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## Taxonomic Study of Species of the Genus (*Heterocaryum* A. DC) in Sar-e-Pol Province of Afghanistan and Some Provinces of Iran

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### ABSTRACT

*Heterocaryum* is an herbaceous annual plant, covered with rough hairs and rarely glabrous, with single or branched stems, raised or lying on the ground, and native to the Gulf States. This study aims to find out taxonomic studies problem of the *Heterocaryum* genus and the new dispersal of species in Sar-e-Pol of Afghanistan and some province of Iran. It is a field and Laboratory research, based on the morphology and micromorphology characteristics like the length and thickness of fruit peduncles, the number of rows of glochids on the achene, and some other traits, on the 26 specimen was conducted at Bu Ali Sina University. According to the examination of the intended features of the collected samples of the genus *Heterocaryum*, a new identification key was provided, and based on the type specimens (*Heterocaryum echinophorum* (Pall.) Brand) was introduced in the flora of Afghanistan for the first time from Sar-e-Pol Province of Afghanistan. The desired traits analysis by Principal coordinates analysis (PCO), and five species of the genus *Heterocaryum* were identified, consisting of three species from Afghanistan (*H. echinophorum*, *H. rigidum*, and *H. x irregular*) and two species from Iran (*H. szovitsianum* and *H. subsessile*). *Suchtelenia* and *Heterocaryum* are very similar plants and it would have been better to put them under one name. Both countries have a high density of species that make up a large part of spring and summer fodder for animals. At the some areas the high density of bushes prevents sudden floods.

### INTRODUCTION

Afghanistan is an infinitely mountainous country with a lot of possibilities of ecological conditions which causes the diversity of the flora of this geography. From the hot deserts to the cold snow areas of Hindu Kush Mountain, with the monsoon climate in the east areas, so more than 4,000 species of vascular plants grow on this beautiful country. The majority of these species are endemic and unique (Breckle, *et al.*, 2010). The Borage family belongs to the Euasterids group which 60 species of them are in Canada and Alaska, 300 species in Turkey, 35 genera (12 native) in Europe, 30 genera (11 native) in North America, 23 genera (10 natives) in South America, 30 genera (9 natives) in Africa and 10 genera (4 natives) in Australia (Akbari, 2022). This family is one of the large families of true dicotyledons known in the world which has 130 genera and 2300 species have been identified (Langstrom, 2002, Luebert 2016, Ranjbar & Almasi, 2013). Initial diversification occurred about 50 million years ago (Chacón *et al.*, 2017). The different species of this genus are mostly found in the temperate and subtropical regions of the world, especially the Mediterranean region, and mostly have a global distribution. All the aerial organs of this genus are often covered with thick and rough hairs. Single cell of epidermal origin that has a cystolithe base and a siliceous or calcareous wall. These hairs often collect free silicic acid. The type of inflorescence is cyme unipare scorioide. The historic study of taxonomic of Boraginaceae family shows that it has different complex groups and genera. The position of groups and species

in this genus has been discussed by many researchers and different opinions have been presented. The number of borage species in flora Iranica is 218 species which are included in 41 genera; the Eritrichieae tribe of this group includes 19 genera and 499 species distributed in Eurasia from the Arctic to the Himalayas and western North America (Langstrom & Chase, 2002. & Ovchinnikova, 2009). Among them, 8 genera (*Heterocaryum*, *Asperugo*, *Lappula*, *Eritrichium*, *Lepechiniella*, *Rochelia*, *Anchusa* L. and *Nonea*) grow in Iran and Afghanistan (Khatamsaz, 2002. & Breckle, *et al.*, 2010). Tribe *Heterocaryeae* Zak. ex *Ovczinnikova* in Bot. was introduced with *Heterocaryum* A. DC and 6 species distributed in South and West Asia (Ovchinnikova, 2009). The available information about the morphological, anatomical and pollinological characteristics of this family is generally the same as other genera of the Borage family. Comprehensive research has been done on the anatomical structure of plants of this family, their pollen grains in other countries. However, some studies were conducted on *Heterocaryum* specie in Iran and no exact research has been done on *Heterocaryum* species in Afghanistan. So the current research is going to focus on *Heterocaryum* specie in Iran and Afghanistan.

### MATERIALS AND METHOD

This is a field research and it was carried out from Apr 2021 to Aug 2022. *Heterocaryum* species were collected from different areas (Balkhab, Sayyad, Sheram, Jarghan, and around the city) of Sar-e-Pol Province. The collected

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samples were processed and preserved in the herbarium of Sar-e-Pol Higher Education Institute. From the comparison of the samples with each other, the ones that showed the most difference were studied and analyzed by the researcher in the laboratory of Bu Ali Sina University (BASU) transferred to Iran. In total 26 samples and nearly thirty traits of the genus *Heterocaryum* based on reproductive and vegetative characteristics like length and width of the (corolla, corolla lobes, anther, fornicules, calyx, calyx teeth) and length of the (hair on the calyx,

gynoecium, glochids on the nutlet, nutlet wing), the number of spines in rows on the nutlet, the age of the plant, plant height, length and width of the (Basal leaves, middle leaves, bract leaves), length of petiole, length of peduncle in flower and fruit state, trichome length on leaves related to their morphological traits were compared and analyzed by PCO. According to the type samples and the mentioned traits, the key for identification was provided. Information related to the studied specimens of the species of this genus is shown in table (1).

**Table 1:** Information about some studied samples in *Heterocaryum* genus

Species	The number of herbarium	Height (m)	Date of collection	Collector	Place of collection
<i>H. echinophorum</i>	44890	625	16/03/2022	Akbari <i>et al.</i>	Afghanistan, Sar- e- Pol, 5 km to Tughany
<i>H. rigidum</i>	44783	625	16/3/2022	Akbari <i>et al.</i>	Afghanistan, Sar- e- Pol, 5 km to Tughany
<i>H. rigidum</i>	44783-1	625	16/3/2022	Akbari <i>et al.</i>	Afghanistan, Sar- e- Pol, 5 km to Tughany
<i>H. subsessile</i>	16788	1540	28/5/2008	Ranjbar	7 km Kalat to Mashhad. 65 km to Mashhad
<i>H. subsessile</i>	24606	2010	3/5/2011	Ranjbar	Kerman, 5 km after Circh
<i>H. subsessile</i>	44582	1186	25/4/2019	Ranjbar	Yazd, Jandagh to Naein, 215 km to Naein
<i>H. subsessile</i>	44778	625	16/3/2022	Ranjbar and Akbari	Afghanistan, Sar- e- Pol, 5 km to Tughany
<i>H. pachypodum</i>	12485	1750	13/05/2006	Ranjbar <i>et al.</i>	Hamedan, Heydareh
<i>H. pachypodum</i>	12494	1700	19/05/2006	Ranjbar	Hamedan, Hamedan Qazvin police way
<i>H. pachypodum</i>	12495	1840	20/02/2006	Ranjbar <i>et al.</i>	Kurdistan, Serishabad, Imam Zadeh Hajar Khaton
<i>H. pachypodum</i>	12553	1638	10/05/2006	Ranjbar <i>et al.</i>	Hamedan, Famenin
<i>H. pachypodum</i> var. <i>kotschyanum</i>	44726	1915	3/5/2016	Ranjbar	Hamedan; Heydareh village
<i>H. pachypodum</i> var. <i>kotschyanum</i>	25550	1535	2/5/2011	Ranjbar	Azarbaijan-e Gharbi; Salmas toward Khoy. Ghareh Tappeh, 21 km to Khoy
<i>H. pachypodum</i> var. <i>kotschyanum</i>	24501	1760	06/05/2011	Ranjbar	Isfahan, Kashan toward Mashhad-e Ardehal
<i>H. pachypodum</i> var. <i>kotschyanum</i>	25809	1515	19/5/2011	Ranjbar	Azarbaijan-e Gharbi, 10 km from Orumiyeh toward Movana, Movana bifurcate
<i>H. pachypodum</i> var. <i>kotschyanum</i>	44073-2	1850	3/5/2016	Ranjbar	Hamedan
<i>H. pachypodum</i> var. <i>kotschyanum</i>	44187	1906	7/7/2019	Ranjbar	Doroud to Azna, 10 km to Azna
<i>H. pachypodum</i> var. <i>kotschyanum</i>	45585	1642	11/5/2019	Ranjbar	Saveh to Hamedan, 110 km to Hamedan
<i>H. szovitsianum</i>	44198-1	1562	2019	Ranjbar	Azarbaijan-e Gharbi, Orumieh-Serdaghi mountain
<i>H. szovitsianum</i>	44198-2	1562	2019	Ranjbar	Azarbaijan-e Gharbi, Orumieh-Serdaghi mountain

<i>H. szovitsianum</i>	44855	1872	28/2/2022	Ranjbar and Akbari	Hamedan; Abshineh village
<i>H. szovitsianum</i>	12491	2095	7/5/2006	Ranjbar <i>et al.</i>	Hamedan, around Shahrestaneh village
<i>H. szovitsianum</i>	44073-1	1850	3/5/2016	Ranjbar	Hamedan
<i>H. szovitsianum</i>	18338	1450	12/5/2009	Ranjbar	Qazvin, 10 km after Abgarm
<i>H. szovitsianum</i>	45583	1809	1/5/2018	Ranjbar and Khelwati	Komijan to Chehrehghan, 15 km to Chehrehghan
<i>H. x irregular</i>	44782	625	16/3/2022	Akbari <i>et al.</i>	Afghanistan, Sar- e- Pol, 5 km to Tughany

**Taxonomic History of the Genus Heterocaryum**

This genus is placed in the family Boraginaceae sub family Cynoglossoidae tribe Rochelieae and sub tribe Heterocaryinae (chacon, *et al.*, 2016). The genus specie’s native to the Gulf States. In flora Iranica, the genus Heterocaryum introduced with 5 species in 2 sections and one hybrid species (Riddle, 1967 & Chacon *et al.*, 2016). The section Heterocaryum which include *H. subsessile*, *H. szovitsianum*, *H. macrocarpum* and *H. rigidum*, while section Laevigata with *H. laevigatum* species, and the Hybrid one is *H. x irregular*. The flora of Iran this genus introduced with 5 species *H. laevigatum*, *H. subsessile*, *H. rigidum*, *H. macrocarpum* and *H. szovitsianum* (Khatemsaz, 2002). This genus consists of 6 species that are distributed in the south of Russia, Turkey, Iran, Central Asia and the Himalayas (Saadati, *et al.*, 2017). Ovczinnikova in (2009) Considered tribe Heterocaryeae for the genus Heterocaryum. Finally, the most recent classification by Saadati *et al.*, (2017) separated the genus Pseudoheterocaryum from Heterocaryum according to phylogeny data, and the genus Heterocaryum remains monotypic with one *H. laevigatum* species.

**Characterstics of Heterocaryum Genus**

Hererocaryum is annual herbaceous plants, covered with rough hairs or rarely glabrous, single or multiple branched stems, erect to lying on the ground. The basal leaves are spoon-shaped, ovate, rectangular or linear (Khoshsokhan-Mozaffar *et al.*, 2018). The inflorescence is cyme, few-flowered, with bractate, calyx five lobed truncate to the base, widened during fruiting, corolla blue and small, short tube with funnel-shaped, five lobed and five separate appendages at the throat. The stamens of flower are very short and indistinct filaments, attached to the corolla tube and enclosed within them. The ovary has four lobes, and the base of the pistil is narrowly columnar with four grooves, short stigma in the corolla tube. The fruits are with four irregularly nutlet, which ventrally attached to the base of the style and inseparable, elliptic or rarely ovate, with distinct margin, two achene larger than the other two winged, commonly the larger nutlets with a shorter winged margin and rarely wingless; the disk of the larger nutlets is striated tubercle and has sharp spines; the disk of the smaller nutlets is smooth

and sometimes without acuminate spines; style hidden among achenes. The pollen grains is elliptical and has six irregular grooves (Khatemsaz, 2002).

**RESULTS AND DISCUSSION**

**Results**

**Identification Key of the Heterocaryum Species**

1. Fruit sessile, whit two winged nutlets and cup shaped, calyx 2-3 mm with scattered hairs, leaves strip lanceolate or lanceolate.....*H. subsessile*
  - Fruit peduncled, up to 20 mm, calyx length 3 to 5 mm with dense hairs.....2
2. Plant glabrous, with scattered hairs, the fruit is dangling, calyx teeth length on the fruit state is 5 to 7 mm.....*H. laevigatum*
  - Plant covered with dense hairs, the fruit is horizontal to erect, calyx teeth length in the fruit state is between 7 to 12 mm.....3
3. Fruit peduncle Short up to 7 mm.....4
  - Fruit peduncle long 7 to 20 mm.....5
4. Fruit peduncle is 5 to 7 mm long, the fruit is 6 to 9 mm long.....*H. szovitsianum*
  - fruit Peduncle 3 to 5 mm, fruit 5 to 7 mm....*H. macrocarpum*
5. Annual plant, short, 5 to 9 cm height, fruit tail 10 to 15 mm.....*H. echinophorum*
  - Biennial plant, more than 15 cm height, fruit tail 10-20 mm.....6
6. Fruit Peduncle 10 to 12 mm, plant up to 40 cm high.....*H. rigidum*
  - Fruit Peduncle 12 to 20 mm, the plant is 15 to 20 cm height.....*H. x irregular*

**The Results of Morphology Studies of Heterocaryum Genus**

Saadati *et al.*, 2017) have considered the distinctive traits for this genus which are: density of hairs, fruits thickness, peduncle in the fruit state straight to curved, nutlets with a row of spines on the margin and connected to the gynobase along its entire length.

According to the length of fruit peduncle and its thickness, plants hairs density, the length of wings in the nutlets and the number of rows glochidiate on the nutlets, height of the plant, age of the plant and some other traits,

five species of the genus *Heterocaryum* were identified. Three species; from Afghanistan *H. echinophorum*, *H. rigidum* and *H. x irregular* and two species; *H. szovitsianum* and *H. subsessile* from Iran. *H. echinophorum* introduced for the

first time in flora Afghanistan under the *Heterocaryum* genus from Sar-e-Pol Province of this country, the distribution location of the collected samples is shown in the following maps (Figure 1).

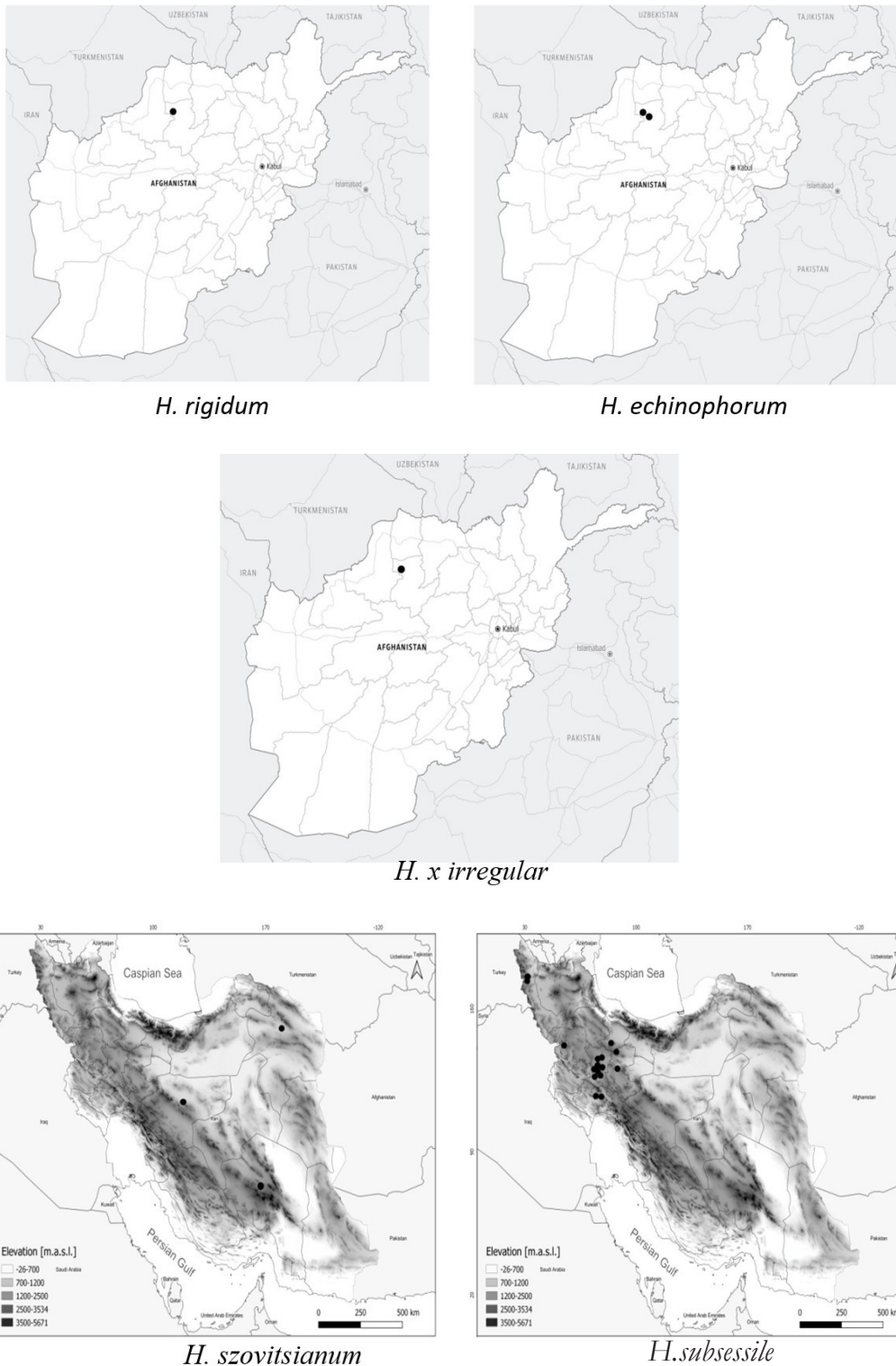
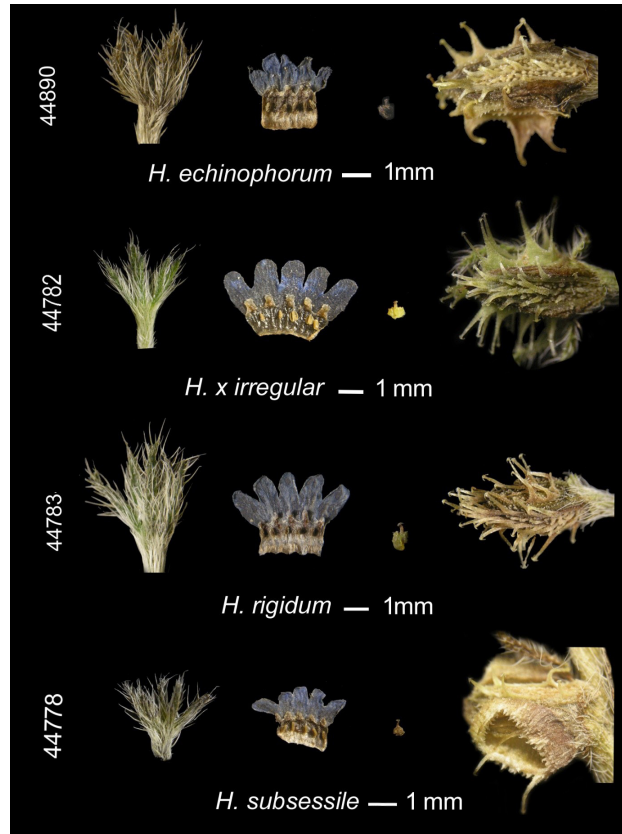


Figure 1: Maps distribution of *Heterocaryum* species in Afghanistan and Iran



**Figure 2:** Reproductive organs of *Heterocaryum* species, *H. echinophorum*, *H. x irregular*, *H. rigidum* and *H. subsessile* from Afghanistan



**Figure 3:** Habitat of *Heterocaryum echniophorum*



Figure 4: Is a picture of a herbarium specimen

**The Analysis and Results of Heterocaryum Genus**

From the principal coordinates analysis (PCO) of the studied species of the Heterocaryum genus, 5 groups were obtained, which are shown in Figure (5). The first group includes 4 populations of Heterocaryum subsessile species. It was distinguished from other species by features such as corolla 1.5 to 2 mm long, calyx teeth 2 to 3 mm long, and the absence peduncle of fruit. The second group consisted of 4 populations related to *H.*

*pachypodum* species. It was identified by diagnostic traits such as the length of the corolla is 2 to 3 mm, the corolla lobes is 0.8 to 1.1 mm long, and the peduncle fruit is 3 to 5 mm long. The third group included *H. echinophorum* and *H. rigidum* species. This group was separated from other species with traits such as short height of plants which is 5 to 15 cm, the peduncle fruit is 10 to 15 mm long, nutlet disc with glochidis and warts. The fourth and fifth groups include 15 populations of *H. szovitsianum* and *H.*

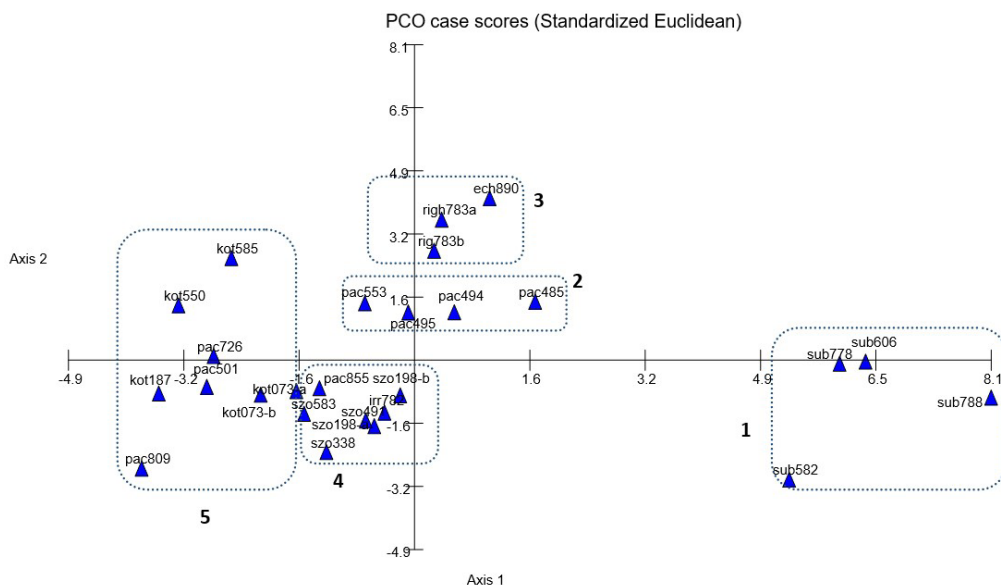


Figure 5: groups outcome from the results of (PCO) of morphological data in the genus Heterocaryum

x irregular. They were separated from other groups with traits such as peduncle fruit that is 5-7 mm long, middle leaves are 2-3.2 cm long and the nutlets are with winged at the base. Populations of species of groups 4 and 5 (except *H. x irregular*) are distributed from the northwest, west to the Mediterranean. The morphological separation of these species is difficult in most floras.

### Discussion

The results of the molecular studies of Saadati *et al.*, (2017) comparing the genus *Suchtelenia* and the genus *Heterocaryum* showed that the genus *Heterocaryum* remains monotypic with one species *H. laevigatum* and the genus *Pseudoheterocaryum* is separated from this genus with 4 species in Iran. Sparsely to densely pubescent covered slightly or glabrous in *H. laevigatum*. The leaves are narrow and long, peduncle absent in *H. subsessile* and often thickened except in *H. laevigatum*, calyx is divided to the base, corolla 5-lobed, nutlets 4 irregular shaped attached to the base of the stamen along its entire length and inseparable; the wings of the nutlet with appendages are often considered in one row and two rows in *H. laevigatum*.

The phylogeny of *Heterocaryum* and *Suchtelenia* was investigated using sequence data from the nuclear rDNA internal transcribed free space area (ITS), the plastid trnL and trnL-trnF intergenic (trnL-F) introns. The results showed that *Heterocaryum* is non-monophyletic due to the inclusion of *Suchtelenia calycina* (C.A.Mey.) A.DC. *Heterocaryum laevigatum* (Kar. & Kir.) A.DC formed a distinct sister clade to *S. calycina* and the remaining *Heterocaryum* species. Therefore, all species of *Heterocaryum* except *H. laevigatum* (the type species of this genus) were transferred to a new genus called *Pseudoheterocarium* (Saadati *et al.*, 2017).

Morphological studies of nutlets of 243 species and 17 genera belonging to the Eritrichieae tribe show the placement of the genera *Suchtelenia* and *Heterocaryum* together. Therefore, both studies (molecular and morphological of nutlets) have a strong inference (Ovchinnikova, 2009).

Chacon *et al.* (2016) considered the following traits in order to fully identify *Heterocaryum*: Plant age, rough or soft hairy covering, linear to ovate stem leaves, calyx almost divided to the base or in a half, small corolla, blue, cylindrical to funnel, small corolla appendages, and some other characters. By viewing the natural images of *Suchtelenia calycina*, from the Russian botanical site of the genus *Suchtelenia*, with characteristics such as plant glabrous, oval leaves, very small bracts, bluish-white corolla, large corolla appendages, calyx teeth cute to half and wide with rounded apex, nutlets are shiny black smooth to slightly warty surface, attached to the gynobase from the base; the ventral surface is raised and the dorsal surface is navicu to the inflated gynobase, the nutlets are separated from the gynobase, introduced.

The present study showed that the placement of *Suchtelenia* genus among *Heterocaryum* species is

inconsistent based on molecular findings. *Heterocaryum* is considered as a similar group to *Lappula* according to its characteristics. Nutlets with glochidid, leaf shape, corolla and calyx, nutlets shape and having gynobase can confirm this (Khoshokhan-Mozaffar *et al.*, 2018). Also, the differences between *H. laevigatum* species and other *Heterocaryum* species are not enough to be considered as two independent genera. On the other hand, the genus *Suchtelenia* is not similar to the two mentioned genera (Ovchinnikova *et al.*, 2021) with characteristics such as smooth stem and leaf surface, black and shiny nutlets with smooth to slightly warty surface, calyx is cut in half and fanner. *H. echinophorum* species was identified by observing and studying the type and herbarium specimens W0050069, M0188090, MW0594366, since the mentioned species is native to Kazakhstan, Russia and Southern Europe, so there is a possibility of its distribution in North of Afghanistan.

Climatic changes, decrease in rainfall and water, and the lack of awareness of the indigenous and rural people of Afghanistan regarding the destruction of the vegetation, have reduced the distribution of the species of the borage family and the genus *Heterocaryum*. For the protection of plant species, it is suggested to the environmental officials of the plant protection department to fence the protected areas. In Kenya, this method was used to protect the forest, and as a result, it reduced the interference of humans and animals to the wildlife and the level of protection of species dispersion. They increased the dispersal of plants species (Kinyili, 2023).

### CONCLUSION

From the conducted research, it is concluded that the number of species and genera of the family *Boraginaceae* in Afghanistan is not known and few research has been done on their identification and introduction. And this has caused a lot of problems from the phylogenetic and taxonomic, which is caused by many similarities between genera and species, and also has caused confusion for researchers. Therefore, some taxonomists have studied morphological traits such as hair, pollen grain, epidermis, chromosomal and molecular characteristics. According to the examination of morphological and micromorphological characteristics of the collected samples of the genus *Heterocaryum*, a new identification key was provided, and based on that, *H. echinophorum* species was identified and introduced for the first time in the flora of Afghanistan from Sar-e-pol Province.

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