Palyno-anatomical Studies of Dicot Phytodiversity in Northern Baluchistan, Pakistan

By

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بسم الله الرحمن الرحيم

In the name of Allah, the Most Merciful, the Most Kind

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Wajia Noor

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ABSTRACT

This is the first study presenting the systematics of the dicots from northern Baluchistan, Pakistan. Field trips were conducted for the collection of dicots from different areas of Northern Baluchistan. Brassicaceae, Fabaceae, and Lamiaceae were the selected families based on their abundance. In total 107 species from three families Brassicaceae, Fabaceae, and Lamiaceae were studied using Light microscopy (LM) and scanning electron microscopy (SEM) to characterize the micromorphological features of the pollen. Light microscopy was used to examine the anatomical characteristics of the transverse sections of petioles prepared via microtomy. The statistical analysis was performed on the quantitative measurements of anatomical and palynological features. The maximum polar axis 52.95µm was noted in *Salvia moorcroftiana* and minimum 15.6µm in *Cardaria draba*. The exine thickness was maximum 4.5µm in *Isatis minima* and minimum 1.3µm in *Crotalaria medicaginea*. The largest petiole length 4995.4µm was observed in *Otostegia limbate* and minimum 261.2µm in *Sisymbrium altissimum.* Maximum length of vascular bundles 211.25µm was observed in *Caragana ambigua*, and minimum 34.5µm in *Cardaria chalepense*. Reticulate, coarsely reticulate, scabrate exine, polygonal and amorphous lumen (regular or irregular shapes), the Number, Position, and Character (NPC) of aperture was $N_3P_4C_3$, and petiole shapes sulcate, flat, oval, or circular with blunt or acute wings, vascular bundles collateral closed, collateral open, bi-collateral, or hadrocentric, arrangements of 1, 1+2, numerous, and trichomes unicellular, uniseriate, and multiseriate were the significant diagnostic features for pollen morphology and petiole anatomy in Brassicaceae. The distinguished palynological and petiole anatomical characters for Fabaceae were exine psilate, verrucate, macroreticulate, microreticulate, reticulate, $N_3P_4C_5$, Amb peritreme, and goniotreme, polar view (triangular obtuse convex, circular, triangular obtuse concave, triangular obtuse convex to straight, circular to elliptic), equatorial view (rectangular obtuse convex, oval, elliptic truncate, quadrangular, rhombic, circular), shapes subprolate, prolate spheroidal, oblate spheroidal, rarely prolate pollen, vascular bundles amphicribral, collateral closed, collateral open, and bicollateral, the number of vascular bundles 1, 1+2, 2, 3, 9, collenchyma angular, lacunar, and lamellar. Significant variations in pollen apertures (tricolpate, tricolporate, hexacolpate, hexa to multi syncolpate), exine (reticulate, macroreticulate, microrteiculate, gemmate, foveolate, gemmate verrucate, perforate, non-perforate), aperture membrane (varruacte, scabrate, gemmate, psilate) amb (ptychotreme, peritreme, goniotreme), shape (oblate, spherical, prolate spheroidal, oblate spheroidal, sub-prolate, sub-oblate), formulas ($N_6P_4C_5$, $N_{3-6}P_4C_3$, $N_{4-6}P_4C_3$, $N_{4-6}P_4C_3$, $N_{3-6}P_4C_3$), petiole shape (oval, flat, sulcate, and round), collenchyma (lacunar, angular, annular, and lamellar), vascular bundles (collateral closed, collateral open, amphicribal) were observed in Lamiaceous species. The taxa of each family were taxonomically differentiated by the Principal Component Analysis (PCA), dendrogram, correlation, box-jatter plot, and normal probability distribution plot. The petiole anatomical and pollen micromorphological characteristics were employed as markers to develop the taxonomic keys for the delimitation of the examined dicot flora. The present findings may provide the baseline for correct identification of the flora of Northern Baluchistan using palyno-anatomical features in order to use for future studies at global perspectives.

Chapter 1

Introduction

1.1 Dicot Phytodiversity Around the Globe

Angiosperm, that contains two leaves, or cotyledons, in the embryo of seed is considered a dicotyledon. Around 175,000 species of dicots have been identified from different areas of the world. The majority of typical garden plants, including shrubs, trees, and broad-leafed flowers like hollyhocks, magnolias, roses, and geraniums, are dicots (Kubitzki et al., 2013). Although there are some exceptions, dicots usually have flower parts (petals, stamens, pistils, and sepals) arranged in multiples of four or five. Most leaves have net veins, which implies that the structures that carry nutrients and water have a pattern resembling a mesh. The vessels in the stems are typically positioned in a continuous ring close to the stem surface (Paterson et al., 2004).

Approximately half of all dicots exhibits a yearly rise in stem diameter due to the cambium, a layer of cells that continue to divide throughout the plant's life, producing new tissue. Taproots and stem branching are common. The stomates are typically dispersed and oriented differently on the surfaces of the leaves (Raikhel and Minorsky, 2001). The pollen grains, except the more primitive families, usually have three germinal furrows or pores (tricolpate condition). In dicots vascular tissue forms a ring around the stem. Eudicots can have woody tissues or be herbaceous. Two-thirds of all flowering plants are eudicots. Some important dicot families are Brassicaceae, Lamiaceae, Boraginaceae, Fabaceae (Beans), Asteraceae (Sunflowers), Papaveraceae (Poppies), Cucurbitaceae (Squash), Fagaceae (Oaks), Rosaceae (Roses) (Kubitzki et al., 2013).

Dicotyledons are significant for several reasons. The food that both humans and animals eat is one of the primary contributions to human life. Among the main food groups that are produced from dicotyledons and are sources of nutrition are pulses, grains, legumes, nuts, fruits, vegetables, berries, tubers, and green leafy vegetables. Plant nuts and seeds are sources of oil. Pulses are a rich source of protein. Iron is abundant in several foods, including brinjal. Carrots are the source of Vitamin A, while vegetables like cabbage, cauliflower, and citrus fruits contain the essential Vitamin C (Raikhel and Minorsky, 2001).

Dicotyledon plants are important to humans not only for food but also for their wood. Timber is utilized for buildings, accessories, equipment, furniture, and transportation. Dicots have made numerous significant contributions to science and medicine. Plants are the source of therapeutic substances used in Ayurvedic, Homoeopathic, and Herbal medicine, as well as Allopathy (Paterson et al., 2004). A significant portion of human, technical, scientific, and pharmaceutical advancements can be attributed to dicots. Dicot herbs were also used to treat a wide range of ailments, such as cancer, skin disorders, joint pain, and heart problems. Important dicots such as *Berberis lycium*, *Ajuga bracteosa*, *Saussurea lapa*, and *Jurinea dolomiaea* are all extremely threatened (Bibi et al., 2015).

1.2 Phytodiversity of Dicot Flora: Present-Day Research

Plant scientists have been trying to find a precise method for classifying and identifying plants. Plant morphology remained the most valuable tool in phylogenetic trees (Khan et al., 2021). The anatomy of plants has proven to be particularly useful in distinguishing between various species and in classifying plants. The anatomical studies resulted in the categorization of various families including Papaveraceae and Capparaceae (Ahmad et al., 2022). The characteristics, including vascularisation, sclarification, cell layers, cavities, and cell diameter, including epidermis, parenchyma, collenchyma, sclerenchyma, chlorenchyma, and pith, have been successfully employed in taxonomy and evolution. The application of palynological investigations have been increased in plant taxonomy since the invention of high-resolution scanning microscopes. Pollen studies have clarified the placement of several species, for example in the genus *Phyrma*. The palynological features such as size, symmetry, apertures, and exine sculpturing have been used in taxonomy (Taia, 2005). Anatomy, palynology, cladistic analysis, ecotaxonomy, chemotaxonomy and serology, karyomorphology, seed morphology, phylogeny, paleobotany, and embryology are the most current trends in plant taxonomy (Khan et al., 2021).

1.2.1 Palynology

Palynology is the scientific study of pollen and spores. Palynomorphs encompass all microfossils, such as spores and pollen grains. Despite contributing to a variety of systematics studies, palynological research assisted in the identification of species via palynomorph examinations (Kailas et al., 2016; Perveen et al., 2004). The palyno-morphological investigation utilizing various microscopic techniques was carried out as an aid to morphological studies and as a crucial tool for plant taxonomists in the classification of species (Kailas et al., 2017). Scanning Electron Microscopy (SEM) has been employed to examine differences between species based on exine ornamentation, apertures, etc (Ragho et al., 2020). In recent years, the use of SEM has established significant approaches in the micro-morphological examinations of pollen (Khan et al., 2021). SEM has greatly visualized the pollen surface because of its high resolution, which provides a comprehensive view of the ultrastructure and permits micromorphological variations that were not practicable with light microscopy. Many studies have examined the pollen morphology of the Brassicaceae family, and all of them have demonstrated the importance of pollen morphology in comprehending the taxonomic status of the family (Kailas et al., 2016).

The term "palynology" originated from the Greek word "Palynein," which means flour or dust (Kayani et al., 2019). According to Halbritter et al. (2018), Europalynous pollens are heterogeneous pollens that differ from other pollens in terms of size, shape, aperture, and exine ornamentation (Kailas et al., 2020). The study of pollen using scanning electron microscopy has also led to the development of innovative terms for defining pollen ornamentation, statistical techniques for pollen sculpture, and ultimately a computational examination of the exine (Qureshi et al., 2019).

1.2.2 Anatomy

Anatomy is one of the important areas of plant research. Anatomy is essential in providing a link between several important fields of modern plant sciences when conducting taxonomic research. Some organs in plants are less affected by environmental influences and are highly significant in systematics (Barthlott, 1981). Comparative plant epidermal studies have shown to be reliable in taxonomy and systematics (Ogunkunle and Oladele, 2008). The significance of anatomical characteristics for taxonomy has been highlighted by Naik and Nigrude (1981), Adedeji (2004), and Metcalfe and Chalk (1950, 1979). These characters help in plant identification and classification when paired with other traits.

The anatomical characteristics of vegetative parts are of great importance (Bahadur et al., 2020). When distinguishing plants, different comparative anatomical characteristics were frequently employed in angiosperms to determine their taxonomic levels and their relationships with other taxa. (Song and Hong, 2018; Stuessy, 2009). From a taxonomic and diagnostic point, comparative anatomical examinations of the petiole have been proven effective at various levels for classifications (Talip et al., 2017). Taxonomy has progressively utilized anatomical approaches in recent years (Ozcan and Eminagaoglu, 2014). The anatomy of petioles has been researched concerning the fossil genus *Heleophyton* (Jehanzeb et al., 2020).

Plant anatomy is important to plant taxonomy. The objective is to develop a categorization system for plants that systematically lists all of the differences and similarities (Okeke et al., 2015). Adedeji (2004) has highlighted the taxonomic significance of anatomical features, which, when paired with other characters, are useful for plant identification and classification. One notable characteristic is the petiole anatomical features, which were used to differentiate plants that belong to different species, genera, and families and are significant for the identification and classification of many plant families (Metcalfe and Chalk, 1979). Furthermore, some authors have emphasized that there is actual taxonomic importance to the arrangement of vascular bundles in distinct petiole parts (Ekeke and Ogazie, 2020).

The petiole's central vascular bundle played a significant role in identifying plants. In the petiole of *R. discoid*, there were fewer vascular bundles than at the base of the leaf (Matias et al., 2007). According to Song and Hong (2018), the petiole vascular bundles in *M. caerulescens* were delimited by the pericycle, comprising a pair of layers of lignified cells. For taxonomic investigations within the tribes, some anatomical traits, such as cuticles, pericyclic fiber patterns, vascular patterns, trichomes, and crystals provide important details. The evolutionary importance of wood anatomy has drawn more inferences. Cell kinds, vessel elements, length and width, types of perforation plates, thickness, and pitting of the lateral wall were the taxonomically important traits (Matias et al., 2007).

Carlquist (1996) worked on the anatomy of the Lamiaceae, sympetalous families, Cucurbitaceae, and Chloranthaceae, in addition to the Aristolochiaceae, Sabiaceae, Caryophylaceae, Rananculaceae, Berberidaceae, Menispermaceac, Resedaceae, Portulaceaceae, and the genera *Rivina* and *Petiveria* of Caryophyllales. They all have far more extensive taxonomy than many other families. They found that anatomy can help with the taxonomy and phylogeny of the studied taxa. Baas et al. (1982) explored the anatomy of the Dicotyledons and their use in the taxonomy of angiosperms and highlighted its significance. Recently anatomical characteristics have been used in the taxonomy (Agbagwa and Ndukwu, 2004; Kharazian, 2007) such as the distinctions between genera and species were made based on the petiole's structure (Olowokudejo, 1987; Shaheen, 2007; Eric et al., 2007). In the Lamiaceae family, the petiole's anatomical structures played a significant role (Metcalfe and Chalk, 1972).

1.3 Dicot Phytodiversity in Pakistan

A flora is a collection of all plant species that are found growing in a certain area. The earth's surface is not evenly covered with plants, and each geographic zone has its specific plant species that are not found in other regions. Floristic surveys are helpful in precisely identifying plant species, enabling their methodical and scientific use. The identification of local flora is important in addition to providing a description of the place, as it can identify specific species found there and provide details about their occurrence, growing season, and distinctive qualities. Additionally, it can be used to detect new species and draw attention to how local vegetation is affected by climatic variables like drought and overgrazing (Ali, 2008). There are currently 414,000 flowering plants in the world, more than 200 families, and about 6000 flowering plant species known to exist in Pakistan. The northern and western highlands of Pakistan are home to over 80% of the country's endemic flowering plants (Ali and Qaiser, 1986). Floristic phytodiversity offers a basis for accurate plant identification and sustainable plant use (Thakur et al., 2012).

In Pakistan, the majority of floristic research has been done in the province of Sindh. Chaudhary et al. (1981) conducted an initial floristic survey of Sindh's Thar Desert. From the research region, 122 species of dicots were reported. According to Rajput et al. (1991), 40 dicot plant species from 23 families in the Thar Desert are used as medicinal herbs to treat a variety of illnesses. Bhatti et al. (2001) carried out a study for the botanical survey of the Nara desert, which is located in the northeastern region of the larger Thar Desert. A total of 149 plant species from 110 genera and 42 families were identified. Qureshi (2004) presented floristic expertise and incorporated numerous floral aspects from the same region. A few other books have also been published by the Nara Desert authors (Qureshi, 2009). The floristic composition of Gorakh hill (Khirthar range) was reported by Perveen and Hussain (2007). Out of 62 genera and 34 families, they identified 74 species. Ansari et al. (1993) created a Floristic List of the District of Khairpur. Their work served as an inventory. From the Potohar range, the published data is scarce regarding dicot phytodiveristy. Furthermore, there is a huge gap, that requires research to update and digitize the entire flora of the country.

1.4 Dicot Phytodiversity: Systematics Research Trends in Pakistan

The higher-level systematic study of dicot Phyto diversity, particularly the interrelationships of tribes, genera, and species, has been the main focus of research conducted in Pakistan. These investigations have revealed evolutionary processes of dicot phytodiversity by determining their morphology, seed chemistry, and chromosome number. These studies helped reconstruct and realign the placements of several tribes in the taxonomic classification scheme that Bentham and Hooker in 1865 had originally established (Shah et al., 2018). According to Akhtar et al. (2021), one of the key methods for differentiating between closely related taxa is a palynological and anatomical study (Albrecht-Buehler, 1994). The concurrent reduction in nonmolecular cell biological investigations has left a gap between the ability to study biology at a lower classification level.

Pollen morphology provided information on the structural characteristics and was widely used in plant taxonomy (Holt et al., 2014). It is beneficial for discrimination as well as identification and categorization of taxa at species and generic levels, and it may also be utilized as a key for taxonomic traits (Mildenhall et al., 2006). Exine sculpture diversity can be used as an aid in the separation of closely related species. Both qualitative and quantitative pollen characteristics can be used to differentiate between taxa at a specific level (Khan et al., 2020). Several important studies on the pollen of various families were carried out by Erdtman (1952), Barth (1964), Barth et al., (1975). These studies showed an extensive variety in pollen morphological characters of Fabaceous species, emphasizing the importance of pollen for identifying different species or groups, as well as aiding in the resolution of problematic taxa in the family (Ahmad et al., 2023).

Petioles anatomical characters played an important role in taxonomic investigations and in studying biodiversity as it helped in the identification of plant species of an area (Muhammad et al., 2006). Anatomical features can help in the separation of morphologically similar plants. Akhtar et al. (2022) conducted a study on the Western Himalayan region's Asteraceae family's petiole anatomy. They discovered that the primary diagnostic anatomical characteristics that aided in the identification of plants at the species level were petiole anatomical traits, such as cell morphologies, petiole form, pith, and trichomes. Researchers have examined the petiole anatomy of plants from a variety of plant groups and floristic zones. Petiole anatomy has been studied in several plant families, including the Cruciferae (Gorovoy et al., 2011), Asteraceae (Akhtar et al., 2022), Cucurbitaceae (Aguoru and Okoli, 2012), Rubiaceae (Kocsis and Borhidi, 2003), Lamiaceae (Jehanzeb et al., 2020), and Euphorbiaceae (Tadavi and Bhadane, 2014), and Rutaceae (Ferreira et al., 2022).

Perveen et al. (2004) carried out extensive research on the pollen morphology of the family Brassicaceae in Pakistan. The Pollen morphology of the family Brassicaceae genera *Arabidopsis*, and *Alyssum* were explored in several ecoregions of Pakistan (Khan, 2004). Since the flora of Baluchistan (44% by land) has not been studied, there is still a huge gap in available knowledge of the pollen morphology of the Brassicaceae family (Umber et al., 2022).

1.5 Brassicaceae, Fabaceae, Lamiaceae

Brassicaceae (mustard family) is dicot group, with 338 genera and 3709 species. It has a monophyletic lineage (Al-Shehbaz, 2012). Around the globe, it is abundant in the Saharo-Sindian, Mediterranean, and Irano-Turanian regions (Hedge, 1976). There are 250 species and 92 genera of Brassicaceae in Pakistan. The Brassicaceae family contains some important species that produce oil. Analytical, palynological, and seed morphological traits analyses aided in the characterization and identification of complex species within the Brassicaceae family (Amina et al., 2020). When morphological data was insufficient, micromorphological and anatomical characters assisted in determining the taxonomical location of the species (Yigit, 2016). Diverse vegetative anatomical traits aid in taxonomic classification and identification by providing boundaries (Olowokudejo, 1987). According to their relatedness, ambiguous taxa were grouped using morphological trait analysis (Amina et al., 2019).

Petiole anatomical analyses of the showed divergence among Brassicaceous species. Its shape varied, ranging from rectangular to half-round (Gorovoy et al., 2011) or subcircular with wings on either side. The vascular bundle might be oval or round, the conductive tissues were distributed collaterally, and the number of parenchyma rows outside the phloem were two to four. The tribe Alysseae had flat, sulcate, or round petiole morphologies. According to Karaismailoğlu (2020), there were 1 (*Berteroa* *mutabilis*) to 9 (*Alyssum strictum*) VBs, with varying numbers of lobes. Studies of the presence or absence of several features in petiole anatomical analysis, such as pith, collenchyma, chlorenchyma, epidermis, and its surface, mesophyll types, were diagnostic in the identification of species in the Brassicaceae family (Qader, 2018). In plant systematics, To precisely define species, taxonomists employ morphology, anatomy, and palynology, among other disciplines (Hameed et al., 2022; Jehanzeb et al., 2020; Shah et al., 2019; Ullah et al., 2021).

The Fabaceae (Leguminosae) family, also commonly termed as pea or bean family, contains approximately 770 genera and 19500 species (Beech et al., 2017). After Asteraceae and Orchidaceae, it constitutes the third-largest angiosperm family concerning diversity and ranks second in the context of revenue to agriculture. It is also recognized as the most represented family in the Neotropics and Africa's tropical rainforests and arid woods (Lattar et al., 2020). Taxonomically, Fabaceae has been subdivided into different subfamilies such as Caesalpinioideae (Mimosoideae), Faboideae (Papilionoideae), Duperquetioideae, Dialioideae, Detarioideae and Cercidoideae (Gomes et al., 2018). Worldwide subfamilies Faboideae (Papilonoideae) and Mimosoideae account for around 9.4% of the eudicot population (Uzma et al., 2012). The diverse family Fabaceae contains predominantly perennial as well as annual herbs, shrubs, and trees that are identifiable by their typical legume (fruit), a diagnostic character of Fabaceae, besides their compound stipulated leaves (Christenhusz et al., 2016).

Members of the Fabaceae family typically exhibit xerophytic characteristics. Their root system predominantly consists of a taproot that often branches extensively. The stem is primarily herbaceous or woody, erect in growth habit, and may possess climbing capabilities facilitated by tendrils. Leaves within this family are characterized as simple, pinnately compound trifoliate, or palmate in arrangement, always positioned alternately, and typically feature leafy stipules. The inflorescence configuration is typically racemose, occasionally solitary (Lashin, 2006). Flowers in Fabaceae are perigynous, displaying zygomorphic symmetry, being bisexual, irregular in shape, and exhibiting complete floral structures. Legumes of the Fabaceae family may either be dehiscent or indehiscent, and they commonly display marginal placentation. Seeds may be present with or without endosperm. The fruit type is typically a legume or pod. Pollen grains in Fabaceae are largely uniform, primarily characterized by radial symmetry, isopolarity, tricolporate apertures, and a prolate shape. Their surface ornamentation typically takes the form of a reticulate, perforate, and regular pattern (Lashin, 2006).

Fabaceae family is considered cosmopolitan because to the large number of species, which are mostly found in tropical climates. It is however most likely to occur in dry woods of America and Africa, while certain varieties of the family are also present in tropical rainforests (Bruneau et al., 2008). They are abundant in Pakistan's temperate, sub-temperate, grassland, and timber grassland habitats, as well as subtropical locations (Ghafoor, 2002). Plants of the Fabaceae family are widely distributed in dry grasslands. In Pakistan, this family holds the third position among flowering plants with 104 genera and 514 species (Jahan et al., 1994). Many plants of the Fabaceae subfamily are significant in agriculture and food, including *Phaseolus* spp. (beans), *Medicago sativa* L. (alfalfa), *Glycine max* L. (soybean), *Cicer arietinum* L. (chickpeas), *Pisum saivum* L. (pea) etc. *Glycyrrhiza glabra* L., *Crotolaria albida* Heyne ex Roth, *Mucuna pruriens* var. *utilis* are medicinal plants that have been used to treat a variety of disorders for years. Few species of this family, for instance, *Lathyrus odoratus* L., *Butea frondosa* (Lam) Taub, *Lupinus hirsutus* L. are ornamental plants of Fabaceae (Wojciechowski et al., 2004; Rahman & Parvin, 2014).

The Lamiaceae is called the mint family one of the biggest families of plants on earth. Mint family representatives are prevalent in temperate warm and climate areas of the world. The mint family is one of the families present widespread in the world. This family comprises about 3000 species and 200 genera. It is recognized by 53 genera and 360 species present in Central Asia (Yusupova, & Baratjon, 2022). The largest genera reported are *Nepeta* (200), *Salvia* (900) *Hyptis* (280), *Coleus* (325), *Thymus* (220), *Teucrium* (250), *Scutellaria* (360), *Plectranthus* (300), and *Clerodendrum* was once contain about genus 400 species (Venkateshappa and Sreenath, 2013), but in year of 2010, it had been reported to about 15 genera (Yuan et al., 2010).

The mint family is frequently common in Mediterranean countries with the fact that many of them release essential oils in maximum amounts which enables them to survive in warm seasons of summer Rama Rao et al. (2015). It is almost cosmopolitan in spreading and is used in culinary, medicinal plants, and as a vegetable all over the world (Naghibi et al., 2022). Traditionally, this family has been considered closely associated with the Verbenaceae (Venkateshappa & Sreenath, 2013). In the 1990s, the studies of phylogeny recommended that many genera classified in the Verbenaceae related to the Lamiaceae (Cantino et al., 1992: Wagstaff et al., 1989). This family member inhabits approximately all climatic conditions. In biochemical studies, mint is characterized by the occurrence of essential oils, contain medicinal properties and have been utilized in the pharmaceutical, perfume industry and cosmetics (Sharma and Bhadange, 2013).

The Lamiaceae medicinal plants have great importance in medicinal, socio-cultural, and spiritual use in tribal and rural people. About 70% to 80% of family used by the global population for their therapeutic and medicinal effects (Venkateshappa and Sreenath, 2013). Many family members are widely cultivated, for aromatic qualities as their cultivation is easy. As well also grown for their decorative and edible leaves, and some are grown for food purposes and foliage such as *Coleus* (Raja, 2012). The members are a great source of biologically active compounds containing saponins, aromatic essential oils, organic acids, and tannins, the family consists of medicinal plants that, have a lot of medicinal usages having antispasmodic, sedative, tonic, diuretic, antifungal, anti-inflammatory and antimicrobial and antiseptic properties (Rao et al., 2015; Sharma and Bhadange, 2013; Rai et al., 2013).

1.6 Northern-Baluchistan Pakistan

Baluchistan's boundaries are located at 24° north latitude and 60° east longitude. The province makes up 347190 km^2, or 44%, of the country's total land area (Provincial Census Report, 1998). It extends from the Gomal River in the northeast to the Arabian Sea in the south, and from the borders of Iran and Afghanistan in the west and northwest to the Sulaiman Mountains and Kirthar Hills in the east. Baluchistan can be divided into two distinct regions. (i) To the northeast, a long range of rocky hills is positioned between Afghanistan and the Indus plains. This area is surrounded by the Sulaiman range in the east and the Toba-Kakar range in the northwest. (ii) The Sulaiman main range, which gradually drops in height from north to south, is the primary geographical feature in northeastern Baluchistan. Baluchistan is blessed with a diverse range of flora and wildlife due to its unique ecological conditions (Anonymous, 1998).

Plate 1. Toba kakari, the southern offshoot of the sulaiman mountains, in Zhob, Baluchistan

There are 33 districts in the province, with Quetta, Pishin, Killa Abdullah, Zhob, Loralai, Harnai, Sibi, Ziarat, and Musakhel making up the northern Baluchistan district. Pakistan's hotspots for indigenous and medicinal plants include the mountains of Northern Baluchistan (Bibi et al., 2015). With more than 44% of its total land area, Baluchistan is Pakistan's largest province. Climate range from arid to semiarid, with tropical coastal regions and mild transitional zones to the north. The principal ecological zones include desert, dry temperate forest, subtropical forest, tropical dry mixed deciduous woodland, and mangrove forest. As a result of its different biological conditions, Baluchistan is fortunate to have a wide range of plants and animals (Ahmed et al., 2020; Saifullah et al., 1997).

Plate 2. Arid-semid arid lands of Loralai, Baluchistan

1.6.1 Dicot Phytodiversity of Northern Baluchistan, Pakistan

The important dicot families of Baluchistan are Asteraceae, Fabaceae, Lamiaceae, Boraginaceae, Polygonaceae, Chenopodiaceae, Ranunculaceae, Berberidaceae, Brassicaceae, Rosaceae, Mimosaceae, Zygophyllaceae, Euphorbiaceae, Malvaceae, and Myrtaceae (Rafay et al., 2013). These families included many important genera such as *Astragalus, Berberis, Launaea, Heliotropium, Alyssum, Lepidium, Nepeta, Salvia, Artemisia, Peganum, Indigofera, Tephrosia, Euphorbia, Malva, Tamarix, Lappula, Withania, Plantago, Cucumis, Centaurea, Cousinia, Jurinea,* and *Pulicaria.* Few studies have been conducted in Baluchistan in the past by different scholars, such as from Quetta city, Hingol National Park, Barkhan, Derabugti, Makran, Musakhel, Barkhan, Kalat, Khuzdar, and southern Baluchistan (Shah et al., 2006; Bibi et al., 2015; Qureshi, 2012; Manzoor et al., 2013). However, none of these studies have included a report on anatomical and palynological determination. *Periploca aphylla*, *Tecoma undulata*, *Prosopis spicigera*, *Withania coagulans*, *Taverniera nummularia*, and *Tamarix gallica* are found in low-lying locations with water availability. In addition, a large number of medicinal plants are also found in Balochistan; some of these are: *Calotropis procera*, *Cynodon dactylon*, *Malvastrum coromandelianum*, *Melilotus alba*, and *Portulaca oleracea* L. (Baloch et al., 2000).

1.7 Background and Justification of the Present Study

Baluchistan is blessed with a diversity of wild plants, but modern systematic studies (e.g., anatomical studies, pollen atlases) are scarce in the literature. The flora of northern Baluchistan is not documented in palynological or anatomical records (Ali, 2008). The morphology of monocot and dicot species in flora and literature is incomplete due to insufficient characters. Keeping in mind, additional characteristics including palynology, anatomy, and seed morphology are required for accurate identification. Plant species have been classified using both the morphological traits of the pollen and the micromorphological traits of the pollen grains (Talebi et al., 2012). Additionally, pollen features such as exine sculpturing, Amb, NPC, aperture details, polarity, symmetry, polar region, apocolpium, and spine details can be useful in the taxonomic studies of dicot families of Baluchistan at both generic and infra-specific ranks because they exhibit certain variations not covered in previous research (Taia, 2005). There are currently no reports on the petiole anatomy of the different plant families in the area under study. The distinction of similarities and differences between the various taxa of the dicot flora based on the petiolar anatomical features, including new insights regarding vascularization and histology (Taia, 2005) is significant taxonomically. According to Akinnubi et al. (2013), petiole anatomy is significant since it may be used to identify and categorize plants as well as track the effects of the environment.

The macromorphological and micromorphological characteristics of the pollen have been used in the taxonomy of plant species (Talebi et al., 2012). Baluchistan is home to a vast array of wild plants. This study intended to collect data on the dicot flora of the Baluchistan region for further investigation. Due to the lack of characteristics, necessary for a more reliable identification of plants belonging to various families, the morphology of the species described in the literature and flora is incomplete. The detailed morphological characteristics is significant in the precise identification. Anatomical approaches are valuable in taxonomic research because they provide insights into petiole anatomy and aid in plant identification and classification (Akinnubi et al., 2013). Since the petiole anatomy of many plant species in the study area has not been investigated before, this research is significant for the systematics of the flora of the area. The research is important in the precise identification of the similarities and variances of the dicots under examination by comparing different species of collected families based on petiole anatomical features, providing new insights on vascularization and sclerification.

1.8 Objectives

- Collection of dicotyleden flora of Baluchistan throughout the flowering and seed-bearing stages. Identification using renowned taxonomists, herbariums, and internet flora, and then deposited at the Herbarium of Pakistan, (ISL) QAU.
- Selection of leading families based on the abundance of species.
- Using LM and SEM to investigate the palynological, petiole/leaf base anatomical aspects of selected families of wild species from Northern Baluchistan.
- Applying palynological, and anatomical features to construct taxonomic keys, dendrograms, correlation and loading plots, and PCA to show relatedness and differences across the taxa of selected families.
- Characterization of palynological and anatomical traits as significant taxonomic aids for identifying, differentiating, and defining wild dicotyledon flora from Northern Baluchistan.

Chapter 2

Material and Methods

The study was conducted in the Plant Systematics and Biodiversity Lab, Department of Plant Sciences, and Herbarium of Pakistan Islamabad (ISL), Quaid-i-Azam University, Islamabad. The selected flora of Northern Baluchistan, Pakistan with special emphasis on palynology and anatomy was the subject of the study. The research was divided into two main sections of systematics studies: (i) Petiole anatomy (LM) and (ii) Palynology (LM and SEM) (qualitative and quantitative).

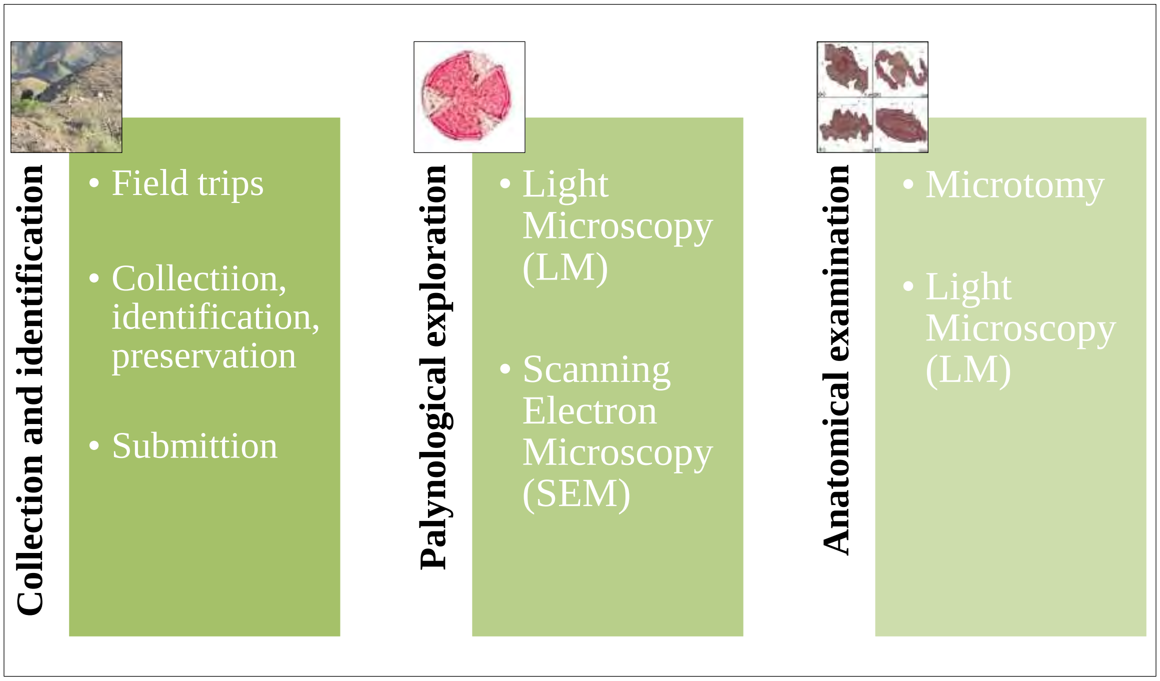

Figure 1. Graphical representation of the steps of methodology

2.1. Northern Baluchistan: An Overview

This research work was carried out across the different areas of Northern Baluchistan Pakistan. Baluchistan is located at 60 degrees east longitude and 24 degrees north latitude. The area of Baluchistan is 44% (347190 km^2) of the total land area of Pakistan (Provincial Census Report, 1998). It stretches from the borders of Iran and Afghanistan in the west, northwest to the Sulaiman Mountains, Kirthar Hills in the east, and from the Gomal River in the northeast to the Arabian Sea in the south. Baluchistan can be separated into two distinct areas. (i) Long ridges of rugged hills stretch to the northeast, sandwiched between Afghanistan and the plains of the Indus. (ii) The Toba-Kakar range in the northwest and the Sulaiman range in the east. The main topographical feature in northeastern Baluchistan is the Sulaiman main range. Baluchistan is blessed with a variety of flora and wildlife (Anonymous, 1998). Northern Baluchistan include Quetta, Pishin, Killa Abdullah, Zhob, Loralai, Harnai, Sibi, and Ziarat (Bibi et al., 2015). Less than 8 inches (20 cm) of rain falls in Baluchistan each year; in the northeast, it rises to around 15 inches (38 cm), and in the northwest, it drops to less than 3 inches (7.6 cm). Due to western disturbances, most of the rain falls, throughout the winter. The summer monsoon is significant in the northeast only. The coastal areas experiences mild summer temperatures, little precipitation, and a constant sea wind (Jan et al., 2021).

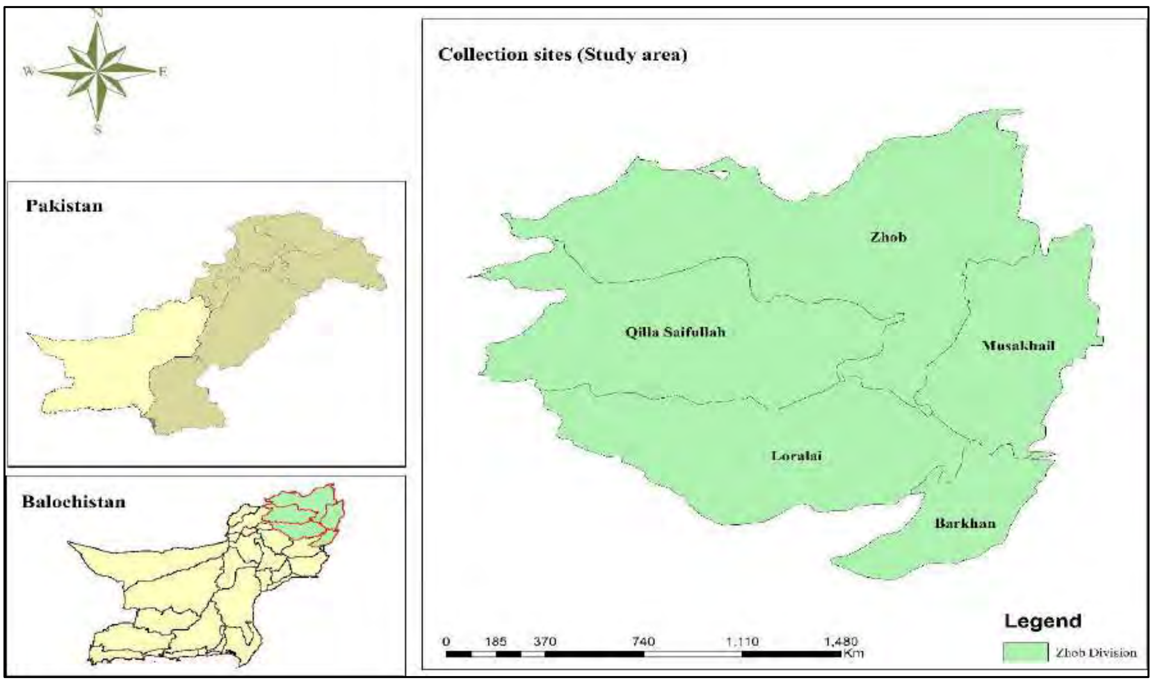

Figure 2. Map of Northern Baluchistan representing the study sites

2.2. Plant specimens: Collection and Preservation

Field trips were carried out from March 2022 to May 2023 to collect specimens from different phytogeographical regions of Northern Baluchistan. Followed the 'Manual of All Taxa Biodiversity Inventories and Monitoring's approved and detailed standard field protocols' during the collection (Eymann et al., 2010). More than two specimens of most species were collected. For a few rare species, only one or two specimens were collected. The mounting and preservation of the specimens were done following the standard protocols of Eymann et al. (2010), and Judd et al. (2002). Quetta, Loralai, Ziarat, Zhob, Sanjavi, Pathankot, Qilla-saifullah, Hanna Lake, Pathankot, Chiltan

National Park, and Otmanzai were the study sites. Fresh plant specimens were collected with both floral and vegetative parts. Plant specimens were dried, compressed, and then placed in blotting sheets and newspapers. A digital camera (Sony, DSC W800) was used to take field photographs of plants and the collection sites. The field notes included comprehensive information about the plants, such as the collection date, voucher number, place, flowering period, geographic coordinates, habitat, and habit. After the collection, the specimens were preserved using ethanol (50 mL absolute) and mercuric chloride before being fumigated.

Plate 3. Fumigation (a) (b), mounting herbarium specimen with details (c) and light microscopic analysis (d)

The plant specimens were identified through comparison and with the help of herbarium specimens from the Quaid-e-Azam University Herbarium (QAU), Islamabad, Pakistan, and other related flora, such as China (Chen and Craven, 2007), North America (Torrey and Grey, 1969), and Pakistan (Nasir and Ali, 1971; Stewart et al., 1972).

Furthermore, the plants were authenticated by expert services of well-known taxonomists Professor Dr. Mushtaq Ahmad (Quaid-e-Azam University) and Dr. Amir Sultan (Director National Herbarium NARC). The Plant List (TPL) and the International Plant Name Index (IPNI) were used to confirm the accepted names of plants. Specimens were then mounted and annotated with the location, date, and name of the collector on standard herbarium sheets. Each specimen was given an accession number and submitted to the Herbarium of Pakistan (ISL) QAU.

2.3 Anatomical Study of Dicot Flora

2.3.1 Tissue Preparation

a) Section Cutting

The petioles were treated for four-hours with a 10% saline formal solution (the solution being changed twice). Subsequently, the samples were treated for one hour with varying concentrations of methanol (70%, 80%, 90%, 100%, and 100% again). Removed the methanol (dealcoholization) with two applications of xylol (one hour each). The samples were then impregnated with wax twice for one hour at 58–62 ^{o} C. The samples were embedded by section cutting using microtomy at 3–5-micron thickness. The melting of the prepared slides was completed at 62 ^{o} C (Akhtar et al., 2022).

b) Staining

The samples were deparaffinized twice for five minutes using xylol. Then rehydrated by applying methanol at 100%, 90%, and 70% concentrations for one minute each. Then washed for one minute with tap water. Haematoxylin, a basic stain, was applied for five minutes and then rinsed with tap water for one minute. After that, the slides were dipped in 1% acid alcohol and washed again for one minute with tap water. The slides were then treated with 1% eosin for 30–60 seconds and washed for one minute with tap water for cleaning. For the final dehydration the slides were treated with 70%, 80%, 90%, 100%, and 100% methanol for 30 seconds. The xylol was applied for one minute to clean the prepared slides (two treatments). The sections were mounted on the slides using Dibutylphthalate Polystyrene Xylene (DPX). Labelled the slides and used light microscopy to examine them (Akhtar et al., 2022).

2.3.2 Light Microscopy (LM) Visualization of Petiole Anatomy

Under light microscope, petiole anatomical were recorded for Brassicaceous, Fabaceous and Lamiaceous species. Using a calibrated eyepiece, the qualitative and quantitative characters were observed (Arnold, 1973). The description of traits was accomplished by Metcalfe and Chalk (1979); Metcalfe (1973); Heneidak and Shaheen (2007); Chen et al. (2008). The length and width of different cell types, cell layers, and other characteristics were noted. Table 2.1 provided the key for the anatomical parameters that were observed.

2.4 Palynological Examinations of dicots in Northern Baluchistan

2.4.1 Light Microscopic Observations of Pollen

The procedure described by Moore et al. (1991) was used for pollen extraction, as well as the chemical preparation methods. The anthers were separated from the flowers and the pollen morphology was visualized under light microscopy. Before the acetolysis process, the pollen grains were boiled in 10% KOH for around 6–10 minutes to help in aperture opening and to assess the aperture features (Reitsma, 1969). With certain modifications, pollen slides were prepared using the standard acetolysis method (Erdtman, 1969) (Zafar et al., 2007). On a glass slide, anthers were placed using needles and forceps. Using a glass rod, smashed the anthers on the glass slides after adding two or three drops of acetic acid for 4–8 minutes to expose the sculpturing patterns and clean the outer pollen surface. The debris was removed with the help of a needle. The samples were dyed and mounted with glycerine jelly. The pollen samples were sealed using the coverslip, and transparent nail polish, and then labeled the slides. The terminologies for the characteristics of pollen were noted provided by Punt et al. (2007) and Grant-Downton (2009).

The prepared slides were observed under LM at different resolutions. The photos were taken with a Leica Light Microscope (Model 1000) equipped with a digital camera, the Infinity 1-5 C-MEL (Canada). For the qualitative and quantitative palynological characters, twenty readings were noted. The terms used to describe the pollen characters were taken from Grant-Downton (2009) and Punt et al. (2007). Many palynological characteristics, including pollen fertility, polar axis, P/E ratio, exine thickness, pore length, pore width, colpus length, and width were observed. The findings were tabulated to provide the maximum, and minimum values along with mean ± standard error.

2.4.2 Scanning Electron Microscopy (SEM) of Pollen

The methodology of Bahadur et al. (2020) was used for the scanning electron microscopy (SEM). Using a needle, two drops of acetic acid were applied to the anthers to remove the debris. A double-coated Scotch tape was used to attach the samples to the counterfoil. An additional gold palladium sputter coating was applied to the specimen. Micromorphological features of the pollen were analyzed under SEM at the University of Peshawar, Pakistan's Central Resource Library (CRL), Department of Physics. A Polaroid P/N 655 film that was placed in the SEM was used to capture the photos of the pollen. Both qualitative and quantitative characters were examined using a standard check sheet (for diagnostic features). Table 2.2 provides the key for the features that have been observed.

P/E ratio (Quantitative analysis)

Ratio of P/E was determined using the given formula below.

P/E × 100

where E is the equatorial diameter and P is the polar axis. The size and shape of pollen were examined based on the P/E ratio (Hussain et al., 2019). The largest axis, which may be the highest value of the polar axis or the equatorial diameter, was used to assign the size of the pollen. The nomenclature used for the pollen size and shape classes was that of Erdtman (1963). Erdtman (1963) classified the grains into six size classes, ranging from very small (<10µm) to giant (>200µm), and eight shape classes.

Viability

Using the provided formula, the vitality of the pollen was calculated quantitatively.

F/F+S*100

F = Fertility

S = Sterility

The number of fertile pollens was represented by F, and the amount of sterile pollen by S.

Number Position Characters (NPC) Classification

The NPC-System classification was used for the name and formula of the pollen, based on its apertures, Number (N-whether single or two or many), Position (P-polar: distal or proximal; global; meridional), and Characters (C-circular or elongated). In this method, keys for the classification of pollen grains have been developed using the term "treme," which denoted an opening. Words like mono, di, and tri were used to describe the number of apertures. The positions of apertures were labeled as cata, ana, zono, and so on. A variety of aperture types, including porate, colpate, colporate, and pororate, were noted in the character (Erdtman, 1969).

2.5 Statistical Analysis

Plant systematics relies heavily on the evaluation of both qualitative and quantitative information in the distinction of species, genera, and tribes. The study employed SPSS-16 to determine the mean and standard values for the quantitative palynological and anatomical parameters. PAST (version 4.11), NCSS (20230), and Origin (2023) were utilized to investigate principal component analysis (PCA), dendrogram Un-weighted Pair Group Clustering Method (UPGMA), and correlation ($p<0.05$) (Iamonico et al., 2023). The general distribution and an analysis of the mean differences are presented in the box plot.

2.6 Taxonomic Key

A dichotomous taxonomic key that aids in species identification was developed using qualitative palynological and anatomical features.

Table 1. Key for the observed anatomical traits

S/No	Qualitative traits	Quantitative traits
1	Wings of petiole	Petiole size
2	Groove in the upper surface	Epidermal cell number of layers
3	Trichomes	Epidermal cell size
4	Shape / outline (culm, petiole)	Collenchyma cell number of layers
5	Cuticle	Collenchyma cell size
6	Epidermal cell shape	Chlorenchyma cell number of layers
7	Subepidermal ring of collenchyma	Chlorenchyma cell size
8	Collenchyma cell shape	Parenchyma cell number of layers
9	Chlorenchyma cell shape	Parenchyma cell size
10	Parenchyma cell shape	Sclerenchyma cell number of layers
11	Sclerenchyma cell shape, presence in hypodermis	Sclerenchyma cell size
12	Vascular bundle arrangement	Vascular bundle number
13	Xylem vessel shape	Xylem vessel size
14	Phloem shape Xylem and phloem parenchyma	Phloem cell size
15	Sclerenchyma presence in vascular bundles	

Table 2. Key for the observed palynological characters

S/ No	Qualitative traits	Quantitative traits
1	Symmetry	Polar axis
2	Polarity	Equatorial diameter
3	Unity	P/E
4	Size class	Mesocolpium
5	Shape class	Number of apertures
6	Operculum	Polar width of colpi
7	Polar view	Polar length of colpi
8	Equatorial view	Equatorial length of colpi
9	Exine sculpturing	Equatorial width of colpi
10	Exine surface	Pore length
11	Colpi apex	Viability
12	Aperture membrane	Exine thickness
13	Pore orientation	
14	Colpi apex	
15	Arrangement of apertures	
16	Lumen shape	
17	AMB	
18	NPC classification (Name and formula)	

Plate 4. The study area Loralai is a range land region, with arid-semiarid weather

Plate 5. The picnic point Pathankot, a comparatively green area with specific flora in contrast to its district Loralai

Plate 6. The study area Sanjavi has a unique flora even in the highly stony soil formations

Plate 7. View of Takht-e-Sulaiman mountains range in district Zhob, Baluchistan

Plate 8. Hazarganji Chiltan National Park is a national park in the Mastung District of western Baluchistan Province of Pakistan

Plate 9. Ziarat, Baluchistan. Some mature trees are thought to be 4,000 –5,000 years old, they are known as living fossils

Chapter 3

Results and Discussion

Section-I

Summary

The northern Baluchistan, Pakistan is blessed with diverse dicot flora. Phytogeographically, it is one of the important floristic regions on earth. Most of the flora of this area is used medicinally. Baluchistan is also home to many endemic plants. This research was carried out in different areas of northern Baluchistan, Pakistan. These areas were explored for the first time for palynological and anatomical studies of dicots.

- In the first part of this section, the details about the collected 107 dicots, belonging to three families, Brassicaceae, Fabaceae, and Lamiaceae are provided. The checklist for each family is given, including the accepted botanical name with author citation, accession number, the areas from where they were collected, their collector's name, habitat, and elevation.
- The second part of this section includes the field photographs of the collected dicots of three selected families. Botanical names are given along with some information on each plant noted during the field.

Table 3. The collected taxa of Brassicaceae from Northern Baluchistan, Pakistan.

S. No	Name of Species	Accession No	Locality	Habitat	Elevation (m)	Collector Name
1.	*Aethionema carneum* (Banks & Soland.) B.Fedtsch.	133144 (ISL)|QAU	Loralai	Sandy soil	850	Wajia Noor
2.	*Alyssum desertorum* Stapf	133145(ISL)|QAU	Loralai	Gravely soil	928	Wajia Noor
3.	*Alyssum turkestanicum* Regel & Schmalh.	133170(ISL)|QAU	Loralai	Stony ground	1555	Wajia Noor
4.	*Brassica oleracea*var. *capitata* Linn	133146(ISL)|QAU	Loralai	Agriculture field	1153	Numan
5.	*Capsella bursa-pastoris* (L.) Medik.	133147(ISL)|QAU	Loralai	Sandy soil	1302	Wajia Noor
6.	*Cardaria chalepensis* (Linn.) Hand.-Mazz.	133148(ISL)|QAU	Loralai	Moist sandy	1433	Wajia Noor
7.	*Cardaria draba* (L.) Desv.	133149(ISL)|QAU	Loralai	Muddy soil	1050	Wajia Noor
8.	*Chorispora tenella* (Pall.) DC.	133171(ISL)|QAU	Ziarat	Stony hill	2123	Wajia Noor
9.	*Clypeola aspera* Turrill	133172(ISL)|QAU	Loralai	Stony ground	1586	Numan
10.	*Coincya tournefortii* Gouan) Alcaraz, T.E.Díaz,	133150(ISL)|QAU	Loralai	Gravely soil	1208	Numan
11.	*Conringia orientalis* (L.) Andrz.	133151(ISL)|QAU	Loralai	Soil crevics	1563	Wajia Noor
12.	*Descurainia sophia (Linn.)*	133152(ISL)|QAU	Zhob	Gravely soil	1497	Wajia Noor
13.	*Dilophia salsa* Thomson	133173(ISL)|QAU	Loralai	Saline	870	Wajia Noor
14.	*Diplotaxis griffithii* (Hook. f. & Thomson) Boiss.	133174(ISL)|QAU	Nushki	Gravelly soil	944	Wajia Noor
15.	*Diplotaxis harra* (Forssk.) Boiss.	133153(ISL)|QAU	Qilla Saifullah	Mountain slope	1280	Wajia Noor
16.	*Draba hystrix* Hook. f. & Thomson	133175(ISL)|QAU	Chaman	Silt	1408	Wajia Noor
17.	*Eruca sativa* Mill.	133154(ISL)|QAU	Zhob	Gravely soil	1366	Wajia Noor

18. *Farsetia hamiltonii* Royle	133155(ISL)|QAU	Zhob	Sandy soil	1421	Wajia Noor
19. *Farsetia heliophila* Bunge ex Coss	133156(ISL)|QAU	Zhob	Gravely soil	1271	Wajia Noor
20. *Goldbachia pendula* Botsch.	133157(ISL)|QAU	Zhob	Gravely soil	1213	Wajia Noor
21. *Isatis minima* Bunge	133176(ISL)|QAU	Pishin	Sandy soil	1496	Sadia
22. *Isatis stocksii* Boiss.	133177(ISL)|QAU	Loralai	Sandy soil	950	Sadia
23. *Lepidium aucheri* Boiss.	133158(ISL)|QAU	Pathankot	Sandy soil	1301	Wajia Noor
24. *Leptaleum filifolium* DC.	133178(ISL)|QAU	Quetta	Mountain slope	2126	Wajia Noor
25. *Matthiola flavida* Boiss.	133159(ISL)|QAU	Zhob	Sandy soil	1130	Wajia Noor
26. *Meniocus heterotrichus* Hadač & Chrtek	133179(ISL)|QAU	Chaman	Sandy clay	1326	Wajia Noor
27. *Meniocus linifolius* (Stephan ex Willd.) DC.	133160(ISL)|QAU	Zhob	Gravely soil	1051	Wajia Noor
28. *Nasturtium officinale* W.T.Aiton	133161(ISL)|QAU	Zhob	Mountain slope	1056	Wajia Noor
29. *Notoceras bicorne* (Aiton) Amo	133162(ISL)|QAU	Loralai	Gravely soil	966	Wajia Noor
30. *Physorrhynchus brahuicus* Hooker f.	133163(ISL)|QAU	Zhob	Gravely soil	988	Wajia Noor
31. *Raphanus raphanistrum* Linn.	133164(ISL)|QAU	Zhob	Sandy soil	833	Wajia Noor
32. *Sisymbrium altissimum* Linn.	133165(ISL)|QAU	Zhob	Gravely soil	761	Wajia Noor
33. *Sisymbrium irio* L.	133180(ISL)|QAU	Ziarat	Stony soil	1788	Wajia
34. *Strigosella africana* (L.) Botsch.	133166(ISL)|QAU	Shirani	Mountain slope	2128	Wajia Noor
35. *Strigosella cabulica* Boiss.	133167(ISL)|QAU	Loralai	Aquatic	697	Wajia Noor
36. *Strigosella intermedia* (C.A. Mey) Botsch.	133168(ISL)|QAU	Zhob	Sandy soil	1064	Wajia Noor
37. *Tetracme stocksii* Boiss.	133169(ISL)|QAU	Loralai	Moist sandy	1525	Wajia Noor

Table 4. The collected taxa of Fabaceae from Northern Baluchistan, Pakistan.

S.No	Name of Species	Accession No	Locality	Habitat	Elevation (m)	Collector Name
1.	*Alhagi maurorum*Medik.	133182(ISL)|QAU	Pishin	Sandy soil	1476	Wajia Noor
2.	*Astragalus anisacanthus* Boiss.	133183(ISL)|QAU	Ziarat	Stony soil	1765	Wajia Noor
3.	*Astragalus brahuicus* Bunge	133184(ISL)|QAU	Kalat	Gravely soil	1930	Sadia
4.	*Astragalus crenatus* Schult.	133185(ISL)|QAU	Sanjavi	Saline marches	890	Wajia Noor
5.	*Astragalus diphtherites* Fenzl	133186(ISL)|QAU	Ziarat	Stony soil	1765	Nauman
6.	*Astragalus hemsleyi* Aitchison & Baker ex Aitchison	133187(ISL)|QAU	Ziarat	Stony soil	1765	Wajia Noor
7.	*Astragalus hypoglottis* Hook.	133188(ISL)|QAU	Loralai	Moist sandy soil	1433	Wajia Noor
8.	*Astragalus ophiocarpus* Benth. ex Boiss.	133189(ISL)|QAU	Quetta	Muddy soil	1532	Wajia Noor
9.	*Astragalus oxyglottis* Steven ex M. Bieb.	133190(ISL)|QAU	Naushki	Stony	918	Wajia Noor
10.	*Astragalus stocksii* Benth. Ex Bunge	133191(ISL)|QAU	Sanjavi	Muddy soil	980	Wajia Noor
11.	*Astragalus subumbellatus* Klotzsch	133192(ISL)|QAU	Ziarat	Stony soil	1796	Wajia Noor
12.	*Astragalus tribuloides* Delile	133193(ISL)|QAU	Loralai	Gravely soil	1208	Wajia Noor
13.	*Astragalus hostilis* Boiss.	133194(ISL)|QAU	Loralai	Soil crevics	1563	Wajia Noor
14.	*Astragalus purpurascens* Bunge	133195(ISL)|QAU	Ziarat	Stony soil	1796	Wajia Noor
15.	*Caragana ambigua* Stocks	133196(ISL)|QAU	Zhob	Gravely soil	1515	Wajia Noor
16.	*Crotalaria burhia* Buch.-Ham. ex Benth.	133197(ISL)|QAU	Sibi	Clay	135	Wajia Noor
17.	*Crotalaria medicaginea* DC.	133198(ISL)|QAU	Sibi	Clay	147	Wajia Noor

18.	*Ebenus stellata* Boiss.	133199(ISL)|QAU	Zhob	Gravely soil	1527	Wajia Noor
19.	*Indigofera cuneifolia* Eckl. & Zeyh.	133200(ISL)|QAU	Hazarganji	Mountain slope	2223	Wajia Noor
20.	*Indigofera intricata* Boiss.	133201(ISL)|QAU	Hazarganji	Mountain slope	2223	Wajia Noor
21.	*Lathyrus oleraceus* Lam.	133202(ISL)|QAU	Zhob	Gravely soil	1271	Wajia Noor
22.	*Lotus corniculatus* L.	133203(ISL)|QAU	Zhob	Gravely soil	1213	Wajia Noor
23.	*Lotus garcinii* DC. ex Ser	133204(ISL)|QAU	Pishin	Sandy soil	1496	Wajia Noor
24.	*Medicago lupulina* L.	133205(ISL)|QAU	Loralai	Sandy soil	950	Wajia Noor
25.	*Medicago polymorpha* L.	133206 (ISL)|QAU	Loralai	Sandy soil	1315	Wajia Noor
26.	*Melilotus indicus* (L.) All.	133207(ISL)|QAU	Loralai	Sandy soil	1299	Wajia Noor
27.	*Onobrychis cornuta* (L.) Desv.	133208(ISL)|QAU	Zhob	Sandy soil	1130	Wajia Noor
28.	*Onobrychis dealbata* Stocks	133209(ISL)|QAU	Ziarat	Stony soil	1788	Wajia Noor
29.	*Onobrychis micrantha* Schrenk ex Fisch. & C.A. Mey**.**	133210(ISL)|QAU	Ziarat	Stony soil	1696	Wajia Noor
30.	*Onobrychis tavernierifolia* Stocks ex Boiss.	133211(ISL)|QAU	Ziarat	Stony soil	1766	Wajia Noor
31.	*Sophora mollis* Span.	133212(ISL)|QAU	Sanjavi	Saline marches	876	Wajia Noor
32.	*Taverniera glabra* Boiss.	133213(ISL)|QAU	Sherani	Clay soil	1671	Wajia Noor
33.	*Tephrosia uniflora subsp. Petrosa* (Blatt. & Hallb.) J. B. Gillett & Ali	133214(ISL)|QAU	Hingol	Muddy, sandy soil	123	Wajia Noor
34.	*Trigonella monantha* C.A. Mey.	133215(ISL)|QAU	Hazarganji	Gravely soil	761	Wajia Noor
35.	*Vicia macrantha* Jurtzev	133216(ISL)|QAU	Loralai	Sandy, Gravely	838	Wajia Noor

Table 5. The collected taxa of Lamiaceae from Northern Baluchistan, Pakistan.

S. No	Name of Species	Accession No	Locality	Habitat	Elevation (m)	Collector Name
1.	*Ajuga alpina* Vill.	133218 (ISL)|QAU	Dhana Sar	Sandy,clay soil	1771	Wajia Noor
2.	*Ajuga bracteosa* Benth.	133219(ISL)|QAU	Sherani	Sandy,clay soil	1571	Wajia Noor
3.	*Clinopodium umbrosum* **(**M.Bieb.) K.Koch	133220(ISL)|QAU	Ziarat	Sandy soil	1799	Wajia Noor
4.	*Eremostachys thyrsiflora* Benth.	133221(ISL)|QAU	Chaman	Silt	1408	Wajia Noor
5.	*Eremostachys vicaryi* Benth.	133222(ISL)|QAU	Pishin	Sandy soil	1496	Wajia Noor
6.	*Isodon rugosus* (Wall.) Codd	133223(ISL)|QAU	Quetta	Mountain slope	2090	Wajia Noor
7.	*Lallemantia royleana* (Benth.) Benth.	133224(ISL)|QAU	Zhob	Gravely soil	1025	Wajia Noor
8.	*Marrubium vulgare* L.	133225(ISL)|QAU	Sanjavi	Gravely	810	Wajia Noor
9.	*Micromeria biflora* (Buch.-Ham. ex D. Don) Benth.	133226(ISL)|QAU	Quetta	Sandy soil	1543	Wajia Noor
10.	*Nepeta bracteata* Benth.	133227(ISL)|QAU	Zhob, Ziarat	Gravely sandy	1000-1850	Wajia Noor
11.	*Nepeta cataria* L.	133228(ISL)|QAU	Ziarat	Gravely soil	1573	Wajia Noor
12.	*Nepeta eriosphaera* Rech. f. & Köie	133229(ISL)|QAU	Quetta	Gravely soil	1583	Wajia Noor
13.	*Nepeta glomerulosa* Boiss.	133230(ISL)|QAU	Quetta	Silt	1520	Wajia Noor
14.	*Nepeta griffithii* Hedge	133252(ISL)|QAU	Barikot	Clay muddy	620	Wajia Noor
15.	*Nepeta Hindostana* (B. Heyne ex Roth) Haines	133231(ISL)|QAU	Quetta	Sandy soil	1490	Wajia Noor
16.	*Nepeta juncea* Benth.	133232(ISL)|QAU	Quetta	Sandy clay soil	1574	Wajia Noor

17.	*Nepeta praetervisa* Rech. f.	133233(ISL)|QAU	Loralai	Gravely soil	1604	Wajia Noor
18.	*Ocimum africanum* Lour.	133234(ISL)|QAU	Quetta	Gravely soil	1540	Wajia Noor
19.	*Origanum majorana* L.	133235(ISL)|QAU	Loralai	Sandy soil	1600	Wajia Noor
20.	*Otostegia aucheri* Boiss.	133236(ISL)|QAU	Sanjavi	Sandy clay soil	1540	Wajia Noor
21.	*Otostegia limbata* (Benth.) Boiss.	133237(ISL)|QAU	Dhana sar	Clay muddy	1790	Wajia Noor
22.	*Phlomis stewartii* Hook. f.	133238(ISL)|QAU	Loralai	Sandy soil	1600	Wajia Noor
23.	*Salvia cabulica* Benth.	133239(ISL)|QAU	Sherani	Clay and sandy	1720	Wajia Noor
24.	*Salvia coccinea* L.f.	133240(ISL)|QAU	Ziarat	Sandy soil	1760	Wajia Noor
25.	*Salvia leucantha* Cav.	133241(ISL)|QAU	Quetta	Sandy soil	1550	Wajia Noor
26.	*Salvia macrosiphon* Boiss.	133242(ISL)|QAU	Ziarat	Sandy soil	1830	Wajia Noor
27.	*Salvia moorcroftiana* Wall. ex Benth.	133243(ISL)|QAU	Sanjavi	Gravely soil	1445	Wajia Noor
28.	*Salvia plebeia* R.Br.	133244(ISL)|QAU	Quetta	Stony soil	1590	Wajia Noor
29.	*Salvia santolinifolia* Boiss.	133245(ISL)|QAU	Makran	Clay to muddy	1520	Wajia Noor
30.	*Scutellaria linearis* Benth.	133246(ISL)|QAU	Loralai	Sandy, gravely	1405	Wajia Noor
31.	*Stachys parviflora* Benth.	133247(ISL)|QAU	Sanjavi	Sandy soil	1590	Wajia Noor
32.	*Teucrium scordium* L.	133248(ISL)|QAU	Ziarat	Sandy soil	1829	Wajia Noor
33.	*Teucrium stocksianum* Boiss.	133249(ISL)|QAU	Sherani	Clay, sandy	1400, 1596	Wajia Noor
34.	*Thymus linearis* Benth.	133250(ISL)|QAU	Ziarat	Sandy soil	1800	Wajia Noor
35.	*Thymus linearis subsp. hedgei Jalas* Benth.	133251(ISL)|QAU	Ziarat	Sandy, gravely	1792	Wajia Noor

Plate 10. Field pictorial view (a) Aethionema *carneum* (b), (c) *Alyssum desertorum* (d) *Alyssum turkestanicum*

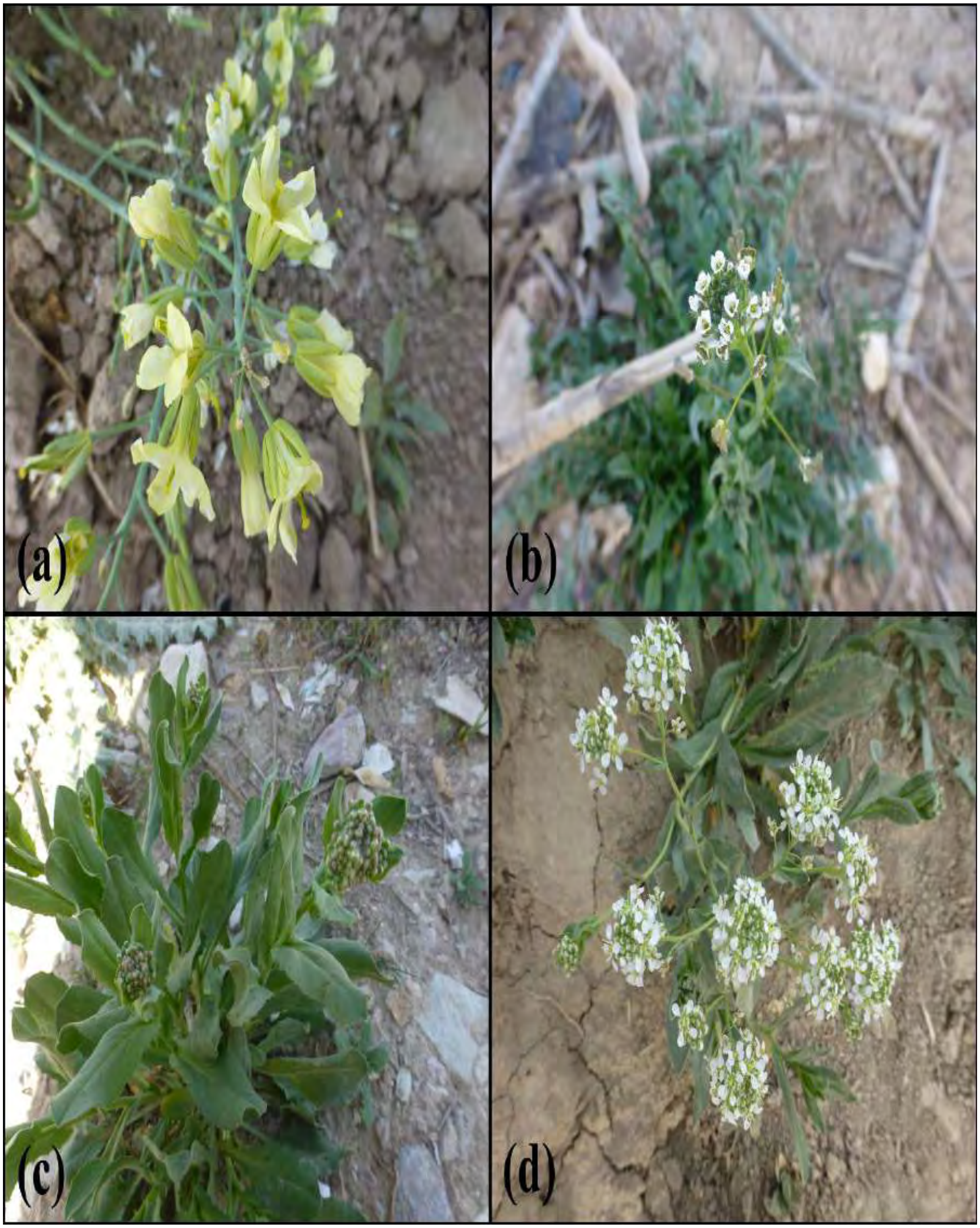

Plate 11. Field pictorial view (a) *Brassica oleracea*var (b)*Capsella bursa-pastoris* (c) *Cardaria chalepensis* (d) *Cardaria draba*

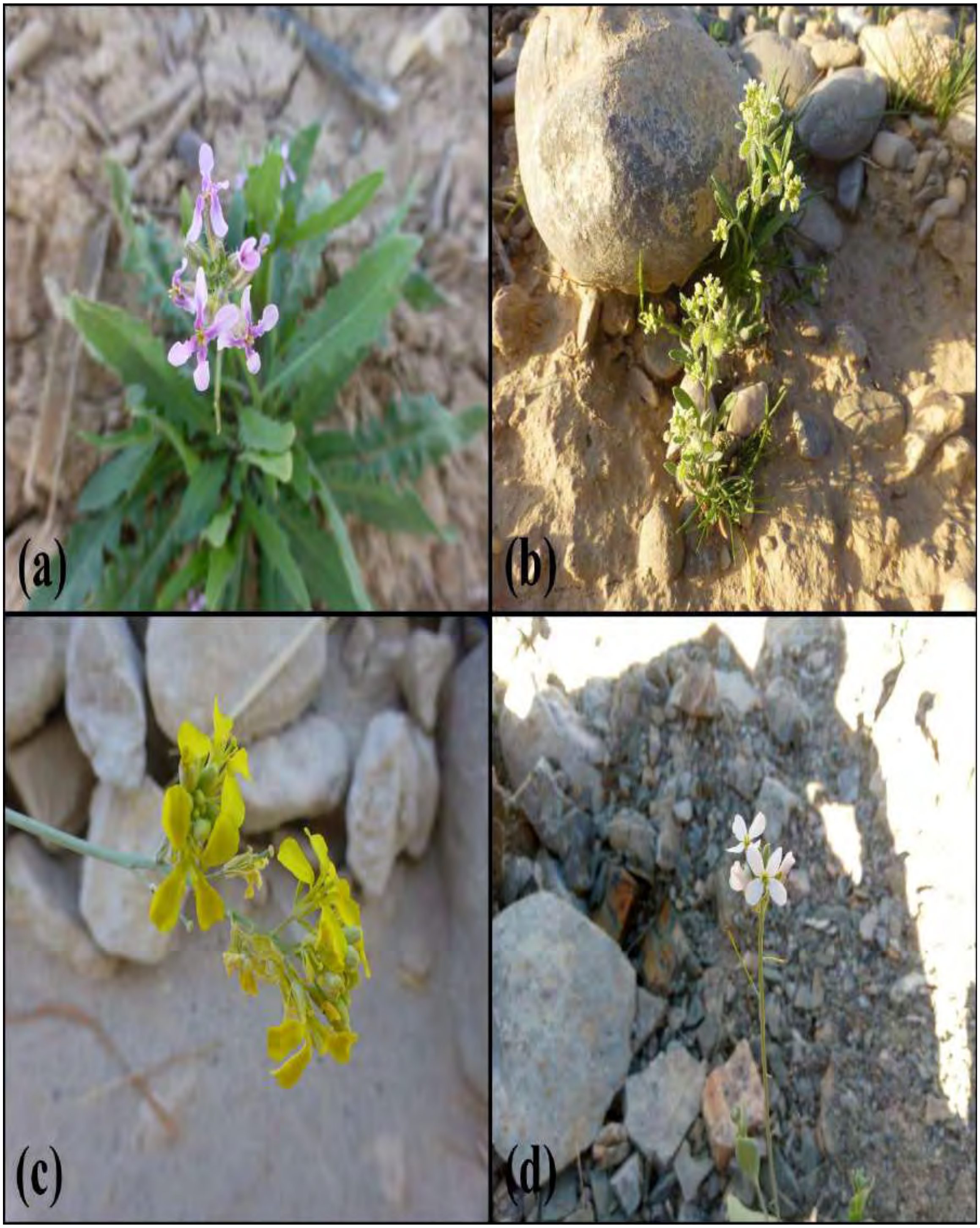

Plate 12. Field pictorial view (a) *Chorispora tenella* (b) *Clypeola aspera* (c) *Coincya tournefortii* (d) *Conringia orientalis*

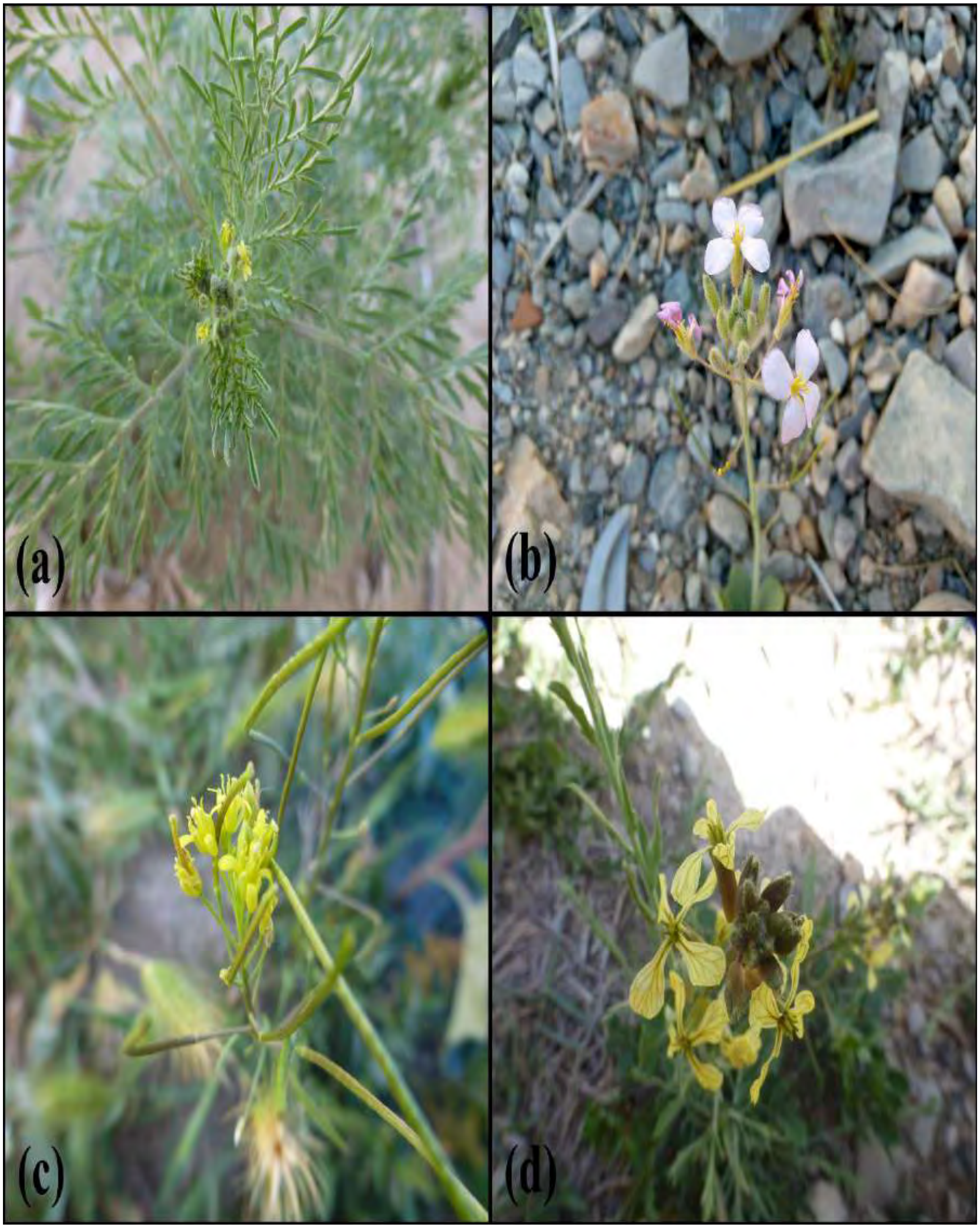

Plate 13. Field pictorial view (a) *Descurainia Sophia* (b) *Diplotaxis griffithii* (c) *Diplotaxis harra* (d) *Eruca sativa*

Plate 14. Field pictorial view (a,b) *Farsetia hamiltonii* (c) *Farsetia heliophila* (d) *Goldbachia pendula*

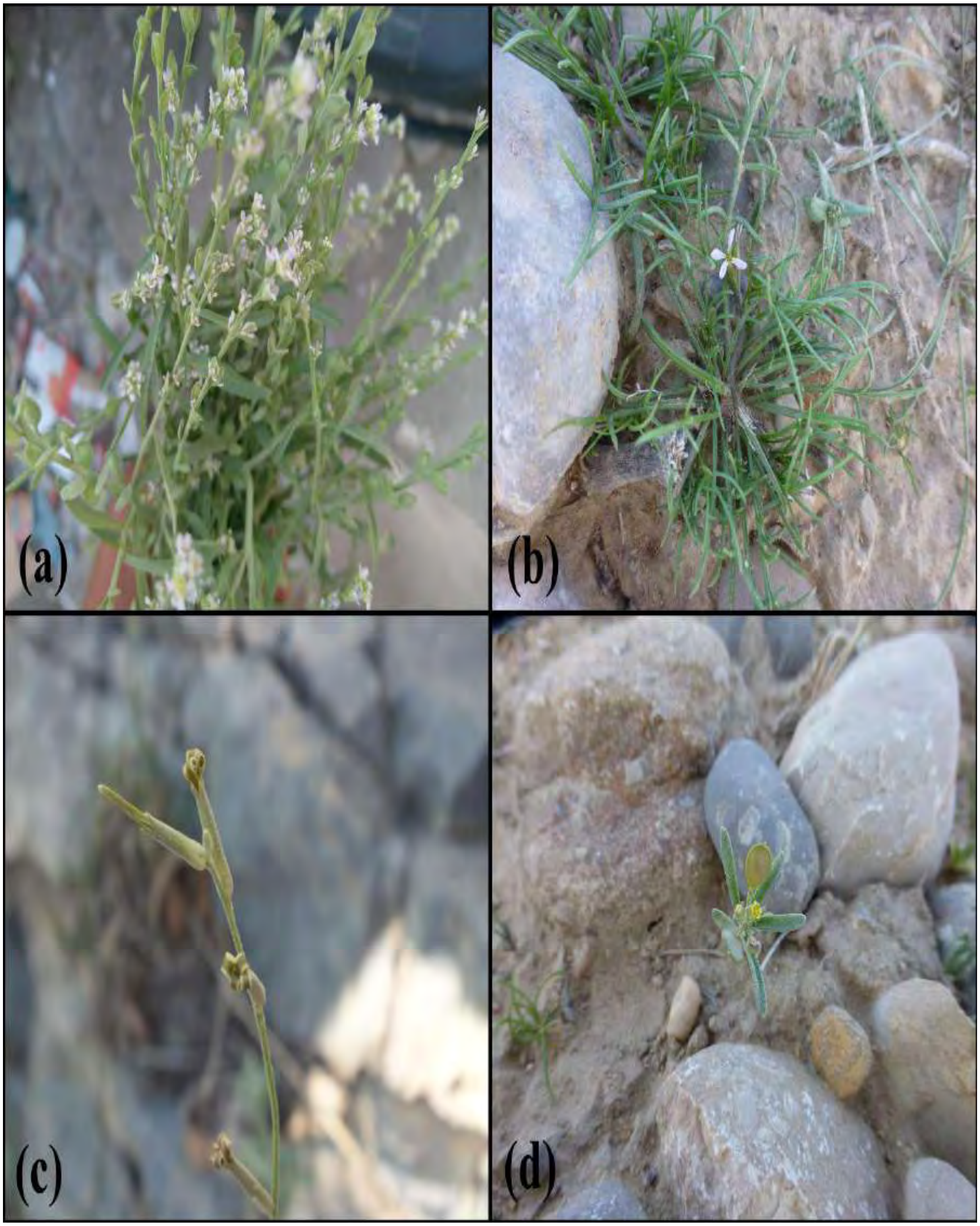

Plate 15. Field pictorial view (a) *Lepidium aucheri* (b) *Leptaleum filifolium* (c) *Matthiola flavida* (d) *Meniocus heterotrichus*

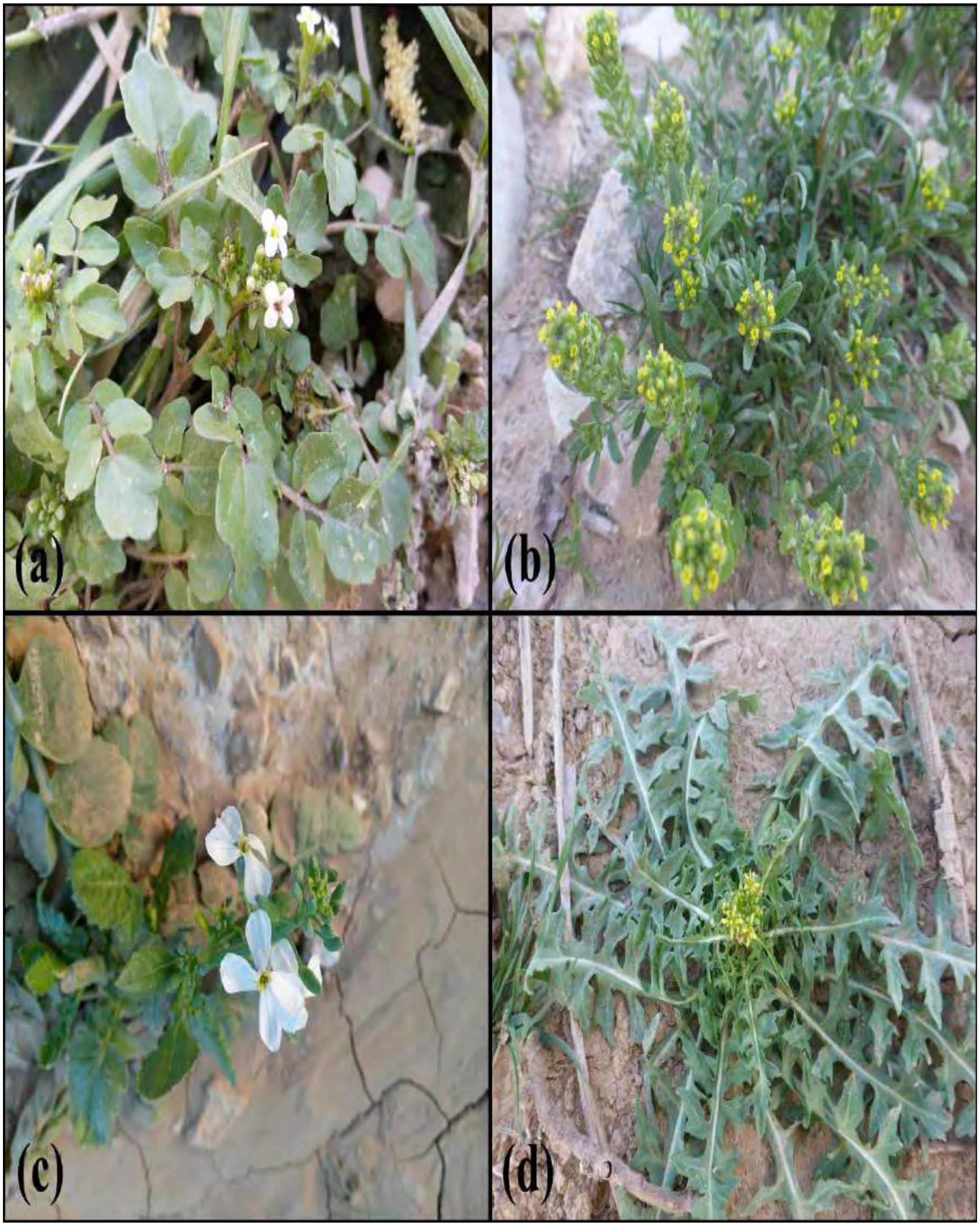

Plate 16. Field pictorial view (a) *Nasturtium officinale* (b) *Notoceras bicorne* (c) *Raphanus raphanistrum* (d) *Sisymbrium altissimum*

Plate 17. Field pictorial view (a) *Sisymbrium irio* (b) *Strigosella africana* (c) *Strigosella cabulica* (d) *Strigosella intermedia*

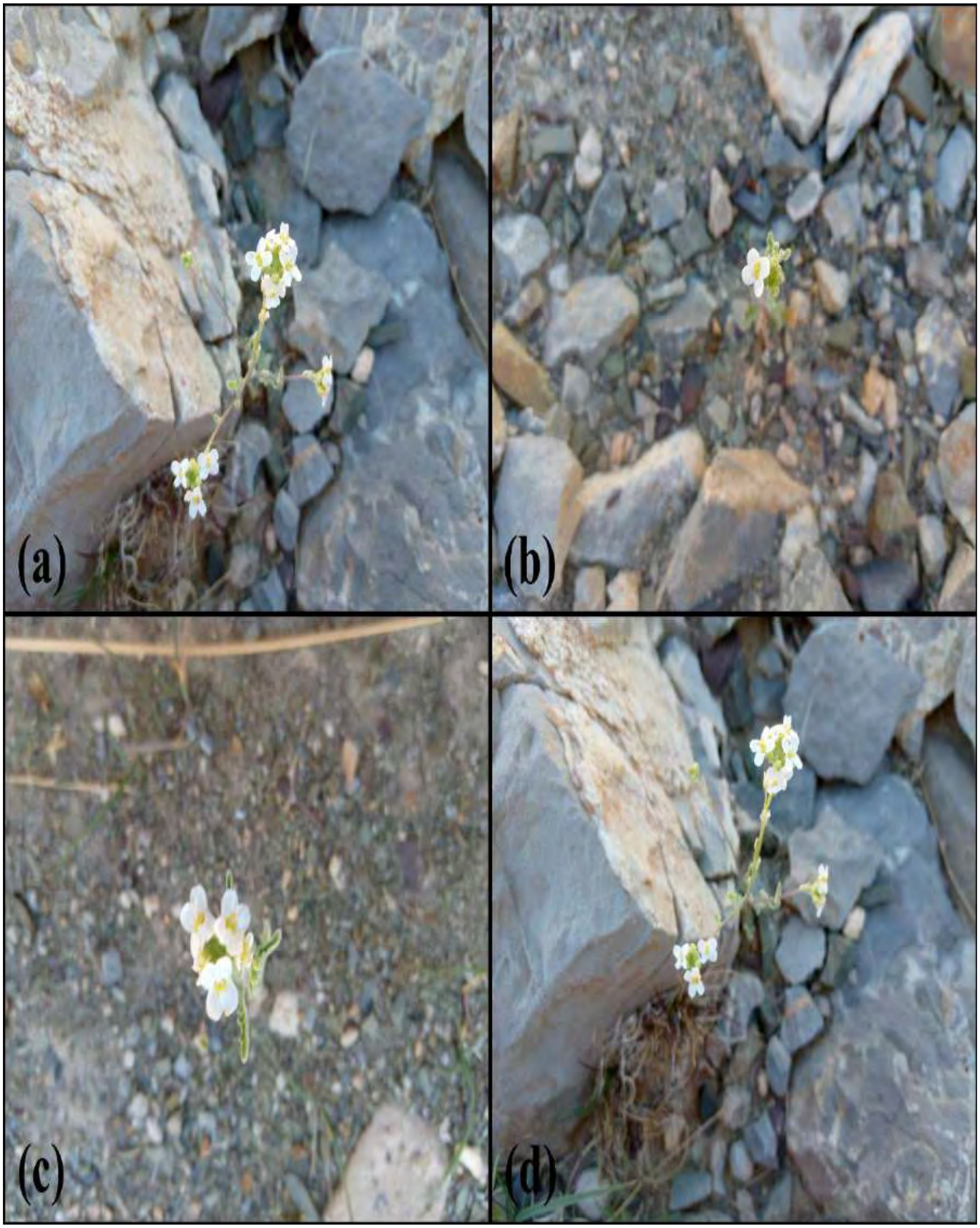

Plate 18. Field pictorial view (a), (b),(c),(d) *Tetracme stocksii*

Plate 19. Field pictorial view (a) *Astragalus anisacanthus* (b) *Astragalus crenatus* (c) *Astragalus hemsleyi* (d) *Astragalus hostilis*

Plate 20. Field pictorial view (a) *Astragalus oxyglottis* (b) *Astragalus stocksii* (c) Astragalus *diphtherites* (d) *Astragalus subumbellatus*

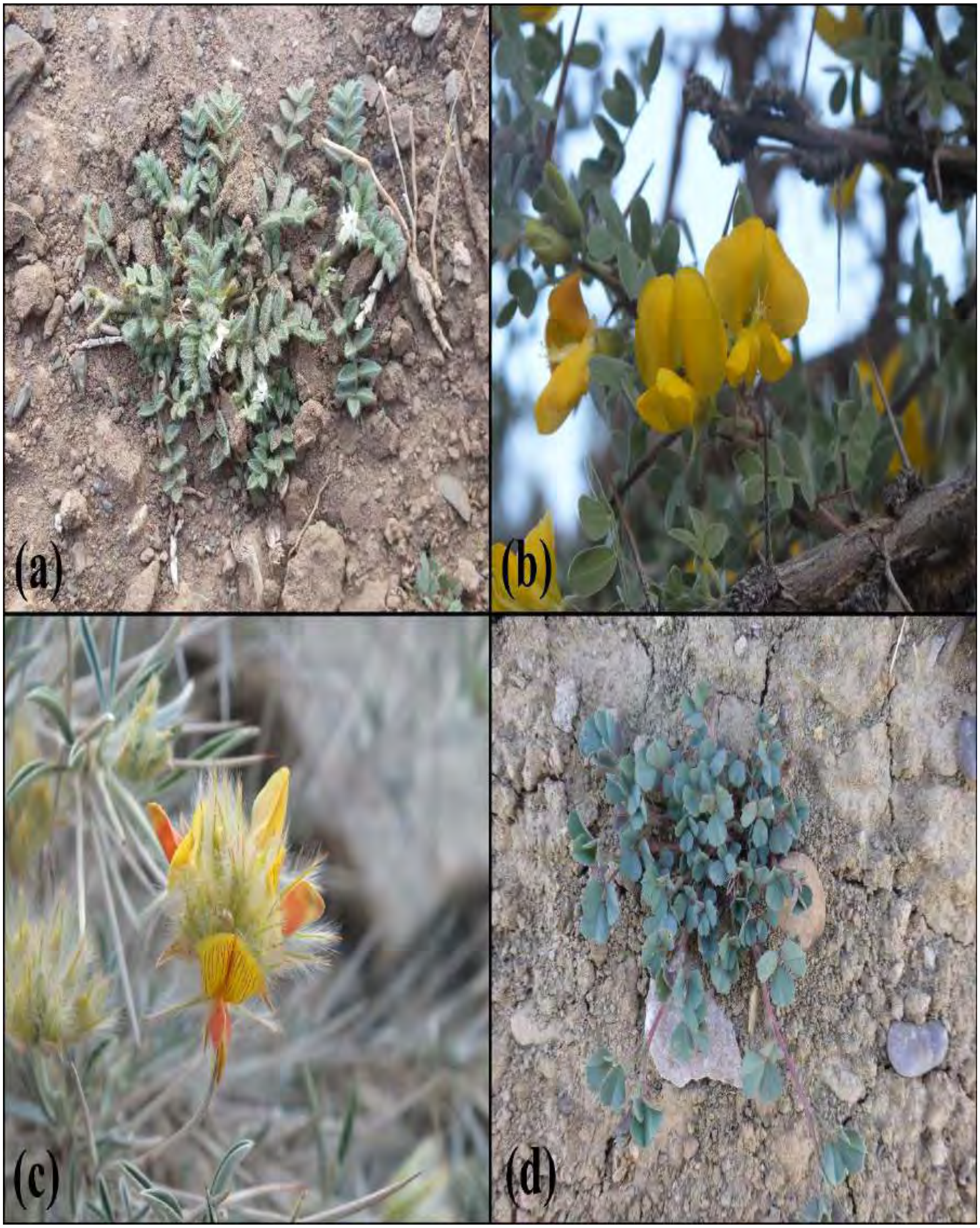

Plate 21. Field pictorial view (a) *Astragalus tribuloides* (b) *Caragana ambigua* (c) *Ebenus stellata* (d) *Indigofera cuneifolia*

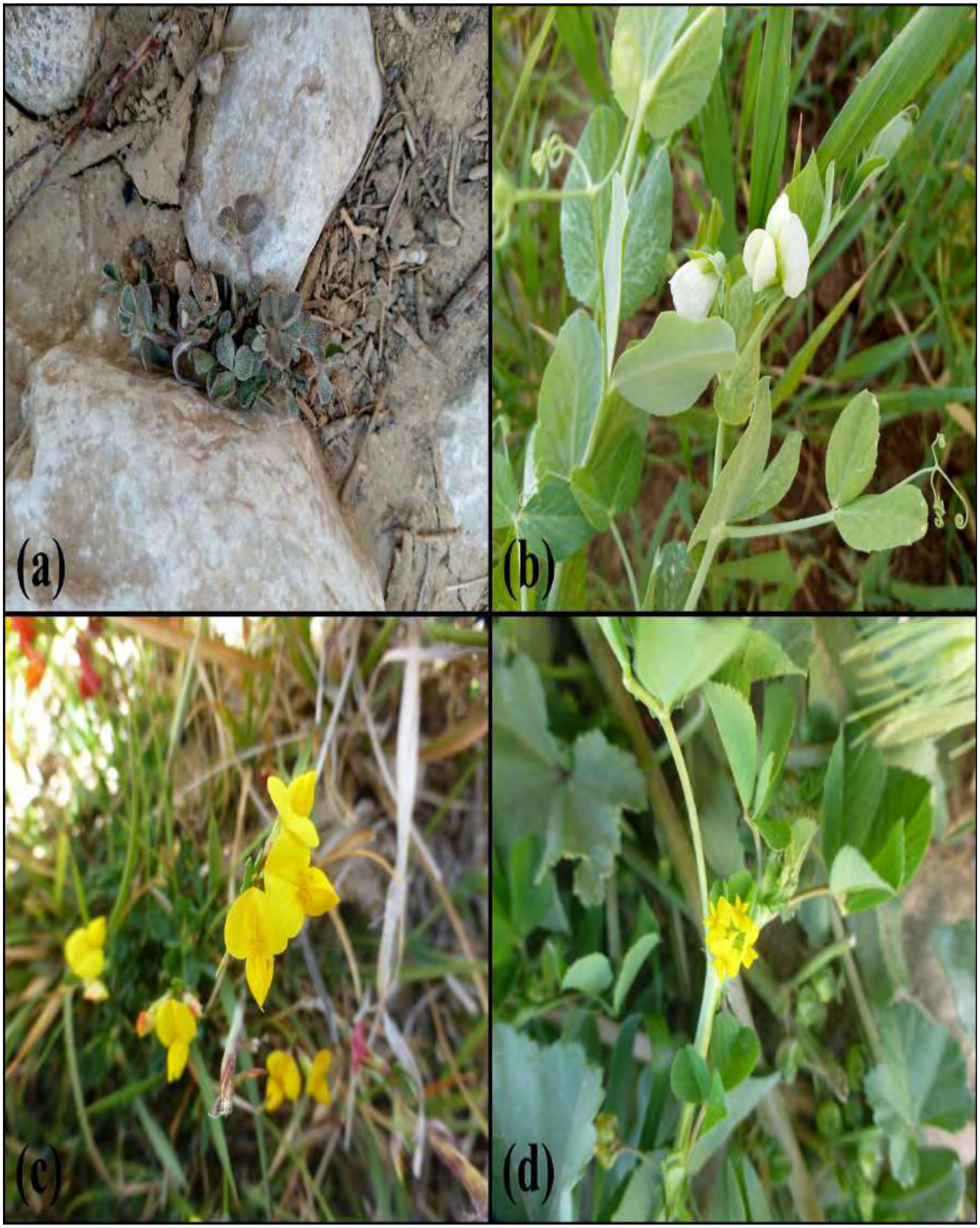

Plate 22. Field pictorial view (a) *Indigofera intricata* (b) *Lathyrus oleraceus* (c) *Lotus corniculatus* (d) *Medicago polymorpha*

Plate 23. Field pictorial view (a) *Melilotus indicus* (b) *Onobrychis cornuta* (c) *Onobrychis tavernierifolia*; (d) *Sophora mollis*

Plate 24. Field pictorial view (a) *Taverniera glabra* (b) *Tephrosia uniflora subsp. Petrosa* (c) Trigonella monantha (d) Vicia *macrantha*

Plate 25. Field pictorial view (a) *Ajuga bracteosa* (b),(c) Clinopodium umbrosum (d) *Eremostachys thyrsiflora*

Plate 26. Field pictorial view (a) *Eremostachys vicaryi* (b) *Isodon rugosus* (c) *Lallemantia royleana* (d) *Marrubium vulgare*

Plate 27. Field pictorial view (a) *Micromeria biflora* (b) *Nepeta bracteate* (c) *Nepeta griffithii* (d) *Nepeta Hindostana*

Plate 28. Field pictorial view (a) *Nepeta praetervisa* (b) *Origanum majorana* (c) *Otostegia aucheri* (d) *Phlomis stewartii*

Plate 29. Field pictorial view (a) *Salvia cabulica* (b) *Salvia coccinea* (c) *Salvia leucantha* (d) *Salvia macrosiphon*

Plate 30. Field pictorial view (a) *Salvia moorcroftiana* (b) *Salvia plebeia* (c) *Salvia santolinifolia* (d) *Scutellaria linearis*

Plate 31. Field pictorial view (a) *Stachys parviflora* (b) *Teucrium scordium* (c),(d) *Teucrium stocksianum*

Plate 32. Field pictorial view (a), (b), (c) *Thymus linearis* (d) *Thymus linearis subsp. hedgei Jalas*

Section-II

Pollen micromorphology

Summary

The second section comprised the pollen micromorphology of dicots from Northern Baluchistan, Pakistan. The study included 35 Brassicaceous, 34 Fabaceous, and 31 Lamiaceous species. Light and scanning electron microscopy were used to analyze the qualitative and quantitative characteristics of pollen.

- Qualitative characters included: symmetry, polarity, unity, size, shape, number of apertures, aperture orientation, exine sculpturing, exine surface, lumen shape, aperture membrane, amb, NPC classification, and formula.
- Quantitative features were: Exine thickness, polar axis (length and width), equatorial diameter (length and width), equatorial width and length of colpi, polar width and length of colpi, mesocolpium, and viability.

The variations in the qualitative features were used to develop taxonomic keys that helped discriminate among the examined species of each family. The distinguished palynological attributes were exine sculpturing, shape, and aperture membrane. The multivariate analysis of the quantitative aspects was carried out by principal component analysis, hierarchal cluster analysis, and correlation plots.

3.1 Pollen Micromorphology of Brassicaceous Flora from Northern Baluchistan

3.1.1 Results and Discussion

The discipline of plant taxonomy gradually evolves from simple to complex (Ranjbar and Karami., 2023; Anjum et al., 2022). In the plant taxonomy, the incorporation of new trends such as seed micromorphology, palynology, petiole, and foliar anatomy proved to be helpful (Jabeen et al., 2023; Ameen et al., 2022). A precise, systematic method to identify the relationship between families, genera, and species levels remained a subject of research (Taia, 2005). Species placement within the tribe can be resolved using pollen characteristics. The Brassicaceous tribe, genus, and species were distinguished by differences in their exine ornamentation (Kownacki et al., 2015). This study documented an atlas of pollen from 35 Brassicaceous plants for the first time, including an endemic plant, *Draba hystrix*.

The observed qualitative characteristics were symmetry, polarity, pollen size, shape, aperture type and orientation, amb view, colpi, exine sculpturing, and lumen shape. Pollen features like shape, size, exine sculpturing, colpi, and lumen were found significant. Pollen characteristics provided additional palynomorphic keys for the separation of Brassicaceous species. The morphological details of the pollen of 35 species of Brassicaceae are summarized in Table 6-9 and illustrated in Plate 33-41. The examined taxa belong to 15 tribes, with the leading tribe; Brassiaceae, Alysseae and Euclidieae.

a) Unit, symmetry, polarity, shape, and size of the Brassicaceous taxa

The size of the pollen of the analyzed species (based on the P/E ratio) was small in 30 plants, and medium in 5, contributing 86% and 14% of the total studied species respectively. It is significant to consider the size and shape of the examined pollen when determining systematic affinities among various taxa. Similar findings were studied for size, shape and symmetry of the pollen of endemic taxa of the Andaman Islands (Kailas et al., 2018). The Brassicaceous taxa in this research were categorized as $N_3P_4C_3$, revealed that all species possessed three apertures (N_3), along the equatorial position (P_4), with aperture type 3-colpate (C_3) (Table 6) (Erdtman, 1960). All plants exhibited radial symmetry (plane passing through central axis of pollen and divides it into similar parts) and were isopolar (both planes are equal and equatorial plane divide the pollen in two equal halves) (Table 6). In this study the observed shapes of the pollen were oblate spheroidal (12 taxa), prolate spheroidal (8 taxa), subprolate (6 taxa), suboblate (5 taxa) and prolate (2 taxa) (Plate 33-41). In polar view the constriction of colpi resulted the appearance of pollen in lobate, triangular or round amb. In polar view the pollen were lobate in 18 species, round in 10 species and triangular in 7 taxa (Table 2).

The size of pollen played a significant role in the plant reproduction since it is important in the dispersal of flowering species (De Storme et al., 2013). Demirpolat (2022) reprted that the *Aethionema membranaceum* was separate species from *Aethionema sancakense* based on the large pollen size. The pollen size has been successfully employed in differentiating the varieties of *Brassica rapa*, *Brassica napus*, and *Brassica juncea* (Saha and Begum, 2020). Kownacki et al. (2015) also reprted oblatespheroidal, prolatespheroidal, subprolate and suboblate shapes of pollen in genus *Clypeola* (Brassicaceae) in Brassicaceae. Previously, *Alyssum desertorum* and *Descurainia sophia* were reported to be prolate to prolatespheroidal from other localities of Pakistan (Kownacki et al., 2015). In the present study, the pollen of *Alyssum desertorum* and *Descurainia sophia* were oblate spheroidal. These insights aided for docmenting changes that can be taken into consideration for taxonomic placement of problematic taxa as well as for determining any potential ecological consequences caused by the differences that have been documented. Amina et al. (2020), studied the pollen morphology of Brassicaceae via LM and SEM and reported prolate, and subprolate to prolate spheroidal pollen shapes in Brassicaceous species.

The polarity, symmetry, and unity were similar in all examined species. Pollen in all studied species were isopolar and radial, and monad. Kownacki et al. (2015), Pinar et al. (2009),Ceter and Tug (2009) documented Brassicaceous taxa with radial symmetry and isopolar polarity. The amb of pollen in polar view categorized the examined taxa into lobate, round and triangular types. Highest numbers of pollen in polar view were observed with lobate amb. Kownacki et al. (2015), reported the lobed and circular amb in Camelineae of Brasicaceae. Amb and polar axis were significant taxonomic parameters in the discrimination of endemic Noccaeae species of Brassicaceae (Atasagun, 2022). Previously, round and elliptical shapes were studied in species of the *Malcolmia* genus (Brassicaceae) (Kızılpınar et al., 2012).

b) Exine sculpturing among the investigated Brassicaceous species

Reticulate sculpturing (resembling a net or network) was the main type of exine ornamentation in Brassicaceae. Three types of reticulation were visualized in this study. Reticulate, coarsely reticulate, and scabrate. Exine and other characters variations were proven to be useful both at generic and specific level (Amina et al., 2020). Simple reticulation was observed in 24 taxa. Similar observations were reported by Kownacki et al. (2015). Coarse reticulation (large and irregular) existed in 10 taxa and scabrate type of exine sculpturing was only observed in *Dilophia salsa.* The lumen shape in most taxa was either regular or irregular polygonal. The irregular amorphous lumen shape was also observed in some species such as *Eruca sativa* and *Strigosella intermedia.* Exine thickness ranged from a minimum of 1.85 µm in *Notoceras bicorne* and *Lepidium aucheri* to a maximum of 4.5 µm in *Isatis minima.* Exine in the genus *Strigosella* ranged from a minimum of 2.15 µm to a maximum of 2.95 µm. The thickness of the exine ranged from 1.95 µm to 3.1 µm in members of the genus *Meniocus*.

The ornamentation of pollen was significant in the taxonomy of species of the genus *Hesperis* (Brassicaceae) reported by Pinar et al. (2009). They reported a large and irregular (coarsely) reticulate type in 13 Brassicaceous taxa. Kownacki et al. (2015), reported 84% simple reticulate and 16% coarsely reticulate types in varieties of the genus *Brassica*. Previously the psilate type was observed in *Eruca vesicaria* from Esakhail, Pakistan (Kownacki et al., 2015). Lumen shapes ranged from regular polygonal (13 species), irregular polygonal (9 species), irregular amorphous (7 species), to irregular amorphous (5 species). Based on the shape of the lumen, the pollen of the Brassicaceae family was separated into three types (Kownacki et al., 2015). Exine ornamentation and lumen shape separated the tribes Brassiceae, Conringieae, Isatideae, and Plagiolobeae. Further, *P. crenulata* belonging to the Zuvanda tribe was placed in the Plagioloba tribe, and *I. planisiliqua* that belongs to the tribe Conringia was moved to the Iljinskaea tribe, based on the changes in the surface micro-sculpturing (Ranjbar and Karami, 2023).

The variations in the shape of lumen shape indicated to be potential diagnostic feature analyzed by Ranjbar et al. (2023) in tribe Camelineae. Similarly taxonomic strength of differences in quantitative data of muri width and lumina diameter was reported by Kownacki et al. (2015). The LM and SEM study of the pollen showed diversity in the colpi orientations. The colpi orientation appeared as sunken concave (2), sunken convex (6), raised convex (12), and raised concave (15). Colpi orientation has been studied for its ability to help identify different Mimosaceous species and weed pollen flora. Apices/ends of colpi were acute in 12 taxa and blunt in 11 taxa, assisting in the building of diagnostic taxonomic keys (Khan et al., 2021). Fused apices were noted in synocolpate pollen. Round apices were only present in 4 species. Separation of Brassicaceous taxa based on pollen morphological attributes and assessing the status of species in particular ecoregions by analyzing pollen viability were significant contributions made by the pollen atlas of Brassicaceous taxa from Northern Baluchistan.

c) Polar axis and Equatorial Diameter

A minimum mean value 15.6 µm of polar axis was observed for *Cardaria draba* and a maximum 33.05 µm in *Dilophia salsa*. Equatorial diameter varied from 14.75 µm in *Diplotaxis griffithii* to 36.75 µm in *Capsella bursa-pastoris* (Table 7).

d) Colpi and mesocolpium

The tricolpate aperture was observed in 26 species. Nine species have tri-syncolpate (three colpi joining at the ends at the polar ends) aperture. The observed pollen in this research were placed in the first and second groups of classification as provided by Dogan et al. (1990). Similar findings were documented by Pinar et al. (2009) in the genus *Hesperis*. In genus *Malcolmia* all studied taxa exhibited tricolpate pollen grains (Kızılpınar et al., 2012). Brassicaceous taxa from central Punjab werfe reported with tricolpate aperture type (Kownacki et al., 2015; Amina et al., 2020). The length and width of colpi were measured both in polar and equatorial views. The minimum and maximum width of colpi in polar view were 2.45 µm *Capsella bursa-pastoris* and 7.65 µm *Coincya tournefortii,* respectively. The length of colpi in polar view ranged from a minimum of 4.05 µm *Strigosella africana* to a maximum of 12.15 µm *Lepidium aucheri*. The width of colpi in equatorial view was minimum in *Strigosella intermedia* 2.15 µm and maximum at 10.95 µm in *Dilophia salsa*. The length of the colpi ranged from 3.7 µm *Meniocus linifolius* to 20.55 µm *Strigosella africana.* The largest mean length of mesocolpium observed was in *Dilophia salsa* (18 µm), while the minimum was in *Meniocus linifolius* (6.05 µm).

e) Viability analysis

Maximum fertility of pollen was calculated for *Meniocus linifolius* (98.1%) and minimum in *Dilophia salsa* (80.1%).

f) Multivariate analysis

The variation in the quantitatively analysed features were analysed statistically. The quantitatively analysed characters were the polar axis, equatorial diameter, length, and width of colpi in polar and equatorial view, exine thickness, and mesocolpium. The principle component analysis (PCA) ordination was observed (Figure 9). The characters significantly differentiated the analysed species. Hierarchical cluster (UPGMA) was consctructed based on the quantitative characters of pollen (Figure 10). The cluster analysis results showed one major and three subclusters. There were three distinct clusters of six species in subclusters. Multiple variable subclusters were distinguished from major clusters. The majority of the variables were found to be positively correlated, with a small number of negatively linked features. Characters that were negatively associated were displayed red, and those that were positively correlated were blue in the plot. The polar axis and equatorial diameter were found to have a positive correlation 0.75. There was also a positive correlation between the length of colpi in the equatorial view and mesocolpium (Figure 11). In the polar view, however, there was a negative correlation between the width and length of the colpi -0.36 and the polar axis -0.32.

g) Systematic implications of current palynological findings in Brassicaceae

There are taxonomically problematic taxa in the Brassicaceae. Palynological information is an additional source for identifying variations among Brassicaceous taxa, in addition to other systematic methods. Colpi orientation was raised and concave with round apices in the species of *Alyssum* i.e. in *Alyssum turkestanicum* and *Alyssum desertorum*. *Alyssum turkestanicum* and *Alyssum desertorum* have regular amorphous and irregular polygonal lumen shapes, respectively, which further distinguish the two species. *Cardaria* species possessed sunken colpi with concave orientation and acute apex (*Cardaria chalepensis*) and convex orientation and blunt end (*Cardaria draba*) types, along with polygonal lumen shape but with regular (*Cardaria chalepensis*) and irregular (*Cardaria draba*) types, providing a scientific approach in systematics. These palynological studies also served as the basis for characterizing species of the same genus i.e., *Diplotaxis, Isatis, Meniocus, Sisymbrium, Strigosella*. Endemic plant *Draba hystrix* pollen has been characterized as small, oblate spheroidal, tricolpate pollen with NPC formula $N_3P_4C_3$, raised and concave colpi orientation, blunt apices of colpi, amb triangular, having reticulate exine sculpturing with irregular amorphous lumen.

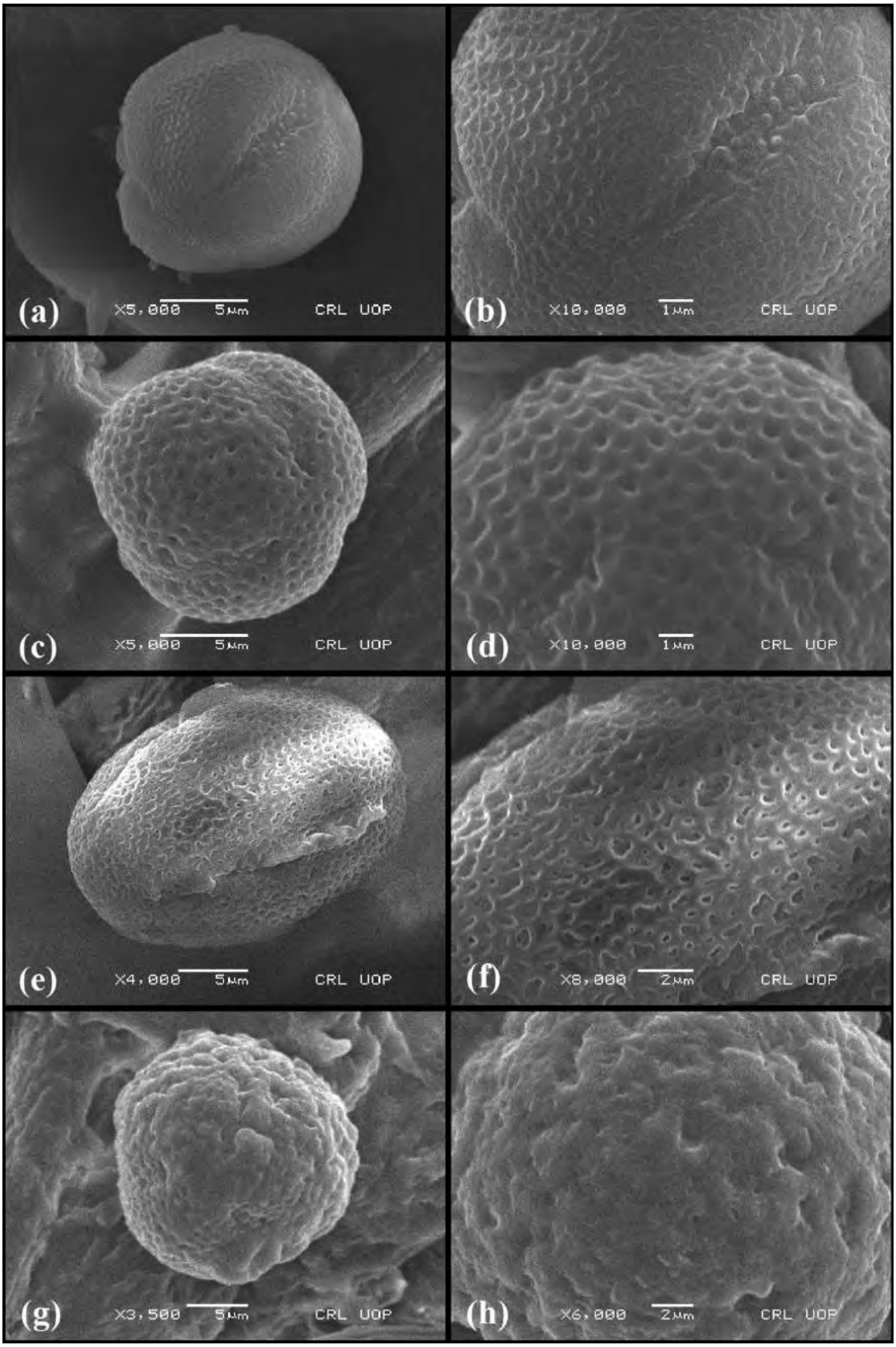

Plate 33. SEM micrographs of pollen (a),(b) *Aethionema carneum,* (c), (d) *Alyssum desertorum,* (e), (f) *Alyssum turkestanicum*, (g), (h) *Brassica oleracea*var. *capitata*

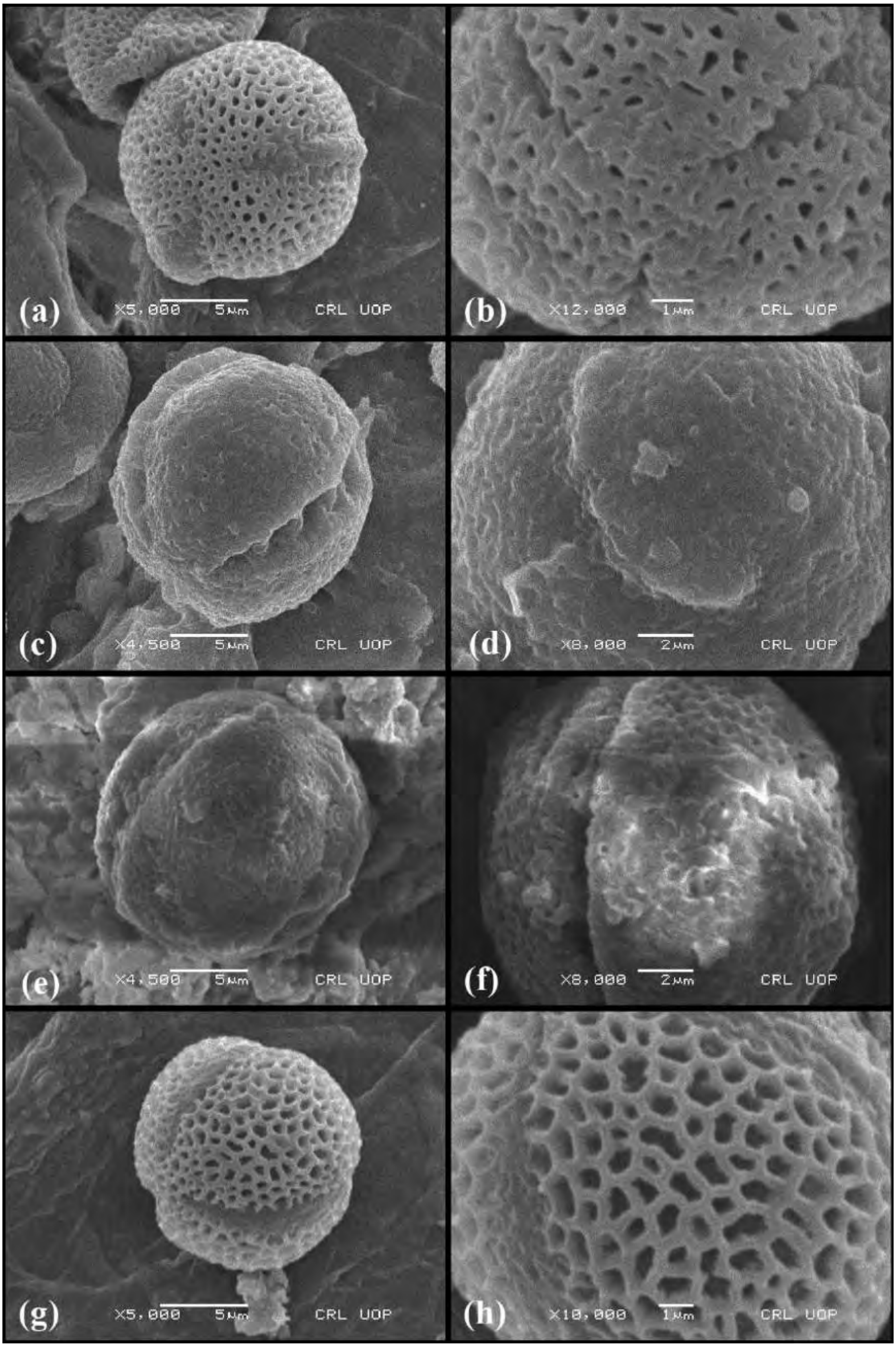

Plate 34. SEM micrographs of pollen (a),(b) *Capsella bursa-pastoris,* (c), (d) *Cardaria chalepensis,* (e), (f) *Cardaria draba*, (g), (h) *Chorispora tenella*

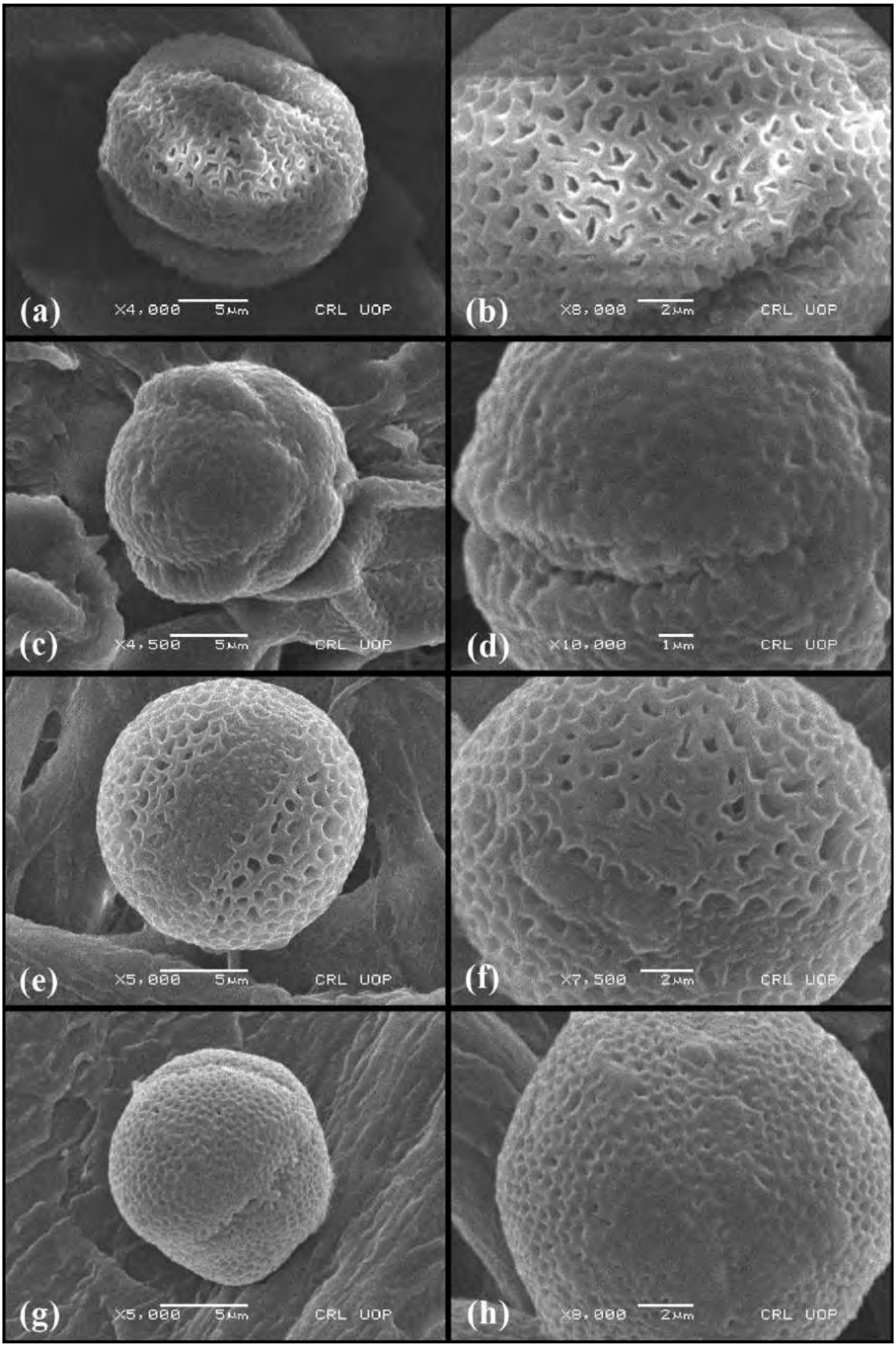

Plate 35. SEM micrographs of pollen (a),(b) *Clypeola aspera,* (c), (d) *Coincya tournefortii,* (e), (f) *Conringia orientalis*, (g), (h) *Descurainia sophia*

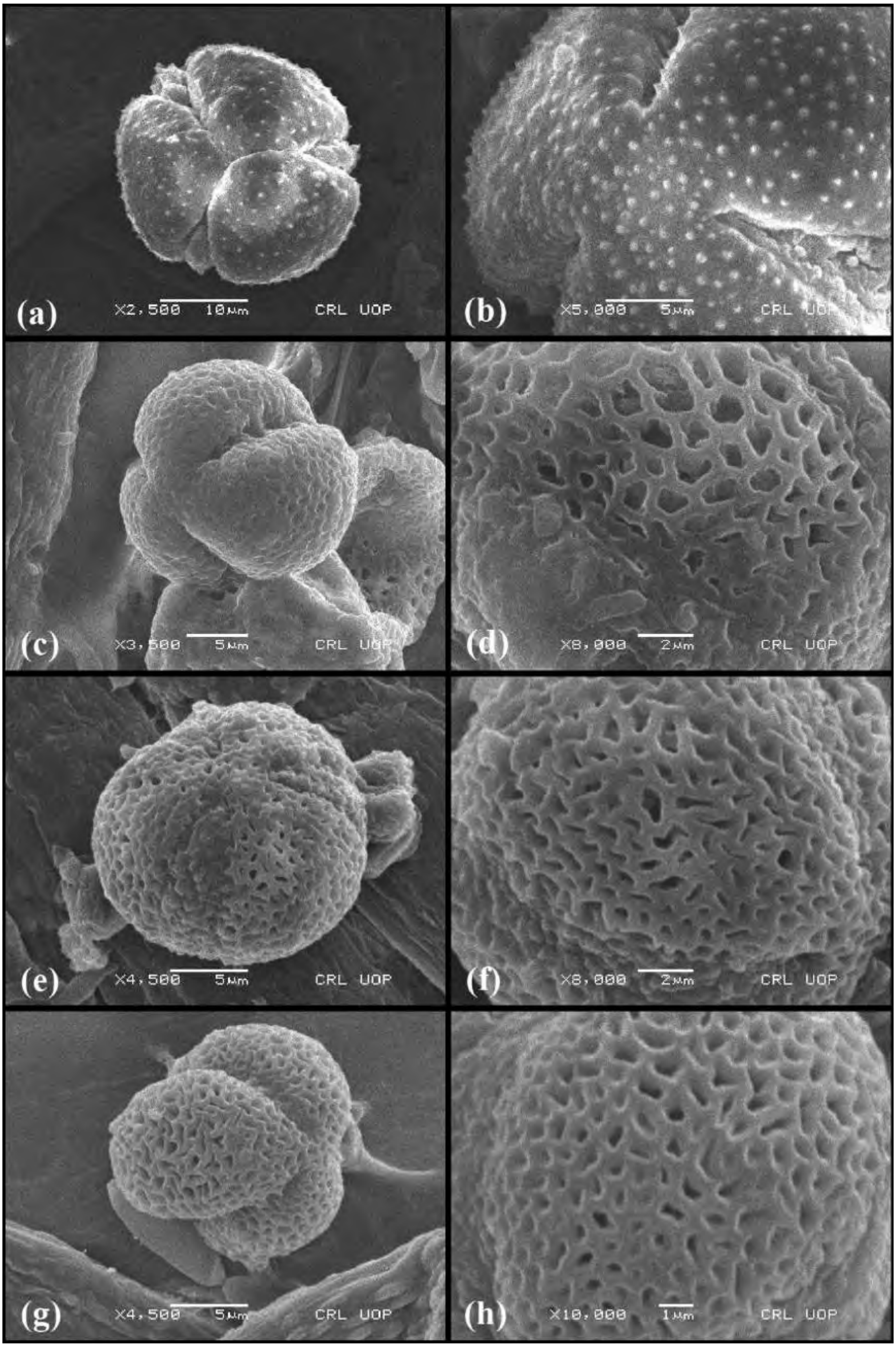

Plate 36. SEM micrographs of pollen (a),(b) *Dilophia salsa,* (c), (d) *Diplotaxis griffithii,* (e), (f) *Diplotaxis harra*, (g), (h) *Draba hystrix*

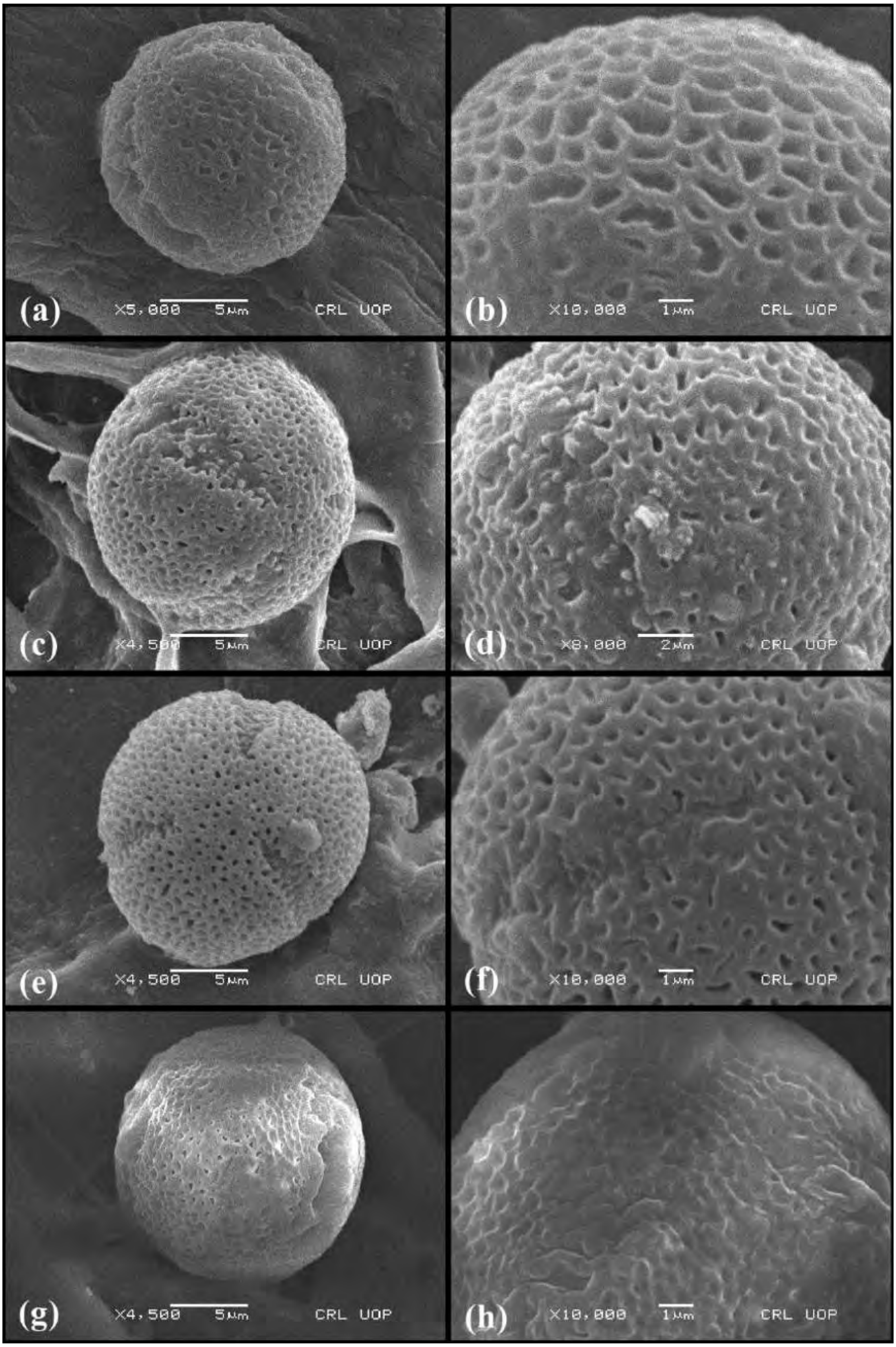

Plate 37. SEM micrographs of pollen (a),(b) *Eruca sativa,* (c), (d) *Farsetia heliophila,* (e), (f) *Goldbachia pendula*, (g), (h) *Isatis minima*

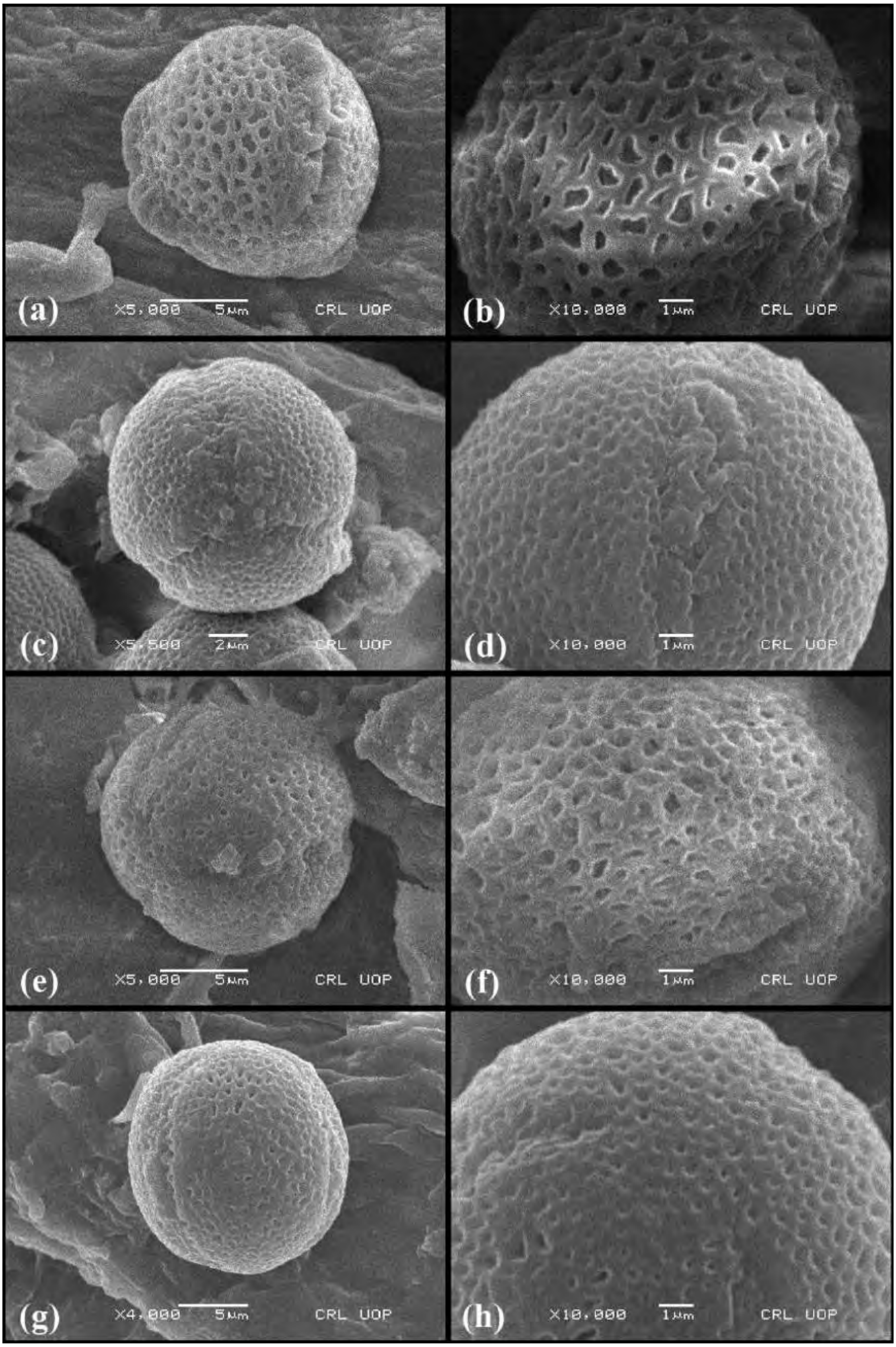

Plate 38. SEM micrographs of pollen (a), (b) *Isatis stocksii,* (c), (d) *Lepidium aucheri,* (e), (f) *Leptaleum filifolium*, (g), (h) *Matthiola flavida*

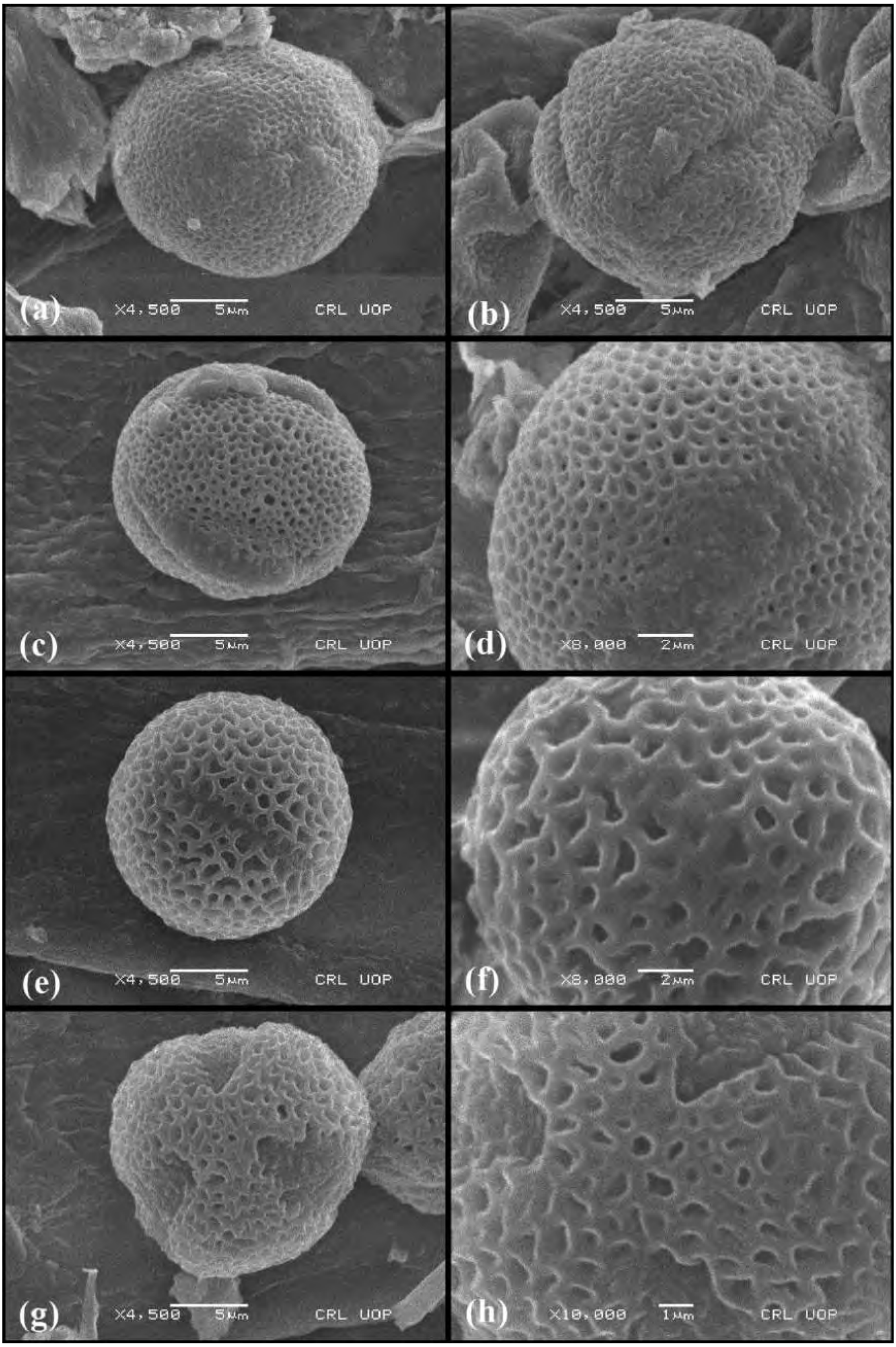

Plate 39. SEM micrographs of pollen (a),(b) *Meniocus heterotrichus,* (c), (d) *Meniocus linifolius,* (e), (f) *Notoceras bicorne*, (g), (h) *Physorrhynchus brahuicus*

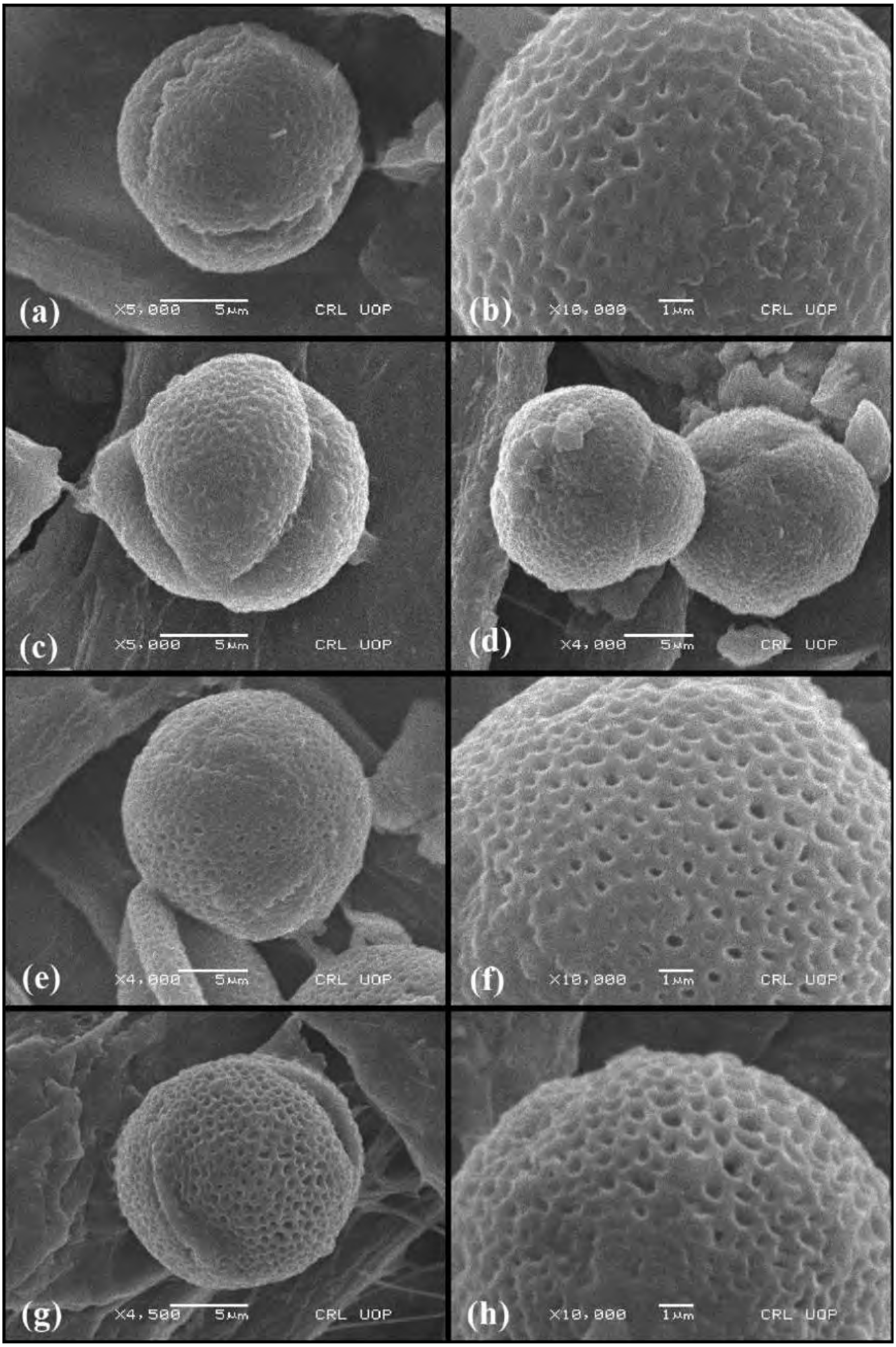

Plate 40. SEM micrographs of pollen (a),(b) *Raphanus raphanistrum,* (c), (d) *Sisymbrium altissimum,* (e), (f) *Sisymbrium irio*, (g), (h) *Strigosella africana*

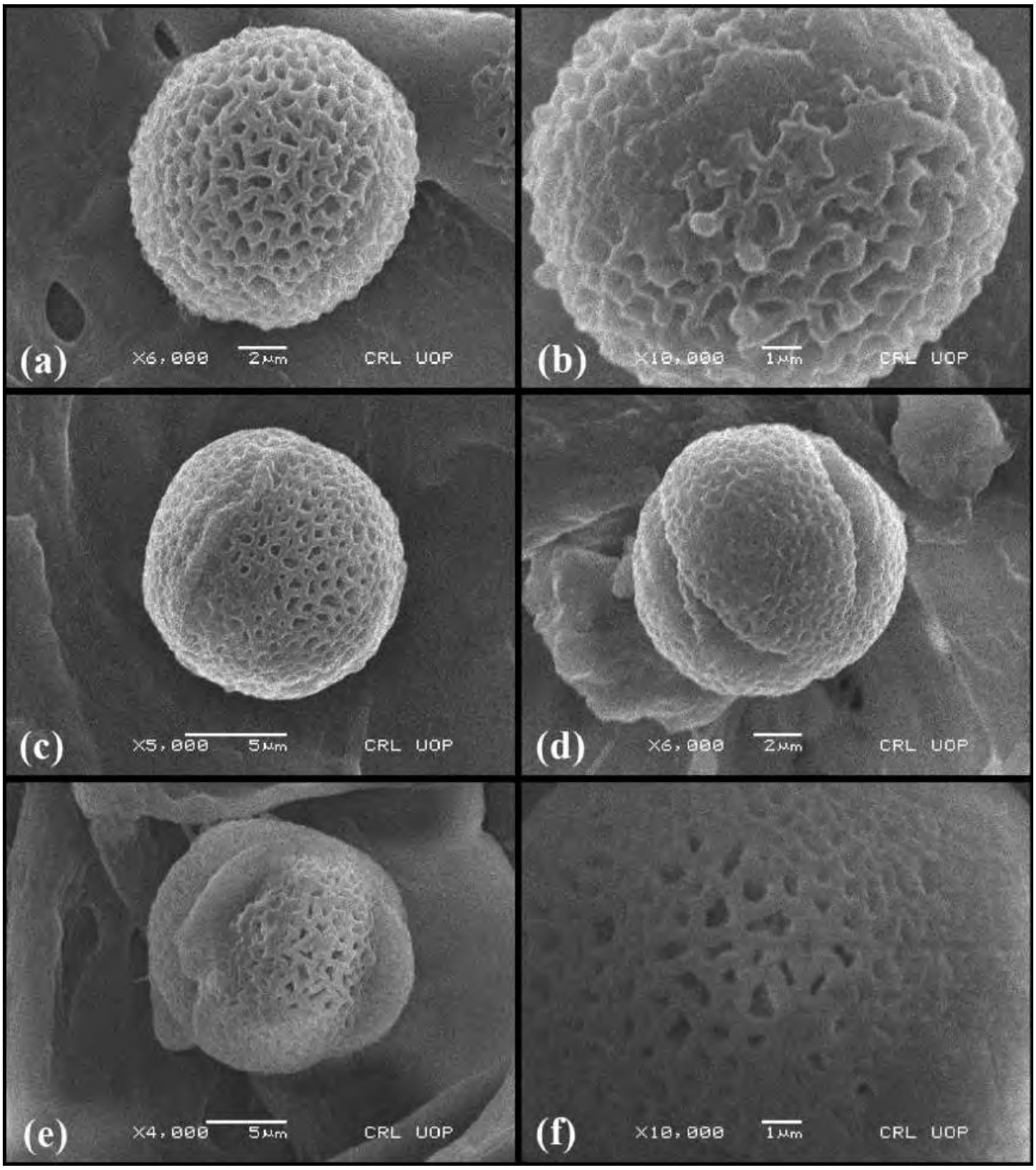

Plate 41. SEM micrographs of pollen (a),(b) *Strigosella cabulica,* (c), (d) *Strigosella intermedia,* (e), (f) *Tetracme stocksii*

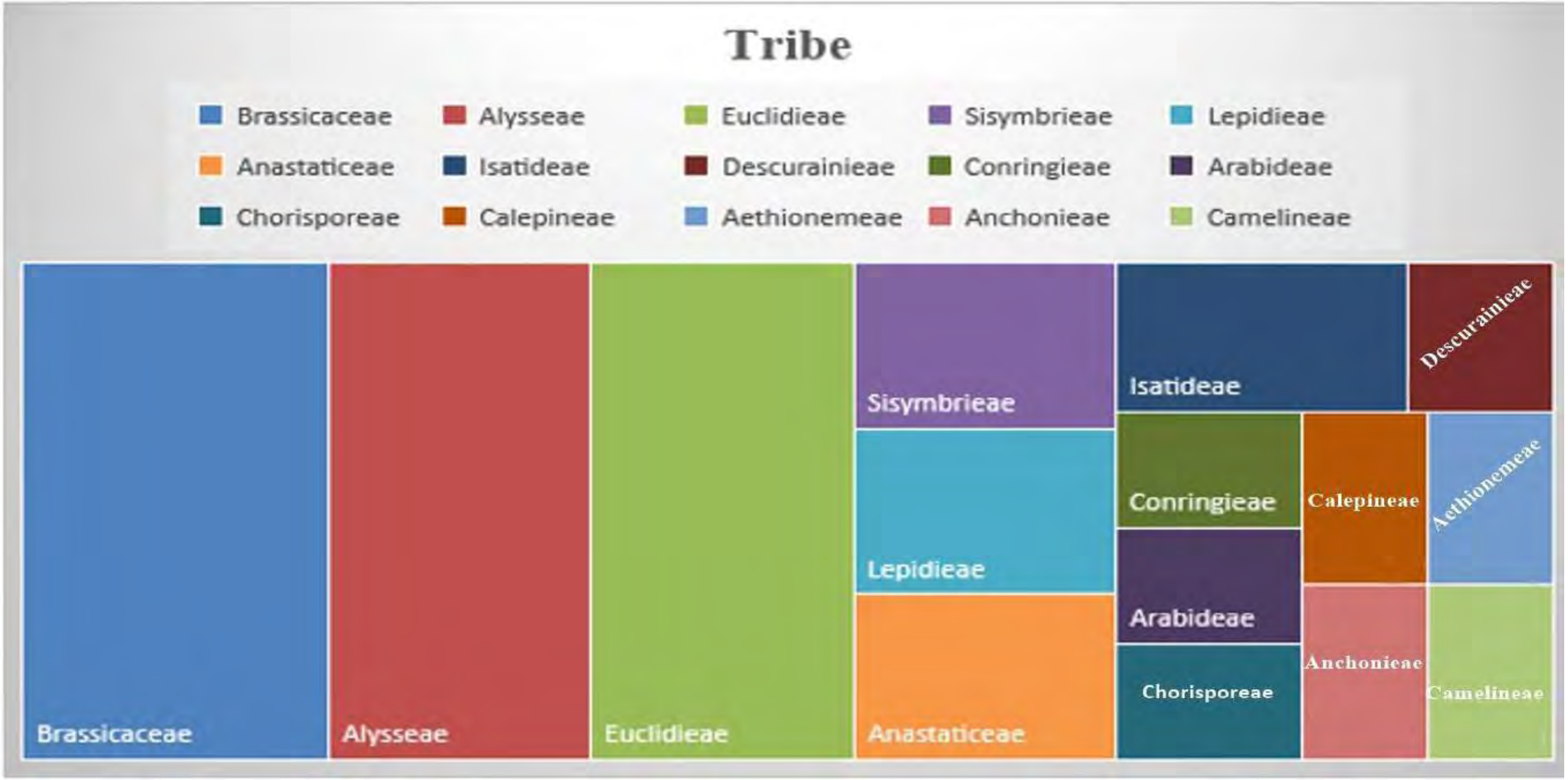

Figure 3. Brassicaceous taxa (size of the box representing of species in this study from each tribe)

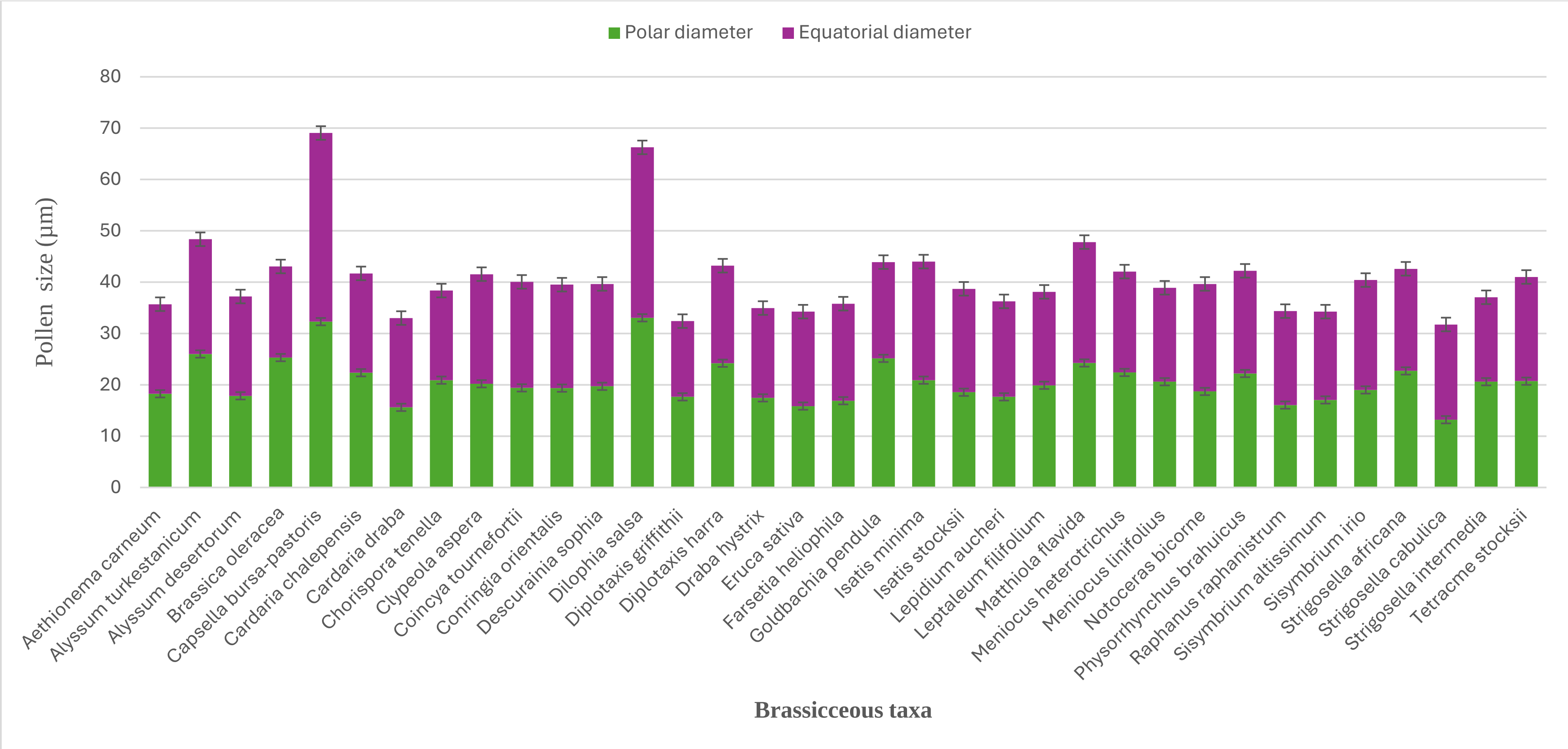

Figure 4. Variations in mean values of pollen size among Brassicaceous taxa (polar diameter and equatorial diameter)

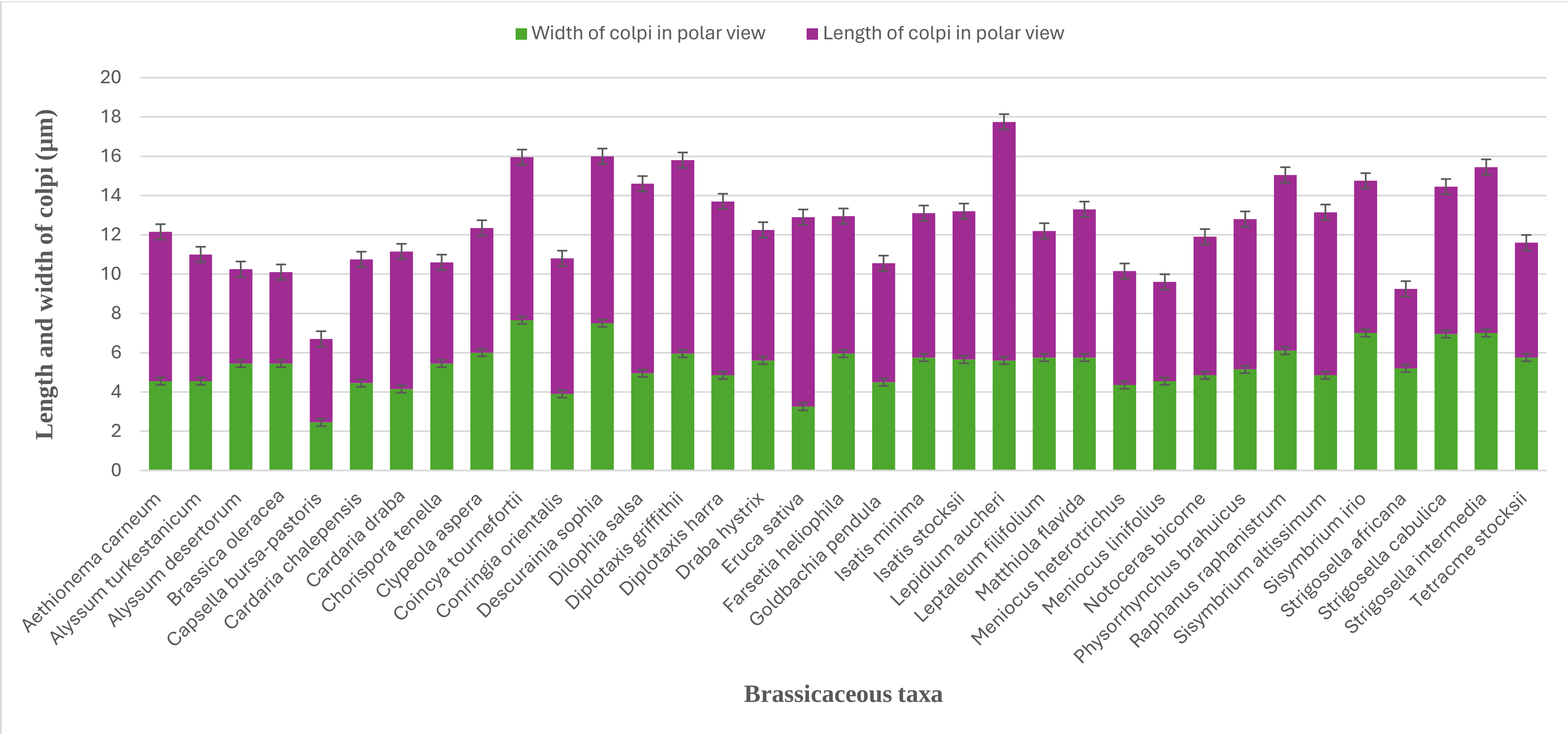

Figure 5. Variations in mean values of length and width of colpi in polar view among Brassicaceous taxa

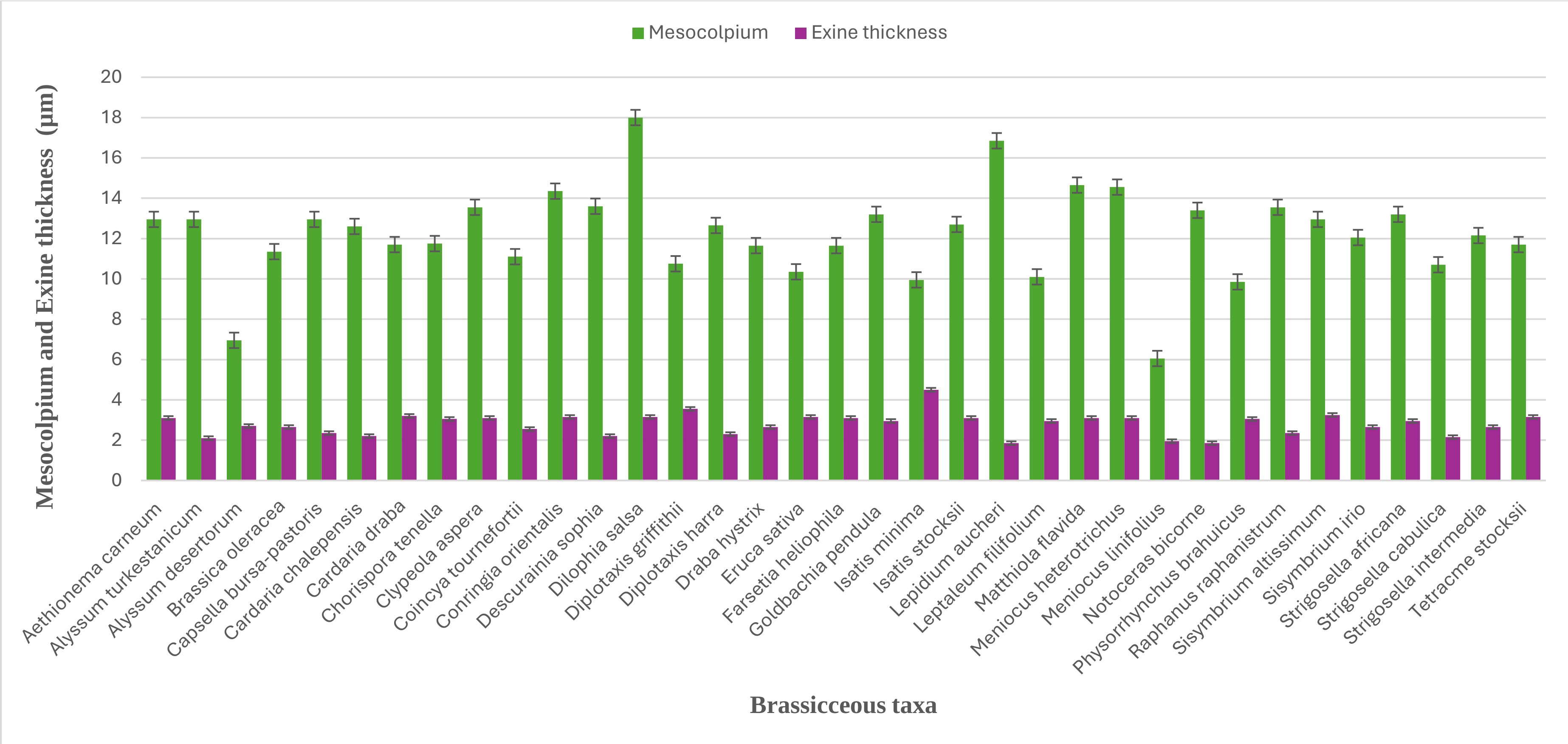

Figure 6. Variations in mean values of mesocolpium and exine thickness among Brassicaceous taxa

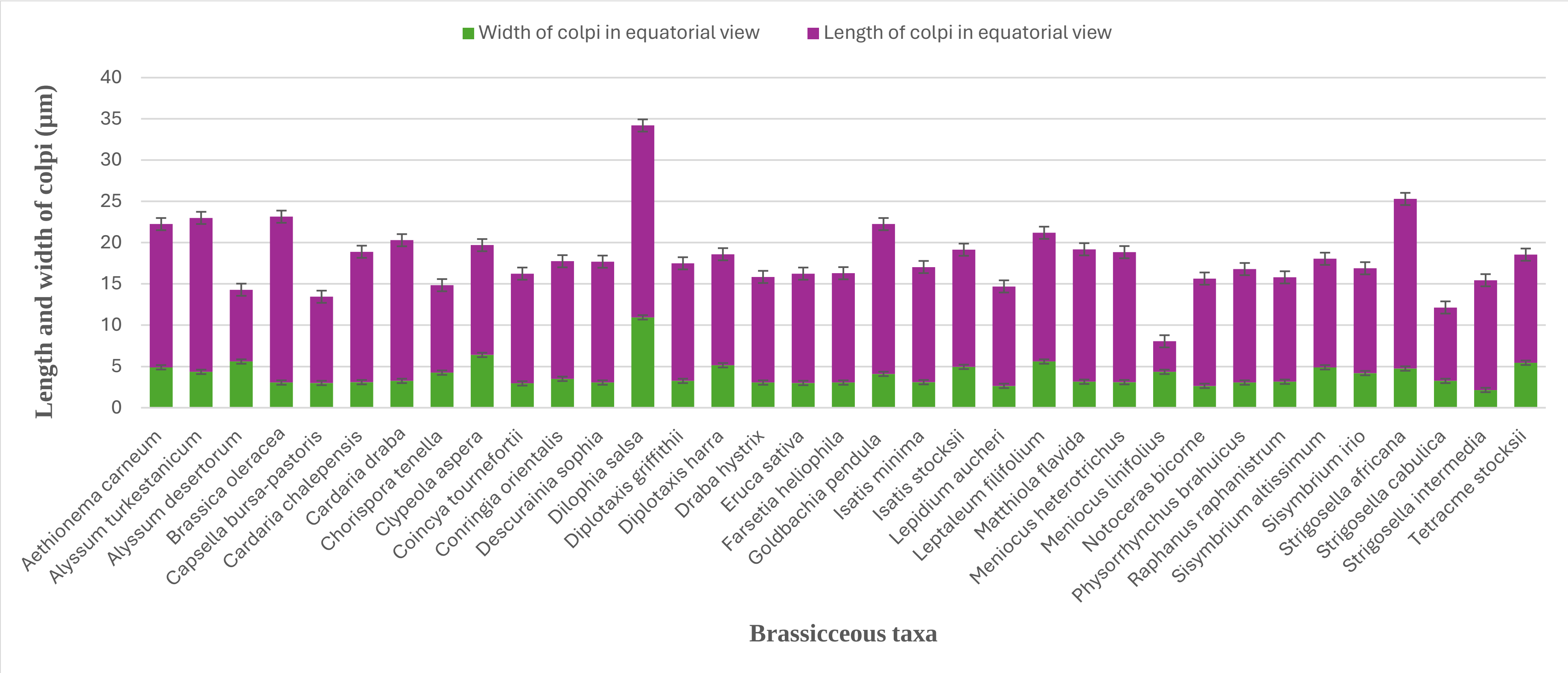

Figure 7. Variations in mean values of colpi length and width in equatorial view

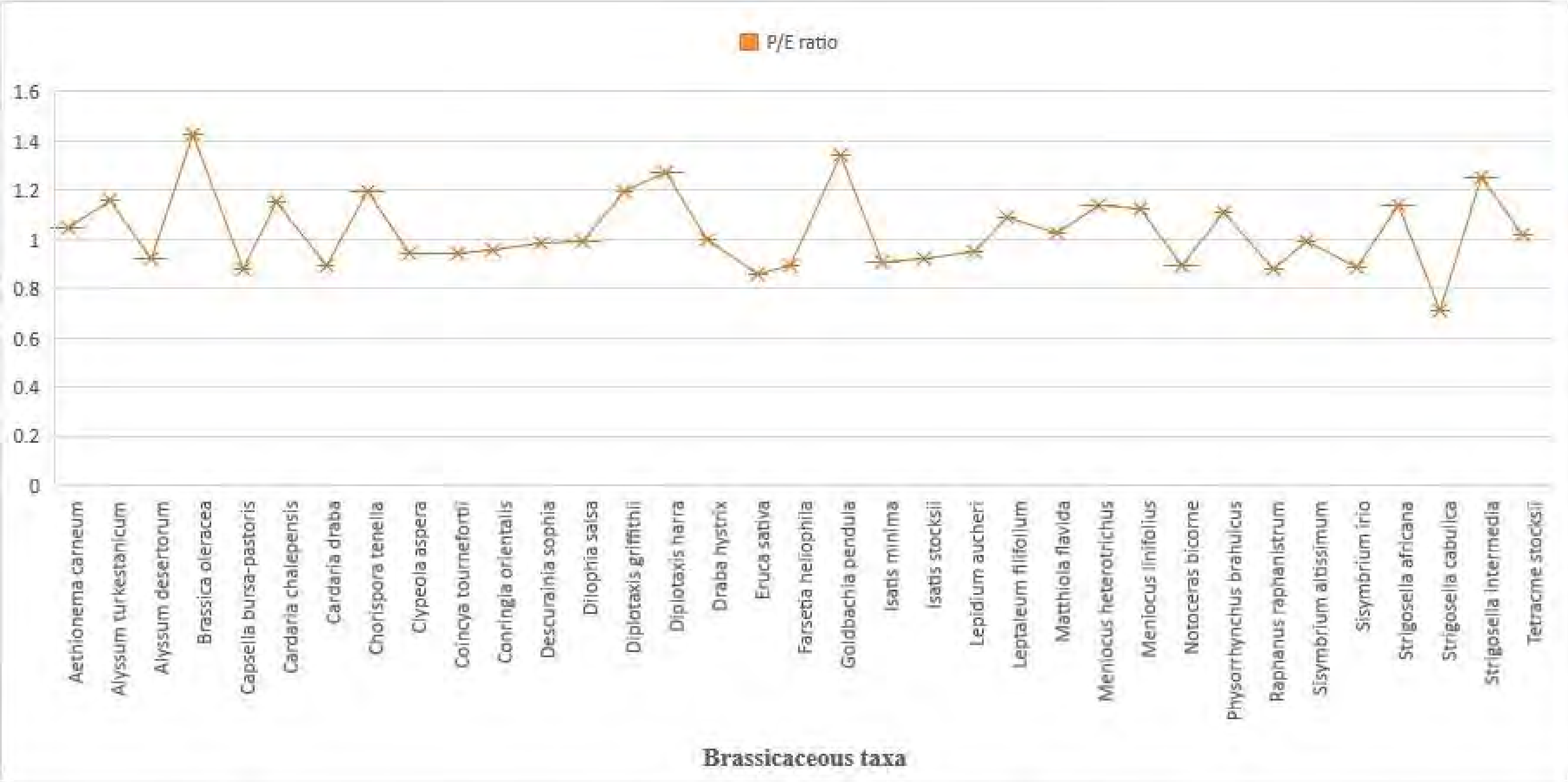

Figure 8. P/E ratio variations among studied Brassicaceous taxa

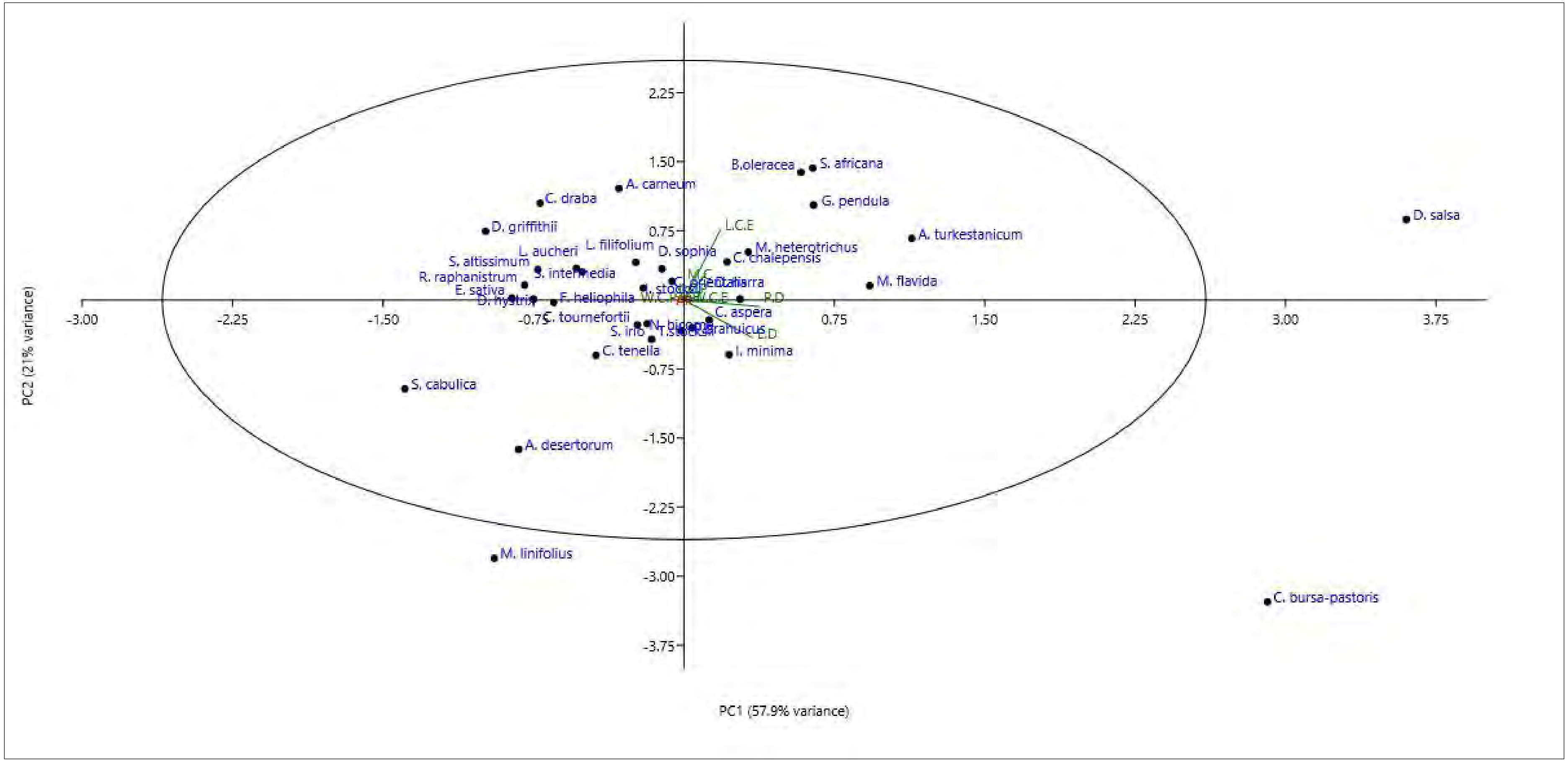

Figure 9. PCA analysis based on quantitative traits; polar axis, equatorial diameter, width and length of colpi in polar view, width and length of colpi in equatorial view, mesocolpium, exine thickness of Brasicaceous flora

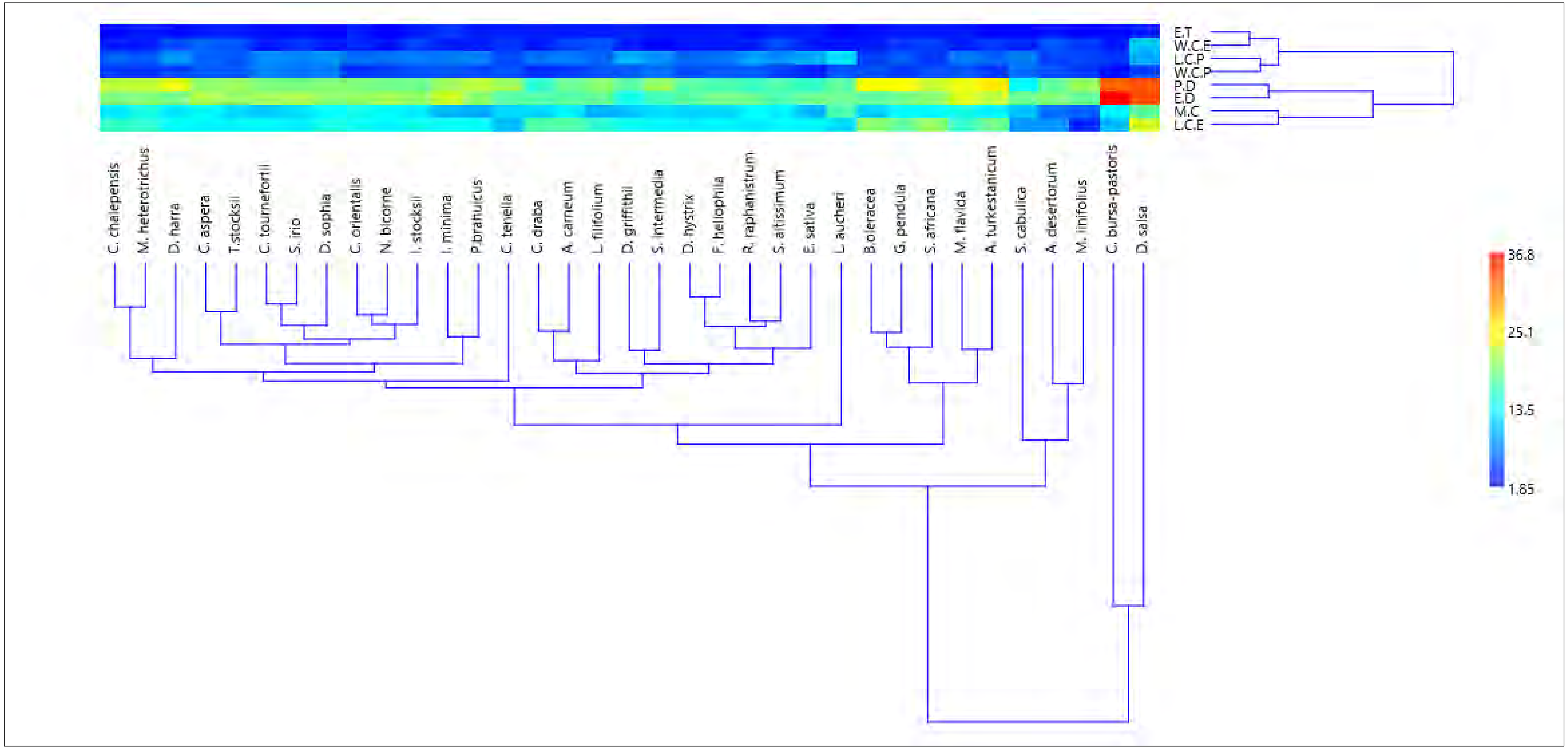

Figure 10. UPGMA cluster analysis based on quantitative traits; polar axis, equatorial diameter, width and length of colpi in polar view, width and length of colpi in equatorial view, mesocolpium, exine thickness of Brassicacoues flora

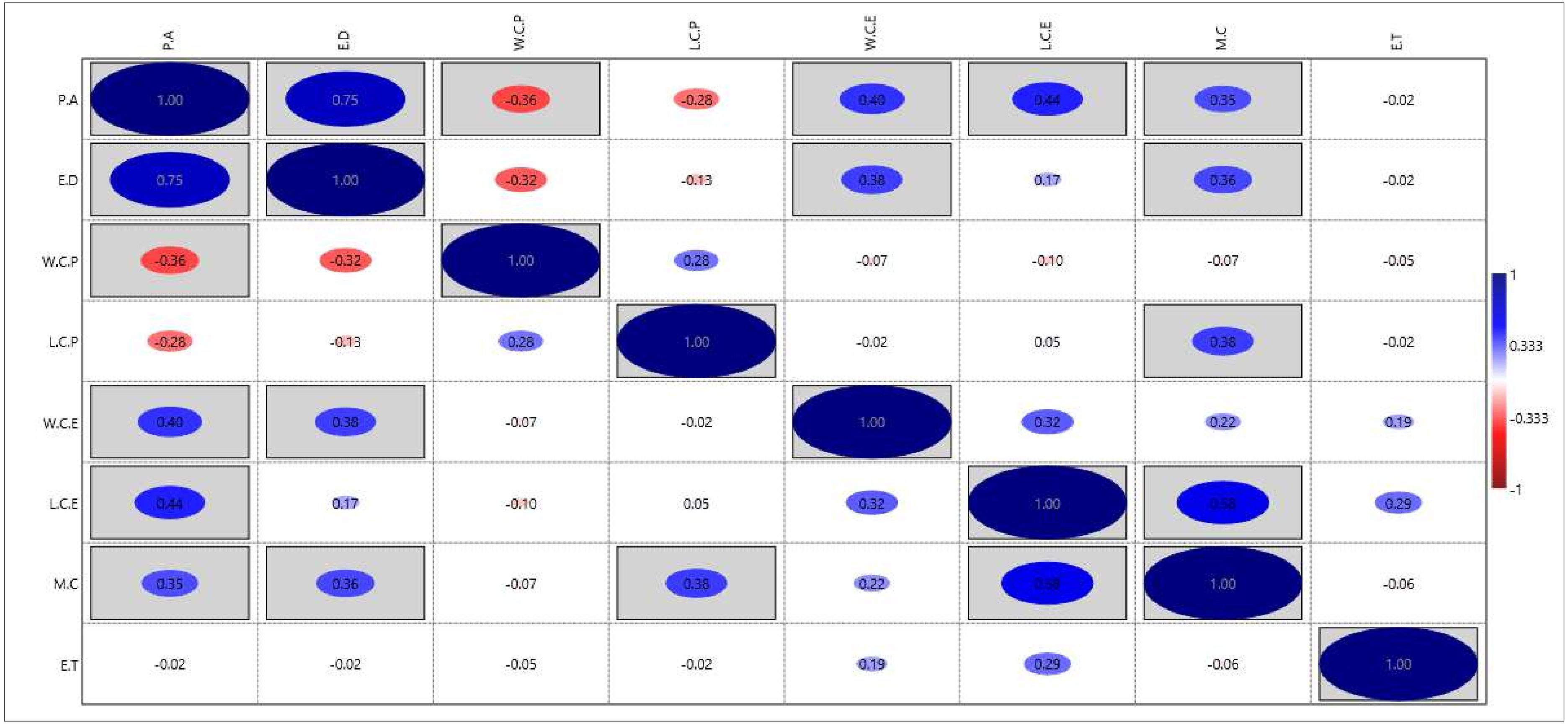

Figure 11. Correlation among the mean values of polar axis (P.A), equatorial diameter (E.D), width and length of colpi in polar view (W.C.P, L.C.P), width and length of colpi in equatorial view (W.C.E, L.C.E), mesocolpium (M), exine thickness (E.T) of Brassicacoues flora

Table 6. Size, shape and viability of pollen for studied Brassicaceous taxa

S. No	Name of Species	Polar diameter (μm)	Equatorial diameter (μm)	Pollen Size	P/E ratio	Pollen Shape	Viability%
1.	*Aethionema carneum*	18.25	17.45	Small	1.045	Prolate spheroidal	92.6
2.	*Alyssum desertorum*	17.85	19.35	Small	0.922	Oblatespheroidal	87.8
3.	*Alyssum turkestanicum*	26	22.35	Medium	1.163	Subprolate	92.6
4.	*Brassica oleracea*	25.3	17.75	Medium	1.425	Prolate	87.8
5.	*Capsella bursa-pastoris*	32.3	36.75	Medium	0.878	Suboblate	90.7
6.	*Cardaria chalepensis*	22.35	19.35	Small	1.155	Subprolate	89.8
7.	*Cardaria draba*	15.6	17.4	Small	0.896	Oblatespheroidal	91.1
8.	*Chorispora tenella*	20.9	17.45	Small	1.197	Subprolate	86.3
9.	*Clypeola aspera*	20.2	21.35	Small	0.946	Oblatespheroidal	88.1
10.	*Coincya tournefortii*	19.4	20.65	Small	0.939	Oblatespheroidal	91.8
11.	*Conringia orientalis*	19.35	20.15	Small	0.960	Oblatespheroidal	89.8
12.	*Descurainia sophia*	19.7	19.95	Small	0.987	Oblatespheroidal	90.3
13.	*Dilophia salsa*	33.05	33.2	Small	0.995	Oblatespheroidal	80.1
14.	*Diplotaxis griffithii*	17.65	14.75	Medium	1.196	Subprolate	90.5
15.	*Diplotaxis harra*	24.2	19	Small	1.273	Subprolate	91.4
16.	*Draba hystrix*	17.45	17.5	Small	0.997	Oblatespheroidal	94.2
17.	*Eruca sativa*	15.85	18.4	Small	0.861	Suboblate	93.5

18.	*Farsetia heliophila*	16.9	18.9	Small	0.894	Suboblate	94.3
19.	*Goldbachia pendula*	25.15	18.75	Small	1.341	Prolate	91.9
20.	*Isatis minima*	20.9	23.1	Medium	0.904	Oblatespheroidal	93.3
21.	*Isatis stocksii*	18.55	20.15	Small	0.920	Oblatespheroidal	90.8
22.	*Lepidium aucheri*	17.65	18.6	Small	0.948	Oblatespheroidal	91.8
23.	*Leptaleum filifolium*	19.9	18.2	Small	1.093	Prolate spheroidal	87.5
24.	*Matthiola flavida*	24.25	23.55	Small	1.029	Prolate spheroidal	94.4
25.	*Meniocus heterotrichus*	22.4	19.65	Small	1.139	Prolates pheroidal	84.6
26.	*Meniocus linifolius*	20.6	18.3	Small	1.125	Prolate spheroidal	98.1
27.	*Notoceras bicorne*	18.7	20.95	Small	0.892	Oblatespheroidal	92.4
28.	*Physorrhynchus brahuicus*	22.2	20	Small	1.11	Prolate spheroidal	88.6
29.	*Raphanus raphanistrum*	16.05	18.3	Small	0.877	Suboblate	92.6
30.	*Sisymbrium altissimum*	17.05	17.2	Small	0.991	Oblatespheroidal	80.8
31.	*Sisymbrium irio*	19	21.4	Small	0.887	Suboblate	84.3
32.	*Strigosella africana*	22.7	19.9	Small	1.140	Prolatespheroidal	90.8
33.	*Strigosella cabulica*	13.2	18.55	Small	0.711	Oblate	89.9
34.	*Strigosella intermedia*	20.6	16.45	Small	1.252	Subprolate	89.9
35.	*Tetracme stocksii*	20.7	20.3	Small	1.019	Prolate spheroidal	92.9

Table 7. Qualitative palynological characteristics of the investigated taxa of Brassicaceae

Taxa	Tribe	No. of apertures	Aperture orientation	Colpi apex	Amb	Exine sculpturing	Lumen shape
Aethionema carneum	Aethionemeae	Tricolpate	Sunken convex	Blunt	Lobate	Reticulate	Regular polygonal
Alyssum desertorum	Alysseae	Tricolpate	Raised concave	Round	Round	Coarsely reticulate	Irregular polygonal
Alyssum turkestanicum	Alysseae	Tricolpate	Raised concave	Round	Round	Reticulate	Regular amorphous
Brassica oleracea	Brassiceae	Tri-Synocolpate	Raised convex	Fused apices	Lobate	Reticulate	Regular polygonal
Capsella bursa-pastoris	Camelineae	Tricolpate	Raised convex	Blunt	Triangular	Coarsely reticulate	Irregular amorphous
Cardaria chalepensis	Alysseae	Tricolpate	Sunken concave	Acute	Lobate	Coarsely reticulate	Regular polygonal
Cardaria draba	Lepidieae	Tricolpate	Sunken convex	Blunt	Round	Coarsely reticulate	Regular polygonal
Chorispora tenella	Chorisporeae	Tricolpate	Sunken convex	Acute	Lobate	Reticulate	Regular polygonal
Clypeola aspera	Alysseae	Tricolpate	Raised concave	Blunt	Lobate	Reticulate	Regular polygonal
Coincya tournefortii	Brassiceae	Tri-Synocolpate	Raised concave	Fused apices	Triangular	Reticulate	Regular polygonal
Conringia orientalis	Conringieae	Tricolpate	Raised convex	Blunt	Round	Reticulate	Regular polygonal
Descurainia sophia	Descurainieae	Tricolpate	Raised convex	Acute	Lobate	Reticulate	Irregular amorphous
Dilophia salsa	Euclidieae	Tricolpate	Raised concave	Round	Round	Scabrate	Regular polygonal
Diplotaxis griffithii	Brassiceae	Tricolpate	Sunken convex	Acute	Round	Reticulate	Irregular amorphous
Diplotaxis harra	Brassiceae	Tricolpate	Raised concave	Acute	Lobate	Coarsely reticulate	Regular polygonal
Draba hystrix	Arabideae	Tricolpate	Raised concave	Blunt	Triangular	Reticulate	Irregular amorphous
Eruca sativa	Brassiceae	Tricolpate	Raised convex	Blunt	Triangular	Coarsely reticulate	Regular amorphous

Farsetia heliophila	Anastaticeae	Tricolpate	Raised concave	Acute	Lobate	Reticulate	Irregular amorphous
Goldbachia pendula	Calepineae	Tricolpate	Raised convex	Acute	Triangular	Reticulate	Regular amorphous
Isatis minima	Isatideae	Tricolpate	Raised convex	Acute	Triangular	Reticulate	Irregular polygonal
Isatis stocksii	Isatideae	Tri-Synocolpate	Raised convex	Fused apices	Round	Reticulate	Regular polygonal
Lepidium aucheri	Lepidieae	Tri-Synocolpate	Raised concave	Fused apices	Lobate	Reticulate	Regular polygonal
Leptaleum filifolium	Euclidieae	Tricolpate	Raised convex	Blunt	Lobate	Reticulate	Irregular polygonal
Matthiola flavida	Anchonieae	Tri-Synocolpate	Raised concave	Fused apices	Lobate	Coarsely reticulate	Irregular polygonal
Meniocus heterotrichus	Alysseae	Tri-Synocolpate	Sunken convex	Fused apices	Lobate	Reticulate	Irregular amorphous
Meniocus linifolius	Alysseae	Tricolpate	Raised convex	Acute	Triangular	Coarsely reticulate	Regular polygonal
Notoceras bicorne	Anastaticeae	Tricolpate	Raised convex	Blunt	Lobate	Coarsely reticulate	-
Physorrhynchus brahuicus	Brassiceae	Tri-Synocolpate	Raised concave	Blunt	Round	Reticulate	Regular polygonal
Raphanus raphanistrum	Brassiceae	Tri-Synocolpate	Sunken concave	Fused apices	Lobate	Reticulate	Regular polygonal
Sisymbrium altissimum	Sisymbrieae	Tri-Synocolpate	Raised concave	Fused apices	Lobate	Coarsely reticulate	Irregular amorphous
Sisymbrium irio	Sisymbrieae	Tricolpate	Raised convex	Blunt	Round	Reticulate	Regular amorphous
Strigosella africana	Euclidieae	Tricolpate	Raised convex	Round	Lobate	Reticulate	Irregular polygonal
Strigosella cabulica	Euclidieae	Tricolpate	Raised concave	Acute	Lobate	Reticulate	Irregular polygonal
Strigosella intermedia	Euclidieae	Tricolpate	Raised concave	Acute	Lobate	Reticulate	Regular amorphous
Tetracme stocksii	Euclidieae	Tricolpate	Raised convex	Acute	Round	Reticulate	Irregular polygonal

Table 8. Quantitative palynological measurements of Brassicaceous taxa

Plant Species	P.D µm	E.D	W.C.P	L.C.P	W.C.E	L.C.E	M	E.T
Min-Max=Mean±SE								
Aethionema carneum	17-19.75=18.5±0.5	16-18.5=17.45±0.4	1.25-4=5.25±4.55	3-5.5=8.5±7.6	1-4.5=5.5±4.9	3-15.75=18.75±17.3	4-10.5=14.5±1.2	12.75=3.7±3.1
Alyssum turkestanicum	24.5-28=26±0.647108955	21-23.75=22.35±0.503736042	1.5-3.75=5.25±4.55	1.75-5.5=7.25±6.45	1.5-3.75=5.25±4.35	3.5-17.25=20.75±18.6	4.25-10.25=14.5±12.95	1-1.75=2.75±2.1
Alyssum desertorum	17-19=17.85 ±0.358817502	17.5-20.25= 19.35±0.49117	5-5.75= 5.45±0.1457	4.5-5=4.8 ±0.093541435	5.25-6.25= 5.6±0.187082	7.75-9.5= 8.7±0.320156212	6.25-7.5= 6.95±0.215	2.25-3.25= 2.7±0.1658
Brassica oleracea var. *capitata*	24.5-26=25.3±0.289	17-18.5=17.75±0.2	4.75-6=5.45±0.215	4.25-5.25=4.65±0.16	2.75-3.5=3.05±0.14	19.5-20.75=20.1±0.21	10.25-13.75= 11.35±0.714	2-3.25= 2.65±0.23
Capsella bursa-pastoris	31-33.25=32.3±0.3	35.5-38=36.75±0.48	2-3=2.45±0.183	3.75-4.75=4.25±0.17	2.75-3.25=3±0.11180	10.25-10.75=10.45±0.09	10.25-15.5=12.95±1	1.75-3=2.35±0.2
Cardaria chalepense	21.75-23=22.35±0.231840462	18-20.5=19.35±0.515994186	3.5-5.25=4.45±0.289395923	5.5-7=6.3±0.266926956	2.75-3.5=3.1±0.127475488	14.75-16.5=15.8±0.310241841	10.25-13.75=12.6±0.610327781	1.75-2.75=2.2±0.16
Cardaria draba	15.25-16.25=15.6±0.1	16.25-18.75=17.4±0.4	1.5-3.25=4.75±4.15	1.75-6.25=8±7	1-2.75=3.75±3.25	2-16=18±17.05	5-2.75= 10.2±13 11.7	1-2.75=3.75±3
Chorispora tenella	20.25-21.5=20.9±0.217944947	17-18=17.45±0.183711731	4.75-6=5.45±0.215058132	4.5-5.75=5.15±0.231840462	3.25-5.25=4.25±0.353553391	9.75-11.25=10.6±0.26925824	10.25-12.75=11.75±0.425734659	2.75-3.5=3.05±0.145773797
Clypeola aspera	18.75-21.75=20.2±0.538516481	20.25-22.75=21.35±0.484767986	1-5.5=6.5±6	1.75-5.5=7.25±6.35	1.75-5.25=7±6.4	1.25-12.75=14±13.3	5.5-10.25=15.75±13.55	0.75-2.75=3.5±3.1
Coincya tournefortii	17.75-20.5=19.4±0.509901951	19.5-21.75=20.65±0.392109679	6.25-8.5=7.65±0.407737661	7.75-9=8.3±0.215058132	2.75-3.25=2.95±0.093541435	12.75-14=13.3±0.215058132	10.25-12.75=11.1±0.444409721	2-3=2.55±0.183711731
Conringia orientalis	17.25-20.75=19.35±0.605185922	19.5-20.75=20.15±0.231840462	3-4.5=3.9±0.257390754	5.25-8=6.9±0.491172068	3-4=3.5±0.176776695	13.5-15.25=14.25±0.306186218	13.5-15.25=14.35±0.302076149	2.75-3.75=3.15±0.16955825

Descurainia Sophia	18.75-20.75=19.7±0.374165739	18.75-20.75=19.95±0.365718471	6.5-8.5=7.5±0.379143772	7.75-9.5=8.5±0.306186218	2.75-3.5=3.05±0.145773797	13.25-15.5=14.65±0.392109679	10.25-15.75=13.6±0.986154146	1.75-2.75=2.2±0.16583124
Dilophia salsa	30.25-35.25=33.05±0.982344135	30.25-35.5=33.2±0.982344135	4.25-5.5=4.95±0.242383993	9.25-10.25=9.65±0.16955825	10.25-11.5=10.95±0.215058132	22.75-23.75=23.25±0.176776695	10.25-24.5=18±2.287192602	2.75-3.5=3.15±0.127475488
Diplotaxis griffithii	17-18.25=17.65±0.231840462	13.75-15.5=14.75±0.306186218	5.25-6.5=5.95±0.215058132	8.75-10.75=9.85±0.331662479	2.75-3.75=3.25±0.176776695	13.25-15.2=14.25±0.353553391	10.25-11.25=10.75±0.176776695	2.75-4.25=3.55±0.254950976
Diplotaxis harra	23.75-24.5=24.2±0.145773797	18-19.75=19±0.306186218	4.25-5.5=4.85±0.231840462	7.75-10.5=8.85±0.539675829	4.5-5.75=5.15±0.231840462	12.75-14.5=13.45±0.310241841	10.25-14=12.65±0.635413251	1.75-2.75=2.3±0.2
Draba hystrix	15.25-19.5=17.45±0.70445014	17.25-18=17.5±0.158113883	4.75-6.25=5.6±0.26925824	5.25-8.5=6.65±0.635413251	2.75-3.25=3.05±0.093541435	12.25-13.25=12.8±0.16583124	10.25-12.75=11.65±0.451386752	2-3.25=2.65±0.231840462
Eruca sativa	15.25-17=15.85±0.302076149	17.75-19.25=18.4±0.26925824	2.75-3.75=3.25±0.176776695	9.25-10.25=9.65±0.16955825	2.75-3.25=3±0.111803399	12.25-14=13.25±0.306186218	9.75-11=10.35±0.20310096	2.75-3.75=3.15±0.16955825
Farsetia heliophila	16.25-17.25=16.9±0.187082869	17.75-20.25=18.9±0.471699057	5.25-6.5=5.95±0.215058132	6.25-7.75=7±0.25	2.75-3.5=3.05±0.145773797	12.75-13.75=13.25±0.176776695	10.25-13.25=11.65±0.578791845	2.75-3.5=3.1±0.127475488
Goldbachia pendula	24.25-26=25.15±0.340954542	18-20.25=18.75±0.395284708	3.5-5.25=4.5±0.285043856	5.5-6.75=6.05±0.215058132	3.25-5.25=4.1±0.35	17.25-18.75=18.15±0.257390754	10.25-15.5=13.2±0.863857627	2.75-3.25=2.95±0.093541435
Isatis minima	19.75-22.25=20.9±0.430116263	22.25-23.75=23.1±0.26925824	5.25-6.25=5.75±0.176776695	6.75-8=7.35±0.231840462	2.75-3.5=3.1±0.127475488	13-14.75=13.95±0.320156212	9.25-10.5=9.95±0.242383993	2.75-10.25=4.5±1.440486029
Isatis stocksii	17.75-19.5=18.55±0.348209707	18-22.25=20.15±0.700892288	1.5-4.75=6.25±5.65	1.25-6.75=8±7.55	1.5-4.25=5.75±4.95	4.25-12.25=16.5±14.2	3.25-10.25=13.5±12.7	0.75-2.75=3.5±3.1
Lepidium aucheri	17-18.25=17.65±0.231840462	17.75-19.5=18.6±0.322102468	3.75-7=5.6±0.610327781	10.75-13.25=12.15±0.430116263	2-3.25=2.65±0.231840462	11.25-13=12.05±0.320156212	15.75-17.75=16.85±0.340954542	1.5-2.25=1.85±0.127475488
Leptaleum filifolium	18.75-20.5=19.9±0.31	17.75-18.75=18.2±0.2	5.25-6.25=5.75±0.17	5.75-7.25=6.45±0.28	5.25-6=5.6±0.1274	15.25-16.25=15.6±0.18	9.5-10.7=10.1±0.2	2.75-3.5=2.9±0.1

Matthiola flavida	23.75-24.5=24.25±0.1	23-24=23.55±0.18	5.25-6.25=5.75±0.1	7-8=7.55±0.183	2.75-3.5=3.15±0.127	14.5-17.25=16.05±0.58	10.25-16.5=14.6±1.1	2.75-3.5=3.1±0.1
Meniocus heterotrichus	21.75-23.25=22.4±0.26925824	18.25-21.25=19.65±0.551135192	3-5.25=4.35±0.375832409	5.5-6.25=5.8±0.145773797	2.75-3.5=3.1±0.127475488	15.25-16.25=15.75±0.176776695	10.25-16=14.55±1.082243041	2.75-3.5=3.1±0.127475488
Meniocus linifolius	20.25-21.25=20.6±0.187082869	18-18.75=18.3±0.145773797	3.75-5.25=4.55±0.242383993	2.75-7.25=5.05±0.73058196	4-4.75=4.35±0.127475488	3-4.25=3.7±0.215058132	4.5-10.25=6.05±1.064776972	1.5-2.25=1.95±0.145773797
Notoceras bicorne	18-19.5= 18.7±0.26692	20.25-22= 20.95±0.310	4.25-5.5= 4.85±0.2318	6.25-7.75= 7.05±0.266	2-3.25= 2.65±0.2318	12.5-13.5= 13±0.176776695	12.25-14.75= 13.4±0.44440	1.5-2.25= 1.85±0.127
Physorrhynchus brahuicus	21.5-23=22.2±0.289395923	18.75-20.75=20±0.353553391	4.5-5.75=5.15±0.231840462	7-8.25=7.65±0.231840462	2.75-3.5=3.05±0.145773797	12.75-14.5=13.75±0.32596012	8.75-10.5=9.85±0.322102468	2.75-3.5=3.05±0.145773797
Raphanus raphanistrum	15.25-17=16.05±0.289395923	18-18.75=18.3±0.145773797	5.25-7=6.1±0.322102468	8-9.75=8.95±0.320156212	2.75-3.5=3.15±0.127475488	12-13.25=12.65±0.231840462	12.75-14.5=13.55±0.320156212	1.75-3=2.35±0.231840462
Sisymbrium altissimum	16-18.25=17.05±0.365718471	15.75-18.25=17.2±0.421307489	1.25-4.25=5.5±4.85	2-7.25=9.25±8.3	1.5-4.25=5.75±4.9	0.75-12.75=13.5±13.15	4-10.25=14.25±12.95	1-2.75=3.75±3.25
Sisymbrium irio	18.5-19.5=19±0.17	20.75-22.25=21.4±0.3	6.25-7.75=7±0.25	7-8.75=7.75±0.32	3.25-5=4.2±0.32	12-13.5=12.7±0.2	11.25-13=12.05±0.36	2-3.25= 2.65±0.231
Strigosella africana	20.5-25.25=22.7±0.92668765	17.75-21.25=19.9±0.696419414	4.25-5.75=5.2±0.254950976	3.25-5.25=4.05±0.365718471	3.75-5.5=4.75±0.306186218	18.25-22.75=20.55±0.81929848	10.25-15.25=13.2±0.815475322	2.75-3.25=2.95±0.093541435
Strigosella cabulica	12.75-14.25=13.2±0.278388218	18-19.25=18.55±0.215058132	6.25-7.75=6.95±0.266926956	6.25-8.75=7.5±0.418330013	2.75-3.75=3.25±0.176776695	8-9.75=8.9±0.340954542	10.25-11.25=10.7±0.2	1.75-2.75=2.15±0.16955825
Strigosella intermedia	19.75-21.5=20.6±0.302076149	15.5-17.75=16.45±0.413823634	6.25-7.75=7 0.25	6.25-7.75=7±0.25	1.75-2.75=2.15±0.16955825	12.75-14=13.3±0.215058132	10.25-13.25=12.15±0.527967802	2-3.25=2.65±0.231840462
Tetracme stocksii	20.25-21.25=20.7±0.16583124	19.-21.25=20.3±0.320156212	3.25-4.=7.75±5.75	0.75-5.5=6.25±5.85	1.75-4.5=6.25±5.45	2-12=14±13.1	2.5-10.25=12.75±11.7	1.25-2.75=4±3.15

(P.D: polar diameter; E.D: equatorial diameter, L.C.P, W.C.P, L.C.E, W.C.E: length and width of colpi in polar and equatorial view, M: mesocolpium, E.T: exine thickness)

Table 9. Dichotomous parallel taxonomic key based on pollen morphological traits of Brassicaceous flora

Link character	Present (+) /absent (-)	Diagnostic characters	Species name
1	+	Small, prolate spheroidal, lumen shape regular polygonal ………………..………………………………..	*Aethionema carneum*
	-	Medium, subprolate, lumen shape regular amorphous………………………………………………………	2
2	+	Reticulate……………………………………………………………………………………………...	*Alyssum turkestanicum*
	-	Small, oblate spheroidal, coarsely reticulate, lumen shape irregular polygonal……………………………..	3
3	+	Tricolpate, Amb round, colpi raised concave, colpi apex round ……………………………………...	*Alyssum desertorum*
	-	Prolate, colpi apex fused, Amb lobate, lumen shape regular polygonal……………..……….………..	4
4	+	Trisynocolpate…………………………………………………………………………………….	*Capsella bursa-pastoris*
	-	Suboblate, colpi apex blunt, Amb triangular, lumen shape irregular amorphous …………………….	5
5	+	Medium, colpi raised convex, ……………………………………………………...............................	*Cardaria chalepensis*
	-	Subprolate, colpi sunken concave, colpi apex acute, Amb lobate …………………………………….	6
6	+	Coarsely reticulate, …………………………………………………………………………………	*Cardaria draba*
	-	Oblate spheroidal, colpi apex blunt, Amb round, …………………………………….	7
7	+	Lumen shape regular polygonal…………………………………………………………………...	*Chorispora tenella*
	-	Subprolate, colpi apex acute ………………………………………………………………………....	8
8	+	Colpi sunken convex………………………………………………………....................................	*Clypeola aspera*
	-	Oblate spheroidal, colpi apex blunt………………………………………………………………………..	9
9	+	Tricolpate, Amb lobate, reticulate…………………………………………………………………..	*Coincya tournefortii*

	-	Trisynocolpate, colpi apex fused, Amb triangular, coarsely reticulate, ……………………………….	10
10	+	Colpi raised concave ………………………………………………………………………………...	*Descurainia sophia*
	-	Amb round, colpi apex blunt, reticulate………………………………………………………….	11
11	+	Lumen shape Regular polygonal…………………………………………………………………..	*Eruca sativa*
	-	Amb lobate, psilate reticulate, lumen shape irregular amorphous…………………………………….	12
12	+	Colpi raised convex…………………………………..	*Farsetia hamiltonii*
	-	Coarsely reticulate, colpi raised concave, colpi apex round,lumen shape regular polygonal………………..	13
13	+	Small, oblate spheroidal…………………………………………………………………………...	*Farsetia heliophila*
	-	Colpi sunken convex, reticulate, lumen shape irregular amorphous ………………………………………..	14
14	+	Medium, Amb round………………………………………………………………………………..	*Goldbachia pendula*
	-	Coarsely reticulate, lumen shape regular polygonal………………………………………………………	15
15	+	Subprolate, colpi apex acute…………………………………………………………………………	*Lepidium aucheri*
	-	Oblate spheroidal, colpi apex blunt, reticulate, lumen shape irregular amorphous …………………………	16
16	+	Colpi raised concave…………………………………………………………………………………	*Matthiola flavida*
	-	Colpi raised convex, lumen shape regular amorphous …………………………………...………..	17
17	+	Amb triangular, colpi apex blunt…………………………………………………………………	*Meniocus linifolius*
	-	Colpi raised concave, Amb lobate, reticulate, lumen shape irregular amorphous……………………..	18
18	+	Suboblate………………………..…………………………………………………………………….	*Nasturtium officinale*
	-	Prolate, lumen shape regular amorphous……………………………………………….	
19	+	Small…………………………………………………………………………………………………	*Notoceras bicorne*
	-	Medium, reticulate, lumen shape irregular amorphous…………………………………………………	20
20	+	Tricolpate, Amb triangular, colpi apex acute, ……………………………..	*Physorrhynchus brahuicus*

	-	Coarsely reticulate, Amb round ………………………………………………………………….	21
21	+	Colpi raised convex………………………………………………………………………………..	*Raphanus raphanistrum*
	-	Colpi raised concave………………………………………………………………………………	22
22	+	Oblate spheroidal, trisynocolpate, colpi apex fused, lumen shape regular polygonal……………................	*Sisymbrium altissimum*
	-	Tricolpate, colpi raised convex, colpi apex blunt…………………………………………………………	23
23	+	Reticulate…………………………………………...………………………………………………..	*Strigosella africana*
	-	Colpi raised concave, coarsely reticulate, ………………………………………………………………..	24
24	+	Lumen shape irregular polygonal, ………………………………………………………………………..	*Strigosella cabulica*
	-	Colpi sunken convex, reticulate, lumen shape irregular amorphous…………………………..................	25
25	+	Trisynocolpate, Amb lobate, colpi apex fused ………………..	*Strigosella intermedia*
	-	Colpi apex acute, Amb triangular, coarsely reticulate, lumen shape regular polygonal……………….	26
26	+	Prolate spheroidal……………………………………………………………………………………….	*Meniocus linifolius*
	-	Oblate spheroidal, Amb lobate, ………………………………………………………………	27
27	+	Tricolpate, colpi raised convex………………………………………………………………………………	*Notoceras bicorne*
	-	Prolate spheroidal, colpi raised concave………………………………………………………………..	28
28	+	Colpi apex blunt………………………………………………………………………………………...	*Physorrhynchus brahuicus*
	-	Suboblate, colpi sunken concave, lumen shape regular polygonal……………………… …………………	29
29	+	Lumen shape regular polygonal…………………………………………………………………………...	*Raphanus raphanistrum*
	-	Oblate spheroidal, colpi raised concave, colpi apex fused, coarsely reticulate, lumen shape irregular amorphous…………………………………………………………………………………………………	30

3.2 Pollen Micromorphology of the Fabaceous taxa in Northern Baluchistan

3.2.1 Results and Discussion

a) Symmetry, Polarity, and Unity of Pollen in Fabaceae

The microscopic visualization of pollen is one of the important techniques for systematics studies. The light and scanning electron microscopy successfully added to the distinction and separation of numerous taxa of different families. The present research work was carried out to determine the palynomorph of Fabaceous taxa for the first time from Northern Baluchistan. The description of variations within characters was noted following Erdtman (1952), Graham et al. (1980), Ferguson and Banks (1994), Silvestre-Capelato and Melhem (1997), Perveen and Qaiser (1998), Banks and Lewis (2009), Buril et al. (2011), and Matos et al. (2020). All the studied taxa were radially symmetrical, isopolar, and monad. The shape and arrangement of the apertures were similar on both poles. Thus, traits such as polarity, symmetry, and unity were non-significant taxonomically.

b) Pollen Size and Shape of Fabaceous taxa

Medium size of pollen was observed in 29 species (Table 11). The pollen in 5 species were small in size. Pollen in three *Astragalus* species were small. *Alhagi maurorum* and *Crotalaria burhia* also observed with small size pollen. The shape of the pollen was determined from the P/E ratio. Variations were observed within the shape and found significant in the distinction of the Fabaceous taxa. The shape of pollen in 18 species was subprolate. The prolate-spheroidal shape was investigated in 9 species including *Astragalus stocksii* and *Astragalus tribuloides.* In 6 species the shape was prolate, including *Astragalus subumbellatus.* Similar results were reported by Liao et al. (2022) for the presence of a range of pollen shapes, including suboblate, spheroidal, subprolate, and prolate in *Astragalus* and *Sophora*. The shape oblate-spheroidal was observed in *Lathyrus oleraceus.*

c) Apertures with Number Position Character (NPC) Classification

Tricolporate, tricolpate, and trisynocolpate apertures were observed in the examined 34 Fabaceous species. Tricolporate aperture was noted in 25 species. The tricolpate aperture was observed in 7 species. Trisynocolpate aperture was present in two species. The edges of the colpi merged at the apices in trisynocolpate aperture. The number, position, and character of aperture (NPC) determined two types trizonocolpate $N_3P_4C_3$ and trizonocolporate $N_3P_4C_5$. The three significant variable traits in this study for the examined species were: polar view, equatorial view, and exine sculpturing. Liao et al. (2022) described the genera such as *Astragalus, Sophora* as taxonomically problematic. In the present study, pollen in all examined (12) species of *Astragalus* were tricolporate.

Although Liao et al., (2022) concluded that palynological analyses can offer some helpful information for identifying these taxa, pollen morphology alone is insufficient to clarify or rebuild taxonomic relationships within these genera. They examined 11 species of *Sophora* as tricolporate (occasionally six-aperture). In this study, it was examined that the additional pollen features such as polar and equatorial view, amb, sculpturing were found significant to aid in the systematics of problematic taxa. The apertures in *Sophora mollis* were tricolporate. The species of genera *Crotalaria, Ebenus, Indigofera, Onobrychis, Vicia* were characterized by tricolpate apertures. The colpi in *Vicia macrantha* were adjoining, created specific trisynocolpate type of aperture.

d) Polar view, Equatorial view, Amb

Significant variations were observed in the polar view, equatorial view, and amb of the pollen of examined Fabaceous species. The circular polar view was observed in pollen of 16 species. Triangular obtuse convex polar view of pollen was present in 7 species. Triangular obtuse concave polar view was noted in the pollen of three species. Other observed types in polar view were circular to triangular obtuse convex, triangular obtuse convex to straight, circular to elliptic, circular to oval, and circular to triangular. Variations were observed in the equatorial view of the pollen in the examined species. 12 species were observed with rectangular obtuse convex equatorial view. Pollen in five species were oval in the equatorial view. In seven species, the appearance of pollen in the equatorial view was elliptic to oval. The elliptic truncate equatorial view was observed in six species. The quadrangular equatorial view was present in the pollen of two species. The rhombic equatorial view was observed in *Sophora mollis.* In *Medicago* *lupulina* the equatorial view was circular. The alterations in the polar and equatorial view of the pollen were significant in the distinction of Fabaceous species.

The amb of the pollen also added to the separation of studied Fabaceous species. The observed amb of pollen was peritreme in 22 species. The goniotreme amb was found in 12 species (Table 10). Though there were only two types of observed amb, but this feature was not genus-specific. The amb varied from species to species. The differences in the palynological traits of *Lotus corniculatus* and *Lotus garcinii* were significant for the separation of these taxa. The pollen in *Lotus corniculatus* was circular to triangular obtuse convex in polar view, elliptic truncate in equatorial view, psilate exine and aperture membrane, with amb goniotreme. In *Lotus garcinii*, the pollen was circular in polar view, elliptic truncate equatorial view, psilate to reticulate exine sculpturing, with psilate aperture membrane and peritreme amb. Similarly, in both of the *Medicago* species the pollen was similar psilate exine and aperture membrane, with goniotreme amb. The polar view and equatorial view were different in *Medicago* species. In *Medicago lupulina*, the pollen were triangular obtuse convex polar and circular in equatorial view. While in *Medicago polymorpha* the pollen were triangular obtuse concave polar and oval in equatorial view.

e) Sculpturing of Exine and Aperture membrane

The ornamentation of the exine was important in the distinction of the species. The exine in three species *Alhagi maurorum, Indigofera cuneifolia, and Indigofera intricata* was microreticulate. The 'reticulate, psilate at the polar end' type of sculpturing was observed in four *Astragalus* species. The simple reticulate sculpturing was present in four species. *Astragalus subumbellatus* was noted with distinct psilate verrucate exine. The macroreticulate exine was present in six species. The combinations of reticulate, psilate, and verrucate exine were also observed within the pollen of the studied species. The observed variations in the exine sculpturing separated the Fabaceous species. Similarly, the aperture membrane was psilate, verrucate, or a combination of these two types of sculpturing (Plate 42-50).

The analysis of qualitative and quantitative features revealed significant variations for taxonomic studies (Ferguson et al., 1994; Zhao et al., 2007; Liao et al., 2022). The family Fabaceae is eurypalynous, with variation in the pollen size, aperture, exine sculpturing and other characters. Lattar et al. (2020) stated one tribe of Fabaceae, Aeschynomeneae as eurypalynous and the second one Adesmieae as stenopalynous. Soares et al. (2021) concluded that the pollen morphology significantly differentiated among the subfamilies of Fabaceae i.e Caesalpinioideae and Detarioideae. Soares et al. (2021) documented that pollen of the Fabaceous species can be distinguished from one another by differences in the following: type of endoaperture (lolongate, circular, or lalongate); position of apertures (angulaperturate, planaperturate, subplanaperturate, or fossaperturate); size (small to large); amb (circular to triangular); shape (oblate to prolate); details of the polar area (very small to large); and exine ornamentation (psilate, scabrate, microreticulate, or reticulate heterobrochate).

The pollen micromorphological traits of the genus *Onobrychis* were relatively homogenous, similar observations were reported by Talebi et al., (2020). *Onobrychis* species slightly differ in the qualitative palynological characters. The polar view varied from circular to oval. Whereas equatorial view appeared as rectangular obtuse convex. The exine sculpturing existed in reticulate to microreticulate, and macroreticulate forms. The *Onobrychis cornuta* was observed as reticulate to microreticulate, oval polar view, rectangular obtuse convex equatorial view. Talebi et al. (2020) reported the *Onobrychis* as obtuse convex in equatorial view and circular in polar view with reticulate ornamentation. Earlier studies specified the *Onobrychis* species with reticulate exine (Pavlova and Manova 2000; Amirabadizadeh and Ghanavati, 2012; Avci et al., 2013).

Crotalaria species were observed with similar palynomorphic features in this study. The difference occurred in the exine structure. In *Crotalaria burhia* the exine was reticulate whereas in *Crotalaria medicaginea* macroreticulate. Bahadur et al., (2023) denoted the variations from reticulate to psilate in the exine sculpturing, and the aperture surface membrane morphology i.e scabrate, gemmate, verrucate as significant variations for the separation of species. *Indigofera cuneifolia* was observed with verrucate to psilate exine while *Indigofera intricata* exine was psilate. The *Astragalus* genus was noted with verrucate or psilate aperture membrane. Ceter et al. (2013) examined the aperture membrane as granulate or verrucate in colpi whereas the operculum membrane as verrucate, granulate, rugulate and reticulate in the *Astragalus* L. section Hololeuce Bunge. They also observed variations in the exine sculpturing from granulate to perforate, reticulate, perforate polar section, and perforate, reticulate, microreticulate, microreticulate to perforate, microrugulate to perforate, perforate to granulate, microrugulate to microreticulate, microreticulate to granulate.

Astragalus species varied in the appearance of polar and equatorial views, ornamentation of exine, surface of aperture membrane and quantitative variables. Ceter et al. (2013), also reported the same that there were differences in the pollen characteristics of *Astragalus*, including pollen form, aperture type, and ornamentation. In particular, pollen surface ornamentation on the polar and equatorial views was useful in differentiating the Fabaceous species. Earlier, Bagheri et al., (2019) reported the genus *Astragalus* as tricolpate and micro reticulate exine. The general morphological traits in the *Astragalus* expressed homogeneity. In this study the variations in the amb, polar and equatorial view, and aperture membrane aid in the discrimination of species. In several species of the genus *Sophora*, echinate exine was previously documented by Liao et al. (2022). In the current study exine in *Sophora* was reticulate to verrucate with psilate aperture membrane.

f) Multivariate Analysis of pollen morphological features of Fabaceae

Principal component analysis identified the variables and aided in the visualization of data set trends (Kim et al., 2015). Based on the PCA plot, the mesocolpium, polar length of colpi, polar width of colpi, and equatorial width of colpi were found significant in the discrimination of species (Figure 12). Uga et al. (2009) determined the link between the micromorphological features using PCA plots. The correlation plots, PCA, and loading plots revealed significant variations in the quantitative features (Kim et al. 2015). A correlation plot helped in determining the relationship and strengthened the principal component analysis. The highest positive correlation 0.85 observed between the polar axis and equatorial diameter. The equatorial length of the colpi and equatorial diameter were also positively correlated 0.61 (Figure 14). A negative correlation -0.24 was observed between mesocolpium and the polar length of the colpi. The UPGMA dendrogram distinguished and highlighted the variations among the taxa of the same genus (Figure 13). *Tephrosia uniflora* subsp. *Petrosa* was distinctly placed from the major cluster. The major cluster included 27 species, whereas 7 species were in the small cluster. The quantitative traits significantly separated the species in the dendrogram. The normal probability plot determined the data distribution along the plot of normal order statistics medians. The exine thickness was normally distributed. The variations were observed in the polar axis, equatorial diameter, polar width of colpi, and equatorial width of colpi (Figure 15). Conclusively there were statistically significant differences between the means of the parameters that were examined, this can be utilized as a methodical approach to taxonomic studies. The combination of statistical analysis with micromorphological pollen features provided valuable insights into the differentiation of the Fabaceous species. The variation in the exine sculpture, surface, operculum, exine thickness, pollen shape, aperture shape, and pollen size significantly aided in the differentiation of Fabaceous species.

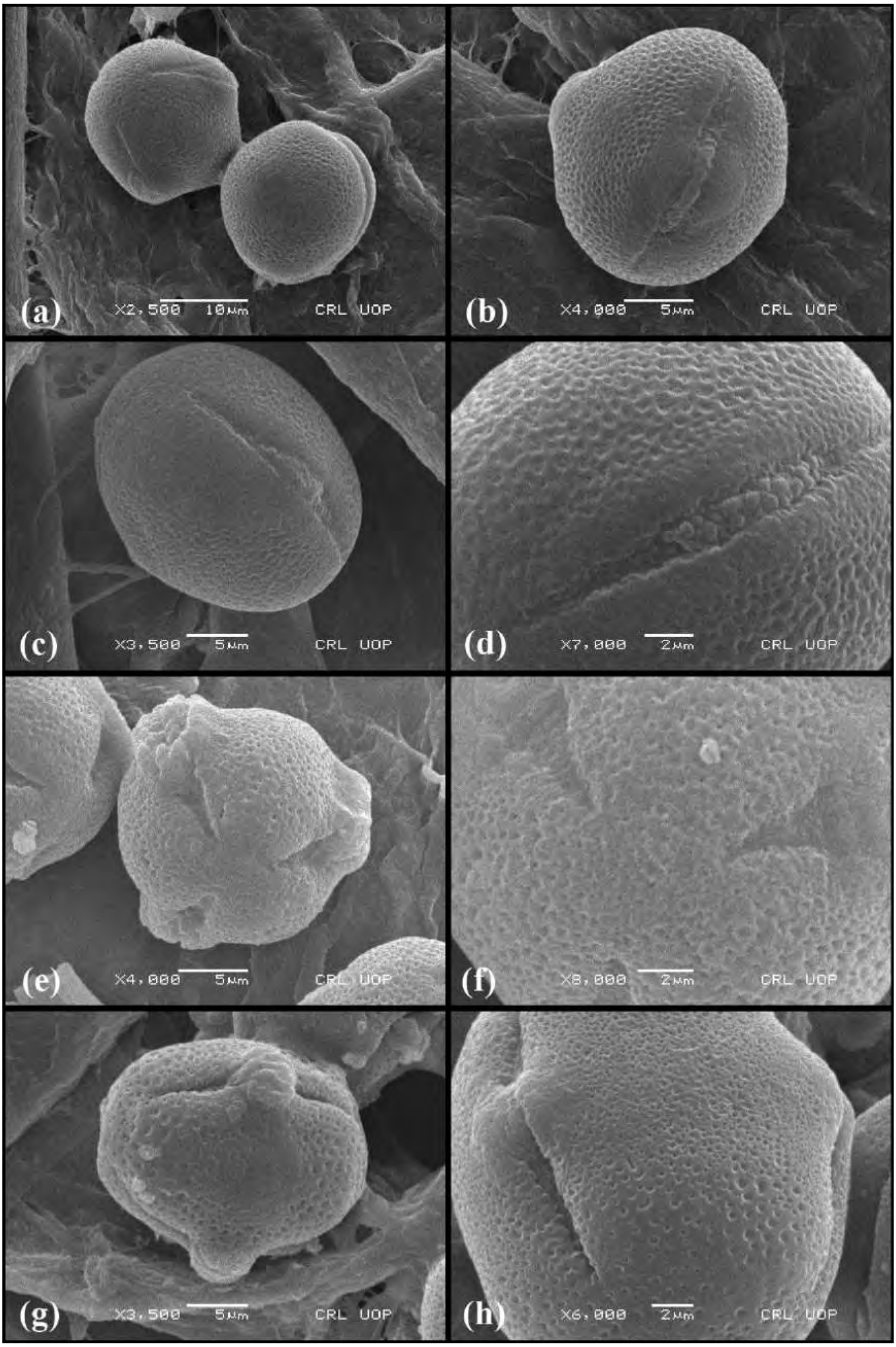

Plate 42. SEM micrographs of pollen (a), (b) *Alhagi maurorum,* (c), (d) *Astragalus anisacanthus,* (e), (f) *Astragalus brahuicus*, (g), (h) *Astragalus crenatus*

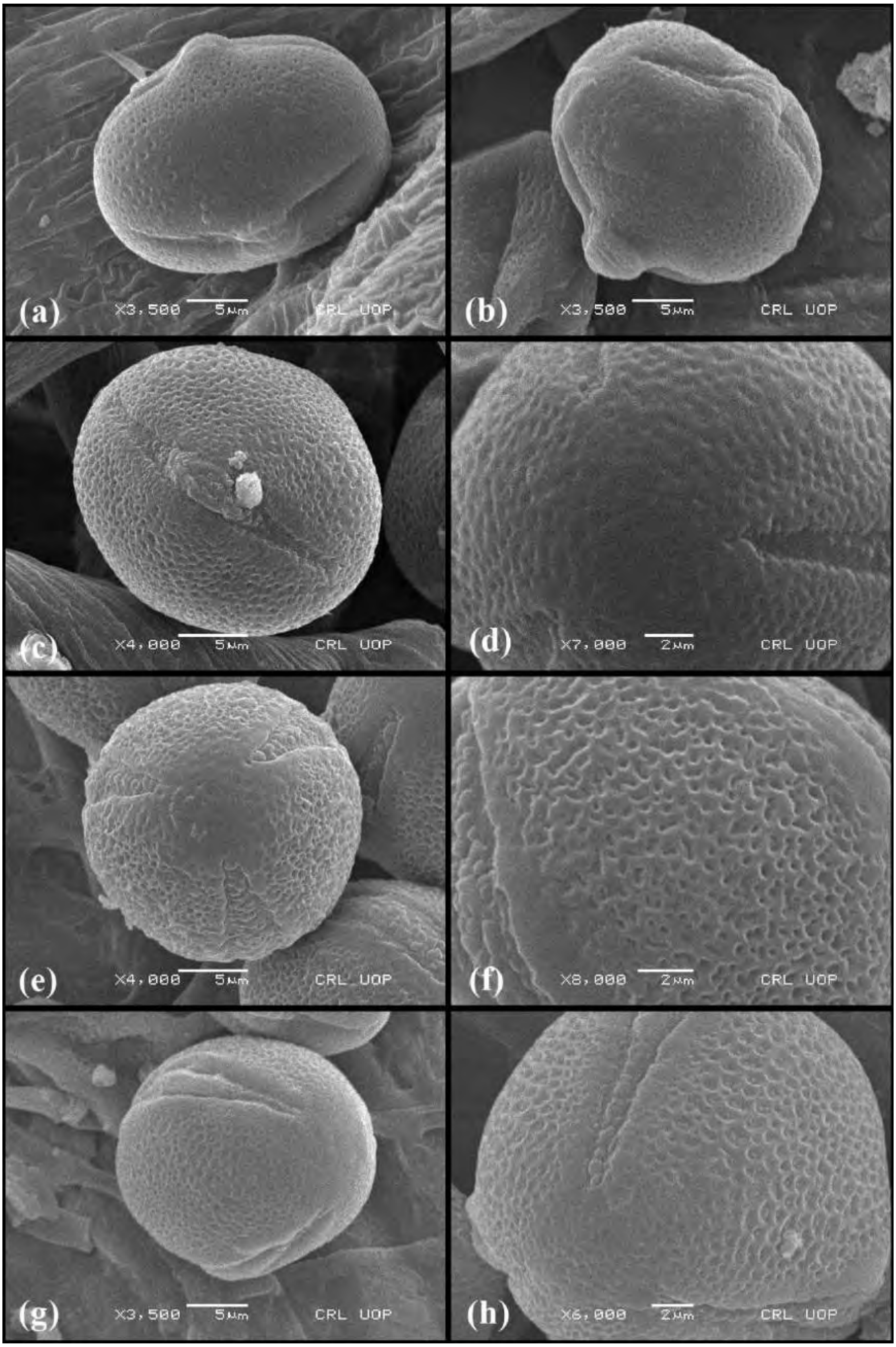

Plate 43. SEM micrographs of pollen (a), (b) *Astragalus diphtherites,* (c), (d) *Astragalus hemsleyi,* (e), (f) *Astragalus hostilis*, (g), (h) *Astragalus hypoglottis*

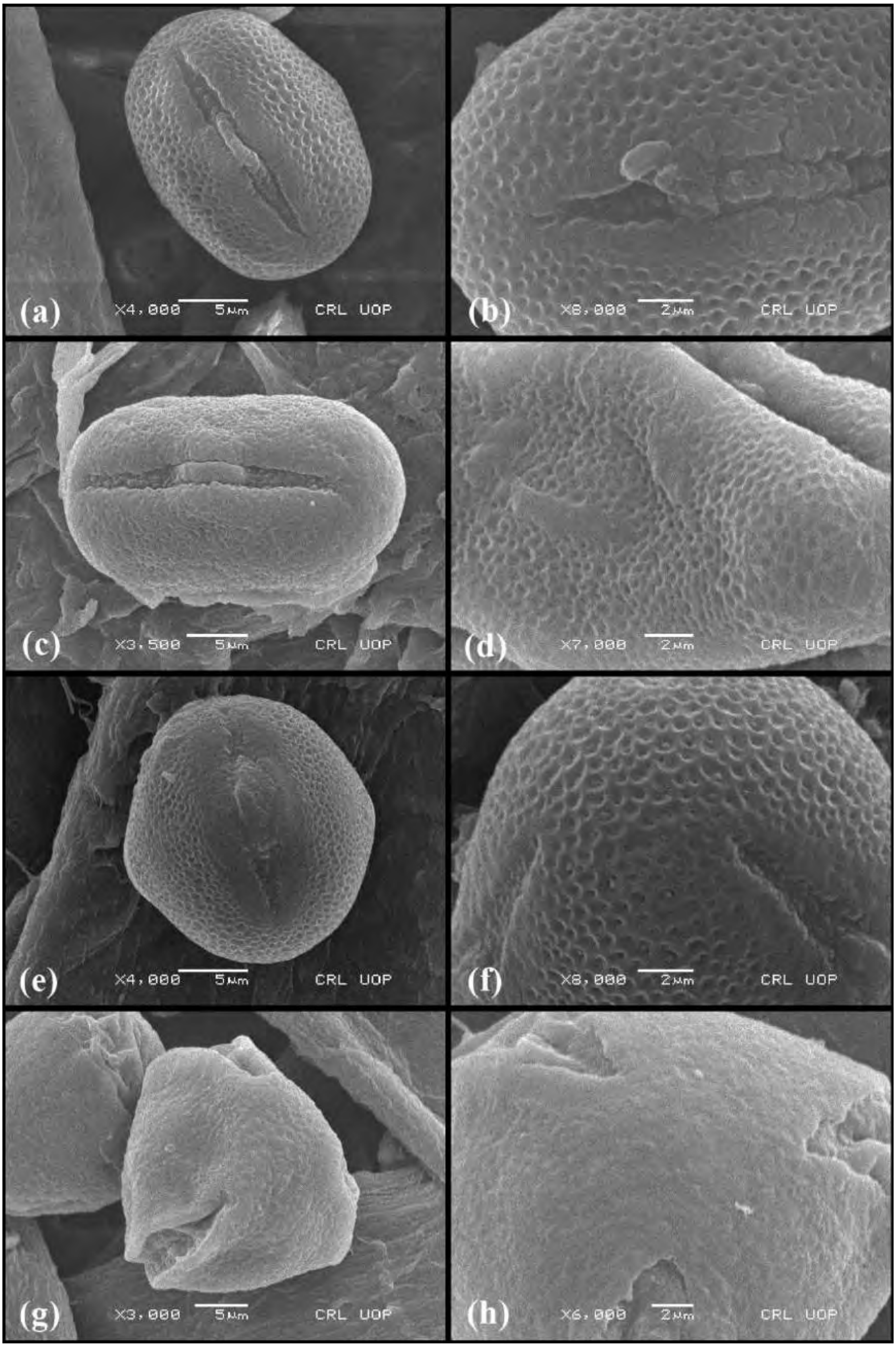

Plate 44. SEM micrographs of pollen (a), (b) *Astragalus ophiocarpus,* (c), (d) *Astragalus oxyglottis,* (e), (f) *Astragalus stocksii*, (g), (h) *Astragalus subumbellatus*

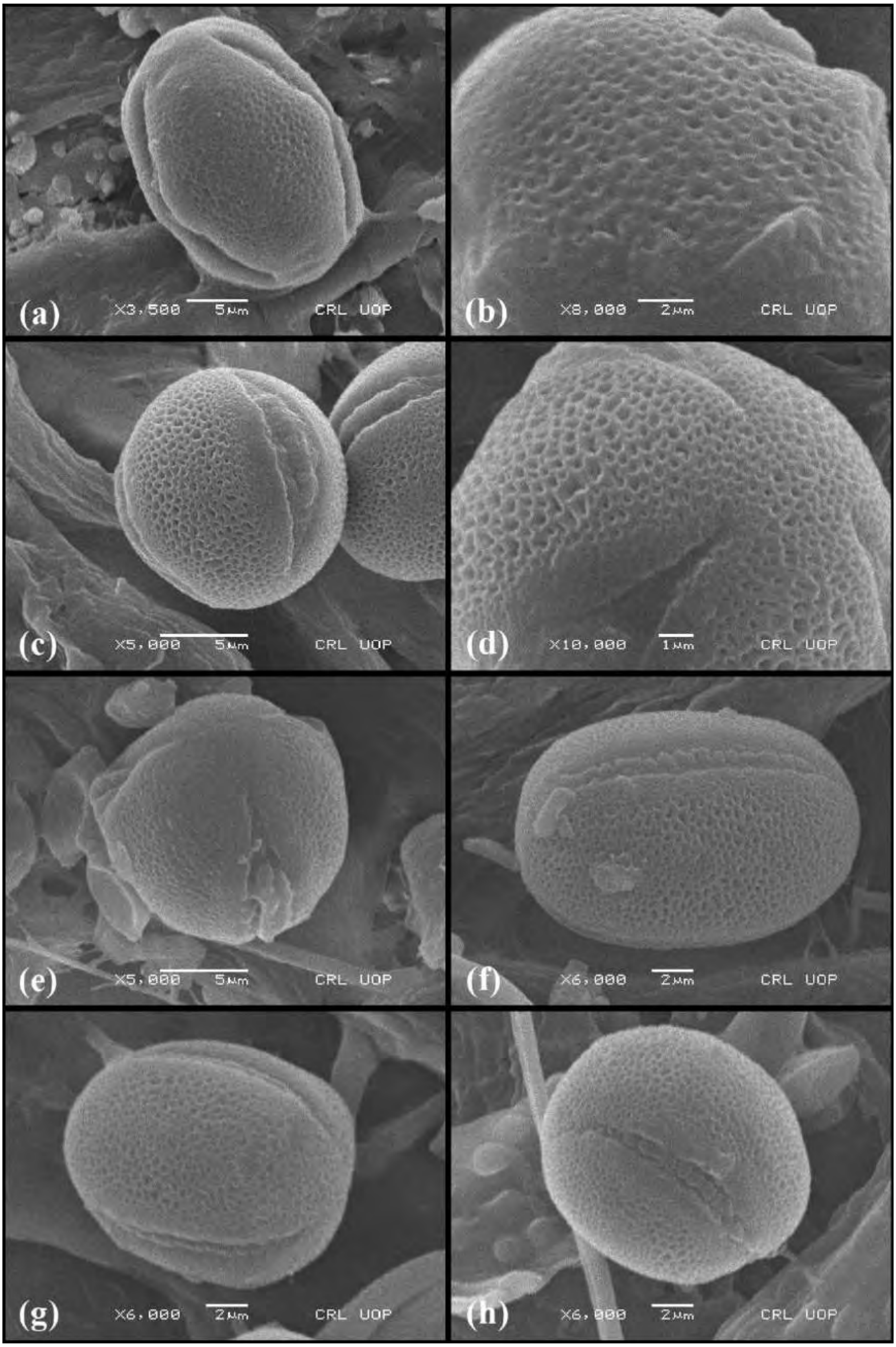

Plate 45. SEM micrographs of pollen (a), (b) *Astragalus tribuloides,* (c), (d) *Caragana ambigua,* (e), (f) *Crotalaria burhia,* (g), (h) *Crotalaria medicaginea*

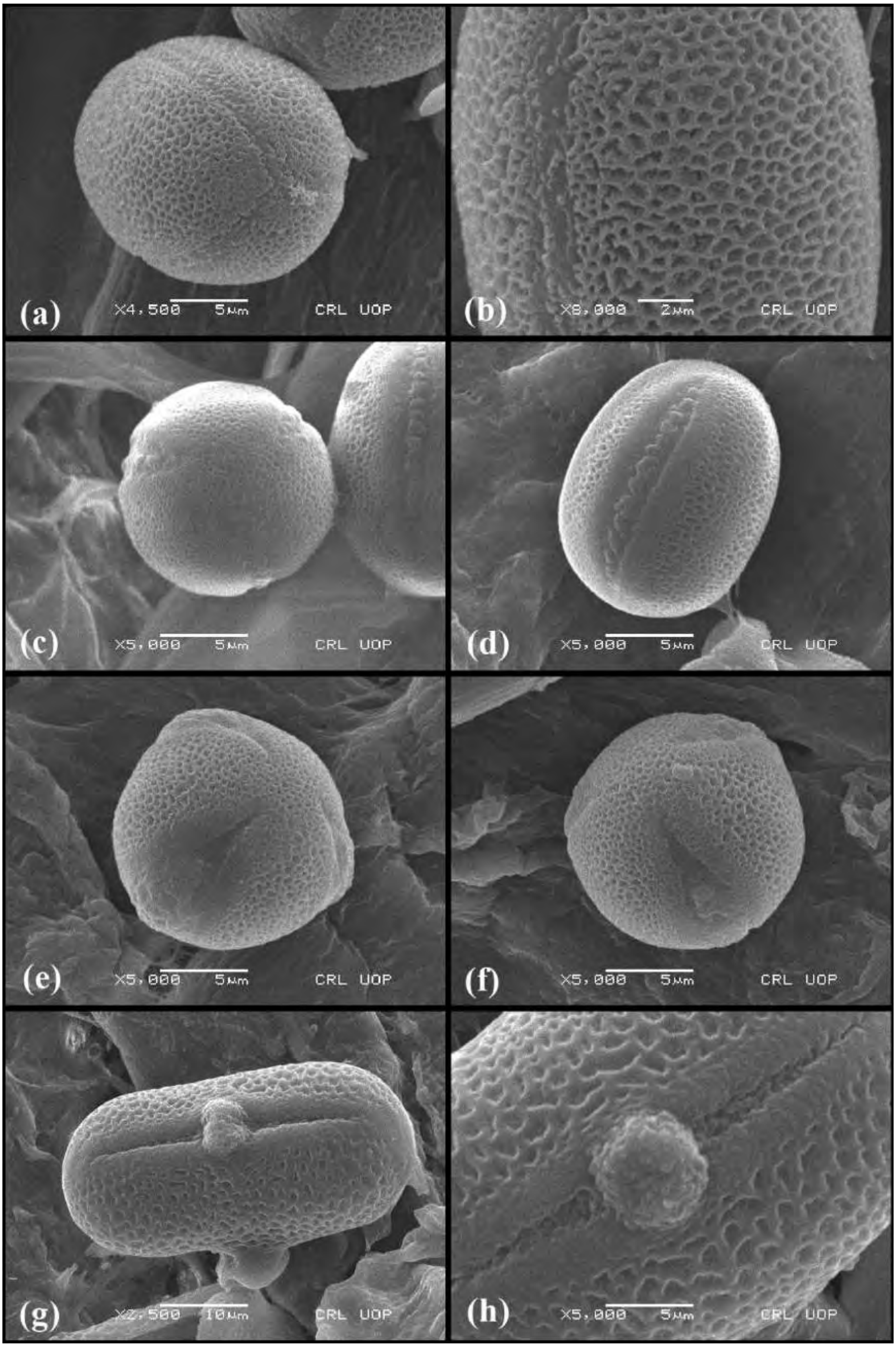

Plate 46. SEM micrographs of pollen (a), (b) *Ebenus stellata,* (c), (d) *Indigofera cuneifolia,* (e), (f) *Indigofera intricata,* (g), (h) *Lathyrus oleraceus*

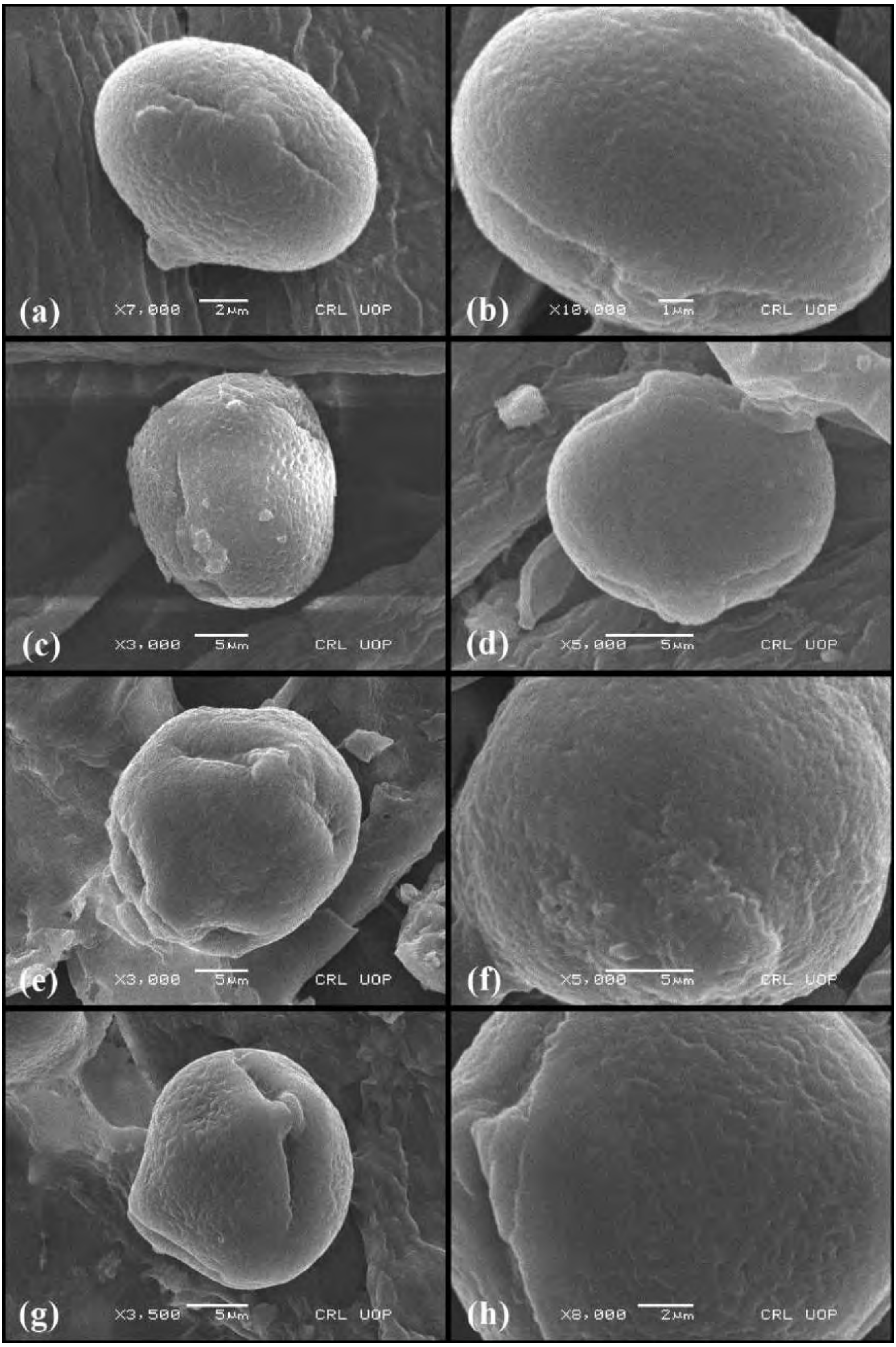

Plate 47. SEM micrographs of pollen (a), (b) *Lotus corniculatus,* (c), (d) *Lotus garcinii,* (e), (f) *Medicago lupulina,* (g), (h) *Medicago polymorpha*

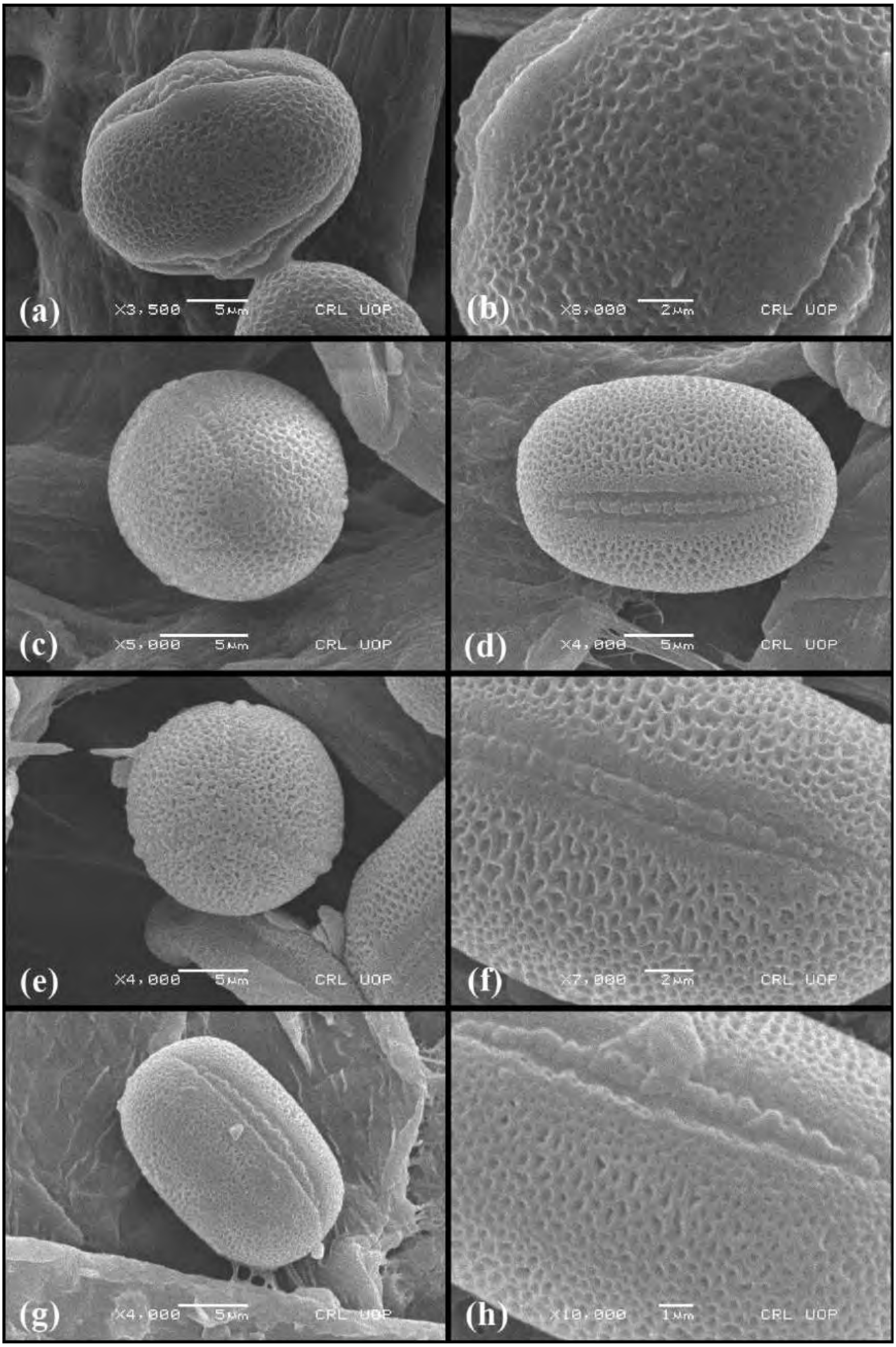

Plate 48. SEM micrographs of pollen (a), (b) *Melilotus indicus,* (c), (d) *Onobrychis cornuta,* (e), (f) *Onobrychis dealbata,* (g), (h) *Onobrychis micrantha*

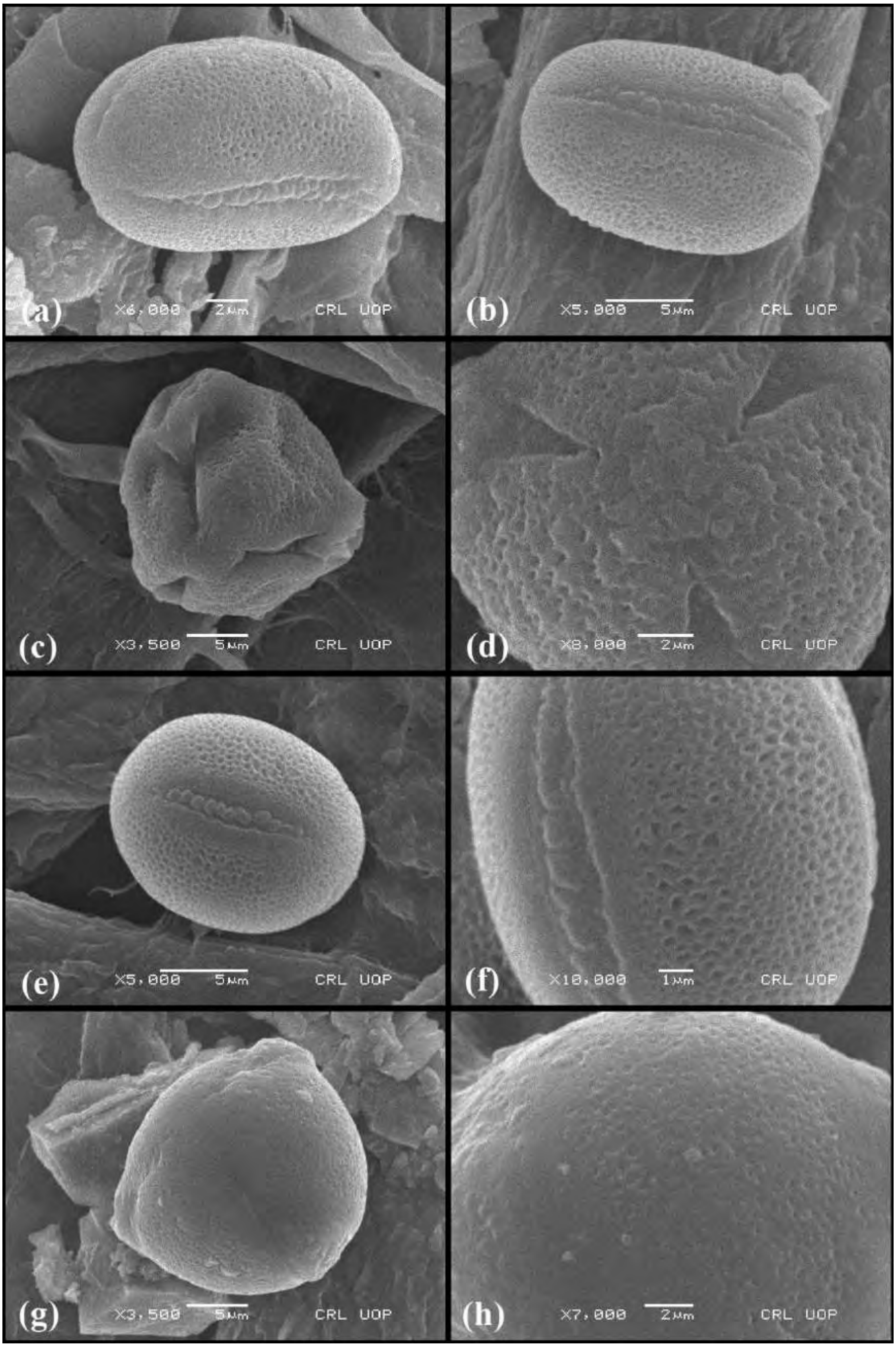

Plate 49. SEM micrographs of pollen (a), (b) *Onobrychis tavernierifolia,* (c), (d) *Sophora mollis,* (e), (f) *Taverniera glabra,* (g), (h) *Tephrosia uniflora subsp. Petrosa*

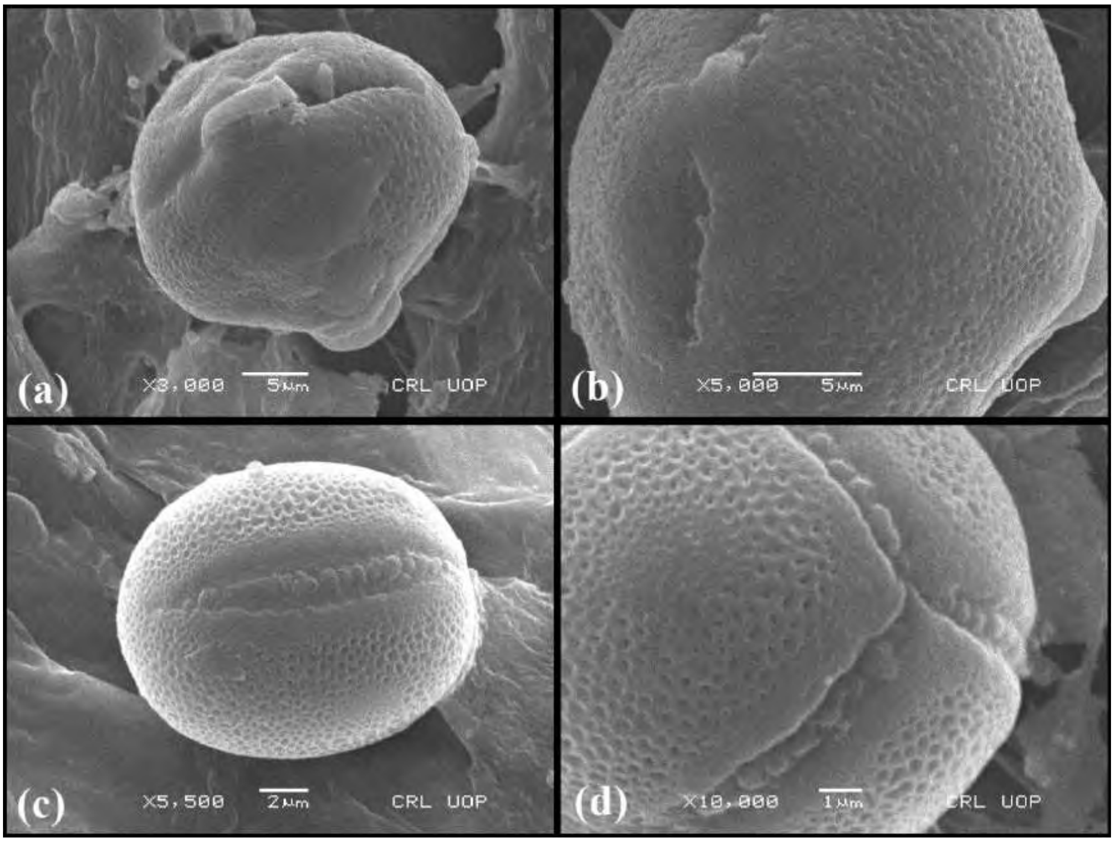

Plate 50. SEM micrographs of pollen (a), (b) *Trigonella macrorrhyncha,* (c), (d) *Vicia macrantha.*

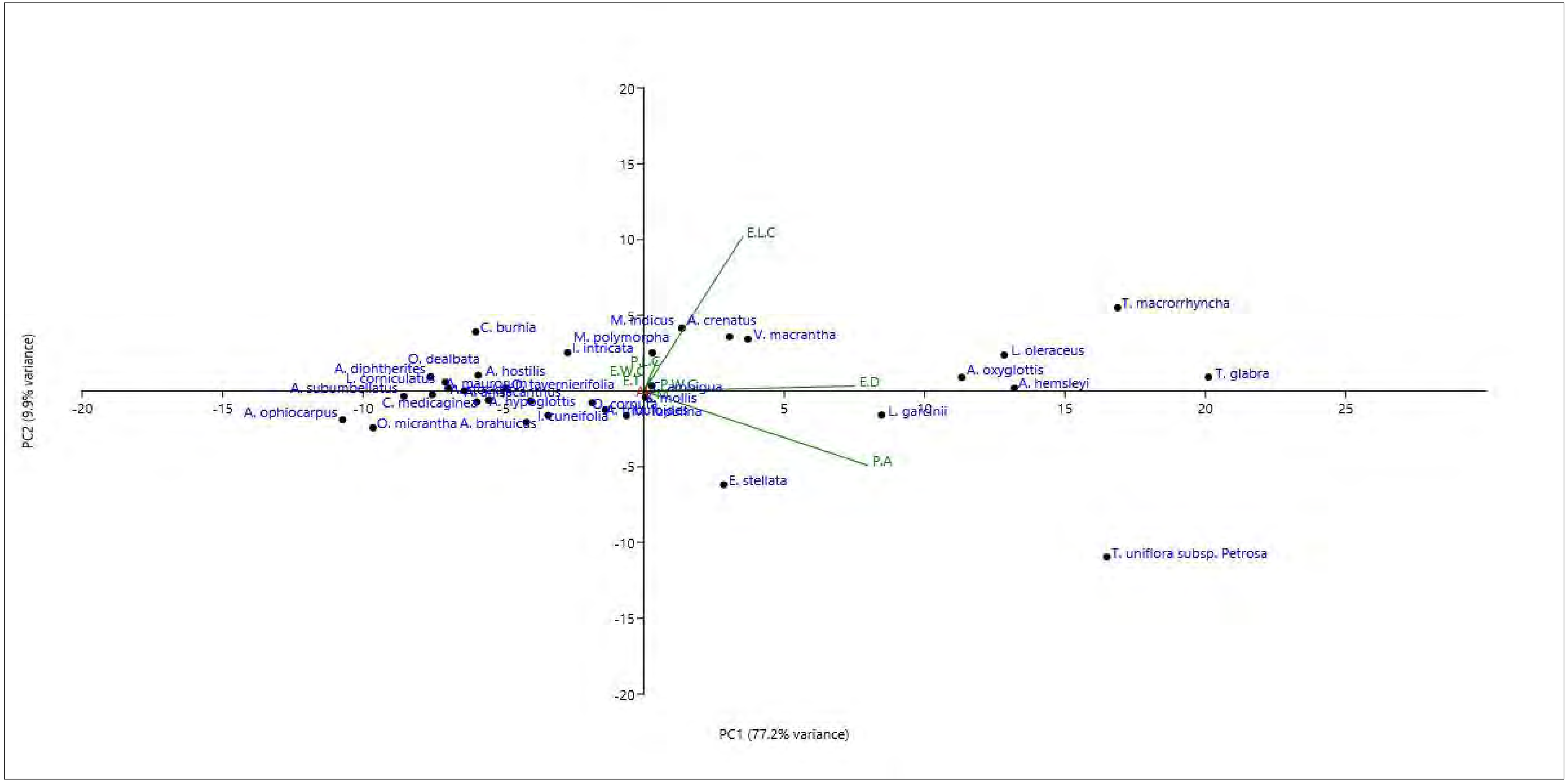

Figure 12. PCA analysis based on quantitative traits; polar axis, equatorial diameter, width and length of colpi in polar view, width and length of colpi in equatorial view, mesocolpium, exine thickness of Fabaceous pollen

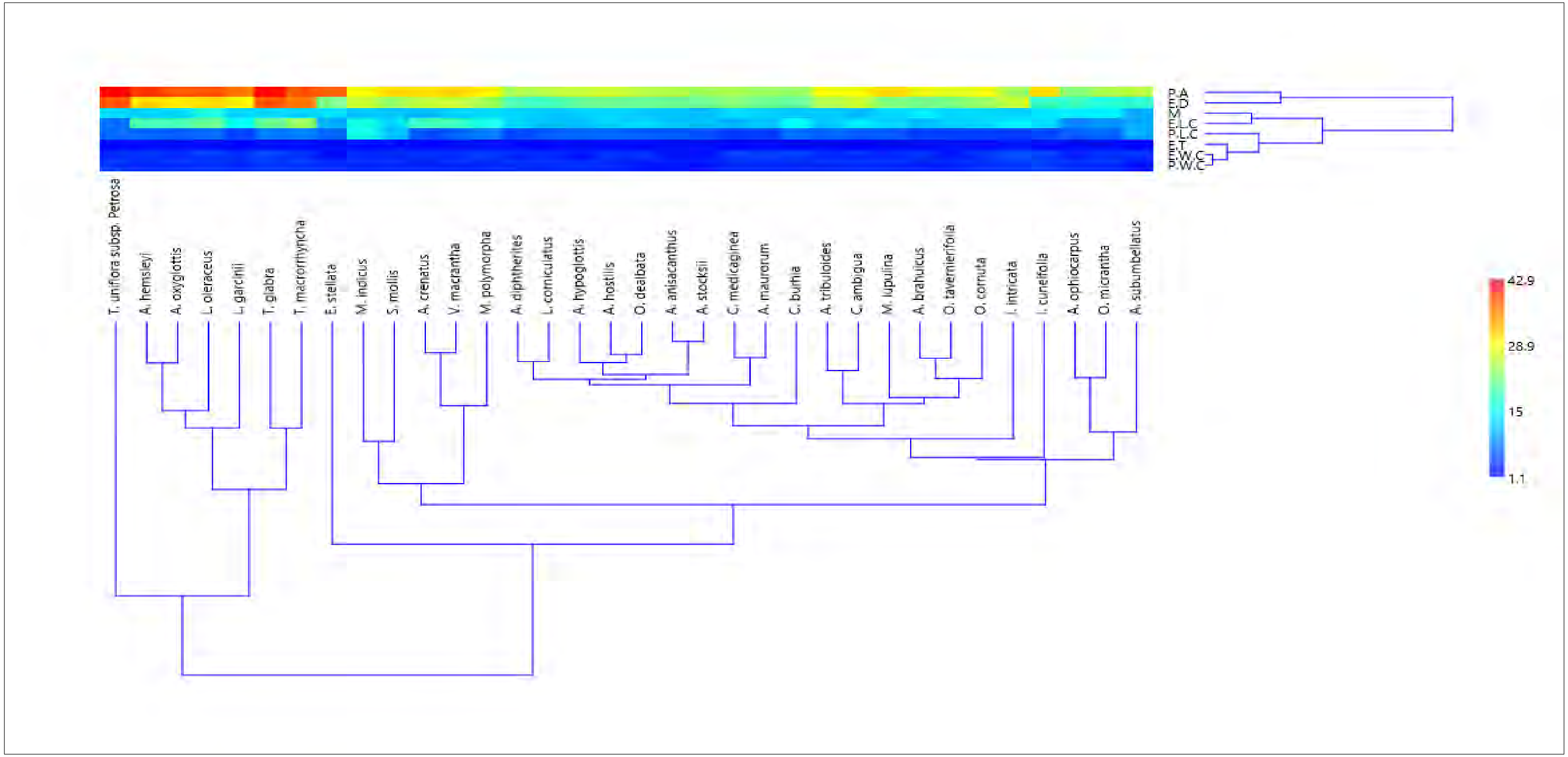

Figure 13. UPGMA cluster analysis based on quantitative traits; polar axis, equatorial diameter, width and length of colpi in polar view, width and length of colpi in equatorial view, mesocolpium, exine thickness of Fabaceous pollen

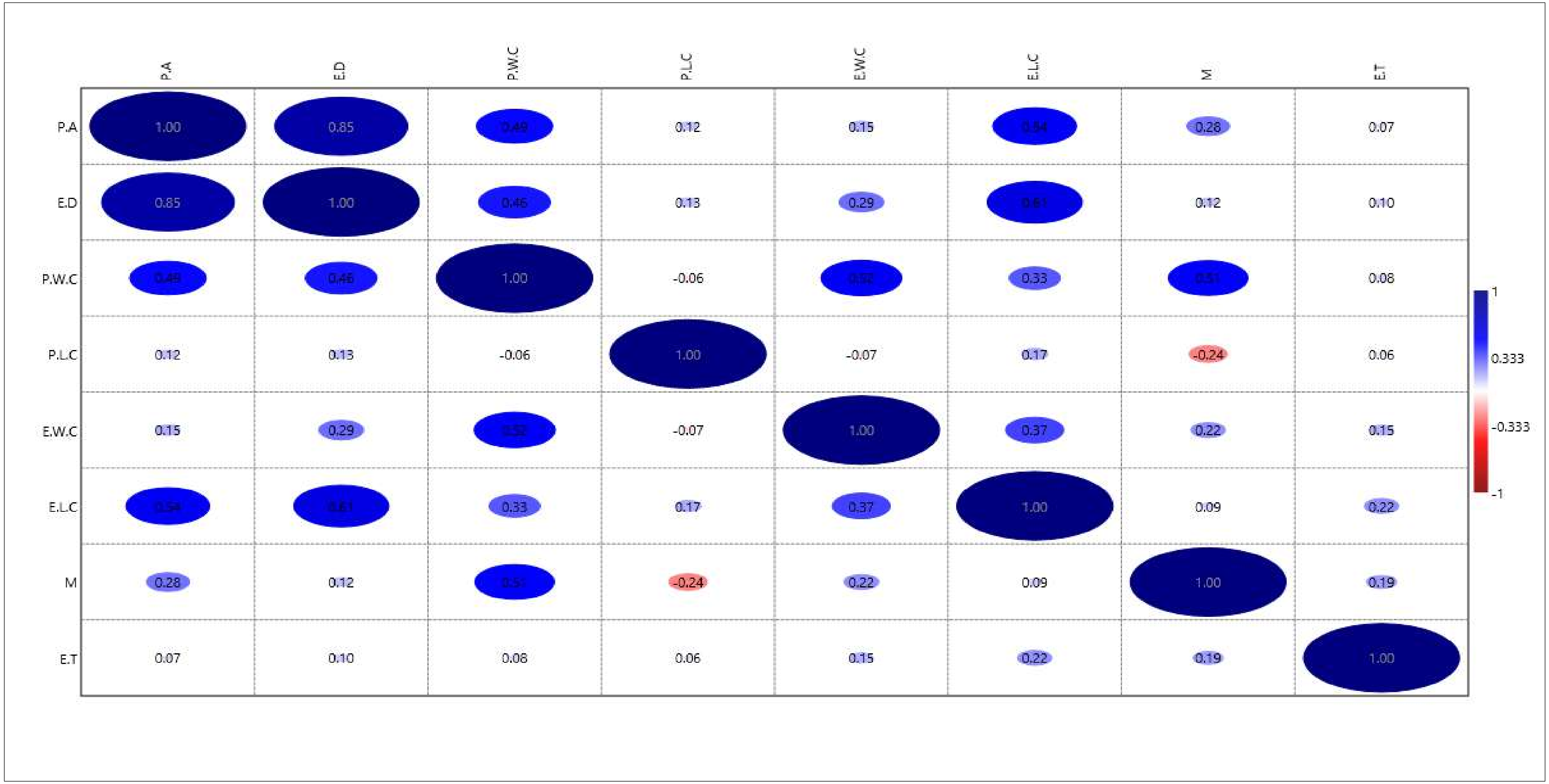

Figure 14. Correlation among the mean values of polar axis (P.A), equatorial diameter (E.D), width and length of colpi in polar view (W.C.P, L.C.P), width and length of colpi in equatorial view (W.C.E, L.C.E), mesocolpium (M), exine thickness (E.T) of Fabaceous pollen

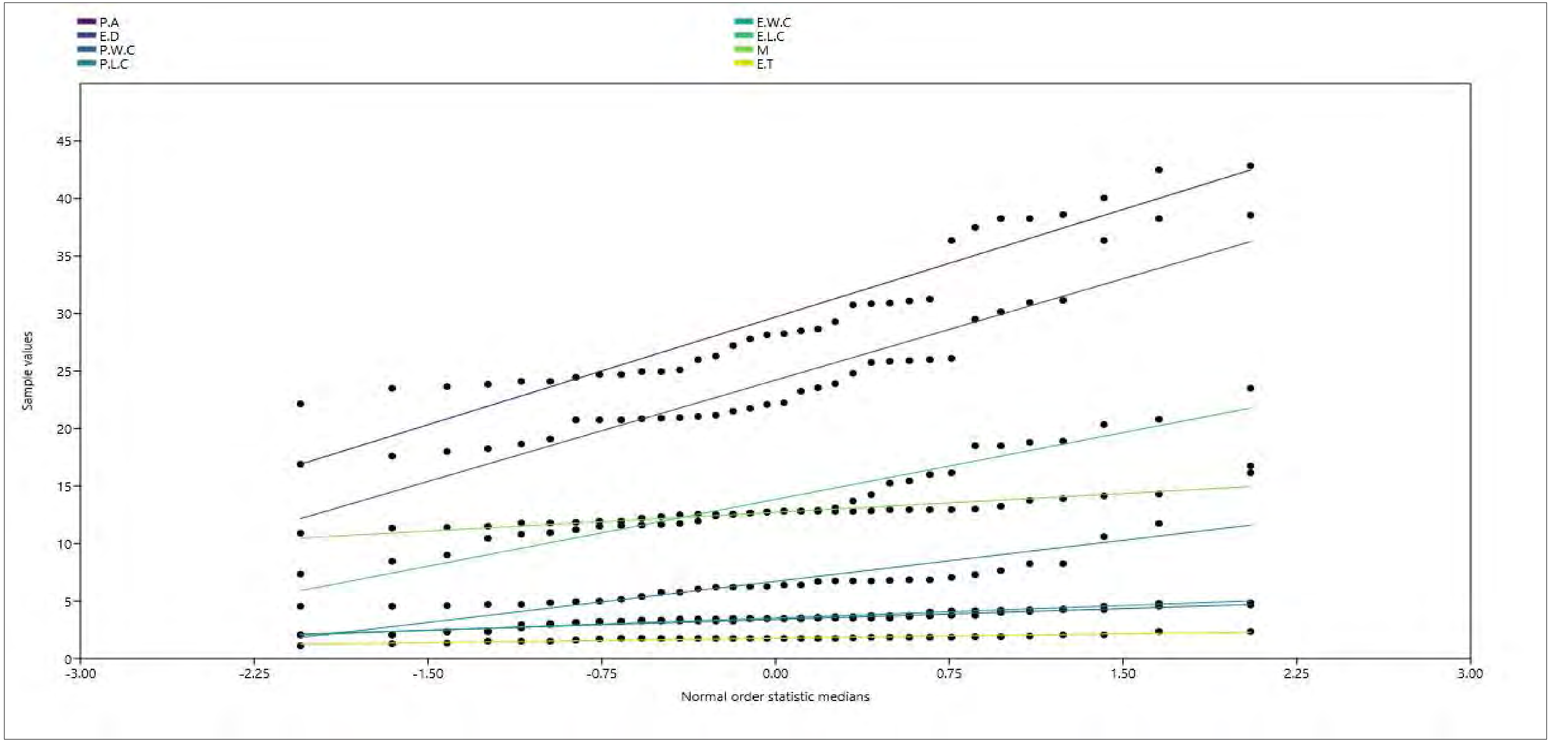

Figure 15. Normal probability distribution among the mean values of polar axis (P.A), equatorial diameter (E.D), width and length of colpi in polar view (W.C.P, L.C.P), width and length of colpi in equatorial view (W.C.E, L.C.E), mesocolpium (M), exine thickness (E.T) of Fabaceous pollen

Table 10. Qualitative palynological characteristics of the investigated taxa of Fabaceae

Taxa	Symmetry	Polarity	Unity	No. of apertures	Polar view	Equatorial view	Exine sculpturing	Aperture membrane	Amb	NPC classification	
										Name	Formula
Alhagi maurorum	Radial	Isopolar	Monad	Tricolporate	Triangular obtuse convex	Oval	Microreticulate	Psilate	Peritreme	Trizonocolporate	$N_3P_4C_5$
Astragalus anisacanthus	Radial	Isopolar	Monad	Tricolporate	Circular	Rectangular obtuse convex	Reticulate, psilate at the polar end	Verrucate	Peritreme	Trizonocolporate	$N_3P_4C_5$
Astragalus brahuicus	Radial	Isopolar	Monad	Tricolporate	Triangular obtuse convex	Elliptic to oval	Reticulate, psilate at the polar end	Psilate	Goniotreme	Trizonocolporate	$N_3P_4C_5$
Astragalus crenatus	Radial	Isopolar	Monad	Tricolporate	Circular	Elliptic to oval	Reticulate psilate	Psilate	Goniotreme	Trizonocolporate	$N_3P_4C_5$
Astragalus diphtherites	Radial	Isopolar	Monad	Tricolporate	Circular	Elliptic to oval	Reticulate to psilate	Psilate	Peritreme	Trizonocolporate	$N_3P_4C_5$
Astragalus hemsleyi	Radial	Isopolar	Monad	Tricolporate	Triangular obtuse convex	Elliptic to oval	Reticulate, psilate at the polar end	Psilate to verrucate	Peritreme	Trizonocolporate	$N_3P_4C_5$
Astragalus hypoglottis	Radial	Isopolar	Monad	Tricolporate	Triangular obtuse convex	Oval	Reticulate to psilate	Verrucate	Peritreme	Trizonocolporate	$N_3P_4C_5$
Astragalus ophiocarpus	Radial	Isopolar	Monad	Tricolporate	Circular	Rectangular obtuse convex	Macroreticulate	Psilate to verrucate	Peritreme	Trizonocolporate	$N_3P_4C_5$
Astragalus oxyglottis	Radial	Isopolar	Monad	Tricolporate	Triangular obtuse concave	Rectangular obtuse convex	Psilate to reticulate and verrucate	Verrucate	Goniotreme	Trizonocolporate	$N_3P_4C_5$
Astragalus stocksii	Radial	Isopolar	Monad	Tricolporate	Triangular obtuse convex	Elliptic truncate	Reticulate	Psilate	Goniotreme	Trizonocolporate	$N_3P_4C_5$
Astragalus subumbellatus	Radial	Isopolar	Monad	Tricolporate	Triangular obtuse convex to straight	Oval	Psilate verrucate	Psilate	Goniotreme	Trizonocolporate	$N_3P_4C_5$

Astragalus tribuloides	Radial	Isopolar	Monad	Tricolporate	Circular to elliptic	Rectangular obtuse convex	Psilate to reticulate	Psilate	Goniotreme	Trizonocolporate	$N_3P_4C_5$
Astragalus hostilis	Radial	Isopolar	Monad	Tricolporate	Circular	Elliptic truncate	Reticulate, psilate at the polar end	Verrucate to psilate	Peritreme	Trizonocolporate	$N_3P_4C_5$
Caragana ambigua	Radial	Isopolar	Monad	Tricolporate	Circular	Oval	Reticulate	Psilate	Peritreme	Trizonocolporate	$N_3P_4C_5$
Crotalaria burhia	Radial	Isopolar	Monad	Tricolpate	Circular	Rectangular obtuse convex	Reticulate	Verrucate	Peritreme	Trizonocolpate	$N_3P_4C_3$
Crotalaria medicaginea	Radial	Isopolar	Monad	Tricolpate	Circular	Rectangular obtuse convex	Macroreticulate	Verrucate	Peritreme	Trizonocolpate	$N_3P_4C_3$
Ebenus stellata	Radial	Isopolar	Monad	Trisynocolpate	Circular	Rectangular obtuse convex	Macroreticulate	Verrucate to psilate	Peritreme	Trizonocolpate	$N_3P_4C_3$
Indigofera cuneifolia	Radial	Isopolar	Monad	Tricolporate	Triangular	Quadrangular	Microreticulate	Verrucate to psilate	Peritreme	Trizonocolpate	$N_3P_4C_3$
Indigofera intricata	Radial	Isopolar	Monad	Tricolporate	Triangular	Quadrangular	Microreticulate	Psilate	Peritreme	Trizonocolpate	$N_3P_4C_3$
Lathyrus oleraceus	Radial	Isopolar	Monad	Tricolporate	Circular	Rectangular obtuse convex	Macroreticulate, psilate at the polar ends	Verrucate to psilate	Peritreme	Trizonocolporate	$N_3P_4C_5$
Lotus corniculatus	Radial	Isopolar	Monad	Tricolporate	Circular to triangular obtuse convex	Elliptic truncate	Psilate	Psilate	Goniotreme	Trizonocolporate	$N_3P_4C_5$
Lotus garcinii	Radial	Isopolar	Monad	Tricolporate	Circular	Elliptic truncate	Psilate to reticulate	Psilate	Peritreme	Trizonocolporate	$N_3P_4C_5$
Medicago lupulina	Radial	Isopolar	Monad	Tricolporate	Triangular obtuse convex	Circular	Psilate	Psilate	Goniotreme	Trizonocolporate	$N_3P_4C_5$
Medicago polymorpha	Radial	Isopolar	Monad	Tricolporate	Triangular obtuse concave	Oval	Psilate	Psilate	Goniotreme	Trizonocolporate	$N_3P_4C_5$
Melilotus indicus	Radial	Isopolar	Monad	Tricolporate	Circular	Elliptic truncate	Macroreticulate	Psilate to verrucate	Peritreme	Trizonocolporate	$N_3P_4C_5$

Onobrychis cornuta	Radial	Isopolar	Monad	Tricolpate	Oval	Rectangular obtuse convex	Reticulate to microreticulate	Verrucate	Peritreme	Trizonocolpate	$N_3P_4C_3$
Onobrychis dealbata	Radial	Isopolar	Monad	Tricolpate	Circular	Rectangular obtuse convex	Macroreticulate	Verrucate	Peritreme	Trizonocolpate	$N_3P_4C_3$
Onobrychis micrantha	Radial	Isopolar	Monad	Tricolpate	Circular	Rectangular obtuse convex	Reticulate to microreticulate	Verrucate	Peritreme	Trizonocolpate	$N_3P_4C_3$
Onobrychis tavernierifolia	Radial	Isopolar	Monad	Tricolpate	Circular to oval	Rectangular obtuse convex	Macroreticulate	Verrucate to psilate	Peritreme	Trizonocolpate	$N_3P_4C_3$
Sophora mollis	Radial	Isopolar	Monad	Tricolporate	Triangular obtuse convex	Rhombic	Reticulate to verrucate	Psilate	Goniotreme	Trizonocolporate	$N_3P_4C_5$
Taverniera glabra	Radial	Isopolar	Monad	Tricolpate	Circular	Elliptic to oval	Reticulate	Verrucate	Peritreme	Trizonocolpate	$N_3P_4C_3$
Tephrosia uniflora subsp. Petrosa	Radial	Isopolar	Monad	Tricolporate	Circular to triangular	Elliptic to oval	Microreticulate to psilate	Psilate to verrucate	Goniotreme	Trizonocolporate	$N_3P_4C_5$
Trigonella macrorrhyncha	Radial	Isopolar	Monad	Tricolporate	Triangular obtuse concave	Elliptic to oval	Psilate to reticulate	Psilate	Goniotreme	Trizonocolporate	$N_3P_4C_5$
Vicia macrantha	Radial	Isopolar	Monad	Trisynocolpate	Circular	Elliptic truncate	Reticulate	Verrucate	Peritreme	Trizonocolpate	$N_3P_4C_3$

Table 11. Size, shape and viability of pollen of studied Fabaceous taxa

S/No	Taxa	P/E ratio	Size	Shape	Viability %
1	*Alhagi maurorum*	1.12	Small	Subprolate	80
2	*Astragalus anisacanthus*	1.18	Medium	Subprolate	78
3	*Astragalus brahuicus*	1.31	Medium	Subprolate	88
4	*Astragalus crenatus*	1.19	Medium	Subprolate	85
5	*Astragalus diphtherites*	1.26	Small	Subprolate	69
6	*Astragalus hemsleyi*	1.28	Medium	Subprolate	78
7	*Astragalus hypoglottis*	1.25	Medium	Subprolate	82
8	*Astragalus ophiocarpus*	1.21	Small	Subprolate	90
9	*Astragalus oxyglottis*	1.26	Medium	Subprolate	80
10	*Astragalus stocksii*	1.13	Small	Prolate-spheroidal	78
11	*Astragalus subumbellatus*	1.41	Medium	Prolate	80
12	*Astragalus tribuloides*	1.13	Medium	Prolate-spheroidal	80
13	*Astragalus hostilis*	1.26	Medium	Subprolate	70
14	*Caragana ambigua*	1.10	Medium	Prolate-spheroidal	88
15	*Crotalaria burhia*	1.13	Small	Prolate-spheroidal	87
16	*Crotalaria medicaginea*	1.10	Medium	Prolate-spheroidal	65
17	*Ebenus stellata*	1.72	Medium	Prolate	68
18	*Indigofera linifolia*	1.19	Medium	Subprolate	88

19	*Indigofera oblongifolia*	1.71	Medium	Prolate	86
20	*Lathyrus oleraceus*	0.93	Medium	Oblate-spheroidal	89
21	*Lotus corniculatus*	1.24	Medium	Subprolate	80
22	*Lotus garcinii*	1.34	Medium	Prolate	70
23	*Medicago lupulina*	1.23	Medium	Subprolate	89
24	*Medicago polymorpha*	1.39	Medium	Prolate	90
25	*Melilotus indicus*	1.22	Medium	Subprolate	86
26	*Onobrychis cornuta*	1.09	Medium	Prolate-spheroidal	89
27	*Onobrychis dealbata*	1.20	Medium	Subprolate	80
28	*Onobrychis micrantha*	1.46	Medium	Prolate	76
29	*Onobrychis tavernierifolia*	1.26	Medium	Subprolate	80
30	*Sophora mollis*	1.32	Medium	Subprolate	88
31	*Taverniera glabra*	1.11	Medium	Prolate-spheroidal	92
32	*Tephrosia uniflora subsp. Petrosa*	1.11	Medium	Prolate-spheroidal	85
33	*Trigonella macrorrhyncha*	1.05	Medium	Prolate-spheroidal	88
34	*Vicia macrantha*	1.19	Medium	Subprolate	78

Table 12. Quantitative palynological measurements of Fabaceous taxa

Plant Species	P.A	E.D	W.C.P	L.C.P	W.C.E	L.C.E	M	E.T
Mean± Stander error								
Alhagi maurorum	23.5±0.30618654	20.95±0.2150545	3.25±0.17677298	4.6±0.34095454214	4.3±0.2284999322	11.95±0.45684422	12.5±0.570087123	1.75±0.17677442
Astragalus anisacanthus	24.7±0.289395923	20.9±0.302076149	2.05±0.215058132	5.75±0.176776695	2.3±0.266926956	11.75±0.176776695	11.8±0.421307489	1.75±0.176776695
Astragalus brahuicus	27.8±0.588430115	21.15±0.340954542	3.05±0.145773797	4.55±0.215058132	3.55±0.215058132	11.2±0.215058132	13.9±0.986154146	1.75±0.176776695
Astragalus crenatus	30.75±0.176776695	25.75±0.176776695	2.95±0.215058132	5.4±0.471699057	3.65±0.322102468	18.5±0.176776695	11.4±0.331662479	1.75±0.176776695
Astragalus diphtherites	24.1±0.231840462	19.1±0.302076149	3.7±0.215058132	4.95±0.183711731	3.15±0.16955825	12.75±0.285043856	12.75±0.637377439	1.5±0.176776695
Astragalus hemsleyi	40.05±0.532681894	31.15±0.231840462	3.25±0.176776695	6.75±0.176776695	3.75±0.176776695	18.8±0.215058132	12.55±0.649037749	1.75±0.176776695
Astragalus hypoglottis	26±0.176776695	20.75±0.176776695	3.75±0.176776695	6.8±0.145773797	2.35±0.231840462	11.55±0.310241841	12.55±0.588430115	1.7±0.145773797
Astragalus ophiocarpus	22.15±0.322102468	18.25±0.306186218	3.45±0.215058132	6.4±0.257390754	2.95±0.215058132	8.45±0.348209707	14.15±1.020416582	1.75±0.176776695
Astragalus oxyglottis	38.25±0.176776695	30.15±0.257390754	3.75±0.176776695	6.2±0.215058132	3.5±0.176776695	18.9±0.340954542	12.8±0.658596994	1.8±0.145773797
Astragalus stocksii	23.85±0.527967802	21.05±0.215058132	2.05±0.215058132	5.75±0.176776695	2.05±0.215058132	11.5±0.353553391	11.35±0.302076149	1.9±0.127475488
Astragalus subumbellatus	24.95±0.365718471	17.6±0.645174395	2.35±0.231840462	10.6±0.269258244	3.65±0.302076149	10.45±0.215058132	10.9±0.331662479	1.35±0.127475488
Astragalus tribuloides	28.15±0.2573907	24.8±0.365718471	3.5±0.176776695	6.75±0.176776695	3.25±0.176776695	11.65±0.257390754	12.2±0.614410286	1.75±0.176776695
Astragalus hostilis	26.3±0.443001129	20.85±0.231840462	3.25±0.176776695	6.75±0.176776695	3.75±0.176776695	12.55±0.242383993	12.8±0.658596994	1.85±0.127475488
Caragana ambigua	28.65±0.231840462	25.9±0.127475488	3.5±0.176776695	6.2±0.348209707	3.45±0.215058132	13.7±0.329772649	11.5±0.353553391	1.1±0.20310096

Crotalaria burhia	23.65±0.231840462	20.75±0.176776695	3.45±0.215058132	7.3±0.538516481	4.15±0.257390754	15.25±0.637377439	11.95±0.48347699	1.5±0.176776695
Crotalaria medicaginea	24.45±0.339116499	22.1±0.430116263	3.5±0.176776695	4.55±0.215058132	4.25±0.176776695	10.95±0.365718471	12.95±0.686476511	1.3±0.215058132
Ebenus stellata	37.5±0.353553391	21.75±0.58630197	3.25±0.176776695	6.85±0.257390754	2.05±0.215058132	10.8±0.2	13.25±0.858778202	1.75±0.176776695
Indigofera cuneifolia	30.9±0.522015325	18±3.877015605	4.65±0.20310096	5.15±0.231840462	4.55±0.215058132	13.1±0.816241386	13.75±0.89791425	1.85±0.15
Indigofera intricate	24.1±0.231840462	25.85±0.257390754	3.65±0.257390754	4.7±0.215058132	4.85±0.231840462	14.25±0.176776695	12.8±0.658596994	2.35±0.231840462
Lathyrus oleraceus	38.6±0.484767986	30.95±0.348209707	4.25±0.176776695	7.05±0.215058132	4.15±0.257390754	20.8±0.588430115	16.75±1.629800601	1.75±0.176776695
Lotus corniculatus	25.1±0.4227883	18.65±0.231840462	3.45±0.215058132	6.05±0.215058132	3.45±0.215058132	11.6±0.26925824	11.95±0.520816666	1.75±0.176776695
Lotus garcinii	36.35±0.257390754	29.5±0.285043856	3.15±0.127475488	5±0.223606798	3.5±0.176776695	15.45±0.215058132	13±0.702673466	1.5±0.176776695
Medicago lupulina	31.1±0.322102468	22.25±0.353553391	3.5±0.25	6.25±0.395284708	3.35±0.127475488	12.85±0.340954542	12.85±0.673609679	1.95±0.145773797
Medicago polymorpha	29.3±0.365718471	23.9±0.231840462	3.25±0.176776695	8.25±0.176776695	4.2±0.413823634	16.15±0.302076149	12.8±0.658596994	2.35±0.231840462
Melilotus indicus	28.5±0.176776695	26±0.176776695	3.25±0.176776695	16.15±0.302076149	3.25±0.176776695	16±0.353553391	11.8±0.421307489	1.9±0.127475488
Onobrychis cornuta	28.25±0.935414347	23.55±0.348209707	4±0.176776695	4.7±0.215058132	4.05±0.348209707	12.65±0.231840462	12.8±0.658596994	1.75±0.176776695
Onobrychis dealbata	24.95±0.365718471	20.75±0.176776695	2.35±0.231840462	6.4±0.26925824	3.6±0.3122499	12.85±0.610327781	12.35±0.562361094	1.85±0.127475488
Onobrychis micrantha	24.7±0.357071421	16.9±0.358817502	3.45±0.16583124	6.85±0.257390754	3.5±0.176776695	9±0.176776695	14.3±1.025914226	1.85±0.127475488
Onobrychis tavernierifolia	27.2±0.428660705	21.5±0.306186218	3.1±0.26925824	4.85±0.231840462	2.65±0.231840462	12.4±0.26925824	12.95±0.686476511	2.05±0.215058132
Sophora mollis	30.85±0.392109679	23.25±0.176776695	3.25±0.176776695	11.75±0.176776695	3.35±0.231840462	12.9±0.187082869	11.85±0.539675829	1.75±0.176776695
Taverniera glabra	42.5±0.353553391	38.25±0.176776695	4.55±0.215058132	7.65±0.231840462	4.8±0.2	20.35±0.407737661	12.95±0.686476511	2.05±0.215058132

Tephrosia uniflora subsp. petrosa	42.85±0.302076149	38.55±0.16583124	4.1±0.26925824	6.7±0.215058132	3.5±0.176776695	7.35±0.231840462	12.95±0.721976454	1.6±0.231840462
Trigonella macrorrhyncha	38.25±0.176776695	36.35±0.322102468	4.25±0.176776695	8.25±0.176776695	3.75±0.176776695	23.5±0.176776695	12.65±0.635413251	1.85±0.127475488
Vicia macrantha	31.25±0.32596012	26.1±0.257390754	3.5±0.176776695	6.25±0.176776695	3.45±0.215058132	18.5±0.176776695	12.55±0.588430115	1.75±0.176776695

(P.A: polar axis; E.D: equatorial diameter, L.C.P, W.C.P, L.C.E, W.C.E: length and width of colpi in polar and equatorial view, M: mesocolpium, E.T: exine thickness)

Table 13. Dichotomous (single access) bracketed / parallel taxonomic key based on pollen morphological traits of Fabaceous flora

Link Character	Leads	Characters	Taxa/ Go to link character
1	a	Trisynocolpate colpi	2
	b	Colpi other than trisynocolpate	3
2	a	Equatorial view rectangular obtuse convex	*Ebenus stellata*
	b	Equatorial view elliptic truncate	*Vicia macrantha*
3	a	Tricolpate pollen	4
	b	Tricolporate pollen	11
4	a	Oval in polar view	*Onobrychis cornuta*
	b	Circular in polar view	*5*
5	a	Reticulate	*6*
	b	Reticulate with combination	*7*
6	a	Rectangular obtuse convex in equatorial view	*Crotalaria burhia*
	b	Elliptic to oval in equatorial view	*Taverniera glabra*
7	a	Aperture membrane verrucate to psilate	*Onobrychis tavernierifolia*
	b	Aperture membrane verrucate	8
8	a	Reticulate to microreticulate	9
	b	Mcroreticulate	10
9	a	Pollen shape prolate-spheroidal	*Onobrychis cornuta*
	b	Pollen shape Prolate	*Onobrychis micrantha*

10	a	Prolate-spheroidal pollen	*Crotalaria medicaginea*
	b	Subprolate	*Onobrychis dealbata*
11	a	Goniotreme amb	12
	b	Peritreme amb	22
12	a	Psilate exine	13
	b	Exine other than psilate	15
13	a	Circular to triangular obtuse convex polar view	*Lotus corniculatus*
	b	Triangular obtuse convex	14
14	a	Circular equatorial view	*Medicago lupulina*
	b	Oval equatorial view	*Medicago polymorpha*
15	a	Aperture membrane verrucate	*Astragalus oxyglottis*
	b	Aperture membrane psilate	16
16	a	Rhombic equatorial view	*Sophora mollis*
	b	Equatorial view not rhombic	17
17	a	Prolate-spheroidal shape	18
	b	Subprolate shape	19
18	a	Circular to triangular polar view	*Tephrosia uniflora subsp. Petrosa*
	b	Triangular obtuse concave polar view	*Trigonella macrorrhyncha*
19	a	Circular to elliptic polar view	20
	b	Triangular obtuse convex polar view	21

20	a	Prolate-spheroidal pollen shape	*Astragalus tribuloides*
	b	Subprolate pollen	*Astragalus crenatus*
21	a	Psilate verrucate exine	*Astragalus subumbellatus*
	b	Psilate exine	*Astragalus brahuicus, Astragalus stocksii*
22	a	Quadrangular equatorial view	23
	b	Equatorial view not quadrangular	24
23	a	Pollen shape subprolate	*Indigofera cuneifolia*
	b	Pollen shape prolate	*Indigofera intricata*
24	a	Triangular obtuse convex polar view	25
	b	Circular polar view	27
25	a	Microreticulate exine	*Alhagi maurorum*
	b	Exine reticulate to psilate or reticulate, psilate at the polar end	26
26	a	Elliptic to oval equatorial view	*Astragalus hemsleyi*
	b	Oval polar view	*Astragalus hypoglottis*
27	a	Small pollen size	*Astragalus ophiocarpus*
	b	Medium pollen size	28
28	a	Prolate pollen shape	*Lotus garcinii*
	b	Pollen shapes other than prolate	29
29	a	Prolate-spheroidal pollen	*Caragana ambigua*
	b	Subprolate pollen	30

30	a	Exine macroreticulate	*Melilotus indicus*
	b	Exine reticulate, psilate at the polar end	31
31	a	Elliptic truncate equatorial view	*Astragalus hostilis*
	b	Rectangular obtuse convex equatorial view	*Astragalus anisacanthus*

3.3 Pollen Micromorphology of the Lamiaceous taxa in Northern Baluchistan

3.3.1 Results and Discussion

The pollen micromorphology of 31 Lamiaceous species was studied via LM and SEM. Qualitative characteristics such as polarity, aperture types, exine sculpturing, amb, and quantitative features like polar axis, equatorial diameter, colpus width and length were observed. The terminologies for pollen features were observed from the standard provided by Erdtman (1969).

a) Symmetry, polarity, and unity of pollen of Lamiaceous species

Symmetry, polarity, and unity of the pollen of Lamiaceous species were homogenous. These characters were found non-significant in the discrimination of taxa. The examined pollen were radial, isopolar and monad (Table 14). Pollen features studied via LM and SEM were genetically specified, consistent, and unique to each species (e.g., exine). These characteristics aided in species differentiation (Mert, 2010). Soares (2018), demonstrated that the pollen traits were highly valuable in the taxonomy of Lamiaceous species. Erdtman (1945) divided the Lamiaceae into the Lamioideae and Nepetoideae groups based on the number of apertures and pollen nuclei.

b) Size, and shapes of pollen of Lamiaceous species

The medium size pollen were noted in 27 species. Small size pollen were observed in *Nepeta praetervisa.* Large pollen were present in three species *Salvia cabulica, Salvia moorcroftiana,* and *Nepeta glomerulosa*. The shapes of the pollen were prolate spheroidal (8), oblate-spheroidal (8), sub- oblate (5), spherical (5), oblate (3), and sub- prolate (2). *Nepeta glomerulosa, Clinopodium umbrosum*, and *Salvia cabulica* pollen were oblate. *Ajuga alpina* was noted with prolate pollen. Pollen in *Scutellaria linearis* and *Salvia leucantha* were sub-prolate (Table 15). Previously, spheroidal pollen were reported with six zonocolpate and three zonocolpate aperture. Doaigey et al. (2017), classified the Lamiaceous pollen into two groups, based on the number of colpi: hexa-zonocolpate group, and tri-zonocolpate group. The pollen of the studied species has a heterocolpate character, which was consistent with previous findings (Erdtman, 1971; Raj, 1974). Kar (1993) concluded that the size of pollen varied greatly between Lamiaceous species from the Indian subcontinent. Earlier studies documented that the Nepetoideae pollen varied in size from small in *S. plebeia* to large in *O. americanum* (Lens et al., 2005). In the present study the size varied from small (*Nepeta praetervisa*), to medium (five species), and large (*Nepeta glomerulosa)* in the *Nepeta* genus. Pollen size varied within the Nepetoideae taxa (Özler et al., 2011).

c) Aperture types of the pollen of Lamiaceous species

Variations were observed in the pollen aperture types in the present study. The number of colpi and pores were varied even within the pollen of a single species. The observed pollen types were tricolpate, tricolporate, hexacolpate, hexacolporate, tetra-hexacolpate, hexa to multi syncolpate, and tri to hexacolpate. Pollen of 8 species of Lamiaceae were tricolpate. *Marrubium vulgare* and *Otostegia limbate* were observed with tricolporate pollen. Hexacolpate aperture was determined in 10 Lamiaceous species. The hexacolporate aperture type was observed in 6 taxa. The pollen in *Salvia santolinifolia* were tri to hexacolpate. Tetra to hexacolpate apertures were observed in 6 taxa. Distinct aperture type hexa to multi syncolpate type was observed in *Salvia leucantha.* This pollen was observed with six to many colpi. The colpi were fused at the apices in synocolpate type (Plate 51-57).

The observed pollen in *Nepeta* genus were tricolpoate, hexacolpate, tetra to hexacolpate. Azizian et al. (2001) also documented the haxacolpate aperture in genus *Nepeta*. Sultan et al. (2021) reported the *Nepeta praetervisa* as hexacolpoate, in the present study, tetra to hexacolpate aperture were noted. Celenk et al. (2008) documented hexacolpate, radially symmetrical, isopolar, microreticulate, bireticulate pollen in *N. congesta var. cryptantha, N. heliotropifolia, N. isaurica, N. cataria, N. meyeri,* and *N. fissa*. Pollen morphology of *N. cataria, N. nuda, N. racemosa,* and *N. viscida* were studied by Moon et al. (2008) and Jamzad et al. (2003). Presently, significant variations were observed in the aperture (hexacolpate, hexacolporate, tricolpate, and tetra to hexacolpate) in the *Nepeta* genus. Similarly, *Stachys parviflora* was observed as trizonocolpate, the results were consistence with *Stachys aegyptiaca*. Hexacolpate, microreticulate, and perorate pollen were observed in *Micromearia biflora.* Reticulate perforate pollen in *Micromeria imbricata* (Doaigey et al., 2018).

Tricolpate, tetracolpate, hexacolpate, heptacolpate, and octacolpate pollen were reported in Lamiaceae earlier by Myoung et al. (2012), Cantino et al. (1992), Boi et al. (2013), Kremer et al. (2014). In the present study, six aperture types were observed. Multisyncolpate aperture was observed in *Salvia leucantha*. In the Lamiaceae, suprareticulate (bireticulate) exine was apomorphic, while in the Lamioidae subfamily, the pollen were plesiomorphic (Cantino, 1992). Pollen in the Lamioidae sub family were documented as psilate, granulate, rugulate, and suprareticulate to rugulate sculpturing patterns (Abu-Asab and Cantino, 1994). In the current study, hexacolpate, and tri to hexacolpate aperture were noted in *Salvia* species. Similar results were reported by Hassan et al. (2009), Fırat et al. (2017). Hexacolpate pollen was prominent in *Salvia* species, with tetra-, penta, hepta, or octacolpate pollen. Aperture heteromorphism was documented in *S. coccinea* (Trudel and Morton, 1992), *S. barrelieri*, *S. eremostachya, S. leucantha, S. splendens,* and *S. uliginosa* (Moon et al., 2008), S. *recognita* (Özler et al., 2011), *S. palaestina* (Moon et al., 2008). Presently, the hetromorphism was observed in *Salvia santolinifolia.* Aperture heteromorphism can be caused by normal ecological conditions.

d) Exine sculpturing, surface, and aperture membrane of Lamiaceous taxa

The macroreticulate (12 species), and reticulate (12 species) exine was observed in the Lamiaceous species. Distinct sculpturing foveolate was present in *Marrubium vulgare* and *Stachys parviflora*, reticulate-gemmate in *Lallemantia royleana,* reticulate-striate in *Thymus linearis*, reticulate and gemmate in *Salvia santolinifolia*, roughly-reticulate in *Phlomis stewartia.* The exine surface was non-perforte, perforate, microperforate, macroperforate (whole pollen), and reticulate in different species. The differences in the aperture membrane of pollen of Lamiaceous species further aided in the discrimination and separation. The aperture membrane was verrucate, scabrate verrucate, gemmate-psilate, scabrate, and psilate (Harley, 1992; Lens et al., 2005; Moon et al., 2008; Schols et al., 2004). The apertures were deeply sunken (in some species) and appeared as a line on the pollen surface (Plate 51-57). Dinç et al. (2009) also reported reticulate (micro and macro). Gemmate-verrucate exine was observed in *Teucrium stocksianum,* consistent with findings by Navarro et al. (2004) for *Teucrium* *oliverianum* granulate pollen. The exine in *Origanum majorana* was reticulate macroperforate, similar was reported for *Origanum syriacum* by Yildiz et al. (2009).

The variations in the shape of primary lumina and muri as well as the number of perforations within the reticulation aided in the taxonomy of Lamiaceous species. Atalay et al. (2016) concluded that *Lamium* species have angular primary lumina and non-undulate muri, with or without perforations. Reticulate tectum was the most prevalent exine type in the Lamiaceae (Perveen and Qaiser, 2003). Earlier studies (Mousavi 2014; Rashid et al., 2018; Ullah et al., 2018) have also documented the significance of SEM of pollen in distinguishing and establishing phylogenetic relationships of various problematic taxa. Pollen morphological variations significantly assisted in the distinction of species and genera within the Lamiaceae (Kandemir et al., 2019; Shah et al., 2019; Ullah et al., 2018). The taxonomic key was developed based on the analyzed pollen features of Lamiaceous species (Table 17). Exine sculpturing of *Ocimum* species was bireticulate-perforate (Harley et al., 1992), these variations resulted in the replacement of various species within the genus. Özler et al. (2011) reported bireticulate exine in *Salvia* species.

e) Polar and equatorial view, amb, and NPC classification of Lamiaceous species

Significant variations were observed within the polar and equatorial view of pollen of studied Lamiaceous species. The terminologies were noted from the types provided by Erdtman (1969). The pollen of Lamiaceae appeared as circular, circular to quinquangular obtuse, circular to quinquangular obtuse, triangular to circular, triangular obtuse, circular to triangular obtuse convex, and triangular shapes in the polar view. The equatorial view was elliptic truncate, elliptic obtuse, circular, circular to elliptic, circular to slightly elliptic, and elliptic to rhombic obtuse truncate. Celenk et al. (2008) reported suboblate, and circular equatorial view in the Lamiaceous species. In the present study, variations were observed in the polar and equatorial views. These variations were successfully employed in the construction of a taxonomic key for the separation of the studied species. The amb of the pollen of Lamiaceous species were ptychotreme in 14 species, ptychotrem- peritreme in 3 taxa, peritreme in 12 members. *Salvia santolinifolia* pollen were peritreme-goinotreme. Pollen amb in *Otostegia* *aucheri* was goniotreme. The *Salvia* genus was noted elliptic to circular in polar view (Sebsebe and Harley, 1992; Lens et al., 2005).

f) Multivariate analysis

The analysis of quantitatively measured features in the form of PCA, dendrogram, correlation plots demonstrated their significance in taxonomic studies (Figure 16). The PCA determined the 62.4% and 23.6% variance between PC1 and PC2 respectively. Most of the examined characters were positively correlated. The highest positive correlation 0.78 was noted between the polar axis and the equatorial diameter. While least positive correlation 0.04 was observed between exine thickness and polar width of colpi. The maximum negative correlation -0.31 was revealed between the exine thickness and equatorial length of colpi (Figure 18). The exine thickness was also negatively correlated -0.01 with the polar length of colpi. The quantitative variables significantly created the phylogenetic association among the taxa via dendrogram. *Salvia leucantha* and *Salvia plebeian* were found closely placed in the phylogenetic tree. Similarly, the two *Otostegia* species were in the same lineated cluster. The box-jatter plot determined the trends in the data set for the quantitative features. The outliers in the data were represented by black dots (Figure 19). The color box represented the mean data values for each trait while the standard error was shown by the straight black line. The quantitative aspects significantly discriminated the analyzed Lamiaceous species.

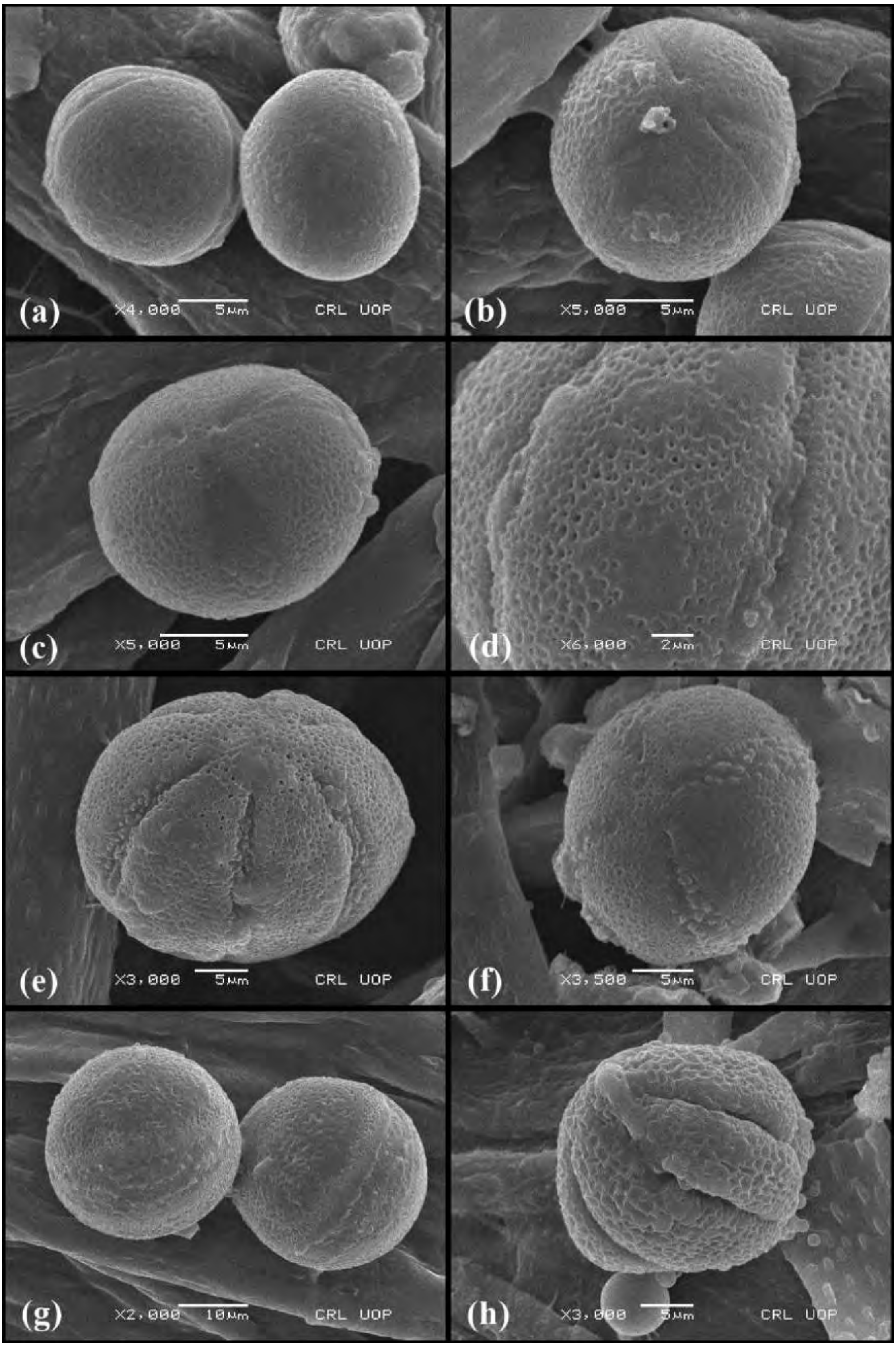

Plate 51. SEM micrographs of pollen; (a), (b) *Ajuga alpina,* (c) *Ajuga bracteosa*, (d), (e)*Clinopodium umbrosum*,(f),(g)*Eremostachys vicaryi*,(h)*Isodon rugosus*

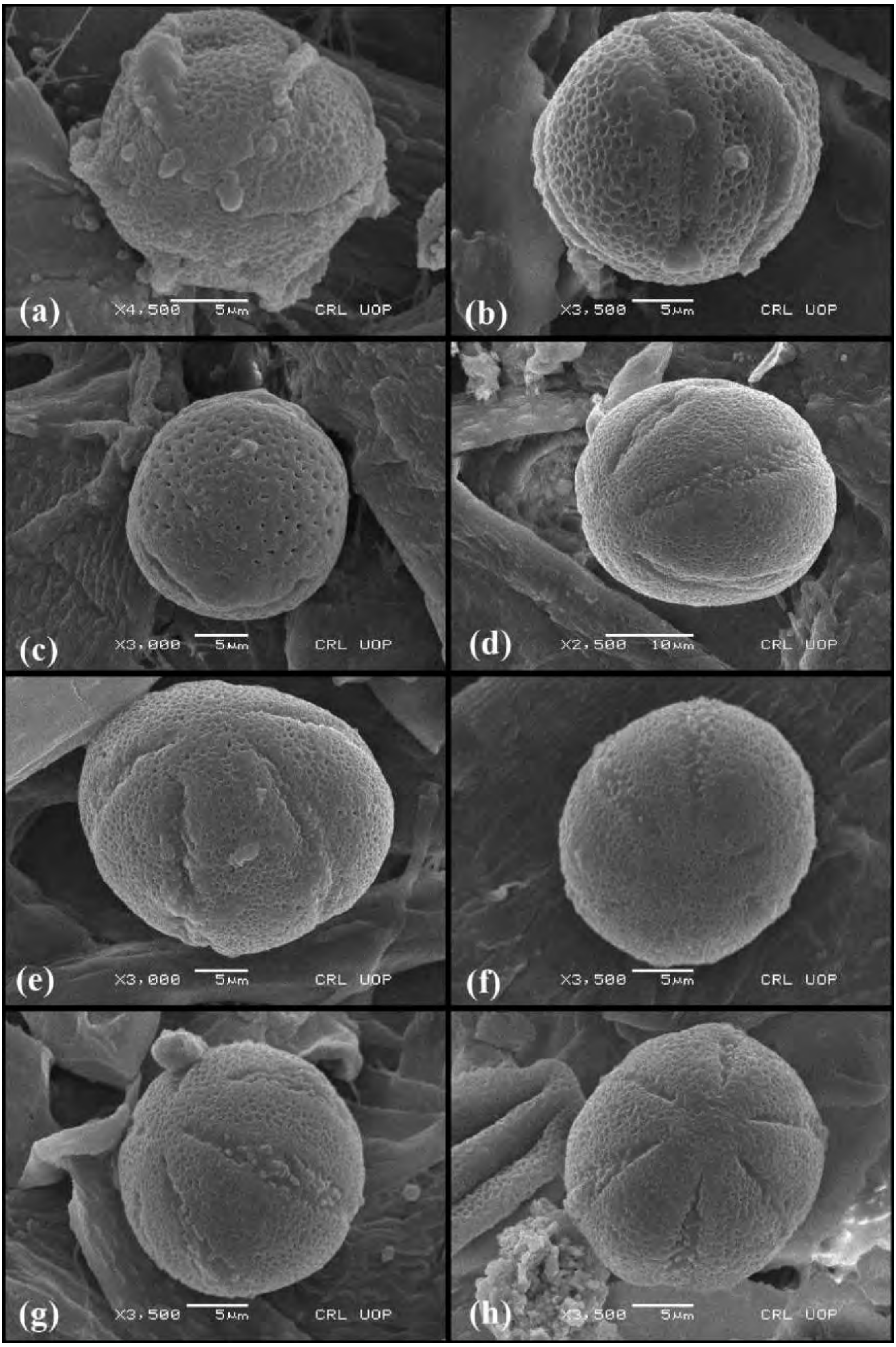

Plate 52. SEM micrographs of pollen (a), (b) *Lallemantia royleana,* (c) *Marrubium vulgare*, (d), (e) *Micromeria biflora*, (f), (g) *Nepeta bracteata*, (h) *Nepeta eriosphaera*

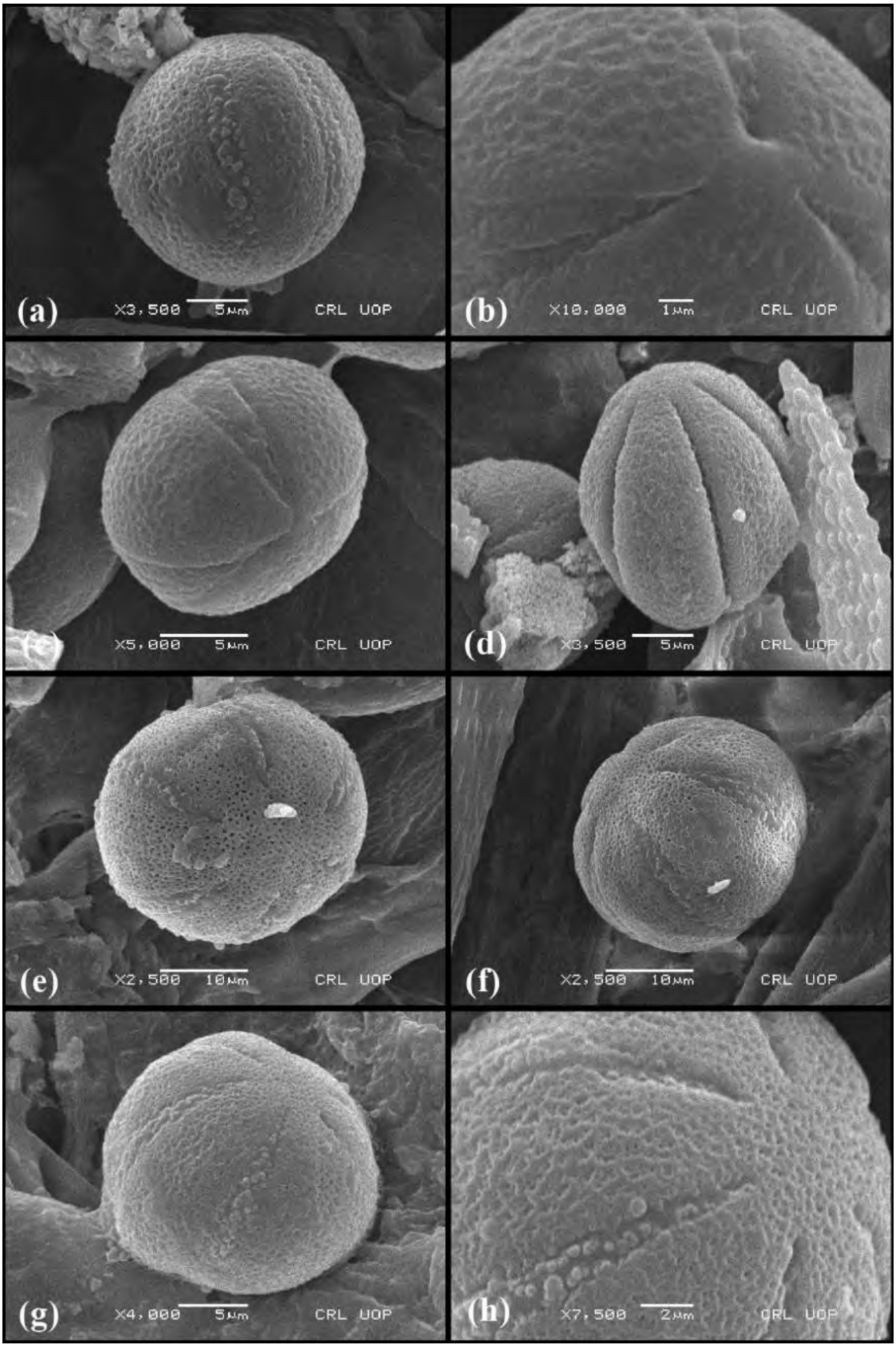

Plate 53. SEM micrographs of pollen (a) *Nepeta eriosphaera*, (b), (c) *Nepeta glomerulosa*, (d) *Nepeta griffithii,* (e), (f) *Nepeta hindostana*, (g), (h) *Nepeta juncea*

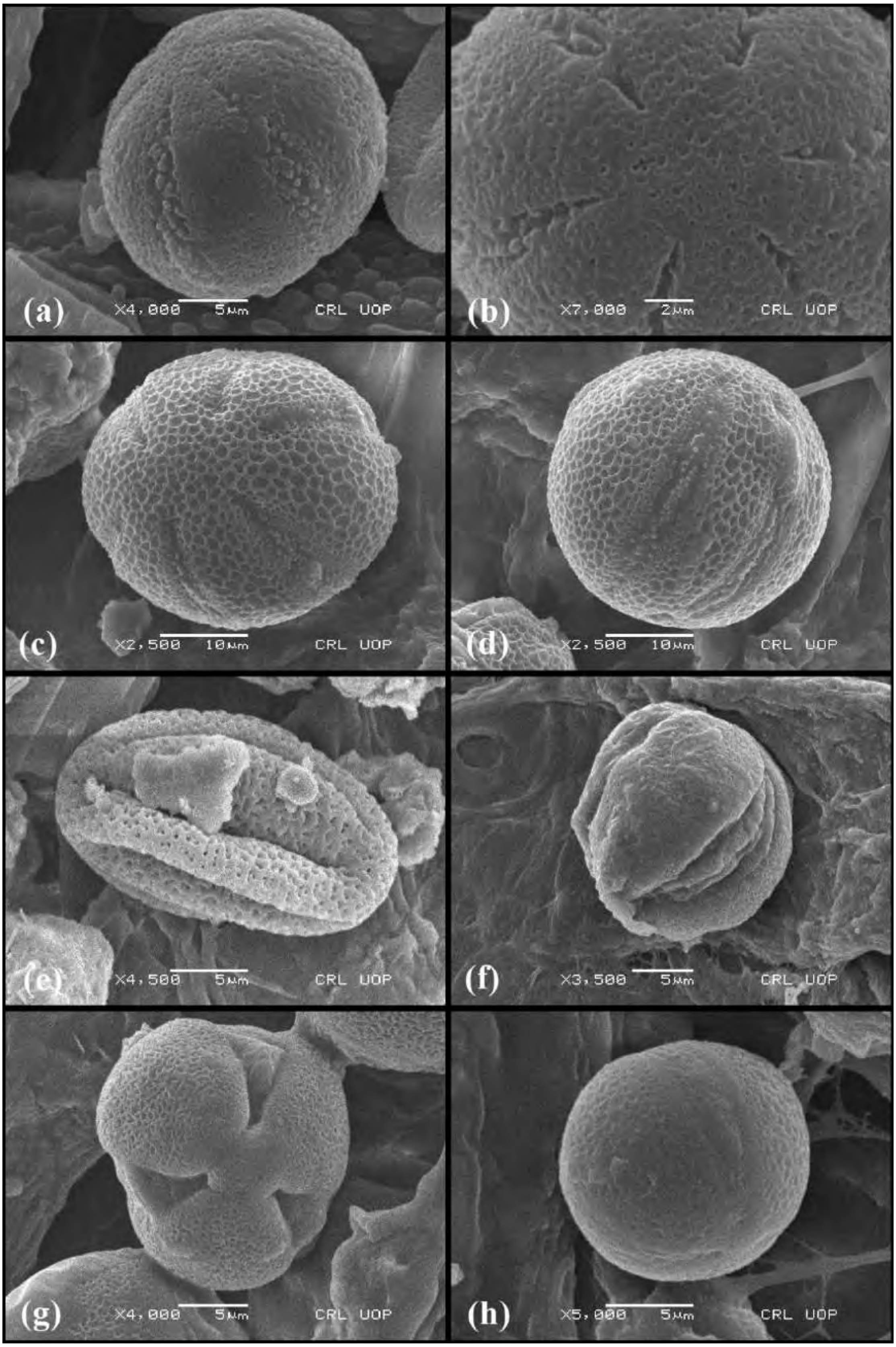

Plate 54. SEM micrographs of pollen (a), (b) *Nepeta praetervisa,* (c), (d) *Ocimum africanum,* (e) *Origanum majorana*, (f) *Otostegia aucheri*, (g), (h) *Otostegia limbata*

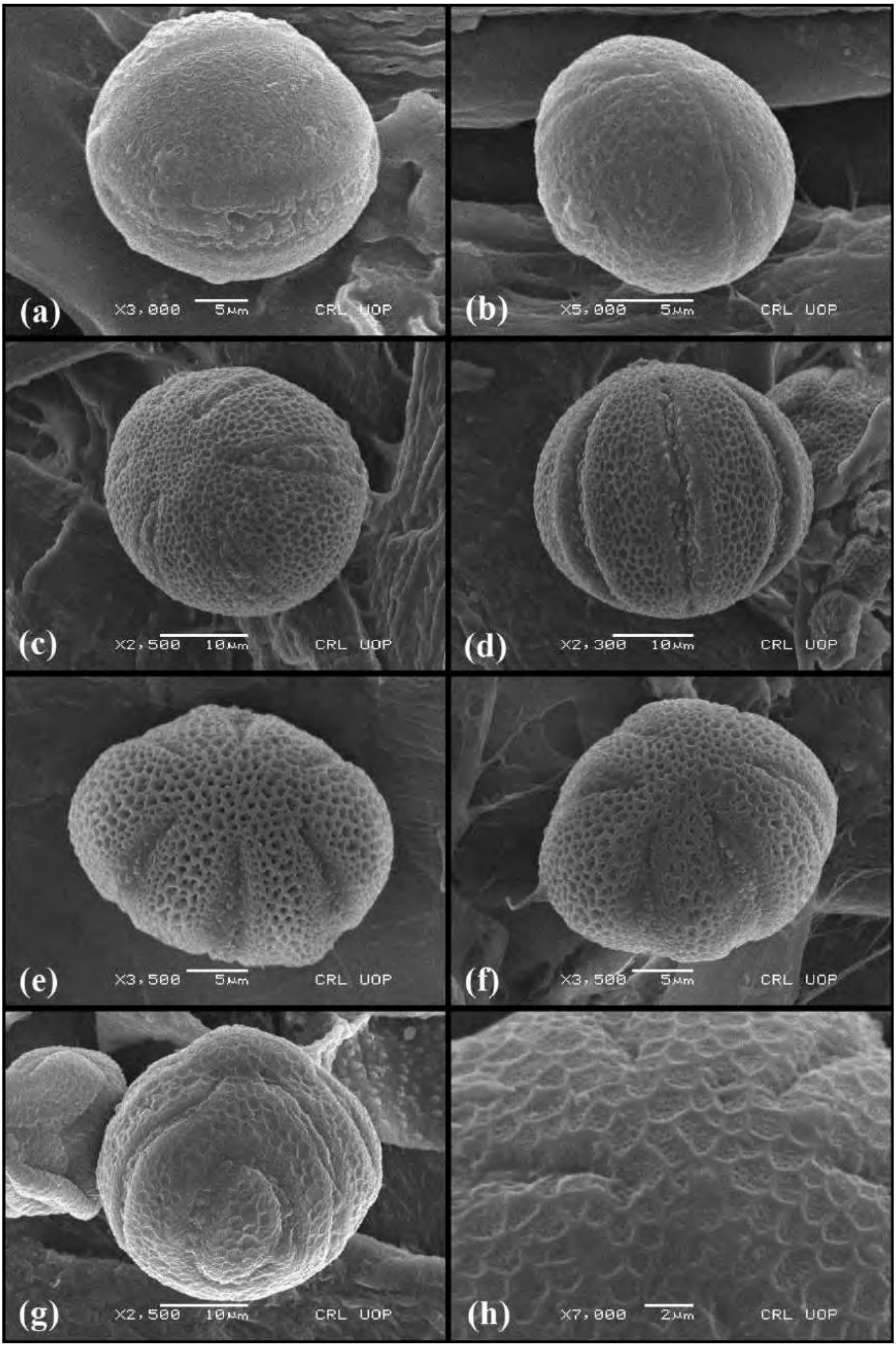

Plate 55. SEM micrographs of pollen (a), (b) *Phlomis stewartii,* (c), (d) *Salvia cabulica,* (e), (f) *Salvia coccinea*, (g), (h) *Salvia leucantha*

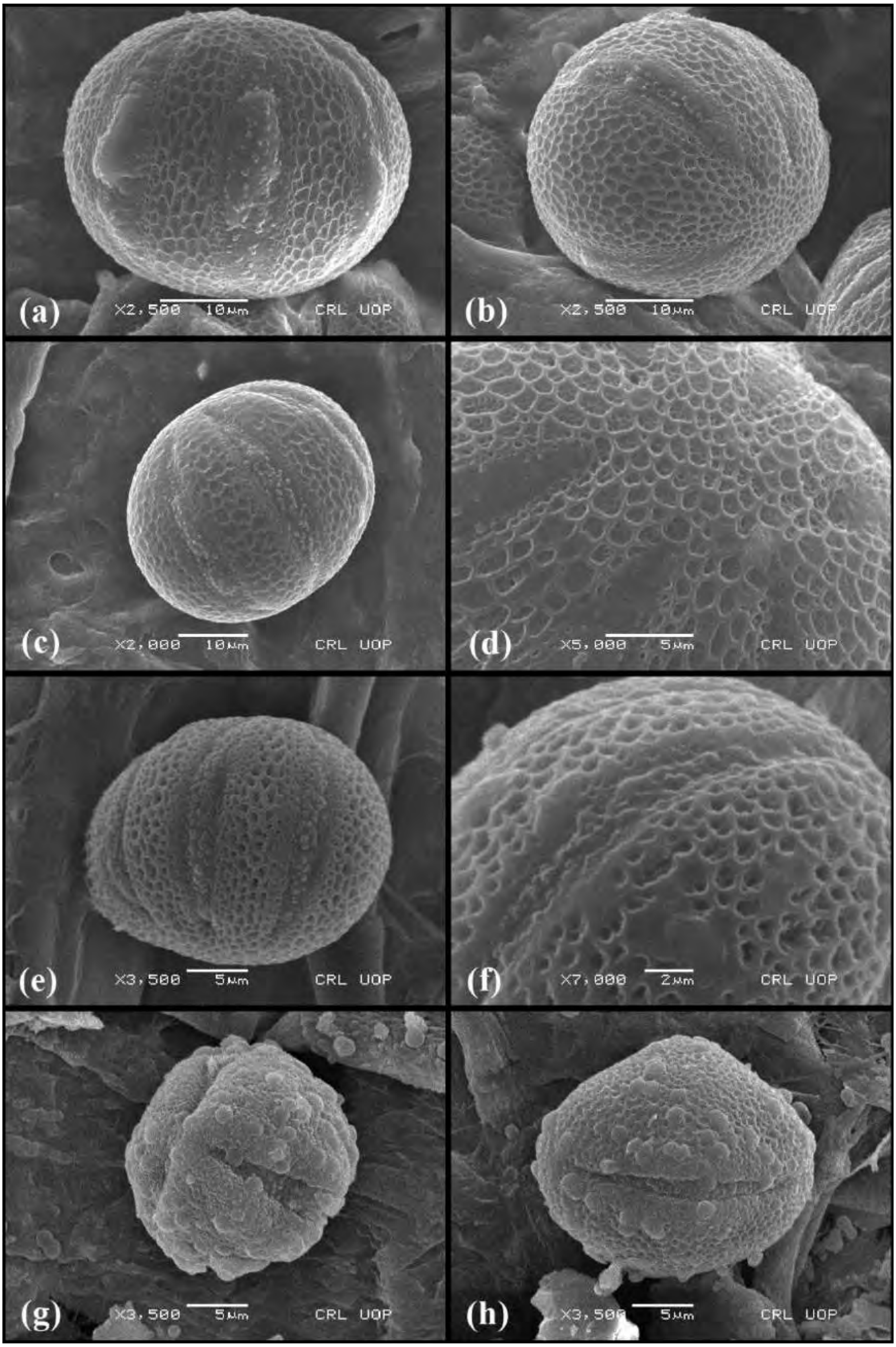

Plate 56. SEM micrographs of pollen (a), (b) *Salvia macrosiphon,* (c), (d) *Salvia moorcroftiana,* (e), (f) *Salvia plebeia*, (g), (h) *Salvia santolinifolia*

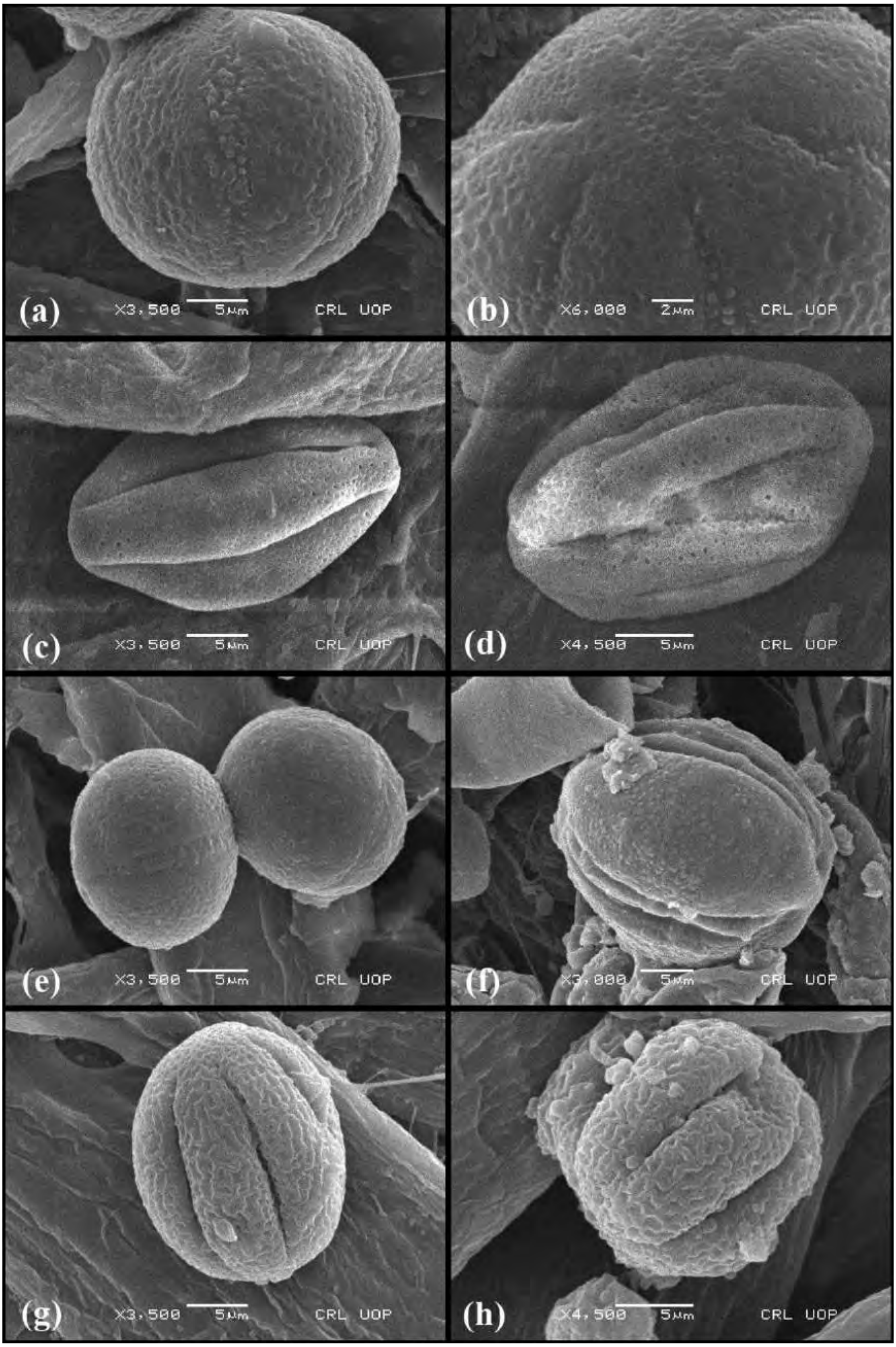

Plate 57. SEM micrographs of pollen (a), (b) *Scutellaria linearis,* (c), (d) *Stachys parviflora,* (e), (f) *Teucrium stocksianum*, (g), (h) *Thymus linearis*

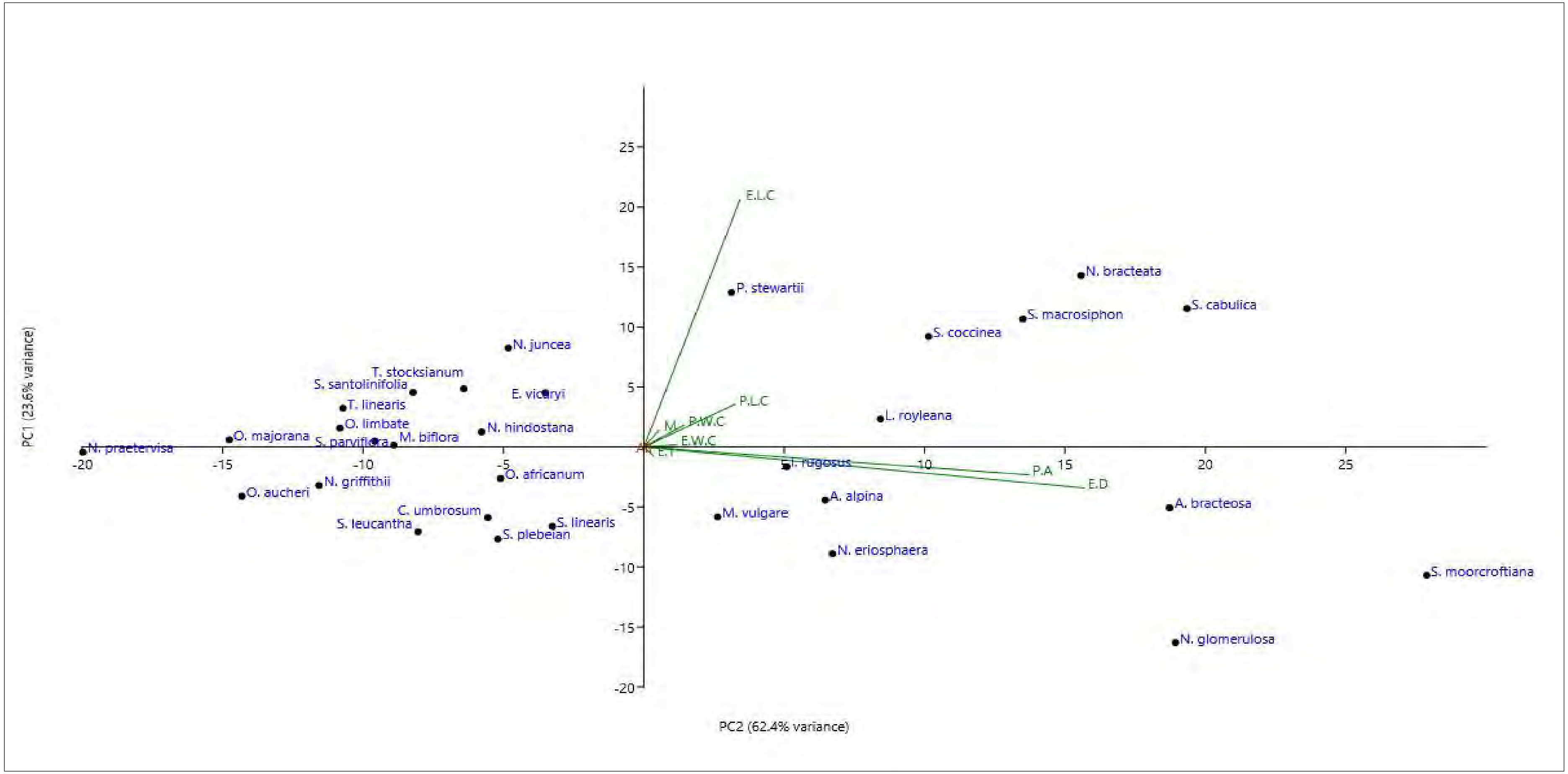

Figure 16. PCA analysis based on quantitative traits; polar axis, equatorial diameter, width and length of colpi in polar view, width and length of colpi in equatorial view, mesocolpium, exine thickness of Lamiaceous pollen

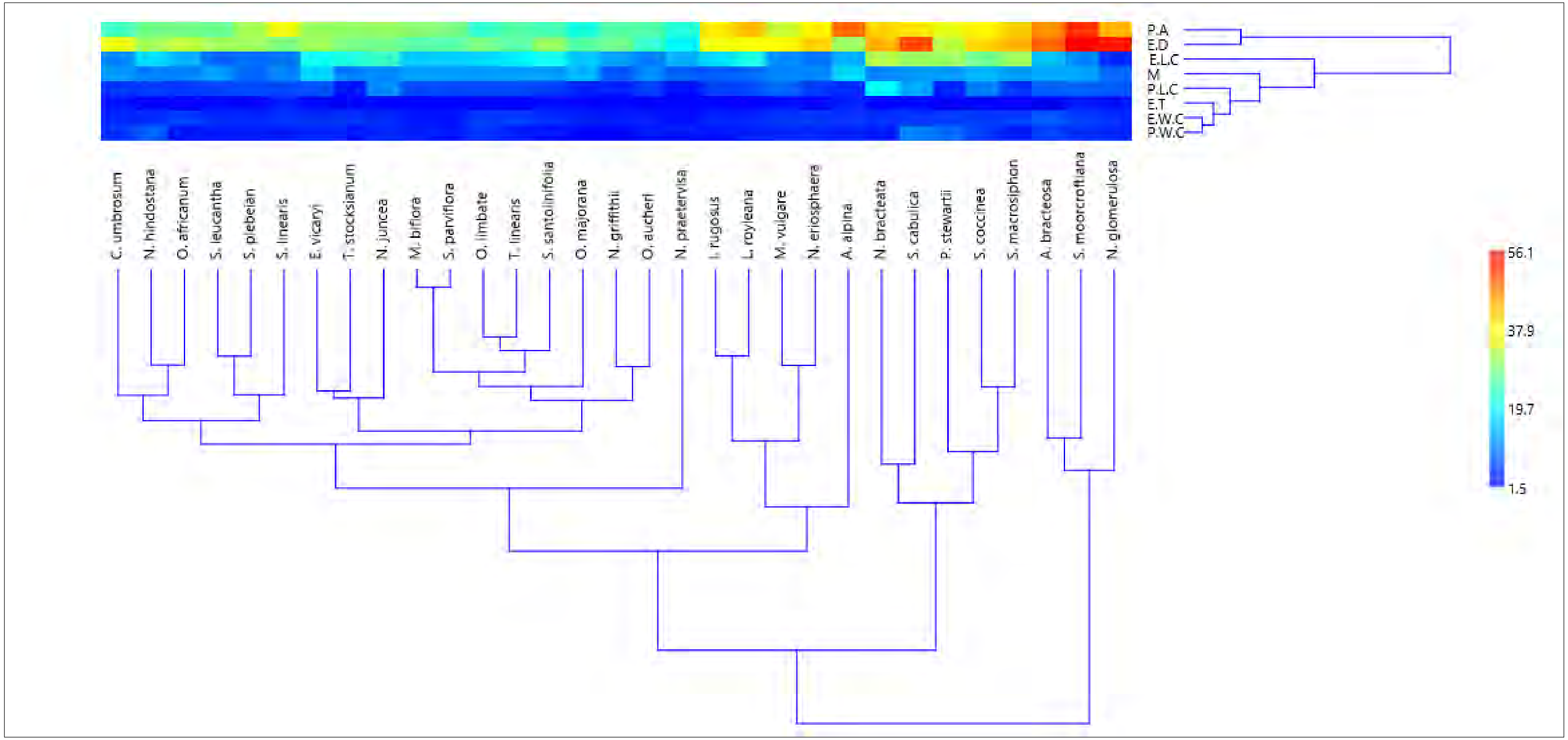

Figure 17. UPGMA cluster analysis based on quantitative traits; polar axis, equatorial diameter, width and length of colpi in polar view, width and length of colpi in equatorial view, mesocolpium, exine thickness of Lamiaceous pollen

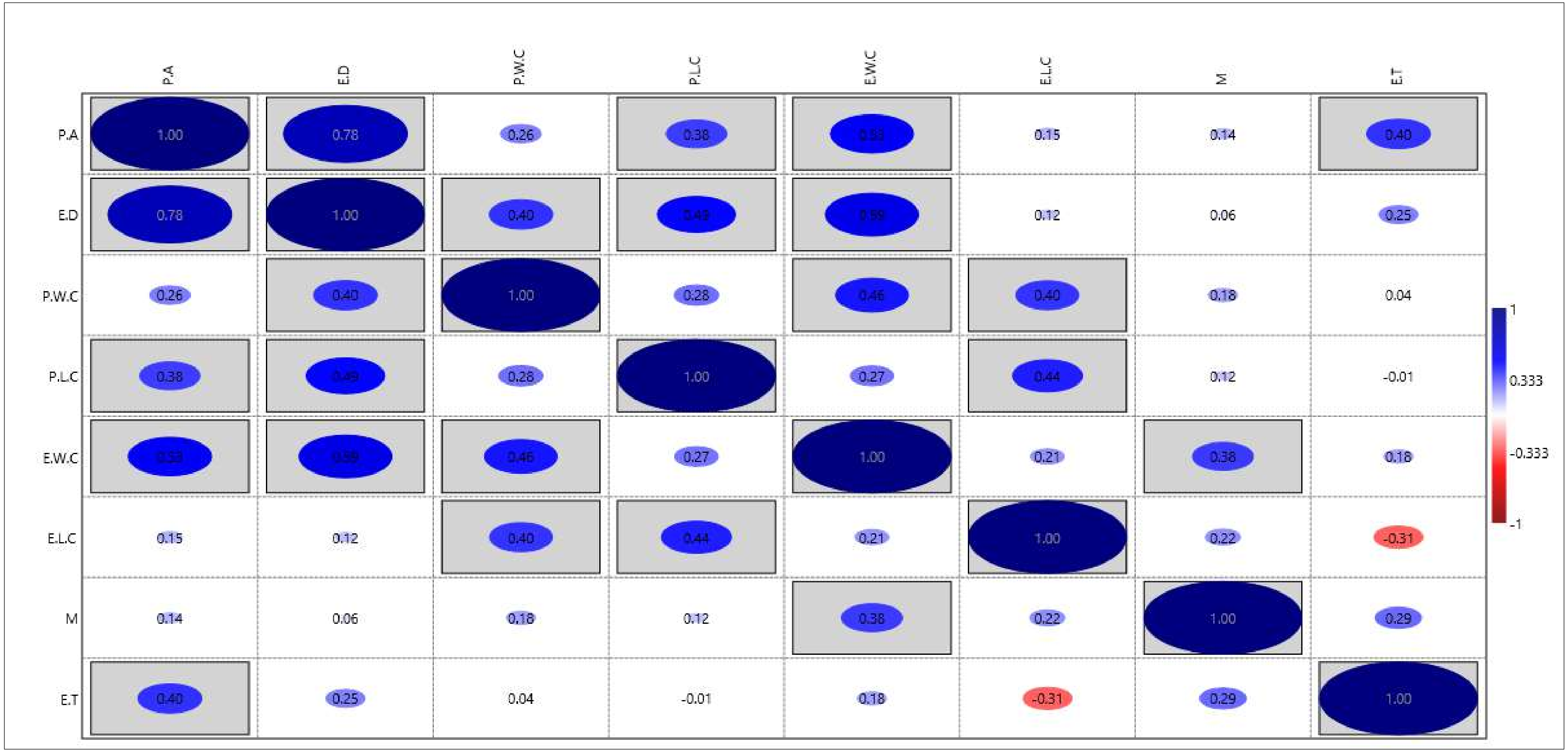

Figure 18. Correlation among the mean values of polar axis (P.A), equatorial diameter (E.D), width and length of colpi in polar view (W.C.P, L.C.P), width and length of colpi in equatorial view (W.C.E, L.C.E), mesocolpium (M), exine thickness (E.T) of Lamiaceous pollen

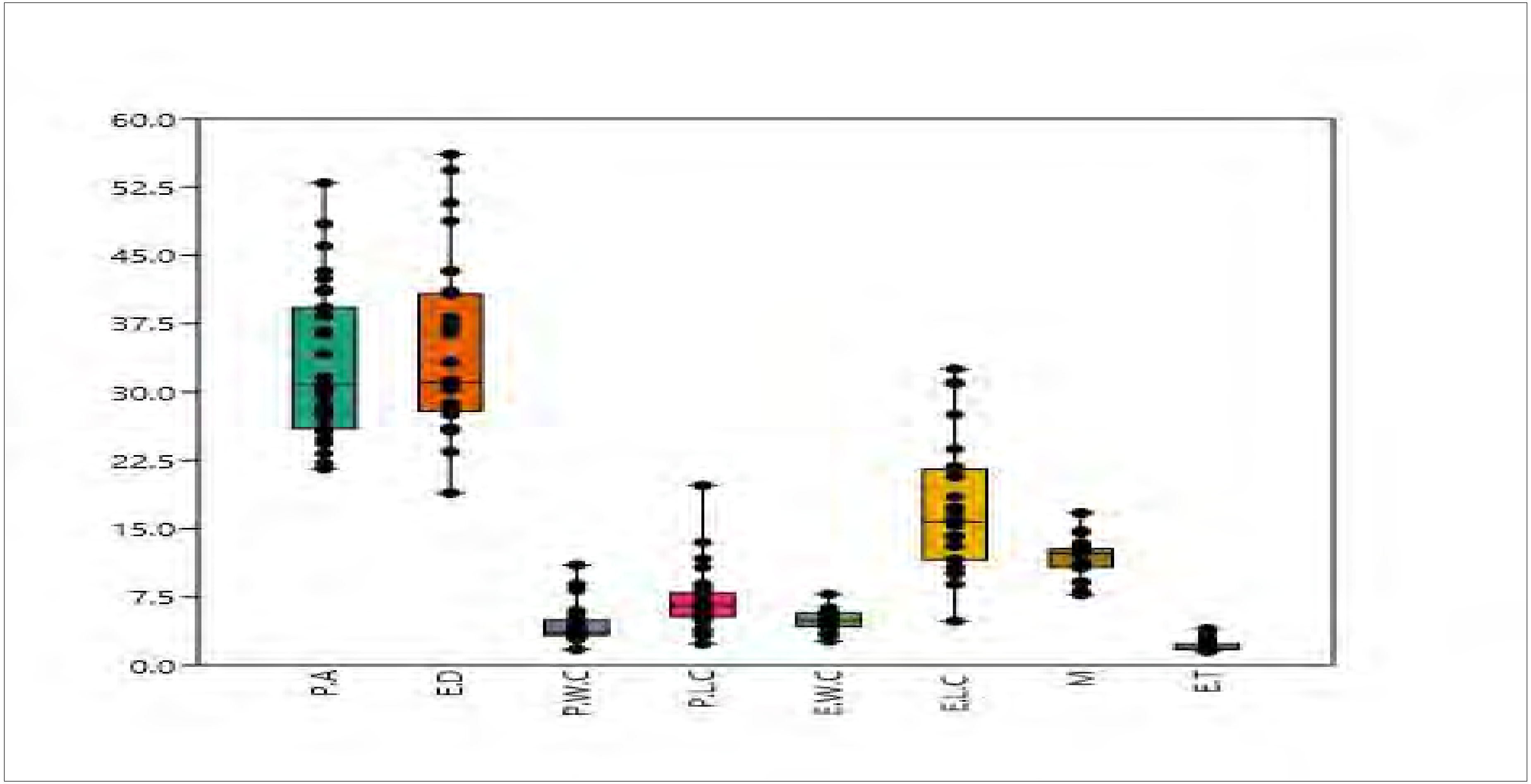

Figure 19. Box-jatter plot for the mean values of polar axis (P.A), equatorial diameter (E.D), width and length of colpi in polar view (W.C.P, L.C.P), width and length of colpi in equatorial view (W.C.E, L.C.E), mesocolpium (M), exine thickness (E.T) of Lamiaceous pollen

Table 14. Size, shape and viability of pollen of Lamiaceous species

Plant name	P. A	E. D	P/E	Size class	Shape class
Ajuga alpina	48.45	31.05	1.5	medium	Prolate
Ajuga bracteosa	46	48.75	0.9	medium	oblate-spheroidal
Clinopodium umbrosum	24.35	36.45	0.6	medium	Oblate
Eremostachys vicaryi	30.75	30.9	0.9	medium	oblate-spheroidal
Isodon rugosus	30.6	27.9	1.0	medium	prolate -spheroidal
Lallemantia royleana	42.45	37.35	1.1	medium	prolate -spheroidal
Marrubium vulgare	34.15	38.15	0.8	medium	sub- oblate
Micromearia biflora	29.75	26	1.1	medium	prolate -spheroidal
Nepeta bracteate	41	43.25	0.9	medium	oblate-spheroidal
Nepeta eriosphaera	38.4	41.05	0.9	medium	oblate-spheroidal
Nepeta glomerulosa	43.25	54.35	0.7	large	Oblate
Nepeta griffithii	25.65	27.5	0.9	medium	oblate-spheroidal
Nepeta Hindostana	27.8	31.25	0.8	medium	sub- oblate
Nepeta juncea	30.6	27.9	1.0	medium	prolate -spheroidal
Nepeta praetervisa	22.2	18.9	1.1	small	prolate -spheroidal
Ocimum africanum	29.75	26	1.1	medium	prolate -spheroidal
Origanum majorana	21.6	25.75	0.8	medium	sub- oblate
Otostegia aucheri	25.75	23.45	1.0	medium	Spherical

Otostegia limbate	23.25	28.5	0.8	medium	sub- oblate
Phlomis stewartii	36.35	33.25	1.0	medium	Spherical
Salvia cabulica	39.25	50.75	0.7	large	Oblate
Salvia coccinea	38.25	40.75	0.9	medium	oblate-spheroidal
Salvia leucantha	27.15	30.95	0.8	medium	sub- prolate
Salvia macrosiphon	41.25	43.25	0.9	medium	oblate-spheroidal
Salvia moorcroftiana	52.95	56.1	0.9	large	oblate-spheroidal
Salvia plebeian	31.55	31.05	1.0	medium	Spherical
Salvia santolinifolia	24.85	30.25	0.8	medium	sub- oblate
Scutellaria linearis	36.65	28.8	1.2	medium	sub- prolate
Stachys parviflora	28.65	26	1.1	medium	prolate -spheroidal
Teucrium stocksianum	30.35	28.25	1.0	medium	Spherical
Thymus linearis	26	26	1	medium	Spherical

Table 15. Qualitative palynological characteristics of pollen of Lamiaceae species

Taxa	Symmetry	Polariy	Unity	No. of apertures	Polar view	Equatorial view	Exine sculpturing	Surface	Aperture membrane	Amb	NPC classification	
											Name	Formula
Ajuga alpina	Radial	Isopolar	Monad	Tricolpate	Circular	Elliptic truncate	Reticulate	Microperforate	Verrucate	Peritreme	Trizoonocolpate	$N_3P_4C_3$
Ajuga bracteosa	Radial	Isopolar	Monad	Tricolpate	Circular	Elliptic truncate	Reticulate	Microperforate	Verrucate	Peritreme	Trizoonocolpate	$N_3P_4C_3$
Clinopodium umbrosum	Radial	Isopolar	Monad	Hexacolpate	Circular to Quinquangular Obtuse	Elliptic Obtuse	Macroreticulate	Perforate	scabrate verrucate	Ptychotreme	Hexazoonocolpate	$N_6P_4C_3$
Eremostachys vicaryi	Radial	Isopolar	Monad	Tricolpate	Circular	Circular	Reticulate	Microperforate	Verrucate	Peritreme	Trizoonocolpate	$N_3P_4C_3$
Isodon rugosus	Radial	Isopolar	Monad	Hexacolpate	Circular	Elliptic Obtuse	Macroreticulate	Microperforate	-	Ptychotreme	Hexazoonocolpate	$N_6P_4C_3$
Lallemantia royleana	Radial	Isopolar	Monad	Hexacolporate	Circular	Circular to Elliptic	Reticulate gemmate	Microperforate	Gemmate psilate	Peritreme	Hexazoonocolporate	$N_6P_4C_5$
Marrubium vulgare	Radial	Iso polar	Monad	Tricolporate	Circular	Circular	Foveolate	Microperforate	Psilate	Peritreme	Trizoonocolporate	$N_3P_4C_5$
Micromearia biflora	Radial	Isopolar	Monad	Hexacolpate	Circular to Quinquangular Obtuse	Elliptic Obtuse	Macroreticulate	Perforate	scabrate verrucate	Ptychotreme	Hexazonocolpate	$N_6P_4C_3$
Nepeta bracteata	Radial	Isopolar	Monad	Tetra-Hexacolpate	Circular	Circular	Reticulate	Macroperforate (whole body)	Scabrate verrucate	Ptychotreme-Peritreme	Tetra-Hexazonocolpate	$N_{4\text{-}6}P_4C_3$
Nepeta eriosphaera	Radial	Isopolar	Monad	Tetra-Hexacolpate	Circular	Circular	Reticulate	Macroperforatee (whole body)	Scabrate verrucate	Ptychotreme-Peritreme	Tetra-Hexazonocolpate	$N_{4\text{-}6}P_4C_3$
Nepeta glomerulosa	Radial	Isopolar	Monad	Tricolpate	Circular	Elliptic truncate	Reticulate	Microperforate	Verrucate	Peritreme	Trizoonocolpate	$N_3P_4C_3$
Nepeta griffithii	Radial	Isopolar	Monad	Hexacolporate	Circular	Elliptic Obtuse	Reticulate	Microperforate	scabrate verrucate	Ptychotreme	Hexazoonocolporate	$N_6P_4C_5$
Nepeta Hindostana	Radial	Isopolar	Monad	Hexacolpate	Circular to Quinquangular Obtuse	Elliptic Obtuse	Macroreticulate	Perforate	scabrate verrucate	Ptychotreme	Hexazoonocolpate	$N_6P_4C_3$
Nepeta juncea	Radial	Isopolar	Monad	Hexacolporate	Circular	Elliptic Obtuse	Reticulate	Microperforate	Scabrate	Ptychotreme	Hexazoonocolpate	$N_6P_4C_3$

Nepeta praetervisa	Radial	Isopolar	Monad	Tetra-Hexacolpate	Circular	Circular	Reticulate	Macroperforate (whole body)	Scabrate verrucate	Ptychotreme-Peritreme	Tetra-Hexazonocolpate	$N_{4-6}P_4C_3$
Ocimum africanum	Radial	Isopolar	Monad	Hexacolporate	Circular	Elliptic truncate	Macroretculat	Microperforate	Scabrate	Ptychotreme	Hexazonocolpate	$N_6P_4C_3$
Origanum majorana	Radial	Isopolar	Monad	Hexacolpate	Circular to Quinquangular	Elliptic truncate	Reticulate	Macroperforate (whole body)	-	Ptychotreme	Hexazoonocolpate	$N_6P_4C_5$
Otostegia aucheri	Radial	Isopolar	Monad	Tricolpate	Triangular to Circular	Circular to slightly Elliptic	Reticulate	Microperforate	Psilate	Goniotreme	Trizoonocolpate	$N_3P_4C_3$
Otostegia limbate	Radial	Isopolar	Monad	Tricolporate	Triangular Obtuse	Elliptic Truncate	Reticulate	Microperforate	Psilate	Peritreme	Trizoonocolporate	$N_3P_4C_5$
Phlomis stewartii	Radial	Isopolar	Monad	Tricolpate	Circular	Circular to Elliptic	Roughly reticulate	Non-perforate	Verrucate	Peritreme	Trizoonocolpate	$N_3P_4C_3$
Salvia cabulica	Radial	Isopolar	Monad	Hexacolpate	Circular	Circular	Macroreticulate	Non-perforate	Verrucate	Peritreme	Hexazoonocolpate	$N_6P_4C_3$
Salvia coccinea	Radial	Isopolar	Monad	Hexacolpate	Circular	Elliptic truncate	Macroreticulate	Macroperforate	scabrate verrucate	Ptychotreme	Hexazoonocolpate	$N_6P_4C_3$
Salvia leucantha	Radial	Iso polar	Monad	Hexa to multi syncolpate	Circular	Circular	Macroreticulate	Microperforate	scabrate verrucate	Peritreme	Hexa-multi-zonocolpate	$N_{4-}.P_4C_3$
Salvia macrosiphon	Radial	Isopolar	Monad	Hexacolporate	Circular	Elliptic truncate	Macroretculiate	Microperforate	Scabrate	Ptychotreme	Hexazonocolporate	$N_6P_4C_5$
Salvia moorcroftiana	Radial	Isopolar	Monad	Hexacolporate	Circular	Elliptic truncate	Macroretculiate	Microperforate	Scabrate	Ptychotreme	Hexazonocolporate	$N_6P_4C_5$
Salvia plebeian	Radial	Isopolar	Monad	Hexacolpate	Circular	Elliptic truncate	Macroreticulate	Macroperforate	scabrate verrucate	Ptychotreme	Hexazonocolpate	$N_6P_4C_3$
Salvia santolinifolia	Radial	Isopolar	Monad	Tri to Hexacolpate	Circular to triangular Obtuse convex	Elliptic to Rhombic Obtuse truncate	Reticulate & gemmate	Perforate	Verrucate gemmate	Peritreme-Goniotrem	Tri-Hexazonocolpate	$N_{3-6}P_4C_3$
Scutellaria linearis	Radial	Isopolar	Monad	Hexacolpate	Circular	Circular	Macroreticulate	Microperforate	Scabrate verrucate	Ptychotreme	Hexazoonocolpate	$N_6P_4C_3$
Stachys parviflora	Radial	Isopolar	Monad	Tricolpate	Tri- angular	Rhombic Obtuse truncate	Foveolate	Reticulate	-	Ptychotreme	Trizonocolpate	$N_3P_4C_3$
Teucrium stocksianum	Radial	Isopolar	Monad	Tricolpate	Tri angular Obtuse convex	Elliptic Obtuse	Gemmate verrucate	Non- perforate	Psilate	Peritreme	Trizonocolpate	$N_3P_4C_3$
Thymus linearis	Radial	Isopolar	Monad	Hexacolpate	Circular	Circular	Reticulate striate	Perforate	Psilate	Peritreme	Hexazoonocolpate	$N_6P_4C_3$

Table 16. Quantitative palynological measurements of Lamiaceous species

Plant Species	**P.A**	**E.D**	**W.C.P**	**L.C.P**	**W.C.E**	**L.C.E**	**M**	**E.T**
Mean± Stander error								
Ajuga alpina	48.45±0.215058132	31.05±0.266926956	3.85±0.231840462	4.9±0.26925824	5.3±0.289395923	14±0.176776695	16.75±1.629800601	4±0.176776695
Ajuga bracteosa	46±0.17678	48.75±0.17678	6±0.17678	8.25±0.17678	7.8±0.2894	15.35±0.30208	13.4±0.81624	1.75±0.17678
Clinopodium umbrosum	24.35±0.831414457	36.45±0.566789202	5.15±0.231840462	5.3±0.289395923	4.25±0.176776695	10.75±0.176776695	12.3±0.538516481	2.95±0.215058132
Eremostachys vicaryi	30.75±0.176776695	30.9±0.527967802	4±0.176776695	7.65±0.231840462	4.95±0.289395923	20.75±0.176776695	16.7±1.620956508	2.05±0.215058132
Isodon rugosus	39.2±0.29993	36.9±0.10293	2.9±0.2001	5.8±0.3893	4±0.24995	17.3±0.1893	7.8±0.289	1.5±0.176
Lallemantia royleana	42.45±0.289395923	37.35±0.231840462	3.75±0.176776695	6.75±0.176776695	4.25±0.176776695	21.55±0.215058132	9.15±0.302076149	1.75±0.176776695
Marrubium vulgare	34.15±0.231840462	38.15±0.16955825	4.25±0.176776695	8.95±0.215058132	5.45±0.215058132	11.676±1.512192448	11.35±0.302076149	2.35±0.231840462
Micromearia biflora	29.75±0.58630197	26±0.176776695	3.5±0.176776695	6.75±0.176776695	3.25±0.176776695	15.75±0.176776695	12.75±0.637377439	1.75±0.176776695
Nepeta bracteate	41±0.176776695	43.25±0.176776695	3.25±0.176776695	19.75±0.467707173	4.9±0.26925824	32.5±0.32596012	10.95±0.215058132	1.75±0.176776695
Nepeta eriosphaera	38.4±0.231840462	41.05±0.266926956	4.25±0.176776695	5.75±0.176776695	5.75±0.176776695	10±0.447213595	11.15±0.257390754	2.05±0.215058132
Nepeta glomerulosa	43.25±0.395284708	54.35±0.430116263	4±0.176776695	7.95±0.215058132	4.55±0.215058132	4.85±0.231840462	9.1±0.331662479	2.65±0.231840462
Nepeta griffithii	25.65±0.257390754	27.5±0.353553391	1.75±0.176776695	3.5±0.176776695	3.5±0.176776695	13.1±0.127475488	7.75±0.684653197	1.75±0.176776695
Nepeta Hindostana	27.8±0.289395923	31.25±0.176776695	8.25±0.176776695	6.05±0.289395923	5.25±0.285043856	17.35±0.231840462	10.95±0.215058132	1.75±0.176776695
Nepeta juncea	30.6±0.26925824	27.9±0.340954542	4.55±0.215058132	11.7±0.310241841	4.7±0.289395923	23.75±0.25	11.15±0.340954542	2.05±0.215058132
Nepeta praetervisa	22.2±0.289395923	18.9±0.257390754	3.5±0.176776695	2.35±0.231840462	2.65±0.231840462	14.2±0.310241841	8.15±0.573367247	2.05±0.215058132

Ocimum africanum	28±0.25	33.25±0.176776695	3.25±0.176776695	5.3±0.289395923	5.75±0.176776695	14.25±0.176776695	10.75±0.176776695	1.75±0.176776695
Origanum majorana	21.6±0.231840462	25.75±0.176776695	1.75±0.176776695	4±0.176776695	3.5±0.176776695	15.75±0.176776695	14.75±1.131923142	1.7±0.215058132
Otostegia aucheri	25.75±0.176776695	23.45±0.215058132	3.25±0.176776695	5.75±0.209165007	3.5±0.176776695	10.75±0.176776695	11.15±0.257390754	1.75±0.176776695
Otostegia limbate	23.25±0.176776695	28.5±0.176776695	5.15±0.231840462	6.75±0.176776695	5.75±0.176776695	16.75±0.176776695	12.6±0.610327781	1.75±0.176776695
Phlomis stewartii	36.35±0.322102468	33.25±0.176776695	8.9±0.302076149	4±0.176776695	5.75±0.176776695	30.95±0.215058132	12.3±0.538516481	1.75±0.176776695
Salvia cabulica	39.25±0.176776695	50.75±0.176776695	11±0.176776695	13.5±0.454147553	5.45±0.215058132	30.95±0.215058132	12.55±0.588430115	2.35±0.231840462
Salvia coccinea	38.25±0.176776695	40.75±0.176776695	5.75±0.176776695	10.75±0.176776695	5.75±0.176776695	27.55±0.365718471	14.55±1.082243041	1.75±0.176776695
Salvia leucantha	27.15±0.302076149	30.95±0.215058132	3.25±0.176776695	6.2±0.215058132	4.85±0.231840462	8.9±0.484767986	12.55±0.588430115	2.2±0.289395923
Salvia macrosiphon	41.25±0.176776695	43.25±0.176776695	3.5±0.176776695	5.75±0.176776695	5.75±0.176776695	31±0.176776695	12.95±0.686476511	2.05±0.215058132
Salvia moorcroftiana	52.95±0.7881	56.1±0.5567	4.95±0.183711	8.65±0.25739	6.3±0.2150	11.6±0.231	13.35±0.7	3.5±0.17
Salvia plebeian	31.55±0.572276157	31.05±0.289395923	3.5±0.176776695	6.7±0.215058132	4.25±0.176776695	8.9±0.127475488	9.05±0.310241841	1.75±0.176776695
Salvia santolinifolia	24.85±0.231840462	30.25±0.306186218	2.95±0.215058132	5.75±0.176776695	4.85±0.231840462	20.75±0.379143772	12.35±0.562361094	2.65±0.231840462
Scutellaria linearis	36.65±0.515994186	28.8±0.266926956	3.6±0.231840462	6.6±0.26925824	5.15±0.231840462	10.15±0.231840462	10.85±0.231840462	3.75±0.176776695
Stachys parviflora	28.65±0.231840462	26±0.176776695	3.25±0.176776695	6.75±0.176776695	3.25±0.176776695	16±0.176776695	12.75±0.637377439	1.75±0.176776695
Teucrium stocksianum	30.35±0.322102468	28.25±0.176776695	3.1±0.127475488	3.25±0.176776695	4.85±0.231840462	21.85±0.257390754	10.75±0.176776695	1.75±0.176776695
Thymus linearis	26±0.176776695	26±0.176776695	3.5±0.176776695	6.75±0.176776695	5.75±0.176776695	18.5±0.176776695	12.8±0.658596994	1.75±0.176776695

P.A: polar axis; E.D: equatorial diameter, L.C.P, W.C.P, L.C.E, W.C.E: length and width of colpi in polar and equatorial view, M: mesocolpium, E.T: exine thickness

Table 17. Dichotomous (single access) bracketed taxonomic key based on pollen morphological traits of Lamiaceous species

Link Character	Leads	Characters	Taxa/ Go to link character
1	a	Small pollen size	*Nepeta praetervisa*
	b	Pollen large or medium	2
2	a	Large pollen	3
	b	Medium pollen	5
3	a	Oblate-spheroidal shape	*Salvia moorcroftiana*
	b	Oblate shape pollen	4
4	a	Tricolptae	*Nepeta glomerulosa*
	b	Hexacolpate	*Salvia cabulica*
5	a	Amb peritreme	*6*
	b	Amb ptychotreme	*15*
6	a	Foveolate sculpturing	*Marrubium vulgare*
	b	Sculpturing other than foveolate	*7*
7	a	Aperture tricolporate	*Otostegia limbate*
	b	Aperture other than tricolporate	8
8	a	Hexa to multi syncolpate	*Salvia leucantha*
	b	Colpi not fused	9
9	a	Hexacolpate / hexacolporate pollen	10
	b	Tricolpate pollen	11

10	a	Hexacolporate pollen	*Lallemantia royleana*
	b	Hexacolpate pollen	*Thymus linearis*
11	a	Exine non-perforate	12
	b	Exine perforate	13
12	a	Aperture membrane psilate	*Teucrium stocksianum*
	b	Verrucate aperture membrane	*Phlomis stewartii*
13	a	Equatorial view circular	*Eremostachys vicaryi*
	b	Elliptic truncate	14
14	a	Oblate-spheroidal	*Ajuga bracteosa*
	b	Prolate shape pollen	*Ajuga alpina*
15	a	Foveolate exine	*Stachys parviflora*
	b	Exine other than foveolate	16
16	a	Exine reticulate	*17*
	b	Exine macrorteiculate	20
17	a	Sub-oblate shape pollen	*Origanum majorana*
	b	Pollen shape not suboblate	18
18	a	Prolate spheroidal pollen	*Nepeta juncea*
	b	Oblate spheroidal pollen	19
19	a	Equatorial view circular to elliptic	*Nepeta bracteate*
	b	Equatorial view circular	*Nepeta eriosphaera*

20	a	Spherical shape pollen	*Salvia plebeian*
	b	Pollen shape not spherical	21
21	a	Oblate pollen	*Clinopodium umbrosum*
	b	Other than oblate shape of pollen	22
22	a	Pollen shape sub-oblate	*Nepeta Hindostana*
	b	Pollen oblate spheroidal or prolate spheroidal	23
23	a	Prolate spheroidal	24
	b	Oblate spheroidal pollen	26
24	a	Hexacolporate aperture	*Ocimum africanum*
	b	Hexacolpate pollen	25
25	a	Polar view circular	*Isodon rugosus*
	b	Circular to quinquangular obtuse polar view	*Micromearia biflora*
26	a	Aperture membrane scabrate	*Salvia macrosiphon*
	b	Aperture membrane verrucate to scabrate	*27*
27	a	Hexacolpate pollen	*Salvia coccinea*
	b	Hexacolporate pollen	*Nepeta griffithii*

Section-III

Anatomy

Summary

The third section included the petiole anatomy of dicots from Northern Baluchistan, Pakistan. The study included 25 Brassicaceous, 15 Fabaceous, and 25 Lamiaceous species. Sections were prepared via microtomy. Petiole cross sections were visualised under Light microscopy and observed the qualitative and quantitative characteristics.

- Qualitative characters included: Petiole outline, wings, cuticle, shape and number of layers of epidermis, collenchyma, parenchyma, chlorenchyma, sclerenchyma, xylem vessels, phloem, vascular bundles arrangement and number.
- Quantitative features were: Length and width of petiole, epidermis cells, collenchyma cells, parenchyma cells, chlorenchyma cells, sclerenchyma cells, xylem vessels, phloem cells, and vascular bundles.

The variations in the qualitative features were used to develop taxonomic keys that helped discriminate among the examined species of each family. The distinguished petiole anatomical characters were petiole outline, shape, and number of layers of collenchyma, parenchyma, vascular bundles arrangement and number. The multivariate analysis of the quantitative features was carried out by principal component analysis, hierarchal cluster analysis, and correlation plots.

3.4 Petiole anatomy of Brassicaceous Flora of Northern Baluchistan

3.4.1 Results and Discussion

The petiole anatomy of the wild Brassicaceous flora of Northern Baluchistan of the Irano-Turanian subregion has not previously been explored. Petiole anatomy of 25 wild species of Brassicaceae belonging to 21 genera were studied and compared. Histological studies were carried out utilizing light microscopy (Plate 58-63). This study included the endemic plant *F. hamiltonii* and the first-time reported species *G. pendula* from the flora of Pakistan. Anatomical studies of petioles significantly provided taxonomic evidence and evolutionary clues and assisted in the classification and identification of plant species (Ozcan et al., 2015). Documentation of taxa based on anatomical studies belonging to specific climatic regions has taxonomic significance. It helps in finding evolutionary patterns and climatic effects controlling anatomical features (Kocsis and Borhidi, 2003).

a) Petiole Outline-based Grouping of Brassicaceous Flora

The shape of the petiole varied from circular to subcircular or dorsoventrally flattened (Karaismailoğlu, 2020). Highly repeated shapes were sulcate (10 species), followed by flat (9 species). Species of the genus *Cardaria* and *Strigosella* possessed similar sulcate shapes, while members of the genus *Farsetia* exhibited different shapes; these results revealed characteristic homogeneity and variations at the genus level. Karaismailoğlu (2020) also reported sulcate, circular and flat-type petioles in Brassicaceae (tribe Alysseae) from Turkey. For *Alyssum desertorum,* similar anatomical features were observed by Karaismailoğlu (2020), but they observed oval shape petiole with an undulated cuticle (Table 19). The outcomes helped in the rearrangement of taxa in different ranks along with marking ecological impacts on anatomical features in various climatic regions. The transverse section of petiole in *B.oleracea, C. bursa - pastoris, D. harra,* and *G. pendula* were circular with slightly convex ends, whereas *F. hamiltonii, P. brahuicus* and *S. altissimum* exhibited a completely circular shape. Petiole margins expressed variations with two major types, blunt end (*D. sophia, F. heliophila, S. africana)* acute margins (*S.cabulica)* and tipped margins *(D. harra)*.

Variations in the petiole shape have been studied in various families like

Lamialies (Akçın et al., 2011), Cruciferae (Olowokudejo, 1987), the results of these studies have been successfully applied in systematics. Based on the outline of the petioles, the Brassicaceous species were separated into various groups. The group (a) plants possessed one large central vascular bundle, (b) plants were observed with one large central and two small vascular bundles towards the wings, (c) included the taxa with many vascular bundles arranged in a circle with a gap/space between them, (d) also observed circular arrangement of numerous vascular bundles but with compact pattern, (e) consist of species with three equal size vascular bundles in the center of the petiole, (f) one central and two comparatively small subsidiary vascular bundles were observed and (g) one central and couple of vascular bundles in each side (Plate 63).

From group (a) *B. oleracea*, *G. pendula* and *S. cabulica* were in the same cluster in dendrogram. A similarly close association was observed for taxa of the group (b) *D. Sophia* with *D. harra* and *M. flavida* with *S. africana* as they shareed the same note of phylogeny. *A. desertorum* and *L. aucheri* (group d) were closest in the phylogenetic cluster. These findings can be effectively manipulated for phylogenetic relationships in Brassicaceous species. A significant phylogenetic association in the studied taxa was investigated by comparing petiole groups and dendrogram results.

b) Epidermis in the Brassicaceous petioles

A single layer of the epidermis was present in all examined species (Kemka-Evans et al., 2021). Rectangular, round, oval, angular, and square shapes of epidermal cells were observed, with square shapes highly repeated (Okanume et al., 2022). A layer of the cuticle (thick or thin) was present on the epidermis in 24 species. In most species, the cuticle was undulated, followed by the smooth surface cuticle. In 9 studied taxa, three types of trichomes or appendages (multiseriate, uniseriate and unicellular) were spotted (Table 19). Presence (in *Cardaria* species) or absence (in *Strigosella* species), as well as types of trichomes in species from the same genus, were found significant tools that assisted in distinguishing taxa at genus and even species level. The presence/absence of trichomes has been exploited proficiently in various families for the delimitation of taxa (Neto et al., 2017). Grove in the upper surface was marked in 10 studied taxa. Prominent pith was present in 9 Brassicaceous taxa *A. carneum, A. desertorum, C. orientalis, E. sative, F. hamiltonii, L. aucheri, M. linifolius, N. bicorne* and *R. raphanistrum*. The highest pith length mean value of 1711.6 µm, and a mean value of width 850.4 µm was recorded for *C. orientalis*.

Similarly largest petiole was also marked in *C. orientalis* with a mean value length of 2498.2 µm and width of 1268.6 µm.

c) Collenchyma in Brassiaceous species

Collenchyma cells were present in all studied taxa, whereas in 15 taxa, the sub-epidermal ring of collenchyma was present. Layers of collenchyma were 2, 3, 4 or 5 in different species. The number of layers and diameter of the collenchyma and the shape of the epidermal cells assisted in the segregation of Amaranthaceae species (Majeed et al., 2022). Two layered collenchyma were marked in 9 taxa. Collenchyma cells in Brassicaceous species possessed various cell shapes like lamellar (12 species), angular (7 species), and lacunar (5 species). The highest length of collenchyma cells recorded for *F. hamiltonii* was 42.1 µm (mean); similarly, the minimum length observed in *S.intermedia* was 18.55 µm (mean). The maximum width observed in *F.hamiltonii* 31.6 µm (mean) while a minimum width of 14.15 µm (mean) was observed in *Coincya tournefortii*. Sclerenchyma was present in some species, in a hexagonal shape. In 11 taxa Sclerenchyma was present in vascular bundles. Sclerenchymatous cells provide strengthening to the petiole in assistance with collenchyma cells.

d) Vascularization of the petiole of Brassicaceous flora

Shapes, numbers, and arrangement of vascular bundles varied in examined species, results were consistent with the outcomes of Karaismailoğlu (2020). Collateral closed, collateral open, bi-collateral, and hadrocentric types were observed in Brassica species. The number of vascular bundles varied from 1 to 20. In 7 species a single central vascular bundle was spotted. Specific arrangements in 1+2 (one central large and two lateral small vascular bundles) existed in 6 species. The collateral closed type was the highly used type (17 species), followed by the bi-collateral type (5 species), and hadrocentric (2 species), while collateral open was possessed by a single species (Table 19). Xylem parenchyma and phloem parenchyma have appeared in all the investigated taxa. Shapes of phloem cells possessed variations from round, oval, rectangular, and angular. Phloem cells include various phloem components performing specific functions. The shape of xylem vessel cells was associated with round, oval, and angular. The shape and wings of the petiole, arrangement, and number of vascular bundles can be significantly employed for the identification of *Flemingia* species in the absence of reproductive features (Thacker et al., 2021).

e) Multivariate analysis of petiolar anatomical features of Brassicaceous species

PCA revealed a 93.7% variance in components 5 and 6, which account for 5.7% and 91.6% of the sum of squares. Species of the genus *Strigosella* were in a close cluster with each other, and a similar relationship existed between *Cardaria* species. PCA mainly linked Ch.L, Ch.W, Ep.L, Ep.W, Co.L, Co.W, Pa.L, Pa.W while a negative correlation was marked for VB.L, VB.W, Pe.L, Pe.W, Pi.L, Pi.W (Figure 25). The results followed the petiole anatomical examination of the Sapindaceae family (Mohtashamian et al., 2022). They also find PCA linked to parenchyma length/width, collenchyma length/width, and VB, but in current research, vascular bundles were negatively correlated. Results from the UPGMA dendrogram showed the two major clades with one large cluster of 20 species and the second one with five species. *D. sophia* and *E. sativa* sharing the closest clade, showed higher similarity based on dendrogram analysis. In the same way, higher relatedness was noted for *M. linifolius* with *M. flavida* and *S. cabulica* with *B. oleracea* (Figure 24). Similar results for diverse taxa were observed by (Akhtar et al., 2021). Overall, two species of *Strigosella* shared the same clade above the two branches of the dendrogram, expressing their closeness in contrast to *S. cabulica*, which shared the nearest clade with *B. oleracea* (Figure 24). A highly positive correlation was observed in epidermis length, epidermis width, collenchyma length, collenchyma width, chlorenchyma length, and chlorenchyma width. Vascular bundle width and length were also positively correlated with each other. Chlorenchyma length, chlorenchyma width, and vascular bundle length were all negatively correlated with epidermis width. Similarly, vascular bundle width was negatively correlated with collenchyma length and chlorenchyma width (Figure 26).

Petiole anatomical features were significant in taxonomical applications in various families like Lamiaceae (Akçın et al., 2011) and Crucifereae (El-Rabiai, 2015). Variations were determined in examined taxa in studied parameters such as vascular bundles number and arrangements, presence or absence of sclerenchyma in vascular bundles, shapes of collenchyma and epidermis, and pith presence/absence. Talip et al. (2017) also found that petiole anatomical features, mainly petiole outline, vascular bundle arrangement, sclerenchyma, and trichomes, were significant in the separation of *Hopea* species. The taxonomic significance of petiolar anatomical traits was reinforced by the outcomes of this research. In the absence of reproductive features, the successful implication of petiole anatomical parameters in the identification of taxa has been carried out (Sheikh and Kumar, 2017).

Variations existed among genera for studied parameters; common characters were the presence of uniseriate epidermis with compactly arranged cells, collenchyma cells were strengthening cells in studied taxa, presence of mature xylem vessels, cuticle, while diversity was observed in petiole shape, vascular bundles shapes and arrangement, presence/absence of petiole wing, trichomes, pith and sclerenchyma, a sub-epidermal ring of collenchyma, groove in the upper surface. Variations in the inspected features of the petiole anatomy found helpful in discrimination at the species level (Thacker et al., 2021). Taxonomic keys based on petiole anatomical characters have been successfully employed for the identification of species (Akinnubi et al., 2013). Taxonomic key evaluates the differences among species by studying petiole anatomical parameters that helped empower the discrimination of complicated species (Table 21).

The correlation was examined among species of the same genus in *Cardaria* and *Strigosella* based on petiolar anatomical examinations. While genus *Farsetia* showed divergence in parameters like pith, sclerenchyma, groove in the upper surface, and petiole wing presence/absence. Variations were observed among studied Brassicaceous taxa for qualitative and quantitative characteristics. Majeed et al. (2022) also reported that qualitative and quantitative petiole anatomical features, petiole length, VB arrangement, collenchyma layers, and trichomes were effectively utilized for taxa discrimination in Amaranthaceae. Conclusively the results can be efficiently incorporated in taxonomical studies in the placement of taxa, evolutionary studies, and microclimatic effects causing changes in anatomical features. Species-level discrimination can be carried out based on anatomical features that were unique and species-specific.

The petiole anatomical investigation of Brassicaceous taxa grouped them into 7 types based on the shape of petiole and the type of vascularization. These outcomes can be effectively implicated in the classification of taxa in association with anatomical studies. The determined correlation among the quantitative features further assisted in the delimitation of taxa as well as providing the base to search for any possible environmental factors that affect these features of anatomy. Thus, the physiological processes can be explored by determining the anatomy of the plant. The structural components of the plants i.e cuticles, cell types, and layers have a direct impact on the physiological aspects such as transpiration rate, and photosynthesis. The derived variations in the anatomical traits were significant for determining the taxonomic relationships. The implications of microscopic visualization of petiole anatomy can be enhanced via a relatively comprehensive study on the tribe and genus level along with the exploration of the complete components of the xylem (parenchyma, fibers, tracheids) and phloem (companion cells, fibers, and parenchyma).

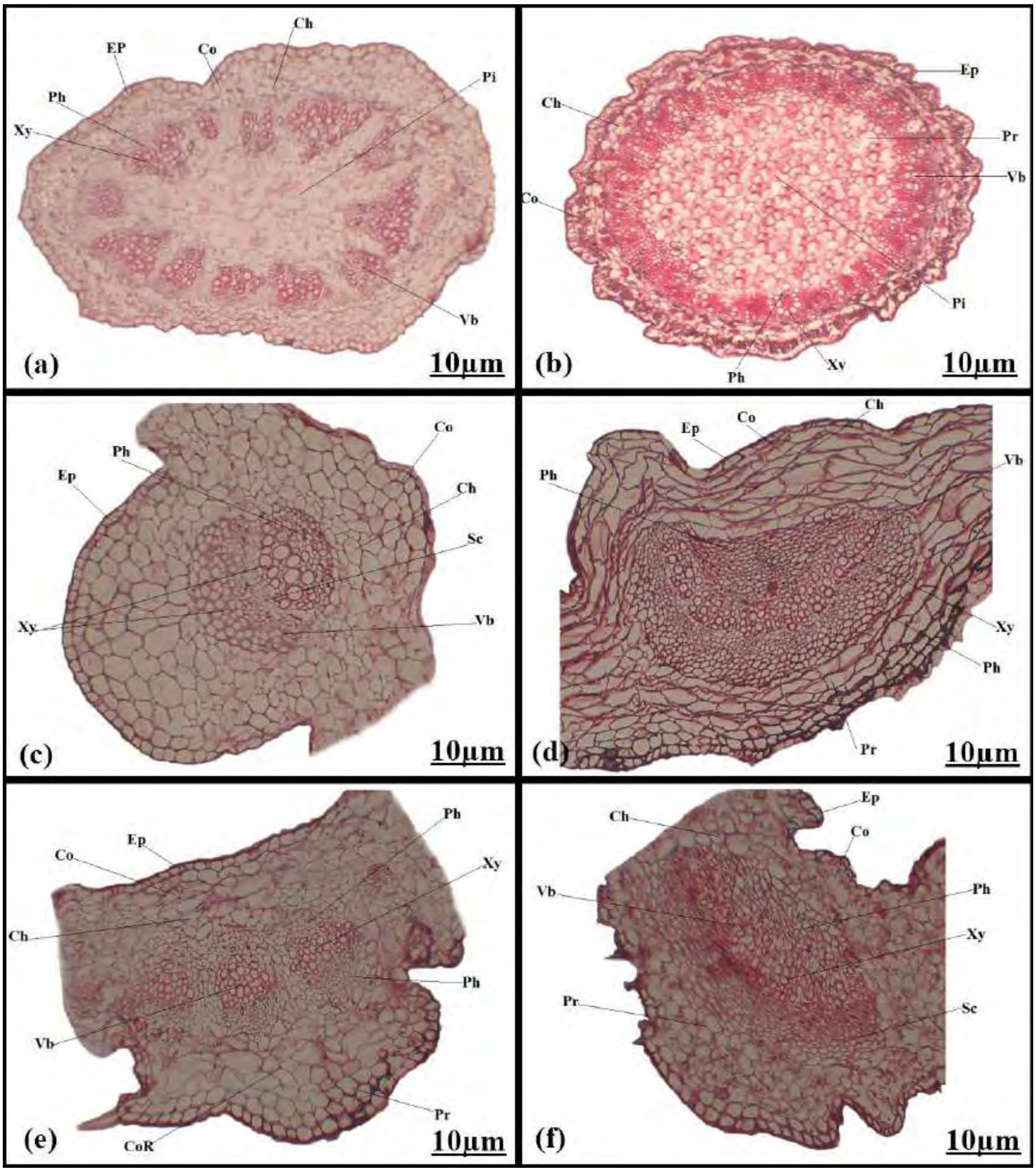

Plate 58. Photomicrographs of petiole anatomy (a) *Aethionema carneum*, (b) *Alyssum desertorum*, (c) *Brassica oleracea*, (d) *Capsella bursa-pastoris*, (e) *Cardaria chalepense*, (f) *Cardaria draba*, (Cu: cuticle, Ep: Epidermis, Pa: parenchyma, Co: collenchyma, Ch: chlorenchyma, Sc: sclerenchyma, Xy: xylem, Ph: Phloem). Scale: 10µm

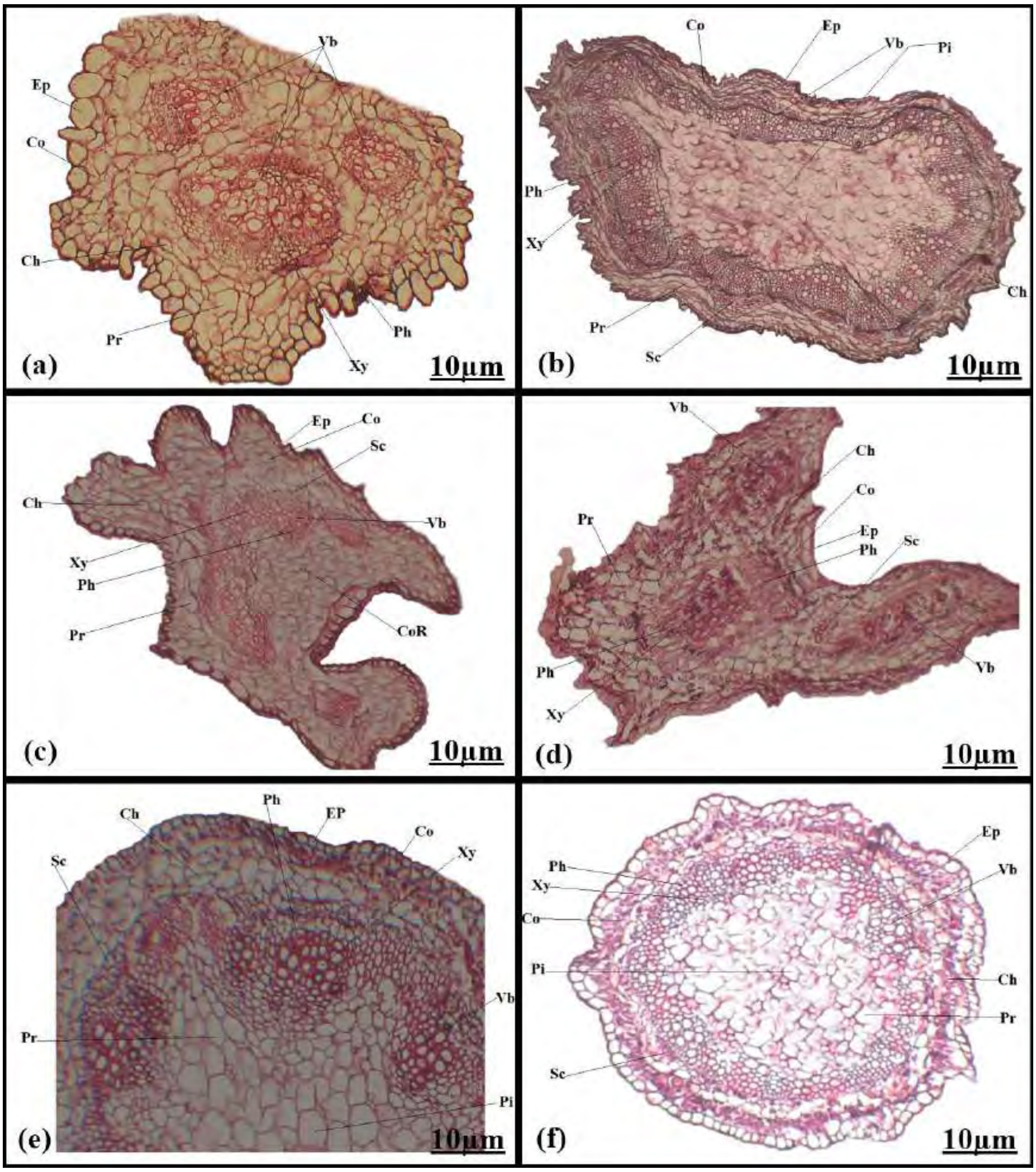

Plate 59. Photomicrographs of petiole anatomy (a) *Coincya tournefortii,* (b) *Conringia orientalis,* (c) *Descurainia Sophia*, (d) *Diplotaxis harra*, (e) *Eruca sativa,* (f) *Farsetia hamiltonii*,(Cu: cuticle, Ep: Epidermis, Pa: parenchyma, Co: collenchyma, Ch: chlorenchyma, Sc: sclerenchyma, Xy: xylem, Ph: Phloem). Scale: 10µm

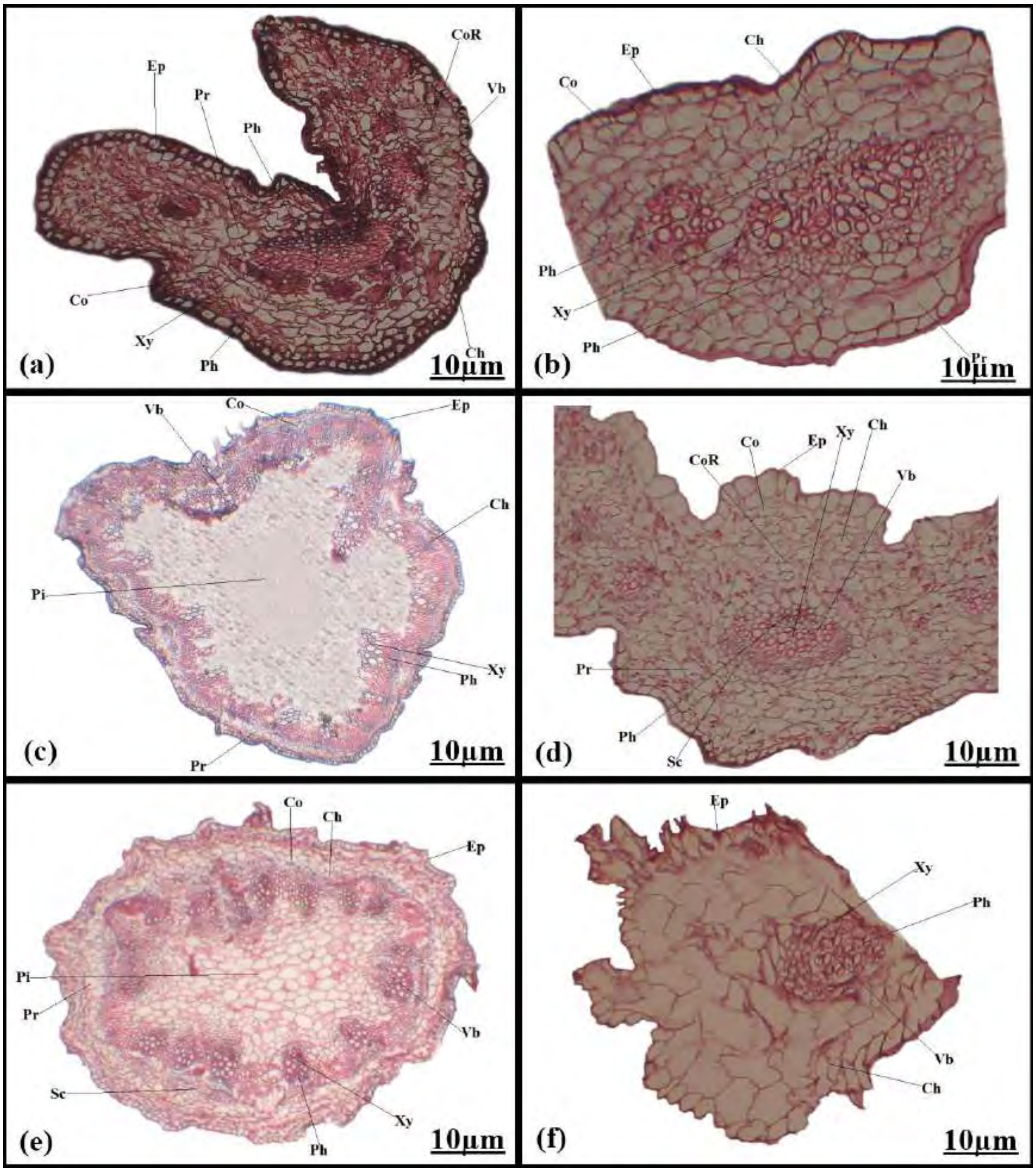

Plate 60. Photomicrographs of petiole anatomy (a) *Farsetia heliophila*, (b) *Goldbachia pendula*, (c) *Lepidium aucheri*, (d) *Matthiola flavida*, (e) *Meniocus linifolius*, (f) *Nasturtium officinale*, (Cu: cuticle, Ep: Epidermis, Pa: parenchyma, Co: collenchyma, Ch: chlorenchyma, Sc: sclerenchyma, Xy: xylem, Ph: Phloem). Scale: 10µm

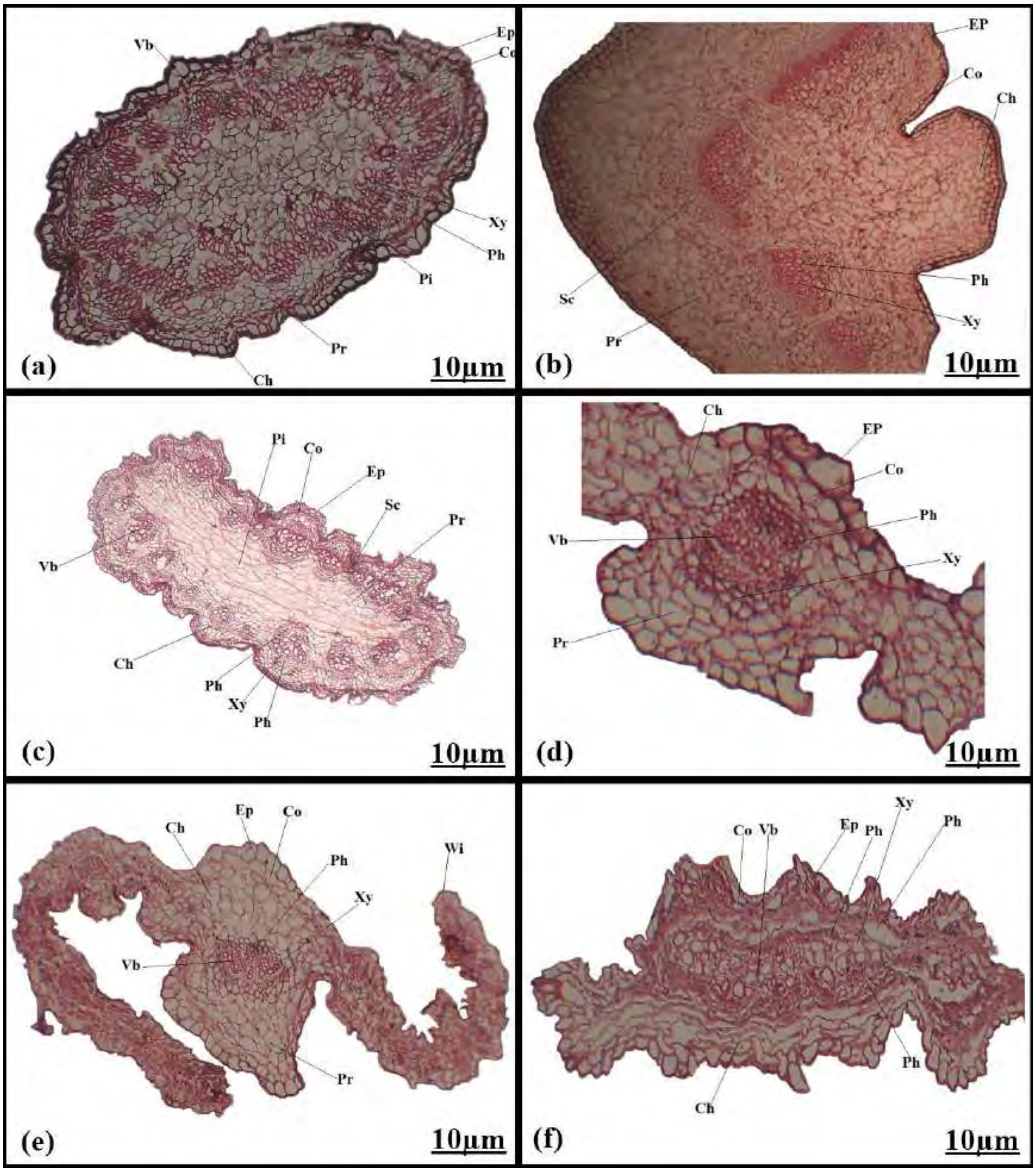

Plate 61. Photomicrographs of petiole anatomy (a) *Notoceras bicorne,* (b) *Physorrhynchus brahuicus,* (c) *Raphanus raphanistrum,* (d) *Sisymbrium altissimum,* (e) *Strigosella africana,* (f) *Strigosella cabulica,* (Cu: cuticle, Ep: Epidermis, Pa: parenchyma, Co: collenchyma, Ch: chlorenchyma, Sc: sclerenchyma, Xy: xylem, Ph: Phloem). Scale:10µm

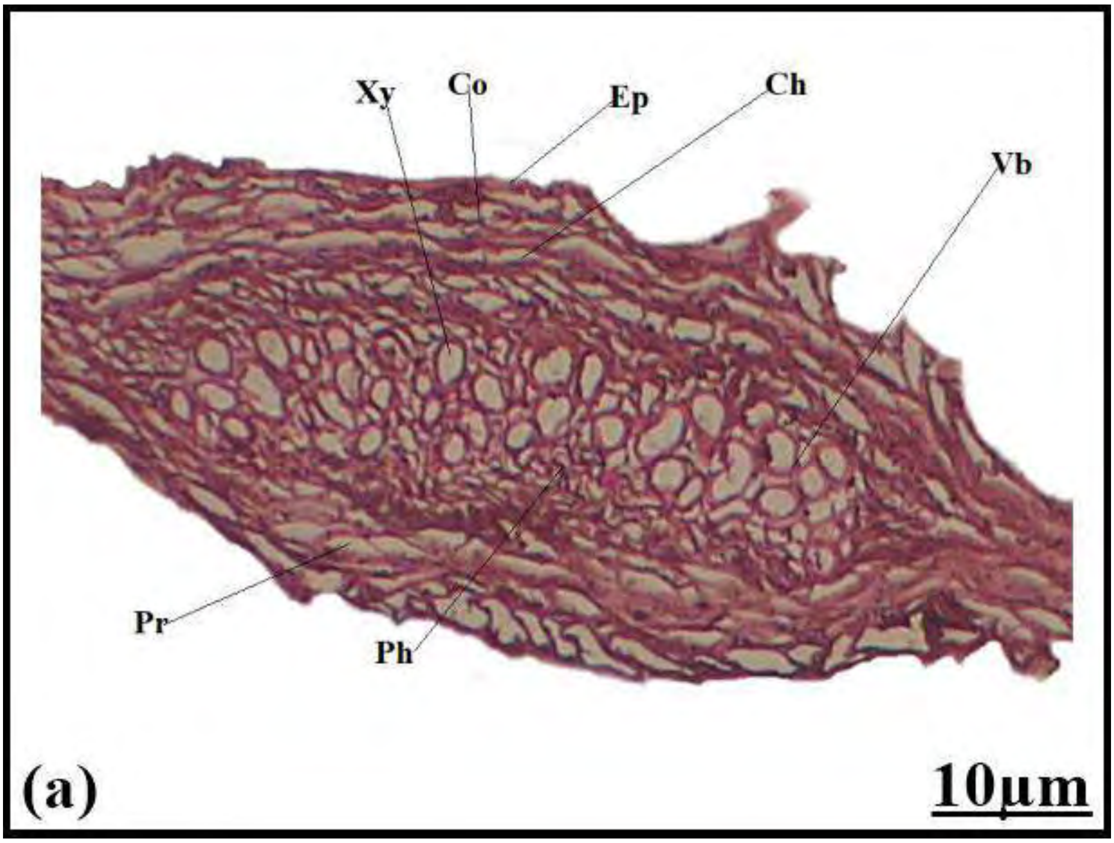

Plate 62. Photomicrographs of petiole anatomy (a) *Strigosella intermedia,* (Cu: cuticle, Ep: Epidermis, Pa: parenchyma, Co: collenchyma, Ch: chlorenchyma, Sc: sclerenchyma, Xy: xylem, Ph: Phloem). Scale: 10µm

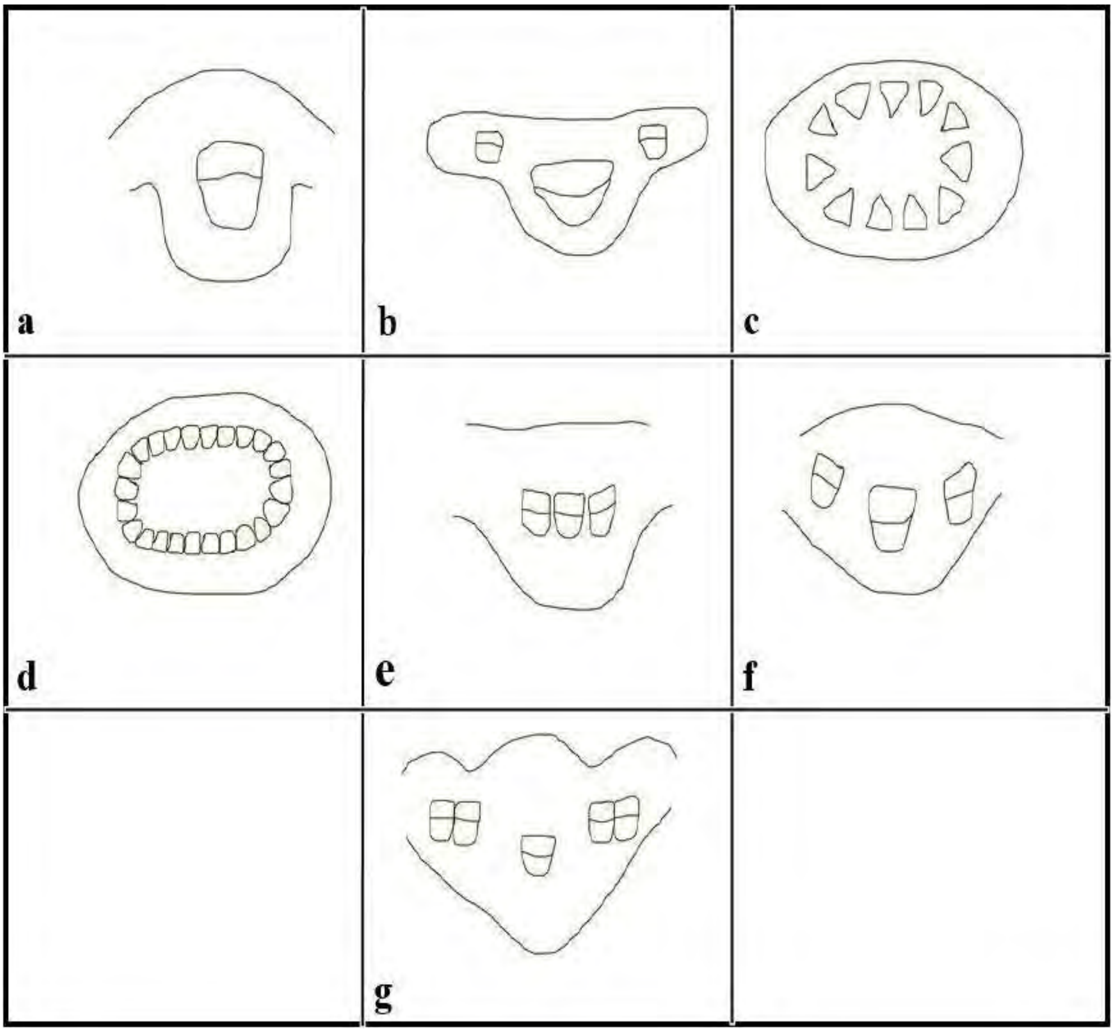

Plate 63. The types of petiole shapes and vascularization in 25 studied Brassicaceous taxa

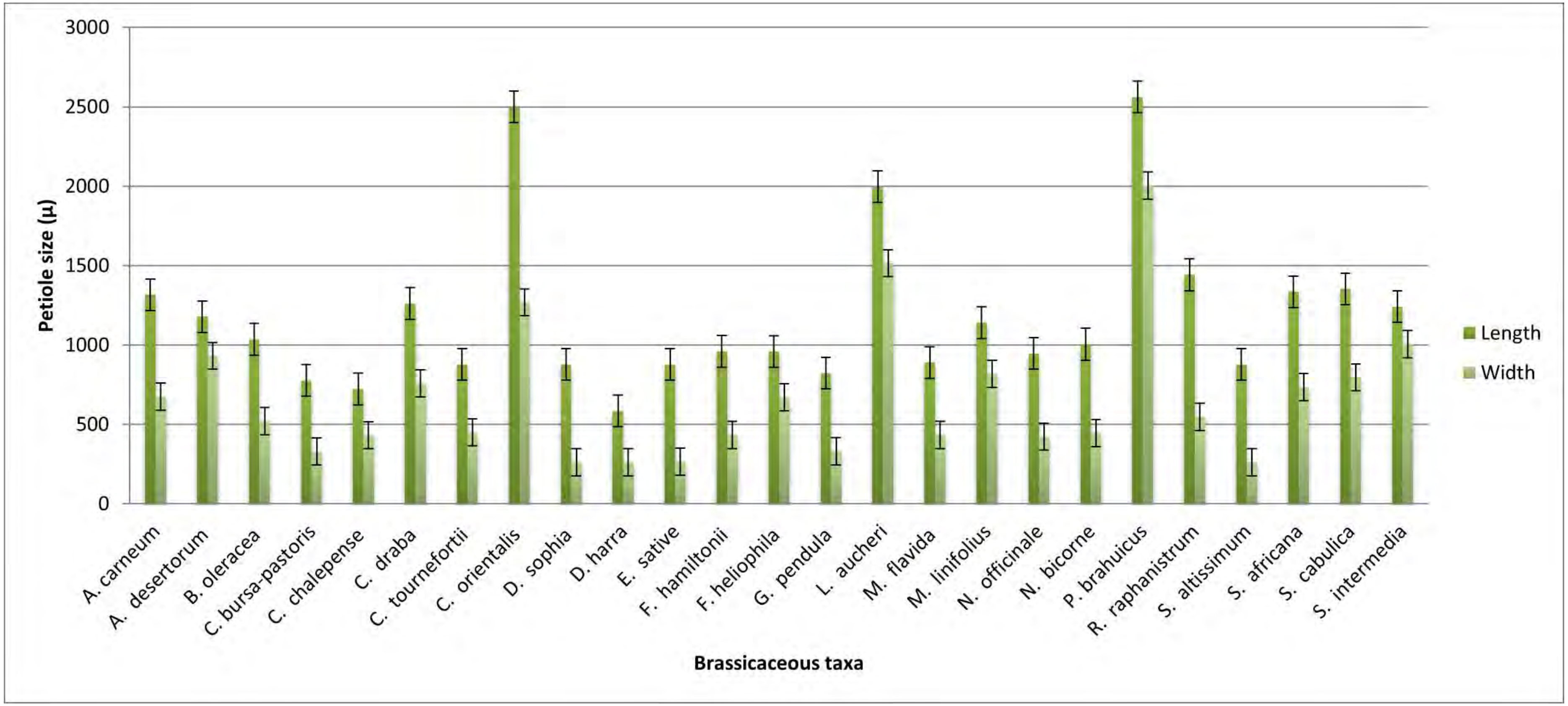

Figure 20. Variations in mean values of petiole length and width

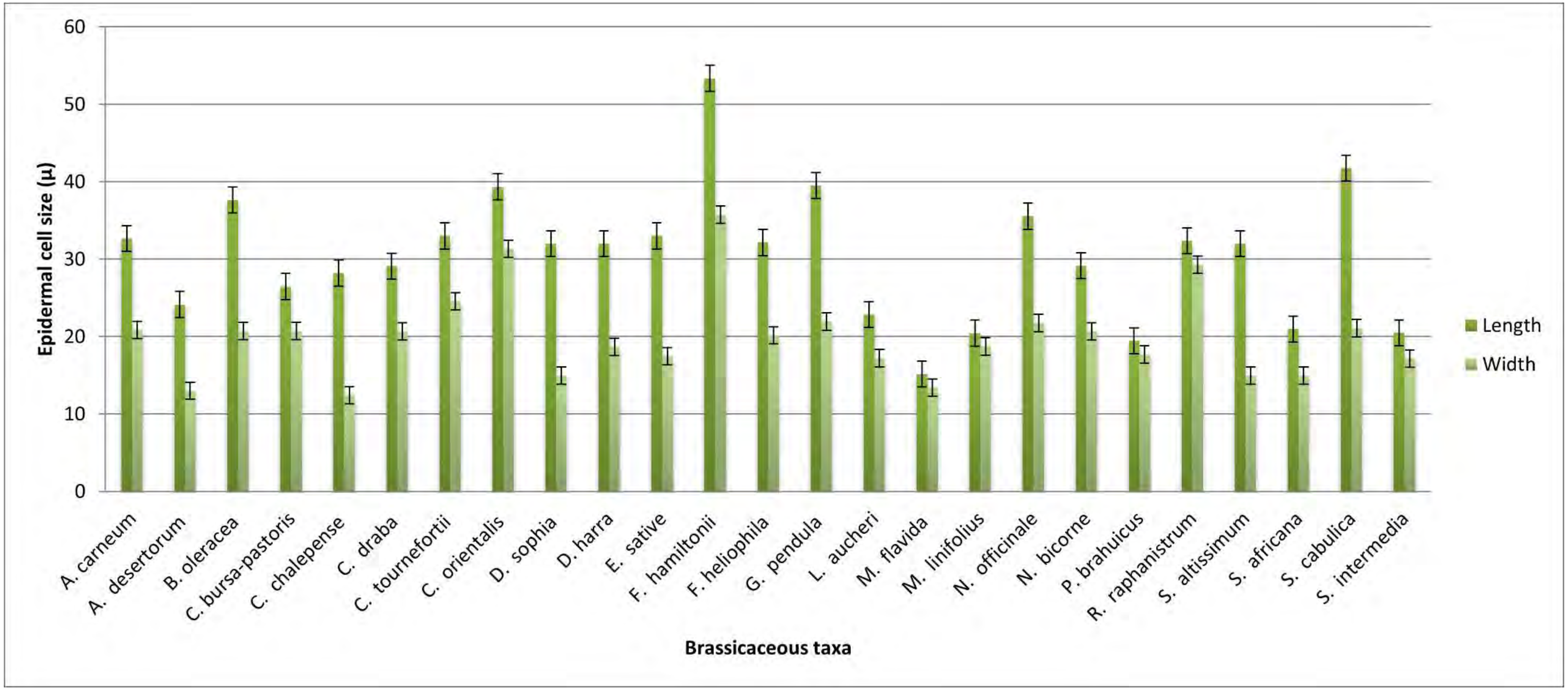

Figure 21. Variations in mean values of epidermal cells length and width

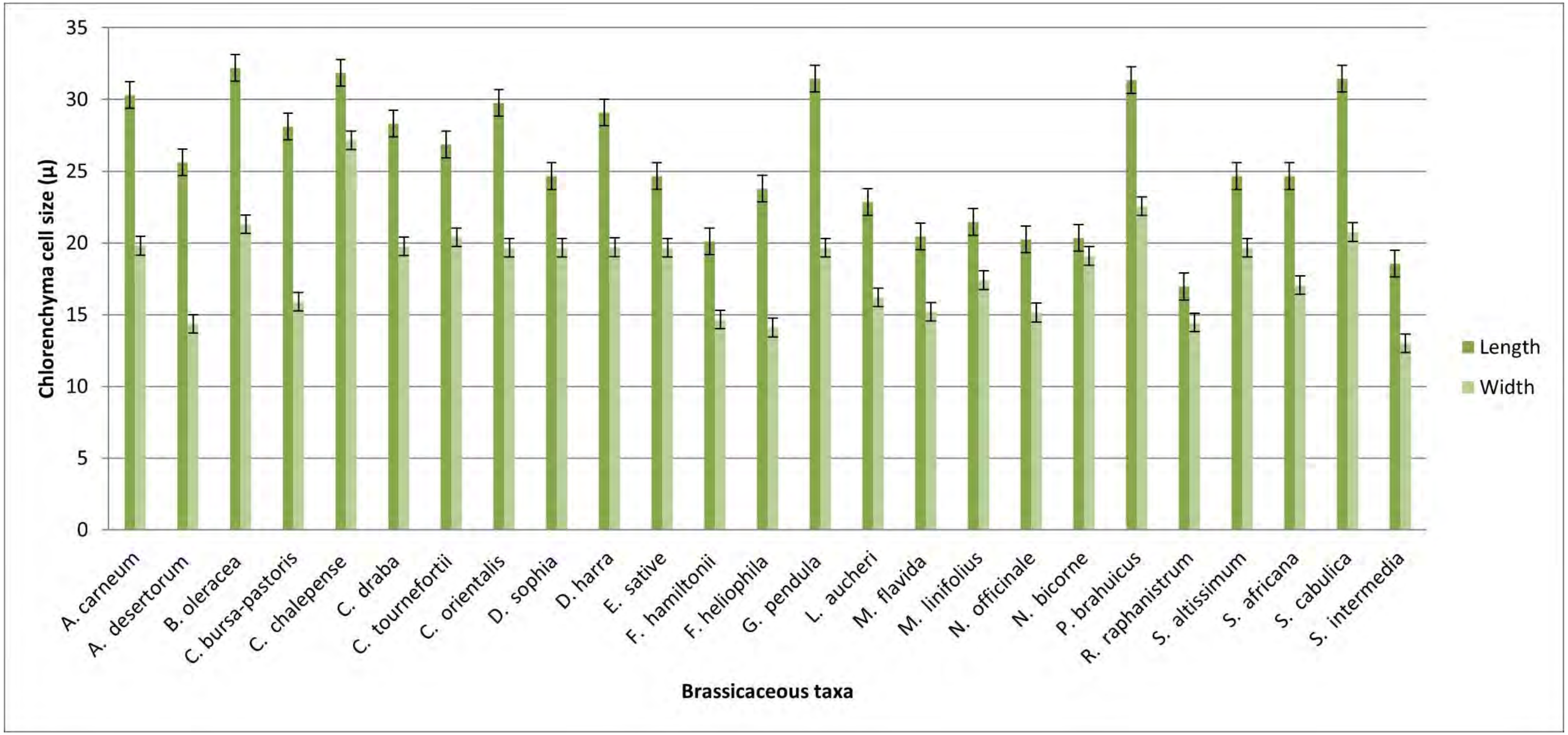

Figure 22. Variations in mean values of chlorenchyma cells length and width

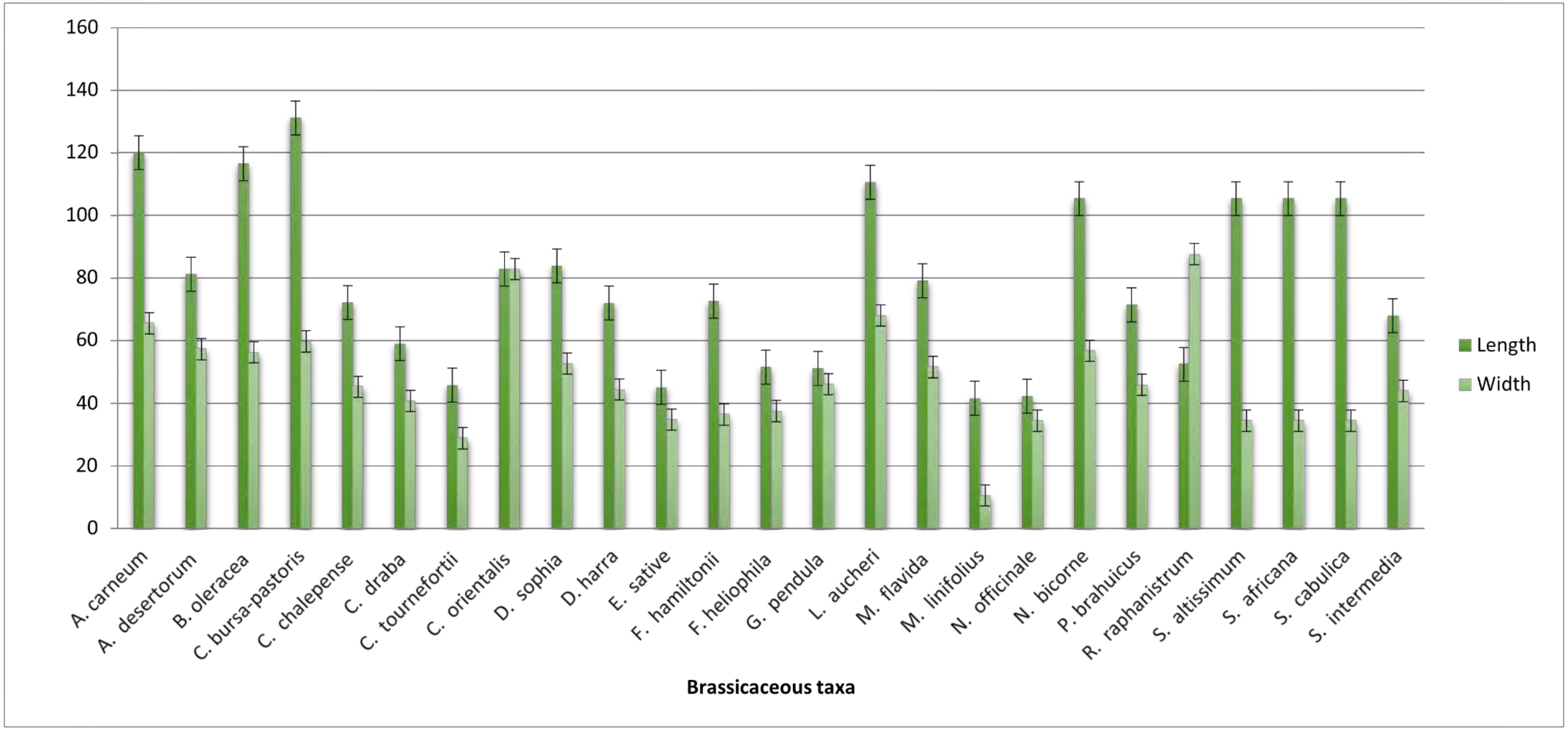

Figure 23. Variations in mean values of vascular bundles length and width

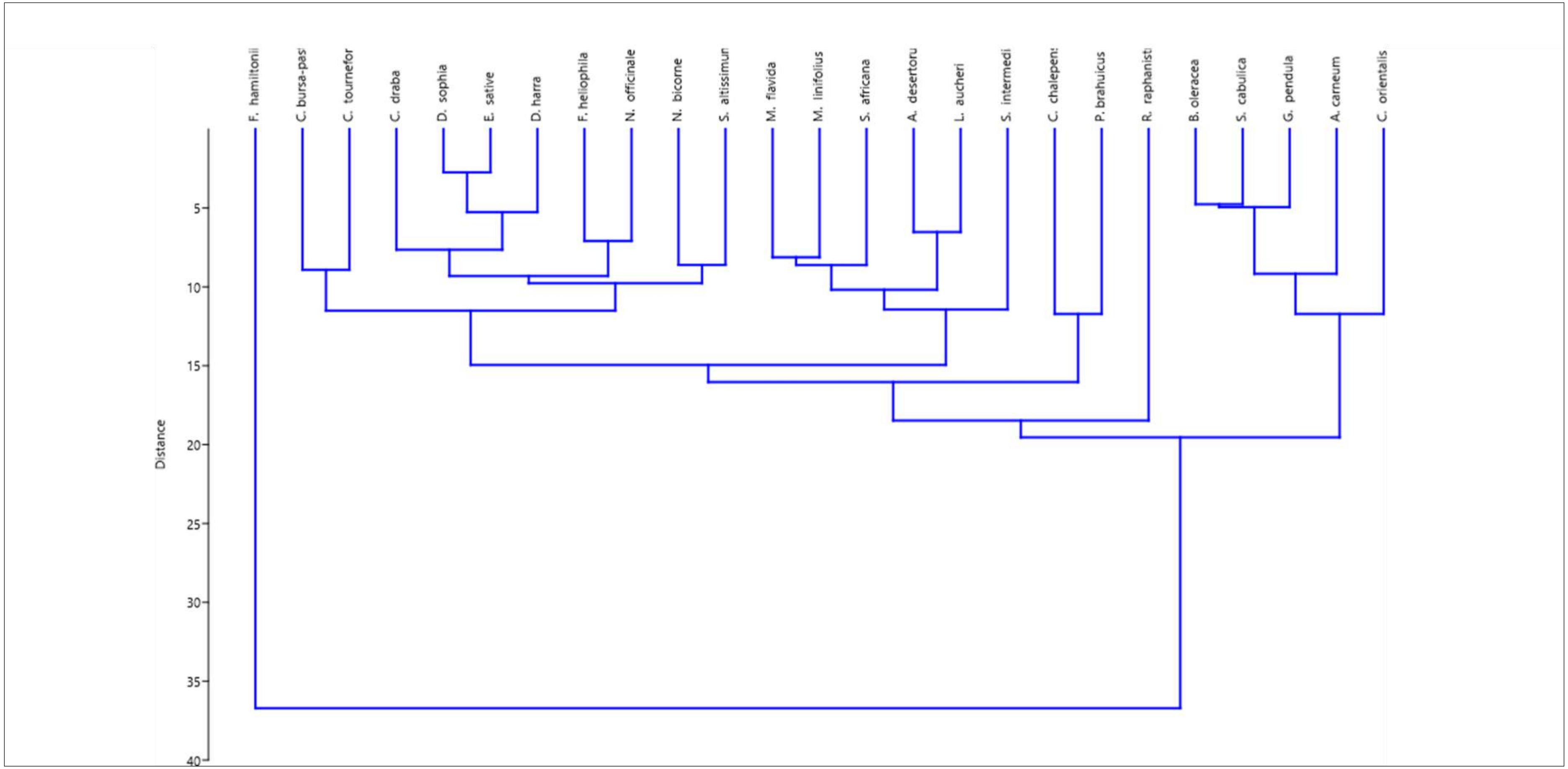

Figure 24. Dendrogram showing the similarity index of Brassicaceae taxa based on quantitative parameters of petiole

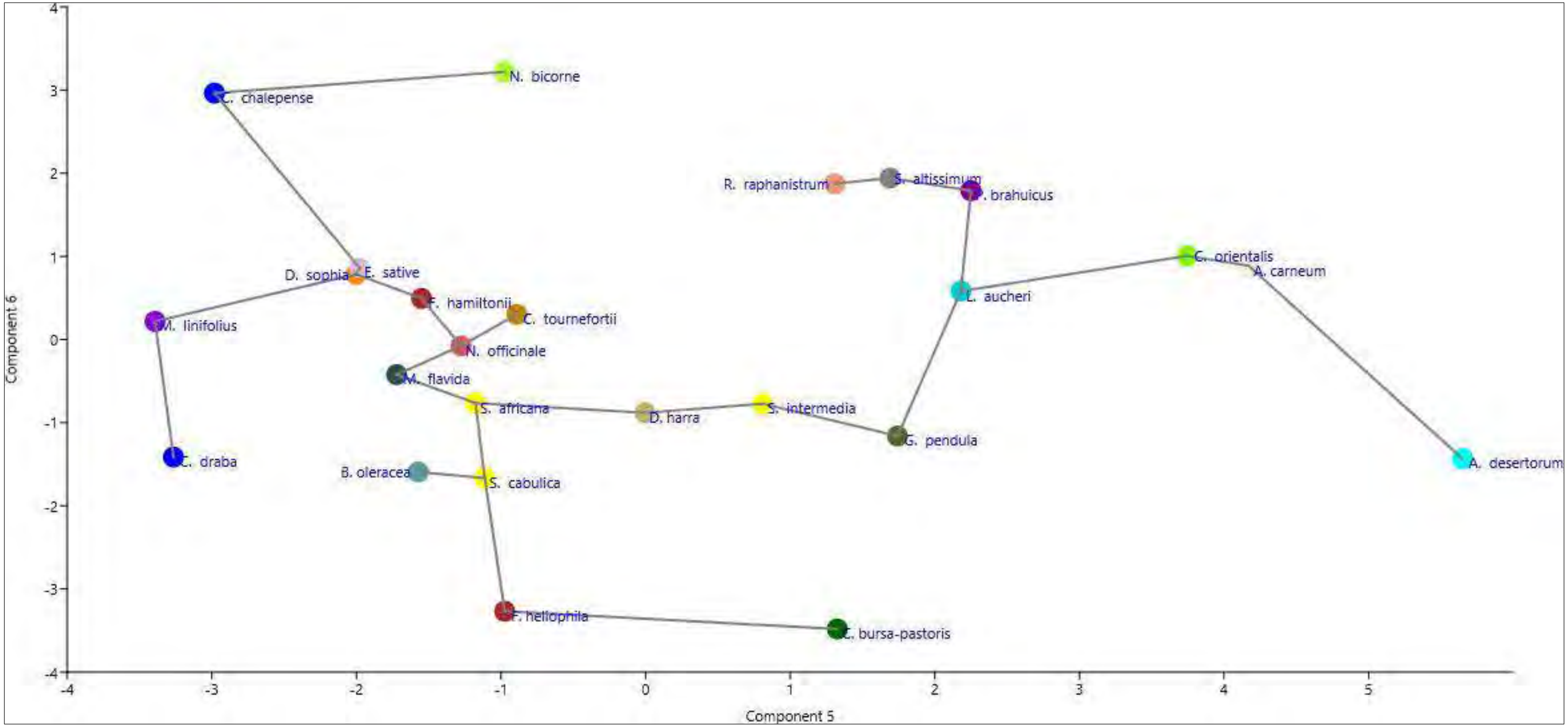

Figure 25. Utility of petiolar features in discriminating among species of Brassicaceae by PCA (epidermis length and width, collenchyma length andwidth, chlorenchyma length and width, parenchyma length and width

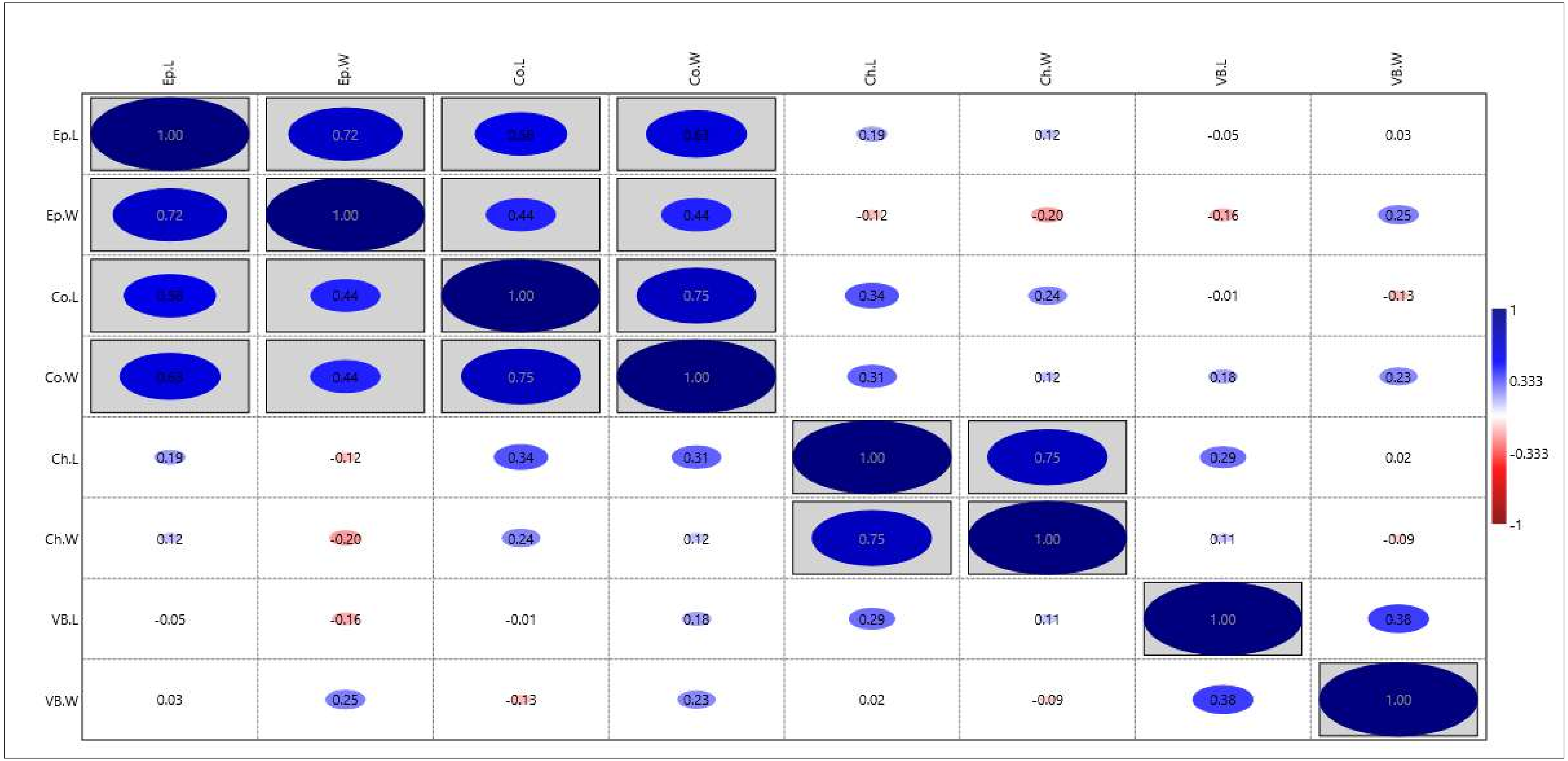

Figure 26. Correlation among the mean values of Ep.L: Epidermal length; Ep.W: Epidermal width; Co.L: Collenchyma length:Co.W: Collenchyma width;Ch.L:Chlorenchyma length; Ch.W: Chlorenchyma width;Vb.L:Vascularbumdle length; Vb.W: Vascular bumdle width

Table 18. Qualitative characteristics based on petiole anatomical features of selected Brassicaceous taxa

Plant Name	No.Co.L	No.Pa.L	No.Ch.L	No.Sc.L:	No.Vbs	Sc.Vbs	Tr	Pi	P.W	G.Up	S.Ep.Co	Xy.Pa	Ph.Pa
Aethionema carneum	3	3	4	0	12	-	-	+	-	-	+	+	+
Alyssum desertorum	3	2	2	2	20	-	-	+	-	-	+	+	+
Brassica oleracea	3	2	3	4	1	+	-	-	+	+	-	+	+
Capsella bursa-pastoris	3	3	3	0	1	-	-	-	+	+	-	+	+
Cardaria chalepense	2	3	1	0	5	-	+	-	+	-	-	+	+
Cardaria draba	3	3	4	7	1	+	+	-	+	-	-	+	+
Coincya tournefortii	3	3	4	5	3	+	-	-	-	+	-	+	+
Conringia orientalis	5	5	5	3	18	+	+	+	-	-	+	+	+
Descurainia sophia	2	2	2	2	1+2	+	+	-	+	-	+	+	+
Diplotaxis harra	2	4	3	2	1+2	+	+	-	+	+	+	+	+
Eruca sativa	2	4	2	7	12	+	-	+	-	-	+	+	+
Farsetia hamiltonii	2	2	2	4	12	+	-	+	-	-	+	+	+
Farsetia heliophila	3	4	3	0	1+2	-	-	-	+	+	+	+	+
Goldbachia pendula	3	3	3	0	1+2	-	-	-	+	+	-	+	+
Lepidium aucheri	4	3	4	7	15	-	+	+	-	-	+	+	+
Matthiola flavida	2	3	2	5	1+2	+	-	-	+	+	-	+	+

Meniocus linifolius	4	2	3	7	14	+	+	+	-	-	+	+	+
Nasturtium officinale	0	0	2	0	1	-	+	-	-	-	-	+	+
Notoceras bicorne	2	2	2	0	14	-	-	+	-	-	+	+	+
Physorrhynchus brahuicus	5	5	4	13	4	-	-	-	-	+	+	+	+
Raphanus raphanistrum	3	3	4	3	14	+	+	+	-	-	+	+	+
Sisymbrium altissimum	2	2	2	2	1	-	-	-	+	-	-	+	+
Strigosella africana	2	4	3	5	1+2	-	-	-	+	-	-	+	+
Strigosella cabulica	2	1	2	5	1	-	-	-	+	+	+	+	+
Strigosella intermedia	2	2	1	7	1	-	-	-	+	+	+	+	+

No.Co.L: number of collenchyma layer, No.Pa.L: Number of perchyenma layer, No.Ch.L: Number of chlorenchyma layer, No.Sc.L: Number of sclernchyma layer No.Vbs: Number of Vascular Bundle (vb), Sc.Vbs: Sclerenchyma presence in the Vbs, Tr: Trichome, Pi: Pith, P.W: Petiole wing, G.Up: Groove in upper surface, S.Ep.Co: Sub-epidermal ring of collenchyma, X. Pa: Xylem Parenchyma, Ph.Pa: Phloem Parenchyma

Table 19. Qualitative characteristics based on petiole anatomical features of selected Brassicaceous taxa

Species	Petiole shape	Epidermal shape	Parenchyma shape	Collenchyma shape	Sclernchyma shape	Vb Arrangments	Trichome shape & no of cells	Cuticle structure	Xylem Vessel	Phloem Shape
Aethionema carneum	Flat	Square to rectangular	Irregular	Lamellar	-	Collateral closed	-	Smooth	Angular	Round
Alyssum desertorum	Oval	Square	Tetra to hexagonal	Lamellar	-	Collateral closed	-	Smooth	Round to angular	Rectamgular to oval
Brassica oleracea	Oval	Square	Hexagonal	Angular	Hexagonal	Collateral open	-	Smooth	Round	Rectamgular to oval
Capsella bursa-pastoris	Sulcate	Rectangular to square	Tetragonal to irregular	Lacunar	-	Bi-Collateral	-	Undulated	Round	Rectangular to hexagonal
Cardaria chalepense	Sulcate	Square to round	Tetra to hexagonal	Lacunar	-	Hadrocentric	Unicellular	Smooth	Round and oval	Rectangular, round, oval
Cardaria draba	Sulcate	Square and round	Tetra to isodiamteric	Lacunar	Hexagonal	Collateral closed	Unicellular	Undulated	Round and oval	Angular
Coincya tournefortii	Sulcate	Round & oval	Tetragonal to irregular	Angular	Hexagonal	Collateral closed	-	Undulated	Round and oval	Angular to oval
Conringia orientalis	Flat	Rectangular	Irregular	Lamellar	Hexagonal	Collateral closed	Uniseriate	Undulated	Round	Rectangular
Descurainia sophia	Sulcate	Square and round to oval	Isodiametric	Angular	Hexagonal	Collateral closed	Unicellular	Undulated	Round	Round and oval
Diplotaxis harra	Flat	Rectangular to round	Isodiametric	Lamellar	Hexagonal	Hadrocentric	Unicellular	Undulated	Round	Round and oval

Eruca sativa	Oval	Square	Hexagonal	Lamellar	Hexagonal	Bicollateral	-	Smooth	Round	Rectangular, round to oval
Farsetia hamiltonii	Circular	Square to round	Irregular	Lamellar	Hexagonal	Collateral closed	-	Smooth	Round to angular	Rectamgular to oval
Farsetia heliophila	Flat	Square	Irregular	Lacunar	-	Bi collateral	-	Smooth	Round	Round
Goldbachia pendula	Sulcate	Square to rectangular	Tetra to hexagonal	Lamellar	Hexagonal	Bi-Collateral	-	Undulated	Round and oval	Rectangular, round
Lepidium aucheri	Flat	Square to round	Isodiametric	Lamellar	Hexagonal	Collateral closed	Multiseriate	Smooth	Angular to round	Rectamgular to oval
Matthiola flavida	Flat	Square to oval	Irregular	Angular	Hexagonal	Collateral closed	-	Smooth	Round	Round, oval
Meniocus linifolius	Sulcate	Square	Isodiametric	Lamellar	Hexagonal	Collateral closed	Multiseriate	Smooth	Round	Rectangular
Nasturtium officinale	Flat	Oval	-	-	-	Collateral closed	Unicellular	-	Round to angular	Rectamgular to oval
Notoceras bicorne	Flat	Square to oval	Hexagonal	Lacunar	-	Collateral closed	-	Undulated	Round and oval	Rectangular and oval
Physorrhynchus brahuicus	Circular	Square	Isodiametric	Lamellar	Hexagonal	Collateral closed	-	Smooth	Round	Rectangular, round to oval
Raphanus raphanistrum	Flat	Square	Hexagonal	Angular	Hexagonal	Bi-Collateral	Multiseriate	Undulated	Round to oval	Rectangular and oval
Sisymbrium altissimum	Circular	Square to oval	Irregular	Angular	Hexagonal	Collateral open	-	Undulated	Round	Round and oval

Strigosella africana	Sulcate	Square to round	Irregular	Angular	-	Collateral closed	Unicellular	Undulated	Round to angular	Rectangular to oval
Strigosella cabulica	Sulcate	Angular	Irregular	Lamellar	-	Hadrocentric	-	Undulated	Round	Rectangular and oval
Strigosella intermedia	Sulcate	Rectangular	Irregular	Lamellar	-	Collateral closed	-	Undulated	Oval to round	Oval, round

Table 20. Quantitative data of histological properties of studied Brassicaceae species

Plant Species	L.W	Epidermal cell (µm)	Collenchyma (µm)	Chlorenchyma (µm)	Parenchyma(µm)	Sclerenchyma (µm)	Vascular bundle (µm)	Xylem (µm)	Phloem (µm)	Pith (µm)	Petiole (µm)
Min-max=Mean±SE											
Aethionema carneum	L	29-35.75=32.65±1.33	29.75-33.25=31.45±0.71	25.75-30.25=28.45±0.878	33.25-36.25=35.05±0.508	-	39.75-43.25=41.6±0.668	54-59.75=56.8±1.088	34.75-42.25=38.2±1.285	921-988=954.4±13.471	1203-1457=1316.6±43.564
	W	19.25-22.25=20.85±0.595	25.75-30.25=28.45±0.88	33.25-36.25=35.05±0.508	18-22.75=19.6±0.903	-	9.75-11.5=10.5±0.285	37-44.75=42.15±1.352	31-34.25=32.45±0.629	321-355=338.8±5.9194	654-691=674.2±6.492
Alyssum desertorum	L	22.75-25.5=24.15±0.451	20.5-22=21.1±0.257	24.526.25=25.6±0.302	24.5-26.25=25.6±0.302	18-20.5=19.35±0.515	113.75-125.25=120.1±2.173	46.75-56.25=51.45±1.727	38.75-44=41.05±0.913	605-677=640.4±12.188	1150-1200=1178±8.602
	W	12.25-13.75=13±0.25	17.75-24.5=22.75±1.262	13.25-15.75=14.35±0.527	15.5-17.75=16.6±0.43	18-18=19.35±0.515	62-73.75=65.55±2.087	33.75-44.25=38±1.732	34.75-47=39.95±2.087	422-478=448.6±9.739	888-967=930.8±14.022
Brassica oleracea var. *capitata*	L	36.75-38.75=37.65±0.407	31.75-33.5=32.5±0.325	31.5-33=32.2±0.289	33-41.25=37.95±1.456	11.75-15.25=13.6±0.68739	106-114=110.6±1.435	39-73.25=59.9±6.024	33.75-38.5=36.25±0.869	-	987-1088=1035.2±18.626
	W	19.75-22=20.75±0.379	19.75-24.5=21.95±0.852	19.75-23=21.3±0.555	20.5-24.75=23.1±0.761	9.5-11=10.2±0.266	63.75-71=68.1±1.228	33.75-38.5=36.25±0.869	31.5-39=34.8±1.30	-	487-555=521±12.806
Capsella bursa-pastoris	L	22-30.5=26.45±1.406	19.75-22.75=21.3±0.532	22-38.25=28.1±2.894	35.5-39.5=36.95±0.708	-	75.25-83.75=79.15±1.700	46.25-59.75=53.9±2.635	42.25-47=44.7±0.888	-	659-852=776.8±32.396

	W	16.25-25.5=20.75±1.816	12.75-17=14.8±0.713	13.25-19.5=15.9±1.097	21-31.25=25.6 ±1.934	-	44.25-62=51.55±3.135	29.75-39.75=34.7±1.750	32.25-39.25=35.8±1.162	-	307-356=328.6±8.096
	L	22-31.25=28.2±1.611	24.5-31=28.05±1.194	29.75-34.25=31.85±0.838	31.75-38.75=35.7±1.178	-	39.5-44.5 =42.25±0.955	54-59.75 =56.8±1.088	34.75-42.25 =38.2±1.285	-	653-781=722.2±24.239
Cardaria chalepense	W	11.25-13.25=12.4±0.384	17.25-21.25=19±0.689	25.5-29.25=27.15±0.691	19.75-23.25=21.45±0.704	-	9.75-11.5=10.5±0.285	37-44.75 =42.15 ±1.352	31-34.25 =32.45±0.629	-	418-455=431±6.610
	L	24.5-34.75=29.1±1.77412	29.75-33.25 =31.4±0.61033	25.75-30.75 =28.3±0.86747	32.25-36.25=34.45±0.75166	16-18.25 =17.25±0.37914	61-75.25 =67.95±2.50674	46-58.5 =51.05±2.80112	42.25-51.75 =45.85±1.64621	-	1249-1278=1260.8±5.462600113
Cardaria draba	W	19.25-22.75=20.65±0.718	14.75-22=17.95±1.307	17.25-22 =19.75±0.832	19-22 =20.7 ±0.577	10.75-15.5 =12.95±0.959	40.75-47 =43.95±1.273	39.5-44 =42.45±0.792	39.75-43 =41.95±0.577	-	713-855=758.2±26.734
	L	26.75-36=33±1.684	19.75-22.25 =21±0.487	24.25-30.5=26.85 ±1.312	33-37.75 =35.15±0.785	17.75-20.5=18.95±0.508	69.25-75.75=72.05±1.099	42.25-47.25=45±0.836	41.5-46=44.25±0.840	-	2487-2509=2498.2±4.374
Coincya tournefortii	W	20.75-28.25=24.55±1.2	12.75-15.5=14.15 ±0.503	17.75-22 =20.4±0.772	18.5-22.25=20.1±0.625	12.75-15.25=14.05±0.463	42.25-47=44.4±0.853	34-40.5=37.95±1.124	29.5-36.25=32.8±1.2	-	1251-1303=1268.6±10.317
Conringia orientalis	L	37-44.75=39.35±1.406	29.5-33.75=32.2±0.755	25.25-33.75=29.75±1.476	38.75-42.25=41.35±0.654	18.25-21.25=20.05±0.514	69.25-75.75=72.05 ± 1.099	42.25-47.25=45±0.836	41.5-46=44.25± 0.840	1699-1734=1711.6±5.988	2487-2509=2498.2±4.374

	W	29.75-32.75=31.35±0.533	24.5-29.75=27.4±0.979	16.75-22=19.65±1.008	31-33.75=32.75±0.506	14.75-17=16.1±0.4	42.25-47=44.4±0.853	34-40.5=37.95±1.124	29.5-36.25=32.8±1.2	838-857=850.4±3.370	1251-1303=1268.6±10.317
Descurainia Sophia	L	30.25-33.75=32±0.602	25.75-27.75=26.65±0.340	23.5-25.75=24.65±0.430	33.5-37.75=35.7±0.803	13.5-16=14.6±0.465	100.25-110.5=105.4±1.940	36.75-43.5=40±1.440	36.5-42=39.25±0.894	-	803-925=876±21.151
	W	13-17.75=14.95±0.796	18-21.75=19.75±0.642	17.75-22=19.65±0.768	23-28.25=25.85±1.0111	9.75-11.5=10.55±0.289	32.25-36.75=34.4±0.722	34.25-40.75=37.6±1.197	36.75-43.25=39.75±1.092	-	223-302=261.2±16.933
Diplotaxis harra	L	30.25-33.75=32±0.602	25.75-27.75=26.65±0.340	26.25-32.75=29.1±1.111	31.23-37.25=40.25±38.55	13.5-16=14.6±0.465	63.75-75.25=71.45±2.014	36-47=40±1.957	36.75-49=41.3±2.113	-	555-608=584.8±9.598
	W	15.75-22=18.65±1.136	1821.75=19.75±0.642	1822=19.7±0.677	27.25-30.75=28.8±0.609	9.7511.5=10.55±0.289	43.548=45.9±0.792	34.2540.75=37.6±1.197	34.75t43.25=38.3±1.593	-	223-302=261.2±16.933
Eruca sativa	L	31.25-35.25=33±0.698	25.75-29=27.15±0.573	23-26=24.65±0.556	33.5-37.75=35.7±0.803	14.5-16.25=15.4±0.375	100.25-110.5=105.4±1.940	36.75-43.25=39.5±1.204	36.5-42=39.25±0.894	394-453=432±10.774	803-925=876±21.151
	W	14.75-20.5=17.45±1.0937	19-20.5=19.75±0.316	17.75-22=19.65±0.768	23-28.25=25.85±1.0111	10.25-12.25=11.05±0.365	32.25-36.75=34.4±0.722	31.75-48.25=38.1±2.801	36.75-43.25=39.75±1.092	185-234=210±7.981	223-302=265.2±15.242
Farsetia hamiltonii	L	50.25-61.25=53.35±2.025	38.75-44.25=42.1±0.966	18.25-21.25=20.1±0.533	46-50.75=47.9±0.83	34.5-38.75=36.65±0.860	54.75-64.25=59.05±1.727	62-69.5=65.1±1.450	59.25-67=62.65±1.271	479-503=493.2±4.565	951-974=959.8±4.851
	W	33.75-38.75=35.75±0.925	29.25-33=31.6±0.635	13-16=14.65±0.503	43-47.25=45.1±0.714	24.5-29.25=26.35±0.808	37.75-43=40.7±0.913	46.25-56=50.75±1.970	39.5-49=43.9±1.578	195-222=210.2±5.228	406-458=433.2±9.041
Farsetia heliophila	L	29.25-36.75=32.15±1.343	19.75-33.75=26.3±2.545	21.25-29.75=23.8±1.595	34.75-37.75=36.2±0.532	15.25-20.25=17.5±1.042	70.5-75.25=72.65±0.982	39.75-49.25=43.9±1.698	34.75-39=37.1±0.785	-	922-1005=959±15.776
	W	18-22=20.15±0.654	13.5-21.75=17.9±1.743	12.75-15.5=14.1±0.478	17.75-21.75=19.55±0.695	12.25-14=13.15±0.322	35.25-37.75=36.4±0.471	36.25-41.75=38.55±1.028	33.5-39.25=36.1±0.998	-	633-777=671.4±26.639

Goldbachia pendula	L	37-42=39.5±0.925	29.75-33.75=31.7±0.751	28.5-33.75=31.45±0.863	47.25-52=49.65±0.875	-	100.25-110.5=105.4±1.940	37.25-45.5=41.15±1.406	37.25-47.25=41.5±1.778	-	805-838=822±5.630
	W	19.25-24.5=21.95±0.878	23.25-28=25.85±0.760	17.75-22=19.65±0.768	37-40.5=38.4±0.635	-	51.5-6356.=1.859771491±4.158	39-47=42.2±1.434834485	33-45.75=38.7±2.072	-	303-351=330.4±8.465
Lepidium aucheri	L	21.75-24.25=22.85±0.451	22-25.5=23.45±0.672	21-24.5=22.85±0.620	30.75-33=32±0.433	16.75-18.25=17.4±0.269	67.75-75.25=72.15±1.226	59.5-79.25=67.85±3.722	54.75-63.5=59.3±1.558	814-853=829±6.730	1984-2009=1996±4.230
	W	14.75-20.5=17.2±0.982	19.5-20.75=20.15±0.231	15.25-17=16.2±0.348	21.75-25.5=23.7±0.639	12.75-15.5=14.3±0.55	43.5-47=45.25±0.575	38.75-49.25=44.25±1.879	38.75-43.5=41.15±0.917	468-491=478.2±3.942	1483-1555=1515±13.524
Matthiola flavida	L	12.25-19.5=15.15±1.393	22-31.25=26.3±1.822	19.25-22.25=20.45±0.514	29.75-39=35.2±2.081	13.5-15.5=14.5±0.395	76-91.25=83.9±2.825	44.75-54.25=48.9±1.6194	39.5-50.75=45.7±2.132	-	843-999=889.6±29.260
	W	11.5-16=13.4±0.816	15.5-18=16.7±0.470	13-17.25=15.2±0.764	24-28=25.55±0.691	8.5-10.75=9.55±0.456	50.25-56.75=52.7±1.192	34.25-42=37.45±1.507	31.25-37=34.25±1.092	-	407-455=432.8±8.742
Meniocus linifolius	L	19.5-22=20.45±0.443	13.75-15.75=14.7±0.374	20.25-23=21.45±0.483	29.5-32.75=30.6±0.578	12-14.25=13.05±0.428	38.75-51.25=45.8±2.118	20.75-28.75=25.25±1.526	18.25-20.25=19.4±0.331	405-447=427.2±7.017	1101-1173=1140.4±12.496
	W	17.25-20.5=18.7±0.614	20.25-23=21.45±0.483	16.5-18.25=17.4±0.322	19.5-20.75=20.15±0.231	9.5-11.25=10.25±0.306	25.75-31.25=28.8±0.902	13.75-21.25=17.2±1.347	12.75-17.75=15.45±0.830	218-267=239.6±9.135	804--845=817.8±7.532
Nasturtium officinale	L	34.5-37.75=35.55±0.577	-	18.5-22.25=20.25±0.794	-	-	44.75-59.5=51.15±2.723	43.75-49.75=47±1.0338	36.5-46.75=41.45±1.743	-	921-966=945.2±7.908
	W	20.5-23=21.5±0.403	-	13.5-16.5=15.15±0.503	-	-	43.549.25=46.05±1.05	34.75-41.5=37.25±1.234	29.75-38.5=33.5±1.433	-	401-442=423±6.833
Notoceras bicorne	L	26.25-30.5=29.15±0.768	22.75-23.75=23.25±0.176	17.25-22.75=20.35±0.950	25.5-30.25=27.95±0.979	-	112-121.25=116.55±1.723	55.25-69.5=61.4±2.396	35.75-44.25=39.85±1.354	601-641=621±6.356	998-1009=1004.4±2.014
	W	19.75-22=20.65±0.375	17.25-19.5=18.2±0.365	15.5-22.75=19.1±1.310	19.75-21=20.45±0.215	-	55.5-57=56.3±0.26	38.75-48.25=44.5±1.581	33.25-40.25=36.85±1.141	247-278=261.6±5.192	428-458=444.8±5.453

Physorrhynchus brahuicus	L	18-20.75=19.45±0.52	25.75-31.25=28.85±1.056	29.5-33.75=31.35±0.785	35.75-38=37.15±0.392	16-20.25=18.35±0.785	42.75-46.75=45.1±0.714	47-53.25=50.6±1.108	47-53.25=50.6±1.108	-	2507-2634=2561.8±25.720
	W	16.75-18.5=17.7±0.320	20.75-24.5=22.25±0.689	21.5-23.5=22.55±0.357	19.75-23.25=21.65±0.664	12.75-15.5=14.25±0.541	33-36=34.8±0.53	44.25-50.5=47.85±1.171	34-43.75=38.45±1.642	-	1988-2016=2004±4.549
Raphanus raphanistrum	L	31.75-33=32.35±0.231	18.25-21=19.7±0.463	16-18.25=16.95±0.428	24.5-32.75=29.3±1.770	13.25-16=14.55±1.164	76.25-88=81.25±1.934	37.25-43.25=39.95±1.022	37.25-44.5=41.7±1.235	854-888-876.8=5.961	1425-1454=1441.2±5.013
	W	27.25-31=29.25±0.647	15.5-18.25=16.95±0.520	13.25-15.5=14.45±0.428	18.25-23.5=20.5±1.009	11-15.25=13.5±0.720	54.75-61.25=57.3±1.223	41.5-50.25=45.35±1.415	37-41.25=38.8±0.792	355-388=370±5.449	531-563=547.8±5.471
Sisymbrium altissimum	L	30.25-33.75=32±0.602	18-21.75=19.75±0.642	23.5-25.75=24.65±0.430	33.5-37.75=35.7±0.803	13.5-16=14.6±0.465	100.25-110.5=105.4±1.940	36.75-43.5=40±1.440	36.5-42=39.25±0.894	-	803-925=8762±1.151
	W	13-17.75=14.95±0.796	18-21.75=19.75±0.642	17.75-22=19.65±0.768	23-28.25=25.85±1.011	9.75-11.5=10.55±0.289	32.25-36.75=34.4±0.722	34.25-40.75=37.6±1.197	29.5-38.25=33.05±1.543	-	223-302=261.2±16.933
Strigosella africana	L	18-24.75=20.95±1.181	28.5-31=30.05±0.443	23.5-25.75=24.65±0.430	33.5-37.75=35.7±0.803	14.5-15.75=15.15±0.231	48.75-56.5=52.45±1.511	36.75-43.5=40±1.44	36.5-42=39.25±0.894	-	1322-1355=1334.8±6.143
	W	13-17.75=14.95±0.79	18-22.75=20.1±0.800	15.5-20.5=17.05±0.902	23-28.25=25.85±1.011	9.75-11.75=10.8±0.357	76.25-94.5=87.7±3.054	31.5-38.75=34.75±1.380	36.75-43.25=39.75±1.092	-	705-756=734.4±8.617
Strigosella cabulica	L	38.75-43=41.75±0.770	29.5-31=30.3±0.28	29.75-32.75=31.45±0.538	37-39=38.1±0.407	19.75-23=21.35±0.594	46.75-55.5=51.55±1.531	37.25-49.5=42.9±2.308	32-48.75=43.4±2.975	-	1257-1423=1352.4±30.793
	W	19.75-23=21.1±0.605	20.5-23=21.85±0.465	19-23=20.75±0.73	20.5-23=21.9±0.465	12-13.75=12.75±0.306	35.75-38.75=37.5±0.5	29.75-38=34.5±1.45	29.5-38.25=33.05±1.543	-	761-882=796±22.340
Strigosella intermedia	L	19.75-21.25=20.5±0.316	15.75-20.5=18.55±0.885	17.25-20.25=18.55±0.496	21.75-25.75=23±0.733	12.25-13.75=13±0.25	76.25-88.75=82.9±2.181	32.25-47=37.95±2.548	32.25-41.25=36.75±1.563	-	1211-1282=1240.8±12.622
	W	15.75-18.75=17.15±0.5	13.75-15.5=14.6±0.341	12-14=13±0.395	16-20.25=18.35±0.682	9.75-13=11.6±0.61	76.25-88.75=82.9±2.181	30.5-37.25=34.1±1.174	24.5-29.5=27.25±0.851	-	1002-1009=1005.2±1.280

Table 21: Dichotomous key based on petiole morphological characters of Brassicaceous flora from Baluchistan

Link character	Present (+) /absent (-)	Diagnostic characters	Species name
1	+	Petiole flat, Epidermal cells square to rectangular, Parenchyma cells irregular, Xylem Vessel angular, Phloem cells round……	*Aethionema carneum*
	-	Parenchyma cells tetra to hexagonal, Xylem Vessel round to angular…………………………………………………………...	2
2	+	Collenchyma cells lamellar, Sclerenchyma absent, Vb arrangement collateral closed…………………………………………..	*Alyssum desertorum*
	-	Parenchyma cells are hexagonal, Collenchyma cells angular, Sclerenchyma cells hexagonal, Vb arrangement collateral open…..	3
3	+	Petiole oval, Epidermal cells square, Cuticle smooth, Phloem cells rectangular to oval……………………………………………	*Brassica oleracea*
	-	Epidermal cells rectangular to square, Cuticle undulated, Phloem cells rectangular to hexagonal, Vb are bi-collateral …………...	4
4	+	Trichome absent, Xylem Vessel round………………………………………………………………………………………….	*Capsella bursa-pastoris*
	-	Cuticle smooth, Phloem cells rectangular, round, oval, Vb are hadrocentric ………………………………………….…………….	5
5	+	Parenchyma cells tetra to hexagonal, Sclerenchyma absent…………………………….……………………………………………	*Cardaria chalepense*
	-	Parenchyma cells tetra to isodiametric, Phloem cells angular………………………………………………………………………..	6
6	+	Epidermal cells Square and round, Collenchyma cells lacunar, Trichome unicellular…………………………………………………………………	*Cardaria draba*
	-	Epidermal cells are round & oval, Parenchyma cells tetragonal to irregular, Collenchyma cells angular, Trichome absent, Phloem cells angular to oval………..……………………………………………………………………………………………………	7

7	+	Petiole sulcate, Xylem Vessel round and oval……..……………………………………………………………………………...	*Coincya tournefortii*
	-	Petiole flat, Trichome uniseriate, Phloem cells rectangular……………………..……………………………………………….	8
8	+	Epidermal cells rectangular, Parenchyma cells irregular, Collenchyma cells lamellar……………………………………………	*Conringia orientalis*
	-	Petiole sulcate, Epidermal cells square and round to oval, Collenchyma cells angular……………………………………………	9
9	+	Vb Arrangement collateral closed, Phloem cells round and oval………………………………………………………………..	*Descurainia Sophia*
	-	Petiole flat, Epidermal cells rectangular to round, Vb arrangement hadrocentric……………………………………………….	10
10	+	Parenchyma cells isodiametric, Trichome unicellular, Cuticle undulated, Phloem cells round and oval……………………………………………	*Diplotaxis harra*
	-	Petiole oval, Epidermal cells square, Parenchyma cells hexagonal, Vb bicollateral…………………………………………………	11
11	+	Xylem Vessel round, Phloem cells rectangular, round to oval………………………………………………………………………..	*Eruca sativa*
	-	Petiole circular, Epidermal cells square to round, Xylem Vessel round to angular, Phloem cells rectangular to oval, Vb collateral closed…………………………………..………………………………………………………………………………….	12
12	+	Collenchyma cells lamellar, sclerenchyma cells hexagonal, Cuticle smooth……………………………………............................	*Farsetia hamiltonii*
	-	Petiole flat, Epidermal cells square, sclerenchyma absent………………………………………………………………………….	13
13	+	Parenchyma cells regular, Collenchyma cells lacunar, Cuticle smooth, Xylem vessel round, Phloem cells round………………….	*Farsetia heliophila*
	-	Petiole sulcate, Epidermal cells square to rectangular, Parenchyma cells tetra to hexagonal, Cuticle undulated, Xylem vessel round and oval, Phloem cells rectangular round……………………………………...………………………………………	14
14	+	Vb arrangement bicollateral, Trichome absent……………………………………………………………….…………………….	*Goldbachia pendula*

	-	Epidermal cells square to round, Parenchyma cells isodiametric, Trichome Multiseriate, Xylem Vessel angular to round, Phloem cells rectangular to oval…………………………………………………………….……………………………………………	15
15	+	Collenchyma cells lamellar…………………….…………………………………………………………………………………...	*Lepidium aucheri*
	-	Trichome absent, Phloem cells round, oval……………………….……………………………………………………………..	16
16	+	Petiole flat, Epidermal cells square to oval, Parenchyma cells irregular, Collenchyma cells angular……………………………….	*Matthiola flavida*
	-	Petiole sulcate, Epidermal cells square, Parenchyma cells isodiametric, Collenchyma cells lamellar, Trichome multiseriate, Phloem cells rectangular…….	17
17	+	Sclerenchyma cells hexagonal, Cuticle smooth, Xylem Vessel round………………………………………………………………	*Meniocus linifolius*
	-	Epidermal cells oval, Parenchyma absent, Collenchyma absent, Trichome unicellular, Cuticle absent…………………………….	18
18	+	Xylem Vessel round to angular………………………………………………………………………………………………	*Nasturtium officinale*
	-	Parenchyma cells hexagonal……………………………………………………………………………………………………….	19
19	+	Petiole flat, Epidermal cells Square to oval, Collenchyma cells lacunar, Sclerenchyma absent, Cuticle undulated, Xylem Vessel round and oval, Phloem cells rectangular and oval………………………………………………………………………………	*Notoceras bicorne*
	-	Petiole circular, Parenchyma cells isodiametric, Collenchyma cells lamellar, Cuticle smooth, Xylem Vessel round, Phloem cells rectangular, round to oval……	20
20	+	Sclerenchyma cells hexagonal, Vb Arrangement collateral closed, Trichome absent…………………………..........................	*Physorrhynchus brahuicus*
	-	Petiole flat, Parenchyma cells hexagonal, Vb arrangement bi-Collateral, Trichome multiseriate, Xylem Vessel round to oval, Phloem cells are rectangular and oval………………………………………………………………………………………..	21

21	+	Epidermal cells square, sclerenchyma cells hexagonal……………….……………………………………….……………………….	*Raphanus raphanistrum*
	-	Petiole circular, Epidermal cells square to oval, Trichome absent, Xylem Vessel round, Phloem cells round and oval, Vb collateral open……...	22
22	+	Sclerenchyma cells hexagonal………………………………………………………….……………………………………….…...	*Sisymbrium altissimum*
	-	Epidermal cells square to round, Trichome unicellular, Xylem Vessel round to angular, Vb arrangement collateral closed……….	23
23	+	Collenchyma cells angular, ………………...………………………………………………...………………………………………	*Strigosella africana*
	-	Epidermal cells angular, Vb Arrangement hadrocentric, Xylem Vessel round……………………………………………………	24
24	+	Phloem cells rectangular and oval ………………………………………………………………………………….……………….	*Strigosella cabulica*
	-	Epidermal cells rectangular, Vb arrangement collateral closed, Xylem Vessel oval to round, Phloem cells Oval, round………....	25
25	+	Petiole sulcate, Parenchyma cells irregular, Collenchyma cells lamellar, Sclerenchyma absent, Trichome absent, Cuticle undulated………..	*Strigosella intermedia*

3.5 Petiole Anatomy of Fabaceous Flora of Northern Baluchistan

3.5.1 Results and Discussion

The current study described the petiole anatomical characteristics of 15 Fabaceous species. The size and morphologies of epidermal cells, collenchyma, chlorenchyma, parenchyma, cuticle layer, vascular bundles, xylem vessels, and phloem cell shape were noted. From a taxonomic perspective, the petiole shape was found to be important since it is less affected by environmental changes (Metcalfe and Chalk, 1957). However, petiole anatomy has been ignored in taxonomic investigations. One of the reasons is that it requires quantitative and qualitative analysis and more than one sample. Anatomical traits have been found significant in distinguishing between higher taxonomic levels, including genera and families. Several anatomical studies were found significant in resolving taxonomic problems within different groups (Carlquist, 1996; Carlsward et al., 1997; Colombo and Spadaro, 2003; Scatena et al., 2005; Satil and Selvi, 2007; Matias et al., 2007; Schweingruber, 2007; Erxu et al., 2009; De la Estrella et al., 2009; Zarrei et al., 2010). The observations from the current study demonstrated their significance in the differentiation of the examined species.

a) Petiole outline, and wings of the Fabaceous species

Winged petioles were present in 8 Fabaceous species. Earlier winged petioles in the Fabaceous species were documented by Mehrabian et al. (2007), Karamian and Ranjbar (2003). Petiole wings were present in *Astragalus purpurascens* and absent in *Astragalus crenatus*. Groove in the upper surface of the petiole was noted in 10 species. The petiole outline was flat, sulcate, round, and oval in the studied species. Oval-shaped petiole was present in *Caragana ambigua*, while round petiole was observed in *Indigofera intricata* and *Lotus garcinii* (Table 23). Petioles in *Taverniera glabra*, were notably conspicuous, with the largest length of 3210µm. In most species, the petiole outline was flat or sulcate. Metcalfe and Chalk (1950) stated that petiole is not greatly affected by different ecological conditions, it is significant taxonomically.

The present petiole cross-section description was generally consistent with earlier research on other *Astragalus* species (Howard, 1979; Haddad and Barnett, 1989;

Pirani et al., 2006). Previously semi-triangular, with convex abaxial sides and either flat or slightly convex adaxial sides petiole outline was reported in *Astragalus spruneri*, (Junković et al., 2021). Studies conducted on *Astragalus* species from Europe have indicated that the central region parenchyma and the amount of collenchyma tissue surrounding vascular bundles were diagnostic in taxonomy for their differentiation (Haddad and Barnett, 1989). In this study, the recommendations of Haddad and Barnett (1989) and Engel (1990) were followed, and cross-sections were taken from the middle of the petiole. Previously, different Fabaceous species have been documented with distinct petiole outlines, from subtriangular to suborbicular (Junković et al., 2021).

The studied species were significantly distinguished based on the anatomy of the petiole. For example, the petiole of *Indigofera cuneifolia* was sulcate, smooth cuticle, bicollateral vascular bundles, and angular to oval xylem vessels, while *Indigofera intricata* round outline, undulated cuticle, amphicribral vascular bundles, and rectangular to round xylem vessels. Four species of *Onobrychis* were the subject of petiole anatomy studies by Karamian et al. (2012). Previously *Onobrychis* sect. Heliobrychis anatomical examinations of the peduncle cross-section showed that different perennial species had petioles with circular, elliptic, pentagonal, and hexagonal form, with the surface of the peduncle smooth, papillo, papillo, or highly papillo (Karamian et al., 2011). They observed the presence of certain cavities in the cortical parenchyma and surrounding the pericyclic fibers. In their investigation, cavities were also found in the stem's cross sections but not in the root.

b) Cuticle, and Epidermal cell shape in the petiole of Fabaceae plants

A smooth and undulated type of cuticle was observed in the petiole of the Fabaceous species. The smooth form was found in seven species, while the undulated type was in ten plants. A thick cuticle was noted in *Melilotus indicus*. A smooth cuticle was observed in the petiole of the *Astragalus* genus. Epidermal cell shape varied from square to angular, irregular, square to rectangular, angular to square, and square (Plate 64-67). Okanume et al. (2022) also observed epidermal cells as rectangular, round, oval, angular, and square in shape. In *Melilotus indicus* square to rectangular, and angular epidermal cells were noted. In the *Astragalus* genus square to angular epidermal cell was observed. The maximum width of epidermal cell was observed in *Lotus garcinii* 27.2 μm (Table 24). In five species, irregular epidermal cells were present. The minimum width of 3.35µm was noted in *Onobrychis dealbata.* Junković et al. (2021) reported that a single-layered epidermis has a denser covering of cuticle, within the *Astragalus* genus. According to Pierani et al. (2006), the epidermis in every member of the *Astragalus* genus was in the form of a single layer of subcircular to sub-rectangular cells. With age, the cortex divides into four to six layers. In our investigation, the epidermis in the *Astragalus* was found square to angular, and irregular. Presently, a single layer of epidermis was present in all studied taxa. Similar results were reported by Kemka-Evans et al. (2021).

c) Collenchyma, Chlorenchyma, and Parenchyma of Fabaceous species

Collenchyma cells were observed in lamellar, lacunar, angular, and lamellar-to-angular shapes. In most species, angular collenchyma was observed. Angular-to-lamellar collenchyma was only noted in *Indigofera cuneifolia*. The lacunar shape was only observed in *Melilotus indicus.* The sub-epidermal ring of collenchyma was present in 11 species. The prominent collenchyma ring was observed in *Trigonella macrorrhyncha* and *Onobrychis tavernierifolia*. The number of layers of collenchyma cells varied from 1-3 in the examined species. Number of layers of chlorenchyma cells ranged from 1-4 (Table 22). The 4 layers of chlorenchyma were observed in *Ebenus stellata.* In the studied Fabaceous species the observed parenchyma cell shapes were triangular to hexagonal, isodiametric, irregular, and hexagonal. The isodiametric parenchyma was noted in six species. The tri-to-hexagonal shape was observed in *Alhagi maurorum*. The irregular form of the parenchyma was noted in five species. In *Onobrychis* genus the parenchyma cells were isodiametric.

The petioles in the *Astragalus* genus comprised one to two collenchyma layers. The subepidermal ring of collenchyma was absent in *Astragalus purpurascens*. In the petioles of Fabaceae species, Nwachukwu et al. (2017) observed 1-2 layers of collenchyma beneath the epidermis, followed by multiple layers of parenchyma. *Alhagi maurorum*, according to Awmack and Lock (2002), had a thick epidermis and a poorly developed cortex. Presently, *Alhagi maurorum* was observed with one layer of collenchyma. Zoric et al. (2009) documented one layer of collenchyma in *T. alpestre, T. montanum,* and *T. velenovskyi.*

Anatomical features of the petiole in the studied *Onobrychis* species differed from each other. *Onobrychis dealbata* and *Onobrychis tavernierifolia,* were differentiated based on the shape of collenchyma cells, xylem vessels, and parenchyma cells. Three to seven, primarily four to six, layers of parenchyma cells were observed previously in Fabaceae petioles (Lana et al., 2011). The parenchyma layers in the cortical region of *Astragalus* species varied from 3-10, including tri to hexagonal, irregular, and isodiametric shapes. *Alhagi maurorum* had a well-developed cortical region with twelve layers of parenchyma cells. *Onobrychis dealbata* and *Onobrychis tavernierifolia* have isodiametric parenchyma cell shapes, whereas the cortical region in the genus *Onobrychis* was composed of three layers of parenchyma.

Astragalus crenatus have three chlorenchyma cells layers. Jušković et al. (2021) documented two to three layers of collenchyma, chlorenchyma and many layers of parenchyma cells in the petiole cross-section of *Astrasgalus*. Sclerenchyma cells and the sclerification between the vascular tissues were absent in all examined species of Fabaeacea. Three species *Caragana ambigua, Sophora mollis,* and *Vicia macrantha* showed evidence of sclerification. A similar was reported by Noor et al. (2023) for the Brassicaceae family from the same study area.

d) Vascularization in the petiole of Fabaceae species

The arrangement of vascular bundles was observed as collateral closed, collateral open, bicollateral, and amphicribral. Bicollateral arrangements was present in *Indigofera cuneifolia*. In ten species collateral closed type of vascular bundle was noted. Amphicribral vascularization was observed in four species. The collateral open type was remarked in *Vicia macrantha.* In *Ebenus stellata* nine vascular bundles were observed. The presence of sclerenchyma between the vascular bundles was present in the three species. Most Fabeaceae species have amphicribral and collateral closed vascular bundles. The vascular bundle of the genus *Onobrychis* and *Astargalus* was distinguished from *Indigofera cuneifolia*, which was a bicollateral arrangement of vascular bundles. In nine species the arrangement of vascular bundles was 1+2. Heneidak and Shaheen (2007) also disclosed the collateral open vascular bundles in *Indigofera* species.

Xylem vessels were observed in various forms, oval to round, angular, round to angular, tri to hexagonal, angular to oval, round to oval, and rectangular to round. Rectangular to round xylem vessels were only found in *Indigofera intricate.* The shape of the phloem cell was tetra to hexagonal in *Melilotus indicus.* In most species the shape of phloem cells was angular. Significant differences in the anatomical features of *Onobrychis* genus, particularly in the xylem vessel diameter, and phloem width, were observed. Zarrabian et al. (2013) examined the petiole anatomy of similar species from different regions and stated that petiole anatomical traits were less affected by different environmental conditions. Al-otraqche (2022) demonstrated that parenchyma encircled the vessels and fibers in the *Onobrychis*. In *Astragalus* species, the vascular bundle size and number differ significantly. Previously, Pirani et al. (2006) reported dense sclerenchymatous sheath made up of extraxylary fibers with exceptionally thick walls surrounding the vascular bundles.

e) Multivariate analysis

The significance of quantitatively measured features in taxonomic investigations was established by their analysis in the form of PCA, dendrograms, correlation, and normal probability plots. The PCA (Figure 27) demonstrated that PC1 and PC2 differed by 72. % and 27.7% variance respectively. Most of the analyzed characteristics had positive correlations. The xylem vessel length and width showed the highest positive association, 0.97. Conversely, the collenchyma width and the vascular bundle width showed the least positive correlation, 0.04. The xylem vessel width and epidermis width showed the highest negative association, which was -0.25 (Figure 29). There was also a negative correlation between vascular bundle length and epidermis length -0.21, xylem vessel length and epidermis width -0.23, chlorenchyma and xylem vessel -0.17. Using a dendrogram, the quantitative variables considerably shaped the evolutionary relationships between the taxa. The dendrogram was delineated into two clusters (Figure 30). One major cluster with 10 taxa, and the second one with 5 taxa. The patterns for the quantitative variables in the data set were identified via the normal probability plot. Black dots in the data reflected the outliers. The trend in the data set was displayed by the straight black line, while the mean data values for each trait were represented by the color. The studied Fabacious species were significantly differentiated based on the petiole anatomical quantitative features.

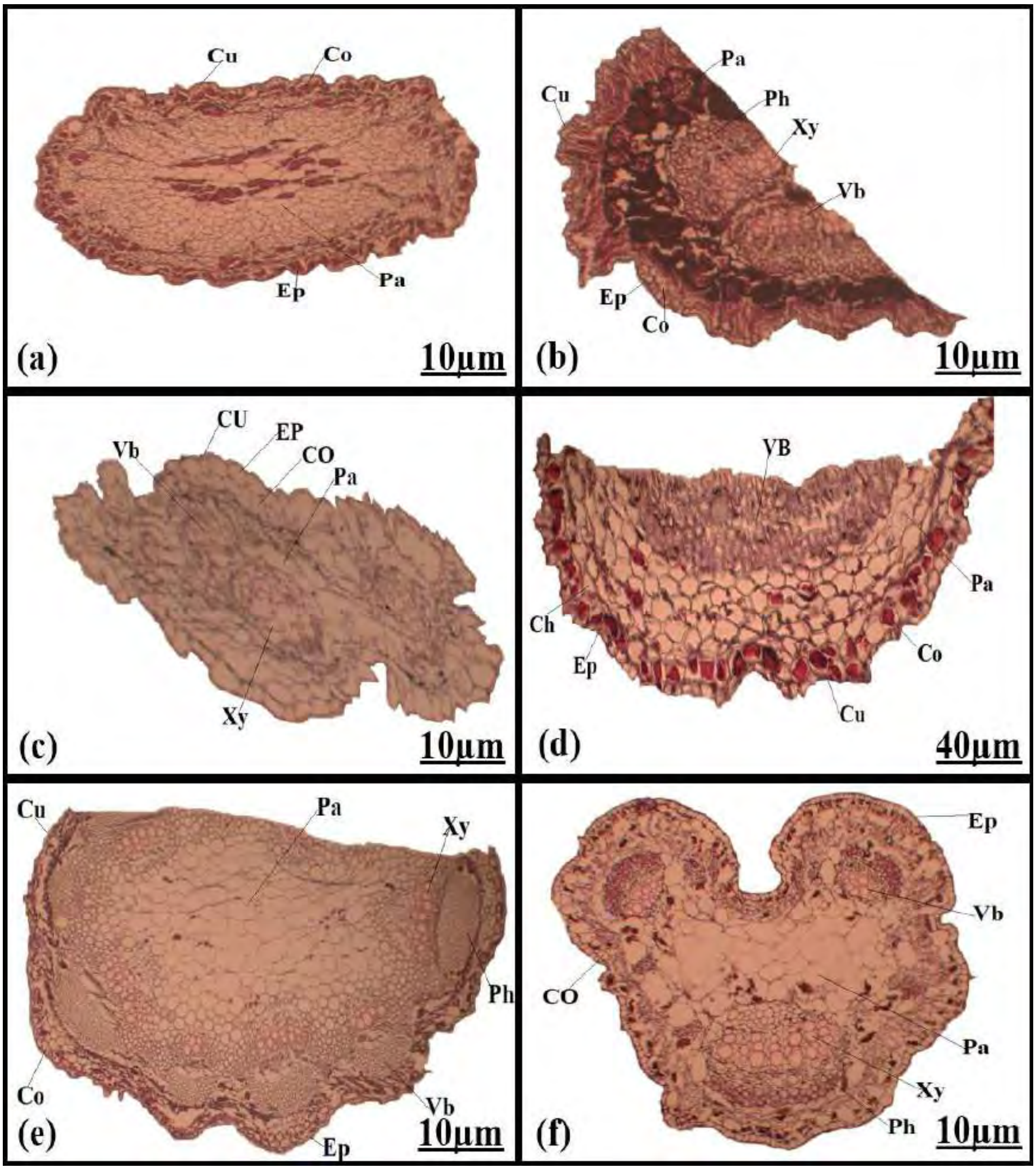

Plate 64. Photomicrographs of petiole anatomy (a) *Alhagi maurorum,* (b) *Astragalus crenatus*, (c) *Astragalus purpurascens*, (d) *Caragana ambigua*, (e) *Ebenus stellata*, (f) *Indigofera cuneifolia,* (Cu: cuticle, Ep: Epidermis, Pa: Parenchyma, Co: Collenchyma, Ch: Chlorenchyma, Sc: Sclerenchyma, Xy: Xylem, Ph: Phloem). Scale:10µm(a,b,c,e,f),40µm (d)

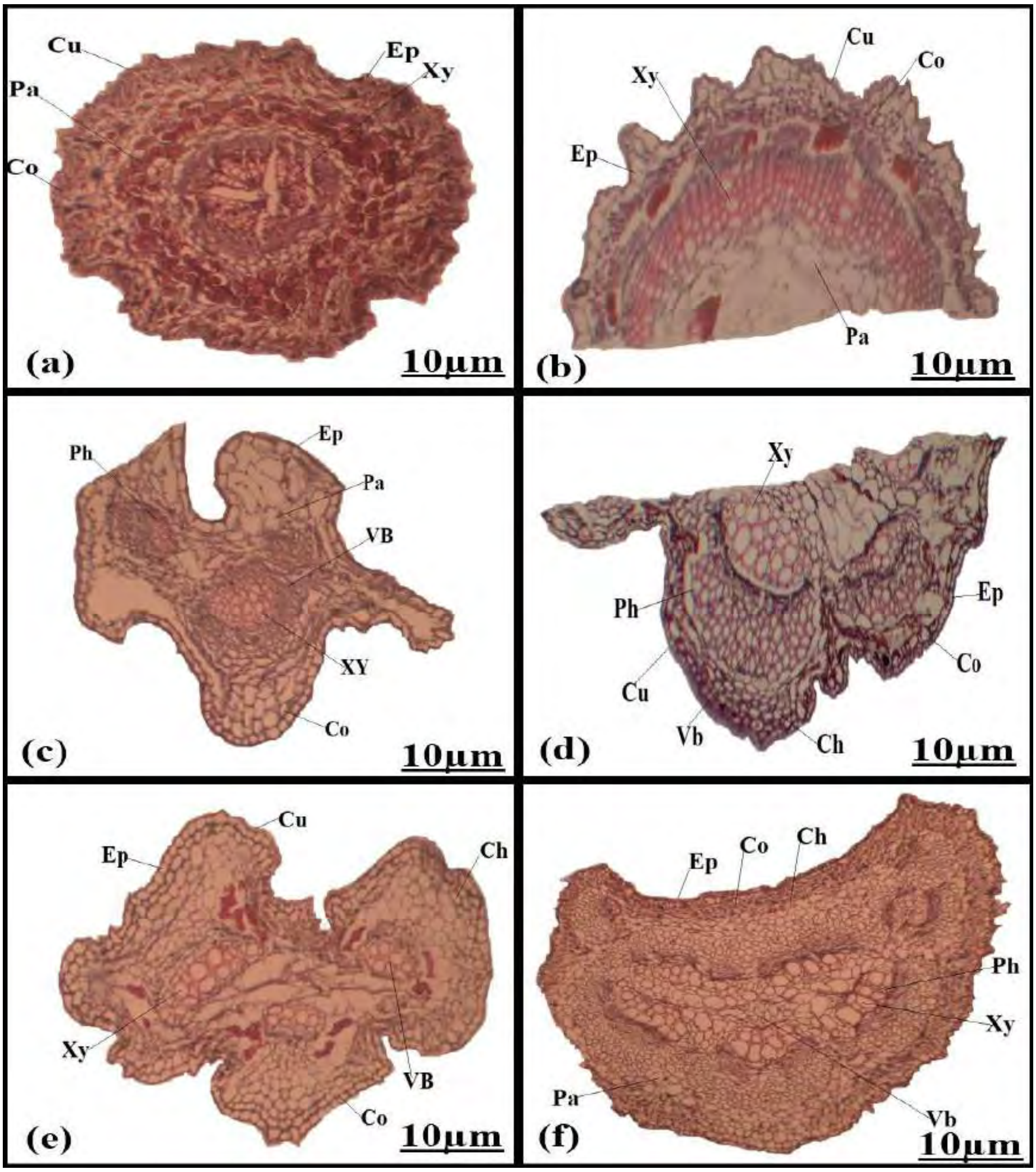

Plate 65. Photomicrographs of petiole anatomy (a) *Indigofera intricata,* (b) *Lotus garcinii,* (c*) Melilotus indicus*, (d) *Onobrychis dealbata*, (e) *Onobrychis tavernierifolia*, (f) *Sophora mollis,* (Cu: cuticle, Ep: Epidermis, Pa: parenchyma, Co: collenchyma, Ch: chlorenchyma, Sc: sclerenchyma, Xy: xylem, Ph: Phloem). Scale:10µm

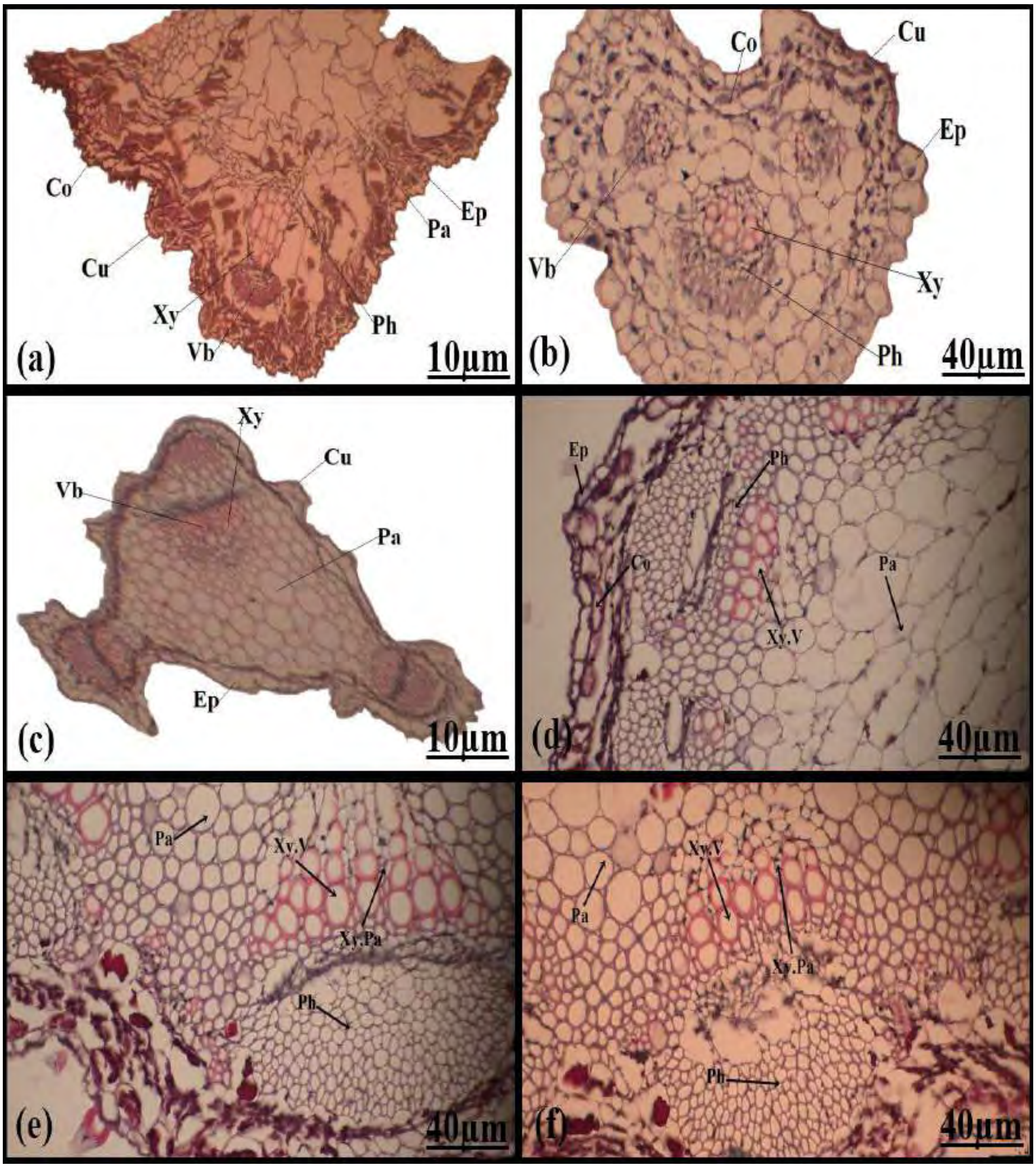

Plate 66. Photomicrographs of petiole anatomy (a) *Taverniera glabra*, (b) *Trigonella macrorrhyncha*, (c) *Vicia macrantha*, (d,e,f), *Ebenus stellata*,(Cu: cuticle, Ep: Epidermis, Pa: parenchyma, Co: collenchyma, Ch: chlorenchyma, Sc: sclerenchyma, Xy: xylem, Ph: Phloem). Scale:10µm(a,c,),40µm (b,d,e,f)

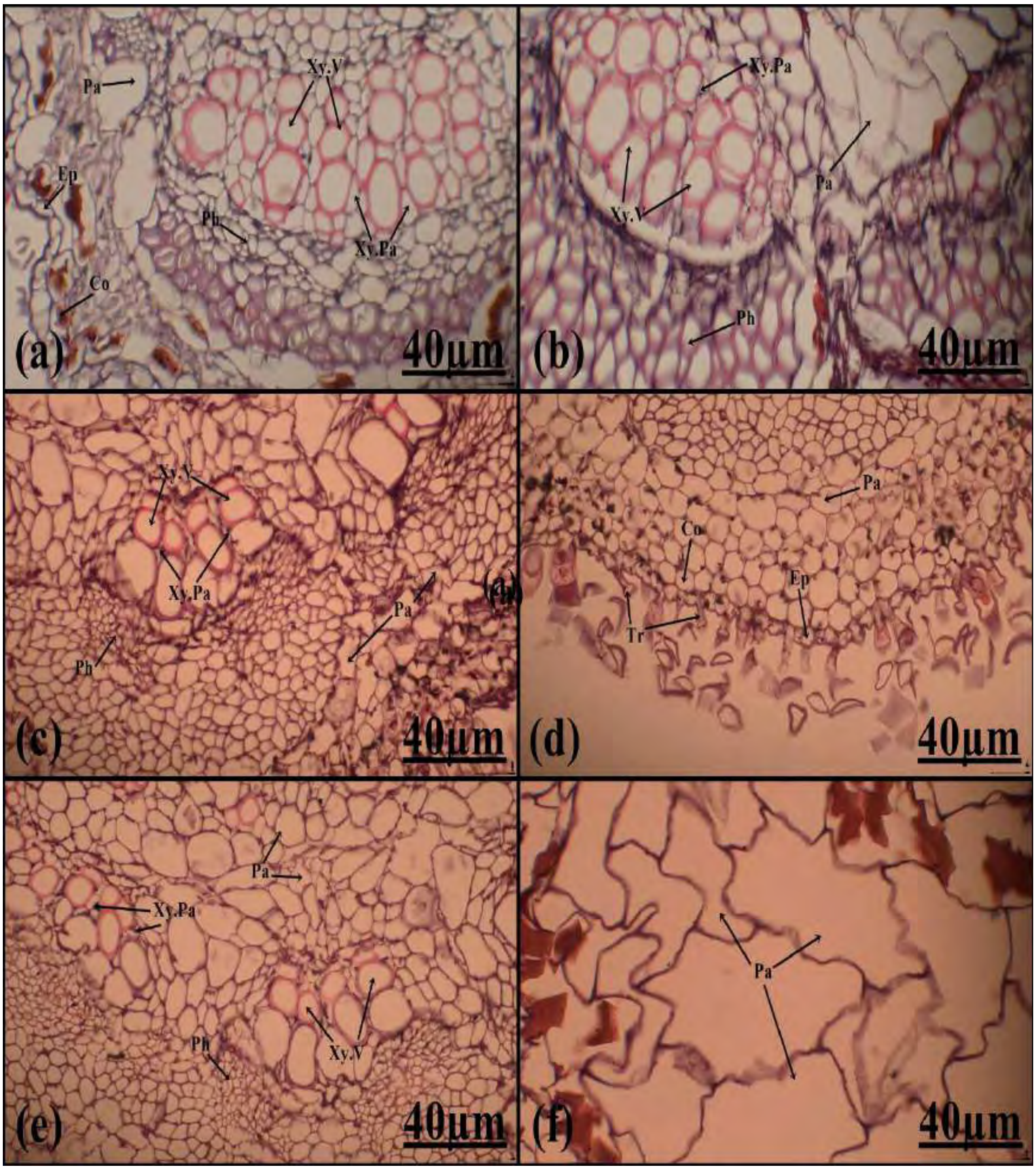

Plate 67. Photomicrographs of petiole anatomy (a) *Indigofera cuneifolia,*, (b) *Onobrychis dealbata*, (c,d,e) *Sophora mollis*, (f) *Taverniera glabra ,* (Cu: cuticle, Ep: Epidermis, Pa: parenchyma, Co: collenchyma, Ch: chlorenchyma, Sc: sclerenchyma, Xy: xylem, Ph: Phloem). Scale:40µm

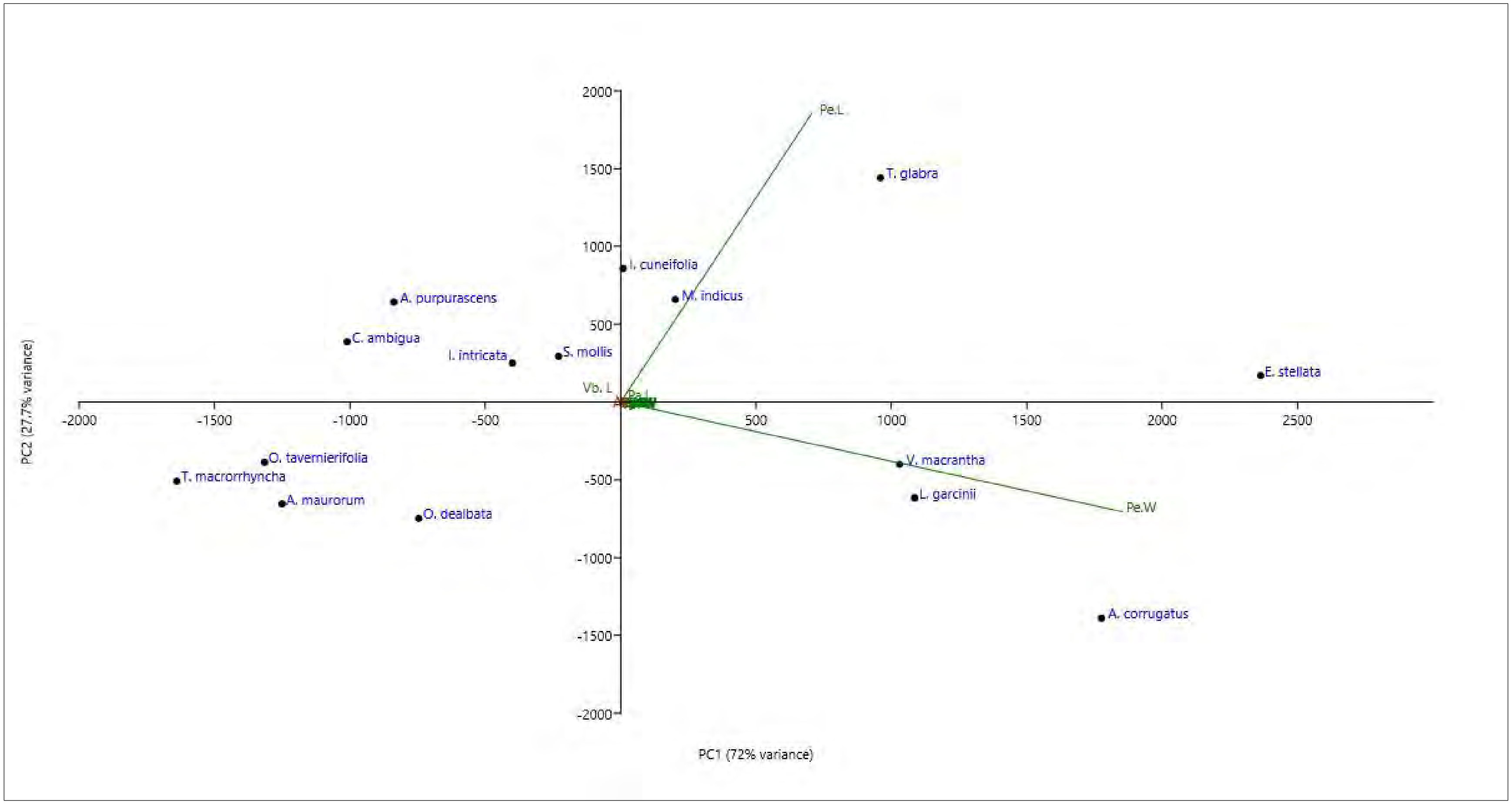

Figure 27. Utility of petiolar features in discriminating among species of Fabaceous by PCA (epidermis length and width, collenchyma length and width, chlorenchyma length and width, parenchyma length and width.

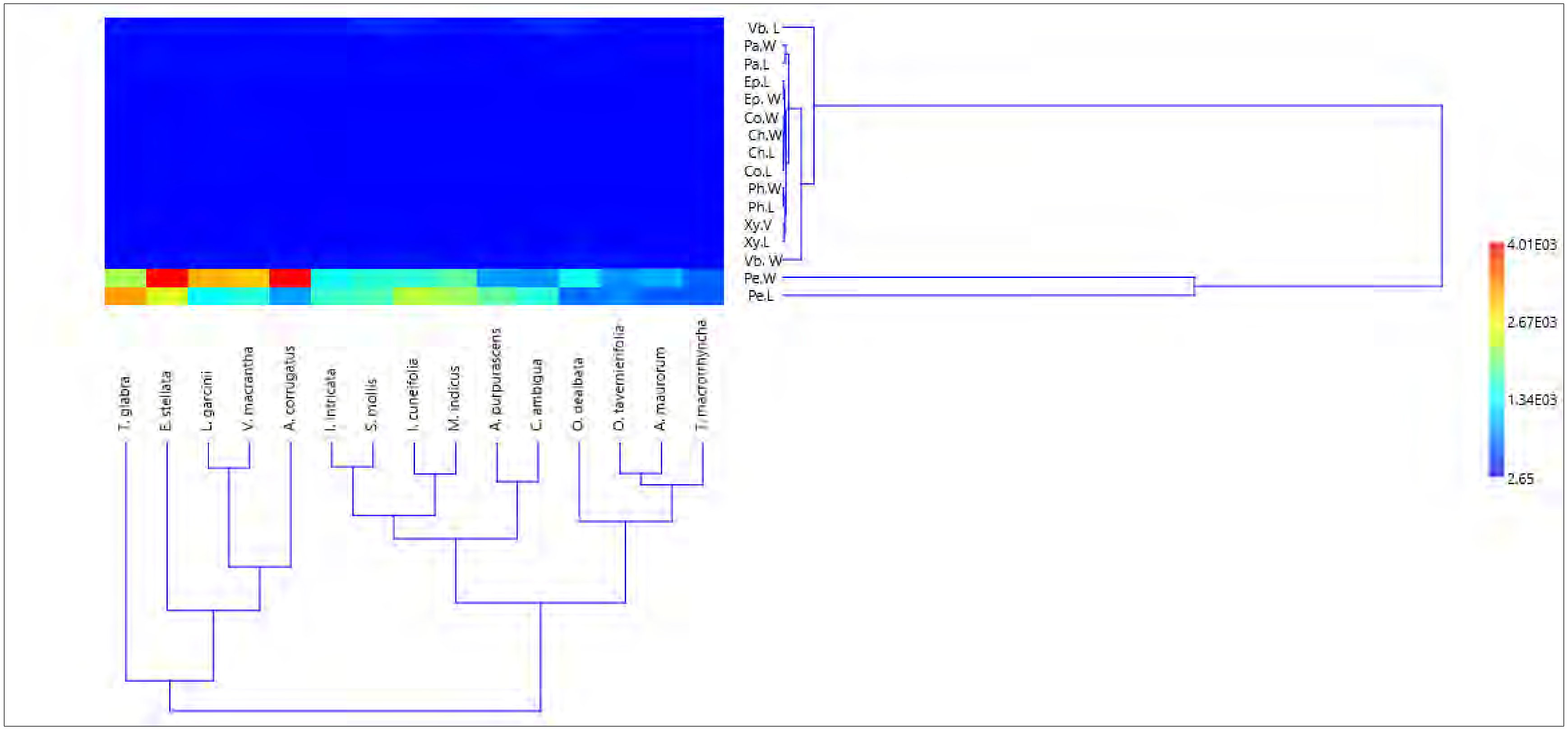

Figure 28. Dendrogram showing the similarity index of Fabaceous taxa based on quantitative parameters of petiole

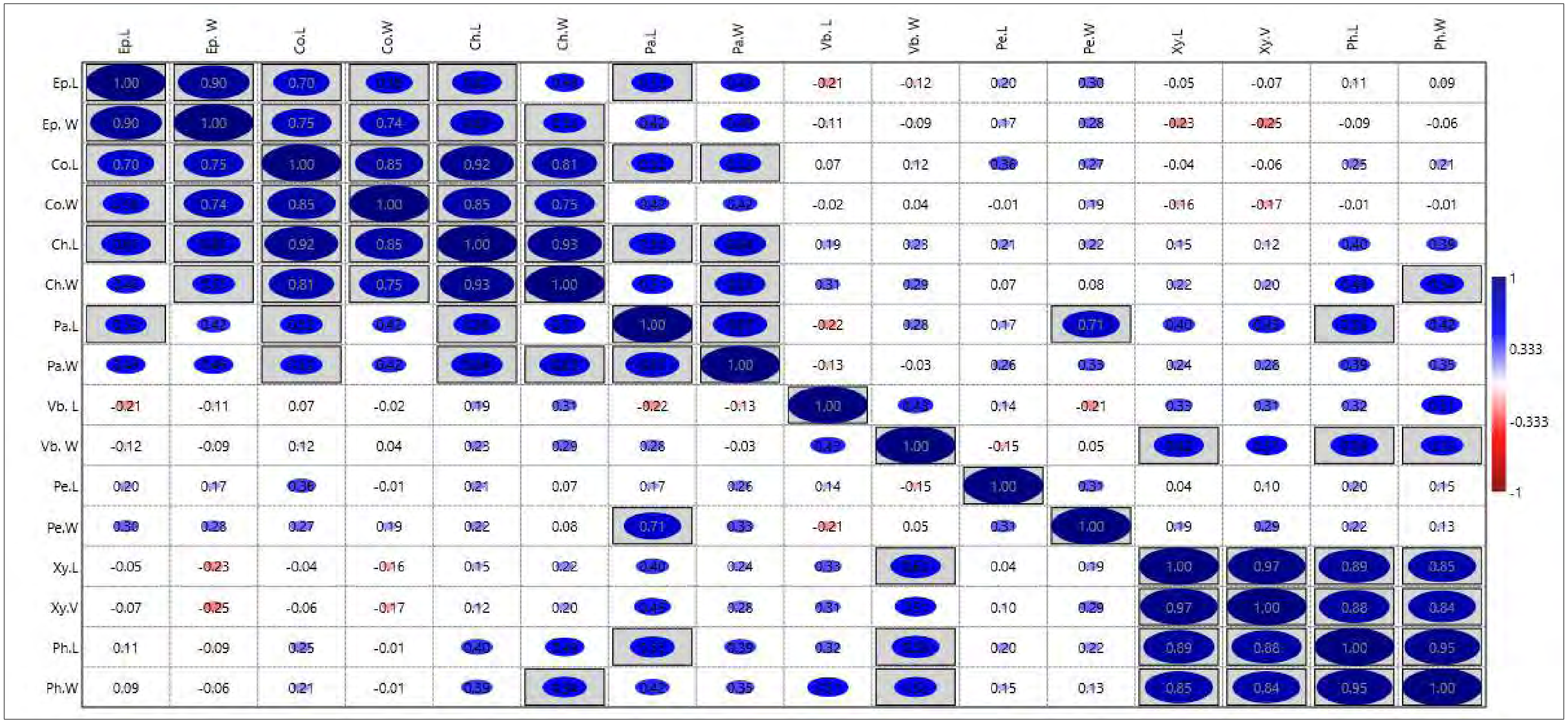

Figure 29. Correlation among the mean values of Ep.L: Epidermal length; Ep.W: Epidermal width; Co.L: Collenchyma length:Co.W: Collenchyma width;Ch.L:Chlorenchyma length; Ch.W: Chlorenchyma width;Vb.L:Vascular bundle length; Vb.W: Vascular bumdle width

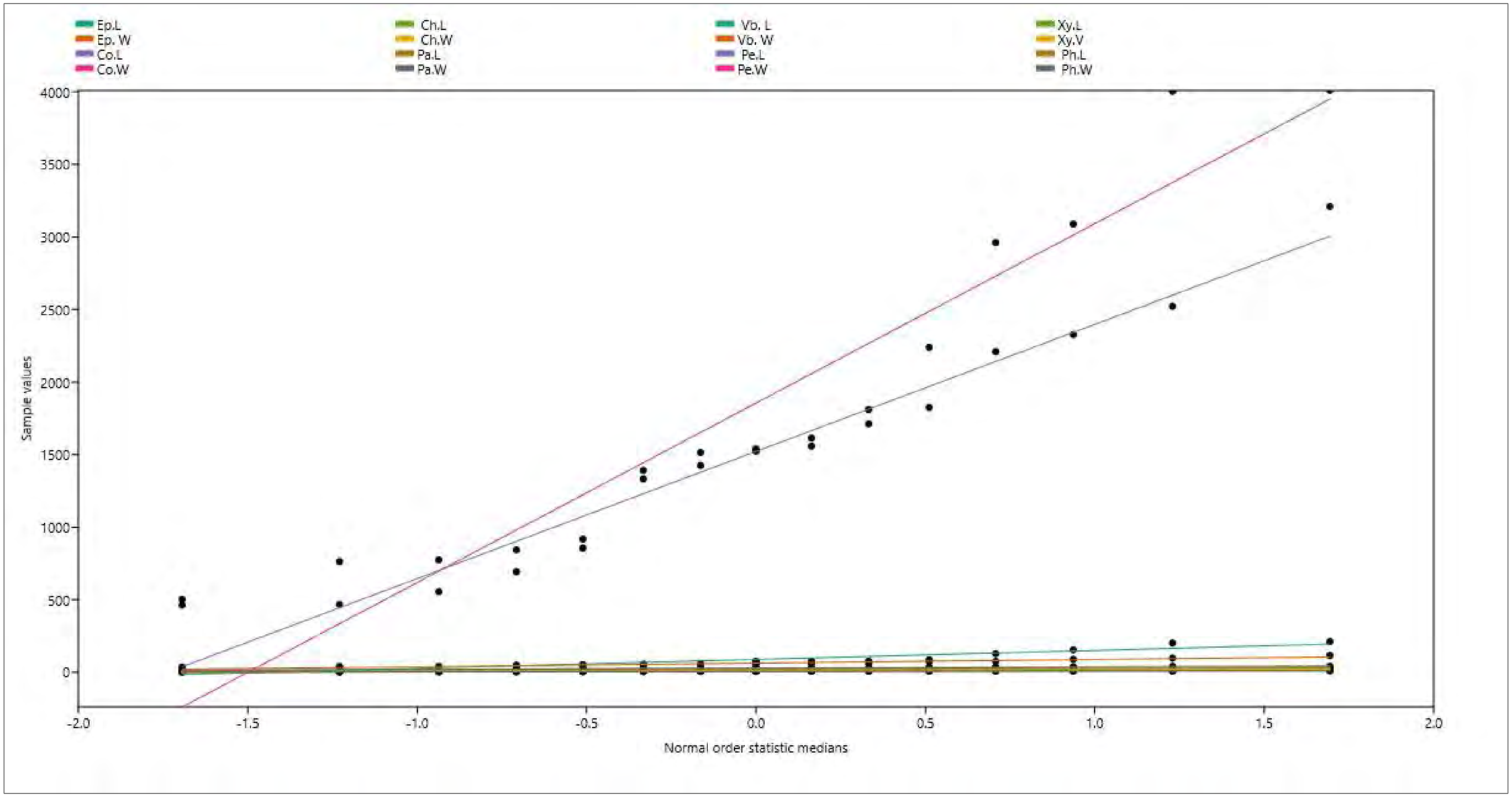

Figure 30. Normal probability distribution among the mean values of Ep.L: Epidermal length; Ep.W: Epidermal width; Co.L: Collenchyma length: Co.W: Collenchyma width; Ch.L: Chlorenchyma length; Ch.W: Chlorenchyma width;Vb.L:Vascular bundle length; Vb.W: Vascular bundle width

Table 22. Qualitative anatomical observations of the Petiole of Fabaceous taxa

Plant	Petiole wing	Grove in the upper surface	Co (No of layers)	Subepidermal ring of Co	Ch (No of layers)	Sc (No of layers)	Sc Presence in VB	Pa layers	No of VB	Xy parenchyma	Ph Parenchyma
Alhagi maurorum	-	-	1	+	3	-	-	12	3	+	+
Astragalus crenatus	-	-	1	+	3	-	-	6	2	+	+
Astragalus purpurascens	+	+	1	-	1	-	-	3	1+2	+	+
Caragana ambigua	-	-	1	+	3	-	+	-	1	+	+
Ebenus stellata	-	+	3	+	4	-	-	7	9	+	+
Indigofera cuneifolia	+	+	2	+	1	-	-	6	1+2	+	+
Indigofera intricata	-	-	2	+	2	-	-	3	1	-	+
Lotus garcinii	-	+	2	+	1	-	-	5	1	+	+
Melilotus indicus	+	+	1	-	1	-	-	3	1+2	+	+
Onobrychis dealbata	+	+	2	-	1	-	-	3	1+2	+	+
Onobrychis tavernierifolia	+	+	3	+	1	-	-	3	1+2	+	+
Sophora mollis	+	+	3	+	1	-	+	2	1+2	+	+
Taverniera glabra	+	+	2	+	1	-	-	5	1	+	+
Trigonella macrorrhyncha	-	+	1	+	1	-	-	2	1+2	+	+
Vicia macrantha	+	-	2	-	2	-	+	3	1+2	+	+

(Co: Collenchyma, Ch: Chlorenchyma, Sc: Sclerenchyma, VB: Vascular bundles, Xy: Xylem, Ph: Phloem

Table 23. Qualitative anatomical observations of the Petiole of Fabaceous taxa

Plant	Outline	Cuticle structure	Ep shape	Pa shape	Co shape	Sc shape	VB arrangement	Xy vessel	Ph shape
Alhagi maurorum	Flat	Undulated	Square to angular	Tri to hexagonal	Lamellar to angular	-	Collateral closed	Oval	Angular
Astragalus corrugatus	Flat	Undulated	Square to angular	Irregular	Lamellar to angular	-	Collateral closed	Oval to round	Angular
Astragalus purpurascens	Flat	Smooth	Irregular	Irregular	Angular	-	Collateral closed	Round to angular	Angular
Caragana ambigua	Oval	Undulated	Square	Isodiametric	Lamellar	-	Collateral closed	Round to angular	Angular
Ebenus stellata	Flat	Undulated	Square to rectangular	Hexagonal	Angular	-	Amphicribral	Angular to oval	Tri to hexagonal
Indigofera cuneifolia	Sulcate	Smooth	Square	Isodiametric	Angular to lamellar	-	Bicollateral	Angular to oval	Angular
Indigofera oblongifolia	Round	Undulated	Irregular	Isodiametric	Lamellar	-	Amphicribral	Rectangular to round	Angular
Lotus garcinii	Round	Undulated	Irregular	Isodiametric	Angular	-	Collateral closed	Round to oval	Angular
Melilotus indicus	Sulcate	Undulated	Square to rectangular and angular	Irregular	Lacunar	-	Collateral closed	Oval	Tetra to hexagonal
Onobrychis dealbata	Sulcate	Smooth	Square to angular	Isodiametric	Angular	-	Collateral closed	Round to oval	Tri to hexagonal
Onobrychis tavernierifolia	Sulcate	Smooth	Square to angular	Isodiametric	Lamellar	-	Collateral closed	Round	Angular
Sophora mollis	Sulcate	Undulated	Square to angular	Irregular	Angular	Angular	Amphicribal	Oval	Tri to Hexagonal
Taverniera glabra	Sulcate	Undulated	Irregular	Irregular	Lamellar to angular	-	Collateral closed	Oval	Angular

Trigonella macrorrhyncha	Sulcate	Undulated	Square	Hexagonal	Lamellar	-	Collateral closed	Angular at edges	Angular
Vicia macrantha	Sulcate	Smooth	Square to angular	Hexagonal	Angular	Tri to Hexagonal	Collateral open	Round to oval	Angular

(Ep: Epidermis, Pa: Parenchyma, Co: Collenchyma, Sc: Sclerenchyma, VB: Vascular bundles, Xy: Xylem, Ph: Phloem

Table 24. Quantitative data of petiole histological properties of studied Fabaceous species

Plant Species	L.W	Ep (µm)	Co (µm)	Ch (µm)	Pa (µm)	Vb (µm)	Pe	Xy (µm)	Ph (µm)
Mean±SE									
Caragana ambigua	L	14.8±0.649037749	23.65±0.562361094	26.55±0.906228448	26.65±0.584166072	211.25±0.612372436	1523.8±10.26839812	11±0.176776695	8.35±0.358817502
	W	17.7±0.266926956	26±0.176776695	30.65±0.257390754	32.5±0.353553391	61.6±1.476058942	775±5.787918451	8.6±0.257390754	7.7±0.266926956
Sophora mollis	L	4.75±0.306186218	4.6±0.257390754	4.45±0.278388218	13.45±0.215058132	202±1.003120132	1713.2±4.851803788	17.35±0.491172068	5.45±0.215058132
	W	4.3±0.145773797	4.25±0.176776695	4.55±0.215058132	16±0.176776695	62.95±0.215058132	1538.4±11.48303096	13.4±0.302076149	5.45±0.215058132
Astragalus purpurascens	L	11.65±0.340954542	12.95±0.215058132	8.7±0.215058132	14.85±0.231840462	51.35±0.640312424	1825.6±6.569627082	4.25±0.176776695	3.65±0.127475488
	W	12.65±0.231840462	13.15±0.127475488	7.35±0.231840462	16.5±0.176776695	38.25±0.176776695	843.8±2.989983278	3.25±0.176776695	2.65±0.231840462
Melilotus indicus	L	12±0.25	18.05±0.348209707	16±0.176776695	20.45±0.215058132	86.55±0.532681894	2209.6±4.53431362	9.7±0.16583124	5.75±0.176776695
	W	12.65±0.231840462	18.45±0.365718471	13.05±0.093541435	18.5±0.176776695	55.95±0.856592085	1810.4±3.668787266	6.75±0.176776695	4.25±0.176776695
Indigofera cuneifolia	L	15.95±0.215058132	18.7±0.215058132	19.2±0.310241841	31.45±0.289395923	129.5±1.092588669	2327.4±9.091754506	20.75±0.176776695	10.75±0.176776695
	W	10.75±0.176776695	13.15±0.257390754	16.7±0.215058132	25.75±0.306186218	98.15±0.503736042	1559±3.860051813	13.25±0.176776695	6.75±0.176776695
Onobrychis dealbata	L	6.75±0.176776695	11.25±0.176776695	13.5±0.176776695	34±0.176776695	155.05±1.417303778	556.6±3.641428291	28.7±0.16583124	14.05±0.60930288
	W	3.35±0.127475488	11.75±0.176776695	16.45±0.289395923	19.85±0.231840462	116.75±0.829156198	1425.4±7.242927585	21.55±0.215058132	10.3±0.502493781
Onobrychis tavernierifolia	L	12.65±0.231840462	13.3±0.215058132	14.85±0.231840462	26.2±0.266926956	38.45±0.348209707	693±2.588435821	20.85±0.231840462	9±0.176776695
	W	11.75±0.176776695	15.75±0.176776695	17.4±0.26925824	32.1±0.491172068	75.9±0.231840462	763.2±3.813135193	14.25±0.176776695	6.5±0.176776695
Taverniera glabra	L	21.1±0.231840462	17.75±0.32596012	16.85±0.257390754	38.35±0.340954542	76.05±0.289395923	3210.4±6.446704585	19.1±0.384057287	10.75±0.176776695

	W	17.35±0.231840462	13.5±0.176776695	15.75±0.176776695	38±0.410791918	37.75±0.285043856	2239.8±5.508175742	16.25±0.480884602	7.65±0.231840462
Indigofera intricata	L	6.6±0.384057287	12.8±0.32015621	8.35±0.23184046	26±0.176776695	77.4±0.831414457	1614.6±5.24022900	8.25±0.17677669	6.15±0.30207614
	W	4±0.176776695	8.55±0.215058132	11±0.176776695	20.75±0.176776695	74.6±0.752495847	1392±6.534523701	6.75±0.176776695	4.25±0.176776695
Vicia macrantha	L	25.05±0.289395923	17.55±0.470372193	16.85±0.322102468	38.25±0.176776695	49.35±0.257390754	1515.2±5.379591063	13.25±0.176776695	8.25±0.176776695
	W	20.85±0.280624304	16.2±0.215058132	18.35±0.231840462	25.9±0.231840462	51.55±0.496235831	2960.2±26.02575647	9.35±0.322102468	6.75±0.176776695
Ebenus stellata	L	5.3±0.145773797	14.45±0.496235831	15.65±0.16955825	40.75±0.176776695	52.85±0.976601249	2522.6±7.567033765	17.3±0.266926956	9.1±0.302076149
	W	4.85±0.231840462	15.45±0.215058132	15±0.353553391	30.75±0.176776695	57.75±0.530330086	4004.2±1.15758369	14.7±0.348209707	6.1±0.422788363
Trigonella macrorrhyncha	L	13.25±0.176776695	13.25±0.176776695	13.1±0.26925824	25.45±0.215058132	36±0.176776695	464±4.5607017	9.05±0.413823634	3.8±0.215058132
	W	9.5±0.25	19.45±0.266926956	11.75±0.176776695	20±0.353553391	34.25±0.176776695	503.6±0.92736185	6.95±0.266926956	2.65±0.231840462
Lotus garcinii Ser	L	20.75±0.176776695	20.9±0.26925824	20.75±0.176776695	40.9±0.231840462	77±0.637377439	1332.8±8.873556221	10.3±0.320156212	5.15±0.231840462
	W	27.2±0.849264388	26.35±0.280624304	18.25±0.176776695	30.75±0.176776695	90.2±0.604152299	3089.4±38.39088433	7.25±0.306186218	3.5±0.176776695
Alhagi maurorum	L	6.5±0.176776695	5.6±0.26925824	7.65±0.231840462	21.75±0.176776695	38.95±0.348209707	466.8±3.39705755	7.35±0.231840462	4.15±0.231840462
	W	5.45±0.215058132	6.25±0.176776695	7.55±0.289395923	26±0.25	41.35±0.645174395	918.8±3.878143886	5.2±0.266926956	3.3±0.215058132
Astragalus crenatus	L	16.55±0.470372193	16.15±0.340954542	14.95±0.183711731	36.25±0.25	57.8±0.780224327	855.6±11.55248891	15.9±0.231840462	7.95±0.215058132
	W	13.45±0.254950976	16.6±0.384057287	13.25±0.176776695	27.2±0.555652769	56.5±0.908295106	4009.8±5.053711507	13.9±0.231840462	5.45±0.215058132

(L: length, W: Width, Ep: Epidermis, Co: Collenchyma, Ch: Chlorenchyma, Pa: Parenchyma, Vb: Vascular bundles, Pe: Petiole, Xy: Xylem, Ph: Phloem)

Table 25. Dichotomous (single access) bracketed / parallel taxonomic key based on petiole anatomical characters of Fabaceous flora

Link Character	Leads	Characters	Taxa/ Go to link character
1	a	Petiole outline oval	*Caragana ambigua*
	b	Petiole outline other than oval	2
2	a	Round shaped petiole	3
	b	Petiole not round	4
3	a	Lamellar collenchyma cells	*Indigofera oblongifolia*
	b	Angular collenchyma cells	*Lotus garcinii*
4	a	Flat petiole	*5*
	b	Sulcate petiole	*8*
5	a	Winged petiole	*Astragalus purpurascens*
	b	Petiole without wings	*6*
6	a	Amphicribral vascular bundles	*Ebenus stellata*
	b	Collateral closed vascular bundles	*7*
7	a	Trigonal to hexagonal parenchyma cells	*Alhagi maurorum*
	b	Irregular parenchyma cells	*Astragalus corrugatus*
8	a	Prominent groove in the surface of the petiole	*Trigonella macrorrhyncha*
	b	Groove not seen in the petiole surface	9

9	a	Lacunar collenchyma	*Melilotus indicus*
	b	Collenchyma other than lacunar	10
10	a	Isodiametric parenchyma	11
	b	Irregular or hexagonal parenchyma	13
11	a	Bicollateral vascular bundles	*Indigofera cuneifolia*
	b	Collateral closed vascular bundles	12
12	a	Angular collenchyma cells	*Onobrychis dealbata*
	b	Lamellar collenchyma cells	*Onobrychis tavernierifolia*
13	a	Oval xylem vessels shape	*Taverniera glabra*
	b	Xylem vessels not oval	14
14	a	Amphicribral vascular bundles	*Sophora mollis*
	b	Collateral open vascular bundles	*Vicia macrantha*

3.6 Petiole anatomy of Lamiaceous species from Northern Baluchistan

3.6.1 Results and Discussion

Petiole anatomy of 25 Lamiaceous species was documented for the first time from Northern Baluchistan. Alosaimi (2023) reviewed the taxonomic significance of the petiole anatomy of Lamiaceous species from various ecological zones. Cantino (1992) conducted a cladistics study based on 85 petiole morpho-anatomical features to demonstrate the Lamiaceae's monophyletic origin (Zhao et al., 2021). Their analysis separated several clades of the Verbenaceae from the Lamiaceae. Petiole outline, wings, trichomes, cuticle, epidermis shape, the subepidermal ring of collenchyma, shape, and number of layers of parenchyma, collenchyma, chlorenchyma, sclerenchyma, vascular bundles numbers and arrangement were observed in the present study.

Various petiole anatomical features, assisted in investigating the restrictions of the evolutionary forces into various lineages among Lamiaceous taxa (Alosaimi, 2023). The current research was carried out phytogeographically in an arid-semiarid ecological region based on chosen Lamiaceae species. The ecologically adapted structural changes in the petiole anatomy were observed in the Lamiaceous species. Variations within the micromorphological features of petiole anatomy assisted in the distinction of Lamiaceous species (Jehanzeb et al., 2020). Howard (1962) stated that families down to the species level can be separated by analyzing the vascularization of petioles among the related genera. Presently, the anatomy of the petiole showed variations in the characteristics, the contour of the petiole, wings, parenchyma cells, collenchyma cells, chlorenchyma, vascular bundle shapes, and arrangement, sclerenchyma between the vascular bundles, and subepidermal ring of collenchyma, trichome, and petiole's surface groove.

a) Petiole outline, cuticle and trichome

Distinct petiole outlines were observed in the Lamiaceous species including sulcate, flat, oval, and round. The sulcate outline was present in 15 species. Flat-shaped petiole was noted in 7 species, and oval in 2 species. A round petiole was noted in *Salvia leucantha*. The petiole in the species of the genus *Nepeta* were sulcate-shaped (Plate

31-33). Shahri et al. (2016) visualized the petiole sections of 10 *Nepeta* species. They documented an open V-shaped contour, creating an elaborate identification key based on petiole morpho-anatomy. The flat form of the petiole was observed in the *Eremostachys* genus. Oval petioles were present in *Salvia moorcroftiana* and *Scutellaria linearis*. The shapes of the petioles varied among genera and within each genus, allowing separation of the examined taxa (Table 26). Alosaimi et al. (2023) stated that to overcome the pressure, put on leaf margins by wind rotation and gravitational forces over time, the micro-petiole structure also evolved an arch shape. Therefore, the variations in petiole shape offer the ecological interpretation of the eco-zones of Lamiaceous species. Reniform, triangular, arc-shape, and half circle varieties of petiole outlines were previously described by Ya'ni et al. (2018) as an important characteristic for identifying Lamiaceous species. Currently, the largest petiole was observed in *Eremostachys vicaryi* with a mean length of 7425.6µm.

Cuticle was present in two forms: smooth and undulated (Akçin et al., 2011). The smooth cuticle was present in 12 species, while the undulated type was noted in 13 species. In *Scutellaria linearis* and *Stachys parviflora* thick cuticle was observed. An undulated cuticle was observed in the members of the *Salvia* genus. Akçin et al. (2011) also reported the undulated cuticle in *Salvia*. Uniseriate, multiseriate, and unicellular forms of trichomes were observed. In the Lamiaceae and related families, the taxonomic relevance of the structure of the trichome was well established (Metcalfe and Chalk, 1972; Kahraman et al., 2010). Uniseriate and unicellular trichomes were present in most of the taxa. The *Salvia* genus was characterized by its uniseriate trichomes. The multiseriate trichomes were noted in *Ajuga alpina*. Previously the genus *Teucrium* faced difficulty in the classification of species based on their morphological attributes. The affinities among some of the members created confusion in finding interspecific relationships and boundaries. The trichome variations in the members of *Teucrium* assisted in the discrimination of the species within this genus (Dinç et al., 2010). Similarly, in the present study, *Teucrium scordium* was distinguished from *Teucrium stocksianum* by its undulated cuticle. The trichomes in the *Scutellaria linearis* were present in unicellular and in *Stachys parviflora* uniseriate form. Kahraman et al., (2010) examined the unicellular, and uniseriate trichomes in *Salvia chrysophylla*.

b) Histology of Lamiaceous species

The layers and shapes of parenchyma, collenchyma, and chlorenchyma were observed in the petiole cross-section. Sclerenchyma layers were not observed in the examined species. Sclerification between the vascular bundles was present in 14 species. *Salvia coccinea* had a prominent sclerenchyma layer between the vascular bundles. There were 2-3 layers of collenchyma along with a subepidermal ring, and 9 layers of parenchyma in the *Eremostachys vicaryi*. All species had distinct xylem parenchyma cells along with the xylem vessels. The morphologies of the epidermal cells varied from round to oval, rectangular to square, and round to oval, and rectangular. The angular epidermis was predominant in petioles the Lamiaceous species. *Otostegia aucheri* had the maximum mean value of epidermis width, 38.25µm. The observed shapes of parenchyma were angular to isodiametric, irregular, and tri to hexagonal. Irregular to isodiametric shape was present in *Thymus linearis* subsp. *hedgei Jalas*.

Collenchyma cell shapes were angular to lamellar, lacunar, and annular. In *Nepeta* genus, angular and annular collenchyma were noticed. In *Stachys parviflora* and *Ajuga bracteosa*, angular to lamellar types were present. The shape of collenchyma and parenchyma in *Thymus linearis* was angular and isodiametric (polygonal), similar was reported by Alosaimi, (2023). *Scutellaria linearis* was observed with lacunar collenchyma and tri-hexagonal parenchyma, whereas Alosaimi, (2023) reported angular and polygonal shapes respectively. The xylem vessels in *Thymus* genus were found in numerous shapes, including round to oval, oval to rectangular, and angular. Variations in the xylem vessels of *Nepeta* species were observed. Xylem vessels were rectangular to oval in *Nepeta glomerulosa*, and rectangular to square in *Nepeta hindostana*. Phloem parenchyma was observed in different shapes i.e. tri to hexagonal, tetra to hexagonal, and angular. The hexagonal parenchyma shape was observed in *Salvia cabulica.*

c) Vascularization in the petiole of Lamiaceous flora

According to Metcalfe and Chalk (1972), the arrangement of vascular bundles in the petioles of Lamiaceae species can serve as a diagnostic feature. Primarily four types of vascular bundles were determined, collateral closed, collateral open, bicollateral, and amphicribral. In 13 species, the collateral closed type was present. The collateral open vascularization was noticed in four species. Bicollateral vascular bundles were present in *Salvia macrosiphon.* The amphicribral type of vascularization was observed in six species. In the genus *Eremostachys* the distribution of vascular bundles were 2+4 and in the *Salvia* genus, it was 1+2. The genus *Salvia* was distinguished specifically for having collateral type of vascular bundles (Celep et al., 2014). A single vascular bundle was present in most of the species including all the members of the genus *Nepeta*. Thus, for *Eremostachys, Salvia,* and *Nepeta* the number of vascular bundles was a characteristic feature taxonomically. In *Salvia leucantha* one small and two large vascular bundles were observed. Akçin et al. (2011) examined the petiole anatomy with special emphasis on the number of vascular bundles in the Lamiaceous taxa. They also documented that the vascular bundles in *Salvia* species existed in the form of 3, 7 and 11.

The vascular system is the most focused feature in the research on petiole anatomy. In *Salvia*, there were vascular bundles in the shape of an arc in the center and one in each of the four corners (Jehanzeb et al., 2020). Besides the number and shapes of vascularization, the addition of the arrangement of the vascular bundles in the current examination helped in providing new insights into the systematics of Lamiaceae taxa. Previous research on two endemic *Salvia* species by Gürdal et al., (2019) revealed collateral type bundles in the petiole cross-section. Information of taxonomic relevance can be found in the larger upper epidermal cells, two to three big vascular bundles in the center, and two to four minor subsidiary bundles in the wings of the petiole in *Salvia chrysophylla* (Kahraman et al., 2010). In the present analysis, the 1+2 arrangement of vascular bundles was prevalent in *Salvia leucantha* and *Salvia macrosiphon.* Previous studies also documented that the structure of the vascular bundles in petioles can be used to identify species in the Lamiaceae family (Metcalfe and Chalk, 1972; Siebert, 2004; Kahraman et al., 2010).

d) Multivariate analysis

A numerical analysis of the length and width of cells of the epidermis, collenchyma, chlorenchyma, parenchyma, vascular bundles, xylem vessels, and phloem was carried out. The principal component analysis accounted for 94.261% and 3.13% variance between PC1 and PC2. The Eigen variables were evaluated to explain the statistical percentage variation among quantitative attributes using PCA clustering of microanatomical petiole features. The xylem vessel width, phloem width, phloem length, and xylem vessel length were the most significant variables. While the vascular bundle length was insignificant among the examined variables (Figure 31). Based on quantitative traits, the two-way dendrogram illustrated the similarity along the Euclidean distance among Lamiaceous species. *Thymus linearis* was placed separately from the major cluster. The dendrogram was separated into two major clades. The A1 cluster consisted of 18 species whereas A2 had 6 taxa (Figure 32). Between *Nepeta Hindostana* and *Ocimum africanum*, the sub-cluster showed the highest phylogenetic association in A1. In the A2 cluster, *Rydingia limbate* and *Salvia cabulica* were placed closest. The quantitative variables significantly separated the examined taxa. The positive and negative correlation between the quantitative variables was determiend in the correlation plot (Figure 33). The highest positive correlation 0.94 was observed between collenchyma width and collenchyma length. A positive association also existed between parenchyma width and collenchyma length, phloem width, and chlorenchyma length. The maximum negative correlation of -0.42 was noted for vascular bundle width and epidermis length. The identification and separation of the Lamiaceous taxa based on the determined qualitative features via taxonomic key is demonstrated in Table 29. In conclusion, the anatomical aspects of petioles such as outline, cell shapes, sizes and layers, vascular bundles number, size, and arrangement, were diagnostic in the recent systematics for the separation of taxa at various taxonomic ranks.

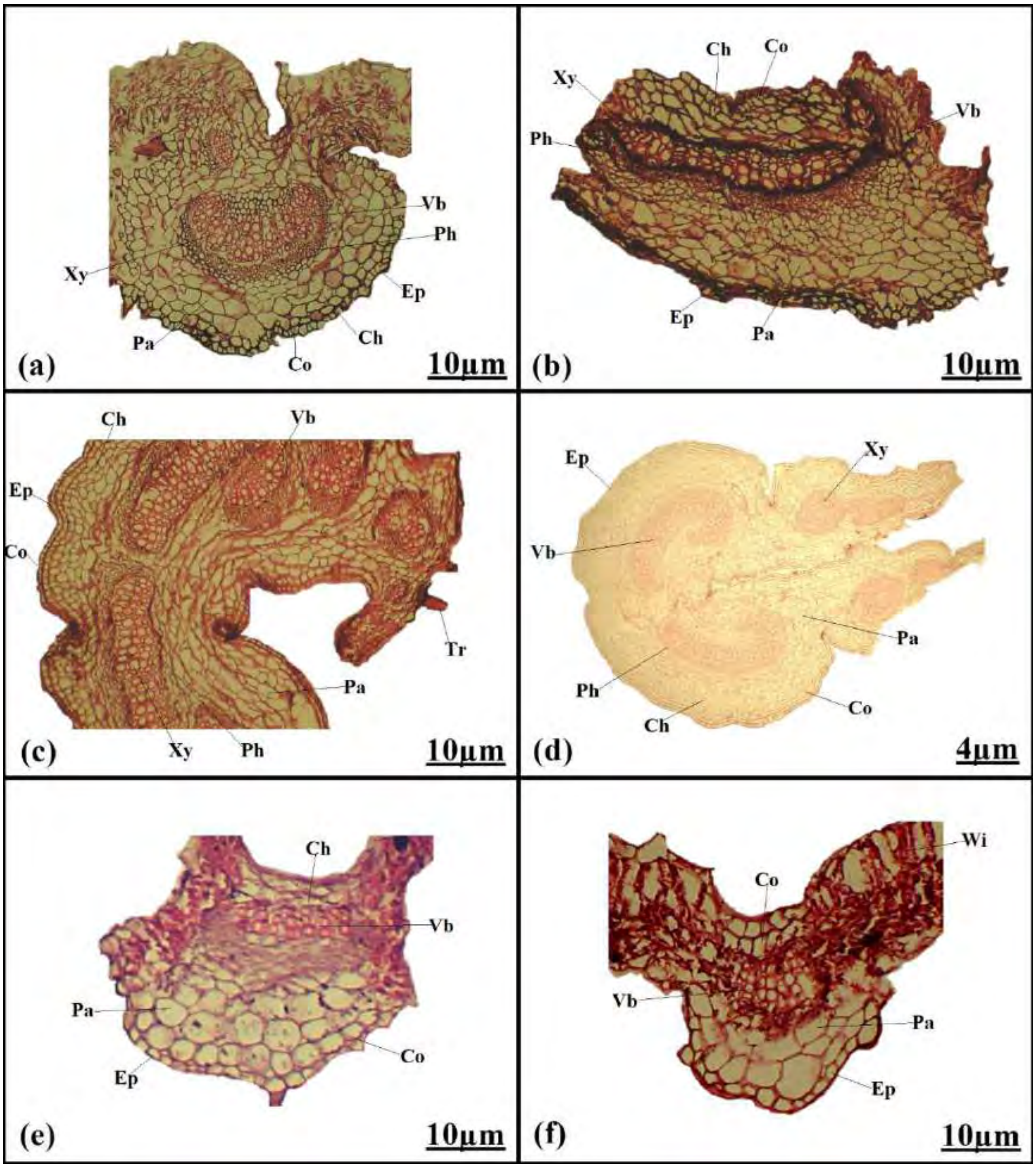

Plate 68. Photomicrographs of petiole anatomy (a) *Ajuga alpina*, (b) *Ajuga bracteosa*, (c) *Eremostachys thyrsiflora*, (d) *Eremostachys vicaryi*, (e) *Nepeta cataria*, (f) *Nepeta eriosphaera,* (Cu: cuticle, Ep: Epidermis, Pa: parenchyma, Co: collenchyma, Ch: chlorenchyma, Sc: sclerenchyma, Xy: xylem, Ph: Phloem). Scale:10µm (a,b,c,e,f),4µm(d)

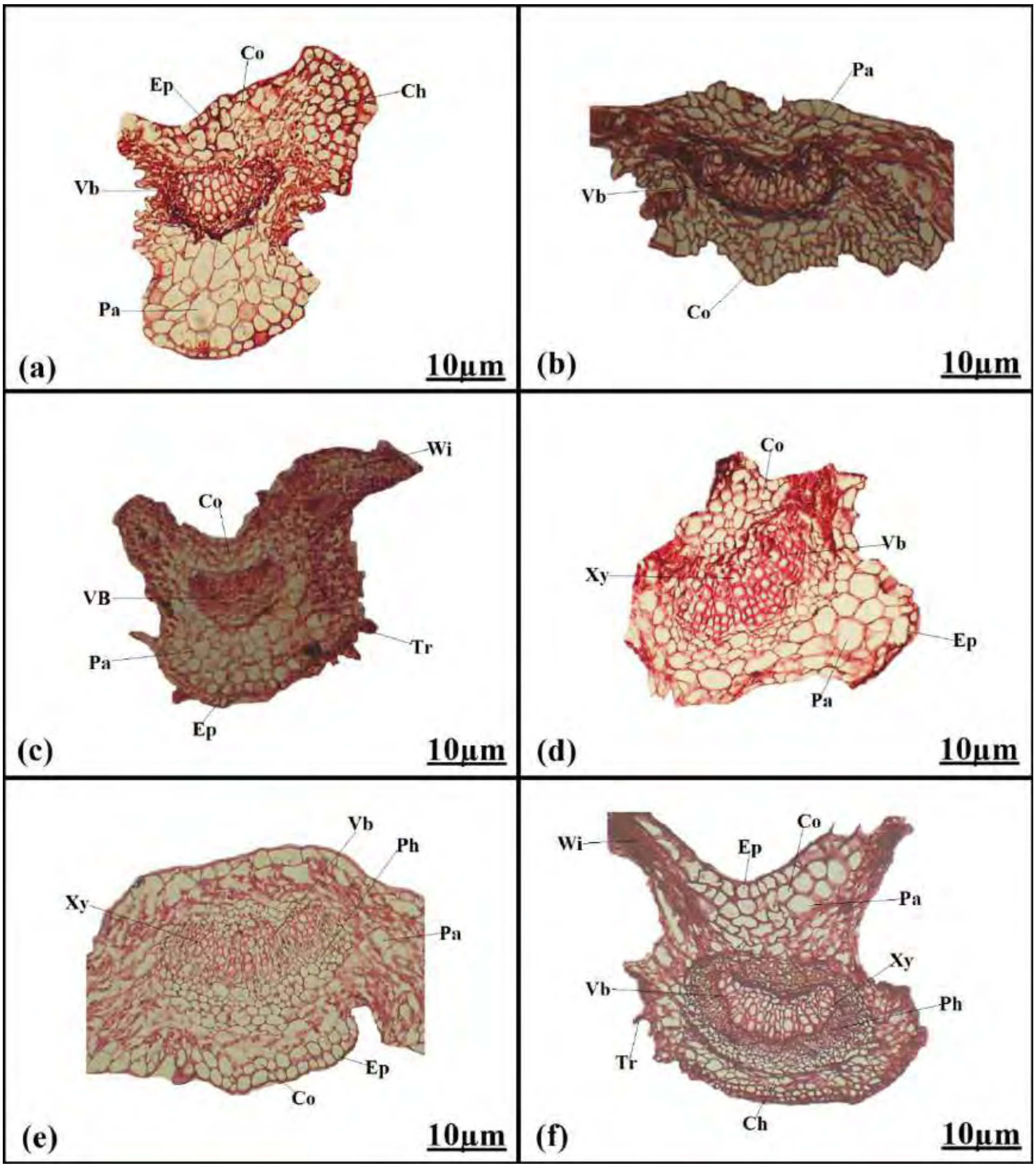

Plate 69. Photomicrographs of petiole anatomy (a) *Nepeta glomerulosa*, (b) *Nepeta Hindostana*, (c) *Nepeta juncea*, (d) *Ocimum africanum*, (e) *Otostegia aucheri,* (f) *Phlomis stewartii,* (Cu: cuticle, Ep: Epidermis, Pa: parenchyma, Co: collenchyma, Ch: chlorenchyma, Sc: sclerenchyma, Xy: xylem, Ph: Phloem). Scale:10µm

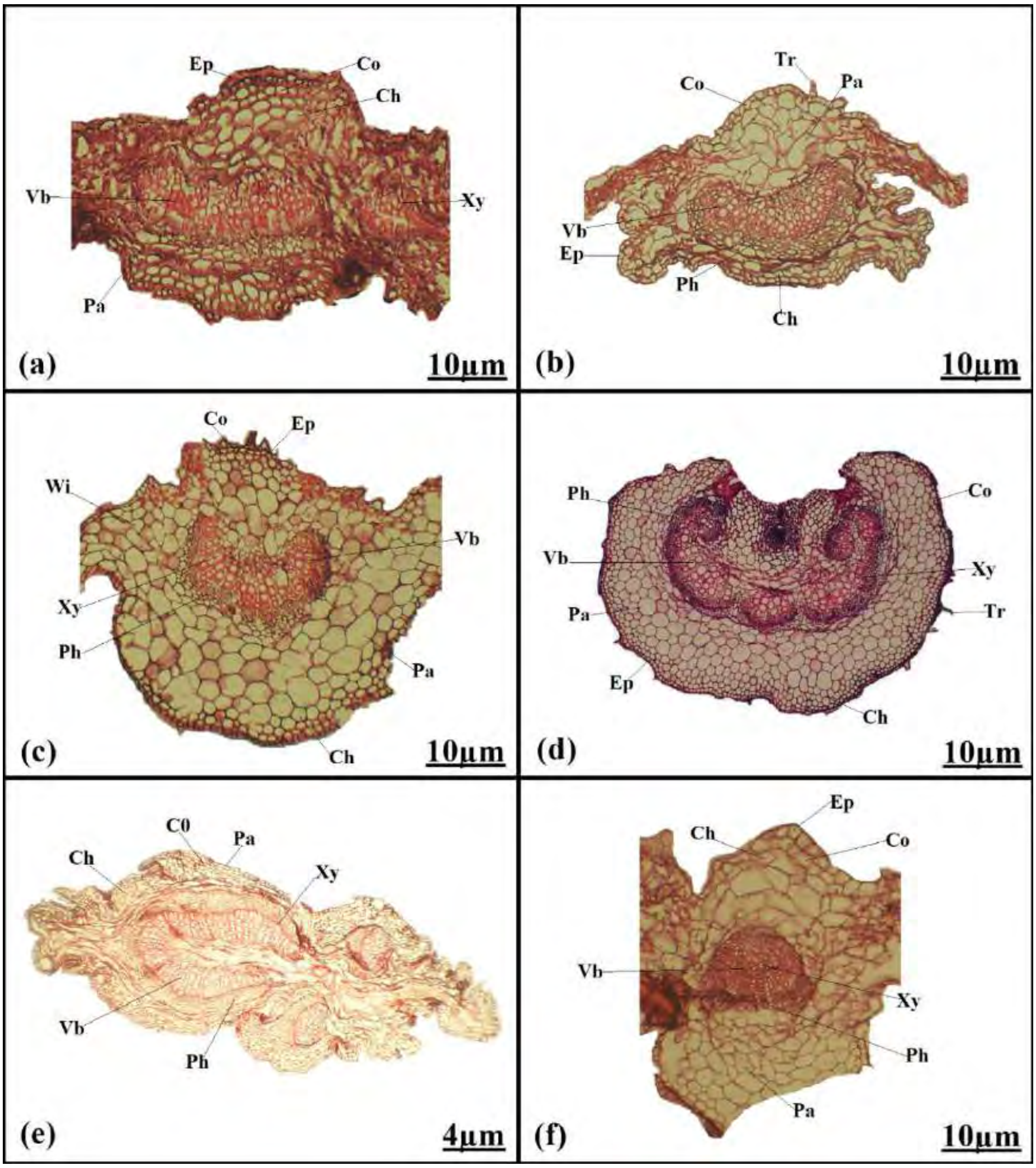

Plate 70. Photomicrographs of petiole anatomy (a) *Otostegia limbata*, (b) *Salvia cabulica*, (c) *Salvia coccinea*, (d) *Salvia leucantha*, (e) *Salvia macrosiphon*, (f) *Salvia moorcroftiana,* (Cu: cuticle, Ep: Epidermis, Pa: parenchyma, Co: collenchyma, Ch: chlorenchyma, Sc: sclerenchyma, Xy: xylem, Ph: Phloem). Scale:10µm (a,b,c,d,,f),4µm(e)

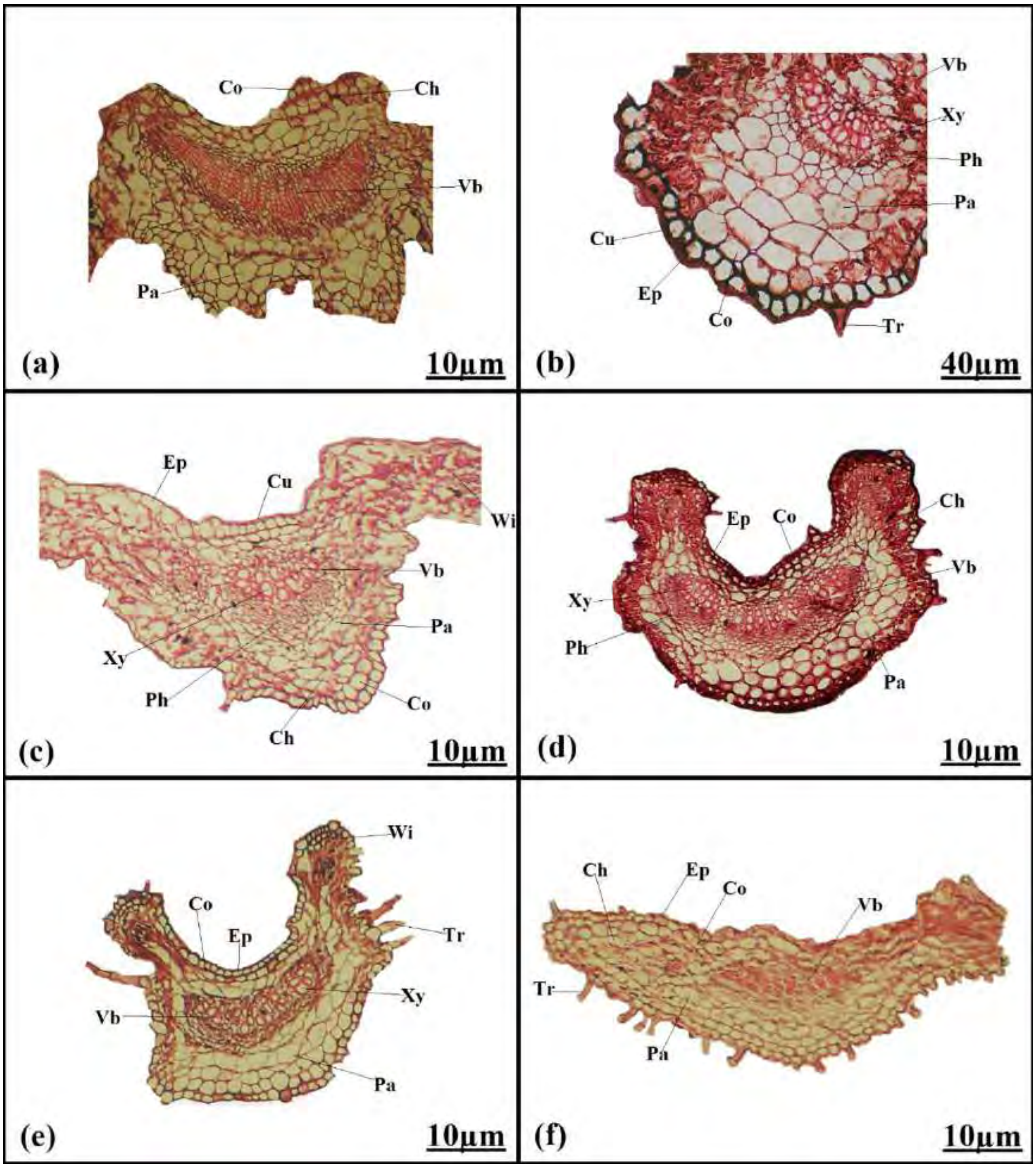

Plate 71. Photomicrographs of petiole anatomy (a) *Salvia plebeia* , (b) *Scutellaria linearis*, (c) Stachys parviflora, (d) *Teucrium scordium*, (e) *Teucrium stocksianum*, (f) *Thymus linearis subsp. hedgei Jalas,* (Cu: cuticle, Ep: Epidermis, Pa: parenchyma, Co: collenchyma, Ch: chlorenchyma, Sc: sclerenchyma, Xy: xylem, Ph: Phloem). Scale:10µm(a,c,e,f),40µm (b)

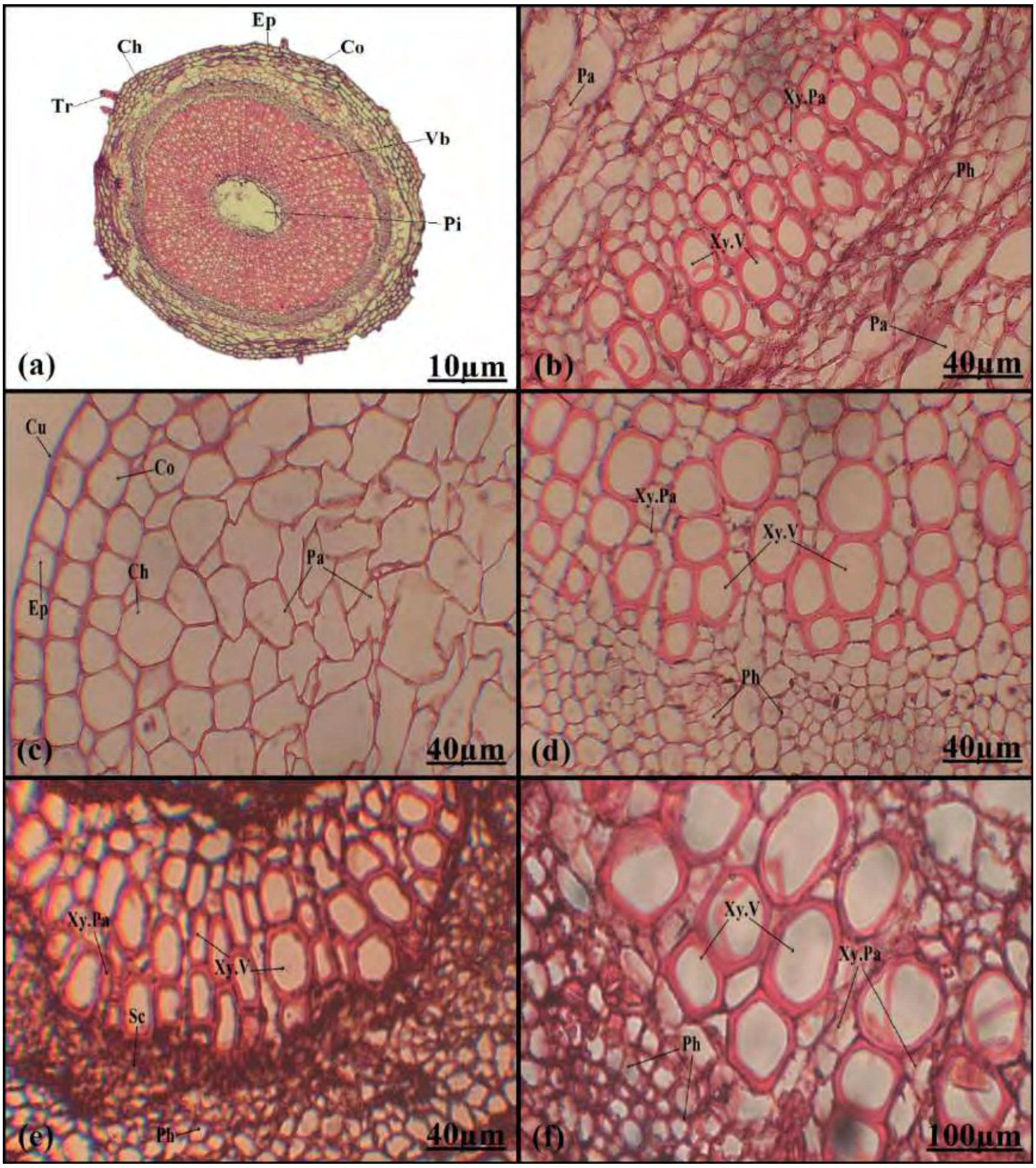

Plate 72. Photomicrographs of petiole anatomy (a) *Thymus linearis*, (b) *Eremostachys thyrsiflora*, (c,d) *Eremostachys vicaryi,* (e) *Phlomis stewartii* , (f) *Salvia leucantha,* (Cu: cuticle, Ep: Epidermis, Pa: parenchyma, Co: collenchyma, Ch: chlorenchyma, Sc: sclerenchyma, Xy: xylem, Ph: Phloem). Scale:10µm(a),40µm (b,c,d,e),100 µm(f)

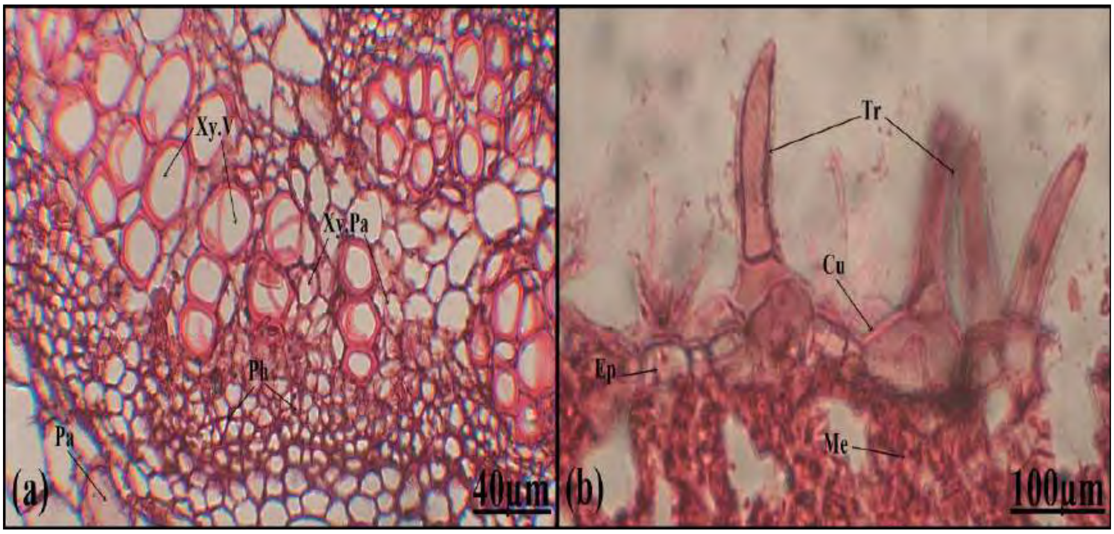

Plate 73. Photomicrographs of petiole anatomy (a) *Salvia leucantha*, (b) *Scutellaria linearis*, (Cu: cuticle, Ep: Epidermis, Pa: parenchyma, Co: collenchyma, Ch: chlorenchyma, Sc: sclerenchyma, Xy: xylem, Ph: Phloem). Scale:40µm(a),100µm (b)

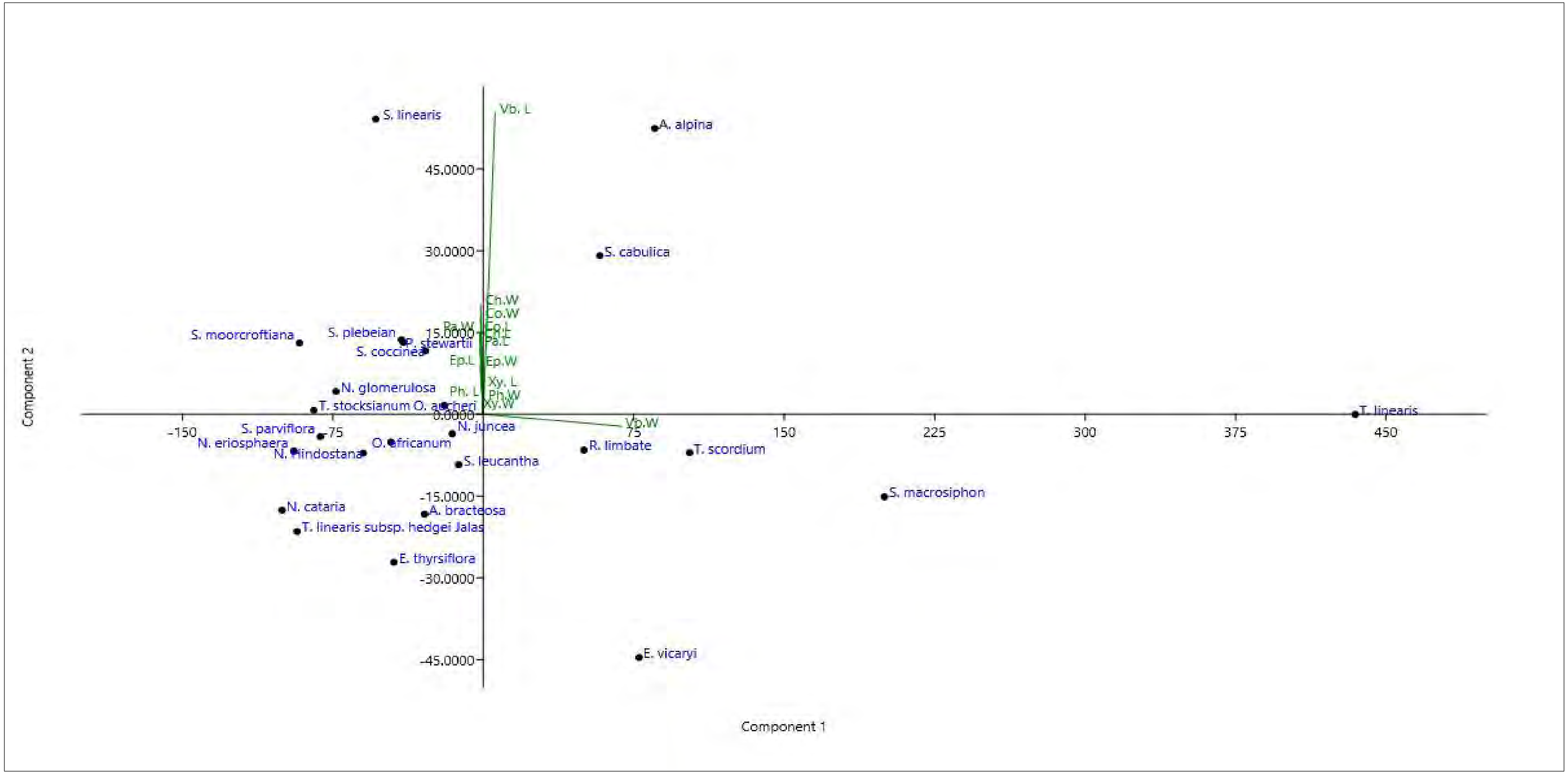

Figure 31. Utility of petiolar features in discriminating among species of Lamiaceae by PCA (epidermis length and width, collenchyma length andwidth, chlorenchyma length and width, parenchyma length and width

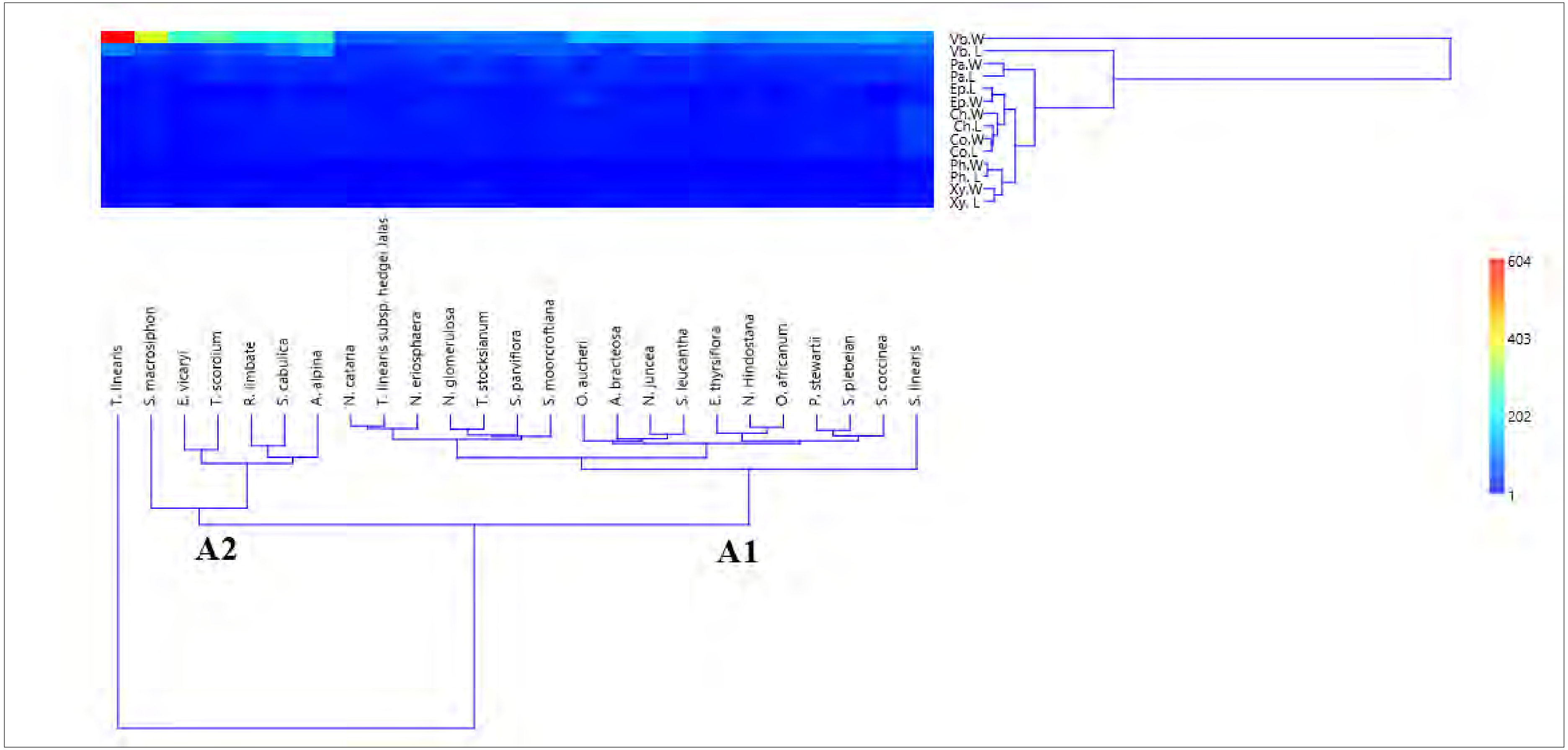

Figure 32. Dendrogram showing the similarity index of Lamiaceae taxa based on quantitative parameters of petiole

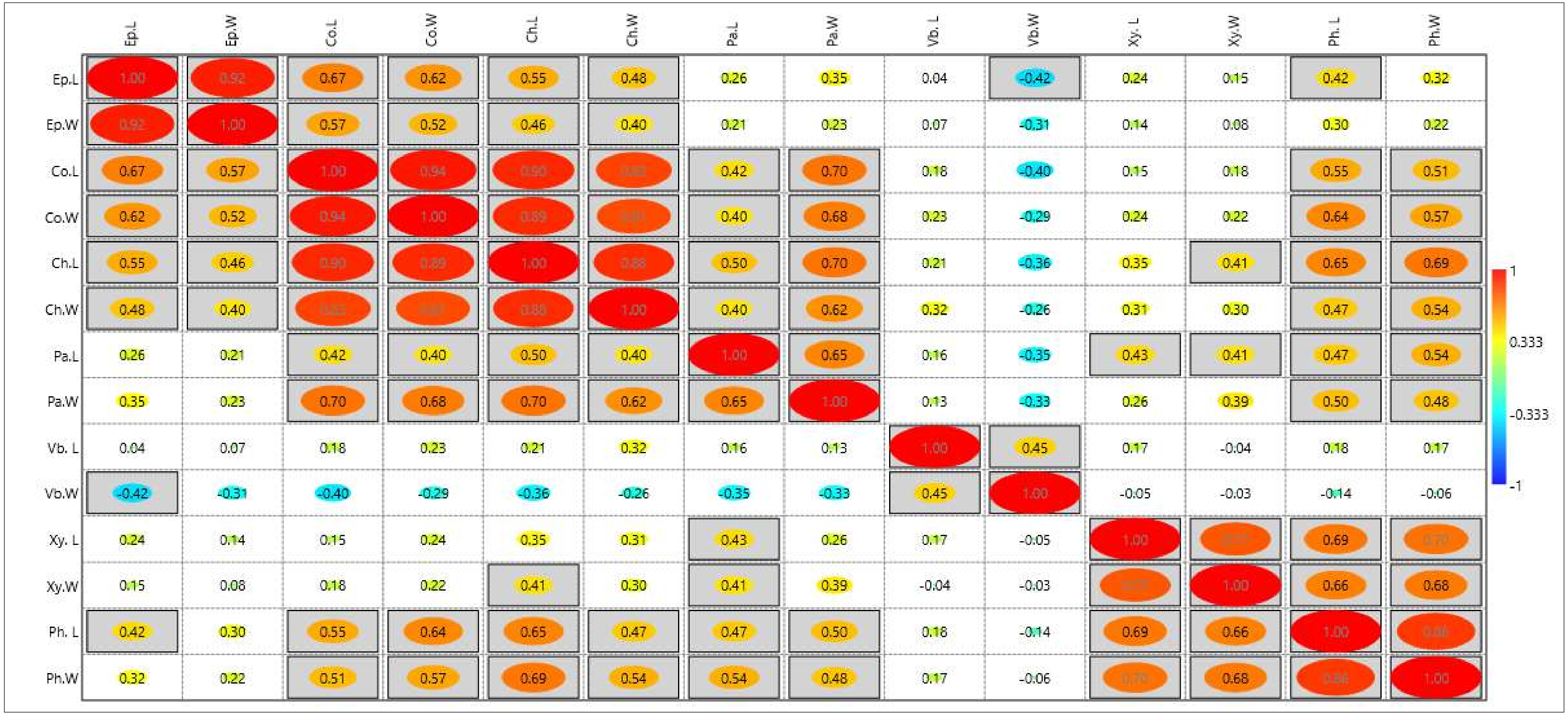

Figure 33. Correlation among the mean values of Ep.L: Epidermal length; Ep.W: Epidermal width; Co.L: Collenchyma length:Co.W: Collenchyma width;Ch.L:Chlorenchyma length; Ch.W: Chlorenchyma width;Vb.L:Vascularbumdle length; Vb.W: Vascular bumdle width

Table 26: Qualitative characteristics of the petiole anatomy of studied Lamiaceous taxa

Plant Name	No. of Co layer	No. of Pa layer	No. of Vbs	Sc in Vbs	Tr	Wing	Groove in surface	Sub-epidermal ring of Co	Xy.Pa
Ajuga alpina	1	3	1+2	+	+	+	+	+	+
Ajuga bracteosa	1	3	1	+	-	+	+	-	+
Eremostachys thyrsiflora	1	5	2+4 (2large, 4 small)	-	-	+	+	+	+
Eremostachys vicaryi	2-3	9	2+4 (2large, 4 small)	-	-	+	+	+	+
Nepeta cataria	-	3	1	-	+	+	+	-	+
Nepeta eriosphaera	-	2	1	-	-	+	+	-	+
Nepeta glomerulosa	1	4	1	+	-	-	-	+	+
Nepeta Hindostana	-	3	1	-	+	+	+	-	+
Nepeta juncea	1	4	1	-	-	+	+	+	+
Ocimum africanum	-	4	1	-	-	+	+	-	+
Otostegia aucheri	-	5	1	+	-	+	-	-	+
Phlomis stewartii	1	3	1	+	+	+	+	-	+
Otostegia limbate	2	4	1	+	-	+	+	+	+
Salvia cabulica	1	4	1	-	+	+	+	-	+
Salvia coccinea	1	4	1	+	+	+	+	-	+

Salvia leucantha	3	6	1+2(1small, 2large	-	+	-	+	+	+
Salvia macrosiphon	2	3	1+2	+	-	-	+	+	+
Salvia moorcroftiana	-	4	1	+	-	+	+	-	+
Salvia plebeian	2	4	1	-	-	+	+	+	+
Scutellaria linearis	1	3	1	+	+	+	-	-	+
Stachys parviflora	1	3	1	+	-	+	+	-	+
Teucrium scordium	1	3	1+2	+	+	+	+	+	+
Teucrium stocksianum	1	3	1	+	+	+	+	+	+
Thymus linearis	1	2	1	+	+	+	+	+	+
Thymus linearis subsp. hedgei Jalas	1	2	1	-	+	+	+	+	+

Table 27. Quantitative characteristics of the petiole anatomy of studied Lamiaceous taxa

Plant Species	L. W	Epidermal cell (µm)	Collenchyma (µm)	Chlorenchyma (µm)	Parenchyma(µm)	Vascular bundle (µm)	petiole	Xylem(µm)	Phloem
Mean±SE									
Scutellaria linearis	L	21.95±0.398434436	40.75±0.379143772	44.4±0.760756203	41.15±0.976601249	81.55±0.348209707	1223.2±8.266801074	18.25±0.176776695	9.25±0.575543222
	W	21.7±0.2	49.6±0.760756203	48.7±0.796084166	57.25±0.866025404	120.65±0.465026881	1815.2±4.066939882	16.05±0.215058132	7.75±0.306186218
Nepeta glomerulosa	L	20.15±0.231840462	13.15±0.257390754	19.4±0.6254998	38.5±0.541987085	61.95±0.588430115	1225.2±7.819207121	18.3±0.215058132	4.5±0.25
	W	20.15±0.231840462	15±0.353553391	22.4±0.527967802	29.65±0.673609679	99.95±0.588430115	640.4±15.29901958	12.65±0.231840462	3.95±0.289395923
Salvia leucantha	L	6.75±0.176776695	9.95±0.398434436	13.25±0.176776695	34.85±0.437321392	60.8±0.902773504	2005.8±1.392838828	20.15±0.231840462	5.75±0.176776695
	W	3.5±0.176776695	9.35±0.635413251	11±0.176776695	31.75±0.176776695	159.45±3.29526175	3053.4±15.71814238	12.65±0.231840462	4.35±0.127475488
Salvia plebeia	L	16.8±0.357071421	20.85±0.302076149	18.4±0.231840462	43.8±0.991841721	71.65±0.673609679	1830±10.16857906	9.75±0.306186218	6.65±0.231840462
	W	15.05±0.289395923	22.05±0.398434436	15.5±0.541987085	40.9±0.682825014	131.45±2.221204628	3230.2±12.9514478	7.5±0.353553391	3.65±0.302076149
Nepeta Hindostana	L	15.45±0.215058132	12.9±0.451386752	17.75±0.285043856	20.75±0.306186218	56.65±0.831414457	1225±7.286974681	11.25±0.176776695	3.35±0.127475488
	W	13.7±0.310241841	13.75±0.262202212	17.65±0.231840462	32.8±0.48347699	112.45±0.508674749	2218.4±8.6	11.1±0.322102468	2±0.25
Nepeta juncea	L	15.75±0.176776695	17.25±0.306186218	17.7±0.435889894	29.3±0.365718471	61.1±0.718505393	963.6±11.68588893	9.15±0.231840462	1.85±0.16955825
	W	14.3±0.413823634	14.85±0.231840462	22.35±0.231840462	21.6±0.231840462	156.8±2.964793416	1418.8±5.435071297	4.25±0.176776695	3.25±0.176776695
Teucrium stocksianum	L	13.55±0.266926956	17.65±0.231840462	20±0.353553391	36.55±0.348209707	57.75±0.728868987	1003±0.707106781	9.25±0.176776695	4.2±0.310241841
	W	12.95±0.215058132	17.15±0.20310096	19.05±0.145773797	37.6±0.4	88.2±0.242383993	1809.6±4.178516483	14±0.176776695	3.15±0.127475488

Salvia cabulica	L	14.9±0.322102468	16.95±0.470372193	20.9±0.367423461	37.05±0.619475585	95.65±0.6254998	1526.4±20.17324961	20.75±0.176776695	4.4±0.127475488
	W	17.95±0.242383993	18.05±0.634428877	18.3±0.215058132	40.55±0.88529656	228.7±1.412887115	2830.6±10.78239306	13.25±0.176776695	4.05±0.289395923
Salvia macrosiphon	L	11.5±0.25	13.4±0.231840462	15.85±0.127475488	15.8±0.215058132	57.3±0.9367497	6006±1	13.25±0.176776695	4.1±0.231840462
	W	10.75±0.176776695	18.35±0.231840462	20.95±0.266926956	29.95±0.577711	373.5±2.467285958	2020.4±10.25962962	11.2±0.339116499	2.65±0.231840462
Otostegia aucheri	L	26.05±1.80520082	20.95±0.413823634	20.75±0.176776695	28.35±0.515994186	58.05±0.619475585	2150.6±60.81414309	11.6±0.231840462	4.6±0.331662479
	W	38.25±0.379143772	19.35±0.331662479	21.25±0.176776695	24.25±0.176776695	154.15±1.621342037	3986.2±20.33568292	9.55±0.348209707	3.15±0.127475488
Teucrium scordium	L	3.75±0.176776695	10.75±0.58630197	13.2±0.145773797	37.45±0.398434436	62.55±1.070630655	918.8±5.969924623	15.85±0.231840462	3.25±0.176776695
	W	6.6±0.26925824	8.8±0.55	20.6±0.127475488	39.2±0.215058132	275.6±1.108489964	4312.8±21.81604914	12.85±0.16955825	3.75±0.32596012
Nepeta eriosphaera	L	16.85±0.792937576	19.65±0.340954542	19.3±0.435889894	23.05±0.266926956	47.6±0.87535707	614.8±6.613622306	7.9±0.392109679	3.1±0.127475488
	W	15.75±0.176776695	15.75±0.176776695	27.1±0.645174395	38±0.379143772	79.05±1.668457371	1521±5.540758071	4.95±0.289395923	2.95±0.093541435
Phlomis stewartii	L	22.05±0.398434436	24.75±0.285043856	15.7±0.456891672	28.2±0.708872344	69.15±1.184799561	2500.6±19.85346317	12.65±0.231840462	3.75±0.176776695
	W	21.5±0.447213595	25.65±1.246495086	24.75±0.285043856	35.9±0.6	132.65±2.007174631	2799.8±3.733630941	6.5±0.176776695	1.75±0.32596012
Otostegia limbate	L	5.15±0.231840462	19.65±0.340954542	19.2±0.215058132	24.8±0.266926956	62.3±0.807774721	2528.6±9.942836617	3.85±0.127475488	2.05±0.215058132
	W	5.1±0.20310096	13.6±0.444409721	19.2±0.215058132	31.6±0.527967802	222.5±1.185854123	4995.4±4.130375286	8.25±0.176776695	1.9±0.187082869
Eremostachys vicaryi	L	3.65±0.302076149	4.25±0.176776695	4.35±0.257390754	18.25±0.176776695	37.3±0.266926956	3001.8±3.307567082	5.15±0.231840462	1.75±0.176776695
	W	5.15±0.231840462	3.7±0.215058132	3.45±0.145773797	20.75±0.176776695	250.55±0.45	7425.6±105.0897711	7.95±0.215058132	1.95±0.266926956
Ajuga alpina	L	12.95±0.374165739	19.95±0.365718471	20±0.353553391	24.85±0.358817502	126.85±0.788986692	1176±11.87434209	8.25±0.176776695	2.65±0.231840462
	W	14.9±0.51599418 6	20.15±0.231840462	33±0.661437828	31.25±0.418330013	253.25±2.007797301	2234±5.865151319	4.95±0.520816666	1.55±0.215058132

Salvia coccinea	L	3.25±0.176776695	13.95±0.348209707	19.3±0.629483916	39.85±0.407737661	75.6±0.358817502	2610.6±15.97059799	8.2±0.183711731	1.75±0.176776695
	W	5.3±0.289395923	16±0.176776695	27±0.684653197	34.9±0.375832409	142.25±0.925337776	3044.4±19.15097909	4.85±0.231840462	1.75±0.176776695
Thymus linearis subsp. hedgei Jalas	L	12.15±0.465026881	16.6±0.384057287	18.25±0.176776695	22.95±0.215058132	37.85±0.302076149	691±7.791020472	5.45±0.215058132	2.35±0.23184046
	W	13.15±0.768927825	15.35±0.340954542	16.3±0.215058132	30.7±0.691013748	80.8±1.2509996	994.6±4.675467891	4.25±0.176776695	1.75±0.17677669
Thymus linearis	L	5.3±0.289395923	6.3±0.289395923	7.05±0.215058132	18.55±0.348209707	103±1.126388033	1754.8±9.248783704	4.25±0.176776695	2.05±0.215058132
	W	7.65±0.231840462	8.5±0.176776695	5.5±0.25	20.75±0.176776695	604.35±1.476058942	1516±6.985699679	2.35±0.231840462	1.75±0.176776695
Nepeta cataria	L	13.5±0.176776695	20.45±0.215058132	18.15±0.257390754	30.5±0.25	34.65±0.322102468	602.8±2.517935662	3.25±0.176776695	1.9±0.127475488
	W	16.85±0.257390754	24.75±0.32596012	17.6±0.444409721	35.1±0.4	74.3±0.320156212	708±2.449489743	2.05±0.215058132	1.75±0.176776695
Stachys parviflora	L	11.65±0.231840462	13.1±0.127475488	13.2±0.215058132	21.55±0.215058132	66.45±0.443001129	1002±1.224744871	10.15±0.6254998	2.65±0.231840462
	W	12.35±0.231840462	14.6±0.257390754	12±0.25	22.05±0.215058132	89.55±1.064776972	1486.4±3.544009029	3.25±0.176776695	1.5±0.176776695
Salvia moorcroftiana	L	17.35±0.231840462	19.05±0.348209707	16.05±0.215058132	31.75±0.637377439	73.25±0.977880361	1315±5.674504384	2.4±0.257390754	1.5±0.176776695
	W	18.95±0.348209707	17.05±0.215058132	16.45±0.463680925	45.45±0.555652769	79.85±0.610327781	1840.2±12.18769872	1.75±0.176776695	1±0.176776695
Ajuga bracteosa	L	15.35±0.302076149	15.9±0.430116263	15.45±0.215058132	34.8±0.614410286	38.35±0.231840462	1000.8±3.411744422	9.1±0.231840462	1.85±0.127475488
	W	16.95±0.456891672	14.45±0.266926956	17.65±0.231840462	40.15±0.231840462	145.35±1.663204738	2419.4±8.078366171	10.75±0.176776695	1±0.176776695
Ocimum africanum	L	8.75±0.32596012	13.85±0.26925824	14.35±0.302076149	23.8±0.348209707	62.85±0.407737661	780±5.941380311	8.45±0.266926956	3.85±0.231840462
	W	11.8±0.708872344	15.15±0.231840462	14.95±0.289395923	29.55±0.215058132	125.45±0.215058132	683.8±8.481745103	6.6±0.302076149	1.8±0.16583124
	L	6.55±0.145773797	11.35±0.257390754	13.45±0.215058132	21.15±0.257390754	40.1±0.678232998	1002.4±2.271563338	11.85±0.302076149	3.25±0.176776695

Eremostachys thyrsiflora	W	6±0.25	11.75±0.32596012	14.35±0.322102468	25.9±0.231840462	128.45±1.379311422	2976.4±24.58780185	8.1±0.127475488	1.6±0.231840462

Table 28. Qualitative features of studied Lamiaceous taxa

Plant Name	Pe shape	Ep shape	Pa shape	Col shape	Vb Arrangem ents	Tr shape	Cu structure	Xy Vessel	Ph Shape
Ajuga alpina	Sulcate	Irregular	Irregular	Angular	Collateral closed	Multiseri ate	Undulated	Round to Oval	Tri to hexagonal
Ajuga bracteosa	Sulacte	Rectangular to square	Angular to isodiametri c	Angular to lamella	Collateral open	unicellul ar	Undulated	Round to angular	Tri to hexagonal
Eremostachys thyrsiflora	Flat	Square to rectangular	Tri to hexagonal	Augular	Collateral closed	unicellul ar	Undulated	Round to Oval	Angular
Eremostachys vicaryi	Flat	Square	Irregular	Lamellar	Amphicribr al	-	Smooth	Round	Angular
Nepeta cataria	Sulcate	Square to angular	Isodiametri c	Lacunar	Collateral closed	-	Smooth	Round to angular	Tri to hexagonal
Nepeta eriosphaera	Sulcate	Rectangular to Oval	Angular	Angular	Collateral closed	-	Undulated	Angular	Angular
Nepeta glomerulosa	Flat	Round to Oval	Irregular	Annular	Collateral closed	-	Smooth	Rectangular to Oval	Tri to hexagonal
Nepeta Hindostana	Flat	Angular	Irregular	Angular	Amphicribr al	Unicellu lar	Smooth	Rectangular to Square	Angular
Nepeta juncea	Sulcate	Angular	Isodiametri c	Annular	Collateral closed	Uniseriat e	Smooth	Round	Tetra to hexagonal
Ocimum africanum	Sulcate	Angular	Isodiametri c	Angular	Collateral closed	-	Undulated	Round	Tetra to hexagonal
Otostegia aucheri	Sulcate	Round to Oval to Rctangula	Irregular	Lacunar	Amphicribr al	-	Smooth	Angular to Oval	Tetra to hexagonal

Phlomis stewartii	Sulcate	Irregular	Irregular	Annular	Amphicribral	Unicellular	Smooth	Oval to rectangular	Angular
Otostegia limbate	Flat	Angular to Rectangular	Isodiametric	Annular	Collateral closed	-	Undulated	Angular	Tri to hexagonal
Salvia cabulica	Sulcate	Angular	Irregular	Lamellar	Collateral open	Uniseriate	Smooth	Round to oval	Hexagonal
Salvia coccinea	Sulcate	Rectangular to Angular	Hexagonal to Angular	Angular	Collateral closed	Uniseriate	Undulated	Oval to round	Tri to hexagonal
Salvia leucantha	Round	Rectangular	Tetra to hexagonal	Angular	Collateral open	Uniseriate	Undulated	Oval to round	Tri to hexagonal
Salvia macrosiphon	Flat	Rectangular	Isodiametric	Lamellar	Bi-Collateral	-	Smooth	Oval to angular	Angular
Salvia moorcroftiana	Oval	Angular	Tri to hexagonal	Angular	Collateral closed	-	Smooth	Round to oval	Tetra to hexagonal
Salvia plebeia	Flat	Squar and Angular	Isodiametric	Lacunar	Collateral open	-	Smooth	Round	Tetra to hexagonal
Scutellaria linearis	Oval	Round to Oval	Tri to Hexagonal	Lacunar	Collateral closed	Unicellular	Undulated	Oval & Round	Angular
Stachys parviflora	Sulcate	Square to angular	Irregular	Lamellar to angular	Collateral closed	Uniseriate	Undulated	Oval to round	Tri to hexagonal
Teucrium scordium	Sulcate	Rectangular to Oval	Isodiametric	Annular	Collateral closed	Uniseriate	Undulated	Oval	Tetra to hexagonal
Teucrium stocksianum	Sulcate	Angular	Tri to Hexagonal	Lacunar	Collateral closed	Uniseriate	Smooth	Oval to Rectangular	Angular
Thymus linearis	Sulcate	Rectangular to angular	Isodiametric	Angular	Amphicribral	-	Undulated	Round to oval	Angular
Thymus linearis subsp. hedgei Jalas	Sulcate	Angular	Irregular to Isodimatric	Angular	Amphicribral	Uniseriate	Undulated	Round to Oval	Angular

Table 29: Dichotomous key based on petiole morphological characters of Lamiacous flora from Baluchistan

Link character	Leads	Character	Taxa/Go to link character
1	+	Petiole shape Oval	2
	-	Petiole shape not Oval	3
2	+	Parenchyma cell shape Angular	*Salvia moorcroftiana*
	-	Parenchyma cell shape Round to oval	*Scutellaria linearis*
3	+	Petiole outline Sulcate	4
	-	Petiole outline not Sulcate	18
4	+	Trichome present	5
	-	Trichome absent	17
5	+	Trichome shape multiseriate	*Ajuga alpina*
	-	Trichome shape not multiseriate	6
6	+	Unicellular	7
	-	Uniseriate	8
7	+	Vascular bundles collateral open	*Ajuga bracteosa*
	-	Vascular bundles amphicribral	*Phlomis stewartii*
8	+	Cuticle smooth	9
	-	Cuticle undulated	13
9	+	Collenchyma annular	*Nepeta juncea*
	-	Collenchyma other than annular	10

10	+	Lamellar collenchyma	*Salvia cabulica*
	-	Lacunar collenchyma	11
11	+	Parenchyma shape isodiametric	*Nepeta cataria*
	-	Parenchyma other than isodiametric	12
12	+	Epidermal cell round to oval and rectangular	*Otostegia aucheri*
	-	Angular epidermal cell	*Teucrium stocksianum*
13	+	Angular xylem vessels	*Nepeta eriosphaera*
	-	Xylem vessels other than angular	14
14	+	Annular collenchyma	*Teucrium scordium*
	-	Angular or lamellar to angular collenchyma	15
15	+	Epidermal cells square to angular	*Stachys parviflora*
	-	Epidermal cells angular or rectangular to angular	16
16	+	Vascular bundles collateral closed	*Salvia coccinea*
	-	Vascular bundles amphicribral	*Thymus linearis subsp. hedgei Jalas*
17	+	Phloem tetra to hexagonal	*Ocimum africanum*
	-	Phloem angular	*Thymus linearis*
18	+	Round petiole outline	*Salvia leucantha*
	-	Petiole outline flat or oval	19
19	+	Oval outline petiole	20
	-	Flat outline petiole	21

20	+	Angular collenchyma	*Salvia moorcroftiana*
	-	Lacunar collenchyma	*Scutellaria linearis*
21	+	Bi-Collateral vascular bundles	*Salvia macrosiphon*
	-	Vascular bundles not bi-Collateral	22
22	+	Collateral open vascularization	*Salvia plebeia*
	-	Amphicribral or collateral closed vascularization	23
23	+	Tri to hexagonal parenchyma	*Eremostachys thyrsiflora*
	-	Irregular or isodiametric parenchyma	24
24	+	Xylem vessels round	*Eremostachys vicaryi*
	-	Xylem vessels not round	25
25	+	Angular collenchyma	*Nepeta Hindostana*
	-	Annular collenchyma	26
26	+	Round to oval phloem	*Nepeta glomerulosa*
	-	Angular to rectangular phloem	*Otostegia limbate*

Conclusion

4. Conclusion

This work represents the first thorough analysis of the anatomy and palynology of 107 dicots of Northern Baluchistan, belonging to Brassicaceae (37), Lamiaceae (35), and Fabaceae (35). The palynological and anatomical analysis found significant taxonomically. Pollen analysis was carried out via light microscopy (LM) and scanning electron microscopy (SEM). For petiole anatomical studies light microscopy (LM) was utilised.

a) Pollen micromorphology

This study revealed significant diagnostic features for each family. The important palynological characters in Brassicaceae pollen were exine reticulate, coarsely reticulate, scabrate, lumen polygonal, amorphous, $N_3P_4C_3$. The Fabaceous pollen were characterised with exine macro-microreticulate, psilate, verrucate, $N_3P_4C_5$, amb peritreme, goniotreme, variations in the polar, and equatorial view, shape subprolate, prolate spheroidal, oblate spheroidal, prolate. The distinguished palynological features in Lamiaceae were tricolpate, tricolporate, hexacolpate, hexcolporate, tetra to hexacolpate, hexa to multi syncolpate, exine reticulate, macro-microrteiculate, gemmate, foveolate, verrucate, perforate, non-perforate, aperture membrane varruacte, scabrate, gemmate, psilate, amb ptychotreme, peritreme, goniotreme, shape oblate, spherical, prolate spheroidal, oblate spheroidal, sub-prolate, sub-oblate, having formula $N_6P_4C_5$, $N_3P_4C_3$, $N_6P_4C_5$, $N_6P_4C_3$, $N_{4-6}P_4C_3$, $N_{4-.}P_4C_3$, $N_{3-6}P_4C_3$.

b) Petiole anatomical micromorphology

The significant diagnostic features for the petiole anatomy in Brassicaceae were petiole shapes sulcate, flat, oval, circular, vascular bundles collateral closed, collateral open, bi-collateral, or hadrocentric, trichomes unicellular, uniseriate, and multiseriate. Significant variations in the Fabaceous petioles were type of vascularisation amphicribral, collateral closed, collateral open, bicollateral, and the number of vascular bundles 1, 1+2, 2, 3, 9, collenchyma cells shape as angular, lacunar, and lamellar. The significant petiole anatomical features in Lamiaceae were variations in petiole shape oval, flat, sulcate, round, collenchyma cell shapes lacunar, angular, annular, lamellar, collateral closed, collateral open, and amphicribal vascular bundles.

The taxonomic keys were constructed based on the observed features, and successfully distinguished the studied taxa. The data for quantitatively analyzed pollen morphological and petiole anatomical traits was subjected to SPSS, Origin, and Past. The dendrogram, PCA, correlation, normal probability, and box-jatter plots demonstrated the separation of species within each family down to the species level. The pollen morphology and petiole anatomy of the studied species assisted in the distinction of taxa at a genus and species level.

Future perspectives

5. Future Perspectives

- The systematics of the examined dicot flora can be further enhanced by conducting DNA barcoding experiments to authenticate species identification at molecular level.
- Examination of the pollen using a transmission electron microscope (TEM) can unveil more taxonomic markers and provide specifics to the existing investigations.
- The endemic species within the arid-semiarid conditions of Baluchistan need to be genetically conserve in context of climate change.
- Future research on the delimitation of dicot flora through sophisticated phylogenetic analyses of taxonomic data using bioinformatics methods will be very helpful.
- It is recommended to connect molecular systematics and classical taxonomy by analyzing the genes that code the studied anatomical and palynological traits.
- The research of adulteration in herbal remedies may benefit greatly from accurate identification using palynological and anatomical documentation.
- Seed atlas on investigated families are recommended for future studies.
- The melissopalynological studies on the flora of Baluchistan are highly recommended, as there is not a single report in this regard.
- The comparative studies of the pollen morphology and petiole anatomy need to be carried out from several ecological zones, to confirm their authenticity and use in the plant systematics studies.

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PUBLISHED PAPERS

QUAID-I-AZAM UNIVERSITY

DEPARTMENT OF PLANT SCIENCES

Subject: **Publication of W – Category Ms. Wajia Noor (Ph.D. Scholar)**

This is in reference to circular regarding the publication requirement for Ph.D. scholars in Department of Plant Sciences, Faculty of Biological Sciences. It is certified that **Ms. Wajia Noor** has published research papers in W-Categories as given below:

S. No.	Paper Title	Year	Impact Factor
1.	Wajia Noor, Muhammad Zafar, Mushtaq Ahmad, Ashwaq T. Althobaiti, Mohamed Fawzy Ramadan, Trobjon Makhkamov, Yusufjon Gafforov, Akramjon Yuldashev, Oybek Mamarakhimov, Omer Kilic, Heba F. Eid, Talip Şahin, Shazia Sultana, Bibi Sadia, Anwer Usma, Amjad Khan, Petiole micromorphology in Brassicaceous taxa and its potential for accurate taxonomic identification, Flora, 303, 152280.	2023	1.9
2.	Noor, W., Zafar, M., Ahmad, M., Sadia, B., Gillani, S. W., Manzoor, M., & Ameen, M. (2024). Taxonomic significance of palyno-morphic markers for the delimitation of some Brassicaceous taxa in Balochistan Province (Pakistan). *Genetic Resources and Crop Evolution*, 1-28.	2024	2.33

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Petiole micromorphology in Brassicaceous taxa and its potential for accurate taxonomic identification

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ABSTRACT

Petiole anatomy is considered one of the important diagnostic characteristics that can be effectively employed in taxa discrimination at the species level. In this study, comprehensive petiole anatomy of 25 wild Brassicaceae taxa from Northern Baluchistan was carried out to examine variations in petiole anatomical features that are significant in the identification and delimitation of species. The current research first time reported *Goldbachia pendula* from Pakistan. The study also includes endemic taxa *Farsetia hamiltonii*. Variations observed among qualitative (shapes of petiole, epidermis, collenchyma, chlorenchyma, sclerenchyma, trichome, cuticle, xylem vessel, phloem, collenchyma, chlorenchyma, sclerenchyma, arrangement and several vascular bundles, presence/absence of pith, the sub-epidermal ring of collenchyma) and quantitative (diameter of petiole, pith, vascular bundles) characteristics of the petiole. Observed shapes of petiole were sulcate, flat, oval or circular with blunt or acute wings. Vascular bundles in petioles were collateral closed, collateral open, bi-collateral, or hadrocentric types with arrangements of single, 1 + 2 (one large central, two lateral) and numerous (2–20). The surfaces of petioles were marked with three types of trichomes unicellular, uniseriate, and multiseriate. Petiole of *Physorrhynchus brahuicus* was the largest (length 2561.8 μm, width 2004 μm). The highest numbers of vascular bundles (20) were observed in the *Alyssum desertorum*. *Conringia orientalis* possessed the largest pith (length 1711.6 μm 850.4 μm). The parameters were collected into a matrix and statistically analyzed the ability of variables to segregate taxa using Past 4.03. PCA clustering, UPGMA dendrogram revealed comparability and negative correlation at genus, among species of the same genus and distinct species. Via documentation of distinct microscopic petiolar anatomical variables, the study will be significant for the characterization and delimitation of taxa at genus and species level in the family Brassicaceae, the exploration of evolutionary and microclimatic effects on designing anatomy of taxa in distinct floristic regions.

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RESEARCH ARTICLE

Taxonomic significance of palyno-morphic markers for the delimitation of some Brassicaceous taxa in Balochistan Province (Pakistan)

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Abstract The pollen morphology of Brassicaceous taxa was analyzed to investigate their reliability as operational taxonomic units (OTUs) down to species level. The derived formula for all observed pollen was $N_3P_4C_3$ based on NPC classification. Documentation of quantitative parameters via statistical analysis included dendrogram, correlation, and loading plots. The studied taxa belong to 15 tribes, Brassiaceae, Alysseae, and Euclidieae being the dominant, including the endemic species *Pseudodraba hystrix*. The assessment of qualitative and quantitative features of pollen grains using LM and SEM aided in the discrimination of closely related species. The size of polar axis ranged from a minimum of 15.85 µm in *Eruca vesicaria* to a maximum of 33.05 µm in *Dilophia salsa*. Values of equatorial axis range from a minimum of 14.75 µm in *Diplotaxis griffithii*. to a maximum of 33.2 µm in *Dilophia salsa*. Exine sculpturing types were reticulate, coarsely reticulate, and scabrate. Lumen was prominent in polygonal and amorphous shapes with irregular and regular types. Lobate round, and triangular ambs were observed in the polar view of pollen. The Polar axis, equatorial axis, and length of colpi in equatorial view exhibited a highly positive correlation. This study describes the micromorphological characteristics of pollen which can aid in accurate identification and delineate boundaries among Brassicaceous members at different taxonomic ranks.

Keywords Colpus orientation · Exine · Lumen · NPC classification · Palynomorphic keys

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Introduction

Palynology is the scientific investigation of pollen grains and spores. Palynomorphs encompass all microfossils containing organic walls, such as spores and grains. In spite of contributing to a variety of systematic concerns, palynological research assists in the identification of species via palynomorph examinations and in determining the frequency of grains that trigger pollinosis (Anjum et al. 2022). Many investigations have examined the pollen morphology of the Brassicaceae family, and all of them have demonstrated the importance of pollen morphology in comprehending the taxonomic status of the family (AbdelKhalik et al. 2002; Perveen et al. 2004; Kailas et al. 2016). The monophyletic family comprising about 338 genera and 3709 species that is collectively referred to as the mustard family is the Brassicaceae (Al-Shehbaz 2012). Several economically valuable vegetables, decorative plants, and high oil-bearing