

Numerical Taxonomic Analysis on Some *Lepidium* L. taxa (Brassicaceae) from Turkey

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Please cite this article as: Bona M. Numerical Taxonomic Analysis on Some *Lepidium* L. taxa (Brassicaceae) from Turkey. Eur J Biol 2020; 79(2): 132-143. DOI: 10.26650/EurJBiol.2020.0020

ABSTRACT

Objective: This study reveals the relationship between *Lepidium campestre, L. spinosum, L. sativum* ssp. *sativum, L. sativum* ssp. *spinescens, L. ruderale, L. virginicum, L. perfoliatum, L. vesicarium, L. caespitosum, L. pumilum, L. cartilagineum, L. latifolium, L. lyratum, L. graminifolium, to determine the effectiveness of the characters used in taxonomic classification, and to help solve taxonomical problems of this large genus at the specific and intraspecific levels by comparing the numerical results with classical taxonomic classification.*

Materials and Methods: This numerical taxonomic study is based on morphological data that come from a wide range of herbarium material and material collected in the wild. For the analyses, 14 taxa were studied. A range of characteristics of sepal, petal, stamen, silicle, pedicel, septum, stigma, and sinus that are considered to be taxonomically important in the genus were investigated. Morphological data, 90 character states, which belong to 55 characters scored as the binary state for each taxon were used in unweighted pair–group method using arithmetic averages and principle components analyses.

Results: *L. caespitosum, L. pumilum* and *L. cartilagineum* are recognised at species rank, not subspecies or varieties. *L. sativum* ssp. *sativum* and *L. sativum* ssp. *spinescens* should be evaluated as two subspecies not synonyms of *Lepidium sativum*. The most effective characters for the delimitation of the studied taxa are seed length, the habitus of plant, sepal length, septum length, seed wings, the presence of swelling leaf residues on the base of the plant, pedicel length.

Conclusion: Numerical analysis studies based on morphological data on *Lepidium* taxa growing in Turkey are a useful tool for solving the taxonomic problems of taxa belonging to the genus *Lepidium*.

Keywords: Brassicaceae, Lepidium, Numerical Taxonomy, PCA, UPGMA

INTRODUCTION

The Brassicaceae is one of the largest families that has major scientific and economic importance (1,2). There are 3660 species and 321 genera in 49 tribes in the family (3). The classification of the Brassicaceae is problematic because the characters traditionally used are variable, even within genera, and may not support natural groups (4).

The first comprehensive taxonomic approach of the Brassicaceae is based on two characteristics: the position of the radicle with cotyledons in the seed and fruit type (5). After almost a century, tribal and

subtribal classification revised mainly based on fruit characters and seed morphology (6). Brassicaceae is represented by 555 species and 91 genera in the Flora of Turkey (6-9). Generic delimitation has been changed according to molecular phylogenetic studies focused on Brassicaceae in the last two decades (10-12). Recent studies show that Turkey is a centre of diversity with 660 taxa belonging to 91 genera, including 571 species, 65 subspecies, and 24 varieties (13).

The genus *Lepidium* L. is primarily distributed in temperate and subtropical regions (14). The genus includes 250 species and one of the largest genera in the Brassicaceae (3). *Cardaria* Desv. was defined as



Corresponding Author: Mehmet Bona E-mail: mehmetbona@gmail.com Submitted: 30.04.2020 • Revision Requested: 05.05.2020 • Last Revision Received: 11.07.2020 • Accepted: 25.09.2020 • Published Online: 22.12.2020

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a section of *Lepidium* (15), later it was accepted as a separate genus (7,14,16). In light of molecular research not only *Cardaria* but also *Coronopus*, *Stroganowia*, *Winklera* Regel, and *Stubendorffia* Schrenk ex Fisch., C.A. Mey. & Avé-Lall. have been classified within *Lepidium* sensu lato (10,12,17-20).

Cardaria, Coronopus, and *Stroganowia* are represented by five species in Turkey: *L. coronopus* (L.) Al-Shehbaz and *L. didymium* L. (formerly *Coronopus*), *L. draba* L. and *L. chalepense* L. (formerly *Cardaria*), *Stroganowia leventii* V.I. Dorofeev (3,21-23)

Lepidium sensu stricto (excluding *Cardaria*, *Coronopus*, and *Strogonovia*) includes 13 species and 2 subspecies in Turkey (7,8,24).

Numerical taxonomy is a grouping method that groups to a taxonomic unit based on their character states using statistical methods (25). In this study, the morphological features of *Lepidium* s.str. taxa distributed in Turkey were examined and their diagnostic characteristics were determined. Detailed measurements based on these characters were used in numerical taxonomic analyses.

This study reveals the relationship between *L. campestre, L. spinosum, L. sativum* ssp. *sativum, L. sativum* ssp. *spinescens, L. ruderale, L. virginicum, L. perfoliatum, L. vesicarium, L. caespitosum, L. pumilum, L. cartilagineum, L. latifolium, L. lyratum, L. graminifolium, to determine the effectiveness of the characters used in taxonomic classification, and to help solve taxonomical problems of this large genus at specific and intraspecific ranks by comparing the numerical results with classical taxonomic classification.*

MATERIALS AND METHODS

The flowering and fruiting material of the genus *Lepidium* were collected from different parts of Turkey during the period May-August 2008, 2009 and 2010. Specimens were collected from as many different parts of the distribution area of the genus as possible in order to thoroughly examine variation patterns. The specimens collected were kept at the Istanbul Uni-

versity, Department of Pharmaceutical Botany Herbarium (ISTE) and compared with the collections of ANK, E, GAZI, HUB, ISTF, ISTE, K, and VAN herbaria.

A range of characteristics of sepal, petal, stamen, silicle, pedicel, septum, stigma, and sinus that are considered to be taxonomically important in the genus were investigated. For these investigations, all parts of the specimens were photographed using MOTIC 2000 camera stereo microscope system, and then measured by using Motic Image Plus 2.0-program. These measurements were used for numerical analyses. For the analyses, 14 taxa (Table 1) and 90 character states, which belong to 55 characters, were scored as the binary state for each taxon (Table 2). To investigate the relationships between the studied taxa,

Table 1. Studied taxa list.			
OTU1	L. campestre		
OTU2	L. spinosum		
OTU3	L. sativum ssp. sativum		
OTU4	L. sativum ssp. spinescens		
OTU5	L. ruderale		
OTU6	L. virginicum		
OTU7	L. perfoliatum		
OTU8	L. vesicarium		
OTU9	L. caespitosum		
OTU10	L. pumilum		
OTU11	L. cartilagineum		
OTU12	L. latifolium		
OTU13	L. lyratum		
OTU14	L. graminifolium		

Table 2. Character list.			
No	Characters	Character states	
C1	Fruit length maximum value	Shorter or longer than 3 mm	
C2		Shorter or longer than 4 mm	
C3		Shorter or longer than 5 mm	
C4	Fruit length minimum value	Shorter or longer than 2 mm	
C5		Shorter or longer than 3 mm	
C6	Fruit width maximum value	Shorter or longer than 4 mm	
C7		Shorter or longer than 3 mm	
C8		Shorter or longer than 4 mm	

Table 2. Continue			
C9	Fruit width minimum value	Shorter or longer than 2 mm	
C10		Shorter or longer than 3 mm	
C11	Pedicel length maximum value	Shorter or longer than 4 mm	
C12		Shorter or longer than 6 mm	
C13	Pedicel length minimum value	Shorter or longer than 2 mm	
C14		Shorter or longer than 3 mm	
C15	Septum length maximum value	Shorter or longer than 2.5 mm	
C16		Shorter or longer than 3.5 mm	
C17	Septum length minimum value	Shorter or longer than 2 mm	
C18		Shorter or longer than 3 mm	
C19	Septum width maximum value	Shorter or longer than 0.7 mm	
C20	Septum width minimum value	Shorter or longer than 0.5 mm	
C21	Stigma length maximum value	Shorter or longer than 0.3 mm	
C22		Shorter or longer than 0.5 mm	
C23	Stigma length minimum value	Shorter or longer than 0.25 mm	
C24	Stigma width maximum value	Shorter or longer than 0.25 mm	
C25		Shorter or longer than 0.35 mm	
C26	Stigma width minimum value	Shorter or longer than 0.15 mm	
C27		Shorter or longer than 0.25 mm	
C28	Sepal length maximum value	Shorter or longer than 1.5 mm	
C29	Sepal length minimum value	Shorter or longer than 1 mm	
C30	Sepal width maximum value	Shorter or longer than 1 mm	
C31	Sepal width minimum value	Shorter or longer than 0.8 mm	
C32	Petal length maximum value	Shorter or longer than 2.5 mm	
C33	Petal length minimum value	Shorter or longer than 2 mm	
C34	Petal width maximum value	Shorter or longer than 1 mm	
C35		Shorter or longer than 1.6 mm	
C36	Petal width minimum value	Shorter or longer than 0.8 mm	
C37	Stamen length maximum value	Shorter or longer than 2 mm	
C38	Stamen length minimum value	Shorter or longer than 1.5 mm	
C39	Seed length maximum value	Shorter or longer than 2 mm	
C40		Shorter or longer than 2.5 mm	
C41	Seed length minimum value	Shorter or longer than 1.5 mm	
C42		Shorter or longer than 2 mm	
C43	Seed width maximum value	Shorter or longer than 1 mm	

Table 2. Continue				
C44	Seed width minimum value	Shorter or longer than 1 mm		
C45	Basal leaves length minimum value	Shorter or longer than 15 cm		
C46	Basal leaves width minimum value	Shorter or longer than 4 cm		
C47	Basal leaves pedicel length minimum value	Shorter or longer than 4 cm		
C48	Plant length maximum value	Shorter or longer than 50 cm		
C49	Life time	Perennial or not		
C50	Habitus	Erect or not		
C51		Single stemmed or not		
C52		Many stemmed or not		
C53	Plant surface	Basal part naked or not		
C54		Upper part naked or not		
C55		Plant waxy or not		
C56	Swelling nodes existence	Present or absent		
C57	Petiola remains existence	Present or absent		
C58	Basal leaves shape	Lyrate basal leaves present or absent		
C59		Pinnatisect basal leaves present or absent		
C60		2-pinnatisect basal leaves present or absent		
C61		3-pinnatisect basal leaves present or absent		
C62		Lanceolate basal leaves present or absent		
C63		Ovate basal leaves present or absent		
C64		Linear basal leaves present or absent		
C65		Lanceolate basal leaves present or absent		
C66	Cauline leaves shape	Pinnatisect cauline leaves present or absent		
C67		2-pinnatisect cauline leaves present or absent		
C68		Lanceolate cauline leaves present or absent		
C69		Ovate cauline leaves present or absent		
C70	Leaves surface	Both surface has trichome or not		
C71		Glabrous above has trichome below		
C72	Stipul existence	Present or absent		
C73	Sepal center color	Purple or yellow		
C74	Sepal margin color	White or yellow		
C75	Sepal surface	Glabrous or not		
C76	Petal color	White or yellow		
C77	Stamen position	Equal or not		
C78	Stamen number	2 or not		

Table 2. Continue			
C79		4 or not	
C80		6 or not	
C81	Pedicel position	Erect or not	
C82	Pedicel surface	Glabrous or not	
C83	Silicle shape	Ovate or not	
C84		Orbicular or not	
C85		Oblong or not	
C86	Silicle wing existence	Present or absent	
C87	Silicle surface	Glabrous or not	
C88	Stilus position	Exceeding sinus or not	
C89	Seed wing existence	Present or absent	
C90	Seed shape	Ovate or not	

two types of numerical analyses were performed using NTSYS– pc 2.1 software (26). The first analysis was the Clustering Analysis (CA) and the second analysis was the Principle Components Analysis (PCA).

RESULTS

The result of the CA is the UPGMA (Unweighted Pair–Group Method using Arithmetic Averages) dendrogram (Figure 1).

The UPGMA dendrogram explains the numerical relationships of the taxa studied. According to the results; *L. sativum* ssp. *sativum* and *L. sativum* ssp. *spinescens* are the closest pair of taxa. *L. spinosum* is grouped with these two taxa and *L. campestre* is the closest species to the group. *L. lyratum* and *L. graminifolium* are the second closest pair of taxa and related to *L. latifolium*. These taxa are grouped with *L. ruderale* and *L. virginicum*. The third closest pair of taxa are *L. caespitosum* and *L. pumilum*, which are grouped with *L. cartilagineum*.

PCA analysis results were coherent with the CA analysis (Figure 2). According to PCA analysis *Lepidium sativum* subsp. *sativum* and *L. sativum* subsp. *spinescens* the closest studied taxa and these taxa were grouped with *L. campestre* and *L. spinosum*. *L. lyratum* and *L. graminifolium* showed a close relation again and these taxa are grouped with *L. ruderale*, *L. virginicum*, *L. latifolium* like they were grouped in UPGMA dendrogram. *L. caespitosum*, *L. pumilum* and *L. cartilagineum* were also grouped. *L. pumilum*





has an equal distance from *L. caespitosum* and *L. cartilagineum*. The pictures of the studied taxa are given in Figures 3-5.

PCA analysis reduces numerous characters to a few number components. Table 3 shows the component's Eigen value and percentage of Eigen value. Eigen vector values of the first 4 components are given in Table 4. The first two components explain 39.61% of the total variation. According to the results of PCA analysis, the first five most effective characters describing the first component are seed length, the habitus of plant, the minimum and maximum length of sepal, and septum length. The first five most effective characters describing the second component are the seeds with or without wings, the presence of swelling leaf residues on the base of the plant, the length of the pedicel, the presence of the plant with a single stem, and the length of the septum.

DISCUSSION

L. spinosum, L. sativum ssp. *sativum* and *L. sativum* ssp. *spinescens* taxa (Figure 3) are clustered in this study and are placed in the section *Lepiacardamon* in classical systematic studies (7). According to the Flora of Turkey (7) *L. campestre*, the only taxon representing the section *Lepia*, is the closest species to the section *Lepiocardamon* (Figure 3). The dendrogram results are compatible with classical taxonomic data in this respect.



Figure 3. A) L. campestre, B) L. spinosum, C) L. sativum subsp. sativum, D) L. sativum subsp. spinescens.



Figure 4. A) L. ruderale, B) L. virginicum.



Figure 5. A) L. perfoliatum, B) L. vesicarium, C) L. caespitosum, D) L. pumilum, E) L. cartilagineum, F) L. latifolium, G) L. lyratum, H) L. graminifolium.

L. sativum is grown as a cultivated plant in many parts of the world. Therefore, it is not easy to draw the boundaries of its geographical distribution. There are different taxonomic approaches in terms of intraspecific classification with the effect of this situation. The species has two subspecies according to the Flora

of Turkey and Flora of Iraq (7,27). In Flora of West Pakistan, the taxonomic level is defined as a variety, not a subspecies (28). According to the revision study conducted in Turkey, because of both the clarity of the morphological differences between the two taxa as well as due to differences in geographical dis-

Table 3. Eigen value and percentage of Eigen value of components.					
Components	Eigen Value	Percentage of Eigen Value	Total Percentage Eigen		
1	21.6700	24.08	24.08		
2	13.9788	15.53	39.61		
3	10.8323	12.03	51.65		
4	8.3728	9.3	60.95		
5	6.4828	7.2	68.16		
6	5.7569	6.39	74.55		
7	4.9996	5.55	80.11		
8	4.4561	4.95	85.06		
9	4.1067	4.56	89.62		
10	3.7017	4.11	93.74		
11	2.5988	2.88	96.62		
12	1.7636	1.95	98.58		
13	1.2713	1.41	100		

Table 4. Component's Eigen vector value.				
	B1	B2	B3	B4
C1	8.1577	1.7598	3.3445	7.6318
C2	7.8622	-3.1628	6.4978	-1.6688
C3	7.3615	-5.525	7.1535	-1.4991
C4	8.1577	1.7589	3.3443	7.6318
C5	7.4498	-5.115	-2.5845	-1.7279
C6	7.4498	-5.115	-2.5845	1.7279
С7	8.1067	6.1259	2.8759	-3.1068
C8	7.0984	-1.7069	2.2081	-3.9225
С9	7.2261	-1.0386	2.8368	1.6263
C10	5.1939	-4.874	1.1398	-1.064
C11	-1.3344	4.5014	4.2963	-1.2953
C12	3.1741	7.5122	4.2598	9.0736
C13	-6.4439	5.5676	3.7611	-1.5546
C14	1.3431	8.4926	-1.4174	-9.391
C15	8.1577	1.7589	3.3443	7.6318
C16	8.2272	-5.4576	9.4618	-2.5207
C17	8.3823	-7.2074	5.6088	-1.4323

Table 4. Continue.				
C18	7.009	-3.8102	-1.0506	-3.4768
C19	7.528	4.4059	2.6295	2.6688
C20	2.801	-1.0181	1.4588	-5.5078
C21	7.528	4.4059	2.6295	2.6688
C22	7.0941	1.9486	-6.6924	-9.7554
C23	5.9917	5.0211	-8.614	1.0578
C24	1.3428	3.1286	-7.9831	3.6451
C25	-1.408	5.7247	2.2524	3.4448
C26	1.051	2.7864	-7.0197	7.8805
C27	-3.313	2.5432	2.5923	5.3754
C28	8.5043	2.5371	-2.1567	-2.4258
C29	8.5043	2.5371	-2.1567	-2.4258
C30	5.5623	5.869	-1.042	-2.678
C31	1.3188	6.2204	-4.0622	-3.1672
C32	6.9054	4.0033	-4.6847	3.6139
C33	3.789	2.8278	-4.1131	5.3833
C34	1.4221	3.924	-3.8543	-1.9705
C35	1.6754	6.7856	3.7243	-1.549
C36	-3.2513	6.5742	-1.7311	1.1972
C37	6.5701	2.4961	-5.0581	-2.4516
C38	4.3287	1.8748	-4.5252	1.7334
C39	8.7965	2.9907	1.1786	1.425
C40	5.6454	-4.5667	1.7145	3.261
C41	8.1577	1.7589	3.3443	7.6318
C42	7.009	-3.8102	-1.0506	-3.4768
C43	8.1577	1.7589	3.3443	7.6318
C44	5.2255	2.7924	3.5573	-2.0302
C45	4.4301	-1.7607	-5.6159	-5.091
C46	3.8871	-6.922	-5.1692	3.2119
C47	3.5547	-2.0308	-3.6233	-5.4515
C48	-2.3959	-4.1107	-4.2069	-7.7936
C49	4.0749	-7.3958	4.1262	-2.0175
C50	8.6528	5.0179	5.8839	-1.687
C51	-4.0749	7.3958	-4.1262	2.0175
C52	4.7507	-6.8027	1.9056	7.5579

Table 4. Continue.				
C53	-1.3428	-3.1286	7.9831	-3.6451
C54	2.3881	5.057	4.4158	-4.327
C55	-2.9336	4.3552	4.4765	-5.122
C56	4.0356	1.1781	2.8518	6.6462
C57	2.1608	8.7231	2.1741	-1.0536
C58	4.4758	2.8737	2.4905	1.7616
C59	-3.9602	5.3022	-3.3933	-2.2854
C60	-1.2301	3.7383	-2.8989	-4.2022
C61	1.0647	-4.7006	-4.6956	-5.4175
C62	2.8898	-5.7685	5.0445	1.737
C63	-4.8731	-3.4476	1.8812	5.6061
C64	-1.1663	-4.6782	1.5965	-4.2597
C65	-1.4111	-4.2019	8.2345	4.1765
C66	-4.7657	2.5251	1.6425	-7.5776
C67	7.7308	-1.9457	-5.1567	-7.1191
C68	-1.6978	-1.2835	3.7654	5.2953
C69	-1.0993	-2.7311	-4.7492	1.0636
C70	2.547	4.2819	-2.3125	4.7474
C71	-4.7657	2.5251	1.6425	-7.5776
C72	-2.6955	-2.2652	-4.9657	4.3641
C73	-2.0825	-3.9243	3.083	4.2735
C74	6.2841	-1.307	5.8645	2.4181
C75	2.1569	-2.6125	2.4595	1.6409
C76	-2.2815	-2.1394	6.5289	3.1386
C77	2.8233	2.3518	-1.0312	6.119
C78	4.2576	3.0021	-4.9163	8.3012
C79	2.0464	2.4789	-3.6733	1.3125
C80	-4.2576	-3.0021	4.9163	-8.3012
C81	-6.4439	5.5676	3.7611	-1.5546
C82	-1.6183	6.0073	4.6361	-6.1571
C83	-2.3005	-3.2699	-3.0227	-5.9666
C84	3.4683	3.9177	6.5477	2.8762
C85	-3.4604	2.2105	1.0309	5.8767
C86	-5.097	-4.4255	-5.8116	-1.3273
C87	-7.2798	-1.711	-5.8173	-3.1014
C88	2.4089	-6.9602	3.6955	7.2506
C89	-5.0479	-9.7737	6.1954	1.0801
C90	-5.9977	-1.4795	2.0557	3.4321

tribution seen between populations, it is stated that the definition should be at the subspecies level. In the Turkey Plant List (Vascular Plants), both subspecies and varieties are listed as a synonym and *L. sativum* is shown as a single species (22). The results of this study support the view that the *L. sativum* species should be better evaluated as two subspecies.

L. latifolium, *L. lyratum*, and *L. graminifolium* (Figure 4) are placed together in section *Lepidium* (7). These species also formed a group in this study and numerical results support the sectional classification. *L. ruderale* and *L. virginicum* (Figure 5), the closest species to the group according to the numerical analysis, are also placed in section *Dileptum* in the Flora of Turkey (7,9).

The last group comprises *L. caespitosum, L. pumilum* and *L. crassifolium. L. caespitosum* (Figure 5) was evaluated as an endemic species in the Flora of Turkey (7). According to Flora of Turkey (7), *L. pumilum* and *L. crassifolium* were represented as two subspecies; *L. cartilagineum* (J. May.) Thell. subsp. *cartilagineum* and *L. cartilagineum* (J. May.) Thell. subsp. *cartilagineum* (Waldst. & Kit.) Thell. Later, Hedge (29) again accepted them as two subspecies but with a new combination. In the Flora of the USSR, they are accepted as separate species (30). These three taxa were listed as subspecies of *L. cartilagineum* by Mutlu (22) while the revision of Turkish *Lepidium* proposed they must be considered as different species (24). Numerical analysis results in this study support the idea of evaluating these three taxa as separate species. It also shows that *L. pumilum* is closer to *L. caespitosum* than to *L. cartilagineum*.

There is a tendency for some of the flower parts to be reduced to the point of absence, and hence flower structure is used in the subgeneric classification in the genus *Lepidium* (31). Flower structure and the characters of vegetative morphology are used in species identification in the genus *Lepidium* (32,33). The results of PCA analysis support the idea that the habitus of plant, the minimum and maximum length of sepal, the presence of swelling leaf residues on the base of the plant, and the presence of the plant with a single stem are important characters.

It has been reported that seed characters tend to have been ignored in *Lepidium*, with the exception of trifid cotyledon of *L. sativum* (31,33). However, the results of the present study showed that seed length, septum length, and the eventual presence and features of the seed wing are diagnostic characteristics for the genus *Lepidium*. This result is coherent with studies using seed characteristics for taxonomic studies in *Lepidium* (31,34,35).

Numerical analysis of *L. sativum* based on 21 morphological traits was performed based on Iranian specimens (36). According to that analysis, the first principal component analysis explained 63.0% of the total variation present in the dataset, besides that, petal length and sepal length and width had the highest positive correlation in PCA analysis (36). The present PCA analysis also shows that the sepal and petal length are two important characteristics that explain the first two components.

CONCLUSION

Numerical analysis studies based on morphological data on *Lepidium* taxa growing in Turkey is a useful tool for solving the taxonomic problems of taxa belonging to the genus *Lepidium*. This study gave significant results as the first step towards more comprehensive studies including more taxa.

Proposed Treatment for Turkish Lepidium L. taxa

Section Lepia (Desv.) DC. L. campestre (L.) Aiton, Hort. Kew. ed. 2, 4: 88 (1812). Section Lepiocardamon Thell. L. spinosum Ard., Animad. Specim. Alt. 2: 34, t. 16 (1764). L. sativum L., Sp. Pl. 2: 644 (1753). subsp. sativum L. sativum L. subsp. spinescens (DC.) Thell. in Vierteljahr. Naturf. Ges. Zürich, 51: 161 (1906) Section Dileptium DC. L. ruderale L., Sp. Pl. 2: 645 (1753). L. virginicum L., Sp. Pl. 2: 645 (1753) Section Lepidium L. perfoliatum L., Sp. Pl. 2: 643 (1753) L. vesicarium L., Sp. Pl. 2: 643 (1753) L. caespitosum Desv. in J. Bot. Agric. 3: 165 & 178 (1815) L. pumilum Boiss. & Bal. in Boiss., Diagn. ser. 2(6): 21 (1859) L. cartilagineum (J. May.) Thell. in Vierteljahr. Naturf. Ges. Zürich 51:173 (1906) L. latifolium L., Sp. Pl. 2: 644 (1753) L. lyratum L., Sp. Pl. 2: 644 (1753).

L. graminifolium L., Syst. Nat. ed. 10, 2: 1127 (1759)

Peer-review: Externally peer-reviewed.

Authors Contributions: Concept: M.B.; Design: M.B.; Supervision: M.B.; Materials: M.B.; Data Collection and/or Processing: M.B.; Analysis and/or Interpretation: M.B.; Literature Search: M.B.; Writing: M.B.; Critical Reviews: M.B.

Conflict of Interest: The authors declare that they have no conflicts of interest to disclose.

Financial Disclosure: There are no funders to report for this submission.

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