PCR amplifications were carried out in a thermocycler type T-Personal 48 (Biometra, Göttingen, Germany), type Primus 96 plus (MWG-Biotech, Ebersberg, Germany), or type 2720 (Applied Biosystems, Carlsbad, California, USA.). Successful PCR reactions were purified with the NucleoSpin Extract II-Kit (Macherey-Nagel) following the manufacturer's instructions, or were reduced to 25 µl and then purified in 4 µl units with 0.025 µl exonuclease I and 0.25 µl shrimp alkaline phosphatase (Sap) in a 5 µl preparation with 0.0725 µl 10× TP buffer (35). Cycle Sequencing was carried out using the BigDye Terminator v.3.1 Cycle Sequencing Kit (Applied Biosystems) in a final volume of 20 µl. Runs were performed on an ABI 3730 48 capillary sequencer (Applied Biosystems). In all cases, the markers were sequenced bi-directionally using the same primers as in PCR reactions.

Alignment, indel coding, and phylogenetic reconstruction

All sequences generated in this study were assembled, edited, and aligned manually using Mesquite v.1.12 (36). Alignments for the phylogenetic analyses from the present study are available as Supplementary Data to the online version of this article (Appendix S2, S3). The beginning and end of the alignments, which contained missing data for many individuals, were trimmed prior to analysis. The plastid and the ITS datasets were first analyzed separately and then combined to one. We did not run any statistical congruence test, but congruence in the tree topologies was considered as the major criterion for combining the two datasets.

Optimal substitution models were estimated using the Akaike information criterion (AIC) in jModelTest v.0.1.1 (37). The general time-reversible model of nucleotide substitution with gamma-shaped rate variation with a proportion of invariable sites (GTR+I+G) and the same model but without proportion of invariable sites (GTR+G) were the estimated best-fit model for ITS and plastid markers, respectively. For Bayesian Inference (BI) number of MCMC generation for each ITS, *rpl32-trnL* and the combined datasets were set to 10.000.000. Trees were sampled every 1000th generation with the default of three "heated" and one "cold" chain, and pre-stationarity MCMC samples were discarded as burn-in (2500 samples). The remaining trees were summarized in a 50% majority-rule consensus tree for each dataset. Maximum parsimony (MP) analyses were performed using PAUP* v.4.0b10 (38) with the following parameters: all characters unordered and equally weighted, heuristic search with random sequence addition, tree-bisection-reconnection branch-swapping, 50 random-addition-sequence replicates, and MAXTREES option set to 10.000. Bootstrapping was done using the following settings: hsearch addseq = random, nchuck = 10, chuckscore = 1, nreps = 50, bootstrap nreps = 5000 (summarized in a 50% majority-rule consensus tree as a cladogram).

Results

After alignment, the ITS and *rpl32-trnL* datasets had a total length of 660 and 1115 bp, respectively. The alignment of ITS dataset (Supplementary file: Appendix S2) did not need introduction of large indels (longer than three bp), except a deletion between the positions 569−572 in *Nuttallanthus texanus* (Scheele) D.A. Sutton, two deletion between the positions 462−466 and 470−475 in *Antirrhinum graniticum* Rothm. and *A. australe* Rothm. and a deletion between the positions 139−155 in *A. graniticum*. However, large indels were proposed in the cpDNA marker (Supplementary file: Appendix S3). Major indels in this fragment are: a deletion between the positions 161−165 in all species of *Linaria* and *Nuttallanthus*, an insertion between the positions 172−183 in *L. thibetica* Franch. and *L. huterti* Lange which overlaps with a shorter insertion of 6 bp in most members of *L.* sect. *Supinae*, a large insertion between the positions 203−222 shown only in *L. tristis* Mill. subsp. m*esatlantica* D.A. Sutton, a large insertion between the positions 223−237 in all species of *Linaria* and *Nuttallanthus*, a large insertion between the positions 288−303 in most species of *L.* sect. *Supinae* subsect. *Supinae*, an insertion between the positions 387−390 in *L. odora* (M.Bieb.) Fisch., an insertion between the positions 482−495 with repetitive motif "TAAA(A)TA" in three accessions of *L.* sect. *Supinae* subsect. *Saxatile* which overlaps with a shorter insertion between the positions 489−495 in some species of *L.* sect. *Supinae* subsect. *Saxatile* and subsect. *Supinae*— this insertion overlaps in turn with a shorter insertion between positions 496−499 shared by all species of the genus *Linaria* and *Nuttallanthus*— a deletion between positions 514−535 in *L. chalepensis* (L.) Mill., a large insertion between positions 542−564 in *L. grandiflora* Desf. which overlaps partly with shorter insertions in some members of sect. *Diffusae* and few other species representing different sections, a large deletion of 266 bp observed in all species of *Linaria* and *Antirrhinum* between the positions 624−890, and a deletion between positions 899−908 shared by almost all species of *L.* sect. *Versicolores* and several species representing various sections.

As ITS and *rpl32-trnL* trees had low resolution with unclear position of some taxa, we combined the two datasets. The congruency of the datasets was checked through topological comparison of the trees obtained as suggested by Pirie (39). Despite few discrepancies between the obtained trees, the results of the combined dataset provide more resolved trees and higher supports compared to the analyses of each dataset alone. Therefore, we present here the results of the species tree which will be also the basis for the discussion too. The differences between the gene trees will follow after description of the combined (species) tree.

In the MP analysis of the combined dataset the number of trees reached the original maximum set of 10,000. The obtained most parsimonious trees were combined to generate a strict consensus (not shown; L= 1743, CI= 0.58, RI= 0.88, RC= 0.51).

Figure 2 presents the semi-strict consensus tree gained from the BI of the combined dataset. *Linaria-Nuttallanthus* forms a monophyletic clade with optimal support (PP 1.00, BS 100). In *Linaria-Nuttallanthus* seven main lineages, including four species-rich clades are recognizable labeled with the sections currently known in the genus. Following clades are highly-supported: (1) *Versicolores* (PP 0.95, BS 100), including two well-supported subclades representing its two subsections: *Versicolores* (PP 0.95, BS 94) and *Elegantes* (Viano) D.A. Sutton (PP 1.00, BS 100); (2) *Supinae* (PP 1.00, BS 70) composed of three subclades: *Supinae* (PP 1.00 and BS 98), *Arvenses* (PP 1.00, BS 98) and *Saxatile* (PP 1.00, BS 99); (3) *Diffusae* (PP 1.00, BS 89); and (4) *Linaria* (PP 1.00, BS 92) which includes the members of sects. *Linaria*, *Speciosae*, and some members of sect. *Diffusae* excluding the type of the section (*L. reflexa* Desf.). *Linaria chalepensis*, *Nuttallanthus texanus* and *L. triornithophora* Willd. as the representatives of sects. *Macrocentrum*, *Lectoplectron* and *Pelisserianae*, respectively, form the most basal grades of the genus.

Main differences between gene trees (Supplementary files: Appendix S4, S5) and the species tree (Fig. 2) are summarized below.

The topology of ITS trees was similar to our species tree except for position of *N. texanus* as sister to sect. *Pelisserianae* but sister to sect. *Macrocentrum* in the species tree, unresolved relationships between the subsections of *L.* sect. *Supinae* in the ITS tree, while resolved in the species tree. Furthermore, in the ITS tree *L. incarnata* (Vent.) Spreng. is placed in L. sect. *Versicolores* subsect. *Elegantes* but in the species tree it is placed in sect. *Versicolores* subsect. *Versicolores*.

Differences between *rpl32-trnL* and the species tree as well as the ITS gene tree are more conspicuous. The main differences observed in the *rpl32-trnL* trees compared to the species tree are: 1- splitting of members of sect. *Supinae* in three separate clades, representing three subsections of this section; 2- position of *L. nivea* Boiss. & Reut. which is nested within *Macrocentrum* clade; 3- division of the *Linaria* clade in a polytomy of three well supported clades plus *L. peloponnesiaca* Boiss. & Heldr., *L. ventricosa* Coss. & Balansa, *L. genistifolia* (L.) Mill. and *L. genistifolia* ssp. *dalmatica* (L.) Maire & Petitm.; and 4- position of *N. texanus* forming a polytomy together with other major clades of *Linaria*.

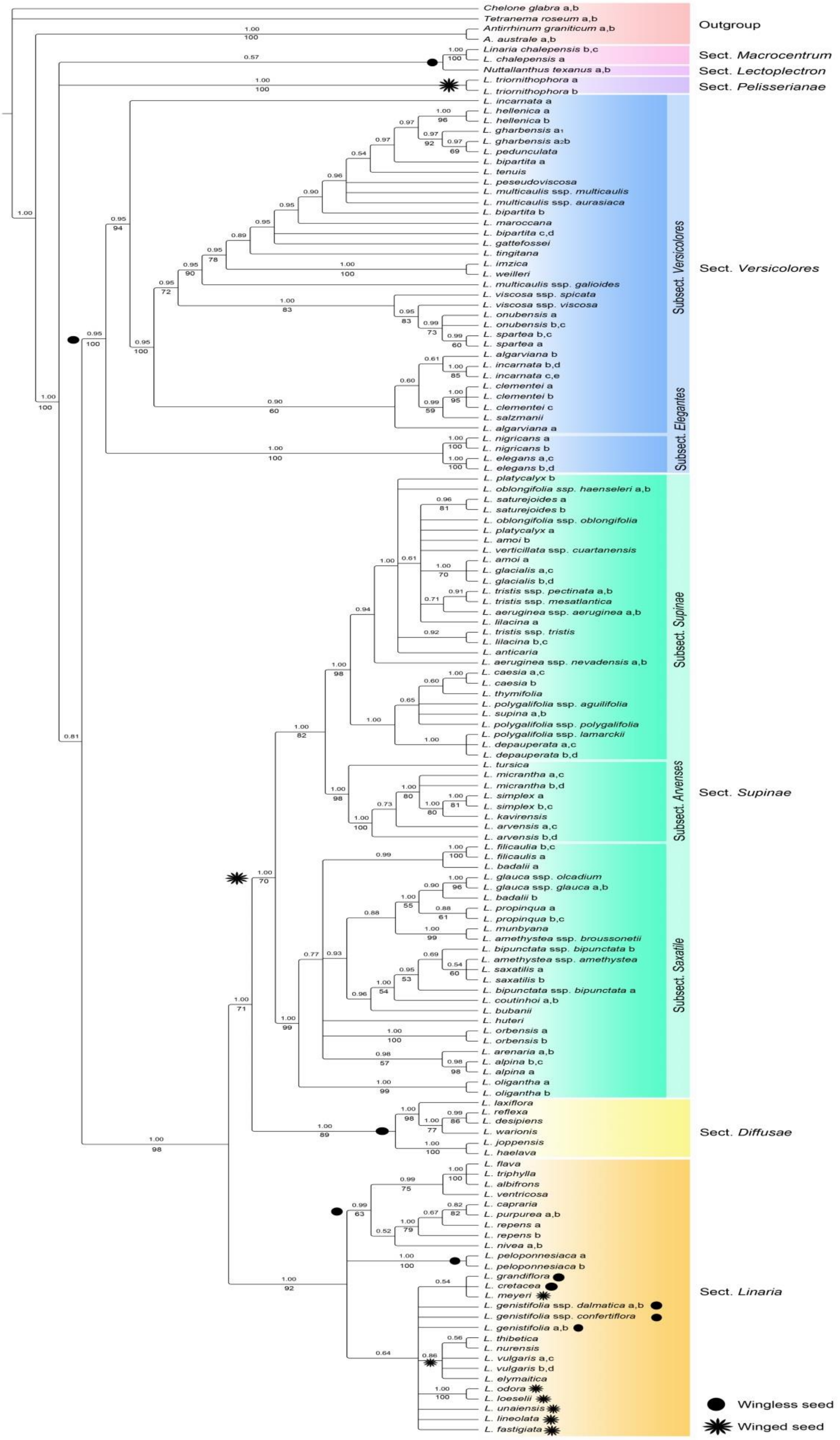

Figure 2. Semi-strict consensus tree obtained from Bayesian Inference for 87 species representing all sections of *Linaria* and four outgroup species: *Chelone glabra* L., *Tetranema roseum* (M. Martens & Galeotti) Standl. & Steyerm, *Antirrhinum graniticum* and *A. australe*. Numbers above branches are Bayesian posterior probabilities, while numbers below branches indicate the maximum parsimony bootstrap values.

Discussion

In accordance with previous phylogenetic studies such as Vargas et al. (19): based on analysis of eight *Linaria* spp.; Fernández-Mazuecos et al. (2): including 94 *Linaria* spp. and Rahmani et al. (3): analyzing 37 *Linaria* spp. using nrDNA ITS sequences, our analyses support the monophyly of *Linaria-Nuttallanthus* strongly. The monophyly of *Linaria* is also supported by other evidence such as basic chromosome number x=6 and a set of morphological characteristics that do not occur together in other members of tribe Antirrhineae. Some important morphological synapomorphies of *Linaria* spp. are: the presence of hypocotylary stems; entire and sessile leaves pinnately veined; terminal, bracteate, racemose inflorescences; and spurred flowers (1, 16).

The species trees gained here from MP and BI analyses (Fig. 2) reveals seven major clades, six of which representing six sections widely accepted by the modern taxonomists (Sect. Macrocentrum, Sect. Lectoplectron, Sect. Pelisserianae, sect. *Versicolores*, sect. *Supinae* and sect. *Diffusae*), although with different taxon composition (see as e.g., Podlech and Iranshar, 25). The seventh clade is subdivided in a polytomy of two weakly supported clades plus *L. peloponnesiaca* Boiss. & Heldr, the Mediterranean *L. chalepensis* as representative species of the bitypic *L.* sect. *Macrocentrum*, forms the most basal grade of *Linaria*, suggesting a Mediterranean origin of the genus. *Linaria chalepensis* and *L. armeniaca* Chav., the two members of *L.* sect. *Macrocentrum*, have been assigned to the unranked group, §*Versicolores*, by Bentham (13) mainly due to the wingless seeds, but some unusual features led Sutton (1) to accept the placement of these taxa in an isolated section. The species of *L.* sect. *Macrocentrum* are morphologically well-characterized by a calyx with adaxial lobes shorter than the four abaxial lobes, and presence of small lateral appendage at the base of the stamen filaments. Thick-walled capsules enclosing tetra- (-poly) hedral seeds which are papillate on surface are other characteristic features of these taxa. The monophyly of this group was also confirmed by Fernández-Mazuecos et al. (2) and Rahmani et al. (3) using ITS sequences and including both species of this section in the analyses. *Nuttallanthus texanus* is sister to *Linaria* sect. *Macrocentrum* with weak support in the Bayesian analysis. From morphological point of view, seeds of *Nuttallanthus* have five or six longitudinal angles. This trait is shared with members of sect. *Macrocentrum* but not with the other sections of *Linaria*. Also flower morphology of *Nuttallanthus* spp. is similar to members of sect. *Macrocentrum* and some species of sect. *Versicolores*. Therefore, based on morphological characters and phylogenetic results, we suggest reducing the species of *Nuttallanthus* to a section under *Linaria* as proposed by Pennell (27), Valdés (16) and Fernández-Mazuecos et al. (2) who treated this group as sect. *Lectoplectron* in *Linaria*.

Linaria sect. *Pelisserianae* is also a bitypic section including *L. triornithophora* and *L. pelisseriana* (L.) Mill. which had been assigned to two unranked groups: *Grandes* or *Arvenses*, respectively, by Bentham (13), but transferred to *L.* sect. *Arvenses* by Wettstein (21). Valdés (16) was the first who described *L.* sect. *Pelisserianae* with both above mentioned species included. Our analyses in accordance with previous molecular phylogenetic studies (2-3) support this treatment. Winged seeds dorsi-ventrally compressed (against other species of *Linaria* with winged seeds which are laterally compressed) caused through their unique placement in the capsule, can be considered as further evidence supporting the monophyly of this small section.

Four other major clades of *Linaria* are discussed below.

Linaria *section* Versicolores

37 accessions in our analyses constituted the monophyletic clade *Versicolores* (PP 0.95 and BS 100). This section is a very distinctive group in *Linaria* by owing bifid styles with discrete stigmatic areas. This character is even unique in the tribe Antirrhineae. Monophyly of this clade was also suggested by Fernández-Mazuecos and Vargas (28) using cpDNA regions (*rpl*32-*trnL* and *trnK-matK*), Fernández-Mazuecos et al. (2) and Rahmani et al. (3) using nrDNA ITS sequences. Two well-supported subclades corresponding to subsections *Versicolores* and *Elegantes* indicate, in turn, the monophyly and naturalness of these two subsections. The main

morphological difference between these two subsections is the stigmatic cleft which is much deeper in members of subsect. *Versicolores* than in subsect. *Elegantes*, defined as emarginated by Sutton (1).

Linaria *section* Supinae

This monophyletic group with high support (PP 1.00 and BS 70) is divided in three subclades: subclade *Supinae* (PP 1.00 and BS 98) is formed of perennial species with long corolla (18-38 mm), and seeds with membranous wing; subclade *Arvenses* (PP 1.00 and BS 98) are annual species with short corolla (2.5-9 mm) and seeds with thick wing; and subclade *Saxatile* (PP 1.00 and BS 99) is represented by annual or perennial species with medium-sized corolla (8-18 mm) and seeds with thick or narrow wing.

The naturalness of section *Supinae* was also intensely supported by Blanco-Pastor et al. (29) using combination of both nuclear and plastid sequences. Nevertheless the monophyly of this group is not supported in other molecular studies of *Linaria* (2-3). Also according to Sutton (1) section *Supinae* is scarcely distinguished from some species of section *Diffusae*.

Valdés (16) in a taxonomic revision of *Linaria* considered sect. *Arvenses* as an independent section because of small flowers and divided the other members of sect. *Supinae* in three subsections: *Supinae* (perennial plants with membranous seed wings), *Amethystea* (annual plants with thick seed wings), and *Saxatile* (annual or perennial plants with ±thin wings). Blanco-Pastor et al. (29) using two nuclear (ITS, AGT1) and two plastid regions (*rpl32-trnL*, *trnS-trnG)* divided this section into three subsections: *Supinae*, *Arvenses* and *Saxatile.*

Linaria *section* Diffusae

Referring to vegetative and floral characters, Chavannes (12) considered *Diffusae* as an informal group in *Linaria*. Bentham (13) considered *Diffusae* as a subgeneric unranked group. Later, Wettstein (49) assigned the rank of section to *Diffusae*. Valdés (16) suggested that section *Diffusae* is probably polyphyletic and Sutton (1) mentioned it as a heterogeneous group of species. In our analysis some species of section *Diffusae* form a monophyletic group (with PP 1.00 and BS 89), while others are nested within *Linaria* clade (see below). Both groups are composed of predominantly annual species (excluding *L. decipiens* Batt.) with wingless seeds. Thus, the monophyly of section *Diffusae* is again not supported by our analysis as well as in previous molecular studies of *Linaria* (2-3). Three species of sect. *Diffusae* (*L. albifrons* Spreng., *L. flava* Desf., and *L. triphylla* Mill.) are placed in clade *Linaria* (with PP 1.00 and BS 100) separated from other species assigned to this section (see below). The seeds of these three species are characterized by prominent anticlinal walls of testa cells plus sunken periclinal walls with no papillae (1).

Linaria *section* Linaria

In accordance with previous molecular phylogenetic studies (2-3), the gained trees indicate that members of sections *Linaria*, *Speciosae* and some members of sect. *Diffusae* constitute a monophyletic clade (PP 1.00 and BS 92). The species of sections *Diffusae* and *Speciosae* have similarities such as wingless seeds, unreduced adaxial lobe of calyx, and simple style. Despite these similarities, members of sect. *Diffusae* constituting this clade are mainly annual plants with procumbent or ascending fertile stems, while the members of sect. *Speciosae* are perennial plants with erect fertile stems. Furthermore, there are several morphological similarities in habit, stem, leaf, flower, and capsule linking the species of sects. *Linaria* and *Speciosae* which are highlighted in Sutton (1). In addition the frequent hybrids reported between the species assigned to these two sections might be evidence indicating close affinity between these two taxa (1). The only major difference between sect. *Linaria* and sect. *Speciosae* is the presence of winged seeds in the former and wingless seeds in the latter. In general these two sections seem to be closely related, and the phylogenetic analyses presented here and previously (2-3) did not provide adequate signals to resolve the relationships among these taxa.

Taxonomic conclusion

When gathering all sources of data, it becomes obvious that the current subgeneric classification of *Linaria* (1, 25) fits in some groups with the molecular phylogeny, but needs substantial revision in other points. Following sections are well defined morphologically: sect. *Lectoplectron*, sect. *Macrocentrum*, sect. *Pelisserianae*, sect. *Versicolores*, and sect. *Supinae*. However, it is necessary to propose a new concept for sect. *Diffusae* by restricting it only to those annual species with wingless seeds that are covered by testa cells with slightly elevated to sunken anticlinal walls plus convex periclinal walls papillate on surface. The main modification should be applied to sect. *Linaria* through expanding it to include sect. *Speciosae* and a part of sect. *Diffusae*. Our results which are in accordance with previous molecular phylogenetic studies (2) suggest classifying sect. *Versicolores* and sect. *Supinae* into subsections, but due to low support and blurred morphological boundaries, such subdivision cannot be applied to the large sect. *Linaria*. Following diagnostic key and the cladogram presented in Fig. 2 summarize the new classification system supported by both morphological and molecular phylogenetic data.

Diagnostic key to the sections of *Linaria*

1a. Abaxial lip of the corolla largely exceeding the adaxial lip, the palate weakly developed, spur very slender to absent…………………. sect. *Lectoplectron*
1b. Adaxial lip of the corolla exceeding the abaxial lip, the palate well-developed, spur large and obvious …………………………………………………... 2
2a. Style bifid………………...…… sect. *Versicolores*
  a. Style with 2 discrete stigmatic areas …………………………...… subsect. *Versicolores*
  b. Style more or less simple with a merely emarginated stigma…………….subsect. *Elegantes*
2b. Style simple, non-divided……………………….3
3a. Seeds dorsi-ventrally compressed, hilum median……….......……………….sect. *Pelisserianae*
3b. Seeds laterally compressed, hilum marginal........ 4
4a. The adaxial lobe of the calyx shorter than the remaining four abaxial lobes; lateral appendage present at the base of each stamen filament ....…………………………….sect. *Macrocentrum*
4b. The adaxial lobe of the calyx longer than or equal to the remaining four abaxial lobes; lateral appendage absent at the base of each stamen filament ……….. 5
5a. Fertile stems usually erect, the primary stem soon degenerates, i.e. stems homomorphic ...... sect. *Linaria*
5b. Fertile primary (epicotylary) stem together with heteromorphic fertile and sterile secondary (hypocotylary) stems present, without adventitious stems from root, fertile stems procumbent, ascending or more rarely erect…………………………………… 6
6a. Seeds discoid with broad, distinct or sometimes narrow wing …………………..………. sect. *Supinae*
  a. Annual plants; corolla small, 2.5-9 mm long; seed with thick wing ……..……. subsect. *Arvense*
  b. Annual or perennial plants; corolla ± large, 8-38 mm long; seed wing narrow or thick…...……….. c
  c1. Perennial plants; corolla large, 18-38 mm long; seed with membranous wing….… subsect. *Supinae*
  c2. Annual or perennial plants; corolla of medium size, 8-18 mm long; seed wing thick or thin but not membranous……………….. subsect. *Saxatile*
6b. Seeds oblongoid, clearly wingless… sect. *Diffusae*

Nomenclature

Linaria **sect.** ***Macrocentrum*** D.A. Sutton, Bot. J. Linn. Soc. 81: 171 (1980) ≡ *L.* sect. *Diffusae* subsect. *Macrocentrum* (D.A. Sutton) Menitsky, Bot. Zhurn. (Moscow & Leningrad) 84(1): 130 (1999). Typus (mentioned in the protologue): *L. armeniaca* Chav.

Linaria **sect.** ***Lectoplectron*** Penn., Monogr. Acad. Nat. Sci. Philadelphia. 1: 302 (1935) ≡ *Nuttallanthus* (D.A. Sutton) Rev. Tribe Antirrhineae 455 (1988). Typus (mentioned in the protologue): *N. canadensis* (L.) D.A.Sutton.

Linaria **sect.** ***Pelisserianae*** Valdés, Rev. Esp. Eur. *Linaria*: 70 (1970) as "sect. *Pelisseriana*". Typus (mentioned in the protologue): *L. pelisseriana* (L.) Mill.

Linaria **sect.** ***Versicolores*** (Benth.) Wettst. in Engler and Prantl, Nat. Pflanzenfam. 4 (3b): 59 (1891) ≡ *L.* [unranked] *Versicolores* Benth. (1846) in DC., Prodr. 10: 275
= *L.* sect. *Corrugatae* Kapan., Bot. Zhurn. (Moscow & Leningrad) 73(10): 1480 (1988)
= *L.* sect. *Bipunctatae* Viano, Candollea 33(2): 214 (1978). Lectotypus (Viano, 1978a: 49): *L. viscosa* (L.) Dumm. Cours.

Linaria **sect.** ***Supinae*** (Benth) Wettst. in Engler and Prantl, Nat. Pflanzenfam. 4 (3b) 59 (1891). Typus: *L. supina* (L.) Chaz.

Linaria **sect.** ***Diffusae*** (Benth.) Wettst. in Engler & Prantl, Nat. Pflanzenfam. 4 (3b) 59 (1891) ≡ *Linaria* [unranked] *Diffusae* Benth. (1846) in DC., Prodr. 10: 284. Lectotypus: (Viano, 1978b: 223): *L. reflexa* Desf. (1798: 42).

Linaria* sect. *Linaria ≡ *L.* sect *Grandes* (Benth.) Wettst. (1891), in Engler & Prantl, Nat. Pflanzenfam. 4, 3b: 59 ≡ *L.* [unranked] *Grandes* Benth. (1846) in DC., Prodr. 10: 27 ≡ *L.* sect *Linariastrum* Chav. (1833) Monogr. Antirrhineae: 114 ≡ *L.* [unranked] *Dicoideae* Boiss. (1879), Fl. Or. 4: 370. Lectotypus (Valdés, 1970: 44): *L. vulgaris* Mill. (1768: no. 1).
= *Linaria* sect. *Speciosae* (Benth.) Wettst. in Engler & Prantl, Nat. Pflanzenfam. 4 (3b) 59 (1891). **Syn. nov.** ≡ *L.* sect. *Linariastrum* Chav. [unranked] *Speciosae* Benth. (1846) in DC., Prodr. 10: 271 = *L.* sect. *Linariastrum* [unranked] *Oblongae* Boiss. (1879) Fl. Or. 4: 364. Lectotypus (Valdés, 1970: 24): *L. genistifolia* (L.) Mill. (1768: no. 14).

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