Available online at www.pelagiaresearchlibrary.com

Pelagia Research Library

European Journal of Experimental Biology, 2014, 4(3):127-134

ISSN: 2248 –9215
CODEN (USA): EJEBAU

A phylogenetic analysis of *Linaria* (Plantaginaceae) species from Iran based on ITS sequence data

Abbas Rahmani[1*], Taher Nejadsatari[1], Seyed Mohammad Mahdi Hamdi[2], Iraj Mehregan[1] and Mostafa Assadi[3]

[1]*Department of Biology, Collage of Basic Science, Science and Research Branch, Islamic Azad University, Tehran, Iran*
[2]*Department of Biology, Garmsar Branch, Islamic Azad University, Garmsar, Iran*
[3]*Research Institute of Forest & Rangelands, Tehran, Iran*

ABSTRACT

Linaria Miller With more than 150 taxa is the largest genus of the tribe Antirrhineae. This study was carried out on the species of Linaria that growing in Iran. Some of these species are native to Iran. Internal transcribed spacer (ITS) sequences were obtained for 37 samples representing 6 sections of Linaria recognized by recent taxonomic treatments from Iran. In addition, we used 82 previously ITS sequences from GenBank to test the monophyly of Linaria. Phylogenetic analysis were conducted using Bayesian inference and maximum likelihood. The results indicate that Linaria species from Iran constituted a monophyletic group within the Antirrhineae. data analysis indicates that the classification of species according winged or wingless seeds so not true, so seed morphology especially seed wing that have been considered as useful for the taxonomy of Linaria species, appears to be a homoplasious character in Linaria. Bayesian inference and maximum parsimony analyses confirmed Two sections of Linaria including Macrocentrum and Versicolores from Iran are monophyletic, while monophyly of sections Linaria, Diffusae, Supinae and Speciosae are unsupported by this results. To determine the evolution of Linaria use of morphological characteristics coupled with molecular data will be most effective.

Keywords: Iran, *Linaria*, phylogeny, ITS.

INTRODUCTION

Linaria Miller With more than 150 taxa is the largest genus of the tribe Antirrhineae [28]. distributed in the Northern hemisphere, its species living in Europe, Asia and North Africa [19]. *Linaria* was identified as a taxonomic being as soon as the time of pre-Linnaean botanists [22, 29]. At first Linnaeus (1753) identified *Linaria* species inner genus Antirrhinum [20]. Miller (1754) first logical description for *Linaria* supplied [21]. *Linaria* species are annual or perennial herbs, with heteromorphic shoots, sessile leaves and racemose inflorescences [26]. for the taxonomy of *Linaria* species, seed morphology was applicable. Small seeds of *Linaria* were encircled by capsules, they were winged seeds or wingless seeds. The wingless seeded species by Viano [32, 33], and the winged seeded species by Valde´s (1970) established [30]. The winged seeds species designated Discoideae and the wingless seeds species introduced Oblongae by Boissier (1879)[3]. Viano's theory called wingless and winged seeds formed

distinct natural sister lineages in *Linaria* species [32, 33]. Sutton(1988) recognized seven sections mainly based on seed morphology within *Linaria* widely accepted today [28]. Species of sections *Linaria*, *Supinae* and *Pelisserianae* have discoid and usually winged seeds. While species of sections *Versicolores*, *Speciosae*, *Diffusae* and *Macrocentrum* have nondiscoid and wingless seeds. In Iran, 35 species of *linaria* belonging to the sections *Linaria* (20 spp.), *Speciosae* (6 spp.), *Supinae* (4 spp.), *Macrocentrum* (2 spp.), *Diffusae* (2 spp.) and *Versicolores* (1 spp.) are present [3, 23, 28, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17]. *Linaria* species that distribution in different regions of Iran, showed in Table 1 [7]. The aims in this study were to test the naturalness of the seven sections of Sutton's (1988) classification that six sections of them are present in Iran and to test the hypothesis of a basal divergence between two lineages of *Linaria*, one with winged seeds and the other with wingless seeds [28].

Table 1. Distribution of Linuria species in Iran

Geographical abbreviations: C, enter Iran; S, southern Iran; SE, south-eastern Iran; N, northern Iran; NE, north-eastern Iran; NW, north-western Iran; E, eastern Iran; W, western Iran [7].

sections	Taxon		Distribution in Iran	Major Features of morphological traits of Linaria [28].
Linaria	*L. vulgaris* Miller		C	Seed form: Discoid Seed wing: Wing Duration: Perennial Stigma: Entire
	L. odora (Bieb) Fischer		C, NE	
	L. khorasanensis S. M. M. Hamdi and M. Assadi		NE	
	L. leptoceras Kuprian		NE	
	L. striatella Kuprian		C, NE	
	L. baminaica Patzak in Koie and Rech. f		NE	
	L. michauxii Chavannes		C, W, NW, E, SE,NE	
	L. farsensis S. M. M. Hamdi and M. Assadi		C	
	L. pyramidalis (Vent) F. G. Dietr.	Subsp. *pyramidalis*	NW	
		Subsp. Koptedaghensis	NE	
	L. fastigiata Chavannes		C, W, NW	
	L. nurensis Boiss. and Hausskn.		C	
	L. remotiflora Patzak in Rech. f.		C	
	L. kurdica Boiss. and Hohen. In Boiss.	Subsp. *kurdica*	W, NW	
		Subsp. Pycnophylla	W, NW	
	L. lineolata Boiss.		C, NW	
	L. karajensis S. M. M. Hamdi and M. Assadi		C	
	L. elymatica (Boiss.) Kuprian		N, W, C	
	L. azerbaijanensis S. M. M. Hamdi and M. Assadi		NW	
	L. khalkhalensis S. M. M. Hamdi and M. Assadi		N, NW	
	L. shahroudensis S. M. M. Hamdi and M. Assadi		C	
	L. guilanensis S. M. M. Hamdi and M. Assadi		N	
Diffusae	*L. bousherensis* S. M. M. Hamdi and M. Assadi		S	Seed form: Nondiscoid Seed wing: No wing Duration: Annual or perennial Stigma: Entire
	L. albifrons (Sibth. and Sm.) Steudel.		S	
Speciosae	*L. mazandaranensis* S. M. M. Hamdi and M. Assadi		N	Seed form: Nondiscoid Seed wing: No wing Duration: Perennial Stigma: Entire
	L. genistifolia (L.) Miller		N, NW	
	L. golestanensis S. M. M. Hamdi and M. Assadi		N	
	L. dalmatica (L.) Miller		N, W, NW	
	L. grandiflora Desf, Ann.		W, NW	
	L. orientalis S. M. M. Hamdi and M. Assadi		N	
Macrocentrum	*L. chalepensis* (L.) Miller	Subsp. *chalepensis*	N, NW, W, C, NE, S	Seed form: Nondiscoid Seed wing: No wing Duration: Annual Stigma: Entire
		subsp. *gorganensis*	N	
	L. armenica Chavannes.		NW	
Supinae	*L. arvensis* (L.) Desf.		S	Seed form: Discoid Seed wing: Wing Duration: Annual or perennial Stigma: Entire
	L. simplex (Willd) DC.		N, NW, C, NE	
	L. kavirensis S. M. M. Hamdi and M. Assadi.		C, NE	
	L. micrantha (Cav.) Hoffmanns. and Link.		W, S	
Versicolores	*L. iranica* S. M. M. Hamdi and M. Assadi		S	Seed form: Nondiscoid Seed wing: No wing Duration: Annual or perennial Stigma: Divided

Table 2. List of taxa investigated in our analysis and herbaria where the vouchers are deposited with Genbank accession numbers
TARI= herbarium of Research Institute of Forests and Rangelands, IAUH= Islamic Azad University Avicennia herbarium

Species	Origin, voucher	ITS Genbank accession numbers
L. vulgaris	Iran: prov. Tehran; Lawasan, Galendoak, 1700m, Mozzafarian, (52344 TARI).	KJ747021
L. odora	Iran: prov. Khorasan: Neshabbour, Dizbade-Olia, 1800m, Pariab and Abbasi, (8825 TARI).	KJ747015
L. korasanensis	Iran: prov. Khorasan, Mashad, SW of Moghan, 2300 m, Assadi and hamdi, (85531 TARI).	KJ747008
L. striatella	Iran: prov. Tehran, 10 km Roudehen to Firouzkouh, 2200m, Rahmani, IAUH without herbariums number.	KJ747020
L. bamianica	Iran: prov. Khorasan: Torbate-Jam, North of Torbate-Jam, 10 km to Bani-Tak, 1050 m, Jouharchi, (30649 TARI).	KJ747001
L. michauxii	Iran: prov. Tehran; 10km Tehran- Karaj highway, 1400m, Rahmani, IAUH without herbariums number.	KJ747013
L. farsensis	Iran: prov. Fars; Abadeh, Soghad, Dashte Ayon, 2100-2270 m, Wendelbo and Foroughi, (17870 TARI).	KJ747003
L. pyramidalis subsp. pyramidalis	Iran: Prov. Azerbaijan; between Urmia and Salmas, 1900m, Assadi, (78929 TARI).	KJ747017
L. pyramidalis subsp. kopetdaghensis	Iran: prov. Khorasan: Bojnurd; 40km western-south Bojnurd, Kuh-Sakuh, 2700, Mehregan, (13934 TARI).	KJ747016
L. fastigiata	Iran; prov. Hamedan; Ghahavand, Biukabad, to Shahbodagh, Aghdash maintain, slope of western, 1800-2300m, Mozzafarian, (64470 TARI).	KJ747004
L. remotiflora	Iran; prov. Kogiloueh and Bouyerahmad; between Yasouj and Dehdasht, Dilgoon, Saverz maintain, 2200-2300m, Assadi and Abouhamzeh, (46411 TARI).	KJ747018
L. nurensis	Iran: prov. Fars; Nourabad, 44km next Fahelian, toward Reshk, 1800m, Mozzafarian, (45932 TARI)	KJ747014
L. kurdica subsp. kurdica	Iran; prov. Kourdestan; Sanandaj, 25km sout-east of Naran village, 2200-2600m, Assadi, (60498 TARI).	KJ747009
L. kurdica subsp. pycnophylla	Iran: Prov. Azerbaijan; Jolfa, 25km sout-east Jolfa, Gheshlagh village, Kuh-Gholenj, 2100-2700m, Assadi and Shahsavari, (65736 TARI).	KJ747010
L. lineolata	Iran: prov. Tehran; Lawasan between Oshan and Lawasan, 1750m, Rahmani, without herbariums number.	KJ747012
L. karajensis	Iran; prov. Tehran; shahrestanac, Kuh- Touchal, 3150 m, Riazi, (8470 TARI).	KJ747006
L. elymatica	Iran; prov. Kerman; Kerman, Lalehzar maintain, 3200 m, Froughi and Assadi, (16360 TARI).	KJ747002
L. azerbaijanensis	Iran: Prov. Azerbaijan; Tabrize, beginning of road to Tehran, near Ghori gol, pass of Shebeli, 2300 m, Assadi(85348 TARI).	KJ747000
L. khalkhalensis	Iran: Prov. Azerbaijan; Khalkhal, 48 km Masooleh to Khalkhal, 1200 m, Assadi (86496TARI).	KJ747007
L. shahroudensis	Iran: Prov. Semnan, Shahroud, 15 km of Tash to Gorgan, 2800m, Assadi and hamdi (85676 TARI).	KJ747019
L. guilanensis	Iran: Prov. Gazvin; 50 km of Gazvin-rasht roude, 1700m, Rahmani, IAUH without herbariums number.	KJ747005
L. bousherensis	Iran: Prov. Boushehr, 43 km to Ganaveh, 180 m, 29.02.1972, Foroughi (3107 TARI).	KJ747025
L. albifrons	Iran: Prov. Boushehr, 30 km to Bandare-Amir, 20 m, Runemark and Mozzafarian (26985 TARI).	KJ747022
L. mazandaranensis	Iran: Prov. Mazandaran; Ghaem-shahr, toward Firouz-Kuh, 8km west-sout of pole-sefid, 1270m, Moosavi (33702 TARI).	KJ747033
L. genistifolia	Iran: Prov. Mazandaran, Ramsar, Javaherdeh, western-south, 2500-2750 m, Wendelbo and Maassoumi, (20889 TARI).	KJ747028
L. golestanensis	Iran: Prov. Golestan; east-south Maraveh-Tapeh, Shalmi mountain 1200m, Faghih-Nia and Zangouii (32889 TARI).	KJ747029
L. dalmatica	Iran: Prov. Azerbaijan; Sarab, Saraban Kouh, Termeh (39071 TARI).	KJ747027
L. grandiflora	Iran: Prov. Azerbaijan; Ahar, 11km North of Azghan, 1840-2200mm Termeh and moosavi (38822 TARI).	KJ747030
L. orientalis	Iran: Prov. Semnan, Shahroud, to azadshahr, Khosh Yielagh, Riazi (8496 TARI).	KJ747035
L. chalepensis	Iran; prov. Kogiloueh and Bouyerahmad; Dogonbadan, 11km to Charam, 1100-1400 m, Assadi and Abouhamzeh, (38592 TARI).	KJ747026
L. armenica	Iran: Prov. Azerbaijan; 5km Khouy to Siah Cheshmeh, 1880m, Hamdi (82225 TARI).	KJ747023
L. arvensis	Iran: Prov. Khuzestan: Dehdez, Gharun-Kuh, 1000-1500m, Mozaffarian, (74494 TARI).	KJ747024
L. simplex	Iran: Prov. Guilan; Bandar Anzali, 30- 50 m, Mozaffarian, (75120 TARI).	KJ747036
L. kavirensis	Iran: Prov. Yazd, Taft, Taft-kuh, 1000-1300m, Dehghanzadeh, (26052 TARI).	KJ747032
L. micrantha	Iran: Prov. Khuzestan: Behbehan, 320m, Mozaffarian, (62494 TARI).	KJ747034
L. iranica	Iran: Prov. Kerman: Jiroft, 1000-1500m, Mirtajeddiny without herbariums number.	KJ747031

MATERIALS AND METHODS

Samples were collected in the field and dried in silica gel or obtained from herbaria in Iran (TARI, IAUH), [18]. Phylogenetic reconstructions were performed in 37 samples of *Linaria* presented in six sections from Iran. Table 2 lists all taxa used in this study and summarizes sources, voucher specimen data and GenBank accession numbers. Total DNA was extracted using the DNeasy Plant Mini kit (Qiagen, Germany). We amplified the ITS region (ITS1-5.8S-ITS2) of the nuclear ribosomal DNA using primer combinations AB101 and AB102 primers: a forward primer AB101annealing, 5'-ACG AAT TCA TGG TCC GGT GAA GTG TTC G-3', and a reverse primer (AB102) annealing, 5'-TAG AAT TCC CCG GTT CGC TCG CCG TTA C-3'. The PCR protocol for ITS region included: 25 cycles of 1 min denaturation (94°C), 1 min annealing (54°C), and 2 min, 30 s elongation (72° C), with two additional seconds elongation per cycle [5].

PHYLOGENETIC ANALYSIS

Phylogenetic reconstructions were performed in 37 sampels of *Linaria* in six sections from Iran. In this study We used the ITS sequence of 34 species of *Linaria*, 37 species of Antirrhinum, 4 species of *Galvezia*, 2 species of *Maurandya* and 2 species of *Kickxia* from GenBank. List of non-Iranian taxa used in our analysis with GenBank accession numbers showed in Table 3. We also used the ITS sequence of *Lafuentea rotundifolia*, from GenBank as the outgroup [1]. Matrices were analyzed with PAUP*4.0b10, with the following options: heuristic search with 1,000 random-addition-sequence replicates; tree bisection-reconnection (TBR) branch swapping; “collapse zero length branches;” saving all most parsimonious trees. Character state changes were treated as equally weighted. Nonoverlapping parsimony informative indels were coded as binary characters and added to the end of the data matrix. Relative clade support was estimated using 1,000 bootstrap, replicates in PAUP* via “full heuristic” searches and simple taxon addition. The consistency index (CI) and retention index (RI) were used to assess the amount of homoplasy present in the data. The best-fitting substitution model (GTR+I+G) was determined under the Akaike Information Criterion (AIC) in Model selected [24]. BI was performed in MrBayes ver. 3.1.2 [25]. A 50% majority-rule consensus tree with Bayesian posterior probabilities (PPs) of clades was calculated after removing the first 10% generations as burn in.

RESULTS AND DISCUSSION

The data set of the ITS region included 611 characters, 224 of them potentially parsimony informative. Strict consensus phylogeny trees, with 1014 steps was included consistency index (CI)=0.4872, retention index (RI)=0.8971 and homoplasy index (HI)= 1.83. Within the *Antirrhineae* four major clades were identified which have been given the name of one typical genus: the Antirrhinum clade (2 genera; PP = 0.99; BS = 60%), the *Maurandya* clade (2 genera; PP = 0.99; BS = 90%), the *Kickxia* clade (2 genera; PP = 1; BS = 100%), and all sampled species of *Linaria* were formed *Linaria* clade (PP = 0.99; BS = 100%). A little supported relationships between major clades were existed. *Linaria* clade included five major clades (A–E). Clade A included two species (*L. chalepensis* and *L. armenica* from Iran) of sect. Macrocentrum (PP = 1; BS = 100%). Clade B included one Iranian species (*L. iranica)* and two other species of sect. *Versicolores* (PP = 0.99; BS = 100%). *Pelisserianae* constituted clade C (PP = 0.69; BS = 71%). *L. triornithophora* is sister to clade B. This is not recognized in Iran. All sampled species of sect. Supinae (*L. arvensis, L. simplex, L. kavirensis* and *L. micrantha* from Iran) and three species of sect. Diffusae were formed Clade D (PP = 0.99; BS = 79%). Clade E (PP = 0.98; BS = 60%) was formed by all sampled species of sect. *Linaria* (*L. vulgaris, L. odora, L. khorasanensis, L. striatella, L. farsensis, L. bamianica, L. michauxii, L. pyramidalis subsp. pyramidalis, L. pyramidalis subsp. kopetdaghensis, L. fastigiata, L. remotiflora, L. nurensis, L. kurdica subsp. kurdica, L. kurdica subsp. pycnophylla, L. lineolata, L. karajensis, L. elymatica, L. azerbaijanensis, L. khalkhalensis, L. shahroudensis and L. guilanensis* from Iran), all sampled species of sect. Speciosae (*L. mazandaranensis, L. genistifolia, L. golestanensis, L. dalmatica, L. grandiflora and L. orientalis* from Iran), and six species of sect. Diffusae (*L. bousherensis1, L. bousherensis2 and L. albifrons* from Iran). A little supported relationships among main clades were existed. Best support was occurred for two sister-group relationship between clades D and E (PP = 0.98; BS = 100%). Relationship between two sister-group clades B and C was supported by BI (PP = 0.69; BS = 71%). On the other side, relationships among other clades remained indefinite in our results.

Table 3. List of non-Iranian taxa used in our analysis with GenBank accession number

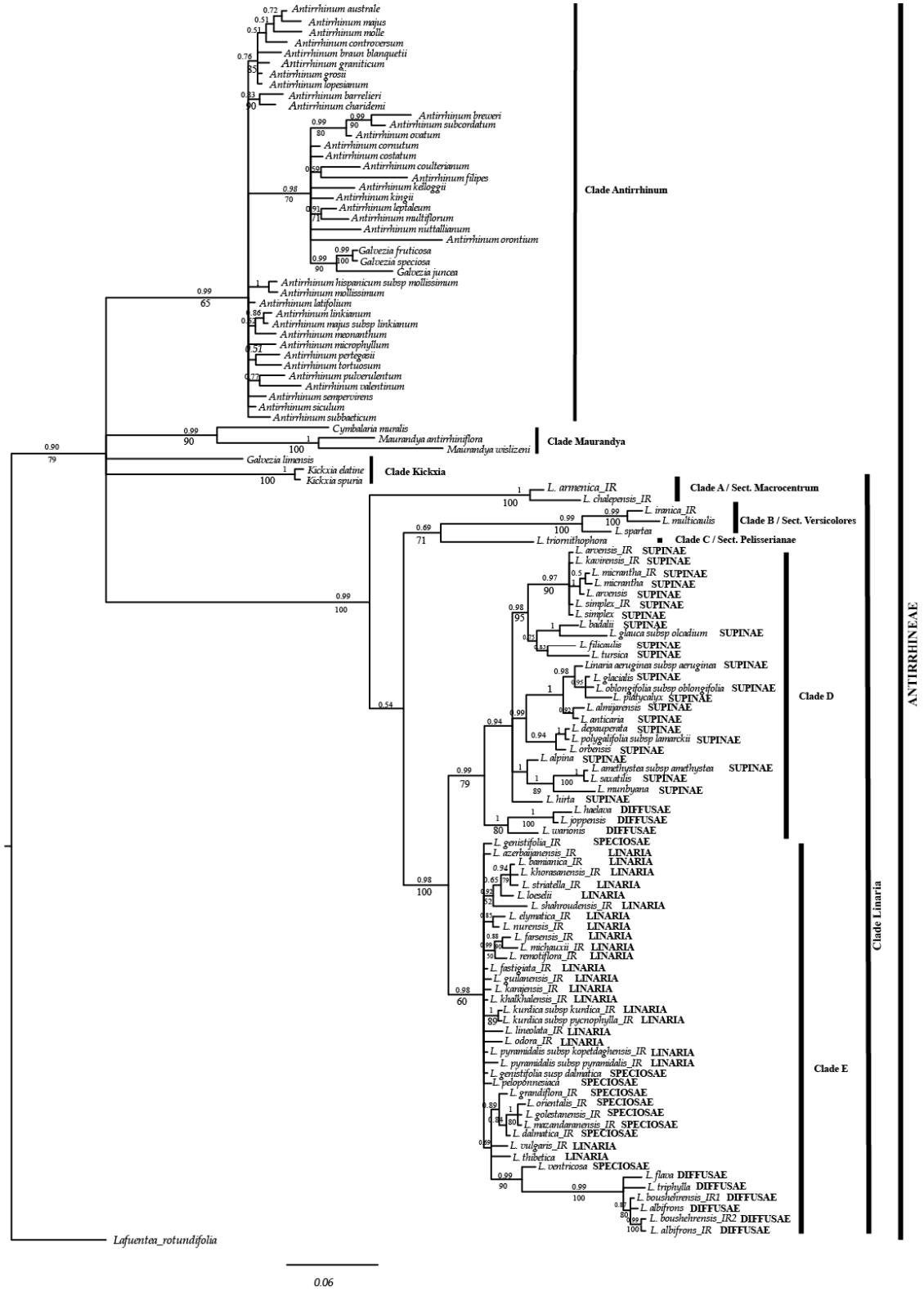
Species	Genbank accession numbers
Antirrhinum_barrelieri	AY880240
Antirrhinum_australe	EU677195
Antirrhinum_braun_blanquetii	EU677196
Antirrhinum_breweri	AY880229
Antirrhinum_charidemi	EU677199
Antirrhinum_controversum	EU677206
Antirrhinum_cornutum	AY880230
Antirrhinum_costatum	AF513893
Antirrhinum_coulterianum	AF513890
Antirrhinum_filipes	AF513896
Antirrhinum_graniticum	EU677209
Antirrhinum_grosii	EU677210
Antirrhinum_hispanicum_mollissimum	EU677224
Antirrhinum_kelloggii	AF513904
Antirrhinum_kingii	AF513903
Antirrhinum_latifolium	EU677212
Antirrhinum_leptaleum	AF513906
Antirrhinum_linkianum	AY731278
Antirrhinum_lopesianum	EU677217
Antirrhinum_majus_linkianum	EU677215
Antirrhinum_majus	AF513888
Antirrhinum_meonanthum	AF513887
Antirrhinum_microphyllum	EU677221
Antirrhinum_molle	AY731268
Antirrhinum_mollissimum	AF513886
Antirrhinum_multiflorum	AY880228
Antirrhinum_nuttallianum	AF513895
Antirrhinum_orontium	AF513889
Antirrhinum_ovatum	AY880233
Antirrhinum_pertegasii	EU677228
Antirrhinum_pulverulentum	EU677230
Antirrhinum_sempervirens	EU677236
Antirrhinum_siculum	AY731276
Antirrhinum_subbaeticum	AY731287
Antirrhinum_subcordatum	AF513902
Antirrhinum_tortuosum	AY731285
Antirrhinum_valentinum	EU677243
Galvezia_fruticosa	AY731252
Galvezia_juncea	AY880243
Galvezia_limensis	AY492104
Galvezia_speciosa	AY316309
Cymbalaria_muralis	AY492101
Kickxia_elatine	AY731265
Kickxia_spuria	AF513880
Maurandya_antirrhiniflora	AY492110
Maurandya_wislizeni	AY878930
Lafuentea_rotundifolia	AF509816
Linaria_triornithophora	AY731248
Linaria_alpina	AY731243
Linaria_hirta	AY731244
Linaria_micrantha	AY731242
Linaria_aeruginea_aeruginea	JQ814486
Linaria_almijarensis	JQ814492
Linaria_amethystea_amethystea	JQ814490
Linaria_anticaria	JQ814491
Linaria_arvensis	JQ814493
Linaria_badalii	JQ814495
Linaria_depauperata	JQ814499
Linaria_filicaulis	JQ814500
Linaria_glacialis	JQ814505
Linaria_glauca_olcadium	JQ814506
Linaria_haelava	JQ814507
Linaria_joppensis	JQ814508

Linaria_loeselii	JQ814511
Linaria_multicaulis	JQ814514
Linaria_munbyana	JQ814515
Linaria_oblongifolia_subsp_oblongifolia	JQ814516
Linaria_orbensis	JQ814518
Linaria_platycalyx	JQ814520
Linaria_polygalifolia_lamarckii	JQ814522
Linaria_saxatilis	JQ814526
Linaria_simplex	JQ814528
Linaria_spartea	JQ814529
Linaria_thibetica	JQ814531
Linaria_triphylla	JQ814532
Linaria_albifrons	JQ814488
Linaria_flava	JQ814501
Linaria_tursica	JQ814533
Linaria_ventricosa	JQ814534
Linaria_warionis	JQ814536
Linaria_genistifolia_dalmatica	JQ814498
Linaria_peloponnesiaca	JQ814519

In this study we provide the first phylogenetic analysis about *Linaria* from Iran. At first the monophyly of *Linaria* on the eight sampled species exponent the seven sections of Sutton's classification, proposed by Vargas et al. [31]. Also in recent phylogenetic study based on ITS sequences by Ferna´ndez et al. (2013), Monophyly of *Linaria* was supported [6]. This analysis include 37 sampels of *Linaria* from Iran. Phylogenetic relationships between *Linaria* species in this study are according to those obtained by Vargas et al. (2004) and Ferna´ndez et al. (2013) based on the ITS sequences [6,31]. In this ITS phylogeny study, our species of *Linaria* organized a intensely supported monophyletic group (fig.1). morphological characters that are not occured elsewhere in the Antirrhineae tribe and basic chromosome number (x=6) affirmed the monophyly of Linaria. [31, 28]. According to our obtained data the genus *Linaria* was determined as a paraphyletic group (fig. 1). This results as Ferna´ndez et al. (2013) finding, Viano's theory, separation of natural sister lineages in *Linaria* species based on with wingless and winged seeds forms, were refused. [32, 33]. Our analysis represent Sections *Macrocentrum* and *Versicolores* to be monophyletic (fig. 1). Other sections including *Linaria*, *Speciosae*, *Supinae* and *Diffusae* do not constitute as monophyletic groups. Species of sect. *Macrocentrum* (*L. chalepensis* and *L. armeniaca*, clade A; fig. 1) describe with some characters including the adaxial lobe of the calyx is shorter than abaxial lobes and lateral appendage present at the base of each stamen filament is small. Sutton (1980), based on these characters are specific between *Linaria*, separated them from sect. *Versicolores*. [27]. Section *Versicolores* is including just one species in Iran (*L. iranica*). Sutton (1988) was defined morphological character for sect. *Versicolores* that not found elsewhere in the Antirrhineae tribe [27]. This character was including separated style with distinct stigmatic region. The species *L. iranica*, that is endemic of Iran, has a bifid style. Champagnat (1961) With specific pattern of seedling progression is affirmed the naturalness of sect. *Versicolores*[4]. Four sections of *Linaria* including *Speciosae*, *Diffusae*, *Supinea* and *Linaria*, conversely sects. *Macrocentrum*, *Pelisserianae* and *Versicolores*, were not determined as monophyletic in this study. Relationships between species of sects. *Speciosae* and *Linaria* were showed in clade E. Previously Sutton (1988) was shown a identical morphological relationship among species of sections *Linaria* and *Speciosae* based on the perennial duration, erect stems, alike leaf, flower, and capsule morphology [28]. But there is a remarkable difference in seed form among species of both sections, also sect. *Linaria* have winged seeds and sect. *Speciosae* have wingless seeds. Three species of sect. *Diffusae* were also included in clade D (*L. haelava*, *L. joppensis* and *L. warionis*). This species formed a monophyletic group within clade D. Clade D also included species of sect. *Supinae* (*L. arvensis*, *L. simplex*, *L. kavirensis* and *L. micrantha* from Iran and other species of sect. Supinae). This results was supported the monophyly of species of sect. *Supinae*. The naturalness of sect. *Supinae* was affirmed union of both plastid and nuclear sequences in a merging-based analysis permitted the discovery of hybridization and imperfect lineage grouping [2]. Another species of sect. *Diffusae*, including *L. bousherensis1*, *L. bousherensis2*, *L. albifrons_IR*, *L. albifrons*, *L. flava*, and *L. triphylla*, constituted a monophyletic group within clade E. The main traits species of sect. *Speciosae* and sect. *Diffusae* including wingless seeds, entire style, normal adaxial lobe of calyx. Actually, separated species from both of sections is difficult. But chiefly morphological differences between species of sect. *Diffusae* and sect. *Speciosae* including annuals duration with ascending stems in sect. *Diffusae* and perennials duration with erect stems in sect. *Speciosae*. At first Valde´s (1970) was proposed that sect. *Diffusae* was apparently polyphyletic, But Sutton (1988) believed that it was constituted a heterogeneous group [28]. Because of low phylogenetic resolution within clade E, we required extra markers for expose the phylogenetic relationships.

Fig. 1 Phylogenetic relationships of 37 sampels of *Linaria* from Iran, 34 species of *Linaria*, 37 species of Antirrhinum, 4 species of *Galvezia*, 2 species of *Maurandya* and 2 species of *Kickxia* on the basis of the analysis of internal transcribed spacer sequences

Numbers above branches are Bayesian posterior probabilities. Numbers below branches are maximum likelihood percentage bootstrap values



CONCLUSION

The results indicate that *Linaria* species from Iran constituted a monophyletic group within the Antirrhineae. data analysis indicates that the classification of species according winged or wingless seeds so not true, so seed morphology especially seed wing that have been considered as useful for the taxonomy of *Linaria* species, appears to be a homoplasious character in *Linaria*. Two sections of Macrocentrum and Versicolores based on conformity special morphological traits and ITS sequences organized separated developmental lineages, therefor should be retained in classification of Linaria. But, naturalness of secttions Supinae, Linaria, Speciosae, and Diffusae were not supported. For carefully determined of Linaria location we need to more molecular markers and more Linaria sampling from all sections.

Acknowledgments
This article is extracted from my Phd thesis. We have finally thank from Islamic Azad University -Tehran Science and Research Branch for providing the facilities necessary to carry out the work.

REFERENCES

[1]Albach, D.C., Meudt, H.M., Oxelman, B. *Am. J. Bot*, **2005**, 92: 297–315.
[2] Blanco-Pastor, J.L., Vargas, P., Pfeil, B.E., *PloS ONE*, **2012**, 7:e39089.
[3]Boissier, E. Flora orientalis, **1879**, Vol 4, AH Georg Geneva.
[4] Champagnat, M ; *Ann Sci Nat Bot Biol Veg*, **1961**, 12: 1–170.
[5] Douzery, E.J.P., Pridgeon, A.M., Kores, P., Linder, H.P., Kurzwell, H., Chase, M.W., *Am. J. Bot*. ,**1999**, 86(6): 887–899.
[6] Ferna´ndez-Mazuecos, M., Blanco-Pastor, J.L. , Vargas, P., *Int. J . Plant . Sci*. ,**2013**, 174(2):234–249.
[7] Hamdi, S.M.M., Islamic Azad University, Research and Science branch – Tehran Iran, **2005**.
[8] Hamdi, S.M.M., Fallahian. F., Assadi, M., Maassoumi, A.A., *J.Bot*, **2005a**, 11: 79–83.
[9] Hamdi, S.M.M., Fallahian. F., Assadi, M., Maassoumi, A.A., Willdenowia, **2005b**, 35: 299–304.
[10] Hamdi, S.M.M., Fallahian. F., Assadi, M., Maassoumi, A.A., *Iran. J. Bot*., **2006**, 11 (2): 251- 258.
[11] Hamdi, S.M.M., Assadi, M., Nejadsatari, T., Linaria farsensis, *Bot. Stud*., **2007a**, 48 (2):233-237.
[12] Hamdi, S.M.M., Assadi, M., Zare, Sh., Aghbeigi, F., *Feddes Reportorium*, **2007b**, 117 (6): 501-508.
[13] Hamdi, S.M.M., Assadi, M., *Iran. J. Bot*., **2007c**, 13 (1).
[14] Hamdi, S.M.M., Assadi, M., Maassoumi, A.A., Attar, F., Jouharchi, M.R.,*Ann.Bot. Fennici*, **2008a**, 45: 74-79.
[15] Hamdi, S.M.M., Assadi, M., Maassoumi, A.A., *Iran. J .Bot*, **2008b**, 14 (1).
[16] Hamdi, S.M.M., Assadi, M., Maassoumi, A.A.,*Botanical Journal of the Linnean Society*, **2008c**, 158, 734–742.
[17] Hamdi, S.M.M., Assadi, M., Maassoumi, A.A., *Kew bulletin*, **2009**, 64: 339–344.
[18] Holmgren, P.K., Holmgren, N.H., (continuously updated): Index herbariorum. Visited Available at http:// sciweb.nybg.org/science2/IndexHerbarium.asp, **2006**.
[19] Hong, D.Y., *Annals of the Missouri Botanical Garden*, **1983**, 70: 701–712.
[20] Linnaeus, C., Species plantarum, Impensis Laurentii Salvii, Stockholm ,**1753**, Vol 2.
[21] Miller, P., J Rivington and J Rivington London 4th ed ,**1754**, Vol 2.
[22] Morison, R., Plantarum historiae universalis Oxoniensis, E Theatro Sheldoniano Oxford, **1680**, Vol 2.
[23] Parsa. A., Scrophulariaceae Flora de lIran, Tehran, **1949**, 4:320-460.
[24] Posada, D., *Mol Biol Evol*, **2008**, 25:1253–1256.
[25] Ronquist, F., Huelsenbeck, J.P., *Bioinformatics*, **2003**, 19: 1572–1574.
[26] Sa´ez, L., Bernal, M., Linaria Mill. in S Castroviejo, A., Herrero, C., Benedı´, E., Rico, J., Gu¨ emes, eds., *Flora iberica*, **2009**, 13:232–324 CSIC Madrid.
[27] Sutton, D.A., *Bot.J .Linn .Soc*, **1980**, 81:169–184
[28] Sutton, D.A., A revision of the tribe Antirrhineae. Oxford University Press, Oxford, 1988.
[29] Tournefort, J.P., Institutiones rei herbariae E Typographia Regia Paris, **1700**.
[30] Valde´s, B., Revisio´n de las especies europeas de Linaria con semillas aladas, An Univ Hipalense, **1970,** 7:1–288.
[31] Vargas, P., Rossello´, J.A., Oyama, R., Gu¨emes, J., *Syst Evol*, **2004**, 249:151–172.
[32] Viano, J., *Versicolores. Candollea*, **1978a**, 33:33–88.
[33] Viano, J., Les linaires a` graines apte`res du bassin me´diterrane´en occidental. 2. Linaria sect. Elegantes, Bipunctatae, Diffusae, Speciosae, Repentes Candollea, **1978b**, 33:209–267.