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Morphological and histological analysis of six Lamiaceae species from the North-Western Indian Himalaya

Estudio morfológico e histológico de seis especies de Lamiaceae del Himalaya noroccidental de la India

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ABSTRACT

The present work presents a morpho-histological study of six Lamiaceae species, namely *Ajuga integrifolia* Buch.-Ham., *A. parviflora* Benth., *Pseudocaryopteris bicolor* (Roxb. ex Hardw.) P.D. Cantino, *Roylea cinerea* Baill., *Salvia cana* Wall. ex Benth. and *S. coccinea* Buc'hoz ex Etl., native to the North-Western Indian Himalaya region. Histological features have been investigated for the first time in *A. parviflora*, *A. integrifolia*, *P. bicolor*, and *S. cana*. *Salvia cana* and *S. coccinea* are reported to have highly reduced connectives, an unusual trait for the *Salvia* genus, which typically features elongated connectives forming a lever mechanism. Three types of stomata, anisocytic, anomocytic, and diacytic were observed among the studied species. Pollen grains identified within the taxa exhibit shapes ranging from prolate to sub-oblate and prolate-spheroidal, accompanied by hexa-zonocolpate aperture. Eleven different types of trichomes were identified in the studied species, categorized as non-glandular (5 types) and glandular (6 types). The new insights gleaned from this study contribute significantly to our comprehension and bridge notable lacunae in botanical understanding pertaining to these taxa, heretofore neglected.

Keywords — Histology; morphology; pollen viability; stomata; trichomes.

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RESUMEN

El presente trabajo realiza un estudio morfo-anatómico de seis especies de Lamiaceae de uso medicinal nativas de la región del Himalaya noroccidental de la India: *Ajuga integrifolia* Buch.-Ham., *A. parviflora* Benth., *Pseudocaryopteris bicolor* (Roxb. ex Hardw.) P.D. Cantino, *Roylea cinerea* Baill., *Salvia cana* Wall. ex Benth. y *S. coccinea* Buc'hoz ex Etl. Se presentan por primera vez las características histológicas de *A. parviflora*, *A. integrifolia*, *P. bicolor* y *S. cana*. Se describen estructuras únicas con conectivos reducidos en las anteras de *S. cana* y *S. coccinea*, un rasgo inusual para el género *Salvia*, que se caracteriza por presentar conectivos alargados formando un mecanismo de palanca. Entre las especies estudiadas se observaron estomas, anisocíticos, anomocíticos y diacíticos. Los granos de polen identificados en los taxones presentan formas que varían desde prolata a suboblata y prolata-esferoidal, con aperturas hexa-zonocolpadas. Se identificaron once tipos de tricomas, los cuales fueron clasificados como no glandulares (5 tipos) y glandulares (6 tipos). Los conocimientos generados en este estudio contribuyen significativamente a la comprensión botánica de estas especies ignoradas hasta el momento.

Keywords — Estomas; histología; morfología; tricomas; viabilidad polínica.

INTRODUCTION

Morpho-histological studies in plant families form a strong backbone in plant science. Phytologists have long been utilizing these observations to fill the knowledge gaps in the botanical literature of various taxa. Additionally, such studies are instrumental in investigating the evolutionary and breeding behaviors, as well as the ecological preferences of plants (Pallvi *et al.*, 2024). They serve as essential subjects for scientific inquiry, shedding light on how plants adapt to shifting environments, thus contributing to the sustainability of ecosystems, and ensuring the availability of vital resources. Notably, within the realm of medicinal plants, the Lamiaceae family has garnered significant attention from the scientific community. This heightened interest is due to the family's well-documented significance in various fields including culinary arts, volatile oil production, cosmetics, aromatics, medicinal and ethnopharmacological applications (Silva *et al.*, 2021; Avasiloaiei *et al.*, 2023; Moshari-Nasirkandi *et al.*, 2023; Ullah *et al.*, 2023).

Traditional healers and ethnobotanists prioritize the study of high-altitude medicinal plants belonging to the Lamiaceae family. Accurate identification and authentication of these plants are crucial for the development of effective crude drugs, facilitating pharmacological research and ensuring safe clinical utilization (Dhawle *et al.*, 2021).

Employing a morpho-histological approach stands as a conventional yet crucial method for the identification and authentication of medicinal plants. These studies provide diagnostic characters that enable the identification of different taxa at level of genera, species, and sub-species; facilitating a deeper understanding of taxonomy, systematics and evolution (Endress *et al.*, 2000; Butt *et al.*, 2021; Thi-Tran *et al.*,

2022). Leveraging the rich data provided by morphological and histological characteristics in plants significantly simplifies the taxonomic identification (Cantino 1992 a,b; Alosaimi, 2023).

Little effort has been expounded to unearth the morpho-histological and microscopic details of high-altitude Lamiaceae plants in the north-west Indian Himalayan region (Srivastava, 2012; Rubal, 2021). Consequently, this study has been undertaken to explore the region focusing on morpho-histological aspects, of six wild Lamiaceae species, namely, *Ajuga integrifolia* Buch.-Ham., *A. parviflora* Benth., *Pseudocaryopteris bicolor* (Roxb. ex Hardw.) P.D. Cantino, *Roylea cinerea* Baill., *Salvia cana* Wall. ex Benth., *and* Salvia *coccinea* Buc'hoz ex Etl., occurring in the vicinity of Baru-Sahib in Himachal Pradesh, situated within the northwestern Indian Himalayas. These species have been extensively documented in the literature regarding their ethno-botanical and medicinal attributes (Natarajan, 2000; Shanmugam, 2013; Yousaf *et al.*, 2018; Gulzar *et al.*, 2020), and are associated with a diverse array of bioactive phytochemical compounds (Pérez *et al.*, 2011; Gulzar *et al.*, 2018; Pundir & Mahindroo, 2019; Afzal *et al*., 2021; Dhiman, *et al*., 2021). Despite numerous taxonomical and ethnomedicinal records, these species have not received comprehensive study regarding their morphological and histological characteristics.

Hence, our research aims to investigate the morphological and histological characteristics of the above-mentioned species of Lamiaceae growing in the North-Western Indian Himalaya, with the objective of identifying diagnostic traits that facilitate accurate species identification providing insights for future taxonomic, evolutionary, and ecological research.

MATERIAL AND METHODS

Plant material

The plant material for this study comprised root, stems, leaves, and inflorescence, collected from six plant species growing naturally at high-altitudes in the North-west Himalayan region of district Sirmaur, Himachal Pradesh, India: *Ajuga integrifolia*, *A. parviflora*, *Pseudocaryopteris bicolor*, *Roylea cinerea*, *Salvia cana*, and *S. coccinea* (Fig. 1, Table 1). The work, conducted during 2020-2023, encompasses field collections, identification, morphological, and histological studies in these six plant species from different populations. A minimum of 4 individuals per population of the species were selected and collected during the flowering stage.

Field characteristics and plant data were meticulously documented during collection. Samples were photographed, and carefully dehydrated using absorbent paper for subsequent herbarium preparation. Preliminary identification was conducted based on live specimens, utilizing various literature sources focusing on the flora of the Western Himalayan region (Hooker, 1884; Mukerjee, 1940; Chowdhery & Wadhwa, 1984; Polunin & Stainton, 1984). Validation of the identification process was ensured by comparing the specimens with authenticated samples preserved at the Herbarium of Botanical Survey of India (BSI), Dehradun and from the Janaki Ammal digital Herbarium of the Indian Institute of Integrative Medicine (IIM),

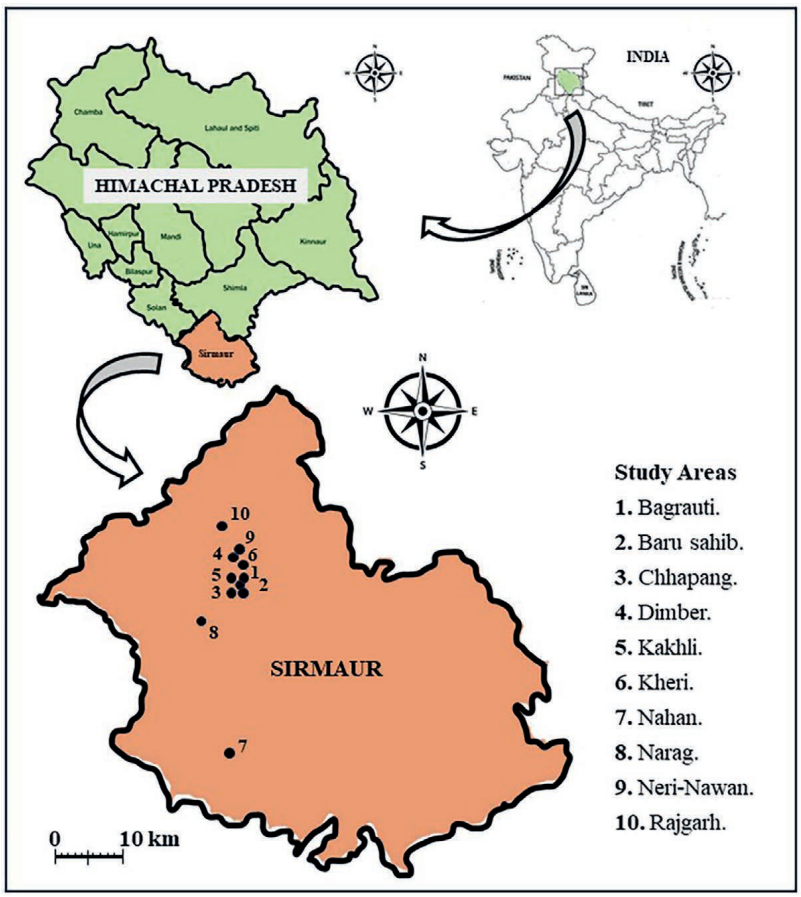

Fig. 1. Map of the study area for plant collection.
Fig. 1. Mapa del área de estudio y colecta de material vegetal.

Jammu. A voucher specimen of each of the taxa was archived at the Department of Botany, Eternal University (Himachal Pradesh) for future reference.

Part of the samples (fragments of roots, stems, leaves, petioles, and inflorescences) were fixed in FAA (formalin, acetic acid, 95% ethanol, distilled water, 10:5:50:35, v/v) until their use in histological techniques.

Macroscopic and microscopic observations

For the study of macroscopic measurements, a meter scale was utilized in the field, while microscopic measurements were performed using a calibrated compound microscope (Coslab-VN-5 Brightfield Microscope) and ocular micrometer, enabling measurements in micrometers (μm). Free-hand transverse sections (TS) were prepared on the samples previously fixed in FAA solution, and histological examinations were conducted at 20X and 40X magnification. Roots were sectioned at 5 cm from the collar, while the stem (or shoot) was sectioned at the level of the 4th internode of an exposed branch in a north orientation for *R. cinerea*, and in the middle part of the main axis for the remaining species. Fully developed leaves in good phytosanitary condition and the petiole were selected and sectioned at their

Table1. Studied species, accession number, location, population sites and their respective GPS co-ordinates.

Tabla 1. Especies estudiadas, número de accesión, localización, sitio de colección y sus respectivas coordenadas GPS.

Species (Accession N°)	Location		Population sites (Altitude m asl)	GPS coordinates*	
	Locality	Google ID name		North	East
A. parviflora (PKAPBS01-02)	Baru sahib	Baru sahib	Baru-1 (1076m)	30.754	77.293
	Kakhli	Lana Machher	Kakh-1 (1050m)	30.756	77.298
	Rajgarh	Dharoti	Raj-1 (1350m)	30.753	77.299
	Dimber	Dimber	Dimr-1 (1020m)	30.794	77.098
			Dimr-2 (960m)	30.794	77.098
A. integrifolia (PKABBS01-02)	Baru sahib	Baru sahib	Baru-2 (1076m)	30.753	77.290
	Kheri	Lana Marg	Khri-1 (800m)	30.773	77.299
	Chhapang	Chhapang	Chap-1 (960m)	30.739	77.313
			Chap-2 (850m)	30.738	77.311
	Nahan	Gugal	Nah-1 (1,400m)	30.685	77.357
P. bicolor (PKPBBS01-02)	Kakhli	Lana Machher	Kakh-2 (1,550m)	30.753	77.299
	Baru sahib	Baru sahib	Baru-3 (1,010m)	30.754	77.293
			Baru-4 (970m)	30.756	77.298
			Baru-5 (1,076m)	30.752	77.290
	Bagrauti	Bagrauti	Bag-1 (990m)	30.753	77.290
	Chhapang	Chhapang	Chap-3 (850m)	30.759	77.297
	Rajgarh	Dharoti	Raj-2 (1,350m)	30.739	77.313
R. cinerea (PKRCBS01-02)	Baru sahib	Baru sahib	Baru-5 (1,076m)	30.752	77.290
	Kakhli	Lana Machher	Kakh-3 (800m)	30.753	77.290
	Neri-Nawan	Neri-Nawan	Neri-1 (970m)	30.759	77.299
	Chhapang	Chhapang	Chap-4 (850m)	30.759	77.293
S. coccinea (PKSCBS01-02)	Dimber	Dimber	Dimbr-1 (1020m)	30.794	77.098
			Dimbr-2 (960m)	30.794	77.098
	Narag	Narag	Nar-1 (980m)	30.754	30.754
	Kheri	Lana Marg	Khri-1 (800m)	30.773	77.299
S. cana (PKSCBS01-02)	Rajgarh	Dharoti	Raj-1 (1,350m)	30.753	77.299
	Baru sahib	Baru sahib	Baru-3 (1,010m)	30.754	77.293

* Longitude and latitude in decimal degrees.

middle region for observation. The obtained sections were stained by employing safranin and fast-green double-stained technique (Johansen, 1940) and mounting it in gelatin-glycerin. Stomata were observed through semi-permanent slides by peeling-off the leaf epidermis, which were stained with 1% safranin, and mounted it in gelatin-glycerin (Mozo *et al*., 2021).

Field and morphological photographs were captured using a Canon Power-Shot A530, while histological photographs were captured using Co-axial trinocular KCM-26 and Zoom stereo ZSM-115 microscopes. Beentje & Williamson (2010) and Lawrence (1955) were consulted for specific terminology.

Pollen study involved fixing of young floral buds in Carnoy's fixative (ethanol: chloroform: glacial acetic acid, 6:3:1, v/v) which were subsequently preserved in 70% ethanol at 4°C. Anthers were squashed from the buds in glycerol: acetocarmine (1:1, v/v) to observe the pollen. Pollen viability (PV) % was calculated as $Vp / N \times 100$ (where, '*Vp*' = numbers of viable pollen, '*N*' = Total number of pollens observed). Viable pollens, appearing as purplish-pink, were photographed, counted, and recorded (Saggoo & Bir, 1983).

RESULTS

Morphological and anatomical observations were made for the six selected species growing naturally in the mountain regions of the North-Western Himalaya at the Sirmaur district of Himachal Pradesh, India.

Ajuga integrifolia

This species occurred as a short-lived perennial herb thriving in moist and shady environments of Baru Sahib, Kheri, Chhapang, and Nahan areas (Fig. 2A). The plant displayed an unbranched structure with a highly tomentose texture. The roots were adventitious, fleshy, and fibrous, exhibiting a cream-white coloration, with a rough, glabrous surface. The stem was highly reduced forming basal nodal stalk from which several inflorescence-forming offshoots emerged. These offshoots typically were unbranched, herbaceous pubescent, presenting a quadrangular to semi-quadrangular shape and light green color. The leaves were decussately arranged, oblanceolate, sub-sessile with a sheathing base, crenate margin, and reticulate veins. The upper-adaxial surface of the leaves appeared greenish compared to lower-abaxial surface, and covered with white-colored trichomes, which were more prominent on the lower-abaxial side (Fig. 2B-C). In the studied area, this species blooms twice in a year in February-March and August-September. The observed inflorescence structure was identified as a verticillaster, with bracteates flowers (two green leafy bracts per flower), perfect bisexual, zygomorphic and hypogynous. The gamosepalous calyx consists of five green sepals covered with dense white trichomes. The corolla was light purple, bi-lipped, with the upper lip, composed of two erect petals, smaller than the lower one; while the lower lip consisted of three spreading petals (Fig. 2D). The androecium consists of four epipetalous didynamous stamens (2+2), with cream white epipetalous filaments and dark red-brown, bean-shaped, dorsifixed anthers (Fig. 2E). Anthers contained sub-oblate, hexa-zonocolpate pollen grains (Fig. 2F), which exhibited 100% viability. The gynoecium presented cream-white gynobasic style, equal in length to the stamen's filament, bifid stigmas with nearly equal fids (Fig. 2G). The Ovary was superior, bi-carpellar, syncarpous and deeply four-lobed (Fig. 2H). Fruits were dry with four reticulated mericarps or nutlets (Fig. 2I) each one containing a brown oval seed.

The root TS displayed a nearly circular outline with secondary growth and wavy margin attributed to a fractured periderm layer and outer dead cork cells (Fig. 2J). Was characterized by the presence of a periderm, comprising 5-6 layers of rectangular cork cells followed by phellogen and a multi-layered (9-13 layers) phelloderm, consisting of rectangular to polygonal parenchyma cells. A well-developed ring of phloem with parenchyma rays was evidenced. Multilayered vascular cambium forms centripetally secondary xylem with pronounced parenchyma rays and diffuse porosity. Finally, a reduced pith was observed.

The shoot section showed early secondary growth with sub-quadrangular outline (Fig. 2K). The epidermis was single-layered, consisting of cuticularized isodiametric cells, with sparsely distributed glandular and non-glandular trichomes.

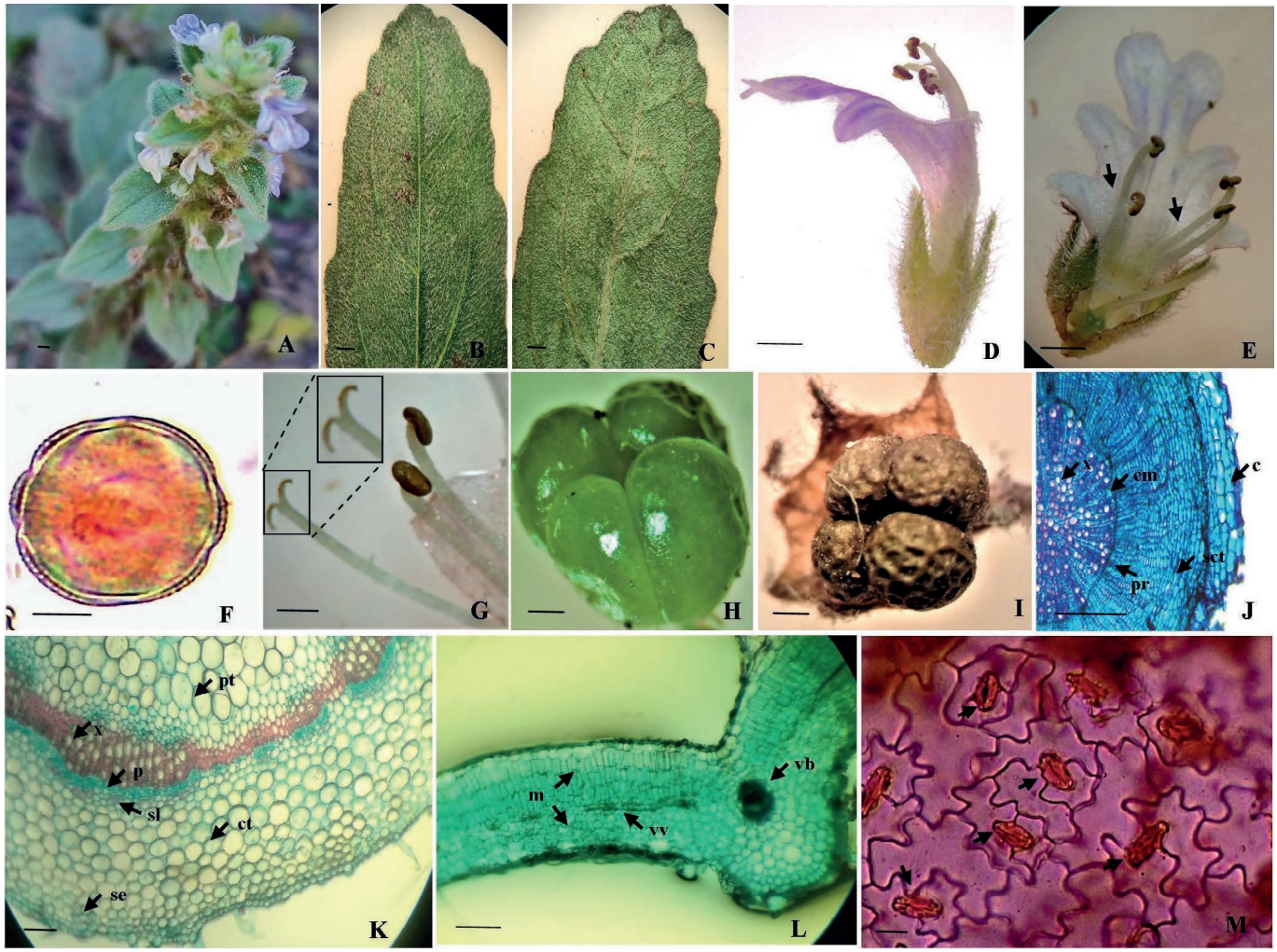

Fig. 2. *Ajuga integrifolia*. A) Natural habit of the species. B-C) Leaf in upper-adaxial and lower-abaxial view, respectively. D) Flower with bi-lipped corolla. E) Dissected corolla showing stamens (arrow). F) Viable pollen grain. G) A bifid stigma. H) Four-lobed ovary. I) Dried fruit with 4 mericarps or nutlets. J) Root TS root. K) Stem TS. L) Leaf TS. M) Leaf surface view with anisocytic stomata (arrow). References: c-cork, cm-cambium, ct-cortex, m-mesophyll, p-phloem, pr-phloem rays, pt-pith, sct-secondary cortex, se-subepidermal collenchyma cells, sl-sclerenchyma, vb-vascular bundle, vv-vascular veins/strands, x-xylem. Scale = A-E, G-I, 1 mm; F, 10 μm; J, 200 μm; K-L, 100 μm; M, 20 μm.

Fig. 2. *Ajuga integrifolia*. A) Hábito natural de la especie. B-C) Vista superficial de la hoja, adaxial y abaxial, respectivamente D) Flor de corolla bi-labiada. E) Corola disectada mostrando los estambres (flecha). F) Grano de polen viable. G) Estigma bífido. H) Ovario tetra lobado. I) Fruto seco con 4 mericarpos o núculas. J) Raíz en sección transversal (ST). K) Tallo en ST. L) Hoja en ST. M) Vista superficial de la epidermis foliar con estomas anisocíticos (flecha). Referencias: c, suber; cm, cambium; ct, cortex; m, mesófilo; p, floema; pr, radios floemáticos; pt, medula; sct, cortex secundario; se, colénquima subepidérmico; sl, esclerénquima; vb, haz vascular; vv, venacion; x, xilema. Escalas = A-E, G-I, 1 mm; F, 10 μm; J, 200 μm; K-L, 100 μm; M, 20 μm.

A prominent subepidermal collenchyma was observed, followed by a multilayered parenchyma cortex. The central cylinder was delimited by perivascular sclerenchyma caps, followed by a continuous ring of phloem, multilayered cambium and secondary xylem. The pith was central and broad, formed by polygonal to oval shaped parenchyma cells.

The leaf TS exhibited one layered epidermis consisting of compactly arranged rectangular to nearly oval shaped cells, covered by a thick cuticle. Both epidermal surfaces showed glandular and non-glandular trichomes sparsely interspersed throughout (Fig. 2L). The mesophyll was dorsiventral with a three to four layered palisade chlorenchyma and spongy parenchyma compactly arranged, interrupted by collateral vascular bundles (VB). The middle vein presented convex-concave contour and a unique collateral VB with collenchyma reinforcements toward the phloem pole.

In surface view, the leaf was amphistomatic featuring polygonal epidermal cells with anticlinal lobulated walls and anisocytic stomata on both epidermal surfaces (Fig. 2M). A higher density of stomata was evidenced on the abaxial surface.

Ajuga parviflora

Ajuga parviflora is an ephemeral perennial plant of herbaceous, unbranched, densely tomentose appearance. It primarily inhabited shaded environments from Baru Sahib, Kakhli, Rajgarh, and Dimber locations (Fig. 3A). The root system was adventitious, fleshy, fibrous, cream white, unbranched to occasionally branched, rough-textured, and devoid of trichomes. The stems were short stout and woody, bearing numerous inflorescences which arises as offshoots. Throughout most of the growing season, the plants maintain a leafy appearance without the presence of ascending or sub-erect offshoots. The species was characterized by presenting foliar dimorphism with basal and cauline leaves. Basal leaves forms rosettes; they were velvety, pilose, and sessile, featuring a spathulate blade with a cuneate base, nearly obtuse apex and entire margins. They exhibited a prominent mid-rib and reticulate vein-framework (Fig. 3B-C) and occasionally dark brown adventitious roots at the lower-abaxial side (Fig. 3D). Cauline leaves were smaller than their basal counterparts. They exhibited an acropetal arrangement on the offshoots with a decussate pattern. Were oblanceolate with an obtuse apex, cuneate base, crenate margins, and reticulate venation. This species blooms in February-March. The flowers were organized in verticillaster inflorescences (Fig. 3E) with an average 11-17 flowers arranged in a whorl at short intervals. The flowers were bracteate, perfect, bisexual, complete, zygomorphic, and hypogynous. The bracts, two in number, exhibited purplish-green, pilose, ovate in shape with an acute apex, entire margin, and a prominent midrib. The external abaxial surface of the bract was pilose and more purplish compared to the inner adaxial surface (Fig. 3F-G). The calyx showed five green, gamosepalous sepals with a campanulate morphology, featured teeth halfway down the tube and dense white trichomes at the external abaxial side (Fig. 3H-I). Interestingly, two were smaller, one longer, and the remaining two equal in size, making the sub-equal calyx lobes. Each sepal displayed a distinct white median rib. The corolla was light purple or white purple, bilipped, gamopetalous; it consisted of five fused petals forming sub-equal lip-lobes and a thin- slender tube equally or slightly longer, and elevated to a higher position than the calyx (Fig. 3J). The corolla upper lip was made up of two erect petals smaller than the ones forming the lower lip, while up to three spread-

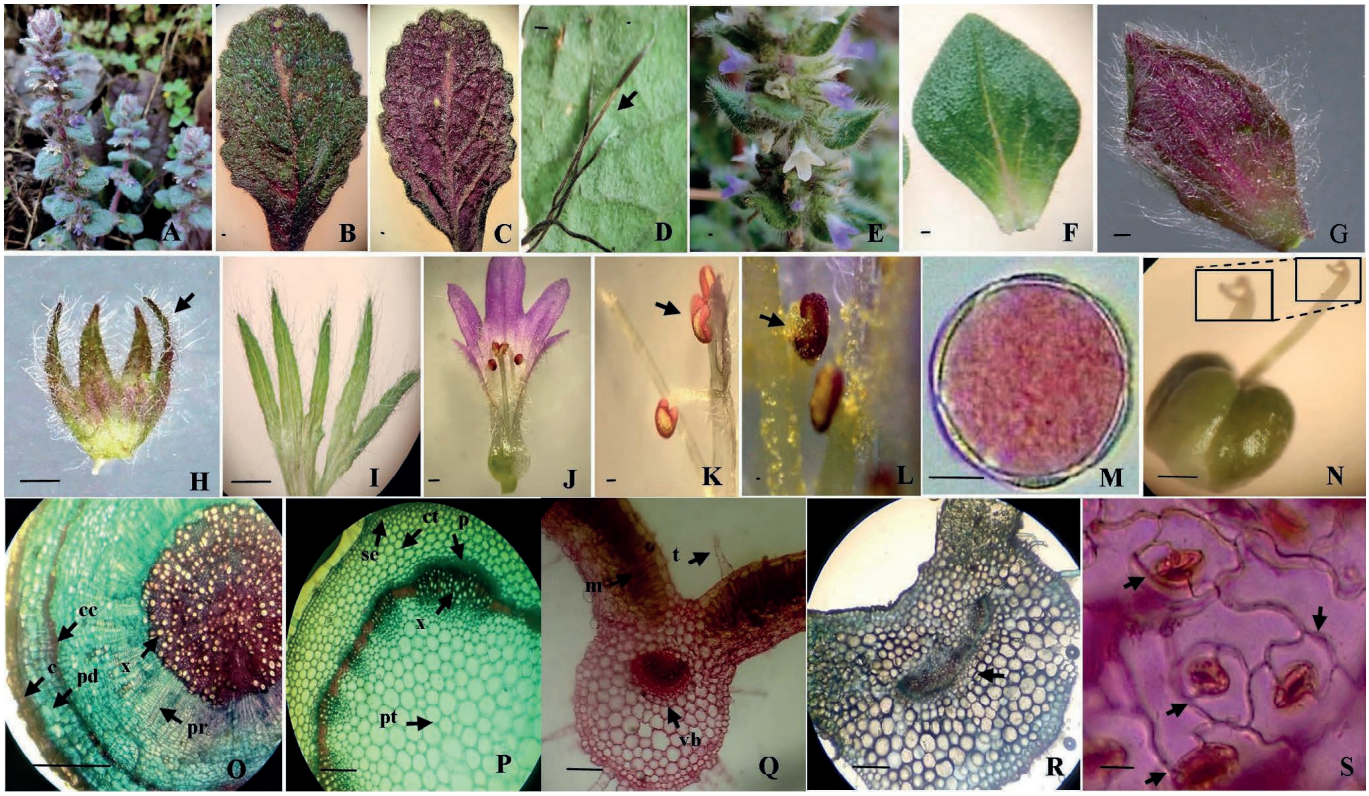

Fig. 3. *Ajuga parviflora*. A) Natural habit of the species. B-C) Cauline leaf in upper-adaxial and lower-abaxial view, respectively. D) Basal leaf with adventitious roots on its lower-abaxial surface (arrow). E) Inflorescence, F-G) Floral bract in upper-adaxial and lower-abaxial view, respectively. H-I) Calyx in outer (lower)-abaxial and inner (upper)-adaxial view, respectively. J) Dissected bi-lipped corolla with sub-equal lip lobes and stamens. K-L) Bean-shaped anthers with bright yellow pollen (arrow). M) Viable pollen grain. N) Four lobed ovary with style and bifid stigma. O) Root TS. P) Offshoot TS. Q) Leaf lamina TS. R) Leaf mid-rib TS. S) Diacytic stomata. References: c, cork; cc, cork cambium; ct, cortex; m, mesophyll; p, phloem; pd, periderm; pr, phloem rays; pt, pith; se, subepidermal collenchyma cells; t, trichome; vb, vascular bundle; x, xylem. Scales= A-J, 1 mm; K-L, N, 0.5 mm; M, 10 μm; O, 200 μm; P, 100 μm; Q-R, 50 μm; S, 20 μm.

Fig. 3. *Ajuga parviflora*. A) Hábito natural de la especie. B-C) Hoja caulinar en vista adaxial y abaxial, respectivamente. D) Hoja basal con raíces adventicias en su superficie abaxial (flecha). E) Inflorescencia. F-G) Bráctea floral, vista adaxial y abaxial, respectivamente. H-I) Cáliz, superficie externa (abaxial) e interior (adaxial). J) Corola bilabiada disectada con lóbulos labiales sub-iguales y estambres. K-L) Anteras arriñonadas con granos de polen amarillo brillante (flecha). M) Grano de polen viable. N) Ovario tetra lobado con estigma bífido. O) raíz en ST. P) Tallos aéreos en ST. Q) Lamina foliar en ST. R) Nervio medio de la hoja en ST. S) Estoma diacítico. Referencias: c, suber; cc, cambium; ct, cortex; m, mesofilo; p, floema; pd, peridermis; pr, radios floemáticos; pt, medula; se, colénquima subepidérmico; t, tricoma; vb, haz vascular; x, xilema. Escalas= A-J, 1 mm; K-L, N, 0.5 mm; M, 10 μm; O, 200 μm; P, 100 μm; Q-R, 50 μm; S, 20 μm.

ing long petals formed the lower lip. The petals showed soft white trichomes and a dark purple median rib. The tooth-tips of the petals were little dark purple when compared to the rest of the lamina. The androecium consisted of didynamous (2+2) epipetalous stamens with cream-white filaments bearing dorsifixed, purplish-brown, bean-shaped anthers (Fig. 3K). The pollen grains were 100% viable, very small, radially symmetrical, sub-prolate to spheroidal, hexa-zonocolpate and displayed a dusty, bright yellow color (Fig. 3L-M). The gynoecium featured a long cream-white style

equal in length to stamens, culminating in a bifid stigma with unequal fids, the large fid was pointed while small one was capitate. The ovary was superior, bicarpellar, syncarpous and four-lobed (Fig. 3N).

The section of the root exhibited secondary growth and circular shape with a slightly wavy segment outline attributed to a fractured periderm and outer dead cork cells (Fig. 3O). The outermost cork comprised 5-6 layers of rectangular cells, followed by a layer of phellogen. The phelloderm and secondary cortex integrated smoothly without any visible boundary between them, and together consist of 9-13 layers of polygonal parenchyma cells. A perivascular ring of lignified cells surrounded the vascular cylinder, phloem layers with traversed parenchyma rays were followed by vascular cambium and secondary xylem. The pith was central, highly reduced and lignified.

The offshoot TS presented early secondary growth with a sub-squarish outline, characterized by a thick cuticle (Fig. 3P) and epidermis composed of a single layer of compactly arranged isodiametric cells with glandular and non-glandular trichomes. Subepidermal collenchyma reinforcements were notably at the corners of the stem section and followed by a multilayered cortex containing chlorenchyma and parenchyma cells. Secondary phloem, vascular cambium and xylem formed concentric continuous rings, exhibiting increased growth towards the angular corners. The central pith consisted of polygonal to rounded parenchyma cells of larger dimensions than those observed in the cortex.

Leaf TS at the primary and secondary veins presented a slightly concave-convex outline (Fig. 3 Q-R). Both, lower and upper epidermis were uniseriated with thick cuticles and glandular as well as non-glandular trichomes. Trichomes were densely arranged on the lower-abaxial surface. The mesophyll was dorsiventral with compact palisade and spongy parenchyma interrupted by collateral VB. The mid vein showed a unique collateral VB arranged in a crescent shape manner (Fig. 3R).

In surface view, the epidermal cells were polygonal with lobulated anticlinal walls. Diacytic stomata were present on both leaf surfaces (Fig. 3S), more abundantly in the abaxial surface in comparison to the adaxial surface.

Pseudocaryopteris bicolor

The taxon is a thornless perennial shrub growing in the area of Kakhli, Baru sahib, Bagrauti, Chhapang and Rajgarh and thrives in partially shaded environments, reaching a lofty height of 2-6 meters during its summer (March-May) bloom (Table 1, Fig. 4A). The vegetative taproot in the species produces multiple branched stems. Young branches were green, soft, sub-quadrangular, and later turn earthy-brown, cylindrical and woody with thick papery bark. The leaves were simple, non-aromatic, petiolated and arranged in a decussate manner. The leaf lamina was ovate-lanceolate to nearly lanceolate, with long cuneate base, acuminate apex, serrate to serrulate margin, pubescent surface, and reticulate venations (Fig. 4B-C). Inflorescence was usually terminal-spike on young branches, while on 3/4 part of older branches they appeared as panicle-like thyrse (Fig. 4D). Flowers were complete, bisexual,

pentamerous, and with leafy bracts. The calyx consisted of 5 fused sepals which were present with single prominent mid-vein (Fig. 4E). The corolla presented by 5 fused petals forming bi-lipped structure, deeply cleft with nearly equal lobes, were light purplish and pubescent outside (Fig. 4F). Four didynamous stamens with long exserted filaments with dorsifixed bi-lobed anthers were recorded. The filaments were nearly equal in length, cream-white, while anthers were bluish-purple (Fig. 4G). Pollen grains were sub-prolate to prolate-spheroidal shaped and observed with hexa-zonocolpate aperture and 100% viability (Fig. 4H). Style was cream white, equal in height to filamented-anthers, with bifid stigma with nearly equal fids (Fig. 4I). Ovary obovate, nearly four lobed, 2/5 part pilose at the base (Fig. 4J), at maturity it forms a septicidal semi-fleshy capsular fruit that seems to be open or splits through four septa and produce four dry one seeded nutlet (Fig. 4K).

Root TS was circular in outline with outer fractured periderm (Fig. 4L). Secondary growth was observed with the presence of periderm, and cork with a few layered phelloderm. The cortical parenchyma region was reduced due to prominently increased wood region. The central region of the section presented scattered vascular tissues throughout, with rows of xylem and medullary rays.

Stem TS was sub-quadrangular shaped and wavy in outline (Fig. 4M), possessed thick cuticularized epidermis with trichomes. The epidermis was succeeded by sub-epidermal collenchyma at the corners, whereas in other regions, it was followed by chlorenchyma cortical cells, observed along with intermittent sclerenchyma patches. Two to four rowed cambium cells delimited the inner radially arranged end-arch xylem from the outer multi (14-21) layered phloem. Medullary rays exhibit radial orientation, traversing through the ring of xylem cells. The pith was prominent at the center of the section with compactly arranged polygonal parenchyma cells.

Leaf TS was wavy in outline with a one layered cuticularized epidermis (Fig. 4N). The epidermis featured stomata and trichomes dispersed throughout on both sides. Below the epidermis, sub-epidermal collenchyma, ground parenchyma and a unique, collateral, open semi-oval shaped VB were observed in the mid-rib region. They were. The mesophyll was dorsiventral with the layeres of palisade and spongy parenchyma partially distinguishable. Leaves were hypostomatic, with anomocytic stomata on the abaxial leaf surface (Fig. 4O).

Petiole TS was wavy and nearly 'C'- shaped, with a concave groove representing its upper adaxial surface (Fig. 4P). With a cuticularized one layered epidermis bearing non-glandular trichomes, followed by 5-7 layers of sub-epidermal collenchyma and ground polygonal parenchyma cells with two strings of dense chlorenchyma cells towards what appeared to be two reduced wings. The VB was horn shaped, collateral and closed.

Roylea cinerea

This taxon was an arborescent, perennial shrub, growing in partially shaded conditions in collection areas Baru-Sahib, Kakhli, Neri-Nawan and Chhapang (Table 1, Fig. 5A). Stem was highly branched, erect, pilose, woody-brown in old branches to

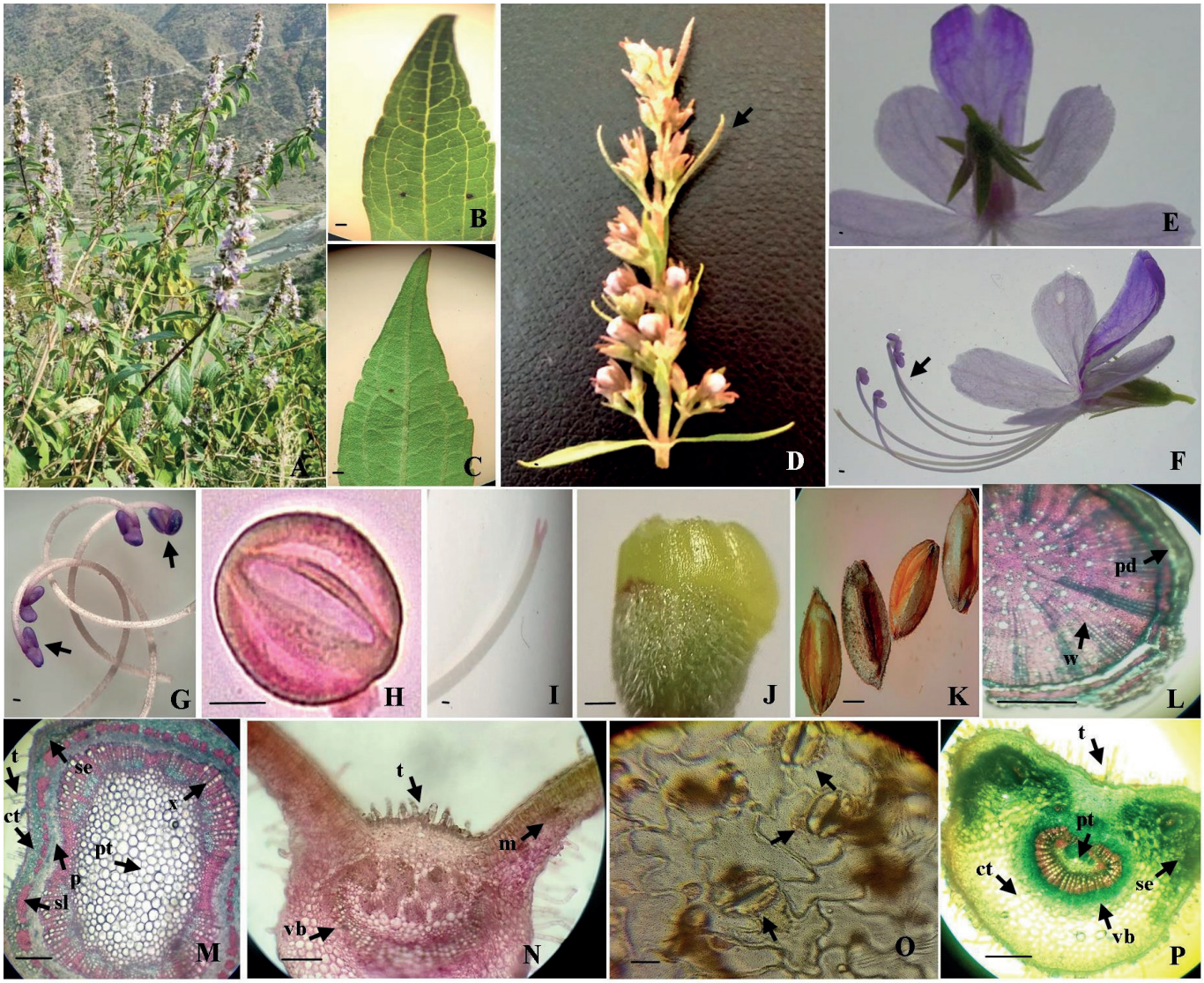

Fig. 4. *Pseudocaryopteris bicolor*. A) Natural habit of the species. B-C) Leaf in upper-adaxial and lower-abaxial view, respectively. D) Inflorescence with leafy bracts (arrow). E) Calyx in lower (outer)-abaxial view. F) Bi-lipped corolla with stamens (arrow). G) Dorsifixed bi-lobed purple anthers (arrow). H) Viable pollen. I) Style with bi-fid stigma. J) Tetra lobed ovary. K) Seeds. L) Root TS. M) Stem TS. N) Leaf lamina TS with mid-rib region. O) Anomocytic stomata (arrow). P) Petiole TS. References: ct, cortex; m, mesophyll; pd, periderm; p, phloem; pt, pith; se, subepidermal collenchyma cells; sl, sclerenchyma; t, trichome; vb, vascular bundle; w, wood region; x, ylem. Scale = A-G, I-K, 1 mm; H, 10 μm; L-M, 200 μm; N, P, 50 μm; O, 20 μm.

Fig. 4. . *Pseudocaryopteris bicolor*. A) Hábito natural de la especie. B-C) Vista superficial de la hoja, adaxial y abaxial, respectivamente. D) Inflorescencia con brácteas foliosas (flecha). E) Cáliz, superficie externa (abaxial). F) Corola bilabiada con estambres (flecha). G) Anteras purpuras dorsifijas (flecha). H) Polen viable. I) Estilo con estigma bífido. J) Ovario tetra lobado. K) Semillas. L) Raíz en ST. M) Tallo en ST. N) Lámina foliar, ST en la región del nervio medio. O) Estoma anomocítico (flecha). P) Pecíolo TS. Referencias: ct, cortex; m, mesófilo; pd, peridermis; p, floema; pt, medula; se, colenquima subepidérmico; sl, esclerenquima; t, tricoma; vb, haz vascular; w, madera; x, xilema. Escalas = A-G, I-K, 1 mm; H, 10 μm; L-M, 200 μm; N, P, 50 μm; O, 20 μm.

green and soft in young ones. Branches were long and of variable lengths, spreading, quadrangular at young stages, opposite, ascending, and herbaceous, densely pilose with greyish white trichomes all over its surface. Old stems were cylindrical, their quadrangular shape seems to be lost with aging, hard, woody, brown, barked, fibrous and glabrous. Leaves were petiolate and decussated. The lamina was dorsiventrally flat with acute apex and broad bases, dark green adaxially and bright green abaxially, ovate with reticulate venation and crenate margin (Fig. 5B-C). Flowers appeared during August-September (Fig. 5D). Six axillary flowers arranged in loose verticils and formed a nodal whorl in a floral spike. Flowers were zygomorphic awl-shaped, bracteate, light purplish-white, bisexual, pentamerous and hypogynous. Bell-shaped calyx consisted of five equally pale-green sepals which were persistent; a gamosepalous pilose tube presented 10-12 ridges (Fig. 5E). Each sepal lobe was obo-elliptic and reticulately veined. The corolla was bi-lipped, pilose-villous, nearly equal in length to calyx, the upper lip was smaller than the lower lip and comprises of two equal lobes (Fig. 5F). The lower lip presented purplish streaks and comprised three unequal (lateral lobes equal while middle larger) lobes (Fig. 5G). A total of four (2+2) stamens determined the didynamous condition of the flower. Filaments were cream-white, puberulent, and epipetalous (Fig. 5H). Anthers were deep brown, bi-lobed (each with a minor groove), dorsifixed, and introrse (Fig. 5I). The pollen grains were prolate-spheroidal, hexa-zonocolpate, and showed 100% viability (Fig. 5J). The gynoecium was bicarpellar, syncarpous with superior tetra-lobed ovary (Fig. 5K) with a simple style, long, glabrous, shiny, bearing a slightly bi-fid stigma (Fig. 5I). The fruit was oval (with narrow base), flat tipped, brown, and pilose with nutlet seeds (Fig. 5L).

Root TS in was circular in outline (Fig. 5M) with secondary growth. A single layered epidermis was replaced by the periderm and a reduced cortex indistinguishable from phloem. At the vascular cylinder the xylem interspersed with radial rows of medullary rays.

The young stem of this species was herbaceous, with quadrangular outline in TS (Fig. 5N). The epidermis features non-glandular trichomes, simple, erect, uni or bicellular, with a multicellular swollen base. At the corners of the section, subepidermal thick-walled collenchyma was present, followed by a multilayered chlorenchyma cortex. A single layered endodermoid strand delimited the central cylinder followed by strands of sclerenchyma which was further followed by a multilayered phloem, cambium and secondary xylem. Finally, the pith broad, consisted of thick-walled polygonal parenchyma cells.

Leaf TS was wavy in outline, with midrib region exhibiting a kidney-shaped, with a slightly concave-convex outline (Fig. 5O). Both adaxial and abaxial surfaces were covered with simple or multicellular, erect, glandular and sometimes non-glandular trichomes. Trichome density was higher on the abaxial surface compared to the adaxial surface. Well-developed substomatal chambers were present in the abaxial epidermis. The mesophyll was dorsiventral. The mid-rib section showed ‘epsilon (□)’ or sometime ‘C-shaped’ collateral, open VB. In superficial view, the epidermis showed isodiametric cells with sinuous to lobulated anticlinal walls and dicytic type of stomata on both surfaces (Fig. 5P).

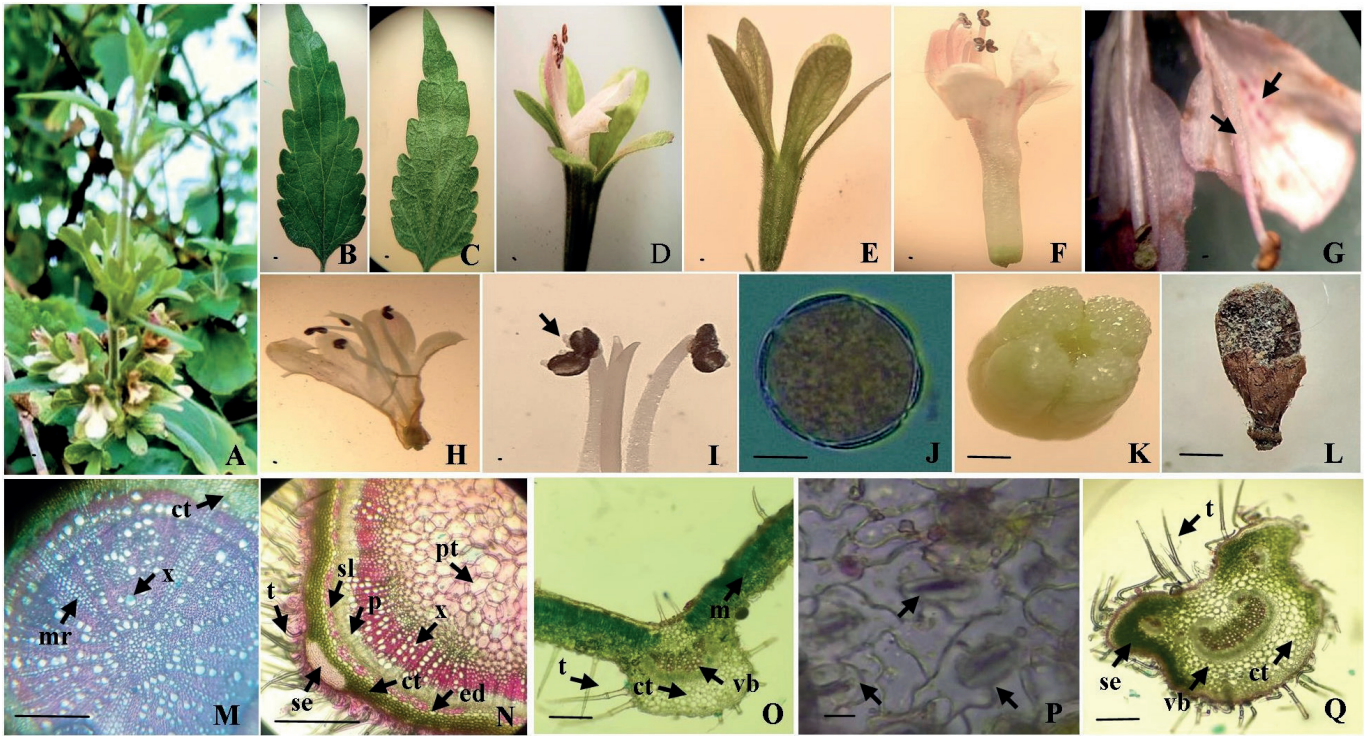

Fig. 5. *Roylea cinerea*. A) Species natural habit. B-C) Leaf in upper-adaxial and lower-abaxial view, respectively. D) Flower. E) Dissected calyx. F) Dissected bi-lipped corolla with removed calyx. G) Lower lip with purple streaks in a dissected corolla (arrow). H) Dissected corolla showing stamens. I) Bi-lobed anthers (arrow). J) Viable pollen. K) Four lobed ovary. L) Flat-tipped pilose nutlet seed. M) Root TS. N) Stem TS. O) Leaf TS with midrib region. P) Diacytic stomata. Q) Petiole TS. References: ct, cortex; ed, endodermis; m, mesophyll; mr, medullary rays; p, phloem; pt, pith; se, subepidermal collenchyma cells; sl, sclerenchyma; t, trichome; vb,,vascular bundle; x, xylem. Scale = A-I, K-L, 1 mm; J, 10 μm; M-N, 200 μm; O, Q, 50 μm; P, 20 μm.

Fig. 5. *Roylea cinerea*. A) Hábito natural de la especie. B-C) Vista superficial de la hoja, adaxial y abaxial, respectivamente. D) Flor. E) Cáliz disectado. F) Corola bilabiada disectada luego de la remoción del cáliz. G) Labio inferior de la corola con líneas purpura (flecha). H) Corola disectada con estambres expuestos. I) Anteras (flecha). J) Polen viable. K) Ovario tetra lobado. L) Núcula pilosa de ápice plano. M) Raíz en ST. N) Tallo en ST. O) ST de hoja en la región del nervio medio. P) Estoma diacítico. Q) Pecíolo en ST. Referencias: ct, cortex; ed, endodermis; m, mesofilo; mr, radios medulares; p, floema; pt, medula; se, colénquima subepidérmico; sl, esclerenquima; t, tricoma; vb, haz vascular; x, xilema. Escalas = A-I, K-L, 1 mm; J, 10 μm; M-N, 200 μm; O, Q, 50 μm; P, 20 μm.

Petiole TS was inverted omega, slightly winged in outline (Fig. 5Q), with cuticularized epidermis and non-glandular multi-celled trichomes. At wings, subepidermal collenchyma followed by multilayered chlorenchyma and a collateral smaller oval-shaped VB were noticed. The central VB was collateral and closed.

Salvia cana

Taxon was a small herbaceous perennial plant, with woody stalked tap root system (Fig. 6A), sparsely observed in a partial-shade and moisture sites at the Rajgarh and Baru Sahib regions of the study area (Table 1). Stem-shoots were many, erect, unbranched, non-woody, squarish, green (light purple to purple coloring along the

edges) and pubescent. Leaves were sub-radical and basal, oblong to oblanceolate, sessile, lamina was abaxially woolly with prominent mid-ribs and lateral veins, margins nearly crenulate with sub-acute apex (Fig. 6B-C). This species blossoms during April-May in the study area (Fig. 6D). The flowers were accompanied by two opposite leafy sessile bracts, pilose, slightly smaller, sometimes equal in length to the calyx, acuminately cordate, green with purple margins at the apex (Fig. 6D-E). Stalkless flowers were observed in separated whorls in the axils of bracts on stem-shoots, forming spikes. At a time, each whorl possessed only 3-4 bloomed flowers along with persistent old dried ones. Calyx was campanulate, bi-lipped with 5 lobed spine-tipped tooths (3/2), externally with prominent veins, sticky trichomes, and purple towards the apex (Fig. 6F).The violet to blue corolla was tubular-campanulate, 5-lobed (2/3), the upper lip was formed by two fused equal lobes (1+1) forming an arc-beak, and a lower lip made of three fused lobes (2+1), with middle lob larger than the lateral ones (Fig. 6G). Two long exserted stamens with glistening white filaments were observed with dorsifixed single-lobed brown anthers (Fig. 6H). The anther connective in both stamens were highly reduced and did not exhibit any lever-like construction. The pollen grains were radially symmetrical, dusty colored, prolate shaped with reticulate ornamentation and recorded with 100% viability (Fig. 6I). The style was slightly longer than the stamens, exerted, and possessed a violet-colored bi-fid (forked) stigma, with unequal fids (Fig. 6H). The ovary was tetra-lobed, superior, bicarpellar, and produced a nutlet brown colored fruit with four oval seeds with veined surface (Fig. 6J-K).

Root TS showed secondary growth with circular outline characterized by irregular wavy shape (Fig. 6L). The peripheral portion was formed by the fractured periderm and dead cork regions. Storage parenchyma cells were observed in the peridermis Cells of cambium and secondary phloem partially remained at some regions. Traversed xylem rays were differentiated running nearly to the half of the root section. A central well-developed pith, formed by polygonal parenchyma cells, was observed.

Stem TS was sub-quadrangular (Fig. 6M) with a single-layered epidermis featuring compactly arranged barrel-shaped cells, glandular and non-glandular trichomes. The subepidermal collenchyma was prominent at the corners, with 7-20 layers of cells, followed by multilayered hexagonal cortical parenchyma cells. Sclerenchyma strands delimited the vascular cylinder region which exhibited a broad central pith, formed by polygonal parenchyma cells.

Leaf TS showed a thick cuticle covering both epidermal surfaces (Fig. 6N). The epidermis was one layered with glandular and non-glandular trichomes dispersed throughout. Mesophyll was dorsiventral with palisade tissues and compactly arranged spongy cells. The mid-rib presented 5-7 collateral open VB symmetrically arranged in an arc (Fig. 6N). Leaf was hypostomatic with diacytic stomata (Fig. 6O).

Fig. 6. *Salvia cana*. A) Species natural habit. B-C) Leaf in upper-adaxial and lower-abaxial view, respectively. D-E) Floral bracts (arrow). F) Dissected calyx. G) Bi-lipped view of corolla. H) Stamens with dorsifixed single-lobed anthers (arrow) and a gynoecium with bi-fid stigma. I) Viable pollen. J) Tetra lobed ovary. K) Seeds. L) Root TS. M) Stem TS. N) Leaf TS with midrib portion. O) Diacytic stomata (arrow). References: c, cork; ct, cortex; m, mesophyll; pd, periderm; p, phloem; pt, pith; sc, idioblast storage cells; se, subepidermal collenchyma cells; sl, sclerenchyma; t, trichome; vb, vascular bundle; xr, xylem ray; x, xylem. Scale = A-H, J-K, 1 mm; I, 10 μm; L-N, 10 μm; O, 20 μm.

Fig. 6. *Salvia cana*. A) Hábito natural de la especie. B-C) Vista superficial de la hoja, adaxial y abaxial, respectivamente. D-E) Brácteas florales (flecha). F) Cáliz disectado. G) Corola bilabiada. H) Estambres con anteras unitecas dosifijas (arrow) y ginecio con estigma bífido. I) Polen viable. J) Ovario tetralobado. K) Semillas. L) Raíz en ST. M) Tallo en ST. N) ST de la hoja en la region del nervio medio. O) Estoma diacítico (flecha). Referencias: c, súber; ct, cortex; m, mesofilo; pd, peridermis; p, floema; pt, medula; sc, idioblastos; se, colénquima subepidérmico; sl, esclerénquima; t, tricoma; vb, haz vascular; xr, radio xilemático; x, xilema. Escalas = A-H, J-K, 1 mm; I, 10 μm; L-N, 10 μm; O, 20 μm.

Salvia coccinea

The species was an annual, small (0.40-0.90 m) herbaceous plant with tap roots, growing along the natural drain or water channels, and wastelands in the mountain area of Dimber valley, Narag, and Kheri (Table 1, Fig. 7A). The stems were sub-quadrangular to nearly cylindrical, soft, pilose, basally dark green to reddish at the apex. Leaves were densely pilose abaxially, simple petiolate, cordate, decussately arranged, deltoid shaped with nearly acute apex; crenate to crenulate margin, and reticulate venation (Fig. 7B-C). Flowering was observed during May-June and the flowers were arranged in terminal racemes. Each flower was pedicellate and exhibited small, lanceolate leafy bracts at the base of the pedicel (Fig. 7D). Calyx was campanulate 4 toothed, green to reddish-purple, pilose and ribbed (Fig. 7E). Corolla tubular-campanulate 5-lobed (2/3), scarlet-red, upper lip 2 lobed (1+1), lower lip 3 lobed (2+1), the middle lobe larger with a rounded to slightly notched apex, while

the lateral lobes were smaller, showy, and wider than the upper lip and horizontally elongated (Fig. 7F). Androecium consisted of 2 long exserted stamens with red filaments and elongated, basifixed brown anthers (Fig. 7G). In *S. coccinea*, as in *S. cana*, the connective tissue of anthers in both stamens was highly reduced. Pollens grains were sub-oblate to prolate-spheroidal, with rough surface ornamentation and exhibited 100% viability (Fig. 7H). Style was long, exerted (Fig. 7I) and equal in length to stamens, pinkish-red with a bifid stigma with fids equal and slightly recurved. The ovary was usually 4 to 5 lobed, superior and bicarpellar, it produced a fruit with 4 (rarely 5) light brown, ovate seeds of nearly uneven surface (Fig. 7J-K).

Root TS was circular in outline (Fig. 7L) with secondary growth. The rizodermis and cortical region were replaced by the periderm. Wood formed by the secondary xylem presented intermediate medullary rays and a central broad pith.

Stem TS was nearly sub-quadrangular to cylindrical in outline (Fig. 7M). The cuticularized epidermis was thick, one layered, with trichomes, both glandular and non-glandular. It was followed by multi (10-13) layered sub/epidermal collenchyma and 5-7 layers of cortical parenchyma cells. Few oily idioblast were observed in subepidermal and cortical layers. A perivascular ring sclerenchyma was evidenced and concentric rings of secondary phloem, cambium and secondary xylem form the vascular cylinder. Finally, a broad central parenchyma pith was present.

Leaf TS was irregular in outline (Fig. 7N). The epidermis was one layered with glandular and non-glandular trichomes. The mesophyll dorsiventral was interrupted by collateral VB. The mid veins showed unique collateral VB arranged in a crescent shape manner. Diacytic stomata were observed on both the leaf surfaces (Fig. 7O).

Petiole TS exhibited a horn-shaped outline (Fig. 7P) with an epidermis consisting of a single layer of densely arranged barrel-shaped cells, with non-glandular trichomes. Five to 7 layers of subepidermal collenchyma and a unique central open collateral VB, accompanied by two minor lateral collateral bundles arranged in each horn (or ear).

Trichomes

Table 2 summarizes the type and distribution of trichomes observed in the different organs of the studied species. Eleven types (α, β, γ, δ, ε, ζ, η, θ, ι, κ, λ) of trichomes were identified and classified into two major groups: Non glandular and glandular trichomes.

Non-glandular trichomes were classified into five types: α, β, γ, δ, and ε, according to their apex shape and cell number and distribution (Fig. 8A-H). Meanwhile glandular trichomes were classified into six types: ζ, η, θ, ι, κ, and λ., according to their stalk cells number and head shapes (Fig. 8I-O).

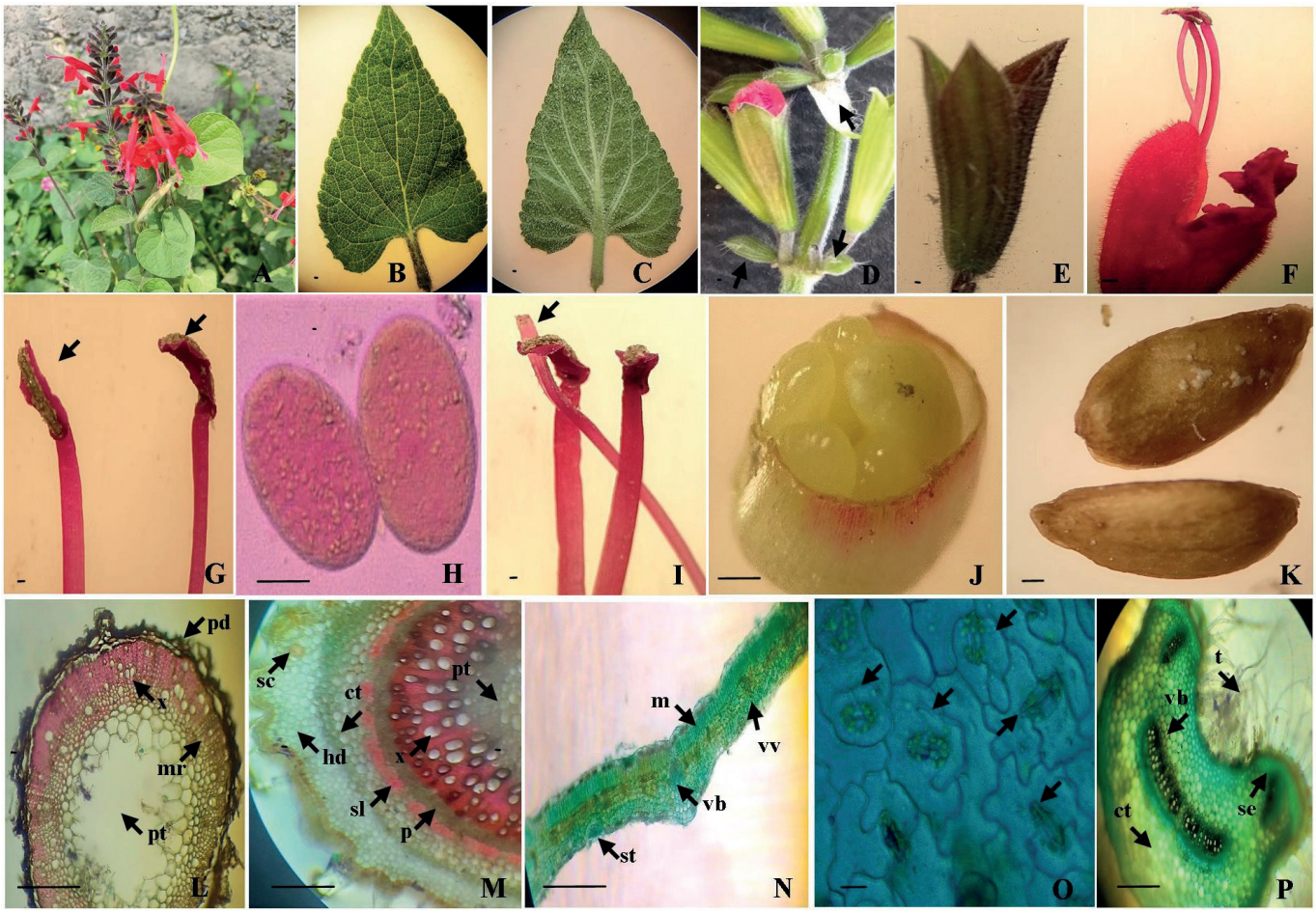

Fig. 7. *S. coccinea*. A) Natural habit of the species. B-C) Leaf in upper-adaxial and lower-abaxial view, respectively. D) Inflorescence with leafy floral bracts (arrow). E) Dissected calyx. F) Bi-lipped view of corolla. G) Stamen filaments with basifixed brown anthers (arrow). H) Viable pollens. I) Anther touching bi-fid stigma (arrow). J) Penta-lobed ovary. K) Seeds. L) Root TS. M) Stem TS. N) Leaf TS. O) Diacytic stomata. P) Petiole TS. References: ct, cortex; m, mesophyll; mr, medullary rays; pd, periderm; p, phloem; pt, pith; sc, idioblast storage cells; se, subepidermal collenchyma cells; sl, sclerenchyma; st, stoma; t, trichome; vb, vascular bundle; vv, vascular veins/ strands, x-xylem. Scale = A-G, J-K, 1 mm; H, 10 μm, L-M, 200 μm; N, P, 50 μm; O, 20 μm.

Fig. 7. *S. coccinea*. A) Hábito natural de la especie. B-C) Vista superficial de la hoja, adaxial y abaxial, respectivamente. D) Inflorescencia con brácteas florales foliosas (flecha). E) Cáliz disectado. F) Corola bilabiada. G) Estambres con anteras basifijas marrones (flecha). H) Polen viable. I) Antera tocando el estigma bífido (flecha). J) Ovario pentalobado. K) Semillas. L) Raíz en ST. M) Tallo en ST. N) Hoja en ST. O) Estoma diacítico. P) Pecíolo en ST. Referencias: ct, cortex; m, mesofilo; mr, radios medulares; pd, peridermis; p, floema; pt, medula; sc, idioblasto; se, colénquima subepidérmico; sl, esclerenquima; st, estoma; t, tricoma; vb, haz vascular; vv, venas, x, xilema. Escalas = A-G, J-K, 1 mm; H, 10 μm, L-M, 200 μm; N, P, 50 μm; O, 20 μm.

Table 2. Characteristics, types, and distribution of Non-glandular and Glandular Trichomes of six Lamiaceae species from the North-Western Indian Himalaya.

Tabla 2. Características, tipos y distribución de tricomas No-glandulares y glandulares de seis Lamiaceae del Himalaya noroccidental de la India.

Trichomes	Types	Cell number and distribution	Tip/head	Species	Plant organs
Non-glandular	A	Simple unicellular	Blunt	*S. coccinea*	Stem, leaf blade
	B	Multicellular uniseriate	Pointed	*A. integrifolia*	Stem, leaf blade and petiole
				A. parviflora	
				P. bicolor	
				R. cinerea	
	Γ	Multicellular dendroid	Bifurcated one or more times,	*A. integrifolia*	Stem, leaf blade and petiole
				P. bicolor	
	Δ	Simple multicellular dendroid	Pointed	*S. cana*	Stem
	E	Simple bicellular uniseriate	Pointed	*A. integrifolia*	Stem, leaf blade and petiole
				A. parviflora	
				P. bicolor	
				R. cinerea	
				S. coccinea	
Glandular	Z	Simple, bicellular uniseriate stalk	Capitate (rounded)	*A. integrifolia*	Stem, leaf blade
				A. parviflora	
				R. cinerea	
				S. cana	
	H	Multicellular, uniseriate stalk	Hooked (rounded)	*S. coccinea*	Stem, petiole
	θ	Simple, unicellular stalk	Capitate (rounded)	*S. coccinea*	Stem
	ι	Multicellular, uniseriate stalk	Capitate (heart-like)	*A. integrifolia*	Stem, leaf blade
	κ	Unicellular, short stalk	Peltate	*S. cana*	Stem
	λ	Multicellular, long stalk	Peltate	*S. cana*	Stem, leaf blade
				S. coccinea	

DISCUSSION

The six species of Lamiaceae analyzed share common taxonomic characters which are widespread in the family, including either shrubby or herbaceous habit, quadrangular stem, decussate phyllotaxy, verticilaster inflorescence, bi-lipped corolla, schizocarpic fruit of four dry one-seeded nutlet, gynobasic style and presence of glandular and non-glandular trichomes, (Ya'ni *et al*., 2018; Venkateshappa & Sreenath, 2013; Zhao *et al.*, 2021). Also, the morphological characters described for the six studied species were similar to the floristic characters documented and described, under synonym names, by Hooker (1884) and Mukerjee (1940).

Considering all the available synonyms of the studied taxa, no literature pertaining to histological features was found for four of the species, namely, A. *parviflora*, *A. integrifolia*, *P. bicolor*, and *S. cana*. Previously an histological study on the genus *Ajuga* was conducted on different plant parts of four species, *A. salicifolia* (L.) Schreber (Ulcay, 2021); A. *orientalis* L. (Cali, 2014); *A. reptans* L. and *A. chamaepitys* (L.) Schreber (Akcin *et al.*, 2006). It has been found that the presence of squarish outline in the offshoots section, broad subepidermal collenchyma and VB arrange

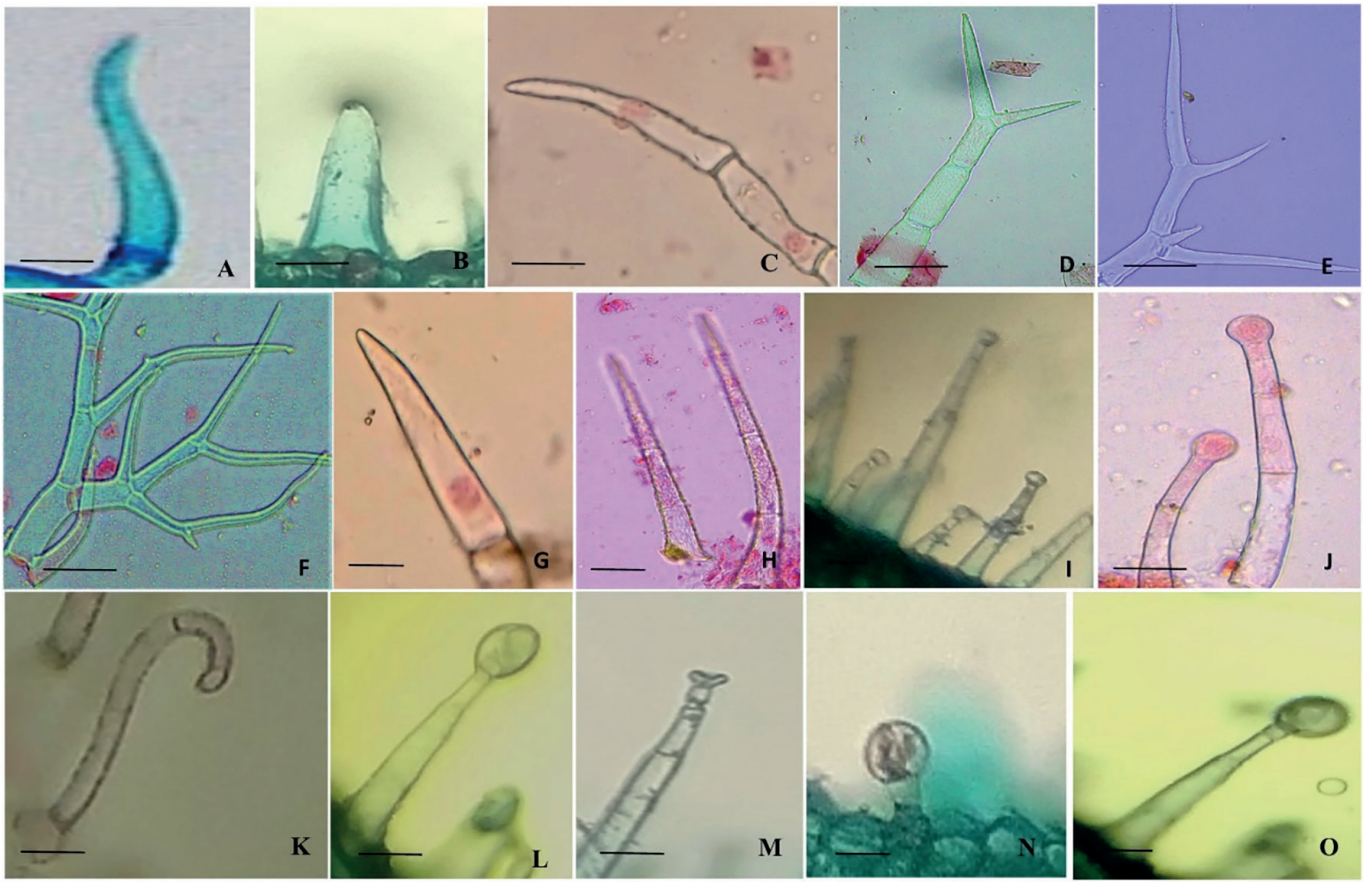

Fig. 8. Non-glandular trichomes (A-H) and Glandular trichomes. (I-O). A-B) Type-α. Simple with blunt tip. C) Type-β. Multicellular uniseriate with pointed tip. D-E) Type-γ. Multicellular with pointed tip, bifurcated one or more times. F) Type-δ. Simple multicellular dendroid with pointed tip. G-H) Type-ε. Simple, bicellular uniseriate with simple pointed tip. I-J) Type-ζ. Simple, bicellular uniseriate stalk with capitate rounded head. K) Type-η. Multicellular with hooked rounded head. L) Type-θ. Unicellular with capitate round head. M) Type-ι. Multicellular uniseriate with capitate heart shaped tip. N) Type-κ. Unicellular short stalk with peltate head. O) Type-λ. Unicellular long stalk with peltate head. Scales = 10 μm.

Fig. 8. Tricomas No-glandulares (A-H) y tricomas Glandulares (I-O). A-B) Tipo-α. Simple con apice rojmo. C) Tipo-β. Multicelular uniseriado de extremo en punta. D-E) Tipo-γ. Multicelular con extremo en punta, bifurcado una o más veces. F) Tipo-δ. Simple multicelular dendroide con extremo en punta. G-H) Tipo-ε. Simple, bicelular uniseriado con extremo en punta. I-J) Tipo-ζ. Simple, bicelular con base uniseriada, capitado con cabeza redondeada. K) Tipo-η. Multicelular con cabeza redondeada, terminado en gancho. L) Tipo-θ. Unicelular capitado con cabeza redondeada. M) Tipo-ι. Multicelular uniseriado, capitado con cabeza acorazonada. N) Tipo-κ. Unicelular de base corta con cabeza peltada. O) Tipo-λ. Unicelular base larga con cabeza peltada. Escalas = 10 μm.

in the vertexes of the stems, as observed in the present work for *A. integrifolia* and *A. parviflora* are common features to the *Ajuga* genus.

The aforementioned studies reveal variations in the number of cell layers within stem tissues of *Ajuga* species, including multilayered collenchyma (e.g., 4-5 layers) and cortex (e.g., 13-14 layers) in the stem, and periderm (e.g., 3-5 layers) in the root indicating intraspecific variability. These histological variations, may be attributed to one or a combination of factors, including developmental differences such as age and growth stage (Carlquist, 2001), environmental or ecological preferences (Sch-

weingruber *et al.*, 2014; Jiménez-Noriega *et al.*, 2017), and genetic factors (Ragni & Greb, 2018; Wang *et al.*, 2022), as documented in other higher plants.

The genus *Pseudocaryopteris* (Briq.) P.D. Cantino consists of three accepted species, namely, *P. bicolor*, *P. foetida* (D. Don) P.D. Cantino, and *P. paniculata* (C.B. Clarke) P.D. Cantino (POWO, 2024). The species *P. bicolor* was previously treated as *Caryopteris bicolor* (Roxb. ex Hardw.) Mabb. under the genus name *Caryopteris* Bunge (Cantino *et al.*, 1998). By considering genus *Pseudocaryopteris*, and the synonyms of *P. bicolor* and *C. bicolor*, no publications are available referred to histological studies. Thus, the present work in *P. bicolor* is a new addition to the knowledge on this taxon.

Regardin *Roylea* Wall., it is monotypic genus, represented solely by *R. cinerea*. The histological characteristics observed in this study are consistent with those previously reported by Upadhyay (2017) in specimens from India.

Among the two studied species of *Salvia*, for *S. coccinea* histological the described characters are in conformity with the previous report by El-Sahhar *et al.* (2017) for plants grown in Egypt, while in *S. cana*, the present histological study is a new addition to the existing knowledge of the taxon. Previous histological studies in the genus *Salvia* have been conducted on various species including *S. ballsiana* (Rech. fil.) Hedge (Kahraman *et al*., 2010), *S. glutinosa* L. (Kahraman, 2009a), *S. hispanica* L. (Abou Zeid *et al.*, 2022), *S. indica* L. (Kahraman., 2009b), *S. quezelii* Hedge & Afzal-Rafii (Celep *et al.*, 2014), *S. rosmarinus* Spenn. (Ahmed., 2023), *S. staminea* Montbret & Aucher ex Bentham (Kahraman, 2009a), and *S. viridis* L. (Özdemir, 2009). The presence of two stamens, as recorded in *S. ballsiana*, *S. hispanica*, *S. indica*, and *S. rosmarinus* (Kahraman *et al*., 2010; Abou Zeid *et al.*, 2022; Kahraman, 2009b; Ahmed, 2023), or sometimes four stamens, as reported in *S. schimpheri* Benth. and *S. majdae* (Rech.f. & Wendelbo) Sytsma, is well-documented within the genus *Salvia* (Ya'ni *et al.*, 2018; Ajani *et al.*, 2022). Presently, we also report the presence of two stamen characters in *S. cana* and *S. coccinia*. Modified lever-like stamens are considered a common character in *Salvia* with few exceptions (Claßen-Bockhoff *et al.*, 2004). However, the two studied taxa, *S. cana* and *S. coccinia* presented highly reduced connective without a lever mechanism.

In the investigated taxa, we identified three distinct types of stomata: anisocytic, anomocytic and diacytic. Among the examined species, four (*A. parviflora, R. cinerea, S. cana, S. coccinea*) exhibited diacytic stomata. These findings align with prior assertions suggesting the prevalence of above all three types of stomata in the family Lamiaceae and diacytic stomata in most of its members (Grozeva *et al.*, 2016; Gul *et al.*, 2019).

Pollen grains within the family Lamiaceae display a diverse range of shapes, including prolate, sub-prolate, oblate, sub-oblate, spheroidal, prolate-spheroidal, and oblate-spheroidal (Saggoo & Bir, 1983). These shapes are commonly accompanied by apertures of trizonocolpate and hexazonocolpate types (Doaigey *et al.*, 2018, Özaltan & Koçyiğit 2022). In the present study species and pollen grains exhibit a range of shapes, including sub-oblate (e.g. *A. integrifolia* and *S. coccinea*), sub-prolate (e.g. *A. parviflora*, *P. bicolar*) to prolate-spheroidal (e.g. *P. bicolar, R. cineria, S. cana* and *S. coccinea*), with corresponding apertures of hexa-zonocolpate types (e.g. *A. integrifolia*, *A. parviflora*, *P. bicolar* and *R. cineria*).

Many researchers have advocated for the examination of trichome morphology in members of family Lamiaceae and its taxonomic relevance (Akcin & Camili 2018; Chatri *et al.*, 2018). Eleven different types of trichomes have been reported together in the currently studied species under two categories i.e. non-glandular and glandular trichomes. Lamiaceae species are acknowledged for hosting diverse types of both glandular and non-glandular trichomes, among which the peltate and capitate glandular trichomes are notably widespread and comprehensively characterized (Bini & Giuliani 2006., Hussin *et al.*, 2024). At present work, peltate trichomes were recorded in *S. cana*, and *S. coccinea* while capitate trichomes in *A. integrifolia*, *S. cana*, and *S. coccinea*.

In addition to the documented characters of the studied taxa by Hooker (1884) and Mukerjee (1940), the following diagnostic combination of characters could be useful in identifying the studied species:

A. integrifolia: roots with secondary growth; basal nodal stalk as reduced stem with secondary growth, unbranched and herbaceous pubescent quadrangular offshoots; sheathing leaf base; amphistomatic leaf with anisocytic stomata; inflorescences blooming twice a year with 2 leafy bracts, light purple bilipped (2/3) corolla; didynamous (2+2), epipetalous, with cream white filaments, and dark reddish-brown bean-shaped, dorsifixed anthers with sub-oblate hexa-zonocolpate pollens; bifid stigmas; superior ovary; nutlets with brown oval 4 seeds; non-glandular (β, γ, ε) as well as glandular (ζ, ι) trichomes.

A. parviflora: adventitious, fleshy, fibrous cream-white roots. Root and stem shoot with secondary growth; amphistomatic leaf with diacytic stomata; basal leaves forming rosettes, velvety and sessile spathulate blades with entire margin, and occasionally with abaxial adventitious roots; and smaller cauline leaves decussate, oblanceolate with crenate margins; 2 leafy bracts (abaxially purplish), sepals with a distinct white median rib; bilipped (2/3) corolla with pilose petals with a dark purple median rib; didynamous (2+2) stamens, purplish-brown anthers with sub-prolate to spheroidal, hexa-zonocolpate dusty- bright yellow pollens; bifid stigma, and superior, four-lobed ovary; reproduce vegetatively without seeds; presence of non-glandular (β, ε) and glandular (ζ) trichomes.

P. bicolor: root with secondary growth with pith absent; sub-quadrangular branches turn earthy-brown, cylindrical and woody with age; young stem soft with prominent pith; non-aromatic simple leaves, ovate-lanceolate to lanceolate with pubescence; leaf with anomocytic stomata on abaxial surface; inflorescences - terminal spikes on young branches and panicle-like thyrses on older branches; sepals with a prominent mid-vein; corolla bi-lipped (deeply cleft with nearly equal lobes); didynamous (2+2) stamens cream-white with equal filaments and bluish-purple dorsifixed bi-lobed anthers producing sub-prolate to prolate-spheroidal hexa-zonocolpate pollens; stigma bifid, 4 lobed ovary forms septicidal semi-fleshy capsular fruit producing four dry, one-seeded nutlets; presence of non-glandular (β, γ, ε) trichomes.

R. cinerea: root with secondary growth; stem quadrangular at young, cylindrical, woody, and brown with age, features subepidermal collenchyma at the corners and a a broad pith; petiole with subepidermal collenchyma at the wings, and a crescent-shaped VB; leaf with dicytic stomata and a central epsilon or C-shaped VB in

TS; terminal spike inflorescences on young branches, and panicle-like thyrses on older branches, flowers in loose verticils forming nodal whorls; flowers awl-shaped, bracteate; ridges on calyx; lower lip of blipped (2/3) corolla with purplish streaks and three unequal lobes; epipetalous condition of didynamous (2+2) stamens; deep brown, bi-lobed, dorsifixed anthers with prolate-spheroidal hexa-zonocolpate pollens; bi-fid stigma, superior tetra-lobed ovary; pilose and flat-tipped oval brown nutlet d fruit, one seede; presence of non-glandular (β, ε) and glandular (ζ) trichomes.

S. cana: woody stalked tap root system, shows secondary growth; stem quadrangular, pubescent; leaves sub-radical, sessile, abaxially woolly, and adaxially rugose and with diacytic stomata; margins of pilose green bracts purple; spine-tipped (3/2) teeth of calyx; exserted condition of 2 stamens, single-lobed brown coloured dorsifixed anther with reduced connectives; prolate shaped dusty coloured pollens with reticulate ornamentation; bi-fid stigma, tetra-lobed superior ovary, nutlet with 4 brown oval seeds; presence of non-glandular (δ) and glandular (ζ, κ, λ) trichomes.

S. coccinea: root showed secondary growth, sub-quadrangular to cylindrical stem, with a red-wine colour in upper half; decussately arranged petiolate leaves, with diacytic stomata in its lower surface; petiole with 4 collateral open VBs, one in each horn (or ear) region, and two in crescent shape at middle of cortex; bracteate flowers in terminal racemes; 4 toothed ribbed calyx; 2/3 lobed scarlet-red blipped corolla; 2 exserted stamens, basifixed brown anthers with sub-oblate to prolate-spheroidal pollens having rough ornamentation; bifid stigma, 4 to 5 lobed superior ovary, 4 to 5 light coloured brown ovate seeds with uneven surfaces; presence of non-glandular (δ) and glandular (ζ, κ, λ) trichomes.

Although the above-mentioned characters are useful in taxonomic characterization, the variations due to genetic and environmental factors cannot be ruled out (Bano *et al*., 2019; Cao *et al*., 2020).

CONCLUSION

The present study offers valuable insights into the morpho-histological characteristics of previously underexplored *A. integrifolia*, *A. parviflora, P. bicolor, R. cinerea*, *S. cana*, and *S. coccinea* (Lamiaceae) from regions of the North-west Himalaya. The new observations in the morpho-histological data of these species add to our understanding and fill existing gaps in botanical knowledge regarding these taxa, which have been hitherto overlooked. Moving forward, the investigation into phytochemical and molecular aspects within these species, particularly in unexplored ones, such as *P. bicolor* and *S. cana* represents a promising and open avenue for future research.

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CONFLICT OF INTEREST

All the authors are aware of and agree for this submission. On behalf of all the authors, the corresponding author states that there is no conflict of interest.

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