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Nutlet Micromorphology and Character Evolution of Some Species of Rochelieae (Boraginaceae) and Its Systematic Implications

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Abstract

Background: The macro/micro-morphology of nutlets in 11 species (and 22 accessions) of the Boraginaceae family was investigated by stereomicroscope and scanning electron microscopy to evaluate the taxonomic relevance of these traits. To evaluate the phylogenetic significance of the character evolution, available DNA sequence data from GenBank were combined with selected original nutlet data, and phylogenetic analysis was performed.

Results: The Rochelieae nutlets' shape varied from ovoid (ovoid, ovoid-triangular, and ovoid-rectangular) to the pyramid. Six major patterns were recognized based on nutlet ultrastructure characters. Rocheliae is characterized by a transition from “without appendage” to “with tubercles and prickles” on the nutlet disk, and also by a shift from “lack of prickles” to “glossy prickles”.

Conclusions: The results indicated that the nutlet ultrastructure pattern of Rochelieae is systematically informative at the genus level, but not at the species level. The results showed that glochid is not an ancestral trait but is a synapomorphy and the transition to this trait occurred in the genus *Lappula*. The Close boundary of nutlet microstructures between *L. barbata* and *L. microcarpa* was discussed.

Background

Boraginaceae s.str. is a sub cosmopolitan family of flowering plants with nearly 90 genera and ca.1600 to 1700 species distributed worldwide (Chacón et al. 2016). Based on the recent molecular phylogeny presented by Chacón et al. (2016), infrafamilial classification of Boraginaceae was recognized into three Subfamilies (Echiochiloideae, Boraginoideae, Cynoglossoideae) and 10 tribes (Boragineae, Lithospermeae, Trichodesmeae, Lasiocaryeae, Asperugeae, Omphalodeae, Rochelieae, Craniospermeae, Myosotideae, Cynoglosseae). Tribe Rochelieae consists of five genera (*Eritrichium*, *Hackelia*, *Lappula*, *Lepechiniella*, and *Rochelia*) and about 207 species and belongs to the subfamily Cynoglossoideae. According to Chacón et al. (2016), Rochelieae can be divided into two subtribes including Eritrichiinae and Heterocaryinae. The genera *Eritrichium*, *Hackelia*, *Lappula*, *Lepechiniella*, and *Rochelia*, belong to sub-tribes Eritrichiinae and the genera *Heterocaryum*, *Suchtelenia*, and *Pseudoheterocaryum* belong to sub-tribe Heterocaryinae (Chacón et al. 2016; Saadati et al. 2017). Three genera in subtribe Eritrichiinae (*Eritrichium*, *Lepechiniella*, and *Lappula*) are recognized as non-monophyletic lineage and *Hackelia* and *Rochelia* comprise monophyletic clades (Khoshsokhan-Mozaffar et al. 2018). The recent molecular phylogeny of Rochelieae tribe provided by Khoshsokhan-Mozaffar et al. (2018) indicated a well-supported clade. *Hackelia* and *Rochelia* are monophyletic while *Lappula, Eritrichium*, and *Lepechiniella* are not. So that *Lappula*, as currently circumscribed, is polyphyletic (Khoshsokhan-Mozaffar et al. 2018). The genus *Eritrichium* is the largest genus of the tribe, with 71 species. The genus *Lappula* contains about 70 species of annual, biennial perennial herbs distributed in Eurasia, Africa, North America, and Australia (Ovchinnikova 2005). Although *Lappula* has a cosmopolitan distribution, the center of diversity is in Siberian and Irano-Turanian provinces of the Holarctic kingdom (Ovchinnikova 2009). Initially, Lehmann (1818) circumscribed 15 species in *Echinospermum* Lehm. (Synonym of *Lappula*).

Echinospermum was divided by de Candolle (1845) into three sections based on nutlet morphology as follows: *Lappula*, *Sclerocaryum*, and *Homalocaryum*. The taxonomical problems of *Lappula* were increased when the number of species in the genus began to expand. Consequently, the reported number of sections, subsections, and series has widely varied by different authors (Popov 1953; Riedl 1967). For the first time, Lehmann (1818) used nutlet characters in the systematics of *Lappula* and showed mericarp characters such as mericarp shape and surface ornamentation to distinguish the species of this genus. Generally, in Boraginaceae, nutlet morphology provides useful systematic characters at various taxonomic levels, such as straight or incurved nutlet, a specialized form of emergence, the position of attachment scar, the distinctive form of prickles or glochids, and epidermal features of nutlets (Johnston 1937; Hilger 1985; Al-Shehbaz 1991; Riedl 1996; Långström and Chase 2002; Moon and Hong 2006; Selvi et al. 2006; Kahraman et al. 2011).

The main goal of this study was to investigate the various varieties of nutlet morphological characters of the tribe Rochelieae s.l. using scanning electron microscopy and the nutlet character evolution with more focus on the *Lappula* genus.

Methods

Morphological study

The plants used in this study were collected from their natural habitats in Iran and deposited in the Herbarium of Azarbaijan Shahid Madani University (ASMUH). Also, a small number of species were taken from herbarium specimens of FUMH (Ferdowsi University of Mashhad Herbarium). The list of voucher specimens and details of localities were given in Table.

Table. List of sampled taxa, locality and their vouchers numbers.

Species (Pop. Code)	Locality	Voucher No.
Lappula barbata (M.Bieb.) Gürke (m)	Tehran, Chalus road, Kooshk	ASMUH0020
L. barbata (c)	Mazandaran, Chalus, Delir vilage	ASMUH0021
L. barbata (ab)	Mazandaran, Noshahr, Kojur, Laregan	ASMUH0022
L. barbata (w)	Tehran, Tuchal	ASMUH0023
L. ceratophora (Popov) Popov	South Khorasan, south-west Sarayan	FUMH46077
L. microcarpa (Ledeb.) Gürke (b)	Mazandaran. Noor, Chamestan, Lavij	ASMUH0024
L. microcarpa (e)	Mazandaran, Neka, Hezarjerib	ASMUH0025
L. microcarpa (g)	Golestan, East of Golestan national park	ASMUH0026
L. microcarpa (i)	Mazandaran, Savadkooh, Veresk	ASMUH0027
L. microcarpa (j)	Mazandaran, Polor to Rine	ASMUH0028
L. microcarpa (p)	Mazandaran, Noshahr, Kojur,	ASMUH0029
L. microcarpa (z)	North Khorasan, Chamanbid	ASMUH0030
L. microcarpa (a)	Tehran, Lavasan, Glucan	ASMUH0031
L. semiglabra (Ledeb.) Gürke	Khorasan Razavi, North of Gonabad	FUMH17236
L. sessiliflora (Boiss.) Gürke	Khorasan Razavi, East of Kashmar	FUMH26636
L. spinocarpus (Forssk.) Asch. ex Kuntze	South Khorasan, Birjand, Shahzile	FUMH30399
Pseudolappula sinaica (A.DC.) Asch. & Schweinf.	Tehran, Chalus road, Morod	ASMUH0032
Asperugo procumbens L.	Mazandaran, Damavand, Sarbandan	ASMUH0034
Heterocaryum rigidum A. DC.	Tehran, Jajroad	ASMUH0035
Myosotis sylvatica Ehrh.	Mazandaran, Sari, Sangdeh forest	ASMUH0036
Rochelia disperma (L. f) C. Koch	Tehran, Lavasan	ASMUH0037

This study was conducted on 9 species of tribe Rochelieae (covering five of the six genera) and two species of tribes Asperugeae and Myosotideae as out-groups. The mature nutlets were selected from materials as herbarium samples. Then air dried nutlets were investigated for shape, size, and other features using stereomicroscope (Dino-Lite) with the help of DinoCapture eye and DinoCapture 2.0 Software (Electronics Corporation).

For SEM observations, the nutlets were mounted onto standard aluminum stubs using double-sided adhesive tape and then photographed using a PHILIPS / FEI XL 20 Scanning Electron Microscope at 15 KV voltages. The measurements are based on 15–20 evaluations from each specimen.

The terminology used to describing the qualitative characters is in accordance with Ma et al. 2010; Selvi et al. 2011; Yu et al. 2012; Hilger 2014. The data were analyzed and examined by WARD dendrogram using PAST software for species decimation of *L. microcarpa* and *L.barbata*. Due to high morphological similarities of *L. microcarpa* and *L.barbata*, to identify them, the Flora of Iran, (Nasseh and Joharchi 2017) and also *Flora Iranica* (Riedl 1996) were used.

Phylogenetic analyses and tracing character evolution

The sequences for the internal transcribed spacer (ITS) region have been obtained from GenBank. Sequence alignments were performed using MUSCLE by the MEGA software ver.7 (Kumar et al. 2016). Poorly aligned positions and divergent regions were eliminated by using Gblocks 0.91b, following the given options for less stringency (Castresana 2000). Phylogenetic analyses were performed using the combined 3-loci data set. The partitioned ML analysis was performed using raxmlGUI 1.1 (Silvestro and Michalak 2012) under the GTR + G model with 1000 bootstrap replicates and with *Asperugo* (tribe Asprugeae) and *Myosotis (*tribe Myosotideae*)* chosen as out-group.

The evolutionary history of characters was traced over an ML tree in Mesquite 3.04 (Maddison and Maddison 2015). The ML approach with the Markov k-state one-parameter (Mk1) model was applied (Lewis 2001).

Result

General description of nutlet micromorphology

The nutlet's morphology and ultrastructure characteristics such as shape size, appendages, and surface sculpturing, varied among the studied taxa. The Rochelieae nutlets' shape was ovoid (ovoid, ovoid-triangular, and ovoid-rectangular) to the pyramid (Fig. 1). As the out-groups, the shape of *Asperugo procumbense* was semicircular, and the *Myosotis sylvatica* one was an ellipse. These two genera belong to Asperugeae and Myosotideae tribes, respectively.

Nine qualitative characters including shape, the centerline of the raphe, base surface of prickles on the desk, the arrangement of glochids, the type of lamellae, tubercles on the desk, appendages on nutlet desk, Number of glochid rows on nutlet edge, and emergence type were selected for morphological evaluation of nutlets. The results obtained from nutlet-ultrastructure investigations are described below and illustrated in Fig. 1. Generally, six different surface types were recognized between studied taxa based on nutlet ultrastructure characters as follow:

Type I: Heterocaryum **and** Pseudolappula **(Syn**: L. siniaca**)**

There is no glochid or appendage on the nutlet disk, but there is a row of glochid (*Heterocaryum*) or glochid-like (*Pseudolappula*) on the nutlet edge. The glochids are distributed in very low numbers in the edges of the nutlets of *Pseudolappula*. The nutlet disk ornament of *Heterocaryum* is “papilla verrucose with verrucae minutely muricate” (called complex papilla) while it is “papilla with aggregate verrucose in the center” in *Pseudolappula*. It appears that each of the microcapillaries found in *Psudolappula* has become complex in the *Heterocaryum*, and each has formed warts (verrucose) that have become more complex and denser.

Type II: Lappula (*L. barbata*, *L. microcarpa* and *L. semiglabra*)

Glochids in different sizes and rows can be seen in the nutlet edge and sometimes on the nutlet disk surface. Glochids have an anchor with 2–4 branches at the apex, and the surface of the glochids is smooth. The ultrastructure of the nutlet emergencies is stellar-aculeate, and sometimes the appendages are prickles or tubercles are seen (scattered or collected) on the surface of the nutlet disk and edge. The glochid stem is composed of fusiform cells, and there are tubercles with 2 to 5 mineralized spines on the stem. These tubercles are also present on the entire surface of the nutlet with a different distribution.

Type III: Rochelia (*R. disperma*, *R. sessiflora* = *L. sessiflora*)

The prickles are stellate and are scattered throughout the surface of the nutlet. The surface of the prickles is not glossy and has verrucose. The tubercles often have more than 2 spines, and the emergencies are stellar-aculeate (similar to type II). Although the nutlet surface of *R. sessiflora* is similar to type II (presence of glochid on the nutlet edge). Moreover, the prickles and verrucose on it and the accumulation of tubercles with more than 5 spines around each prickle show more similarity to type III.

Type IV: L. ceratophora **and** L. spinocarpus

There is not any glochid, tubercle, or prickles on the nutlet surface. The nutlet surface of *L. ceratophora* is not smooth, and the papilla appears as a verrucose-like. While the ultrastructure of the nutlet in *L. spinocarpus* seems papilla with flowerlike verrucose. Also, the tubercles appear as verrucose and lack any spines.

Type V: Asperugo

The nutlet surface lacks any glochid and prickles. Papilla appears as dome-shaped in different sizes, and it is verrucose at the base of them.

Type VI: Myosotis

The surface of the nutlet is smooth, and there is not any ornamentation (nonexpressiate).

a–h = Type I; (a-d) *Heterocaryum rigidum*; **a, b**: An overview photograph of nutlet with stereomicroscope and SEM. **c, d**: The close up views of nutlet disk with “papilla verrucose with verrucae minutely muricate”.

(e-h) *Pseudolappula siniaca*; **e, f**: An overview photograph of nutlet with stereomicroscope and SEM. **g, h**: The close up views of nutlet disk with “papilla with aggregate verrucose in center”.

i-o Type II. (**i, j)** *Lappula. barbata*; **(k, l)** *Lappula. Microcarpa*; (The more detail of these two species were described between different specimens in Fig. 3). *Lappula semiglabra* **m, n**: An overview photograph of nutlet with stereomicroscope and SEM. **o**: The close up views of nutlet disk.

p-v = Type III; (p-r) *Rochelia sessiflora*; **p, q**: An overview photograph of nutlet with stereomicroscope and SEM. **r**: The close up views of nutlet disk with prickles and verrucose on it. **(s-v)** *Rochelia disperma* **s, t**: An overview photograph of nutlet with stereomicroscope and SEM. **u, v**: The close up views of stellare-aculeate emergencies in nutlet disk.

w-ac = Type IV; (w-y) *Lappula spinocarpus*; **w, x**: An overview photograph of nutlet with stereomicroscope and SEM. **y**: The close up views of nutlet disk with papilla with flowerlike verrucose on it. **(z-ab)** *Lappula ceratophora* **z, aa**: An overview photograph of nutlet with stereomicroscope and SEM. **ab**: The close up views of nutlet disk with papilla appears as a verrucose-like on it.

ac-af = Type V; *Asperugo procumbense*; **ac-ad**: the overview photograph of nutlet with stereomicroscope and SEM. **af**: The close up views of nutlet disk with Papilla appears as dome-shape on it.

ag-aj = Type VI; *Myosotis sylvatica*; **ag-ai**: the overview photograph of nutlet with stereomicroscope and SEM. **aj**: The close up views of nutlet disk with smooth surface.

3.2. Evolution of microstructural characters of nutlet

The resulting ancestral state reconstruction and the proportional likelihoods for character states are shown in Fig. 2. The out-group species *Asperugo* and *Myosotis* were unique regarding the bilaterally flattened and ellipse with a smooth surface, respectively. Tracing the evolution of nutlet micromorphology indicated that the glochids were not an ancestral character.

Arrangement of glochid character:

The status of ancestral taxa (with or without glochids) was unclear, and the proportional likelihoods of any three characters were almost equal (node A). Transition to the glochids character occurs in the genus *Lappula* (*L. semiglabra*, *L. microcarpa*, and *L. barbata*) (node H).

The appendage on nutlet disk character:

The status “without appendage” and “dump-shape papilla” in *Myosotis* and *Asperugo* (the proportional likelihoods 1) were differentiated these two tribes from each other and Rocheliea tribe. The tubercle and prickles on the disk were ancestral characters (the proportional likelihoods 0.43). While the ancestor of these characters is unclear in node A, the status “tubercle and prickles on disk” had more proportional likelihoods in A group and then C, D, F. Transition to the “lack of appendage” status occurred in the *L. ceratophora*, *L. spinocarpus;* node G).

Prickles surface character:

Tracing of character “prickles surface” showed the ancestral status of “lack of prickles” in node A that to be followed with less proportionality in nodes B, C, and D. Transition to the “glossy prickles” status occurred in the genus *Lappula* in node H. Moreover, the transition to the simple and complex “verrucose prickles” status was observed in genus *Rochelia* in node F.

Surface emergence:

Tracing of surface emergence character was unclear in node A. However, the proportional likelihoods of “stellar-aculeate” status had the highest node C ratio (0.72).

In node G, the transition to “verrucose-subverrucose” status (the proportional likelihoods 0.99) was stabilized as a synapomorphy.

Other traits were studied regarding evolutionary tracing that did not show clear evolutionary signals in the nodes, such as the shape of the nutlet, the lamella type, the shape of the nutlet, the lamella type, and the centerline of the nutlet disk.

3.3. Close boundary of nutlet microstructures between L. barbata and L. microcarpa

Both *L. microcarpa* and *L.barbata* had high micro-morphological similarities (Fig. 3). Different clustering and ordination methods produced similar results; therefore, only WARD tree of micro-morphological characters is presented here (Fig. 4). In general, plant samples of each species did not group and formed a separate group. This result shows that the micro-morphological characters studied could not delimit these two species.

Discussion

As in other Boraginaceae genera (Ovchinnikova 2009; Weigend et al. 2009; Selvi et al. 2011; Yu et al. 2012; Hilger 2014), the infrageneric taxonomic significance of nutlet characteristics in tribe Rochelieae were found to be obvious when investigated under stereomicroscope and SEM. In this study, it was found that the appendages on the nutlet varied between different genera. Although the fruit type of Boraginaceae is relatively constant, the variation in nutlet ornamentation has quickly occurred in some tribes like Cynoglosseae sensu lato (including tribe Rocheliea) and Trichodesmeae (Cohen 2014). It is not surprising that *Lappula*, one of the largest genera in Rochelieae, shows considerable diversity in nutlet characters. Recent molecular evidence suggested that *Lappula* is polyphyletic (Khoshsokhan-Mozaffar et al. 2018). In this way, Khoshsokhan-Mozaffar et al. indicated that species of the *Lappula* genus are scattered across the Eritrichiinae clade and forming three distinct lineages. *Lappula sinaica*, as a new genus, was segregated from *Lappula* and established as genus *Pseudolappula* (Khoshsokhan-Mozaffar et al. 2018). Moreover, as shown in our study, a distinct generic delimitation based on nutlet characters alone could be detected for *Pseudolappula* (syn: *Lappula siniaca*). Nutlet micromorphology of *Pseudolappula* (syn: *L. siniaca*) provides valuable data in separating it from *Lappula* genus; these

characters are “no glochid or appendage on nutlet disk” and the type of nutlet ornamentation including “papilla with aggregate verrucose in the center”. The features of disk ornamentation indicated more close affinity of *Pseudolappula siniaca* to *Heterocaryum* than *Lappula* genus, especially in the evolution of microcapillaries.

Current data are also in agreement with molecular phylogenies provided by Khoshsokhan-Mozaffar et al. (2018) that transferred closely related species *L. sessiliflora* to *Rochelia* genus. This conclusion has previously been suggested by various studies (Khoush et al. 2010; Huang et al. 2013; Mozaffar et al. 2013; Rolfsmeier 2013; Weigend et al. 2013). Moreover, the flowers and nutlet features (two of them undeveloped) indicated more affinity of the species to *Rochelia* genus than *Lappula* (Popov 1974). Moreover, our result indicated the features like prickles and verrucose on the nutlet and the accumulation of tubercles with more than five spines around each prickle show more similarity to genus *Rochelia*.

Our study convincingly provided a clear distinction between two species *L. ceratophora* and *L. spinocarpus* belonging to sect. *Sclerocaryum* (Riedl 1967; Ovchinnikova 2009) from other *Lappula* genus. The lack of glochid, tubercle, or prickles on the nutlet surface and specific types of nutlet ornamentation (papilla with verrucose like or flowerlike-verrucose) characterize the clade that includes them regarding recent molecular phylogenetic analysis (Khoshsokhan-Mozaffar et al. 2018).

We identified four different types of nutlet surface ornamentation among studied taxa. According to our study and the other available record (Cohen 2014), the ancestral type is ambiguous for the family and Rochelieae tribe. Given the matrix of cpDNA and nutlet surface analysis, Cohen (2014) indicated that smooth nutlets as ancestral for the clade that includes Boragineae and Lithospermeae tribes, and Nutlets with glochids as a synapomorphy for Cynoglosseae sensu lato (including tribe Rocheliea). The results of our study confirmed Cohen assertion that glochid is not ancestral trait but is a synapomorphy so that in node H, the transition to this trait occurred in the genus *Lappula*. Rocheliae is characterized by a transition from “without appendage” to “with tubercles and prickles” on the nutlet disk, and also by a shift from “lack of prickles” to “glossy prickles”. Also, a transition from “nonexpressiate” status of surface emergence to “stellar-aculeate” status occurred in this tribe. interestingly, in this tribe, smooth nutlets are a synapomorphy (Cohen 2014). Considering this point, the transition to “lack of appendage” that occurred in node G could indicate a synapomorphy of two species of sect. *Sclerocaryum* (*L. ceratophora* and *L. spinocarpus*).

In Boraginaceae, nutlets need to develop strategies to achieve dispersal ability. In previous studies, nutlets with glochids or wings have implied adaptive traits for additional dispersal types, such as epizoochory or anemochory (Ma et al. 2010; Selvi et al. 2011). The presence of glochid on neutlet could be an explanation of the widespread geographic distribution of Cynoglosseae sensu lato (including Rochelieae) (Cohen 2014). According to Weigend et al. (2016), two species of sect. *Sclerocaryum* applies “the whole-plant dispersal by wind or flash-floods” as a dispersal mechanism which could explain the synapomorphy of “lack of appendage” observed in our results. Indeed, this mechanism caused the separation of nutlets from the mother plant to become unnecessary.

Nutlet micromorphology results in this study provide no clear distinction among species *L.barbata* and L. *microcarpa*. The morphological complexities of these two species have already been addressed by different taxonomists (Popov 1953; Akhani 1998). A revision of the *Lappula* genus by Nasseh and Joharchi (2017) suggested that the two species may be synonymous and more molecular studies be conducted. Moreover, according to the molecular results (Khoshsokhan-Mozaffar et al. 2018) in the nr-DNA ITS tree of Rochelieae, the clade delimiting these two species was not well-supported. The variety observed in the nutlet of *L.microcarpa* and *L.barbata* could be related to seed heteromorphism that previously is known to occur in a few Boraginaceae genera Boraginaceae, e.g. *Eritrichium, Lappula* (*L. duplicicarpa* and *L. semiglabra*) and *Heterocaryum* (Wang et al. 1989).

Conclusions

In this study, the nutlet ultrastructure pattern of Rochelieae is systematically informative at the genus level, but not at the species level. The results showed that glochid is not an ancestral trait but is a synapomorphy and the transition to this trait occurred in the genus *Lappula*. Nutlet micromorphology results in this study provide no clear distinction among species *L.barbata* and L. *microcarpa*.

Declarations

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Authors' contributions

SN designed the study project, ME & SN performed experiments and data analysis and drafted the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The data used and analyzed for the current study can be obtained from the corresponding author.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Akhani H (1998) Plant biodiversity of Golestan National Park, Iran. Biologiezentrum des OÖ Landesmuseums
2. Al-Shehbaz IA (1991) The genera of Boraginaceae in the southeastern United States. Journal of the Arnold Arboretum. Suppl Ser 1:1-169
3. Castresana J (2000) Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. Mol biol evol 17:540-552
4. Chacón J, Luebert F, Hilger HH, Ovchinnikova S, Selvi F, Cecchi L, Guilliams CM, Hasenstab-Lehman K, Sutorý K, Simpson MG (2016) The borage family (Boraginaceae s. str.): A revised infrafamilial classification based on new phylogenetic evidence, with emphasis on the placement of some enigmatic genera. Taxon 65:523-546
5. Cohen JI (2014) A phylogenetic analysis of morphological and molecular characters of Boraginaceae: evolutionary relationships, taxonomy, and patterns of character evolution. Cladistics 30:139-169
6. de Candolle A (1845) Prodromus Systematis naturalis regni vegetabilis, Paris.
7. Hilger H (1985) Ontogenie, Morphologie und systematische Bedeutung geflugelter und glochidientragender Cynoglosseae-und Eritricheae-Fruchte (Boraginaceae). Bot Jahrb Syst, Pflanzengesch Pflanzengeogr
8. Hilger HH (2014) Ontogeny, morphology, and systematic significance of glochidiate and winged fruits of Cynoglosseae and Eritrichieae (Boraginaceae). Plant Divers Evol 131:167-214
9. Huang JF, Zhang ML, Cohen JI (2013) Phylogenetic analysis of Lappula Moench (Boraginaceae) based on molecular and morphological data. Plant syst evol 299:913-926
10. Johnston I (1937) Studies in the Boraginaceae XII. 2. Novelties and critical notes. J Arnold Arbor 18:10-25
11. Kahraman A, Celep F, Doğan M, Guerin GR, Bagherpour S (2011) Mericarp morphology and its systematic implications for the genus Salvia L. section Hymenosphace Benth.(Lamiaceae) in Turkey. Plant syst evol 292:33-39
12. Khoshsokhan-Mozaffar M, Sherafati M, Kazempour-Osaloo S (2018) Molecular phylogeny of the tribe Rochelieae (Boraginaceae, Cynoglossoideae) with special reference to Lappula. Ann Bot Fenn BioOne 293-308
13. Khoush SMM, Kazempour OS, Saadatmand S, Atar F (2010) Molecular phylogeny of Rochelia (Boraginaceae) based on nrDNA ITS and cpDNA trnL-F sequences. 22-29

14. Kumar S, Stecher G, Tamura K (2016) MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol biol evol 33:1870-1874
15. Långström E, Chase M (2002) Tribes of Boraginoideae (Boraginaceae) and placement of Antiphytum, Echiochilon, Ogastemma and Sericostoma: a phylogenetic analysis based on atpB plastid DNA sequence data. Plant Syst Evol 234:137-153
16. Lehmann JGC (1818) Plantae e familia Asperifoliarum nuciferae. Dummler, Berlin, p 78
17. Lewis PO (2001) A likelihood approach to estimating phylogeny from discrete morphological character data. Syst biol 50:913-925
18. Ma W, Zhao X, Tan D, Baskin C, Baskin J, Xue J (2010) Nutlet dimorphism in individual flowers of two cold desert annual Lappula species (Boraginaceae): implications for escape by offspring in time and space. Plant ecol 209:361-374
19. Maddison W, Maddison D (2015) Mesquite: a modular system for evolutionary analysis. Version 3.04. 2015
20. Moon HK, Hong SP (2006) Nutlet morphology and anatomy of the genus Lycopus (Lamiaceae: Mentheae). J Plant Res 119:633-644
21. Mozaffar MK, Osaloo SK, Oskoueiyan R, Saffar KN, Amirahmadi A (2013) Tribe Eritrichieae (Boraginaceae s. str.) in West Asia: a molecular phylogenetic perspective. Plant Syst Evol 299:197-208
22. Nasseh Y, Joharchi MR (2017) Revision of the genus Lappula Moench based on morphological characters in Khorassan Provinces (Iran). Nova Biol Rep 4:66-73
23. Ovchinnikova S (2005) The system of the subtribe Echinosperminae (Boraginaceae). Bot Zhurn 90:1153-1172
24. Ovchinnikova S (2009) On the position of the tribe Eritrichieae in the Boraginaceae system. Bot Serbica 33:141-146
25. Popov M (1953) Boraginaceae. Flora SSSR 19:97-691
26. Riedl H (1967) Boraginaceae in, Rechinger KH. Flora Iranica. Graz, Akademische Druck_u. Verlagdanstalt 48:215
27. Riedl H (1996) Studies in the genus Lappula (Boraginaceae) I. Lappula in the" Flora Iranica" region. Serie B für Botanik und Zoologie. Ann Naturhist Mus 79-86
28. Rolfsmeier SJ (2013) Taxonomy and phylogeny of the genus Lappula Moench (Boraginaceae) in North America. Kansas State University.
29. Saadati N, Mozaffar MK, Sherafati M, Osaloo SK (2017) Pseudoheterocaryum, a new genus segregated from Heterocaryum (Boraginaceae) on the basis of molecular data. Aust Syst Bot 30:105-111
30. Selvi F, Bigazzi M, Hilger HH, Papini A (2006) Molecular phylogeny, morphology and taxonomic re-circumscription of the generic complex Nonea/Elizaldia/Pulmonaria/Paraskevia (Boraginaceae-Borageneae). Taxon 55:907-918

31. Selvi F, Coppi A, Cecchi L (2011) High epizoochorous specialization and low DNA sequence divergence in Mediterranean Cynoglossum (Boraginaceae): Evidence from fruit traits and ITS region. Taxon 60:969-985
32. Silvestro D, Michalak I (2012) raxmlGUI: a graphical front-end for RAxML. Org Divers Evol 12:335-337
33. Weigend M, Gottschling M, Selvi F, Hilger HH (2009) Marbleseeds are gromwells–Systematics and evolution of Lithospermum and allies (Boraginaceae tribe Lithospermeae) based on molecular and morphological data. Mol Phylogenetics Evol 52:755-768
34. Weigend M, Luebert F, Selvi F, Brokamp G, Hilger HH (2013) Multiple origins for Hound's tongues (Cynoglossum L.) and Navel seeds (Omphalodes Mill.)–The phylogeny of the borage family (Boraginaceae s. str.). Mol phylogenetics evol 68:604-618
35. Weigend M, Selvi F, Thomas D, Hilger H (2016) Boraginaceae. Flowering Plants. Eudicots. Springer, pp. 41-102
36. Yu WT, Jacques FM, Chen ST, Zhou ZK (2012) Nutlet micro-morphology of the genus Microula (Boraginaceae) from the Qinghai–Tibetan Plateau, and its systematic implications. Nord J Bot 30:596-612
37. Wang WC, Liu YL, Zhu GL, Lian YSh, Wang JQ, Wang QR (1989) Lappula V. Wolf. In: Kong XW, Wang WC (eds) Flora Reipublicae Popularis Sinicae, vol 64. Science Press, Beijing, pp 177-207 (in Chinese)

Figures

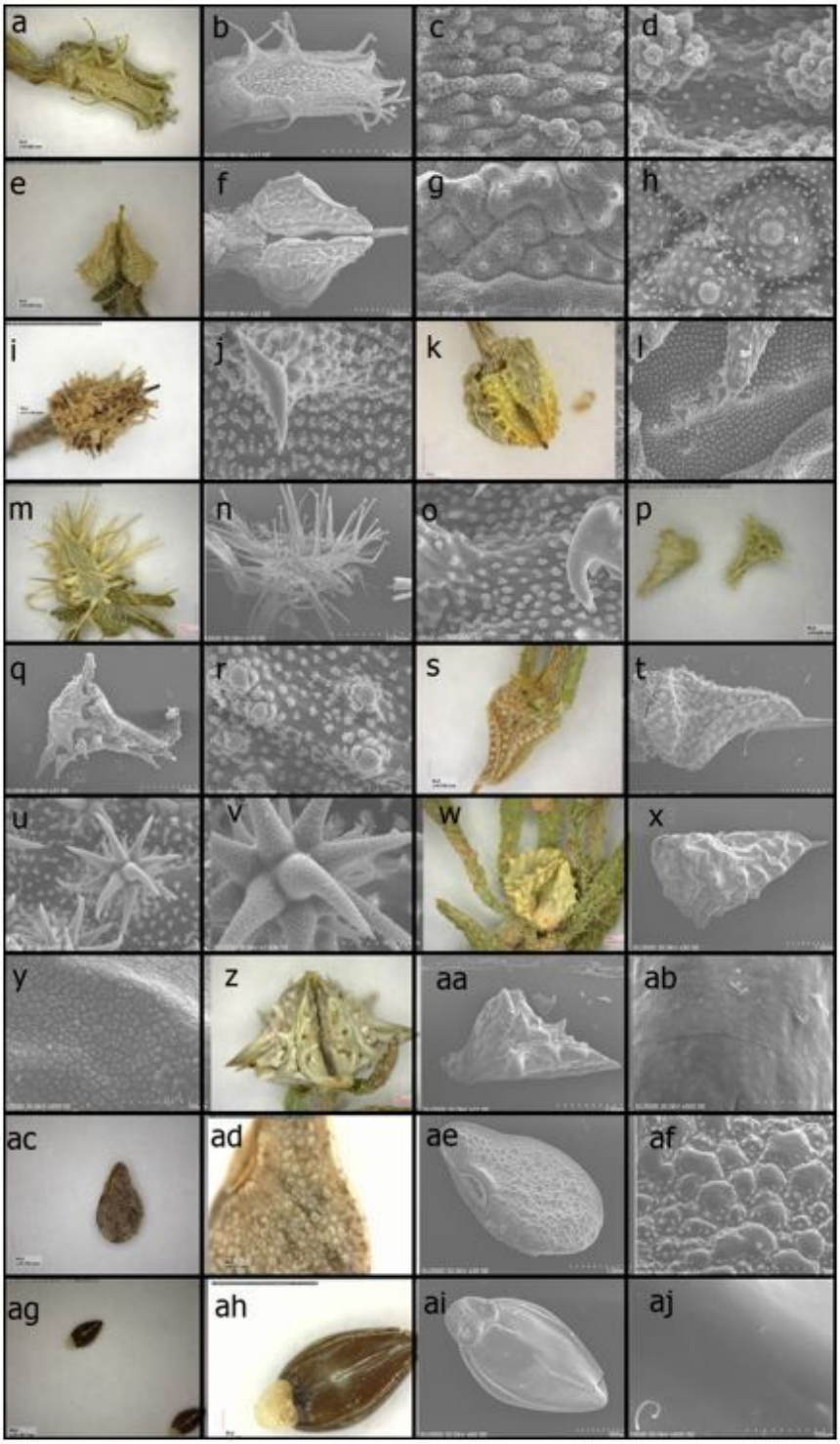

Figure 1

Six types of nutlet morphology in the studied species.

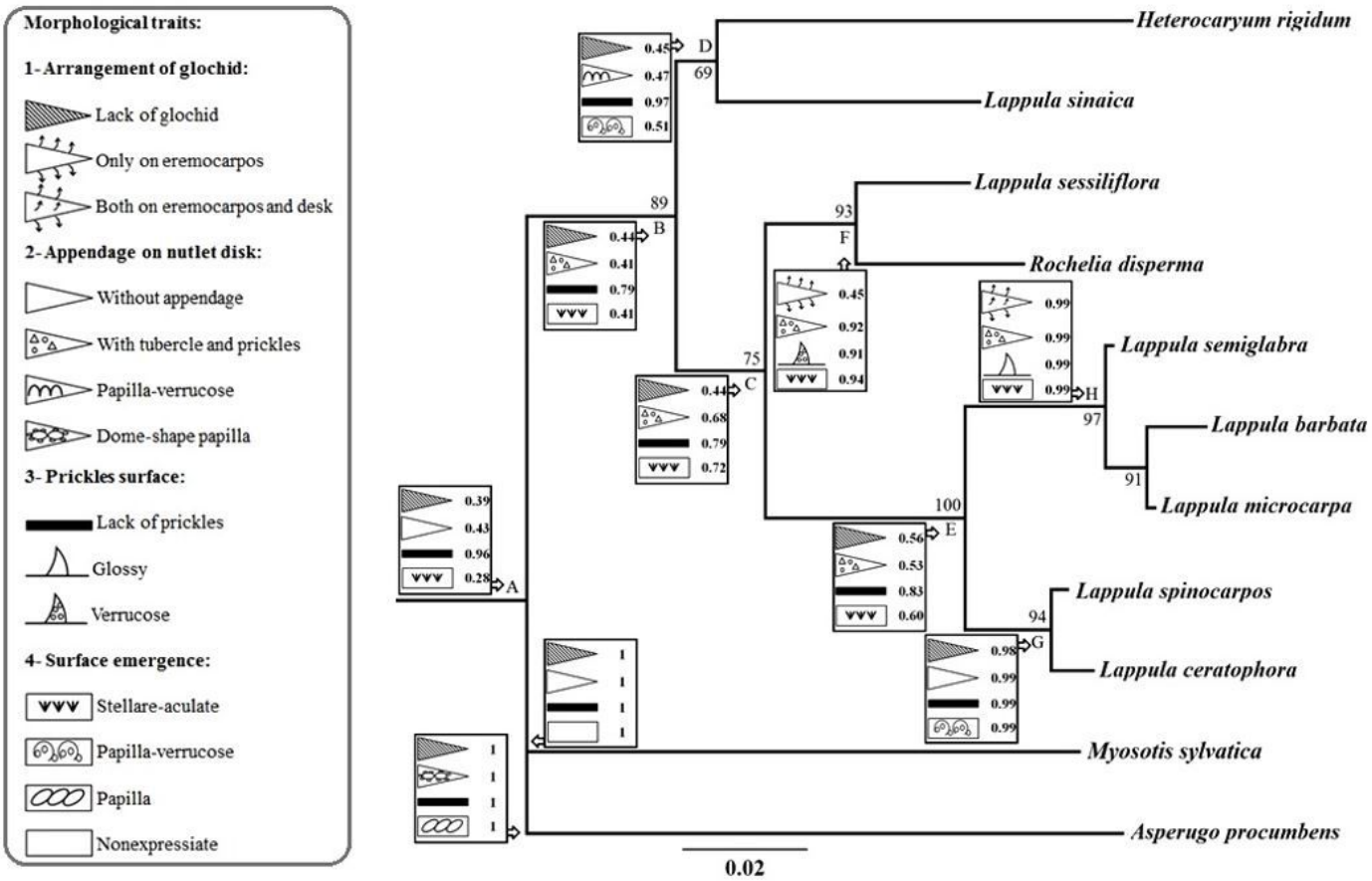

Figure 2

Results of nutlet character evolution shown on the Maximum Likelihood tree of tribe Rochelieae (based on the internal transcribed spacer). Numbers on branches are Maximum Likelihood bootstrap support (only shown when ≥ 65).

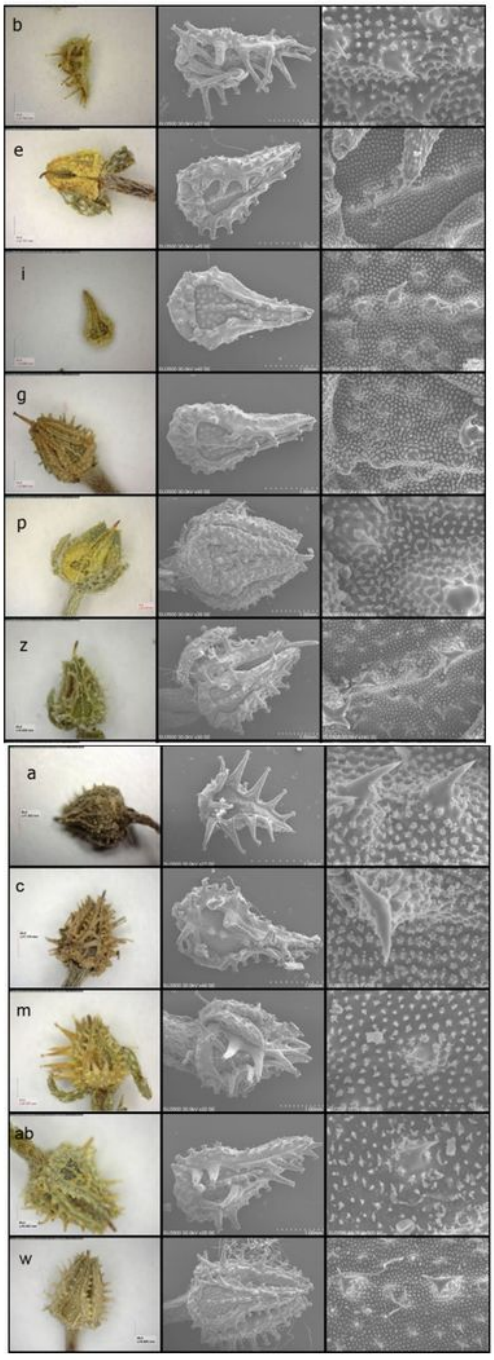

Figure 3

nutlet morphology in the different specimens of Lappula microcarpa and lappula barbata. The details of the studied specimens are in accordance to the Table (L. microcarpa codes: b, e, I, g, p, z, a; L. barbata: c, m, Ab, w)

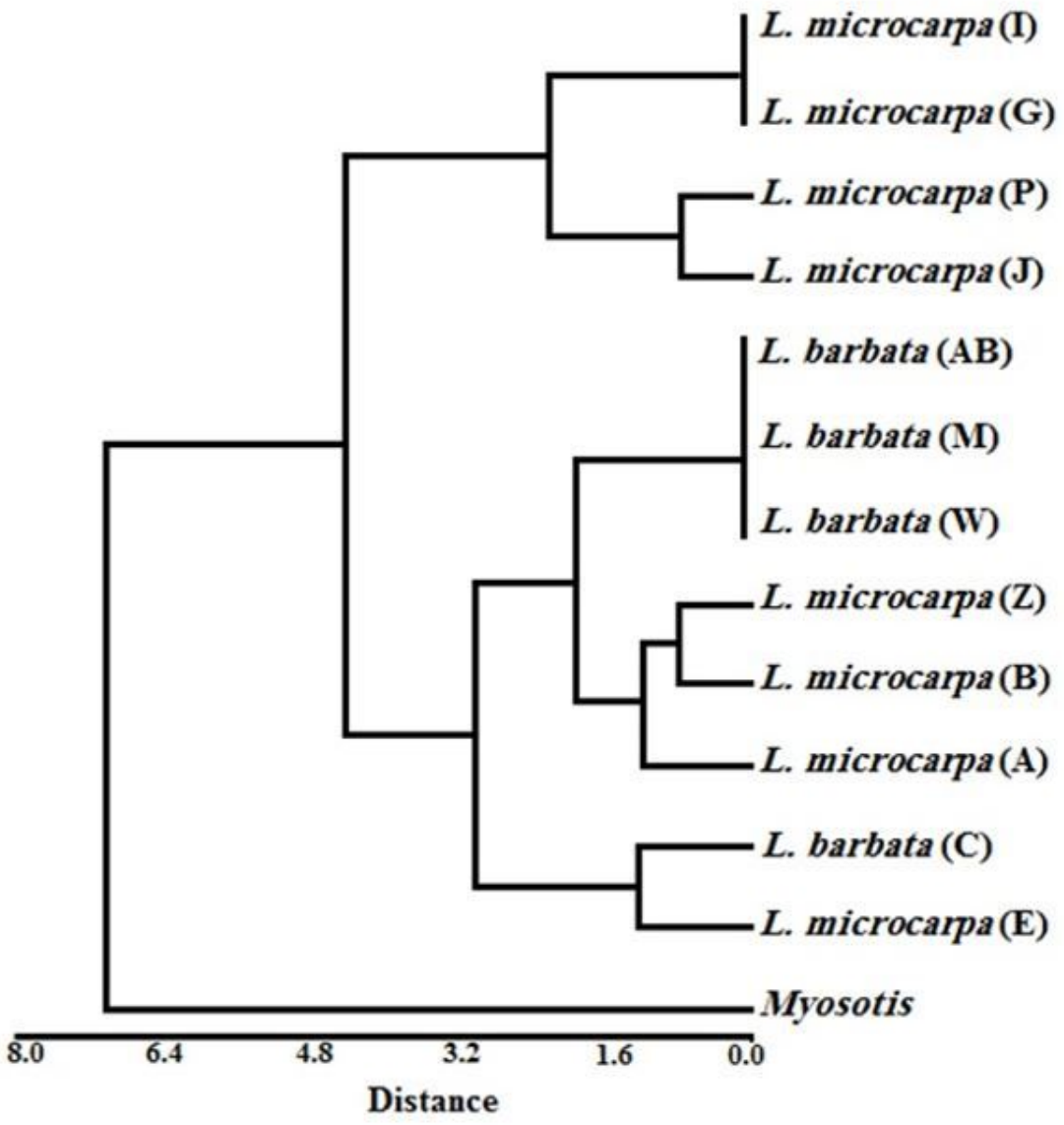

Figure 4

WARD dendrogram showing the relationship among different specimens belong to L. microcarpa and L. barbata based on nutlet characters.