*http://dergipark.org.tr/trkjnat*
*Trakya University Journal of Natural Sciences, 24(1): 71-76, 2023*
*ISSN 2528-9691*
*DOI: 10.23902/trkjnat.1252980*

**Research Article**

# THE IMPORTANCE OF trnL/trnF IGS REGION IN THE TAXONOMY OF THE GENUS *Potentilla* L.

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**Cite this article as:**

Yılmaz A. 2023. The importance of trnL/trnF IGS region in the taxonomy of the genus *Potentilla* L. *Trakya Univ J Nat Sci*, 24(1): 71-76, DOI: 10.23902/trkjnat.1252980

Received: 18 February 2023, Accepted: 12 April 2023, Published: 15 April 2023

***Edited by:***
*Yerlan Turuspekov*

***Key words:***
*DNA barcoding*
*MP dendrogram*
*Phylogenetic relationships*

**Abstract:** This study was performed to determine the phylogenetic relationships in the genus *Potentilla* L. and to assess the importance of the trnL/trnF intergenic spacer (IGS) region. The selection of DNA barcoding region to provide for more effective, accurate and consistent results in the analysis is one of the most important points in success of the study. For this purpose, 71 *Potentilla* taxa were examined based on trnL/trnF IGS sequence data of chloroplast DNA acquired from the National Center for Biotechnology Information (NCBI) and a Maximum Parsimony (MP) dendrogram was drawn. The trnL/trnF IGS region has the convenient sequence length for barcoding region, and it is also proposed the use of this region which has highly variable and parsimony informative sites. When the probabilities of substitutions from one base to another base for trnL/trnF IGS region were examined, it was assigned that the rate of transitional substitutions with 57.13% is higher than the transversional substitutions. The MP dendrogram revealed that the studies taxa were placed in six groups as Fragarioides, Reptans, Anserina, Alba, Himalayan and Argentea. Phylogenetic relationships within the Argentea clade, represented by the highest species number in this study, were observed as quite confusing compared to other clades. In order to get the most accurate results, it is necessary to determine the regions giving the best results, and then to use these regions together with as many taxa as possible. In conclusion, the using of trnL/trnF IGS sequence information that have high variable sites and grouping ability for studies taxa is strongly recommended.

**Özet:** Bu çalışma, *Potentilla* cinsindeki filogenetik ilişkileri belirlemek ve trnL/trnF IGS bölgesinin önemini değerlendirmek amacıyla yapılmıştır. Analizlerde daha etkili, doğru ve tutarlı sonuçlar elde etmek için DNA barkodlama bölgesinin seçimi çalışmanın başarılı olmasındaki en önemli noktalardan biridir. Bu amaçla, NCBI'den elde edilen kloroplast DNA'ya ait trnL/trnF IGS sekans bilgisi temelinde 71 *Potentilla* taksonu incelendi ve MP dendrogram çizildi. trnL/trnF IGS bölgesi barkodlama bölgesi olarak uygun sekans uzunluğuna sahiptir ve ayrıca yüksek varyasyon ve parsim info bölgelere sahip bu bölgenin kullanılması önerilir. trnL/trnF IGS bölgesi için bir bazdan başka bir baza değişim olasılıkları incelendiğinde, %57,13 ile transisyonel baz değişimlerinin, transversiyonel değişimlere oranla daha yüksek olduğu belirlenmiştir. MP dendrogram, incelenen taksonların Fragarioides, Reptans, Anserina, Alba, Himalayan ve Argentea olarak altı gruba yerleştirildiğini ortaya çıkardı. Bu çalışmada en fazla türle temsil edilen Argentea'da filogenetik ilişkilerin diğerleri ile karşılaştırmada oldukça karmaşık olduğu görülmüştür. En doğru sonuçların alınabilmesi için, en iyi sonucu veren bölgelerin belirlenmesi, daha sonra mümkün olduğu kadar çok takson ile belirlenen bu bölgelerin birlikte kullanılması gerekir. Sonuç olarak, çalışılan taksonlar için yüksek varyasyon gösteren bölgelere ve guruplama yeteneğine sahip olan trnL/trnF IGS sekans bilgilerinin kullanılması tavsiye edilir.

## Introduction

The genus *Potentilla* L. belonging to the family Rosaceae, which is well known for its economically important crops, is represented by over 400 species (Soják 2009, Bean 2015, Persson *et al.* 2020a). Rosaceae family contains subfamilies, tribes and subtribes within these subfamilies (Xiang *et al.* 2017, Zhang *et al.* 2017, Persson 2021). The genus *Potentilla* evaluated in the subfamily Rosoideae, tribe Potentilleae and subtribe Potentillinae has the highest species diversity in the temperate and boreal regions of the northern hemisphere (Bean 2015).

Six major clades, Anserina, Argentea, Alba, Fragarioides, Ivesioid and Reptans, in the genus *Potentilla* were identified by Töpel *et al.* (2011) based on chloroplast (cpDNA) and nuclear DNA. Although similar results were obtained in the study of Feng *et al.* (2017), it can still be stated that there exist some problems in the relationships between clades, and clades are not well supported every time.

Another fact that makes it difficult to better understand the genus phylogenetically and taxonomically is polyploidization. Polyploidization, in addition to hybridization, is a situation commonly observed in *Potentilla*. Besides their contribution to the complexity of understanding the genus, both are important processes for the speciation and the evolution processes of its members (Dobeš & Paule 2010, Paule *et al.* 2012, Persson *et al.* 2020a). Ploidy levels within the genus changes from diploid to hexadecaploid, but diploidy is represented with only a few number of species (Kalkman 2004, Persson *et al.* 2020a). Chromosome counts in many species of the genus were revealed during the last five decades and the basic chromosome number of the genus was reported as n=7 (Goswami & Matfield 1975, Ilnicki & Jeremi 2008, Töpel *et al.* 2011). However, different chromosome numbers based on different ploidy levels in some species within the genus are also seen (Müntzing 1931, Skalinska 1950, Müntzing 1958, Ilnicki & Jeremi 2008, Töpel *et al.* 2011).

Different ploidy levels or doubling of chromosomes can produce hybrid individuals that have reproductive barriers, which in turn may lead to evolution of new species (Persson *et al.* 2020b). In the study of Töpel *et al.* (2011) who used chloroplast and nuclear DNA to identify topological incongruences for the purpose of understanding the hybridization in *Potentilla,* the authors stated that they identified four well-supported incongruences in the genus, of which four include polyploid taxa, which are considered candidates for further investigations of allopolyploid origin. These findings make it relatively hard to understand the taxonomic and phylogenetic position of genus. In order to overcome such difficulties, molecular techniques, especially those based on the sequence information belonging to chloroplast and nuclear DNA, are frequently used to explain the phylogenetic relationships of the species and genetic status within the genus. However, different results on the phylogenetic relationships for some taxa, depending on whether the chloroplast or nuclear data were used, were also obtained (Eriksson *et al.* 1998, Eriksson *et al.* 2003, Dobeš & Paule 2010, Töpel *et al.* 2011, Persson *et al.* 2020b).

In this study, 71 *Potentilla* taxa were examined based on trnL/trnF intergenic spacer (IGS) sequence data of chloroplast DNA acquired from the National Center for Biotechnology Information (NCBI) ***i)*** to evaluate the taxonomy of the genus and the phylogenetic relationships between species in the most accurate way by using as many taxa as possible, ***ii)*** to provide data about the region studied for a proper species identification and discrimination in further studies, and ***iii)*** to make an accurate evaluation in the taxonomy of the genus by comparing the data obtained with previous studies.

## Materials and Methods

All sequence data belonging for trnL/trnF IGS regions were acquired from the NCBI database, and the data for each taxon were analysed in point of compatibility of sequence information and the sequences which is compatible were determined and examined in the study (Eriksson *et al.* 2003, Lundberg *et al.* 2009, Töpel *et al.* 2011, Töpel *et al.* 2012, Feng *et al.* 2015, Feng *et al.* 2017). For this purpose, the sequence data obtained by different researchers at different periods were evaluated (generally partial trnL gene/trnL-trnF IGS/partial trnF gene) and trnL-trnF IGS sequences that are compatible in all researches and contain all sequence information were extracted and used in the analysis in order to use the *Potentilla* taxa examined by different researchers harmoniously, and thus to determine the most comprehensive taxonomic relationships in the genus by using as many taxa as possible (Supplementary Material Table S1).

As a result, 71 taxa were investigated based on sequence information of trnL-trnF IGS from chloroplast DNA. The multiple sequence alignments for the investigated taxa were performed using the Molecular Evolutionary Genetics Analysis (MEGA 11) (Tamura *et al.* 2021).

The information containing variable and parsim-info sites of the taxa were computed. The probabilities of substitution from one base to another base, transitional substitutions (%), transversional substitutions (%) and transition/transversion ratios for purines and pyrimidines were determined. Nucleotide frequencies of trnL-trnF IGS regions were computed and showed as A+T/U % and G+C %.

The evolutionary history was inferred using the Maximum Parsimony (MP) method. The MP dendrogram showing bootstrap values on branches (with the option of hide values lower than 50%) was provided to determine the species identification ability of the region examined and to evaluate the phylogenetic relationships and taxonomic statues of the genus. Gaps evaluated as missing data after analyses in the comparison of examined sequences were eliminated with the complete deletion option of the program for more effective analyses.

## Results

trnL/trnF IGS regions were extracted from trnL/trnF gene regions of chloroplast DNA acquired from the NCBI database to make compatible all sequence information and to use as many taxa as possible in order to obtain more comprehensive and meaningful results for the genus *Potentilla*. Genbank codes for trnL/trnF IGS sequences acquired from the NCBI database are presented in Supplementary Material Table S1. The alignment length of the investigated taxa was determined as 582 bp. The variable sites and parsimony informative sites, which are very important indicators in phylogenetic relationships and species identification for selected barcoding regions, were observed in 133 and 91 nucleotides, respectively. The chloroplast DNA regions are known, in general, to have highly conserved sequences and to show low variations. However, the region examined exhibits high variation (Supplementary Material Table S2). The probabilities of substitutions from one base to another base were examined and the highest base substitutions were observed in the rate of 23.43% from C to T and 18.08% from G to A (Table 1).

Transitional and transversional substitutions were computed as 57.13% and 42.87%, respectively, showing that the rate of transitional substitutions caused by the substitutions between the same base groups (purines: A ↔ G or pyrimidines: C ↔ T) is higher than the transversional substitutions for trnL/trnF IGS region. The transition/transversion rate for purines ($k_1$) and pyrimidines ($k_2$) were determined as 2.66 and 2.66, respectively, in addition to the overall transition/transversion rate (R=1.08). Nucleotide frequencies for trnL/trnF IGS sequences were analysed as 72.67% (A+T/U) and 27.33% (C+G) (Table 2).

**Table 1.** The probability of substitution (r) from one base (row) to another base (column) )Transitional substitutions are shown in bold).

| | A | T | C | G |
|---|---|---|---|---|
| **A** | - | 8.8 | 3.19 | **7.12** |
| **T** | 6.78 | - | **8.5** | 2.67 |
| **C** | 6.78 | **23.43** | - | 2.67 |
| **G** | **18.08** | 8.8 | 3.19 | - |

**Table 2.** The information of taxa examined based on trnL/trnF intergenic spacer sequences.

| **Taxon** | **Alignment** | **Variable** | **Parsim-info** | **Transitional** | **Transversional** | **Transition/Transversion rate** | | | **Nucleotide** | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **length (bp)** | **site** | **site** | **substitutions** | **substitutions** | **Purines** | **Pyrimidines** | **Overall** | **freq.** | **(%)** |
| | | | | (%) | (%) | (k1) | (k2) | (R) | A+T/U | G+C |
| 71 | 582 | 133 | 91 | 57.13 | 42.87 | 2.66 | 2.66 | 1.08 | 72.67 | 27.33 |

The results showed that the trnL/trnF IGS sequences for *Potentilla* taxa consists of highly A and T/U bases. The MP dendrogram was drawn to evaluate the phylogenetic relationships among the taxa examined and the taxonomy of the genus *Potentilla*, and to better understand the species identification ability of the region studied for the genus (Fig. 1). The dendrogram showing the phylogenetic relationships of 71 investigated taxa based on trnL/trnF IGS sequences separated the taxa in six groups as Alba, Anserina, Argentea, Fragarioides, Himalayan and Reptans.

## Discussion

Morphological characters are very important tools used in species identification and classification studies. However, they can also be reasons for misclassifications, and in turn systematic problems due to the variations in most of the morphological characters relied on. The members of the genus *Potentilla* spread over wide areas with different ecological conditions and geographical factors. Such a distributional pattern, in addition to plants displaying intermediate morphological characters caused by hybridization, has the possibility to cause variations in morphological characters, which in turn lead to obtaining insufficient morphological data to base the phylogenetic details and taxonomic status of members of the genus on. Rieseberg & Ellstrand (1993) stated that hybridization can result in new character combinations not found in the parental species. As a result, morphological variations caused by various factors like hybridization, genetic drift, gene flow, and different epigenetic mechanisms force researchers to determine more stable diagnostic characteristics. The new groups within the genus *Potentilla* were defined by Wolf (1908) according to style shape and position. Style morphology as a morphological character is very important especially in the evaluation of the phylogenetic relationship between the species of *Potentilla* (Töpel *et al.* 2011). Töpel *et al.* (2011) stated that style characters are also informative in the aspect of the evaluation of different clades.

The classical taxonomic system based on the morphological similarity of individuals may be insufficient for species identification and phylogeny especially in a genus like *Potentilla*, which is hard to understand. Therefore, DNA barcoding has been often used to solve taxonomic problems and to explain the genetic status and phylogenetic relationships of different taxa. The sequence information belonging to the chloroplast and nuclear genomes have been used in various molecular studies for plant groups especially with taxa whose relationships not clearly revealed and taxonomically problematic. However, there are regions that do contribute to adequate taxonomic discrimination or low species identification ability based on high variation. As a result, it can be stated that the determination of successful barcoding regions in improving of available taxonomic problems and in the evaluation of phylogenetic relationships would have a considerable effect.

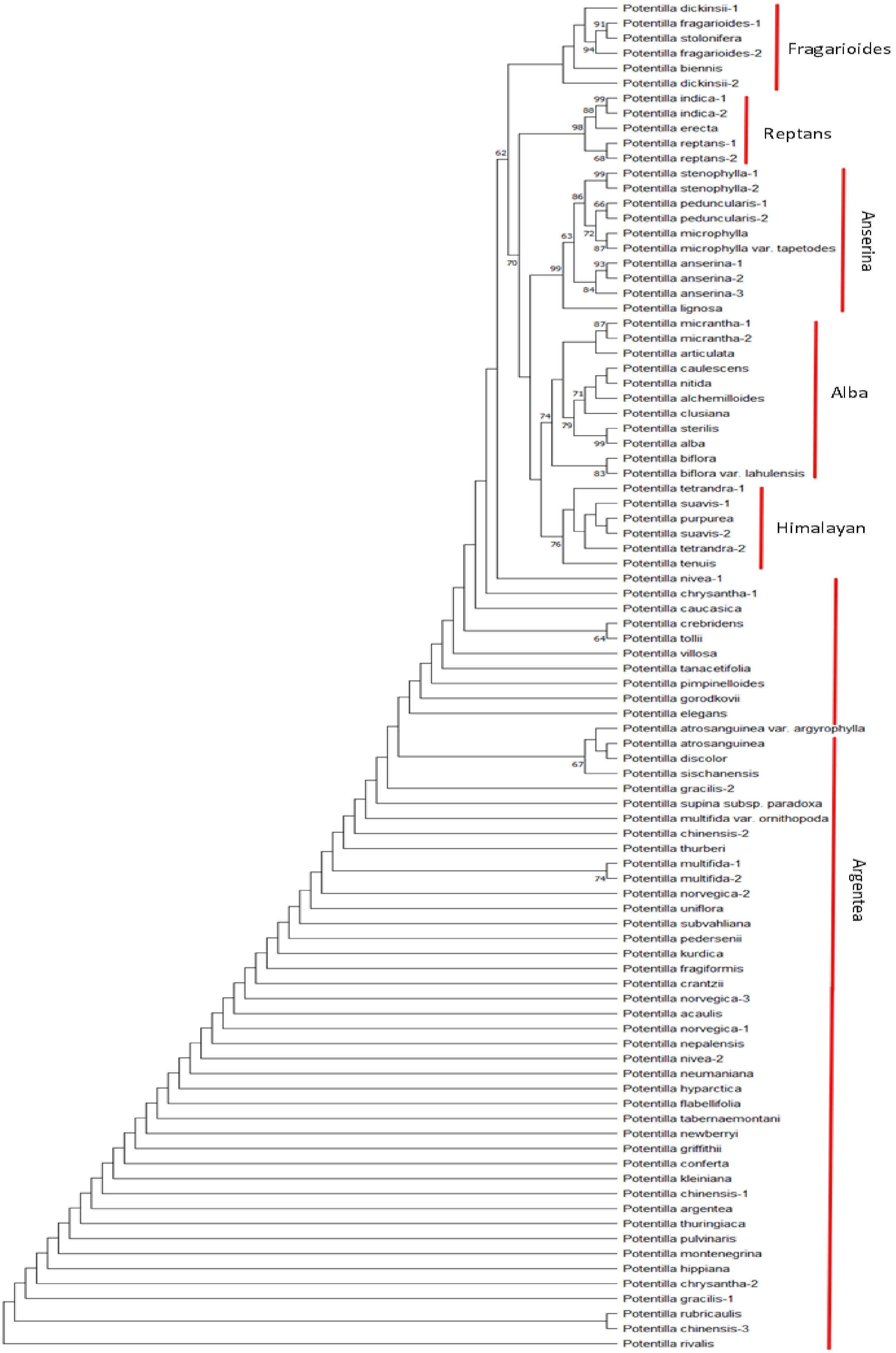

**Fig. 1.** Maximum Parsimony tree provided from trnL/trnF intergenic spacer sequences of *Potentilla* taxa.

When the information of taxa based on trnL/trnF IGS sequences was examined (Tables 1, 2), it can be said that the region selected exhibits high variation and has enough sequence length for barcoding.

The phylogenetic relationships of the *Potentilla* taxa were examined by many researchers and Töpel *et al.* (2011) identified six major clades in the genus as Anserina, Argentea, Alba, Fragarioides, Ivesioid, and Reptans. Similar results were obtained in studies performed by using molecular data (Dobeš & Paule 2010, Feng *et al.* 2017).

In our study, five clades except for Ivesioid and an extra clade (Himalayan) were determined. Feng *et al.* (2017) reported that the Himalayan clade contains former *Sibbaldia* L. species, which is resolved as a sister to other *Potentilla* taxa according to the plastid tree. The taxa evaluated in the Himalayan clade were found to be nested in Alba clade according to the nuclear tree. The present study supports the Himalayan clade with the taxa *P. suavis* Soják, *P. purpurea* (Royle) Hook.f., *P. tetrandra* (Bunge) Hook.f. and *P. tenuis* (Hand.-Mazz.) Soják. Moreover, this clade is resolved as close to the Alba clade.

Himalayan and Alba clades resolved as close with each other were observed as next to Anserina clade, consisting *P. stenophylla* (Franch.) Diels, *P. peduncularis* D. Don, *P. microphylla* D. Don, *P. anserina* L. *and P. lignosa* Willd. in MP dendrogram (Fig. 1). Afterwards, Reptans and Fragarioides clades showed proximity to them, respectively. The Reptans clade in this study consists of *P. erecta* (L.) Raeusch, *P. indica* (Jacks.) Th. Wolf. and *P. reptans* L. taxa studied by different researchers and at different times (Eriksson *et al.* 2003, Töpel *et al.* 2011, Feng *et al.* 2017). The Fragarioides clade was represented by specimens belonging to *P. dickinsii* Franch. & Sav., *P. fragarioides* L., *P. stolonifera* Lehm. and *P. biennis* Greene.

The relationship of *P. indica* and *P. reptans* with *P. dickinsii* and *P. fragarioides* in the study of Eriksson *et al.* (2003) shows similarity to this study in an aspect of the taxonomic relationships of Reptans and Fragarioides clades. *Potentilla biennis* that is resolved with the Ivesioid clade (Persson *et al.* 2020b) was grouped in Fragarioides clade here.

The Argentea clade in the present study was represented by the highest species number. However, the phylogenetic relationships of the species within this clade seem quite confused when compared to other clades. While *P. norvegica* L. is evaluated in the Argentea clade in analyses based on chloroplast DNA, it is evaluated in the Ivesioid clade with studies based on nuclear DNA (Eriksson *et al.* 1998, Eriksson *et al.* 2003, Dobeš & Paule 2010, Töpel *et al.* 2011). This situation is interpreted as a possible connection between these two clades on the basis of previous studies. In this study, which used nucleotide sequences belonging to trnL-trnF IGS regions, 3 taxa belonging to *P. norvegica* were grouped in the Argentea clade. *Potentilla norvegica*, *P. rivalis* Nutt., *P. newberryi* A.Gray, and *P. supina* L. were resolved with the Argentea clade in the study of Persson *et al.* (2020b) based on chloroplast data. The similar result was observed in the present study based on the relations between the taxa stated.

Hybridization and, the following introgression and polyploidy are important mechanisms in the evolution of the genus *Potentilla*. The different ploidy levels and species containing multiple ploidy levels is remarkable for the genus. *Potentilla norvegica* and *P. intermedia* have more than one ploidy level (Rice *et al.* 2014). Persson *et al.* (2020b) showed that *P. norvegica* and *P. intermedia* are allopolyploids and share an evolutionary history containing parental lineages from the Argentea and Ivesioid clades. In other words, it can be said that many evolutionary mechanisms have effects on the genus.

## Conclusion

Although many barcoding regions belonging to chloroplast and nuclear genome are used to contribute to species identification, solution of taxonomic problems, and evaluation of phylogenetic relationships, the selection of DNA region preferred for analysis is one of the most important points in success of the study in an aspect of providing the most accurate and consistent results. Furthermore, the abilities of species identification and separation of regions selected can show variability in plant groups. For this reason, it is necessary to reveal the properties of preferred DNA sequence regions in different plant groups. The use of trnL/trnF IGS sequences is strongly recommended in taxonomical and phylogenetic evaluation of the genus *Potentilla*.

We aimed to evaluate the taxonomy and the phylogenetic relationships of the genus *Potentilla* by using as many taxa as possible. In this concept, the result of the present study using trnL/trnF IGS sequence information support the presence of five clades (Anserina, Argentea, Alba, Fragarioides, Reptans) and an extra clade (Himalayan).

### Acknowledgement

The author would like to thank to NCBI for sequence information used in this study and the authors sharing the sequence information in NCBI.

**Ethics Committee Approval:** Since the article does not contain any studies with human or animal subject, its approval to the ethics committee was not required.

**Data Sharing Statement:** The author confirm that the data supporting the findings of this study are available within the supplementary material of the article.

**Conflict of Interest:** The authors have no conflicts of interest to declare.

**Funding:** The author declared that this study has received no financial support.

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