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Original Research

Systematic Significance of Achene Macro-Micromorphological Characteristics and Associated Flora of Genus *Persicaria* from Temperate Himalayan Region of Kashmir

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Abstract

Macromorphological-micromorphological achene characteristics of genus *Persicaria* from temperate Himalayas were studied using scanning electron microscopy, stereomicroscopy, and light microscopy to investigate their correct identification, taxonomic implications and classification. The achene ultrastructure of *Persicaria* exhibits significant variations, with a diverse array of micromorphological characteristics observed. Achene micromorphological attributes were studied by different microscopic techniques, including achene size, color, shape, outline, texture, sculpturing, and periclinal and anticlinal wall patterns. Achene shapes were classified into different categories as trigonous, circular-biconvex, trigonous-biconvex, trigonous-bigonous, elliptic, biconvex, biconvex-trigonous, broadly ovate, and ovate-trigonous. Achene colors were black, black-dark brown, dark brownish, light brown-dark brownish, and black brown. SEM-based micromorphology features include achene sculpturing as finely vereucate, striate, psilate, reticulate to papillate, rugose, reticulate to granulate, striate to regulate, puncticulate, and striate to puncticulate. The range of achene length varied from 1.62–4.35 mm, maximum length was observed in *P. amplexicaulis* (3.51–4.35=4.02±0.18) mm, and minimum was in *P. sinuata* (1.62–1.99=1.86±0.08) mm. Epidermal walls included slightly regular to regular arrangements. A taxonomic key was created using micro- and macro-morphological characteristics to aid in distinguishing species boundaries and facilitating efficient identification within the *Persicaria* genus. The achene morphology of *Persicaria* along an altitudinal gradient of 1300–2700

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m was described with precise diagnostic importance of characters using SEM. *P. sinuata* was recorded from a narrow altitudinal range of 2400–2700 m with a restricted habitat within the upper temperate zone. This research work seeks to determine the taxonomic implications of achene morphology for the first time in the selected *Persicaria* species from the temperate Himalayas.

Keywords: altitude, GIS, northern hemisphere, SEM, taxonomic key

Introduction

Persicaria is a genus with about 120 species, the majority of which are weeds found in disturbed areas and croplands [1–4] and are easy to identify due to the presence of thin filaments of stamens and rectangular cells of the tepal outer layer [5, 6]. It is found in the northern temperate zones of both hemispheres and in Pakistan, represented by 26 species (flora of Pakistan). It is distinguished by a simple leaf, ciliate or entire chartaceous ochre, stamens 4–8 and 4–5 tepals, and an inflorescence with many flowers that are capitate, spicate, and panicle-shaped [7, 8]. *Persicaria* is frequently thought to be a member of the *Polygonum* L. [9, 10]. Based on various morphological and molecular data, the genus *Persicaria* is distinct from *Polygonum* s.l. as shown by the ochrea type or filament type, tepal venation, and inflorescence [5, 7, 11–14]. The Kashmir Himalayas, located in the Himalayan biodiversity hotspot, is a significant ecological mountainous region that is rich in biodiversity. It is home to large areas of temperate forests that are unique to the Himalayas [15–18]. The Himalayas, a mountain range stretching from Pakistan to Bhutan, are renowned for housing some of the highest and steepest peaks in the world. The Eastern Himalayas, which encompass Nepal and Bhutan, possess distinct characteristics that set them apart from other regions. The Central Himalayas are in India, while the Western Himalayas, which include regions of Pakistan, India, and Kashmir, complete the Himalayan range. Seed and fruit morphology have been regarded as vital indicators of flowering plant identification, and particularly surface sculpturing and ornamentation have been suggested to have systematic significance [19–25]. Many researchers have studied achene morphology in the Polygonaceae [26–34]. The *Polygonum* section's achene morphology studied in detail in Canada for delineation of the taxa revealed four distinct achene types: smooth, papillose, roughened, and papillose-striate [35]. Decraene et al. [11] also mentioned the significance of the achene morphology and anatomy for the systematic implications of the e Polygoneae and *Persicaria* tribes.

Achene and seed morphological characteristics have been considered important attributes in species classification, presenting reasonably stable characters that are relevant for comparative study at every level of the taxonomic hierarchy [36, 37]. Seed micromorphology includes characteristics that demonstrate morphological significance in evolutionary and phylogenetic relationships as well as in the identification of species [38, 39]. Taxonomic identification can be supported by the variety of seeds' form, color, and size [40]. Seed features have been found to be an effective method for identifying species at the family level and their classification at the genus level [41]. Seed ultrastructural properties, particularly cell shape and seed surfaces, are regarded as important distinguishing characteristics at intra-specific and intra-generic levels [42]. Seed morphology is an effective tool for analyzing seeds, including all members of different plant families, despite being a very time-consuming and labor-intensive process [43]. The study of seed morphology using a scanning electron microscope (SEM) has considerably advanced understanding of interactions among genera and families at a taxonomic level [44-47]. Although the majority of stereo microscopically evaluated features focus on seed size and shape instead of a detailed investigation of surface ornamentation [48], SEM uses extremely high magnification and incredibly detailed ultrastructure characteristics to identify qualitative variations that optical microscopy cannot [49].

We collected achenes from 10 wild species in the genus *Persicaria*, as micromorphological features are important for the taxonomic identification of species. This is the first comprehensive study of the morphological and microscopic characteristics of achenes of *Persicaria* from the Himalayan region of Kashmir. The objective of this study is to investigate the micromorphological features of achenes of *Persicaria amplexicaulis* (D.Don) Ronse Decr, *Persicaria barbata* (L.) H.Hara, *Persicaria longiseta* (Bruijn) Kitag, *Persicaria nepalensis* (Meisn.) Miyabe, *Persicaria sinuata (*Royle ex Bab.) Rajbh. & R.Joshi, *Persicaria capitata* (Buch.-Ham. ex D.Don) H.Gross, *Persicaria hydropiper* (L.) Delarbre, *Persicaria orientalis* (L.) Spach, *Persicaria maculosa* Gray and *Persicaria lapathifolia* (L.) Delarbre. To provide detailed information about their micromorphological features for precise, reliable, and proper evaluation of their taxonomic and systematic relationships, macro-morphological characteristics such as shape, color, size, external surface texture, length-width of base and apex, ultra-sculpturing of seed, surface ornamentation, including anticlinal-periclinal wall pattern, and general surface were examined

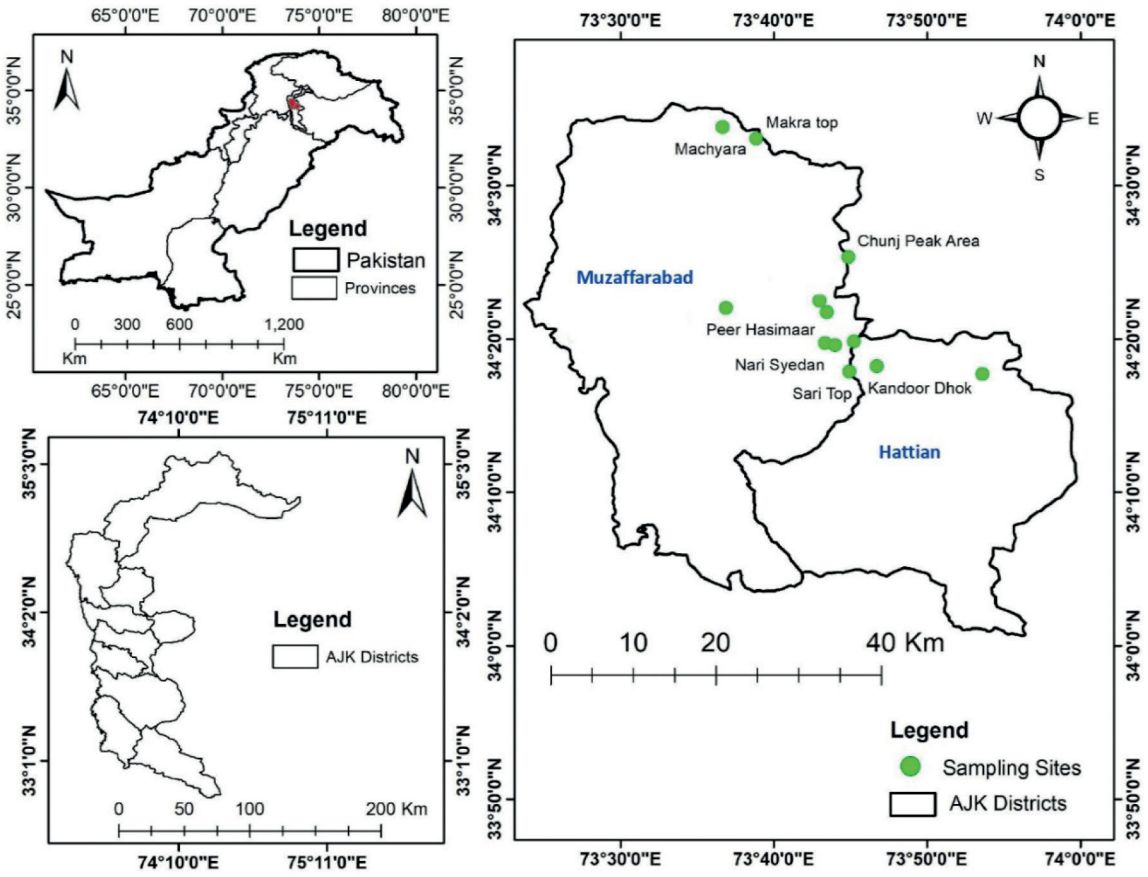

Fig. 1. Study area map of genus *Persicaria.*

by using light microscopy (LM), stereomicroscopy, and scanning electron microscopy (SEM). These traits are used to identify species at the genus level, as well as to assist both locals and scientists in making accurate identifications and assessing the extent of variability in the achene features of the genus *Persicaria* for future taxonomic studies.

Materials and Methods

Study Area

Persicaria species were collected from the temperate zone of the Kashmir Himalayan region. The studied area lies between 33°–35°N latitude and 73°–75°E longitude in the western Himalayan Mountain state of Jammu and Kashmir (AJK), Pakistan (Government of AJK 2019), as shown in (Fig.1). Sampling sites were selected according to the microhabitat preferences of the genus *Persicaria*, geography, and elevation range. The investigated *Persicaria* habitat sites are located at an elevation of 1300–2700 m in the temperate zone, as shown in elevation classes (Table 1, Fig. 1).

Plant Sampling, Collection, and Identification

To collect Polygonaceae species from the temperate zone, field trips were organized from June to October 2022 to several localities in the western Himalayas region. Comprehensive field visits were made to various localities in the western Himalayan region based on genus *Persicaria* diversity in moist and dry temperate forests along the elevation gradient for the true representation of diversity in the study area. The Global Positioning System (Garmin GPS, Model Oregon 700) was used to record the geographic data of the selected sites, such as altitude, latitude, and longitude, as listed in Table 1. Plants were collected with their ground and aerial parts, and renowned taxonomists identified the collected specimens and compared them to specimens that had already been deposited at the Herbarium of Pakistan (ISL) and compared them with the flora of Pakistan. A total of ten species of *Persicaria* were collected, identified, and preserved in the Herbarium of Pakistan Quaid-i-Azam University, Islamabad. Five to six achenes per species were examined in terms of their color, shape, size, and surface ornamentation.

Table 1. Checklist of the *Persicaria* species.

Taxon	Synonym	Status	Habitat	Life Form	Leaf Form	Locality	Altitude (m)	Latitude	Longitude	Accession No.
Persicaria orientalis (L.) Spach	*Polygonum orientale* L.	Wild	Near water or in open forests	Ther	Mac	Gydran area	2150	34°17’40.91”N	73°47’37.34”E	ISL-1333387
Persicaria hydropiper (L.) Delarbre	Polygonum hydropiper L.	Wild	Water and fully moist conditions	Hem	Mic	Kandoor	1841	34°18’31.89”N	73°48’13.15”E	ISL-1333382
Persicaria barbata (L.) H.Hara	*Polygonum barbatum* L.	Wild	In or along water bodies	Ther	Mic	Byla panjkot	1321	34°21’29.65”N	73°43’18.86”E	ISL-1333380
Persicaria longiseta (Bruijn) Kitag.	*Polygonum longisetum* Bruijn	Wild	Shady and moist areas	Ther	Nan	Leepa valley	2028	34°17’42.93”N	73°53’36.47”E	ISL-1333384
Persicaria nepalensis (Meisn.) Miyabe	*Cephalophilon nepalense* (Meisn.) Tzvelev	Wild	On the sides of water bodies in stones	Ther	Mic	Sritop	2676	34°18’52.57”N	73°44’22.06”E	ISL-1333386
Persicaria sinuata (Royle ex Bab.) Rajbh. & R.Joshi	*Polygonum sinuatum* Royle ex Bab.	Wild	Along the banks of streams	Ther	Mic	Chunj peak	2381	34°18’13.80”N	73°46’43.01”E	ISL-1333389
Persicaria capitata (Buch.-Ham. ex D.Don) H.Gross	*Polygonum capitatum* Buch.-Ham. ex D.Don	Wild	Moist and shady areas	Ther	Mic	Narri syedan top	2192	34°17’09.63”N	73°47’57.86”E	ISL-1333381
Persicaria maculosa Gray	*Persicaria fusiformis* (Greene) Greene	Wild	Shady areas	Hem	Mic	Khapi	2486	34°18’10.52”N	73°43’46.51”E	ISL-1333385
Persicaria lapathifolia (L.) Delarbre	*Dioctis maculatum* Raf.	Wild	Disturbed habitats, meadows and fields, shores of rivers or lakes	Hem	Mic	Hill	1407	34°20’56.64”N	73043’28.80”E	ISL-1333383
Persicaria amplexicaulis (D.Don) Ronse Decr.	*Bistorta amplexicaulis* (D.Don) Greene	Wild	Rocky mountains, shady places	Geo	Mic	Tlah area	2587	34°18’37.85”N	73°44’18.53”E	ISL-133379

Abbreviations: Ther = Therophytes, Hem = Hemicryptophytes, Geo = Geophytes, Mac = Macrophylls, Mic = Microphylls, and Nan = Nanophylls.

Light Microscopy (LM) and Stereomicroscopy

The external macro-morphological characters of the mature achenes were investigated with the aid of a stereomicroscope. For seed size (length and width), the value of 5–6 seeds of each species were measured by a micrometer. Micro morphological readings of the seed apex and base were taken by using LM (OPTIKA Microscope Italy) at 10X by following the methodology of Jabeen et al. [50].

Scanning Electron Microscopy (SEM)

Achenes were dipped in alcohol for 3–4 minutes to get rid of any dirt on the surface, then SEM was applied to the dry achenes. The achenes samples were then directly adhered to the stubs with tape. The samples were coated with gold-palladium sputtering before being analyzed with a SEM (JEOL JSM-5910) at the Central Resource Laboratory of the University of Peshawar. Polaroid P/N 665 film was used to capture micrographs. The samples were analyzed for indicative seed surface traits, including form, color, size, surface sculpturing, hilum, epidermal cells, cell boundaries, anticlinal and periclinal walls by following the methodology of Jabeen et al. [50].

GIS Mapping

Geographic information system software Arc GIS 10.8.2 was used for the geospatial analysis of species of genus *Persicaria*, including mapping of the study area and digital elevation modeling by following the protocols of [51, 52]. The GIS data was converted into Geo-TIFF format, the data sheets were divided into smaller sections, and a digital boundary was created. The map indicating the inventory of *Persicaria* specimens was developed using GPS information recorded from each of the sampling sites. The World Geodetic System 1984 (WGS84) coordinate system was used to set reference points for locating the selected study sites by Geo Referencing.

Statistical Data Analysis

The length, width, and length-width ratio of each of the mentioned characteristics, as well as the recorded features, were used to analyze the achene morphology. For each parameter, five readings were recorded. Statistical analysis of the data was done using the SPSS software, version 16.0 (Table 2), following the technique of [53]. The quantitative features were summarized using the minimum-maximum (mean) and standard error. The length-to-width ratio was calculated using the formula L/W, where L is the seed's length and W is its width. The SPSS 16.0 statistical software mean values were applied in the cluster analysis, which was carried out using the PAST 4.03 version [54]. The validity of seed macro- and micromorphological features was examined using principal component analysis (PCA) [15, 55].

Results

The seed characteristics and associated flora of ten *Persicaria* species along the altitudinal gradient were studied (Tables 2 and 3). The taxonomic features of the seeds' surface indicated some taxonomic significance in both quantitative and qualitative characters. Macro- and micromorphological features such as color, texture, seed shape, base, apex, seed outline, hilum presence or absence, position and level, achene sculpturing, and anticlinal and periclinal wall pattern were found to be quite different within *Persicaria* taxa (Table 3). SEM and stereomicroscope micrographs of all taxa studied are shown in (Table 3). These taxa were dominated by therophytic life forms and microphyllous leaf forms (Table 1).

Associated Flora and Altitudinal Range of the *Persicaria* Species

The *Persicaria* species were collected from an altitudinal range of 1300–2700 m in the study area, showing a strong influence of altitude on the distribution pattern of the species. The geographic distribution showed that the *Persicaria* genus is mostly restricted to aquatic and moist habitats. *P. barbata* and *P. lapathifolia* were recorded and distributed in a range of 1300–1700 m in the lower temperate zone. *P. sinuata* was recorded from a narrow altitudinal range of 2400–2700 m with a restricted habitat within the upper temperate zone. *P. longiseta, P. hydropiper, P. orientalis and P. capitata* were recorded from an altitudinal range of 1700–2200 m. *P. amplexicaulis* was recorded and studied at more than 2000 m in the upper temperate zone of the Kashmir Himalayan. *P. nepalensis* and *P. maculosa* had a broad altitudinal range starting from 1300–2700 m from lower to upper temperate zone. *P. barbata* and *P. lapathifolia* mostly found in association with *Ilenthis altisema, Populus alba, Salix tetrasperma, lonicera quinquelocularis, Daphne oleoides, Bracharia ramosa, Oenothera rosea, Erioscirpus comosus,* and *Adiantum cappilus- veneris*. *P. sinuata* formed association with *Aesculus indica, Pinus wallichiana, vibernum grandiflorum,* and *Rumex nepalensis*. *P. nepalensis* and *P. maculosa* associated with *Pinus wallichiana, Abies pindrow, Pronus padus, Viburnum grandiflorum, Rumux acetosa, Mentha arvensis,* and *Hedera nepalensis*. *P. longiseta, P. hydropiper, P. orientalis, and P. capitata* associated with species *Pinus wallichiana, Berberis lycium, Rumux hastatus, Indegofera hetarantha,* and *Mentha longifolia,* and *P. amplexicaulis* associated with *Taxus wallichiana*, *Abies pindrow, Pinus wallichiana, Parrotiopsis jacquemontii*, *Allium humile, Bergenia ciliata, Oxyria digyna, Rumex acetosa,* and *Rumex nepalensis*.

Achene Shape and Color

The achene morphology of studied taxa varied significantly from each other. Eight different shapes were observed (Table 3). Achene shapes were classified

Table 2. Achene micro and macro morphological quantitative data.

Taxa	Seed Length (min-max=mean±Serr) mm	Seed Width (min-max=mean±Serr) mm	Seed L/ Seed W mm	Base Length (min-max=mean±Serr) µm	Base Width (min-max=mean±Serr) µm	Base L/ BaseW µm	Apex Length (min-max=mean±Serr) µm	Apex Width (min-max=mean±Serr) µm	Apex L/ Apex W µm
Persicaria orientalis	2.90-3.29=3.07±0.08	2.50-3.19=2.94±0.15	1.04	8.7-9.43=9.04±0.15	34.79-35.17=34.98±0.10	0.25	22.36-23.1=22.65±0.16	12.93-13.39=13.18±0.09	1.71
Persicaria hydropiper	2.53-2.98=2.69±0.10	1.75-2.23=1.93±0.11	1.39	27.36-27.76=27.57±0.08	44.36-44.64±44.53±0.06	0.61	24.96-25.47=25.23±0.12	13.84-14.5=14.17±0.13	1.78
Persicaria barbata	2.29-2.51=2.40±0.05	117-1.76=1.40±0.13	1.71	10.65-11.16=10.81±0.11	30.86-31.36=31.16±0.10	0.34	20.16-20.92=20.48±0.18	22.79-23.12=22.93±0.07	0.89
Persicaria longiseta	1.76-2.12=1.93±0.08	1.08-1.61=1.27±0.12	1.51	19.95-20.48=20.16±0.11	56.97-57.31=57.14±0.06	0.35	29.45-29.81=29.58±0.08	37.94-38.74=38.43±0.17	0.76
Persicaria nepalensis	2.25-2.52=2.39±0.06	1.75-2.21=2.01±0.10	1.18	34.37-34.86=34.60±0.12	51.24-52.97=51.86±0.38	0.66	32.89-33.47=33.20±01.12	54.75-55.14=54.93±0.08	0.6
Persicaria sinuata	1.62-1.99=1.86±0.08	0.99-1.25=1.15±0.06	1.61	26.45-26.63=26.55±0.03	27.75-28.11=27.9±0.07	0.95	14.99-15.57=15.25±0.12	18.73-19.13=18.89±0.09	0.8
Persicaria capitata	2.52-2.86=2.69±0.05	1.97-2.28=2.11±0.08	1.27	36.89-37.19=37.5±0.06	64.76-65.45=65.08±0.15	0.57	29.18-30.18=29.54±0.22	45.39-46.1=45.77±0.14	0.64
Persicaria maculosa	2.53-3.01=2.57±0.10	1.12-1.50=1.27±0.09	1.66	25.84-26.42=26.13±0.11	43.91-44.5=44.23±0.13	0.59	22.8-23.31=23.05±0.13	26.55-27.11=26.82±0.11	0.85
Persicaria lapathifolia	2.59-3.12=2.79±0.12	1.50-2.12=1.84±0.13	1.51	30.39-30.98=30.72±0.12	46.1-46.85=46.40±0.16	0.66	39.86-43.25=40.94±0.77	46.32-46.94=46.6±0.14	0.87
Persicaria amplexicaulis	3.51-4.35=4.02±0.18	2.59-2.98=2.84±0.09	1.41	25.17-26.35=25.86±0.27	64.98-65.61=65.29±0.15	0.39	19.35-20.6=20.03±0.27	8.67-9.46=9.02±0.19	2.22

Abbreviations: Min = Minimum, Max = Maximum, L =Length, and W = Width.

Table 3. Qualitative characteristics of achene morphology among the studied *Persicaria* species.

Taxa	Seed shape	Seed texture	Color	Hilum (+,-)	Hilum position	Hilum level	Seed apex occurrence	Seed base shape	Seed apex shape	Seed outline	Achene sculpturing	Anticlinal wall Pattern	Periclinal wall Pattern	Epidermal cell arrangement
Persicaria orientalis	Circular-biconvex	Granulate to striate	b	+	Basal	R	V	Cuniate	Acute	S	Finely vereucate	Thick and raised	Thick and depressed	Irregular
Persicaria hydropiper	Trigonus-biconvex	Pubescent	db	+	Basal	R	V	Cuniate	Acute	S	Striate	Raised and granulate	Thick and depressed	Irregular
Persicaria barbata	Trigonus	Glabrous, shining	b	+	Basal	D	V	Obtuse	Acute	S	Psilate	Concave	Irregularly thickened	Irregular
Persicaria longiseta	Trigonus-bigonous, elliptic	Shining	lb-db	+	Basal	R	V	Cuneate	Acuminate	S	Striate	Not visible	Not visible	Irregular
Persicaria nepalensis	Biconvex-trigonus	Shining	b-db	+	Basal	R	V	Obtuse	Acute	S	Reticulate to pappilate	Convex granulate	Straight and granulate	Slightly irregular
Persicaria sinuata	Trigonus	Shining glabrous	b	+	Basl	R	V	Cuneate	Acute	S		Thick and depressed	Convex and thickened	Irregular
Persicaria capitata	Trigonus	Shining-glabrous, striate	b-db	+	Basal	R	V	Obtuse	Acute	S	Reticulate and granulate	Thick and granulate	Straight and granulate	Slightly irregular
Persicaria maculosa	Biconvex	Shining glabrous	b-db	+	Basal	D	V	Obtuse	Acuminate	S	Striate to rugulate	Depressed		Irregular
Persicaria lapathifolia	Circular to broadly ovate	Shining	bb	+	Basal	R	V	Obtuse	Acuminate	S	Puncticulate	Thick and convex	Buttressed	Irregular
Persicaria amplexicaulis	Ovate, trigonus	Glabrous, shining	db-b	+	Basal	R	V	Cuneate	Acute	S	Striate and puncticulate	Thick and depressed	Irregularly thickened and raised	Irregular

Abbreviations: b = Black, db = Dark brown, lb-db = Light brown to dark brown, b-db = Black to dark brown, bb = Black brown, + = Present, - = Absent, R = Raised, V= Visible, D = Depressed, S = Smoo.

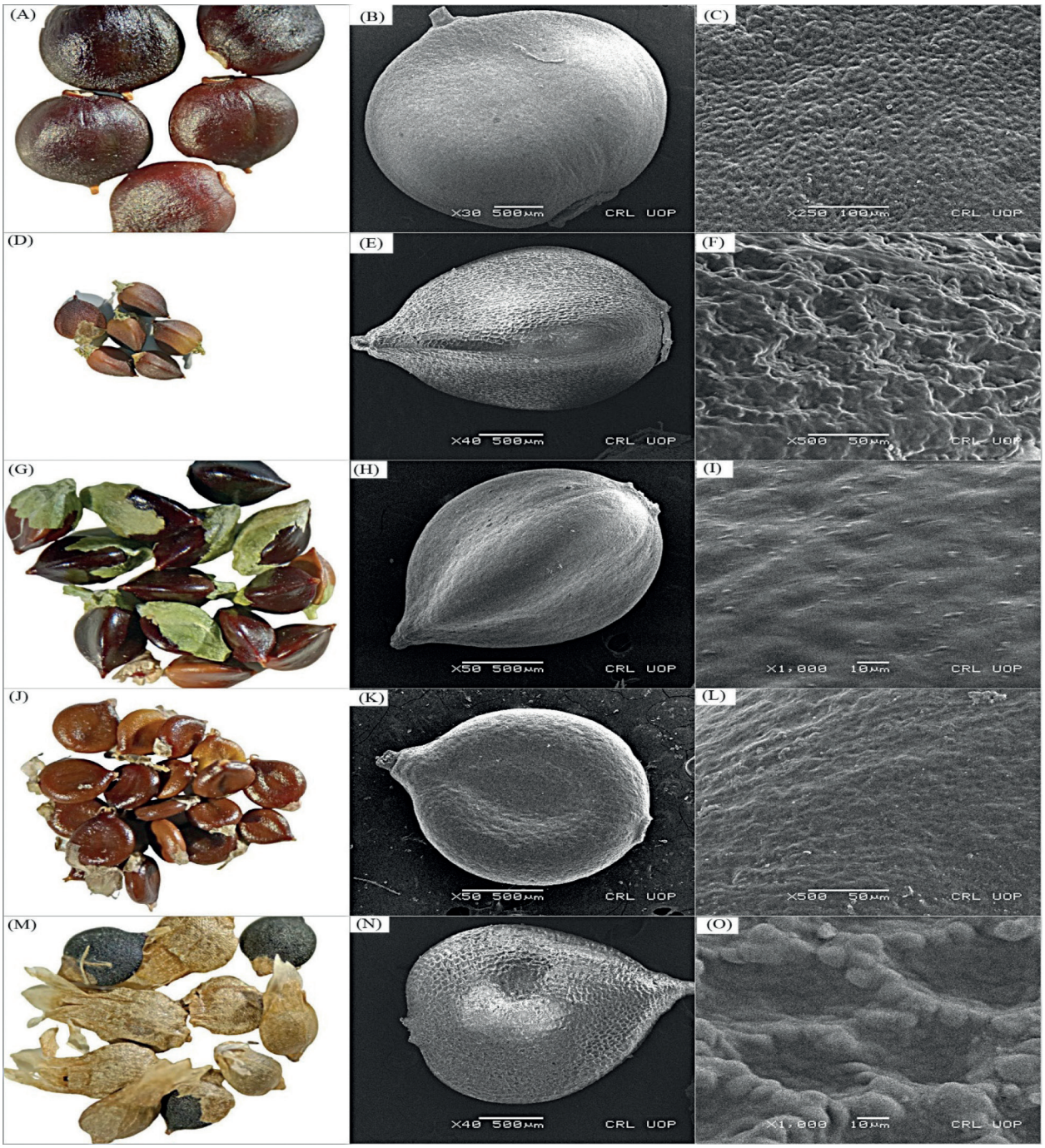

Fig. 2. SEM of achene morphology of genus *Persicria*. (A, B, C) *P. orientalis* circular-biconvex achene with finely vereucate surface sculpturing; (D, E, F) *P. hydropiper* trigonous-biconvex achene with striate sculpturing; (G, H, I) *P. barbata* trigonous achene with psilate sculpturing; (J, K, L) *P. longiseta* trigonous-bigonous, elliptic achenes with striate sculpturing; (M, N, O) *P. nepalensis* biconvex-trigonous achene with reticulate to pappilate sculpturing.

into different categories as trigonous, circular-biconvex, trigonous-biconvex, trigonous-bigonous, elliptic, biconvex, biconvex-trigonous, broadly ovate, and ovate-trigonous. Achene colors were black, black-dark brown, dark brownish, light brown-dark brownish, and black brown. Seed shape is trigonous in *P. barbata, P. sinuate,* and *P.* capitate, circular-biconvex in *P. orientalis* (Fig. 2A), trigonus-biconvex in *P. hydropiper* (Fig. 2D), trigonus in *P. barbata* (Fig. 2G), trigonous-bigonous and elliptic in *P. longiseta* (Fig. 2J), biconvex-trigonous in *P. nepalensis* (Fig. 2M), biconvex in *P. maculosa* (Fig. 3G), circular to broadly ovate in *P. lapathifolia* (Fig. 3J), and ovate-trigonous

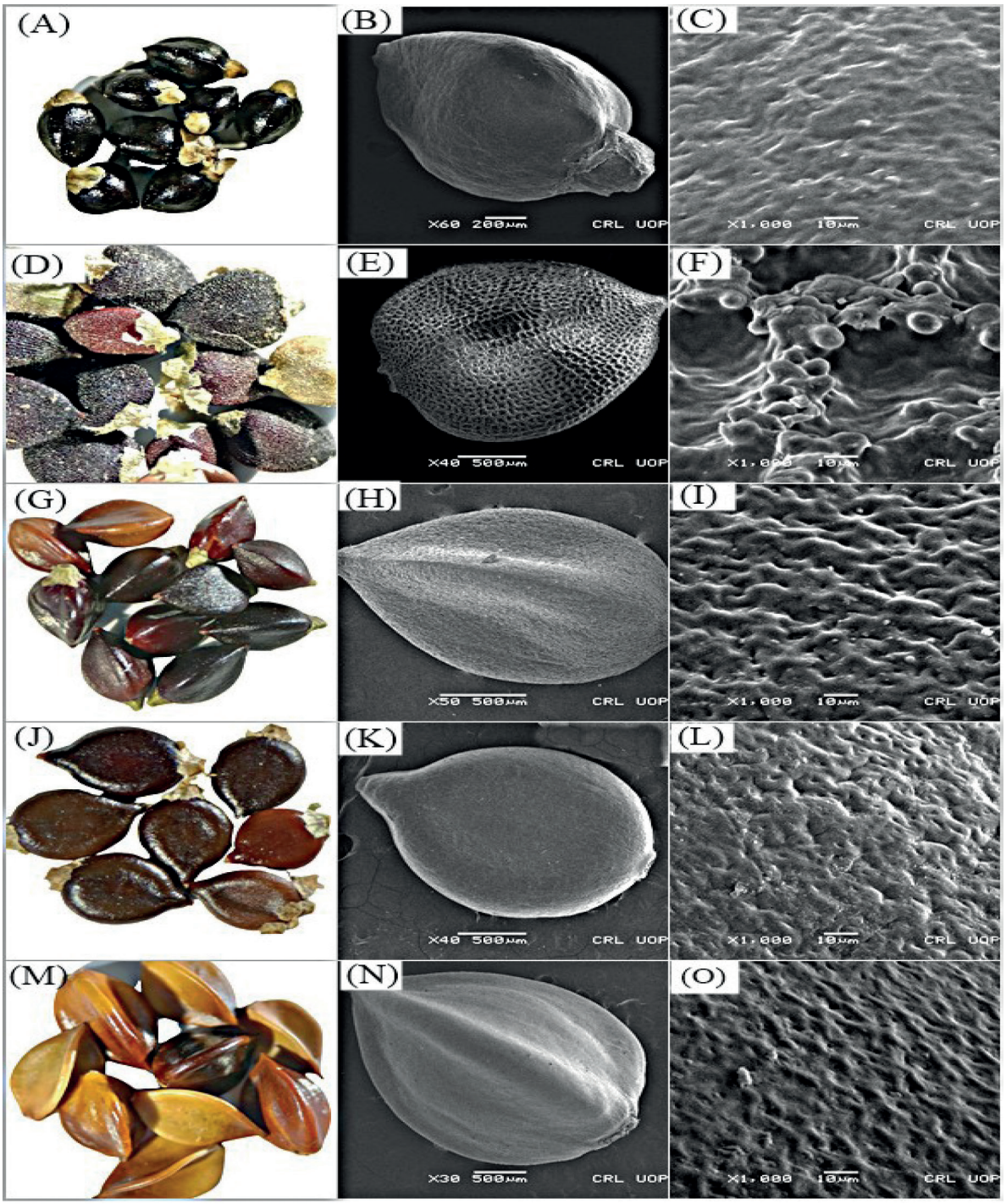

Fig. 3. SEM of achene morphology of genus *Persicria*. (A, B, C) *P. sinuata* trigonous achene with rugose sculpturing; (D, E, F) *P. capitata* trigonous achene with reticulate to granulate sculpturing; (G, H, I) *P. maculosa* biconvex achene with Striate to rugulate sculpturing; (J, K, L) *P. lapathifolia* circular to broadly ovate achene with puncticulate sculpturing; (M, N, O) *P. amplexicaulis* ovate to trigonous achene with striate and puncticulate surface sculpturing.

in *P. amplexicaulis* (Fig. 3M). Five different colors were observed (Table 3); black in *P. orientalis, P. barbata,* and *P. sinuata* (Figs. 4A and 4G, and Fig. 3A), black-dark brown in *P. maculosa, P. amplexicaulis, and P. nepalensis,* dark brownish in *P. capitata* and *P. hydropiper,* light brown-dark brownish in *P. longiseta* (Fig. 2J) and black brown in *P. lapathifolia* (Fig. 3J).

Achene Size and Texture

Achenes size and texture showed great variations among the studied species of genus *Persicaria*. Range of achenes length varied from 1.62–4.35 mm, maximum length was observed in *P. amplexicaulis* (3.51–4.35=4.02±0.18) mm, and minimum was in *P. sinuata* (1.62–1.99=1.86±0.08)

mm. Achenes width ranged from 0.99–3.19 mm, maximum width was measured in *P. orientalis* (2.50–3.19=2.94±0.15) mm, minimum was in *P. sinuata* (0.99–1.25=1.15±0.06) mm. The length–width ratio of achenes ranged from 1.04–1.71 mm, maximum was in *P. barbata* (1.71) mm and minimum was in *P. orientalis* (1.04) mm. Achene base length ranged from 8.7–37.19 µm, maximum was in *P. capitata* (36.89–37.19=37.5±0.06) µm, minimum in *P. orientalis (*8.7–9.43=9.04±0.15) µm. Base width ranged from 27.75–65.61 µm, maximum was calculated in *P. amplexicaulis* (64.98–65.61=65.29±0.15) µm, minimum was in *P. sinuata* (27.75–28.11=27.9±0.07). The length–width ratio of the base ranged from 0.25–0.95 µm, maximum was observed in *P. sinuata* (*0.9)* µm, minimum was in *P. orientalis* (0.25) µm. Achene apex length ranged from 14.99–43.25 µm, maximum was observed in *P. lapathifolia* (39.86–43.25=40.94±0.77) µm and minimum in *P. sinuata* (14.99–15.57=15.25±0.12) µm. Apex width ranged from 8.67–55.14 µm, maximum was in *P. nepalensis* (54.75–55.14=54.93±0.08) µm, minimum in *P. amplexicaulis* (8.67–9.46=9.02±0.19) µm, and the maximum length–width ratio of apex was in *P. amplexicaulis* (2.22) µm and minimum in *P. nepalensis* (0.6) µm (Table 2). Achenes' texture also showed great variations. Six different textures were observed in this study (Table 3). Glabrous to shining were studied in *P. barbata, P. sinuata*, *P. amplexicaulis,* and *P. maculosa*, shining texture in *P. longiseta*, *P. nepalensis,* and *P.* lapathifolia, granulate to striate in *P. orientalis* (Fig. 2A), pubescent in *P. hydropiper*, shining– glabrous (Fig. 2 D), and striate in *P. capitata* (Fig. 3D).

Achene Base and Apex Shape

Two different kinds of bases were observed in this research work (Table 3). Cuniate base was in *P. orientalis, P. hydropiper, P. longiseta, P. sinuata* and *P. amplexicaulis*, and obtuse base in *P. barbata, P. nepalensis, P. capitata, P. maculosa* and *P. lapathifolia*, and two different kinds of apex shapes were studied; acute and acuminate (Table 3).

Achene Outline, Hilum Position and Level

In the studied taxa, no as-such variations were seen in achene outline, hilum position, and level**.** All examined species have smooth outlines of achenes (Table 3) and basal and raised hilum, with the exception of *P. barbata* and *P. maculosa*; hilum was in a depressed condition in these two taxa.

Achene Sculpturing and Epidermal Wall Arrangements

The surface sculpturing and epidermal cell arrangement of the genus *Persicaria* showed significant diversity. Eight sculpturing patterns results were observed: Finely vereucate, striate, psilate, reticulate to papillate, rugose, reticulate and granulate, striate to regulate, puncticulate and striate to puncticulate (Table 3, Fig. 2 and 3). SEM results showed two types of epidermal cell arrangements: Most of the taxa with irregular epidermal cell arrangement and only two species *P. capitata* and *P. nepalensis* with slightly irregular epidermal cell arrangement.

Anticlinal and Periclinal Wall Pattern

In this study, anticlinal wall patterns were described into seven classes (Table 3, Fig. 2 and 3): thick and depressed in *P. sinuata* and *P. amplexicaulis,* thick and raised in *P. orientalis*, raised and granulate in *P. hydropiper,* concave in *P. barbata,* convex granulate in *P. nepalensis,* thick and granulate in *P. capitata,* depressed in *P. maculosa,* thick and convex *P. lapathifolia,* and not visible in *P. longiseta*. Periclinal wall patterns were described into seven classes (Table 3, Fig. 2 and 3): thick and depressed in *P. orientalis* and *P. hydropiper*, straight and granulate in *P. nepalensis and P. capitata,* buttressed in *P. maculosa and P. lapathifolia,* irregularly thickened in *P. barbata,* convex and thickened in *P. sinuata,* irregularly thickened, raised in *P. amplexicaulis,* and not visible in *P. longiseta* (Fig. 2 and 3).

Principle Component Analysis

P. capitata, P. nepalensis, and *P. lapathifolia* are more correlated than other *Persicaria* species in the results of the PCA performed on the examined species of the genus and *P. orientalis* and *P. barbata* showed an isolated position due to the lowest and highest length-width ratios of 1.04 and 1.71, respectively (Table 2, Fig. 4). The variations in the characters of *P. amplexicaulis*, *P. sinuata* and *P. barbata* led to their isolation. The achene and apex lengths of *P. amplexicaulis* were 4.02 mm and 65.29 µm, which was the largest of the studied taxa, while *P. sinuata* also showed a distinct position due to its smallest achene length (1.86) mm. *P. orientalis* with the lowest base length-width ratio of 0.25 and the highest 0.95 in *P. sinuata*. PC1 accounted for 41.29% of the overall variations in the studied quantitative data, followed by PC2 contributed 31.09% of the total variations. The quantitative features that helped in the formulation of different groups in PCA were achene length, width, base and apex length, width, and their length-width ratios. Qualitative characteristics, such as achene color, texture, shape, hilum position, achene outline, surface sculpturing, periclinal wall pattern, anticlinal wall pattern and epidermal arrangement of the cells, were the main characteristics in this study.

Discussion

In this study, we investigated the macro- and micromorphological features of the achenes by using a stereomicroscope, light microscope, and scanning electron microscope. With the use of SEM micrographs of seeds, several taxonomical problems associated with plant identification and authentication have been resolved in the modern era [50, 55–58]. SEM as a technique in plant

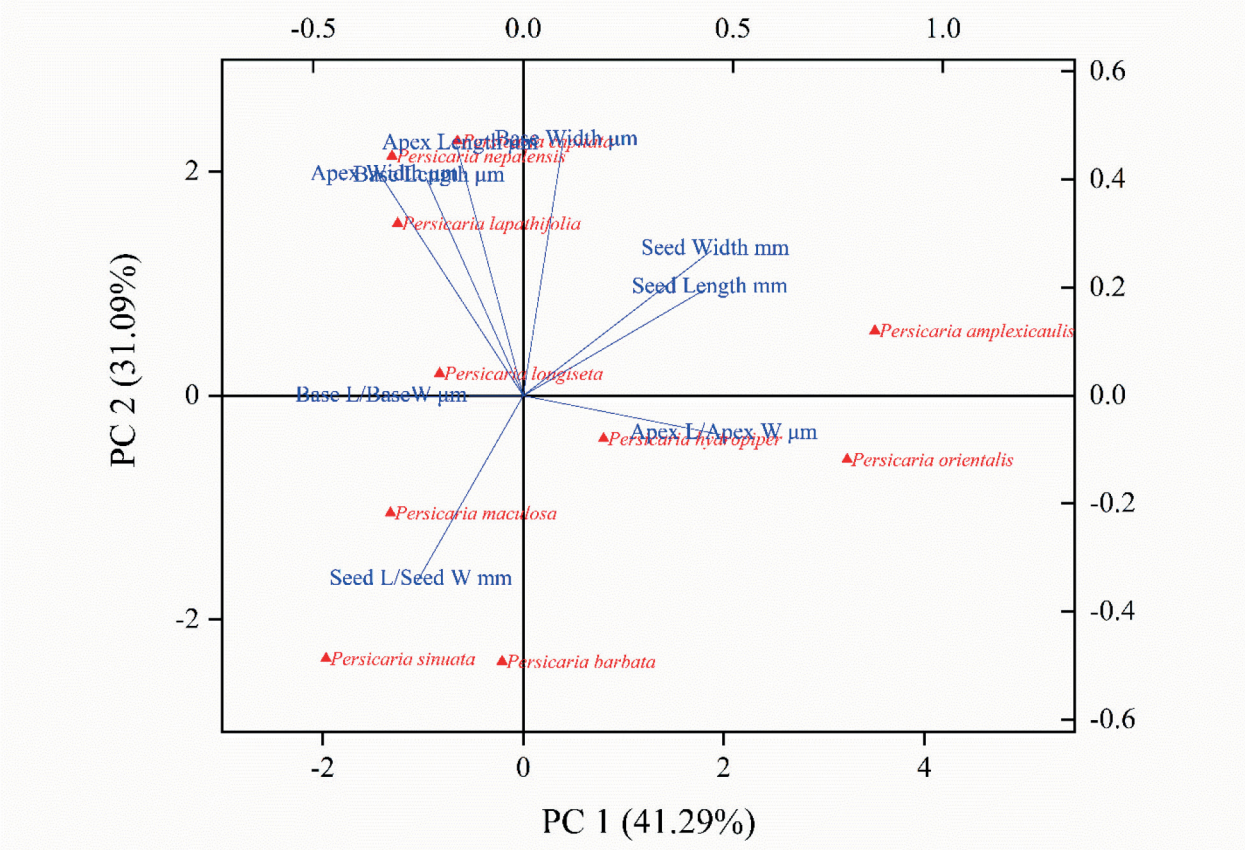

Fig. 4. PCA analysis based on quantitative achene morphological features.

systematics for studying micromorphological variations is a reliable source because of its high accuracy [59-63]. At various taxonomic levels, the *Persicaria* achene morphology showed significant variations in distinct characters [3, 4, 6, 64, 65]. The study of achene micromorphological features revealed many variations that could be very useful in the proper identification of species at the genus level. Within *Persicaria*, achene color, shape, hilum, cell surface anticlinal and periclinal wall arrangements, epidermal cell shape, outline, length, width, apex, and base shape all differed significantly. In this study, achene varied significantly in size, shape, color, and sculpturing. Achene morphology, particularly micromorphological characteristics, has previously been confirmed to be significant in Polygonaceae, including the genus *Persicaria*, demonstrating the taxonomic importance in the identification of closely related taxa. This is the first systematic report of achene micromorphology for the genus *Persicaria* from the Kashmir Himalaya. Trigonous achenes were the dominant achene shape, followed by circular-biconvex, trigonous-biconvex, trigonous-bigonous, elliptic, biconvex-trigonous, biconvex, circular to broadly ovate and ovate-trigonous. The collection season of seeds has implications on their shape [66, 67] i.e., *P. hydropiper* has either trigonous or convex achenes [68]. Black was the most dominant achene color, followed by black-dark brown, dark brownish, light brown-dark brownish, and the least observed black brown. [67] reported only triangular achenes in *P. barbata, P. hydropiper,* and *P. capitata,* which deviated from our current results. Achene texture was mostly glabrous to shining followed by granulate to striate, pubescent, shining- glabrous and striate. Mostly hilum was visible in the basal position, either in a depressed or raised condition. The largest achene length was in *P. amplexicaulis* and the lowest length was in *P. sinuata*; the largest width in *P. orientalis* and the lowest in *P. sinuate;* the largest achene length/width ratio in *P. barbata* and the lowest was in *P. nepalensis*. Martin (1954) provided achene numerical data for *P. orientalis* and *P. hydropiper*, which varied from 2.75–3.5 mm and 2.25–3 mm (length), respectively, and 3–3.5 mm and 1.75–2 mm (width), respectively, which in terms of size, are comparable to those evaluated in this study. The achene size of the studied species was also similar to that recorded in previous results [64, 67]. The achenes' shape and size matched those seen in past studies [6, 11, 29, 30, 67, 69].

Cuniate achene bases were the dominant base shape, followed by obtuse. The largest achene base length was in *P. capitata* and lowest in *P. barbata.* The largest base width was in *P. amplexicaulis* and the lowest in *P. sinuata*. Acute apex was the dominant apex shape followed by acuminate. The largest apex length was in *P. lapathifolia*

and the lowest in *P. sinuate*; the largest apex width in *P. nepalensis* and the lowest in *P. amplexicaulis* (Table 2). This is the first study to deeply study the base and apex quantitative and qualitative features in detail. Our qualitative results, specifically base and apex shape, were closely related to the findings published in 2016 [30]. The Achene outline was smooth in all studied taxa. Basal and raised hilum in all species except for *P. barbata and P. maculosa*, hilum was in depressed condition in these two taxa. Micromophological and ultra-structural study of seeds is a reliable source of information in the evolution of angiosperms and their taxonomic classification in the current synthetic systems [62, 70–72]. This SEM-based study revealed eight discernable types of surface sculpturing of the genus *Persicaria*, varied from finely vereucate, striate, psilate, reticulate to pappilate, rugose, reticulate and granulate, striate to regulate, puncticulate, and striate to puncticulate. The achene surface results of this study more or less deviated from [64, 73]. The most dominant epidermal cell arrangement was irregular, followed by slightly irregular in two species: *P. capitata* and *P. nepalensis.* The anticlinal walls pattern was mostly thick and depressed followed by thick and raised, raised and granulate, concave, convex granulate, thick granulate, depressed, and not visible in *P. longiseta.* Periclinal wall pattern dominantly showed thick and depressed, followed by straight and granulate, buttressed, irregularly thickened, convex and thick, and not visible in *P. longiseta.* This study demonstrated that analyzing achene morphological features, particularly at the taxon level, could yield much taxonomic information in the systematic identification of closely related taxa at the genus level.

The PCA analysis based on the studied features showed a high degree of similarity between *P. capitata, P. nepalensis,* and *P. lapathifolia* (Fig. 4). *P. orientalis* and *P. barbata* revealed an isolated position due to the lowest and highest length-width ratios. *P. amplexicaulis*, *P. sinuata,* and *P. barbata* led to their isolation position due to more variations in quantitative data as compared to other studied species. *P. sinuata* also showed a distinct position due to its smallest achene length, *P. orientalis* with the lowest base length-width ratio and some other distinct qualitative features lead to an isolated position.

Kanwal et al. [30] investigated seed atlas of family Polygonaceae from Pakistan in which some Persicaria species were also studied, results revealed that achenes with 1.5-3x1-2.5 mm, trigonous, bigonous-trigonous, bigonous, obovate, ovate and deltoid-elliptic, achene apex acuminate-acute, base obtuse-cuneate, light brown, dark brown to black and shiny, copper brown, surface ruminate, foveolate, rugose, puncticulate, alveolate-tuberculate, and basal hilum. *P. amplexicaulis* was characterized by elliptic achenes with a rugose surface. *P. maculosa, P. nepalensis* and *P. lapathifolia,* were characterized by showing some common features, like ovate, obovate-deltoid achenes. These results were also supported by the past study of [74], where similar achenes were observed in *P. nepalensis* and *P. lapathifolia* with the presence of dark-brown achenes with an obtuse base and acuminate apex. *P. maculosa* also falls in the same category, by having dark brown to black achenes with obtuse base and acute apex but remaining distinct by different achene surfaces. *P. longiseta* with light-dark brown achenes remains distinct because of different achene surfaces and shapes. In the present study, *Persicaria* species were similar in our and the aabove-mentionedstudies, i.e. *P. amplexicaulis, P. maculosa, P. nepalensis,* and *P. lapathifolia, P. longiseta* had more or less similar quantitative results, achenes shape, color, base, and apex shapes but our results remain distinct in variations in achene surfaces. The achene surface of *P. amplexicaulis* is rugose; the surface of *P. lapathifolia* is ruminate and puncticulate; the surface of *P. longiseta* is faintly ruminate and puncticulate; the surface of *P. maculosa* ruminate and puncticulate; the surface of *P. nepalensis* is smooth centrally depressed. In our present study, differences in achene surface from the previous results, i.e. *P. amplexicaulis* had a striate and puncticulate surface; *P. longiseta* had a striate surface, *P. maculosa* had a striate to regulate surface; *P. nepalensis* had a reticulate to pappilate surface and only *P. lapathifolia* had a puncticulate surface, which is similar to the previous findings [30]. The research results partly corroborate those previous findings [4, 75, 76].

Kong et al. [43] studied the achene morphology of 45 taxa of Korean Polygonaceae. Most of the species of *Persicaria* (*P. hydropiper, P. lapathifolia, and P. orientalis*), had trigonous achenes and biconvex achenes were studied in *P. amplexicaulis.* In the present study *P. orientalis*, *P. lapathifolia* and *P. amplexicaulis* deviated from previous results by having circular-biconvex, circular to broadly ovate, and ovate-trigonous shapes, respectively. The size and color of the previous and this study revealed similar results, but achene sculpturing in different Korean taxa of Persicaria delineated from our results by having smooth sculpturing in *P. orientalis, P. hydropiper* and *P. longiseta;* shallow in *P. lapathifolia;* tubercle in *P. nepalensis.* According to our study, *P. orientalis, P. hydropiper, P. longiseta, P. lapathifolia,* and *P. nepalensis* had finely vereucate, striate, reticulate to pappilate, and puncticulate surface sculpturing. SEM uses extremely high magnification and finely detailed ultrastructure characteristics to reveal qualitative distinctions that optical microscopy cannot [49, 56, 62, 71, 77].

Kantachot et al. [67] studied the achene morphology of 20 species of *Polygonum* in five sections from Thailand, including the genus *Persicaria;* smooth- undulate surface ornamentation (P. capitatum, *P. barbata*, *P. hydropiper* and *P. lapathifolia*), which is dissimilar to this study except reticulate-papillose (*P. nepalensis*) and triangular or lenticulate shape.

Mosaferi and Keshavarzi [78] investigated the morphological features of Polygonaceae from Iran and proposed that achene morphology of this family has taxonomic significance for the delimitation of taxa at the genus level; our findings are mostly similar to theirs but show significant differences with previous achene sculpturing results by having smooth with scattered dots (*P. lapathifolia*), fossulate (*P. maculosa*) and share similarity in *P. hydropiper* by having studied striate sculpturing in both present and past studies.

According to the present research, one of the key characteristics of the Himalayan Mountains is elevation, and species diversity decreases along the altitudinal gradient in the temperate to subalpine forest of the Kashmir Himalayas. Current results are similar to the past results; the number of species decreases as altitude increases [16, 51, 52, 79–82]. The present study was conducted in the temperate zone, which is dominated by tree conifer species, this is further supported by various studies [51, 83–85]. *Pinus wallichiana, Abies pindrow, Berberis lycium, Vibernum grandiflorum, Mentha longifolia, Bergenia ciliata, Persicaria amplexicaulis,* and *Rumex nepalensis* were the most dominant species in the study area. *P. wallichiana* has the largest range, stretching from lower to upper temperate and nearly touching the tree line in the study area, similar to the findings of [86]. The present study showed the distribution of the genus *Persicaria* along the altitudinal gradient in temperate Himalayas; *P. nepalensis* and *P. maculosa* had broad altitudinal ranges starting from 1300 to 2700 m and *P. sinuata* with a narrow altitudinal range of 2400–2700 m. Most of the studied taxa were collected from an elevation of 1500–2500 m, supported by earlier studies, as the medium altitude range has a higher number of species [16, 18, 51, 52, 82, 87–89]. When we compare our results with the above-mentioned studies, there are a lot of similarities in achene shape, color, and size, but there are also many differences in micromorphological traits, such as surface sculpturing and periclinal and anticlinal wall pattern, within the *Persicaria* genus. Nevertheless, the combination of various morphological characters in achenes may help to identify, categorize, and describe the species at the genus level. Further comprehensive molecular-based research is needed to analyze the evolutionary and phylogenetic trends in the complex micromorphology of *Persicaria* species.

Taxonomic Key

(1) + Seed circular-biconvex, texture granulate to striate, black, achene finely vereucate, anticlinal wall thick and raised.....*P. orientalis*.
– Seed trigonous-biconvex, texture pubescent, dark brownish, achene striate, anticlinal raised and granulate.....2.

(2) + Hilum raised, base cuniate, periclinal thick and depressed.....*P. hydropiper.*
– Seed trigonous, texture glabrous, shining, black, hilum depressed, base obtuse, achene psilate .....3.

(3) + Apex acute, anticlinal concave, periclinal irregularly thickened.....*P. barbata.*
– Seed trigonous-bigonous, elliptic, light brown-dark brownish, hilum depressed, base cuneate, achene striate, anticlinal and periclinal wall not visible .....4.

(4) + Epidermal cell irregular, apex acuminate, irregular epidermal cell arrangement.....*P. Longiseta.*
– Seed biconvex-trigonous, brown to black-dark, base obtuse, achene reticulate to pappilate, anticlinal convex granulate, periclinal straight and granulate..5.

(5) + Texture shining.....*P. nepalensis.*
– Texture shining glabrous, base cuneate, achene rugose, anticlinal thick and depressed, periclinal convex and thick ..... 6.

(6) + slightly irregular epidermal cell arrangement .....*P. sinuata.*
– Texture Shining- glabrous, striate, achene reticulate and graniulate, anticlinal thick and granulate, periclinal straight and granulate, slightly irregular epidermal cell arrangement.....7.

(7) + Seed trigonous, hilum raised, apex acute..... ..... *P. capitata.*
– Seed Biconvex, texture shining glabrous, hilum depressed, achene striate to regulate, anticlinal striate to regulate, periclinal depressed ..... 8.

(8) + Black-dark to brown.....*P. maculosa.*
– Seed circular to broadly ovate, texture shining, black-brown, achene pappilate, anticlinal thick and convex, periclinal buttressed.....9.

(9) + Base obtuse, apex acuminate.....*P. lapathifolia.*
– Seed ovate, trigonous, texture glabrous, shining, dark brown to black, apex acute, achene striate and pappilate, ant thick and depressed, per irregularly thick and raised .....10.

(10)+ Hilum present, basal, hilum raised, seed apex visible, base cuneate, outline smooth, irregular epidermal cell arrangement.....*P. amplexicaulis.*

Conclusions

This study demonstrates that analyzing the micromorphological characterization of achenes morphology is useful for species identification and delimitation in the genus *Persicaria.* The results of the SEM study revealed significant variations in the structure of achene surface morphology among different species, indicating important taxonomic distinctions. The findings also provide useful information on this aspect of the specimens analyzed. The present investigation has shown that achene color, shape, surface, hilum, anticlinal wall and periclinal wall arrangements, cell boundaries, epidermal cell shape, width, length, and length/width ratio can be used to differentiate species at the generic level. The range of achene length varied from 1.62–4.35mm, maximum length was observed in *P. amplexicaulis* (3.51–4.35=4.02±0.18) mm, and minimum was in *P. sinuata* (1.62–1.99=1.86±0.08) mm. The *Persicaria* species were collected from an altitudinal range of 1300–2700 m in the study area, showing a strong influence of altitude on the distribution pattern of the species. We use GIS elevation modeling to identify the altitudinal range of different species of the genus *Persicaria*. Taxonomic keys were used to differentiate the complex's taxa. The PCA analysis further highlights the relationship between the investigated taxa. The present study implies that more comparative research and sampling on achene morphological and micromorphological characterization in the genus *Persicaria* using various

microscopic techniques would help to improve the existing systematic delimitation of this complicated genus.

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Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethics approval and consent to participate

All the experiments were performed in accordance with relevant guidelines and regulations.

Author Contributions

Syed Waseem Gillani: Field Collection, Writing- Original draft preparation, Mushtaq Ahmad: Supervision and Review Editing, Muhammad Manzoor: Statistical analysis, Syed Waseem Gillani: Methodology and Data analysis, Hamayun Shaheen: Methodology and English editing, Shazia Sultana and Lixin Zhang: Investigation, Shaista Jabeen: Conceptualization, Syed Waseem Gillani and Muhammad Manzoor: Interpretation of Results, Mona S Alwahibi and Mohamed Farouk Elsadek: Funding acquisition & data curation.

References

1. MCDONALD C.B. A biosystematic study of the *Polygonum hydropiperoides* (Polygonaceae) complex. American Journal of Botany. **67** (5), 664, **1980**.
2. KIM S.-T., DONOGHUE M.J. Molecular phylogeny of *Persicaria* (Persicarieae, Polygonaceae). Systematic Botany. **33** (1), 77, **2008**.
3. UZUN S.P., ALPER U., ALICI E. Seed Morphology of Some Selected Species of Fabaceae, Polygonaceae, Primulaceae, Violaceae, Rosaceae and Phyllanthaceae. Journal of the Institute of Science and Technology. **10** (3), 2028, **2020**.
4. KESKIN M., SEVEROĞLU Z. The genus *Persicaria* (Polygonaceae) in Turkey with a new taxon record. EMU Journal of Pharmaceutical Sciences. **3** (2), 97, **2020**.
5. DECRAENE L.-P.R., AKEROYD J. Generic limits in *Polygonum* and related genera (Polygonaceae) on the basis of floral characters. Botanical Journal of the Linnean Society. **98** (4), 321, **1988**.
6. AL-NEWANI H., ALIWAY S., AL-MASOUDI R. The taxonomical significant of computerd phylogenetic analysis and morphological data in some species of Polygonaceae. The Iraqi Journal of Agricultural Science. **51** (6), 1517, **2020**.
7. HARALDSON K. Anatomy and taxonomy in Polygonaceae subfam. Polygonoideae Meisn. emend. Jaretzky. Acta Universitatis Upsaliensis. Symbolae botanicae Upsalienses. **1978**.
8. FREEMAN C., REVEAL J. Polygonaceae. Vol. 5. Flora of North America. Oxford University Press. **2005**.
9. ZHU L., LU F., HOU Y., LI F. Micro-morphology of leaf epidermis and its taxonomical significance of *Polygonum* section Cephalophilon in China. Journal of Wuhan Botanical Research. **25** (2), 136, **2007**.
10. MAHMOUDI M., BOUGHALLEB F., PELLEGRINO G., ABDELLAOUI R., NASRI N. Flower, seed, and fruit development in three *Tunisian* species of *Polygonum*: Implications for their taxonomy and evolution of distyly in Polygonaceae. PLos One. **15** (1), e0227099, **2020**.
11. DECRAENE L.P.R., HONG S.-P., SMETS E. Systematic significance of fruit morphology and anatomy in tribes Persicarieae and Polygoneae (Polygonaceae). Botanical Journal of the Linnean Society. **134** (1-2), 301, **2000**.
12. FRYE A.S.L., KRON K.A. rbcL phylogeny and character evolution in Polygonaceae. Systematic Botany. **28** (2), 326, **2003**.
13. YURTSEVA O.V., DEVIATOV A.G., SOKOLOFF D.D. Exocarp structure in the genus *Atraphaxis* (Polygonaceae, Polygoneae). Plant Systematics and Evolution. **308** (5), 32, **2022**.
14. KOLAGANI C., SHUKLA P.N., DUDIAPALA R. editors. *Polygonum chaturbhujanum* (Polygonaceae), a New Species from Pench Tiger Reserve, Maharashtra, India. Annales Botanici Fennici. **2024**.
15. HAQ S.M., CALIXTO E.S., KUMAR M. Assessing biodiversity and productivity over a small-scale gradient in the protected forests of Indian Western Himalayas. Journal of Sustainable Forestry. **40** (7), 675, **2021**.
16. GILLANI S.W., AHMAD M., ZAFAR M., HAQ S.M., WAHEED M., MANZOOR M., SHAHEEN H., SULTANA S., REHMAN F.U., MAKHKAMOV T. An Insight into Indigenous Ethnobotanical Knowledge of Medicinal and Aromatic Plants from Kashmir Himalayan Region. Ethnobotany Research and Applications. **28**, 1, **2024**.
17. KAYANI S., AHMAD M., GILLANI S.W., MANZOOR M., REHMAN F.U., JABEEN S., BUTT M.A., BABAR C.M., SHAH S.A.H. Ethnomedicinal appraisal of the medicinal flora among the sub-alpine and alpine Iindigenous communities of Palas Valley Kohistan, Northern Pakistan. Ethnobotany Research and Applications. **28**, 1, **2024**.
18. MANZOOR M., AHMAD M., ZAFAR M., GILLANI S.W., SHAHEEN H., PIERONI A., AL-GHAMDI A.A., ELSHIKH M.S., SAQIB S., MAKHKAMOV T. The local medicinal plant knowledge in Kashmir Western Himalaya: a way to foster ecological transition via community-centred health seeking strategies. Journal of Ethnobiology and Ethnomedicine. **19** (1), 56, **2023**.
19. ROTH I. Fruits of angiosperms. Encyclopedia of Plant Anatomy. **1977**.
20. OLOWOKUDEJO J.D. Scanning electron microscopy of fruits in the genus *Biscutella* L.(Cruciferae). Phytomorphology. **1986**.
21. BARTHLOTT W. Scanning Electron Microscopy of the Epidermal Surface in Plants. Claugher D. Ed., Scanning Electron Microscopy in Taxonomy and Functional Morphology, Clarendon Press, Oxford, pp 69-94. **1990**.

22. ZHANG Z.-Y., YANG D.-Z., LU A.-M., KNAPP S. Seed morphology of the tribe Hyoscyameae (Solanaceae). Taxon. **54** (1), 71, **2005**.
23. HAREESH V.S., SABU M. Significance of seed morphology and anatomy in the systematics of Musaceae. Botanical Journal of the Linnean Society. **201** (1), 1, **2023**.
24. BIENIEK A., BIENIEK A., BIELSKA N. Variation in Fruit and Seed Morphology of Selected Biotypes and Cultivars of *Elaeagnus multiflora* Thunb. North-Eastern Europe, Agriculture. **13** (2), 495, **2023**.
25. PENG Q.Y., XIE M.H., PAN X.K., LI Y., GAO L., XU F., WU C.X., YANG M.F. Morphology and distribution of sensilla on antennae and mouthparts of the adult bruchid beetles, *Bruchidius coreanus* (Coleoptera: Bruchidae). Microscopy Research and Technique. **2024**.
26. MARTIN A.C. Identifying *polygonum* seeds. The Journal of Wildlife Management. **18** (4), 514, **1954**.
27. OH I.C., HONG S.P. A taxonomic study of achene microstructure in the genera *Polygonum* L. s. str. and *Polygonella* Michx. (Polygoneae-Folygonaceae). Korean Journal of Plant Taxonomy. **29**. (2), 123, **1999**.
28. AYODELE A.E., ZHOU Z.K. Scanning electron microscopy of fruits in the West African Polygonaceae. Journal of Systematics and Evolution. **48** (5), 336, **2010**.
29. GHIMIRE B., CHOI G.E., JEONG M.J., LEE H., LEE K.M., SON S.W., LEE C.H., SUH G.U. Fruit morphology and anatomy of *Persicaria chinensis* (Polygonaceae). Journal of Japanese Botany. **91** (4), 218, **2016**.
30. KANWAL D., ABID R., QAISER M. The seed atlas of Pakistan-XIV. Polygonaceae. Pakistan Journal of Botany. **48**, (5), 1833, **2016**.
31. SUMBEMBAYEV A.A., LAGUS O.A., NOWAK S. Seed morphometry of *Rheum* L.(Polygonaceae) species from Kazakhstan and its implications in taxonomy and species identification. Biodiversitas: Journal of Biological Diversity. **24** (9), **2023**.
32. ESTRADA-RUIZ E. A new species of winged fruits of Podopterus (Caryophyllales, Polygonaceae) from the Miocene amber, Chiapas, Mexico. Palaeoworld. **32** (1), 188, **2023**.
33. BHATT A., PHARTYAL S.S., DAIBES L.F., CHEN X. Interpopulation variation in seed traits of five Polygonaceae. Frontiers in Ecology and Evolution. **11**, 1093285, **2023**.
34. CAO D.L., ZHANG X.J., QU X.J., FAN S.J. Phylogenomics, divergence time estimation, and adaptive evolution in the Polygonoideae (Polygonaceae). Journal of Systematics and Evolution. **2023**.
35. KESKIN M., YILMAZ A., SEVEROĞLU Z. Micromorphological description of *Polygonum istanbulicum* (Polygonaceae). Acta Microscopica. **30** (2), 111, **2021**.
36. HEYWOOD V.H. Scanning electron microscopy in the study of plant materials. Micron. **1** (1), 1, **1969**.
37. BARTHLOTT W. Epidermal and seed surface characters of plants: systematic applicability and some evolutionary aspects. Nordic Journal of Botany. **1** (3), 345, **1981**.
38. ATTAR F., KESHVARI A., GHAHREMAN A., ZARRE S., AGHABEIGI F. Micromorphological studies on *Verbascum* (Scrophulariaceae) in Iran with emphasis on seed surface, capsule ornamentation and trichomes. Flora-Morphology, Distribution, Functional Ecology of Plants. **202** (2), 169, **2007**.
39. JOHNSON L.A., HUISH K.H., PORTER J.M. Seed surface sculpturing and its systematic significance in *Gilia* (Polemoniaceae) and segregate genera. International Journal of Plant Sciences. **165** (1), 153, **2004**.
40. NINKAEW S., PORNPONGRUNGRUENG P., BALSLEV H., CHANTARANOTHAI P. Seed morphology of nineteen *Crotalaria* L.(Fabaceae) species in Thailand. Thai Forest Bulletin (Botany). **45** (1), 47, **2017**.
41. SPETA F. Hyacinthaceae, Flowering Plants Monocotyledons: Lilianae (except Orchidaceae). Springer Science & Business Media. **1998**.
42. KUBITZKI K., ROHWER J.G., BITTRICH V. Flowering Plants·Dicotyledons: Magnoliid, Hamamelid and Caryophyllid Families. Springer Science & Business Media. **2013**.
43. DAVITASHVILI N., KARRER G. Taxonomic importance of seed morphology in *Gentiana* (Gentianaceae). Botanical Journal of the Linnean Society. **162** (1), 101, **2010**.
44. AHMAD M., ZAFAR M., BAHADUR S., SULTANA S., TAJ S., CELEP F., MAJEED S. Palynomorphological diversity among the Asteraceous honeybee flora: An aid to the correct taxonomic identification using multiple microscopic techniques. Microscopy Research and Technique. **85** (2), 570, **2022**.
45. YANG X., BASKIN J.M., BASKIN C.C., HUANG Z. More than just a coating: ecological importance, taxonomic occurrence and phylogenetic relationships of seed coat mucilage. Perspectives in Plant Ecology, Evolution and Systematics. **14**. (6), 434, **2012**.
46. ULLAH I., KIM D. IoT resource management using direct discovery mechanism in OCF framework. International Journal of Grid and Distributed Computing. **11**, **2018**.
47. ULLAH F., PAPINI A., SHAH S.N., ZAMAN W., SOHAIL A., IQBAL M. Seed micromorphology and its taxonomic evidence in subfamily Alsinoideae (Caryophyllaceae). Microscopy Research and Technique. **82** (3), 250, **2019**.
48. UTAMI E.S.W., HARIYANTO S., MANUHARA Y.S.W. In vitro propagation of the endangered medicinal orchid, *Dendrobium lasianthera* JJ Sm. through mature seed culture. Asian Pacific Journal of Tropical Biomedicine. **7** (5), 406, **2017**.
49. BELHADJ S., DERRIDJ A., AIGOUY T., GERS C., GAUQUELIN T., MEVY J.P. Comparative morphology of leaf epidermis in eight populations of Atlas pistachio (*Pistacia atlantica* Desf., Anacardiaceae). Microscopy Research and Technique. **70** (10), 837, **2007**.
50. JABEEN S., ZAFAR M., AHMAD M., ALTHOBAITI A., OZDEMIR F., KUTLU M., MAKHKAMOV T., SULTANA S., AMEEN M., MAJEED S. Ultra-sculpturing of seed morphotypes in selected species of genus *Salvia* L. and their taxonomic significance. Plant Biology. **25** (1), 96, **2023**.
51. ALAM N.M., SHAHEEN H., MANZOOR M., TINGHONG T., ARFAN M., IDREES M. Spatial Distribution and Population Structure of Himalayan Fir (Abies pindrow (Royle ex D. Don) Royle) in Moist Temperate Forests of the Kashmir Region. Forests. **14** (3), 482, **2023**.
52. SHAHEEN H., ATTIQUE A., RIAZ M.T., MANZOOR M., KHAN R.W.A., RIAZ M.T. From biodiversity hotspot to conservation hotspot: assessing distribution, population structure, associated flora and habitat geography of threatened Himalayan Yew in temperate forest ecosystems of Kashmir. Biodiversity and Conservation. 1, **2024**.
53. MAJEED S., ZAFAR M., AHMAD M., ZAFAR S., GHUFRAN A., AYOUB M., SULTANA S., YASEEN G., RAZA J. Morpho-palynological and anatomical studies in desert cacti (*Opuntia dillenii* and *Opuntia monacantha*) using light and scanning electron microscopy. Microscopy Research and Technique. **85** (8), 2801, **2022**.

54. HAMMER Ø., HARPER D.A., RYAN P.D. PAST: Paleontological statistics software package for education and data analysis. Palaeontologia electronica. **4** (1), 9, **2001**.
55. ALVES M.D.C., POZZA E.A. Scanning electron microscopy applied to seed-borne fungi examination. Microscopy Research and Technique. **72** (7), 482, **2009**.
56. BAI R.Z., ZHAO F., DREW B.T., XU G., CAI J., SHEN S.K., XIANG C.L. Seed morphology of *Hypericum* (Hypericaceae) in China and its taxonomic significance. Microscopy Research and Technique. **2023**.
57. AHMAD S., ZAFAR M., AHMAD M., ALI M.I., SULTANA S., RASHID N., BUTT M.A., SHAH S.N., OZDEMIR F.A., KUTLU M.A. Seed morphology using SEM techniques for identification of useful grasses in Dera Ghazi Khan, Pakistan. Microscopy Research and Technique. **83** (3), 249, **2020**.
58. DOSSOU S.S.K., LUO Z., WANG Z., ZHOU W., ZHOU R., ZHANG Y., LI D., LIU A., DOSSA K., YOU J. The dark pigment in the sesame (*Sesamum indicum* L.) seed coat: isolation, characterization, and its potential precursors. Frontiers in nutrition. **9**, 858673, **2022**.
59. ABEYSINGHE P.D. LM, Micromorphology, SEM of Leaf and Molecular Characterization of the Genus *Cinnamomum* (Family Lauraceae) Found in Sri Lanka: Taxonomic Significance. In book: Cutting Edge Research in Biology, Vol. 9, pp.17-28, BP International. **2023**.
60. DAS P., CHETTRI V., GHOSH S., GHOSH C. Micromorphological studies of the leaf and stem of Camellia sinensis (L.) Kuntze with reference to their taxonomic significance. Microscopy Research and Technique. **86** (4), 465, **2023**.
61. ZAMAN W., PARK S. Taxonomic significance of anatomical and micro-morphological characteristics of *Silene takesimensis* Uyeki & Sakata. Microscopy Research and Technique. **86** (11), 1542, **2023**.
62. JABEEN S., ZAFAR M., AHMAD M., ALI M.A., ELSHIKH M.S., MAKHKAMOV T., MAMARAKHIMOV O., YULDASHEV A., KHAYDAROV K., GAFFOROV Y. Micrometer insights into *Nepeta* genus: Pollen micromorphology unveiled. Micron. **177**, 103574, **2024**.
63. ALHOMAIDI E. Scanning electron microscopic exploration of intricate pollen morphology and antimicrobial potentials of gourd family. Microscopy Research and Technique. **2024**.
64. BHANDARI K.B., KUTAL D., KUNWAR R.M., JAN H.A., BUSSMANN R.W., PANIAGUA-ZAMBRANA N.Y. *Persicaria amphibia* (L.) Delarbre *Persicaria barbata* (L.) H. Hara *Persicaria capitata* (Buch.-Ham. ex D. Don) H. Gross *Persicaria hydropiper* (L.) Spach *Persicaria nepalensis* (Meisn.) H. Gross Polygonaceae. Ethnobotany of the Himalayas: Springer; p. 1477. **2021**.
65. VERLOOVE F., OTTO R., JANSSENS S., KIM S.-T. A Cryptic Invader of the Genus *Persicaria* (Polygonaceae) in La Palma and Gran Canaria (Spain, Canary Islands). Diversity. **13** (11), 551, **2021**.
66. YURTSEVA O. Ultrasculpture of achene surface in *Polygonum* section *Polygonum* (Polygonaceae) in Russia. Nordic Journal of Botany. **21** (5), 513, **2001**.
67. KANTACHOT C., CHANTARANOTHAI P. Achene morphology of *Polygonum* sl (Polygonaceae) in Thailand. Tropical natural history. **11** (1), 21, **2011**.
68. LEE T. Polygonaceae, Coloured flora of Korea. **1**, 245, **2003**.
69. ÖZKAN N.G., YAZLIK A. *Polygonum orientale* (*Persicaria orientalis*; Polygonaceae) in Turkey re-discovered after 73 years and considerations about its status. Eurasian Journal of Forest Science. **8** (3), 302, **2020**.
70. FATIMA A., ZAFAR M., AHMAD M., YASEEN G., SULTANA S., GULFRAZ M., KHAN A.M. Scanning electron microscopy as a tool for authentication of oil yielding seed. Microscopy Research and Technique. **81** (6), 624, **2018**.
71. JABEEN S., ZAFAR M., AHMAD M., ALTHOBAITI A.T., OZDEMIR F., KUTLU M., MAKHKAMOV T.K., SULTANA S., AMEEN M., MAJEED S. Ultra-sculpturing of seed morphotypes in selected species of genus Salvia L. and their taxonomic significance. Plant Biology. **2022**.
72. MUNIR R., KHAN B., DASTAGIR G., UZA N.U. Implication of scanning electron microscopy and light microscopy for morphology of some selected seed drugs: As a tool for authentication. Journal of Microscopy and Ultrastructure. **11** (1), 60, **2023**.
73. KANTACHOT C., CHANTARANOTHAI P. Achene Morphology of *Polygonum* sl, Polygonaceae). In Tropical Natural History. **11** (1), 21, **2011**.
74. ANJEN L., BOJIAN B., GRABOVSKAYA-BORODINA A., HONG S.-P., MCNEILL J., MOSYAKIN S., OHBA H., PARK C.-W. Polygonaceae. Flora of China. **5**, 277, **2003**.
75. WILSON K. Some widespread species of *Persicaria* (Polygonaceae) and their allies. Kew bulletin. 621, **1990**.
76. RAO J.P., SATISH K. *Persicaria perfoliata* (L.) H. Gross (Polygonaceae): A species new to Eastern Ghats of India. Tropical Plant Research. **3** (2), 249, **2016**.
77. WAHEED A., AHMAD M., GHUFRAN M.A., JABEEN A., OZDEMIR F.A., ZAFAR M., SULTANA S., SHAH M.A., MAJEED S., KHAN A.S. Implication of scanning electron microscopy in the seed morphology with special reference to three subfamilies of Fabaceae. Microscopy Research and Technique. **84** (9), 2176, **2021**.
78. MOSAFERI S., KESHAVARZI M. Micromorphological study of Polygonaceae tribes in Iran. Phytologia Balcanica. **17** (1), 89, **2011**.
79. STEVENS G.C. The elevational gradient in altitudinal range: an extension of Rapoport's latitudinal rule to altitude. The American Naturalist. **140** (6), 893, **1992**.
80. BRITTON N.L., BROWN A. An Illustrated Flora of the Northern United States: Canada and the British Possessions from Newfoundland to the Parallel of the Southern Boundary of Virginia, and from the Atlantic Ocean Westward to the 102d Meridian. C. Scribner's sons, 2d Edition. **1913**.
81. AHMAD M., UNIYAL S.K., BATISH D.R., SINGH H.P., JARYAN V., RATHEE S., SHARMA P., KOHLI R.K. Patterns of plant communities along vertical gradient in Dhauladhar Mountains in Lesser Himalayas in North-Western India. Science of The Total Environment. **716**, 136919, **2020**.
82. AHMED M.J., MURTAZA G., SHAHEEN H., HABIB T. Distribution pattern and associated flora of *Jurinea dolomiaea* in the western Himalayan highlands of Kashmir: an indicator endemic plant of alpine phytodiversity. Ecological Indicators. **116**, 106461, **2020**.
83. DAR J.A., SUNDARAPANDIAN S. Patterns of plant diversity in seven temperate forest types of Western Himalaya, India. Journal of Asia-Pacific Biodiversity. **9** (3), 280, **2016**.
84. BHATT I., NEGI V.S., RAWAL R. Promoting nature-based solution (NbS) through restoration of degraded landscapes in the Indian Himalayan Region. Nature-based Solutions for Resilient Ecosystems and Societies. 197, **2020**.

85. SHRESTHA A.B., WAKE C.P., MAYEWSKI P.A., DIBB J.E. Maximum temperature trends in the Himalaya and its vicinity: an analysis based on temperature records from Nepal for the period 1971–94. Journal of climate. **12** (9), 2775, **1999**.
86. SHAHEEN H., ULLAH Z., KHAN S.M., HARPER D.M. Species composition and community structure of western Himalayan moist temperate forests in Kashmir. Forest Ecology and Management. **278**, 138, **2012**.
87. RAHBEK C. The elevational gradient of species richness: a uniform pattern? Ecography. 200, **1995**.
88. GRYTNES J.A., VETAAS O.R. Species richness and altitude: a comparison between null models and interpolated plant species richness along the Himalayan altitudinal gradient, Nepal. The American Naturalist. **159** (3), 294, **2002**.
89. ANWAR S., KHAN S.M., AHMAD Z., ULLAH Z., AFZA R., ABBAS Z., ABDULLAH A., HUSSAIN M. Plant diversity and communities pattern with special emphasis on the indicator species of a dry temperate forest: A case study from Liakot area of the Hindu Kush mountains, Pakistan. Tropical Ecology. **64** (1), 37, **2023**.