

Molecular Phylogenetic Analyses of *Vicia* L. (Fabaceae) Taxa Growing in the Southeastern Anatolia Region Based on Chloroplast *TrnL* Sequences

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Abstract

In the current study, some natural *Vicia* L. taxa growing naturally in the Southeast Anatolia Region were investigated from the point of view of molecular phylogenetic. For this purpose, transfer ribonucleic acid Leucine (*trnL*) was sequenced in order to study the phylogenetic relationships of the *Vicia* L. species. *Lathyrus inconspicuus* L. var. *inconspicuus* and *Lathyrus cassius* Boiss. were used as an outgroup. The length of the *trnL* area was determined approximately as 269-534 bp. The *trnL* sequences were submitted to the NCBI database and accession numbers received. We obtained information about the point mutations, as well as the protected and the changing characters. The taxa distinguished in the section level were separated and also grouped in terms of proximity on the phylogenetic tree (sect. Narbonensis, sect. Lathyroides, sect. Peregrinae, sect. *Vicia*, sect. Lentopsis). While, sect. Hypechusa, sect. Ervilia and sect. Cracca were not detailed to a great level of informativity. The data acquired were observed to be greatly reliable in terms of their ability to be used in solving taxonomical problems of the *Vicia* L. taxa.

Key words: cpDNA, Fabaceae, molecular phylogeny, sequencing, *Vicia* L.

1. Introduction

The legume family includes 720-730 genera and about 19.500 species. There are 69 genera in Turkey containing 1128 taxa and including 375 endemic taxa (Simpson, 2012). The genus *Vicia* is a member of the Viciae legume tribe of Papilionoideae, and the genus itself has proven to be a popular group to study (Frediani et al., 2004). *Vicia* L. comprises about 210 species, is considered to have 22 divisions in two subgenus (*Vicia* and *Vicilla*), and is widely distributed throughout Europe and Asia (~110 species) North America (~ 20 species) and Africa (~15 species) (Kupicha, 1976; Weber and Schifino-Wittmann, 1999; Roze and Rurane, 2013). The genus

was originally localized to the Mediterranean and Irano-Turanian regions (Kupicha, 1981). The Mediterranean region is the main diversity center (Gunn, 1980; Naranjo et al., 1998). In Turkey, 64 species, 22 subspecies and 18 varieties of this genus have been recorded (Davis, 1970; Vural, 2000; Inceer and Ayaz, 2005; Başbağ et al., 2013).

Vicia L. species are morphologically diverse and the morphological approach find it quite difficult to account for all the genetic variation that exist. Previous papers have reported morphological, anatomical, cytological, karyological and biochemical and molecular (although this has not been sufficient) data in order to elucidate the relationships within the *Vicia* genus (Frediani et al., 1992; Cremonini et al., 1998 a, b; Maggini et al., 1991; Venora et al., 2000; Tabur et al., 2002; Navratilova et al., 2003; Bryant and Hughes, 2011; Kahraman et al., 2013). Recently, plant organelle genomes (chloroplast and mitochondrial DNA) have been widely used for population genetics studies and in order to reveal phylogenetic relationships (Soltis et al., 1992). Non-coding regions in the chloroplast genome are often preferred for molecular systematic and plant-related population genetic studies (Shaw et al., 2006). DNA regions that are not coded show a high degree of mutation. For this reason, they are widely used to show evolutionary relationships (Taberlet et al., 1991). One of the non-coding regions in the chloroplast DNA is tRNA and this is used in determining evolutionary relationships. The most frequently used of these regions are *trnT* (UGU) - *trnL* (UAA) IGS, *trnL5'*-*trnL3'* intron and *trnL* (UAA) - *trnF* (GAA) IGS regions (Taberlet et al., 1991; Kelchner, 2000). This study aimed to use transfer ribonucleic acid Leucine (*trnL*) as the differential region to examine the relationships within the *Vicia* genus growing in the Southeast Anatolian Region.

2. Materials and Methods

2.1. Plant material

Plant material was collected from different localities of the study area from April to May, which is the vegetation periods of plants from natural habitats and identified according to the diagnostic morphological characteristics described in the Flora of Turkey and the East Aegean Islands (Davis, 1970). The study area is located in the Southeastern Anatolia Region. (Adiyaman, Batman, Diyarbakır, Gaziantep, Mardin, Siirt, Şanlıurfa) covers the area. Species were included in the eight different sections; *Cracca* S. F. Gray, *Ervilia* (DC) Link, *Lentopsis* Kupicha, *Peregrinae* Kupicha, *Lathyroides* (Buchenau) Tzvel, *Hypechusa* (Alef.) Aschers and Graebner, *Vicia* L. and *Narbonensis* (Radzhi) Maxted. To construct the phylogenetic relationships of the *Vicia* genus, we analyzed sequences of *trnL* regions from a total of 22 specimens and 25 taxa. *Lathyrus cassius* Boiss. and *Lathyrus inconspicuous* L. were used as out-group. DNA sequences of the studied taxa were entered into the NCBI database and an accession number was obtained for each species. Plant deposited under suitable conditions at the Herbarium of the Department of Biology (DUF), Faculty of Science and Art, Dicle University.

Table 1. The taxa of *Vicia* L. studied and their collecting sites, section, accession number and voucher name and herbarium number.

| No | Taxon names | Section | Location | Accession number | Voucher name and herbarium number |
|----|---|--------------|--|------------------|-----------------------------------|
| 1 | <i>V. narbonensis</i> L. var. <i>narbonensis</i> | Narbonensis | Batman, Batman University, central campus, 570 m. | MN862284 | A.Kaplan DUF 90 |
| 2 | <i>V. hybrida</i> L. | Hypechusa | Batman, Batman University, central campus, 570 m. | MN862285 | A.Kaplan DUF 88 |
| 3 | <i>V. villosa</i> Roth subsp. <i>varia</i> (Host) Corb. | Cracca | Adiyaman, Bozova to Adiyaman, 18.5 km, 589 m. | MN862286 | A.Kaplan DUF 48 |
| 4 | <i>V. mollis</i> Boiss. & Hausskn. | Hypechusa | Adiyaman, Hilvan to Bozova, Külünçe village, 5 km, 600 m | MN862287 | A.Kaplan DUF 40 |
| 5 | <i>V. galeata</i> Boiss. | Hypechusa | Adiyaman, Hilvan to Bozova, Külünçe village, 5 km, 600 m. | MN862288 | A.Kaplan DUF 44 |
| 6 | <i>V. sativa</i> L. subsp. <i>sativa</i> | Vicia | Diyarbakir, Dicle University campus, Bakbako stream around, 630-640 m. | MN862289 | A.Kaplan DUF 82 |
| 7 | <i>V. sativa</i> L. subsp. <i>nigra</i> L. (Ehrh.) var. <i>nigra</i> L. (Ehrh.) | Vicia | Diyarbakir, Çermik to Çüngüş, 6-7 km, 703 m. | MN862290 | A.Kaplan & A.S.Ertekin DUF 35 |
| 8 | <i>V. michauxii</i> Sprengel. var. <i>stenophylla</i> Boiss. | Peregrinae | Şanlıurfa, Şanlıurfa to Suruç, 18km, 666 m. | MN862291 | A.Kaplan DUF 08 |
| 9 | <i>V. monantha</i> Retz subsp. <i>monantha</i> Retz. | Cracca | Şanlıurfa, Akçakale, 382 m. | MN862292 | A.Kaplan DUF 09 |
| 10 | <i>V. anatolica</i> Turrii. | Hypechusa | Mardin, Exit of Mardin 2 km, 1095m. | MN862293 | A.Kaplan DUF 12 |
| 11 | <i>V. peregrina</i> L. | Peregrinae | Siirt, Çatılı village, 1475 m. | MN862294 | A.Kaplan DUF 73 |
| 12 | <i>V. noeana</i> Reuter ex Boiss var. <i>noeana</i> Reuter ex Boiss. | Hypechusa | Diyarbakir, Ergani to Çermik, 13 km, 870-875 m. | MN862295 | A.Kaplan & A.S.Ertekin DUF 16 |
| 13 | <i>V. sericocarpa</i> Fenzl. var. <i>sericocarpa</i> Fenzl. | Hypechusa | Diyarbakir, Ergani to Çermik 13 km, 870-875 m. | MN862296 | A.Kaplan & A.S.Ertekin DUF 18 |
| 14 | <i>V. lathyroides</i> L. | Lathyroides | Diyarbakir, Çermik to Çüngüş, 11.5-12 km, 905 m. | MN862297 | A.Kaplan & A.S.Ertekin DUF 39 |
| 15 | <i>V. ervilia</i> (L.) Willd. | Ervilia | Diyarbakir, Ergani to Çermik, 16-17 km, 880m | MN862298 | A.Kaplan & A.S.Ertekin DUF 21 |
| 16 | <i>V. galilaea</i> Plitm. Et. Zoh. | Narbonensis | Diyarbakir, Çermik to Siverek, 1 km, 732m | MN862299 | A.Kaplan & A.S.Ertekin DUF 32 |
| 17 | <i>V. cracca</i> L. subsp. <i>stenophylla</i> Vel. | Cracca | Adiyaman, Ferryboat to Siverek, 4 km, 588m | MN862300 | A.Kaplan DUF 56 |
| 18 | <i>V. palaestina</i> Boiss. | Cracca | Diyarbakir, Çermik to Çüngüş 11.5-12 km, 905 m. | MN862301 | A.Kaplan & A.S.Ertekin DUF 38 |
| 19 | <i>V. lutea</i> var. <i>hirta</i> (Balbis) Lois. | Hypechusa | Diyarbakir, Devegeçidi picnic area, 732 m. | MN862302 | A.Kaplan & A.S.Ertekin DUF 59 |
| 20 | <i>V. esdraelonensis</i> Warb. & Eig | Hypechusa | Diyarbakir, the way of Bilgekişla, 3.5 km, 740 m. | MN862303 | A.Kaplan & A.S.Ertekin DUF 60 |
| 21 | <i>V. villosa</i> Roth subsp. <i>eriocarpa</i> (Hausskn) P.Ball. | Cracca | Adiyaman, Hilvan to Bozova, Külünçe village, 5 km, 600 m. | MN862304 | A.Kaplan DUF 41 |
| 22 | <i>V. sativa</i> L. subsp. <i>amphicarpa</i> (Dorth.) Aschers. & Graebn. | Vicia | Diyarbakir, Siverek Dicle University campus, 630-640 m. | MN862305 | A.Kaplan DUF 80 |
| 23 | <i>V. assyriaca</i> Boiss. | Hypechusa | Şanlıurfa, Siverek Avurtepe village, Otlu avenue, 1080 m. | MN862306 | A.S.Ertekin DUF 79 |
| 24 | <i>V. montbretii</i> Fisch. & C.A.Mey. | Lentopsis | Şanlıurfa, Siverek Avurtepe village, Otlu avenue, 1080 m. | MN862307 | A.S.Ertekin DUF 78 |
| 25 | <i>V. caesarea</i> Boiss. & Bal. | Lentopsis | Siirt, Şirvan to Madenköy, 4-5 km, 1020 m. | MN862308 | A.S.Ertekin DUF 184 |
| 26 | <i>Lathyrus cassius</i> Boiss. | Lathyrus | Diyarbakir, the way of Bilgekişla, 3.5 km, 740 m. | MN862309 | A.S.Ertekin DUF 4241 |
| 27 | <i>Lathyrus inconspicuus</i> L. | Linearcarpus | Gaziantep, Islahiye Bahçe to Gaziantep, 22 km. | MN862310 | A.S.Ertekin DUF 5249 |

2.2. DNA extraction, PCR, sequencing, sequence alignment and phylogenetic analyses

Total genomic DNA were isolated from dried and stored at room temperature leaf tissues using the Lefort et al. (1998) method. The purity and quantity determinations of extracted DNAs were determined by NanoDrop 1000c UV-Vis Spectrophotometer (Thermo Scientific) at A_{260} / A_{280} nm. The isolated DNA samples were checked in 1 % agarose gel electrophoresis (Figure 1). Specific primer pairs were used to amplify (amplification) the targeted region on genomic DNA. Amplification of *trnL* region was carried out by using primer

pairs *trnc* 5' CGA AAT CGG TAG ACG CTA CG 3' (Forward) / *trnd* 5' GGG GAT AGA GGG ACT TGA AC 3' (Reverse) (White et al., 1990).

DNA amplification was performed in a 50 µl volume mixture containing genomic DNA (100 ng/µl), 10X PCR Buffer, MgCl₂ (25 mM), dNTP mixture (25 mM), selected primer pair (50 µM), Taq polymerase (5u/µl) and sterile water (ddH₂O). PCR reaction of each region consisted of almost same amount of Buffer (5 µl), DNA (1 µl), MgCl₂ (1,5 µl), dNTP (1 µl), each primer (forward and reverse) (1,5 µl) and Taq polymerase (0.3 µl). PCR amplification was started with 2 min initial denaturation at 94 °C, and terminated with 5 min at 72 °C. Each reaction ended with a final 4°C hold step and consisted of 35 cycle numbers. Each reaction cycle consisted of denaturation step at 94 °C for 20 sec (1 min), annealing step at 57 °C for 30 sec and elongation step at 72 °C for 30 1 min. Amplicons were visualized by electrophoresis on 2 % agarose gels (Figure 2). After purification, products were sequenced in both directions using Applied 3130 Genetic Analyzer automated sequencer. All sequence chromatograms were opened using DNA dragon software. Ambiguous sites were checked manually and corrected by comparing the strands. Sequences of *trnL* region have been deposited in NCBI database for further studies.

Molecular Evolutionary Genetics Analysis software (MEGA 5.1; [Beta 2]) is used to measure total nucleotide length (base pair, bp) (Table 2). DNA sequences were aligned via the Clustal W program. The sequence data was analyzed by using the UPGMA method (Figure 3) based on the Kimura model (Kimura, 1980) and bootstrap analysis with 1000 replications (Felsenstein, 1985). The bootstrap value ranges from 0 % to 100 %. According to the Kress et al. (2005), bootstrap support values, it was defined as > 85% very strong, 70-85 % strong, 50-70 % weak and <50 % very weak. 70 % or greater bootstrap support is often associated with the identification of the correct phylogeny. If the bootstrap support for a certain branch is below 50 %; It is concluded that the relationship between the species is not fully clarified and the branching model is not determined and as a result, this branch will be determined in the tree as a fork (polytomy = uncertainty point) rather than a single node (Freeman and Herron, 1999).

3. Results

3.1. Observation of amplification products

When PCR was performed on gDNA samples of targeted species, the presence of bands was visualized by 1% agarose gel electrophoresis (Figure 1). And then amplicons (*trnL* region) were visualized by electrophoresis on 2 % agarose gels (Figure 2).

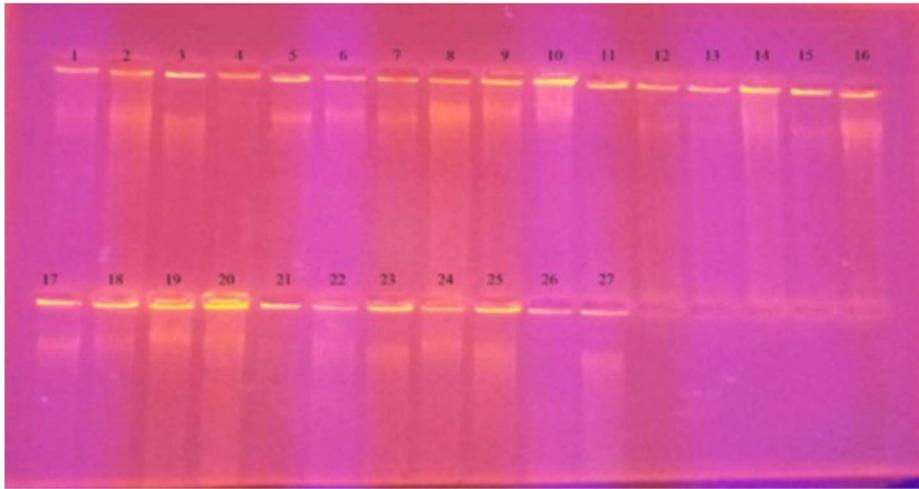


Figure 1. 1 % gel image of *Vicia* species PCR products; 1) *V. narbonensis* var. *narbonensis* 2) *V. hybrida* 3) *V. villosa* subsp. *varia* 4) *V. mollis* 5) *V. galeata* 6) *V. sativa* subsp. *sativa* 7) *V. sativa* subsp. *nigra* var. *nigra* 8) *V. michauxii* var. *stenophylla* 9) *V. monantha* subsp. *monantha*. 10) *V. anatolica* 11) *V. peregrina* 12) *V. noeana* var. *noeana* 13) *V. sericocarpa* var. *sericocarpa* 14) *V. lathyroides* 15) *V. ervilia* 16) *V. galilaea* 17) *V. cracca* subsp. *stenophylla* 18) *V. palaestina* 19) *V. lutea* var. *hirta* 20) *V. esdraelonensis* 21) *V. villosa* subsp. *eriocarpa* 22) *V. sativa* subsp. *amphicarpa* 23) *V. assyriaca* 24) *V. montbretii* 25) *V. caesarea* 26) *Lathyrus cassius* 27) *Lathyrus inconspicuus*.

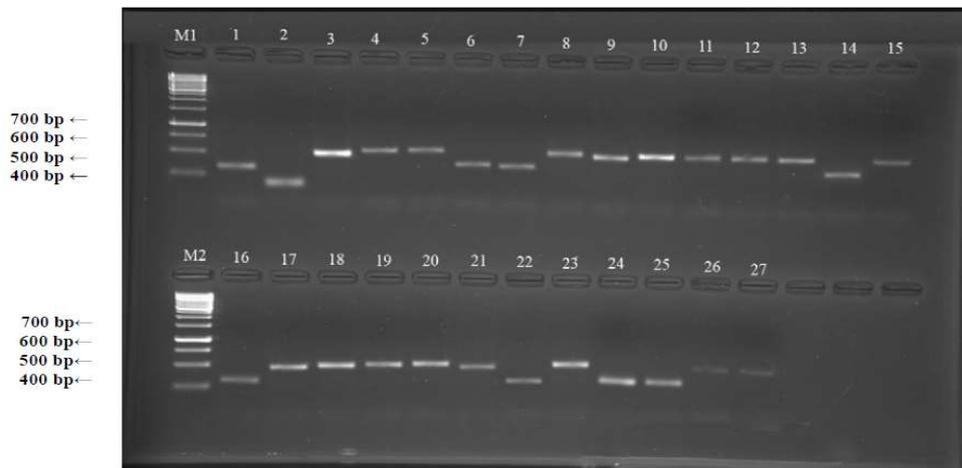


Figure 2. Nuclear DNA of the *Vicia* species *trnL* region image of agarose gel (2%); 1) *V. narbonensis* var. *narbonensis* 2) *V. hybrida* 3) *V. villosa* subsp. *varia* 4) *V. mollis* 5) *V. galeata* 6) *V. sativa* subsp. *sativa* 7) *V. sativa* L. subsp. *nigra* var. *nigra* 8) *V. michauxii* var. *stenophylla* 9) *V. monantha* subsp. *monantha* 10) *V. anatolica* 11) *V. peregrina* 12) *V. noeana* var. *noeana* 13) *V. sericocarpa* var. *sericocarpa* 14) *V. lathyroides* 15) *V. ervilia* 16) *V. galilaea* 17) *V. cracca* subsp. *stenophylla* 18) *V. palaestina* 19) *V. lutea* var. *hirta* 20) *V. esdraelonensis* 21) *V. villosa* subsp. *eriocarpa* 22) *V. sativa* subsp. *amphicarpa* 23) *V. assyriaca* 24) *V. montbretii* 25) *V. caesarea* 26) *Lathyrus cassius* 27) *Lathyrus inconspicuus*.

3.2. Nucleotide sequences of the *trnL* region

The base ratios obtained from approximately 408 bp of which *trnL* gene were analyzed in the series are given in Table 1. When Table 2 is examined *trnL* region sizes varied from 269 bp (*Vicia narbonensis* var. *narbonensis* and *Vicia caesarea*) to 534 bp (*Vicia esdraelonensis*). It appears to be A: 27,3 %, G: 16,4 %, C: 17,2 %, T (U):39,1 %. DNA sequences related to the samples are G - C ratio (33,6 %), A - T ratio (66,4 %). The DNA sequences of the samples are relatively rich in A - T, the G - C ratio is less.

Table 2. *trnL* region sizes in *Vicia* L. and *Lathyrus* L. (outgroup) taxa.

| Species name | T(U) | C | A | G | Total |
|---|------|------|------|------|-------|
| <i>Vicia narbonensis</i> var. <i>narbonensis</i> | 36,1 | 19,0 | 27,9 | 17,1 | 269,0 |
| <i>Vicia hybrida</i> | 40,3 | 16,7 | 26,7 | 16,3 | 461,0 |
| <i>Vicia villosa</i> subsp. <i>varia</i> | 38,8 | 17,4 | 29,1 | 14,7 | 443,0 |
| <i>Vicia mollis</i> | 41,0 | 15,0 | 27,9 | 16,2 | 427,0 |
| <i>Vicia galeata</i> | 41,1 | 14,7 | 28,7 | 15,4 | 428,0 |
| <i>Vicia sativa</i> subsp. <i>sativa</i> | 36,3 | 19,0 | 25,9 | 18,7 | 347,0 |
| <i>Vicia sativa</i> subsp. <i>nigra</i> var. <i>nigra</i> | 38,4 | 19,5 | 25,3 | 16,8 | 292,0 |
| <i>Vicia michauxii</i> var. <i>stenophylla</i> | 39,7 | 17,4 | 27,8 | 15,1 | 478,0 |
| <i>Vicia monantha</i> subsp. <i>monantha</i> | 38,7 | 17,2 | 28,8 | 15,3 | 431,0 |
| <i>Vicia anatolica</i> | 40,6 | 17,0 | 27,5 | 15,0 | 488,0 |
| <i>Vicia peregrina</i> | 41,0 | 15,7 | 28,1 | 15,2 | 402,0 |
| <i>Vicia noeana</i> var. <i>noeana</i> | 41,4 | 16,1 | 26,9 | 15,6 | 435,0 |
| <i>Vicia sericocarpa</i> var. <i>sericocarpa</i> | 41,0 | 15,4 | 28,2 | 15,4 | 415,0 |
| <i>Vicia lathyroides</i> | 36,0 | 18,9 | 26,3 | 18,9 | 350,0 |
| <i>Vicia ervilia</i> | 40,3 | 16,6 | 27,1 | 15,9 | 409,0 |
| <i>Vicia galilaea</i> | 34,9 | 19,0 | 27,5 | 18,7 | 327,0 |
| <i>Vicia cracca</i> subsp. <i>stenophylla</i> | 38,7 | 16,7 | 28,6 | 16,0 | 426,0 |
| <i>Vicia palaestina</i> | 39,2 | 16,2 | 28,4 | 16,2 | 444,0 |
| <i>Vicia lutea</i> var. <i>hirta</i> | 39,8 | 16,7 | 27,8 | 15,7 | 497,0 |
| <i>Vicia esdraelonensis</i> | 39,1 | 16,7 | 27,3 | 16,9 | 534,0 |
| <i>Vicia villosa</i> subsp. <i>eriocarpa</i> | 37,6 | 17,6 | 28,6 | 16,3 | 490,0 |
| <i>Vicia sativa</i> subsp. <i>amphicarpa</i> | 38,0 | 19,2 | 25,7 | 17,0 | 276,0 |
| <i>Vicia assyriaca</i> | 41,2 | 15,7 | 26,9 | 16,2 | 413,0 |
| <i>Vicia montbretii</i> | 35,7 | 20,9 | 24,3 | 19,1 | 325,0 |
| <i>Vicia caesarea</i> | 38,3 | 20,8 | 23,0 | 17,8 | 269,0 |
| <i>Lathyrus cassius</i> | 38,0 | 17,4 | 26,8 | 17,8 | 511,0 |
| <i>Lathyrus inconspicuus</i> | 40,0 | 16,3 | 26,3 | 17,4 | 448,0 |
| Avg. | 39,1 | 17,2 | 27,3 | 16,4 | 408,7 |

3.3. Molecular phylogeny

Phylogenetic associations and genetic diversity data were made with Mega (Molecular Evolutionary Genetics Analysis) 5. 1 Beta 2 software using sequence analysis results. Unweighted pair group method with arithmetic mean (UPGMA) method was used during the drawing of the phylogenetic tree (Figure 3). In order to increase the reliability of the obtained tree, bootstrap analysis with 1000 replications was preferred. The percentage of each branch shows how much that branch is supported. If the branch's reliability rate is below 50 %, the program qualifies this branch as very weak and collapses that branch, and the empty branch resulting from the collapsed branch is called a polytomy. Since the branching model cannot be determined in collapsed branches, no interpretation can be made about the relationship between taxa. It can be said that only this branch has created a polytomy.

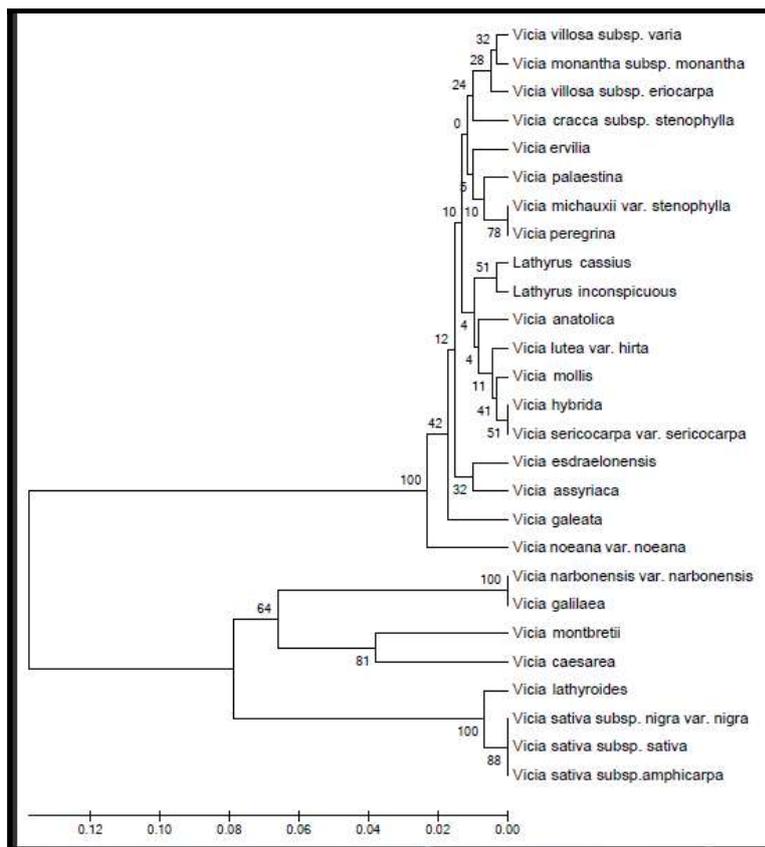


Figure 3. Phylogenetic tree of *Vicia* species based on (UPGMA) analysis of the *trnL* region. *Lathyrus cassius* and *Lathyrus inconspicuus* were used as a outgroup. Bootstrap analysis of UPGMA was based on 1000 replicates. Percentage of each branch shows the support rate of that branch (Bootstrap value).

4. Discussion

DNA sequencing technologies and recent advances in molecular biology enable us to characterize the genomes of organisms and many ongoing genome projects for various species are providing valuable insight into their biology and uses. In this context, many molecular studies of the *Vicia* genus have been carried out. Different coding and non-coding regions from both nuclear and chloroplast DNAs have been studied. For instance, phylogenetic relationships between *Vicia faba* (Fabaceae) and related species *trnL* sequence (Fennel et al., 1998), nuclear ribosomal DNA internal transcribed spacer (*ITS*) (Foladi et al., 2013) and *matK* (Steele and Wojciechowski, 2003) are, some of the molecular systems which have been extensively used for molecular systematic studies.

In this study, we chose to use the transfer ribonucleic acid Leucine (*trnL*) region of nuclear DNA. The reasons for using chloroplast in plant phylogenetic studies are as follows: the structural stability of cpDNA, that it is haploid (n), that it generally has a uniparental transfer, that it is non-recombinant (Small et al., 2004). In the unweighted pair group method with arithmetic mean (UPGMA) tree obtained in the present study (Figure 3), it was observed that *Lathyrus* species used as an outgroup were nested within other species and this branch was supported with a bootstrap value of 51 %. The ingroup consists of two main clades. Using the data from Figure 3, the branch with *V. narbonensis* var. *narbonensis* and *V. galilaea* (sect. *Narbonensis*) species was supported with a bootstrap value of 100 %. Molecular data is based on sequences of the *trnL* with high bootstrap values. In many

studies (Enneking and Maxted, 1995; Fennel et al., 1998; Potokina, 1999; Schaefer et al., 2012), the species belonging to sect. *narbonensis* have been studied in the same branch. These results are in line with those of the current study. Schaefer et al. (2012), reported that the section was monophyletic. Our study supports the assertion that section is monophyletic. The species belonging to sect. *Lentopsis* (*V. montbretii*, *V. caesarea*) were supported with a bootstrap value of 81 %. Schaefer et al. (2012), in their study, sect. *lentopsis* examined the species in the tree of maximum likelihood in the same branch. Binzaf (2012), Foladi, et al. (2003), Schaefer et al. (2012) also studied the molecular phylogeny of *Vicia* species in parallel with our work. The branch where *V. sativa* subsp. *sativa*, *V. sativa* subsp. *nigra* var. *nigra*, *V. sativa* subsp. *amphicarpa* species are found is supported a bootstrap value with 88 % and this value shows us that we have achieved a meaningful result. Molecular data based on sequences of the *trnL* with high bootstrap values, support the placement of these species in section *V. lathyroides*, which was connected to this branch with a high bootstrap value, for example a 100 %. Van de Wouf et al. (2001), found that, *V. sativa* subsp. *amphicarpa* and *V. sativa* subsp. *nigra* var. *nigra* were located close to each other, while *V. sativa* subsp. *sativa* was located farther away in the UPGMA phylogenetic tree they obtained. Potokina, (1999) reported that the *V. sativa* complex coexists in the dendograms they obtained in their study. Leht, (2009), Potokina, (1999) and Schaefer et al. (2012) reported that this section is not monophyletic but polyphyletic. However, Potokina et al. (1999), examined *V. lathyroides* species as a separate branch in their study and supported the branch where this species was located with a value of 53 %. They also found it close to the *Vicia* section in all the trees they created. Jaaska, (2008) and Leht, (2009) reported that they found the species to be close to the *Vicia* section. Although the bootstrap value of the *V. lathyroides* species is low, it has established a close connection with the sect. *Vicia* as in the studies mentioned above. Schaefer et al. (2012) determined that the section was not monophyletic. The results we obtained in our study support the current studies.

The branch with the highest bootstrap value in the group was the branch with *V. michauxii* var. *stenophylla* and *V. peregrina* species, which was supported with a bootstrap value of 78 %. Molecular data based on sequences of the *trnL* with high bootstrap values, supports the placement of these species in this section. In many studies (Fennel et al., 1998; Potokina et al., 1999; Leht, 2009; Emre, 2011; Schaefer et al., 2012), these species have been found in the same branch and examined in the *Peregrinae* section. Schaefer et al. (2012) in their study, they found that the section was monophyletic. This result is in line with our study.

As is well known, unrooted trees are obtained with bootstrap method. The values on the branches of the trees are used to determine the most statistically reliable branches. The percentage ratio of each branch indicates how much that branch is supported. If the reliability rate of the branch is below 50 %, the program considers this branch very weak and collapses that branch, and the empty branch resulting from the collapsed branch is called polytomy. Since the branching model in collapsed branches cannot be determined, no comment can be made on the relationship between taxa. It can only be said that this branch has created a polytomy. In the phylogenetic tree that we have obtained, *V. villosa* subsp. *varia*, *V. villosa* subsp. *eriocarpa*, *V. cracca* subsp. *stenophylla*, *V. monantha* subsp. *monantha*, *V. palaestina* (sect. *Cracca*), *V. ervilia* (sect. *Ervilia*) *V. lutea* var. *hirta*, *V. galeata*, *V. assyriaca*, *V. esdraelonensis*, *V. noeana* var. *noeana*, *V. hybrida*, *V. mollis*, *V. anatolica*, *V. sericocarpa* var.

sericocarpa (sect. *Hypechusa*) were species branches where the species were found to have collapsed. Polytoomy was observed since the reliability value was below 50 %. Therefore, the parser did not provide any information. In the current study, these taxa were found to be problematic, and showed a random distribution in phylogenetic trees, and were thus supported by a low bootstrap value. The tree that we created was not successful in separating these species. This may be because the taxa are actually differentiated from a single ancestral species or because of conflicting or missing data. The occurrence of polytomies in phylogenetic analysis may indicate an interesting evolutionary history that needs further research. In addition, molecular characters are more objective than morphological characters, but they are not accurate. Polymorphisms and uncertainties may arise in determining the basis for DNA sequence data. It may not be easily understood, especially if the sequences between taxa are very different when making sequence alignments. Also, the possibilities of paralogy arising from ancestral gene duplication or hybridization can cause confusion when comparing homologous sequences (Simpson, 2012). The genetic diversity of the species is already due to the variation in genes and their recombination (Lorenzetti, 1994, Lorenzetti, 1996). In the current study, such mutations occurring in species were indicative of genetic diversity and such differences manifested themselves in the dendrogram we obtained. In the future, we propose that only studies on these taxa (various molecular marker systems such as biochemical, AFLP, RFLP, ISSR, SSR) should be conducted in detail. We also consider that such studies could study the *MatK* region in the chloroplast genome. It should not be forgotten that each method often leads to a different result. Expecting a specific and accurate result (a tree) is not appropriate anyway; there are no perfect trees, but some are useful.

This study attempted to examine *Vicia* L. species morphologically and molecularly in a flexible way and to eliminate the deficiencies in the literature. It is our duty to carefully conserve the gene resources of the many *Vicia* L. species, which are almost as old as human history are and which are extensively cultivated, and pass them on to future generations. We hope that the economic integration studies found at the end of the inventory and the documentation studies conducted in basic sciences worldwide will now be carried out more effectively in Turkey and that the information presented will serve this purpose.

Conflicts of Interests

Authors declare that there is no conflict of interests

References

- Başbağ, M., Hoşgören, H., & Aydın, A. (2013). *Vicia* Taxa in the Flora of Turkey. *Anadolu Tarım Bilimleri Dergisi*, 28(1), 59-66.
- Binzat, O. K. (2012). Revision of *Vicia* L. (Leguminosae) in Central Anatolia, Turkey. Ph.D. Thesis, Middle East Technical University, Institute of Natural and Applied Sciences, September, Ankara.
- Bryant, J. A., & Hughes, S. G. (2011). *Vicia*, Chapter 14, C. Kole (Ed.). *Wild Crop Relatives: Genomic and Breeding Resources, Legume Crops and Forages*, Heidelberg: Springer Verlag.

- Cremonini, R., Miotto, D., Api Ngu, M., Tota, D., Pignone, D., Blangiforti, S., & Venora, G. (1998a). Cytology of *Vicia* species 5: nuclear chromatin structure, karyomorphological analysis and DNA content in newly discovered relatives of *Vicia faba* L.: *Vicia kalakhensis* Khattab, Maxted & Bisby and *Vicia eristalioides* Maxted. *Cytologia*, 63, 371-379.
- Cremonini, R., Ruffini, Castiglione, M., Venora, G., Blangiforti, S., Lo Savio, F. P., & Pignone, D. (1998b). Cytology of *Vicia* species 6: nuclear chromatin organization, karyomorphological analysis and DNA amount in *Vicia serratifolia* Jacq. *Caryologia*, 51, 195-205.
- Davis, P. H. (1970). *Vicia* L. *Flora of Turkey and East Aegean Islands* (Vol 3). Edinburgh, U.K.: Edinburgh University Press.
- Emre, İ. (2011). Determination of Genetic Diversity in the *Vicia* L. (section *Vicia*) by Using SDS-PAGE. *Pakistan Journal of Botany*, 43(3), 1429-1432.
- Enneking, D., & Maxted, N. (1995). *Narbon bean (Vicia narbonensis* L.). *Evolution of Crop Plants*, 2. Ed. Smartt, J., Simmonds, N. W., London: Longman, 316-321.
- Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution*, 39, 783-791.
- Fennel, S. R., Powell, W., Wright, F., Ramsay, G., & Waugh, R. (1998). Phylogenetic relationships between *Vicia faba* (Fabaceae) and related species inferred from chloroplast *trnL* sequences. *Plant Systematics and Evolution*, 212, 247-259.
- Frediani, M., Sassoli, O., & Cremonini, R. (1992). Nuclear DNA characterization of two species of *Vicia*: *V. bithynica* L. and *V. narbonensis* L. *Biologia Plantarum*, 34, 335-344.
- Frediani, M., Maggini, F., Gelati, M. T., & Cremonini, R. (2004). Repetitive DNA sequences as probes for phylogenetic analysis in *Vicia* genus. *Caryologia*, 57(4), 379-386.
- Freeman, S., & Herron, J. C. (1999). *Evrimsel Analiz*. Çıplak, B., Başıbüyük. H. H., Karaytuğ, S., & Gündüz, İ. (Eds.). Palme Publishing, 28-29, 438-449.
- Foladi, F. Z., Salimpour, F., Sharifnia, F., & Ghanavati, F. (2013). Phylogenetic study of tribe Vicieae based on Internal Transcribed Spacer (ITS). *Annals of Biological Research*, 4(1), 75-79.
- Gunn, C. R. (1980). *Genus Vicia with notes about Tribe Vicieae (Fabaceae) in Mexico and Central America*. United States Department of Agriculture, Printing Office Washington D.C. 20402, 1-48.
- Inceer, H., & Hayırlıoğlu-Ayaz, S. (2005). Giemsa C-banded karyotypes of *Vicia cracca* L. subsp. *cracca* and *V. bithynica* L. *Turkish Journal of Botany*, 29, 311-316.
- Jaaska, V. (2008). Isozymes Evidence on the Specific Distinctness and Phylogenetic Position of *Vicia incisa* (Fabaceae). *Central European Journal of Biology*, 3(2), 169-176.
- Kahraman, A., Binzat, O. K., & Doğan, M. (2013). Pollen morphology of some taxa of *Vicia* L. subgenus *Vicia* (Fabaceae) Turkey. *Plant Systematics and Evolution*, 299, 1749-1760.
- Kelchner, S. A. (2000). *The evolution of non-coding chloroplast DNA and its application in plant systematics*. *Annals of the Missouri Botanical Garden*, 87, 482-498.

- Kimura, M. A. (1980). Simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution*, 16, 111-120.
- Kress, W. J., Liu, A.-Z., Newman, M., & Li, Q. J. (2005). The molecular phylogeny of *Alpinia* (Zingiberaceae): a complex and polyphyletic genus of gingers. *American Journal of Botany*, 92(1), 167-178.
- Kupicha, F. K. (1976). The infrageneric structure of *Vicia* L. *Notes Roy Bot Garden Edinburgh*, 34, 287-326.
- Kupicha, F. K. (1981). *Vicieae*. In: Polhill, R. M. & Raven, P. H. (Eds). *Advances in Legume Systematics, part 1*. London: Ministry of Agriculture, Fisheries and Food, pp. 377-381.
- Lefort, F., Lally, M., Thompson, D., & Douglas, G. C. (1998). Morphological traits, microsatellite fingerprinting and genetic relatedness of a stand of elite oaks (*Q. robur* L.) at Trullynally, Ireland. *Silvae Genetica*, 47, 257-262.
- Leht, M. (2009). Phylogenetics of *Vicia* (Fabaceae) based on morphological data. *Feddes Repertorium*, 120(7-8), 379-393.
- Lorenzetti, F., Falcinelli, M., & Veronesi, F. (1994). *Miglioramento Genetico delle Piante Agrarie*. Italy: Edagricole, p. 328.
- Lorenzetti, F., Salvatore, C., & Veronesi, F. (1996). *Genetica Agraria*. Italy: Patron, 456.
- Maggini, F., Cremonini, R., Zolfino, C., Tucci, G. F., D'Ovidio, R., Delre, V., DePace, C., Scarascia Mugnozza, G. T., & Cionini, P. G. (1991). Structure and chromosomal localization of DNA sequences related to ribosomal subrepeats in *Vicia faba*. *Chromosoma*, 100, 229-234.
- Naranjo, C. A., Ferrari, M. R., Palermos, A. M., & Poggio, L. (1998). Karyotype, DNA content and meiotic behaviour in five South American species of *Vicia* (Fabaceae). *Annals of Botany*, 82, 757-764.
- Navratilova, A., Neumann, P., & Macas, J. (2003). Karyotype Analysis of Four *Vicia* species using *in situ* Hybridization with Repetitive Sequences. *Annals of Botany*, 91, 921- 926.
- Potokina, E. K., Tomooka, N., Vaughan, D. A., Alexandrova, T., & Xu, R. Q. (1999). Phylogeny of *Vicia* (Fabaceae) based on analysis of RAPDs and RFLP of PCR amplified chloroplast genes. *Genetic Resources and Crop Evolution*, 46, 149-161.
- Roze, I., & Rurane, I. (2013). Revision of genus *Vicia* L. in the flora of Latvia, *Acta Biologica Universitatis Daugavpiliensis*, 13(1), 85-93.
- Schaefer, H., Hechenleitner, P., Santos-Guerra, A., Menezes de Sequeira, M., Pennington, T. R., Kenicer, G., & Carine, A. M. (2012). Systematic, biogeography, and character evolution of the legume tribe Fabeae with special focus on the middle-Atlantic island lineages. *BioMed Central Evolutionary Biology*, 12, 250.
- Shaw, J., Lickey, E. B., Schilling, E. E., & Small, R. L. (2006). Comparison of whole chloroplast genome sequences to choose noncoding regions for phylogenetic studies in angiosperms: the tortoise and the hare III. *American Journal of Botany*, 94(3), 275-288.
- Simpson, M. G. 2012. *Plant Systematics* (2. Ed.), Aytaç, Z., & Kaptaner İğci, B. (Eds.), Ankara: Nobel Publishing.
- Small, R. L., Cronn, R. C., & Wendell, J. F. (2004). Use of nuclear genes for phylogeny reconstruction in plants. *Australian Systematic Botany*, 17, 145-170.

- Soltis, D. E., Soltis, P. S., & Milligan, B. G. (1992). *Intra specific chloroplast DNA variation: systematics and phylogenetic implications*. In: Soltis, P. S., & Soltis, D. E. (Eds.) New York: Molecular plant systematics, Chapman and Hall, pp. 117-150.
- Steele, K. P., & Wojciechowski, M. F. (2003). *Phylogenetic analyses of tribes Trifolieae, Viciae, based on sequences of the plastid gene (matK) (Papilionoidae: Leguminosae)*. In: Klitgaard, B. B., & Bruneau, A. (Eds). *Advance in Legume Systematic, part 10, Higher Level Systematics*, Royal Botanic Garden, Kew.
- Taberlet, P., Gielly, L., Pantaou, G., & Bouvet, J. (1991). Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Molecular Biology*, 17, 1105-1109.
- Tabur, S., Civelek, Ş., & Bağcı, E. (2002). Cytotaxonomic studies on some *Vicia* L. Species Growing in the Eastern Mediterranean and Southern Aegean Regions II. *Acta Botanica Hungarica*, 44(1-2), 185-204.
- Van de Wouw, M., Maxted, N., Chabane, K., & Ford-Lloyd, B. V. (2001). Molecular taxonomy of *Vicia* ser. *Vicia* based on amplified fragment length polymorphisms. *Plant Systematics and Evolution*, 229, 91-105.
- Venora, G., Blangiforti, S., Frediani, M., Maggini, F., Gelati, M. T., Ruffini, C. M., & Cremonini, R. (2000). Nuclear DNA contents, rDNAs, chromatin organization and karyotype evolution in Faba section of the *Vicia* genus. *Protoplasma*, 213, 118-125.
- Vural, M. (2000). *Vicia* L. In: Güner, A., Özhatay, N, Ekim, T., & Başer, K. H. C. (Eds.). *Flora of Turkey and the East Aegean Islands* (Vol. 11), 89-92, Edinburgh: Edinburgh University Press.
- Weber, L. H., & Schifino-Wittmann, M. T. (1999). The *Vicia sativa* L. *aggregate* (Fabaceae) in Southern Brazil. *Genetic Research and Crop Evolution*, 46, 207-211.
- White, T. J., Burns, T., Lee, S., & Taylor, J. (1990). *Amplification and direct sequencing of fungal RNA genes for phylogenetics. PCR Protocols: A Guide to Methods and Application*, Innis, M., Gelfand, D., Sninsky, J., & White, T. (Eds.). San Diego: Academic Press, 315-322.