Morphotaxonomic Studies of the Genus *Mazus* Lour. from Hazara Division, Pakistan

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Abstract: *Mazus* Lour. belongs to the family Scrophulariaceae, is a small genus with 35 species. This genus is distributed in various parts of Asia, Australia, and New Zealand. In current study, this genus has been morphologically revised from Hazara division, in the province of Khyber Pakhtunkhwa, Pakistan. Frequent field visits were conducted from the month of July till end of September. Plant samples were collected and properly identified, and voucher specimens were deposited in the Hazara University Herbarium Pakistan (HUP). Ripened seeds were studied under SEM. Three native species are recognized from the area of study, mostly confined to moist plain areas. Plants are annual herbs, and these species have been classified mainly based on stem characters and abundance and nature of trichomes, while seeds were brown, oblong, with colliculate surface.

Index Terms: *Mazus,* Taxonomy, Hazara, Trichomes, bilabiate, colliculate, didynamous, verrucate

I. INTRODUCTION

Mazus Lour. is a genus of small, annual or perennial plants. It was formerly placed in the tribe Gratioleae of subfamily Gratioloideae of family Scrophulariaceae, while recently in the family Mazaceae [1]. It is a genus of 35 species (25 species from China, including 4 varieties), generally found in moist habitats in the regions of China, East and Southeast Asia, Russia, Australia and New Zealand, [2]. Plants are herbs with erect or procumbent stem, having leaves in basal rosettes and winged petioles. Flowers have bilabiate corolla with didynamous stamens. Fruit is a locucidal capsule with numerous small seeds, [2].

Varghese [3] studied twenty species of the tribe Gratioleae of Scrophulariaceae, (including *Mazus* Lour.) and discussed in detail different floral parts and their anatomy. Heenan [4] described *Mazus arenarius*, as a new species from New Zealand and distinguished it from *Mazus radicans* on the basis of gross morphological characters, like leaf, peduncle, flower, fruit and seeds. Hsieh, [5] revised genus *Mazus* on the basis of its morphological, palynological and cytological studies from Taiwan, by comparing six species belonging to the genus. Deng [6] recognized two major lineages in *Mazus*, corresponding to their distribution in Australia and Asia. Li, [7] described a new and endemic species of *Mazus*, i-e., *Mazus danxiacola* from eastern China; they also discussed its conservation status.

Pennell [8] described 04 species of *Mazus* from Western Himalayas. Stewart [9] reported 03 species of *Mazus* from Pakistan and Kashmir. The genus has been represented by three species in Pakistan, while two species have been reported from Hazara region [10].

AREA OF STUDY

Hazara is the part of Khyber Pakhtunkhwa (KP) province of Pakistan. It lies between 33º-44' and 35º-35' North latitude and 72º-45' and 73º-75' East longitude. Gigit-Baltistan and Azad Kashmir are located in North and East of Hazara. To the South is the Capital Islamabad and the province of Punjab, whilst to the West lays the rest of K.P. The total area of Hazara is 18,013 km², including agricultural, industrial, wasteland, forest and alpine range [11].

Altitudes range from 1100 feet on the Indus River near Tarbela to more than 15,000 feet at Alai, Battagram district. Hazara is mostly mountainous having Himalayan ranges, while some prominent plain areas are Pakhli, Mangal, Rash, Haripur and Khanpur [12]. Climate varies with altitude, rainfall, snowfall and temperature. Hazara is the wettest part of Pakistan as it lies immediately South of the main Himalaya Range, and is exposed to moist winds from the Arabian Sea. Due to its location, Hazara has a bimodal rainfall regime, with one peak in February or March and another monsoonal peak in July and August with temperature around 41°C in summers. While in winters, minimum temperature is around 0°C and much lower at the high mountain peaks. Snowfalls are common in winter, [13].

Vegetation of Hazara varies within the region. Dry subtropical sub-mountainous vegetation is found in Garhi-Habibullah, Terbela, Khanpur and foothills of Kaghan valley. Dry temperate forests are found in Kohistan. Moist temperate forests are present in Galiat and Shogran. Alpine and sub-alpine vegetation is found in upper Kaghan valley and Kohistan [14].

II. MATERIALS AND METHODS

This study was conducted in the following phases:

2.1. Literature review: As a first step, different relevant literatures were studied to gather information regarding the habit and habitat of different species belonging to genus *Mazus*. For this purpose, different floras were consulted, and herbaria were visited.

2.2. Planning: In the light of information gathered through literature review, study trips were scheduled to all parts of the region according to the blooming periods of the plants. The first trip was made in the last week of February 2015. Further trips were made at an interval of 15-20 days, till November 2017.

2.3. Field visits and Material Collection: Plant specimens (Voucher specimens of each plant) with mature flowers and fruits were collected, properly pressed, dried and mounted on herbarium sheets. Information was recorded. The plant specimens were identified by comparing it with already identified specimens in the herbarium or going through available literature, especially with Flora of Pakistan, family Scrophulariaceae [10]. Plants mounted on the Herbarium sheets and were deposited in the herbarium of Hazara University, Mansehra.

2.4. Photography: Pictures of plants belonging to genus *Mazus*. from Hazara division were taken with Canon 16.0 MP digital camera, and USB digital microscope, and are reproduced here.

2.5. Deposition of Voucher Specimens: At the end of research work, collected plant material was deposited in the Herbarium Hazara University Pakistan (HUP) for future records.

2.6. Seed Morphology

Mature, dried, and healthy seeds of all three species were collected from the specimens. Morphological characters of seeds like, size, shape, colour, and testa ornamentations were observed by using scanning electron microscope. Mostly 10-15 seeds per plant were studied and examined under scanning electron microscope (JSM-5910, JEOL, Japan), at CRL, Peshawar university, to clearly examine the ornamentation of testa. Dry seeds were directly mounted on metallic plate using adhesive tape. Then these seeds were coated with gold by putting them in sputtering chamber (SPI Module Sputter Coater, Model No.11430) for a period of 6 minutes. After that, seeds were observed under SEM and statistical data like, size, shape, length, and width of seeds was recorded.

The terminology used is in accordance with [15] and [16].

III. RESULTS

Mazus Loureiro, Fl. Cochinch. 2:385. 1790.

Annual or perennial herbs. Stem branched, ascending or sub-erect, sometimes stoloniferous, hairy with glandular or eglandular hairs. Basal leaves in rosette form, spathulate to oblanceolate, margin toothed. Cauline leaves smaller than basal leaves, alternate, lamina suborbicular, margin crenate. Inflorescence racemose, somewhat secund, few to many flowered. Flowers pedicellate, bracteate. Calyx bell-shaped, 5-lobed. Corolla bilabiate, upper lip short, triangular, lower lip spreading, 3-lobed, middle lobe with 2 ridges at centre having glandular hairs on ridges. Stamens 4, didynamous, anthers dorsifixed. Capsule ovoid to globose, glabrous with pointed apex, loculicidal. Seeds brown, small, many.

Three species are found to occur in area of study.

Key to species

1 + Stem stoloniferous.....3. *Mazus surculosus*

\- Stolons absent..... ….....2

2 + Plants densely hairy.....1. *Mazus delavayi*

\- Plants almost glabrous..... ....2. *Mazus pumilus*

1. *Mazus delavayi* Bonati in Bull. Herb. Boiss. ser. 2, 8:530. 1908; Edinburgh *et al.*, Scroph. in Fl. Pak. 119:220. 2015. Fig. 1.A

Small annual herb, upto 28 cm tall, not stoloniferous, deep-rooted, hairy. Stem 1 to many, branched, ascending or sub-erect, hairy with glandular and eglandular hairs. Basal leaves in rosette form, spathulate to oblanceolate, hairy, margin toothed, decurrent at base, short-petioled, lamina upto 50 mm long. Cauline leaves smaller than basal leaves, sessile, lamina suborbicular, hairy on both surfaces, cuneate at the base, apex obtuse or rounded, margins crenate. Inflorescence elongated racemes, somewhat secund, hairy, few to many flowered. Flowers pedicellate, bracteate. Pedicels shorter than calyx, upto 2 mm long, densely hairy with glandular hairs. Bracts linear-triangular, green, sparsely hairy. Calyx 4 mm, bell-shaped, 5-lobed, tube usually shorter than lobes, lobes with apex acute, glandular hairy. Corolla 8 mm long, off-white, yellow markings on lower lip, bilabiate, upper lip short, triangular, lower lip spreading,

3-lobed, tube hairy on outside. Stamens 4, didynamous, anthers dorsifixed. Ovary glabrous. Capsule 4 mm long, ovoid to globose, glabrous with pointed apex, apiculate, loculicidal. Seeds brown, small, 0.4 mm long, many, oblong.

Specimen Examined: Battagram Distt., Govt. Girls College Battagram, white flowers, 1063 m, 20.03.2014, *Shamila Firdous* 14 (HUP), Mansehra Distt., Shinkiarai, near river Siran, 1073 m, 14.05.2017, *Shamila Firdous* 87 (HUP).
Flowering period: March- May.
Fruiting period: April- June.

2. *Mazus pumilus* (Burm.f.) van Steenis, Nova Guinea n.s. 9(1) 31. 1958; Edinburgh *et al.*, Scroph. in Fl. Pak. 120:220. 2015. Fig. 1.B, D-F, I
Small annual herb, upto 15 cm tall, not stoloniferous. Stem many, ascending, branched, slightly flexuous in area of inflorescence, sparsely hairy with glandular and eglandular hairs. Basal leaves clustered, spathulate to oblanceolate, glabrous, margin toothed, decurrent at base, short-petioled, lamina upto 25 mm long. Cauline leaves sessile, lamina suborbicular, glabrous, cuneate at the base, apex obtuse or rounded, margin dentate. Inflorescence racemose, secund, hairy, few to many flowered. Flowers pedicellate, bracteate. Pedicels longer than calyx in fruits, densely hairy. Bracts linear-lanceolate, hyaline, glabrous. Calyx 6 mm long, bell-shaped, 5-lobed, tube usually shorter than lobes, lobes ovate, apex acute, becoming stellate patent, 8 mm long in fruits, hairy. Corolla 11 mm, purplish white, orange spots and hairs on throat, bilabiate, upper lip short, triangular, lower lip spreading, tube hairy on outside. Stamens 4, didynamous, anthers dorsifixed. Ovary glabrous. Capsule 4.5 mm long, globose, glabrous with pointed apex, apiculate, loculicidal, brown. Seeds brown, oblong, small, 0.4 mm long, many.

Specimen Examined: Battagram Distt., Govt. Girls College Battagram, light purple flowers, 1051 m, 16.03.2014, *Shamila Firdous* 16 (HUP); Mansehra Distt., Manda-gucha, 1410 m, 30.10.2016, *Shamila Firdous* 88 (HUP); Haripur Distt., near Khanpur Dam, 565 m, 28.02.2017, *Shamila Firdous* 89 (HUP); Abbottabad Distt., Bilal town, Abbottabad, 1400 m, 07.12.2016, *Shamila Firdous* 90 (HUP).
Flowering period: February- December.
Fruiting period: March- December.
Plant is newly reported from the area of study, as it was previously reported from Punjab and Sindh.

3. *Mazus surculosus* D. Don., Prodr, Fl. Nepal 87.1825 (*surculosa*'); Edinburgh *et al.*, Scroph. in Fl. Pak. 117:220. 2015.Fig.1.C, G-H
Annual herb, upto 10 cm tall, stoloniferous. Stem with aerial stolons having long internodes, few erect branches, hairy with white eglandular hairs. Basal leaves in rosette form, ptiolate, petioles upto 15 mm long, lamina spathulate to obovate, tapering towards base, upto 30 mm long, hairy on both surfaces, margin crenate, lobed near base, apex obtuse. Cauline leaves opposite, smaller than basal leaves, short-petioled, lamina suborbicular to rounded, hairy on both surfaces, apex obtuse or rounded, margin lobed. Inflorescence terminal racemes, hairy. Flowers pedicellate, bracteate. Pedicels longer than calyx, 9 mm long in flowers, 12 mm long in fruits, hairy. Bracts linear, 3 mm long. Calyx campaulate, 7 mm long, 5-lobed, lobes 1/3 as long as calyx, lobes with apex acute, glandular hairy. Corolla 15 mm long, bilabiate, lips white, tube purple, yellow markings on palate, hairy near throat, upper lip short, 2-lobed, lower lip spreading, 3-lobed, lateral lobes orbicular, quite wider than middle ovate lobe, tube hairy on outside. Stamens 4, didynamous, anthers dorsifixed. Style long, white, stigma capitate. Capsule ovoid, 5 mm long, glabrous. Seeds small, 0.3 mm long and many.

Specimen Examined: Mansehra Distt., Shinkiari, Shahzeb lake, purple and white flowers, growing in moist soil, 1072 m, 14.05.2017, *Shamila Firdous* & *Jan Alam* 66 (HUP).
Flowering period: May- June.
Fruiting period: June- July.

Seed morphology of *Mazus* Lour.
Seeds are brown, usually oblong, surface colliculate (having tiny projections), finely verrucate, hilum is basal.
Three species are described below.

1. *Mazus delavayi* Bonati in Bull. Fig. 2.A-B
Seeds are pale brown, 0.40 mm long, 0.22 mm broad, oblong to cuneate, apex rounded, base truncate.

2. *Mazus pumilus* (Burm.f.) Steenis Fig. 2.C-D
Seeds are brown, 0.35 mm long, 0.21 mm broad, oblong, round at apex and base.

3. *Mazus surculosus* D. Don. Fig. 2.E-F
Seeds are brown, 0.45 mm long, 0.25 mm broad, oblong to cuneate, apex obtuse, base cuneate, surface finely reticulate-verrucate.
List of the collected species and their seed morphology is presented as Table-I.

IV. DISCUSSION

In current study three species of *Mazus* (including 1 new report i.e. viz., *M. pumilus*) have been recorded from the area of study. These species are all annual herbs, while no perennial herb was found in the area. Plants were classified mainly on the basis of vegetative characters like, stem (stoloniferous or not) and hairs (dense or sparse). Similarly, trichomes were specific at specific level, like their secretory nature and intensity on the surface of leaves and stems.

Seeds belonging to three species of *Mazus* Lour. are observed from the area of study with seeds of two species are described for the first time. Seeds are oblong with colliculate and finely verrucate surface. These observations are in accordance with Ghimire [17] who described seeds of *M. pumilus* as ovoid in shape with colliculate surface. While Hsieh, [5] described seeds of *M. pumilus* as having ellipsoid shape with reticulate surface and convex shaped cells at surface.

V. CONCLUSION

Macro-morphological characters of plants like habit, vegetative and floral characters, are helpful in delimitation of genus as well as species. Especially characters of stem and trichomes and shape and hairy palate of corolla are crucial at specific level. Similarly, seed-coat surface is found to be unique feature of genus.

VI. RECOMMENDATIONS

Beta taxonomy needs special emphasis with respect of floristics of Hazara, as there is a possibility of discovery of more species, new to science as well as new records to the country and the study area as well. Hence, further thorough field investigations are required in the study area, particularly focusing districts of Torghar and Upper and Lower Kohistan. As anatomy plays an important role in modern taxonomy, so anatomical characters are needed to be investigated in these medicinally important plants.

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Data availability statement: All data were found in Hazara Division of the Province of Khyber-Pakhtunkhwa, Pakistan. All voucher specimens are present at Herbarium of Hazara University.

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Table-I: Morphology of seeds

S. No	Name of specimen	Colour	Size (mm)		Shape	Apex	Base	Hilum	Ornamentation	
			Length	Width					Primary	Secondary
1	*Mazus delavayi*	Pale Brown	0.40	0.22	Oblong	Rounded	Truncate	Basal	Colliculate	Verrucate
2	*M. pumilus*	Brown	0.35	0.21	Oblong	Rounded	Rounded	Basal	Colliculate	Verrucate
3	*M. surculosus*	Brown	0.47	0.27	Oblong	Obtuse	Cuneate	Basal	Colliculate	Reticulate-Verrucate

Fig. 1: A, *Mazus delavayi,* habit; **B**, *Mazus pumilus,* habit; **D**, capsule, **E,** stamen, **F**, pedicel along with bract, **I**, seeds; **C**, *Mazus surculosus,* habit; **G**, flower, **H**, calyx.

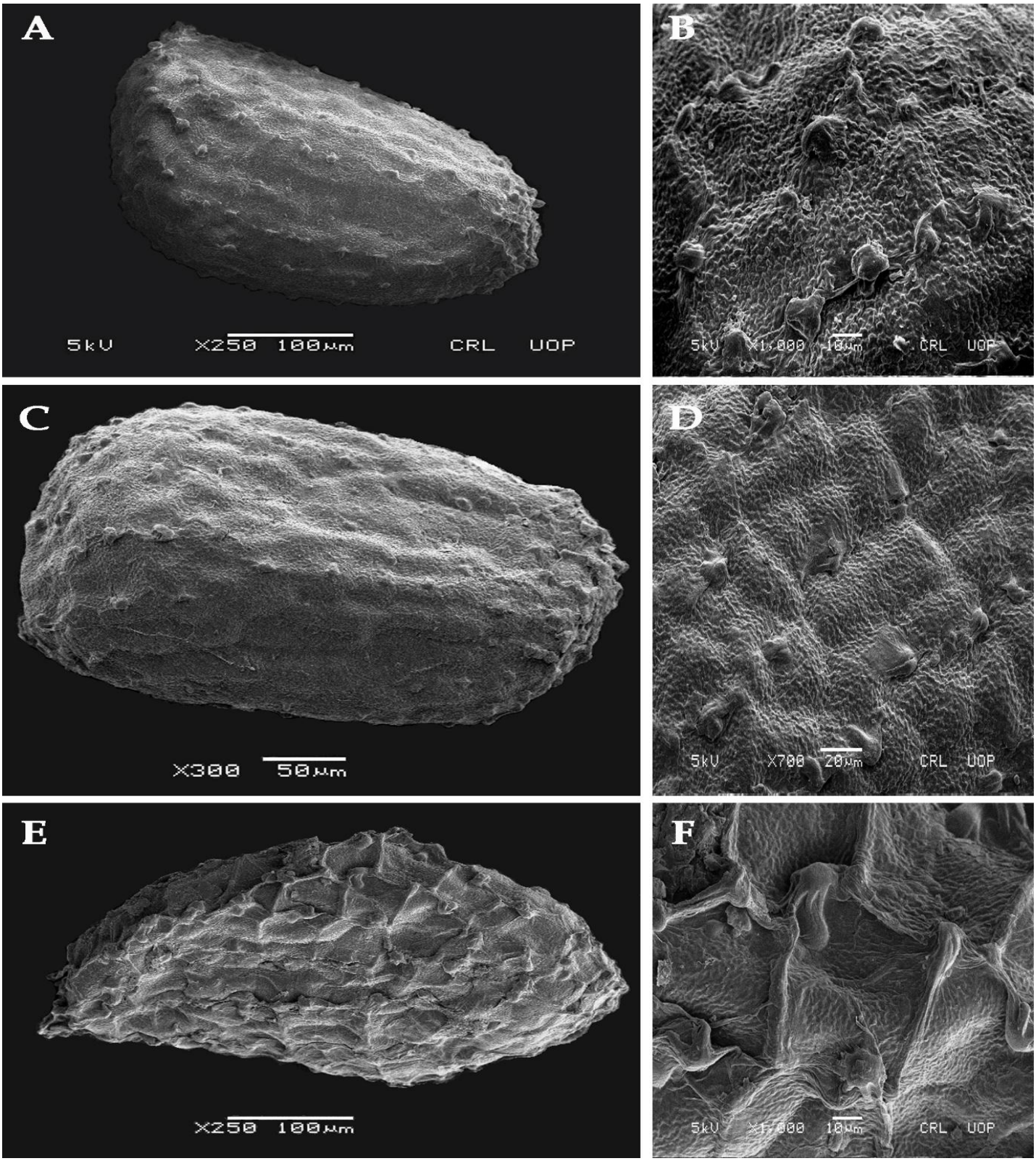

Fig. 2: *M. delavayi:* **A**, seed, **B**, seed-coat ornamentation; *Mazus pumilus:* **C**, seed, **D,** seed-coat ornamentation; *M. surculosus:* **E**, seed, **F,** seed-coat ornamentation.

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