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ARTICLE

# Systematic significance of micromorphological and palynological characteristics in *Lagochilus* Bunge ex Benth. (Lamiaceae) in Iran

**Fatemeh Azimishad[1]*, Masoud Sheidai[1], Maryam Keshavarzi[2], Seyed Mehdi Talebi[3], Zahra Noormohammadi[4]**

[1]Faculty of Life Sciences and Biotechnology, Shahid Beheshti University, Tehran, Iran
[2]Department of Plant Sciences, Faculty of Biological Science, Alzahra University, Tehran, Iran
[3]Department of Biology, Faculty of Sciences, Arak University, Arak 38156-8-8349, Iran
[4]Biology Department, Islamic Azad University, Sciences and Research Branch, Tehran, Iran

**ABSTRACT** *Lagochilus* is a genus with ten taxa (species, subspecies and variety) in Iran, which nine of them are endemic. This is the first micromorphological investigation of this genus. Micromorphological features of trichomes on the stems, leaves and calyces, and also pollen morphology of 19 populations were investigated by scanning electron microscopy. Two types of trichome (glandular and non-glandular) including 14 forms were described. Here, among the non-glandular trichomes, cell number and size of trichomes are considered as valuable characteristics, while the glandular trichomes are observed as stalked, sessile and peltate. *Lagochilus* pollen grains are tricolpate and small to medium sized. The basic shape of the pollen grains in most taxa is prolate, however prolate-subprolate pollen grains was recorded for *L. aucheri* ssp. *aucheri* var. *aucheri* 2. Four types of exine sculpture patterns were distinguished: bireticulate, reticulate, microreticulate and incomplete reticulate. Quantitative and qualitative characteristics were examined by multivariate analysis. The results indicated that the studied taxa were separated from each other; however varieties of *L. aucheri* did not grouped together. The results support the existence of known varieties in *L. aucheri*. In general, our investigations reveal the usefulness of micromorphological characteristics in taxon delimitation at the specific and infraspecific levels.
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***Corresponding author**
E-mail: ailarazimishad3@gmail.com

## Introduction

The genus *Lagochilus* Bunge ex Benth. (1834), belong to family Lamiaceae, subfamily Lamioideae (Bendiksby et al. 2011), comprises about 44 species, from which six species occur in Iran (Jamzad 1988; Zhang et al. 2017). *Lagochilus* species are mostly distributed in dry slopes, valleys and deserts from Iran to Mongolia, Russia (south Siberia), northwest China and north Pakistan, but have wide distribution in Central Asia (Harley et al. 2004; Zhang et al. 2017).

The chemical components of several *Lagochilus* species were surveyed due to their medicinal uses. Lagochilin is a diterpene which was found in various species of the genus, and is thought to be responsible for the sedative, hypotensive and hemostatic effects of these species (Chizhov et al. 1970; Jiao et al. 2014). Taban et al. (2009) have reported the antibacterial activity of flowers and leaves of *L. kotschyanus* Boiss. (1848) oils against four Gram-positive and two Gram-negative bacteria.

These plants are subshrubs or perennial herbs with woody root and green-white, rigid stem with spiny bracteoles. Leaf blade is rhombic, palmatipartite or pinnatipartite with campanulate-tubular calyx and villous-pillose corolla. Nutlets flattened-obconical, oblong-obovoid or oblongovid (Jamzad 1988).

Ikramov (1976) investigated the classification, distribution, and community ecology of *Lagochilus* species in Central Asia of former USSR. Recently, Zhang et al. (2017) have studied *Lagochilus* species using chloroplast sequence data to reveal the species relationships as well as the date of divergence. However only three species sampled from Iran was included in their investigation and they were considered as a complex and very much different clade within the genus *Lagochilus* (Zhang et al. 2017).

Boissier (1879) mentioned 6 species, Parsa (1949) listed 6 species and two varieties and Rechinger (1982) recognized 5 species from Iran. Jamzad (1988) identified 6 species through revision of the genus *Lagochilus* in Iran, these

are *L. alutaceus* Bunge (1873), *L. aucheri* Boiss. (1844), *L. lasiocalyx* (Stapf) Jamzad (1988), *L. macranthus* Fisch. & C.A. Mey. (1841), *L. quadridentatus* Jamzad (1988) and *L. cabulicus* Benth. (1848). The first five species are endemic to Iran and are considered as the endangered species (Jalili and Jamzad 1999).

Jamzad (1988) described *L. quadridentatus* and 3 infraspecific taxa as *L. aucheri* ssp. *heterophyllus, L. aucheri* ssp. *aucheri* var. *elegans* and *L. aucheri* ssp. *aucheri* var. *tomentosus*. She found a complexity in naming of *L. aucheri* group. Morphological characteristics as calyx teeth number, calyx width, shape of leaves and indumentum type of different parts of calyx, nutlet and stem traits were used in identification key for specific and infraspecific level. However, according to the Plant List, there are five species in Iran and known subspecies and varieties by Jamzad are synonym names (Govaerts et al. 2013).

The taxonomic value of trichomes density and types and also pollen morphology in identification, delimitation and phylogenetic reconstruction of some members of the family Lamiaceae have been elucidated by many authors (Erdtman 1945; Wunderlich 1967; Abu-Asab and Cantino 1989; 1992; 1993a,b; 1994; Cantino 1990; Doaigey1991; Bini Maleci and Servettaz, 1991; Harley et al. 1992; Wagstaff 1992; Navarro and El Qualidi, 2000; Moon and Hong 2003; C¸elenk et al. 2008 a,b; Moon et al. 2008a,b,c; Salmaki et al. 2008; Hassan et al. 2009; Gairola et al. 2009; Salmaki et al. 2009; Özler et al. 2011; 2013; Firdous et al. 2015 ; Atalay et al. 2016a,b; Talebi et al. 2018a,b). Besides of systematic significance, trichomes have several roles in plants. For example, there are assumed to attraction of pollinators (Wagner 1991), protection from herbivores and pathogens (Xiao et al. 2017) and decrease the leaf temperature and transpiration (Peter and Shanower 1998).

Pollen morphology is generally supported segregation of some genera of Lamiaceae (Abu-Asab and Cantino 1994). Palynological study of the genus *Lagochilus* have been poorly investigated and is still lacking (Abu-Asab and Cantino 1994; Talebi and Rezakhanlou 2012; Badamtsetseg et al. 2012), so their work comprises only a few *Lagochilus* species.

Whereas phylogenetic and molecular studies of most *Lagochilus* species have not been investigated yet and also there is no comprehensive micromorphological and palynological study covering all species of *Lagochilus* in Iran and Asia, therefore, the main objectives of the present study are to provide a detailed investigation and description of trichomes and pollen micromorphology of the genus *Lagochilus*, mainly by scanning electron microscopy (SEM), to determine whether these data can be of value in the taxonomy of the genus and delimitation of the species. Furthermore, trichomes and pollen morphology of most species was described for the first time.

**Table 1.** Voucher specimens of the genus *Lagochilus* examined in the present study. Ns: number of plant samples.

| Taxon | Ns | Locality | Latitude | Longitude | Altitude (m) | Voucher code |
|---|---|---|---|---|---|---|
| *L. aucheri* ssp. *aucheri* var. *aucheri* 2 | 5 | Zanjan, Dash kasan Mt. | 36° 43′ 58″ | 48° 79′ 20″ | 1700 | HSBU2018650 |
| *L. aucheri* ssp. *aucheri* var. *aucheri* 2 | 5 | Zanjan, 90 km of Zanjan-Bijar road | 35° 75′ 79″ | 48° 48′ 22″ | 1500 | HSBU2018651 |
| *L. aucheri* ssp. *aucheri* var. *aucheri* 2 | 5 | Zanjan, Bulamaji village | 36° 56 ′ 31 ″ | 48° 48 ′ 79 ″ | 1500 | HSBU2019100 |
| *L. aucheri* ssp. *aucheri* var. *aucheri* 1 | 5 | Qazvin, Abegarm | 35° 80′ 03″ | 49° 31′ 19″ | 1500 | HSBU2018652 |
| *L. aucheri* ssp. *aucheri* var. *aucheri* 3 | 5 | Alborz, Karaj, Dizin | 35° 82′ 00″ | 50° 97′ 00″ | 2500 | HSBU2018653 |
| *L. aucheri* ssp. *aucheri* var. *tomentosus* | 5 | Tehran, Firuzkuh, Sarbandan | 35° 75′ 50″ | 52° 77′ 24″ | 2000 | HSBU2018654 |
| *L. aucheri* ssp. *aucheri* var. *tomentosus* | 5 | Tehran, Damavand, Voleyran | 35° 68′ 84″ | 52° 06′ 38″ | 2000 | HSBU2019101 |
| *L. aucheri* ssp. *aucheri* var. *tomentosus* | 3 | Tehran, Damavand, Tar lake | 35° 73′ 17″ | 52° 21′ 00″ | 1950 | HSBU2019102 |
| *L. aucheri* ssp. *aucheri* var. *kotschyanus* | 4 | Markazi, Delijan | 34°06′ 38.7″ | 50° 32′ 23″ | 1511 | HSBU2018658 |
| *L. aucheri* ssp. *aucheri* var. *kotschyanus* | 4 | Markazi, Mahallat | 34°00′ 43.2″ | 50° 32′ 14.2″ | 1910 | HSBU2019103 |
| *L. aucheri* ssp. *aucheri* var. *elegans* | 5 | Qazvin, Takestan | 36° 07′ 21″ | 49° 70′ 13″ | 1450 | HSBU2018660 |
| *L. alutaceus* | 5 | Razavi Khorasan, Chenaran | 36°30′ 52.3″ | 59°02′ 42 ″ | 1450 | HSBU2018655 |
| *L. cabulicus* | 5 | North Khorasan, Esfarayen | 36°56′ 0.23″ | 57° 44′ 3″ | 1535 | HSBU2018656 |
| *L. cabulicus* | 5 | Razavi Khorasan, Chenaran | 36°30′ 52.3″ | 59°02′ 42 ″ | 1450 | HSBU2018661 |
| *L. macracanthus* | 5 | Alborz, Eshtehard, Jafar abad village | 35° 72′ 44″ | 50° 36′ 62″ | 1300 | HSBU2018657 |
| *L. macracanthus* | 5 | Alborz, Eshtehard, Nekujar village | 35° 67′ 84″ | 50° 39′ 29″ | 1450 | HSBU2019104 |
| *L. macracanthus* | 5 | Tehran, Vardavard Mt. | 35° 73′ 60″ | 51° 10′ 30″ | 1500 | HSBU2019105 |
| *L. macracanthus* | 4 | 20-kilometer after Saveh, Samavak village. | 35° 03′ 85″ | 50° 08′ 42″ | 1500 | HSBU2019106 |
| *L. lasiocalyx* | 4 | Isfahan, Bagherabad | 33°51′ 12.4″ | 50° 33′ 9″ | 1638 | HSBU2018659 |

## Materials and Methods

### Plant samples

Totally 19 accessions of 10 taxa (species, subspecies and variety) were examined. In each populations, three to five plants were sampled for the scanning electron microscopy (SEM). Vouchers were collected from nature (2016-2018) and identified based on the descriptions provided in Flora Iranica (Rechinger 1982) and Flora of Iran (Jamzad 2012). Accessions studied and voucher details are listed in Table 1. All vouchers of the current study were deposited in Herbarium of Shahid Beheshti University (HSBU).

### Micromorphological characters

Micromorphological features of leaves, stems and calyces were assessed using SEM. One to five mature leaves, stems and calyces were selected from fresh plant materials from each population. Middle parts of the samples were mounted directly on aluminum stubs using double-sided adhesive tape. The samples were coated with gold by a magnetron, sputtering device for about 10 nanometer thickness and observed with a SU 3500 SEM (Hitachi).

The terminology used is based on Cantino (1990) and Navarro and El Qualidi (2000) for trichomes. Size measurements on SEM images were made using UTHSCSA Image Tool Ver.3.0. Trichomes density was calculated by dividing the hair number per $mm^2$ by the samples area (Gonzales et al. 2008). The distribution and types of trichomes were determined (Table 2).

### Pollen grains

Fully matured anthers were obtained from the specimens and were prepared for study by SEM. Pollen grains were placed directly to aluminum stubs with double-sided cellophane tape and coated with gold by a magnetron sputtering device for about 10 nm thickness, then observed in a Hitachi SU 3500 SEM (in the Central Laboratory of Shahid Beheshti University) at 15 kV and photographed at magnifications ranging from×600 to ×10,000 to determine the exine ornamentation. Fully developed pollen grains (15-30 pieces) were selected and the following pollen measurements were taken: polar axis (P), equatorial axis (E), colpus length (Clg), mesocolpium thickness, apocolpium diameter and P/E ratio (Table 3). All of the measurements were performed using UTHSCSA Image Tool Ver. 3.0. The pollen terminology follows that of used by Faegri and Iversen (1975), Abu-Asab and Cantino (1994) and Hesse et al. (2009).

### Statistical analysis

For multivariate analysis, the mean of quantitative characteristics was used, while qualitative characters were coded as binary/multistate features. Standardized variables (mean = 0, variance = 1) were used in the statistical analysis. For grouping the studied taxa, cluster analysis using UPGMA (Unweighted Paired Group with Arithmetic Average) methods was performed using Euclidean and taxonomic distance among the species. Principal Coordinate Ordination (PCO) and Principal Coordinate Analysis (PCA) were performed (Podani 2000). Moreover, the one-way analysis of variance (ANOVA) was used to compare the trichomes numbers among the studied species. PAST (Paleontological statistics software package) version 2.17 was used for statistical analysis (Podani 2000).

## Results

Different trichome features as trichome type, size and density on the stems, leaves and calyces of the studied taxa along their palynological data are summarized in Tables 2 and 3, respectively. Selected SEM micrographs of different trichome types and pollen grains studied were presented in Figs. 1-3 and Figs. 4-6, respectively.

### Micromorphological characters

In general, two main types of trichomes were observed in the studied taxa: non-glandular (NG) and glandular (G). Both non-glandular and glandular trichomes occur on the same organ, particularly in the leaves and calyces.

#### *Non-glandular trichomes*

Non-glandular type trichomes were simple and unbranched in the examined specimens. However, this type varied in density, size, cell number, thickness of cell wall and presence of papillae on the trichome surface, therefore they were classified into 4 types (A-D, Table 2):

#### *Unicellular trichomes (A).*

A1: prickles-hairs. These were the unicellular epidermal appendages. In terms of size, this form was shorter than 50 μm on the leaves of *L. cabulicus*, *L. macracanthus* and *L. aucheri* ssp. *aucheri* var. *elegans* (Fig. 1A-B) to 200 μm in the leaves and calyces of most specimens (except *L. aucheri* ssp. *aucheri* var. *aucheri* 3), which have papillate surface (Fig. 1C). The highest number of this kind of trichomes was seen on the leaves of *L. aucheri* ssp. *aucheri* var. *tomentosus* and its lowest amount was found on the leaves of *L. macracanthus* and *L. aucheri* ssp. *aucheri* var. *aucheri* 1 (Table 2).

A2: thin-walled trichomes. This form was triangular and very thin-walled hairs with ridges. In the term of size, thin-walled non-glandular trichomes can be subdivided into two types; short (i): shorter than 50 μm, which was found on the stems and calyces of *L. macracanthus* and *L. cabulicus* (Fig 1D) to hairs ranged from 50-300 μm on

**Table 2.** Indumentum characteristics of the *Lagochilus* taxa examined. Numbers refer to mean ± standard deviation of trichomes number. NG: non-glandular. A1: prickles-hairs. A2-i: short thin-walled trichomes. A2-ii: long thin-walled trichomes. B1: short bicelled trichomes densely covered by micropapillae. B2: short bicelled trichomes, the basal cell is without micropapillae. B3: long bicelled trichomes. C: long tricelled trichomes. D: long four-celled trichomes. G: glandular. G1: sessile capitate. G2: short-stalked capitate. G3: peltate.

| Species | Leaf | | | | Stem | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | NG | | G | | NG | | | | | | | G | | |
| | A1 | A2-i | G1 | G3 | A1 | A2-i | A2-ii | B1 | B2 | B3 | C | G1 | G2 | G3 |
| *L. aucheri* ssp. *aucheri* var. *aucheri* 1 | 55.33 ± 4.5 | 0.00 ± 0.00 | 0.00 ± 0.00 | 10.5 ±1 | 0.00 ± 0.00 | 350 ± 90.7 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| *L. aucheri* ssp. *aucheri* var. *elegans* | 117.3 ± 4.48 | 0.00 ± 0.00 | 35 ± 8.65 | 10 ± 0.00 | 0.00 ± 0.00 | 326.66 ± 43.15 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 3 ± 0.8 | 10 ± 4.00 | 8 ± 2.5 | 0.00 ± 0.00 |
| *L. aucheri* ssp. *aucheri* var. *kotschyanus* | 195 ± 72.23 | 0.00 ± 0.00 | 2.25 ± 0.55 | 10 ± 0.00 | 0.00 ± 0.00 | 183.35 ± 21.24 | 3 ± 0.00 | 142.66 ± 8.35 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 5.8 ± 1.2 | 2 ± 0.5 |
| *L.aucheri* ssp. *aucheri* var. *tomentosus* | 223.33 ± 23.5 | 0.00 ± 0.00 | 9.8 ± 0.57 | 10 ± 0.2 | 120 ± 2.8 | 138.66 ± 15.6 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 18.75 ± 4.32 | 1.9 ± 1 | 0.00 ± 0.00 |
| *L. aucheri* ssp. *aucheri* var. *aucheri* 2 | 140.67 ± 25.1 | 0.00 ± 0.00 | 0.00 ± 0.00 | 10 ± 0.00 | 0.00 ± 0.00 | 367.66 ± 52.33 | 0.00 ± 0.00 | 25.24 ± 6.33 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 15.5 ± 6.33 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| *L. aucheri* ssp. *aucheri* var. *aucheri* 3 | 0.00 ± 0.00 | 282.33 ± 113.8 | 1.5 ± 0.3 | 30 ± 6.67 | 0.00 ± 0.00 | 250 ± 10.65 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 5.5 ± 2.2 | 0.00 ± 0.00 | 4.5 ± 1.8 |
| *L. alutaceus* | 110 ± 35.61 | 0.00 ± 0.00 | 5.2 ± 1.20 | 28.33 ± 3.2 | 0.00 ± 0.00 | 20 ± 7.67 | 0.00 ± 0.00 | 0.00 ± 0.00 | 290.1 ± 48.88 | 0.00 ± 0.00 | 18.67 ± 3.5 | 7.5 ± 3.67 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| *L. cabulicus* | 60 ± 2.65 | 0.00 ± 0.00 | 0.00 ± 0.00 | 26.33 ± 4.5 | 0.00 ± 0.00 | 293.33 ± 57.67 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 1.9 ± 0.5 | 1.2 ± 0.3 | 0.00 ± 0.00 | 2.66 ± 0.5 |
| *L. lasiocalyx* | 70.66 ± 3.55 | 0.00 ± 0.00 | 0.00 ± 0.00 | 5.3 ± 1.5 | 0.00 ± 0.00 | 201.66 ± 53.2 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 2 ± 0.00 | 3 ± 0.00 | 8.5 ± 2.33 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| *L. macracanthus* | 55.33 ± 5.66 | 0.00 ± 0.00 | 15.8 ± 4.5 | 10 ± 1.5 | 185 ± 55.33 | 276.67 ± 65.5 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 5.8 ± 3.42 | 0.00 ± 0.00 | 0.00 ± 0.00 |

**Table 2.** Continued.

| Species | Calyx | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | NG | | | | | | G | |
| | A1 | A2-i | B1 | B3 | C | D | G1 | G3 |
| *L. aucheri* ssp. *aucheri* var. *aucheri* 1 | 310 ± 77.33 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 9 ± 3.7 | 22.51 ± 7.8 |
| *L. aucheri* ssp. *aucheri* var. *elegans* | 202.66 ± 13.56 | 0.00 ± 0.00 | 75.2 ± 20 | 0.00 ± 0.00 | 0.00 ± 0.00 | 24.5 ± 4 | 7 ± 2 | 23.84 ± 5.66 |
| *L. aucheri* ssp. *aucheri* var. *kotschyanus* | 633.33 ± 158.55 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 17.67 ± 3.66 | 0.00 ± 0.00 | 17.6 ± 3.33 | 45 ± 10.15 |
| *L.aucheri* ssp. *aucheri* var. *tomentosus* | 236.77 ± 27.6 | 0.00 ± 0.00 | 30.67 ± 12.33 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 12.54 ± 4.47 | 35 ± 3.6 |
| *L. aucheri* ssp. *aucheri* var. *aucheri* 2 | 136 ± 14.53 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 6.2 ± 3.33 | 7.9 ± 2.1 |
| *L. aucheri* ssp. *aucheri* var. *aucheri* 3 | 0.00 ± 0.00 | 200 ± 35.7 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 8.8 ± 2.2 | 23.33 ± 5.3 |
| *L. alutaceus* | 122.3 ± 15.66 | 0.00 ± 0.00 | 0.00 ± 0.00 | 223.33 ± 102.2 | 0.00 ± 0.00 | 0.00 ± 0.00 | 5.5 ± 0.44 | 25 ± 6.8 |
| *L. cabulicus* | 110.2 ± 21.33 | 185.57 ± 28.63 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 1 ± 0.3 | 8.35 ± 4.45 |
| *L. lasiocalyx* | 125 ± 18.88 | 0.00 ± 0.00 | 0.00 ± 0.00 | 3 ± 1.5 | 5 ± 2.33 | 3 ± 0.9 | 9 ± 3.25 | 21.67 ± 5.00 |
| *L. macracanthus* | 210.15 ± 11.66 | 200 ± 52.5 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 22.2 ± 6.47 | 33.33 ± 6.74 |

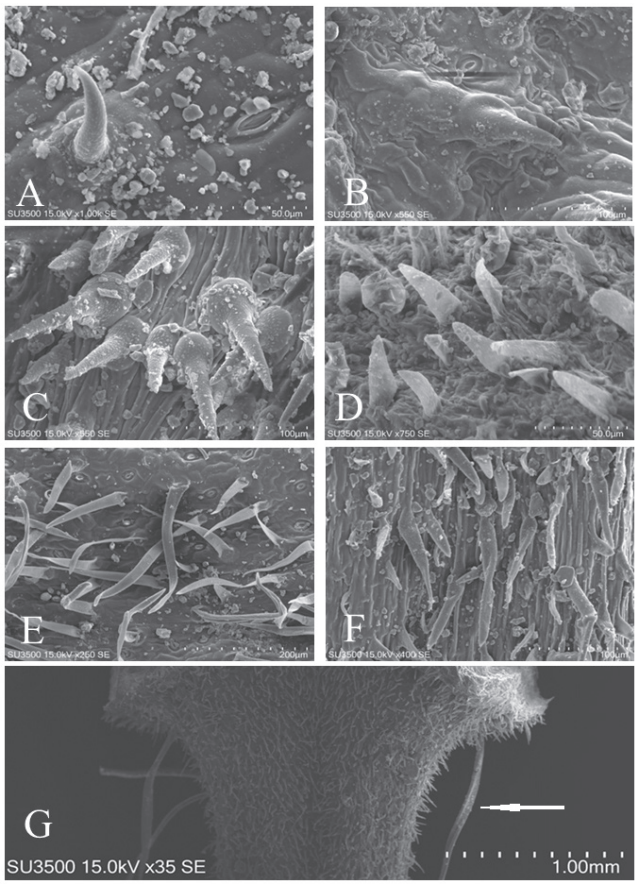


**Figure 1.** SEM micrographs of non-glandular unicellular trichomes in selected *Lagochilus* taxa. Prickles trichomes on the leaf (A-C). A: *L. aucheri* ssp. *aucheri* var. *elegans*. B: *L. cabulicus*. C: *L. aucheri* ssp. *aucheri* var. *aucheri* 2. Short thin-walled trichomes (D-F). D: on the stem of *L. macracanthus*. E: on the calyx of *L. aucheri* ssp. *aucheri* var. *aucheri* 3. F: on the stem of *L. aucheri* ssp. *aucheri* var. *aucheri* 1. Long thin-walled trichomes on the stem of *L. aucheri* ssp. *aucheri* var. *kotschyanus* (G).

the stems, leaves and calyces of *L. aucheri* ssp. *aucheri* var. *aucheri* 3 (Fig. 1E), and also on the stems of most specimens (e.g., *L. lasiocalyx*, *L. aucheri* ssp. *aucheri* var. *aucheri* 1 and *L. aucheri* ssp. *aucheri* var. *aucheri* 2, Fig. 1F). The stems of *L. aucheri* ssp. *aucheri* var. *aucheri* 2 and *L. alutaceus* had highest and lowest amounts of these types of trichomes, respectively (Table 2). Long thin-walled trichomes (ii): as long as 1000- 2000 μm only on the stems of *L. aucheri* ssp. *aucheri* var. *kotschyanus* (Fig. 1G).

*Bicelled trichomes (B).*

B1: short (50-200 μm in size), bicelled trichomes, densely covered by micropapillae; the apical cell is triangular and acute. This form was observed on the stems of *L. aucheri* ssp. *aucheri* var. *kotschyanus* and *L. aucheri* ssp. *aucheri* var. *aucheri* 2 (Fig. 2A) and on the calyces of *L. aucheri* ssp. *aucheri* var. *elegans* and *L. aucheri* ssp. *aucheri* var. *tomentosus* (Fig. 2B).

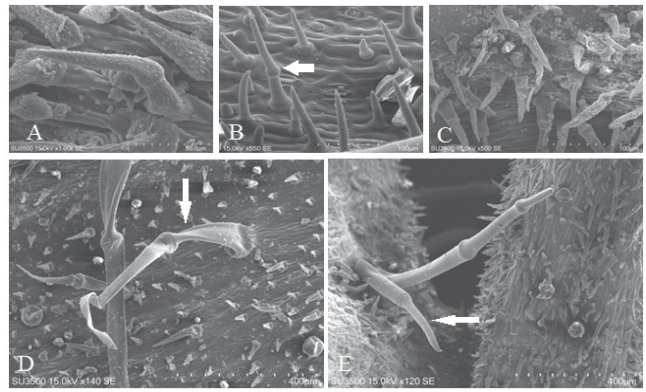


**Figure 2.** SEM micrographs of non-glandular trichomes in selected *Lagochilus* taxa. Bicelled trichomes type B1 on the stem of *L. aucheri* ssp. *aucheri* var. *aucheri* 2 (A) and on the calyx of *L. aucheri* ssp. *aucheri* var. *elegans* (B). Bicelled trichomes type B2 on the stem of *L. alutaceus* (C). Thin-walled bicelled trichomes type B3 on the calyx of *L. alutaceus* (D). Thick-walled bicelled trichomes type B3 on the stem of *L. lasiocalyx* (E).

B2: short (50-200 μm in size), bi- celled trichomes, the basal cell is without micropapillae. This form can distinguishable on the stem of *L. alutaceus* (Fig. 2C).

B3. Long (1000-1500 μm in size), bicelled trichomes with rare micropapillae, this form was thin – walled on the *L. alutaceus* calyx (Fig. 2D) and thick-walled on *L. lasiocalyx* stem (Fig. 2E).

*Long, tricelled trichomes (1000-2000 μm)(C).*

Elongated and thin-walled trichomes with rare micropapillae, can be observed on the stems of *L. aucheri* ssp. *aucheri* var. *elegans*, *L. cabulicus* and *L. alutaceus* (Fig. 3A-B), and thick-walled on the stems and calyces of *L. lasiocalyx* and *L. aucheri* ssp. *aucheri* var. *kotschyanus*, respectively (Fig. 2E and Fig. 3C).

*Long, four-celled trichomes (D).*

This type is found on the calyces of *L. lasiocalyx* and *L. aucheri* ssp. *aucheri* var. *elegans* (Fig. 3D-E).

*Glandular trichomes*

Three types of glandular trichomes were revealed by SEM micrographs, which all types were found in all studied specimens. The first type (G1) represented the sessile capitate glandular trichomes, (e.g., *L. aucheri* ssp. *aucheri* var. *aucheri* 1 and *L. macracanthus*; Fig. 3F). The second type (G2) represented the short-stalked capitate glandular trichomes (length of the stalk up to 10 μm) which were only registered on the stems of *L. aucheri* ssp. *aucheri* var. *elegans, L. aucheri* ssp. *aucheri* var. *kotschyanus* and *L.aucheri* ssp. *aucheri* var. *tomentosus* (Fig. 3G). The dominant glandular hairs, peltate glandular trichomes (G3), consist of a

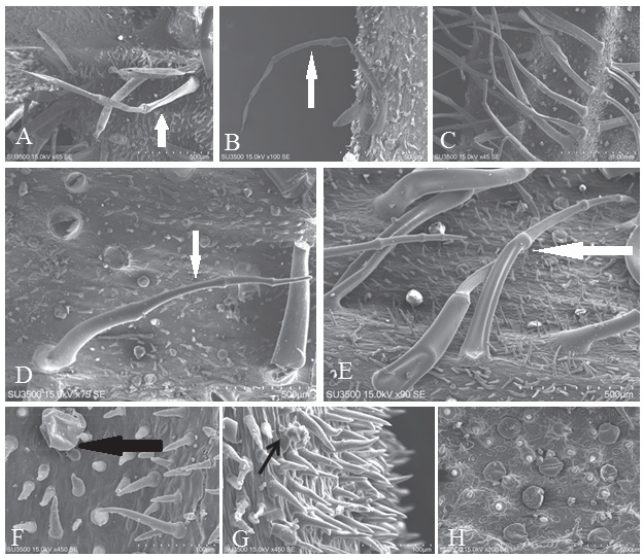

**Figure 3.** SEM micrographs of non-glandular and glandular trichomes in selected *Lagochilus* taxa. Long tricelled thin- or thick-walled trichomes of type C (A-C). Thin-walled trichomes on the stems *L. alutaceu*s (A) and *L. cabulicus* (B). Thick-walled trichomes on the calyx of *L. aucheri* ssp. *aucheri* var. *kotschyanus* (C). Long, four-celled trichomes on the calyx of *L. lasiocalyx* (D) and *L. aucheri* ssp. *aucheri* var. *elegans* (E). Sessile capitate glandular trichomes on the calyx of *L. aucheri* ssp. *aucheri* var. *aucheri* 1. (F). Short stalked capitate glandular trichomes on the stem of *L. aucheri* ssp. *aucheri* var. *tomentosus* (G). Peltate glandular trichomes on the leaf of *L. alutaceus* (H).

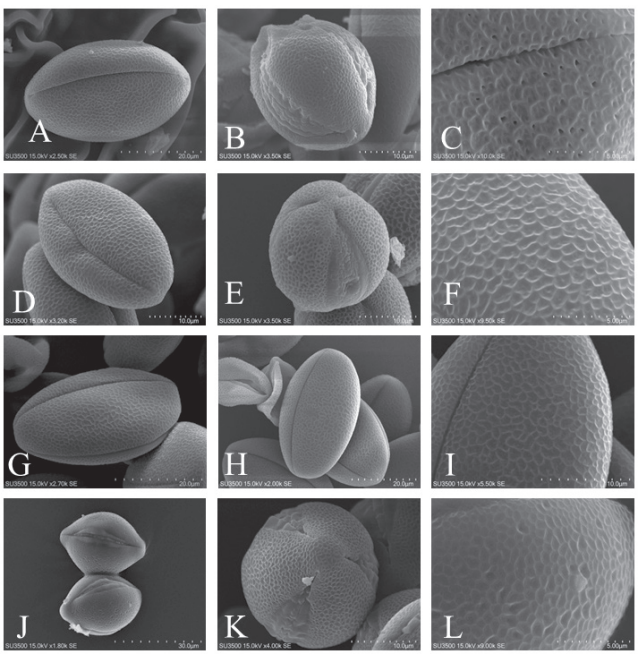

**Figure 5.** SEM micrographs of pollen grains in the *Lagochilus* taxa examined. A-C: *L. macracanthus*. D-F: *L. aucheri* ssp. *aucheri* var. *kotschyanus.* G-I: *L. aucheri* ssp. *aucheri* var. *aucheri* 1. J-L: *L. aucheri* ssp. *aucheri* var. *aucheri* 2.

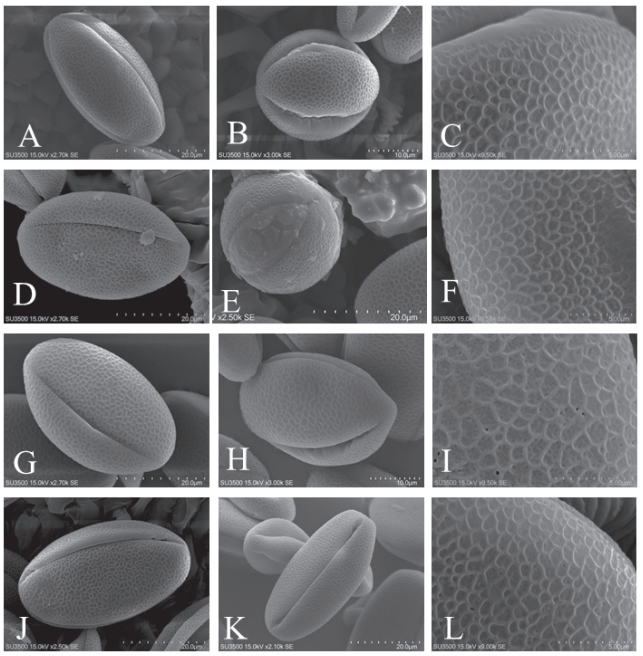

**Figure 4.** SEM micrographs of pollen grains in the *Lagochilus* taxa examined. A-C: *L. alutaceus*. D-F: *L. cabulicus*. G-I: *L. aucheri* ssp. *aucheri* var. *elegans*. J-L: *L. aucheri* ssp. *aucheri* var. *aucheri* 3.

basal cell, a short stalk cell and a multicellular head (4-8 cells). Its highest number was recorded on the leaves of *L. aucheri* ssp. *aucheri* var. *aucheri* 3, *L. cabulicus* and *L. alutaceus,* and also on the calyx of *L. aucheri* ssp. *aucheri* var. *kotschyanus* (Table 2; Fig. 3H).

### *General pollen features*

#### *Pollen size and shape*

The pollen grains were monads and mostly small to medium in size. The polar axis length varied from 29.35 μm in *L. aucheri* ssp. *aucheri* var. *aucheri* 2 to 43.91 μm in *L. aucheri* ssp. *aucheri* var. *aucheri* 1, while the equatorial diameter length varied from 17.6 μm in *L. aucheri* ssp. *aucheri* var. *kotschyanus* to 24.59 μm in *L. aucheri* ssp. *aucheri* var. *aucheri* 3 (Table 3). The shape of all pollen grains was prolate (P/E = 1.57-1.9) in equatorial view, except for *L. aucheri* ssp. *aucheri* var. *aucheri* 2, which was prolate-subprolate (P/E = 1.34) (Table 3). In polar view, the shape of all pollen grains was circular.

#### *Apertures*

Pollen grains were isopolar and tricolpate in all examined taxa. Simple colpi were elongated and narrowing at the poles. Colpus length ranges from 25.84 μm in *L. aucheri* ssp. *aucheri* var. *aucheri* 2 to 41.46 μm in *L. aucheri* ssp. *aucheri* var. *aucheri* 3 (Table 3). Mesocolpium value varied from 12.5 μm in *L. aucheri* ssp. *aucheri* var. *kotschyanus* to 19.55

**Table 3.** Summary of pollen morphological data for the *Lagochilus* taxa examined. All sizes are in µm. Numbers refer to mean ± standard deviation (minimum-maximum). Clg: colpus length. P: polar axis. E: equatorial axis. Sc: sculpturing type 1a, 1b, 1c = bireticulate; 2: reticulate; 3: microreticulate; 4: incomplete reticulate. All measurements are in µm.

| Taxon | P | E | P/E | Clg | Mesocolpium | Apocolpium | Sc | Shape |
|---|---|---|---|---|---|---|---|---|
| *L. aucheri* ssp. *aucheri* var. *aucheri 1* | 43.91 ± 1.7 (40.9-46.83) | 23 ± 2.2 (20.17-26.26) | 1.90 | 41 ± 1.9 (37.65-43.19) | 15.68 ± 1.41 (14.36–17.95) | 4.2 ± 1.04 (3.69–5.41) | 1c | Prolate |
| *L. aucheri* ssp. *aucheri* var. *elegans* | 41.31 ± 1.24 (35.68-42.94) | 22.8 ± 1.42 (20.93-25.18) | 1.81 | 38.51 ± 1.06 (37.05-40.04) | 16.53 ± 1.8 (15.21-17.85) | 4.35 ± 1.7 (5.7-6.2) | 1a | Prolate |
| *L. aucheri* ssp. *aucheri* var. *kotschyanus* | 33.08 ± 1.41 (31.22-34.29) | 17.6 ± 0.62 (16.97-18.16) | 1.87 | 30.56 ± 1.64 (29.50-32.44) | 12.5 ± 1.36 (10.75-15.3) | 3 ± 0.72 (2.4–4.03) | 1c | Prolate |
| *L. aucheri* ssp. *aucheri* var. *tomentosus* | 39.82 ± 0.91 (38.57-40.98) | 22.95 ± 0.97 (21.21-24.35) | 1.73 | 37.81 ± 1.15 (35.5-39.49) | 15.61 ± 2.29 (13.69–18.72) | 4.7 ± 0.45 (4.04–5.13) | 4 | Prolate |
| *L. aucheri* ssp. *aucheri* var. *aucheri 2* | 29.35 ± 0.92 (27.62-30.34) | 21.78 ± 0.84 (20.73-22.67) | 1.34 | 25.84 ± 0.74 (25.20-26.59) | 14 ± 0.78 (12.8-15.21) | 4.6 ± 1.1 (3.91-5.22) | 2 | Prolate-subprolate |
| *L. aucheri* ssp. *aucheri* var. *aucheri 3* | 43.71 ± 0.96 (42.66-44.78) | 24.59 ± 0.99 (24.36-25.65) | 1.77 | 41.46 ± 0.45 (41.05-41.92) | 19.55 ± 1 (19.1-20.2) | 4.8 ± 1.03 (3.30–6.02) | 1a | Prolate |
| *L. alutaceus* | 35.59 ± 2.27 (33.12-39.93) | 19.21 ± 1.81 (15.46-21.70) | 1.85 | 31.56 ± 2.09 (30.16-35.07) | 14.72 ± 1.35 (13.15-15.47) | 2.45 ± 0.21 (2.34–2.78) | 1a | Prolate |
| *L. cabulicus* | 38.96 ± 1.38 (36.30-40.77) | 21.74 ± 1.71 (19.36-23.03) | 1.79 | 36.57 ± 0.23 (36.52-36.85) | 16.26 ± 0.96 (15.53-17) | 4.15 ± 0.98 (3.5-4.8) | 1a | Prolate |
| *L. lasiocalyx* | 33.73 ± 1.54 (30.03-36.14) | 21.43 ± 1.5 (19.08-23.64) | 1.57 | 31.2 ± 1.62 (29.38-34.57) | 16.13 ± 1.17 (15-18.11) | 3.2 ± 0.83 (2.5-3.92) | 3 | Prolate |
| *L. macracanthus* | 39.83 ± 0.74 (39.08-40.69) | 23.66 ± 1.5 (22.42-24.51) | 1.68 | 37.78 ± 0.95 (36.05-39.98) | 16.03 ± 1.73 (13.75-18.06) | 4.85 ± 0.63 (4.11-5.63) | 1b | Prolate |

µm in *L. aucheri* ssp. *aucheri* var. *aucheri* 3. Apocolpium diameter ranges from 2.45 µm in *L. alutaceus* to 4.85 µm in *L. macracanthus* (Table 3).

### *Exine ornamentation*

Exine sculpture displayed four distinct types of surface ornamentation: bireticulate or suprareticulate (Fig. 4A-L, Fig. 5A-F, and Table 3), reticulate (Fig. 5G-L, Table 3), microreticulate (Fig. 6A-C, Table 3) and incomplete reticulate (Fig. 6D-F, Table 3). Bireticulate patterns can be subdivided into three subtypes, based on the detailed configuration of the exine ornamentation patterns.

*Bireticulate sculpture pattern.* The most common (seven taxa) sculpture pattern among the studied taxa was the bireticulate sculpture pattern (special type of reticulate ornamentation, where a two-layered reticulum consisting of a suprareticulum supported by a microreticulate layer). According to the number of perforations per 25 µm$^2$, it can be divided into three subtypes (a-c). In subtype 1a, the number of perforations was <5 (*L. alutaceus, L. cabulicus, L. aucheri* ssp. *aucheri* var. *elegans* and *L. aucheri* ssp. *aucheri* var. *aucheri* 3, Fig. 4A-L). In subtype 1b, the number of perforations was >5 (Fig. 5A-C, *L. macracanthus*). Bireticulate subtype 1c was determined with prolonged primary lumina without perforations, which was reported from *L. aucheri* ssp. *aucheri* var. *kotschyanus* (Fig. 5D-F) and *L. aucheri* ssp. *aucheri* var. *aucheri* 1 (Fig. 5G-I).

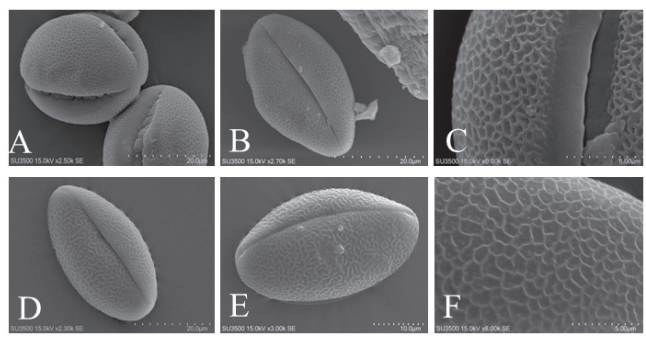


**Figure 6.** SEM micrographs of pollen grains in the *Lagochilus* taxa examined. A-C: *L. lasiocalyx*. D-F: *L. aucheri* ssp. *aucheri* var. *tomentosus*.

*Reticulate sculpture pattern.* Among the examined taxa, *L. aucheri* ssp. *aucheri* var. *aucheri* 2 had reticulate sculpture pattern (Fig. 5J-L).

*Microreticulate sculpture pattern.* The microreticulate sculpture pattern is observed only in *L. lasiocalyx* (Fig. 6A-C). In this pattern diameter of lumina is smaller than 1 µm.

*Incomplete reticulate sculpture pattern.* Incomplete reticulate sculpture pattern (network like pattern formed by exine elements, is incomplete), occurs for *L. aucheri* ssp. *aucheri* var. *tomentosus* (Fig. 6D-F).

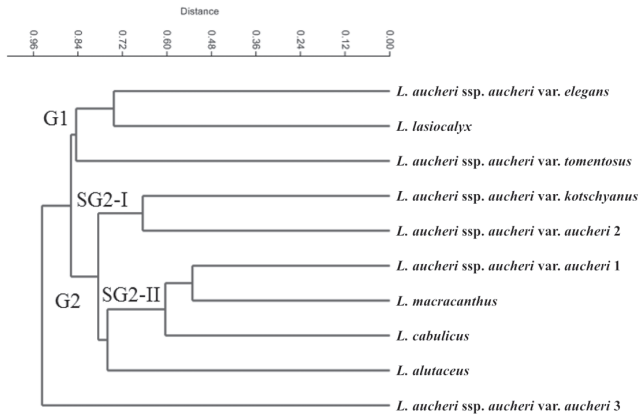


**Figure 7.** UPGMA dendrogram of the studied *Lagochilus* taxa based on micromorphological and palynological characteristics. G: group. SG: subgroup.

### *Statistical analysis*

The UPGMA dendrogram based on the micromorphological and palynological features showed the existence of two main clusters (Fig. 7). Group1 (G1) contained specimens with three- or four- celled trichomes on the stems or calyces (*L. lasiocalyx*, *L. aucheri* ssp. *aucheri* var. *elegans* and *L. aucheri* ssp. *aucheri* var. *tomentosus*), while Group 2 (G2) was subdivided into two subgroups: subgroup I (SG2-I) contained specimens with two-celled trichomes of type B1 (*L. aucheri* ssp. *aucheri* var. *aucheri* 2 and *L. aucheri* ssp. *aucheri* var. *kotschyanus*). Subgroup II (SG2-II) contained specimens with short unicellular thin-walled trichomes on the stems and calyces (*L. aucheri* ssp. *aucheri* var. *aucheri* 1, *L. macracanthus*, *L. cabulicus* and *L. alutaceus*). *L. aucheri* ssp. *aucheri* var. *aucheri* 3 was sister to all *Lagochilus* species (Fig. 7). As seen in Figure 7, known varieties of *L. aucheri* were distributed through the tree.

The ANOVA result did not show significant differences ($p \leq 0.05$) in the number between all observed types of trichomes, however, PCA-biplot showed that some of species had distinct type of trichomes that were useful in identification of them (Fig. 8). For example, the number of trichomes type A1 on the calyces is a distinguishing trait for identification of *L. aucheri* ssp. *aucheri* var. *kotschyanus*, however, *L. aucheri* ssp. *aucheri* var. *tomentosus* were identified by highest number of trichomes of type A1on the leaves. In addition, trichomes of type B2 and C on the stems was significant trait for *L. alutaceus*.

## Discussion

### *Micromorphological characters*

Both glandular and non-glandular trichomes were existed on the stems, leaves and calyces of all investigated taxa. The trichomes types presented on the studied species were mostly similar to those of family Lamiaceae reported by Navarro and El Qualidi (2000), Cantino (1990) and Atalay et al. (2016a). Generally, number of non-glandular trichomes was higher than glandular ones, which is a common feature in Lamiaceae family (Metcalfe and Chalk 1950).

Our study showed that the size and cell number of trichomes were valuable and could be applied to classify the non-glandular trichomes into four types including 10 forms; therefore, they can be used as taxonomic tools in species and intraspecific identification.

Two main types of glandular trichomes were capitate and peltate. The studied taxa had two types of capitate trichomes which differed from each other in their stalk length. Talebi and Rezakhanlou (2012) reported the capitate trichomes with a basic and two apical cells in *L. macracanthus*. The peltate trichomes of Lamiaceae have mostly a secretory head of four central and 6-14 peripheral cells (Werker 1993). Peltate glandular hairs with eight-celled head were previously reported in *L. macracanthus* (Talebi and Rezakhanlou 2012). The distribution of glandular trichomes is obviously correlated with their role in pollination (Navarro and El Qualidi, 2000). In *Lagochilus* genus, this type totally appears on the outer side of the calyces and sparse on the stems.

### *Pollen grain morphology*

Erdtman (1945) classified the Lamiaceae family into two subfamilies based on the number of apertures and nuclei in the mature pollen grains. The first group comprises the subfamily Lamioideae with tricolpate pollen grains and the second group contains the subfamily Nepetoideae characterized by hexacolpate pollen grains. According to the latest molecular phylogenetic studies by Bendiksby et al. (2011) the genus *Lagochilus*, included in the subfamily Lamioideae and tribe Leonureae. The present study showed that the all the examined taxa have tricolpate pollen grains similar with the other genera of subfamily Lamioideae (Abu-Asab and Cantino 1994; Atalay et al. 2016b).

The shapes of the pollen grains in equatorial view is prolate except for *L. aucheri* ssp. *aucheri* var. *aucheri* 2, with prolate-subprolate shape (P/E = 1.34). The state of hydration and/or fixation could be affecting the pollen shape (Demissew and Harley 1992; León-Arencibia and La-Serna Ramos 1992; Moon et al. 2008a). Thus, differences in shape among the pollen grains may not be significant or even applicable in their taxonomy (Xiang et al. 2013). Moon et al. (2008a) suggested, in order preserving a more natural form, careful processing such as critical point drying of the fresh material is required.

The observed exine sculpture patterns in *Lagochilus* were determined as bireticulate, reticulate, microreticulate and also incomplete reticulate. Reticulate and microreticulate exins are common in several species of subfamily Lamioideae and in other genera of Lamiaceae (Wagstaff 1992; Abu-Asab and Cantino 1992, 1994; Çelenk et al. 2008 a,b; Moon et al. 2008 a,b,c; Özler et al. 2011, 2013; Atalay et al. 2016b). Bireticulate exin are a plesiomorphic in subfamily Lamioidae, while it is apomorphic in Lamiaceae (Cantino 1992 a,b). Derived states as psilate, granulate, rugulate and suprareticulate-rugulate forms of sculpturing pattern and branched columellae occur in some members of Lamioids. Similar pollen features suggest relationships within and between certain genera in Lamioidae (Abu-Asab and Cantino 1994; Atalay et al. 2016b). Incomplete reticulate sculpture pattern occured for *L.aucheri* ssp. *aucheri* var. *tomentosus* (Fig. 6D-F). This pattern not been reported previously in Lamiaceae.

Among the investigated taxa, palynological properties of *L. aucheri* have been provided by Abu-Asab and Cantino (1994). Characteristics investigated by them correlate with our current results, except for the P/E ratios, which are possibly due to differences in procedure of preparation.

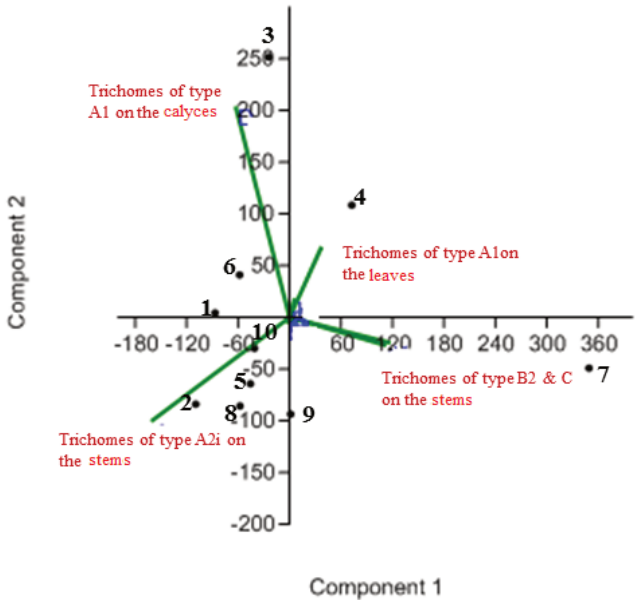


**Figure 8.** PCA-biplot of the studied taxa and their trichome features. 1: *L. aucheri* ssp. *aucheri* var. *aucheri* 1. 2: *L. aucheri* ssp. *aucheri* var. *elegans*. 3: *L. aucheri* ssp. *aucheri* var. *kotschyanus*. 4: *L. aucheri* ssp. *aucheri* var. *tomentosus*. 5: *L. aucheri* ssp. *aucheri* var. *aucheri* 2. 6: *L. aucheri* ssp. *aucheri* var. *aucheri* 3. 7: *L. alutaceus*. 8: *L. cabulicus*. 9: *L. lasiocalyx*. 10: *L. macracanthus*.

### *Systematic significance of trichome and pollen grains micromorphology*

#### *L. aucheri complex*

Jamzad (1988), in her taxonomic revision of the genus *Lagochilus* in Iran, recognized two subspecies for *L. aucheri*: ssp. *heterophyllus* and ssp. *aucheri*. Moreover, she identified four varieties for ssp. *aucheri*. She stated that the indumentum is one of the characteristics that can be used in distinguishing varieties. Results of our investigation showed that the trichomes micromorphology can be used in distinguishing varieties that presented by Jamzad (1988). She has reported that *L. aucheri* ssp. *aucheri* var. *aucheri* was characterized by glabrous stem and calyx, while we observed short unicellular trichomes on the stem and calyx of this taxon. Moreover, leaves of this variety had lowest number of prenominated trichomes.

*L. kotschyanus* Boiss. was originally described as a species but was reduced to a variety of *L. aucheri* by Bornmüller (1907). *L. aucheri* ssp. *aucheri* var. *kotschyanus* has long unicellular trichomes on the stems and tricelled trichomes on the calyces, which were not observed in any other varieties of the species.

*L. aucheri* ssp. *aucheri* var. *elegans* and *L. aucheri* ssp. *aucheri* var. *tomentosus*, are two new infraspecific taxa that were described by Jamzad (1988). In our study, *L. aucheri* ssp. *aucheri* var. *elegans* was characterized by four-celled trichomes on the calyces, and *L. aucheri* ssp. *aucheri* var. *tomentosus* was identified by short unicellular trichomes on the stems and bicelled trichomes on the calyces. In addition, *L. aucheri* ssp. *aucheri* var. *tomentosus* identified by highest number of trichomes of type A1on the leaves.

In carrying out of determination of specimens, we identified plants which have basic traits of *L. aucheri* but differed from known species in some morphological characteristics such as ratio of teeth length /calyx tube or calyx teeth numbers. This holds true for plants of Zanjan (*L. aucheri* ssp. *aucheri* var. *aucheri* 2) and Dizin (*L. aucheri* ssp. *aucheri* var. *aucheri* 3) populations. Detailed micromorphological analysis revealed that trichomes of type A1 were observed on the leaves and calyces of all specimens, except *L. aucheri* ssp. *aucheri* var. *aucheri* 3, while, unicellular thin-walled trichomes with 200-300 µm in size (A2 i type), present only on the leaves, stems and calyces of *L. aucheri* ssp. *aucheri* var. *aucheri* 3. Similarly, bicelled trichomes of type B1 were observed on the stems of *L. aucheri* ssp. *aucheri* var. *aucheri* 2 and *L. aucheri* ssp. *aucheri* var. *kotschyanus* , however, latter vary from *L. aucheri* ssp. *aucheri* var. *aucheri* 2 in having trichomes of type A2ii on the stems and trichomes of type C on the calyces. The basic shape of the pollen grains in most taxa was prolate, however prolate-subprolate pollen grains was recorded for *L. aucheri* ssp. *aucheri* var. *aucheri* 2. Moreover, reticulate sculpturing pattern of pollen grains were observed only in *L. aucheri* ssp. *aucheri* var. *aucheri*

2. *L. aucheri* ssp. *aucheri* var. *aucheri* 3 had highest value of colpus length and mesocolpium area among all taxa examined. Therefore, it seems to be new intraspecific ranks in *L. aucheri* complex that need to additional and detailed morphological and molecular studies.

In our UPGMA tree of micromorphological and palynological characteristics, the examined taxa are separated from each other, but varieties of *L. aucheri* do not grouped together and not nested in the same clade as the members of *L. aucheri*. Therefore, *L. aucheri* seems to be a polyphyletic and this genus needs to revise of morphological characteristics for identifying of species.

### *Other Lagochilus species*

Jamzad (1988) described *L. lasiocalyx* as the new combination, based on *L. aucheri* var. *lasiocalyx* Stapf., and treated *L. aucheri* var. *perhispidus* Bornm. as a synonym of *L. lasiocalyx*. In flora of Iran (Jamzad 2012), *L. lasiocalyx* has placed toward known varieties of *L. aucheri*. Result of UPGMA tree of micromorphological characteristics corroborates her morphology-based conclusion; *L. lasiocalyx* showed a close relationship with *L. aucheri* ssp. *aucheri* var. *elegans* and *L. aucheri* ssp. *aucheri* var. *tomentosus*.

According to Jamzad (1988), there was close affinity and the high degree of morphological similarities between *L. cabulicus* and *L. aucheri*, in so far as it is difficult to distinguish them. We could differentiate them by trichomes and pollen grains characteristics. The trichomes size on the leaves of *L. cabulicus* was very short (< 50μm) while it varied from 100-200 μm in *L. aucheri* 1, also we could differentiate them by the shorter size of the pollen grains in *L. cabulicus*. The polar and equatorial axis ranged from 36.30-40.77 μm and 19.36-23.03 μm, respectively in *L. cabulicus*, while they varied from 40.9-46.83 μm and 20.17-26.26 μm in *L. aucheri* 1. Despite bireticulate sculpture pattern observed in both species, they varied from each other according to the number of perforations per 25 $\mu m^2$.

In the UPGMA tree, a close relationship could be identified among *L. cabulicus*, *L. macracanthus* and *L. alutaceus*, which these results, generally were corroborate with Rechinger classification (1982).

The result of statistical analysis showed that trichomes characteristics such as density and cell numbers of non-glandular trichomes could be used as a relevant features for identification of the investigated taxa concerning they stable position between studied populations of each species.

### ***Key to studied taxa according to non-glandular trichomes***

Based on our results of trichomes morphology and density of *Lagochilus* species, we proposed that the type of indumentum can be used as a significant trait for identification of the examined taxa. Among non-glandular trichomes, size, cell number and density are considered as valuable, therefore, a key to the studied taxa of *Lagochilus* according to the result of the non-glandular trichomes are given below.

1 a. Leaves only with unicellular trichomes shorter than 50 μm..... ***L. cabulicus***
b. Leaves with unicellular trichomes both shorter and longer than 50 μm ..... 2

2 a. Leaves with unicellular trichomes range 50-200 μm..... 3
b. Leaves with unicellular trichomes range 200-300 μm..... ***L. aucheri* ssp. *aucheri* var. *aucheri* 3**

3 a. Stems only with unicellular trichomes..... 4
b. Stems both with uni- and multi-cellular trichomes..... 5

4 a. Stems with unicellular trichomes shorter than 50 μm..... ***L. macracanthus***
b. Stems with unicellular trichomes longer than 50 μm..... 6

5 a. Stems both with uni- and bicelled trichomes..... 7
b. Stems both with uni- and tricelled trichomes..... 8

6 a. Calyces with short unicellular trichomes up to 100 μm..... ***L. aucheri* ssp. *aucheri* var. *aucheri* 1**
b. Calyces with bicelled trichomes range 100-200 μm..... ***L. aucheri* ssp. *aucheri* var. *tomentosus***

7 a. Stems with short unicellular trichomes (50-200 μm), calyces only with unicellular trichomes..... ***L. aucheri* ssp. *aucheri* var. *aucheri* 2**
b. Stems with long unicellular trichomes (1000-2000 μm), calyces with uni- and tricelled trichomes..... ***L. aucheri* ssp. *aucheri* var. *kotschyanus***

8 a. Calyces with uni- and bicelled trichomes..... 9
b. Calyces with uni- and tricelled trichomes..... ***L. lasiocalyx***

9 a. Calyces with bicelled trichomes range 500-1000 μm, without four-celled trichomes..... ***L. alutaceus***
b. Calyces with bicelled trichomes ranges 100-200 μm, with four-celled trichomes..... ***L. aucheri* ssp. *aucheri* var. *elegans***

## Conclusion

According to the Plant List (Govaerts et al. 2013), some of species and infraspecific taxa currently recognized as synonyms (e.g., *L. lasiocalyx*, , *L. aucheri* ssp. *aucheri* var. *elegans* and *L. aucheri* ssp. *aucheri* var. *tomentosus*), while, based on micromorphological results were presented here, all known species and infraspecific taxa were delimited.

In conclusion, the present study provides micromorphological and palynological characteristics of the genus *Lagochilus* that would be applicable in identification,

delimitation and classification of the genus; however, Phylogenetic and morphological studies of *Lagochilus*, based on complete sampling will be necessary to confirm existence of new taxa and to illuminate the intraspecific relationships of the genus.

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