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# The Evaluation of psbA-trnH IGS Sequences in The Genus *Potentilla* L. as Barcoding Region

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## ABSTRACT

With more than 400 species, the genus *Potentilla* has a wide distribution causing morphological variations. Besides the wide distribution, many conditions such as hybridization, introgression, autopolyploidy, and allopolyploidy are observed in the genus. These can lead to misidentifications and taxonomic problems. DNA barcoding studies are important for species identification and solving taxonomic problems. However, preferred DNA sequences may have different effects among plant groups. Therefore, which DNA regions should be preferred is important to obtain more comprehensive results. In this research, psbA-trnH IGS sequences belonging to the *Potentilla* taxa were examined based on their compatibility and analyzed variable sites, passim-info sites, and nucleotide frequencies (%) for the region relevant by using Molecular Evolutionary Genetics Analysis (MEGA 11). Finally, a Maximum Parsimony (MP) dendrogram was performed to evaluate the *Potentilla* taxa taxonomically and phylogenetically. As a result of this study, it can be stated that the taxa belonging to the genus *Potentilla* were well grouped in the dendrogram and the use of psbA-trnH IGS sequences is strongly recommended for further studies.

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# Barkodlama Bölgesi Olarak *Potentilla* L. Cinsinde psbA-trnH IGS Sekanslarının Değerlendirmesi

## ÖZET

*Potentilla* cinsi 400'den fazla türle, morfolojik değişimlere neden olan geniş bir yayılışa sahiptir. Geniş yayılışın yanı sıra, cinsteki hibridizasyon, introgression, otopoliploidi ve allopoliploidi gibi birçok durum gözlemlenir. Bunlar, yanlış tanımlamalara ve taksonomik problemlere yol açabilir. DNA barkodlama çalışmaları, tür tanımlama ve taksonomik problemlerin çözümünde önemlidir. Ancak, tercih edilen DNA sekansları, bitki grupları arasında farklı etkilere sahip olabilir. Bu nedenle, hangi DNA bölgelerinin tercih edilmesi gerektiği daha kapsamlı sonuçlar elde etmek için önemlidir. Bu çalışmada, *Potentilla* taksonlarına ait psbA-trnH IGS sekansları uyumlulukları açısından incelendi ve variable bölgeler, parsim-info bölgeler ve ilgili bölge için nükleotit frekansları (%) Molecular Evolutionary Genetics Analysis (MEGA 11) kullanılarak analiz edildi. Son olarak, Maximum Parsimony (MP) dendrogram taksonomik ve filogenetik olarak *Potentilla* taksonlarını değerlendirmek için oluşturuldu. Çalışmanın sonucu olarak *Potentilla* cinsine ait taksonların dendrogramda iyi gruplandığı ve psbA-trnH IGS sekanslarının daha sonraki çalışmalarda kullanımının fayda sağlayacağı söylenebilir.

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## INTRODUCTION

The genus *Potentilla* L. (Rosaceae) comprises more than 400 species with a wide distribution area in the world (Sojak, 2008; Sojak, 2009; Persson et al., 2020a; Yılmaz, 2023a,b). Especially temperate zones of the northern hemisphere and its boreal parts are the regions where the genus *Potentilla* has the highest species number and diversity (Bean, 2015; Yılmaz, 2023a).

There are still many problems in the evaluation of the genus *Potentilla* taxonomically and aspect of its species identification concept. In other words, species

names, and their positions as taxonomically and phylogenetic relationships based on variations determined are controversial for the genus and continue the discussions based on the many genera circumstances within the family Rosaceae by the taxonomists (Lundberg et al., 2009; Dobes & Paule, 2010; Eriksson et al., 2022). Besides the changes in species names, many taxa have been transferred to other genera or described within different genera every day. Especially, the relationships between the genus *Potentilla* and *Sibbaldia* L. based on the identification of some species, in addition to their taxonomic positions of taxa evaluated within *Potentilla* or *Sibbaldia* are highly variable and complex (Lundberg et al., 2009; Eriksson et al., 2015). Similar relationships are observed between the genus *Potentilla* and *Argentina* Hill. in the studies by Sojak (2010) and Feng et al. (2014).

Essentially, the mistakes in the definition and taxonomic status of species are the most important reasons for making problematic and hard to understand the genus. Although morphological characters are very useful and important in the species description and even determination of variations, it is inadequate in plant groups such as *Potentilla* that have species showing high variations. Polyploidy is a common observed and well-known phenomenon for *Potentilla* species. Furthermore, this process is very significant to understanding the speciation and later in the evolution of the *Potentilla* taxa. It is stated that many species evolved by hybridization and polyploidization (Töpel et al., 2011; Persson et al., 2020b). In other words, it can be stated that both hybridization and polyploidization are important processes in the evolution of *Potentilla* taxa (Potter et al., 2007; Paule et al., 2011, 2012; Persson et al., 2020a). As a result, many *Potentilla* taxa have variable chromosome numbers from diploid to hexadecaploid (16x) (Kalkman, 2004). Besides the *Potentilla* taxa with variable chromosome numbers, it is observed that Potentilla species are exhibiting different chromosome numbers in different locations (IPCN; Kechaykin et al., 2016; Schanzer et al., 2020).

Another case increasing the complexity of *Potentilla* taxa in the aspect of taxonomics and phylogenetics is species diversity and their distributions. The genus *Potentilla* is one of the biggest plant groups evaluated within the family Rosaceae according to the species number and distribution. In other words, the genus is represented by the habitats showing distribution in many parts of the world. All of these increase hybridization, polyploidy, and the presence of taxa that exhibit intermediate morphological characters between parent plants in the genus, as a result of introgression and genetic drift.

The inadequacy of morphological characters caused by all these stated cases makes it necessary to determine the new characters. Determination of molecular characters and their use for the description of problematic taxa in the aspect of specified features especially gives very important results to overcome such difficulties besides a more detailed evaluation of the genus as phylogenetically. Especially, molecular studies based on DNA sequence information of nuclear or chloroplast genomes have been frequently used in different plant groups. However, species identification and separation ability of the barcoding region preferred may exhibit variability in different plant groups. For this reason, the using separately of different DNA sequences containing gene and spacer regions with as many taxa as possible provides important information about which DNA sequences must be preferred and which region combinations are more useful for analysis. One of these which is been the most important and frequently preferred for efficient identification is a psbA-trnH region.

In this study, psbA-trnH intergenic spacer (IGS) sequence information acquired from the National Center for Biotechnology Information (NCBI) was examined for 77 *Potentilla* taxa

i. to understand the species identification and discrimination abilities
ii. to evaluate phylogenetic relationships of *Potentilla* taxa based on the region preferred
iii. to make a comparison with previous studies
iv. finally to make recommendations about the DNA barcoding region preferred for further studies.

## MATERIALS and METHODS

The sequence information of the psbA-trnH region for all *Potentilla* taxa was first acquired from the NCBI database. After all, data belonging to the psbA-trnH sequence which are shared by different researchers were collected, and it was observed the regions for sequences related such as psbA gene/psbA-trnH IGS/trnH gene, psbA-trnH IGS and psbA gene/psbA-trnH IGS. Finally, the psbA-trnH IGS sequences were extracted from these regions to make the most accurate analysis using as many taxa as possible and thus to reveal the discrimination ability of the region examined more clearly. Although the sequence lengths of many *Potentilla* taxa for the region examined are compatible, it was observed the presence of incompatibility in some taxa uploaded to NCBI by different researchers. Essentially, it is commonly observed that situations do not match the sequences in the aspect of their lengths in many plant groups (Yılmaz & Yeltekin, 2022). Therefore, the sequences for each *Potentilla* taxon were analyzed based on their compatibility, and compatible sequences were mostly preferred for analysis (Appendix). Moreover, some *Potentilla* taxa whose sequences were uploaded by different researchers were represented by a few

samples in the phylogenetic tree to show the accuracy of the data in NCBI. In total, 77 *Potentilla* taxa belonging to 91 samples for the psbA-trnH IGS region were investigated to evaluate the phylogenetic relationships and to understand the species identification ability of the region preferred.

The analysis for the determination of variable sites, passim-info sites, base substitutions, and nucleotide frequencies, besides the phylogenetic tree, was performed by using Molecular Evolutionary Genetics Analysis (MEGA 11) (Tamura et al., 2021). Firstly, all sequences for the *Potentilla* taxa were aligned and then variable sites and parsim-info sites were computed. Base substitution probabilities were determined and shown in Table 1. Furthermore, transitional and transversional base substitutions (%), in addition to transition/transversion ratios for purines and pyrimidines were determined and shown in Table 2. Nucleotide frequencies % as A+T/U and G+C of psbA-trnH IGS sequences belonging to *Potentilla* taxa were also computed.

Finally, the Maximum Parsimony (MP) dendrogram that bootstrap values are reported on branches with the option of hide values lower than 50% was provided to evaluate the genus *Potentilla* as taxonomically and phylogenetically, besides species identification and separation abilities of the region examined for *Potentilla* taxa. The positions with gaps and missing data appearing as a result of multiple sequence alignments were eliminated with the complete deletion option of the program for more effective analyses, so it is aimed to provide more meaningful and comprehensive results.

## RESULTS and DISCUSSION

psbA-trnH IGS sequences belonging to the *Potentilla* taxa were extracted from psbA/trnH gene regions provided by the NCBI database. In this study, a total of 91 samples representing 77 *Potentilla* taxa were analyzed in the aspect of species identification and separation ability of psbA-trnH IGS sequences. Some taxa were represented by a few samples and these taxa were preferred from the data set loaded by different times and researchers to evaluate the sequence compatibilities and discrepancies in the NCBI database. For this aim, firstly all psbA-trnH IGS sequences of *Potentilla* taxa were aligned and then variable and parsimony informative nucleotides were determined. Variable sites and parsimony informative sites were observed in 344 and 115 nucleotides, respectively. In addition to the determination of variable and parsimony informative nucleotides of *Potentilla* taxa for the psbA-trnH IGS region, the probabilities of base substitution were computed. The highest base substitution was observed at the rate of 25.4% from C to T. After that, the second highest base substitution was observed at the rate of 14.67% from G to A (Table 1). Moreover, the rates (%) of transitional and transversional base substitutions were computed as 53.31% and 46.69%, respectively, by using the substitution rate from one base to another base from Table 1.

Table 1. The probability of substitution (r) from one base (row) to another base (column) for psbA-trnH IGS sequences (Transitional substitutions are shown in bold)

*Çizelge 1. psbA-trnH IGS sekansları için bir bazdan diğerine değişim olasılıkları (Transisyonel baz değişimleri koyu renkli gösterilir)*

| | A | T | C | G |
|---|---|---|---|---|
| **A** | - | 9.01 | 1.97 | **7.69** |
| **T** | 8.11 | - | **5.55** | 4.25 |
| **C** | 8.11 | **25.4** | - | 4.25 |
| **G** | **14.67** | 9.01 | 1.97 | - |

The transition/transversion rate was determined as 1.80 for purines ($k_1$), and 2.81 for pyrimidines ($k_2$), besides the overall transition/transversion rate (R=0.82). Finally, nucleotide frequencies for psbA-trnH IGS sequences belonging to the *Potentilla* taxa were analyzed as 73.34% (A+T/U) and 26.66% (G+C). As a result, it can be stated that psbA-trnH IGS sequences for *Potentilla* taxa consist of A and T/U bases at high levels.

All information such as alignment length, variable site, passim-info site, transitional substitutions, transversional substitutions, transition/transversion rates, and nucleotide frequencies were shown in Table 2.

Table 2. The information of taxa examined based on psbA-trnH IGS sequences

*Çizelge 2. psbA-trnH IGS sekansları temelinde incelenen taksonların bilgisi*

| Taxon | Alignment length (bp) | Variable site | Parsim-info site | Transitional substitutions (%) | Transversional substitutions (%) | Transition/Transversion rate Purines ($k_1$) | Pyrimidines ($k_2$) | Overall (R) | Nucleotide freq. (%) A+T/U | G+C |
|---|---|---|---|---|---|---|---|---|---|---|
| 77 | 802 | 344 | 115 | 53.31 | 46.69 | 1.80 | 2.81 | 0.82 | 73.34 | 26.66 |

MP dendrogram was drawn to show the phylogenetic relationships of the taxa examined and to determine the species identification ability for the *Potentilla* taxa of sequences (Figure 1).

Many researchers in their studies based on phylogenetic relationships of the *Potentilla* taxa state

the presence of six major clades (Anserina, Argentea, Alba, Fragarioides, Ivesioid, and Reptans) in the genus (Dobes & Paule, 2010; Töpel et al., 2011; Feng et al. 2017). The dendrogram separated the taxa into seven groups based on psbA-trnH IGS sequences. The taxa resolved in Group I (*P. caulescens*, *P. nitida*, *P. Biflora*and *P. alba*) are evaluated in the Alba clade. In other words, taxa belonging to the Alba clade were grouped and formed a distinct group in the phylogenetic tree. Similarly, it was observed that the taxa evaluated in the Reptans clade (*P. indica*, *P. erecta,* and *P. reptans*) were clustered together in Group II.

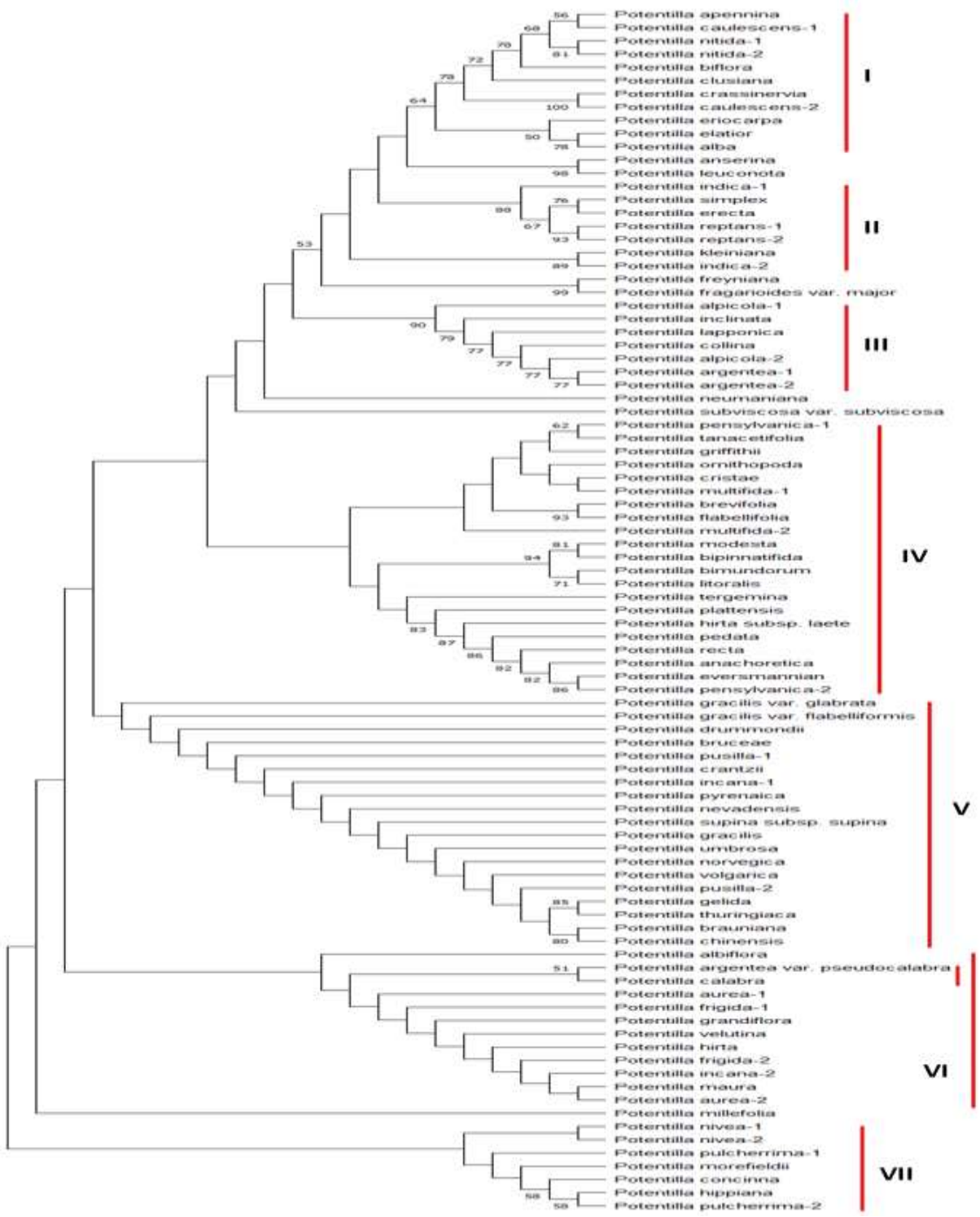



Figure1. MP tree provided from psbA-trnH IGS sequences of *Potentilla* taxa
*Şekil 1. Potentilla taksonlarının psbA-trnH IGS sekanslarından elde edilen MP ağacı*

The taxa belonging to the Argentea clade were represented by the highest species number in the MP dendrogram and clustered in other groups. Furthermore, the taxa evaluated in the Argentea clade formed the outmost groups in the dendrogram. Paule et al. (2012) state in their study based on implications of hybridization and cytotypic differentiation in the speciation of *P. alpicola* that "Populations of *P. collina* have been regarded rather as recent derivatives of the hexaploid *P. argentea*. The observation of clones within both *P. alpicola* and *P. collina* suggested a possible apomictic mode of reproduction". Moreover, the case study of *P. alpicola* made by Paule et al. (2012) shows that processes such as apomixis play a significant role in the creation of polymorphism in the genus *Potentilla*. In this study, when the relationships among *P. alpicola*, *P. collina,* and *P. argentea* were investigated, it was observed that the five samples of these taxa were clustered together in Group III and separated from other taxa belonging to the Argentea clade. In other words, it can be stated that these three taxa are closely related to each other phylogenetically.

*P. argentea* var. *pseudocalabra* was evaluated as an intermediate between hexaploid *P. argentea* and *P. calabra* by Paule et al. (2011). Here, *P. calabra* and *P. argentea* var. *pseudocalabra* were clustered very closely in Group VI and showed similarity phylogenetically based on psbA-trnH IGS sequences.

Especially, molecular studies based on DNA sequence information of nuclear or chloroplast genomes have been frequently used in different plant groups. Furthermore, combined analysis containing two or more genes and spacer regions has been used by many researchers to provide better phylogenetic resolution and identification in plant groups. However, the species identification and discrimination capabilities of DNA sequences preferred in the relevant plant group should be known to obtain maximum benefit from the DNA region used and then to use the right region combinations, because the DNA regions preferred are not equally effective in different plant groups.

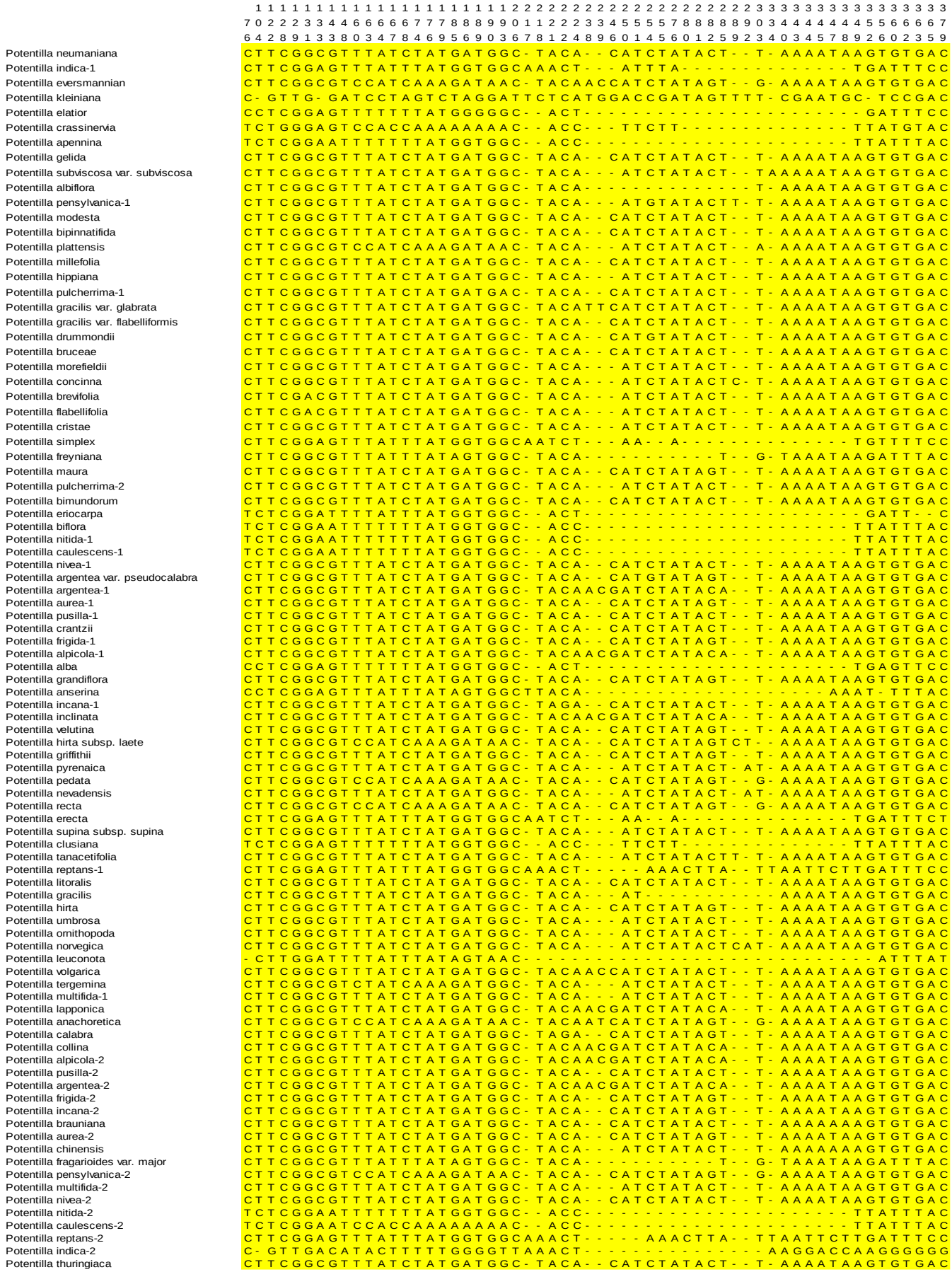
Santos and Pereira (2018) state in their study that cpDNA sequences are very important in species identification and phylogenetic analysis in plants, in addition to the importance of the region and region combinations. Furthermore, they used the SPInDel (Species Identification by Insertions/Deletions) approach to achieve better identification of plant species by using the combination of variable length sequences in cpDNA. As a result, when considered alone, the regions having low discrimination ability increased the separation ability with right region combinations. Similarly, Gontcharov et al. (2004) state that combined analysis is better than single-gene analysis in the aspect of phylogenetic resolution and is supported by morphological information.

In this concept, the studies based on species identification abilities of different DNA sequences have high importance to get better results in the future with the right region combinations. It is stated in the study on the importance of trnL/trnF IGS region in the taxonomy of the genus *Potentilla* L. by Yılmaz (2023a) that the region preferred has high variable sites and grouping ability, in addition to strongly recommended for further studies. Similarly, both ITS1 and ITS2 sequences between rDNA genes in the study made by Yılmaz (2023b) are strongly recommended in the phylogenetic evaluation of *Potentilla* taxa, besides their determination of contribution levels. However, rbcL sequences were insufficient in the identification and grouping of some *Potentilla* taxa according to the phylogenetic tree (Yılmaz, 2023c). In other words, it can be stated that rbcL sequences in the comparison of other DNA regions provide less information in the solving of taxonomic problems and analysis of phylogenetic relationships.

Here, psbA-trnH IGS sequences were examined in the aspect of species identification and separation abilities for *Potentilla* taxa, besides phylogenetic evaluation.

Loera-Sanchez et al. (2020) state in their study based on the identification of forage legumes and grasses using trnH-psbA sequences that the barcoding region examined is a promising candidate for efficient identification and plant species richness assessments. However, Yılmaz (2021) states in the study based on comparisons of nuclear and chloroplast DNA regions in the aspect of their species identification abilities for *Crocus* L. taxa that the DNA region belonging to partial psbA gene-psbA/trnH IGS-partial trnH gene have not enough sequence variations (30 nucleotides) for the identification of species, although it phylogenetically separated some *Crocus* taxa.

As a result of this study, it can be stated that the psbA-trnH IGS region separated the many *Potentilla* taxa from each other and grouped them based on the clades according to the phylogenetic tree. However, it was insufficient in the complete solution of still existing problems and the discrimination of all taxa phylogenetically. The determinations of variable and parsimony informative nucleotides give very important information based on the phylogenetic and taxonomic relationships for plant groups analyzed, besides the species identification ability of the region examined. Furthermore, the inconsistency between both of them may show the sequence incompatibility for the taxa analyzed. In this study, 344 variable sequences and 115 parsimony informative sites were determined for psbA-trnH IGS sequences of *Potentilla* taxa. When the DNA sequences of each taxa were examined, it was observed the variability in the nucleotide sequences and sequence lengths of some taxa. This is the main reason for the presence of a high


Potentilla neumaniana
Potentilla indica-1
Potentilla eversmannian
Potentilla kleiniana
Potentilla elatior
Potentilla crassinervia
Potentilla apennina
Potentilla gelida
Potentilla subviscosa var. subviscosa
Potentilla albiflora
Potentilla pensylvanica-1
Potentilla modesta
Potentilla bipinnatifida
Potentilla plattensis
Potentilla millefolia
Potentilla hippiana
Potentilla pulcherrima-1
Potentilla gracilis var. glabrata
Potentilla gracilis var. flabelliformis
Potentilla drummondii
Potentilla bruceae
Potentilla morefieldii
Potentilla concinna
Potentilla brevifolia
Potentilla flabellifolia
Potentilla cristae
Potentilla simplex
Potentilla freyniana
Potentilla maura
Potentilla pulcherrima-2
Potentilla bimundorum
Potentilla eriocarpa
Potentilla biflora
Potentilla nitida-1
Potentilla caulescens-1
Potentilla nivea-1
Potentilla argentea var. pseudocalabra
Potentilla argentea-1
Potentilla aurea-1
Potentilla pusilla-1
Potentilla crantzii
Potentilla frigida-1
Potentilla alpicola-1
Potentilla alba
Potentilla grandiflora
Potentilla anserina
Potentilla incana-1
Potentilla inclinata
Potentilla velutina
Potentilla hirta subsp. laete
Potentilla griffithii
Potentilla pyrenaica
Potentilla pedata
Potentilla nevadensis
Potentilla recta
Potentilla erecta
Potentilla supina subsp. supina
Potentilla clusiana
Potentilla tanacetifolia
Potentilla reptans-1
Potentilla litoralis
Potentilla gracilis
Potentilla hirta
Potentilla umbrosa
Potentilla ornithopoda
Potentilla norvegica
Potentilla leuconota
Potentilla volgarica
Potentilla tergemina
Potentilla multifida-1
Potentilla lapponica
Potentilla anachoretica
Potentilla calabra
Potentilla collina
Potentilla alpicola-2
Potentilla pusilla-2
Potentilla argentea-2
Potentilla frigida-2
Potentilla incana-2
Potentilla brauniana
Potentilla aurea-2
Potentilla chinensis
Potentilla fragarioides var. major
Potentilla pensylvanica-2
Potentilla multifida-2
Potentilla nivea-2
Potentilla nitida-2
Potentilla caulescens-2
Potentilla reptans-2
Potentilla indica-2
Potentilla thuringiaca

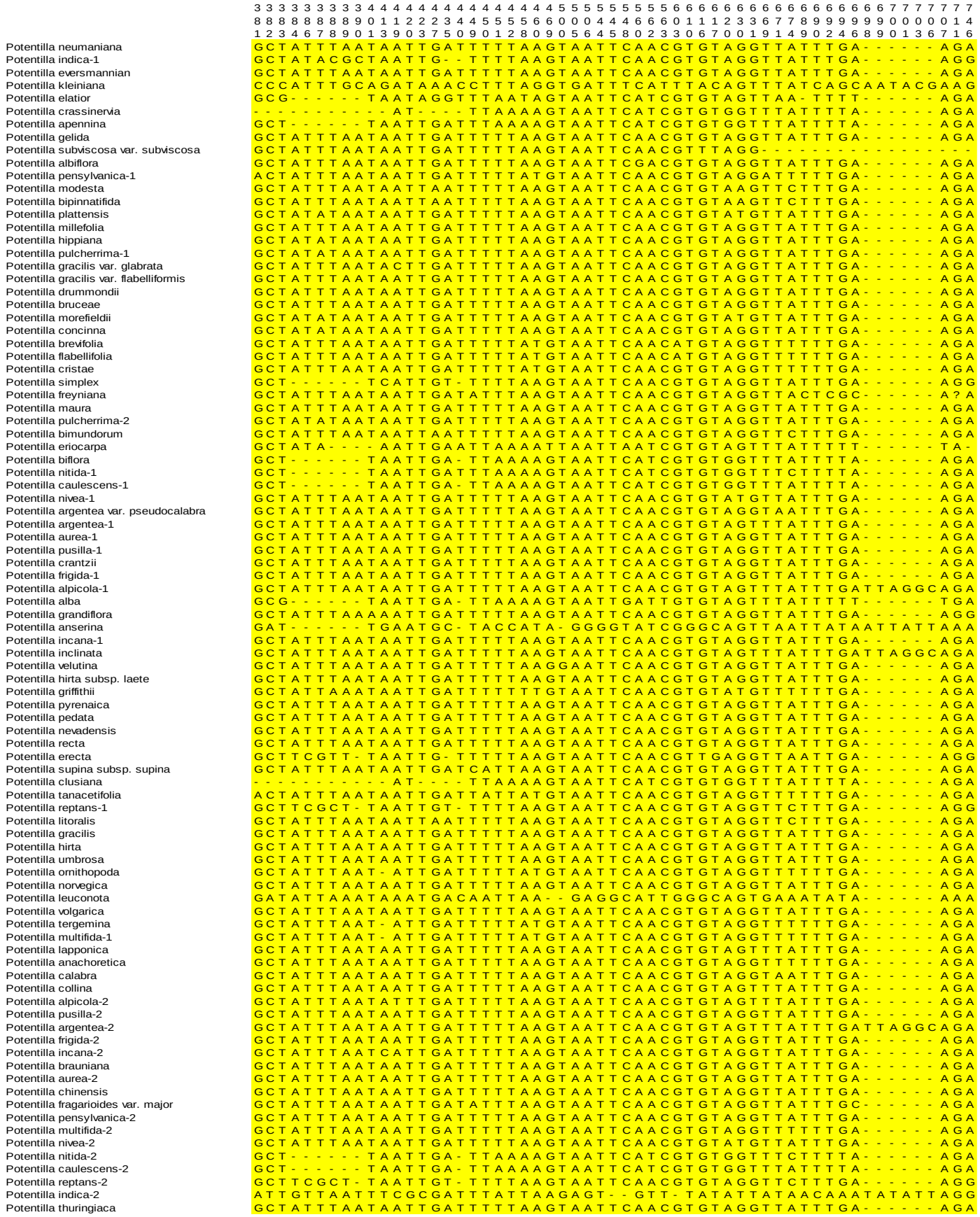



Figure2. *Potentilla* taxa and Parsimony informative sites belonging to psbA-trnH IGS sequences (The numbers show variable nucleotides)

*Şekil 2. Potentilla taksonları ve psbA-trnH IGS sekanslarına ait Parsimony informative bölgeler (Sayılar değişken nükleotitleri gösterir)*

level of the variable nucleotide number. In other words, most of the sequence variations between *Potentilla* taxa examined are caused by the change of only a nucleotide. Parsimony informative sites observed in 115 nucleotides show the accuracy of this (Figure 2).

## CONCLUSION

Although morphological characters are very important tools in the identification and classification of species, many times they can be also reasons for misclassifications in some plant groups because of some situations such as wide geographical distribution, geomorphological structure and climatic changes, hybridization, introgression, autopolyploidy, and allopolyploidy. The genus *Potentilla* with species over 400 exhibits all situations that may cause changes in morphological characters and thus misidentification. In other words, the discrepancies observed in the MP dendrogram can be caused by situations such as misidentifications of the taxa, missing data caused by sequencing, or labeling errors in accessions. Although combined analysis containing different DNA sequences is very important in the solving of problems stated, it is necessary to have knowledge about which DNA regions are more useful and then, which region combinations will provide more advantage.

Finally, it can be stated that the taxa belonging to the genus *Potentilla* were well grouped in the dendrogram and the using of psbA-trnH IGS sequences is strongly recommended for further studies with the combination of the regions with the ability to reveal the phylogenetic relationships.

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### Author's Contributions [Century12 bold]

The contribution of the author is 100 %.

### Statement of Conflict of Interest

The author has declared no conflict of interest.

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## APPENDIX

GQ384973, LC632299, MN871351, JF708223, GQ385036, GQ385035, GQ385033, GQ385029, GQ385027, GQ385025, GQ385023, GQ385022, GQ385021, GQ385020, GQ385017, GQ385016, GQ385014, GQ385013, GQ385012, GQ385010, GQ385009, GQ385008, GQ385007, GQ385006, GQ385005, GQ385004, GQ384999, GQ384998, GQ384997, GQ384995, GQ384993, GQ384992, GQ384991, GQ384990, GQ384989, GQ384988, GQ384987, GQ384986, GQ384984, GQ384983, GQ384982, GQ384981, GQ384980, GQ384976, GQ384975, GQ384974, GQ384972, GQ384970, GQ384969, GQ384968, GQ384964, GQ384963, GQ384962, GQ384961, GQ384960, GQ384959, GQ384958, GQ384957, GQ384956, GQ384955, GQ384954, GQ384952, GQ384951, GQ384950, GQ384948, GQ384947, GQ384946, MN871395, MN871365, MN871363, MN871352, MN871335, HM776571, JX276877, JX276876, JX276873, JX276869, JX276827, JX276825, JX276784, JX276782, OQ161262, LC703124, MF543685, MF543674, DQ778819, HG800560, HG800559, HE966756, HE966755, HQ433307