Available online at www.garmian.edu.krd

Passer Journal

Passer

Passer 6 (Issue 1) (2024) 115-123

http://passer.garmian.edu.krd/

Delimitation of Iraqi *Scorzonera* L. (Cichorieae, Asteraceae) species in Sulaimani District (MSU), Kurdistan Region, based on morphological characterization

Hamza Rezhin Ghafoor[1], Muhammed Salih Rubar[1*], Ahmad Nariman Salih[1]

[1]*Department of Biotechnology and Crop Science, College of Agricultural Engineering Sciences, University of Sulaimani, Sulaimani, Kurdistan Region, Iraq.*

Received 06 October 2023; revised 22 December 2023;
accepted 23 December 2023; available online 12 February 2024

DOI: 10.24271/PSR.2023.419571.1402

ABSTRACT

The genus *Scorzonera* L. is one of the largest genera of the subtribe Scorzonerinae, tribe Cichorieae, and family Asteraceae. This genus is characterized by complicated variation patterns present among its species. An extensive sampling characterization of macro- and micro-morphology was conducted for Scorzonera species (eight species, including one variety) distributed in the MSU district of the Kurdistan Region-Iraq to clarify inter-sub-generic and interspecies delimitations. We examined all morphological features of the collected samples in detail, along with their anatomical and distribution status. There were several morphological and anatomical characteristics that could be used to distinguish complicated and overlapped species. Analysis of principle components (PCA) and cluster analysis (AHC) were used to determine relationships between taxa. As a result of PCA and HCA, the species were divided into three clusters, in accordance with the taxonomic treatment of Scorzonera based on its morphological characteristics. Variables such as the shape of outer phyllaries, achenes, stigma, anther, stem base collar, leaf shape, leaf margin, leaf tips and surface, and root shape explained most of the total variations. PCA analysis suggests that the characters mentioned above may also be helpful in delineating species groups. Among the species being studied, *S. mollis* and one variety (S. *cana* var. *jacquiniana*) were discovered and examined in the MSU district for the first time. In particular, the results of this study can be used to update and improve the flora of Iraq and the Kurdistan Region for Scorzonera spp.

Keywords: Asteraceae, Cichorieae, Scorzonera; Taxonomy; PCA; Kurdistan-Region.

1. Introduction

The genus *Scorzonera* L. is one of the largest genera of subtribe Scorzonerinae, tribe Cichorieae, and family Asteraceae[1], Linnaeus records it as one of the most primitive taxa[2]. First detailed arrangement and complete classification of the genus *Scorzonera* was given by de Candolle[3]. Then, Boissier updated the genus once again[4-6]. Among these revisions, the work of Lipschitz[5] is considered the most comprehensive.

The genus comprises approximately 180–190 species in the world[7], most of which are perennial herbaceous plants. It originates in the Ancient Mediterranean. The *Scorzonera* species mainly inhibits temperate and arid regions of Central Europe, Central Asia, and Northern Africa[8], with the significant center of diversity in the arid and Irano-Turanian regions.

There are 28 *Scorzonera* species distributed in Europe[9-11], and 29 species and three varieties mainly inhabit arid regions, deserts, and the high plateau of north-western China[12-15]. In the flora of Turkey, 35 genera belonging to the tribe Cichorieae have been recorded[16]; including 59 *Scorzonera* species found in different regions of Turkey[17] including 28 endemic species to Turkey. There are 38 genera in the tribe Cichorieae of the flora Iranica, in addition to 39 species of *Scorzonera*.[16, 18]. Four new species [19–21] and one new variety [22] have been identified and added to the flora of Iranica since Rechinger[18] updated the genus *Scorzonera*[19-21, 23]. The Asteraceae family, which has three subfamilies and 16 tribes, is the biggest dicotyledonous plant family recorded in the flora of Iraq. These subfamilies include 123 genera and 433 species (including 14 cultivars); of them, 71 species of 26 genera of the tribe Cichorieae include15 species of *Scorzonera* genus were recorded in Kurdistan region; of which eight species distributed in the MSU district[24, 25].

Scorzonera species are characterized by complicated variation patterns present among species. According to previous molecular studies, to reconstruct the evolutionary relationships among the members of the Cichorieae tribe using nuclear ribosomal Internal Transcribed Spacer (nrITS), External Transcribed Spacer (ETS), and morphological features of the achene[7, 8, 26], it is revealed that *Scorzonera* is polyphyletic, consisting of four separate clades

* Corresponding author
E-mail address: rubar.salih@univsul.edu.iq (Instructor).
Peer-reviewed under the responsibility of the University of Garmian.

(*Scorzonera* s. str., *S. purpurea*, *S. albicaulis* and *Podospermum)*. These studies also, resulted in relocating some *Scorzonera* species (i.e. *S. latifolia* and *S. cinera*) to different genus such as *Gelasia* genus. In flora of Iraq, the genus is classified to three subgenera, Podospermum, Pseudopodospermum, and Scorzonera. This classification was based on a few morphological traits (root shape, leaf type and shape, phyllaries indumentum, achene shape)[25, 27].

Despite the presence of few studies of anatomical[28-30], phytochemical[31], ethnopharmacology characterization[32, 33], and floristic study[34-36] of *Scorzonera* species, no comprehensive study has been carried out yet, clarifying the nomenclature, intergeneric and interspecific diagnostic characters of *Scorzonera* in the genera, subgenera, and specific level in Iraq. A detailed morphological study was carried out for *Scorzonera* distributed in the Kurdistan Region of Iraq, Sulaymaniyah district (MSU) in order to clarify the circumscription of species belonging to the *Scorzonera* genus and give current insight into their lineages in Iraq.

Understanding the morphological (macro and micro-morphological features) boundaries and diagnostic characteristics between the subgenera and within their species was, thus, the goal of the current study. Additionally, it offers a taxonomic approach that clarifies species circumscription, distribution, and the morphological delimitations amongst Scorzonera taxa.

2. Materials and Methods

2.1. Plant materials and identification

Mature plants bearing flowers and fruit were collected in two years of 2021–2022 and 2022–2023, starting from the mid of March to the end of September.

2.2. Distribution and morphological studies of Scorzonera L. species

Species identification was performed using different resources, including flora Iranica[18], flora of Turkey[16], and flora of Iraq[25], and some other references[37-39].

Macro-morphological studies were conducted using a dissecting microscope (MOTIC). Micromorphological studies (trichomes, leaf epidermis, stomatal complex, and palynological study) were examined and visualized using a stereo compound microscope (MOTIC).

Depending on the availability of the specimens, a thorough morphological analysis was performed on around 20–25 samples. For the fresh materials, morphological measurements and a comprehensive photographic records were also taken. Elevation and global positioning system coordinates were recorded for all the specimen's collected points using Garmin Rino GPS device. A geographical map was created using QGIS software. The resources mentioned above and herbarium specimens were examined to determine the distribution, location, and flowering time of *Scorzonera* species in the MSU district. For references and further investigation, specimens were deposited in the herbarium of the University of Sulaimani, College of Agricultural Engineering Sciences (SUFA, acronym according to Thiers, 2021).

2.3. Palynological studies

Pollen grain material was obtained from fresh flower specimens to be used in palynological studies. The morphology of the pollen grains was investigated using a compound microscope (MOTIC). Eye piece graticule was used to measure the size (polar [P], equatorial [E], and P/E ratio) of the pollen gains. The measurements were based on a total of 20 pollen grains per plant sample, stained with 1% safranin dye.

2.4. Coding and analysis of morphological characteristics

A total of 80 qualitative and quantitative traits (67 macro- and 13 micro-morphological traits) from eight different *Scorzonera* taxa were used in the coding and analysis of morphological attributes. These morphological traits were coded with binary or multiple-ordered categorical states. The definition characters and their state coding are provided in Supplementary Table 1. The selected characters were subjected to principal component analysis (PCA) and cluster analysis (HCA) in order to identify the most variable morphological characters according to Ahmad *et al.*[40], using XLSTAT V4.01.20780 software. The PCA was conducted for the species using a rank dissimilarity matrix of two-way data from all 80 studied criteria. Cluster analysis was also performed based on the squared Euclidean distance of Ward's agglomeration method to classify the taxa relatedness.

3. Results

3.1. Scorzonera species delimitation using taxonomic approaches

Of the studied species of *Scorzonera*, *S. mollis* had many similar morphological characteristics to *S. mucida*. The same situation was observed with *S. phaeopappa* and *S. semicana*. The non-tuberose root system of several varieties of *Scorzonera cana* var. *jacquiniana* and *Scorzonera incisa* facilitated their differentiation from the other species and varieties (Figure 1, Supplementary Table 1).

The stem base collar, which is an above-ground feature that connects the stem to the root, was also among the most crucial characteristics for intraspecific species identification. For example, *S. mucida* had a longer stem base collar compared to *S. mollis*; however, they both have similar morphological appearances for other characteristics. *S. lanata* had a long base collar, making this species have a similar appearance to the two previous species. Other traits have to be considered for this species to be identified to indicate its identity (*see Supplementary* Table 1).

In general, individuals of *Scorzonera* species have both basal and cauline leaves, except for *S. lanata*, which has only basal leaves. With the exception of *S. lanata*, which has a basal leaf with a highly lanate indumentum at the surface, the blade surfaces of all the species could not be distinguished from either the cauline or basal leaf simply by visual inspection. *S. papposa* appeared with a broad white midrib on the basal and cauline leaves. This characteristic was considered a taxonomic-important trait, being a unique attribute for this species. Another significant similarity between the species was the venation on their leaves, the basal leaves of *S. mollis* and *S. mucida* might be used to distinguish them. The leaf venation of *S. mollis* appeared parallel, whereas *S. mucida* had palmately reticulate venation. Additionally, the cauline leaf venations of *S. mollis* were palmately reticulate, while for *S. mucida* they were pinnately reticulate. However, *phaeopapa* and *S. semicana* are both similar in their cauline leaf venation; the venation pattern of *S. phaeopappa* was palmately reticulate in comparison to the parallel venations of *S. semicana*. Additionally, among the species being studied, petiolate cauline leaves might be used to distinguish S. semicana from other species within the same taxon, particularly from S. phaeopappa, (Figure 1, Supplimentary Table 1).

Figure 1: Root stock and leaf of Scorzonera under study (1. S.cana var. jacquiniana, 2. S.incisa, 3. S.lanata, 4. S.mollis, 5. S.mucida, 6. S.papposa, 7. S.phaeopapa, 8. S.semicana). The black bar on the right represents the scale bar equal to 1cm.

At maturity and the fruiting stage, distinct characteristics of peduncles occurred in *S. mollis, S. phaeopappa*, and *S. semicana*. The uppermost end of the peduncle (at the base of the capitula) becomes flattened (incrassate) at fruiting time, unlike the rest of the species with a non-incrassate peduncle (Supp. Table 1).

The inflorescence of *Scorzonera* was mainly a solitary liguliflorous capitulescence. Among the capitula characteristics, the receptacle surface plays a crucial role in identifying both *S. mollis* and *S. mucida*. *S. mollis* held a foveolate, and *S. mucida* showed a scaly receptacle. Besides, *S. phaeopappa* had a fovate receptacle and *S. semicana* had a foveolate receptacle; they appeared rather different. The number of capitula per plant was an important characteristic for diagnosing *S. phaeopappa* from *S. semicana* in the fruiting stage. The capitula number of *S. phaeopappa* did not exceed five per plant; however, in *S. semicana*, 12 capitula numbers per plant were recorded. The number of florets per capitula was used to diagnose each of *S. semicana* and *S. phaeopappa*. The number of florets did not exceed 35 florets/capitula in *S. semicana*; however, in *S. phaeopappa*, 40 florets/capitula were recorded (Figure 2, Supp. Table 1).

The form and arrangement of phyllaries were of great taxonomic value in Asteraceae. Phyllaries were arranged from one to many seriate and from equal or sub-equal in length to strongly graduated. Each of *S. cana* var. *jacquiniana*, *S. incisa*, and *S. papposa* was characterized by four seriate phyllaries, in comparison with the three seriate phyllaries recorded for the rest of the species under study. Further, *S. cana* var. *jacquiniana* was characterized and could be differentiated from the rest of the species by the presence of corniculate (appendage-like structure) on the first seriate (outermost) phyllaries. Additionally, the phyllary traits were important to distinguish each of *S. mollis* and *S. mucida*, as they were different in the outermost and third seriate phyllary number, size, surface, and shape.

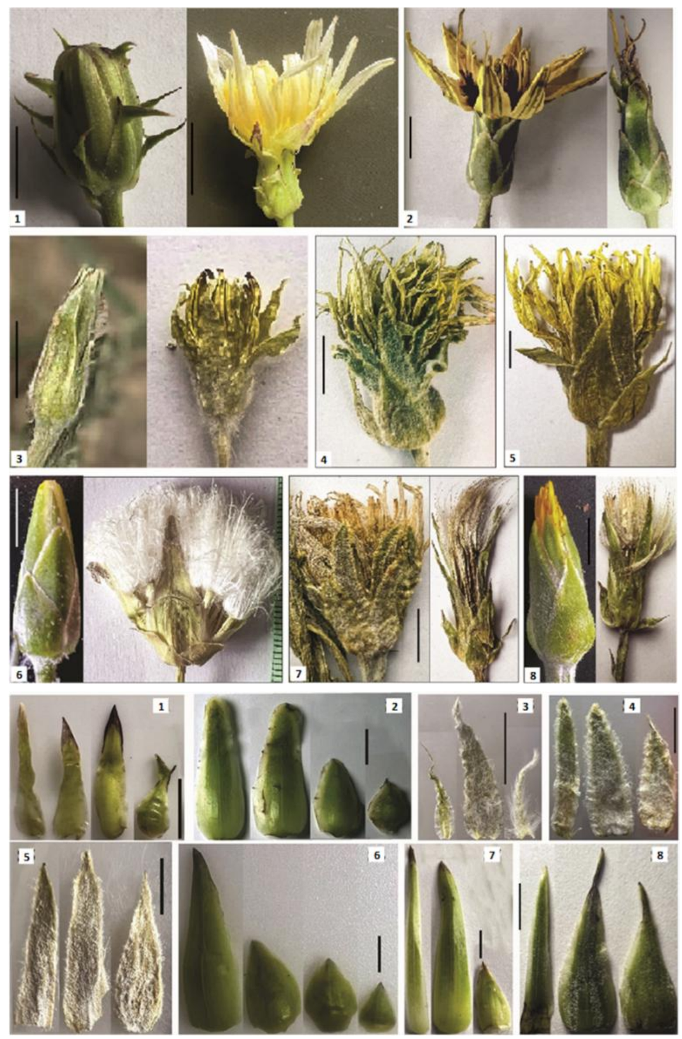

Figure 2: Capitula and phyllaries characteristics (1. S.cana var. jacquiniana, 2. S.incisa, 3. S.lanata, 4. S.mollis, 5. S.mucida, 6. S.papposa, 7. S.phaeopapa, 8. S.semicana). The black bar on the right represents the scale bar equal to 1cm.

Similarly, the phyllaries' characteristics were also important for the identification of *S. phaeopappa* and *S. semicana*; their phyllaries differed when investigating their size, surface, and shape. At maturity and fruiting time, the phyllary's margin in *S. phaeopappa* was not reflexed or curled at maturity. Conversely, in *S. semicana*, the first and second seriate phyllaries became reflexed, and the third seriate curled outward. Additionally, the phyllary margins of a third row of S. semicana and a fourth row of S. incisa curled outward throughout the time of maturity and fruiting, (Figure 1, Supp. Table 1).

Floret was important for the identification of some closely morphologically related species, such as *S. mollis* and *S. mucida*. The floret of *S. mollis* was 23.1 mm on average, which was much longer than that presented in *S. mucida* (15.1 mm on average). The limb of *S. mollis* was differentiated from *S. mucida* limbs by observing an entirely bright yellow color in *S. mollis*; a bright yellow from the central became darker yellow toward the base of the limb (claw) in *S. mucida*, (Figures 3 and 4, Supp. Table 1).

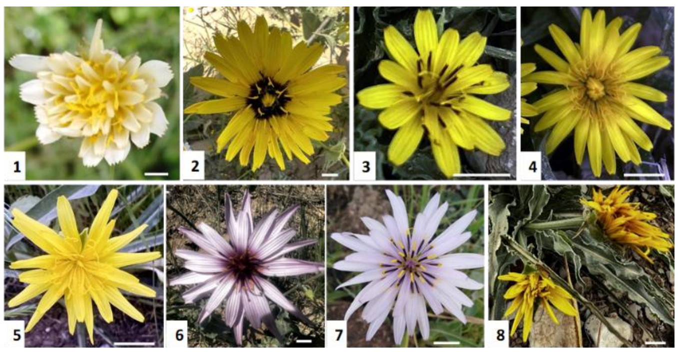

Figure 3: Flower of Scorzonera specied under study (1. S.cana var. jacquiniana, 2. S.incisa, 3. S.lanata, 4. S.mollis, 5. S.mucida, 6. S.papposa, 7. S.phaeopappa, 8. S.semicana). The black bar on the right represents the scale bar equal to 1cm.

Figure 4: Natural habitat of Scorzonera specied under study (1. S.cana var. jacquiniana, 2. S.incisa, 3. S.lanata, 4. S.mollis, 5. S.mucida, 6. S.papposa, 7. S.phaeopappa, 8. S.semicana). The black bar on the right represents the scale bar equal to 1cm.

In the same manner, *S. semicana* and *S. phaeopappa* were similar enough to be distinguished by the color of their ligules. The limb of *S. phaeopappa* was bright purple to pink and became darker toward the claw in comparison with the purply-tinged tips and golden-yellow presented by *S. semicana*. Additionally, *S. phaeopappa's* floret length was less than *S. semicana's* (Figures 3 and 4, Supp. Table 1).

The androecium characters of *Scorzonera* species for both *S. mollis* and *S. mucida* had different lengths of filaments. The filament of *S. mucida* (0.7 mm) was much longer than that of *S. mollis* (0.2 mm). Unlike *S. phaeopappa* and *S. semicana*, both had similar filament lengths (0.9 mm), although their anther and filament were different colors, as the anther color of *S. phaeopappa* was dark violet, while *S. semicana* had a dark brown anther color. Besides, the filament color of *S. phaeopappa* was yellow, whereas it was dark brown in *S. semicana* (Supp. Table 1).

For *S. phaeopappa* and *S. semicana*, clear differences in stigma length and color were observed, as the stigma of *S. mucida* (5.5 mm) was much longer than that presented in *S. mollis* (2.8 mm). Additionally, the stigma color of *S. mollis* was bright brown, and for *S. mucida*, it was dark brown (Supp. Table 1).

Each of the *S. mucida*, *S. phaeopappa*, and *S. semicana* were similar in achenes characteristics; they all had dimorphic achene. The achenes from the margins were distinct in length, diameter, colors, and surfaces from the achenes located at the center of the capitula. In contrast, the rest of the species had monomorphic achenes. Another important achene character was the rib number on the surface of the achene. *S. phaeopappa* and *S. semicana* were rather similar in the rib number (12–14), in contrast to *S. mucida* (8–10 ribs). While each of *S. lanata*, *S. mollis*, and *S. papposa* was characterized as unribbed achenes (Supp. Table 1).

Color was the most important character to separate the achenes of *S. mucida*, *S. phaeopappa*, and *S. semicana*. *S. mucida* was rather similar to *S. semicana* in terms of the marginal achene color (dark brown and bright brown, respectively). However, they had a significant difference from *S. phaeopappa*, as it has a grayish-colored marginal achene. Due to their comparable narrowly-cylindrical achene shapes, *S. cana* var. *jacquiniana*, *S. mollis*, *S. mucida*, *S. phaeopappa*, and *S. semicana*, might be distinguished from other *Scorzonera* species, such as *S. incisa* (fusiform), *S. lanata* (straight), and *S. papposa* (oblanceolate). The achene sculpture characteristic differentiated the *Scorzonera* species under study into three groups: glabrous surfaces (*S. cana* var. *jacquiniana*), hairy (*S. lanata*), and muricate-lamellate in the remaining species. The absence of carpopodium led to a difference between each of *S. incisa* and *S. papposa* and the rest of the species (Supp. Table 1).

S. phaeopappa was distinguished from the other *Scorzonera* species under study by its pappus color. The species was greyish pappus, while the others were of similar pappus color (pure to dirty white); however, they were different with tiny color tones. Furthermore, *S. papposa* was distinguished from the rest of the species by bearing a plumose bristle pappus type, whereas the others had a dimorphic pappus type, plumose bellow, and 4-5 longer scabrous bristles above (Supp. Table 1).

3.2. Micro-morphological diagnostic characteristics

Scrozonera lanata was identified with micro-morphological characteristics by the presence of lanate indumentum on the achene surface, which is the reason behind its name. While the results of the pollen grain investigation for the species under study showed diversity in the size and shape of pollen grains, *S. mucida* and *S. lanata* were recognized from the rest of the species, more specifically from their most similar species (*S. mollis*, based on morphological characteristics). The recognition was mainly based on the smaller size of the pollen grains compared to the others. Further, pollen shape was also important in separating *S. phaeopappa* (subprolate) from *S. semicana* (prolate spheroidal) and *S. mollis* (prolate spheroidal) from *S. mucida* (oblate spheroidal), (Figure 5, Supp. Table 1).

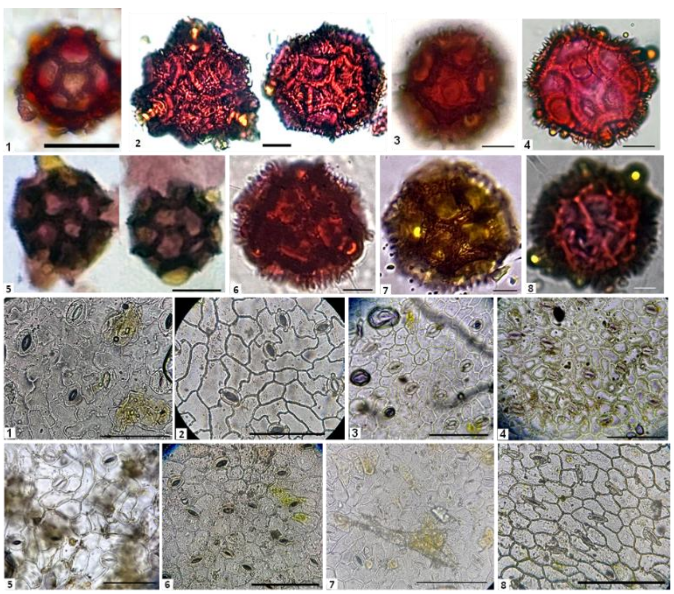

Figure 5: Pollen grains and epidermal complex of Scorzonera species under study (1. S.cana var. jacquiniana, 2. S.incisa, 3. S.lanata, 4. S.mollis, 5. S.mucida, 6. S.papposa, 7. S.phaeopappa, 8. S.semicana). The black bar on the right represents the scale bar equal to 1 µm.

Species of *S. mollis, S. papposa,* and *S. phaeopappa* appeared similar in terms of subsidiary cell wall shape. They were presented in straight, slightly curved, or wavy-shaped subsidiary cell wall shapes. Whereas, *S. incisa, S. lanata*, and *S. semicana* represented similar curved shapes of subsidiary cell walls on both epidermal surfaces. However, *S. cana* var. *jacquiniana* differed from the rest of the species by displaying only wavy-shape subsidiary cell walls abaxially and adaxially.

Although the species under study were all represented by large subsidiary cells from the lower surfaces, *S. mollis* and *S. lanata* were exceptions because *S. mollis* subsidiary cells from the upper surface were larger compared to the lower surface subsidiary cell size. *S. lanata* was different from the rest of the species under investigation by the similar size of subsidiary cells abaxially and adaxially (Figure 5, Supp. Table 1).

3.3. Ecology and Geographical Distribution

Recordings of *Scorzonera* species were generally conducted at the blooming stage in April (*S. lanata, S. mollis, S. mucida*, and *S. phaeopappa*), April-May (*S. incisa, S. papposa*, and *S. semicana*), and May (*S. cana* var. *jacquiniana*). Fruiting time started from April to June (*S. semicana*), May-June (*S. mucida, S. papposa*, and *S. phaeopappa*), and June-July (*S. incisa*).

According to the results obtained from the collection of field trips to different geographical regions in Sulaymaniyah (MSU) district, these species were different based on geographical distribution, location, and elevation level, in addition to the soil types and water resources of the sites of their presence. Some of the species under study were represented as common species with a large population (*S. incisa* and *S. semicana*). Some others were represented as a small population in their geographical locations (*S. lanata*, *S. mollis*, and *S. papposa*). The other studied species were distributed as individual or scattered plants (*S. mucida* and *S. phaeopappa*).

Regarding the exhibition of the species to geographical location, *S. papposa* presented at most of the locations for all the trips during the study period. In contrast, *S. cana* var. *jacquiniana*, *S. lanata*, *S. mollis*, and *S. mucida* were considered rare species because they were found only in one location during the study period (Table 1, Supp. Table 1). Among the species under study, one species (*S. mollis*) and a variety (*S. cana* var. *jacquiniana*) were recorded and investigated for the first time in the MSU district.

3.4. Taxonomic relationships based on PCA and cluster analysis

The measured macro- and micromorphological characters of *Scorzonera* species are summarized in Supplementary Table 1. The Principal Components Analysis (PCA) was performed to determine the extent of distance among the species based on the morphological characters. Most variable characters among the *Scorzonera* taxa were also specified for each of the species.

This study demonstrates that the examined species were divided into different groups based on macro- and micro-morphological characteristics. The agglomerative hierarchical clustering (AHC) analysis was also performed to validate and identify the species clustering based on the studied characteristics (Figures 7 and 8). A total of 80 phenetic variables, corresponding to eight taxa, were able to cluster the species into three main groups (Figure 7). The first group (labeled 1) contained two species (*S. cana* var. *jacquiniana* and *S. incisa*). The second cluster (labeled 2) consisted of only one species (*S. lanata*), and the rest of the examined five species were clustered in the third group. The last cluster (3) was further divided into two sub-groups to include two species (*S. phaeopappa* and *S. semicana*) in sub-group (3a), and the other three species (*S. mollis, S. mucida*, and *S. papposa*) were clustered into the second subgroup (3b) cluster 3.

Based on the variable morphological characteristics, the PCA analysis showed the distance and the extent of relationships between the species under study. The scatter plot demonstrated that the species were able to sort into three distant and dissimilar groups (Figure 8).

Table 1: Ecological, geographical distribution, and altitude for species under study.

No.	Species	MSU regions															Occurrence				Population size	Elevation above sea level (m)
		Sulaimania	Baziyan - Chamchamal	Azmar-Goizha	Sharbazher	Sharazur	Qaradagh	Garmiyan	Zewe	Hawraman	Penjwen	Gmo	Piramagrun	Qaiwan	Sartak-Bamo	Dukan	Common	Frequent	Occasional	Rare		
1	***S.cana var. jacquiniana***										✓								✓		individual	
2	***S . incisa***		✓				✓					✓			✓				✓		Large	900–1700
3	***S . lanata***		✓	✓					✓	✓		✓	✓	✓		✓	✓				small	1050–1500
4	***S .mollis***								✓			✓								✓	small	1050–1500
5	***S .mucida***		✓						✓				✓			✓			✓		individual	1050–1500
6	***S .papposa***	✓	✓		✓		✓			✓		✓				✓	✓				small	850–1750
7	***S .phaeoppapa***						✓			✓	✓	✓		✓		✓			✓		individual	800–1700
8	***S .semicana***		✓	✓	✓		✓	✓	✓	✓	✓	✓	✓		✓	✓	✓				large	600–1800

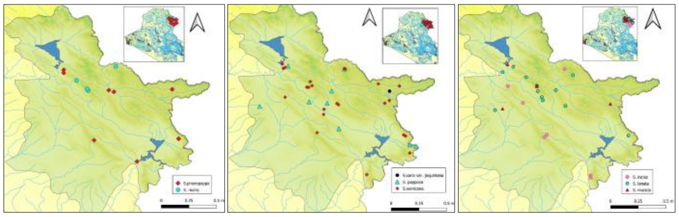

Figure 6: Distribution map of Scorzonera species under study.

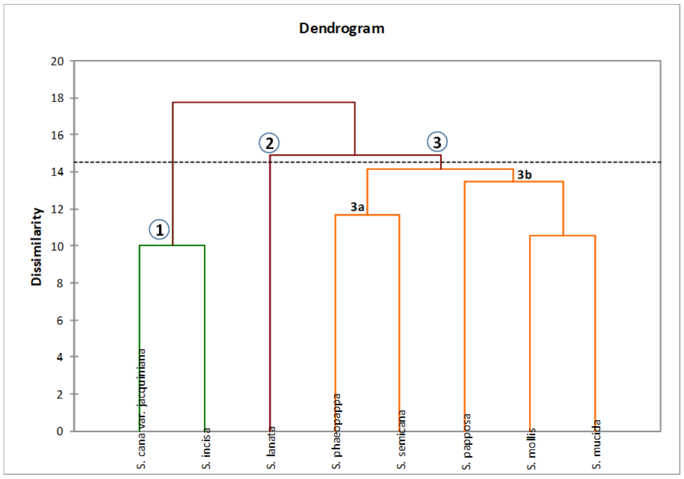

Figure 7: AHC dendrogram of Scorzonera species based on morphological characters.

4. Discussions

The investigation outcomes might be an update and improved version for the flora of Iraq and the Kurdistan Region for *Scorzonera* spp., particularly in MSU district. The study confirmed the divergence of *Scorzonera* and proposed a revised classification of the subgenera. The present work intensively focuses on the classification of morphologically complicated and overlapped species belonging to the *Scorzonera* genus. The findings would contribute several new critical traits of vegetative, reproductive, and anatomical aspects that were highly varied across the species to diagnose, identify, and classify the Scorzonera subgenera and species. The outcomes also provide a taxonomic reassessment of *Scorzonera* species based on broad taxonomic sampling, morphological, and micro-morphological data. The study includes intensive morphological characteristics of 80 macro- and micro-traits. Relying on such size and traits allowed for an intensive investigation into the relationships of eight *Scorzonera* species for the first time. The species variations, similarity distances, and overlapping species were successfully examined.

Morphological characters of plant height, leaves, and phyllaries were able to diagnose *S. cana* var. *jacquiniana* and separate the species from the others. Characteristics of leaf margin, leaf division, color of ligule, stamen, and corolla tube were highly useful to identify *S. incisa* and *S. papposa*. The most powerful diagnostic characteristics were lower leaf shape, ligule color, pappus color, and type. However, *S. phaeopappa* could be identified using the characteristics of ligules, pappus color, and carpopodium appearance. Additionally, *S. lanata* was different from the other species and would be differentiated from the closely related species (*S. mollis* and *S. mucida*) based on root tuber size, leaf margin, and hairy ovary. Further, *S. semicana* was different in terms of rootstock shape, size of cauline leaves, achene beak, and carpopodium appearance. The important diagnostic characters for *S. mucida* and *S. mollis* could be rootstock shape, stem basal collar, leaf shape, leaf size, achene beak, and carpopodium appearance, and these traits could be relied on in further investigation.

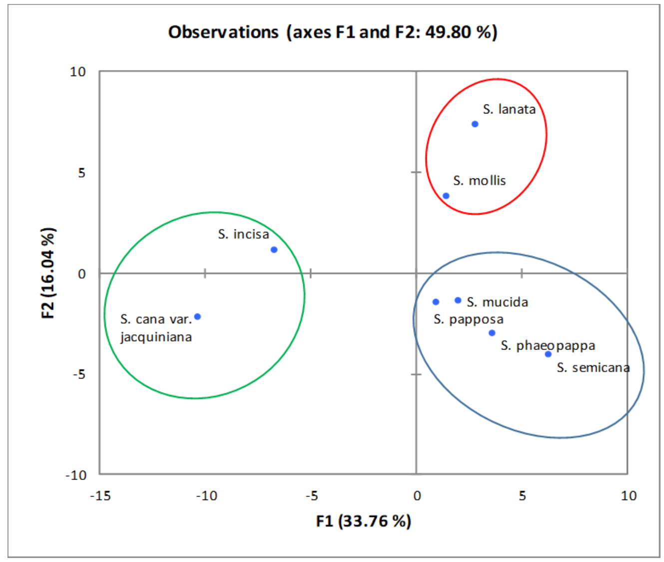

Figure 8: PCA scatter plot of Scorzonera species based on morphological characters. The dots indicated on the biplot demonstrate the distances and relationships between the genotypes based on their morphological characteristics.

The genus *Scorzonera* has long been considered to have complicated variation patterns that make it difficult to distinguish among its species, and still, their infrageneric classification is preliminary. Although efforts have been made to classify the genus[7, 8, 26, 39] into three to four polyphyletic subgenera, no comprehensive numerical taxonomic research of *Scorzonera* species has been conducted before. The results of the numerical examination of PCA and AHC analyses confirmed three subgeneric ranks of the genus. These subgenera classifications were based on the data of 81 morphological characteristics. The results of PCA and AHC corresponded with the results of previous studies, which grouped *Scorzonera* genera into three subgenera: Podospermum, Pseudopodospermum, and Scorzonera[18, 38, 41, 42]. The Podospermum subgenera includes *S. cana* var. *jacquiniana* and *S. Incisa. The species S. Incisa* was previously classified under the subgenera Pseudopodospermum, but in this study it was classified under the subgenera Podospermum. It was obvious that *S. incisa* was closer to *S. cana* var. *jacquiniana* than those classified under the Pseudopodospermum subgenera. The subgenera Pseudopodospermum includes *S. mucida*, *S. mollis*, *S. papposa*, *S. phaeopappa*, and *S. Semicana*, while the subgenera Scorzonera includes only *S. lanata*. The *Scorzonera* classification in the Flora of Iraq is not in congruence with the current and other previous studies[39]. In the flora of Iraq, each of *S. incisa*, *S. lanata*, and *S. papposa was* classified under the subgenus Pseudopodospermum[25].

Members of subg. *Podospermum* were distinguished by a non-tuberose root, pinnatifid leaves, distinct carpopodium, and smooth achene surfaces. Members of subg. *Pseudopodospermum* were characterized by tuberose roots, entire leaves, achenes with or without conspicuous carpopodium, and various types (sulcate, lamellate-muricate, or dentate-muricate) achene surfaces. While members of subg. Scorzonera were distinguished by tuberose roots, entire leaves, and achenes without a carpopodium, the presence of completely concealed long and dense hairs on achene surfaces.

The position of the eight examined species on the PCA (Figure 7) shows that the species are distinguished into three distinct clusters. The present study is a preliminary step in the analysis of morphological characters by means of numerical analysis. The results basically agree with the traditional taxonomic treatments of *Scorzonera*. Qualitative variables such as the shape of outer phyllaries, achenes, stigma, anther, stem base collar, leaf shape, leaf margin, leaf tips and surface, and root shape explain most of the total variation. In addition, PCA analysis suggests that the characters mentioned above could be used in species group delimitation. Binary characters seem to be more important than quantitative characters in separating *Scorzonera* taxa. A molecular investigation of specific genes or whole chloroplast sequencing would be required to support and confirm the current classification based on morphological characterization.

5. Conclusion

In conclusion, Finally, the delimitation of Scorzonera species was investigated in Iraqi Kurdistan's MSU district, utilizing a variety of macro- and micro-morphological traits. Many important key characters have been proven to distinguish closely related and overlapped morphological species. These characteristics included the shape of the outer phyllaries, achenes, stigma, anther, stem base collar, leaf shape, leaf margin, leaf tips and surface, and root shape. Principle Component Analysis (PCA) and cluster analysis (AHC) showed that the species were distinguished into three distinct clusters. These results were in accordance with the taxonomic treatments of Scorzonera using morphological characteristics.

Further, PCA analysis suggests that the characters mentioned above could be successfully used in the delimitation of species groups. Among the species being studied, S. mollis and one variant (*S. cana* var. *jacquiniana*) were discovered and examined in the MSU district for the first time. Future investigations to study these species based on nuclear and cytoplasmic-specific gene markers could further affirm the current results.

Conflict of interests

None

Author Contribution

All authors designed and implemented the research, analyzed the results, and wrote the manuscript.

Acknowledgements.

The authors are highly grateful to the University of Sulaimani, College of Agricultural Engineering Sciences, Departments of Biotechnology and Crop Science, for accessing the laboratory facilities and technical support.

References

1. Kamelin, R. and I. Tagaev, *A review of species of the genus Scorzonera (Asteraceae).* Botanicheskii zhurnal, 1986.
2. Linnaeus, C., *Species plantarum, exhibentes plantas rite cognitas, ad genera relatas, cum differentiis specificis, nominibus trivialibus, synonymis selectis, locis natalibus, secundum systema sexuale digestas.* 1753.
3. de Candolle, A., *Flora of France.* 1805. **2**.
4. Boissier, E.P., *Flora orientalis: sive, Enumerato plantarum in Oriente a Graecia et Aegypto ad Indiae fines hucusque observatarum. Calyciflorae, Gamopetalae. Volumen Tertium*. 1875: Apud H. Georg, Bibliopolam.
5. Lipschitz, S.U.l., *Fragmenty k monografii roda Scorzonera*. 1935: Onti, Glavnai͡a Redakt͡sii͡a Khimicheskoĭ Literatury.
6. Lipschitz, S., *Fragmenta monographiae generis*. 1939.
7. Zaika, M., et al., *Scorzonera sensu lato (Asteraceae, Cichorieae)–taxonomic reassessment in the light of new molecular phylogenetic and carpological analyses.* PhytoKeys, 2020. **137**: p. 1.
8. Mavrodiev, E.V., et al., *Phylogenetic relationships in subtribe Scorzonerinae (Asteraceae: Cichorioideae: Cichorieae) based on ITS sequence data.* Taxon, 2004. **53**(3): p. 699-712.
9. Chater, A., *Scorzonera L.* Flora Europaea, 1976. **4**: p. 317-322.
10. Paraschos, S., et al., *Three New Dihydroisocoumarins from the Greek Endemic Species Scorzonera cretica.* Journal of natural products, 2001. **64**(12): p. 1585-1587.
11. Granica, S. and C. Zidorn, *Phenolic compounds from aerial parts as chemosystematic markers in the Scorzonerinae (Asteraceae).* Biochemical Systematics and Ecology, 2015. **58**: p. 102-113.
12. Liu, G., Y. Zhao, and J. Xu, *Study on classification and ecology geographical distribution of plants from the genus Scorzonera in the Mongolian Plateau.* Grassland of China, 2001. **23**(2): p. 12-18.
13. Duran, A. and E. Hamzaoglu, *A new species of Scorzonera (Asteraceae) from South Anatolia, Turkey.* Biologia-Bratislava, 2004. **59**(1): p. 47-50.
14. Xueli, H., *Study on the genus Scorzonera L. from China.* Journal of Hebei University (Natural Science Edition), 2004. **24**(1): p. 65-73.
15. Karaer, F. and F. Celep, *Rediscovery of Scorzonera amasiana Hausskn and Bornm-a threatened endemic species in Turkey*. Bangladesh Journal of Botany, 2007. **36**(2): p. 139-144.
16. Davis, P., *Flora of Turkey*. 1965, Turkey.
17. Sarı, A., et al., *Phenolic compounds and in vitro antioxidant, anti-inflammatory, antimicrobial activities of Scorzonera hieraciifolia Hayek roots.* South African Journal of Botany, 2019. **125**: p. 116-119.
18. Rechinger, K., H. Lack, and J. Van Soest, *Compositae: Lactuceae*. 1977, Verlagsanstalt: Akademische Druck u.
19. Safavi, S., *A New Record and an Interesting Species of the Genus Scorzonera L. from Iran.* The Iranian Journal of Botany, 2004. **10**(2): p. 159-162.
20. Safavi, S., *Scorzonera tomentosa L. (Asteraceae), a new record for the Flora of Iran.* The Iranian Journal of Botany, 2019. **25**(1): p. 40-43.
21. Safavi, S., *Scorzonera incisa (Asteraceae), as a new record from NW Iran.* Nova Biologica Reperta, 2019. **6**(3): p. 334-337.
22. Safavi, S., *Notes on the Genera Scorzonera L. and Scolymus L. (Asteraceae-Lactuceae) in Iran.* The Iranian Journal of Botany, 2006. **12**(1): p. 59-69.
23. Dianat-Nejad, H., V. Mozaffarian, and S. Safavi, *Scorzonera koelpinioides (Asteraceae), a New Record for the Flora of Iran.* The Iranian Journal of Botany, 1998. **7**(2): p. 265-267.
24. Townsend, C., E. Guest, and A. Al-Rawi, *Flora of Iraq*, Compositae. 1966, Baghdad: Ministry of Agriculture and Agrarian Reform.
25. Ali, S., et al., *Flora of Iraq. Volume 6, Compositae*, ed. S.A. Ghazanfar, J. Edmondson, and D. Hind. Vol. Volume 6. 2019, Kew, UK: Published on behalf of the Ministry of Agriculture, Republic of Iraq by Royal Botanic Gardens, Kew. viii, 1-458.
26. Kilian, N., B. Gemeinholzer, and H. Lack, *Cichorieae.* Systematics, evolution, and biogeography of Compositae, 2009: p. 343-383.
27. Guest, E. and C. Townsend, *Flora of Iraq*. Vol. 2. 1966: Ministry of Agriculture of the Republic of Iraq.

28. Susa, A., *Anatomy syudy of epidermal cells for genus Scorzonera L. Compositae (Asteraceae) in Iraq.* Al-Qadisiyah Journal of Pure Science, 2015. **20**(1): p. 117-145.
29. Musa, M., *Anatomical Comparison for Inflorescence Peduncles for Some Species of Compositae (Asteraceae).* Iraqi Journal of Desert Studies, 2018. **8**(2).
30. Susanna, A., et al., *The classification of the Compositae: A tribute to Vicki Ann Funk (1947–2019).* Taxon, 2020. **69**(4): p. 807-814.
31. Amin, H., et al., *Phytochemistry and ethnopharmacology of some medicinal plants used in the Kurdistan Region of Iraq.* Natural product communications, 2016. **11**(3).
32. Ahmad, S. and A. Askari, *Ethnobotany of the Hawraman region of Kurdistan Iraq.* Harvard papers in botany, 2015. **20**(1): p. 85-89.
33. Galalaey, A., et al., *Ethnobotanical study of some wild edible plants in Hujran Basin, Kurdistan Region of Iraq.* Zanco Journal of Pure and Applied Sciences, 2021. **33**(s1).
34. Ahmad, S. and S. Salih, *Azmar_Goizha Plant Field Guide*. 2016, Sulaymaniyah, Kurdistan Region-Iraq: Kurdistan Botanical Foundation.
35. Ahmad, S. and S. Salih, *Qara Dagh Mountain Plant Field Guide*. 2019, Sulymaniyah, Kurdistan Region-Iraq: American University of Iraq, Sulaimani (AUIS) Press.
36. Ahmad, S. and S. Salih, *Qaiwan Mountain Plant Field Guide*. 2021, Sulaymaniyah, Kurdistan Region-Iraq: American University of Iraq, Sulaimani (AUIS) Press.
37. Mavrodiev, E., et al., *Phylogeny of Tragopogon L. (Asteraceae) based on internal and external transcribed spacer sequence data.* International Journal of Plant Sciences, 2005. **166**(1): p. 117-133.
38. Çoşkunçelebi, K., et al., *A conspectus of Scorzonera sl in Turkey.* Turkish Journal of Botany, 2015. **39**(1): p. 76-87.
39. Hatami, E., et al., *Delimitation of Iranian species of Scorzonera subg. Podospermum and S. subg. Pseudopodospermum (Asteraceae, Cichorieae) based on morphological and molecular data.* Willdenowia, 2020. **50**(1): p. 39-63.
40. Ahmad, N., M. Abid, and A. Al-Assie, *Profiling of foxtail millet (Setaria italica L.) germplasm using in vitro drought screening and SSR markers.* Plant Genetic Resources, 2022. **20**(4): p. 277-289.
41. Lipschitz, S., *Scorzonera.* Flora of the USSR, 1964. **29**: p. 27-111.
42. Safavi, S., et al., *Flora of Iran: Asteraceae tribe Cichorieae*. 2013, Islamic Republic of Iran: Ministry of Jihad-e-Agriculture, Agricultural Research and Education Organization, Research Institute of Forests and Rangelands.