Classification of Turkish *Plantago* L. Species Using Numerical Taxonomy

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Abstract: Twenty-three *Plantago* L. taxa belonging to 2 subgenera (*Euplantago* Harms and *Psyllium* (Juss.) Harms) were classified by numerical taxonomic methods. A total of 36 morphological and ecological characters were utilised for numerical taxonomic analysis of *Plantago* taxa. The classification obtained by numeric taxonomic methods was compared to that obtained by conventional methods. In general, both of the classifications agreed well, except for the section *Hymenopsyllium* Pilger being clustered under the subgenus *Psyllium* contrary to the conventional classification.

Key Words: Plantago, Numerical Taxonomy, Turkey, Discriminant Function Analysis

Türkiye Plantago L. Türlerinin Sayısal Taksonomi Kullanılarak Sınıflandırılması

Özet: Bu çalışmada Türkiye'de dağılım gösteren *Euplantago* Harms ve *Psyllium* (Juss.) Harms altcinslerine ait 23 *Plantago* taksonu sayısal taksonomi yöntemleri kullanılarak sınıflandırılmıştır. *Plantago* taksonlarının numerik taksonomik analizi için toplam 36 morfolojik ve ekolojik karakter kullanılmıştır. Sayısal taksonomik yöntemler sonucunda elde edilen sınıflandırma geleneksel sınıflandırma ile karşılaştırılmıştır. Genelde iki sınıflandırma uyum göstermekle birlikte, *Hymenopsyllium* Pilger seksiyonu geleneksel sınıflandırmanın aksine *Psyllium* altgenusu ile kümelenmiştir.

Anahtar Sözcükler: Plantago, Sayısal Taksonomi, Türkiye, Ayrışım Fonksiyon Analizi

Introduction

Plantago L. species belong to the family *Plantaginaceae*, order *Plantaginales*, superorder *Asteridae* and subclass *Dicotyledoneae*. The name comes from the Latin "planta", meaning "sole of the foot", a reference to the broad leaves lying touching the ground that are found in basal rosettes in some species.

Plants in the genus *Plantago*, more commonly known as plantains, have been considered valuable for their herbal properties for centuries. In the Gray Herbarium Index (Anonymous, 1968), around 200 *Plantago* species are named, along with 56 subspecies, 33 forms, 1 subform, 188 varieties and 9 subvarieties. In total 483 *Plantago* taxa were named worldwide. Nineteen sections were described in 2 subgenera, namely *Euplantago* and *Psyllium*. Only one section, *Psyllium*, was found in the subgenus *Psyllium*. All other sections were clumped in the subgenus *Euplantago* (Table 1) (Pilger, 1937).

The genus *Plantago* contains 21 species distributed throughout Turkey (Tutel, 1971b, 1982). The genus is of considerable importance in terms of conservation studies and biological diversity. Tutel (1993) listed 23

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Table 1. Plantago subgenus and secuons in the world (Pliger, 1957).	Table 1.	Plantago subgenus a	and sections in the	world (Pilger,	1937).
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Subgenus: Euplantago	Subgenus: Psyllium
Sections:	Section:
1- <i>Plantag</i> o*	19- Psyllium*
2- Micropsyllium	-
3- Paleopsyllium	
4- Holopsyllium	
5- Oliganthos	
6- Microcalyx	
7- Coronopus*	
8- Oreophytum	
9- Novorbis	
10- Mesembrynia	
11- Lamprosantha*	
12- Eremopsyllium	
13- Oreades*	
14- Gentianoides*	
15- Bamphula	
16- Arnoglossum*	
17- Leucopsyllium*	
18- Hymenopsyllium*	

* present in Turkey

Plantago taxa found in Turkey in the "Flora of Turkey". Among these 23 *Plantago* taxa, 7 were important in environmental conservation studies. According to the "Red Data Book" by IUCN (1982) 2 species were endemic to Turkey, 5 species were rare, 2 of them were in danger and 1 species was not known well. Others show a similar dispersal all over Turkey.

In the "Flora of Turkey", Tutel (1982) described *Plantago* L. species in 9 sections and 23 taxa in Turkey. Later the section *Plantago* L. was revised and, within this section, 2 subspecies were described: *Plantago major* L. subsp. *major* and P. *major* subsp. *intermedia* (Gilib.) Lange. In the section *Coronopus* DC. *Plantago coronopus* was divided into *P. coronopus* L. subs*P. coronopus* and *P. coronopus* L. subs*P. coronopus* and *P. coronopus* L. subsp. *Plantago gentianoides* Pilger, one subspecies, *Plantago gentianoides* Sm. subsp. gentianoides, was described instead of *Plantago gentianoides* Sm. (Tutel, 1993) (Table 2).

Two species, *Plantago anatolica* Tutel et R.Mill (Tutel et al., 1980) and *Plantago euphratica* Decne. ex Barnéoud (Tutel, 1978), were endemic to Turkey and categorised as rare and endangered (Lucas and Synge, 1978; Ekim et al., 2000). According to the IUCN Plant Red Data Book (Lucas and Synge, 1978), *P. crassifolia* and *P. squarrosa*

were vulnerable, and *P. albicans*, *P. loeflingii* and *P. sempervirens* were rare in Turkey; however, there is not much information about *P. argentea*.

Medical importance of some Plantago species:

Some of the *Plantago* species were used as medicines and some as vegetables (Steinmetz, 1954; Baytop, 1984). For example:

Plantago major:

All of the parts can be used for pharmaceutical purposes. The name of the drug was *Herba Plantaginis majoris*, and the dried green parts were used for asthma and toothache (Baytop, 1984). The anti-toxic, anti-inflammatory and expectorant characteristics suggest why the plant is useful in lung disorders. The leaves of this species are used in salads in Eastern Anatolia.

Plantago lanceolata:

This is used as a drug called *Herba Plantaginis lanceolatae* for coughs and asthma; it is used as a tea, syrup or pills. It is also used for treating injuries or opening pustules (Baytop, 1984). *Plantago lanceolata* contains iridoid glycosides and phenols and has mucilage rich anti-inflammatory activity. Its leaves are traditionally used topically as an adjunctive, emollient, and itch-relieving treatment in dermatological conditions, and in cases of eye irritation.

Plantago afra:

This species known to be *Semen Psyllii* or *Semen Plantaginis* in medicine. Their seeds have the following effects: they are used for softening in chronic astringency. At the same time they are used in the silk industry and in silk production (Baytop, 1984). Medicinal preparations are made from the seeds or the seeds are taken orally; preparations based on *Plantago afra* are categorised as bulk laxatives. Their effect is purely mechanical and linked to their mucilage: the polysaccharide macromolecules absorb a large volume of water and form a gel.

Numerical taxonomy has been applied earlier in the classification of plant taxa in Turkey (see Togan et al., 1983; Kence, 1988, Kence et al., 1988, Doğan et al., 1992). In this study, we tried to classify 23 *Plantago* taxa on the basis of morphological and ecological characters by numeric taxonomic methods and compared the resulting classification with that of traditional methods.

Table 2. *Plantago* taxa belonging to 9 sections distributed in Turkey and their IUCN status.

GENUS	PLANTAGO
Subgenus: <i>Euplantago</i> Harms	Subgenus: Psyllium (Juss.) Harms
 Sect. 1. Plantago L. 1. P. major L. subsp. major 2. P. major L. subsp. intermedia (Gilib.) Lange Sect. 2. Coronopus DC. 3. P. coronopus L. subsp. coronopus 4. P. coronopus L. subsp. commutata (Guss.) Pilger 5. P. crassifolia Forsskål "vulnerable". 6. P. maritima L. 7. P. holosteum Scop. 	 Sect. 9. <i>Psyllium</i> (Juss.) Barnéoud 19. <i>P. squarrosa</i> Murray "vulnerable". 20. <i>P. scabra</i> Moench 21. <i>P. afra</i> L. 22. <i>P. sempervirens</i> Crantz "rare". 23. <i>P. euphratica</i> Decne. ex Barnéoud "Endemic, rare, endangered".
Sect. 3. <i>Lamprosantha</i> Decne. 8. <i>P. media</i> L.	
Sect. 4. <i>Oreades</i> Decne. 9. <i>P. atrata</i> Hoppe	
Sect. 5. <i>Gentianoides</i> Pilger 10. <i>P. gentianoides</i> Sm. subsp. <i>gentianoides</i> 11. <i>P. anatolica</i> Tutel et R.Mill "Endemic, Rare, endangered",	
Sect. 6. Arnoglossum Decne. 12. P. lanceolata L. 13. P. argentea Chaix "not well known". 14. P. lagopus L.	
Sect. 7. <i>Leucopsyllium</i> Decne. 15. <i>P. albicans</i> L. "rare" 16. <i>P. loeflingii</i> L. "rare"	
Sect. 8. <i>Hymenopsyllium</i> Pilger 17. <i>P. cretica</i> L. 18. <i>P. bellardii</i> All.	

Materials and Methods

The ecological and morphological characteristics of the genus *Plantago* (Tutel, 1971a) used in this study were grouped as follows:

Altitude at which species are found

Formation types

Indumentum properties

Qualitative and quantitative leaf properties

Scape characteristics

Bract characteristics

Spike characteristics

Flower organ characteristics

Fruit characteristics

Seed characteristics

During the data preparation, one of the authors (Betûl Tutel) also used materials from different herbaria (ISTF, ATA, EGE, DUF, ANK, ISTE and ISTO) in Turkey and (E) in Scotland. The data matrix formed from 36 characters (Table 3, Appendix 1) belonging to *Plantago* taxa was standardised, in other words, the characters were transformed and the distribution of all the characters were converted into new distributions with mean 0 and standard deviation 1. Similarity coefficients were calculated and phenograms were constructed using the UPGMA method in the NTSYS-pc package (Rohlf, 1992). According to the cophenetic correlation coefficient, the phenogram that causes the least distortion to the original data was chosen. Discriminant function analysis (DFA) (Canonical correlation analysis) was performed using the

		states used in	numerical taxonomic analysis.
1	ALTITUDE OF SPECIES Present at sea level (0) Present between sea level and 1000 m (1) Present between 1000-2000 m (2) Present >2000 m (3)	22	LEAF CHARACTERISTICS Leaf is not one of the below (0) Leaf linear (1) Leaf linear-lance (2) Leaf lanceolate (3)
2	FORMATION TYPES Dwarf shrub (0) Not dwarf shrub (1)		Leaf narrow elliptic (5) Leaf elliptic-ovate, rotundate-ovate (6) Leaf spatulate-ovate (7)
3	Not form cushion (0)	23	Leaf length (cm)
1	INDUMENTUM CHARACTERISTICS	24	Leaf width (cm)
4	Glandular hairs not present on the body (0) Glandular hairs not present on the body (1) Dense long bairy (0)	25	Leaf vein number
J	Not dense long hairy (1)	26	Leaf base cordate (0) Leaf base not cordate (1)
6	SCAPE CHARACTERISTICS Scapes without sulcate (0) Scapes with mild sulcate (1)	27	Leaf with petiole (0) Leaf without petiole (1)
	BRACT CHARACTERISTICS	28	Leaf alternate (0) Leaf not alternate (1)
7 8	Apex of bract is caudate or long acuminate (0) Apex of bract is not caudate or long acuminate (1) Bract length (mm)	29	SPIKE CHARACTERISTICS Peduncle length (cm)
9	Upper bract not purple (1)	30	Spike length (cm)
10	FLOWER STRUCTURE Anterior sepals are united (0) Anterior sepals are not united (1)	31	Spike cylindrical (1) Spike ovoid (2) Spike conic (3)
11 12	Anterior sepals are asymmetric (0) Anterior sepals are not asymmetric (1) Posterior sepals are winged (0) Posterior sepals are not winged (1)	32	FLOWERING TIME First flowering time February (1) First flowering time March (2)
13 14	Anterior sepal length (mm) Petal bright (0) Petal not bright (1)		First flowering time April (3) First flowering time May (4) First flowering time June (5)
15	Petal length less than 1 mm (0) Petal length greater than 1 mm (1)	22	First flowering time July (6)
10	Corolla tube hairy (1) Corolla tube hairy (1)	22	Last flowering time June (1) Last flowering time June (2)
18	Corolla tube not wrinkled (1) Anther purple (0)		Last flowering time Suly (3) Last flowering time September (5)
19	Anther not purple (1) Anther big apiculate (0) Anther not big apiculate (1)		Last flowering time October (6) Last flowering time November (7) Last flowering time December (8)
20	FRUIT CHARACTERISTICS Fruit ovoid (1)	34	SEED CHARACTERISTICS Seeds trigonous (0) Seeds not trigonous (1)
	Fruit conic (2) Fruit ellipsoid (3)	36	Seed number
	Fruit semi-global (4) Fruit global (5) Fruit ovoid-conic (6) Fruit ovoid-ellipsoid (7) Fruit conic-ellipsoid (8)	36	Seed length (mm)
21	Fruit length (mm)		

Table 3. List of characters and character states used in numerical taxonomic analysis.

SYNTAX package for the same variables (Podani, 1993 SYNTAX-pc: Computer programs for multivariate data analysis in ecology and systematics. Version 5.0. Budapest.) except for the all or none traits (namely variables coded as 1 or 0). Prior to DFA, the data were subjected to ANOVA (Sokal and Rohlf, 1981).

Results

Numerical taxonomic analysis of the genus *Plantago* in Turkey was carried out using 36 ecological and morphological characteristics (Table 3). The results of the numerical classification were compared to what is already known about *Plantago* sections distributed in Turkey.

Principle Component Analysis:

Principle component analysis (PCA) utilising correlation matrices among the characters resulted in 5

groups. The distribution of taxa among the groups is as follows: *P. major* subsp. *major* and *P. major* subsp. *intermedia* are in group 1; *Plantago coronopus* subs*P. coronopus* and *Plantago coronopus* subsp. *commutata* are in group 2; *Plantago media*, *Plantago anatolica*, *Plantago lanceolata*, *Plantago gentianoides* subsp. *gentianoides*, and *Plantago lagopus* are in group 3; *Plantago crassifolia*, *Plantago maritima*, *Plantago holosteum*, *Plantago argentea*, *Plantago atrata*, *Plantago loeflingii*, and *Plantago albicans* are in group 4; *Plantago cretica*, *Plantago bellardii*, *Plantago squarrosa*, *Plantago scabra*, *Plantago afra*, *Plantago sempervirens* and *Plantago euphratica* are in group 5 (Figure 1).

The first 3 principal components explained 49.10% of total morphometric variation. The first axis explained 26.26\%, the second axis 11.89% and the third axis 10.95% of total variation (Table 4).



Figure 1. Scatter diagram of *Plantago* taxa on the first 2 principle components.

Table 4.	Eigenvalues	of 1	10 vecto	rs, p	percent	eigenvalues	and	cumulative	percent	variation
	explained by	each	n vector	(eige	envalues	>1 shown in	n Tab	ole).		

Vector	Eigenvalue	Percent Eigenvalue	Cumulative Percent
1	9.455	26.26	26.26
2	4.280	11.89	38.15
3	3.942	10.95	49.10
4	3.060	8.50	57.60
5	2.995	8.32	65.92
6	2.007	5.57	71.49
7	1.735	4.82	76.31
8	1.369	3.80	80.11
9	1.221	3.39	83.50
10	1.031	2.86	86.36

When the sections were imposed on the 2-dimensional scatter (Figure 2) only the section *Oreades* (*P. atrata*) was positioned within the section *Coronopus* and the section *Leucopsyllium* (*P. albicans* and *P. loeflingii*) was positioned between 2 subgenera of *Plantago*.

PCA indicated that different sets of characters displayed high loadings in clustering *Plantago* taxa in 3 axes (Table 5). Corolla tube shape, leaf length and width, leaf vein number and spike length had high loadings on the first axis. Altitude, anterior sepal shape, fruit shape, first flowering time and seed length characteristics were highly loaded on the second axis. Posterior sepal shape, petal length, corolla tube hairy, corolla tube texture (wrinkled or smooth), anther colour, and seed shape

were the characters showing high loadings on the third axis.

The phenogram resulting from the UPGMA clustering of similarity matrix is presented in Figure 3. Similar types of grouping with PCA analysis were obtained; however, in general 2 major groups were formed (at -0.100 phenon line); one of the major groups contained *Euplantago* sections and the second group consisted of *Hymenopsyllium* and *Psyllium* sections. In the latter group, the section *Psyllium* was well separated from the other sections. Two species belonging to the section *Leucopsyllium* were positioned between the sections *Euplantago* and *Psyllium* as was seen in PCA. When the phenogram resulting from the UPGMA clustering (Figure



Figure 2. Scatter diagram of 9 sections in the genus Plantago.

Table 5. Summary of character (numbers correspond to character list in Table 3) loadings (highest 5 loadings) on the first 3 axes.

Characters	Axis 1	Axis 2	Axis 3
1- Altitude		0.598	
10- Anterior sepal united		-0.578	
12- Posterior sepals winged			-0.615
15- Petal length			0.448
16- Corolla tube hairy			0.767
17- Corolla tube wrinkled	-0.746		0.482
18- Anther colour			-0.649
20- Fruit shape		-0.619	
23- Leaf length	-0.816		
24- Leaf width	-0.899		
25- Number of leaf veins	-0.811		
30- Spike length	-0.836		
32- First flowering time		0.553	
34- Seed shape			0.448
36- Seed length		0.695	



Figure 3. UPGMA dendogram showing the relationships within the genus *Plantago*. (○: Arroglossum, ●: Leucopsyllium, □: Gentianoides, ■: Plantago, Δ: Oreades, ▲: Lamprosantha, +: Coronopus, *: Pysllium, ⊞: Hymenopsyllium)

3) was studied, the previously described sections *Plantago*, *Coronopus*, *Arnoglossum*, *Hymenopsyllium* and *Psyllium* at the 0.100 phenon line were visualised.

Discriminant Function Analysis

Characters used in the discriminant function were analysed with ANOVA. Out of 15 characters 6 (bract length, sepal length, leaf length, number of leaf veins, peduncle length, and spike shape) were significantly different (Table 6).

The same data set was later subjected to DFA to find the relative position of 2 sections namely, *Hymenopsyllium* and *Leucopsyllium*. Both were positioned close to the subgenus *Psyllium* in DFA. In particular, *Hymenopsyllium* was very close to *Psyllium*, whereas *Leucopsyllium* was far from this group (Figure 4).

Total variation in DFA was explained by 3 components. The first canonical variate explained most of

the variation (73.36%). The second and third variates explained the rest of the variation, 19.61% and 7.03%, respectively (Table 7).

On the first axis *Euplantago* and the rest of the groups (*Psyllium*, *Hymenopsyllium* and *Leucopsyllium*) were separated. On the second axis, however, the section *Leucopsyllium* was separated from *Psyllium* and *Hymenopsyllium*. The significantly different characters were also the ones which have high loadings compared to other characters used in DFA. Six characters (fruit length, leaf width, number of leaf veins, spike shape, first flowering time and seed length) on the first axis, 5 (fruit shape and length, leaf width, number of leaf veins and spike shape) on the second axis and 6 (sepal length, leaf width, number of leaf veins, spike shape, first flowering time and seed length) had high loadings on the third axis. It is noted that the leaf width, number of leaf veins and spike shape characters were highly loaded on all 3 axes.

Table 6. Summary of analysis of variance of 16 *Plantago* characters.

Variables	Among Group SSQ	Within Group SSQ	F ratio	
1 - Bract length	17.83	1.71	10.428***	
2- Sepal length	1.82	0.41	4.437**	
3- Fruit shape	3.27	4.36	0.750ns	
4- Fruit length	0.10	0.32	0.301ns	
5- Leaf shape	4.73	3.75	1.259ns	
6- Leaf length	121.73	27.85	4.372**	
7- Leaf width	5.34	2.87	1.858ns	
8- Number of leaf veins	12.27	2.51	4.886**	
9- Peduncle length	393.16	83.88	4.687**	
10- Spike length	44.29	26.11	1.696ns	
11- Spike shape	1.31	0.41	3.233*	
12- First flowering time	1.41	1.55	0.912ns	
13- Second flowering time	9.55	5.17	1.847ns	
14- Number of seeds	11.1	20.26	0.563ns	
15- Seed length	0.14	0.30	0.475ns	

ns not significant

*P < 0.05; **P < 0.01;***P < 0.001



Figure 4. Discriminant function analysis of the subgenera *Euplantago* and *Psyllium*, and the sections *Hymenopsyllium* and *Leucopsyllium*. Triangles: *Psyllium*, Squares: *Leucopsyllium*, Circles: *Hymenopsyllium*, and Diamonds: *Euplantago* subgenera.

Eigenvalue	Eigenvalue as percentages
10.86 2.90	73.36 19.61
	Eigenvalue 10.86 2.90 1.04

Table 7. Eigenvalues of the first 3 canonical variates and explained percent variation by these canonical variates in discriminant function analysis.

Discussion

The existence of 2 *Plantago* subgenera, *Euplantago* and *Psyllium*, was corroborated by the numerical methods used in this study. It seems that the selected characters reflect the taxonomic relationships well. However, the classification obtained by UPGMA shown in the dendogram did not agree with the traditional classification of the sections *Lamprosantha*, *Gentianoides*, *Oreades* and *Leucopsyllium* (Figure 1). The latter sections could not be separated as the others could.

The section *Hymenopsyllium* section was originally classified within the subgenus *Euplantago*. However, both in UPGMA clustering and PCA, *Hymenopsyllium* was clumped with the section *Psyllium*. In an additional DFA, 2 different subgenera (*Euplantago* and *Psyllium*) were defined as 2 groups and *Hymenopsyllium* as the third group (only including metric variables) and the fourth group was *Leucopsyllium*. In this analysis, *Hymenopsyllium* (and *Leucopsyllium*) was far from the

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subgenus *Euplantago*. This was a new finding in the taxonomy of the genus *Plantago*. This could result from giving more weight to some of the traits in traditional taxonomy. Numerical taxonomy uses a large number of characters and gives equal weight to all of the characters used to construct a classification. Thus, the classification obtained by numerical taxonomy gave more information than did conventional methods on the relationship between the *Plantago* taxa used in the present study. The results of this study showed the usefulness of numerical methods in resolving the obscured relationships between taxonomic units.

Although this study added new findings to the literature, it is somewhat limited to the known genera, sections, species and subspecies distributed in Turkey. A comprehensive study covering all *Plantago* species seems to be necessary to construct a more satisfactory classification and also it would be much better if further studies use molecular data other than the ones (ecological and morphological characters) used in this study.

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Appendix

Species/subspecies	1	(T)	4	2	9	7	8	6	10 1	112	13	14	15	16	17 1	8 1	9 2(0 21	22	23	24	25	26	27 2	8	е е	31	32	33	34	35	36
P. major subsp. major	0	-	-	-	-	-	1.8	0	-	-	2.3	-	0	0	-	_	9	m	9	20	6.25	7	0	-	1 16	ы N		Ŋ	4	0	10	1.5
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P. cretica	-	-	-	0	0	-	6.3	0	1	-	3.5	-	-	0	0	_	ш 0	2	2	5.75	0.3	-	-	0	1 0.7	75 0.	7 2	m	-	-	N	1.8
P. bellardii	-	-	-	0	0	-	5.3	0	1	-	3.5	-	-	0	0	_	ш 0	2	2	2.75	0.25	÷	-	0	1	÷.	m N	N	m	-	2	1.5
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P. sempervirens	-	1	-	-	0	0	7.5	0	-	-	5.3	-	-	0	0	_	N.	ŝ	-	3.75	0.1	-	-	0	2 0	5 0.	9	N	N	-	N	2.2
P. euphratica	с м	1	-	-	0	-	4.8	0	1	-	4	-	-	0	0	_	9	5	-	5.25	0.23	m	-	0	0 3.7	75 2	-	9	m	-	N	с. З