Systematics and Phytogeography of Angiospermic Floral Diversity in Baluchistan, Pakistan

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بسم الله الرحمن الرحيم

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

Dedicated

to

Wajia Noor

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This is to certify that the research work presented in this thesis, entitled " Systematics and Phytogeography of Angiospermic Floral Diversity in Baluchistan, Pakistan" was conducted by Ms. Bibi Sadia under the supervision of Prof. Dr. Mushtaq Ahmad. No part of this thesis has been submitted anywhere else for any other degree. This thesis is submitted to the Department of Plant Sciences in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Field of Plant Sciences (Plant Systematics and Biodiversity), Department of Plant Sciences, Quaid-i-Azam University, Islamabad, Pakistan.

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Bibi Sadia

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ABSTRACT

The systematics of angiospermic flora of Baluchistan, Pakistan was first time investigated in this study. The collection of the angiosperms was carried out via field trips in different areas of Baluchistan including Loralai, Uthal, Bela, Hingol National Park, Hazar Ganji Chiltan National Park, Quetta, Ziarat, Quetta, Kalat, Mastung, Pathankot, Qillasaifullah, Sanjavi, and Zhob. The details of phytogeography with photographs were documented for 108 angiosperms belonging to selected three families including Asteraceae, Boraginaceae, and Poaceae, as leading groups based on the number of species collected. The pollen micromorphological characterization was carried out via light microscopy (LM) and scanning electron microscopy (SEM). The petiole and culm transverse sections were prepared using microtomy, and their anatomical traits were studied via light microscopy. The data of quantitative measurements of the palynological and anatomical traits was collected into a matrix and analyzed statistically via SPSS (2016), Past (2021), Origin (2023), and NCSS (2023). The polar axis was maximum 88.15µm in *Chloris barbata* and minimum 13.2µm in *Paracaryum intermedium var. intermedium.* The largest equatorial diameter 90.75µm was observed in *Chloris barbata. Gymnarrhena micrantha* pollen was noted with minimum 3.3µm equatorial diameter. Among the examined petioles, the minimum length 18.3µm and width 13.75µm of vascular bundles were noted in *Leuzea repens*. The maximum width 302.1µm and length 250.85µm of vascular bundles were observed in *Alkanna tinctoria* subsp. *Tinctoria* and *Launaea aspleniifolia* respectively. The significantly varied palynological features among Asteraceous taxa were shape (prolate spheroidal, oblate spheroidal, suboblate, subprolate, and prolate), heterogeneity in the number of apertures (tricolpate, tricolporate, tetracolpate, tetracolporate), variations in polar and equatorial views from circular to triangular obtuse convex and elliptic), exine (echinate, lophate, scabrate, perforate or non-perforate), lacuna shape, number of spines per pollen, number of spines between colpi, spine length, and width. Boraginaceae was found eurypalynous family in terms of pollen traits variations, and the significant palynological features such as differences in polarity (isopolar or heteropolar), colpi type (isocolpate or porocolpate), exine (psilate, scabrate, gemmate, and foveolate), aperture membrane (smooth, granulate, operculate), Amb (goniotreme, peritreme, and ptychotreme), and shape (prolate spheroidal, oblate spheroidal, suboblate, subprolate, and prolate) were found taxonomically important in the identification of taxa. While

the Poaceous pollen were stenopalynous when characterized, although there were differences in the shape (prolate spheroidal, oblate spheroidal, suboblate, subprolate, and prolate), degree of harmomegathy, annulus appearance, and pore orientation sunken or prominent. The largest polar axis was observed in *Chloris barbata* 88.15µm. *P. intermedium var. intermedium* was observed with smallest polar axis of 13.2 µm. The systematically useful petiole anatomical features that differentiated the taxa up to the species level were petiole outline (sulcate, round, flat, oval), vascular bundles number and arrangement (collateral closed, collateral open bicollateral and amphicribral), collenchyma (angular, lamellar, annular, and lacunar), cuticle (smooth or undulated), trichomes (unicellular, uniseriate, and multiseriate), wings, and groove in the surface. The important culm anatomical traits of grasses were culm shape (terete, quadrangular, semi-terete, elliptical, bundle sheath composition, number, and arrangement of major and peripheral vascular bundles, central and marginal cavities, and sclerenchymatous hypodermis. The Principal Component Analysis (PCA), dendrogram, correlation, and normal probability distribution plot successfully separated the taxa of each family. The pollen micromorphological and petiole/culm anatomical traits were used as taxonomic markers to develop the taxonomic keys for the delimitation of studied angiospermic flora. The distinguished palynological and anatomical characters provide the base for the identification of angiospermic flora.

Chapter 1

Introduction

1.1 Angiosperms floral diversity

The interaction of speciation and extinction, which determines the net diversification of many lineages, leads to the incredible diversity of angiosperms (Magallón and Castillo, 2009). Angiosperms, or flowering plants, have amassed an extraordinary species diversity through the interaction of evolutionary and ecological forces. This diversity encompasses a wide range of morphological, functional, and ecological versatility and forms the structural and energetic basis of the vast majority of modern terrestrial ecosystems. The exceptional diversity and richness of angiosperms have promoted the diversification of other biological lineages and allowed for the formation of rich and complicated interactions within and among trophic levels (Schneider et al., 2004).

A crucial component of the global ecosystem, angiosperms, provide ecosystem services such as fuel, oxygen, medicines, soil regeneration, erosion and flood control, and other advantages that are necessary for human survival. Our ability to support modern human populations and foster human social development has been greatly influenced by the "domestication" of roughly 200 angiosperms to supply the majority of the world's supply of food, feed, and fiber. Comparing members of the two main angiosperm subclasses, monocots, and dicots, is of particular interest in comparative biology as it provides significant insight into divergence at numerous taxonomic levels (Paterson et al., 2004).

Angiosperm species richness must be understood in terms of the interaction of origination and extinction, which controls the rate of species diversification, at the most fundamental level. Sims and McConway (2003), among others, showed that species diversity is unevenly distributed among angiosperm lineages. Through independent but complementary quantitative investigations of leaf macroflora and palynofloras, the dynamics of early angiosperm diversification and accompanying changes in the composition of the land flora were revealed. These studies provided evidence of the quick rise in angiosperm variety (Magallón and Castillo, 2009).

1.2 Systematics Phytogeography of Angiosperms: Present Era

Plant experts have been searching for an exact technique to accurately identify and categorize plants. Plant morphology is still the most useful tool in phylogenetic trees when it is properly weighted. Anatomical data is the next step that researchers take; these investigations resulted in a reorganization of many classifications, such as Papaveraceae and Capparaceae. In classifying plants and comprehending the relationships between different taxa, the internal structure of the plants has proved very helpful. Taxonomy and evolution have successfully inferred the following parameters: vascularity, sclerification, cell layers, cavities, and diameter of cells, including epidermis, parenchyma, collenchyma, sclerenchyma, chlorenchyma, and pith. Since the development of high-resolution scanning microscopes, palynological investigations have gained popularity as a technique in plant taxonomy. Studies on pollen have made it clearer where certain species fit in, such as in the genus *Phyrma*. These pieces make use of exine ornamentation, apertures, symmetry, form, and size as palynological elements (Taia, 2005). The most recent trends in plant taxonomy are Anatomy, palynology, diversity, and adaptation, cladistic analysis, ecotaxonomy, chemotaxonomy and serology, karyomorphology, seed morphology, phylogeny, paleobotany, and embryology.

1.2.1 Anatomy

Understanding the relationships between the taxa has been greatly enhanced by the internal structures of the plants. Important features in phylogeny and taxonomy include the arrangement of the vascular system in the stem and leaf, the anatomy of the petiole and nodal structures, the vasculature and architecture of the leaf, and epidermal investigations. Barthlott (1994) employed the epicuticular wax secretions in the systematics of Caryophyllales. Additionally, he proceeded to classify angiosperms and Rununculiflorae using the epicuticular secretions. Gibson (1994) used the internal features of the stem to delineate the genera in the family Cactaceae, whereas Al-Shammary (1991) employed the trichome architecture in the systematic of Saxifragaccae. Jansen et al. (2001) investigated the anatomical variations in the Myrtales, Gentianales, and Fabales. On the other hand, fewer studies were conducted on the interior structure of petioles and leaves than on stems. Nonetheless, a few publications about cuticles, stomata, and trichomes have been helpful in taxonomic relationships.

Baranova (1992) has studied the epidermis of Austrobaileya (a) and comparative stramatographic studies of angiosperms. Wilkinson (1994) has studied the anatomy of leaves in Pittosporaceae and Pterostemonaceae; Al-Shammary (1991) has studied the trichomes in Saxifragaceae, stomatal myrosin cells in Cariaceae, vascular tissue differentiation and pattern development in plants. Taia (2005) determined the micro characteristics of the leaves in the tribe Trifoleae, the primary vasculature in the leaves of the family Chenopodiaceae, and its applications for systematics and evolution. For phylogenetic reasons, anatomy collected inferences in taxonomy. The following characteristics were important to the taxonomy of this field: cell kinds, vessel elements, length and width, perforation plate types, and thickness and pitting of the lateral wall. Carlquist's (1970; 1977; 1981; 1990) worked on the anatomy of the Lamiaceae, sympetalous families, Cucurbitaceae, and Chloranthaceae, as well as Eupomatia, Aristolochiaceae, Sabiaceae, Caryophylaceae, Rananculaceae, Berberidaceae, Menispermaceac, the genus *Pentaphragma, Resedaceae, Portulaceaceae, Rivina* and *Petiveria* of Caryophyllales. In addition to other studies, all of these have greatly expanded taxonomy. The taxonomy and phylogeny of the angiosperms can be aided by wood type and structure. The anatomy of the Dicotyledons and its use in the taxonomy of angiosperms have been examined by Baas (1982), who emphasized the role that anatomy plays in biodiversity.

In Asteraceae, the anatomical studies were of systematic importance. There were studies on the petiole, stem, and leaf of the Asteraceous taxa (Łotocka and Geszprych, 2004; De las Mercedes Sosa et al., 2014; Akhtar et al., 2021; Ozcan et al., 2014; Melo-de-pinna and Menezes, 2002; Mabel et al., 2013; Al-Suboh et al., 2019; Janaćković et al., 2021; Ekeke and Ogazie, 2020; Rabiae and Elbadry, 2020; Tekin and Kartal, 2016) that employed the anatomical characters in the taxonomy. Systematics of various genera such as *Centaurea* was problematic, and their taxonomy mainly relied on the morphological features (Al-Suboh et al., 2019).

Different anatomical structures were reported in the Asteraceae based on its diverse habits. In some cases, ecological changes may take place, but the structure of the petiole remains not altered (Kamel and Loutfy, 2001; Metcalfe and Chalk, 1979). Thus, petiole anatomy was used as a supplementary tool in plant taxonomy with growing success in employing this line of evidence in the classification of taxa. Such as the placement of vascular bundles in the various areas of the petiole has taxonomic significance. When compared to the other sections of the petiole, the distal end (the area directly beneath the lamina) possesses more taxonomic characteristics (Ekeke and Ogazie, 2020). The micromorphological traits in the Asteraceae were studied significantly in the taxonomy of taxa at the generic and species level (Janaćković et al., 2019).

Petiolar anatomical characteristics were one of the significant factors in the identification and classification of many plant families. They have been used to distinguish between plants of various species, genera, and families, but were not well explored. The use of petiole anatomy as a supplemental tool in plant taxonomy has attracted more attention in recent years, and there has been tremendous advancement in the utilization of this line of evidence (Bercu and Popoviciu, 2014). The placement of vascular bundles in various areas of the petiole has also been thought to have taxonomic significance by various writers.

Plant anatomy has been found to be very important in plant taxonomy. The purpose was to develop a system of classifying plants in a way that all the differences and similarities are set out in an ordered manner (Okeke et al., 2015). Mabel et al. (2013) and Adedeji (2004) have all stressed the taxonomic importance of anatomical features, which along with other characters were useful for the identification, and classification of plants. Taxonomists use various aspects and disciplines within the field of taxonomy to classify species into relevant categories. One significant aspect is petiolar anatomical features which are the key parameter used in the identification and classification of many plant families and have been used in differentiating plants of different species, genera, and families (Metcalfe and Chalk 1979). In recent times, there has been increased interest and research on the use of petiole anatomy as a complementary tool in plant taxonomy and significant progress has been made in using this taxonomic marker in plant classification. Different authors have also considered the arrangement of vascular bundles in different portions of the petiole to have taxonomic relevance (Ekeke and Ogazie 2020).

Noraini et al. (2016) highlighted the potential of petiole vascular patterns in distinguishing certain taxa. The features found in petiole transverse sections include vascular tissue patterns, the presence/absence of sclerenchyma cells around bundles, medullary vascular bundles, and types of trichomes. Several studies demonstrated the utility of petiole anatomy in grouping genera and identifying species, as evidenced by (Kocsis and Borhidi, 2003: Noraini and Cutler, 2009). While limited research exists on petiole anatomy within Boraginaceae. In Boraginaceae species, variations in petiole anatomy could potentially be linked to their habitat preferences, water availability, and overall growth strategies. Exploring the internal cellular organization, vascular bundles, and associated tissues within the petioles could shed light on the mechanisms these plants employ to adapt to different environmental conditions.

Plant histology was essential in classifying two major groups (vascular and non-vascular plants) (Mousa et al., 2021). Petiole anatomical features were successfully manipulated in the separation of borassoid palms possessed undivided phloem in petiole from coryphoid palms which have two separated phloem strands in vascular bundles. The presence or absence of vessels, the development of their end walls, and distribution in different parts of the plant were studied to be significant parameters in phylogeny and taxonomy (Erwin and Stockey, 1991). Previously, the classification of Poaceae members into tribes completely relied on the morphology of inflorescence, now, the systematics distribution is heavily based on cryptic characteristics. Characters based on the anatomy of plants are the most important among these cryptic features. Variations in stem microhairs and nodal vascularisation assisted in the delimitation of Poaceae species into major groups. Macro spikes and the shapes and diameters of vascular bundles, and epidermis were found significant in distinguishing the taxa in Poaceae (Al-Khafaji and Al-Bermani, 2014).

The anatomy of different organs of grasses has been studied. Comparative leaf anatomical features of grasses that have significant taxonomic value (Ellis, 1979) were studied to indicate adaptations in different ecological regions (Rafique et al., 2021). Leaf blade morpho-anatomical studies of bamboo and anatomical adaptations in stem, root, and leaf to survive in ecological constraints were investigated (Rafique et al., 2021; Leandro et al., 2020; Mousa et al., 2019). Qualitative and quantitative detailed taxonomic study of the stem of Poaceae species helped in the characterization of the taxa (Al-Khafaji and Al-Bermani, 2014). The Stem of the Poaceous taxa of range lands was comparatively analyzed based on sclerenchyma and vascular bundles (Makesh et al., 2022). Anatomical evolution in leaves and stems concerning arid zones was analyzed by Shamah et al. (2019). The effects of climatic variations and weather conditions on stem anatomical features during summer and monsoon were studied by Makesh et al., (2022). The findings were found positive in the delimitation of studied Poaceae taxa. The number of vascular bundles, cortical layers, and xylem vessel length helped separate the species. Variations existed in the anatomy of grasses in various climatic conditions, these changes helped in the characterization up to the genus level in grasses (Makesh et al., 2022).

1.2.2 Palynology

Another important aspect is palynology, which involves the study of palynomorphs to identify and classify closely related species (Umber et al., 2022). The term "palynology" originates from the Greek word "Palynein," meaning dust or flour (Kayani et al., 2019). Eurypalynous refers to heterogeneous pollen that differ in shape, size, aperture, and exine ornamentation compared to others (Halbritter et al., 2018). Palynology has become increasingly captivating in scientific research. Various characteristics of pollen, including symmetry, shape, apertural pattern, and exine sculpturing, played a significant role in plant taxonomy. Today, the study of pollen grains is widely used for taxonomic identification of flowering plants. Taxonomists and botanists have extensively studied pollen morphology to classify flowering plants at the species and variety levels (Bahadur et al., 2019; Hameed et al., 2020).

Since the development of high-resolution power microscopes such as scanning and transmission electron microscopes, palynological research has gained popularity as a technique in plant taxonomy (Erdtman, 1963). The most insightful recent studies in this field are those by Cantino et al. (1998) who studied the genus *Caryopteris* of the Labiatae; Ferguson and Skvarla (1982) studied the family Leguminosae. Additional taxa for which pollen analyses have shed light on the group's evolutionary position include those in the genus *Phyrma* and its related species, Globularieae and Selagineae, the families Apocynaceae and Periplocaceae, Ranunculaceae, the Palaeobotanical work on the early Angiosperm pollen from the Cretaceous, genus *Triplostegia* of the Valerianaceae, members of the Calyceraceae, the pollen morphology of the genus *Oryza* (Poaceae), and genera of the family Caryophyllaceae and tribe Trifolieae (Taia, 2005). Four distinct pollen types within the Caryophyllaceae have been identified; they can be applied to the family's classification, three pollen groupings that were useful for deciphering the family's phylogeny. Also examined the pollen grains of the *Atriplex* species, characterizing them as eurypalynous. Taia (2005) had investigated the morphology of pollen in Amaranthaceae and Trifolieae species. Within the Trifolieae tribe, she discovered three pollen types and five subtypes that can be used to understand the group's evolutionary history. In the phylogenetic order of the examined group, these works employ pollen features such as wall structure and ornamentation, polarity, symmetry, shape, and aperture.

Pollen analysis of different members of Asteraceae has been investigated from different regions of the world. 19 texa of genus *Centura* from Iran examined by Shabestari et al. (2013), 8 tribes of Asteraceae from Indonesia (Salamah et al., 2019) have been investigated. Various features like symmetry of different types such as radiosymmetrical (Shabestari et al., 2013), radioasymmetrical, tricolpate aperture, spheroidal, prolate to subprolate, micoechinate to regulate (Telleria and Katinas, 2009) and scabrate sculpturing with dense acute and sparse spinules (Shabestari et al., 2013) have been studied in the members of Asteraceae. Pollen of seven species of the *Chuquiraga* genus were examined morphologically. Several species of the *Mutisia* genus from Asteraceae were analyzed for pollen using TEM (Telleria and Katinas, 2009). Radiosymmetrical and radioasymmetrical types of pollen were recognized using LM and SEM techniques. Lophate pollen with and without depressions were found in Barnadesiodideae (Urtubey and Tellería, 1998).

Harmomegathy is a fascinating adaptation observed in the pollen in the Boraginaceae family used to stay healthy in different environments. This involves the pollen folding themselves when they lose water, helping them avoid drying out too much. The pollen has a strong outer layer and a more flexible inner layer. This flexibility allows the pollen to fold up and protect itself. The inner layer also helps the folding process and is important for keeping the pollen alive. Analyzing the harmomegathy mechanism in various Boraginaceae species can provide valuable information about the evolutionary trajectory of this family, shedding light on how pollen morphology and harmomegathy have shaped their ecological success. The Boraginaceae family has many types of pollen with different shapes. This diversity in pollen shapes and folding mechanisms helps these plants survive in different places like dry and wet areas (Volkova et al., 2013).

The pollen morphology and phylogeny of the orders Restionales and Poales were studied by Ferguson and Skvarla (1982). Siddiqui and Qaiser (1988) also looked at the pollen morphology of Karachi-based Poaceae plants. Salgado-Labouriau and

Rinaldi (1990) investigated the pollen morphology of 60 species of the family Poaceae from Venezuelan mountains. Skvarla et al. (2003) examined the link between the annulus and pore in Poaceae pollen. Liu et al. (2004) observed the pollen morphology of 75 species and 42 genera of Chloroiodeae. Ozler et al. (2009) investigated the pollen morphology of the Turkish genus *Agropyron*.

Palynology finds applications in forensic, genetic, and systematic studies. In modern taxonomy, pollen are studied along with other morphological characters to distinguish plant species (Sufyan et al., 2018). The study of pollen has implications in agriculture, biotechnology, forestry, horticulture, and plant breeding (Yousaf et al., 2022). Additionally, pollen can be used to monitor the cytotoxic effects of chemicals like herbicides and insecticides. Palynologists have investigated the significance of palynological features in different plant groups, finding them highly effective in species identification. The species within the Boraginaceae family displaed a range of pollen morphologies, allowing for the recognition of many different genera and species (Volkova et al., 2013).

1.3 Angiospermic Flora of Pakistan

Pakistan is located between 23°–37° N and 61°–81° E, with a total land area of 804,152 km^2. Temperatures fluctuate from well below zero in the high, glacier-clad mountains to 52°C (125°F) at Sibi on the plains. Altitudes range from sea level to 8,611 m (at K2, the second highest summit on Earth). The average yearly precipitation varies from approximately 50 mm in Baluchistan's Nok Kundi to 2032 mm in Kashmir's monsoonal uplands (Ali, 2008). A variety of biotic communities and a rather rich flora comprising at least 5,700 species of flowering plants have been produced as a result of the extreme fluctuation in height, temperature, precipitation, and other physical characteristics (Ali, 2008). Pakistan boasts an abundant flora, with over 6,000 vascular plants, 12 percent of which are utilized by the locals for traditional and medical uses (Ullah, 2022).

Northern Baluchistan in the Western Asiatic Subregion is one of the important floristic provinces in Pakistan (Irano-Turanian Region of Tethyan Subkingdom, Holoarctic Kingdom). Numerous of indigenous species can be found in northern Baluchistan and the montane areas of northern Pakistan, especially in the districts of Chitral and Kashmir. Nonetheless, these areas are very little understood and are probably home to a sizable number of novel and potentially endemic species (Chaudhary et al., 1981; Frodin, 2010).

1.4 Angiosperms: Systematics Research in Pakistan

In Pakistan, the previous research has focused on the higher-level systematic study of the Angiosperms, primarily the tribe circumscriptions and interrelationships. These studies have shed light on the anatomy, pollen morphology, phytogeography, seed chemistry, and chromosome count of Angiosperms, improving our understanding of their evolutionary processes. These investigations assisted in realigning and recreating the positions of different tribes within the taxonomic categorization system that Bentham and Hooker (1865) had previously delineated (Shah et al., 2018). Anatomical examination is one of the important tools for the discrimination of closely related taxa (Akhtar et al., 2021).

In Asteraceae, the anatomical studies were of systematic importance. There are studies on the petiole, stem, and leaf of the Asteraceous taxa (Zafar et al., 2007; Qureshi et al., 2009; Akhtar et al., 2022; Umber et al., 2022) that employed the anatomical characters in the taxonomy. Pollen morphology of Asteraceae has been evaluated in previous studies from Pakistan. Umber et al. (2022) observed a palynomorph of twelve species of Asteraceae and Brassicaceae from Esa Khel, Mianwali, Pakistan. They documented tetracolporate, tricolporate, and trizonocolporate types in Asteraceae concluding that pollen morphological examination helped in identification as well as classification of plants at species, generic, and family levels. Similarly, 46 Asteraceous species were checked for their pollen viability by Qureshi et al. (2009).

Zafar et al. (2007) examined pollen fertility along with morphology for the taxonomic description of Asteraceous flora of Rawalpindi. Shape, and exine ornamentation were significant features for the separation of studied taxa. Fertility data (90-98%) expressed the well-established Asteraceous species in the Rawalpindi area (Zafar et al., 2007). In Pakistan, palynology has been utilized to examine the pollen morphology of medicinally important plants (Bahadur et al., 2019; Yousaf et al., 2022). On the pollen morphology of Poaceae, there are few reports from Pakistan. In Karachi, Sindh, Siddiqui & Qaiser (1988) analyzed the pollen of 60 species, and

Perveen (2006) looked into the pollen morphology of 22 species of Pakistan's Poaceae family.

Angiosperms fossils pollen was examined by Khan et al. (2017) utilizing LM techniques. The palynomorphs found in the Bara Formation bore striking similarities to contemporary groups, including the Arecaceae, Liliaceae, Myricaceae, and Nymphaeaceae. The location of vegetation along the shore during sedimentation was disclosed by the rich collection of ancient pollen from the Arecaceae family. Fossil pollen from the Nymphaeaceae and Liliaceae groups revealed the presence of freshwater environments. The occurrence of pollen types with connections to the Myricaceae family suggested that brackish water mangrove swamps were common across the coastal zone. Majeed et al. (2023) characterized the dicot angiosperms from Pakistan's Thal desert using micromorphological analysis.

1. 5 Phytogeography of Angiosperms in Baluchistan

Baluchistan is endowed with a wide variety of flora and animals, due to its varied ecological conditions (Tareen et al., 2010). The focal points of Pakistan's significant flora, which includes indigenous and medicinal species, are the highlands of Baluchistan. Baluchistan is third in terms of the number of endemic taxa, after the Sino-Japanese region of Kashmir and the northern areas of Pakistan (Ali and Qaiser, 1986). Researchers from around the world have been studying Baluchistan's biodiversity, which has been widely recognized for millions of years as a significant source of traditional medicines. It is a key hub for herbal remedies, a diverse range of cultures and traditions, and an abundance of rare and indigenous plants. Baluchistan's rural populations rely heavily on biological resources for their livelihood (Bibi et al., 2015).

Asteraceae, Poaceae, Fabaceae, Brassicaceae, Lamiaceae, Chenopodiaeae, Boraginaceae, Lilaceae, Rosaceae, Caryophyllaceae, and other prominent families were among those gathered in 1891 by Lace and Hemsley. Baluchistan is home to a variety of native plants, including *Amaranthus viridis, Calendula arvensis, Calotropis procera, Cynodon dactylon, Cyperus rotundus, Carthamus oxyacantha, Medicago polymorpha, Melilotus alba, Acanthodium, Spicatum, Eleusine flagellifera,* and *Alhagi maurorum* (Baloch et al., 2000). *Heliotropium remotiflorum, Allium baluchistanicum, Tetracme stocksii, Viola makranica, Berberis baluchistanica,* *Blepharis sindica,* and *Seriphidium quettense* are a few of the endemic species found in Baluchistan (Bibi et al., 2015).

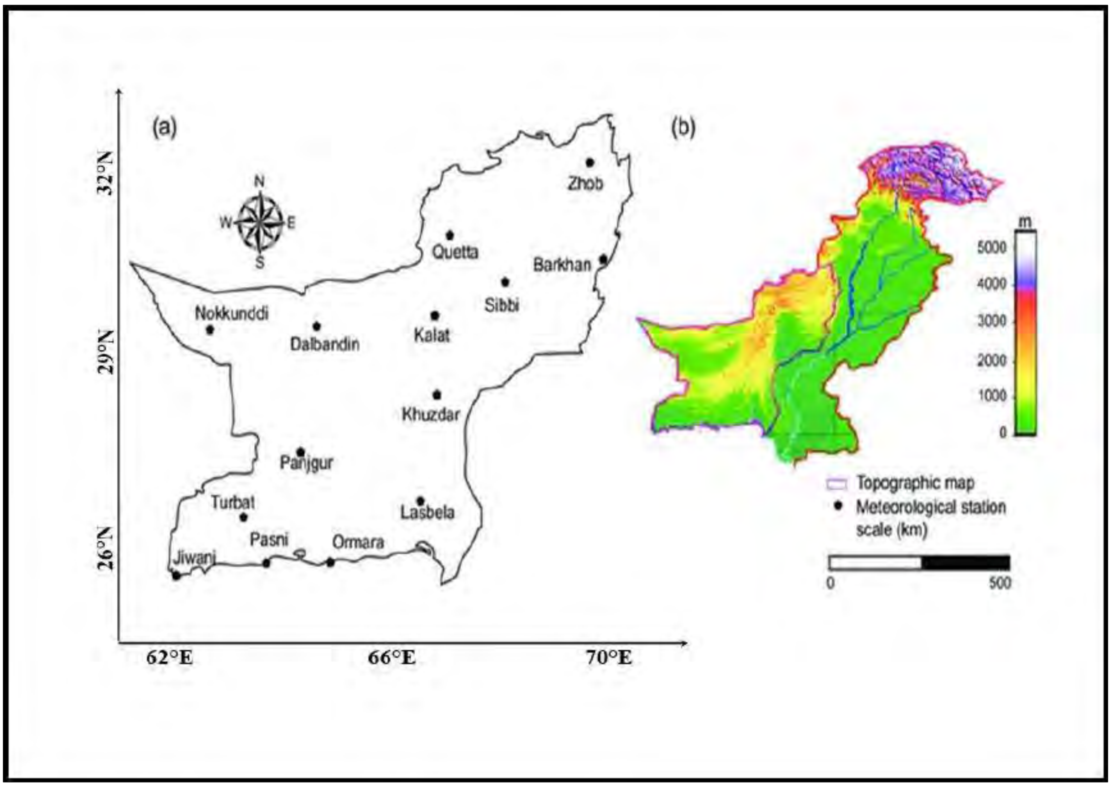

Figure 1. Topography and geographic position of Baluchistan (Ahmed et al., 2018)

1.6 Asteraceae, Boraginaceae, Poaceae

One of the largest plant families, Asteraceae contains 25,000 species and 950–1500 genera (Souza et al., 2017). Presented by 770 species and 188 genera, Asteraceae in Pakistan is the largest Angiospermic family. A total of 19 tribes have been recognized in Pakistan, distributed in five sub-families (Jeffrey and Kadereit, 2007). The Asteraceae family is published in six volumes. The total Asteraceous taxa from Baluchistan consists of 230 species. The distribution of the species in tribes is still not in the placement having a high possibility of refreshing the classification. This research gap in taxonomic studies such as palynology, anatomy, and seed morphology is required to be investigated for the correct classification of the species. Numerous studies have been conducted on secretory structures that are restricted in their distribution. Some members of this family's anatomical traits, including their epidermal and laminal properties, have been described as having various types of glandular or covering trichomes, hydathodes, hypoderm, and vascular bundles with a parenchymatic sheath made up of big cells. Additionally, because of the production of essential oils, this family contains species that are very significant in the domains of nutrition, cosmetics, and pharmacy (Adedeji and Jewoola, 2008). It is one of the important families in industry, pharmacy, and food. Anatomical examination is one of the important tools for the discrimination of closely related taxa (Akhtar et al., 2022).

The family Boraginaceae also called the Borage or forget me not family, is one of the largest and most valuable families in the world in terms of anatomical, morphological, ecological, and medicinal characteristics (Rabizadeh et al., 2020; Yousaf et al., 2022). This family can be found worldwide in temperate regions and is characterized by its vast diversity, with approximately 130 genera and 2,300 species (Buys et al., 2003; Attar et al., 2019; Yousaf et al., 2022). In Pakistan, there are 32 genera, and 135 species represented, including cultivated varieties such as *Anchusa* and *Cordia*. Common systematic features of this family species include scorpioid inflorescences, gynobasic style, and an ovary with two carpels, which are divided into four nutlets. The Boraginaceae family is divided into four subfamilies Boraginoideae, Cordioideae, Ehretioideae, and Heliotropioideae (Rabizadeh et al., 2020). Boraginaceous species also have significant roles in the fields of cosmetology and pharmacology (Yousaf et al., 2022).

Grasses belong to the economically important family Poaceae (Makesh et al., 2022). They are soil stabilizers as they play a key role in the recycling of eroded soil. Physical and chemical properties of the soil define the grass types in specific floristic regions (Ahmad et al., 2009). 23% of the earth's vegetation cover is occupied by grasses (Singh and Parabia, 2003). Worldwide, 10000 species and 620 genera of Poaceae have been reported including 492 species and 158 genera gathered in 26 tribes from Pakistan (Flora of Pakistan). It is a homogenous group of monocots and easily recognizable family in the field (Anjum and Muhammad, 2012). Poaceae ranks first in abundance among the families. According to the classification of Barnhart (1895), in Pakistan it is represented by five subfamilies comprising of annual or perennial herbs, rarely shrubs and trees (Raees et al., 2017). To archaeobotanists, grasses are significant for the examination of taxonomic history as they provide various resources for humans, including main food crops (Al-Khafaji and Al-Bermni, 2014). They can survive varied climatic conditions due to their greater adaptability (Nazish and Althobaiti, 2022). The family includes species surviving in saline, arid, and other harsh environmental conditions. It includes medicinally important plants e.g *C. dactylon* (anabolic, antiseptic), *S. italica* (treat chicken pox) (Shamah et al., 2019).

1.7 Background and Justification of the Present Study

The phytogeography of Baluchistan is of immense importance. It is a vast region from the Gomal River in the northeast to the Arabian Sea in the south, as well as from the Iranian and Afghani borders in the west and northwest to the Sulaiman Mountains and Kirthar Hills in the east. Two separate regions can be distinguished within Baluchistan. Long ranges of rugged hills extend to the northeast, hemmed in between Afghanistan and the Indus Valley. The Sulaiman range and the Toba-Kakar range from the eastern and western boundaries of this region, respectively (Bibi et al., 2015). Baluchistan boasts an abundance of wild flora; yet there is a dearth of literature about this productive region concerning modern systematic research such as pollen atlas and anatomical studies, as well as records of vegetation. In Baluchistan, the research on floral enlisting is an area of investigation for taxonomists. There is no literature on the area's Angiospermic flora concerning palynology or anatomy. Leporatti and Lattanzi (1999) reported traditional phytotherapy on coastal areas of Makran; Goodman and Ghafoor (1992) investigated the ethnobotany of Southern Baluchistan, with particular reference to medicinal plants; and Shah et al. (2006) conducted an ethnobotanical study of the flora of district Musakhel and Barkhan in Baluchistan.

Tareen et al. (2010) highlighted the traditional knowledge of folk remedies held by the women in the Baluchistan districts of Kalat and Khuzdar. The medicinal flora of Baluchistan's Hingol National Park was documented by Qureshi (2012). Manzoor et al. (2013) reported on Quetta residents' use of fruits, vegetables, and plants to cure diabetes. The sole book that was accessible at the time of partition, Flora of British India (Hooker, 1879), did not have the records for significant regions like Baluchistan. The first collector in Baluchistan and the Khyber Pass region in 1838 was William Griffith (1847). Recently, there have been foreign organisations operating in the area. The plants have also been collected by a few local botanists. Some regions, like Baluchistan, have not yet been fully investigated (Ali, 2008).

The morphology of the species reported in flora and literature, together with the monocot and dicot categories, are incomplete since features alone are insufficient for accurately identifying plants belonging to different families. Keeping in mind, additional characteristics including palynology and anatomy are needed to guarantee accurate identification (Taia, 2005). The flora of Baluchistan is very unique because of its specific arid to semiarid conditions. Besides ethnobotanical and phytochemical studies, there are currently no reports on the petiole anatomy of the different plant families in Baluchistan. The flora of Baluchistan has to be related to one another using petiolar anatomical traits, such as new understandings of vascularization and elucidation. This would enable precise identification of the similarities and differences between the taxa under study. It is impossible to dispute the importance of anatomical approaches in taxonomic research. 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.

Similarly, palynology is a fundamental component of taxonomy, and the information it produces is valued as significant taxonomic standards (Bahadur et al., 2018). Plant species have been classified using the micromorphological traits of the pollen (Talebi et al., 2012). Because they exhibit certain variations not covered in earlier research, additional pollen features like exine sculpturing, Amb, NPC, aperture details, polarity, symmetry, polar region, apocolpium, and spine details can be useful taxonomic tools in the classification of different angiosperm plant families found in Baluchistan at both generic and infra-specific ranks. When it comes to systematic modifications, the proposed study is significant. There isn't a single study on the micro-morphological features of angiospermic flora of Baluchistan to assure appropriate identification and the morphology of species recorded in flora and literature is incomplete since characters alone are insufficient for correctly identifying plants of different families. The petiole anatomy and pollen atlas of the majority of the plant species in the area under consideration have not previously been recorded, making the proposed research endeavor extremely important. The planned research contributes to finding similarities and differences between the taxa of these families to define boundaries for different taxonomic ranks.

There is a need for accurate identification and distinction of these plant species from one another because species (Asteraceae, Boraginaceae, Poaceae) of the same genus and different genera of the same tribe frequently have similar general appearances. Additionally, it has been observed that taxa belonging to the Asteraceae, Boraginaceae, and Poaceae have various names in different local regions, leading to confusion and misidentification of species for researchers. For accurate identification and delimitation, morphological, anatomical, and palynological documentation of features become necessary, necessitating a thorough research of these taxa. The study included new specific characteristics such as the number of major and peripheral vascular bundles, cavities/air spaces, xylem vessel shape, sclerenchyma presence in the hypodermis, groove in the upper surface of the petiole (anatomical), harmomegathy, colpi type, edges of apertures, NPC classification, number of spines per pollen and between colpie (palynological). Therefore, this work offers baseline data that can help future researchers identify and classify angiospermic flora. As far as we are aware, no thorough investigation has been done on the systematic criteria of the angiospermic flora of Baluchistan.

1.8 Objectives

- Collection (via field trips during the flowering season), identification (via well-known taxonomists, herbarium, and online flora) and deposition (to Herbarium of Pakistan, (ISL) QAU) of wild Angiospermic flora of Baluchistan, and separation of the leading collected families.
- Taxonomic phytogeography of Angiospermic flora (Asteraceae, Boraginaceae, Poaceae) of Baluchistan, Pakistan, based on qualitative and quantitative anatomical (LM and SEM) and palynological (LM and SEM) attributes for the identification and classification.
- Annotation of variations based on anatomy and palynology at the various levels of classification.
- Development of taxonomic keys and statistical analysis (dendrogram, correlation, and loading plots, boxplots, PCA) based on qualitative and quantitative palynological, anatomical characters to highlight similarities and variations among taxa of each family.

Plate 1. Ziarat (Baluchistan) home the second largest juniper forest in the world

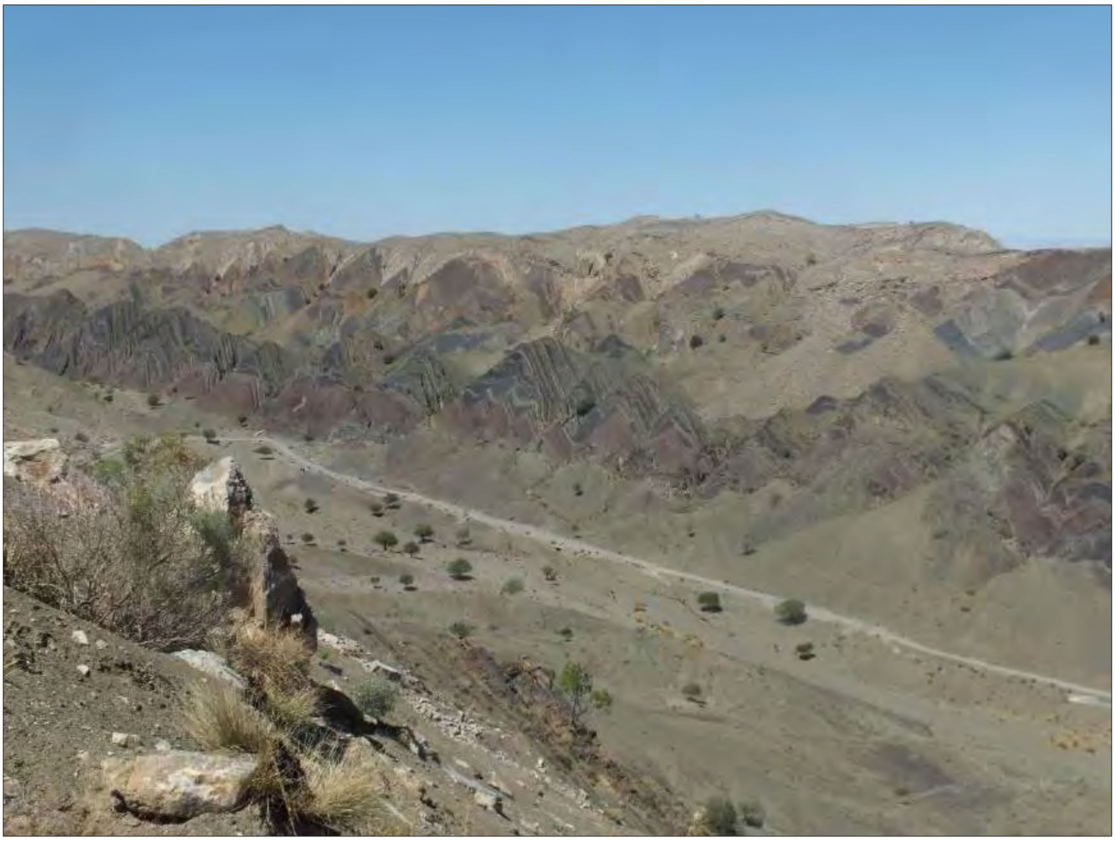

Plate 2. Loralai 4700m above the sea level: arid rangeland region of Baluchistan

Plate 3. Takht-e-Sulaiman Zhob, Baluchistan

Plate 4. Hingol National Park, Baluchistan, covers an area of about 6100km^2

Chapter 2

Material and Methods

The study was conducted in the Plant Systematics and Biodiversity Lab, Department of Plant Sciences, Herbarium of Pakistan Islamabad (ISL), Quaid-i-Azam University, Islamabad. The systematics of Angiosperms of Baluchistan, Pakistan was the exclusive focus of the investigation. The research was divided into two primary sections: (i) Anatomical studies, which included qualitative and quantitative descriptions (Microtomy and LM) and (ii) Palynological determinations both qualitative and quantitative descriptions (LM and SEM) (Figure 2).

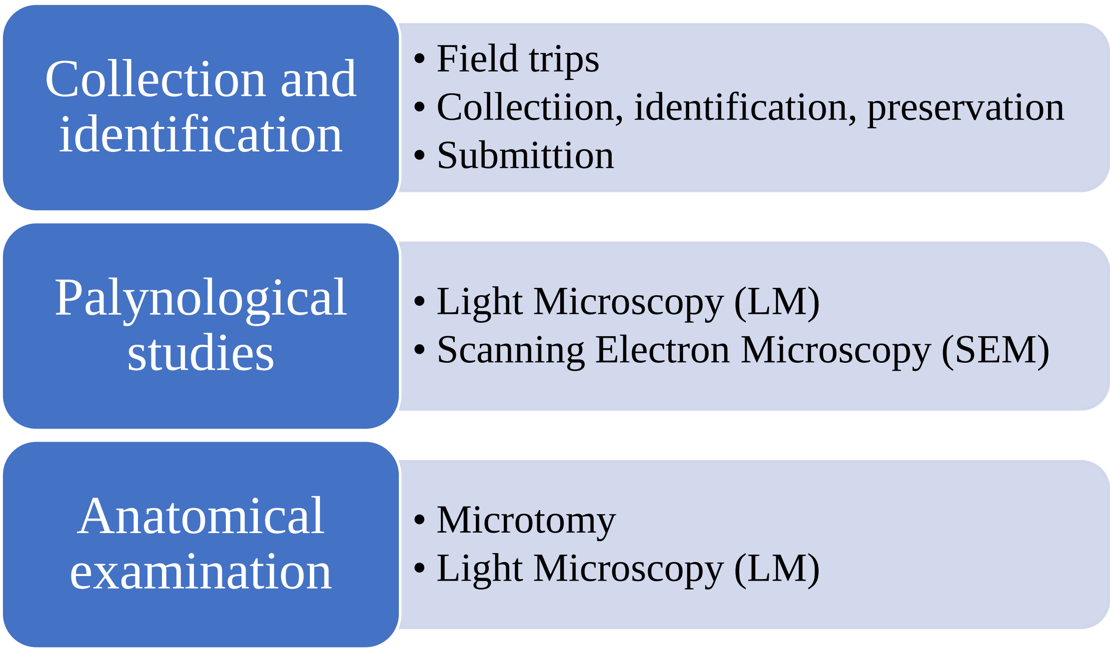

Figure 2. Summary of steps of methodology

2.1 Study area overview

As per the study's aims, this exploratory work was carried out throughout different areas in Baluchistan such as Quetta, Khuzdar, Loralai, Mastung, Ziarat, Zhob, Makran, Sanjavi, Pathankot, Kundmalir, Qillasaifullah, Hanna Lake, Pathankot, Kharan, Chiltan National Park, Las Bela, Otmanzai, Beela, Kalat, Hingol, Othal, Hubchowki, Sibbi (Figure 3).

2.1.1 Geography of Baluchistan

The largest province, Baluchistan, makes up 44% of Pakistan's total land with an area of 347193 km^2. With fewer than 5% of the total population, it is the smallest province of Pakistan (Manzoor et al., 2013). Situated in the southwest, it borders the Pakistani provinces of Sindh to the southeast and Khyber Pakhtunkhwa and Punjab to the northeast. In addition to the Arabian Sea to the south, it borders Iran to the west and Afghanistan to the north internationally (Spooner, 1988). Baluchistan is a vast, mountainous plateau with basins separated by hills that are craggy enough and high enough. Natural resources, particularly its natural gas deposits, are the main drivers of the province's economy. The province's second-biggest city, after Quetta, is Turbat in the south.

The largest deep-water port in the world, Gwadar Port, is located on the Arabian Sea and is a significant commercial hub. The exceptionally arid desert environment of Baluchistan is well-known (Ahmed et al., 2019). 94% of the area of Baluchistan consists of range lands, more than half of the range land area is covered by grasses *Cymbopogon* and *Chrysopogon*, grazed by a variety of herbivores. Rainfall of western depression is prevalent in Baluchistan. The climate of Baluchistan is favorable for rearing livestock and fruit production. The availability of water has a direct impact on the production of wild flora. Due to its arid climate and high exposure, Baluchistan province is among the most vulnerable to droughts (Jamro et al., 2020).

2.1.2 Climate

The climate ranges from coastal tropical to moderate temperate in the north (arid to semi-arid) conditions with an annual mean of 250mm precipitation (Khan et al., 2021). According to Jan et al. (2021), the main ecological zones include desert and mangrove forests, tropical dry mixed deciduous forests, subtropical forests, and dry temperate forests. The top highlands experience extremely cold winters and hot summers. The winters in the lower highlands are not all the same. In the northern districts of Ziarat, Quetta, Kalat, Muslim Baagh, and Khanozai, temperatures are as low as -20 °C. In contrast, conditions are moderate near the Makran coast. The plains experience moderate winters, with temperatures that never drop below freezing. Particularly in the arid districts of Chagai and Kharan, summers are hot and dry. According to Ahmed et al. (2019), summertime temperatures on the plains can reach 50 °C, making them extremely hot. Minimum and maximum temperatures range from -10 to 50 ^{o}C (Khan et al., 2021).

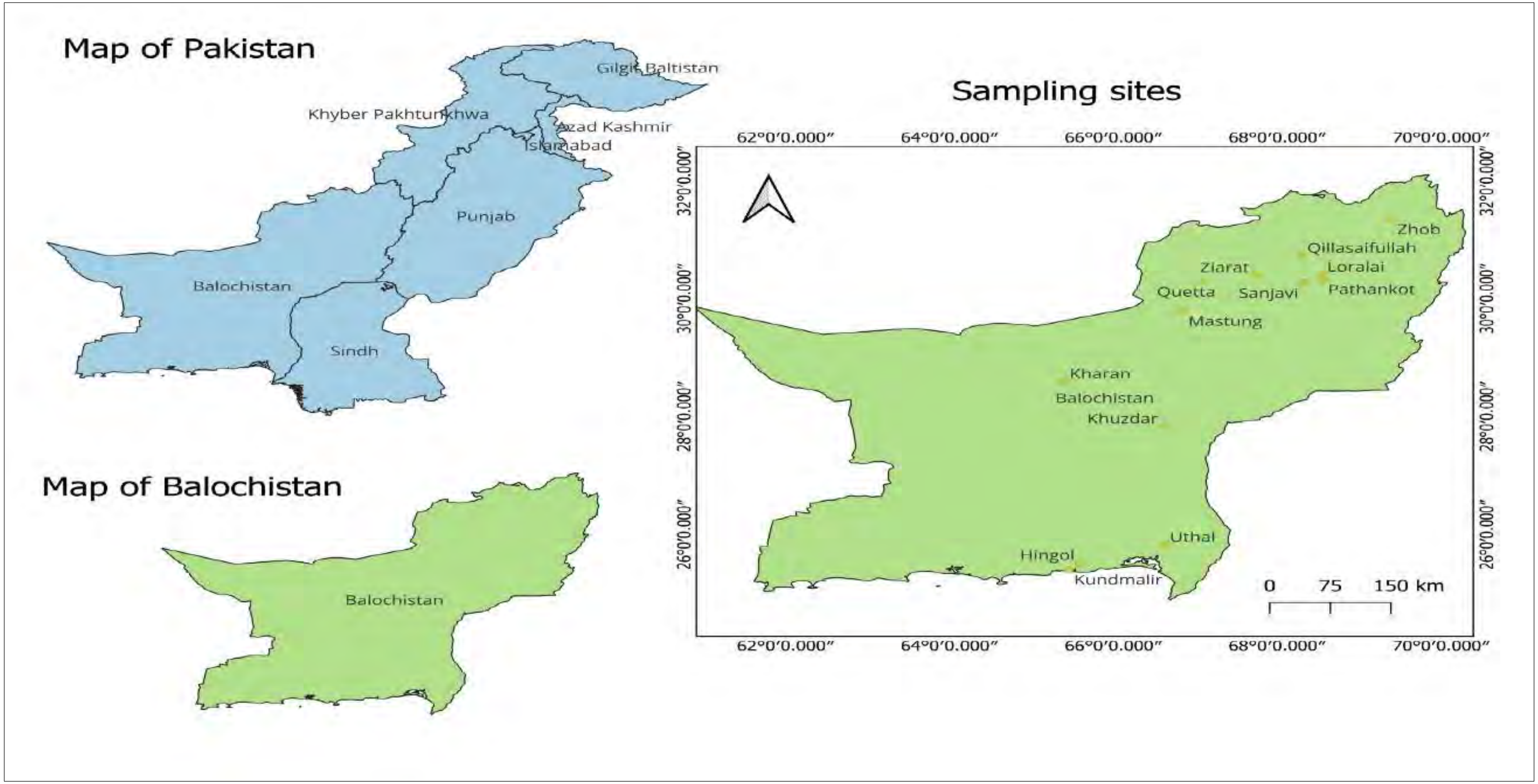

Figure 3. Map of Pakistan showing study areas of Baluchistan

Plate 5. The world's second-largest juniper forest Ziarat, Baluchistan

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

Plate 6. Hingol National Park, Baluchistan

It is situated in the three districts Gwadar, Lasbela, and Awaran. It is different from other national parks since it has six separate ecosystems and is made up of a combination of deserts, mountains, valleys, and water bodies.

Plate 7. Landscape view of study area Uthal (Lasbela), Baluchistan

Plate 8. Lower Mountain region of Takht-e-Sulaiman Zhob, Baluchistan

Plate 9. Encompassing view of Loralai, Baluchistan

Plate 10. Rangeland areas of Sanjavi, Baluchistan

Plate 11. Huge stony mountains of study area Pathankot Baluchistan

Plate 12. Hazarganji Chiltan National Park, Mastung Baluchistan, Pakistan

2.2 Collection and Preservation of Plant Material

Field trips were conducted for the collection of specimens from the various phytogeographically important localities of Baluchistan from March 2022 to May 2023 (Plate 13). The field explorations employed standard field procedures, as recommended, and detailed in the Manual of All Taxa Biodiversity Inventories and Monitoring (Eymann et al., 2010). For most species, more than two specimens were gathered. For several uncommon species, there were just one or two samples that could be obtained. The standard procedures of (Eymann et al., 2010; Judd et al., 2002) were followed for specimen preservation and mounting. The study districts were Quetta, Khuzdar, Loralai, Mastung, Ziarat, Zhob, Makran, Sanjavi, Pathankot, Kundmalir, Qillasaifullah, Hanna Lake, Pathankot, Kharan, Chiltan National Park, Las Bela, Otmanzai, Beela, Kalat, Hingol, Othal, Hubchowki, Sibbi. Fresh plant specimens were collected including both floral and vegetative components. Plant specimens were dried, pressed, and arranged in newspapers and blotting sheets. Using a digital camera (Sony, DSC W800), field photos of plants and collection locations were captured. In-depth plant information, including collection date, voucher number, locality, flowering period, geographic coordinates, habitat, and habit was recorded in field notes. The collected specimens were preserved with mercuric chloride and ethanol (50 mL absolute) and finally fumigated.

The identification of the plant specimens was confirmed by comparison and with the help of; herbarium specimens (herbarium of Pakistan (ISL) QAU Quaid-i-Azam University Herbarium, Islamabad, Pakistan) and other related flora, including the flora of China (Chen and Craven, 2007), North America (Torrey and Grey, 1969), and Pakistan (Nasir and Ali, 1971; Stewart et al., 1972), well-known taxonomist Professor Dr. Mushtaq Ahmad (Quaid-e-Azam University), Dr. Amir Sultan (Director National Herbarium NARC). International Plant Name Index (IPNI) and the Plant List (TPL) were utilized for plant name confirmation. Specimens were then mounted on standard herbarium sheets, numbered, labelled, and annotated with the location, date, and collector's name. Assigned the accession numbers to each specimen and submitted them to the Herbarium of Pakistan (ISL) QAU (Plate 14).

Plate 13. Exploring the angiospermic flora in the world's second-largest juniper forest, Ziarat (Baluchistan), with field documentation from local communities

Plate 14. Fumigation (a, b) and mounting (c, d) of the specimen on herbarium sheets

2.3 Anatomical Study of Angiospermic Flora

2.3.1 Histological Studies

a) Section Cutting

The petioles /culms were separated and treated with 10% saline formal solution (four hours) for fixation (changed the solution two times). Then dehydrated the samples by treating them with different concentrations of methanol (70%, 80%, 90%, 100%, and again 100 %) each for one hour. For dealcoholisation (methanol removal), xylol was applied twice for one hour each. Then impregnated the samples using wax at 58-62 ^{o} C (two times for one hour). At 3-5 micron thickness the samples were embedded by section cutting using microtomy. Finally at 62 ^{o} C the melting of the prepared slides was accomplished (Akhtar et al., 2022).

b) Staining

Deparaffinization of the samples was carried out for five minutes with xylol (repeated two times). Applied the methanol in 100 %, 90%, and 70% concentrations each for one minute, to rehydrate the samples. Then washed with tap water for one minute. The basic stain haematoxylin was applied for five minutes and washed for one minute with tap water. Then dipped the slides in 1% acid alcohol and washed again for one minute with tap water. For 30-60 seconds, treated the slides with 1% eosin, and clean them with tap water for one minute. For final dehydration 70%, 80%, 90%, 100% & 100% methanol was applied for 30 seconds each. Cleared the prepared slides with xylol for one minute (two treatments). Dibutylphthalate Polystyrene Xylene (DPX) was employed for mounting of the sections on the slides. Labelled the slides and observed them utilizing light microscopy (Akhtar et al., 2022).

2.3.2 Petiole/Culm Anatomy via Light Microscopy (LM)

Petiole and culm anatomical examination under the light microscope was documented for Asteraceous, Boraginaceous, and Poaceous flora. The qualitative and quantitative characters were observed with calibrated eyepiece according to the method of Arnold (1973). The description of traits was accomplished by Metcalfe and Chalk (1979); Metcalfe (1973); Heneidak and Shaheen (2007), and Chen et al. (2008). The standard Kellogg, (2015) terminology of anatomy was also followed. For the cavities, the descriptions of Yang et al. (2011) were followed. The length and width of various cells, layers of cells, and other features were noted. The key for observed parameters is given in the Table 1.

2.4 Palynological Studies

Light microscopy was utilized to examine the morphology of pollen using samples taken from anthers of flowers during anthesis. According to Moore et al. (1991), pollen were washed with 70% alcohol to remove oily compounds. Then, acetolysis was carried out according to a standard technique (Erdtman, 1963) with some changes (Zafar et al., 2007). The pollen were acetolyzed for four to eight minutes, to reveal the sculpturing patterns and clean the exterior pollen surface. Before LM observations, glycerine jelly was used for mounting. The terminologies for pollen characteristics were derived from Grant-Downton (2009) and Punt et al. (2007).

2.4.1 Pollen Exploration via Light Microscopy (LM)

The process outlined by Moore et al. (1991) was used for the chemical preparation techniques as well as the flower and pollen extraction. The pollen were boiled in 10% KOH for approximately 6–10 minutes before the acetolysis procedure to facilitate aperture opening and make it easier to evaluate aperture features (Reitsma, 1969). The conventional acetolysis procedure (Erdtman, 1969) was used to prepare pollen slides along with some modifications (Zafar et al., 2007). Utilizing forceps and needles, anthers were placed on a glass slide. Poured two to three drops of acetic acid over anthers on the glass slides and crushed them using a glass rod. The debris was removed with a needle and then samples were mounted in glycerine jelly once the acetolysis process was complete and then dyed with glycerine jelly. The cover slip, transparent nail polish, labelling, and cover slip were used to seal the pollen samples.

Using the prepared slides and LM at various resolutions, both qualitative and quantitative characteristics were observed. Leica Light Microscope (Model 1000) embedded with the Infinity 1-5 C-MEL (Canada) digital camera was used to take the photomicrographs. Twenty readings for each attribute were recorded. Different palynological traits such as exine thickness, pore length, pore width, colpus length, colpus width, polar axis, P/E ratio, pollen fertility, etc. The minimum, maximum, and mean ± standard error of the results were tabulated.

2.4.2 Scanning Electron Microscopy (SEM) of Pollen

Scanning electron microscopy (SEM) was conducted using the methodology of (Bahadur et al., 2019). Two drops of acetic acid were added to the anthers to remove the debris (with a needle). The samples were mounted to the stub using double-coated Scotch tape. The specimens were provided with an additional gold palladium sputter coating. SEM experiments were carried out on micromorphological traits at the Department of Physics, Central Resource Library (CRL), University of Peshawar, Pakistan. The pollen images were taken using Polaroid P/N 655 film that was put in the SEM. A standard check sheet was used to examine both qualitative and quantitative characters (for diagnostic features). The key for observed traits is given in Table 2.

P/E ratio (Quantitative analysis)

The ratio of P/E was determined using the given formula.

P/E × 100

Where P is the polar axis and E is the equatorial diameter. Based on the P/E ratio, the size and shapes of pollen were investigated (Hussain et al., 2019). The size of the pollen was calculated from the longest axis which may be a maximum value of polar axis or equatorial diameter. The terminology of Erdtman (1963) was followed for the size and shape classes of the pollen. Six size classes ranging from very small grain (<10µm) to gigantic grains (>200µm) and eight shape classes have been categorized by Erdtman (1963).

Viability

The viability of the pollen was determined mathematically by the given formula.

F/F+S*100

F = Fertility

S = Sterility

The value of F is calculated as the number of fertile pollen and S as the number of sterile pollen.

Number Position Characters (NPC) Classification

Referring to the pollen, the NPC-System classification based on the apertures, their Number (N-whether single or two or many), Position (P-polar: distal or proximal; global; meridional), and Characters (C-circular or elongated) was employed for the name and formula. The word "treme," which refers to an aperture, has been used in this approach to prepare keys for the classification of pollen. For the number of apertures, terms such as mono, di, and tri were used. The position of the apertures was designated as cata, ana, zono, and so on. In the character, the type of the aperture was observed such as porate, colpate, colporate, pororate, etc (Erdtman, 1969).

2.5 Statistical Analysis

Evaluation of qualitative and quantitative details is an important component of plant systematics for determining the boundaries of species, genus, and tribes. Using SPSS-16, the mean and standard values for the quantitative palynological and anatomical features under study were calculated. PAST (ver. 4.11), NCSS (20230), Origin (2023) were used to research correlation ($p<0.05$), dendrogram Un-weighted Pair Group Clustering Method (UPGMA), and principal component analysis (PCA)

(Iamonico et al., 2023). In the box plot the overall distribution and analysis of differences between the means was analysed.

2.6 Taxonomic Key

The palynological and anatomical qualitative attributes were then used to establish dichotomous taxonomic keys, that help in species identification.

Table 1. The key to 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	Ground parenchyma	Vascular bundle number
13	Vascular bundle arrangement	Number of major and peripheral vascular bundles
14	Bundle sheath	Xylem vessel size
15	Xylem vessel shape	Phloem cell size
16	Phloem shape Xylem and phloem parenchyma	
17	Sclerenchyma presence in vascular bundles	
18	Cavities / air spaces (central, marginal)	

Table 2. The 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	Harmomegathy	Polar width of colpi
7	Annulus	Polar length of colpi
8	Operculum	Equatorial length of colpi
9	Polar view	Equatorial width of colpi
10	Equatorial view	Pore length
11	Exine sculpturing	Viability
12	Exine surface	Exine thickness
13	Edges of the aperture	Number of spines per pollen
14	Aperture membrane	Number of spines between colpi
15	Pore orientation	Spine length
16	Colpi type	
17	Arrangement of apertures	
18	Lacuna shape	
19	AMB	
20	NPC classification (Name and formula)	

Chapter 3

Results and Discussion

Section-I

Summary

Phytogeographically Baluchistan is one of the important floristic regions. The region is home to wild medicinal plants. This research was carried out in different areas of Baluchistan, Pakistan. These areas were explored for the first time for palynological and anatomical studies of angiospermic flora.

- In the first part of this section, the details of some collection sites such as Quetta, Loralai, Hingol and others are given.
- The second part includes information of collected 109 angiosperms, belonging to three families, A,steraceae, Boraginaceae, and Poaceae. 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 third part of this section consists of the field photographs of the collected angiosperms of three selected families. Botanical names are given along with some information on each plant noted during the field.

Table 3. The documented taxa of Asteraceae from Baluchistan, Pakistan

S/ No	Accepted name	Tribe	Elevation (m)	Location	Accession Number
1	*Achillea cretica* L.	Anthemideae	1800	Hanna lake	133289 (ISL)|QAU
2	*Artemisia biennis* Hook.f.	Anthemideae	2100	Ziarat	133290 (ISL)|QAU
3	*Atractylis carduus* C.Chr.	Cardueae	1319	Loralai	133291 (ISL)|QAU
4	*Blumea sinuata* (Lour.) Merr.	Inuleae	1500	Sanjavi	133292 (ISL)|QAU
5	*Centaurea iberica* Trevir. ex Spreng.	Cardueae	2000	Ziarat	133293 (ISL)|QAU
6	*Cirsium arvense* (L.) Scop.	Cardueae	1400	Loralai	133294 (ISL)|QAU
7	*Cousinia haeckeliae* Bornm.	Cardueae	1900	Zhob	133295 (ISL)|QAU
8	*Cousinia prolifera* Jaub. & Spach	Cardueae	724	Kharan	133296 (ISL)|QAU
9	*Crepis kotschyana* Boiss.	Cichorieae	1490	Sanjavi	133297 (ISL)|QAU
10	*Erigeron bonariensis* L.	Astereae	1320	Loralai	133298 (ISL)|QAU
11	*Filago hurdwarica* (Wall. ex DC.) Wagenitz	Gnaphalieae	1600	Quetta	133299 (ISL)|QAU
12	*Flaveria trinervia* (Spreng.) C.Mohr	Tageteae	1450	Loralai	133300 (ISL)|QAU
13	*Gymnarrhena micrantha* Desf.	Gymnarrheneae	1800	Qillasaifullah	133301 (ISL)|QAU
14	*Hertia intermedia* Kuntze	Senecioneae	1780	Loralai	133302 (ISL)|QAU
15	*Heteroderis pusilla* Boiss.	Cichorieae	1920	Hanna lake	133303 (ISL)|QAU
16	*Himalaiella afghana* (Lipsch.) Raab-Straube	Cardueae	1017	Sanjavi	133304 (ISL)|QAU

17	*Himalaiella heteromalla* (D.Don) Raab-Straube	Cardueae	1886	Zhob	133305 (ISL)|QAU
18	*Iphiona grantioides* (Boiss.) Anderb.	Inuleae	280	Kundmalir	133306 (ISL)|QAU
19	*Jurinea berardioidea* Diels	Cardueae	1490	Sanjavi	133307 (ISL)|QAU
20	*Jurinea carduiformis* (Jaub. & Spach) Boiss.	Cardueae	1735	Chiltan National Park	133308 (ISL)|QAU
21	*Koelpinia linearis* Pall.	Cichorieae	1865	Sanjavi	133309 (ISL)|QAU
22	*Koelpinia turanica* Vassilcz.	Cichorieae	1400	Loralai	133310 (ISL)|QAU
23	*Lactuca dissecta* D.Don	Cichorieae	1600	Loralai	133311 (ISL)|QAU
24	*Lactuca orientalis* Boiss.	Cichorieae	1400	Zhob	133312 (ISL)|QAU
25	*Lactuca serriola* L.	Cichorieae	1400	Zhob	133313 (ISL)|QAU
26	*Lasiopogon muscoides* (Desf.) DC.	Gnaphalieae	1560	Sanjavi	133323 (ISL)|QAU
27	*Launaea acanthodes* (Boiss.) Kuntze	Cichorieae	1560	Loralai	133315 (ISL)|QAU
28	*Launaea aspleniifolia* (Willd.) Hook.f.	Cichorieae	1130	Pathankot	133316 (ISL)|QAU
29	*Launaea fragilis* subsp. *Fragilis*	Cichorieae	1960	Zhob	133317 (ISL)|QAU
30	*Launaea intybacea* (Jacq.) Beauverd	Cichorieae	1600	Sanjavi	133318 (ISL)|QAU
31	*Launaea oligocephala* Bornm.	Cichorieae	2200	Zhob	133319 (ISL)|QAU
32	*Launaea procumbens* (Roxb.) Amin	Cichorieae	1590	Mastung	133320 (ISL)|QAU
33	*Launaea stenocephala* (Boiss.) Kuntze	Cichorieae	1240	Khuzdar	133321 (ISL)|QAU
34	*Leuzea repens* (L.) D.J.N.Hind	Cardueae	400	Hannalake	133322 (ISL)|QAU

35	*Microcephala lamellata* (Bunge) Pobed.	Anthemideae	1766	Quetta, Ziarat	133342 (ISL)|QAU
36	*Pallenis hierochuntica* (Michon) Greuter	Inuleae	1350	Pathankot	133324 (ISL)|QAU
37	*Pentanema divaricatum* Cass.	Inuleae	1490	Loralai	133325 (ISL)|QAU
38	*Phagnalon schweinfurthii* Sch.Bip. ex Schweinf.	Gnaphalieae	2022	Shirani	133326 (ISL)|QAU
39	*Pterachaenia stewartii* (Hook.f.) R.R.Stewart	Cichorieae	1550	Loralai	133327 (ISL)|QAU
40	*Pulicaria undulata* (L.) Kostel.	Inuleae	1350	Loralai	133328 (ISL)|QAU
41	*Pulicaria angustifolia* DC.	Inuleae	1320	Loralai	133329 (ISL)|QAU
42	*Reichardia tingitana* Roth	Cichorieae	1550	Sanjavi	133330 (ISL)|QAU
43	*Scorzonera koelpinioides* Rech.f.	Cichorieae	1524	Loralai	133331 (ISL)|QAU
44	*Scorzonera raddeana* C. Winkl.	Cichorieae	1750	Hazarganji	133332 (ISL)|QAU
45	*Senecio glaucus* L.	Senecioneae	660	Kundmalir	133333 (ISL)|QAU
46	*Seriphidium maritimum* (L.) Poljakov	Anthemideae	2200	Ziarat	133334 (ISL)|QAU
47	*Sonchus arvensis* L.	Cichorieae	1500	Quetta	133335 (ISL)|QAU
48	*Sonchus oleraceus* L.	Cichorieae	1230	Sanjavi	133336 (ISL)|QAU
49	*Symphyotrichum subulatum* (Michx.) G.L.Nesom	Astereae	1530	Loralai	133337 (ISL)|QAU
50	*Takhtajaniantha pusilla* (Pall.) Nazarova	Cichorieae	1750	Quetta	133338 (ISL)|QAU
51	*Xylanthemum macropodum* (Hemsl. & Lace) K.Bremer & Humphries	Anthemideae	1446	Loralai	133340 (ISL)|QAU
52	*Zoegea purpurea* Fresen.	Cardueae	1550	Loralai	133341 (ISL)|QAU

Table 4. The documented taxa of Boraginaceae from Baluchistan, Pakistan

S. No	Accepted name	Elevation (m)	Location	Habitat	Accession Number
1	*Alkanna tinctoria* subsp. *Tinctoria*	490	NARC	Muddy soil	133344 (ISL)|QAU
2	*Buglossoides arvensis* (L.) I.M.Johnst.	1400	Loralai	Sandy soil	133345 (ISL)|QAU
3	*Caccinia mucronanthera* Desf.	1650	Loralai	Sandy-gravely soil	133346 (ISL)|QAU
4	*Cynoglossum lanceolatum* Forssk.	1500	Pathankot	Sandy soil	133347 (ISL)|QAU
5	*Gastrocotyle hispida* (Forssk.) Bunge	1380	Loralai	Sandy soil	133348 (ISL)|QAU
6	*Heliotropium bacciferum* Forssk.	160	Kundmalir	Clay type	133349 (ISL)|QAU
7	*Heliotropium campanula* (Forssk.) Bunge	1750	Quetta, Zhob	Sandy-gravely	133350 (ISL)|QAU
8	*Heliotropium crispum* Desf.	300, 1350	Kundmalir, Beela, Loralai	Muddy, sandy soil	133351 (ISL)|QAU
9	*Heliotropium curassavicum* Desf.	204	Kundmalir	Clay-muddy	133352 (ISL)|QAU
10	*Lappula spinocarpos* (Forssk.) Asch. ex Kuntze	1400-1650	Otmazai-Zhob	Clay type	133353 (ISL)|QAU
11	*Lappula spp* (Popov) Y.J. Nasir	1350	Quetta	Sandy soil	133354 (ISL)|QAU
12	*Lappula spinocarpos* subsp. *ceratophora* (Popov) Y.J. Nasir	1280-1980	Quetta	Gravely soil	133355 (ISL)|QAU
13	*Onosma limitanea* var. *limitanea* I.M. Johnst.	1800	Loralai	Sandy soil	133356 (ISL)|QAU
14	*Onosma limitanea* var. *major* I.M. Johnst.	1750	Quetta	Sandy, gravely	133357 (ISL)|QAU
15	*Paracaryum intermedium* var. *intermedium* YASIN J. NASIR	1350	Loralai	Sandy soil	133358 (ISL)|QAU

16	*Paracaryum intermedium var. calathicarpum* (Stocks) Y.J. Nasir	1600	Quetta	Gravely soil	133359 (ISL)|QAU
17	*Rochelia disperma* (L.f.) K.Koch	1530	Loralai	Sandy-gravely	133360 (ISL)|QAU
18	*Rochelia sessiliflora* (Boiss.) Khoshsokhan & Kaz.Osaloo	252	Kundmalir	Clay-muddy soil	133361 (ISL)|QAU
19	*Trichodesma indicum* (L.) Sm.	320	Las Bela	Sandy clay	133362 (ISL)|QAU

Table 5. The documented taxa of Poaceae from Baluchistan, Pakistan

S/N	Accepted name	Elevation (m)	Location	Habitat	Accession number
1	*Aristida adscensionis* L.	1633	Makran	Muddy soil	133255 (ISL) | QAU
2	*Aristida cyanantha* Steud.	1440	Mastung	Silty soil	133256 (ISL) | QAU
3	*Aristida funiculata* Trin. & Rupr.	1040	Yateabad	Gravely soil	133257 (ISL) | QAU
4	*Arundo donax* L.	1100	Sanjavi	Muddy soil	133258 (ISL) | QAU
5	*Avena sativa* L.	1500	Pathankot	Sandy soil	133281 (ISL) | QAU
6	*Boissiera squarrosa* (Sol.) Nevski	1800	Kalat	Sandy soil	133259 (ISL) | QAU
7	*Bromus lanceolatus* Steud.	1540	Pathankot, Sanjavi	Sandy to gravely soil	133282 (ISL) | QAU
8	*Cenchrus divisus* (Gmel.) Henr.	960	Makran, Panjgur	Sandy rocky soil	133275 (ISL) | QAU
9	*Cenchrus flaccidus* Griseb.	1700-700	Kalat, Kharan	Muddy soil	133276 (ISL) | QAU
10	*Cenchrus orientalis* Steud.	1530-88	Loralai, Uthal	Sandy-muddy soil	133283 (ISL) | QAU
11	*Cenchrus setigerus* Vahl	65	Hingol, Bela	Lower hills and plains	133260 (ISL) | QAU
12	*Chloris barbata* Sw.	850	Othal, Bela	Muddy soil	133261 (ISL) | QAU
13	*Chrysopogon aucheri* (Boiss.) Stapf	1800	Hingol, Kalat, Makran	Rocky slopes	133262 (ISL) | QAU
14	*Chrysopogon serrulatus* Sw.	70	Hingol	Muddy-sady soil	133284 (ISL) | QAU
15	*Cymbopogon martini* (Roxb.) W.Watson.	853	Khuzdar, Hingol	Muddy soil	133263 (ISL) | QAU

16	*Dactyloctenium aristatum* Link, Hort.	850	Bela	Sandy soil	133264 (ISL) | QAU
17	*Dactyloctenium scindicum* Boiss.	60	Bela Othal	Gravely soil	133265 (ISL) | QAU
18	*Desmostachya bipinnata* Desf.	1400	Sanjavi	Sandy soil	133285 (ISL) | QAU
19	*Dichanthium foveolatum* Desf.	90	Hingol	Sandy soil	133286 (ISL) | QAU
20	*Diplachne fusca* (L.) P. Beauv.	20	Hubchowki	Muddy soil	133266 (ISL) | QAU
21	*Eleusine indica* Desf.	1580	Pathankot	Sandy-gravely soil	133287 (ISL) | QAU
22	*Enneapogon persicus* Boiss.	1650	Mastung, Hingol	Sandy soil	133267 (ISL) | QAU
23	*Eragrostis curvula* (Schrad.) Nees	1700	Kalat, Mastung	Silty soil	133268 (ISL) | QAU
24	*Eremopyrum bonaepartis* (Spreng.) Nevski	1800	Kalat	Muddy soil	133269 (ISL) | QAU
25	*Eremopyrum distans* (K. Koch) Nevski	2500	Mastung, Sibbi	Sandy soil	133270 (ISL) | QAU
26	*Hordeum marinum* subsp. gussoneanum (Parl.) Thell.	1500	Mastung	Gravely soil	133271 (ISL) | QAU
27	*Imperata cylindrica* (L.) Raeusch	2000	Makran	Semi-sandy soil	133272 (ISL) | QAU
28	*Leptothrium senegalense* (Kunth) Clayton	960	Kund maleer	Sandy soil	133273 (ISL) | QAU
29	*Ochthochloa compressa*	95-1400	Uthal, Loralai	Semi-sandy soil	133288 (ISL) | QAU
30	*Panicum antidotale* Retz.	63	Bela	Sandy soil	133274 (ISL) | QAU
31	*Phalaris minor* Retz.	1750	Hingol	Gravely soil	133277 (ISL) | QAU
32	*Piptatherum baluchistanicum* Freitag	2500	Kalat, Hingol	Sandy soil	133278 (ISL) | QAU
33	*Poa annua* Desf.	1450	Loralai	Sandy soil	133363 (ISL) | QAU

34	*Poa infirma* Boiss.	1590	Quetta	Sandy soil	133364 (ISL) | QAU
35	*Saccharum griffithii* Desf.	2200	Ziarat	Sandy-gravely soil	133343 (ISL) | QAU
36	*Schismus arabicus* Nees	2124	Makran, Kalat	Mountains	133279 (ISL) | QAU
37	*Tetrapogon villosus* Desf.	1751	Makran, Hingol	Mountain slopes	133280 (ISL) | QAU
38	*Vulpia persica* Boiss.	1400	Pathankot	Sandy soil	133314 (ISL) | QAU

3.1 Phytogeography of Studied Angiospermic Flora

Baluchistan is a province in Pakistan that has a rich and diverse flora including many endemic plants. Many wild plants of Baluchistan have medicinal properties. This study included comprehensive field trips in the different localities of Baluchistan and collected 109 plant specimens. The plants were then separated into their respective families. The three most prominent families were Asteraceae, Poaceae, and Broaginaceae.

3.1.1 Areas

a) Quetta

The climate of Quetta District is arid. Summers are warm, and winters are moderate to extremely cold. Snow can fall in January, February, and December. Since Quetta is not in the monsoon zone, it does not experience frequent, heavy rainfall. District Quetta is steep topographically, rising to a height of 5510 feet above sea level (Ali et al., 2023). Quetta is the largest city and capital of Baluchistan. The Quetta Sub-basin covers 603 km^2 and extends from 30° 0′ to 30° 30′ N and from 66° 40′ to 67°15′ E (Khan et al., 2012). With a population of more than 3.0 million and being the most affected by the water resources available, Quetta could soon be facing a serious issue. The Quetta Valley experiences significant temperature variations between summer and winter because to its semi-arid environment. Usual summer temperatures are between 24 and 26 °C, typical autumn temperatures are between 12 and 18 °C, average winter temperatures are between 4 and 5 °C, and average spring temperatures are between 15 and 20 °C. January had the greatest monthly precipitation average (56.7 mm), while September had the lowest monthly precipitation average (0.3 mm) (Qureshi et al., 2022).

b) Ziarat

Ziarat holds the second-biggest juniper reserves in the world; the largest reserves are in California. Because of the longevity of the trees in these reserves, some mature trees are thought to be 4,000 to 5,000 years old, they are known as living fossils. Because they have positive effects on the environment and the economy both locally and globally, juniper forests are very important. These distinctive and important ecosystems provide various benefits to the local communities, including firewood, bark for fences, cattle feed, and fencing materials. The largest juniper forest block, however, is located close to Ziarat and spans a vast area of over 2800 km^2. It is one of the world's biggest, most distinctive, and oldest juniper forests (Achakzai et al., 2016).

c) Hingol

The largest national park in Pakistan is called Hingol National Park (HNP), and it is situated in the Makran coastline region. Positioned at 65º 32' 12" East and 25º 42' 16" North, the park spans an approximate area of 6,100 square kilometers and is situated in the three districts of Gwadar, Lasbela, and Awaran in Baluchistan, 190 km west of Karachi. In 1988, the Hingol was designated as a national park (Khan et al., 2010). The southern portion of the Hangul River, which hugs the Arabian Sea's coast and supports a wide variety of species, is the source of the park's name. The HNP is different from other national parks in Pakistan since it has six separate ecosystems and is made up of a combination of deserts, mountains, valleys, and water bodies. HNP is surrounded by tributaries of the Hangul River, a barren mountain range to the south, and a lush forest to the north. To the south lie the Arabian Sea and the Gulf of Oman. Baluchistan, Pakistan's HNP is a natural sanctuary for threatened species of wildlife (Buzdar et al., 2023). According to Aslam et al. (2022) and Khan et al. (2010), it is home to over 257 plant and 289 animal species, including hundreds of rare species. This region has an arid, moderate subtropical climate. Summertime temperatures typically reach around 40ºC during the day, with evenings seeing a drop in temperature due to dampness and sea winds. Temperature swings throughout the winter season range from 5º C to 35º C, respectively, for days and nights (Khan et al., 2010). The low recorded yearly rainfall ranges from 50 to 150 mm.

d) Uthal

Uthal is situated 13 to 26 km landward from Miani Hor in the southern region of Baluchistan. It's a marshy lagoon on Lasbela district shore. Uthal is located in Baluchistan's Porali Plain and approximately 20 to 75 metres above mean sea level (Khan et al., 2020). The population of this semi-arid region has grown significantly during the past few decades. Uthal spans from 66.5◦ to 66.6◦ E longitude and from 25.7◦ to 25.8◦ N latitude. The Porali Plain, an alluvial plain of the Porali River, is where Uthal is situated. The Bela Plain lies to the north, the Harro Range to the west, and the Mor and Pab Ranges to the east. The Porali Plain's lower regions, which terminate at Sarinda Lake, make up the southern portion. Uthal's soil is made up of loose clay and fine sand. Uthal experiences hot summers and mild, dry winters due to its arid climate (Mahmud et al., 2022).

e) Loralai

The province of Baluchistan has 32 districts, including Loralai. The principal city of the Loralai district is City Loralai. It is situated in the northeast of Baluchistan, Pakistan. The name "Bori" was long known in the city of Loralai (Noor et al., 2020). This arid area is located 4,700 feet above sea level on a 9,830 km^2 land area. This area is made up of both rangelands used for uncontrolled grazing and agricultural. Wheat, corn, apricots, apples, grapes, pomegranates, melons, and almonds are the principal crops (Younas et al., 2022). Pathankot is one of the picnic spots of Loralai. This area is rich in angiosperm biodiversity.

f) Zhob

Regionally Zhob is the tenth-largest district of the province Baluchistan. It is 12,400 km^2 in size. The district is situated in in the northeast of Quetta, Baluchistan Pakistan. The district Loralai is in the south and Afghanistan is to the north of the Zhob. Summertime brings considerable rainfall, with an annual mean rainfall of approximately 320.5 mm. Zhob District has an annual average temperature of 5 to 35 °C, and its steep terrain sees a lot of snowfall. The amount of rainfall, the depth of the weathered material, and the existence of rock joints and cracks all affect the availability of ground and surface water; in this case, the average depth of groundwater is 49 feet (Chandio et al., 2020).

g) Kund Malir

Kund Malir is a desert beach located around 145 km from Zero-Point on the Makran Coastal Highway in Hingol, Balochistan, Pakistan. The region is a component of Pakistan's largest national park, Hingol National Park. The most well-known feature of Kund Malir Beach is its sandy texture (Kassi et al., 2014). Large dunes may also be found in this area, and with time, they created sandy deserts. The Arabian Sea is situated on one side of the RCD road, but only various outcrops of various rock formations are visible on the other. As a result, there are several prawn and lobster breeding grounds

(Bano et al., 2015). The Kund Malir shoreline is a little village of Baloch fishermen, perched on a hill with the sea flowing beneath it. It is located near the Hingol River. The Makran Coastal Highway connects this seashore to Karachi. The coasts of Sindh and Balochistan "experience almost the same range of temperature and rainfall, with short, mild winters (10° to 20° C) and long, warm summers (21° to 39° C), and low rainfall (less than 250 mm/annum) (Shahzad et al., 2022).

Plate 15. Field pictorial view (a) *Achillea cretica*; pale green colour, leaves apex narrow, (b) *Artemisia biennis*; non-aromatic herb, erect, unbranched, pale-green, (c) *Atractylis carduus*; leaves coriaceous, linear–lanceolate or oblong-ellliptic, (d) *Centaurea iberica*; purplish capitulum, spinulose calyx

Plate 16. Field pictorial view (a), (b) *Cirsium arvense*; branches suberect, upper leaves gradually smaller, corolla pale purplish, (c) *Cousinia haeckeliae*; biennial or perennial shrublet, with long, laxly cobwebby hairy, (d) *Cousinia prolifera*; leaves oblong-lanceolate towards, corolla yellow

Plate 17. Field pictorial view (a) *Erigeron bonariensis*; Capitula numerous in racemes or panicles, (b) *Filago hurdwarica*; leaves crowded below the glomerules, capitula (c) *Flaveria trinervia*; Leaves opposite, ray florets, yellow, fertile, (d) *Gymnarrhena micrantha*; dwarf annual herb

Plate 18. Field pictorial view (a) *Hertia intermedia*; glabrous to glaucous undershrub, ligule of ray florets yellow (b) *Heteroderis pusilla*; annual, small herb, (c) (d) *Himalaiella heteromalla*; leafy in lower and middle part, uppermost part with very small leaves

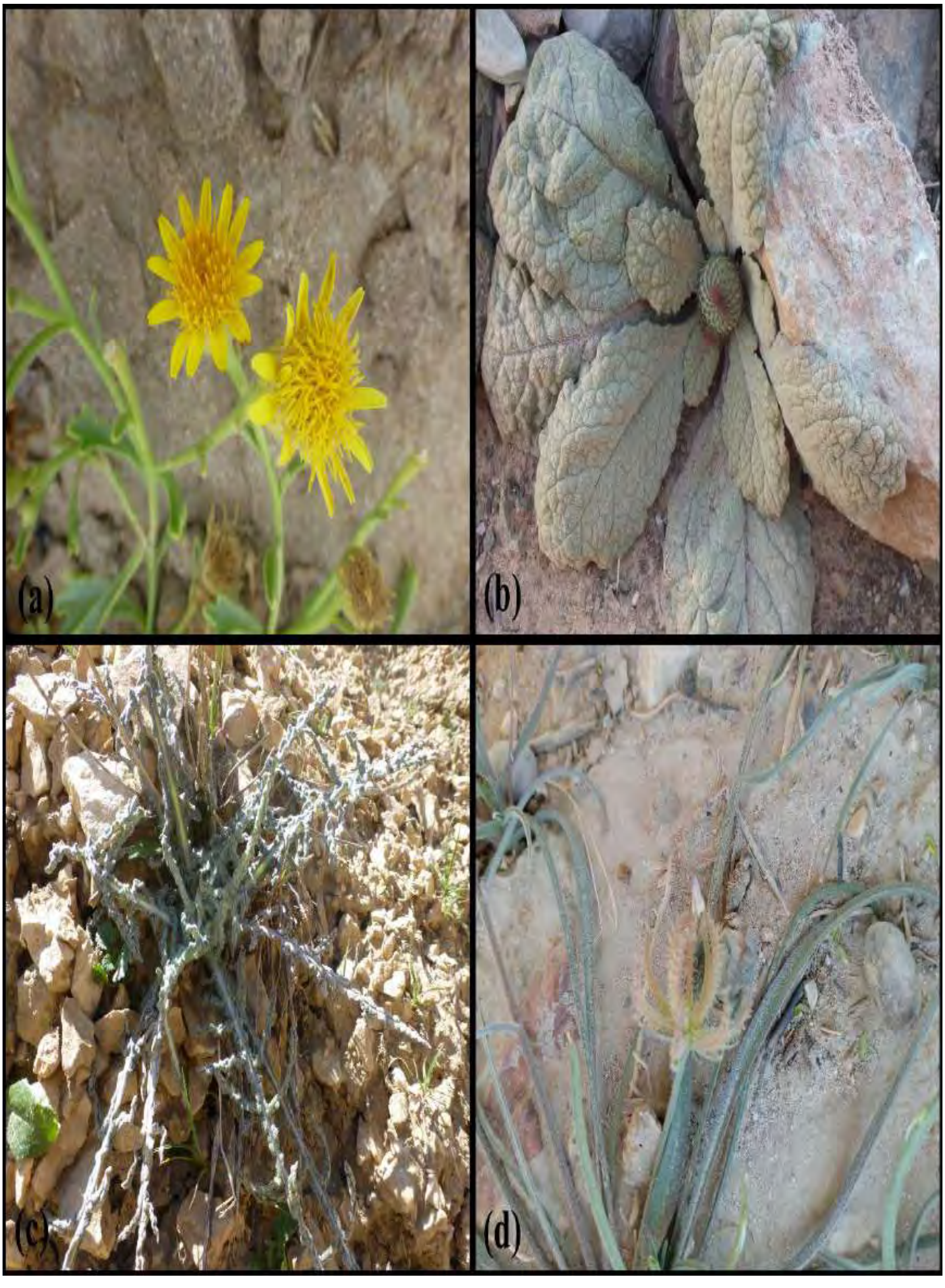

Plate 19. Field pictorial view (a) *Iphiona grantioides*; branch end yellowish single capitulum, leaves sessile, (b) *Jurinea berardioidea*; Leaves undivided to pinnatisect, capitula solitary or several in a corymbiform synflorescence, (c) *Jurinea carduiformis*; mid rib thin light green to pale yellow, (d) *Koelpinia linearis*; leaves dense, sessile

Plate 20. Field pictorial view (a) *Lactuca dissecta*; dichotomously branched above. basal leaves mostly rosulate, glabrous, sessile-narrowed at the base, (b) *Lactuca orientalis*; Plants perennial, semi-shrub and glabrous, virgates-flexuous branchlets with green striations of decurrent bases of leaves, (c) *Lactuca serriola*; biennial or perennial herbs, Radical leaves sessile, (d) *Lasiopogon muscoides*; annual herbs, capitula terminal

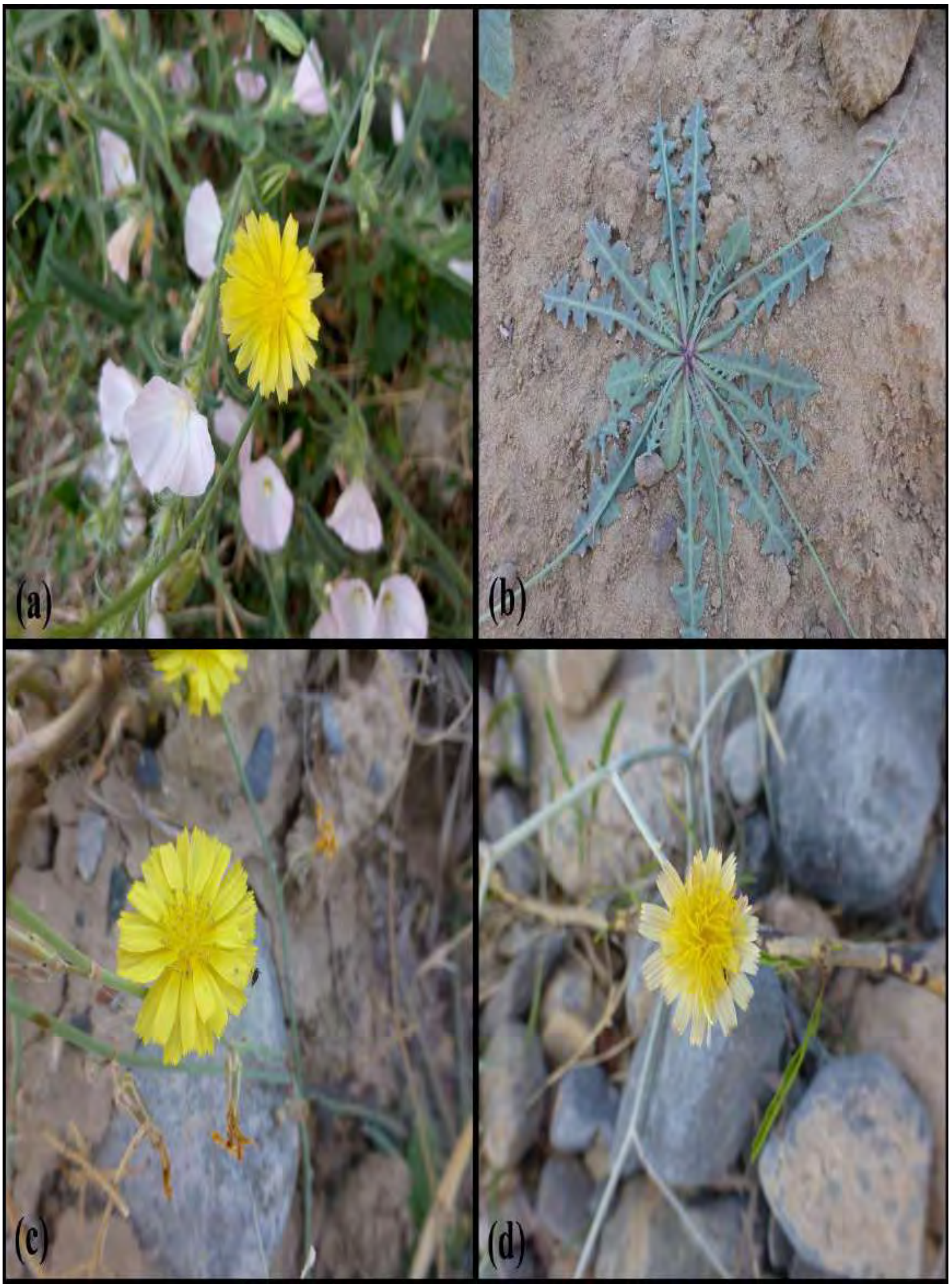

Plate 21. Field pictorial view (a) *Launaea oligocephala*; leaves rosulate bright yellow ligules, (b), (c) *Launaea procumbens*; Leaves rosulate, variable, yellow ligules (d) *Launaea acanthodes*; erect, dichotomously branched

Plate 22. Field pictorial view (a) *Leuzea repens*; leaves simple, subsessile, stiff, oblong–elliptic to ± linear, (b) *Pallenis hierochuntica*; ray floret tri dentate, hairy leaves (c) *Pentanema divaricatum*; Erect branches, capitulum yellow to white, (d) *Phagnalon schweinfurthii*; Woody perennials

Plate 23. Field pictorial view (a) *Pulicaria undulata*; perennial, leaves covered in white woolly hair, (b) *Reichardia tingitana*; tall herb, basally branched or unbranched or stemless herb. (c) *Scorzonera koelpinioides*; perennial, non-tuberous, Stems many, erect, grayish-green, (d) *Scorzonera raddeana*; Perennial, tuberous herb

Plate 24. Field pictorial view (a) *Senecio glaucus*; Leaves sessile, (b) *Seriphidium maritimum*; A subshrub with aromatic leaves are covered in hairs giving a soft silvery appearance and smell pleasantly medicinal, (c) *Sonchus arvensis*; perennial sow-thistle, (d) *Sonchus oleraceus*; erect herb with glaucous

Plate 25. Field pictorial view (a) *Sonchus oleraceus*; basal and lower leaves winged petioled, (b) *Symphyotrichum subulatum*; annual to biennial florets few, yellow, (c) *Xylanthemum macropodum*: erect shrublet, (d) *Zoegea purpurea*; annual, Florets white or purplish

Plate 26. Field pictorial view (a) *Alkanna tinctoria* subsp. *Tinctoria*; sessile alternate leaves, flower white, (b) (c) *Caccinia mucronanthera*; A leafy perennial lanceolate to oblanceolate, (d) *Cynoglossum lanceolatum*; much-branched biennial plant

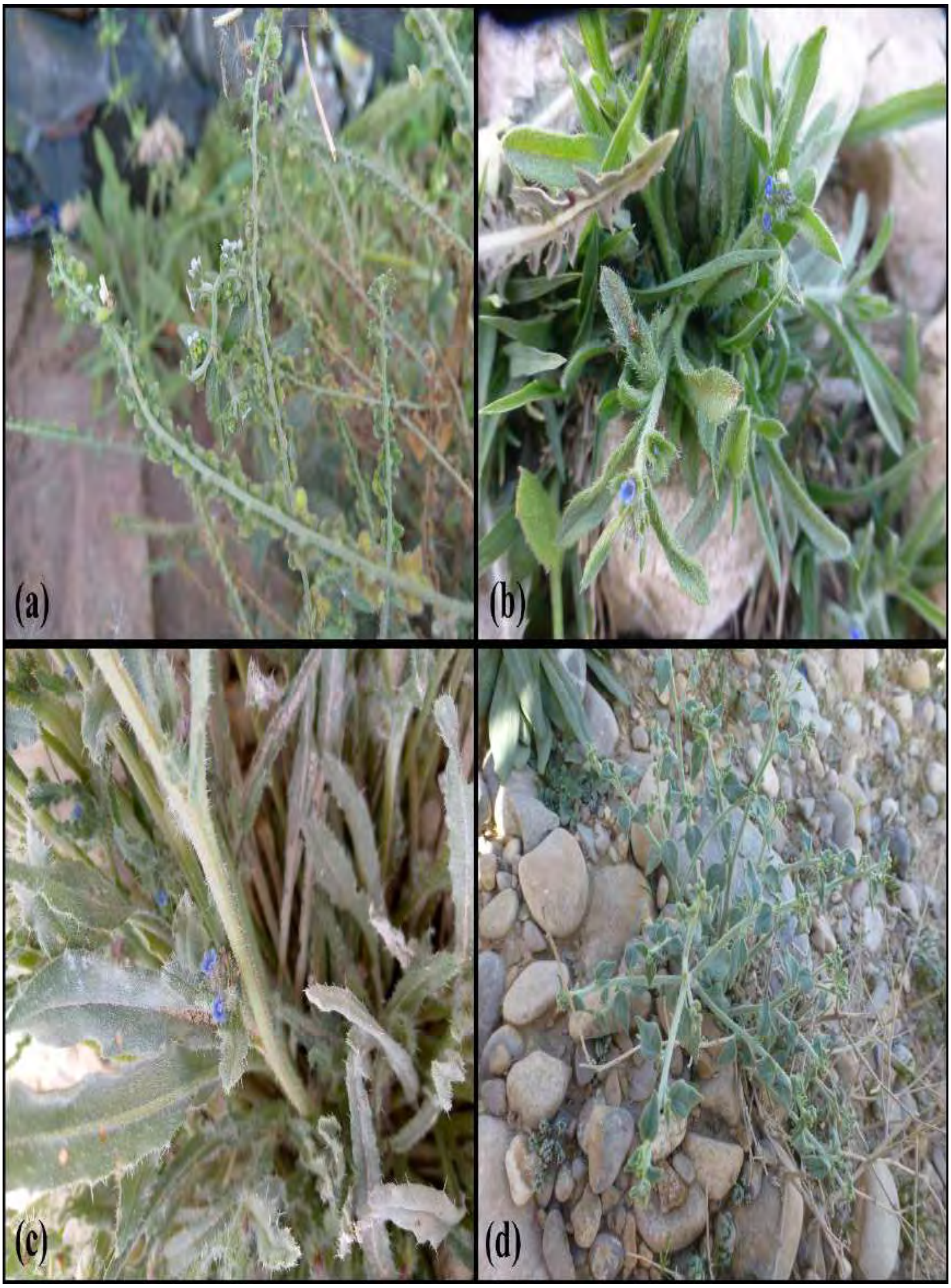

Plate 27. Field pictorial view (a) *Cynoglossum lanceolatum*; erect much branched, biennial, (b)**,** (c) *Gastrocotyle hispida*; leaves hairy on both surfaces, corolla purplish blue to blue, (d) *Heliotropium bacciferum*; perennial, decumbent or procumbent with a woody base

Plate 28. Field pictorial view (a) *Heliotropium campanula*; tetracyclic flowers and actinomorphic corollas, (b) *Heliotropium crispum*; uniseriate flowers, (c) *Lappula spp*; sessile, oblong, elongated leaves, flower white, (d) *Lappula spinocarpos*; branches hairy with appressed to sub appressed hairs, cauline leaves similar but smaller

Plate 29. Field pictorial view (a) *Lappula spinocarpos* subsp. *ceratophora*; greyish hairy oblong leaves with light purplish flower, (b) (c) *Onosma limitanea* var. *limitanea*; spiny leaves, oblong red and yellow corolla, (d) *Onosma limitanea* var. *major*: non woody herb, spiny leaves

Plate 30. Field pictorial view (a) *Paracaryum intermedium var. calathicarpum*; larger ovoid nutlet, (b) *Paracaryum intermedium* var. *intermedium*; biennials cauline leaves sessile, smaller. Inflorescence lax, (c) *Rochelia disperma*; distinguished by the pedicels which never exceed the calyx, (d) *Trichodesma indicum*; plants with amplexicaul leaves and a glabrous undersurface to leaves, corolla whitish

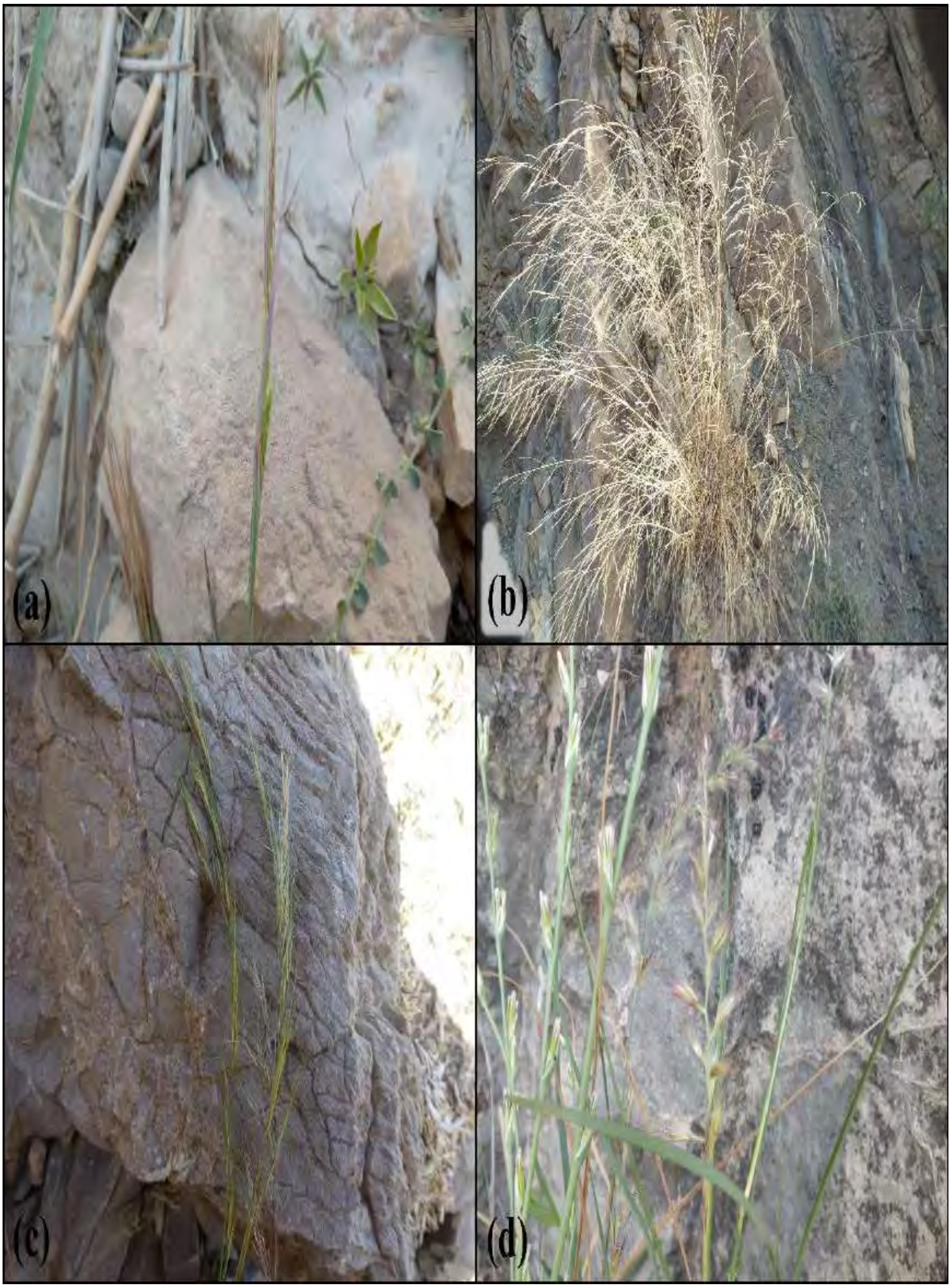

Plate 31. Field pictorial view (a) *Aristida adscensionis*; annual or short-lived perennial, leaf-blades linear, (b) *Aristida cyanantha*; densely tufted, the branches capillary, spikelets purplish, (c) Aristida funiculata; tufted annual with wiry culms, (d) *Cenchrus divisus*: muddy green leaves with alternate spikelets

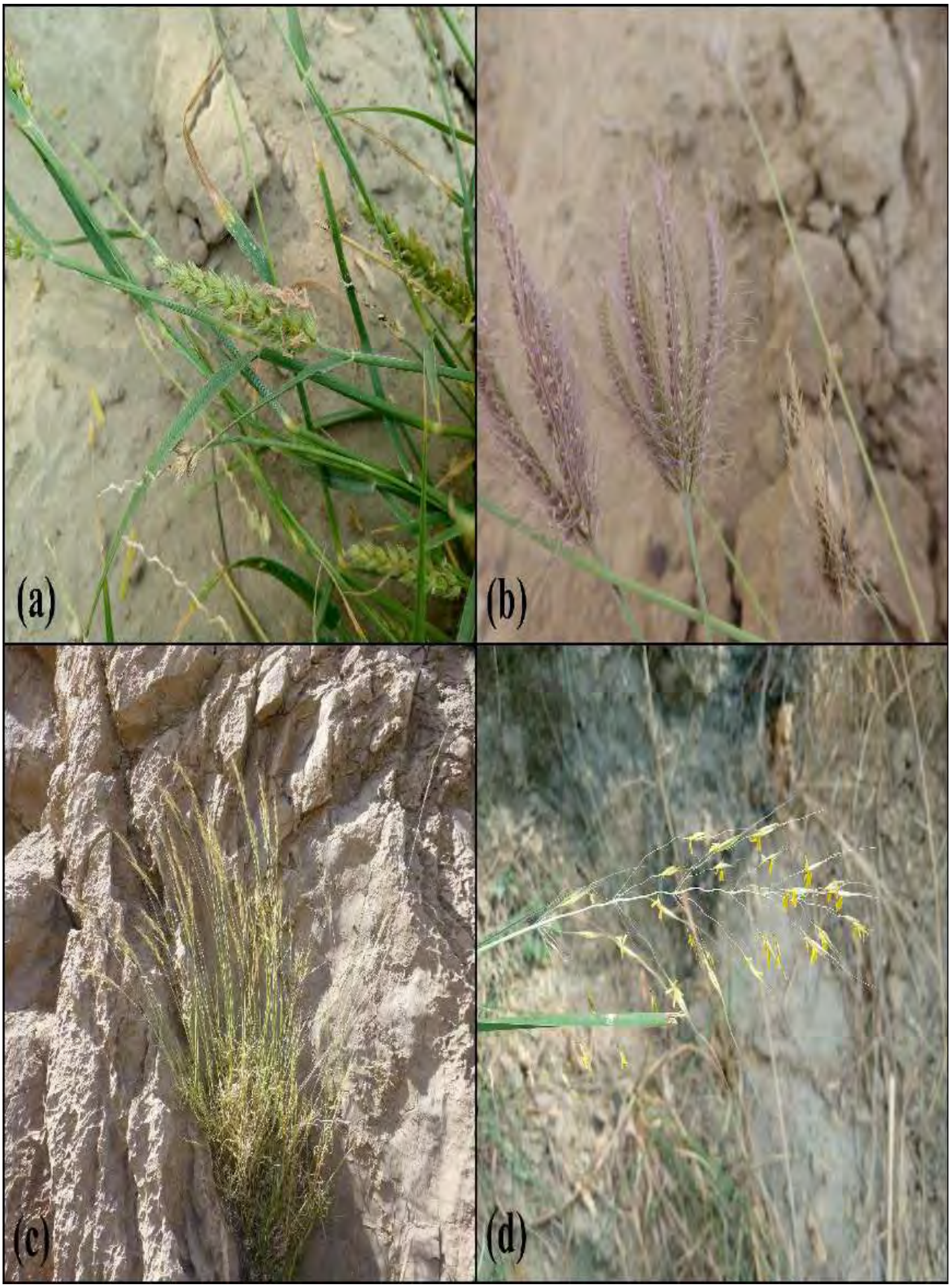

Plate 32. Field pictorial view (a) *Cenchrus setigerus*; birdwood grass (b) *Chloris barbata*; loosely tufted, leaf-blades flat, spikelets 3-flowered (c) (d) *Chrysopogon aucheri*; tufted glaucous perennial often with silky villous

Plate 33. Field pictorial view (a) *Chrysopogon serrulatus*; erect, robust, leaf-blades mostly cauline (b) *Cymbopogon martini*; leaf-blades linear-lanceolate or lanceolate, usually dark green above (c) (d) *Dactyloctenium aristatum*; sprawling tufted annual, spikelets 3-5-flowered

Plate 34. Field pictorial view (a) *Dichanthium foveolatum*; ufted perennial with silky hairy basal sheaths, leaf-blades very narrow, green or glaucous, (b) *Enneapogon persicus*; tufted wiry perennial, (c) *Eragrostis curvula*; spikelets 4-13-flowered, grey-green (d) *Eremopyrum distans*; leaf-blades flat

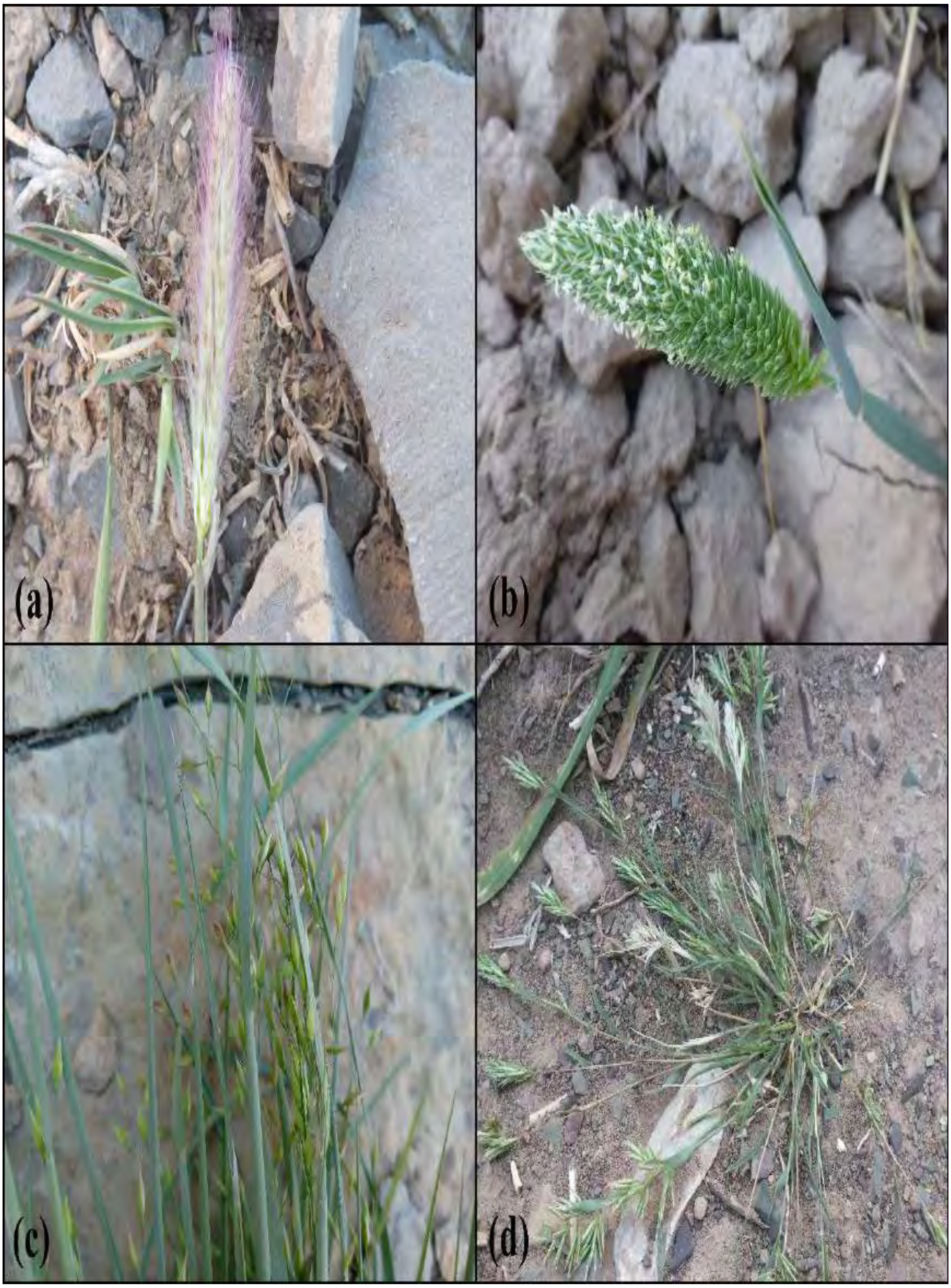

Plate 35. Field pictorial view (a) *Hordeum marinum* subsp. *gussoneanum*; spike oblong to ovate, (b) *Phalaris minor*; broadly winged, the wing margin usually erose-denticulate, sterile floret (c) *Piptatherum baluchistanicum*; leaf-blades flat or rolled, greyish, densely hairy on the upper surface (d) *Schismus arabicus*; leaf-blades linear

Plate 36. Field pictorial view (a) (b) *Tetrapogon villosus*; spikes exserted, paired, dorsally adpressed, rarely separating at maturity, purple-tinged (c) *Ochthochloa compressa*; sprawling stoloniferous perennial, clustered at the top of the culm, spikelets 4-8-flowered (d) *Cenchrus orientalis*: perennial herb, Inflorescence fluffy, nearly white inflorescence with pink tones

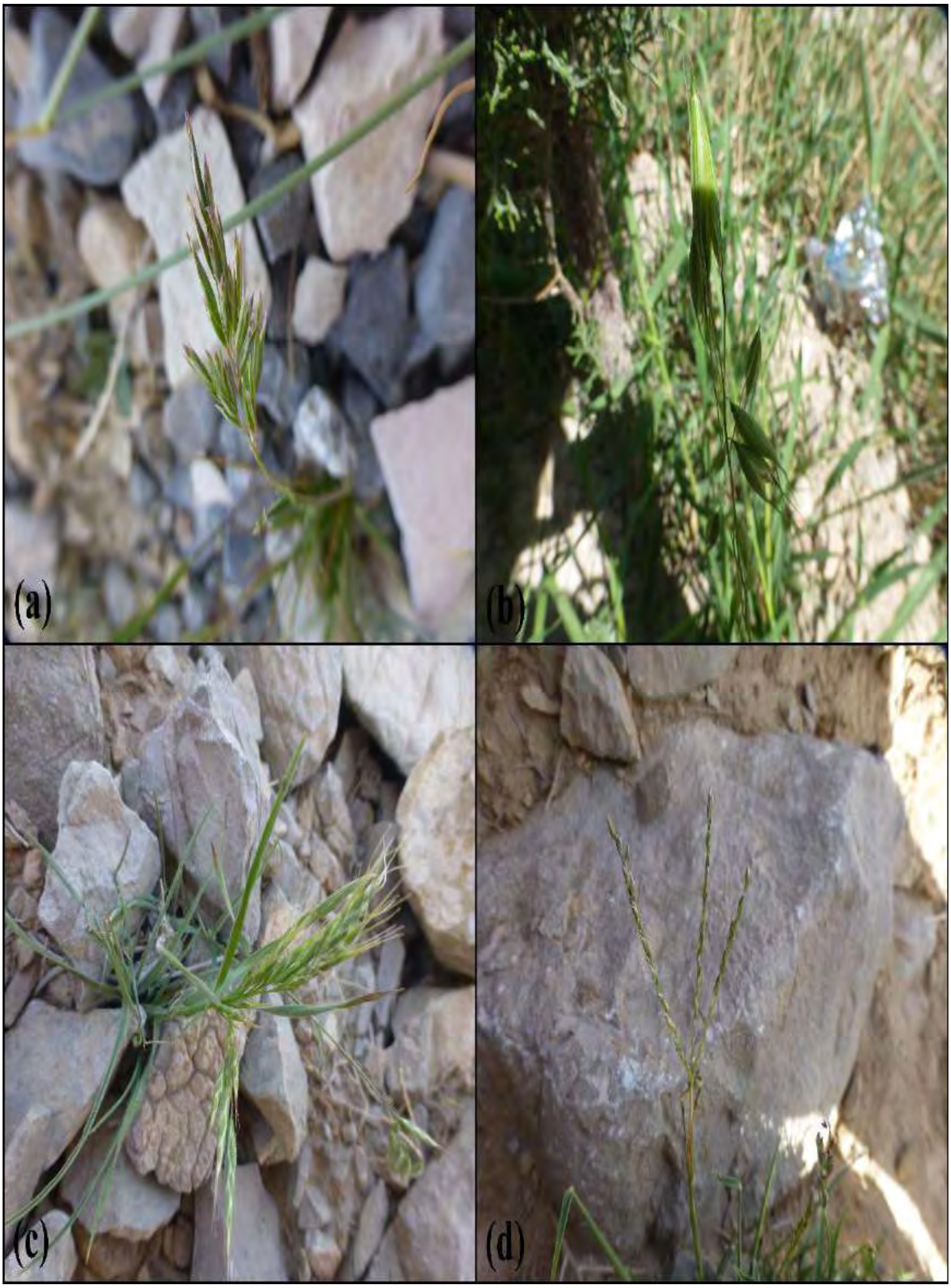

Plate 37. Field pictorial view (a) *Poa annua*; tufted annual or short-lived perennial, (b) *Avena sativa*; have blue leaves, and blooms are usually green spikelets (c) *Bromus lanceolatus*; pikelets narrowly elliptic (d) *Eleusine indica*; leaf-blades usually folded

Plate 38. Field pictorial view (a) *Vulpia persica*; annual, inflorescence a simple, erect or slightly nodding raceme (b) *Poa infirma*; loosely tufted annual, (c) *Desmostachya bipinnata*: harsh tussocky rihozomatous perennial, (d) *Saccharum griffithii*; perennial herb, almost petiolate at the base

Section-II

Pollen micromorphology

Summary

The second section comprised the pollen micromorphology of Angiosperms from different areas of Baluchistan, Pakistan. The study included 52 Asteraceae, 17 Boraginaceous, and 38 Poaceous 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, lacuna shape, spines, 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, number of spines per pollen, number of spines between colpi, and viability.

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

3.2 Pollen Micromorphology of Asteraceous Flora from Baluchistan

3.2.1 Results

a) Symmetry, Polarity, and Unity

Radial symmetry of pollen was observed in all 50 examined Asteraceous species. Similarly, in all the studied species, pollen have the analogous appearance of proximal and distal halves (isopolar) (Plate 39-50). The observed pollen in all studied species were in the monad form both in LM and SEM observations.

b) Size, Shape, and View

The maximum polar and equatorial diameter was observed in *Launaea procumbens* 43.5 µm and 56.3 µm. Minimum values of polar and equatorial diameter were observed in *Jurinea berardioidea* 19.65 µm and *Centaurea iberica* 14.52 µm. The size determined for Asteraceous pollen based on the longest axis (polar/equatorial) (Joujeh et al., 2019) were small (6 species), medium (43 species), and large (1 species). The shape classes of the pollen were categorized on the ratio between the polar axis and equatorial diameter. The pollen shapes were prolate-spheroidal, oblate spheroidal, sub-oblate, sub-prolate, oblate, prolate, and perprolate. The sub-prolate shape was observed in eighteen taxa. The per-prolate pollen was observed only in *Centaurea iberica*. In the polar view, the pollen appeared as triangular obtuse convex, triangular obtuse concave, circular, quadrangular obtuse convex, and trilobate (Table 6). The triangular obtuse convex view was observed in a maximum number of species (31 taxa). Quadrangular obtuse convex and trilobate views were noted only in *Jurinea carduiformis* and *Sonchus oleraceus*. Shapes observed in the equatorial view were elliptic, circular, elliptic truncate, rectangular obtuse, and obtuse. *Cousinia prolifera* was the only species with a rectangular obtuse equatorial view. *Jurinea carduiformis* was observed as elliptic obtuse in equatorial view.

c) Amb and NPC Classification

The circumference of the pollen in polar view in a way that the poles are directed towards the observer, is called amb. For non-constricted pollen, the amb is the same with the equatorial view whereas for equatorially constricted grain the amb is not correlated with the equator. The amb types observed in this research were goniotreme, peritreme, and ptychotreme. The goniotreme contour was possessed by the pollen of 35 Asteraceous taxa. Pollen with peritreme outline possessed almost uniformly distributed apertures along circular amb. In the goniotreme orientation, the amb was angular and the apertures were at the angles of the pollen. The ptychotreme amb had lobate or concave sides and the apertures were situated halfway between the angles. Number (N), position (P), and character (C) called NPC classification proposed by Erdtman (1969) was used to determine pollen type with the NPC formula. NPC classification determined tritreme ($N_{3:}$ three apertures) / tetratreme (N_4: four apertures), zonotreme ($P_{4:}$ aperture at equatorial zone), colpate (C_3: Colpate) and colportae ($C_{5:}$ colpus + pore) type named as tri-zono-colporate, tetra-zono-colporate and tri-zono-colpate (Table 6). The formula for the determined types were $N_3P_4C_{5,}$ $N_4P_4C_5$ and $N_3P_4C_{3.}$

d) Apertures

The examined pollen were tricolpate, tricolporate and tetracolporate concerning number of apertures. The leading type was tricolporate, which included 47 taxa. Tetracolporate aperture reported for *Blumea sinuata* and *Sonchus oleraceus.* The tricolpate aperture type was observed only in *Jurinea carduiformis*. Pores were covered with operculum in the pollen of all the observed species. A maximum width of 5.7 µm of colpi was found for polar view in *Scorzonera koelpinioides* and a length 10.65 µm in *Erigeron bonariensis* and *Koelpinia linearis*. In the equatorial view, *Launaea procumbens* exhibited a maximum width of 6.25 µm, and *Cirsium arvense* was noticed with a maximum length of 22.1 µm.

e) Exine Sculpturing and Surface

SEM imparts the in-depth exploration of the spatial arrangements in the intricate exoskeleton of Asteraceous pollen (Vincent and Norris, 1989). The examined Asteraceous taxa were categorized as echinate, echinate lophate, scabrate, and gemmate types based on exine ornamentation. Maximum number of species were echinate (20 species) and echinate lophate (21 species). The largest spines were present in *Launaea acanthodes* with a mean length of 4.55 µm. Scabrate ornamentation of exine was present in 9 species. *Zoegea purpurea* was the only taxa with gemmate exine. The surface of exine was observed with distributed pores (perforate) or without pores (non-perforate). The perforate exine surface was noticed in 22 species whereas the non-perforate type in 18 taxa. The exine in the echinate lophate pollen was raised creating a pattern of ridges called lophae (lophate). The space surrounded by the lophae is a lacuna. The shapes of lacuna in the lophate pollen were noted with the following types, irregular convex hexagon, irregular convex pentagon, pentagon to hexagon, irregular hexagon, irregular convex pentagon to hexagon, pentagon, regular convex pentagon to hexagon, hexagon.

f) Multivariate analysis

Pollen quantitative data of studied taxa was subjected to hierarchical cluster analysis (UPGMA) (Figure 4). There was one major and three small clusters. Six species were in 3 separate small clusters. Major clusters were delineated into several variable subclusters. A similarity coefficient was observed among the length and width of colpi in equatorial and polar views with polar axis and equatorial diameter. The number of spines between colpi was also positively correlated with number of spines per pollen. While number of spines per pollen was negatively correlated with the width and length of colpi in polar view. The normal probability statistics revealed the highest variations among the Asteraceous taxa for the number of spines per pollen and several spines between colpi (Figure 5). The principal component analysis (PCA) ordination of 9 tribes of Asteraceous species was observed with grouping by correspondence to the polar axis, equatorial diameter, length, and width of colpi in polar and equatorial view, exine thickness, mesocolpium, spine length, number of spines and number of spines between colpi (Figure 6). Significant variations were observed among and between PC1, PC2, PC3, PC4, PC5, and PC6 with eigenvalues from 1.65 to 438.17. The correlation among and between the mean values of studied variables revealed that most of the variables were positively correlated. The positively correlated characters were labelled with blue while negative correlated were in red (Figure 7). The ridge line plot compared and visualized the overall distribution among the micromorphological traits of Asteraceous species (Figure 8). Analysis of variance (Multiple sample ANOVA) determined the variations among the mean values of a data set of more than two independent variables. In this study, the p-value was less than 0.01 level of significance and the obtained value was greater than 0.01, so the null hypothesis was rejected. Hence it is concluded that there exists a highly statistically significant difference among the means of analysed traits.

3.2.2 Discussion

Palynological studies are significant in the determination of systematics and evolutionary associations of different groups of angiospermic flora (Stephen et al., 2017). Taxonomy is dynamic to date, as the problems are present in nomenclature and ranking of species. In solving these problems, palynological, molecular, and karyological investigations are very useful. Morphological attributes of pollen have been proven useful in the taxonomy of Asteraceous taxa (Shabestari et al., 2013). Joujeh et al. (2019) described that pollen wall characters were taxonomically significant in the separation of species of one of the difficult genera (regarding their taxonomy) i.e *Centaurea*. Heterogeneity in the observed features of pollen can be utilized as a key tool to identify genera and species. In the present study, palynological exploration of 50 taxa of the family Asteraceae belonging to 9 tribes Cichorieae (21 taxa), Cardueae (11 taxa), Inuleae (6 taxa), Anthemideae (4 taxa), Gnaphalieae (2 taxa), Astereae (2 taxa), Senecioneae (2 taxa), Gymnarrheneae (1 taxon), Tageteae (1 taxon) was carried out from Baluchistan.

a) Cichorieae

Pollen of 21 studied taxa of tribe Cichorieae were echinate and echinate lophate types. The perforate and non-perforate both types of exine surface was observed. Tri-zono-colporate aperture type possessed by 20 taxa. *Sonchus oleraceus* was the only member of the tribe with a tetra-zono-colporate type. Goniotreme and peritreme amb were observed in this tribe. Species of the genus *Lactuca* possessed alterations in the amb, pollen, and lacuna shape, and perforations in the exine surface. The members of *Lactuca* were distinguished based on the palynological description. The pollen types correlated with the general morphology of the plants within the genus *Lactuca* of Cichorieae (Abid and Qaiser, 2023). The polar area, the extent of spines in the polar area, the polar axis, and the equatorial diameter were regarded as highly variable features among *Lacutca* species (Abid and Qaiser, 2023; Wang et al., 2009). Abid and Qaiser et al. (2023) reported the *Lactuca dissecta* as a small pollen type, whereas in the current examination, it was observed as medium, sub-prolate, goniotreme amb with irregular convex pentagon to hexagon lacuna. *Crepis bodinieri, Crepis nepifera* (Peng et al., 2013), *Crepis biennis,* and *Crepis elongate* (Wang et al., 2009) were reported as oblate to oblate spheroidal with pentagon lacuna. In the present study, *Crepis kotschyana* pollen was observed as an oblate shape but with an irregular convex hexagon-shaped lacuna. The shape was related at the genus level in *Crepis* with lacuna the distinguishing feature for the species differentiation. *Melanoseris* genus and its varieties were classified into two groups based on the pollen's qualitative and quantitative aspects (Abid and Qaiser, 2022). *Koelpinia turanica* (medium, sub-oblate, elliptic, triangular obtuse convex, lophate, irregular hexagon, goniotreme) and *Koelpinia linearis* (small, oblate spheroidal, circular, non-lophate, peritreme) revealed clear differences in pollen microfeatures. The qualitative features amb, sculpturing, lacuna shape, and polar and equatorial view were not enough for the separation of *Launaea, Scorzonera,* and *Sonchus* species (Table 6). The variations in the shape of the pollen, polar axis, equatorial diameter, colpi length and width, mesocolpium, spine length, and abundance can be employed in the delimitation of members of *Launaea, Scorzonera,* and *Sonchus.*

b) Cardueae

The pollen ornamentation of observed Cardueae members were scabrate, echinate, echinate lophate, and gemmate types. The scabrate sculpturing being the dominant type in Cardueae, was present in 7 members, echinate in 2 species, and echinate lophate and gemmate were observed in a single species. The Cardueae tribe was represented by a single type of aperture i.e tri-colporate. The characters' shape, polar and equatorial view, and lacuna shape were variable among the examined members and can be implicated in the specification of species. Cardueae tribe was represented by oblate spheroidal to suboblate with prolate spheroidal to subprolate (Osman, 2009). Two pollen types were recognized: *Jurinea* and *Himalaiella.* Osman (2009) recognized four pollen types: *Onopordum, Notobasis, Carthamus,* and *Carlina* pollen type. The genus *Ptilostemon* was reported with echinate and scabrate ornamentation (Ferrauto et al., 2017). The variations in the species of the same genus in the Cardueae were because of the adaptation of mesophilous taxa to arid and semi-arid environmental conditions to accommodate harmogametic variations for the intense fluctuation of humidity and temperature (Hidalgo et al., 2008). Similar variations were observed in the species of genus *Cousinia* from the arid-semiarid area of Baluchistan, Pakistan, in the shape, size, sculpturing, and amb.

c) Inuleae

Tribe Inuleae was represented by perforated exine in all examined species in this research with echinate sculpturing (Osman, 2006) in 5 taxa and echinate lophate in one species. Goniotreme and peritreme amb types were noticed in the studied taxa. The variations in most of the features (size, shape, amb, polar, and equatorial view) in the species of the same genera did not allow to assignment of the pollen types for the genus *Pulicaria*. The abundance of spines per pollen differentiates the members of *Pulicaria*. *Pulicaria-type* pollen (Osman, 2006) was recognized based on pollen class, exine, and spine morphology (Coutinho et al., 2011). Zarin et al. (2010) concluded that micromorphological features of pollen in Inuleae were valuable in resolving the generic level taxonomic problems in *Amblyocarpum* and *Carpesium*. Besides echinate pollen type, the four genera of Inuleae, were grouped based on spine rows between the apertures into type 1; Anisopappus, and type 2; *Blumea*, *Duhaldea*, and *Pentanema* (Pornponggrungrueng and Chantaranothai, 2002). The tectum pattern between the spines was recognized as the *Pentanema* type (Dawar et al., 2002). The variations in the quantitative traits' spine length, number of spines per pollen, and number of lacunae were recommended for delimitation of species in Inuleae (Osman, 2006; Zarin et al., 2010). This study revealed that pollen features such as shape, view, size, exine, and amb were not sufficient for the delimitation of taxa within this tribe. However, the number of the spines, their arrangement, and length could further prove as operational taxonomic markers down to species level (Padrón-Mederos et al., 2011). The naturality of the Inuleae was enhanced by the relative homogeneity of the morphological features of the pollens (Pereira-Coutinho and Dinis, 2007).

d) Anthemideae

The palynomorphic data of the *Achillea cretica* and *Artemisia biennis* was not significant for the separation of these Anthemideae taxa (not marked by the taxonomic key Table 8). Whereas the *Seriphidium maritimum* and *Xylanthemum macropodum* were observed with distinct variations in the exine, shape, amb, polar and equatorial view, and number of spine rows (Meo and Khan, 2006). The study demonstrates that pollen microfeatures separate some taxonomic groups within the Anthemideae. The extent of variations in the thickness of the exine was one of the strengthening features of the species distinction (Meo and Khan, 2009). The spine length in *Artemisia* was a significant taxonomic marker well correlated with other morphological and molecular traits (Martin et al., 2001).

e) Implications of Palynomorphic Markers in the Systematics of Asteraceae

The taxonomy of Asteraceae is important because of its divergent macromorphological characteristics. The distribution of the species in tribes and subfamilies is difficult owing to diverse acquired characteristics in discrete environmental conditions. These adaptations even affect the number of chromosome sets. Unity, shape, and symmetry of the pollen distinguish Asteraceae from other families as they were stated to be operational taxonomic units at subdivision levels (higher). It was inferred that polarity, symmetry, and unity in Asteraceae cannot be employed in species delimitation (Salamah et al., 2019). The taxonomic and morphological studies helped determine the similarities and evolutionary relationship among Asteraceous species e.g characteristic of lophate pollen in Asteraceous tribes was found to be convergent evolution (Blackmore, 1986). Morphological evidences were used to find the evolutionary relationship among the species of Asteraceae from Indonesia including *Zinnia elegans*, and *Tagetes patula*.

Genera such as *Gundelia*, *Brachylaena*, *Tarchonanthus* (Wang et al., 2009), *Adenocaulon* were problematic to be placed in a particular tribe. Wang et al. (2009) suggested that pollen was one of the distinguishing characters found to be highly useful in the classification of Asteraceae as this character has been used as a source of discriminating information. Based on the morphological studies of pollen, the classification of different species has been rearranged; 1. The new genera *Tarlmounia* and *Strobocalyx* were arisen. 2. The phylogenetics of *Warioina* and *Gundelia* was also revised. Differences in the size, shape, exine sculpturing, surface, polar and equatorial view, amb, and aperture type of pollen, a lacuna was regarded as heteromorphy in Asteraceous pollen. Features such as the shape of the spine, orientation of the apertures, and number of perforations at the spine and spine rows have been reported as significant morphological traits. Exine layer was a significant phylogenetic feature for cladistic analysis in Barnadesioideae (Zhao et al., 2000).

Structure and pattern of exine ornamentation were found significant in the taxonomy of genus *Centaurea*. The species of the same genus were observed with similarity in some structural features, this fact confirmed their interspecific relationship. While the variations in quantitative characteristics provided species-level distinction. Therefore, to target *Centaurea* species some of the pollen features were of significant taxonomic value (Joujeh et al., 2019). Exine thickness varied in the different specimens of similar species and even within the single specimen, thus based on this feature deviant forms cannot be formed, because of continuous range variation. Pollen shape, exine, and sculpturing were helpful in the classification of the genus *Centura* (Shabestari et al., 2013). Similar conclusions were obtained by Tellería and Katinas (2009) for exine thickness, endo-aperture features, and size of pollen in the genus *Mutisia*. Salamah et al. (2019) reported the diversity of pores within 8 tribes of Asteraceae and concluded that this feature was beneficial for the delimitation of taxa up to the species level within each Asteraceous tribe.

The morphology of a trait might indicate its ecological purpose, the preservation of ancestral features, or its adaptability to evolution. Morphology differs greatly throughout clades (Mander et al., 2021). Among other aspects, the shape of angiosperm pollen has changed during evolutionary time towards an increasing number of openings. From a neo-Darwinian perspective, this implies that: (i) the aperture number must exhibit some polymorphism, and (ii) increasing the aperture number must result in an improvement in fitness. Different aperture numbers of pollen types were frequently found in the same plant. The pollen grains with four apertures germinated more quickly than those with three, but the four-apertured grains also had additional weaknesses (Dajoz et al., 1991). In the present study echinate, echinate lophate, scabrate, and gemmate pollen types were observed. The variable morphology in terms of size, exine stratification, surface ornamentation, and apertures play a crucial role in ecological adaptation. The number of apertures varied from trocolpate, tricolporate to tetracolporate. The variations in the morphology and size of pollen of different taxa determine the degree of pollination. Pollinator behaviour and morphology combine to determine the likelihood of successful pollination, which in the end is significant for the survival of that taxa. Bees' desire to gather pollen with a certain morphology was reflected in the variations in the morphology of pollen delivered by pollinators. To help pollinating partners complete the pollination process, pollen shape has varied with time (Hasegawa et al., 2023). Wind-pollinated Asteraceous genera *Ambrosia* and *Artemisia* have generally smooth dry pollen in contrast to the more widespread insect-pollinated genera of the family which have ornamented, often sticky pollen (Mander et al., 2021).

The characteristic documentation and visualization strengthened the abstraction that the pollen morphology of the studied tribes was significant in the separation and delimitation of taxa at a generic and specific level. A taxonomic key was generated to evaluate the heterogeneity in several features of the pollen, this finally defined the species boundaries for the distinction of the Asteraceous taxa. The analysis of mesoporia (distance between the pores) and ektexiniuos bodies (formation on the pore surface) in the future will further strengthen the studies to generate more detailed taxonomic markers for Asteraceous species.

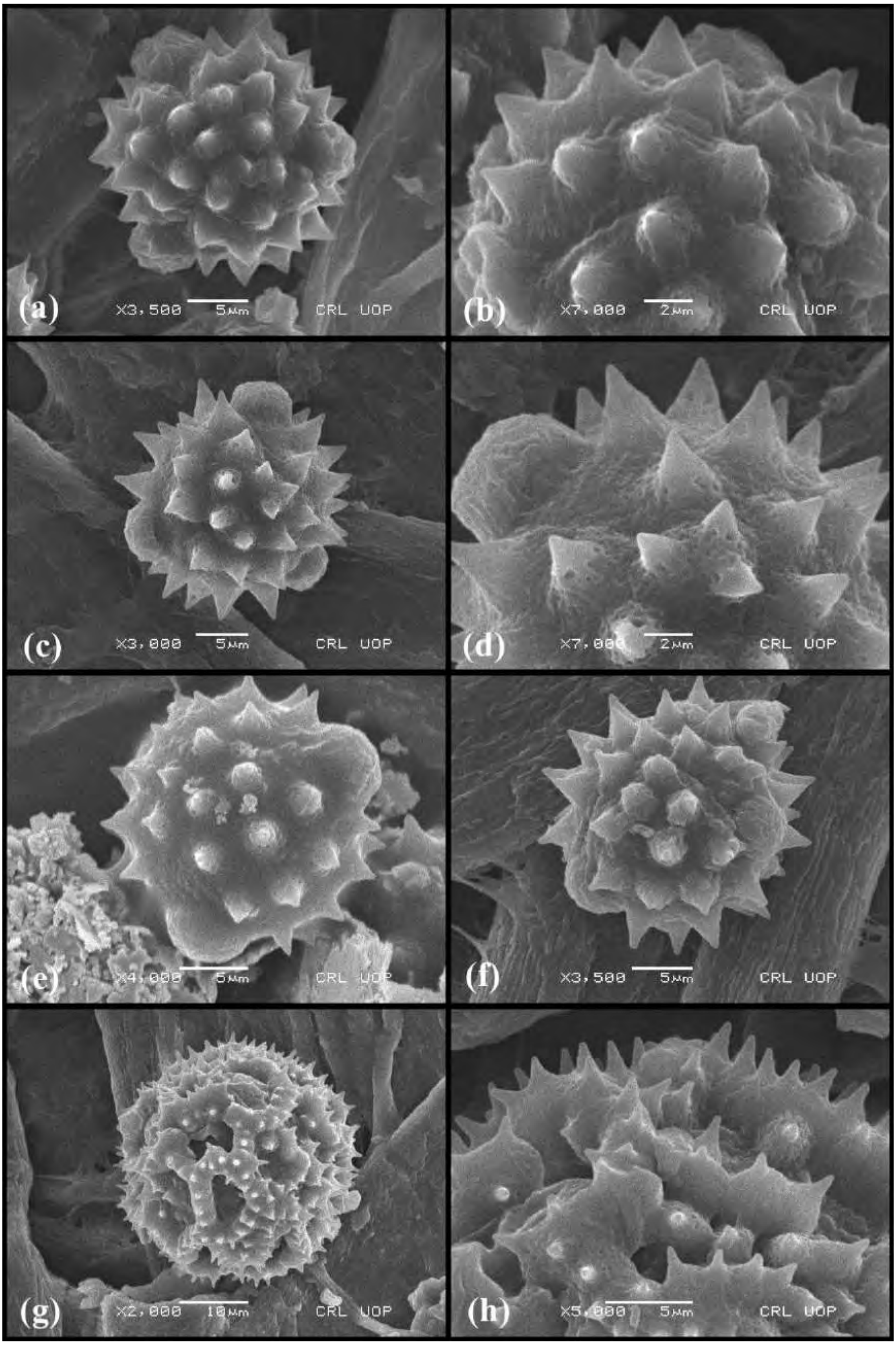

Plate 39. SEM micrographs of pollen (a),(b) *Achillea cretica,* (c),(d) *Artemisia biennis,* (e),(f) *Atractylis carduus,* (g),(h) *Blumea sinuata*

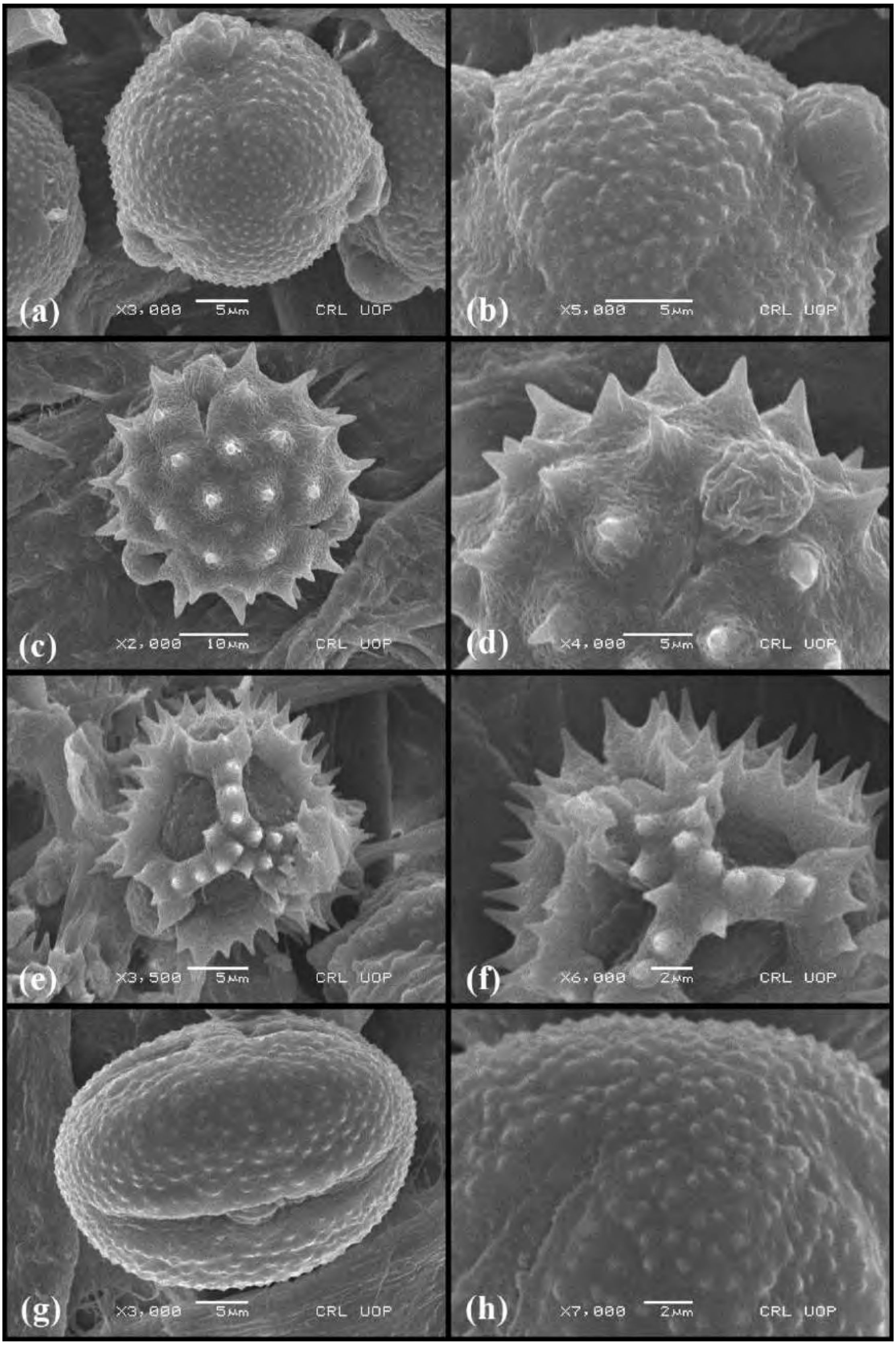

Plate 40. SEM micrographs of pollen (a),(b) *Centaurea iberica,* (c),(d) *Cirsium arvense*, (e),(f) *Cousinia haeckeliae*, (g),(h) *Cousinia prolifera*

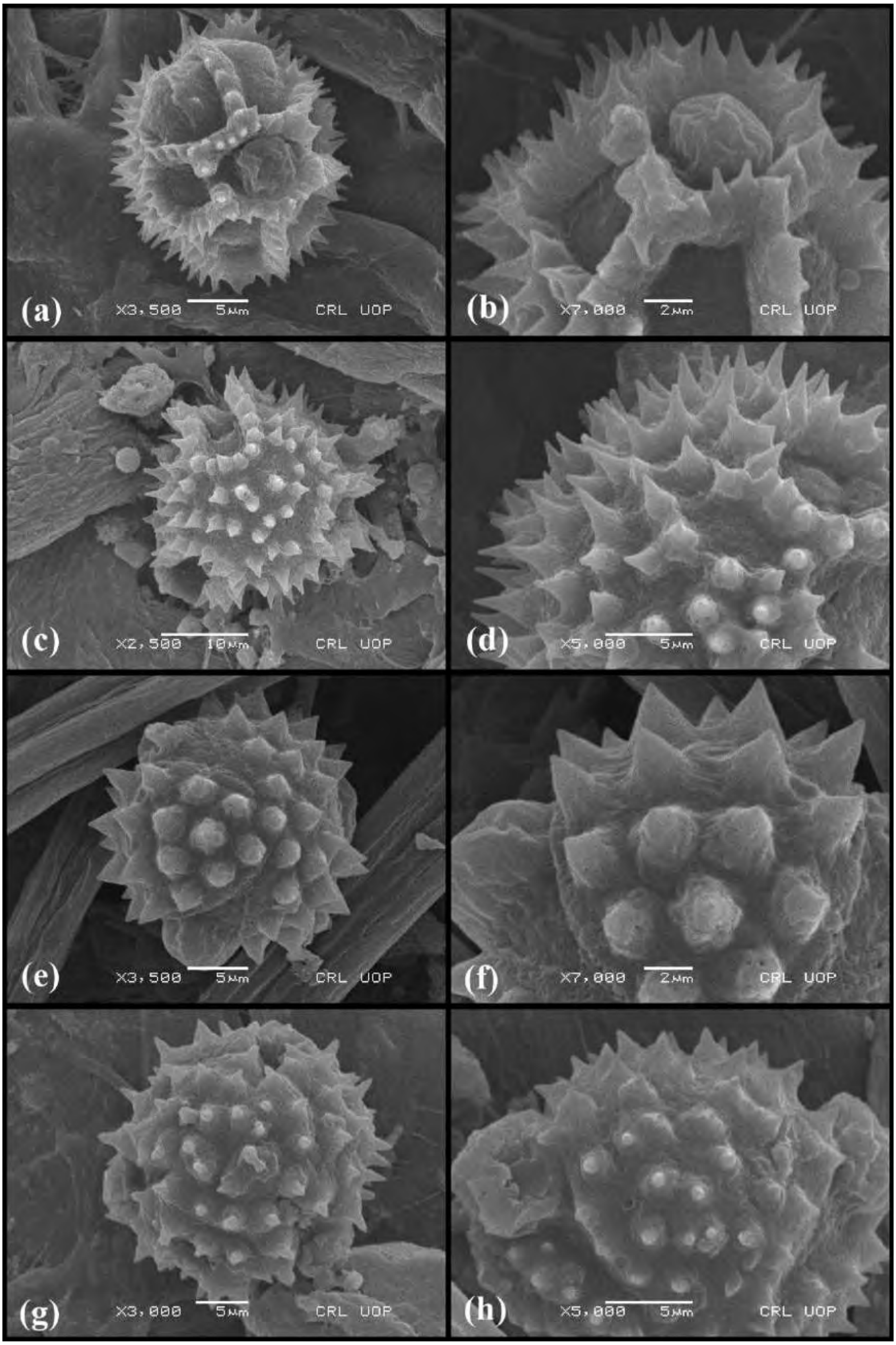

Plate 41. SEM micrographs of pollen (a),(b) *Crepis kotschyana*, (c) *Erigeron bonariensis*, (d) *Filago hurdwarica*, (e),(f) *Flaveria trinervia*, (g),(h) *Gymnarrhena micrantha*

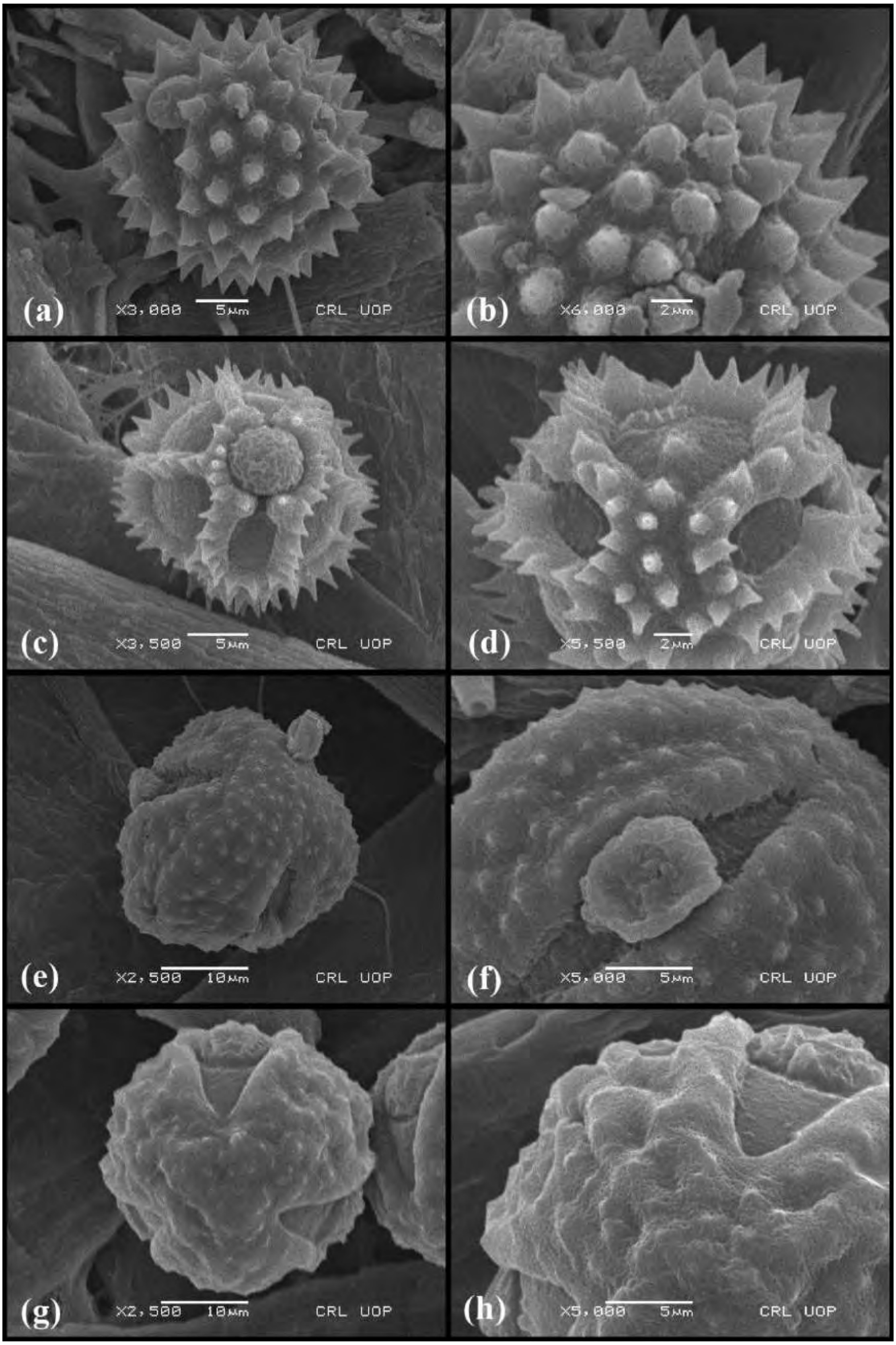

Plate 42. SEM micrographs of pollen (a),(b) *Hertia intermedia*, (c),(d) *Heteroderis pusilla*, (e),(f) *Himalaiella afghani*, (g),(h) *Himalaiella heteromalla*

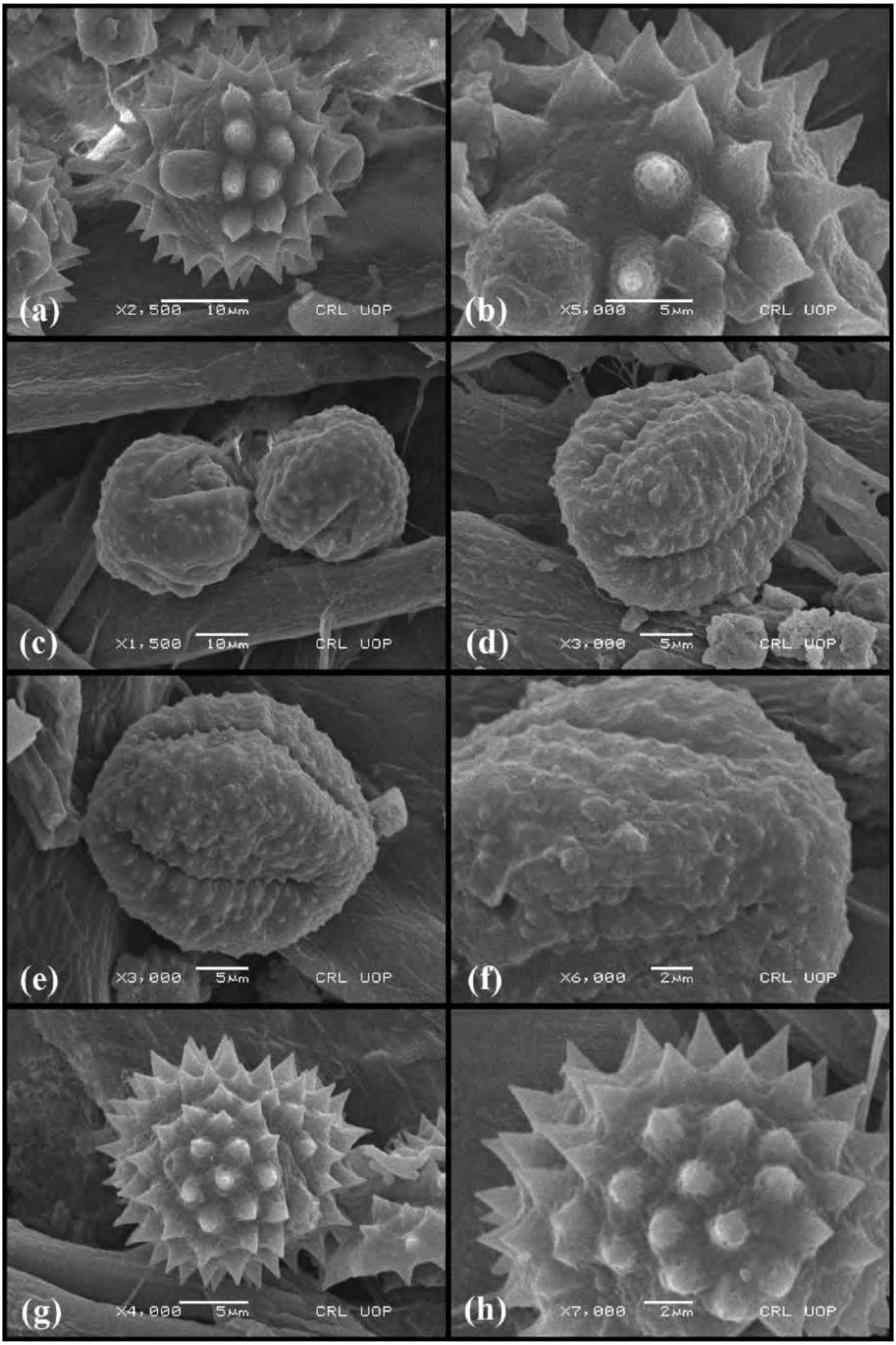

Plate 43. SEM micrographs of pollen (a),(b) *Iphiona grantioides*, (c) *Jurinea berardioidea*, (d),(e),(f) *Jurinea carduiformis*, (g),(h) *Koelpinia linearis*

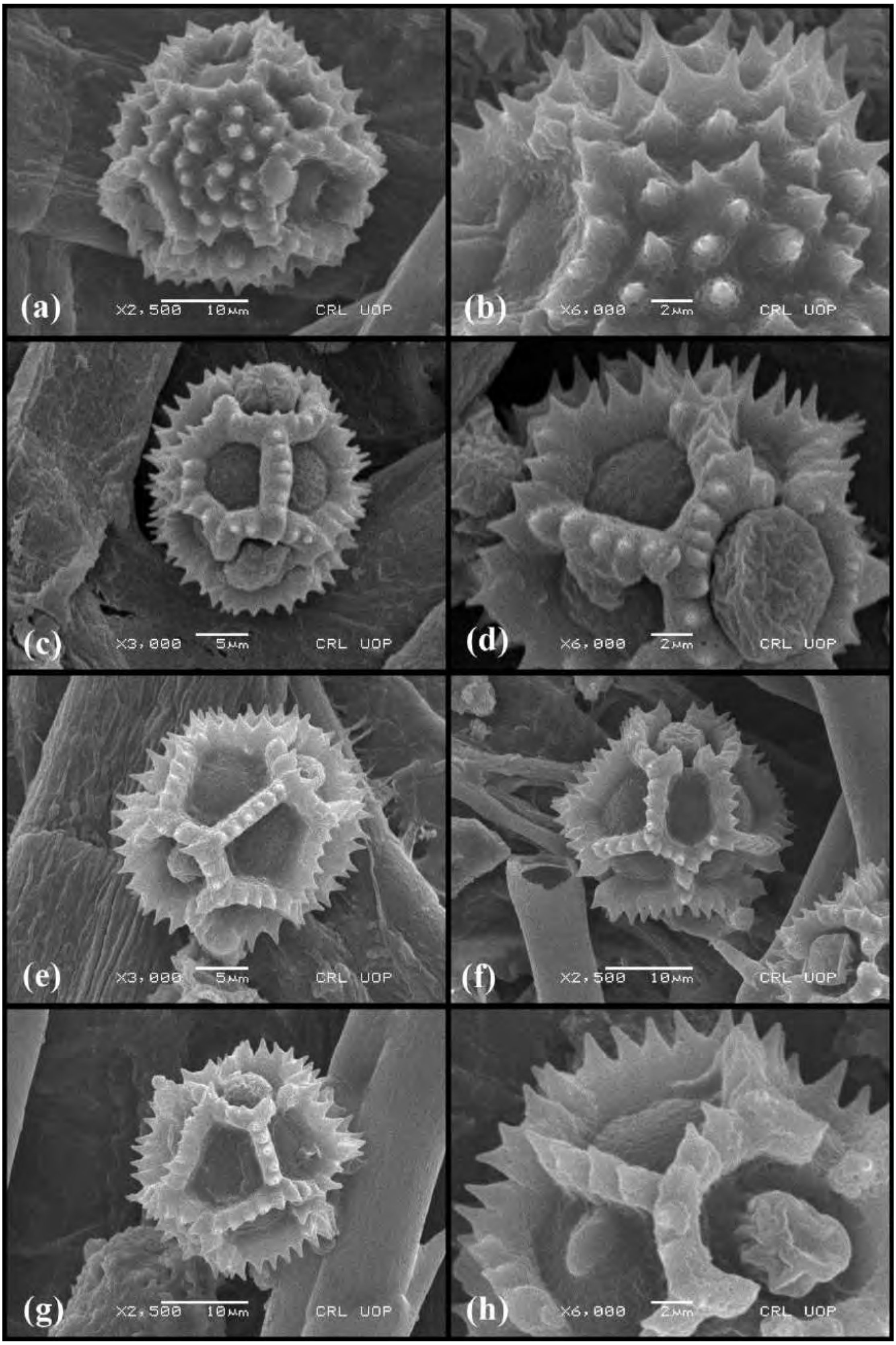

Plate 44. SEM micrographs of pollen (a),(b) *Koelpinia turanica*, (c),(d) *Lactuca dissecta*, (e),(f) *Lactuca orientalis*, (g),(h) *Lactuca serriola*

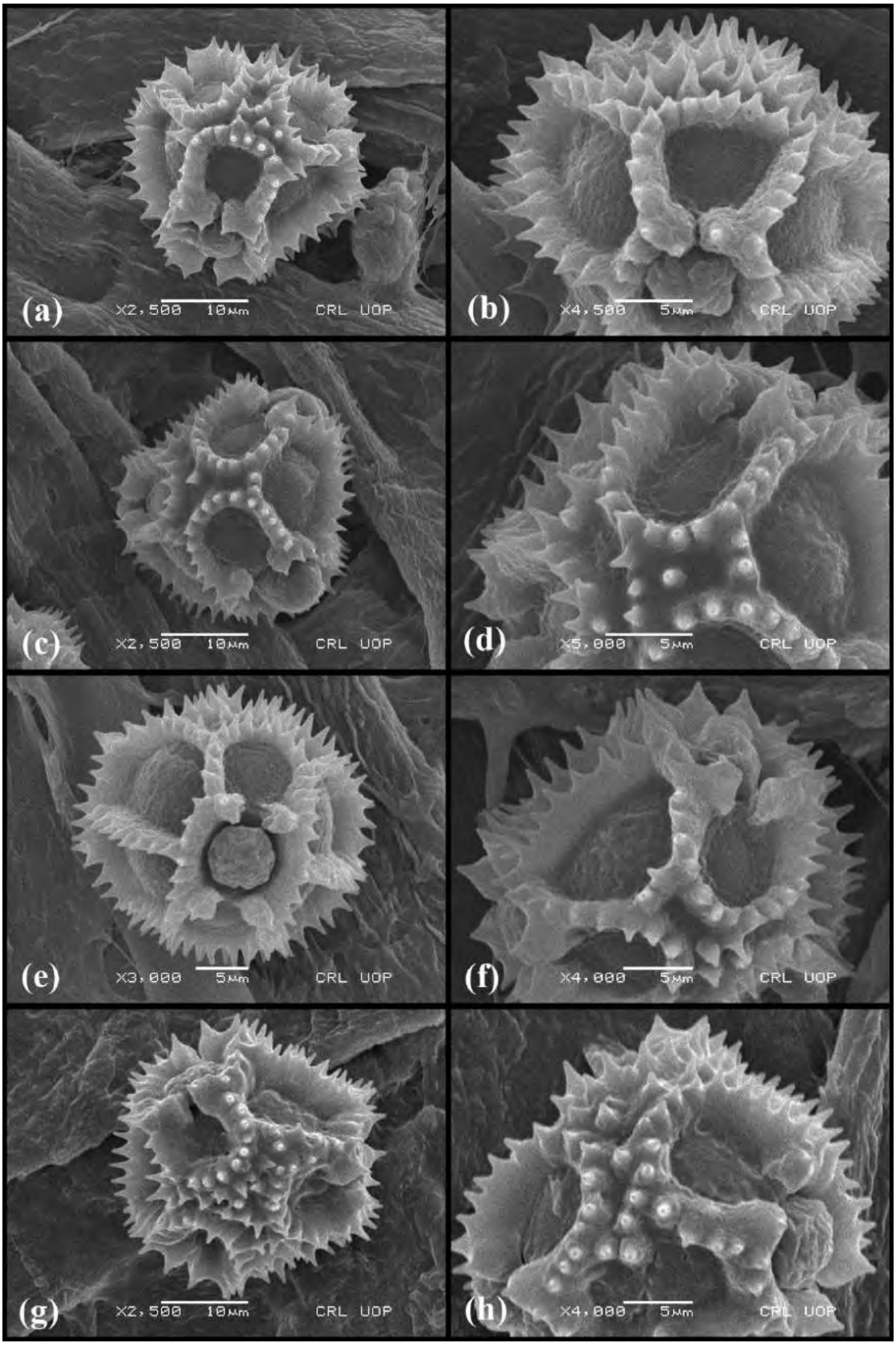

Plate 45. SEM micrographs of pollen (a),(b) *Launaea acanthodes,* (c),(d) *Launaea aspleniifolia,* (e),(f) *Launaea fragilis* subsp. *Fragilis,* (g),(h) *Launaea intybacea*

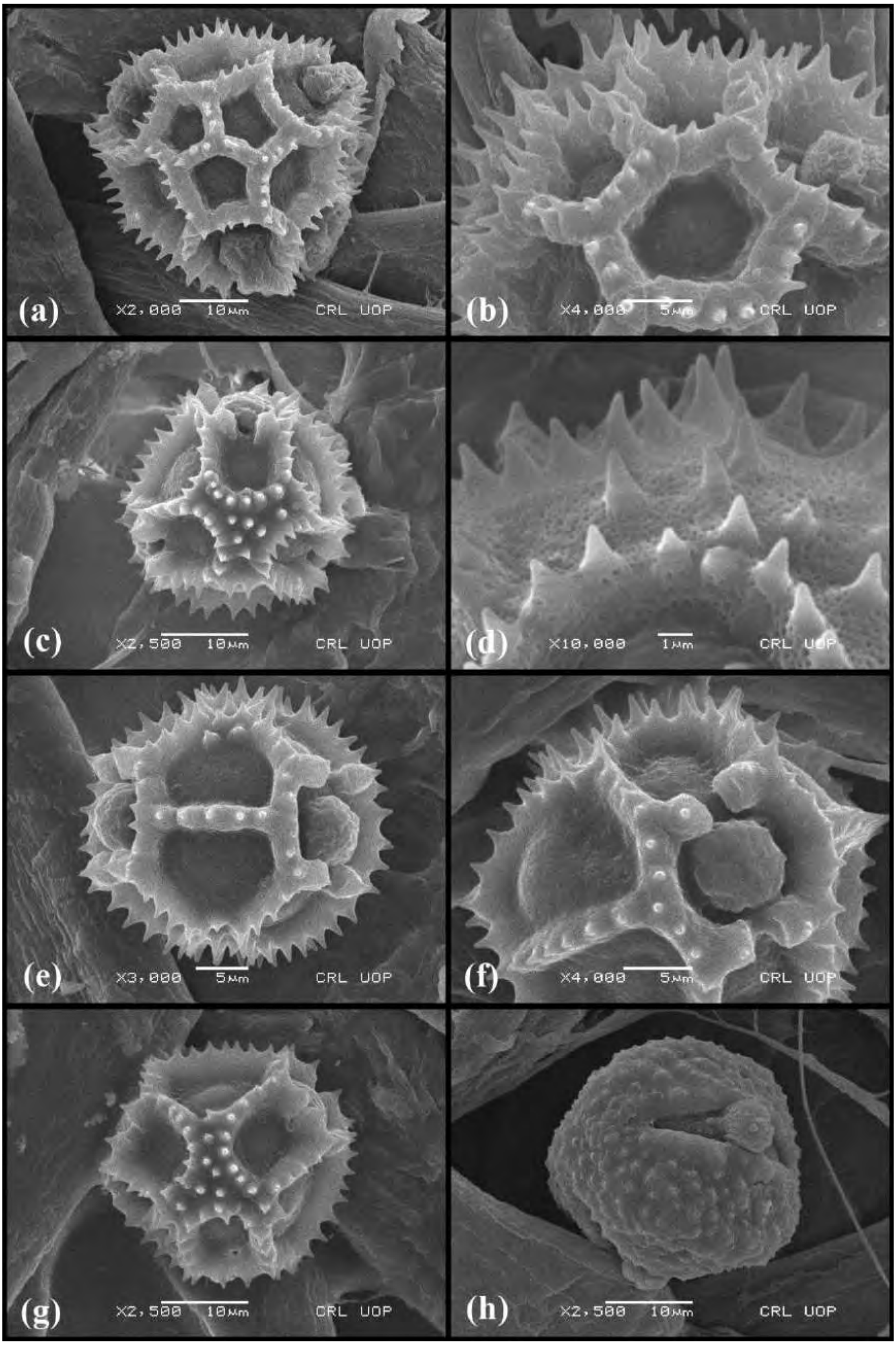

Plate 46. SEM micrographs of pollen (a),(b) *Launaea oligocephala*, (c),(d) *Launaea procumbens*, (e),(f),(g) *Launaea stenocephala*, (h) *Leuzea repens*

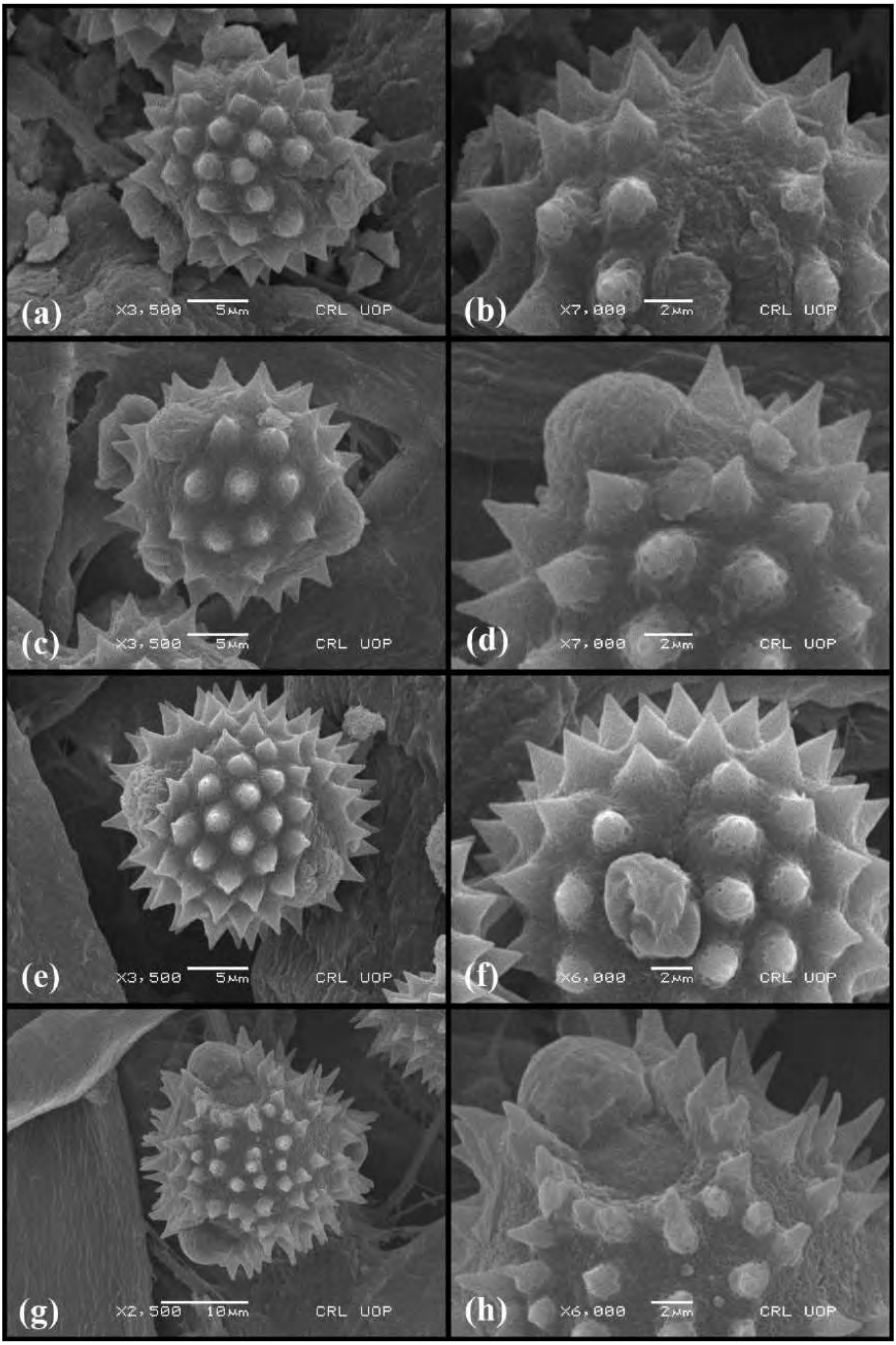

Plate 47. SEM micrographs of pollen (a),(b) *Pallenis hierochuntica*, (c),(d) *Pentanema divaricatum*, (e),(f) *Phagnalon schweinfurthii*, (g),(h) *Pterachaenia stewartii*

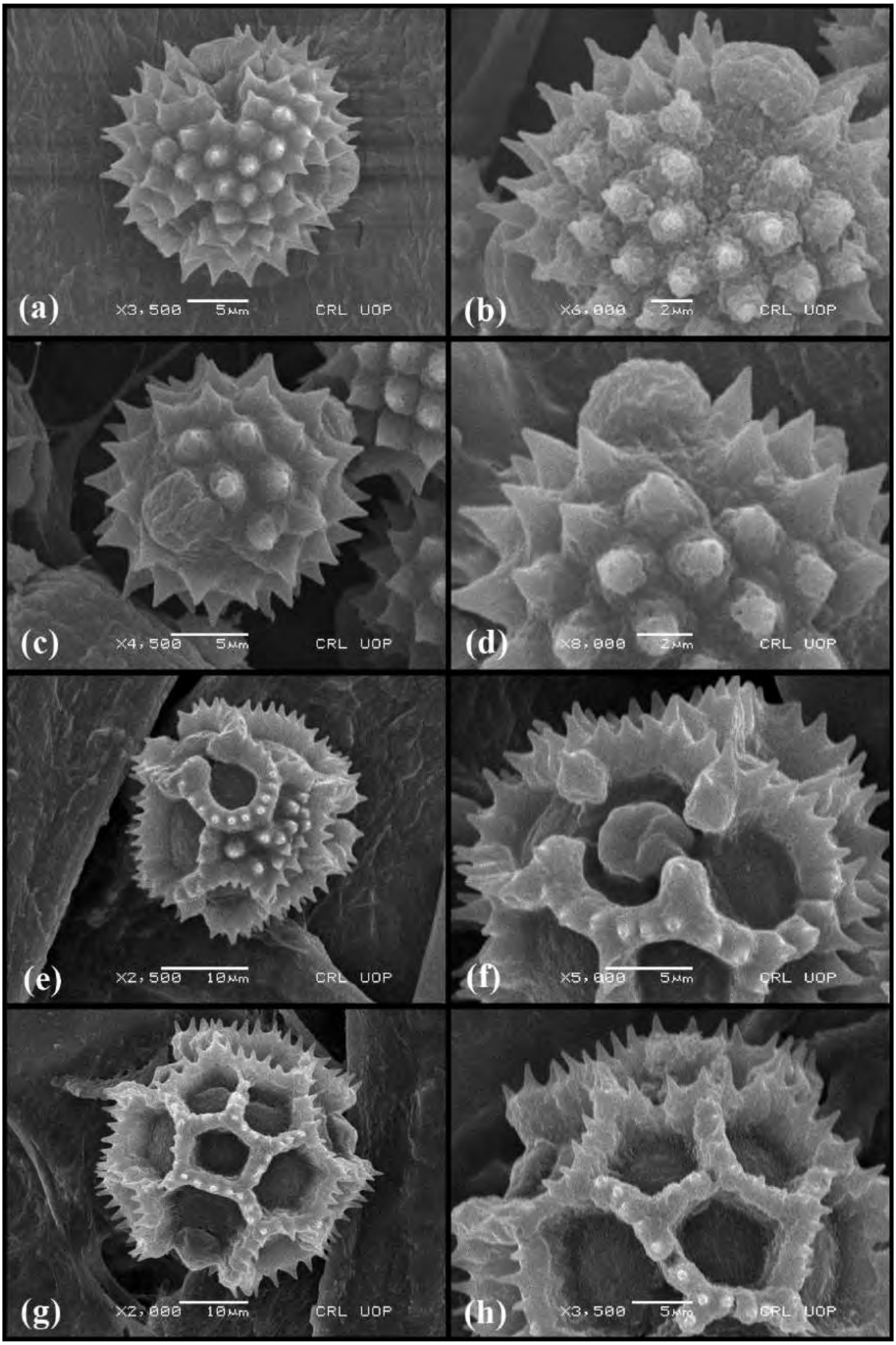

Plate 48. SEM micrographs of pollen (a),(b) *Pulicaria angustifolia*, (c),(d) *Pulicaria undulata*, (e),(f) *Reichardia tingitana*, (g),(h) *Scorzonera koelpinioides*

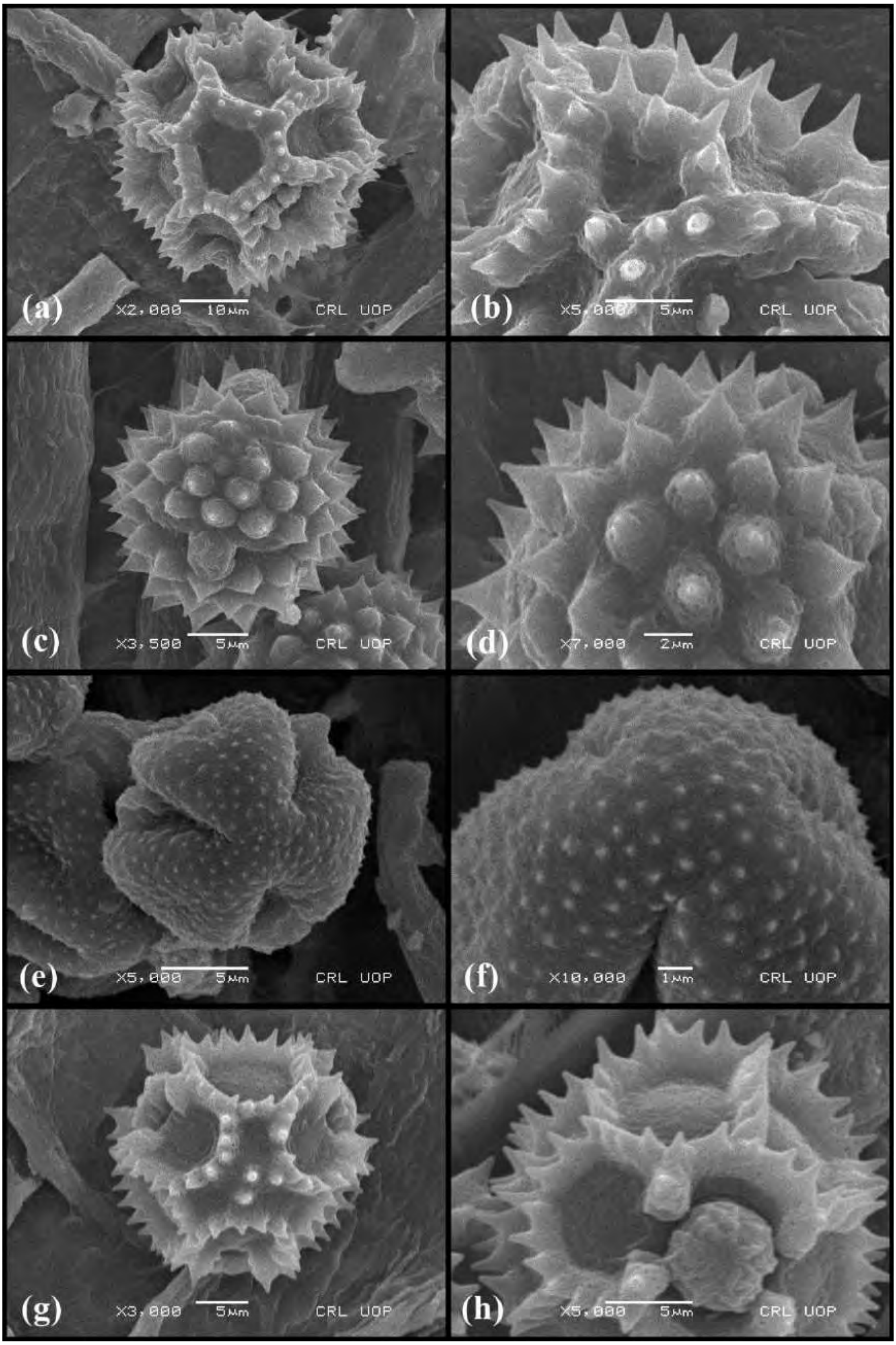

Plate 49. SEM micrographs of pollen (a),(b) *Scorzonera raddeana*, (c),(d) *Senecio glaucus*, (e),(f) *Seriphidium maritimum*, (g),(h) *Sonchus arvensis*

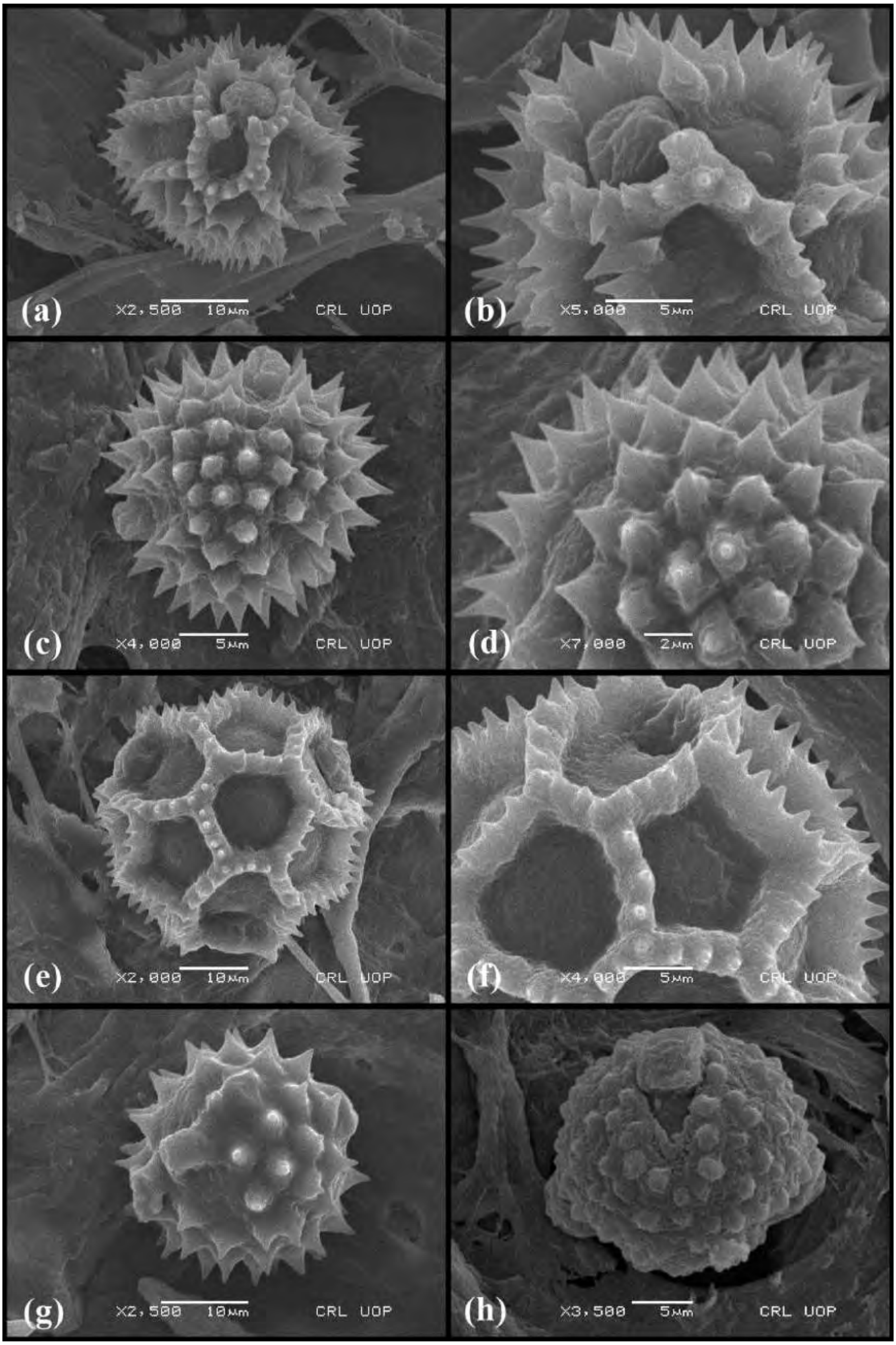

Plate 50. SEM micrographs of pollen (a),(b) *Sonchus oleraceus*, (c),(d) *Symphyotrichum subulatum*, (e),(f) *Takhtajaniantha pusilla*, (g) *Xylanthemum macropodum*, (h) *Zoegea purpurea*

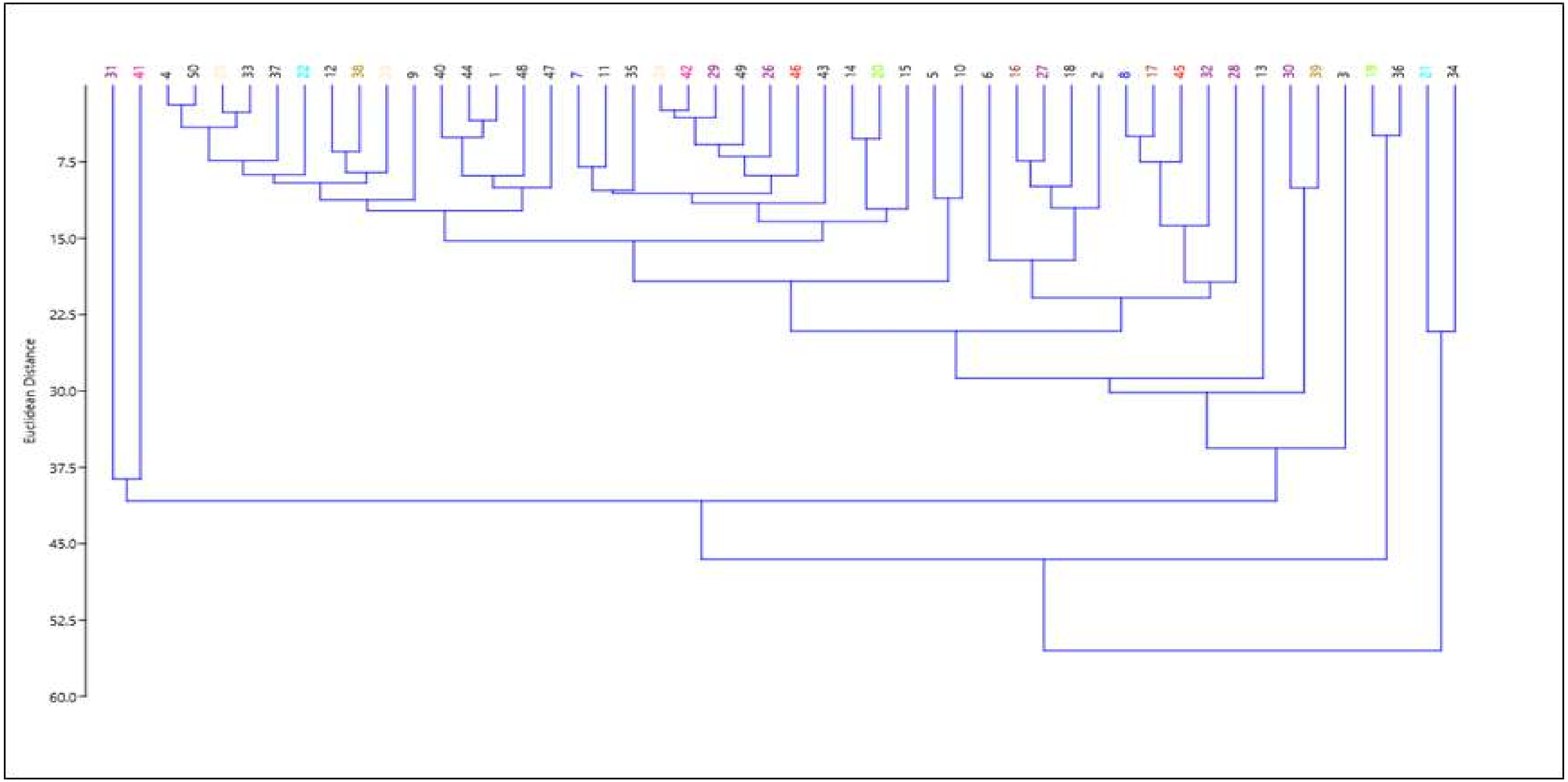

Figure 4. UPGMA cluster analysis based on quantitative traits (Polar Diameter, Equatorial Diameter, Width of colpi in polar view, Length of colpi in polar view, Width of colpi in equatorial view, Length of colpi in equatorial view, Mesocolpium, Exine thickness, Number of spines per pollen, Number of spines between colpi, Spine length of Asteraceous flora (Numbering represents the taxa number in qualitative Table)

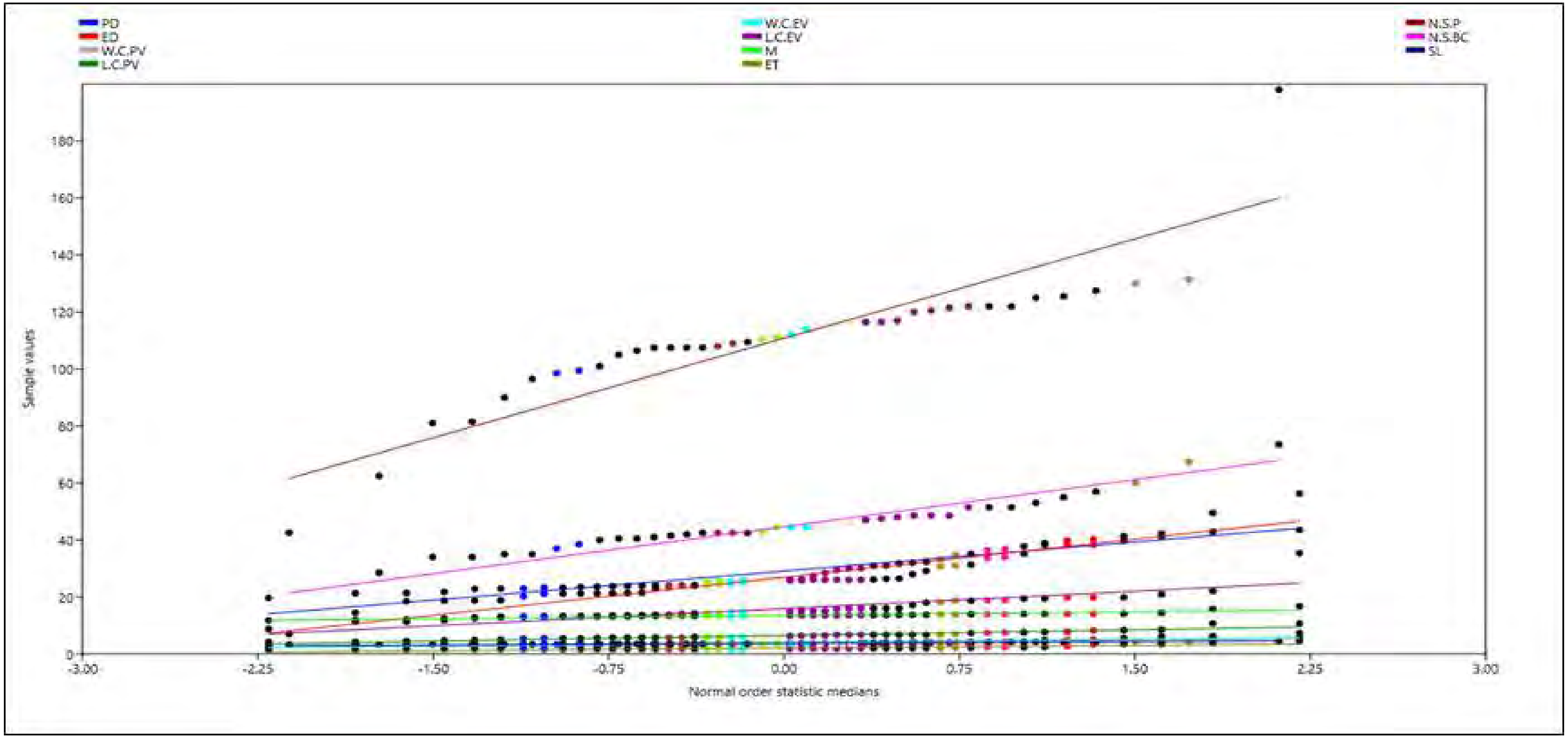

Figure 5. Normal probability distribution quantitative traits (PD: Polar Diameter, ED: Equatorial Diameter, W.C.PV: Width of colpi in polar view, L.C.PV: Length of colpi in polar view, W.C.EV: Width of colpi in equatorial view, L.C.EV: Length of colpi in equatorial view, M: Mesocolpium, ET: Exine thickness, N.S.P: Number of spines per pollen, N.S.BC: Number of spines between colpi, SL: Spine length) of Asteraceous flora

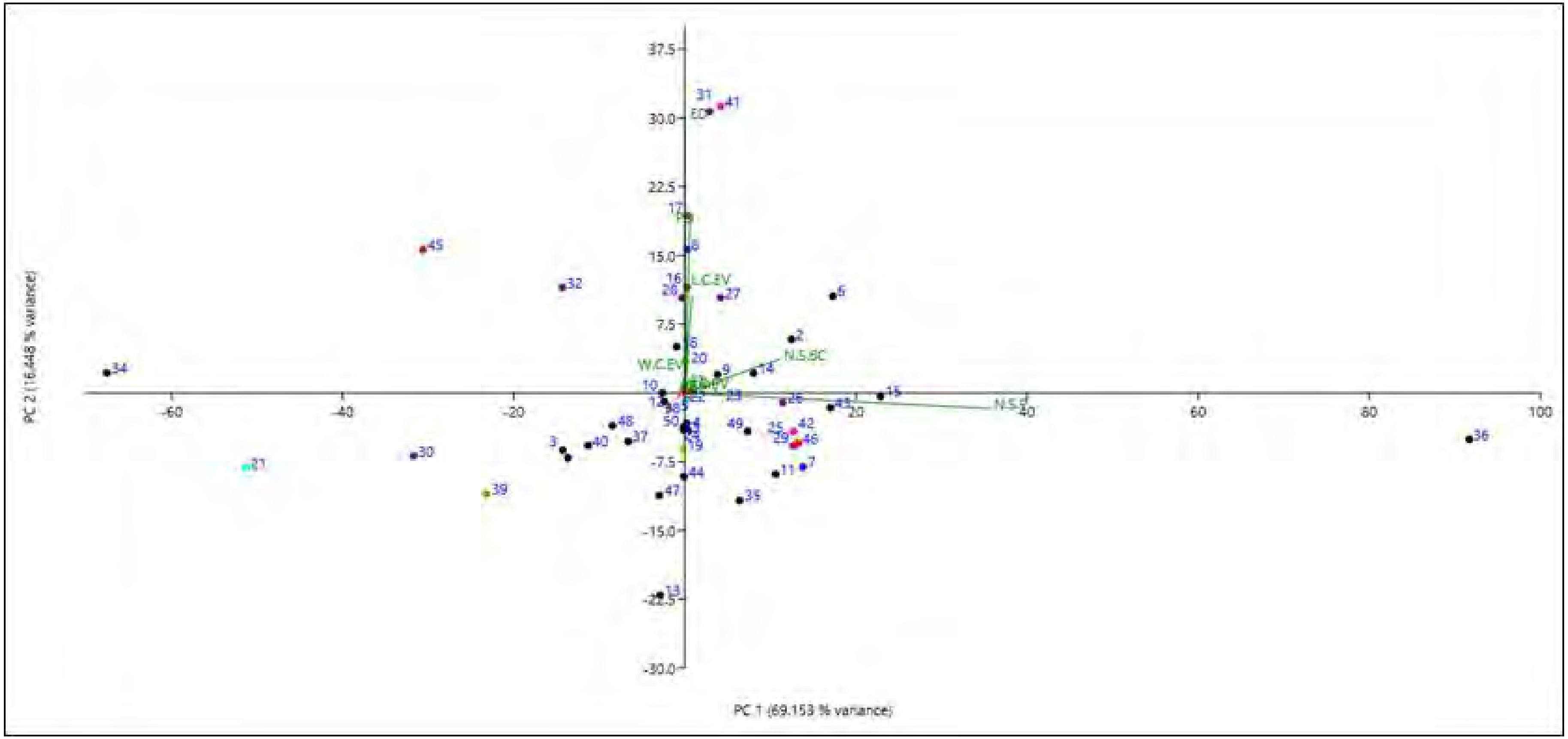

Figure 6. PCA analysis of PD: Polar Diameter, ED: Equatorial Diameter, W.C.PV: Width of colpi in polar view, L.C.PV: Length of colpi in polar view, W.C.EV: Width of colpi in equatorial view, L.C.EV: Length of colpi in equatorial view, M: Mesocolpium, ET: Exine thickness, N.S.P: Number of spines per pollen, N.S.BC: Number of spines between colpi, SL: Spine length (Numbering represents the taxa number in qualitative Table)

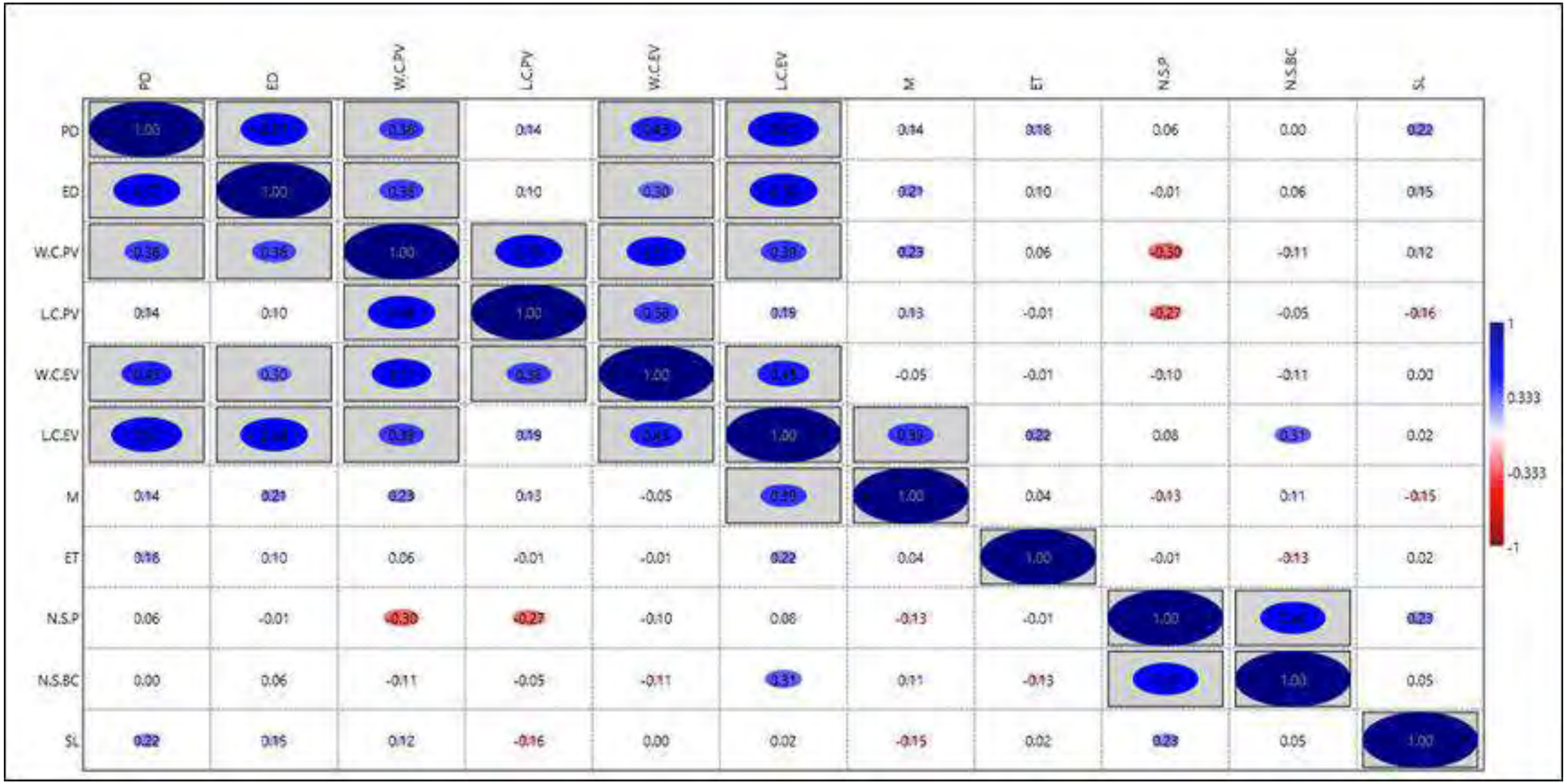

Figure 7. Correlation among the mean values of PD: Polar Diameter, ED: Equatorial Diameter, W.C.PV: Width of colpi in polar view, L.C.PV: Length of colpi in polar view, W.C.EV: Width of colpi in equatorial view, L.C.EV: Length of colpi in equatorial view, M: Mesocolpium, ET: Exine thickness, N.S.P: Number of spines per pollen, N.S.BC: Number of spines between colpi, SL: Spine length)

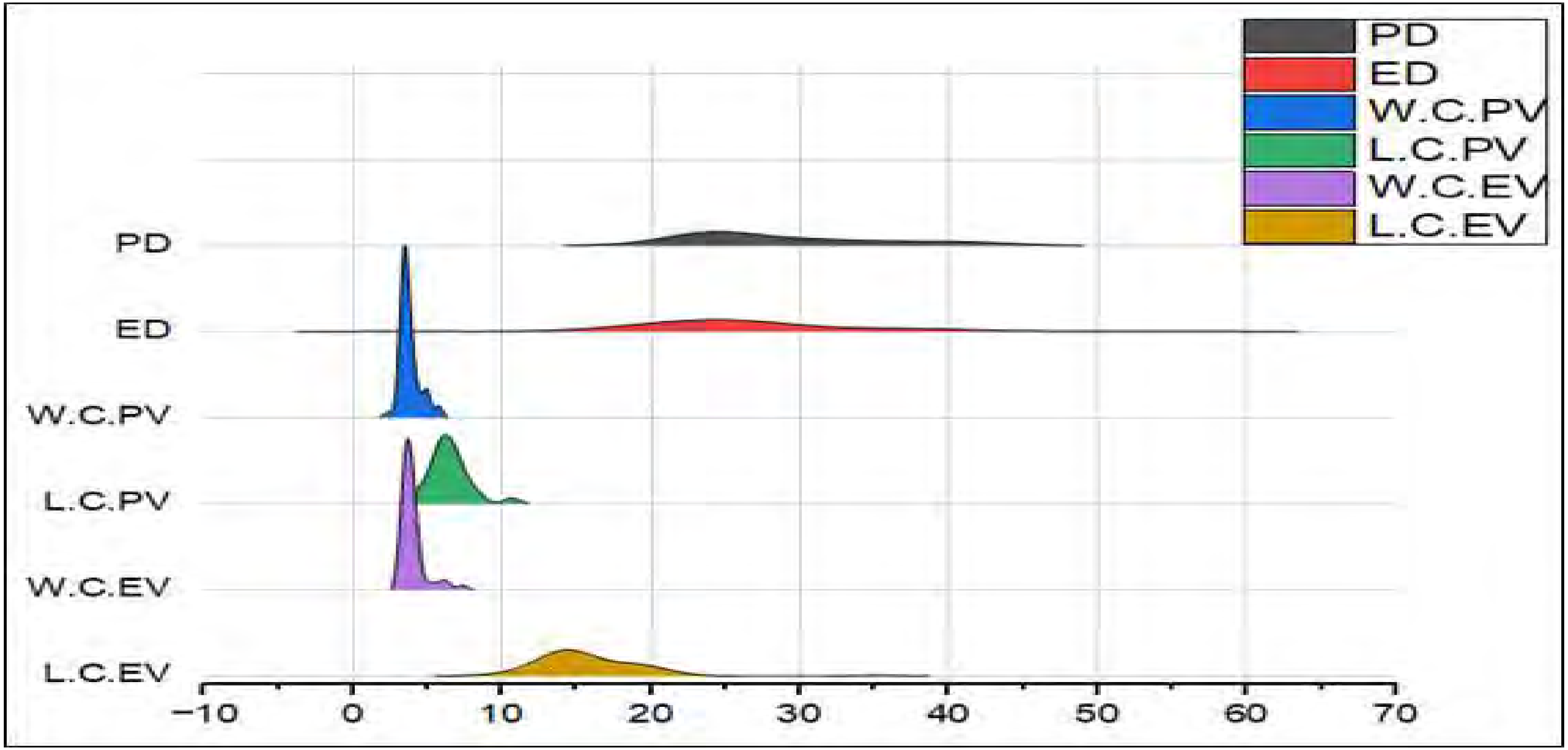

Figure 8. Ridgeline plot for paired traits compared and visualized the overall distribution of Palynological traits (PD: Polar Diameter, ED: Equatorial Diameter, W.C.PV: Width of colpi in polar view, L.C.PV: Length of colpi in polar view, W.C.EV: Width of colpi in equatorial view, L.C.EV: Length of colpi in equatorial view

Table 6. Qualitative characters based on LM and SEM of pollen of Asteraceous species

Taxa	Symmetry	Polarity	Unity	Size class	Shape class	No. of apertures	Polar view	Equatorial view	Exine sculpturing	Exine surface	Edges of apertures	Lacuna shape	Amb	NPC classification		Viability F/F+S *100
														Name	Formula	
A. cretica	Radial	Isopolar	Monad	Medium	Sub-prolate	Tricolporate	Triangular obtuse convex	Circular	Echinate	Perforate	Operculum	-	Goniotreme	Trizonocolporate	$N_3P_4C_5$	89.47
A. biennis	Radial	Isopolar	Monad	Medium	Sub-prolate	Tricolporate	Triangular obtuse convex	Circular	Echinate	Perforate	Operculum	-	Goniotreme	Trizonocolporate	$N_3P_4C_5$	83.33
A. carduus	Radial	Isopolar	Monad	Medium	Sub-prolate	Tricolporate	Triangular obtuse convex	Circular	Echinate	Perforate	Operculum	-	Goniotreme	Trizonocolporate	$N_3P_4C_5$	81.81
B. sinuata	Radial	Isopolar	Monad	Medium	Oblate spheroidal	Tetracolporate	Circular	Circular	Echinate lophate	Perforate	Operculum	Irregular convex hexagon	Peritreme	Tetrazonocolporate	$N_4P_4C_5$	93.75
C. iberica	Radial	Isopolar	Monad	Medium	Per-prolate	Tricolporate	Circular	Elliptic truncate	Scabrate	Perforate	Operculum	-	Peritreme	Trizonocolporate	$N_3P_4C_5$	85
C. arvense	Radial	Isopolar	Monad	Medium	Sub-oblate	Tricolporate	Triangular obtuse convex	Circular	Echinate	Perforate	Operculum	-	Goniotreme	Trizonocolporate	$N_3P_4C_5$	88
C. haeckeliae	Radial	Isopolar	Monad	Small	Sub-prolate	Tricolporate	Triangular	Elliptic	Echinate lophate	Perforate	Operculum	Irregular conve	Goniotreme	Trizonocolporate	$N_3P_4C_5$	95.45

							obtuse convex					x hexagon				
C. prolifera	Radial	Isopolar	Monad	Medium	Prolate spheroidal	Tricolporate	Triangular obtuse concave	Rectangular obtuse	Scabrate	Non-perforate	Operculum	-	Ptychotreme	Trizonocolporate	$N_3P_4C_5$	89.47
C. kotschyana	Radial	Isopolar	Monad	Medium	Oblate	Tricolporate	Triangular obtuse convex	Elliptic	Echinate lophate	Non-perforate	Operculum	Irregular convex hexagon	Goniotreme	Trizonocolporate	$N_3P_4C_5$	84.2
E. bonariensis	Radial	Isopolar	Monad	Medium	Prolate	Tricolporate	Circular	Circular	Echinate	Perforate	Operculum	-	Goniotreme	Trizonocolporate	$N_3P_4C_5$	81.81
F. hurdwarica	Radial	Isopolar	Monad	Small	Sub-prolate	Tricolporate	Triangular obtuse convex	Elliptic	Echinate	Perforate	Operculum	-	Goniotreme	Trizonocolporate	$N_3P_4C_5$	67.85
F. trinervia	Radial	Isopolar	Monad	Medium	Sub-prolate	Tricolporate	Triangular obtuse convex	Elliptic	Echinate	Perforate	Operculum	-	Goniotreme	Trizonocolporate	$N_3P_4C_5$	80
G. micrantha	Radial	Isopolar	Monad	Medium	Oblate	Tricolporate	Triangular obtuse convex	Elliptic	Echinate	Non-perforate	Operculum	-	Goniotreme	Trizonocolporate	$N_3P_4C_5$	68
H. intermedia	Radial	Isopolar	Monad	Medium	Sub-prolate	Tricolporate	Triangular obtuse convex	Circular	Echinate	Perforate	Operculum	-	Goniotreme	Trizonocolporate	$N_3P_4C_5$	75
H. pusilla	Radial	Isopolar	Monad	Medium	Sub-prolate	Tricolporate	Triangular obtuse convex	Circular	Echinate lophate	Non-perforate	Operculum	Irregular Convex	Goniotreme	Trizonocolporate	$N_3P_4C_5$	66.66

												Pentagon				
H. afghana	Radial	Isopolar	Monad	Medium	Prolate spheroidal	Tricolporate	Triangular obtuse convex	Elliptic	Scabrate	Non-perforate	Operculum	-	Ptychotreme	Trizonocolporate	$N_3P_4C_5$	68.18
H. heteromalla	Radial	Isopolar	Monad	Medium	Prolate spheroidal	Tricolporate	Circular	Elliptic truncate	Scabrate	Perforate	Operculum	-	Peritreme	Trizonocolporate	$N_3P_4C_5$	88
I. grantioides	Radial	Isopolar	Monad	Medium	Prolate	Tricolporate	Circular	Circular	Echinate	Perforate	Operculum	-	Peritreme	Trizonocolporate	$N_3P_4C_5$	90.9
J. berardioidea	Radial	Isopolar	Monad	Medium	Sub-oblate	Tricolporate	Circular	Circular	Scabrate	Non-perforate	Operculum	Penta to hexagon	Goniotreme	Trizonocolporate	$N_3P_4C_5$	72
J. carduiformis	Radial	Isopolar	Monad	Medium	Sub-prolate	Tricolpate	Trilobate	Elliptic obtuse	Scabrate	Non-perforate	-	-	Ptychotreme	Trizonocolpate	$N_3P_4C_3$	73.07
K. linearis	Radial	Isopolar	Monad	Small	Oblate spheroidal	Tricolporate	Circular	Circular	Echinate	Perforate	Operculum	-	Peritreme	Trizonocolporate	$N_3P_4C_5$	80
K. turanica	Radial	Isopolar	Monad	Medium	Sub-oblate	Tricolporate	Triangular obtuse convex	Elliptic	Echinate lophate	Perforate	Operculum	Irregular hexagon	Goniotreme	Trizonocolporate	$N_3P_4C_5$	83.33
L. dissecta	Radial	Isopolar	Monad	Medium	Sub-prolate	Tricolporate	Triangular obtuse convex	Elliptic	Echinate lophate	Perforate	Operculum	Irregular convex penta to Hexagon	Goniotreme	Trizonocolporate	$N_3P_4C_5$	76.92

L. orientalis	Radial	Isopo lar	Mo nad	Medi um	Prolat e sphero idal	Tricolpor ate	Circular	Elliptic	Echinat e lophate	Non- perfo rate	Opercu lum	Penta gon	Peritre me	Trizonocolp orate	N_3P_4 C_5	95.45
L. serriola	Radial	Isopo lar	Mo nad	Medi um	Oblate sphero idal	Tricolpor ate	Triangul ar obtuse convex	Elliptic	Echinat e lophate	Perfo rate	Opercu lum	Penta gon	Goniotr eme	Trizonocolp orate	N_3P_4 C_5	90
L. acanthod es	Radial	Isopo lar	Mo nad	Medi um	Sub- prolat e	Tricolpor ate	Triangul ar obtuse convex	Elliptic	Echinat e lophate	Perfo rate	Opercu lum	Irregu lar conve x Hexa gon	Goniotr eme	Trizonocolp orate	N_3P_4 C_5	95
L. aspleniif olia	Radial	Isopo lar	Mo nad	Medi um	Prolat e	Tricolpor ate	Triangul ar obtuse convex	Elliptic truncat e	Echinat e lophate	Non- perfo rate	Opercu lum	Irregu lar conve x Hexa gon	Goniotr eme	Trizonocolp orate	N_3P_4 C_5	69.23
L. fragilis	Radial	Isopo lar	Mo nad	Medi um	Oblate sphero idal	Tricolpor ate	Triangul ar obtuse convex	Circula r	Echinat e lophate	Non- perfo rate	Opercu lum	Irregu lar conve x Hexa gon	Goniotr eme	Trizonocolp orate	N_3P_4 C_5	75
L. intybacea	Radial	Isopo lar	Mo nad	Medi um	Sub- prolat e	Tricolpor ate	Triangul ar obtuse convex	Circula r	Echinat e lophate	Non- perfo rate	Opercu lum	Irregu lar conve x Hexa gon	Goniotr eme	Trizonocolp orate	N_3P_4 C_5	79.16
L. oligocep hala	Radial	Isopo lar	Mo nad	Medi um	Sub- prolat e	Tricolpor ate	Triangul ar obtuse convex	Circula r	Echinat e lophate	Non- perfo rate	Opercu lum	Regul ar conve x	Goniotr eme	Trizonocolp orate	N_3P_4 C_5	80

												penta to hexagon				
L. procumbens	Radial	Isopolar	Monad	Large	Sub-oblate	Tricolporate	Triangular obtuse convex	Circular	Echinate lophate	Perforate	Operculum	Irregular convex Hexagon	Goniotreme	Trizonocolporate	$N_3P_4C_5$	73.33
L. stenocephala	Radial	Isopolar	Monad	Medium	Sub-oblate	Tricolporate	Triangular obtuse convex	Circular	Echinate lophate	Non-perforate	Operculum	Irregular convex hexagon	Goniotreme	Trizonocolporate	$N_3P_4C_5$	80.76
L. repens	Radial	Isopolar	Monad	Medium	Sub-oblate	Tricolporate	Triangular obtuse convex	Elliptic	Scabrate	Perforate	Operculum	-	Goniotreme	Trizonocolporate	$N_3P_4C_5$	80
P. hierochuntica	Radial	Isopolar	Monad	Medium	Sub-oblate	Tricolporate	Triangular obtuse convex	Circular	Echinate	Perforate	Operculum	-	Goniotreme	Trizonocolporate	$N_3P_4C_5$	96.15
P. divaricatum	Radial	Isopolar	Monad	Medium	Prolate spheroidal	Tricolporate	Triangular obtuse convex	Circular	Echinate	Perforate	Operculum	-	Goniotreme	Trizonocolporate	$N_3P_4C_5$	91.66
P. schweinfurthii	Radial	Isopolar	Monad	Medium	Sub-oblate	Tricolporate	Triangular obtuse convex	Elliptic	Echinate	Perforate	Operculum	-	Goniotreme	Trizonocolporate	$N_3P_4C_5$	65.62
P. stewartii	Radial	Isopolar	Monad	Small	Oblate spheroidal	Tricolporate	Triangular obtuse convex	Elliptic	Echinate	Perforate	Operculum	-	Goniotreme	Trizonocolporate	$N_3P_4C_5$	88.46

P. undulata	Radial	Isopolar	Monad	Medium	Prolate spheroidal	Tricolporate	Triangular obtuse convex	Elliptic	Echinate	Perforate	Operculum	-	Goniotreme	Trizonocolporate	$N_3P_4C_5$	95
P. angustifolia	Radial	Isopolar	Monad	Small	Sub-prolate	Tricolporate	Circular	Circular	Echinate	Perforate	Operculum	-	Peritreme	Trizonocolporate	$N_3P_4C_5$	90
R. tingitana	Radial	Isopolar	Monad	Medium	Sub-prolate	Tricolporate	Triangular obtuse convex	Circular	Echinate lophate	Non-perforate	Operculum	Irregular convex Hexagon	Goniotreme	Trizonocolporate	$N_3P_4C_5$	57.142
S. koelpinioides	Radial	Isopolar	Monad	Medium	Sub-oblate	Tricolporate	Triangular obtuse concave	Circular	Echinate lophate	Non-perforate	Operculum	Penta to hexagon	Goniotreme	Trizonocolporate	$N_3P_4C_5$	83.33
S. raddeana	Radial	Isopolar	Monad	Medium	Prolate spheroidal	Tricolporate	Triangular obtuse convex	Circular	Echinate lophate	Perforate	Operculum	Hexagon	Goniotreme	Trizonocolporate	$N_3P_4C_5$	91.66
S. glaucus	Radial	Isopolar	Monad	Medium	Sub-oblate	Tricolporate	Circular	Circular	Echinate	Perforate	Operculum	-	Peritreme	Trizonocolporate	$N_3P_4C_5$	80
S. maritimum	Radial	Isopolar	Monad	Medium	Prolate	Tricolporate	Triangular obtuse concave	Elliptic	Scabrate	Non-perforate	Operculum	-	Ptychotreme	Trizonocolporate	$N_3P_4C_5$	87.5
S. arvensis	Radial	Isopolar	Monad	Medium	Oblate spheroidal	Tricolporate	Triangular obtuse convex	Circular	Echinate lophate	Perforate	Operculum	Irregular convex Hexagon	Goniotreme	Trizonocolporate	$N_3P_4C_5$	83.33
S. oleraceus	Radial	Isopolar	Monad	Medium	Sub-prolate	Tetracolporate	Quadrangular	Elliptic	Echinate lophate	Perforate	Operculum	Irregular conve	Goniotreme	Tetrazonocolporate	$N_4P_4C_5$	66.66

							obtuse convex					x hexagon				
S. subulatum	Radial	Isopolar	Monad	Small	Sub-prolate	Tricolporate	Circular	Circular	Echinate	Perforate	Operculum	-	Peritreme	Trizonocolporate	$N_3P_4C_5$	73.07
T. pusilla	Radial	Isopolar	Monad	Medium	Oblate spheroidal	Tricolporate	Circular	Circular	Echinate lophate	Non-perforate	Operculum	Penta to hexagon	Peritreme	Trizonocolporate	$N_3P_4C_5$	58.62
X. macropodum	Radial	Isopolar	Monad	Medium	Sub-prolate	Tricolporate	Circular	Elliptic	Echinate	Perforate	Operculum	-	Goniotreme	Trizonocolporate	$N_3P_4C_5$	72
Z. purpurea	Radial	Isopolar	Monad	Medium	Oblate spheroidal	Tricolporate	Circular	Circular	Gemmate	Non-perforate	Operculum	-	Peritreme	Trizonocolporate	$N_3P_4C_5$	77.77

amb: circumference, NPC: Number, Position, Characteristic

Table 7. Quantitative measurements of pollen of Asteraceae

Taxa	PD	ED	W.C.PV	L.C.PV	W.C.EV	L.C.EV	M	ET	N.S.P	N.S.BC	SL
Min-Max=Mean±SE											
A. cretica	24.5-26.5= 25.65±0.35	20.5-22= 21.25±0.25	3-4.25= 3.6±0.23	6.25-7.25= 6.75±0.17	3-4= 3.5±0.17	12.75-14= 13.3±0.21	12.75-14.25= 13.55±0.26	1.25-2.25= 1.75±0.17	92.5-105= 98.5±2.31	32.5-47.5= 38.5±2.57	3-4= 3.5±0.17
A. biennis	36-37.75= 36.85±0.32	30.25-32.25 =31.05±0.44	3.25-4.25= 3.75±0.17	4.5-5.5= 5.05±0.18	3.5-4.75= 4.15±0.23	13.5-15.5= 14.55±0.39	12.75-14.25= 13.65±0.25	1.5-2.25= 1.8±0.14	105-140= 125±6.37	37.5-45= 40.5±1.45	3-4.25= 3.6±0.23
A. carduus	28.25-28.75= 28.5±0.26	23.75-24.75 =24.25±0.17	3-4.75= 3.9±0.32	5.25-6.25= 5.75±0.17	3.75-4.75= 4.25±0.17	14.25-15.75 = 14.9±0.26	12.75-14= 13.3±0.21	1.75-3.25= 2.4±0.26	100-112.5= 107.5±2.62	2.5-12.5= 7±1.65	3-4= 3.5±0.176
B. sinuate	23-24.75= 23.75±0.3	25.5-26.5= 26±0.17	3-4= 3.5±0.17	5.25-7.25= 6.3±0.37	3-4.75= 3.95±0.34	14-14.75= 14.35±0.12	13.5-14.75= 14.1±0.23	1.5-2.25= 1.9±0.12	105-117.5= 112±2.15	37.5-47.5= 42.5±1.76	3.75-4.75= 4.25±0.76
C. iberica	37.75-43.25= 39.8±0.98	13.1-= 15.3.52±0.41	3-4.75= 4.1±0.3	5.5-7.25= 6.4±0.32	5.25-7= 6.15±0.34	18.75-20.5= 19.75±0.3	11.25-13.25= 12.45±0.36	1.5-2.25= 1.85±0.12	-	-	-
C. arvense	30.25-31.75= 31±0.25	37.75-38.75 =38.25±0.17	2.75-3.75= 3.25±0.17	5.75-6.75= 6.2±0.18	3.5-4.75= 3.9±0.23	21-23.5= 22.1±0.49	12.75-14.5= 13.75±0.32	3-4= 3.5±0.17	122.5-137.5 = 130±2.5	35-45= 40.5±1.83	3-4= 3.5±0.17
C. haeckeliae	25.5-27.25= 26.15±0.3	20.5-21.75= 21.15±0.23	2.75-4.5= 3.6±0.32	8-9= 8.5±0.17	2.75-3.75= 3.25±0.17	10.75-11.75= 11.25±0.17	13.5-15.25= 14.15±0.3	1.5-2.75= 2.05±0.21	112.5-137.5 =125.5±4.63	37.5-50= 44.5±2.15	3-4= 3.5±0.17

C. prolifera	38.5-42.25= 40.25±0.74	37.75-39.5= 38.25±0.32	2.75-3.75= 3.25±0.17	5.5-7.75= 6.85±0.37	2.75-4= 3.45±0.21	18.25-19.75 =18.85±0.25	13-14= 13.5±0.17	1.25-2= 1.6±0.12	-	-	-
C. kotschyana	22.75-24.5= 23.6±0.32	2.75-4.5= 33.65±0.32	2.75-3.75= 3.35±0.16	4-5.25= 4.55±0.21	2.75-4= 3.4±0.23	13-14= 13.5±0.17	12.75-14= 13.5±0.2	1.25-2.25= 1.75±0.17	105-122.5= 114±3.22	40-55= 47.5±2.5	2.75-3.75= 3.25±0.17
E. bonariensis	34.5-35.75= 35.15±0.23	20.25-22.25 = 21.35±0.4	4.25-5.5= 4.85±0.23	10.25-11= 10.65±0.12	6.75-8= 7.35±0.23	18.75-20.5= 19.45±0.31	12.25-14= 13.05±0.28	1.5-2.75= 2.05±0.21	95-127.5= 108±5.66	37.5-50= 44.5±2.15	2.75-3.75= 3.25±0.17
F. hurdwarica	22.75-24.25= 23.45±0.28	19.5-20.75= 20.15±0.23	3-4= 3.5±0.17	3.75-4.75= 4.25±0.17	3-4= 3.5±0.17	15.25-17= 16±0.3	12-13.75= 12.85±0.32	1.5-2.25= 1.95±0.14	112.5-130= 122±2.89	37.5-55= 44.5±3.48	4-4.75= 4.4±0.12
F. trinervia	28.75-30.75= 30±0.35	23.5-25.75= 24.65±0.43	3-3.75= 3.4±0.12	5.25-6.25= 5.75±0.17	3-3.75= 3.35±0.12	15.25-16.75 = 16±0.25	13-14.75= 14±0.3	4-5.25= 4.55±0.21	92.5-125= 107.5±6.07	37.5-55= 47±2.89	3.25-4.25= 3.85±0.2
G. micrantha	22.25-23.5= 22.95±0.21	3-3.75= 3.3±0.145	3-3.75= 3.3±0.14	7-8.5= 7.7±0.26	3.25-4= 3.65±0.12	14.25-15.5- =14.85±0.23	13.5-14.75= 14.1±0.23	1.75-2.75= 2.15±0.16	87.5-137.5= 107.5±8.32	40-55= 46±2.57	3.25-4.25= 3.75±0.17
H. intermedia	33-33.75= 33.3±0.14	25.5-26.5= 26±0.17	3-4= 3.5±0.17	6.25-7.25= 6.75±0.17	6.25-7.25= 6.75±0.17	16.5-18= 17.1±0.25	12-14= 12.85±0.35	1.5-2.75= 2.05±0.21	110-127.5= 117±3.1	45-57.5= 51.5±2.31	3.25-4= 3.6±0.12
H. pusilla	30.75-34.75= 32.3±0.81	24.25-26= 25.15±0.34	3.5-4.5= 4±0.17	4.75-6= 5.45±0.21	3.5-4.5= 4±0.17	12.75-14.25 =13.55±0.28	12.25-14= 13.05±0.28	1.25-2.25= 1.75±0.17	122.5-140 =131.5±3.22	50-60= 55±1.76	3-4.25= 3.6±0.23
H. afghana	37-38.75= 37.8±0.32	33.25-34.75 =33.95±0.28	3.25-4.25= 3.7±0.16	5.5-6.5= 6±0.17	3-4= 3.5±0.17	20.25-21.75 = 20.9±0.26	12.75-14= 13.45±0.21	3.25-4.5= 3.9±0.23	-	-	-

H. heteromalla	41.5-44.75= 42.85±0.56	34.75-43.75= 41.25±1.66	3.75-4.75= 4.25±0.17	6.25-7.25= 6.75±0.17	2-5.5= 4.35±0.62	18-19.5= 18.6±0.25	12.25-13.75= 13.1±0.26	1.5-3= 2.1±0.25	-	-	-
I. grantioides	40.75-41.75= 41.25±0.17	25.5-26.5= 26±0.17	3.75-4.75= 4.25±0.17	5.25-6.25= 5.75±0.17	3-3.75= 3.45±0.14	15.5-16.5= 16±0.17	13-14.5= 13.85±0.26	1.25-2= 1.65±0.12	100-117.5= 111±3.02	35-45= 40±1.76	4-4.5= 4.25±0.21
J. berardioidea	18.25-22= 19.65±0.63	25.5-26.5= 26±0.17	3-4= 3.5±0.17	4.75-6= 5.45±0.21	3.25-4.5= 3.8±0.21	13.25-14.25= 13.75±0.17	13.5-14.75= 14.1±0.23	1.25-2.25= 1.75±0.17	-	-	-
J. carduiformis	33-37= 34.75±0.68	25.25-27= 26.1±0.32	4.25-5.5= 4.85±0.23	6.75-8= 7.35±0.231	3.25-4.25= 3.75±0.17	18.75-20.5= 19.45±0.31	15.25-16.25= 15.75±0.17	3-3.5= 3.3±0.093	-	-	-
K. linearis	20.25-22.25= 21.35±0.4	22.75-23.75 =23.25±0.17	5.25-6.25= 5.75±0.17	10.25-11= 10.65±0.12	3-4= 3.5±0.17	2.5-10.75= 8.75±1.57	12.75-13.75= 13.25±0.17	1.25-2.25= 1.75±0.17	57.5-70= 62.5±2.62	22.5-37.5= 28.5±2.57	3.5-4.5= 4±0.17
K. turanica	22.75-23.75= 23.25±0.17	27.25-28.5= 27.95±0.21	2.75-3.75= 3.35±0.16	6-7.25= 6.7±0.21	3.5-4.75= 4.15±0.23	19.25-20.5= 19.85±0.23	13-14= 13.5±0.17	1-2.25= 1.55±0.21	80-125= 110.5±7.92	42.5-50= 46±1.27	3-3.75= 3.45±0.14
L. dissecta	30.5-31.25=30.9±0.12	25.25-26.25 =25.75±0.17	2.75-3.75= 3.25±0.17	6-7= 6.5±0.17	2.75-3.75= 3.25±0.17	14.25-15.5= 14.85±0.23	12.75-14= 13.45±0.21	1.75-3= 2.35±0.23	100-125= 116±4.51	37.5-47.5= 42.5±1.76	2.75-3.75= 3.25±0.17
L. orientalis	24.75-26.25= 25.55±0.26	23.25-24.5= 23.85±0.23	3.25-4.75= 3.95±0.26	6.5-7.25= 6.95±0.14	3-4.25= 3.65±0.23	13.75-16= 15.05±0.38	13-14.75= 13.95±0.32	1.5-2.75= 2.05±0.21	-	-	-
L. serriola	23.75-24.75= 24.25±0.17	24.5-26= 25.3±0.28	3-4.5= 3.7±0.28	6.25-7.25= 6.75±0.17	3-4= 3.5±0.17	12.25-15.75 =14.25±0.62	12.25-13.75= 13.05±0.26	1.5-2.2= 1.95±0.14	112.5-137.5 =120.5±4.43	37.5-50= 44.5±2.15	3.5-4.5= 4±0.17

L. acanthodes	28.75-31.25= 30.05±0.4	23.5-26.25= 25.1±0.5	2.75-3.75= 3.25±0.17	3.75-4.75= 4.25±0.17	3.5-4.75= 4.15±0.23	15.25-16.75 =16.05±0.28	12.75-14.75= 13.8±0.37	1.25-2.25= 1.75±0.17	112.5-127.5 =121.5±2.57	40-62.5= 48.5±4.07	4-5.25= 4.55±0.21
L. aspleniifolia	41-43.75= 42.2±0.52	29.75-32.25 =31.35±0.43	2.75-4= 3.3±0.21	4.75-6.5= 5.6±0.3	2.75-4.5= 3.45±0.31	17.25-18.5= 17.95±0.21	13-14= 13.5±0.17	1.25-2.25= 1.75±0.17	112.5-120= 115.5±1.45	37.5-47.5= 42.5±1.76	3.75-4.75= 4.25±0.17
L. fragilis	30.25-33.5= 32.05±0.69	34.5-35.75= 35.15±0.23	3-4.75= 3.8±0.34	6.25-8= 7.3±0.32	3-4.5= 3.85±0.26	18-19.5= 18.75±0.28	12.75-14= 13.3±0.21	1.25-2.25= 1.75±0.17	100-112.5= 106.5±2.31	37.5-67.5= 57±5.14	3.8-4.2= 4±0.17
L. intybacea	25.5-26.75= 26.05±0.21	21-22= 21.5±0.17	3.25-4= 3.6±0.12	6.25-7.25= 6.75±0.17	3-4.5= 3.9±0.25	15.25-16.25= 15.75±0.17	12.75-13.75= 13.25±0.17	1.75-3= 2.35±0.23	102.5-137.5 = 122±6.34	45-57.5= 51.5±2.31	3-4= 3.5±0.17
L. oligocephala	23.5-26.25= 25.05±0.58	20.25-22= 21.2±0.28	3-4.5= 3.6±0.25	5.25-6.25= 5.75±0.17	3.75-4.75= 4.25±0.17	13-14.5= 13.9±0.25	12.75-14= 13.3±0.21	1.25-2.25= 1.75±0.17	72.5-90= 81±3.4	25-45= 34±3.4	2.75-4= 3.3±0.21
L. procumbens	41.25-45.25= ±43.50.7	54.75-58= 56.3±0.66	4.5-5.5= 4.95±0.18	7.25-9.75= 8.35±0.43	5.25-7.25= 6.25±0.39	17.75-19.5= 18.65±0.32	10.25-15.75= 14.1±0.98	1.5-2.25= 1.9±0.12	102.5-137.5= 116.5±6.54	30-40= 35±1.76	3.75-4.75= 4.25±0.17
L. stenocephala	31.25-32.25= 31.75±0.17	37.75-38.75= 38.25±0.17	3-4= 3.5±0.17	6.75-8.25= 7.55±0.28	3.5-4.5= 4±0.17	17.25-19= 18.1±0.30	12.75-14= 13.3±0.21	1.25-2.25= 1.75±0.17	80-107.5= 96.5±4.65	30-50= 42±3.65	3.25-4.25= 3.75±0.17
L. repens	22.25-23.75= 22.9±0.24	25.25-28= 26.5±0.57	3-3.75= 3.4±0.12	6.75-8.5= 7.45±0.31	3.5-4.5= 4±0.17	14.25-16.5= 15.15±0.42	12.75-14= 13.35±0.23	1.5-2.75= 2.05±0.21	-	-	-
P. hierochuntica	23.25-24.75= 24±0.28	30.25-31.25= 30.75±0.17	3-4= 3.5±0.17	5.5-6.75= 6.1±0.23	3.5-4.5= 4±0.17	13-13.75= 13.35±0.12	13-14.25= 13.7±0.21	1.25-2.25= 1.75±0.17	37.5-47.5= 42.5±1.7	30-45= 37±2.66	3-3.75= 3.35±0.12

P. divaricatum	20.5-22=21.25±0.25	18-19.75= 18.65±0.30	1.75-3= 2.35±0.23	4.25-5.25=4.8±0.2	2.75-4= 3.45±0.21	11-12.25= 11.8±0.21	13.5-14.25= 13.9±0.12	1.5-2.25= 1.9±0.12	100-127.5= 116.5±4.78	37.5-57.5= 48.5±3.58	3-4= 3.75±0.17
P. schweinfurthii	20.25-24.75= 22.8±0.86	24.5-28.5= 26.4±0.78	2.75-4= 3.45±0.21	5.25-6.25= 5.7±0.17	3.25-4.25= 3.75±0.17	13.75-16.25= 15.15±0.43	2.5-14.75= 11.8±2.33	1.5-2.25= 1.85±0.12	162.5-225= 198±13.16	47.5-95=73.5±8.314	4-4.75=4.4±0.12
P. stewartii	23-24.5= 23.85±0.26	23.75-26= 24.75±0.39	3.5-4.75= 4.1±0.23	5.25-7.25= 6.25±0.39	3.5-4.75= 4.15±0.23	11.5-14.5= 12.85±0.57	12.25-13.5= 12.95±0.21	1.25-2.25= 1.75±0.17	97.5-112.5= 105±2.85	35-50= 41.5±2.69	3-4= 3.5±0.17
P. undulata	28-30.75=29.5±0.5	25.25-26.25= 25.75±0.17	3.5-4.25= 3.85±0.12	4.75-6= 5.45±0.21	3-3.75= 3.45±0.14	12.75-14= 13.3±0.21	12.25-13.75= 13.1±0.26	1.25-2.25= 1.75±0.17	102.5-115= 109.5±2.15	37.5-47.5= 42.5±1.76	3-4.25= 3.6±0.23
P. angustifolia	20.25-23.25= 21.75±0.58	18.5-19.25= 18.8±0.14	2.75-3.75= 3.25±0.17	6.25-7.25= 6.75±0.17	3-4= 3.5±0.17	13-14.5= 13.6±0.25	11.25-12.75= 12±0.25	1.5-2.75= 2.05±0.21	72.5-112.5= 90±6.7	27.5-40= 34±2.31	3-4= 3.5±0.17
R. tingitana	25.5-26.5= 26±0.17	20.75-21.75= 21.25±0.17	3-4= 3.5±0.17	5.5-6.5= 6±0.17	3.25-4.25= 3.75±0.17	15.5-16.5= 16±0.17	13.25-14= 13.6±0.12	1.25-2.25= 1.75±0.17	87.5-112.5= 99.5±4.28	35-50= 43±2.89	3.5-4.5= 4±0.17
S. koelpinioides	37.25-39.75= 38.95±0.44	48.25-50.5= 49.5±0.41	4.75-6.5= 5.7±0.32	7.75-8.75= 8.25±0.17	3.75-6.5= 5.05±0.45	34.25-36= 35.35±0.3	16.25-17.25= 16.75±0.17	1.25-2.25= 1.75±0.17	95-137.5= 107.5±7.7	57.5-75= 67.5±3.25	3-3.75= 3.35±0.12
S. raddeana	24.5-28= 26.25±0.57	23.25-24.5= 23.85±0.23	2.75-3.75= 3.25±0.17	4.75-5.75= 5.35±0.16	3.25-4.25= 3.75±0.17	13.25-15.25 = 14.3±0.35	12.75-14.25= 13.65±0.25	1.25-2.25= 1.75±0.176	110-137.5= 122±5.2	45-57.5= 51.5±2.31	3-4= 3.5±0.17
S. glaucus	23.5-24.75= 24.2±0.21	28.5-30.25= 29.15±0.3	2.75-3.75= 3.25±0.17	4.5-5.5= 4.95±0.18	3.5-4.5= 4±0.17	13.75-14.75 =14.25±0.17	12.75-13.75=13.25±0.17	1.25-2.25= 1.75±0.17	112.5-137.5 =127.5±4.25	42.5-55= 48.5±2.31	3.25-4= 3.6±0.12

S. maritimum	25.5-26.5= 26±0.17	18-19= 18.5±0.17	3-4= 3.5±0.17	5.25-6.75= 5.95±0.28	2.75-4= 3.45±0.21	12-14.25= 13.1±0.45	13.25-14.75= 14±0.28	1.25-2.25= 1.75±0.17	-	-	-
S. arvensis	32.75-38.25= 36.3±1.03	37.75-43.25= 39.8±0.98	4.5-5.5= 4.95±0.18	5.5-7.25= 6.4±0.32	5.25-6= 5.55±0.14	18.75-20.5= 19.75±0.3	10.75-13= 12.1±0.45	2.75-4= 3.45±0.21	62.5-100= 81.5±6.4	30-40= 35±1.76	3.75-4.75= 4.2±0.18
S. oleraceus	25.25-26.75= 26.15±0.25	20.5-21.5= 21.05±0.18	2.75-3.75= 3.25±0.17	7-8.25= 7.65±0.23	2.75-3.75= 3.25±0.17	14-15.25= 14.55±0.21	12.75-14= 13.3±0.21	1.25-2.25= 1.75±0.17	112.5-137.5= 120±4.6	50-75= 60±4.25	2.75-4.25= 3.5±0.25
S. subulatum	22.75-23.75= 23.25±0.17	18-19.5= 18.7±0.26	2.75-3.75= 3.25±0.17	5.25-6.25= 5.75±0.17	2.75-4.25 =3.55±0.28	10.75-11.75 =11.25±0.17	13.5-15.5= 14.45±0.33	1.25-2.25= 1.75±0.17	102.5-115= 109±2.31	37.5-4= 41±1.27	3-3.9= 3.45±1.27
T. pusilla	23-24.5= 23.9±0.25	24.5-25.75= 25.15±0.23	4.25-6= 4.85±0.3	5.75-6.75= 6.25±0.17	3-4= 3.5±0.17	13.75-15.5= 14.75±0.3	13-14= 13.5±0.17	1.5-2.25= 1.9±0.12	82.5-112.5 =101±6.59	40-55= 48±2.66	3.25-4.25= 3.75±0.17
X. macropodum	26-28.5= 27.4±0.52	22.25-23.5= 22.95±0.21	3-4= 3.5±0.17	5.75-7.75= 6.75±0.32	3.25-4.25= 3.75±0.17	13-14= 13.35±0.18	10.75-13.25= 12.4±0.44	1.75-3= 2.35±0.23	100-130= 116±5.62	45-62.5= 53±3.2	3-3.75= 3.3±0.14
Z. purpurea	23.5-24.75 =24.1±0.23	25.5-26.5 =26±0.17	3-4=3.5± 1080.176776695	5.5-6.5= 6±0.17	3.25-4.25= 3.75±0.17	2.5-16.5= 13.2±2.68	12.75-14= 13.4±0.23	2-3.25= 2.65±0.23	-	-	-

Polar diameter (PD), equatorial diameter (ED), width of colpi in polar view (W.C.PV), length of colpi in polar view (L.C.PV), width of colpi in equatorial view (W.C.EV), length of colpi in equatorial view (L.C.EV), mesocolpium (M), exine thickness (ET), number of spines per pollen (N.S.P), number of spines between colpi (N.S.BC), spine length (SL)

Table 8. Dichotomous (single access) bracketed / parallel taxonomic key based on pollen morphological traits of Asteraceae taxa

Link Character	**Leads**	**Characters**	***Taxa/ Go to link character***
1	a	Colpate	*Jurinea carduiformis*
	b	Colporate	2
2	a	Tetrazonocolporate	3
	b	Trizonocolporate	4
3	a	Amb Peritreme	*Blumea sinuata*
	b	Amb Goniotreme	*Sonchus oleraceus*
4	a	Shape per-prolate	*Centaurea iberica*
	b	Shape non-per-prolate	*5*
5	a	Shape sub-prolate	*6*
	b	Shape non-subprolate	*31*
6	a	Exine echinate	*14*
	b	Exine non-echinate	*7*
7	a	Exine Gemmate	*Zoegea purpurea*
	b	Exine Scabrate	8
8	a	Surface perforate	9
	b	Surface non-perforate	10
9	a	Polar view circular	*Himalaiella heteromalla*
	b	Polar view triangular obtuse conevx	*Leuzea repens*
10	a	Amb Goniotreme	*Jurinea berardioidea*
	b	Non-Goniotreme	11
11	a	Amb Peritreme	*Lactuca orientalis*
	b	Amb Ptychotreme	12
12	a	Polar view Triangular obtuse convex	*Himalaiella afghana*
	b	Polar view Triangular obtuse concave	13
13	a	Equatorial view rectangular obtuse	*Cousinia prolifera*

	b	Equatorial view elliptic	*Seriphidium maritimum*
14	a	Exine echinate lophate	15
	b	Exine echinate non-lophate	26
15	a	Surface non-perforate	16
	b	Surface perforate	24
16	a	Amb goniotreme	17
	b	Amb peritreme	*Takhtajaniantha pusilla*
17	a	Polar view triangular obtuse concave	*Scorzonera koelpinioides*
	b	Polar view triangular obtuse convex	18
18	a	Shape oblate	*Crepis kotschyana*
	b	Shape non-oblate	19
19	a	Equatorial view elliptic truncate	*Launaea aspleniifolia*
	b	Equatorial view circular	20
20	a	Lacuna shape regular convex penta to hexagon	*Launaea oligocephala*
	b	Lacuna shape irregular convex	21
21	a	Lacuna pentagon	*Heteroderis pusilla*
	b	Lacuna hexagon	22
22	a	Shape oblate spheroidal	*Launaea fragilis*
	b	Shape sub-prolate	23
23	a	Exine thickness 13.25 um	*Launaea intybacea*
	b	Exine thickness 13.6	*Reichardia tingitana*
24	a	Shape prolate spheroidal	*Scorzonera raddeana*
	b	Shape sub-prolate	25
25	a	Lacuna irregular convex penta to hexagon	*Lactuca dissecta*
	b	Lacuna irregular convex hexagon	*Cousinia haeckeliae*
26	a	Exine surface perforate	27
	b	Exine surface non-perforate	*Gymnarrhena micrantha*
27	a	Polar view triangular obtuse convex	28
	b	Polar view triangular obtuse concave	29
28	a	Equatorial view elliptic	*Filago hurdwarica*

	b	Equatorial view circular	*Atractylis carduus*
29	a	Pollen size small	*Pterachaenia stewartii*
	b	Pollen size medium	30
30	a	Pollen shape prolate spheroidal	*Pentanema divaricatum*
	b	Pollen shape sub-oblate	*Pallenis hierochuntica*
31	a	Polar and equatorial views circular	*Erigeron bonariensis*
	b	Polar and equatorial view triangular obtuse concave and elliptic	*Seriphidium maritimum*

3.3 Pollen Micromorphology of Boraginaceous Flora from Baluchistan

3.3.1 Results

a) Size and Shape

Pollen size was mostly small in the examined species in the present study. Family Boraginaceae has previously been documented with the smallest pollen. The *Onosma*, *Rochelia*, and *Paracaryum* species were observed with small-sized pollen, except the *Heliotropium* species, which were medium-sized. *A. tinctoria subsp. tinctoria* and *T. indicum* were also observed with medium size pollen. Whereas in *C. mucronanthera* the pollen size ranged from small to medium. *L. spinocarpos* was separated based on medium-sized pollen, as the *L. spinocarpos subsp. ceratophora* and *Lappula spp* were recorded as small-sized pollen. The abstracted shapes of the pollen based on the P/E ratio were prolate spheroidal (8), oblate spheroidal (3), suboblate (2), prolate (2), and subprolate (2). All three *H. bacciferum* (prolate), *H. campanula* (oblate spheroidal), and *H. crispum* (sub-oblate) species can be classified based on the pollen shape, as all possessed distinct shapes. The species of *Rochelia* and *Paracaryum* were also observed with distinct shapes (Plate 1, 2, 3, and 4). *P. intermedium var. calathicarpum* was prolate spheroidal, whereas the pollen in *P. intermedium var. intermedium* was prolate in shape. Suboblate pollen was found in *R. disperma*, and subprolate-shaped pollen in *R. sessiliflora*. Being oblate spheroidal *L. spinocarpos* was also separated from the other two *Lappula spp* species, as the two were prolate spheroidal.

b) Symmetry, Polarity, Unity

The pollen qualitative attributes symmetry, polarity, unity, number of apertures, polar view, equatorial view, exine sculpturing, exine surface, aperture membrane, colpi type, arrangement of apertures, Amb, Number, Position and Character (NPC) type of Boraginaceae were investigated and documented to incorporate them into the systematics of this family. The symmetry and unity of all the studied species were radial and monad. Heteropolarity is one of the taxonomically significant features of Boraginaceae. The heteropolarity of Boraginaceous taxa was not only caused by the difference between the size of the two poles but may exist because of the difference in the arrangement of apertures concerning the poles. Pollen in 12 studied taxa were isopolar, and 5 were heteropolar. The heteropolarity differentiated among the members of the same genus and between the subspecies and variety of the same species. Among the 3 *Heliotropium* species *H. bacciferum* was heteropolar. Whereas pollen of *L. spinocarpos subsp. Ceratophora* and *O. limitanea var. major* were heteropolar, while of *L. spinocarpos* and *O. limitanea var. limitanea* were isopolar. For other genera, such as *Paracaryum* and *Rochelia* the pollen polarity was the same for the examined species.

c) Aperture Characteristics

The aperture type, arrangement, number, aperture membrane, and colpi type were found to be influential systematically. The porocolpate and heterocolpate arrangement of apertures were noted in 9 taxa. In porocolpate type, around the equator, pollen have a pattern of apertures where pores and colpi alternate. While in the heterocolpate, simple and compound colpi were present in pollen. These were the specific features of some Boragenous taxa. All the studied *Heliotropium* and *Paracaryum* species were porocolpate and heterocolpate. Meanwhile, the species of genera *Rochelia* and *Lappula* species were separated based on the above two traits (Table 9, 10). The number of apertures has existed in five distinguished types. This trait was assessed to be eminently significant in the delimitation of these Boraginaceous taxa. Five types tricolpate, tricolporate, trisynocolporate, hexocolporate, and hexotricolporate were observed. *L. spinocarpos subsp. ceratophora* was hexotricolporate whereas *L. spinocarpos* is tricolporate. *O. limitanea var. limitanea* was tricolporate while *O. limitanea var. major* was trisynocolporate. *R. disperma* was tricolporate but the *R. sessiliflora* was hexotricolporate type. So, the aperture numbers were also a major palynological characteristic systematically. In the case of genera *Heliotropium* and *Paracaryum,* the number of apertures was significant up to the genus level only. The names of NPC classification for the examined Boraginaceous species were trizonocolpate, trizonocolporate, hexozonocolporate, and hexotrizonocolporate with formula $N_{6,3}P_4C_5$ and $N_{6,3}P_4C_5$.

d) Exine Sculpturing

The exine sculpturing of pollen in 12 species was psilate. Other observed types were scabrate, foveolate, and gemmate. Sculpturing was found helpful in the discrimination of *L. spinocarpos subsp. ceratophora* psilate from *L. spinocarpos* scabrate. Similarly, *O. limitanea var. limitanea* gemmate and *O. limitanea var. major* scabrate were significantly distinguished. For the genera *Heliotropium, Rochelia,* and *Paracaryum,* exine ornamentation was systematically nonsignificant. The foveolate exine was present singly in *C. mucronanthera.* Perforation in the exine surface was observed in 4 taxa. *H. campanula* has perforated exine among the three *Heliotropium* species. Other species with perforated exine were *A. tinctoria subsp. tinctoria, C. mucronanthera,* and *T. indicum*. The aperture membrane was smooth, granular, operculate, and granular-operculate types. Considerable variations occurred in the studied taxa's polar, equatorial view, and Amb. The Amb was found in three forms i.e., peritreme (8), ptychotreme (7), and goniotreme (2). *Onosma* and *Lappula* species were separated based on the Amb. Pollen appeared as circular, triangular obtuse convex, and quinquangular obtuse convex in the polar view. While the equatorial view was observed in the form of rectangular obtuse convex, elliptic truncate, circular, rectangular obtuse concave, and rhombic obtuse truncate.

e) Multivariate Analysis

For the hierarchical cluster analysis, the two-way dendrogram was created based on quantitative data on pollen (Figure 9). The dendrogram separated *A. tinctoria* subsp. *tinctoria* being the largest. The 2 major clusters were then further delineated into two subclusters each. The *Heliotroipium* species were in the same single cluster. Similarly, the species of *Paracaryum* were also in the same cluster. The *Onosma* and *Rochelia* species were found in different clusters, which quantitatively revealed the palynological traits' variations. The *Lappula spinocarpos* was separate from two other *Lappula* species. The principal component analysis ordination (PCA) of 17 Boraginaceous species was observed with grouping by correspondence to the polar axis, equatorial diameter, polar and equatorial length, and width of colpi, exine diameter, mesocolpium (Figure 10). Score 1 and Score 2 accounted for the variance among the mean values of quantitative parameters. The overall data distribution was statistically presented in a box plot (Figure 11). The data dispersion range was given along the Y-axis, and the analyzed characters were represented along the X-axis. The outliers in each group were represented as dots. The correlation plot determined the possible association among the means of different traits. The maximum correlation was found between two pairs of mesocolpium and the polar length of colpi and the polar axis and equatorial diameter. Meanwhile, a negative correlation was observed between the exine thickness and equatorial width of the colpi (Figure 12).

3.3.2 Discussion

Palynology is a new field of plant research, however, it has made significant contributions by offering helpful data for phylogenetic analysis (Perveen, 2000). In systematics, all pollen characteristics were essential. Generally, many pollen characteristics, including the aperture type, exine sculpturing, size, shape, polarity, and symmetry were used to categorize pollen. Understanding linkages across tribes and genera based on pollen features can also be done by looking at the patterns of pollen evolution (Mazari and Liu, 2019). The palynological characteristics of the Boraginaceae were heterogeneous (Umber et al., 2022), and it was found eurypalynous family; many different species can be identified by this aspect (Díez and Valdés, 1991). For the identification of morphotypes, palyno-anatomical data was frequently used. In numerous recent pollen morphological and petiole anatomical studies, it has been demonstrated useful in the taxonomic delimitation of species. These characteristics have been used to classify many species in Boraginaceae (Teke and Binzet, 2017). For Boraginaceae taxa, the existence of pollen that were both heterocolpate and isocolpate was a distinguishing palynological characteristic. The Boraginaceae's heterocolpate pollen were often dumbbell or rectangular, either with or without equator-based constriction (Mazari et al., 2018).

Heliotropium pollen were examined by Perveen et al. (1995), Scheel et al. (1996), Quiroz-Garcıa et al. (1997), Gasparino et al. (2014) and Kamel *et al.* (2018). Kasem (2015), and Yousaf et al. (2021) used the palynological and anatomical data using LM to discriminate the *Heliotropium* species. Mazari et al. (2018) distinguished the pollen of *Heliotropium* according to the presence or absence of constriction at the equatorial region. In this study, 4 *Heliotropium* species have been distinguished based on the differences in both palyno-anatomical features. The exine sculpturing, apertures numbers, and Amb were found to be non-significant in the distinction of *Heliotropium* species. All species were observed with psilate exine, amb ptychotreme, hexotricolporate type apertures. The same was reported by Landi et al. (2022) that *Heliotropium L*. and *Myriopus*, the two Heliotropiaceae species, do not exhibit morphological variety in their pollen concerning the kind and quantity of their apertures or sculpturing of exine.

However, among the Heliotropiaceae taxa, differences in pollen's exine surface and the number of apertures were found useful in the taxonomic identification. The same results were reported by Landi et al. (2022), that Boraginaceous species displaed differences in the size, shape, type, and number of apertures (heterocolpate, 3-porate, 3-colporate, and 3-colpate) on the pollen, along with their exine ornamentation (rugulate, echinate, psilate, reticulate, and microechinate-verrucate), allowed the *Heliotropium* species to be separated. Smaller diameter values, aperture, and exine ornamentation distinguish the Heliotropiaceae species. Currently, the polarity and shape of the pollen proved to be important taxonomic markers for the studied *Heliotropium* species. Pollen in *H. bacciferum* was heteropolar prolate, whereas in *H. campanula* oblate spheroidal and *H. crispum* suboblate.

Koyuncu et al. (2013) stated that the systematics of the unique genus *Onosma* is challenging to investigate. The two studied *Onosma* varieties of the same species *O. limitanea*, differed clearly in palyno-anatomical traits. The variety *limitanea* was isopolar, gemmate, and peritreme pollen with the angular epidermis, and round xylem vessel. The variety *major* was heteropolar, scabrate, goniotreme, angular to isodiametric epidermis, and round to angular xylem vessel. The colpi were Trisyncolporate in the variety *major*. Teke and Binzet (2017) differentiated that pollen of *O. discedens* were spheroidal while in *O. nana* subprolate. As demonstrated by the investigations on the genus *Onosma*, the information collected from palynological studies was sufficient to identify species. Koyuncu et al. (2013) identified a new *Onosma* species *Onosma atila-ocakii sp. nova* The morphological identity was difficult from the closely related species *O. roussaei* and *O. aucheriana*. However, the distinct palynological features heteropolar, syncolporate, subprolate, smallest pollen and granulate-scabrate exine separated this new species.

Maggi et al. (2008) reported five *Onosma* species as tiny, 3-syncolporate, subprolate, heteropolar, and have tectums that were microechinate, round to triangular polar shapes, and ovate equatorial contours. They concluded that the genus *Onosma* was highly homogenous, there were no taxonomically significant differences seen in the micromorphology of pollen across the *Onosma* taxa studied, except pollen size. In contrast, substantial differences in the characteristics of pollen were discovered by Perveen et al. (1995) to identify Boraginaceous taxa.

The *Lappula* and *Rochelia* belong to the tribe Eritrichieae, whereas *Paracaryum* is a member of Cynoglosseae. The two tribes were studied by Díez and Valdés (1991). Currently, the three *Lappula* members studied were *Lappula* spp, L. *spinocarpos*, and *L. spinocarpos subsp. ceratophora.* Although the classification of the genus is difficult, the palynological examination determined clear differences among them. This knowledge was significant in the taxonomic placement and separation of species within the genus *Lappula*. The *L. spinocarpos* was isopolar, whereas the *subsp. ceratophora* was heteropolar. Other differences were that *L. spinocarpos* was tricolporate, scabrate, and peritreme Amb, while *L. spinocarpos subsp. ceratophora* was hexotricolporate, psilate, ptychotreme Amb. The one unidentified *Lappula spp* was completely similar to *subsp. ceratophora,* but the variations occurred in the polar view. Triangular obtuse convex polar view was observed in the *Lappula spp,* but *subsp. ceratophora* appeared circular in a polar view. The *Rochelia* species *R. disperma* and *R. sessiliflora* were isopolar, psilate, operculate, and ptychotreme Amb, with non-perforated exine. *R. disperma* was tricolporate, isocolpate, suboblate in contrast *R. sessiliflora* was hexotricolporate, heterocolpate, porocolpate, subprolate. Both highly differed in the polar and equatorial views. In the former, the polar and equatorial views were triangular obtuse convex and rectangular obtuse concave, while in the latter, quinquangular obtuse convex and elliptic truncate. The members of the Eritrichieae tribe can be distinguished based on palynological investigations. In contrast, Díez and Valdés (1991) reported that Eritrichieae taxa cannot be separated on the pollen shape and apertures, the distinction can only be carried up to the generic level.

The members of the Cynoglosseae tribe have been separated based on their micromorphological characteristics (Attar et al., 2018). The *Paracaryum* varieties *P. intermedium var. calathicarpum* and *P. intermedium var. intermedium* shared all the palynological traits besides the pollen shape. Similar results were documented by Díez and Valdés (1991). The rearrangements of *Paracaryum* and *Cynoglossum* species were accomplished utilizing pollen traits (Ovchinnikova et al., 2021). Cynoglosseae cannot be separated based solely on characteristics discovered in pollen due to the similarities in shape and apertural system observed in some genera, including certain species. The generic boundaries persisted in *Cynoglossum,* and in Myosotis, the infrageneric categorization prevailed (Díez and Valdés, 1991).

Furthermore, one of the accepted methods in palynology is numerical analysis. In contrast to classical taxonomy, Binzet et al. (2018) emphasized the value of numerical taxonomy and recommended using it for taxa with comparable morphologies (like *Onosma*). They highlighted that the best method for determining the identities and morphological correlations among the genus *Onosma* is through quantitative taxonomy. Several techniques were used in numerical analysis, but PCA, and dendrogram were the most popular. PCA evaluates the best qualities for taxonomy and permits multicollinear statistics to identify the traits. Heteropolar, and trisynocolporate, were more advanced traits (Mazari and Liu, 2019; Teke and Binzet, 2017). The multivariate analysis of equatorial diameter, exine thickness, polar axis, mesocolpium, colpus polar and equatorial length, and width via PCA, phylogeny, correlation, and boxplot. The variations in the data separated the taxa and varieties.

PCA is the most popular quantitative technique for identifying pollen from different plant species and investigating the most important pollen characteristic with the highest proportion of variability. PCA is typically represented graphically as two-dimensional or occasionally three-dimensional axes-based plans of sample data. Using parameters such as mesocolpium, polar axis, equatorial diameter, exine thickness, colpus length, and width, Attar et al. (2018) used PCA to examine pollen variability in 16 different plant species from different families. Among the angiosperms the smallest pollen grains ca. 5 × 2 mm were found in the Boraginaceae, *Myosotis,* and *Trigonotis*. The largest pollen 55 × 40 mm observed in *Anchusa*, Boragineae. The variations in the pollen size significantly differentiated the examined species. The *A. tinctoria subsp. tinctoria* was observed with the largest sizes, 47.5 µm, and 43.85µm. Previously, pollen size was the most significant feature for separating *Onosma* species (Maggi et al., 2008). The boxplot determined the outliers in the data and the deviation. The evolutionary association among the quantitative variables was captured in the phylogenetic tree. The positive and negative association among the data set was determined via a correlation plot. Taxonomic key based on the palynological traits (Table 12) efficiently distinguished the Boraginaceous taxa up to the species level. The variations in the polarity, number of apertures, arrangement of apertures, Amb, polar and equatorial views, sculpturing, and pollen shape proved to be diagnostic for the taxonomic distinction of the examined species.

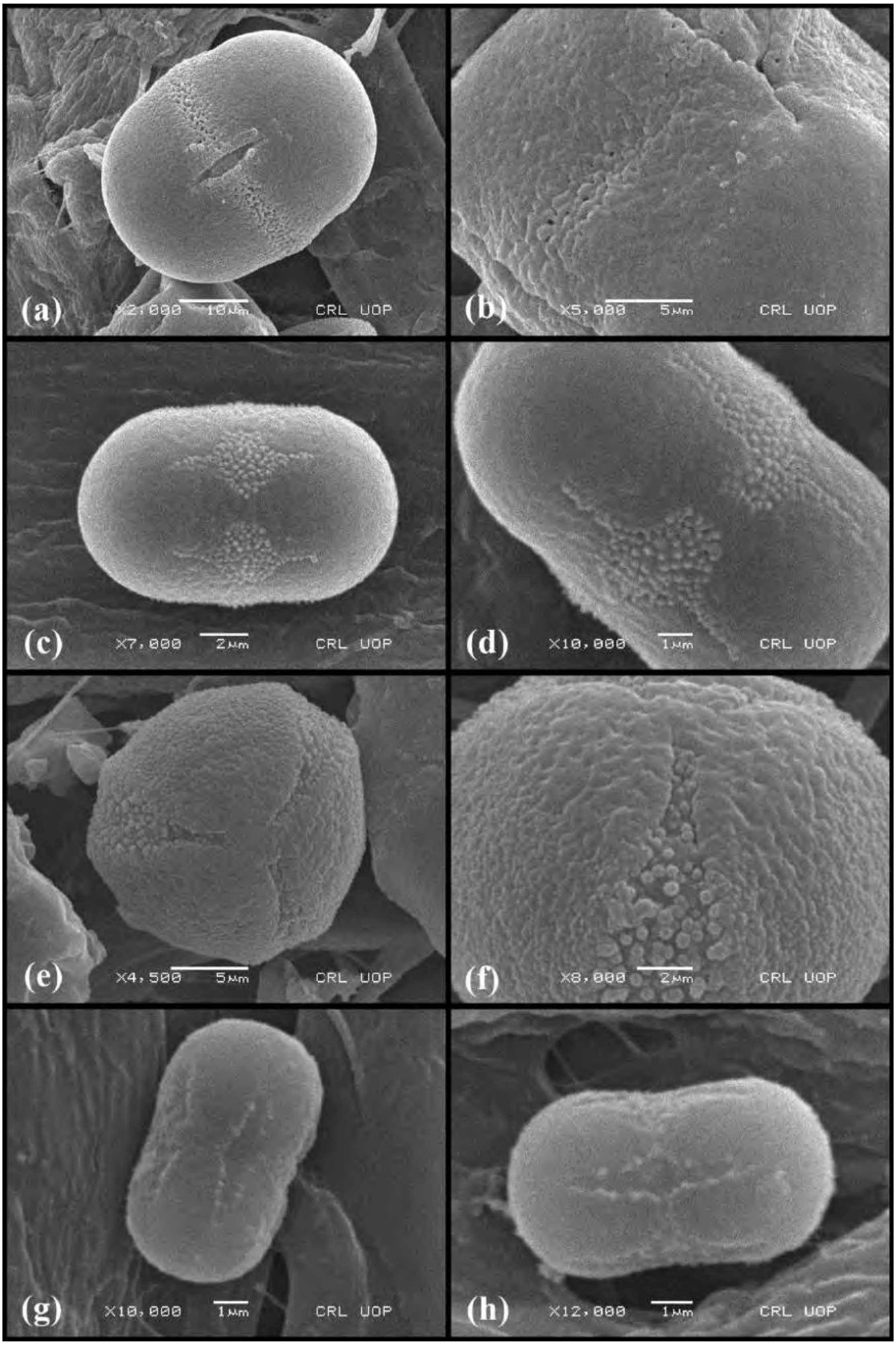

Plate 51. SEM micrographs of pollen (a),(b) *Alkanna tinctoria* subsp. *Tinctoria,* (c),(d) *Buglossoides arvensis,* (e),(f) *Caccinia mucronanthera,* (g),(h) *Cynoglossum lanceolatum*

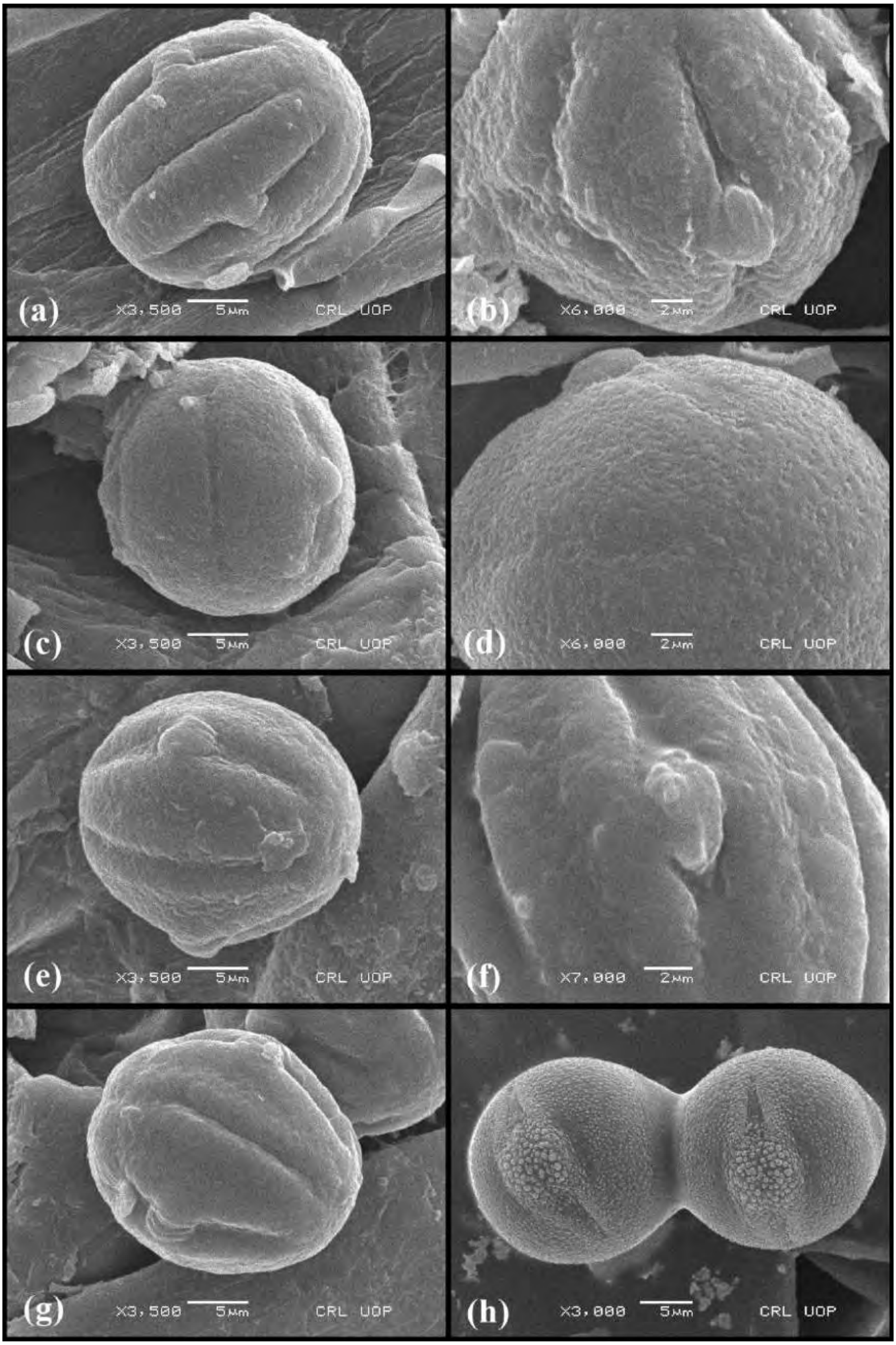

Plate 52. SEM micrographs of pollen (a),(b) *Heliotropium bacciferum,* (c),(d) *Heliotropium campanula,* (e),(f) *Heliotropium crispum,* (g),(h) *Lappula spinocarpos*

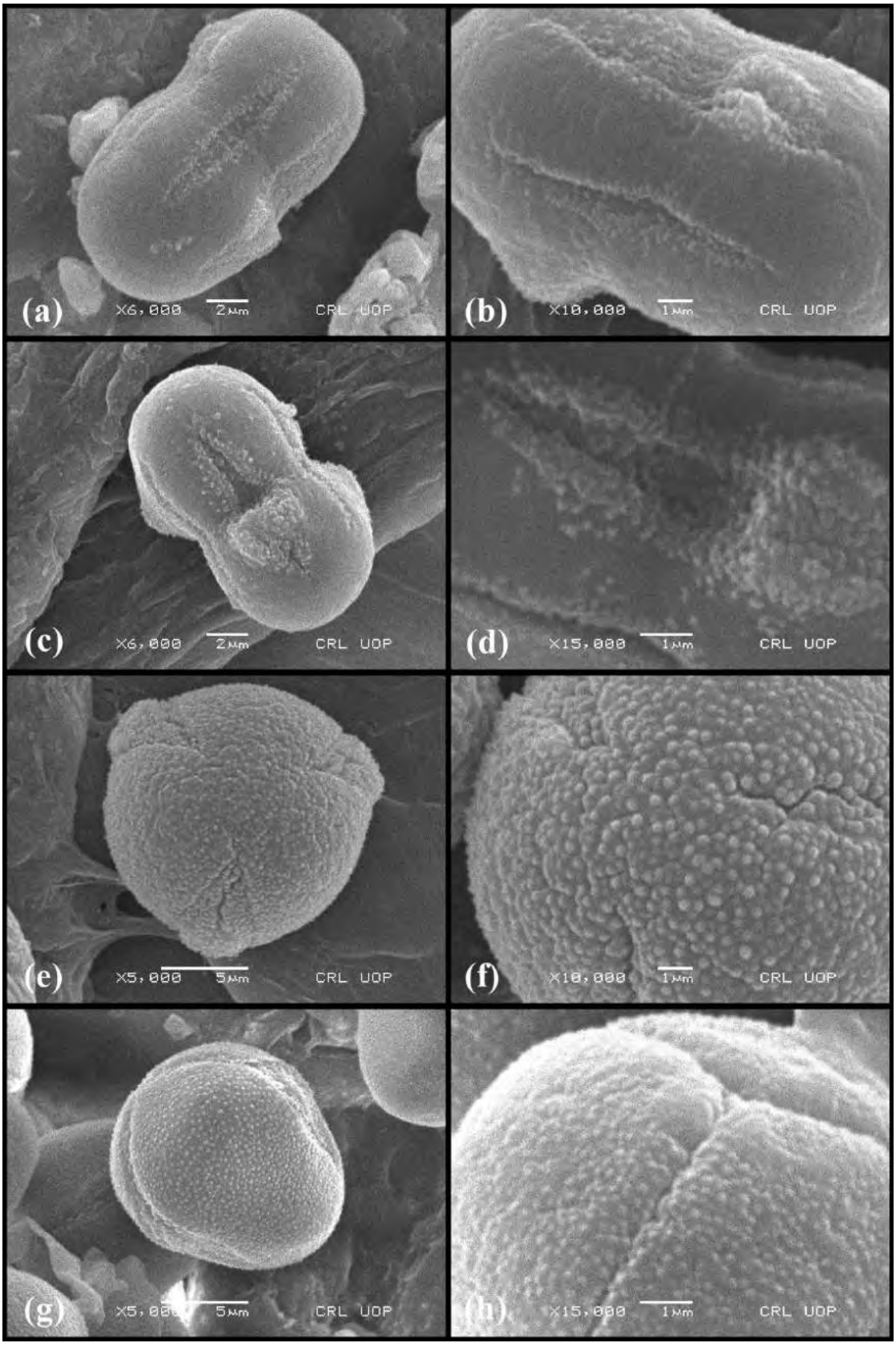

Plate 53. SEM micrographs of pollen (a),(b) *Lappula spinocarpos* subsp. *ceratophora,* (c),(d) *Lappula spp,* (e),(f) *Onosma limitanea* var. *limitanea,* (g),(h) *Onosma limitanea* var. *major*

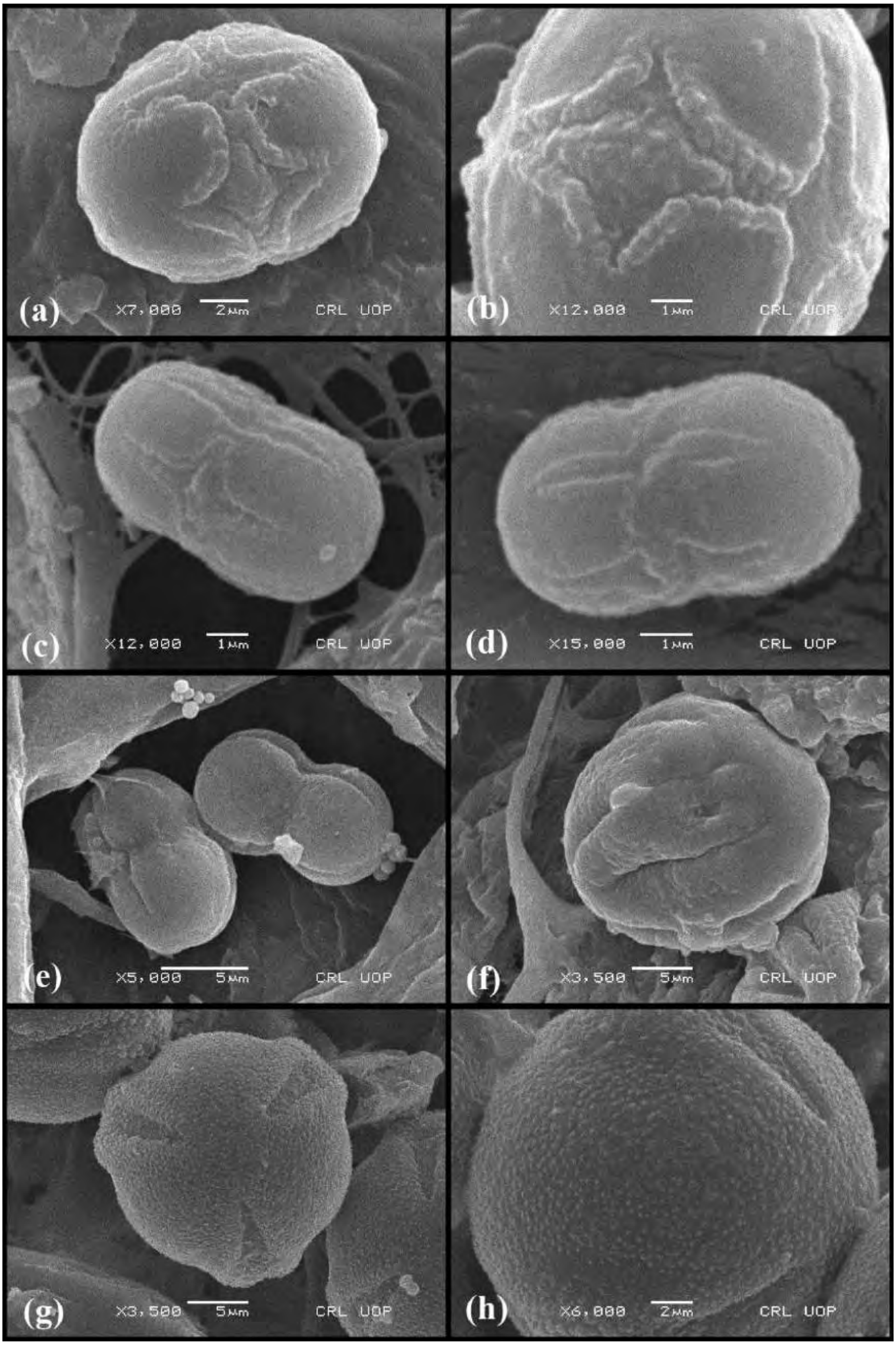

Plate 54. SEM micrographs of pollen (a),(b) *Paracaryum intermedium var. calathicarpum*, (c),(d) *Paracaryum intermedium* var. *intermedium*, (e) *Rochelia disperma*, (f) *Rochelia sessiliflora*, (g),(h) *Trichodesma indicum*

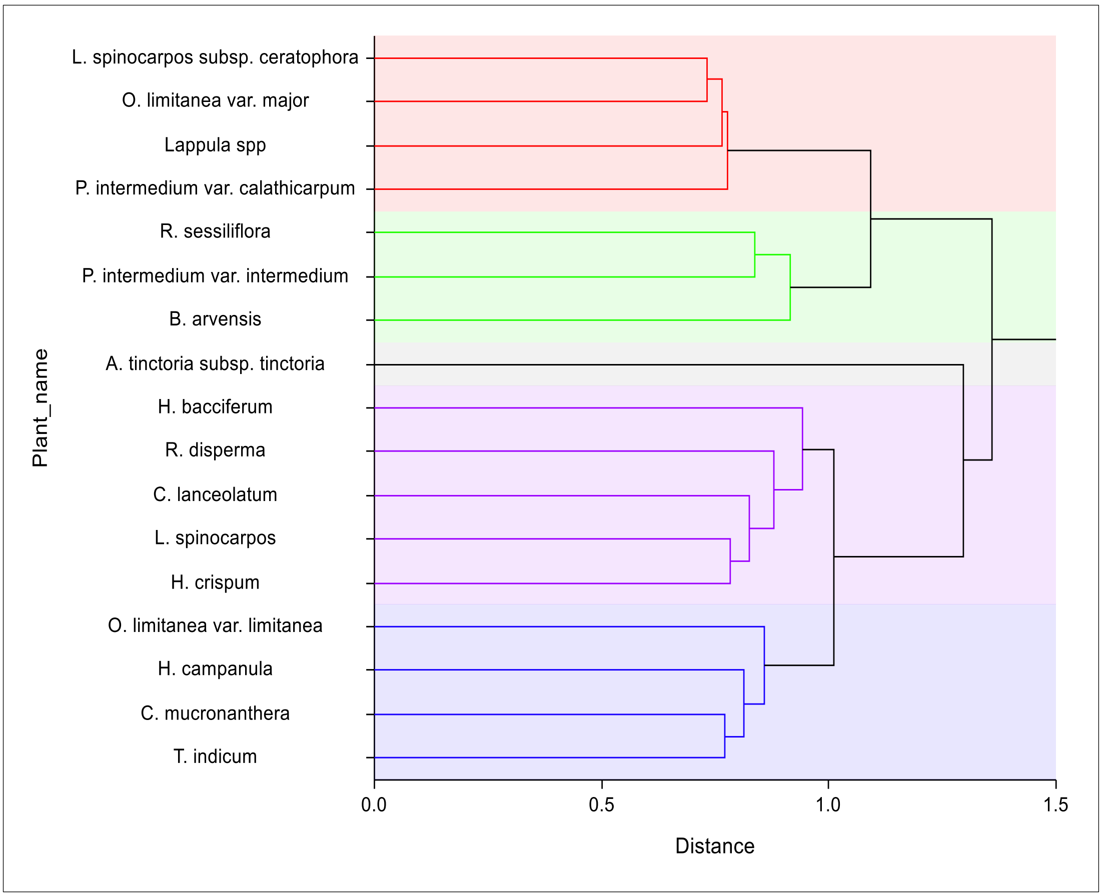

Figure 9. UPGMA cluster analysis based on Polar axis, Equatorial Diameter, polar width and length of colpi, equatorial width and length of colpi, Mesocolpium, and Exine thickness

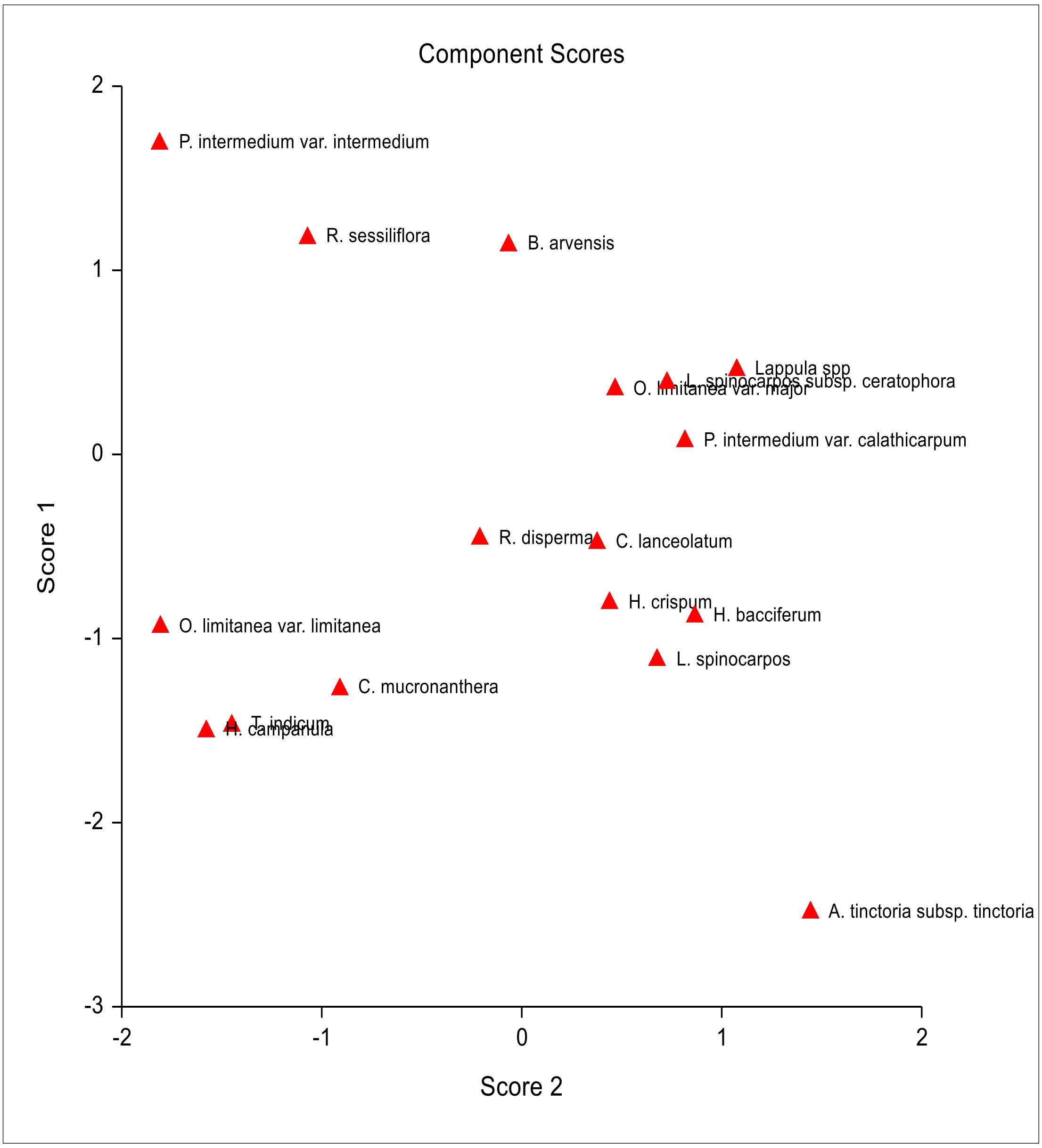

Figure 10. Multivariate analysis via PCA plot for Polar axis, Equatorial Diameter, polar width and length of colpi, equatorial width and length of colpi, Mesocolpium, and Exine thickness

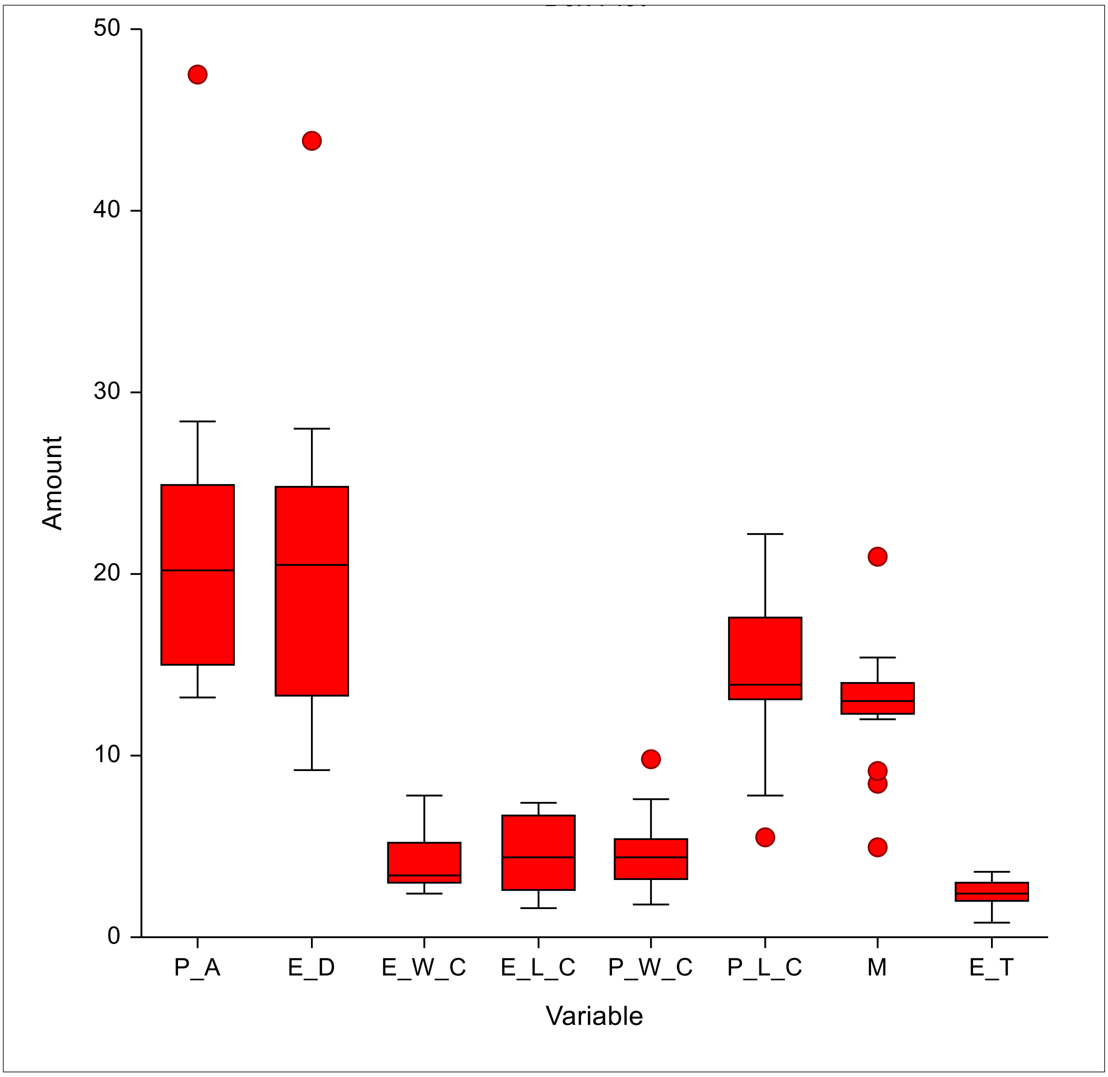

Figure 11. Statistical analysis via boxplot of the mean values of Polar axis (P.A), Equatorial Diameter (E.D), polar width and length of colpi (P.W.C, P.L.C), equatorial width and length of colpi (E.W.C, E,L,C), Mesocolpium (M), Exine thickness (E.T)

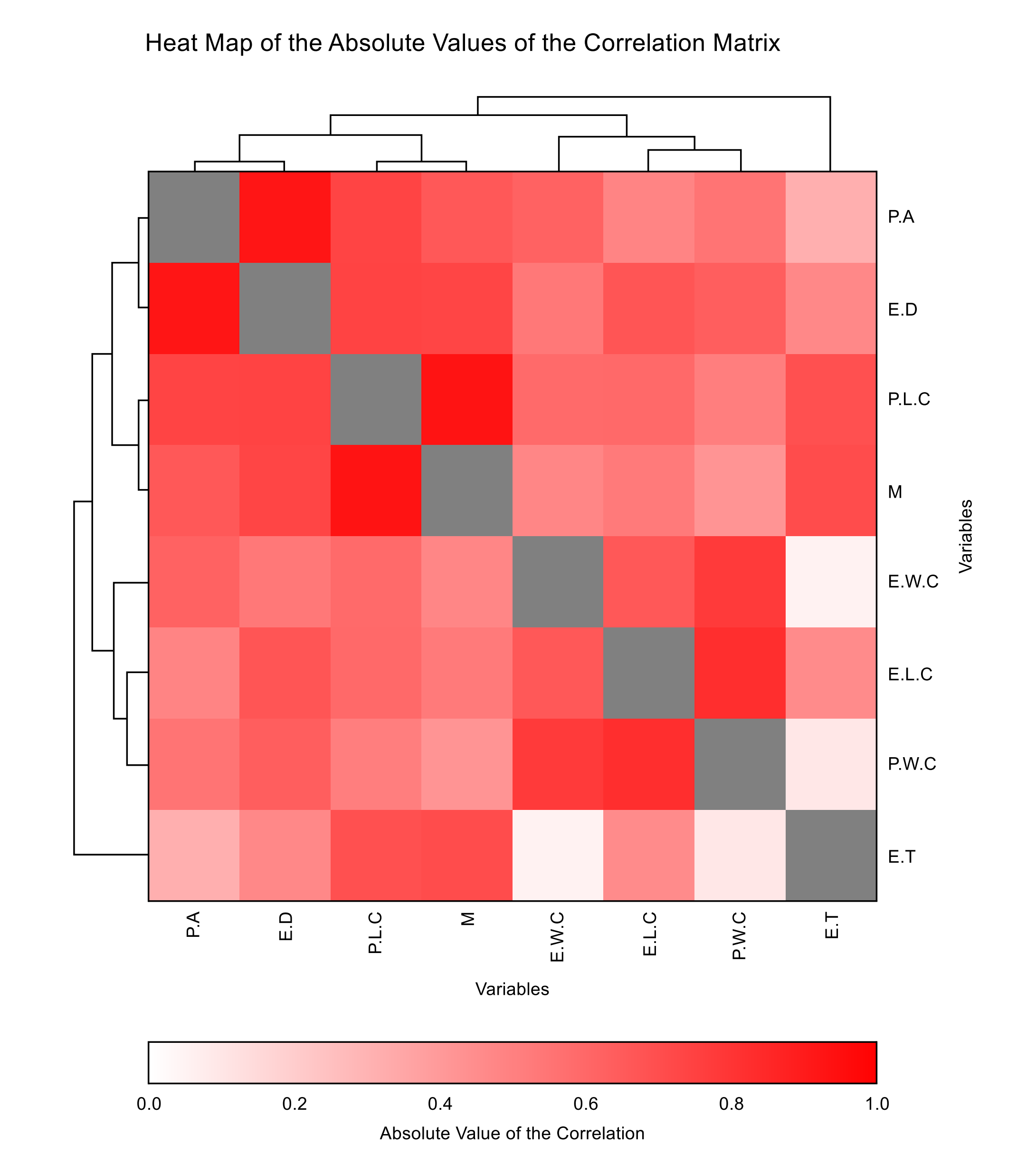

Figure 12. Correlation among the mean values of Polar axis (P.A), Equatorial Diameter (E.D), polar width and length of colpi (P.W.C, P.L.C), equatorial width and length of colpi (E.W.C, E,L,C), Mesocolpium (M), Exine thickness (E.T)

Table 9. Qualitative characters derived from the quantitative data of LM of pollen of Boraginaceous taxa

Plant name	Polar axis (µm)	Equatorial diameter (µm)	Size class	P/E ratio	Shape class
A. tinctoria subsp. tinctoria	47.5	43.85	Medium	1.083	Prolate spheroidal
B. arvensis	15.85	12.4	Small	1.278	Subprolate
C. lanceolatum	20.15	22.5	Small	0.895	Oblate spheroidal
C. mucronanthera	24.85	24.55	Small to Medium	1.012	Prolate spheroidal
H. bacciferum	28.45	20.5	Medium	1.387	Prolate
H. campanula	24.1	25.15	Medium	0.958	Oblate spheroidal
H. crispum	23.95	27.05	Medium	0.885	Suboblate
L. spinocarpos	25	27.95	Medium	0.894	Oblate spheroidal
L. spinocarpos subsp. ceratophora	15.2	14.1	Small	1.078	Prolate spheroidal
Lappula spp	15	13.3	Small	1.127	Prolate spheroidal
O. limitanea var. limitanea	22.65	20.65	Small	1.096	Prolate spheroidal
O. limitanea var. major	14.9	14	Small	1.064	Prolate spheroidal
P. intermedium var. calathicarpum	14.15	13.3	Small	1.063	Prolate spheroidal
P. intermedium var. intermedium	13.2	9.2	Small	1.434	Prolate
R. disperma	15.4	19.75	Small	0.779	Suboblate
R. sessiliflora	14.95	13	Small	1.15	Subprolate
T. indicum	26	22.85	Medium	1.137	Prolate spheroidal

Table 10. Qualitative characters based on SEM of pollen of Boraginaceous taxa

Taxa	Symmetry	Polarity	Unity	No. of apertures	Polar view	Equatorial view	Exine sculpturing	Exine surface	Aperture membrane	Colpi type	Arrangement of apertures	Amb	NPC classification	
													Name	Formula
A. tinctoria subsp. tinctoria	Radial	Isopolar	Monad	Tricolporate	Circular	Rectangular obtuse convex	Psilate	Perforate	Smooth	Isocolpate	-	Peritreme	Trizonocolporate	$N_3P_4C_5$
B. arvensis	Radial	Heteropolar	Monad	Hexacolporate	Circular	Rectangular obtuse convex	Psilate	Non-perforate	Granular	Isocolpate	-	Peritreme	Hexazonocolporate	$N_{6,3}P_4C_5$
C. lanceolatum	Radial	Isopolar	Monad	Hexotricolporate	Circular	Rectangular obtuse convex	Psilate	Non-perforate	Smooth	Heterocolpate	Porocolpate	Peritreme	Hexotrizonocolporate	$N_{6,3}P_4C_5$
C. mucronanthera	Radial	Isopolar	Monad	Tricolpate	Triangular obtuse convex	Elliptic truncate	Foveolate	Perforate	Granular	Isocolpate	-	Goniotreme	Trizonocolpate	$N_3P_4C_5$
H. bacciferum	Radial	Heteropolar	Monad	Hexotricolporate	Quinquangular obtuse convex	Elliptic truncate	Psilate	Non-perforate	Operculate	Heterocolpate	Porocolpate	Ptychotreme	Hexotrizonocolporate	$N_{6,3}P_4C_5$
H. campanula	Radial	Isopolar	Monad	Hexotricolporate	Quinquangular obtuse convex	Circular	Psilate	Perforate	Operculate	Heterocolpate	Porocolpate	Ptychotreme	Hexotrizonocolporate	$N_{6,3}P_4C_5$
H. crispum	Radial	Isopolar	Monad	Hexotricolporate	Quinquangular obtuse convex	Elliptic truncate	Psilate	Non-perforate	Operculate	Heterocolpate	Porocolpate	Ptychotreme	Hexotrizonocolporate	$N_{6,3}P_4C_5$
L. spinocarpos	Radial	Isopolar	Monad	Tricolporate	Circular	Circular	Scabrate	Non-perforate	Granular	Isocolpate	-	Peritreme	Trizonocolporate	$N_3P_4C_5$
L. spinocarpos subsp. ceratophora	Radial	Heteropolar	Monad	Hexotricolporate	Circular	Rectangular obtuse concave	Psilate	Non-perforate	Granular operculate	Heterocolpate	Porocolpate	Ptychotreme	Hexotrizonocolporate	$N_{6,3}P_4C_5$

Lappula spp	Radial	Heteropolar	Monad	Hexotricolporate	Triangular obtuse convex	Rectangular obtuse concave	Psilate	Non-perforate	Granular operculate	Heterocolpate	Porocolpate	Ptychotreme	Hexotrizonocolporate	$N_{6,3}P_4C_5$
O. limitanea var. limitanea	Radial	Isopolar	Monad	Tricolporate	Circular	Circular	Gemmate	Non-perforate	Granular operculate	Isocolpate	-	Peritreme	Trizonocolporate	$N_3P_4C_5$
O. limitanea var. major	Radial	Heteropolar	Monad	Trisynocolporate	Triangular obtuse convex	Rhombic obtuse truncate	Scabrate	Non-perforate	Granular	Isocolpate	-	Goniotreme	Trizonocolporate	$N_3P_4C_5$
P. intermedium var. calathicarpum	Radial	Isopolar	Monad	Hexotricolporate	Circular	Elliptic truncate	Psilate	Non-perforate	Operculate	Heterocolpate	Porocolpate	Peritreme	Hexotrizonocolporate	$N_{6,3}P_4C_5$
P. intermedium var. intermedium	Radial	Isopolar	Monad	Hexotricolporate	Circular	Rectangular obtuse convex	Psilate	Non-perforate	Operculate	Heterocolpate	Porocolpate	Peritreme	Hexotrizonocolporate	$N_{6,3}P_4C_5$
R. disperma	Radial	Isopolar	Monad	Tricolporate	Triangular obtuse convex	Rectangular obtuse concave	Psilate	Non-perforate	Operculate	Isocolpate	-	Ptychotreme	Trizonocolporate	$N_3P_4C_5$
R. sessiliflora	Radial	Isopolar	Monad	Hexotricolporate	Quinquangular obtuse convex	Elliptic truncate	Psilate	Non-perforate	Operculate	Heterocolpate	Porocolpate	Ptychotreme	Hexotrizonocolporate	$N_{6,3}P_4C_5$
T. indicum	Radial	Isopolar	Monad	Tricolporate	Circular	Circular	Scabrate	Perforate	Granular	Isocolpate	-	Peritreme	Trizonocolporate	$N_3P_4C_5$

amb: circumference, NPC: Number, Position, Characteristic

Table 11. Quantitative characeters based on light microscopic observation of pollen of Boraginaceous taxa

Plant Species	**P.A**	**E.D**	**W.C.P**	**L.C.P**	**W.C.E**	**L.C.E**	**M**	**E.T**
Mean±SE								
Alkanna tinctoria subsp. *tinctoria*	47.5±1.892419087	43.85±1.310057251	4.8±0.2	2.2±0.16583124	4.75±0.306186218	22.15±0.322102468	20.95±2.742489745	2.8±0.16583124
Buglossoides arvensis	15.85±0.515	12.4±0.4	2.55±0.183	1.65±0.127475488	1.75±0.176776695	9.95±0.310241841	9.15±0.375832409	1.6±0.127475488
Coccinia mucronanthera	24.85±0.2318404	24.55±0.3741657	7.1±0.43	6.7±0.390	5.15±0.231	17.5±0.353	15.35±1.329	1.95±0.145
Cynoglossum lanceolatum	20.15±0.605185922	22.5±0.840386816	4.1±0.231840462	4.45±0.145773797	4.55±0.348209707	14.5±0.25	13±0.707106781	3±0.111803399
Heliotropium bacciferum	28.45±0.92668765	20.5±0.25	4.85±0.231840462	4.05±0.266926956	3.35±0.127475488	18.95±0.374165739	14.1±1.047616342	3.05±0.145773797
Heliotropium campanula	24.1±0.527967802	25.15±0.231840462	5.65±0.127	6.85±0.451	9.8±0.266	18.45±0.310	13.9±1.017	2.1±0.187
Heliotropium crispum	23.95±0.544288526	27.05±1.052971984	3.05±0.145	6.3±0.289	5.15±0.358	13.75±0.5	13.05±0.768	3.25±0.176
Lappula spinocarpos	25±0.575543222	27.95±1.116355678	3.85±0.231840462	7.35±0.231840462	4.4±0.430116263	16.05±0.691013748	13.15±0.735696948	3.55±0.289395923
Lappula spp	15±0.734	13.3±0.5631	2.4±0.9138	2.8±0.2781	2.4±0.8288	13.9±0.4993	12±0.3337	2.8±0.7188
Lappula spinocarpos subsp. ce ratophora	15.2±0.3829	14.1±0.6732	3.25±0.6942	2.7±0.939	2.5±0.185	13±0.91	13±0.2936	2.5±0.2859
Onosma limitanea var. limitanea	22.65±0.231840462	20.65±0.16955825	7.75±0.136	6.5±0.285	6.75±0.176	13.25±0.176	12.95±0.834	2.05±0.215
Onosma limitanea var. major	16.85±0.375832409	11.75±0.77055175	4.45±0.145773797	3.4±0.127475488	2.6±0.20310096	10.65±0.322102468	8.4±0.471699057	2.2±0.16583124
Paracaryum intermedium var. intermedium	13.2±0.681909085	9.2±0.514781507	3.05±0.145773797	2.2±0.16583124	3.25±0.111803399	5.5±0.111803399	4.95±1.330883165	0.75±0.32596012
Paracaryum intermedium var. calathicarpum	14.15±0.340954542	13.3±0.215058132	3.25±0.176776695	4.75±0.306186218	3.6±0.331662479	13.6±0.4	12.6±0.654790043	3.25±0.176776695

Rochelia disperma	15.4±0.358817502	19.75±0.32596012	3.35±0.375832409	6.85±0.302076149	5.75±0.553398591	14±0.693721846	13.95±0.985520167	2.45±0.183711731
Rochelia sessiliflora	14.95±0.470372193	13±0.306186218	2.4±0.15	2.45±0.183711731	3.65±0.358817502	7.8±0.289395923	8.45±1.007472084	1.05±0.215058132
Trichodesma indicum	26±0.661437828	22.85±0.846315544	7.7±0.254	6.65±0.562	7.55±0.289	17.75±0.2850	13.95±0.9725	2.3±0.2

P.A: Polar Axis, E.D: Equatorial Diameter, W.C.P.V: width of colpi in polar view, L.C.P.V: length of colpi in polar view, W.C.E.V: width of colpi in equatorial view, L.C.E.V: length of colpi in equatorial view, M: mesocolpium, E.T: exine thickness

Table 12. Dichotomous key based on pollen morphological characters of Boraginaceous taxa

Link Character	**Leads**	**Characters**	***Taxa/ Go to link character***
1	+	Heteropolar	2
	-	Isopolar	6
2	+	Amb peritreme	*B. arvensis*
	-	Amb, not peritreme	3
3	+	Goniotreme Amb	*O. limitanea var. major*
	-	Ptychotreme	4
4	+	Aperture membrane non granular operculate	*H. bacciferum*
	-	Aperture membrane granular operculate	5
5	+	Polar view circular	*L. spinocarpos subsp. ceratophora*
	-	Polar view triangular obtuse convex	*Lappula spp*
6	+	Exine sculpturing foveolate	*C. mucronanthera*
	-	Exine sculpturing non foveolate	7
7	+	Gemmate exine	*O. limitanea var. limitanea*
	-	Exine not gemmate	8
8	+	Scabrate ornamentation of exine	9
	-	Pislate ornamentation of exine	10
9	+	Oblate spheroidal	*L. spinocarpos*
	-	Prolate spheroidal	*T. indicum*
10	+	Tricolporate	11
	-	Hexotricolporate	12
11	+	Circular polar view	*A. tinctoria subsp. tinctoria*
	-	Triangular obtuse convex polar view	*R. disperma*
12	+	Perforated exine surface	*H. campanula*
	-	Non-perforated exine surface	13
13	+	Oblate spheroidal	*C. lanceolatum*
	-	Shape class not oblate spheroidal	14

14	+	Amb ptychotreme	15
	-	Amb peritreme	16
15	+	Suboblate	*H. crispum*
	-	Subprolate	*R. sessiliflora*
16	+	Prolate spheroidal	*P. intermedium var. calathicarpum*
	-	Prolate	*P. intermedium var. intermedium*

3.4 Pollen Micromorphology of Poaceous Flora from Baluchistan

3.4.1 Results

a) Homogenous traits

The pollen of grasses were generally stenopalynous. Among most traits, such as number of apertures, symmetry, polarity, unity, Amb, and operculate pores, homogeneity was observed. The pollen in all studied species were radially symmetrical. The Poaceous taxa appeared as heteropolar, based on the presence of a single pore on one pole. Both in light microscopy and scanning electron microscopy, the pollen was observed in the form of single entities, monad. The features symmetry, polarity, and unity were not observed with variations, these traits were determined up to the family level and were not significant for finding the distinctions among the grasses at other ranks such as tribe, genus, and species. Similarly, a large pollen was recorded for all the studied species, except the *Vulpia persica* which was medium in size. The Amb of pollen in all of the studied grasses was peritreme. Single pore was observed in all of the grass species, so the NPC-acquired name for them was monoporate. The NPC formula for the poaceous species was $N_1P_0C_4$.

b) Heterogeneity in the traits of Poaceous pollen

These traits can be classified into less varied and significantly varied traits. The polar view, equatorial view, exine surface, NPC classification, and pore orientation were observed with less variation. These traits were present in fewer forms and combinations (Table 13).

i) Less heterogeneous traits

The polar view was circular or circular to elliptic. Similarly, the equatorial view was also observed as circular or circular to elliptic. There were little changes in these features from species to species. The exine surface was micro-perforate, non-perforate, and non-perforate at the annulus. These characters in combination can add to the distinction of the grass species. In the same way, pore orientation was observed in two forms sunken and prominent. In sunken the pore and operculum were observed as a deep depression in the pollen. The prominent form appeared as a completely visibly emerged pore, the annulus, pore, and operculum. The characters with fewer variations were also significant in the distinction of the species. These characters in combination provide a base for the construction of taxonomic keys.

ii) Significantly varied traits

The characteristics such as shape class, harmomegathy, exine sculpturing, annulus degree of prominence, and viability were significantly varied traits (Table 13).

1: Shape class

The shape class was derived from the P/E ratio. The shape class was observed in the form of prolate spheroidal, prolate, subprolate, oblate spheroidal, suboblate, and spherical. Prolate spheroidal pollen was the highly observed shape. 16 grass species were prolate spheroidal. The prolate shape was noted only in *Aristida adscensionis* and *Piptatherum baluchistanicum.* Subprolate type was present in eight species. Two *Aristida* species *Aristida cyanantha*, and *Aristida funiculate* were subprolate. Among the *Cenchrus* species only *Cenchrus divisus* pollen was subprolate. Nine Poaceous species were oblate spheroidal. This shape separated the *Cenchrus flaccidus, Chrysopogon serrulatus, Dactyloctenium scindicum, Eremopyrum bonaepartis,* and *Poa infirma* from the species of similar genera studied in this research. *Panicum antidotale* was the only species with a spherical pollen. The suboblate pollen was noted in *Eragrostis curvula.*

2: Harmomegathy

Besides the presence or absence of harmomegathy, the extent of harmomegathy varied from species to species. The degree of presence of this trait was expressed as + signs. This trait was also important from ecological and environmental aspects, specifically the availability of water. Harmomegathy was completely absent in *Aristida funiculata* and *Panicum antidotale.* In 12 species maximum harmomegathy was seen (+++), whereas in the other 12 species minimum expression was observed (+).

3: Exine sculpturing

The variations in the exine sculpturing significantly added to the separation of species based on pollen attributes. The exine sculpturing was scabrate, psilate verrucate, microscabrate verrucate, scabrate verrucate, verrucate, psilate, and scabrate regulate.

Ochthochloa compressa was observed with a very distinct type of sculpturing i.e. psilate to foveolate. In *Aristida adscensionis* the psilate verrucate ornamentation was observed. Whereas psilate to verrucate type was also rare, noted in 3 species only. In 8 grasses scabrate verrucate exine was examined. Microscabrate verrucate exine was noted in 5 species. All 5 of them were non-perforate, except the *Phalaris minor,* which was also non-perforate, but at the region of the annulus, there were perforations. The degree of appearance of the annulus or the extent of its presence also varied from species to species. This character was also labelled as + signs. In *Bromus lanceolatus* and *Dactyloctenium aristatum* the annulus was not seen visible.

4: Viability

The viability of Poaceous pollen was recorded in different percentages for each species. The high fertility of pollen was observed in the taxa such as *Chrysopogon aucheri*, and *Cymbopogon martini*. Few species like *Dactyloctenium scindicum* were represented by low fertility. Although the viability could not directly add to the taxonomic exploration of species, these variations demonstrate the ecological, environmental, and status of these species within their biological habitat.

3.4.2 Discussion

The monoporate pollen of Poaceae have a relatively homogeneous shape. Although it is a diagnostic feature for Poaceae, the stenopalynous condition with monoporate (Raees et al., 2017), annulate pollen limits the discrimination between taxa within this family based on exine and shape. Except for some cultivated grasses, this makes it challenging to differentiate between them at the genus and species levels. However, the aggregate results of these investigations have clarified the challenges associated with utilizing light microscopy (LM) to distinguish between pollen (Guimarães et al., 2018). Scanning electron microscopy (SEM) has been used in recent studies to shed light on the surface ornamentation and taxonomic differentiation of Poaceae's lower taxa. Based on ornamentation under SEM, *Oryza, Saccharum, and Sorghum,* however, might be qualitatively distinguished at lower taxonomic levels (Mander et al., 2013).

The systematics of Poaceae was supported and strengthened by the examination of novel palynological properties and changes in pollen micromorphological features

(Nazish and Althobaiti, 2022). The identification of fossil wild rice was carried out by comparing it with wild rice marsh utilizing scanning electron microscopy. The size of the rice pollen was significant in the discrimination of fossil pollen, but the complete identification was carried out by examining the exine sculpturing from SEM. The micromorphology completely identified the fossil pollen as wild rice (Lee et al., 2004). Shaheen et al. (2022) carried out the palynological assessment of the taxonomically problematic genus *Paspalum*. In this study, 38 Poaceous species from arid to semi-arid regions of Baluchistan were examined under LM and SEM to investigate the micromorphological traits of the pollen. These traits were significant in the distinction of species.

Pollen in all examined species were radially symmetrical, monad, and monoporate. These characters revealed their restricted taxonomic significance at the family level (Harun et al., 2022). The palynological studies do not align with tribe and genus-level classifications. Palynology, however, is quite beneficial at that specific level. Parveen and Qaiser (2012) reported typically apolar, monoporate to diporate, and sporadically triporate pollen. In contrast in the current studies, the heteropolar pollen were observed. The presence of a single pore on one pole makes the grass pollen heteropolar. Per our results, Baser et al. (2009) also reported monoporate, heteropolar pollen in grasses.

The grass species from the arid-semiarid areas of Baluchistan were all large except *Vulpia persica.* It was suggested that the large pollen in the grasses were the result of polyploidy (Muller, 1979). In contrast medium pollen, rarely large pollen were observed by Perveen and Qaiser (2012). In the genus *Eremopyrum,* Baser et al. (2009) reported prolate-spheroidal, and operculate-annulate pollen that had mixed scabrate-type exine. Nazish and Althobaiti (2022) found all the poaceous pollen a sub-spheroidal shape, in contrast to our study. The variations observed in this research in the shape of the Poaceous pollen, were prolate spheroidal, subprolate, oblate spheroidal, prolate, spherical, and suboblate, that added in the taxonomic studies of Poaceous species. *Aristida adscensionis* pollen were observed as prolate shaped, different from the other two studied *Aristida* species. The shape in this study successfully separated the species of genera *Cenchrus*, *Chrysopogon*, *Dactyloctenium*, *Eremopyrum,* and *Poa.* Perveen and Qaiser (2012) only examined spheroidal shape in most of the grasses from Pakistan.

The five pollen types categorized by them were heterogeneous, and the different species of the same genus were in the same pollen type.

Prolate, prolate-spheroidal, and sub-oblate pollen shapes were the most common ones (Ullah et al., 2021). The previous studies on the shape, polar view, and equatorial view simply documented them, lacking the standard for the types of shapes recorded from the P/E ratio given by Erdtman (1952). Similarly, the polar and equatorial views were not drawn from the polar and equatorial views categories. *Phalaris minor* was found as prolate spheroidal, whereas the previous study stated it as spheroidal-subprolate in the equatorial view. The variations in the shapes of species of genera *Aristida, Cenchrus, Chrysopogon, Dactyloctenium, Eremopyrum,* and *Poa,* significantly differentiated the members under the same genus.

Along with a few other characteristics pollen shape, and polar and equatorial perspectives were significant systematically. There were notable differences between the polar and equatorial views (Harun et al., 2022). The polar view appeared as circular, (Raees et al., 2017), or circular to elliptic in the Poaceous pollen. The circular polar and equatorial views were observed in *Dactyloctenium aristatum,* in contrast, the semi-angular views were documented by Harun et al. (2022) in the same species. The exine in *Eragrostis curvula* was microscabrate verrucate with non-perforate surface. Previously (Perveen and Qaiser., 2012) the species *Eragrostis termula* was observed as medium scabrate tectum. Similarly, only scabrate exine was reported in the grass pollen by Raees et al. (2017). Perveen and Qaiser (2012) carried out a comprehensive study on 54 Poaceous pollen from Pakistan. Five different pollen kinds have been identified by them, based on the exine ornamentations. Tectum can be areolate, areolate cum scabrate, or just scabrate in general. Psilate, verrucate, scabrate, microscabrate, and macroscabrate type of exine was observed in our examinations. Harun et al. (2022) examined mostly scabrate exine followed by verrucate type. Ullah et al. (2021) reported the highly used exine types among the grass species were gemmate-verrucate, scabrate-reugulate, verrucate-reticulate, gemmate-scabrate, fine-gemmate, gemmate-rugulate, gemmate-reticulate, gemmate-scabrate, scabrate-verrucate, and scabrate ornamentations.

Wei et al. (2023) created a reference framework for pollen surface ornamentation morphotypes computationally. The surface ornamentation of grass pollen varied greatly. A data collection of 223 species was established by identifying nine new categories. Computational research revealed that the two quantitative characteristics of pollen sculptural pieces (size and density) provide strong support for morphotypes. The particular data set and phylogenetic mapping verified that pollen morphological sculpting is independent of biotic factors.

Differentiating between the grass species was made possible by using multivariate data analysis in conjunction with digital image processing of the ornamentation under SEM. The PCA accounted for 83.7% and 15.2% variance for PC1 and PC2 respectively (Figure 13). The polar axis and equatorial diameter were positively correlated (Figure). Exine thickness, pore length, and width were the least positive traits. Whereas equatorial diameter was negatively -0.26 correlated with pore length. While pore width was negatively associated -0.21 with the polar axis (Figure 15). Ullah et al. (2021) studied *Cenchrus ciliaris* has the biggest equatorial diameter (45.00 μm) whereas *C. ciliaris* had the lowest exine thickness (1.50 μm). The pollen shape, exine groups, (Özler et al., 2009), harmomegathy, aperture membrane, polar view, equatorial view, pore orientation, exine surface, aperture membrane sculpturing (Harun et al., 2022), and annulus appearance were all in the combination significantly differentiated up to the species level.

Therefore, it can be concluded that these traits have considerable taxonomic value and play a substantial role in their separation and identification. For the creation of pollen atlas, this study offers important information on how to classify different pollen types within their designated categories and how to talk about different pollen properties (Ullah et al., 2021). The pollen morphology was observed with some significant variations in the traits such as shape, polar and equatorial view, harmomegathy, and exine sculpturing.

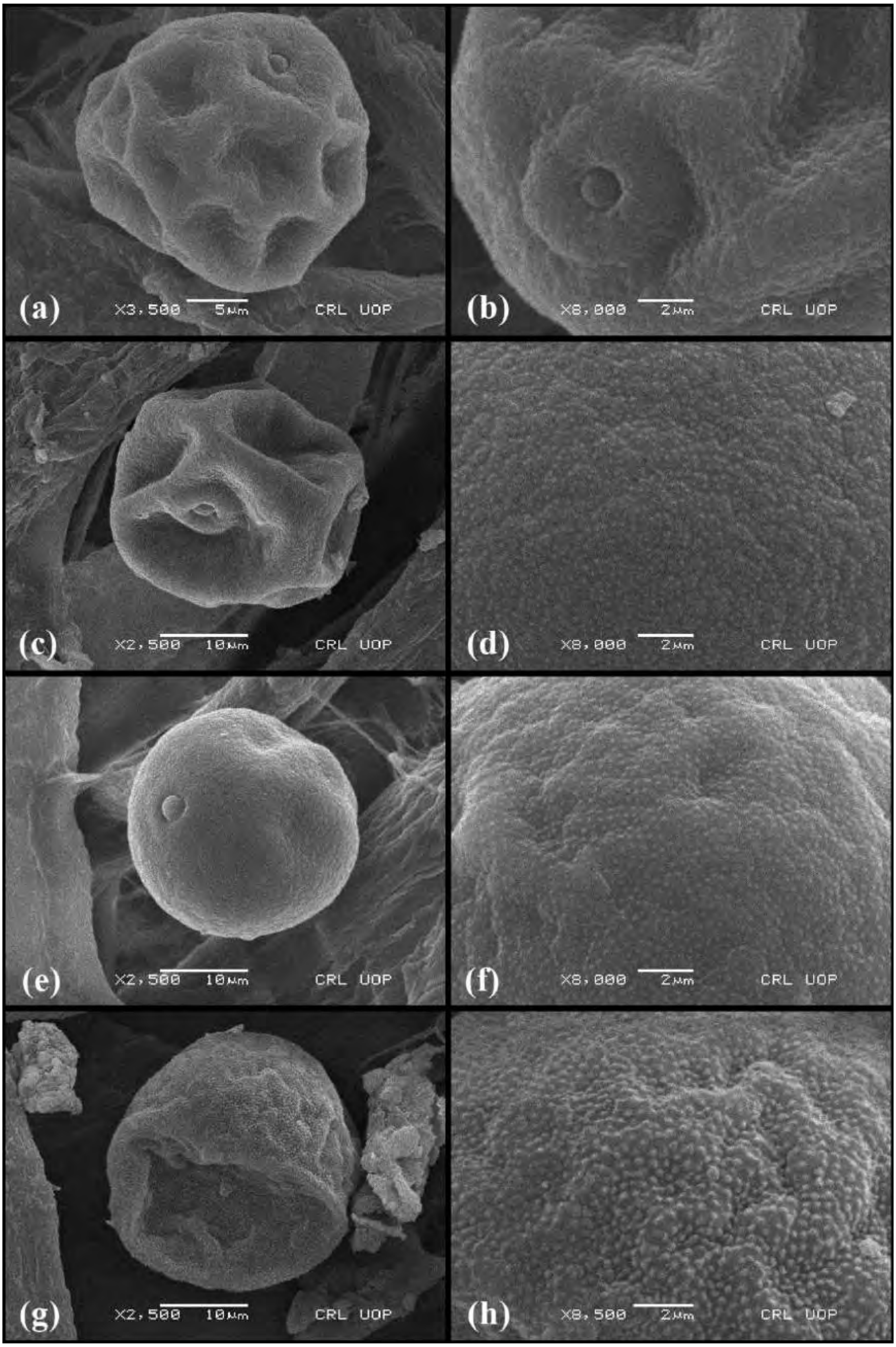

Plate 55. SEM micrographs of pollen (a),(b) *Aristida adscensionis*, (c),(d) *Aristida cyanantha,* (e),(f) *Aristida funiculata*, (g),(h) *Arundo donax*

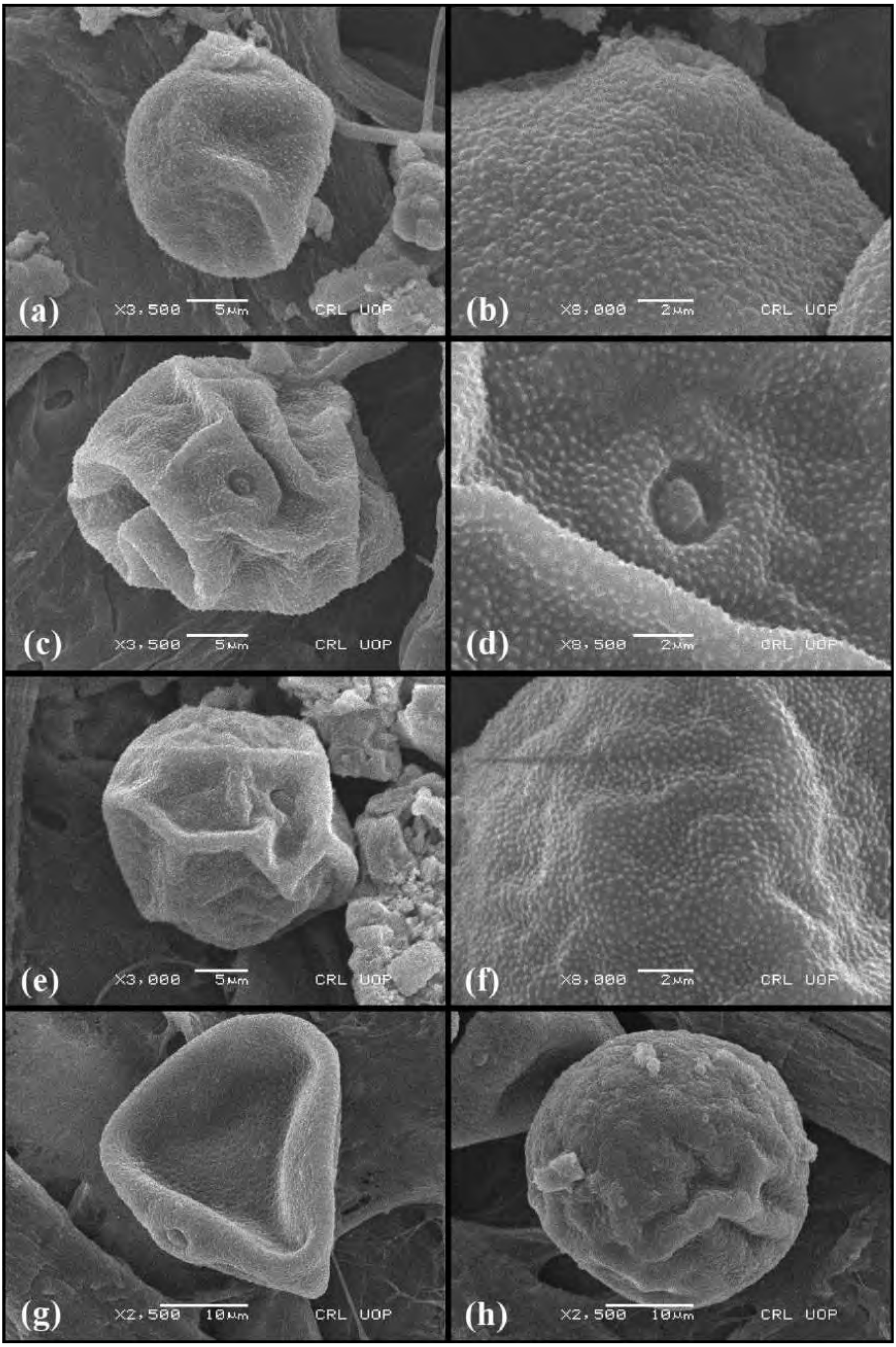

Plate 56. SEM micrographs of pollen (a),(b) *Avena sativa*, (c),(d) *Boissiera squarrosa,* (e),(f) *Bromus lanceolatus,* (g),(h) *Cenchrus divisus*

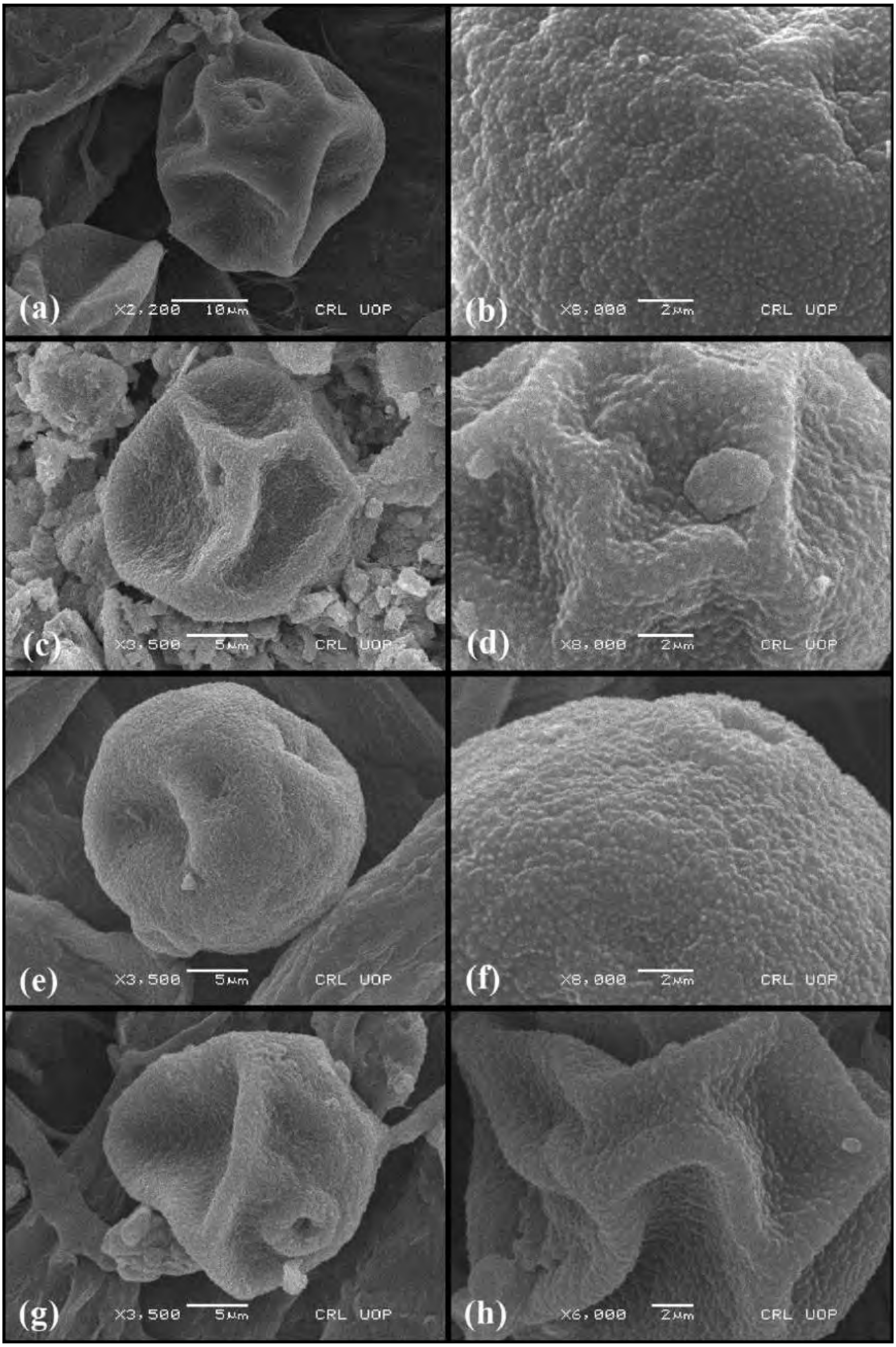

Plate 57. SEM micrographs of pollen (a),(b) *Cenchrus flaccidus*, (c),(d) *Cenchrus orientalis*, (e),(f) *Cenchrus setigerus*, (g),(h) *Chloris barbata*

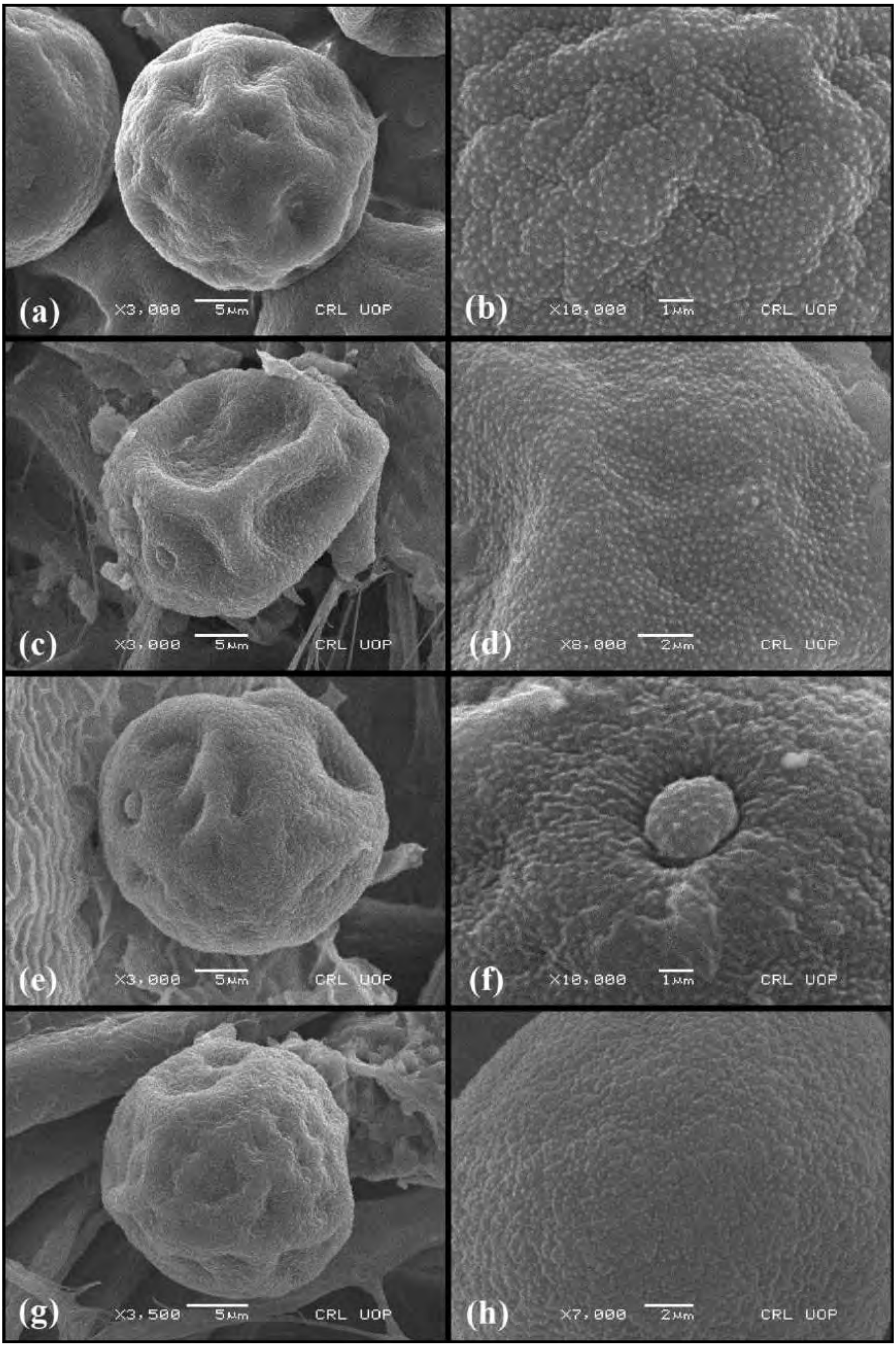

Plate 58. SEM micrographs of pollen (a),(b) *Chrysopogon aucheri*, (c),(d) *Chrysopogon serrulatus*, (e),(f) *Cymbopogon martini*, (g),(h) *Dactyloctenium aristatum*

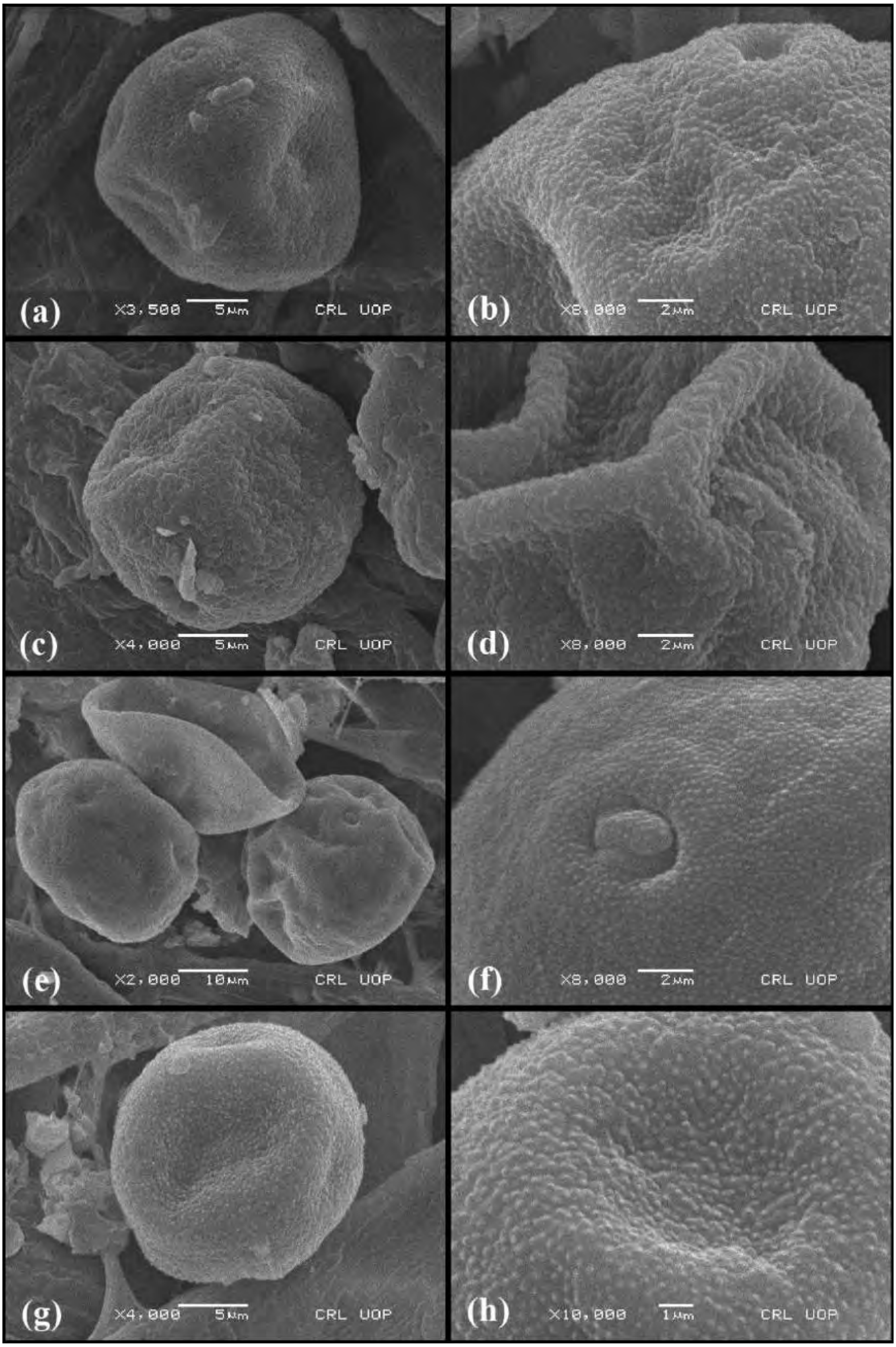

Plate 59. SEM micrographs of pollen (a),(b) *Dactyloctenium scindicum*, (c),(d) *Desmostachya bipinnata*, (e),(f) *Dichanthium foveolatum*, (g),(h) *Diplachne fusca*

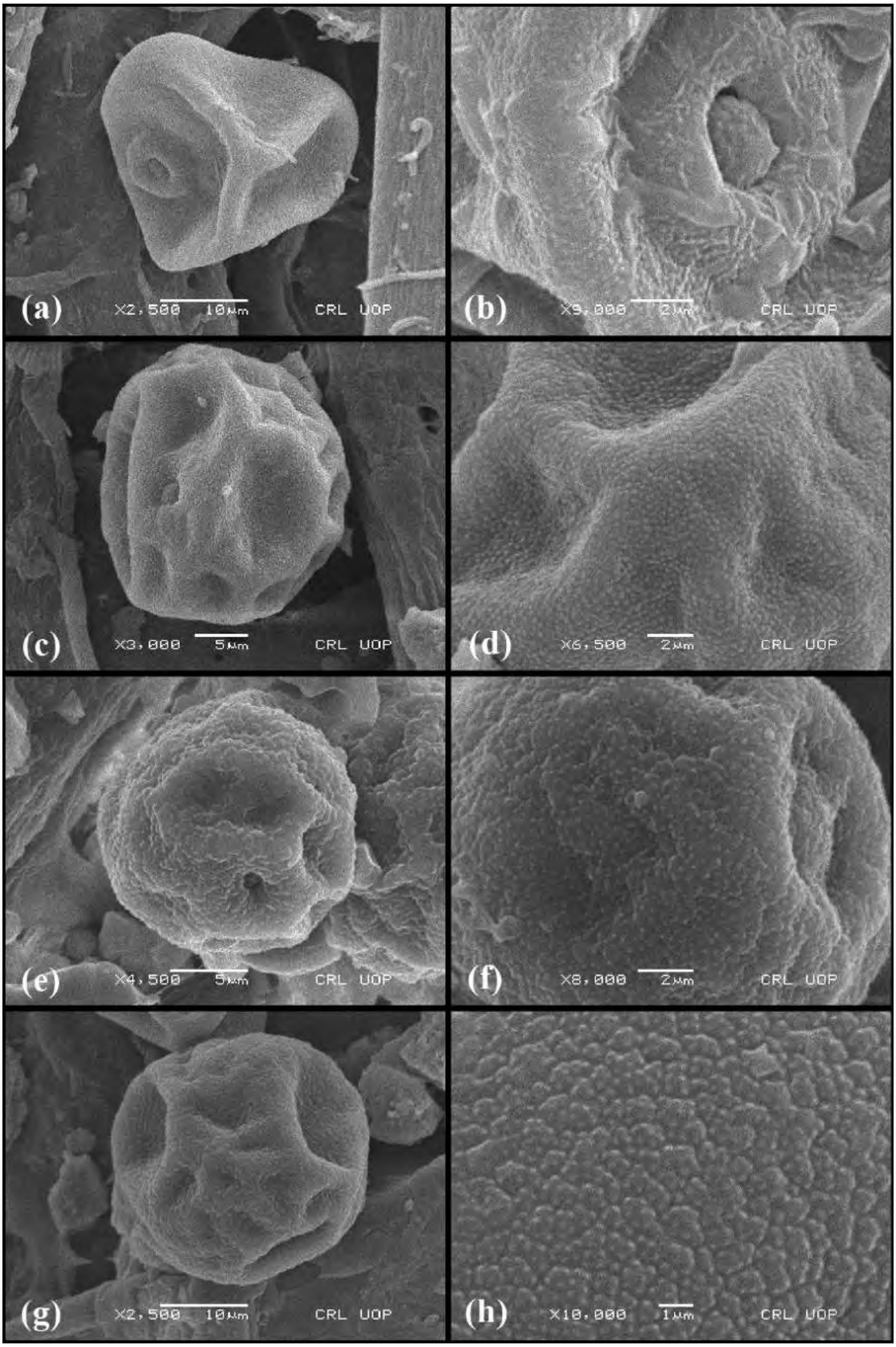

Plate 60. SEM micrographs of pollen (a),(b) *Eleusine indica*, (c),(d) *Enneapogon persicus*, (e),(f) *Eragrostis curvula*, (g),(h) *Eremopyrum bonaepartis*

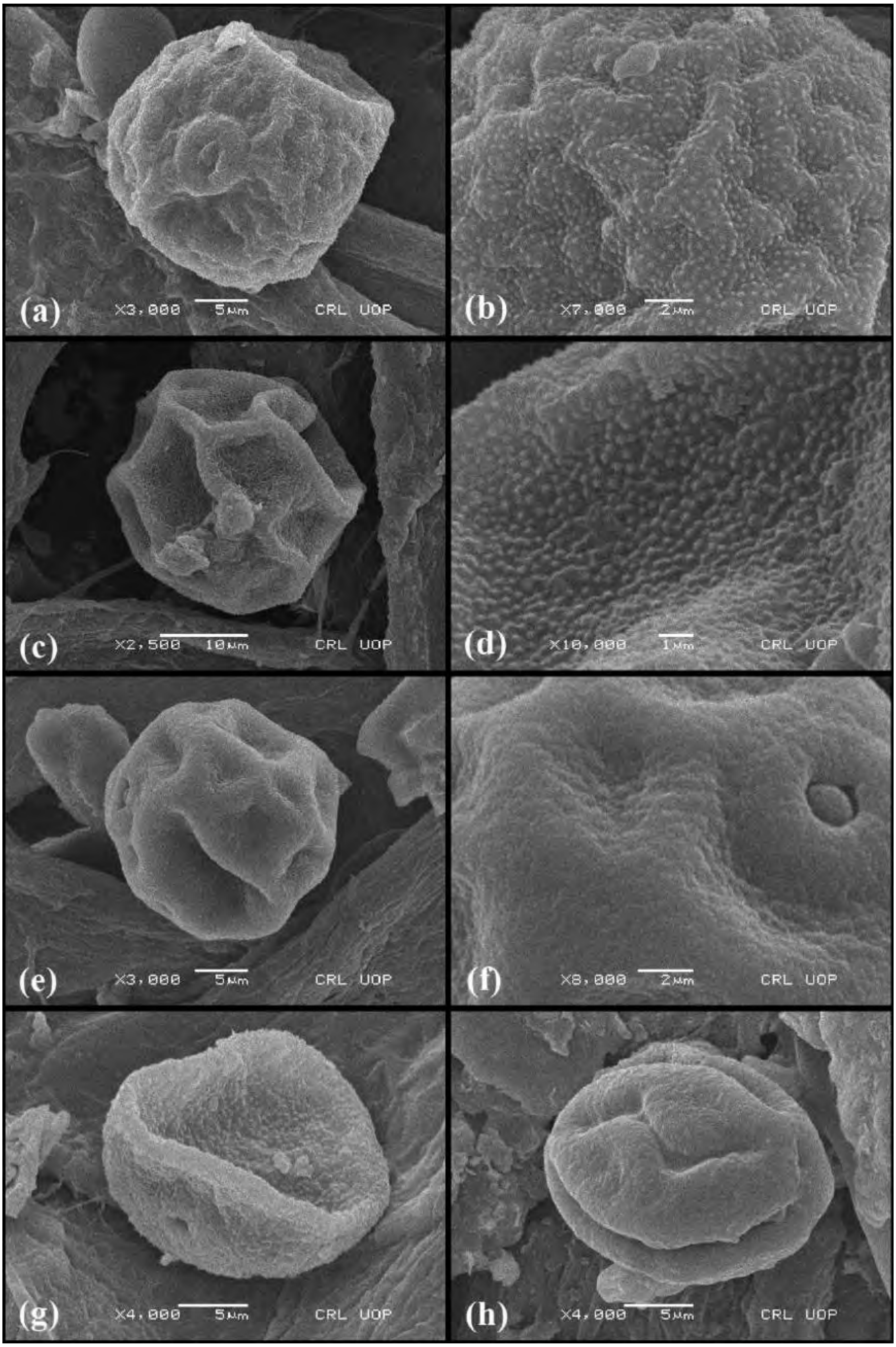

Plate 61. SEM micrographs of pollen (a),(b) *Eremopyrum distans*, (c),(d) *Hordeum marinum* subsp. gussoneanum, (e),(f) *Imperata cylindrica*, (g),(h) *Leptothrium senegalense*

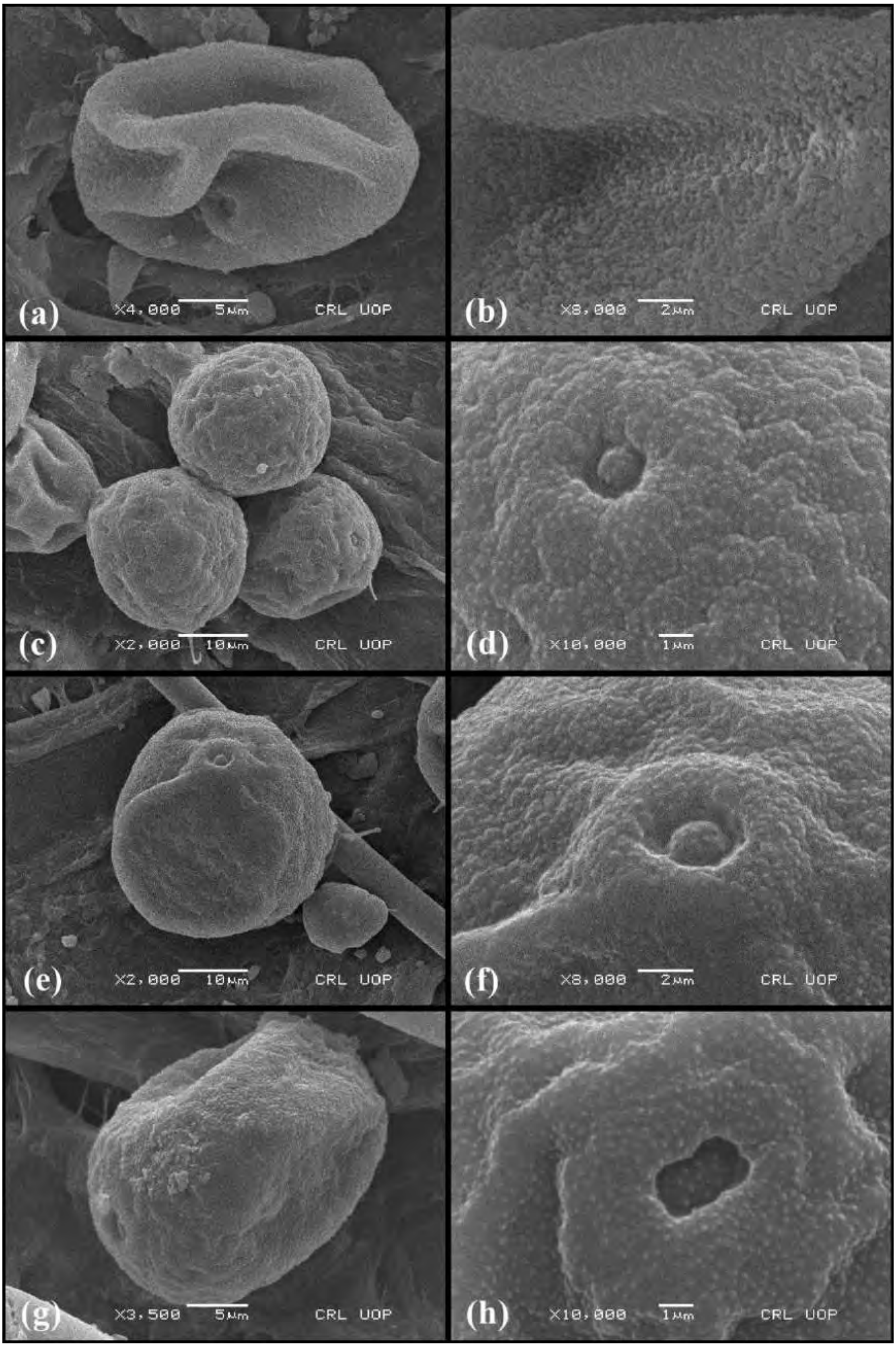

Plate 62. SEM micrographs of pollen (a),(b) *Ochthochloa compressa*, (c),(d) *Panicum antidotale*, (e),(f) *Phalaris minor*, (g),(h) *Piptatherum baluchistanicum*

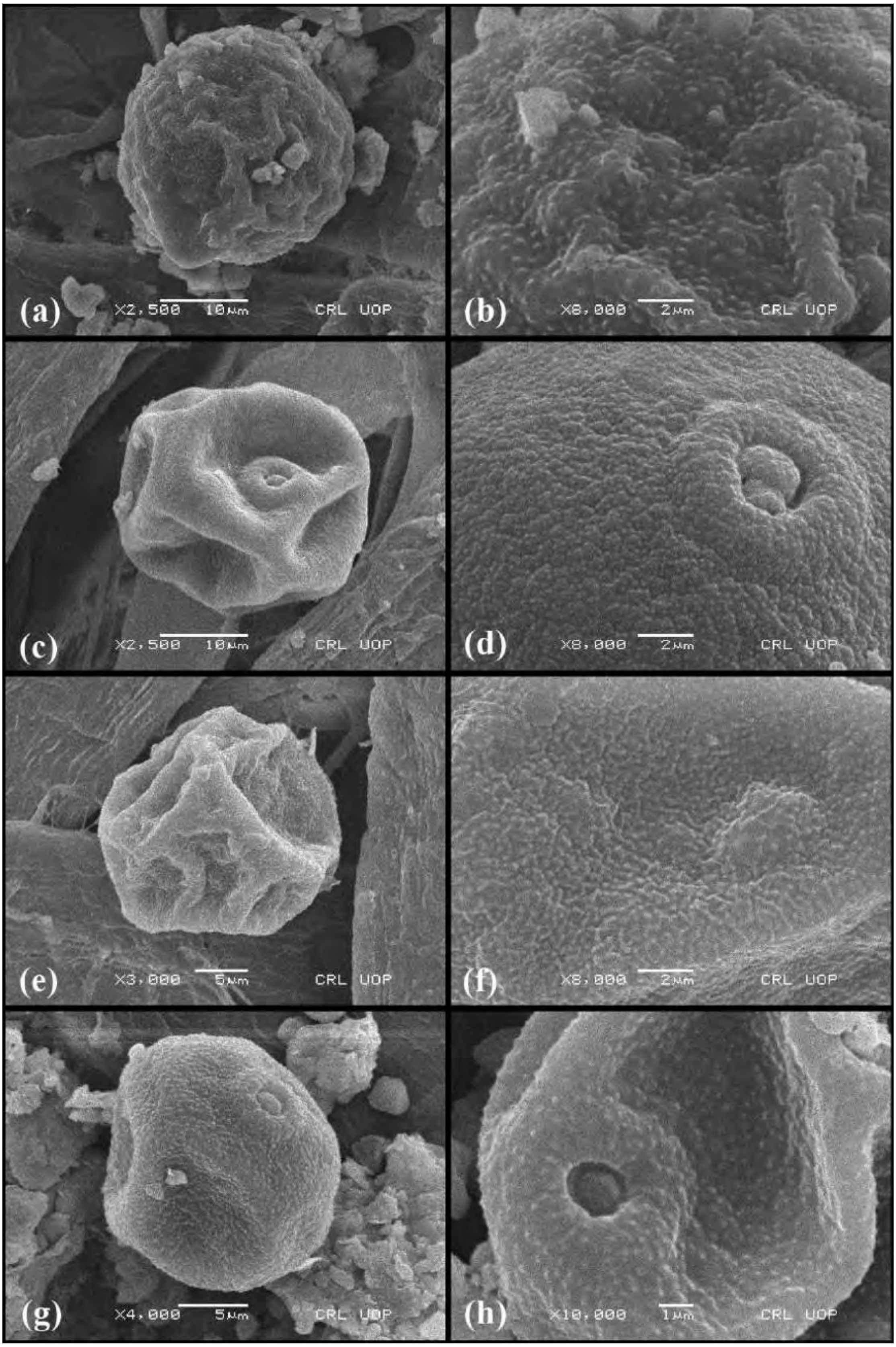

Plate 63. SEM micrographs of pollen (a),(b) *Poa annua,* (c),(d) *Poa infirma,* (e),(f) *Saccharum griffithii,* (g),(h) *Schismus arabicus*

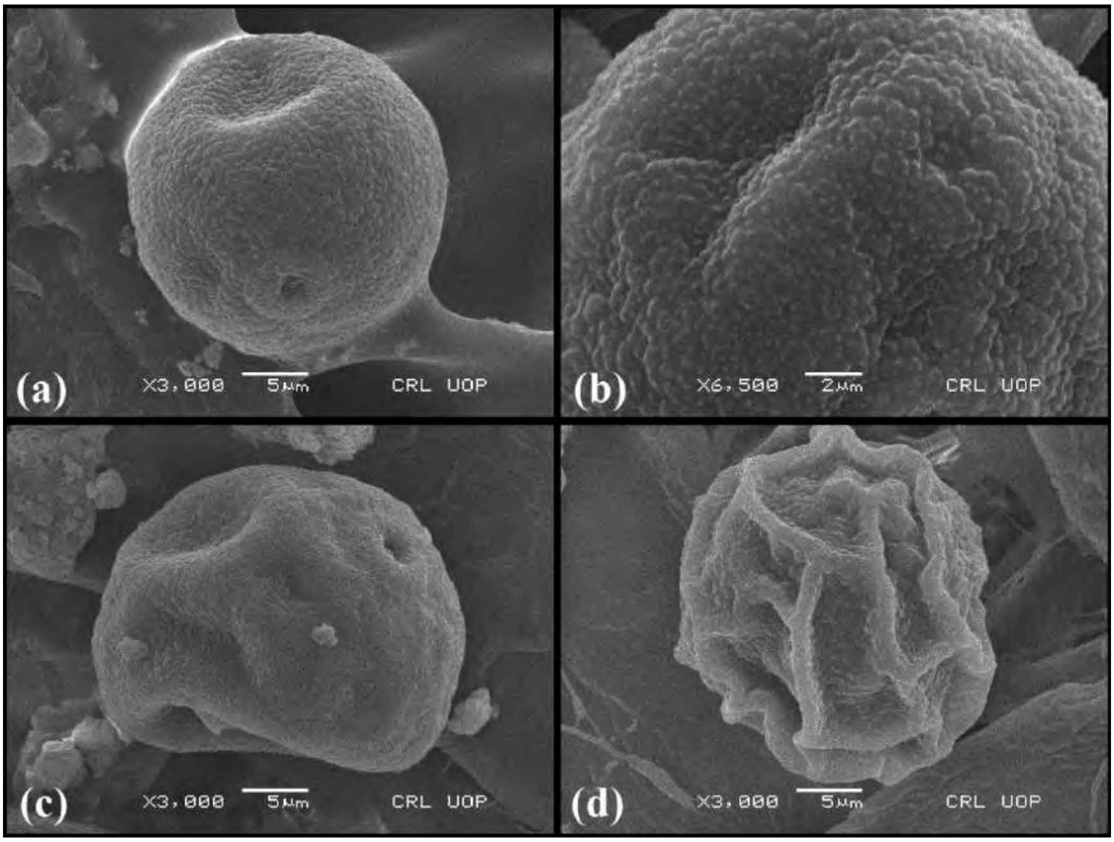

Plate 64. SEM micrographs of pollen (a),(b) *Tetrapogon villosus* , (c),(d) *Vulpia persica*

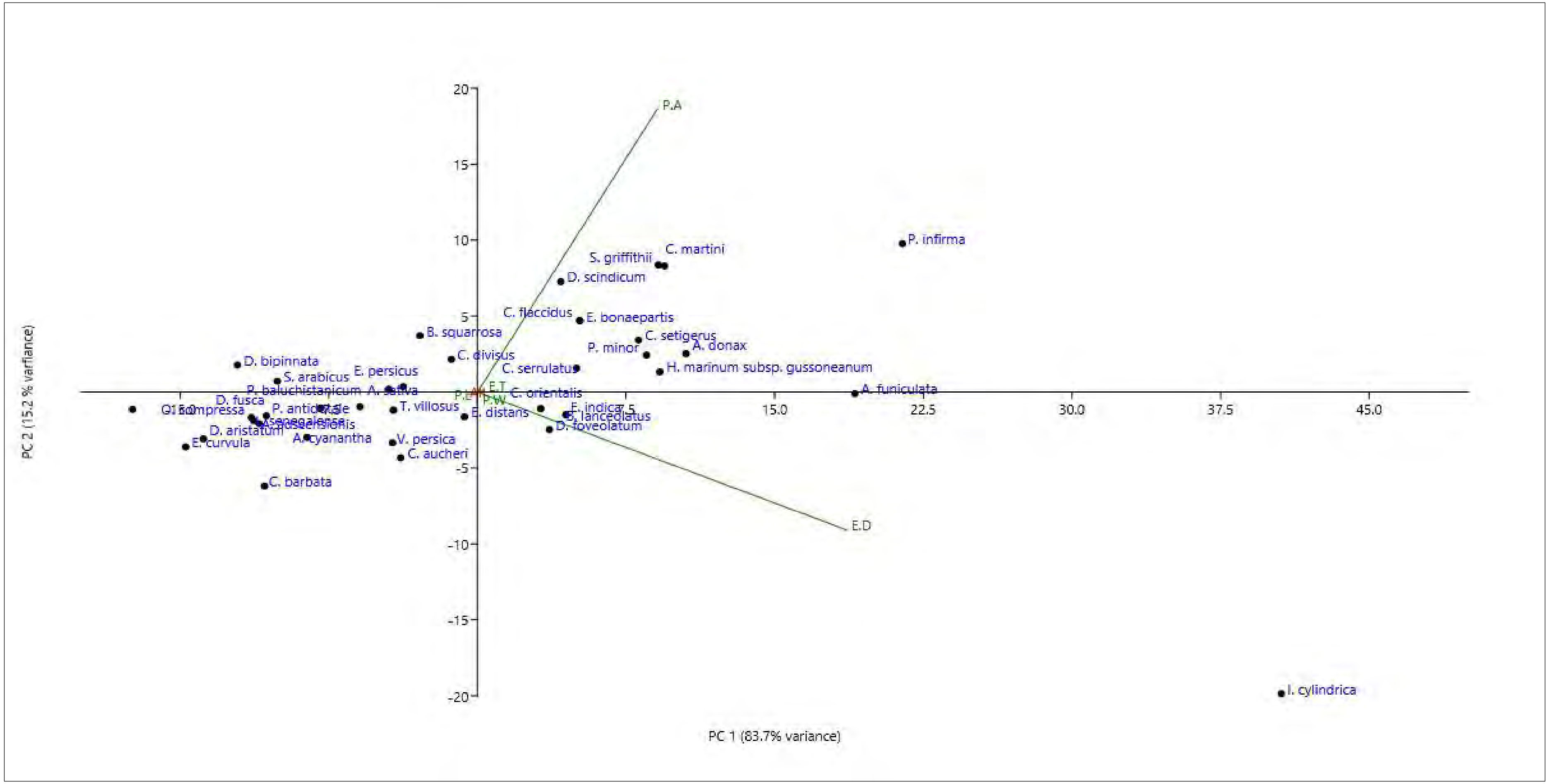

Figure 13. PCA analysis of P.A: Polar axis, E.D: Equatorial Diameter, E.T: Exine thickness, P.L: pore length, P. W: pore width

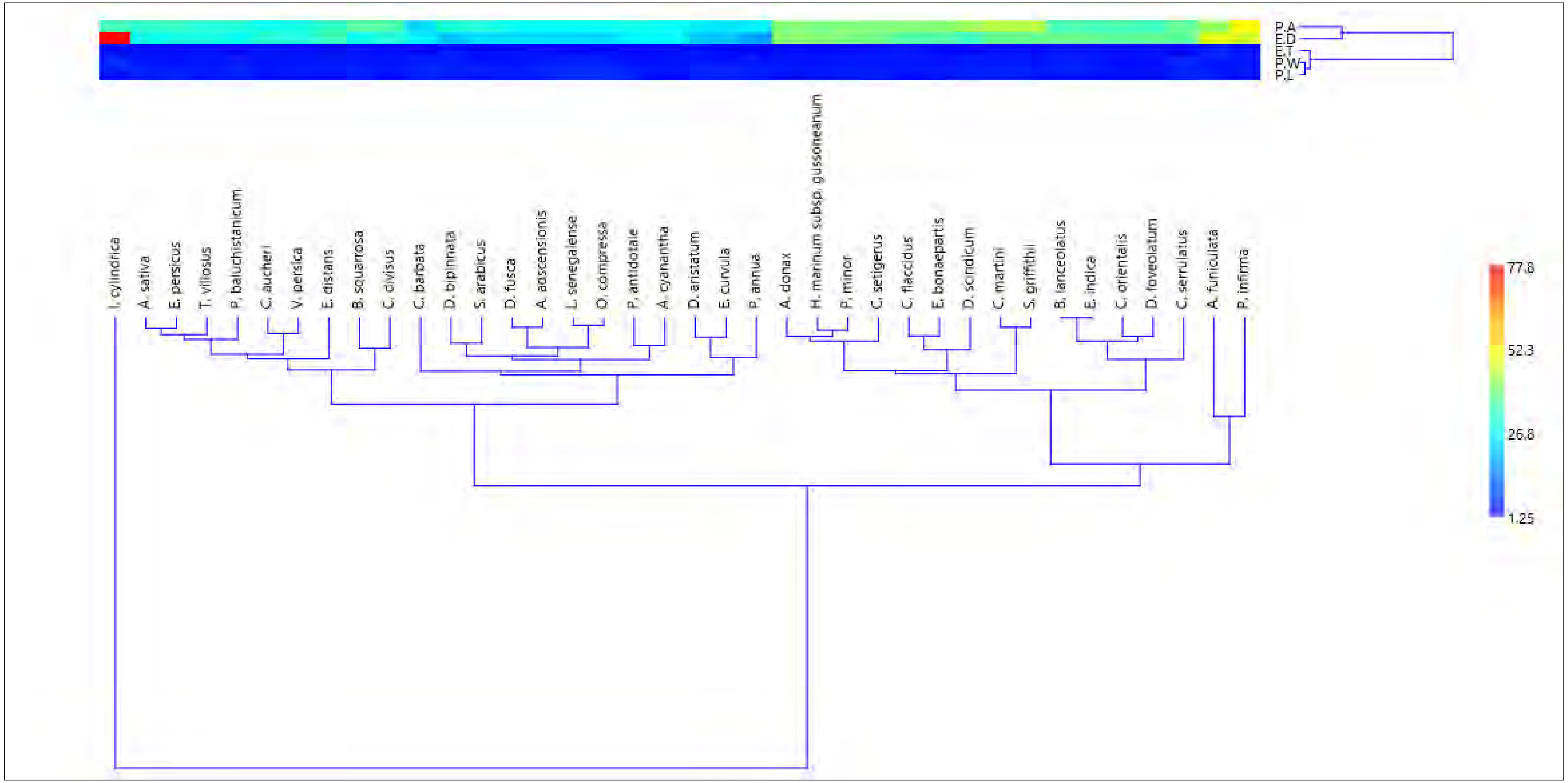

Figure 14. UPGMA cluster analysis based on P.A: Polar axis, E.D: Equatorial Diameter, E.T: Exine thickness, P.L: pore length, P. W: pore width

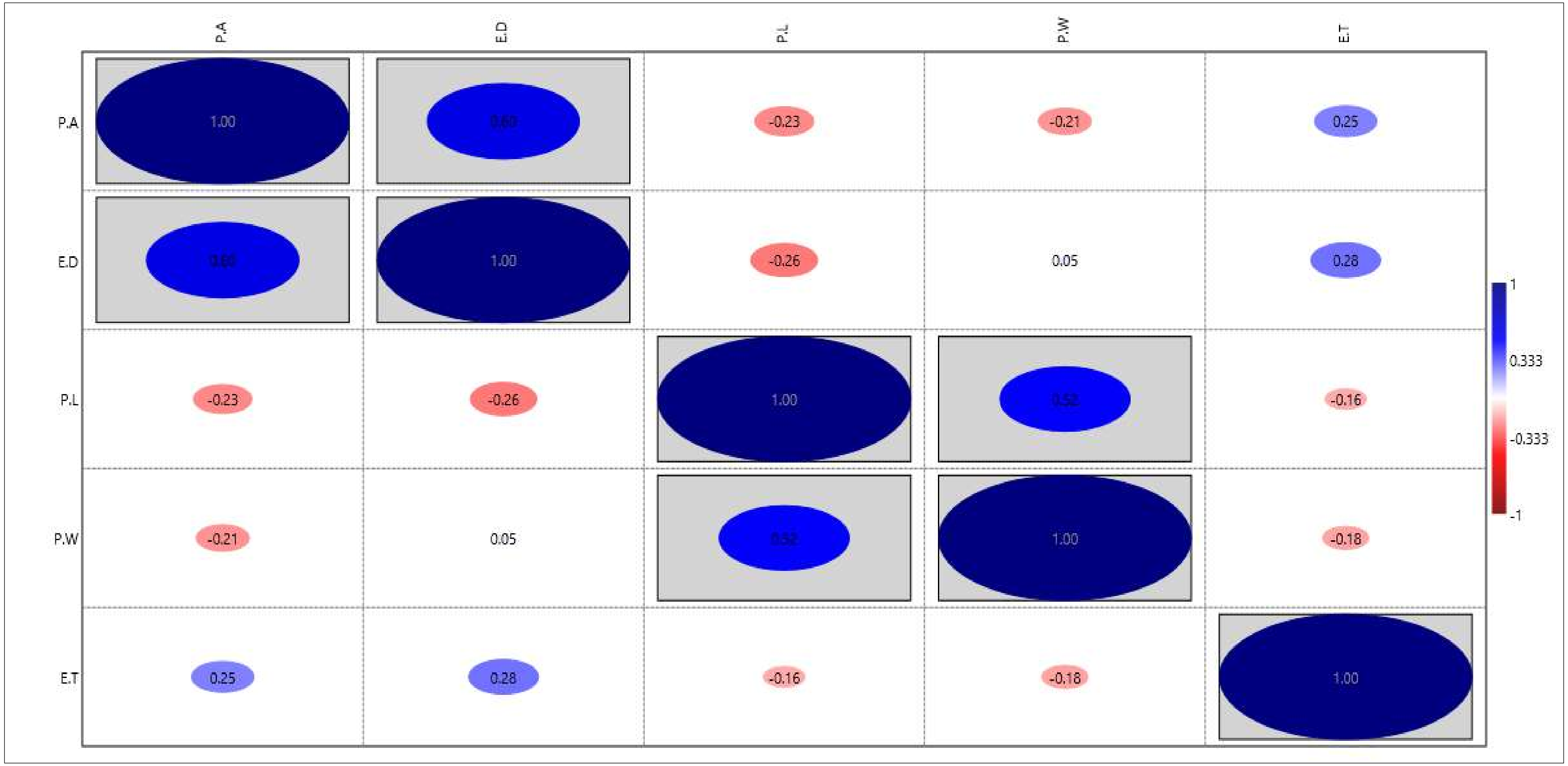

Figure 15. Correlation among the mean values of P.A: Polar axis, E.D: Equatorial Diameter, E.T: Exine thickness, P.L: pore length, P. W: pore width

Table 13. Qualitative characters based on LM and SEM of pollen of Poaceous flora

Taxa	Symmetry	Polarity	Unity	Size class	Shape class	No. of apertures	Polar view	Equatorial view	Harmomegathy	Exine sculpturing	Exine surface	Annulus prominence	Operculum	Pore orientation (sunken/ prominent,	Amb	NPC classification	
																Name	Formula
Aristida adscensionis	Radial	Heteropolar	Monad	Large	Prolate	Monoporate	Circular	Circular to elliptic	++	Psilate verrucate	Microperforate	++	Operculate	Prominent	Peritreme	Monoporate	$N_1P_0C_4$
Aristida cyanantha	Radial	Heteropolar	Monad	Large	Subprolate	Monoporate	Circular to elliptic	Circular to elliptic	++	Microscabrate verrucate	Non-perforate	++	Operculate	Prominent	Peritreme	Monoporate	$N_1P_0C_4$
Aristida funiculata	Radial	Heteropolar	Monad	Large	Subprolate	Monoporate	Circular	Circular	-	Scabrate verrucate	Non-perforate	+	Operculate	Prominent	Peritreme	Monoporate	$N_1P_0C_4$
Arundo donax	Radial	Heteropolar	Monad	Large	Oblate spheroidal	Monoporate	Circular	Circular	+++	Macroscabrate verrucate	Non-perforate	++	Operculate	Prominent	Peritreme	Monoporate	$N_1P_0C_4$
Avena sativa	Radial	Heteropolar	Monad	Large	Oblate spheroidal	Monoporate	Circular	Circular	+	Macroscabrate	Non-perforate	+	Operculate	Prominent	Peritreme	Monoporate	$N_1P_0C_4$
Boissiera squarrosa	Radial	Heteropolar	Monad	Large	Prolate spheroidal	Monoporate	Circular	Circular	+++	Scabrate	Non-perforate	++	Operculate	Prominent	Peritreme	Monoporate	$N_1P_0C_4$
Bromus lanceolatus	Radial	Heteropolar	Monad	Large	Prolate spheroidal	Monoporate	Circular	Circular	+++	Scabrate	Non-perforate	-	-	Sunken	Peritreme	Monoporate	$N_1P_0C_4$
Cenchrus divisus	Radial	Heteropolar	Monad	Large	Subprolate	Monoporate	Circular	Circular	+++	Psilate to verrucate	Non-perforate	++	Operculate	Prominent	Peritreme	Monoporate	$N_1P_0C_4$

Cenchrus flaccidus	Radial	Hetero polar	Mo nad	Larg e	Oblate sphero idal	Monop orate	Circ ular	Circul ar	++	Scabrate verrucat e	Non-perforate	++	Opercu late	Sunke n	Peritr eme	Monop orate	$N_1P_0 C_4$
Cenchrus orientalis	Radial	Hetero polar	Mo nad	Larg e	Prolat e sphero idal	Monop orate	Circ ular	Circul ar	++	Scabrate verrucat e	Non-perforate	++	Opercu late	Sunke n	Peritr eme	Monop orate	$N_1P_0 C_4$
Cenchrus setigerus	Radial	Hetero polar	Mo nad	Larg e	Prolat e sphero idal	Monop orate	Circ ular	Circul ar	+	Verrucat e scabrate	Non-perforate	++	Opercu late	Promi nent	Peritr eme	Monop orate	$N_1P_0 C_4$
Chloris barbata	Radial	Hetero polar	Mo nad	Larg e	Oblate sphero idal	Monop orate	Circ ular	Circul ar	+++	Verrucat e	Non-perforate	++	Opercu late	Sunke n	Peritr eme	Monop orate	$N_1P_0 C_4$
Chrysopogon aucheri	Radial	Hetero polar	Mo nad	Larg e	Subob late	Monop orate	Circ ular	Circul ar	+	Scabrate verrucat e	Non-perforate	+	Opercu late	Sunke n	Peritr eme	Monop orate	$N_1P_0 C_4$
Chrysopogon serrulatus	Radial	Hetero polar	Mo nad	Med ium	Oblate sphero idal	Monop orate	Circ ular	Circul ar	++	Scabrate	Non-perforate	++	Opercu late	Promi nent	Peritr eme	Monop orate	$N_1P_0 C_4$
Cymbopogon martini	Radial	Hetero polar	Mo nad	Larg e	Prolat e sphero idal	Monop orate	Circ ular to ellip tic	Circul ar to elliptic	+++	Scabrate verrucat e	Perforate at the annulus	++	Opercu late	Promi nent	Peritr eme	Monop orate	$N_1P_0 C_4$
Dactyloctenium aristatum	Radial	Hetero polar	Mo nad	Larg e	Prolat e sphero idal	Monop orate	Circ ular	Circul ar	++	Verrucat e	Non-perforate	-	Opercu late	Promi nent	Peritr eme	Monop orate	$N_1P_0 C_4$
Dactyloctenium scindicum	Radial	Hetero polar	Mo nad	Larg e	Oblate sphero idal	Monop orate	Circ ular	Circul ar	+	Microsc abrate verrucat e	Non-perforate	++	Opercu late	Promi nent	Peritr eme	Monop orate	$N_1P_0 C_4$
Desmostachya bipinnata	Radial	Hetero polar	Mo nad	Larg e	Prolat e sphero idal		Circ ular	Circul ar	+	Verrucat e	Non-perforate	+	Opercu late	Promi nent	Peritr eme	Monop orate	$N_1P_0 C_4$
Dichanthium foveolatum	Radial	Hetero polar	Mo nad	Larg e	Oblate sphero idal	Monop orate	Circ ular	Circul ar to elliptic	++	Scabrate to psilate	Non-perforate	++	Opercu late	Promi nent	Peritr eme	Monop orate	$N_1P_0 C_4$

Diplachne fusca	Radial	Heteropolar	Monad	Large	Prolate spheroidal	Monoporate	Circular	Circular	+++	Scabrate	Non-perforate	++	Operculate	Prominent	Peritreme	Monoporate	$N_1P_0C_4$
Eleusine indica	Radial	Heteropolar	Monad	Large	Subprolate	Monoporate	Circular	Circular to elliptic	+++	Psilate	Non-perforate	++	Operculate	Prominent	Peritreme	Monoporate	$N_1P_0C_4$
Enneapogon persicus	Radial	Heteropolar	Monad	Large	Subprolate	Monoporate	Circular	Circular	++	Scabrate	Non-perforate	+	Operculate	Prominent	Peritreme	Monoporate	$N_1P_0C_4$
Eragrostis curvula	Radial	Heteropolar	Monad	Medium	Sub oblate	Monoporate	Circular	Circular	+	Microscabrate verrucate	Non-perforate	+	Operculate	Sunken	Peritreme	Monoporate	$N_1P_0C_4$
Eremopyrum bonaepartis	Radial	Heteropolar	Monad	Large	Oblate spheroidal	Monoporate	Circular	Circular to elliptic	+	Scabrate verrucate	Non-perforate	+	Operculate	Sunken	Peritreme	Monoporate	$N_1P_0C_4$
Eremopyrum distans	Radial	Heteropolar	Monad	Large	Prolate spheroidal	Monoporate	Circular	Circular	+	Scabrate verrucate regulate	Non-perforate	++	Operculate	Prominent	Peritreme	Monoporate	$N_1P_0C_4$
Hordeum marinum subsp. gussoneanum	Radial	Heteropolar	Monad	Large	Prolate spheroidal		Circular	Circular	+++	Scabrate	Non-perforate	+	Operculate	Prominent	Peritreme	Monoporate	$N_1P_0C_4$
Imperata cylindrica	Radial	Heteropolar	Monad	Large	Prolate spheroidal	Monoporate	Circular	Circular	++	Psilate to verrucate	Non-perforate	++	Operculate	Sunken	Peritreme	Monoporate	$N_1P_0C_4$
Leptothrium senegalense	Radial	Heteropolar	Monad	Large	Prolate spheroidal	Monoporate	Circular	Circular to elliptic	+++	Psilate to verrucate	Non-perforate	+	Operculate	Sunken	Peritreme	Monoporate	$N_1P_0C_4$
Ochthochloa compressa	Radial	Heteropolar	Monad	Large	Prolate spheroidal	Monoporate	Circular	Circular	+++	Psilate to foveolate	Non-perforate	++	Operculate	Prominent	Peritreme	Monoporate	$N_1P_0C_4$
Panicum antidotale	Radial	Heteropolar	Monad	Large	Spherical	Monoporate	Circular	Circular	-	Scabrate verrucate	Non-perforate	++	Operculate	Prominent	Peritreme	Monoporate	$N_1P_0C_4$

Phalaris minor	Radial	Heteropolar	Monad	Large	Prolate spheroidal	Monoporate	Circular	Circular	+		Microscabrate verrucate	Perforate at the annulus	++		Operculate	Prominent	Peritreme	Monoporate	$N_1P_0C_4$
Piptatherum baluchistanicum	Radial	Heteropolar	Monad	Large	Prolate	Monoporate	Circular	Circular to elliptic	+		Scabrate rugulate	Non-perforate	++		Operculate	Prominent	Peritreme	Monoporate	$N_1P_0C_4$
Poa annua	Radial	Heteropolar	Monad	Large	Prolate spheroidal		Circular	Circular	++		Scabrate rugulate	Non-perforate	+		Operculate	Prominent	Peritreme	Monoporate	$N_1P_0C_4$
Poa infirma	Radial	Heteropolar	Monad	Large	Oblate spheroidal	Monoporate	Circular	Circular	+		Microscabrate verrucate	Non-perforate	++		Operculate	Prominent	Peritreme	Monoporate	$N_1P_0C_4$
Saccharum griffithii	Radial	Heteropolar	Monad	Large	Subprolate		Circular	Circular to elliptic	++		Scabrate to psilate	Non-perforate	+		Operculate	Sunken	Peritreme	Monoporate	$N_1P_0C_4$
Schismus arabicus	Radial	Heteropolar	Monad	Large	Subprolate	Monoporate	Circular	Circular	+++		Macroscabrate	Non-perforate	++		Operculate	Prominent	Peritreme	Monoporate	$N_1P_0C_4$
Tetrapogon villosus	Radial	Heteropolar	Monad	Large	Subprolate	Monoporate	Circular	Circular	+		Verrucate	Non-perforate	++		Operculate	Prominent	Peritreme	Monoporate	$N_1P_0C_4$
Vulpia persica	Radial	Heteropolar	Monad	Medium	Prolate spheroidal	Monoporate	Circular	Circular	++		Scabrate verrucate	Non-perforate	++		Operculate	Sunken	Peritreme	Monoporate	$N_1P_0C_4$

amb: circumference, NPC: Number, Position, Characteristic

Table 14. Quantitative measurements of pollen characters of Poaceous taxa

Plant Species	Polar. Diameter	Equatorial. Diameter	P/E ratio	Pore length	Pore width	Intine thickness	Exine thickness	Viability F/F+S* 100
Aristida adscensionis	54.9±0.444409721	40.7±0.672681202	1.35	4.55±0.215058132	2.35±0.231840462	1.25±0.176776695	4.35±0.257390754	92
Aristida cyanantha	62.35±0.302076149	48.75±0.32596012	1.28	5.15±0.231840462	2.65±0.231840462	1.5±0.176776695	4.7±0.16583124	80
Aristida funiculata	50.5±0.25	38.7±0.289395923	1.3	3.9±0.127475488	2.35±0.231840462	1.5±0.176776695	4.8±0.2	88
Arundo donax	58.35±0.257390754	58.7±0.289395923	0.99	3.95±0.145773797	1.75±0.176776695	1.05±0.215058132	4.25±0.176776695	79
Avena sativa	83.6±0.322102468	86.15±0.257390754	0.97	4.65±0.16955825	3.75±0.176776695	1±0.176776695	4.55±0.215058132	80
Boissiera squarrosa	57.85±0.340954542	54.8±0.649037749	1.06	4±0.176776695	2.65±0.231840462	1.25±0.176776695	4.6±0.257390754	88
Bromus lanceolatus	51.4±0.331662479	48.35±0.6254998	1.1	4.85±0.231840462	2.35±0.231840462	1.5±0.176776695	4.25±0.176776695	86
Cenchrus divisus	75.35±0.785015923	64±0.5	1.18	4.95±0.289395923	4.25±0.176776695	1.35±0.231840462	4.85±0.231840462	90
Cenchrus flaccidus	56.4±0.322102468	60.1±0.26925824	0.94	4.25±0.176776695	1±0.176776695	1.5±0.176776695	4.85±0.231840462	80
Cenchrus orientalis	79.85±0.231840462	68.35±0.407737661	1.2	4.9±0.176776695	4±0.215058132	1.75±0.176776695	4.8±0.215058132	92
Cenchrus setigerus	81.15±0.257390754	75.75±0.176776695	1.07	4.85±0.231840462	3.25±0.176776695	1.25±0.176776695	5.15±0.231840462	79

Chloris barbata	88.15±0.331662479	90.75±0.176776695	0.97	4.55±0.215058132	3.5±0.176776695	1.3±0.145773797	4.55±0.215058132	88
Chrysopogon aucheri	42.95±0.21506	50.8±0.16583	0.85	5.15±0.23184	1.75±0.32596	1.5±0.17678	5.15±0.23184	95
Chrysopogon serrulatus	45.15±0.231840462	49.1±0.471699057	0.92	3.5±0.176776695	1.75±0.176776695	1±0.176776695	3.85±0.231840462	92
Cymbopogon martini	58.85±0.302076149	55.85±0.527967802	1.05	5.45±0.215058132	2.65±0.231840462	1.5±0.176776695	5.3±0.289395923	91
Dactyloctenium aristatum	61.2±0.320156212	55.6±0.407737661	1.101	4.55±0.215058132	3.5±0.176776695	1.25±0.176776695	4.55±0.215058132	88
Dactyloctenium scindicum	83.4±0.187082869	88.5±0.176776695	0.94	4.55±0.215058132	3.25±0.306186218	1.25±0.176776695	4.35±0.127475488	76
Desmostachya bipinnata	56±0.25	52.25±0.778620575	1.07	4.25±0.176776695	1.9±0.127475488	1.25±0.176776695	4.55±0.215058132	78
Dichanthium foveolatum	78.85±0.231840462	52.35±0.407737661	1.3	4.2±0.176776695	4.55±0.215058132	1.5±0.176776695	4±0.215058132	82
Diplachne fusca	51.05±0.348209707	48.4±0.231840462	1.06	4±0.176776695	2.95±0.215058132	1.75±0.176776695	4.55±0.215058132	78
Eleusine indica	71.3±0.421307489	56±0.25	1.27	4.85±0.231840462	2.35±0.231840462	1.5±0.176776695	4.5±0.25	65
Enneapogon persicus	74.85±0.231840462	62.35±0.407737661	1.2	4±0.176776695	4.55±0.215058132	1.75±0.176776695	4.55±0.215058132	96
Eragrostis curvula	38.65±0.407737661	45.9±0.415331193	0.84	3.55±0.215058132	1.75±0.176776695	0.75±0.176776695	4±0.176776695	80
Eremopyrum bonaepartis	63.9±0.231840462	68.75±0.440170422	0.93	5.25±0.285043856	2.05±0.215058132	1.75±0.176776695	5.15±0.231840462	94
Eremopyrum distans	58.25±0.176776695	57.85±0.340954542	1.01	4.55±0.215058132	2.65±0.231840462	1.5±0.176776695	4.55±0.215058132	90

Hordeum marinum subsp. gussoneanum	78.95±0.348209707	73.05±0.619475585	1.08	5.15±0.231840462	3.75±0.176776695	1.85±0.127475488	4.85±0.231840462	82
Imperata cylindrica	71.05±0.28939592	68.65±0.358817502	1.04	5.15±0.231840462	4±0.176776695	1.25±0.176776695	4.55±0.215058132	86
Leptothrium senegalense	63.05±0.913099118	58.45±0.266926956	1.08	4.25±0.176776695	2.65±0.231840462	1.4±0.26925824	5.1±0.20310096	85
Ochthochloa compressa	72.65±0.231840462	71.05±0.348209707	1.02	4.85±0.231840462	3.4±0.231840462	1±0.176776695	4.85±0.231840462	90
Panicum antidotale	50.85±0.231840462	50.85±0.800780869	1	3.3±0.145773797	4±0.176776695	1.5±0.176776695	4.85±0.231840462	90
Phalaris minor	68.5±0.285043856	63.25±0.176776695	1.08	4.85±0.231840462	3.75±0.176776695	1.15±0.231840462	5.15±0.231840462	79
Piptatherum baluchistanicum	65.75±0.26220221	46.5±0.176776695	1.41	3.5±0.176776695	2.35±0.231840462	1.5±0.176776695	4.55±0.215058132	74
Poa annua	76.45±0.721976454	73.25±0.176776695	1.04	4.85±0.231840462	3.5±0.176776695	1.5±0.176776695	5.15±0.231840462	84
Poa infirma	65.2±0.266926956	65.35±0.923985931	0.99	4.85±0.231840462	3.75±0.176776695	1.75±0.176776695	4.85±0.231840462	88
Saccharum griffithii	72.35±0.816241386	61.8±0.508674749	1.17	5.15±0.231840462	3.25±0.176776695	1.65±0.127475488	4.85±0.231840462	78
Schismus arabicus	63.9±0.384057287	53.2±0.768114575	1.2	3.5±0.176776695	3.75±0.176776695	1±0.176776695	4.85±0.231840462	95
Tetrapogon villosus	75.75±0.176776695	63.75±0.176776695	1.19	4.85±0.231840462	5.1±0.20310096	1.5±0.176776695	4.7±0.289395923	82
Vulpia persica	38.35±0.20310096	35.55±0.145773797	1.1	2.95±0.215058132	1.5±0.176776695	1±0.176776695	3.5±0.176776695	88

Section-III

Anatomy

Summary

The third section included the petiole/culm anatomy of angiosperms from Baluchistan, Pakistan. The study included 39 Asteraceous, 14 Boraginaceous, and 26 Poaceous species. Sections were prepared via microtomy. Petiole/culm cross sections were visualized under Light microscopy and observed the qualitative and quantitative characteristics.

- Qualitative characters included: Petiole/culm outline, wings, cuticle, shape and number of layers of epidermis, collenchyma, parenchyma, chlorenchyma, sclerenchyma, xylem vessels, phloem, cavities, 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 characteristics 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.

3.5 Petiole anatomy of Asteraceous Flora from Baluchistan

3.5.1 Results

a) Cichorieae

The petiole anatomy of 18 members of Chicorieae was determined. All members exhibited petiole wings except *H. pusilla*. Flat, sulcate, oval, and round petiole shapes were present. Flat-shaped petioles were highly observed (13 taxa). The sulcate shape was present in three species. Oval and round shapes of the petiole were noted in *H. pusilla* (Plate 66) and *L. dissecta* respectively. Trichomes appeared in five taxa. Unicellular, multiseriate, uniseriate, and uniseriate to multiseriate trichomes types were observed in this tribe. In the Cichorieae undulated cuticle was examined in 13 taxa. The epidermal cells were observed in six shapes, square, angular, rectangular, square to oval, square to rectangular, square to angular, and rectangular to oval. The square shape was the highly repeated shape i.e. in six taxa. Parenchyma cells possessed irregular, isodiametric, angular to isodiametric, tetra to hexagonal, angular to isodiametric, and irregular to isodiametric shapes. Irregular-shaped parenchyma was the prominent shape. Three species of this tribe revealed the isodiametric parenchyma.

Angular, lamellar, lacunar, and angular to lamellar collenchyma arrangements were noted. Angular collenchyma was present in nine taxa of this tribe. Collenchyma existed in single or double layers. The subepidermal ring of collenchyma was present in *L. orientalis, L. oligocephala, L. procumbens, R. tingitana, S. arvensis,* and *S. oleraceus*. Chlorenchyma layers varied from 2-4. In nine species three layers of chlorenchyma were observed. Among the members of the Chichorieae single sclerenchyma layer was possessed by *C. kotschyana*. Whereas sclerenchyma in the vascular bundle was observed in 11 taxa. Vascular bundle types were collateral closed (8 taxa), bicollateral (8 taxa), and collateral open (2 taxa). Round, oval, and angular shapes of xylem vessels were determined. Phloem cells were mostly trigonal to hexagonal, and angular in shape. The number of vascular bundles ranged from 1 to 7, with 1+2 arrangement in 7 species (Table 15). Air spaces in the cross section were observed in four species of *Launaea, C. kotschyana, H. pusilla, K. linearis, P. stewartii,* and *S. koelpinioides.*

b) Cardueae

Petiole wings were observed in all the ten studied members of Cardueae. Sulcate, flat, and oval shape of the cross-section was present. *C. haeckeliae* have oval whereas *J. carduiformis* and *Z. purpurea* possess flat shapes. Six species of this tribe were noted with trichomes. *A. carduus* exhibited uniseriate while others have multiseriate trichomes (Table 16). In Cardueae members the cuticle was observed undulated in six and smooth in 2 taxa. The epidermal cells were square (in three taxa) to oval, polygonal, rectangular, and angular in shape. Tetra to hexagonal parenchyma observed in *A. carduus, C. haeckeliae, J. berardioidea* (Plate 65, 67), and *Z. purpurea* (Plate 71). Irregular and isodiametric shapes were observed in two, two species respectively. Collenchyma cells were angular (three taxa), lamellar (three taxa), and annular to angular in shape. Tri to hexagonal-shaped sclerenchyma cells were observed in *C. arvense*. In all other species, no prominent sclerenchyma was noted. The vascular bundle in five members was bicollateral in arrangement. Amphicribral and collateral closed types were observed in two taxa each. Collateral open vascular bundle was present in *Centaurea iberica*. The 1+2 types were noted in 3 species, in others the number varied from 1-8. Xylem vessels were round, round to oval, and angular shapes. Phloem cells were angular, hexagonal, rectangular to oval in shape.

c) Inuleae

In the cross-sections, the sulcate and flat petiole observed. *I. grantioides* possessed uniseriate trichomes. Undulated cuticle was present in *B. sinuate, P. angustifolia*, and *P. undulata*. Epidermal cells were different in each member, square to oval in *B. sinuata*, rectangular in *I. grantioides*, angular in *P. angustifolia*, and angular to oval in *P. undulata*. Irregular parenchyma was observed in *P. undulata,* whereas all others possessed tetra to hexagonal shapes. Similarly, all other taxa were observed with angular collenchyma whereas *P. undulata* with lacunar type. Vascular bundles were collateral closed and bicollateral. The number of vascular bundles was 4 in *B. sinuate*, 2 in *I. grantioides,* and 1+2 in *Pulicaria* species.

d) Anthemideae

In the transverse cross sections, *A. biennis* appeared circular, *M. lamellate* sulcate, *S. maritimum* flat, *X. macropodum* oval in shape. Uniseriate trichomes were noted in *M. lamellate* and *S. maritimum*. The undulated cuticle was present in M. lamellate and *S. maritimum,* whereas the smooth type was observed in *X. macropodum* and *A. biennis*. The shapes of epidermal cells were rectangular, rectangular to square and ova, angular to oval, and square. The parenchyma cells were mostly irregular with angular shape. Collenchyma cells were angular in shape. Sclerenchyma was present only in *X. macropodum* with hexagonal type. The members of this tribe were observed with bicollateral, collateral open, and collateral closed arrangements of vascular bundles. All members possessed angular phloem cell shape. While the xylem vessels were oval to round and angular shapes. Number of vascular bundles varied from 1 to 4. M. lamellate observed with 1+2 type of vascularization. Air spaces were observed in *A. biennis* and *S. maritimum.*

e) Gymnarrheneae

Gymnarrhena micrantha was observed with an oval shape in the cross-section having a grove in the upper surface. It was observed with undulated cuticle having uniseriate trichomes. Epidermal cells were square to rectangular, and parenchyma was isodiametric in shape. Collenchyma cells were angular in shape and present in a single layer. Four layers of chlorenchyma cells were observed, while sclerenchyma was absent. Vascular bundles were bicollateral with 1+2 in arrangement having round to oval xylem vessels and, round shape phloem cells. Both xylem and phloem parenchyma were noted.

f) Gnaphalieae

Lasiopogon muscoides have sulcate shape cross-section, and the trichomes were multiseriate with undulated cuticle. The epidermal and collenchyma cells were angular in shape. The vascular bundle was bicollateral with oval and round xylem vessels and angular phloem cells. The number of vascular bundles was 3. Prominent air spaces were observed in *L. muscoides*. Sclerenchyma was absent.

g) Astereae

Symphyotrichum subulatum appeared as a sulcate shape without trichomes. The cuticle was undulated. Epidermal cells were angular to round. Parenchyma cells were irregular while collenchyma cells were annular. Two layers of collenchyma were observed. Chlorenchyma cells were distributed in three layers. Sclerenchyma was observed in vascular bundles. Bicollateral vascular bundles were present in a 1+2 arrangement. Oval-to-round xylem vessels were noted with angular to round phloem cells.

3.5.2 Discussion

This is the first research on the investigation of anatomical traits with their implications in the separation of the Asteraceous taxa from the arid to semiarid region of Baluchistan. Previously the significance of anatomical characters in modern taxonomy were documented by various researchers from other ecological regions. Petiole anatomy of Amaranthaceous taxa from Pakistan has been recently studied by Majeed et al. (2022). Al-Suboh et al. (2019) reported the significance of anatomical traits in the systematics of Asteraceous species from Iraq. Similarly, De las Mercedes Sosa et al. (2014) stated that anatomical features are the source of systematically useful tools in the genus Chrysolaena of the tribe Vernonieae. The petiole anatomical traits have been used for systematics implications in families such as Brassicaceae (Noor et al., 2023), Malvaceae (Nurul-Aini et al., 2013). The anatomical traits analyzed here were petiole outline, trichomes, cuticle, shapes, and number of layers of epidermal cells, chlorenchyma cells, parenchyma cells, vascular bundles arrangement and number, air spaces, along with quantitative measurement of length and width of these parameters.

The highly acquired shapes for the studied Asteraceous species were flat in 18 and sulcate in 15 taxa. The tribe Cichorieae was highly represented by the flat shape whereas Cardueae was by sulcate outline. The oval outline was noted in *H. pusilla*. Among the three *Lactuca* species, *L. dissecta* distinguished for its round outline. The *S. arvensis* sulcate shape, and *S. oleraceus* flat shape can also be separated based on petiole outline. Similarly, in Cardueae, the two *Jurinea* species can significantly be differentiated based on outline (Table 17). Two *Pulicaria* species of Innuleae were also distinguished by distinct shapes flat and sulcate. The petiole outline proved to be of taxonomic significance among the members of the various tribes (Ekeke and Ogazie, 2020). In contrast, the taxa of genus *Launaea* (Plate 68, 69) (all flat) and *Himalaiella* (all sulcate) exhibited no variations, so these genera can only be specified up to genus level based on the outline.

The separation of the taxa of Anthemideae, Gymnarrheneae, Gnaphalieae and Astereae wass established on the petiole outline in combination with the other anatomical traits. Ekeke and Ogazie (2020) specified eight types based on the petiole outline and vascular bundles types, reported the fair variations among the species. Noor et al. (2023), Noraini et al. (2016), Chia (2000) reported that petiole shape was of taxonomic significance in Brassicaceae, Dipterocarpaceae and Tiliaceae respectively. The largest size of petiole was observed in *J. carduiformis* 4006 µm length and 2544.2 µm width. In the Anthemideae most of the members were noted with no grove in the petiole surface. The eminent leaf-like wings were present in 28 species collectively in all tribes. The leaf-like wings have been described as one of the Asteraceae main traits (Metcalfe and Chalk, 1979).

In the present study four distinct types of trichomes were observed. The trichomes in *L. oligocephala* were uniseriate to multiseriate. The presence of more than one type of trichome can be found in single species in Asteraceae was reported by De las Mercedes Sosa et al. (2014). The multiseriate trichome type was associated with Cardueae. For the rest of the studied tribes and their species, this character can be used for the delimitation within the genera (Table 15). The detailed scanning electron microscopy (SEM) of trichomes of these Asteraceous species in the future will be applicable in their systematics. Conversely, the structure of the cuticle was either smooth or undulated in the investigated taxa on 10 µm. On this resolution, it is an additional feature for the documentation, but the in-depth examination was required on higher magnification and resolution, which may lead to better specification of the taxa. Plates 72, 73, 74 showed the magnified views of *C. arvense, J. carduiformis, S. oleraceus, K. linearis* and *Z. purpurea*. The smooth type of cuticle was either completely smooth, or with depressions along the junction of two epidermal cells as in *S. oleraceus* (Plate 74). The undulations of the cuticle in the Asteraceous species may be close and elongated for example in *K. linearis* or distend with shorter length for example *C. arvense* (Plates 72, 73). The former may be termed as frequent whereas later one was less frequent with a larger wavelength. The cuticle in addition to the vascular bundles type could be used for the identification of Asteraceous species (Ekeke and Ogaize 2020).

The variations in the shapes, number of layers of epidermal, parenchyma, collenchyma, sclerenchyma cells further assisted in the identification of studied taxa. The same was reported by Tekin and Kartal (2016) that shape of the petiole, the structure of the cortical parenchyma, and the arrangement of the vascular bundles, there were notable differences which served as a helpful aid for classifying the endemic taxa of Tanacetum. Here the epidermal cells were observed with numerous types such as square, rectangular, angular, polygonal, square to oval, square to rectangular, and square to round. The epidermal cells shapes were not specific to tribe or genera so they can be used particularly for species-level delimitation. In the same way, parenchyma cells shape variations among the species significantly used for the taxa separation. The highly acquired shapes were irregular, isodiametric, angular, tetra to hexagonal. In the studied plants, collenchyma was observed in all four types, angular, annular, lamellar and lacunar. Angular collenchyma was frequently observed. In *S. maritimum* no prominent collenchyma was observed. The cell sizes were not significantly different for the separation of the taxa among the studied Asteraceous members. Al-Suboh et al. (2019) documented that Centaurea species have been characterized based on the comparative anatomical traits. The development and arrangement of collenchyma along with the vascular bundles number and arrangement of trichomes types were significant features.

Currently the collenchyma layers were 1 to 2 in all the tribes, except Cardueae, in which the number of layers exceeded up to 4. Similarly, the Cardueae was noted for the presence of subepidermal ring of collenchyma in most of the members. Whereas the variations in the number of chlorenchyma layers, the existence of air spaces and the presence and absence of sclerenchyma in vascular bundles in all the studied tribes may be attributed to the identification down to species level (Table 15). Similar conclusions were documented by Mabel et al. (2013), that the petiole shapes, the layers of their collenchyma, the parenchyma cells, and the arrangements of their vascular bundles vary between species and were important for the classification and delimitation of the species in the Asteraceae. Janaćković et al. (2019) characterized the *Artemisia umbelliformis* as concave form outline, single-layered epidermis, and collenchyma, one large and two small vascular bundles, trichomes are absent. These features were found to be significant in the Asteraceae taxonomy. In the present study, the observed shapes of sclerenchyma were angular, hexagonal, trigonal to hexagonal, and angular to hexagonal.

The arrangement, size and number of vascular bundles are genetically coded. These characters when combined with other traits such as cuticle type, number of layers of different cells and their shapes can be significant in the delimitation of taxa at various levels of classification (Ekeke and Ogazie, 2020). The number of vascular bundles varied from 1 to 8, with 1+2 distribution in 15 taxa. The arrangement of vascular bundles included bicollateral, collateral closed, collateral open, and amphicribral. Largest vascular bundle was observed in *I. grantioides* 449.1µm length and 337.8 µm width. The arrangement, size, and number of vascular bundles varied from species to species. Thus, they were of high taxonomic significance in the identification of Asteraceous species at various levels of classification. The xylem vessels were noted in the oval, round, combination of oval and round, rarely angular. Whereas phloem cells were the combination of angular, irregular, rectangular, oval, hexagonal, trigonal, rarely round. Sclerenchyma was present in vascular bundles in 21 species.

Six types were determined based on the petiole outline and vascular bundles shapes and arrangement (Figure 16). Type 1 is observed in 11 plants with larger central vascular bundles towards the prominent outwards basal end of the petiole and two lateral vascular bundles were in the midline. In Type 2, all three vascular bundles were arranged along the central line of the petiole with no prominent basal end. This type was observed in 9 species. Type 3 is simple vascularization, having one central vascular bundle. This was present in 6 studied species. Inter-spread vascular bundles are existed in a curve line single row in six species, this type was referred as Type 4. The basal end was not prominent, and they have elongated concave shape outline. A similar feature was observed by Ekeke and Ogazie (2020) and Metcalfe and Chalk (1972) for Asteraceae and other dicot taxa respectively. Types 5 and 6 were the less occurring types. In type 5, vascular bundles were arranged in a row in a triangular petiole outline. This was documented in 3 species. Type 6 was also present in 3 taxa. This included the arrangement of vascular bundles in a row. The petiole outline was heart-shaped. The basal end was prominent.

In a novel system of axes, PCA score plots determine clusters of the variables, and categories and help to visualize trends in the data set (Kim et al., 2015). PCA determined that the length and width of vascular bundles, width of phloem cells, width of xylem vessels were found significant in the discrimination among the taxa (Figure 17). Correlation loading plots for the determination of association among the mean values, were necessary and strengthened the principal component analysis. Uga et al. (2009) studied the relationship among the root anatomical traits via PCA plots to the identification of trees. Loading plots determined the positive and negative correlation while correlation loading plots help to interpret the correlation between PCs and variables by presenting significant levels and visualization of explained variance (Kim et al., 2015). The highest positive correlation from 0.87 to 0.98 was found in xylem vessels length and width with phloem cells length and width. The least positive correlation 0.07 was observed between vascular bundles length with xylem vessels and phloem cells (Figure 18). Similarly, the UPGMA dendrogram revealed the differences and separated the taxa of the same genera (Figure 19). The ridge line plot compared and visualized the overall distribution among the pairs of petiole anatomical traits (Figure 20). Hence it is concluded that there existed statistically significant differences among the means of studied characteristics, this can be used as a systematics approach for the distinction of taxa.

The adaptive divergence is promoted by morphological and anatomical variations because of phenotypic plasticity. The species in dry areas adapt to xeromorphic nature. The anatomical features linked to the environment indicate the ecological adaptations for unfavourable conditions (De las Mercedes Sosa et al., 2014). The examined species were observed with variations in the combination of studied qualitative and quantitative characters. Petiole shape, and variations in the number, shapes, and arrangement of vascular bundles were the distinguishing characteristics of taxonomic value (Mabel et al., 2013). These characters were successfully manipulated in the construction of a taxonomic key for the identification of the studied Asteraceous species (Table 18).

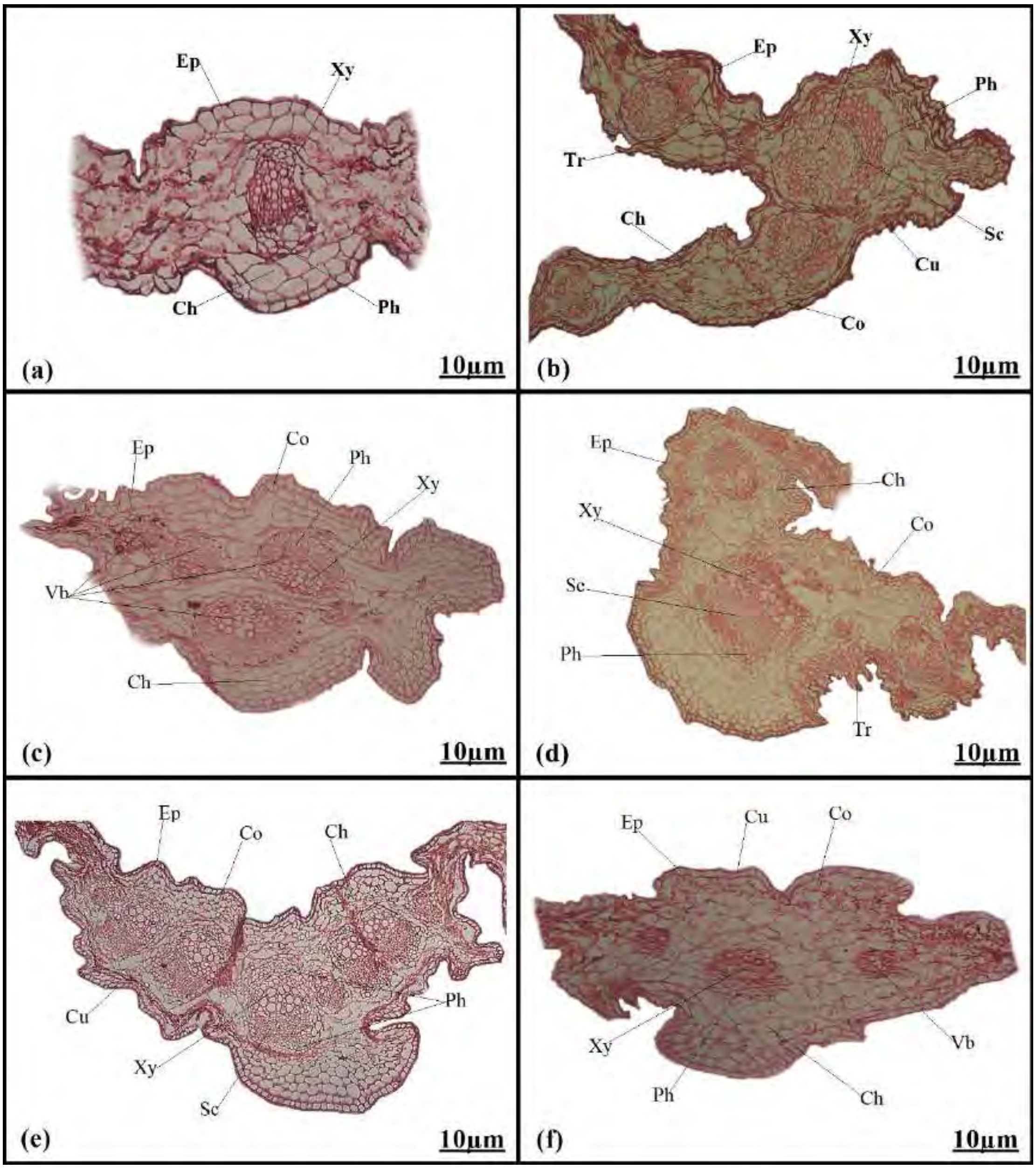

Plate 65. Photomicrographs of petiole anatomy of (a) *Artemisia biennis*, (b) *Atractylis carduus*, (c) *Blumea sinuate*, (d) *Centaurea iberica*, (e) *Cirsium arvense*, (f) *Cousinia haeckeliae* (Cu: cuticle, Ep: Epidermis, Pa: parenchyma, Co: collenchyma, Ch: chlorenchyma, Sc: sclerenchyma, Xy: xylem, Ph: Phloem). Scale: 10µm

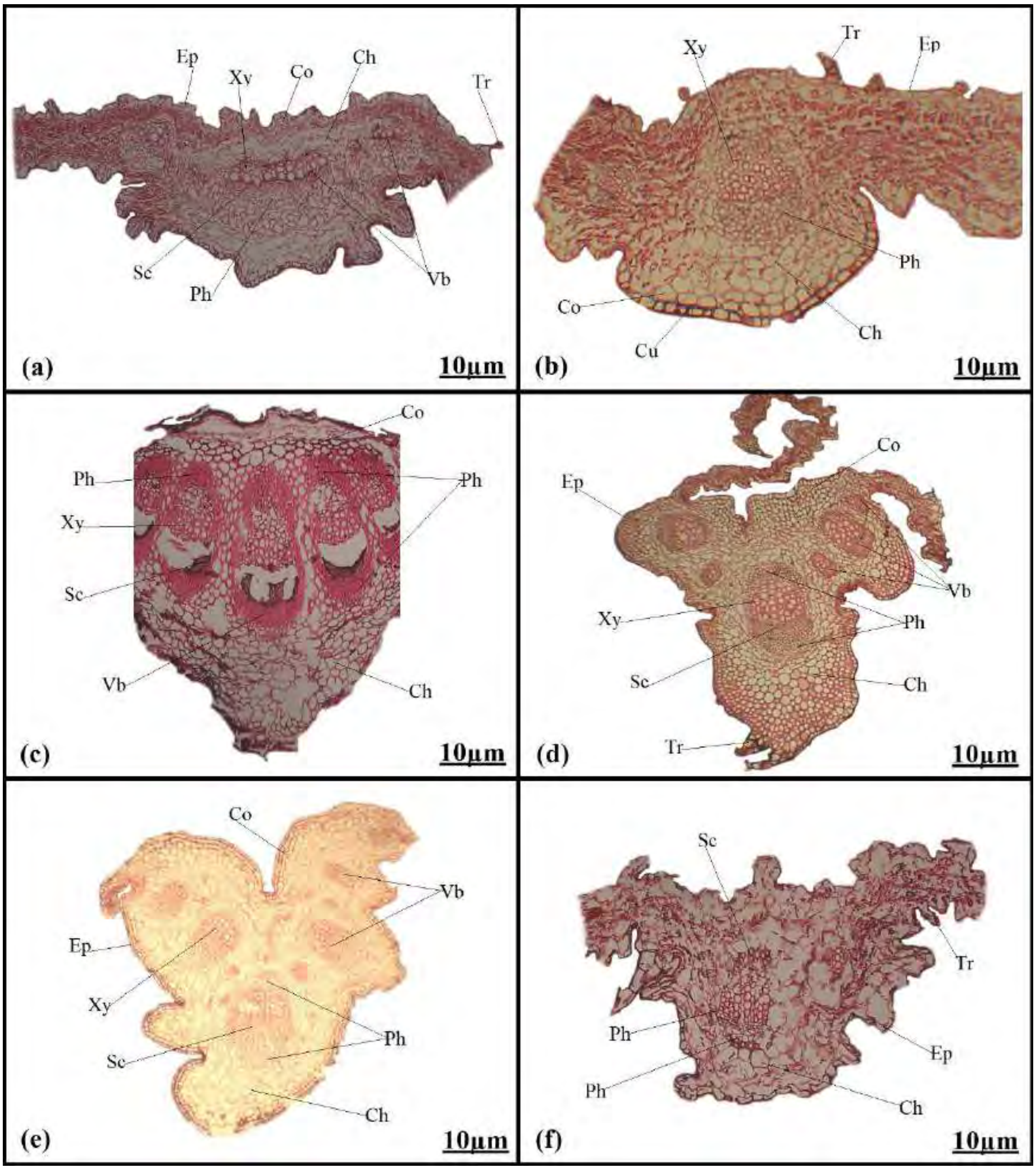

Plate 66. Photomicrographs of petiole anatomy of (a) *Crepis kotschyana*, (b) *Gymnarrhena micrantha,* (c) *Heteroderis pusilla*, (d) *Himalaiella afghani,* (e) *Himalaiella heteromalla*, (f) *Iphiona grantioides* (Cu: cuticle, Ep: Epidermis, Pa: parenchyma, Co: collenchyma, Ch: chlorenchyma, Sc: sclerenchyma, Xy: xylem, Ph: Phloem). Scale: 10µm

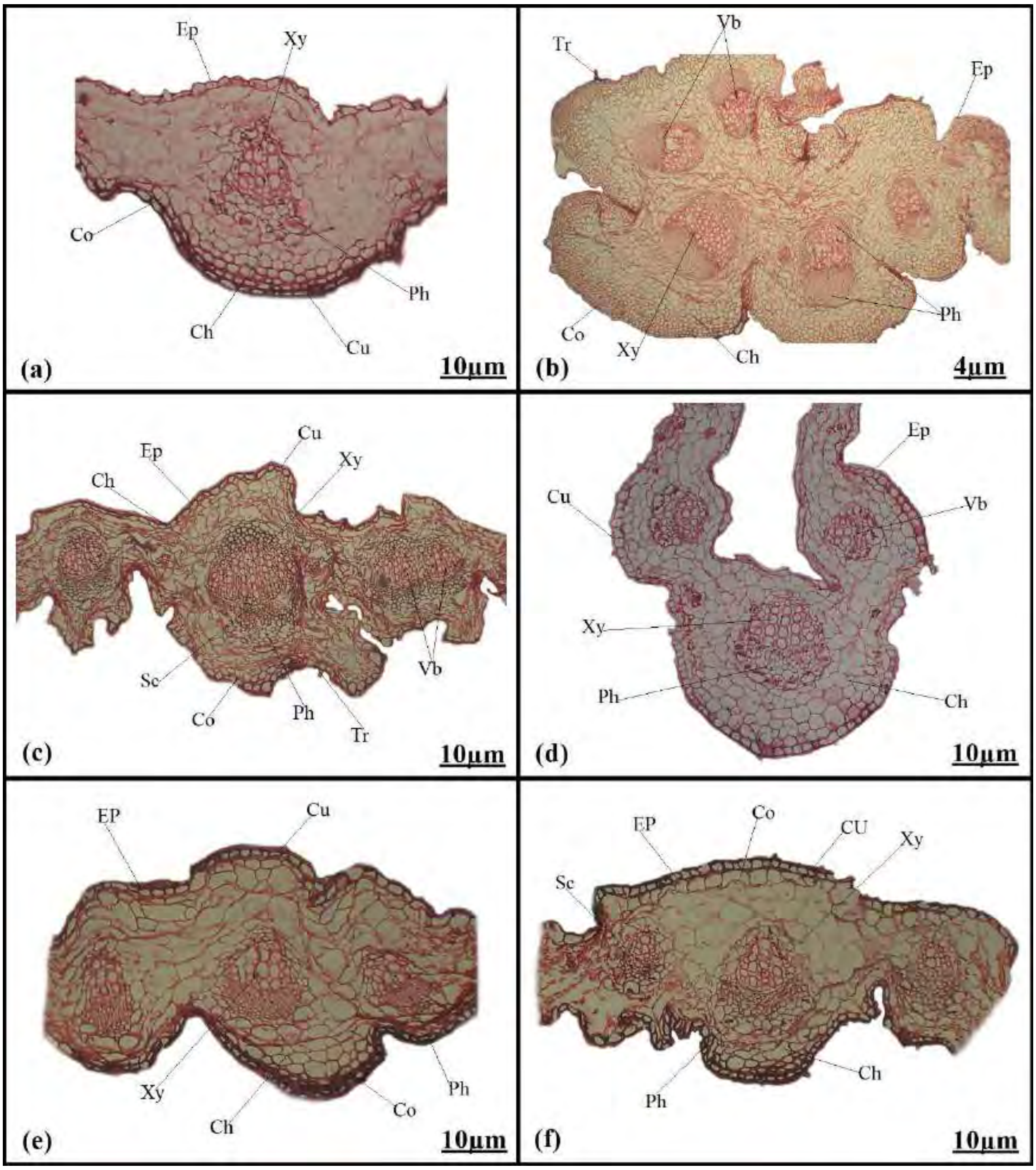

Plate 67. Petiole anatomy of (a) *Jurinea berardioidea*, (b) *Jurinea carduiformis*, (c) *Koelpinia linearis*, (d) *Lactuca dissecta*, (e) *Lactuca orientalis*, (f) *Lactuca serriola* (Cu: cuticle, Ep: Epidermis, Pa: parenchyma, Co: collenchyma, Ch: chlorenchyma, Sc: sclerenchyma, Xy: xylem, Ph: Phloem). Scale: 10µm (a, c, d, e, f), 4 µm (b)

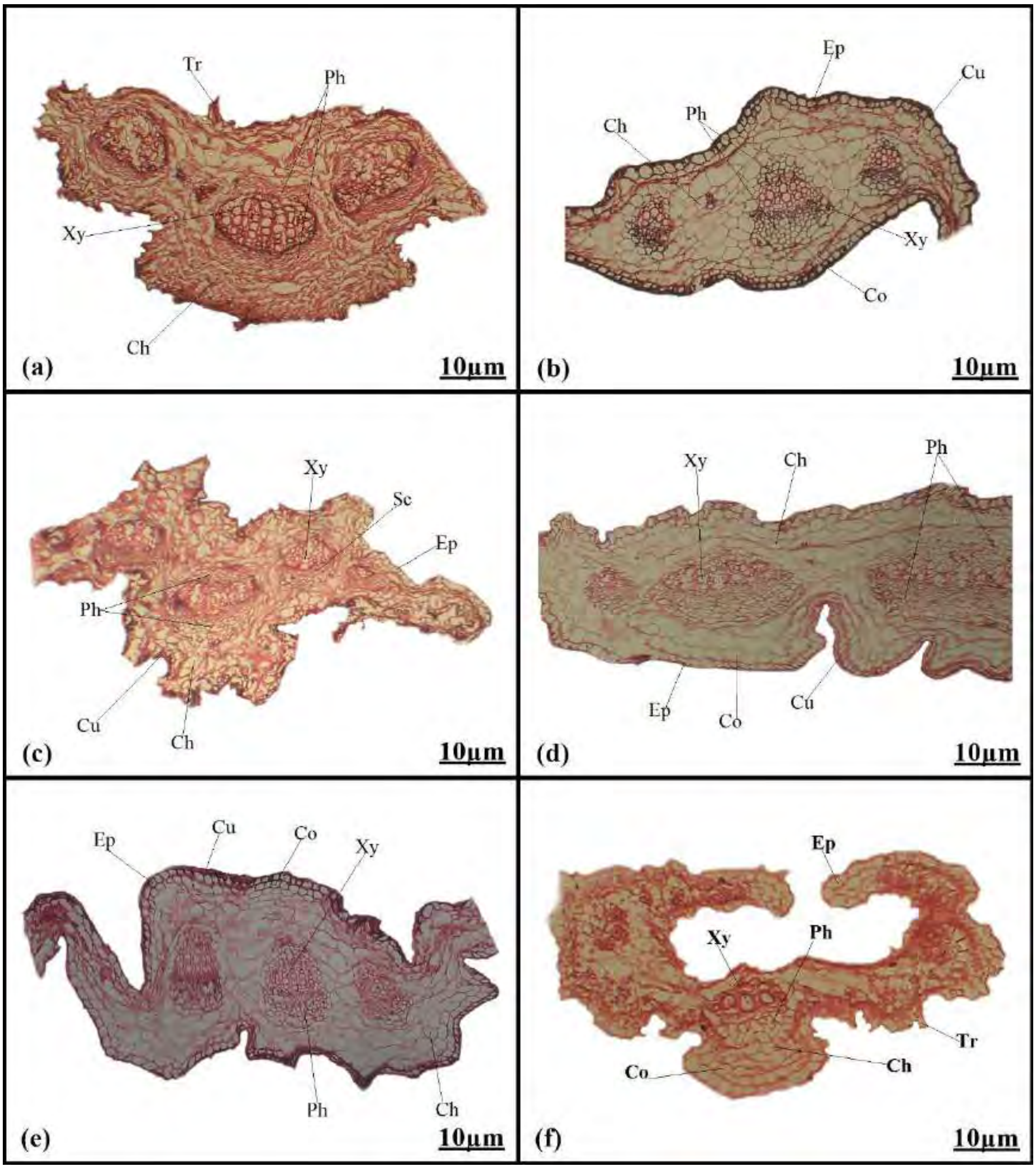

Plate 68. Petiole anatomy of (a) *Lasiopogon muscoides,* (b) *Launaea acanthodes,* (c) *Launaea aspleniifolia*, (d) *Launaea fragilis* subsp. *Fragilis,* (e) *Launaea intybacea*, (f) *Launaea oligocephala* (Cu: cuticle, Ep: Epidermis, Pa: parenchyma, Co: collenchyma, Ch: chlorenchyma, Sc: sclerenchyma, Xy: xylem, Ph: Phloem). Scale: 10µm

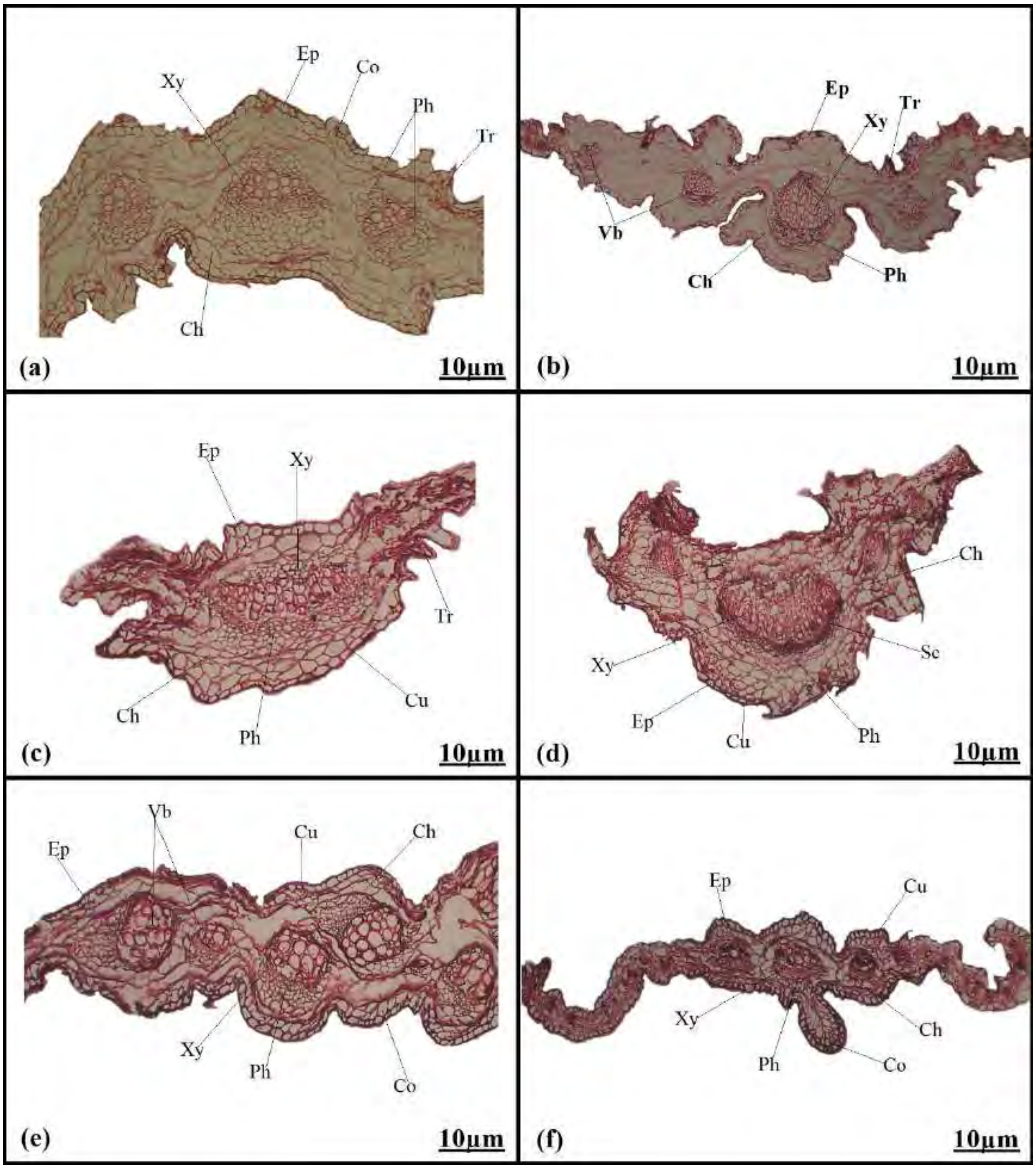

Plate 69. Petiole anatomy of (a) *Launaea procumbens*, (b) *Launaea stenocephala*, (c) *Leuzea repens*, (d) *Microcephala lamellate*, (e) *Pterachaenia stewartia*, (f) *Pulicaria angustifolia* (Cu: cuticle, Ep: Epidermis, Pa: parenchyma, Co: collenchyma, Ch: chlorenchyma, Sc: sclerenchyma, Xy: xylem, Ph: Phloem). Scale: 10µm

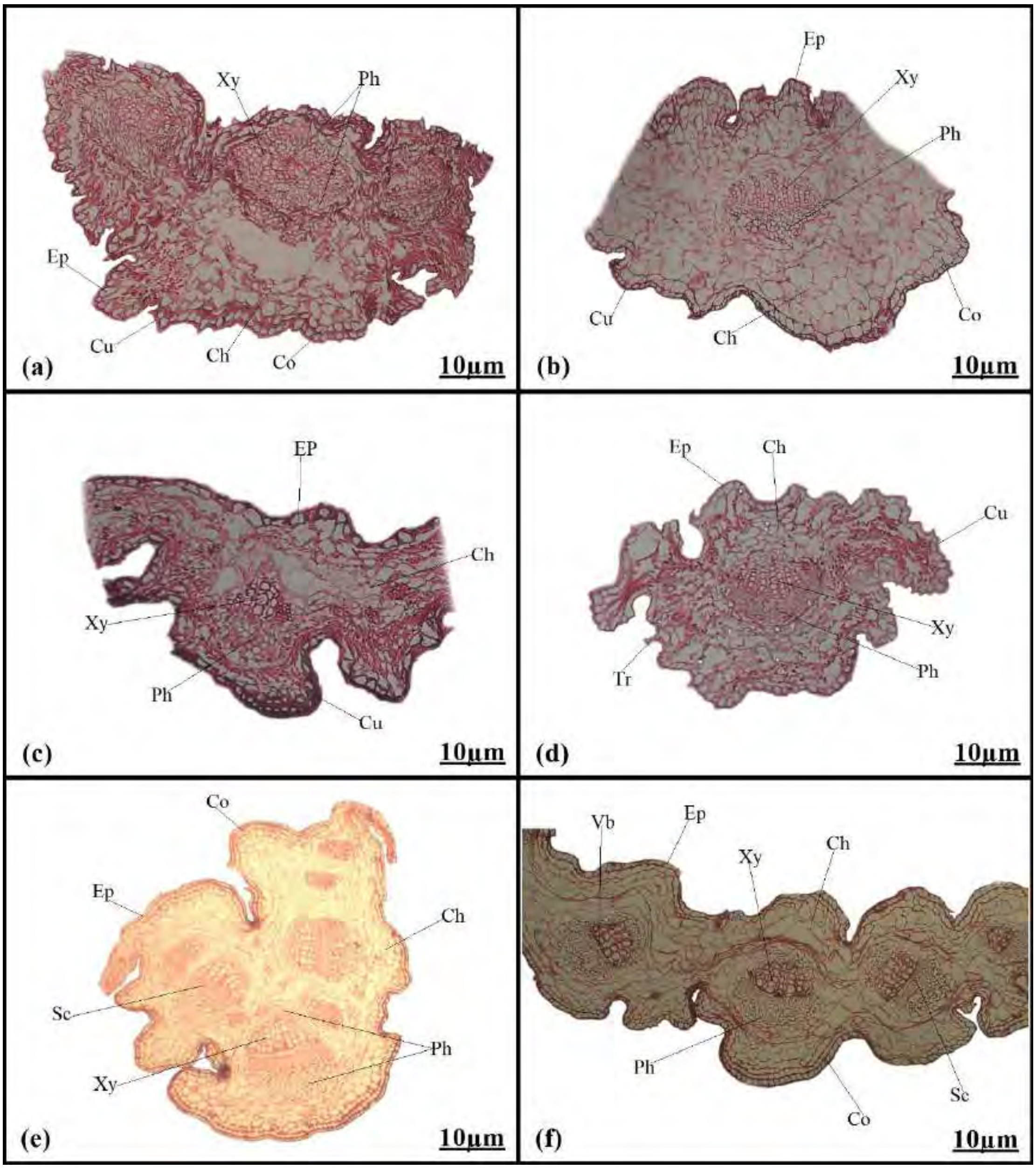

Plate 70. Petiole anatomy of (a) *Pulicaria undulata* (b) *Reichardia tingitana*, (c) *Scorzonera koelpinioides*, (d) *Seriphidium maritimum*, (e) *Sonchus arvensis*, (f) *Sonchus oleraceus* (Cu: cuticle, Ep: Epidermis, Pa: parenchyma, Co: collenchyma, Ch: chlorenchyma, Sc: sclerenchyma, Xy: xylem, Ph: Phloem). Scale: 10µm

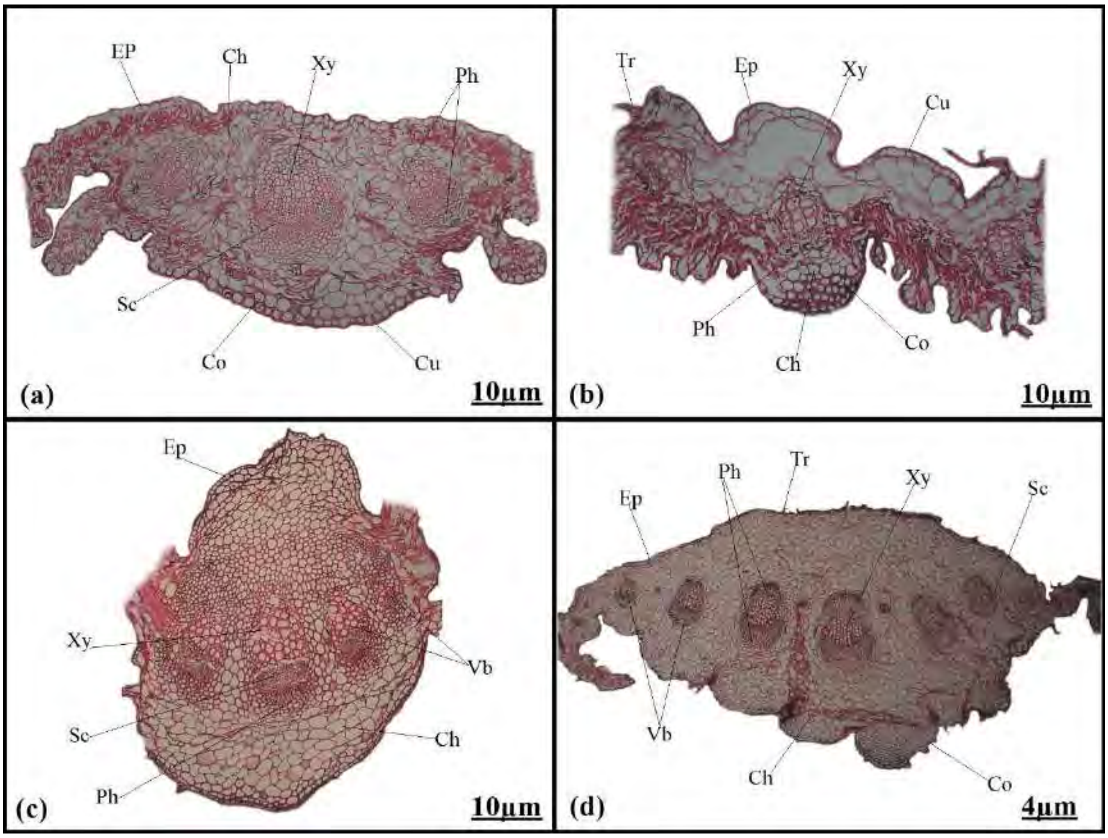

Plate 71. Petiole anatomy of (a) *Symphyotrichum subulatum*, (b) *Takhtajaniantha pusilla* (c) *Xylanthemum macropodum*, (d) *Zoegea purpurea* (Cu: cuticle, Ep: Epidermis, Pa: parenchyma, Co: collenchyma, Ch: chlorenchyma, Sc: sclerenchyma, Xy: xylem, Ph: Phloem). Scale: 10µm (a, b, c), 4µm (d)

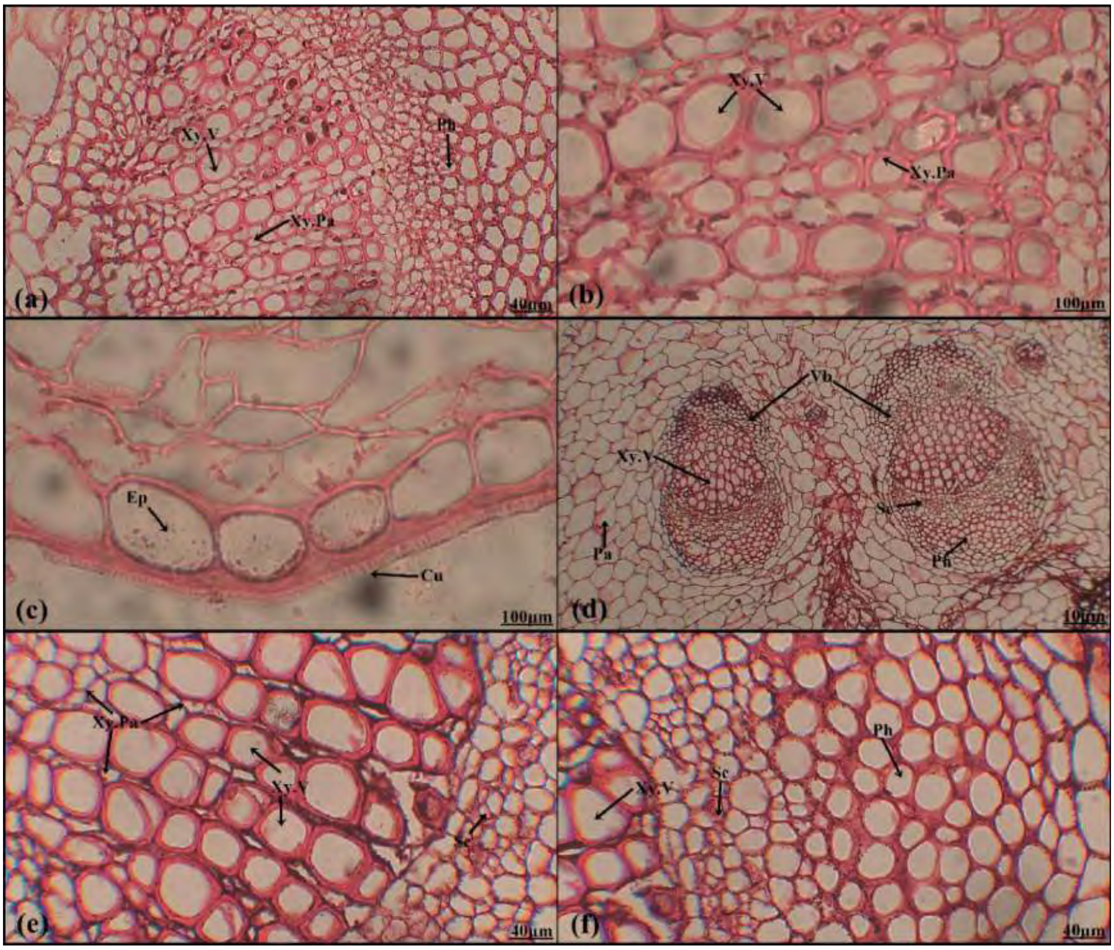

Plate 72. Petiole magnified photomicrographs of *Koelpinia linearis* (a) vascularization (b) xylem vessel and parenchyma (c) cuticle and epidermis and *Zoegea purpurea* (d) vascularization (e) xylem vessel and parenchyma (f) phloem (Xy.V:xylem vessels, Ph: Phloem, Cu: cuticle, Ep: Epidermis, Pa: parenchyma). Scale: 40 µm (a, e, f), 100 µm (b, c), 10 µm (d)

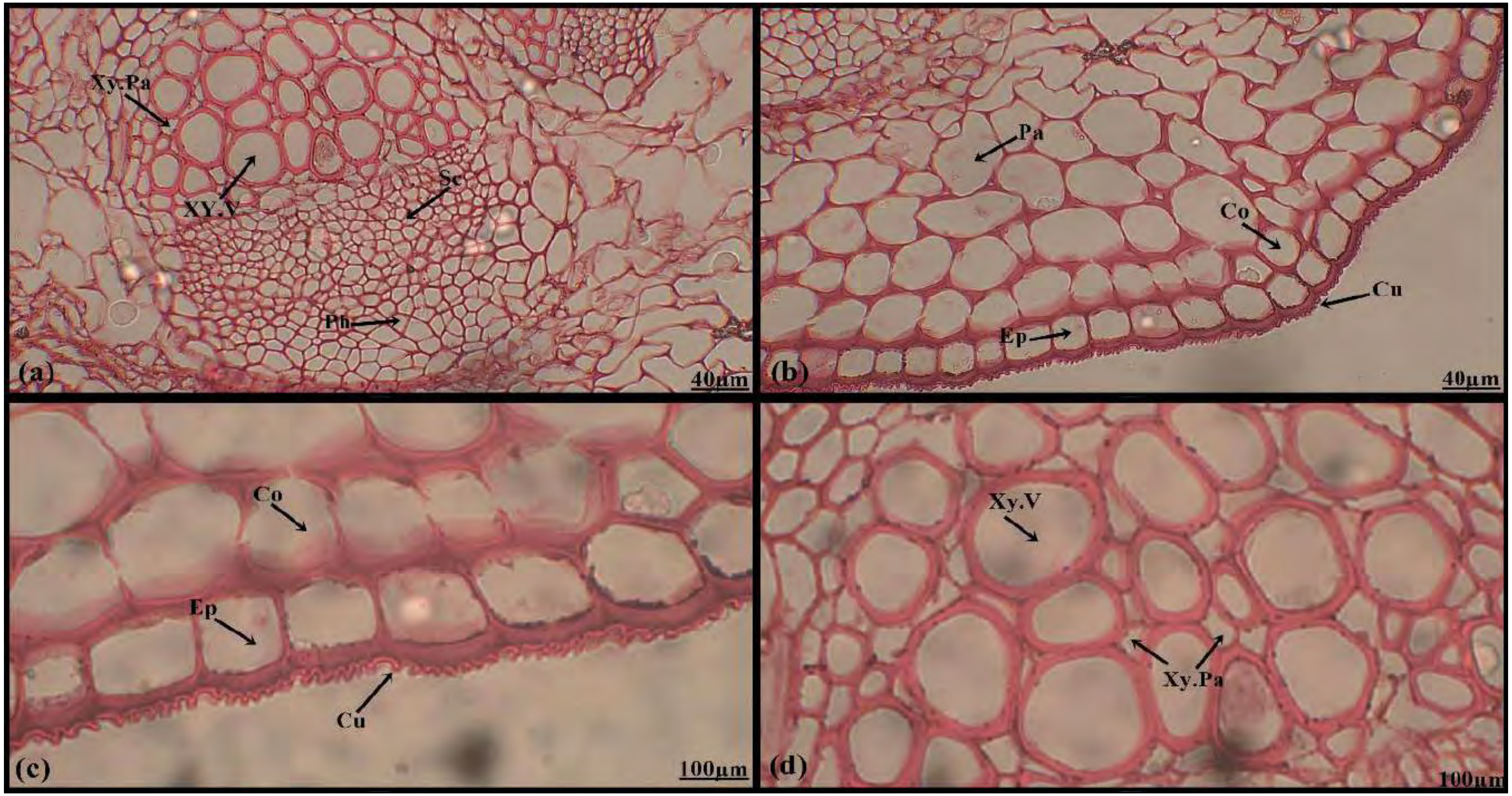

Plate 73. Magnified photomicrographs of petiole cross-section of *C. arvense* (a) vascular bundles (b) parenchyma and collenchyma (c) epidermis and cuticle (d) xylem vessels (Xy.V: xylem vessels, Ph: Phloem, Co: collenchyma, Cu: cuticle, Ep: Epidermis, Pa: parenchyma). Scale: 40 µm (a, b), 100 µm (c, d)

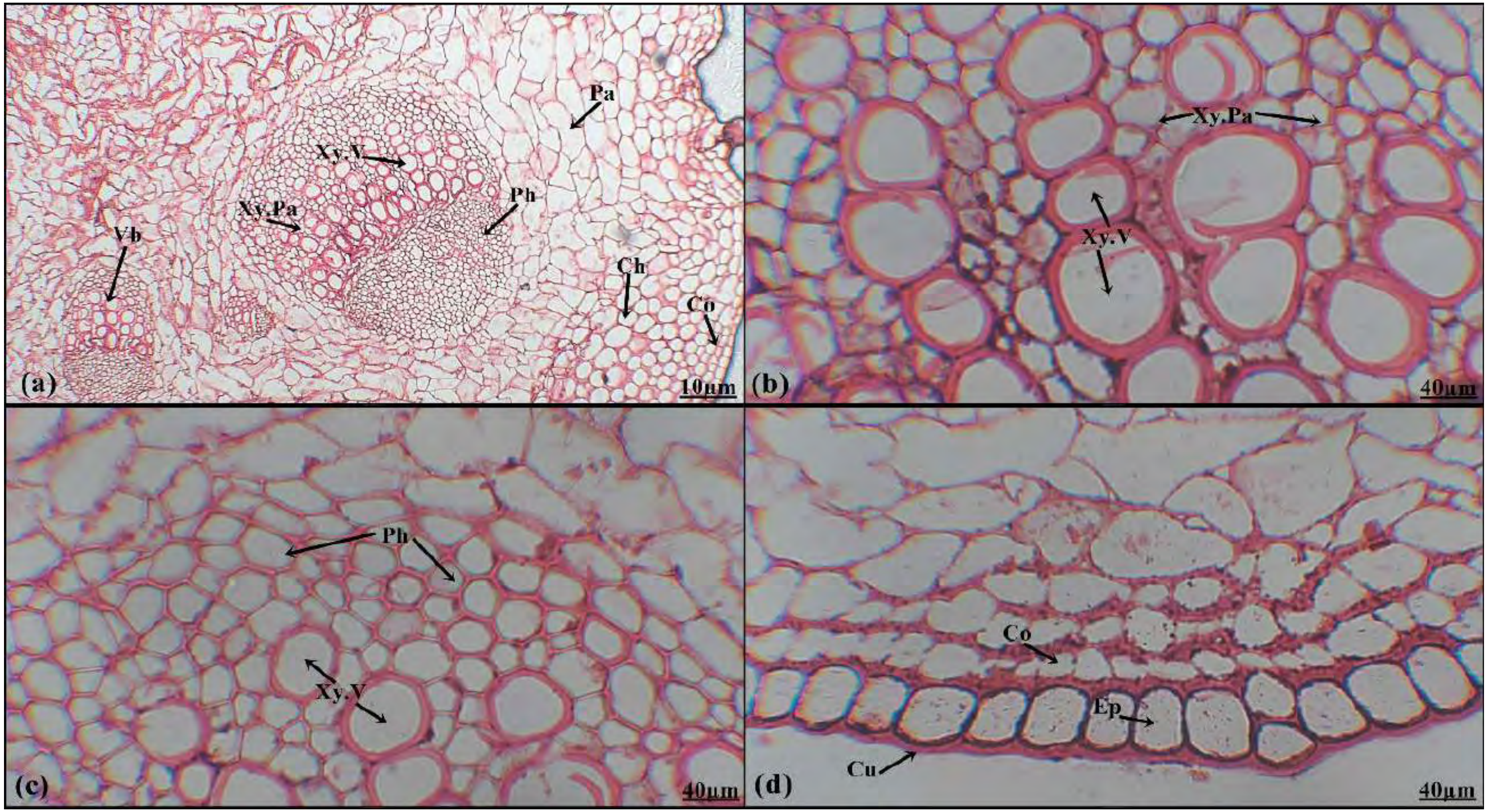

Plate 74. Photomicrographs of petiole cross-section of *J. carduiformis* (a) vascularization (b) xylem vessels and parenchyma (c) phloem and *S. oleraceus* (d) cuticle and epidermis (Xy.V: xylem vessels, Ph: Phloem, Co: collenchyma, Ch: chlorenchyma, Cu: cuticle, Ep: Epidermis, Pa: parenchyma). Scale: 10 µm (a) 40 µm (b, c, d).

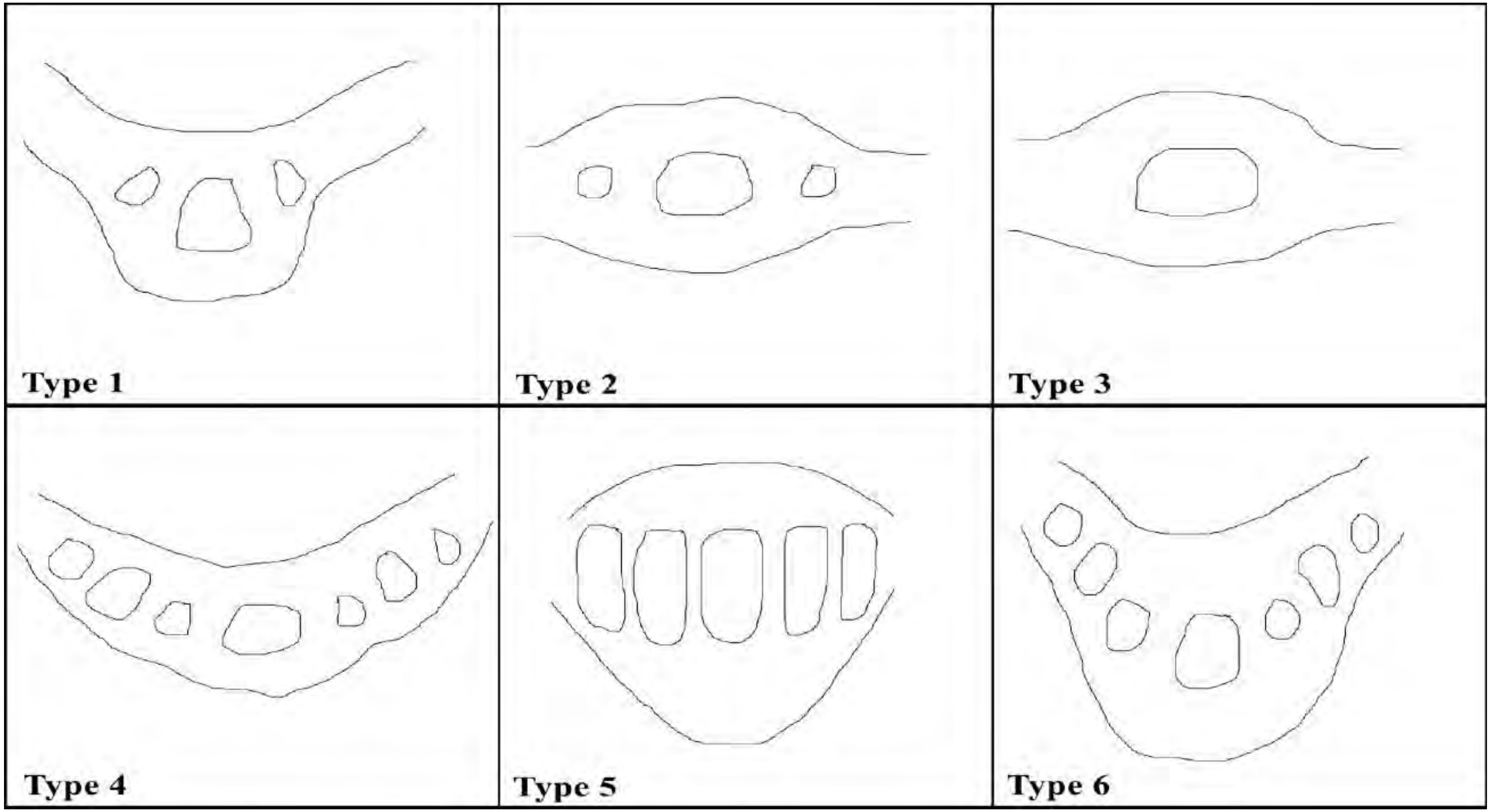

Figure 16. The observed petiole types of Asteraceae based on the contour and vascularization.

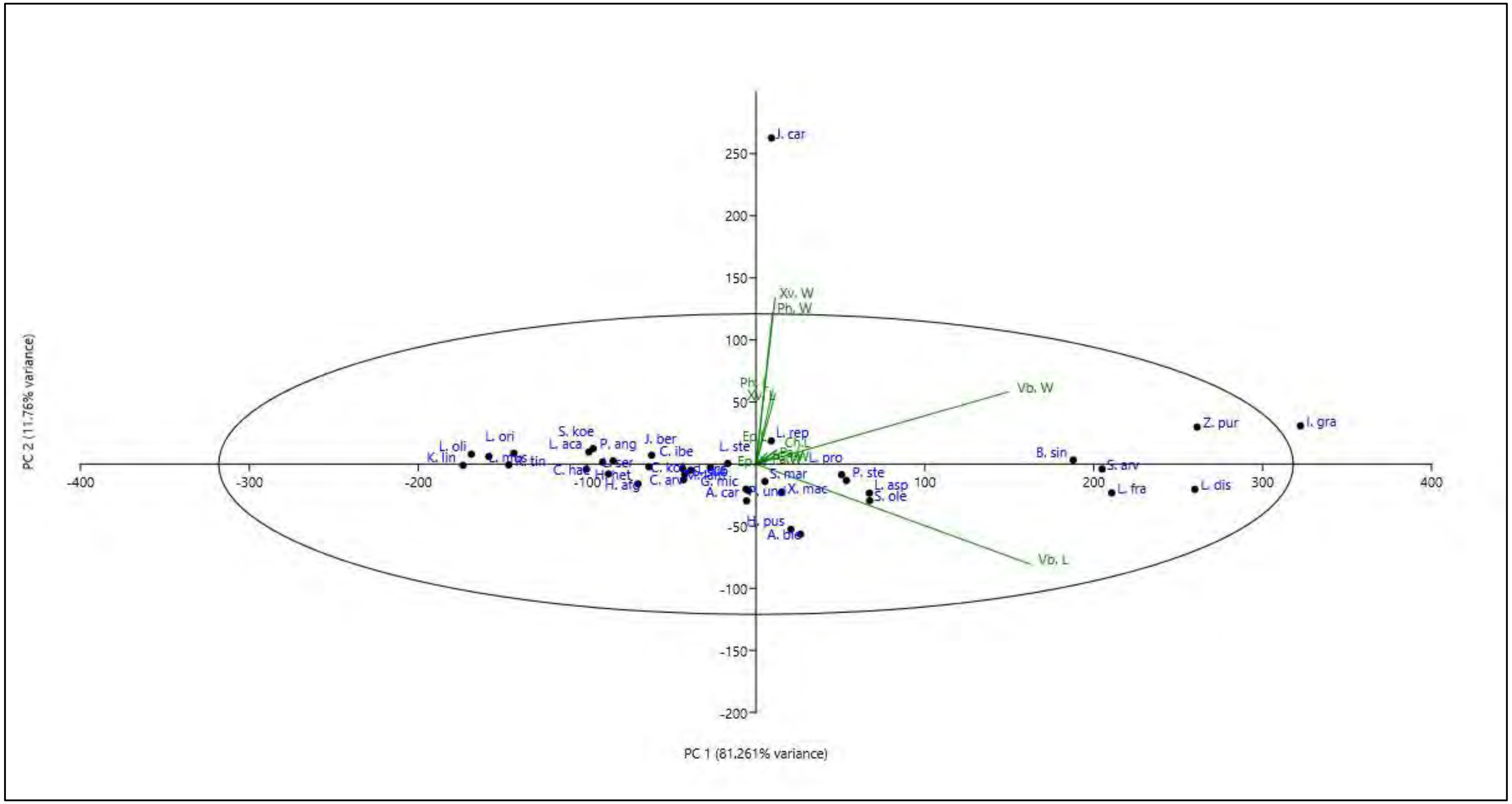

Figure 17. Utility of petiole features in discrimination among the species of Asteraceae by PCA (length and width of epidermis, parenchyma, chlorenchyma, xylem, phloem, vascular bundles)

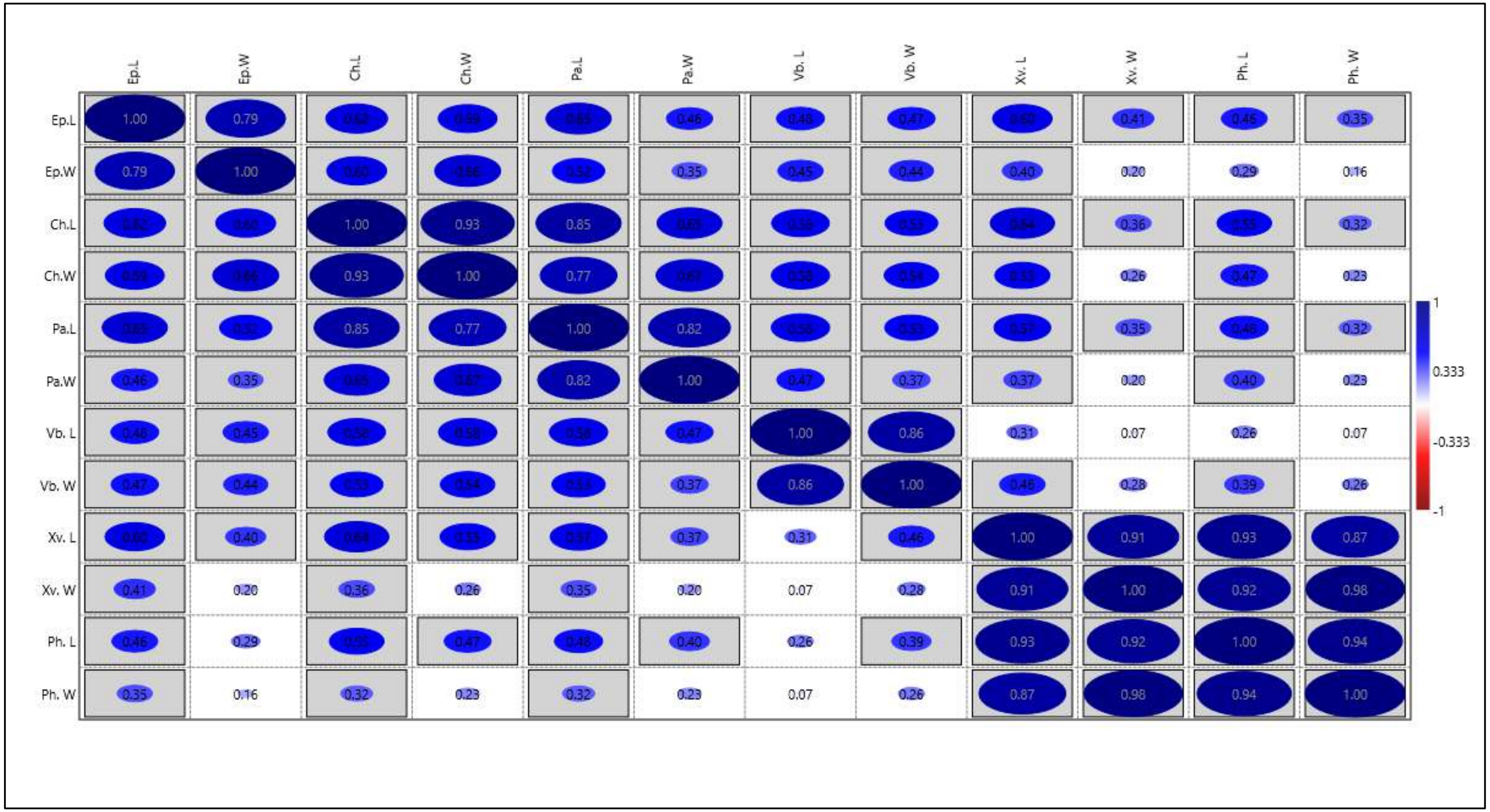

Figure 18. Correlation among mean values of length and width of L: length, W: Width, Ep: Epidermis, Ch: Chlorenchyma, Pa: Parenchyma, Vb: Vascular bundles, Xv: Xylem vessel, Ph: Phloem

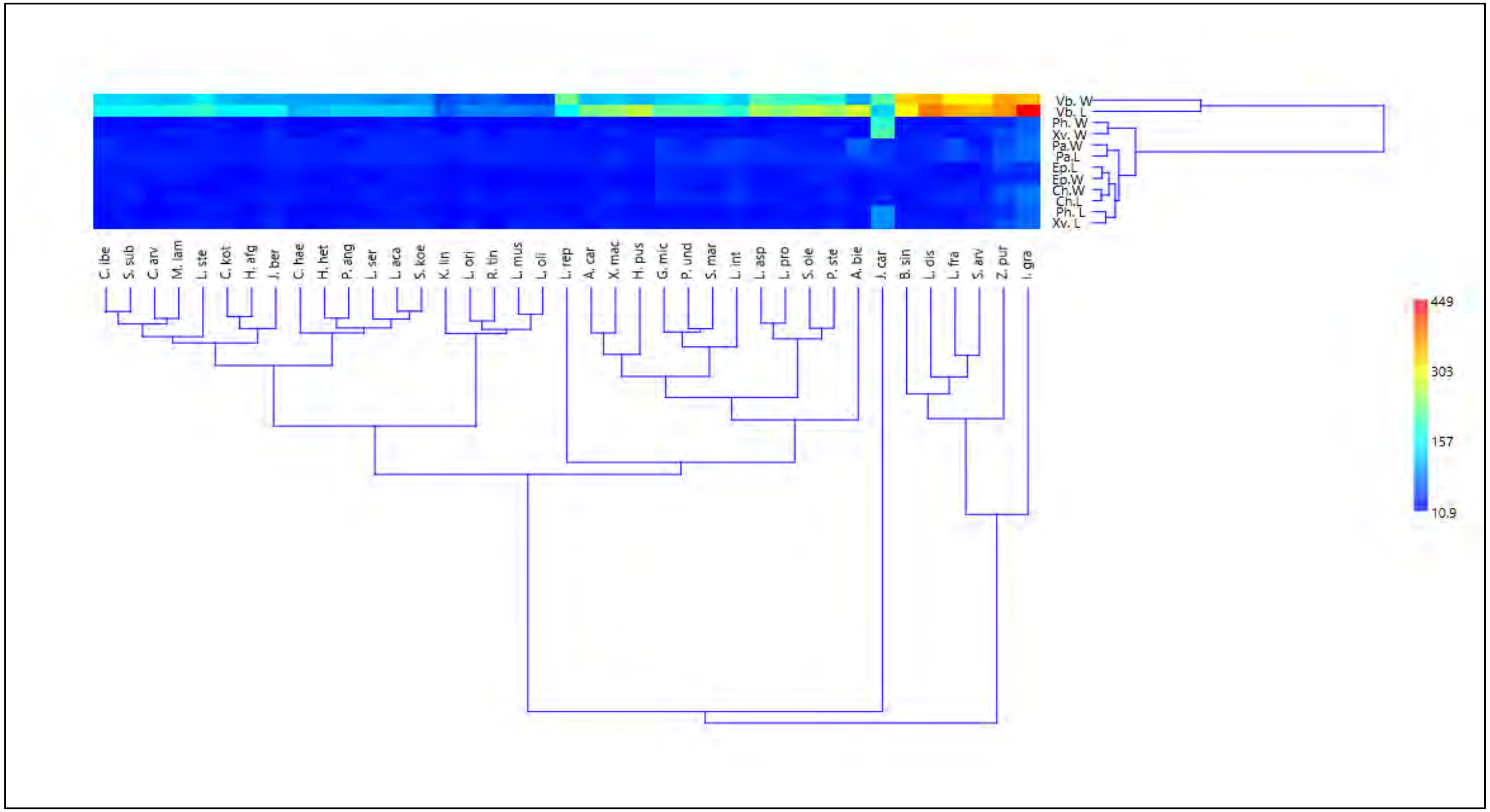

Figure 19. Dendrogram showing the similarity index of Asteraceous taxa based on quantitative parameters of the petiole

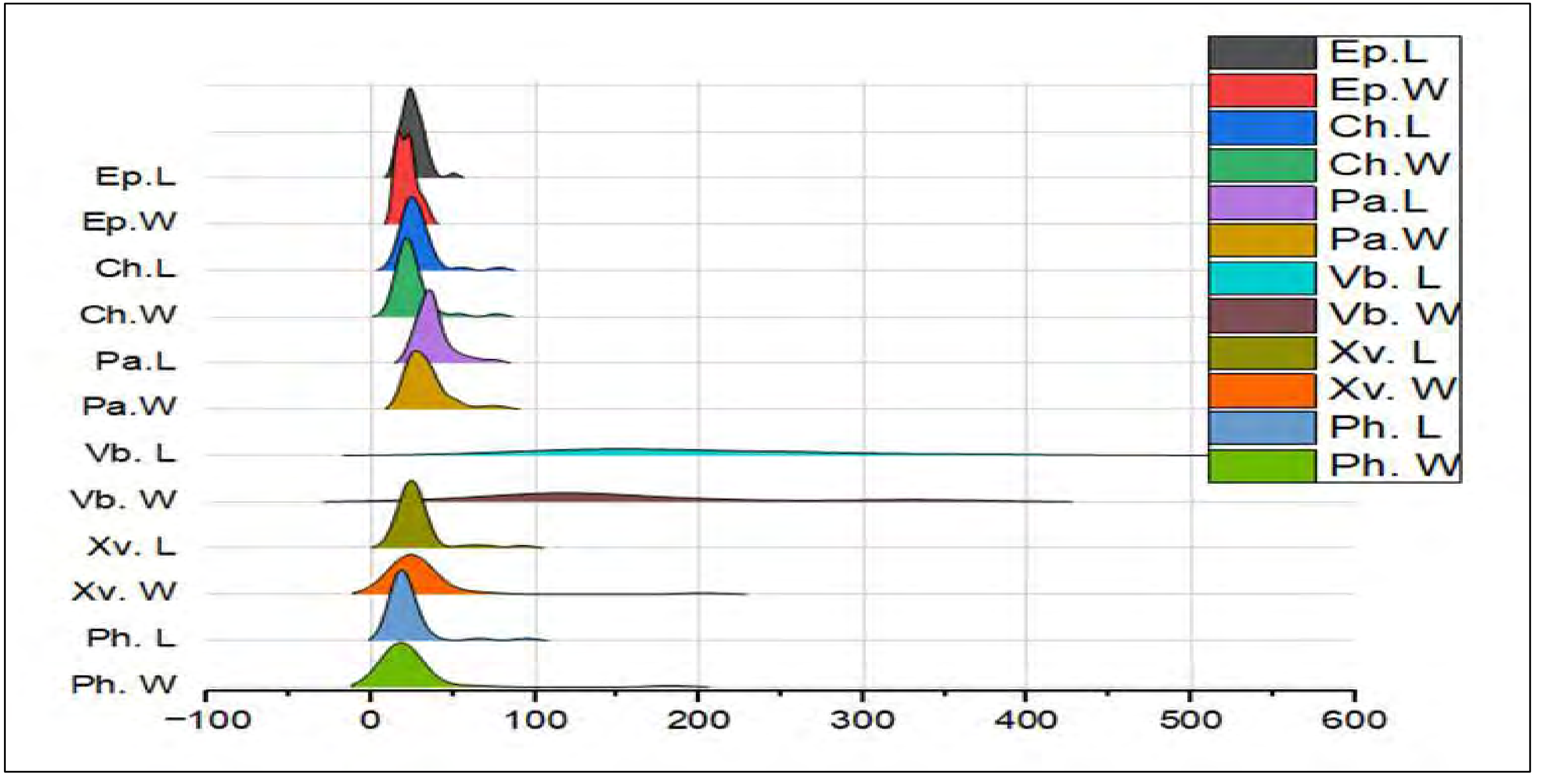

Figure 20. Ridge line plot for paired traits compared and visualised the overall distribution of petiole anatomical traits (L: length, W: Width, Ep: Epidermis, Ch: Chlorenchyma, Pa: Parenchyma, Vb: Vascular bundles, Xv: Xylem vessel, Ph: Phloem

Table 15. Qualitative anatomical observations of the Petiole of Asteraceous 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	Air spaces as	No of VB	Xy parenchyma	Ph Parenchyma
					Cichorieae						
Crepis kotschyana	+	+	2	-	2	1	+	+	1+2	+	+
Heteroderis pusilla	-	+	2	-	5	-	+	+	5	+	+
Koelpinia linearis	+	+	2	-	3	-	+	+	4	+	+
Lactuca dissecta	+	+	2	-	3	-	-	-	1+2	+	+
Lactuca orientalis	+	+	1	+	3	-	-	-	3	+	+
Lactuca serriola	+	-	1	-	3	-	-	-	1+2	+	+
Launaea acanthodes	+	+	1	-	3	-	+	-	1+2	+	+
Launaea aspleniifolia	+	+	2	-	4	-	+	-	1+2	+	+
Launaea fragilis subsp. *Fragilis*	+	+	1	-	2	-	+	+	5	+	+
Launaea intybacea	+	+	2	-	3	-	+	-	3	+	+
Launaea oligocephala	+	+	1	+	3	-	-	+	1+2	+	+
Launaea procumbens	+	+	2	+	3	-	+	+	1+2	+	+
Launaea stenocephala	+	+	1	-	2	-	+	+	5	+	+

Pterachaenia stewartia	+	+	1	-	2	-	-	+	5	+	-
Reichardia tingitana	+	+	1	+	3	-	+	-	1	+	-
Scorzonera koelpinioides	+	+	1	-	3	-	-	+	1	+	+
Sonchus arvensis	+	+	2	+	4	-	+	-	7	+	+
Sonchus oleraceus	+	+	2	+	4	-	-	-	4	+	+
Cardueae											
Atractylis carduus	+	+	2	+	2	2	+	+	1+2	+	+
Centaurea iberica	+	+	2	+	3	-	+	+	1+2	+	+
Cirsium arvense	+	+	3	+	4	2	+	+	8	+	+
Cousinia haeckeliae	+	+	1	-	2	-	-	-	1+2	+	+
Himalaiella afghani	+	+	3	+	3	-	-	-	6	+	+
Himalaiella heteromalla	+	+	2	+	3	-	+	-	5	+	+
Jurinea berardioidea	++	+	2	-	3	-	-	+	1	+	+
Jurinea carduiformis	+	+	4	+	6	-	-	-	5	+	+
Leuzea repens	+	+	-	-	2	-	+	-	1	+	-
Zoegea purpurea	+	-	2	-	6	-	+	-	7	+	+
Inuleae											
Blumea sinuate	+	+	2	+	3	-	-	+	4	+	+
Iphiona grantioides	+	+	1	-	2	-	+	+	2	+	-
Pulicaria angustifolia	+	+	1	-	2	-	-	-	1+2		
Pulicaria undulata	+	+	1	-	3	-	-	+	1+2	-	-

Anthemideae											
Artemisia biennis	+	-	-	-	2	-	-	+	1	-	+
Microcephala lamellate	+	-	1	-	3	-	+	-	1+2	+	+
Seriphidium maritimum	+	+	-	-	3	-	-	+	1	+	-
Xylanthemum macropodum	+	-	2	-	6	3	+	-	4	+	+
Gymnarrheneae											
Gymnarrhena micrantha	+	+	1	-	4	-	-	-	1+2	+	+
Gnaphalieae											
Lasiopogon muscoides	+	-	2	-	4	-	-	+	3	+	+
Astereae											
Symphotrichum subulatum	+	+	2	-	3	-	+	-	1+2	+	+

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

Table 16. Qualitative anatomical observations of the Petiole of Asteraceous taxa

Plant	Outline	Trichome shape	Cuticle structure	Ep shape	Pa shape	Co shape	Sc shape	VB arrangement	Xy vessel	Ph shape
					Cichorieae					
Crepis kotschyana	Sulcate	Multiseriate	Smooth	Angular	Irregular	Lamellar	Angular	Collateral open	Round	Irregular
Heteroderis pusilla	Oval	-	Undulated	Angular	Irregular	Angular	-	Collateral open	Round to oval	Angular
Koelpinia linearis	Flat	Unicellular	Undulated	Rectangular to oval	Irregular	Lacunar	-	Bicollateral	Oval and round	Oval to angular
Lactuca dissecta	Round	-	Undulated	Square	Tetra to hexagonal	Angular	-	Collateral closed	Round	Tri to hexagonal
Lactuca orientalis	Flat	-	Smooth	Square to rectangular	Isodiametric	Angular	-	Collateral closed	Oval	Rectangular to angular
Lactuca serriola	Flat	-	Undulated	Square to rectangular	Isodiametric	Lamellar	-	Collateral closed	Angular to oval	Angular
Launaea acanthodes	Flat	-	Smooth	Angular	Irregular	Angular	-	Bicollateral	Round	Tri to hexagonal
Launaea aspleniifolia	Flat	-	Undulated	Square to angular	Angular to isodiametric	Angular	-	Bicollateral	Angular	Angular to round
Launaea fragilis subsp. *Fragilis*	Flat	-	Undulated	Square to oval	Tetra to hexagonal	Lamellar	-	Bicollateral	Round	Angular to oval
Launaea intybacea	Flat	-	Undulated	Square	Tetra to hexagonal	Lamellar	-	Bicollateral	Round	Round and angular
Launaea oligocephala	Flat	Uni to multiseriate	Undulated	Square	Isodiametric	Lacunar	-	Collateral closed	Oval	Oval
Launaea procumbens	Flat	Unicellular	Undulated	Square	Tetra to hexagonal	Lamellar	-	Bicollateral	Round to oval	Tri to hexagonal
Launaea stenocephala	Flat	Uniseriate	Undulated	Square to rectangular	Irregular to isodiametric	Angular	-	Collateral closed	Oval to angular	Angular to oval

Pterachaenia stewartia	Flat	-	Undulated	Square to angular	Irregular	Lacunar	-	Collateral closed	Round to oval	Angular
Reichardia tingitana	Sulcate	-	Undulated	Square to rectangular	Isodiametric to irregular	Angular	-	Collateral closed	Round	Tri to hexagonal
Scorzonera koelpinioides	Flat	-	Undulated	Angular	Tetra to hexagonal	Angular	-	Collateral closed	Round to angular	Angular to oval
Sonchus arvensis	Sulcate	-	-	Square	Irregular	Annular	-	Bicollateral	Oval and round	Angular
Sonchus oleraceus	Flat	-	-	Square	Irregular to isodiametric	Angular to lamellar	-	Bicollateral	Oval to angular	Tri to hexagonal
Cardueae										
Atractylis carduus	Sulcate	Uniseriate	Undulated	Rectangular	Tetra to hexagonal	Lamellar	Angular to hexagonal	Amphicribral	Angular	Angular
Centaurea iberica	Sulcate	Multiseriate	Undulated	Square	Irregular	Angular	-	Collateral open	Round	Hexagonal
Cirsium arvense	Sulcate	-	Undulated	Square	Irregular	Annular	Tri to hexagonal	Amphicribral	Round and oval	Angular
Cousinia haeckeliae	Oval	-	Smooth	Square	Tetra to hexagonal	Lamellar	-	Collateral closed	Oval	Angular to hexagonal
Himalaiella afghani	Sulcate	Multiseriate	Smooth	Square	Irregular to isodiametric	Angular to annular	-	Bicollateral	Oval and round	Rectangular to angular
Himalaiella heteromalla	Sulcate	-	-	Square to oval	Isodiametric	Angular	-	Bicollateral	Round	Round and angular
Jurinea berardioidea	Sulcate	-	Undulated	Rectangular to polygonal	Tetra to hexagonal	Lamellar	-	Collateral closed	Round to oval	Angular
Jurinea carduiformis	Flat	Multiseriate	-	Rectangular to square	Angular to isodiametric	Annular and angular	-	Bicollateral	Round	Round and oval
Leuzea repens	Sulcate	Multiseriate	Undulated	Angular to square	Isodiametric	-	-	Bicollateral	Angular to oval	Angular

Zoegea purpurea	Flat	Multiseriate	Undulated	Square to angular	Tetra to hexagonal	Angular	-	Bicollateral	Angular to oval	Angular to oval
Inuleae										
Blumea sinuate	Sulcate	-	Undulated	Square to oval	Tetra to hexagonal	Angular	-	Collateral closed	Oval to round	Round to angular
Iphiona grantioides	Flat	Uniseriate	-	Rectangular	Tetra to hexagonal	Angular	-	Bicollateral	Round	Tri to hexagonal
Pulicaria angustifolia	Flat	-	Undulated	Angular	Tetra to hexagonal	Angular	-	Collateral closed	Oval	Angular
Pulicaria undulata	Sulcate	-	Undulated	Angular to oval	Irregular	Lacunar	-	Bicollateral	Angular	Angular and oval
Anthemideae										
Artemisia biennis	Circular	-	Smooth	Rectangular to square	Irregular	-	-	Bicollateral	Oval to angular	Angular
Microcephala lamellate	Sulcate	Uniseriate	Undulated	Rectangular to oval	Angular	Angular	-	Collateral open	Oval to angular	Angular
Seriphidium maritimum	Flat	Uniseriate	Undulated	Angular to square and oval	Irregular	-	-	Collateral closed	Round to angular	Angular
Xylanthemum macropodum	Oval	-	Smooth	Rectangular to square	Irregular	Angular	Hexagonal	Collateral open	Round to oval	Angular
<u>Gymnarrheneae</u>										
Gymnarrhena micrantha	Oval	Uniseriate	Undulated	Square to rectangular	Isodiametric	Angular	-	Bicollateral	Round to angular	Round
Gnaphalieae										
Lasiopogon muscoides	Sulcate	Multiseriate	Undulated	Angular	Irregular	Angular	-	Bicollateral	Oval and round	Angular
<u>Astereae</u>										

Symphyotrichum subulatum	Sulcate	-	Undulated	Angular to round	Irregular	Annular	-	Bicollateral	Oval and round	Angular to round

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

Table 17. Quantitative measurement of anatomical characters of the Petiole of Asteraceous taxa

Plant Species	L. W	Epidermal cell (µm)	Collenchyma (µm)	Chlorenchyma (µm)	Parenchyma(µm)	Sclerenchyma (µm)	Vascular bundle (µm)	Cross section (µm)	Xylem(µm)	Phloem
Min-max=Mean±SE										
Artemisia biennis	L	29.75-32.25=31.3±0.4636809	34.75-40.75=38.85±1.07703296	20.25-21.25=20.75±0.176776	50.25-56.5=52.8±1.41288711	16.25-18=17.25±0.306186218	149.75-151.25=150.5±0.25	1005-1208=1066.8±40.32790	465-498=479±6.268971207	37-39.25=38.25±0.4677071
	W	15.5-18.25=16.95±0.566789	20.75-22=21.3±0.215058132	15.25-16.25=15.7±0.1837117	66.25-69.75=68.3±0.7881941	15.25-17=16.15±0.322102468	79.5-98.75=88.9±4.0098316	807-888=848.2±13.868669	555-597=585.2±7.690253572	33.75-34.75=34.25±0.1767766
Atractylis carduus	L	23-24.5=23.6±0.25739075	20.5-22.25=21.6±0.302076149	23-24=23.5±0.176776695	23-25.25=24.15±0.375832	13.25-15.25=13.85±0.35881750	225.25-226.25=225.75±0.176	1108-1206=1165.8±23.40170	88.75-101=98.25±2.378287199	98-99.75=98.65±0.3020761
	W	20.75-22=21.3±0.215058132	22.75-23.75=23.25±0.17677669	20.25-21.25=20.75±0.176776	20.25-21.75=20.95±0.266926	9.5-12.25=11.05±0.58843011	120.75-123.75=121.95±0.514	836-889=861.6±10.371113	75.5-78=76.3±0.443001129	69.25-75.25=71.95±0.9598176
Blumea sinuata	L	31-32=31.5±0.176776695	21-22=21.5±0.176776695	13.25-16.75=14.85±0.654790043	25.25-27=26.1±0.302076149	18-19.5=18.9±0.257390754	112-128.25=120.15±3.233225943	1352-1369=1360.8±3.168595904	34-39.5=37.2±1.010569147	38.25-39.25=38.75±0.176776695
	W	24-26=24.9±0.407737661	17.75-19=18.35±0.231840462	20.25-21.75=21.05±0.289395	27.75-32.25=30.85±0.831414	12.75-13.5=13.2±0.145773797	75.5-87=81.4±2.089557369	703-801=769.4±18.451558	39.75-42.25=41.15±0.509901	32-50.25=44.15±3.288806
Centaurea iberica	L	23.5-24.5=24±0.176776695	26.25-28=27.1±0.340954542	19-22=20.1±0.515994186	30.75-36=33±0.876783896	20.25-22=21.1±0.322102468	157-163.75=160.4±1.3313	1304-1343=1317±7.422937	34-39.5=37.2±1.010569147	33.25-38.5=36.3±1.07354552
	W	24-25.75=24.8±0.348209707	23.75-24.5=24.2±0.145773797	23-25.25=23.95±0.413823634	36.75-38=37.35±0.231840462	15.25-17.75=16.45±0.463680925	126-138.75=134.9±2.315167381	893-935=910.8±7.825599019	39.75-42.25=41.15±0.509901951	32-34.5=33.75±0.447213595

Cirsium arvense	L	25.25-29.5=27.4±0.853668554	17.75-19.25=18.65±0.257390754	12.75-13.5=13.1±0.15	30.25-32.25=31.35±0.375832409	13.25-14=13.6±0.127475488	94.25-100.5=98.1±1.152714188	1352-1369=1360.8±3.168595904	47-50.5=48.95±0.755810823	40.25-43.25=41.85±0.551135192
	W	20.75-22=21.35±0.231840462	15.25-17=15.9±0.302076149	9.5-12=10.85±0.407737661	22.25-25.25=24.1±0.497493719	9.25-12.25=10.45±0.544288526	77.75-84.75=80.75±1.159202312	756-801=788±8.300602388	34-39.5=37.2±1.010569147	33.25-38.5=36.3±1.073545528
Cousinia haeckeliae	L	30.25-31.25=30.75±0.176776695	24.75-26.25=25.5±0.25	22.75-23.75=23.25±0.176776695	36.75-38.25=37.6±0.26925824	12.75-14.75=13.6±0.331662479	100.25-112=104.3±2.152614689	796-805=801±1.643167673	20.5-22.25=21.3±0.348209707	22.75-23.75=23.25±0.176776695
	W	2324.5=23.9±0.257390754	27.75-28.75=28.25±0.176776695	12.75-13.75=13.25±0.176776695	29.75-33.5=32.00.668019461	9.25-13.25=11.35±0.796868873	75.25-83.75=78.25±1.704772712	455-468=461.4±2.58069758	27-28.75=28.1±0.302076149	31.75-34=32.75±0.467707173
Crepis kotschyana	L	23.75-27.75=25.4±0.678232998	23-24.5=23.8±0.289395923	18.5-22=20.25±0.651920241	33-34.5=33.45±0.278388218	13-14=13.45±0.16583124	150.25-151.25=150.75±0.176776695	984-1004=996.8±3.42636834	57.25-63.75=61±1.294217911	69.75-75.25=72.75±0.977880361
	W	15.75-17.25=16.65±0.257390754	15.25-17.25=16.55±0.348209707	15.5-16.75=16.05±0.215058132	22.75-24.5=23.5±0.306186218	11-12.25=11.6±0.231840462	113.75-123=117.6±2.013392659	550-588=570.2±6.183849933	77-83.5=80.25±1.398660073	86.75-89.5=87.9±0.562361094
Gymnarrhena micrantha	L	55.25-59.5=57.5±0.883883476	38.75-44.5=41.25±0.965012953	41.5-47=44±1.110742995	30.5-32.25=31.3±0.348209707	13.5-14.5=13.95±0.2	202-222=210.05±3.613689251	1005-1123=1071.8±26.95069572	64.25-73.75=70.1±1.617482612	88.75-97=92±1.547174845
	W	69.25-73=71.85±0.673609679	24.25-26=24.8±0.310241841	43.5-47.25=45.85±0.668954408	24.25-25.75=24.95±0.289395923	9.25-12.25=10.2±0.538516481	126.25-138.75=132.8±2.344408241	756-805=791.4±8.969949833	75.75-88.75=83.85±2.205957842	198.75-203.75=200.8±0.940079784

Heteroderis pusilla	L	21.75-24.25=22.65±0.451386752	20.25-21.25=20.75±0.176776695	20.25-22=21.2±0.289395923	30.25-33.75=32.15±0.744143803	12.75-14.75=13.75±0.32596012	87.25-92.25=90.4±1.011187421	756-801=788±8.300602388	34-39.5=37.2±1.010569147	75.25-82=79.85±1.221167474
	W	24-26=25.15±0.358817502	15.25-17.25=16.15±0.340954542	18.25-19.25=18.75±0.176776695	21.25-24.5=22.75±0.596866819	9.25-12.25=10.2±0.538516481	50.25-55.75=53.85±1.020416582	301-355=333.6±9.031057524	50.25-57.25=54.75±1.329943608	50.25-55.5=53.45±0.966307405
Himalaiella afghana	L	33.25-34.25=33.75±0.176776695	37.75-38.75=38.25±0.176776695	32-33.25=32.65±0.231840462	44.25-47.25=46.5±0.570087713	17.75-19.25=18.65±0.257390754	126.25-138.75=133.05±2.140969407	4001-4009=4006±1.414213562-	89.5-94.25=91.9±0.808548081	88.75-99.25=94.15±1.875166659
	W	20.25-22.25=21.35±0.407737661	26.75-29.5=27.65±0.491172068	23-24.75=23.8±0.289395923	36.75-38.75=37.5±0.353553391	15.25-16.5=15.95±0.215058132	202-211.25=206±1.701102584	2509-2567=2544.2±11.74478608	200.25-204.5=201.8±0.834415963	175.25-189.5=181.9±2.842314198
Himalaiella heteromalla	L	34.25-37=35.3±0.520816666	37-39=38±0.395284708	23.75-24.75=24.25±0.176776695	31.75-33.25=32.4±0.26925824	12.75-13.5=13.1±0.127475488	125.5-139=131.8±2.756129533	666-688=676±3.728270376	36.5-47=40.2±1.780800382	42.75-45.5=43.95±0.588430115
	W	23.5-24.5=24±0.176776695	24.5-25.75=25.15±0.231840462	20.25-21.25=20.75±0.176776695	23.75-27.75=24.85±0.735696948	10.25-12=11.05±0.289395923	92.75-95.5=93.95±0.588430115	755-808=783.4±9.867117107	32-33.75=32.85±0.340954542	50.25-53.75=52.75±0.637377439
Iphiona grantioides	L	24.5-25.75=25.25±0.209165007	23-24.5=23.6±0.257390754	23.25-24.5=23.8±0.215058132	21.5-25.25=23.35±0.691917625	12.75-14.75=13.45±0.348209707	245.5-251.25=249.1±1.190063024	1352-1469=1408.8±24.55280025	97.25-101.75=99.9±0.800780869	104.25-108.75=105.8±0.792148976
	W	25.25-28=26.45±0.588430115	25.25-28.25=27±0.62249498	19.5-20.75=20.15±0.231840462	18.25-21.75=19.75±0.612372436	10.25-12.25=11.1±0.422788363	131.75-140.75=137.8±1.80520082	756-801=788±8.300602388	58.75-64.25=61.7±1.061838029	110.25-114.75=111.9±0.756637298

Jurinea berardioidea	L	25.25-27.75=26.2±0.421307489	22.75-24=23.4±0.231840462	22.75-24.25=23.65±0.257390754	17.75-19.25=18.6±0.26925824	12.75-13.5=13.1±0.127475488	150.25-156.25=154.75±1.131923142	2001-20057=9224.6±4421.696582	68.75-72.25=70.35±0.744143803	65.5-68=66.75±0.506211418
	W	20.75-23=21.6±0.407737661	15.25-16.75=16.15±0.257390754	20.25-21.5=20.95±0.215058132	10.25-11.75=11.15±0.257390754	9.25-10.25=9.7±0.16583124	75.75-83=80.95±1.316719408	405-488=452.6±13.37385509	34-39.5=37.2±1.010569147	41.25-44.75=42.9±0.589491306
Jurinea carduiformis	L	25.25-27=26.35±0.302076149	27.75-29.5=28.7±0.289395923	20.25-21.25=20.75±0.176776695	30.25-33.75=31.8±0.634428877	12.75-14.25=13.35±0.257390754	136.75-138.5=137.65±0.340954542	756-801=788±8.300602388	34-39.5=37.2±1.010569147	37.25-39.5=38.4±0.430116263
	W	18-19.5=18.6±0.257390754	23.5-24.5=24±0.176776695	11.25-12.75=12±0.25	18.25-21.75=20.1±0.567890835	9.25-10.5=9.85±0.231840462	99.75-11=104.15±2.22289226	552-557=554.4±0.92736185	39.75-42.25=41.15±0.509901951	34-36.75=35.15±0.491172068
Koelpinia linearis	L	12.75-15.25=13.75±0.440170422	20.25-22=20.95±0.310241841	15.25-17=16.45±0.329772649	24-28=25.55±0.73058196	12.75-13.5=13.2±0.145773797	62.25-64=63.1±0.302076149	1201-1222=1209±3.535533906	34-39.5=37.2±1.010569147	34.25-38.5=36.65±0.860958768
	W	15.25-16.25=15.75±0.176776695	15.25-16.25=15.75±0.176776695	17.75-19.25=18.5±0.25	32.75-35.25=33.65±0.437321392	9.25-12.25=10.2±0.538516481	49.5-51.75=50.35±0.392109679	641-653=648.2±2.39582971	33-34.25=33.7±0.215058132	38.25-39.75=39.15±0.257390754
Lactuca dissecta	L	49.5-50.5=49.9±0.20310096	31.5-32.75=32.05±0.215058132	22-27.25=25.25±0.876783896	64-66.5=64.8±0.443001129	12.75-16.25=14.75±0.693721846	60.25-63.25=61.95±0.649037749	2001-2009=2004.8±1.496662955	34-39.5=37.2±1.010569147	33.25-38.5=36.3±1.073545528
	W	34.5-35.75=35.15±0.231840462	24.25-25.5=24.85±0.231840462	22.75-24.25=23.35±0.257390754	49.75-52=51.05±0.443001129	12.25-13.5=12.95±0.215058132	50.25-55.75=52.9±1.15	1469-1505=1496.6±6.918092223	39.75-42.25=41.15±0.509901951	32-34.5=33.75±0.447213595

Lactuca orientalis	L	21.75-23=22.35±0.231840462	23-24.5=23.8±0.289395923	18.5-20.25=19.4±0.302076149	75.25-82.25=78.7±1.511208126	12.75-13.5=13.2±0.145773797	98.25-105.5=100.6±1.268857754	808-815=812.4±1.208304597	36.5-41.75=39.35±0.838898087	33.25-38.5=36.3±1.073545528
	W	12.75-13.75=13.25±0.176776695	20.25-22=21.15±0.302076149	20.25-22=21.15±0.302076149	25.25-26.25=25.75±0.176776695	9.25-12.25=10.2±0.538516481	43.5-47.25=45.75±0.869626357	1001-1005=1003±0.707106781	40.25-43.5=42.1±0.610327781	31.25-34.25=32.75±0.58630197
Lactuca serriola	L	18-20.25=19.25±0.395284708	20.5-24.5=23.3±0.73058196	18.5-20.25=19.4±0.302076149	37.75-38.75=38.25±0.176776695	12.25-13.5=13±0.237170825	76-99.25=86.9±4.567274899	808-815=812.4±1.208304597	35.75-39.75=37.7±0.713267131	37-38.25=37.45±0.242383993
	W	16-13.75=13.3±0.2	20.25-22=21.15±0.302076149	17-22-21.15=0.302076149	32-26.25=25.75±0.176776695	8.75-12.25=9.9±0.640312424	42.75-47.25=44.85±0.944060379	1001-1005=1003±0.707106781	40.25-43.5=42.1±0.610327781	31.25-34.25=32.75±0.58630197
Lasiopogon muscoides	L	20.25-22=21.2±0.320156212	19-20.5=19.75±0.285043856	17.75-19=18.3±0.215058132	32.75-48.25=41.95±3.705907446	12.75-13.5=13.2±0.145773797	125.25-130.5=127.75±0.883883476	903-912=906.6±1.691153453	57.25-63.75=60.95±1.121940284	58.25-61.5=60.05±0.598957428
	W	12.75-13.75=13.25±0.176776695	14.25-16.25=15.5±0.353553391	16.75-18=17.35±0.231840462	25.25-26.25=25.75±0.176776695	9.25-12.25=10.2±0.538516481	97-100.5=98.7±0.70445014	401-404=402.8±0.583095189	48.25-54.75=50.55±1.110742995	37.25-38.5=37.95±0.215058132
Launaea acanthodes	L	20.25-22.25=21.25±0.395284708	20.5-22=21.3±0.289395923	15.25-17.25=16.4±0.358817502	50.25-52=51.35±0.302076149	12.75-13.5=13.2±0.145773797	75.5-98.25=84.5±4.10411379	704-815=773.8±20.60922124	36.5-41.75=39.35±0.838898087	34-39.5=36.25±1.277203977
	W	17.23-16.25=15.75±0.176776695	20.25-22=21.15±0.302076149	17.88-22=21.15±0.302076149	32.77-33.75=30.7±1.496662955	9.25-12.25=10.2±0.538516481	43.5-47.25=45.6±0.816241386	1001-1005=1003±0.707106781	40.25-43.5=42.1±0.610327781	30.25-32=31.15±0.280624304

Launaea aspleniifolia	L	17.75-18.75=18.25±0.176776695	35.75-38=37.15±0.392109679	36.75-38.5=37.45±0.310241841	55.5-59.75=57.85±0.853668554	12.75-13.5=13.2±0.145773797	250.25-251.5=250.85±0.231840462	129-1125=915±196.5408355	112-113.25=112.65±0.231840462	100.25-109.25=104.85±1.528070679
	W	22.75-23.75=23.25±0.176776695	22.75-24.5=23.6±0.340954542	22.75-24.5=23.7±0.348209707	50.25-57.75=53.75±1.464155046	9.75-12.25=10.75±0.425734659	200.5-205.75=202±0.961769203	808-815=812.4±1.208304597	132.25-138.75=135.75±1.106797181	97.75-99.25=98.5±0.25
Launaea fragilis subsp. *Fragilis*	L	26.25-30.5=29.15±0.768927825	22.75-23.75=23.25±0.176776695	17.25-22.75=20.35±0.950657667	25.5-30.25=27.95±0.979157801	16.25-17.75=17±0.25	112-121.25=116.55±1.723731418	55.25-69.5=61.4±2.396090566	35.75-44.25=39.85±1.354621718	38.75-48.25=44.5±1.58113883
	W	19.75-22=20.65±0.375832409	17.25-19.5=18.2±0.365718471	15.5-22.75=19.1±1.310057251	19.75-21=20.45±0.215058132	12.75-14=13.4±0.231840462	55.5-57=56.3±0.266926956	38.75-48.25=44.5±1.58113883	33.25-40.25=36.85±1.141818725	50.25-69.5=61.4±2.396090566
Launaea intybacea	L	37-44.75=39.35±1.40668049	24.5-29.75=27.4±0.979795897	24.5-29.75=27.4±0.979795897	16.75-22=19.65±1.008092258	38.75-42.25=41.35±0.654790043	18.25-21.25=20.05±0.514781507	62.175-62.725=62.455±0.109373214	68.25-83.25=74.2±2.520416632	47-55.25=49.85±1.509552914
	W	29.75-32.75=31.35±0.533853913	29.5-33.75=32.2±0.755810823	25.25-33.75=29.75±1.476905549	18.75-22=19.65±1.008092258	31-33.75=32.75±0.506211418	14.75-17=16.1±0.4	31.275-32.575=31.715±0.257948638	47-56.25=51.6±1.870160421	33.75-47=41.6±2.224297642
Launaea oligocephala	L	21.75-24.25=22.85±0.451386752	19.5-20.75=20.15±0.231840462	15.25-17=16.2±0.348209707	30.75-33=32±0.433012702	16.75-18.25=17.4±0.26925824	67.75-75.25=72.15±1.226274847	49.6-50.225=49.9±0.105770979	59.5-79.25=67.85±3.7220626	54.75-63.5=59.3±1.558043645
	W	14.75-20.5=17.2±0.982344135	22-25.5=23.45±0.672681202	21-24.5=22.85±0.620483682	21.75-25.5=23.7±0.639335593	12.75-15.5=14.3±0.55	43.5-47=45.25±0.575543222	37.075-38.875=37.875±0.338101316	38.75-49.25=44.25±1.879162047	38.75-43.5=41.15±0.917196816

Launaea procumbens	L	50.25-61.25=53.35±2.025771458	38.75-44.25=42.1±0.96695398	18.25-21.25=20.1±0.533853913	46-50.75=47.9±0.838898087	34.5-38.75=36.65±0.860958768	54.75-64.25=59.05±1.727353467	23.775-24.35=23.995±0.121295095	62-69.5=65.1±1.45043097	59.25-67=62.65±1.271318214
	W	33.75-38.75=35.75±0.925337776	29.25-33=31.60.635413251	13-16=14.65±0.503736042	43-47.25=45.1±0.714142843	24.5-29.25=26.35±0.808548081	37.75-43=40.7±0.913099118	10.15-11.45=10.83±0.226025441	46.25-56=50.75±1.97008883	39.5-49=43.9±1.578369412
Launaea stenocephala	L	19.5-22=20.45±0.443001129	19.75-33.75=26.5±2.891798748	20.25-23=21.45±0.48347699	29.5-32.75=30.6±0.578791845	12-14.25=13.05±0.428660705	38.75-51.25=45.8±2.11896201	27.525-29.325=28.51±0.312409987	20.75-28.75=25.25±1.526843148	18.25-20.25=19.4±0.331662479
	W	17.25-20.5=18.7±0.614410286	13.75-15.75=14.7±0.374165739	16.5-18.25=17.4±0.322102468	19.5-20.75=20.15±0.231840462	9.5-11.25=10.25±0.306186218	25.75-31.25=28.8±0.902773504	20.1-21.125=20.445±0.188314896	13.75-21.25=17.2±1.347219359	12.75-17.75=15.45±0.830662386
Leuzea repens	L	19.75-21.25=20.5±0.316227766	13.75-15.5=14.6±0.340954542	17.25-20.25=18.55±0.496235831	21.75-25.75=23±0.733143915	12.25-13.75=13±0.25	16-20.25=18.35±0.682825014	76.25-88.75=82.9±2.181742423	32.25-47=37.95±2.548774215	32.25-41.25=36.75±1.56324982
	W	15.75-18.75=17.15±0.573367247	15.75-20.5=18.55±0.88529656	12-14=13±0.395284708	16-20.25=18.35±0.682825014	9.75-13=11.6±0.610327781	13.25-13.75=13±0.25	44.75-56.25=50.6±2.19317122	30.5-37.25=34.1±1.174201857	24.5-29.5=27.25±0.851469318
Microcephala lamellata	L	11.5-16=13.4±0.816241386	22-31.25=26.3±1.822429697	19.25-22.25=20.45±0.514781507	29.75-39=35.2±2.081766077	13.5-15.5=14.5±0.395284708	76-91.25=83.9±2.82577423	21.075-24.975=22.24±0.731505297	44.75-54.25=48.9±1.619413474	39.5-50.75=45.7±2.132193706
	W	12.25-19.5=15.15±1.393287479	15.5-18=16.7±0.470372193	13-17.25=15.2±0.764035339	24-28=25.55±0.691013748	8.5-10.75=9.55±0.456891672	50.25-56.75=52.7±1.192161902	10.175-11.2=10.68125±0.218034544	34.25-42=37.45±1.507066687	31.25-37=34.25±1.092588669

Pterachaenia stewartii	L	26.75-36=33±1.684488053	19.75-22.25=21±0.487339717	24.25-30.5=26.85±1.312440475	33-37.75=35.15±0.785015923	17.75-20.5=18.95±0.508674749	69.25-75.75=72.05±1.099431671	33.05-33.875=33.37±0.153582226	42.25-47.25=45±0.836660027	41.5-46=44.25±0.840386816
	W	20.75-28.25=24.55±1.2	12.75-15.5=14.15±0.503736042	17.75-22=20.4±0.772981242	18.5-22.25=20.1±0.6254998	12.75-15.25=14.05±0.463680925	42.25-47=44.4±0.853668554	17.625-18.9=18.36±0.215435605	34-40.5=37.95±1.124722188	29.5-36.25=32.8±1.2
Pulicaria angu stifolia	L	16.75-18.5=17.7±0.320156212	25.75-31.25=28.85±1.05652733	29.5-33.75=31.35±0.785015923	35.75-38=37.15±0.392109679	16-20.25=18.35±0.785015923	42.75-46.75=45.1±0.714142843	62.675-65.85=64.045±0.643010498	47-53.25=50.6±1.108489964	48-53.25=50.6±1.108489964
	W	18-20.75=19.45±0.520816666	20.75-24.5=22.25±0.689202438	21.5-23.5=22.55±0.357071421	19.75-23.25=21.65±0.664266513	12.75-15.5=14.25±0.541987085	33-36=34.8±0.532681894	49.7-50.4=50.1±0.113743132	44.25-50.5=47.85±1.171537451	34-43.75=38.45 - ±1.642026187
Pulicaria undulata	L	24.5-34.75=29.1±1.7741195	29.75-33.25=31.4±0.610327781	25.75-30.75=28.3±0.867467579	19-22=20.7±0.577711	16-18.25=17.25±0.379143772	61-75.25=67.95±2.506740912	31.225-31.95=31.52±0.136565003	46-58.5=51.05±2.801115849	42.25-51.75=45.85±1.646207763
	W	19.25-22.75=20.65±0.718505393	14.75-22=17.95±1.307191646	17.25-22=19.75±0.832916562	32.25-36.25=34.45±0.751664819	10.75-15.5=12.95±0.959817691	40.75-47=43.95±1.273283158	17.825-21.375=18.955±0.668365544	39.5-44=42.45±0.792148976	39.75-43=41.95±0.577711
Reichardia tingitana	L	29.25-36.75=32.15±1.3430376	19.75-33.75=26.3±2.5450933	21.25-29.75=23.8±1.5956973	34.75-37.75=36.2±0.5326818	15.25-20.25=17.5±1.0428326	70.5-75.25=72.65±0.98298016	15.825-19.425=16.785±0.6659861	39.75-49.25=43.9±1.698528775	34.75-39=37.1±0.785015923
	W	18-22=20.15±	13.5-21.75=17.9±1.743918003	12.75-15.5=14.1±	17.75-21.75=19.5	12.25-14=13.15±0.322102468	35.25-37.75=36.4	11.225-11.95=11.6	36.25-41.75=38.55	33.5-39.25=36.1

		0.654790043		0.478278162	5±0.695521387		±0.471699057	1±0.126144758	±1.028955781	±0.998749218
Scorzonera koelpinioides	L	38.75-43=41.75±0.77055175	29.5-31=30.3±0.289395923	19.75-23=21.1±0.605185922	37-39=38.1±0.407737661	19.75-23=21.35±0.594768863	46.75-55.5=51.55±1.531747368	31.425-35.575=33.81±0.769845764	37.25-49.5=42.9±2.308408543	32-48.75=43.4±2.975525164
	W	20.5-23=21.85±0.465026881	29.75-32.75=31.45±0.5385164	19-23=20.75±0.733143915	20.5-23=21.9±0.465026881	12-13.75=12.75±0.3061862	35.75-38.75=37.5±0.5	19.025-22.05=19.9±0.558513652	29.75-38=34.5±1.451292527	29.5-38.25=33.05±1.54393976
Seriphidium maritimum	L	34.5-37.75=35.55±0.577711	25.5-33.75=29±1.541103501	18.5-22.25=20.25±0.794512429	34.5-38=36.8±0.619475585	17-19.75=17.95±0.48347699	44.75-59.5=51.15=2.723279273	23.025-24.15=23.63±0.197705589	43.75-49.75=47±1.033803656	36.5-46.75=41.45±1.743559577
	W	20.5-23=21.75±0.403112887	19.25-25.25=21.8±1.2708265	13.5-16.5=15.15±0.5037360	18.25-23=20.35±0.768927825	14.75-16=15.45±0.215058132	43.5-49.25=46.05±1.05	10.025-11.05=10.575±0.17084349	34.75-41.5=37.25±1.234908904	29.75-38.5=33.5±1.43396304
Sonchus arvensis	L	36.75-38.75=37.65±0.4077376	31.75-33.5=32.5±0.32596012	19.75-23=21.3±0.555652769	33-41.25=37.95±1.4564511	11.75-15.25=13.6±0.6873863	106-114=110.6±1.435270009	24.675-27.2=25.88±0.465658136	39-73.25=59.9±6.024221941	42.75-46=44.55±0.60930288
	W	19.75-22=20.75±0.379143772	19.75-24.5=21.95±0.8529361	31.5-33=32.2±0.289395923	20.5-24.75=23.1±0.760756	9.5-11=10.2±0.266926956	63.75-71=68.1±1.228820573	12.175-13.875=13.025±0.3201562	33.75-38.5=36.25±0.869626357	31.5-39=34.8±1.307191646

Ep: Epidermis, Pa: Parenchyma, Co: Collenchyma, Sc: Sclerenchyma, VB: Vascular bundles, Xy: Xylem, Ph: Phloem, L: Length, W: Width

Table 18. Dichotomous key based on petiole anatomical characters of Asteraceous taxa

Link character	**Leads**	**Characters**	***Taxa/ Go to link character***
1	+	Oval outline	*Heteroderis pusilla*
	-	Outline not oval	2
2	+	Round outline	*Lactuca dissecta*
	-	Outline other than round	3
3	+	Sulcate outline	*4*
	-	Flat outline	*6*
4	+	Trichome present	*Crepis kotschyana*
	-	Trichome not observed	5
5	+	Collenchyma angular	*Reichardia tingitana*
	-	Collenchyma annular	*Sonchus arvensis*
6	+	Vascular bundles collateral closed	7
	-	Vascular bundles bicollateral	12
7	+	Epidermal cells square shape	*Launaea oligocephala*
	-	Epidermal cells other shapes	8
8	+	Angular epidermal cells	*Scorzonera koelpinioides*
	-	Epidermal cells not angular	9
9	+	Parenchyma square to angular	*Pterachaenia stewartia*
	-	Parenchyma square to rectangular	*10*
10	+	Lamellar collenchyma	*Lactuca serriola*
	-	Angular collenchyma	11
11	+	Cuticle smooth	*Lactuca orientalis*
	-	Cuticle undulated	*Launaea stenocephala*
12	+	Lacunar collenchyma	*Koelpinia linearis*
	-	Collenchyma other than lacunar	*13*
13	+	Xylem vessel angular	*Launaea aspleniifolia*
	-	Xylem vessel not angular	*14*
14	+	Round and angular phloem	*Launaea intybacea*
	-	Phloem cells other than round and angular	15

15	+	Angular to oval phloem cells	*Launaea fragilis* subsp. *Fragilis*
	-	Tri to hexagonal phloem cells	16
16	+	Epidermal cells angular	*Launaea acanthodes*
	-	Epidermal cells square	*17*
17	+	Tetra to hexagonal parenchyma	*Launaea procumbens*
	-	Irregular to isodiametric parenchyma	*Sonchus oleraceus*

3.6 Petiole anatomy of Boraginaceous Flora from Baluchistan

3.6.1 Results

The petiole anatomy of 14 Boraginaceous taxa was examined. The characteristics both qualitative and quantitative were studied.

a) External characteristics

Most studied petioles were observed with wings (Plates 75, 76, and 77). Among the four studied *Heliotropium* species, *H. curassavicum* and *H. bacciferum* have winged petioles. However, the petioles of *H. campanula* and *H. crispum* were without winged structures. *P. intermedium var. intermedium* and *R. sessiliflora* were also lacking the winged petioles. The presence or absence of grooves in the petiole discriminated the taxa. The groove was present in all studied species except *H. crispum, H. curassavicum,* and *O. limitanea var. major.* The outline of the petiole was noted with sulcate (8), flat (4), and oval (2) shapes. In *H. bacciferum* and *H. curassavicum,* the sulcate shape was observed. In contrast, distinct flat and oval-shaped petioles were present in *H. campanula* and *H. crispum* respectively. Both *O. limitanea var. limitanea* and *O. limitanea var. major* had sulcate shape petioles. The second species with an oval outline was *R. sessiliflora*.

Prominent trichomes were observed in 6 species. Unicellular trichomes were present in 3 species, *O. limitanea var. limitanea, P. intermedium var. intermedium,* and *T. indicum*. Uniseriate types of trichomes were present in *H. crispum* and *O. limitanea var. major*. In species *C. mucronanthera* the multiseriate trichomes were observed. The cuticle was either undulated or smooth in Boraginaceous species. In *Heliotropium crispum* (Plate 78) the undulated type of cuticle was visible under light microscope on magniffication100 µm. The length of the petiole varied from 553.4 µm in *H. curassavicum* to 12004.8 µm in *Lappula spp.* The width of the petiole was minimum in *H. curassavicum* 953.2 µm and maximum in *H. crispum* 9003.8 µm.

b) Cellular composition

In the internal structures, the shapes and number of layers of epidermal cells, collenchyma cells, chlorenchyma cells, and parenchyma cells were investigated. The sclerenchyma presence in vascular bundles, number, and arrangement of vascular bundles, air spaces, and sub-epidermal ring of collenchyma were also examined. The epidermal cells were round, oval, rectangular, angular, and isodiametric, and the combination of these shapes. In most of the species, the angular shape was present along with other shapes. There was a single layer of collenchyma in the 11 taxa. In *A. tinctoria* subsp. *Tinctoria, H. crispum,* and *Lappula spp,* there were 2 collenchyma layers. The subepidermal ring of collenchyma was observed in *H. crispum* and *H. campanula.* Angular collenchyma was present in 9 plants. Followed by lamellar (2), annular (2), and lacunar (1). *H. campanula* was distinguished from other *Heliotropium* species for lamellar collenchyma. In both varieties *O. limitanea var. limitanea* and *O. limitanea var. major* similar angular collenchyma were observed. The lacunar type of collenchyma was present only in *C. mucronanthera*.

The parenchyma number of layers ranged from 1 to 7. The maximum parenchyma layers, 7, were present in *H. campanula,* whereas the minimum,1, was observed in *O. limitanea var. limitanea.* The shape of the parenchyma was mostly irregular to angular and isodiametric. Chlorenchyma was present in 1 or 2 layers in the studied Boraginaceous plants. There was no prominent layer in *C. lanceolatum.* In most species, the sclarification was not seen as prominent. In *A.tinctoria* subsp. *tinctoria* irregular sclerenchyma and in *T. indicum* angular sclerenchyma was present. The presence of air spaces or gaps or air cavities was present in five species *C. lanceolatum, G. hispida, O. limitanea var. major, P. intermedium var. intermedium,* and *T. indicum.*

c) Vascularization

In the anatomy of the petiole, the arrangement of vascular bundles, their number, and size were important in the distinction of taxa. In the examined species the vascular bundles were arranged in collateral closed (7), amphicribral (5), bicollateral (1), and collateral open (1) patterns. The vascular bundle in *H. bacciferum* was amphicribral, while the collateral closed type was present in the remaining three *Heliotropium* taxa. The two varieties *O. limitanea var. limitanea* and *O. limitanea var. major* were also separated based on the distinct vascularization i.e amhpicribral in the former and collateral closed type in the later one. The number of vascular bundles was 1 in all the studied species. This feature cannot be used to delimit the studied Boraginaceous taxa. The variation in the size of the vascular bundle can be utilized for the differentiation among the species. The xylem vessel shapes were round, oval to square, rectangular, and angular. The observed phloem cell shapes were mostly angular, trigonal to hexagonal (Table 4, 5).

3.6.2 Discussion

The anatomical and palynological data is commonly employed for morphotype identification. It has been shown in multiple recent studies to be useful in the taxonomic delimitation of species, including pollen morphology and petiole anatomical research. These traits have been applied to the classification of numerous Boraginaceae species (Teke and Binzet, 2017). The petiole anatomy of the examined Boraginaceous species provided distinct traits for the characterization of these species. There were differences in the qualitative and quantitative anatomical features which added to the taxonomic exploration of the examined species. The petiole's structure especially the median area that contains vascular bundles, can serve as a taxonomic characteristic (Akcin et al., 2004).

Light microscopy was utilized by Kasem (2015) and Yousaf et al. (2021) to distinguish between different *Heliotropium* species based on anatomical and palynological data. In the present study, light microscopy was successfully employed in the comparative examination of internal petiolar structures. Akcin et al. (2004) studied the petiole anatomy of *Trachystemon orientalis*. There was a single epidermal cell layer, with a thick cuticle. They observed a central large vascular bundle along with three small vascular bundles towards the sides or wings. There were well-developed parenchymatous and cortical regions.

Mazari et al. (2018) distinguished the 4 *Heliotropium* species based on the differences in both palyno-anatomical features. Currently, the amphicribral vascular bundles were noted singly in *H. bacciferum.* Other significantly different anatomical traits were petiole outline and collenchyma shapes. *H. curassavicum* and *H. bacciferum* were sulcate in outline. *H. campanula* is flat, while *H. crispum* was an oval-shaped outline. Kandemir et al. (2020) studied the leaf and stem anatomy of seven *Heliotropium* species. They concluded that for this genus, the stomatal size, number of hypodermis layers, collenchyma, cortex cells, palisade, and spongy layers, and trichomes were important for species identification and have taxonomic significance. They emphasized the importance of stem and leaf anatomical characteristics for determining the degree of taxonomic similarity amongst *Heliotropium* species. The anatomy of *Heliotropium* species was highly significant in the taxonomy of the genus (Abbasi et al., 2011). They suggested that *H. transoxanum* may be regarded as a subgroup of *H. dasycarpum* and inferred that two species, *H. aucheri* and *H.*

carmanicum, were separate species. Stem anatomy, in conjunction with data on trichomes and pollen, suggested useful taxonomic methods for distinguishing between this genus' species. *H. longiflorum* was distinguished for its noteworthy anatomical details. The findings additionally demonstrated a perfect affinity between the two *H. jizanense* and *H. lasiocarpum* species (Kasem, 2015).

Systematics study in the rare genus *Onosma* is difficult (Koyuncu et al., 2013). Teke and Binzet (2017) studied the foliar anatomy of three endemic species of *Onosma*. Daironas et al. (2014) examined the morphology, leaf, and stem anatomy for the distinction of medicinally important *Onosma caucasica*, and *Onosma sericea*. They reported that the anatomical features of both species were similar to those of Boraginaceae anatomy. Each variety may be distinguished by the corolla's and the downy leaf blade's severity as well as the hair's structure. In the present investigation, different petiolar anatomical features clearly distinguished the two examined *Onosma* variants of the same species, *O. limitanea*. The variety *limitanea* have amphicribral vascular bundles arrangement whereas the variety *major* have collateral closed vascular bundles. The distinct vascular bundles proved highly useful in taxonomic studies (Elkiran, 2023).

The petiole of *P. intermedium var. intermedium* was flat in shape, with unicellular trichomes. The cuticle was undulated. The vascular bundles were closed collaterally. The petiole anatomy from different floristic regions revealed more than one vascular bundle. AL-Hadeethi et al. (2016), and Akcin et al. (2004) observed more than one vascular bundle in the petiole cross-section. The presence of a single vascular bundle in the petiole of the Boraginaceous species from the dry lands of Baluchistan was a distinct feature of this family in contrast to the members of the same family from other floristic regions. This feature might be an evolutionary adaptation in the study area. The area was scarce in the availability of water. There was little literature on the anatomical features especially the petiole anatomy of Boraginaceae. There was no documentation on the shapes of the various types of cells and other important aspects such as vascular bundle arrangement, number, and petiole shape.

PCA score plots on a unique axis system identify variable clusters and categories and aid in the visualization of data set trends (Kim et al., 2015). Principal component analysis was strengthened by correlation loading plots, which were essential for determining the link between the mean values. To identify phylogeny, Uga et al. (2009) investigated the relationships between the root anatomical features using PCA plots. While correlation loading plots, which displayed significant levels and visualized explained variance, aid in the interpretation of correlation between PCs and variables, loading plots identify positive and negative correlations (Kim et al., 2015). PCA accounted for 74.04% and 25.59% sum of square variance between PC1 and PC2 (Figure 19). The quantitative anatomical traits were found significant in species discrimination. The dendrogram was delineated into two major clusters (Figure 20). *H. crispum* and *H. campanula* were in the same cluster. In the same way, the two *Onosma* varieties were in a single cluster. The xylem cells' width and length were negatively correlated with epidermis length, width, and petiole length. Similarly, the vascular bundles were negatively correlated with petiole length and width. While parenchyma width was positively associated with vascular bundle width (Figures 21 and 22). As a result, it is determined that there were statistically significant differences between the means of the parameters that were examined; this can be utilized as a methodical approach to taxonomic delimitation.

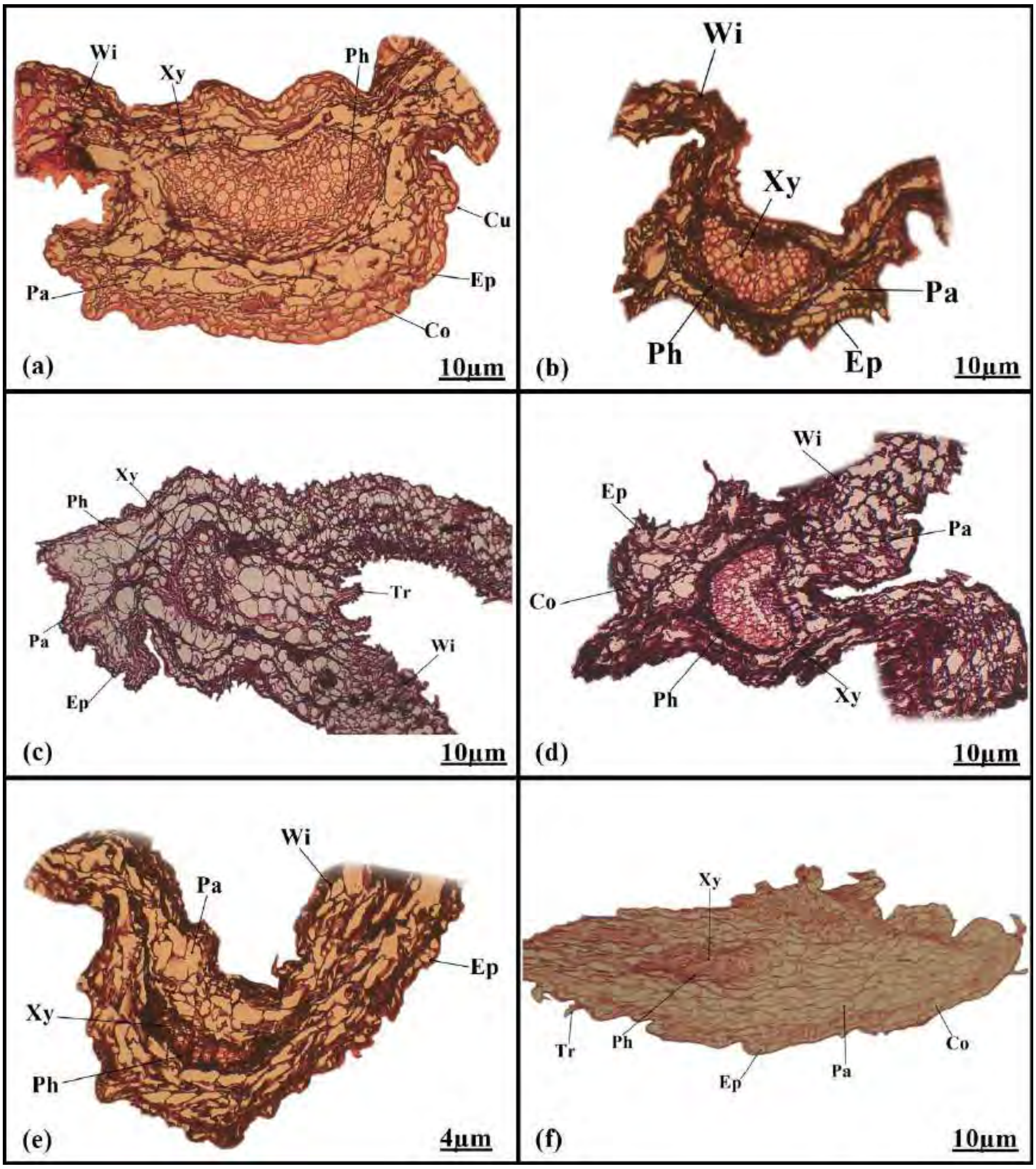

Plate 75. Petiole anatomy of (a) *Alkanna tinctoria* subsp. *Tinctoria*, (b) *Cynoglossum lanceolatum*, (c) *Caccinia mucronanthera*, (d) *Gastrocotyle hispida*, (e) *Heliotropium bacciferum*, (f) *Heliotropium campanula* (Cu: cuticle, Ep: Epidermis, Pa: parenchyma, Co: collenchyma, Ch: chlorenchyma, Sc: sclerenchyma, Xy: xylem, Ph: Phloem). Scale: 10µm (a, b, c, d, f), 4µm (e)

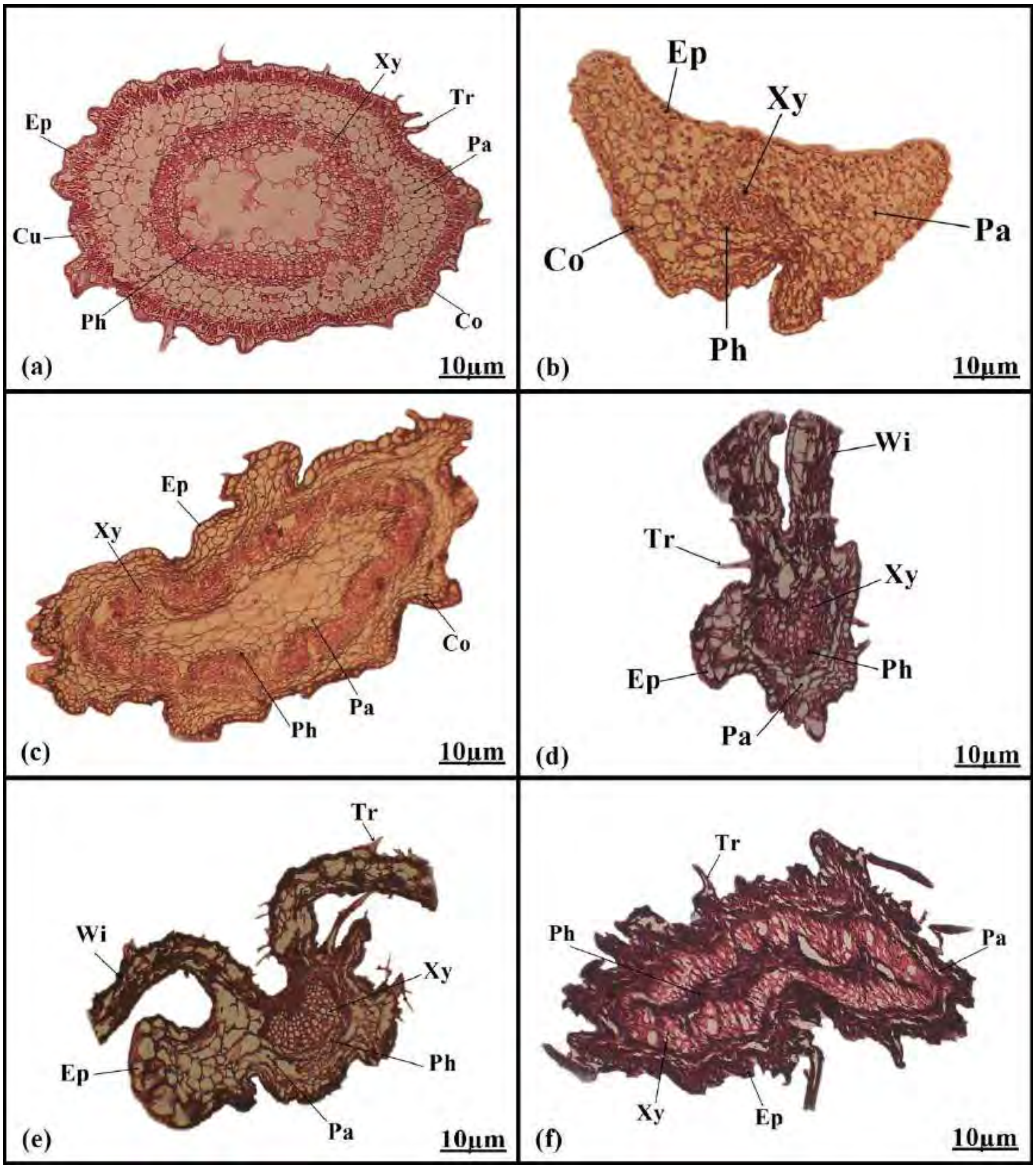

Plate 76. Petiole anatomy of (a) *Heliotropium crispum,* (b) *Heliotropium curassavicum*, (c) *Lappula spp*, (d) *Onosma limitanea* var. *limitanea*, (e) *Onosma limitanea* var. *major*, (f) *Paracaryum intermedium* var. *intermedium* (Cu: cuticle, Ep: Epidermis, Pa: parenchyma, Co: collenchyma, Ch: chlorenchyma, Sc: sclerenchyma, Xy: xylem, Ph: Phloem). Scale: 10µm (a, b, c, d, e, f)

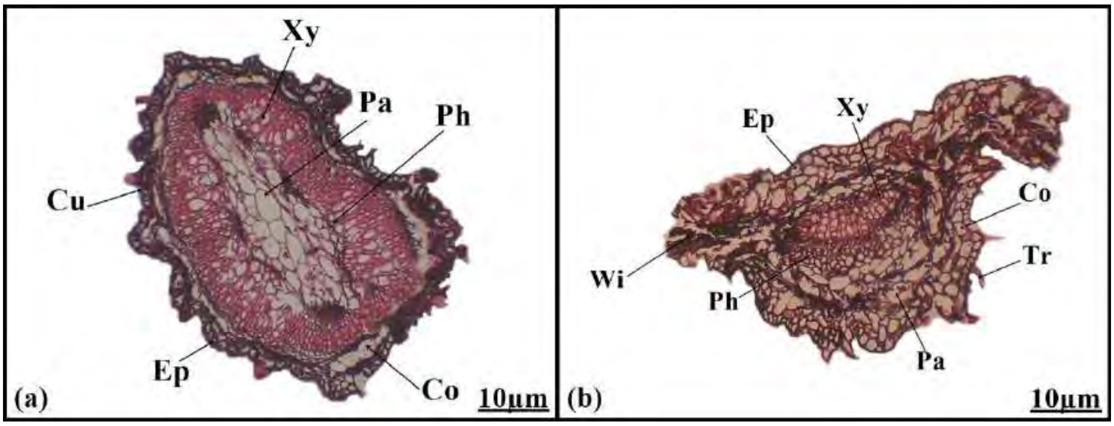

Plate 77. Petiole anatomy of (a) *Rochelia sessiliflora*, (b) *Trichodesma indicum* (Cu: cuticle, Ep: Epidermis, Pa: parenchyma, Co: collenchyma, Ch: chlorenchyma, Sc:sclerenchyma, Xy: xylem, Ph: Phloem). Scale: 10µm (a,b)

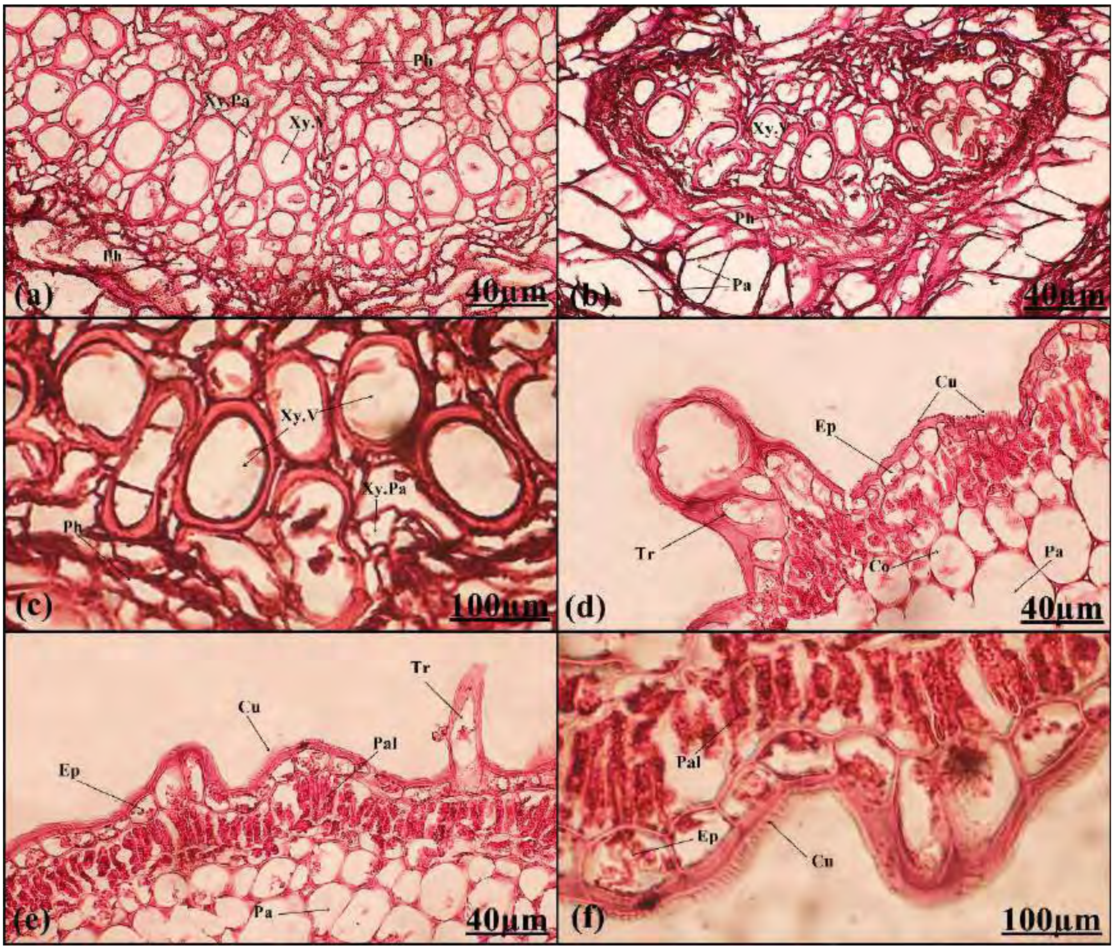

Plate 78. Petiole magnified photomicrographs of vascularization in (a) *Alkanna tinctoria* subsp. *Tinctoria* (b) *Caccinia mucronanthera*; xylem vessels and phloem with phloem parenchyma (c) *Caccinia mucronanthera*; epidermis, trichomes and cuticle in (d), (e), (f) *Heliotropium crispum* (Xy.V: xylem vessels, Ph: Phloem, Cu: cuticle, Ep: Epidermis, Pa: parenchyma). Scale: 40 µm (a, b, d, e), 100 µm (c, f)

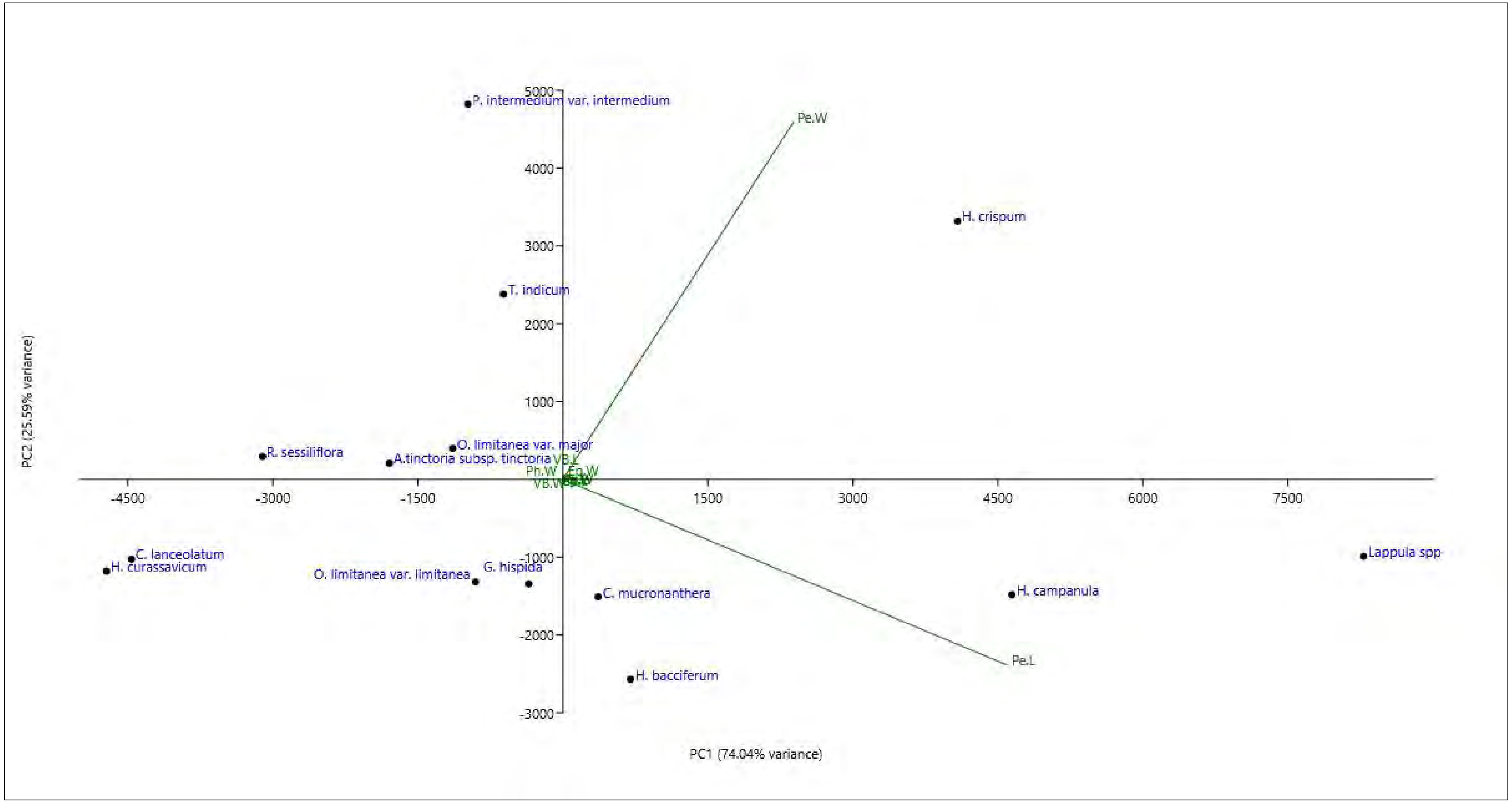

Figure 21. Utility of petiole features in discrimination among species of Boraginaceae by PCA (length and width of epidermis, parenchyma, chlorenchyma, xylem, phloem, vascular bundles)

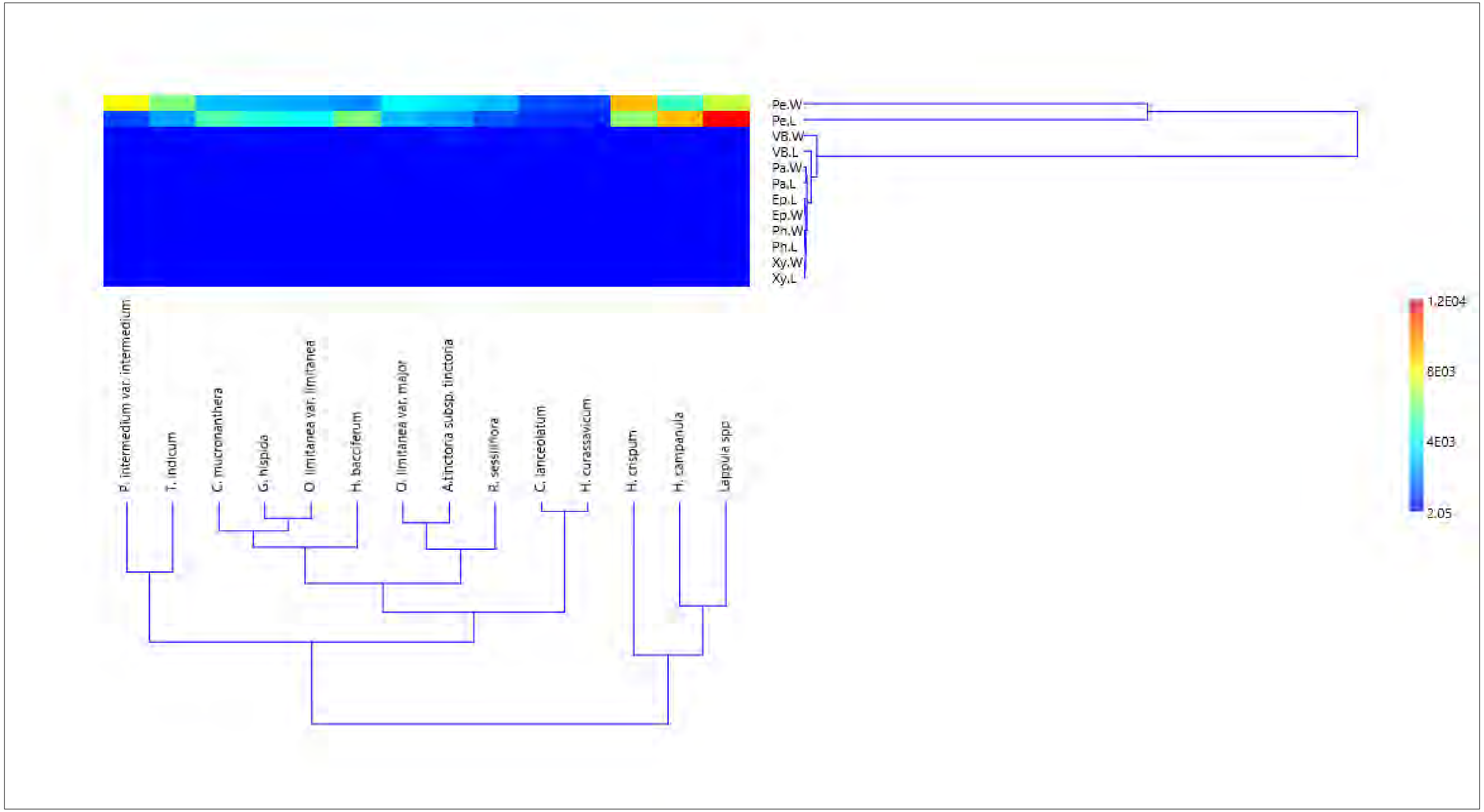

Figure 22. Dendrogram showing the similarity index of Boraginaceous taxa based on quantitative parameters of the petiole

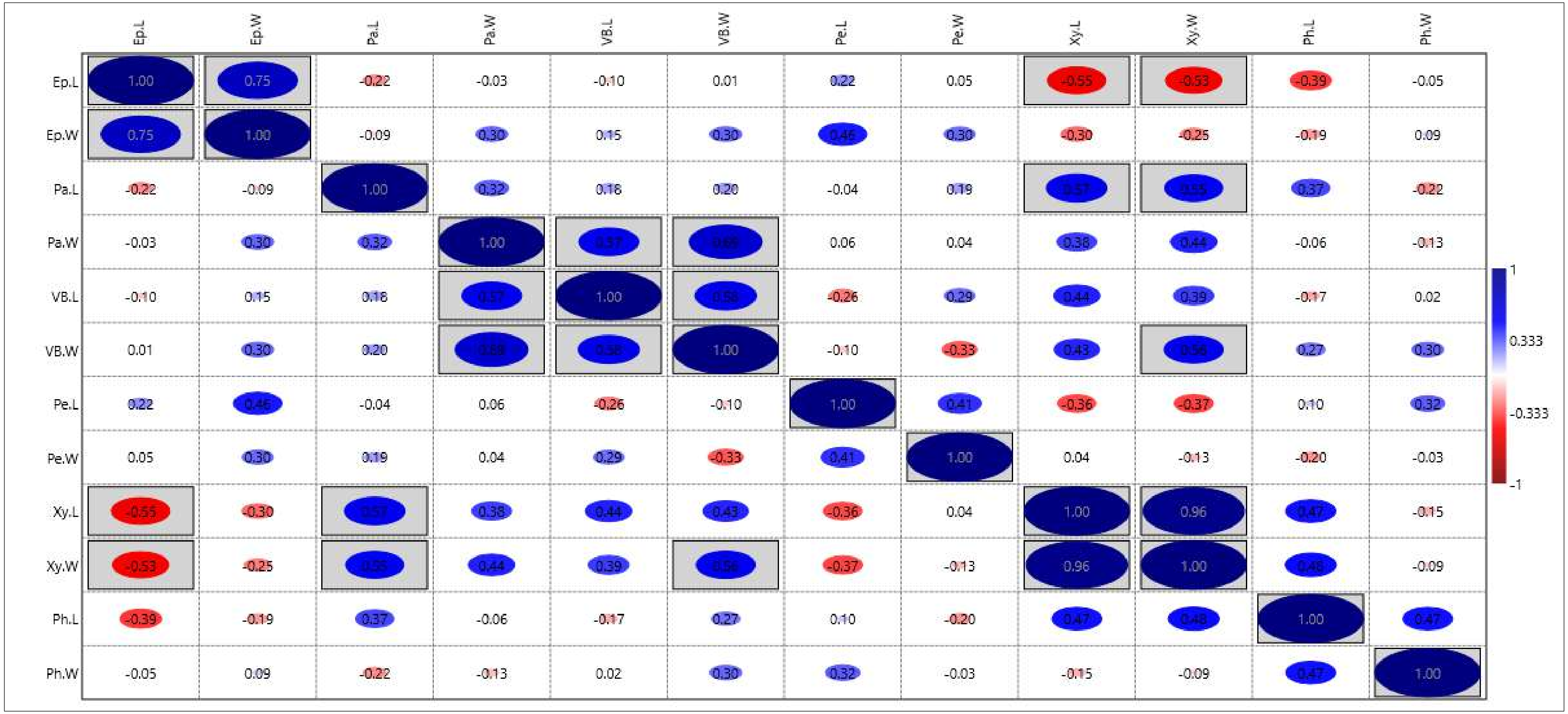

Figure 23. Correlation among mean values of length and width of petiole anatomical characters (L: length, W: Width, Ep: Epidermis, Pe: Petiole, Pa: Parenchyma, Vb: Vascular bundles, Xy: Xylem vessel, Ph: Phloem)

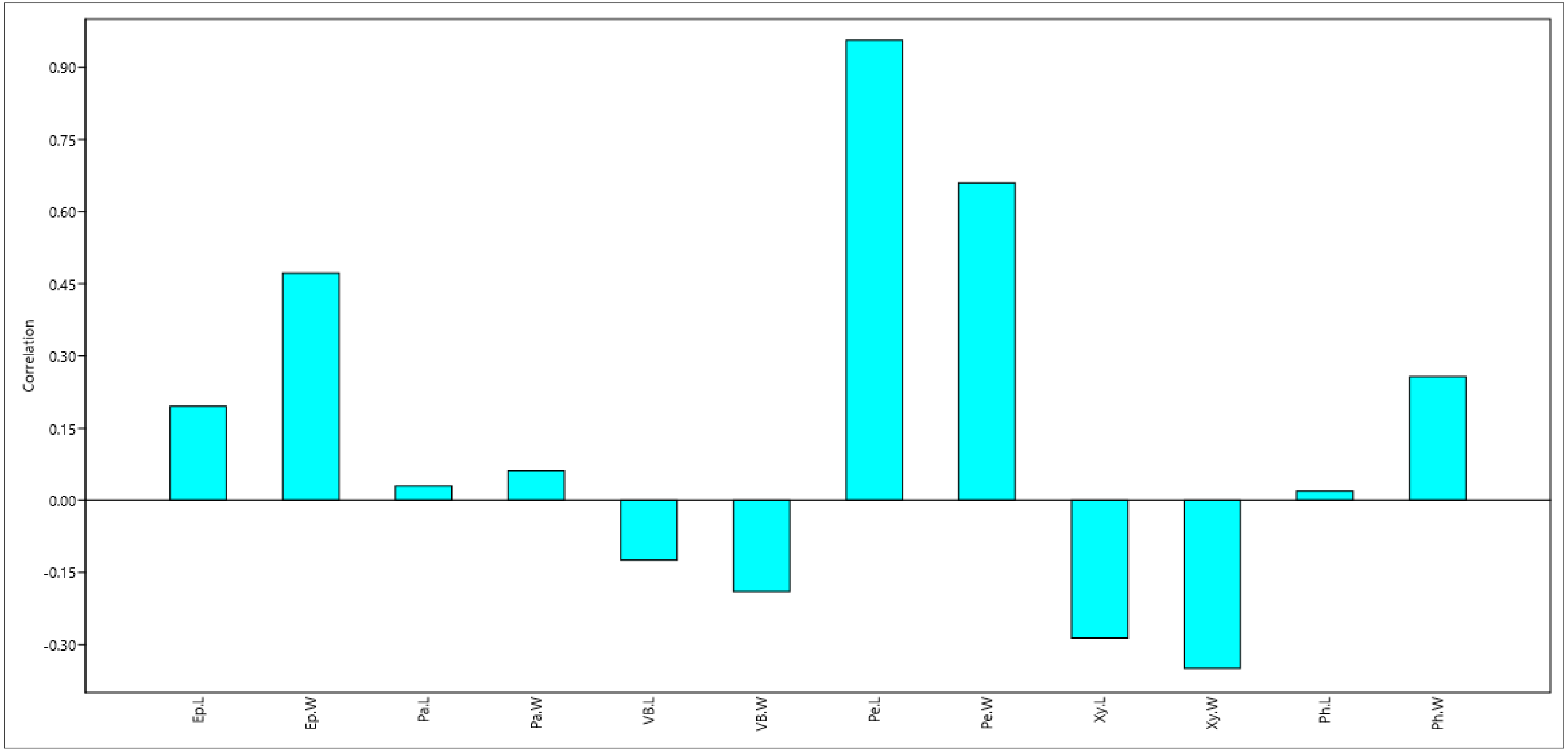

Figure 24. Correlation loading plot for the mean values of length and width of petiole anatomical characters (L: length, W: Width, Ep: Epidermis, Pe: Petiole, Pa: Parenchyma, Vb: Vascular bundles, Xy: Xylem vessel, Ph: Phloem)

Table 19. Qualitative anatomical observations of the Petiole of Boraginaceous taxa

Plant	**Petiole wing**	**Grove in the upper surface**	**Co (No of layers)**	**Subepidermal ring of Co**	**Ch (No of layers)**	**Pa (No of layers)**	**Sc Presence in VB**	**Air spaces As**	**No of VB**
A.tinctoria subsp. *tinctoria*	+	+	2	-	2	2	+	-	1
C. lanceolatum	+	-	1	-	-	2	-	+	1
C. mucronanthera	+	-	1	-	1	3	-	-	1
G. hispida	+	+	1	-	1	4	-	+	1
H. bacciferum	+	+	1	-	1	3	-	-	1
H. campanula	-	+	1	+	2	7	-	-	1
H. crispum	-	-	2	+	1	3	-	-	1
H. curassavicum	+	-	1	-	2	4	-	-	1
Lappula spp	-	+	2	-	2	4	-	-	1
O. limitanea var. limitanea	+	+	1	-	1	1	-	-	1
O. limitanea var. major	+	-	1	-	1	2	-	+	1
P. intermedium var. intermedium	-	+	1	-	1	2	-	+	1
R. sessiliflora	-	+	1	-	2	3	-	-	1
T. indicum	+	+	1	-	2	4	+	+	1

Co: Collenchyma, Ch: Chlorenchyma, Sc: Sclerenchyma, VB: Vascular bundles, Pa: parenchyma

Table 20. Qualitative anatomical observations of the Petiole of Boraginaceous taxa

Plant	Outline	Trichome	Ep Shape	Pa shape	Co Shape	Sc shape	VB arrangement	Xy vessel shape	Ph shape
A.tinctoria subsp. *tinctoria*	Sulcate	-	Oval to angular and irregular	Irregular	Annular	Irregular	Bicollateral	Isodiametric to round	Angular to irregular
C. lanceolatum	Sulcate	-	Square to angular	Irregular	Lamellar	-	Collateral closed	Round	Tri to hexagonal
C. mucronanthera	Sulcate	Multiseriate	Irregular	Isodiametric	Lacunar	-	Collateral closed	Round to oval	Angular
G. hispida	Sulcate	-	Angular to irregular	Irregular	Angular	-	Amphicribral	Round to oval	Angular
H. bacciferum	Sulcate	-	Angular to irregular	Irregular	Angular	-	Amphicribral	Round to angular	Angular
H. campanula	Flat	-	Rectangular to square and round	Irregular	Lamellar	-	Collateral closed	Round to oval	Angular
H. crispum	Oval	Uniseriate	Square to angular	Isodiametric	Annular	-	Collateral closed	Round	Angular
H. curassavicum	Sulcate	-	Square to angular	Angular to isodiametric	Angular	-	Collateral closed	Round to angular	Tri to hexagonal
Lappula spp	Flat	-	Square to oval	Tri to hexagonal	Angular	-	Amphicribral	Round to angular	Angular
O. limitanea var. limitanea	Sulcate	Unicellular	Angular	Angular to irregular	Angular	-	Amphicribral	Round	Tri to hexagonal
O. limitanea var. major	Sulcate	Uniseriate	Isodiametric to angular	Irregular to isodiametric	Angular	-	Collateral closed	Round to angular	Tri to hexagonal
P. intermedium var. intermedium	Flat	Unicellular	Angular	Irregular	Angular	-	Collateral closed	Round to oval	Angular

R. sessiliflora	Oval	-	Angular	Tri to hexagonal	Angular	-	Amphicribral	Round, angular, oval	Tri to hexagonal
T. indicum	Flat	Unicellular	Round to angular	Angular to isodiametric	Angular to lamellar	Angular	Collateral open	Rectangular to square	Irregular

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

Table 21. Quantitative anatomical observations for the histological characters of Petiole of Boraginaceous taxa

S/No	Taxon	L. W	Ep (µm)	Co (µm)	Pa (µm)	VB (µm)	Pe (µm)	Xy (µm)	Ph (µm)
Mean±S.E									
1	*A.tinctoria subsp. Tinctoria*	L	13.65±0.594768863	21.4±0.257390754	38.5±0.353553391	130.6±1.116915395	2510.4±4.822862221	21±0.176776695	4.25±0.176776695
		W	19.45±0.266926956	18±0.25	64.15±0.231840462	302.1±0.709753478	3536.8±8.410707461	19.2±0.215058132	3.45±0.215058132
2	*C. lanceolatum*	L	13.25±0.176776695	-	15.85±0.231840462	71.55±0.215058132	711±3.78153408	13.25±0.176776695	4±0.176776695
		W	13.25±0.176776695	-	24.6±0.257390754	137.95±0.215058132	1210±3.082207001	12.65±0.231840462	3.65±0.257390754
3	*C. mucronanthera*	L	7.05±0.215058132	-	36.75±0.176776695	49.95±0.183711731	5218±5.186520992	23.25±0.176776695	6.75±0.176776695
		W	12.95±0.21505	-	21.6±4.781	161.7±0.53268	3005.4±1.630	20.75±0.1767	4±0.30618
4	*G. hispida*	L	20.15±0.231840462	17.1±0.331662479	17.25±0.306186218	94.15±0.816241386	4505.6±2.158703314	9.55±0.215058132	4.25±0.176776695
		W	19.65±0.20310096	14.85±0.231840462	25.35±0.392109679	200.95±0.215058132	2822.8±6.102458521	6.75±0.176776695	4.4±0.302076149
5	*H. bacciferum*	L	18.25±0.176776695	-	35.85±0.231840462	38.25±0.176776695	6003.6±0.92736185	7.35±0.231840462	4.85±0.231840462
		W	12.95±0.215058132	-	25.75±0.176776695	88.5±0.285043856	2219.8±6.836665854	6.4±0.257390754	3.75±0.176776695
6	*H. campanula*	L	15.45±0.215058132	22.35±0.231840462	18.6±0.231840462	50.75±0.467707173	9004±1.140175425	10.25±0.306186218	5.15±0.231840462
		W	18.8±0.215058132	15.85±0.231840462	33.8±0.289395923	81.9±0.187082869	5003±0.7071066781	6.75±0.176776695	3.5±0.176776695

7	*H. crispum*	L	17.65±0.231840462	24.85±0.231840462	36.05± 0.242384	88.9±0.302076149	6292.8±3.056141358	12.35±0.231840462	3.25±0.176776695
		W	21±0.353553391	16.3±0.26692695	25.45±0.2150581	42.35±0.23184046	9003.8±1.2409673	7.65±0.23184046	2.05±0.2150581
8	*H. curassavicum*	L	13.35±0.231840462	6.75±0.176776695	18.5±0.176776695	50.15±0.407737661	553.4±1.029563014	3.25±0.176776695	3.25±0.176776695
		W	11.75±0.176776695	4.1±0.231840462	13.4±0.20310096	49.85±0.407737661	953.2±0.860232527	2.95±0.215058132	3.05±0.145773797
9	*Lappula spp*	L	14.25±0.176776	13.25±0.17	20.75±0.176	51.35±0.302076	12004.8±1.496	3.7±0.2150581	3.55±0.2893
		W	18.55±0.215058132	11.5±0.25	25.95±0.215058132	75.95±0.215058132	7117.2±5.276362383	3.25±0.176776695	4.85±0.231840462
10	*O. limitanea var. limitanea*	L	18.5±0.306186218	-	25.15±0.231840462	48.65±0.437321392	4004.2±1.15758369	13.4±0.231840462	3.25±0.176776695
		W	13.25±0.176776695	-	21.1±0.257390754	77.4±0.831414457	2591.2±1.15758369	10.8±0.215058132	2.35±0.231840462
11	*O. limitanea var. major*	L	20.75±0.176776695	-	26±0.176776695	39.05±0.649037749	3005.2±1.356465997	10.75±0.176776695	3.25±0.176776695
		W	21.5±0.1767766	-	35.75±0.395	92.05±0.39843	4005.4±1.363	9.55±0.21505	2.35±0.231
12	*P. intermedium var. intermedium*	L	8.75±0.176776695	-	25.75±0.176776695	101.15±0.231840462	1104±0.707106781	18.35±0.231840462	3.5±0.176776695
		W	7.95±0.2150581	-	27.45±0.398	51.2±0.2150581	8004.4±0.9273	12.95±0.09354	3.25±0.1767
13	*R. sessiliflora*	L	5.65±0.127475488	20.75±0.176776695	33.65±0.257390754	56.45±0.863857627	1306.6±0.92736185	18.25±0.176776695	5.15±0.231840462
		W	3.5±0.176776695	25.75±0.176776695	24.25±0.176776695	63.45±0.215058132	3006±1.224744871	13.5±0.176776695	3.1±0.127475488
14	*T. indicum*	L	20.15±0.231840462	25.85±0.231840462	28.5±0.176776695	63.45±0.266926956	2557.4±15.24663897	11.75±0.176776695	5.1±0.26925824
		W	19.2±0.2150581	23.5±0.17	11.5±0.1767	81.65±0.23	6005.6±1.07	8.45±0.2150	4.55±0.21

L: Length, W: Width, Ep: Epidermis, Pa: Parenchyma, Co: Collenchyma, Sc: Sclerenchyma, VB: Vascular bundles, Xy: Xylem, Ph: Phloem, Pe: Petiole

Table 22. Dichotomous key based on petiole anatomical characters of Boraginaceous taxa.

Link character	Leads	Characters	Taxa/ Go to link character
1	+	Outline flat	2
	-	Outline oval or sulcate	4
2	+	Unicellular trichome	*P. intermedium var. intermedium*
	-	Trichome not visible	3
3	+	Epidermal cells rectangular to square and round	*H. campanula*
	-	Epidermal cells square to oval	*Lappula spp*
4	+	Outline oval	5
	-	Sulcate outline	6
5	+	Parenchyma cells isodiametric	*H. crispum*
	-	Trigonal to hexagonal parenchyma cells	*R. sessiliflora*
6	+	Trichomes present	7
	-	Trichomes not present	9
7	+	Multiseriate trichomes	*C. mucronanthera*
	-	Unicellular or uniseriate trichomes	8
8	+	Unicellular trichomes	*O. limitanea var. limitanea*
	-	Uniseriate trichomes	*O. limitanea var. major*
9	+	Vascular bundles bicollateral	*A.tinctoria* subsp. *tinctoria*
	-	Vascular bundles not bicollateral	10
10	+	Collateral closed vascular bundles	11
	-	Amphicribral vascular bundles	12
11	+	Lamellar collenchyma	*C. lanceolatum*
	-	Angular collenchyma	*H. curassavicum*
12	+	Air spaces present	*G. hispida*
	-	Air spaces not present	*H. bacciferum*

3.7 Culm Anatomy of Poaceous Flora from Baluchistan

3.7.1 Results

The details of anatomical examinations of the culm of studied species are given below.

a) Cuticle and Epidermis

Thick cuticle was present in *A. adscensionis, C. setigerus, D. scindicum, D. fusca, L. senegalense, C. divisus, P. baluchistanicum* and *T. villosus* (Plates 79-83). Epidermal cells were compactly arranged in a single row in 21 species (Table 25). In *A. funiculata, B. squarrosa, C. barbata, E. curvula,* and *I. cylindrica* epidermis was two-layered. The shape of epidermal cells was square, rectangular, angular, and round (Table 26). The maximum size of the epidermis was *D. fusca* 21.25 µm length and 18.15 µm width. The smallest epidermal cell was noted in *T.* villosus 4.85 µm length and 5.55 µm width (Table 27).

b) Hypodermis and sclerenchyma

Hypodermis was sclerenchymatous in studied taxa with a maximum number of layers of 10 in *E. curvula* and a minimum of one layer in *D. fusca* (Plates 80, 81). Sclerenchyma cell shapes were from tri, tetra to hexagonal, and isodiametric. *E. distans* and *H. marinum* sclerenchyma strands were found with each vascular bundle. The maximum size of sclerenchyma cell was observed in *C. aucheri* 18.25 µm length and 15.75 µm width. Sclerenchyma cell was smallest in *A. cyanantha* with 6.6 µm length and *B. squarrosa* 5.15 µm width (Table 27).

c) Parenchyma and chlorenchyma

Ground parenchyma was densely present in *A. cyanantha, E. curvula and L. senegalense*. Round, angular, isodiameteric, hexagonal and irregular shapes of parenchyma were observed. Parenchyma cells were smallest in size in *E. bonaepartis* with length 12.55 µm and width 11.2 µm. *L. senegalense* has maximum cells length 38.25 µm and *B. squarrosa* 34.55 µm width. Chlorenchyma were found in these shapes; lamellar, annular, isodiameteric, lacunar, angular and hexagonal (Table 26). *C. divisus* was noted with maximum chlorenchyma cell length of 20.05 µm length and width 20.45

µm in *C. flaccidus*. Smallest chlorenchyma cell was observed in *A. adscensionis* 12.35 µm (length) and *E. curvula* 9.25 µm (width) (Table 27).

d) Vascularization and Cavities

Vascular bundles were collateral closed. Protoxylem lacuna (lysigenous cavity) arised at the inner side of the protoxylem. Bundle sheath surrounds the vascular bundles. It was sclerenchymatous with parenchyma layer. Vascular bundles were arranged in single to four circles or in some plants somewhat scattered arrangement. The vascularization was categorized as major and peripheral vascular bundles. Maximum number of major VB is 42 noted in *C. divisus* whereas minimum number was five observed in *E. bonaepartis*. *A. funiculate* has maximum number of peripheral vascular bundles 23, whereas *Cymbopogon martini* has minimum number six. Among the studied taxa nine species have hollow culm (central cavity) in cross section, and ten species have marginal/cortical cavities (Table 25).

e) Statistical analysis

Statistical examination of anatomical parameters disclosed that component 1 and 2 accounted for 89.746% and 10.023% sum of squares variance in PCA analysis. Species of similar genus were clustered closed with exception of *Eremopyrum*. Members of *Aristida* were in close cluster with each other. PCA positively linked Ep.L, Ep.W, Ch.L, Ch.W, Pa.L, Pa.W, Vb.L, Vb.W, Cu.L, Cu.W in cluster while Xy.L, Xy.W and Ph.L, Ph.W were negatively corelated. Outcomes were in the agreement of Mohtashamian *et al.,* (2022), they determined that family Sapindaceae anatomical features (parenchyma length/width, collenchyma length/width, VB) were significant for discrimination and analysing variations. UPGMA dendrogram resulted in two clades, one major cluster with 24 and second with two taxa. Akhtar *et al.,* (2021) observed that diverse taxa expressed more correlation with each other. *C. setigerus* and *C. flaccidus* were marked with differences in quantitative anatomical features from *C. divisus*. *Aristida* species were gathered in the same cluster above the two clades. *Eremopyrum* species were in the different clusters (Figures 30, 31).

3.7.2 Discussion

Culm anatomy was documented in Poaceous taxa for the first time from Baluchistan, Pakistan. The microanatomical structures were significant for differentiating the studied grasses (Apóstolo et al., 2022). Hassan et al. (2022) examined 18 anatomical attributes in the delimitation of seven *Aegilops* species. Anatomy of stem and internodes impart a judgemental significance on the classification of *Aegilpos* taxa. Shape of the culm in the current studied grasses was terete twelve species, elliptical nine, quadrangular three, and semiterete in two species (Kellogg, 2015). Al-Khafaji and Al-Bermani, (2014) documented terete and crescent shapes of the stem in grasses. Culm diameter, vascular bundle shape, diameter, number, distribution, and thickness of sclerenchyma were taxonomic tools successfully implicated in the discrimination of species of the same genus in the examined Poaceae taxa (Al-Khafaji and Al-Bermani, 2014). The comparative examination revealed that the grasses of the arid regions adapted terete shape culm.

Similarly, in this study, the studied taxa of genus *Aristida, Cenchrus, Dactyloctenium* and *Eremopyrum* were observed with differences in culm shapes, shapes of parenchyma, sclerenchyma, chlorenchyma, number of major and peripheral vascular bundles, number of chlorenchyma layers, central and marginal cavities. These variations were specific to each taxon, thus highly significant in the identification and delimitation of studied taxa. The distinct qualitative and quantitative features for all the studied taxa are given in Tables 25, 26, and 27.

In the current study majority of the grasses possessed smooth cuticle in comparison to undulated (Wooller, 2002). Thickness and structure of the cuticle were the additional parameters in the delimitation of taxa. Thick cuticle was present in *A. adscensionis, C. setigerus, D. scindicum, D. fusca, L. senegalense, C. divisus, P. baluchistanicum* and *T. villous.* Epidermal or endodermal cells in compact arrangement beneath the cuticle were present in Poaceous taxa in single or double layers in square, rectangular, angular and round shapes. Previously thicker epidermis in *Eleusine indica, Paspalidium flavidum* and *Setaria pumila* reported as strengthening character in their identification (Rafique et al., 2021). In poaceous species photosynthetic tissue chlorenchyma was prominent. Layers of chlorenchyma cells and their diameter were found significant in the specification of taxa. Shamah et al. (2019) reported spherical to oval epidermal cells with thick cuticle in stem and a continuous cylinder formed in ground tissues consisting of chlorenchyma strands followed by sclerenchyma surrounding vascular bundles. Sclerenchyma layer in the culms of most of the Poaceae was bounded to epidermis either continuous or discontinuous pattern (Al-Khafaji and Al-Bermani, 2014). Maximum thickness in terms of number of layers of sclerenchyma was observed in *E. curvula.* Whereas *E. bonaepartis, A. donax, C. divisus, C. flaccidus* were also observed with thick sclerenchyma. In the studied species, sclerenchymatous hypodermis was present. The examined plants showed variations in sclerenchyma strands and number of layers. Sclerenchyma was marked in cortex region, below the epidermis, with vascular bundles. *E. distans* and *H. marinum* were observed with dense sclerenchyma strands in vascular bundles. This trait may be resulted of less water availability in the arid and semiarid regions. Anatomical traits employed as tools for taxonomy in Bambusa species as strong relation was recorded for microstructures and delimitation of taxa. Dense sclerification in the culm specifically in vascular region and inner side of epidermis were distinctive anatomical characters in Bamboos (Apóstolo et al., 2022).

Vascular bundles in the studied grasses were scattered in rings, each surrounded by bundle sheath. Proto and meta xylem (present in Y shape) were distinguished. Protoxylem lacuna or lysigenous cavity was prominent. Vascular bundles were categorized in major and peripheral. Major vascular bundles were larger in size and present towards centre whereas peripheral fwere arranged at margins and comparatively smaller in size. The number of vascular bundles, their size and arrangement were diagnostic characters for the distinction of examined grass species (Yang et al., 2014). Bundle sheath surrounding the vascular bundles generally made up of sclerenchyma called as mestome sheath, it was accompanied by parenchymatous sheath. Shamah et al. (2019) studied that each vascular bundle surrounded by a single sclerenchyma bundle sheath, scattered in ground tissue. Larger vascular bundles in *E. tenella* and *D. bipinnata* were significant for their distinction. The key adaptability is phenotypic plasticity which is taxonomic, environmental, and ecological significance of that trait (Wells et al., 2000). Air spaces were referred as aerenchyma (highly organised with distinct patterns of development) or cavities (large irregular, mostly lysigenous). In grasses these cavities were present in the cortex or pith. The studied Poaceous taxa were marked and distinguished for the presence of distinct type of air spaces. Central cavities were characterized in nine while marginal / peripheral cavities were in ten grasses. *Schismus arabicus* possessed largest central cavity followed by *Piptatherum baluchistanicum. Aristida cyanantha* and *Diplachne fusca* are marked with highest number of marginal cavities. Grasses develop central cavities at internodes, and may remain solid or a combination of solid and hollow (McKim, 2019).

PCA score plots determine clusters of the variables categories and helps to visualize trends in data set in a novel system of axes (Kim et al., 2015). Correlation loading plots for determination of correlation among the mean values, are necessary and strengthen principal component analysis. Uga et al. (2009) studied relationship among the root anatomical traits via PCA plots. These plots contributed to the identification of studied trees. Loading plots determine the positive and negative correlation while correlation loading plots help to interpret correlation between PCs and variables via presenting significant levels and visualization of explained variance (Kim et al., 2015). The highest correlation was found in culm diameter, chlorenchyma, parenchyma, sclerenchyma, vascular bundles and epidermis respectively. Phloem was negatively correlated with other anatomical traits; little correlation was exhibited with xylem. Xylem was the least positively correlated parameter (Figures 28-29). Variance among the means was determined via multiple samples ANOVA. Analysis of variance (Multiple sample ANOVA) determines the variations among the data set of more than two independent variables. In this study the p-value was less than 0.05 alpha level, while the obtained value was greater than this value. Hence it was concluded that there exists a highly statistically significant difference among the means of studied characteristics, this can be used as a systematics approach for the delimitation of taxa.

Culm anatomical features have significant implications in modern taxonomy and ecology. Siqueiros-Delgado (2007) provided application of culm anatomy in *Bouteloua* and relatives (Gramineae) stated that there are very few valuable characters. Characterization of the culm anatomy of *Guadua angustifolia* determined its significance in taxonomy of Poaceae (Londoño et al., 2002). Quantitative characterization and morpho-anatomical studies were successfully employed in correct characterization of each studied Bamboos species (Apóstolo et al., 2022). Depending on the cross-sectional diameter and type (solid or hollow) of the stem, the quantitative and qualitative traits have substantially helped to isolate the species. The sclerenchyma tissue thickness varied among studied species. Similar differences exist between the parenchyma tissues in terms of thickness, tissue type and structure, ranging from regular annular to semi-annular or annular tissue types and between tiny, medium, and large clusters of tissue. The number of vascular bundles, their diameters, and the diameter of a single vessel differed significantly between species (Mousa et al., 2021). A taxonomic key based on anatomical characteristics has been effectively used to identify species (Mabel et al., 2013). By examining anatomical features that aid in enabling the classification of complex species, taxonomic keys analyse the differences between species. In this study the anatomical features of the culm were successfully employed in the separation of studied grasses (Table 28).

Genus's level positive correlation was determined in *Aristida, Dactyloctenium, Cenchrus* based on quantitative features such as diameters of epidermis, chlorenchyma, parenchyma, xylem phloem, vascular bundles, and culm. While *Eremopyrum* species expressed variations and were divergent in the cluster and principal component analysis. Instead, features such as marginal and central cavities, vascular bundles number and arrangement, shapes, and number of layers of epidermis, chlorenchyma, and sclerenchyma were varied both at the genus and species level. These will help in the identification of grass species in the absence of morphological and floral evidence. Previously morpho-anatomical features were successfully employed in identification, differentiation, and characterization of *Bambusa* species utilizing both qualitative (cells shapes, types, surface) and quantitative (cell layers, diameter) features (Apóstolo et al., 2022). The studies have shown that culm anatomy characters can be used to distinguish between different subfamilies, tribes, and genera within Poaceae. For instance, the arrangement of vascular bundles were diagnostic feature that can be used to distinguish between the subfamilies Panicoideae and Chloridoideae (Banan et al., 2019). Similarly, the presence or absence of bulliform cells in the epidermis and the arrangement of vascular bundles can be used to distinguish between different genera within the subfamily Pooideae (Kumar and Nautiyal, 2017). In conclusion, this study documents the significant anatomical features of studied grasses. The findings can be effectively applied to taxonomical investigations in the taxa placement, evolutionary studies, and the ecological impacts on the anatomical features. Distinctive anatomical characteristics and species explicit can assist in carrying out species-level discrimination.

a) Ecological implications of culm anatomy

Environmental gradients along with functional traits shape the distribution of species and differences in their occurrence. In arid areas with water scarcity, grass cuticle is one of the significant features that help in survival. The thicker yet differential permeable cuticle in the stem helps grasses grow in various conditions as it prevents evaporation of water and acts as barrier to pathogens (Shamah et al., 2019). It is regarded as paleocological trait for the study of grasses in lake sediments. Cuticle analysis from fossil record is alternative to reconstruct the grass flora previously dominated from the area (Wooller, 2002). Thick epidermis indicates the adaptability of grasses in saline and drought conditions by playing significant role in conservation of water (De Micco and Aronne, 2012). Similarly compact arrangement of cells in epidermis and its thickness is a mark for limited water supply and it as an adaptive feature. Drought and salinity design thicker epidermis in grasses. Thicker epidermis in *Eleusine indica*, *Paspalidium flavidum* and *Setaria pumila* reported as an adaptation to salinity and drought stress (Rafique et al., 2021).

Shamah et al. (2019) characterized drought tolerance adaptations in anatomy, helping the survival in arid and semiarid regions. Increased and developed sclerenchyma in grasses help in mechanical support and tolerate stress conditions (Rafique et al., 2021). Increased sclerification is significant feature in stress tolerant species. It provides strength, resist water loss, and protect plant tissues (Rafique et al., 2021). Structural modifications in microanatomical features are used to assess the tolerance to environmental stresses. In dry environments plants with these adaptations can be used to pasture areas and increase the vegetation cover (Apóstolo et al., 2022). These variations help to evaluate the grasses and their adaptability (Rafique et al., 2021). Salinity resulted in an increased number of vascular bundles, sclerification, and decreased metaxylem, phloem, and sheath in *Leptochloa fusca* (Ola et al., 2012). Cavities in the roots of *Paspalum distichum* are an advantage in flooded conditions (Yang et al., 2011). Air spaces help the plants to survive in stressed conditions. Pith cavities and small marginal/cortical cavities studied in four grasses are regarded as adaptive features to tolerate flood conditions and these types of species may help to restore the degraded ecosystems (Yang et al., 2011).

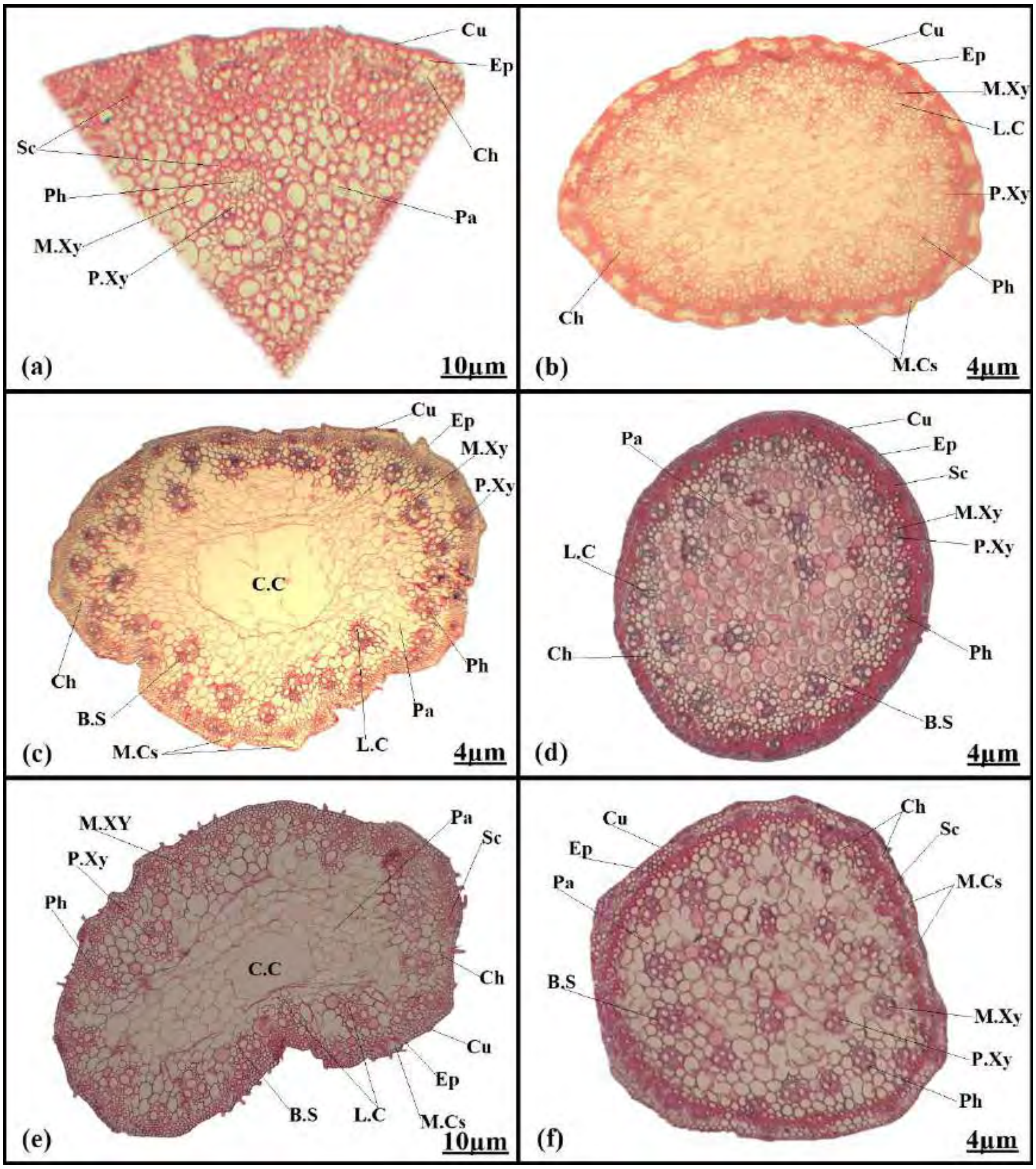

Plate 79. Culm anatomy of (a) *Aristida adscensionis* (b) *Aristida cyanantha* (c) *Aristida funiculata* (d) *Arundo donax* (e) *Boissiera squarrosa* (f) *Cenchrus setigerus* (Ph: Phloem, M.Xy: Meta Xylem, P.Xy: Proto Xylem, Ch: Chlorenchyma, Cu: Cuticle, Ep: Epidermis, C.C: Central cavity, Sc: Sclerenchyma, Pa: Parenchyma, M.Cs: Marginal cavities, B.S: Bundle sheath, L.C: Lysigenous cavity)

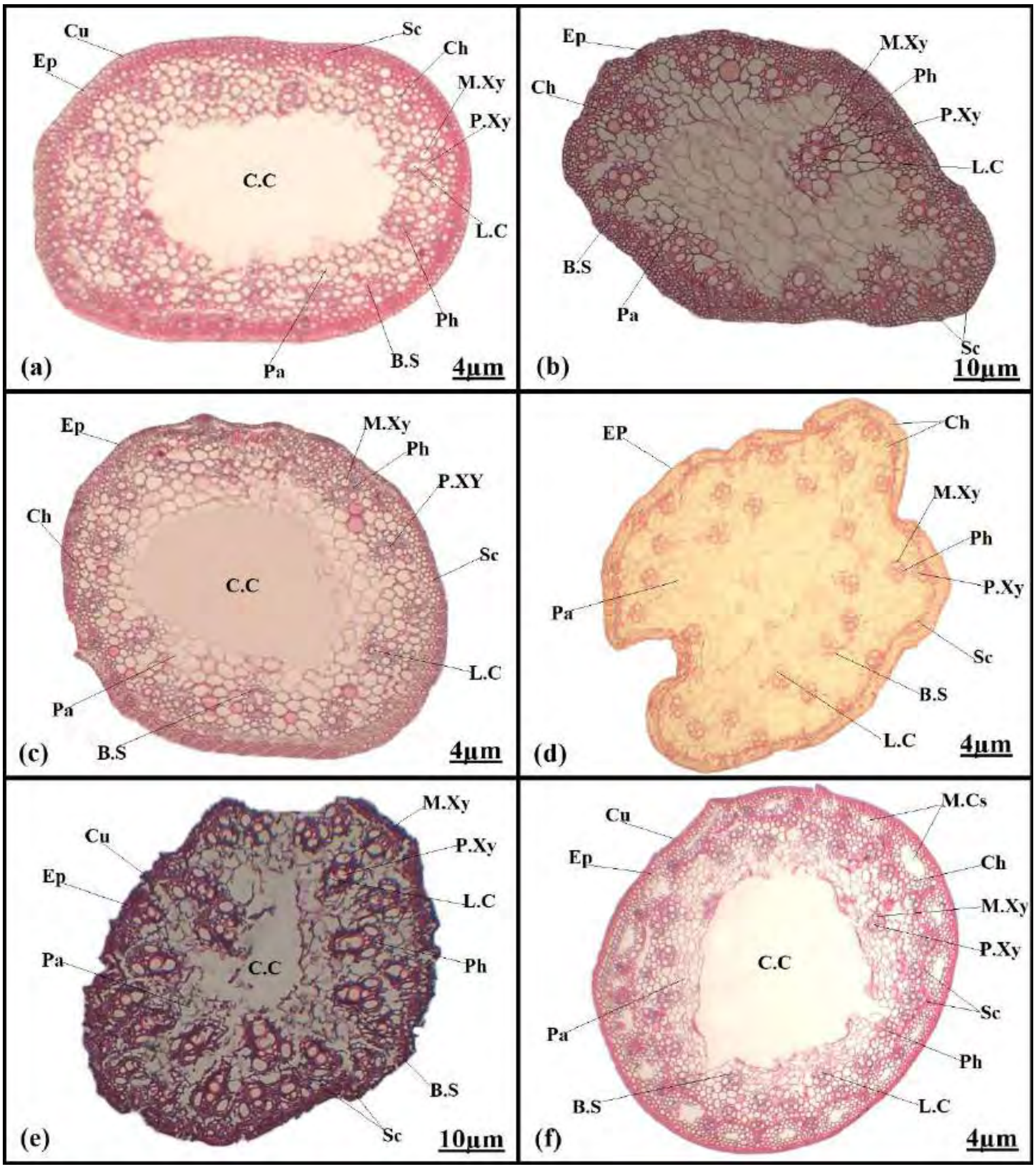

Plate 80. Culm anatomy of (a) *Chloris barbata* (b) *Chrysopogon aucheri* (c) *Cymbopogon martini* (d) *Dactyloctenium aristatum* (e) *Dactyloctenium scindicum* (f) *Diplachne fusca* (Ph: Phloem, M.Xy: Meta Xylem, P.Xy: Proto Xylem, Ch: Chlorenchyma, Cu: Cuticle, Ep: Epidermis, C.C: Central cavity, Sc: Sclerenchyma, Pa: Parenchyma, M.Cs: Marginal cavities, B.S: Bundle sheath, L.C: Lysigenous cavity)

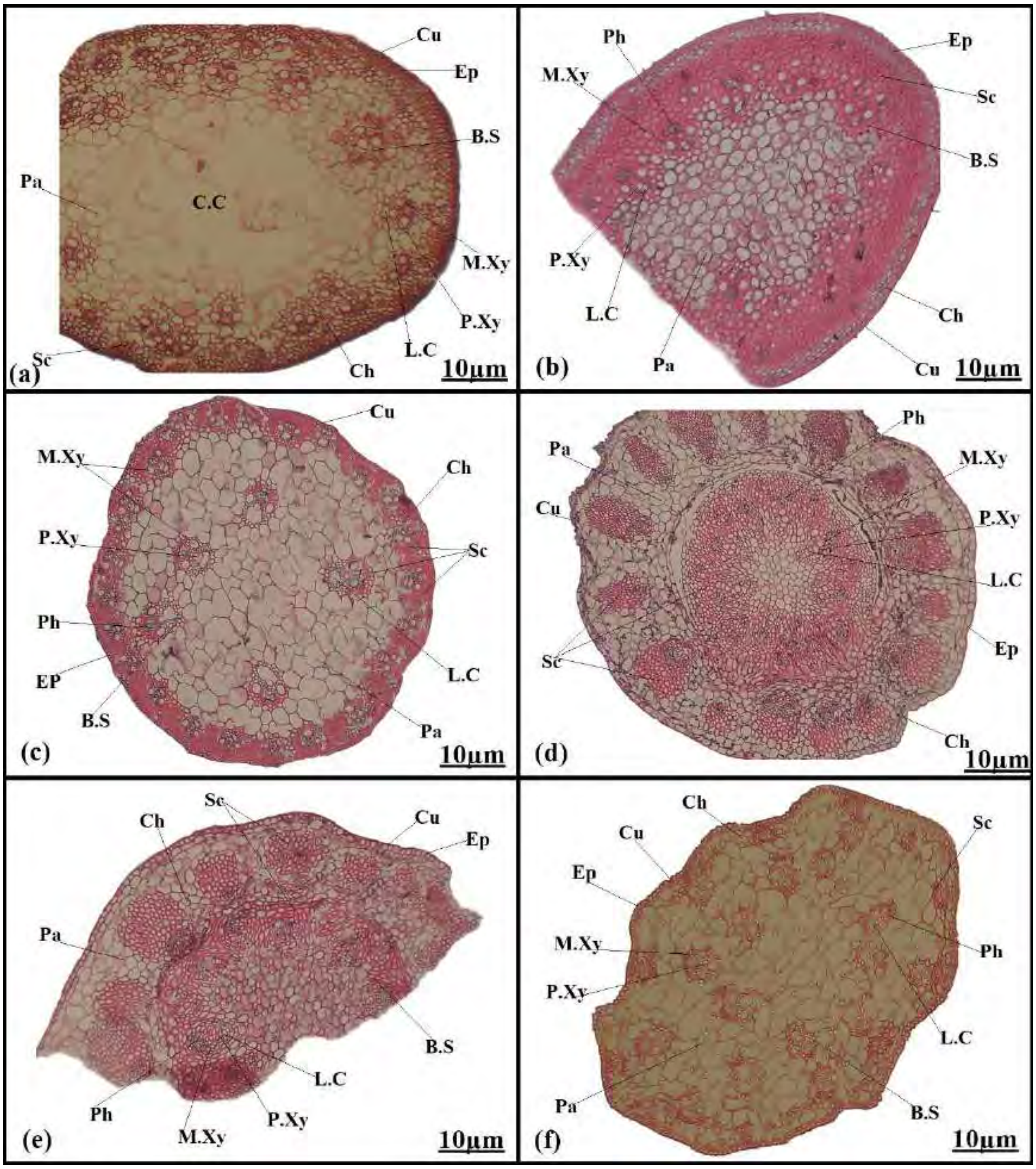

Plate 81. Culm anatomy of (a) *Enneapogon persicus* (b) *Eragrostis curvula* (c) *Eremopyrum bonaepartis* (d) *Eremopyrum distans* (e) *Hordeum marinum* (f) *Imperata cylindrica* (Ph: Phloem, M.Xy: Meta Xylem, P.Xy: Proto Xylem, Ch: Chlorenchyma, Cu: Cuticle, Ep: Epidermis, C.C: Central cavity, Sc: Sclerenchyma, Pa: Parenchyma, M.Cs: Marginal cavities, B.S: Bundle sheath, L.C: Lysigenous cavity)

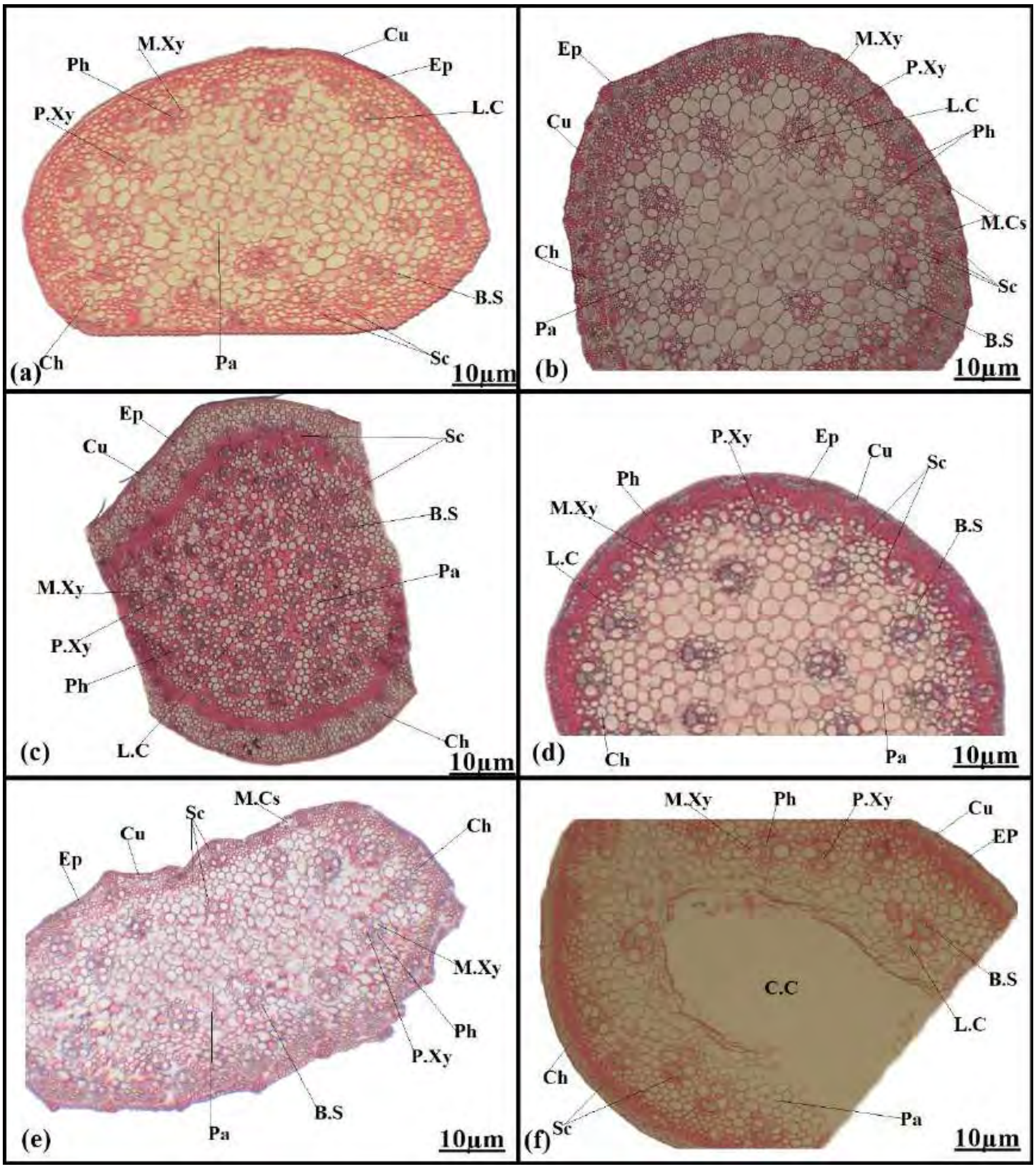

Plate 82. Culm anatomy of (a) *Leptothrium senegalense* (b) *Panicum antidotale* (c) *Cenchrus divisus* (d) *Cenchrus flaccidus* (e) *Phalaris minor* (f) *Piptatherum baluchistanicum* (Ph: Phloem, M.Xy: Meta Xylem, P.Xy: Proto Xylem, Ch: Chlorenchyma, Cu: Cuticle, Ep: Epidermis, C.C: Central cavity, Sc: Sclerenchyma, Pa: Parenchyma, M.Cs: Marginal cavities, B.S: Bundle sheath, L.C: Lysigenous cavity)

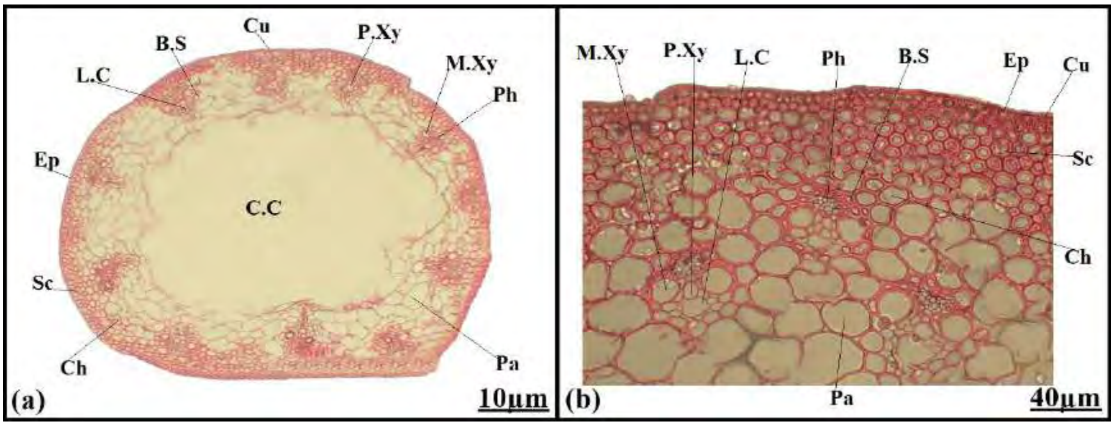

Plate 83. Culm anatomy of (a) *Schismus arabicus* (b) *Tetrapogon villosus* (Ph: Phloem, M.Xy: Meta Xylem, P.Xy: Proto Xylem, Ch: Chlorenchyma, Cu: Cuticle, Ep: Epidermis, C.C: Central cavity, Sc: Sclerenchyma, Pa: Parenchyma, M.Cs: Marginal cavities, B.S: Bundle sheath, L.C: Lysigenous cavity)

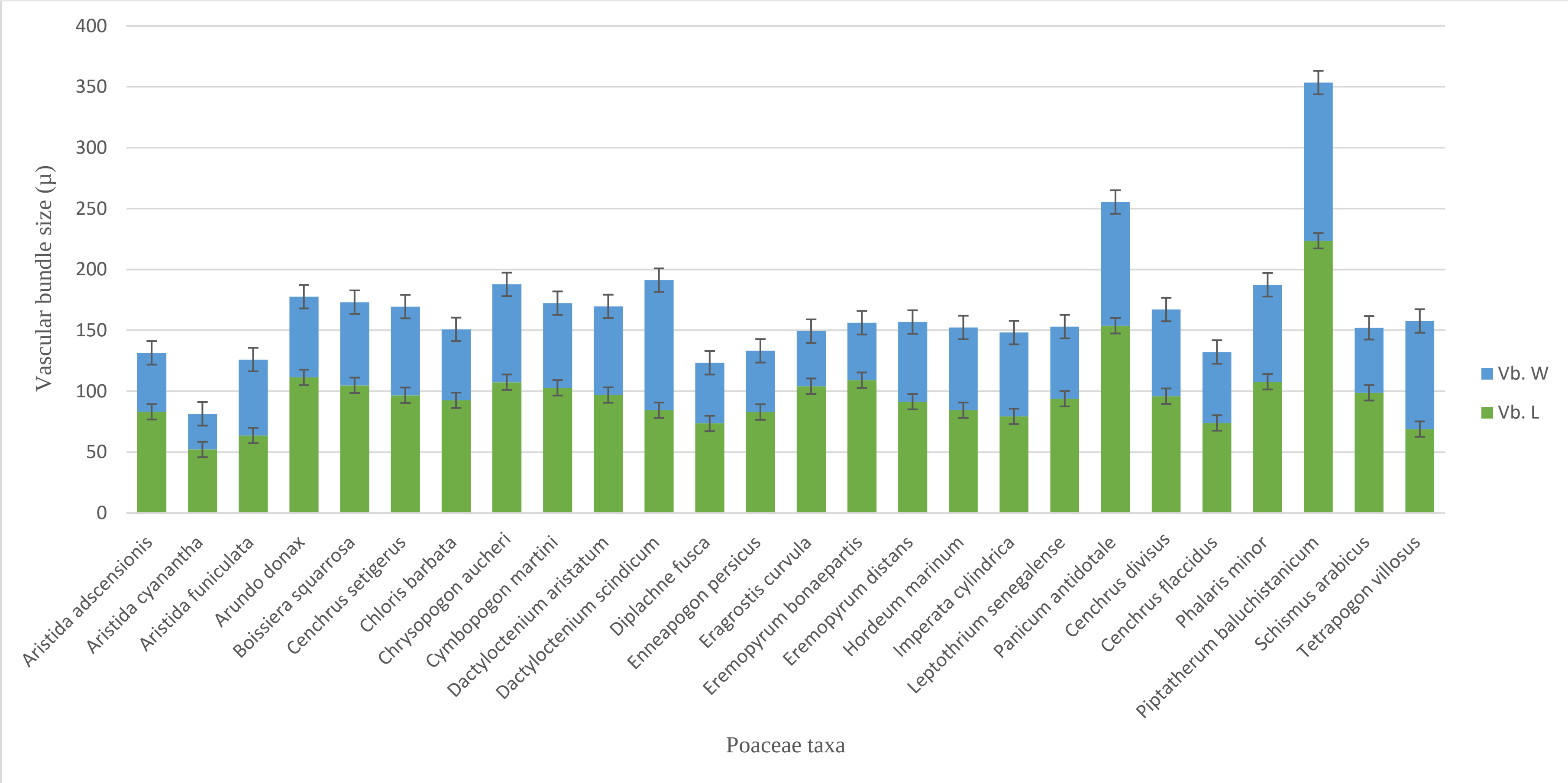

Figure 25. Variations among the mean values of vascular bundles length and width of Poaceous taxa

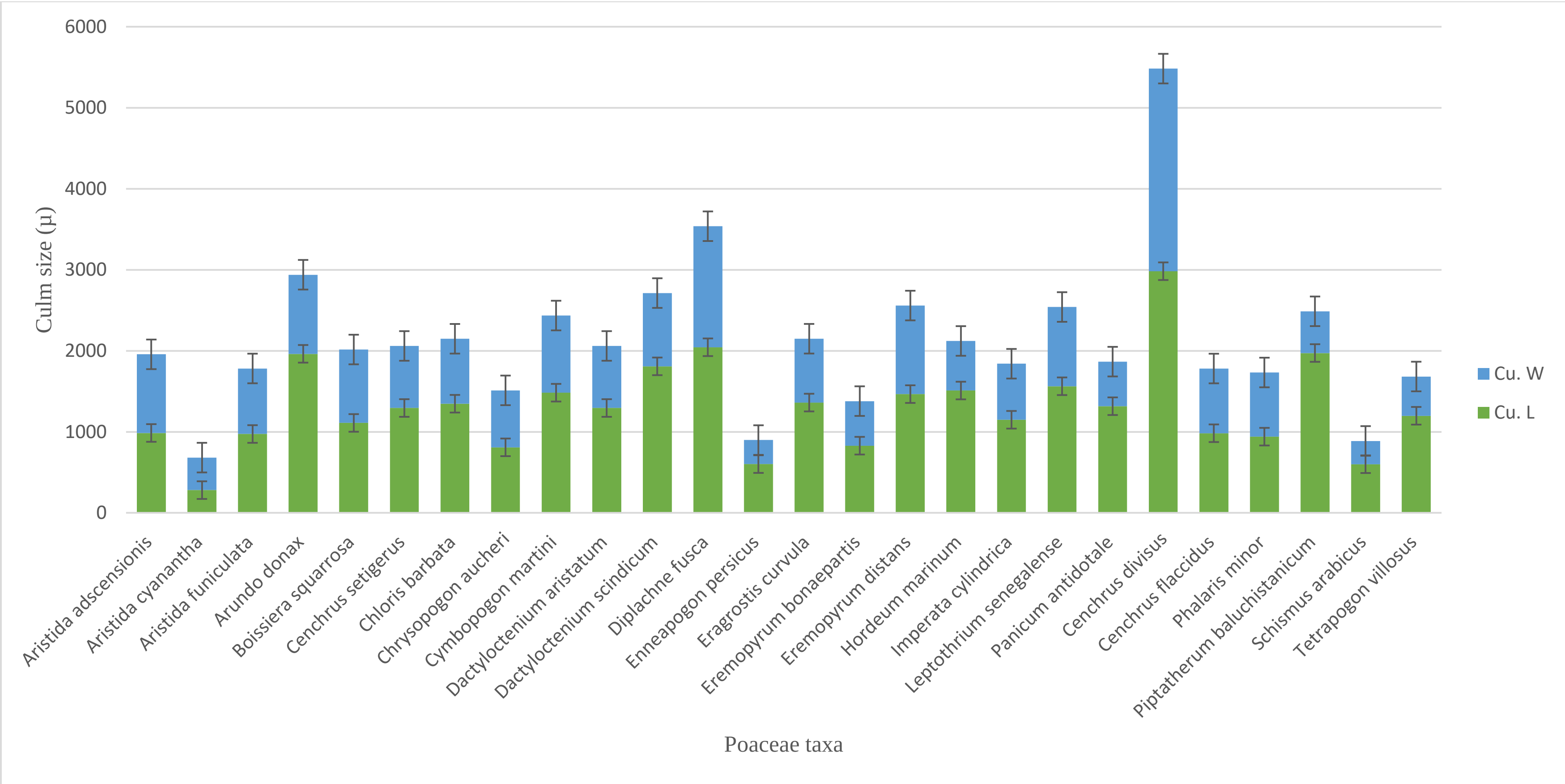

Figure 26. Variations in mean values of culm size length and width of Poaceous taxa

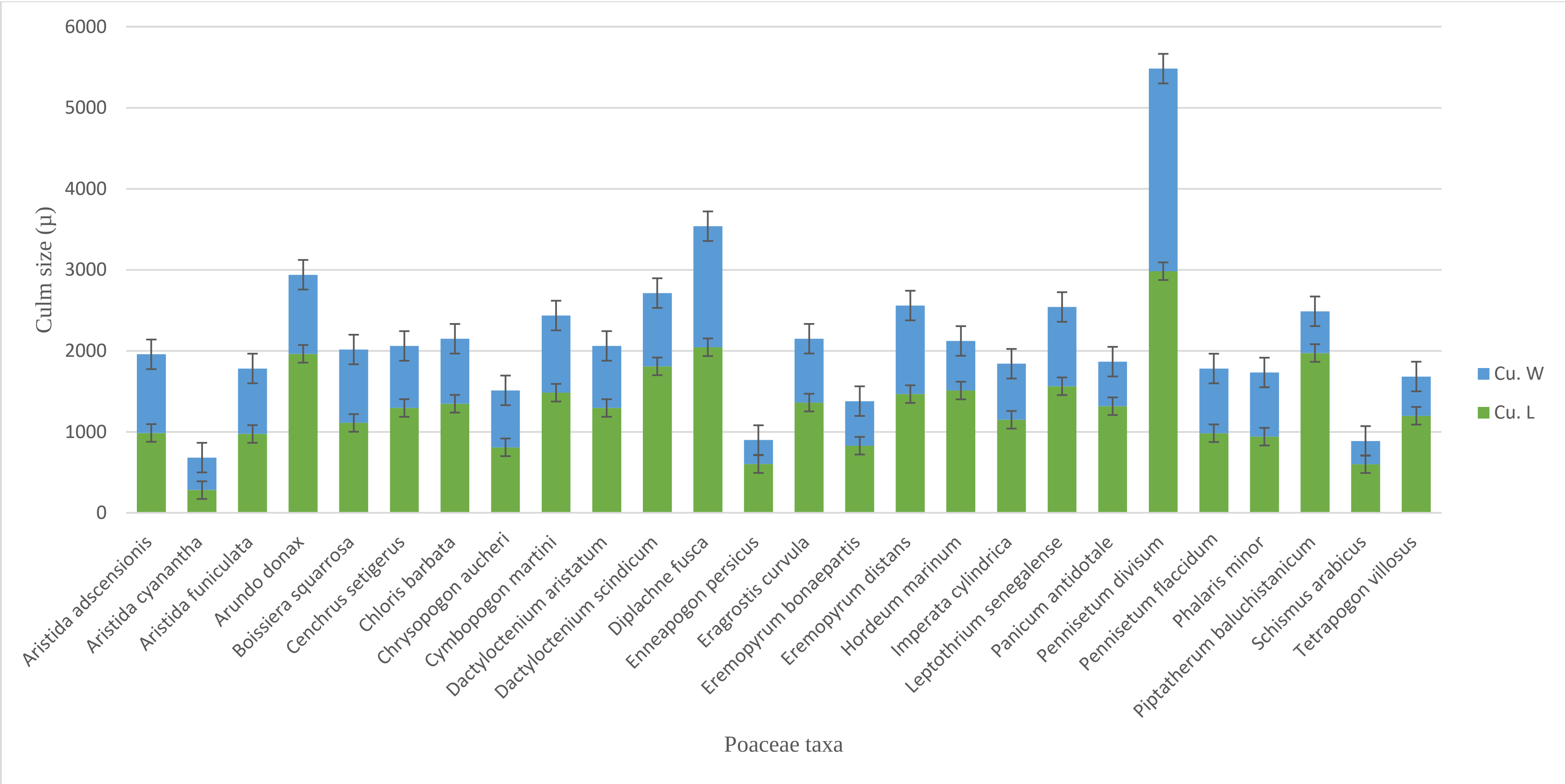

Figure 27. Variations among the mean values of culm length and width

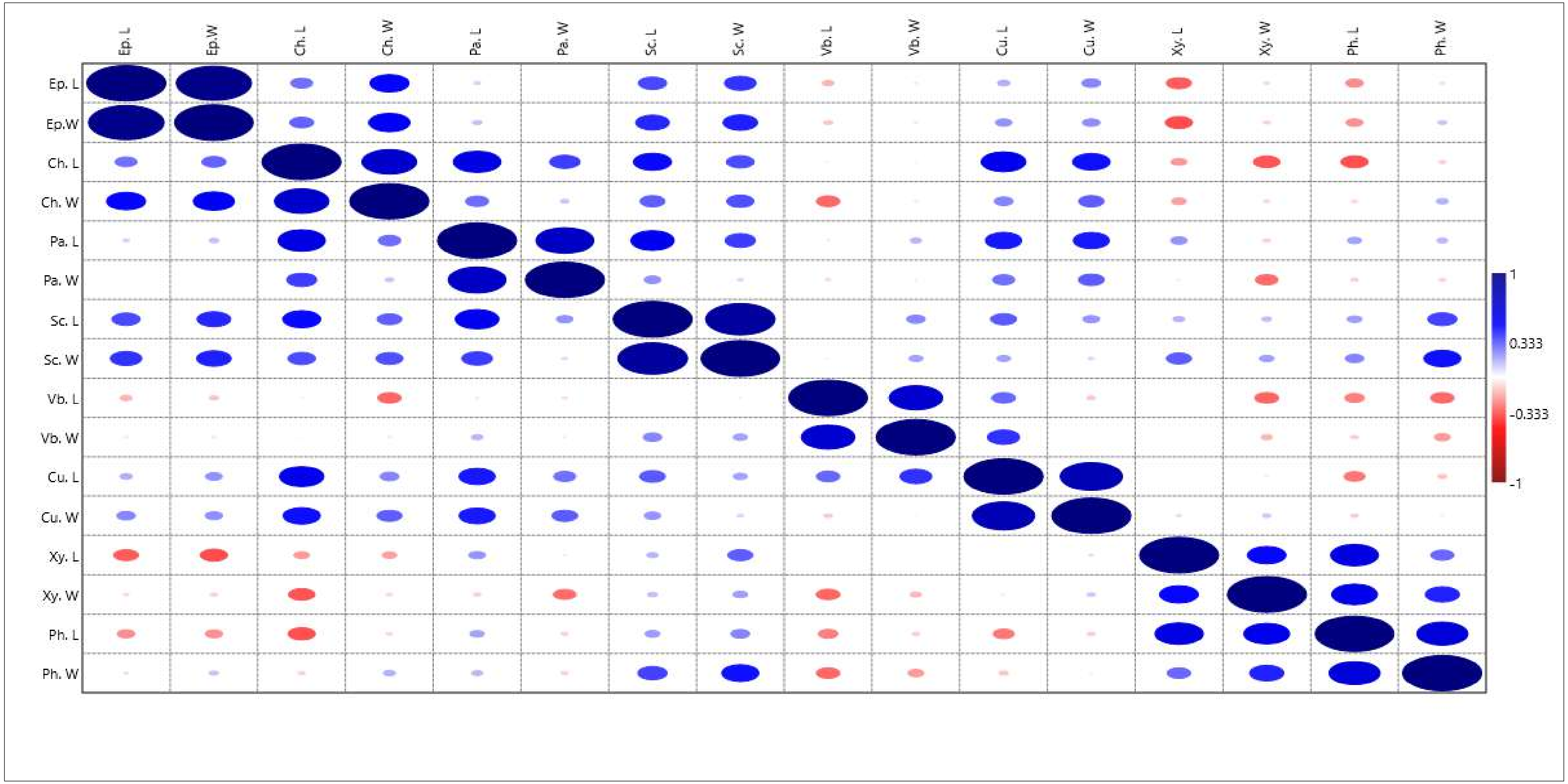

Figure 28. Correlation among mean values of length and width of Ep: Epidermis, Ch: Chlorenchyma, Pa: Parenchyma, Sc: Sclerenchyma, Vb: Vascular bundle, Cu: Culm, Xy: Xylem, Ph: Phloem (Red: negative, Blue: positive correlation)

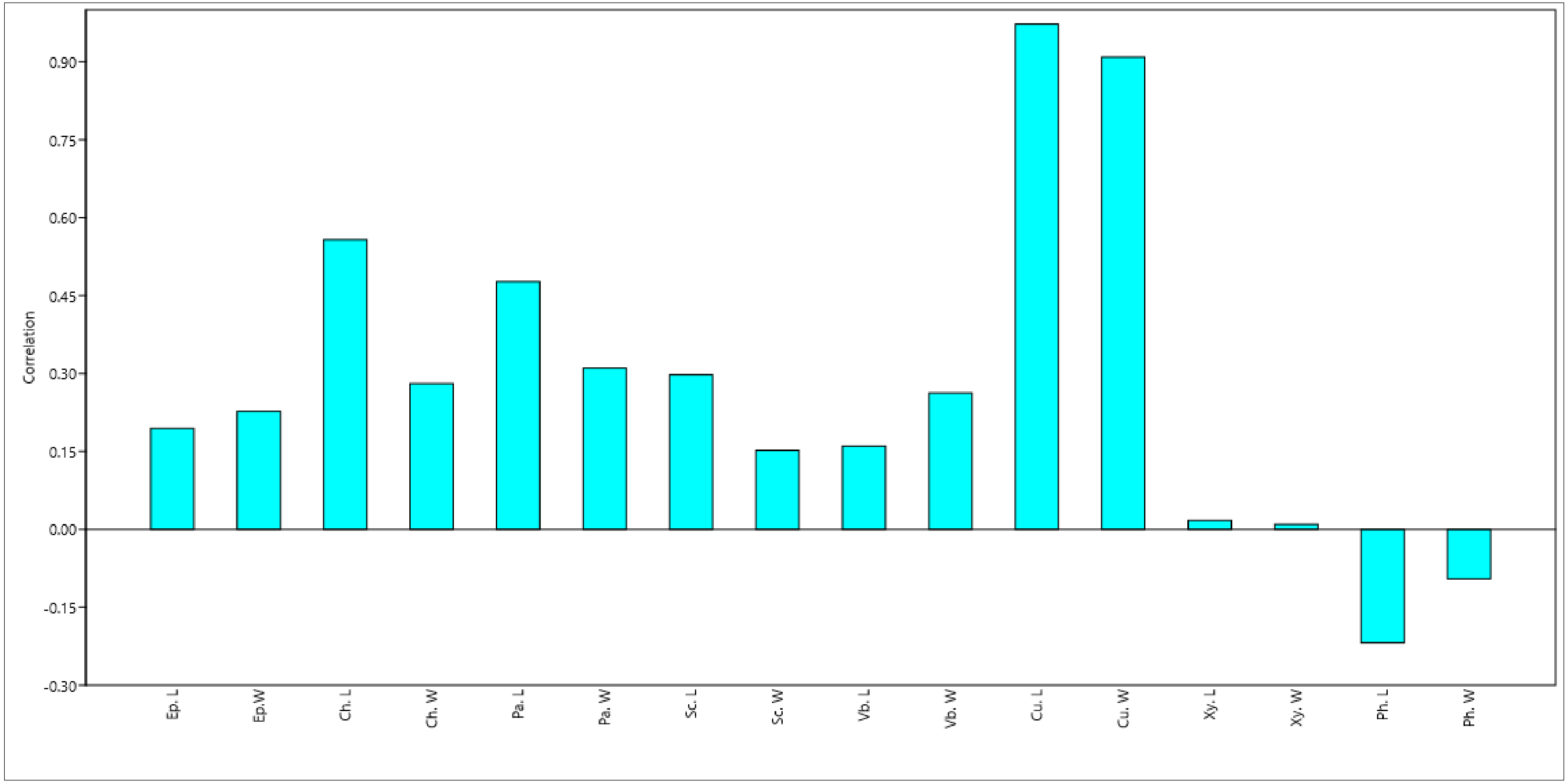

Figure 29. Loading plot based on positive and negative correlation among mean values of length and width of Ep: Epidermis, Ch: Chlorenchyma, Pa: Parenchyma, Sc: Sclerenchyma, Vb: Vascular bundle, Cu: Culm, Xy: Xylem, Ph: Phloem

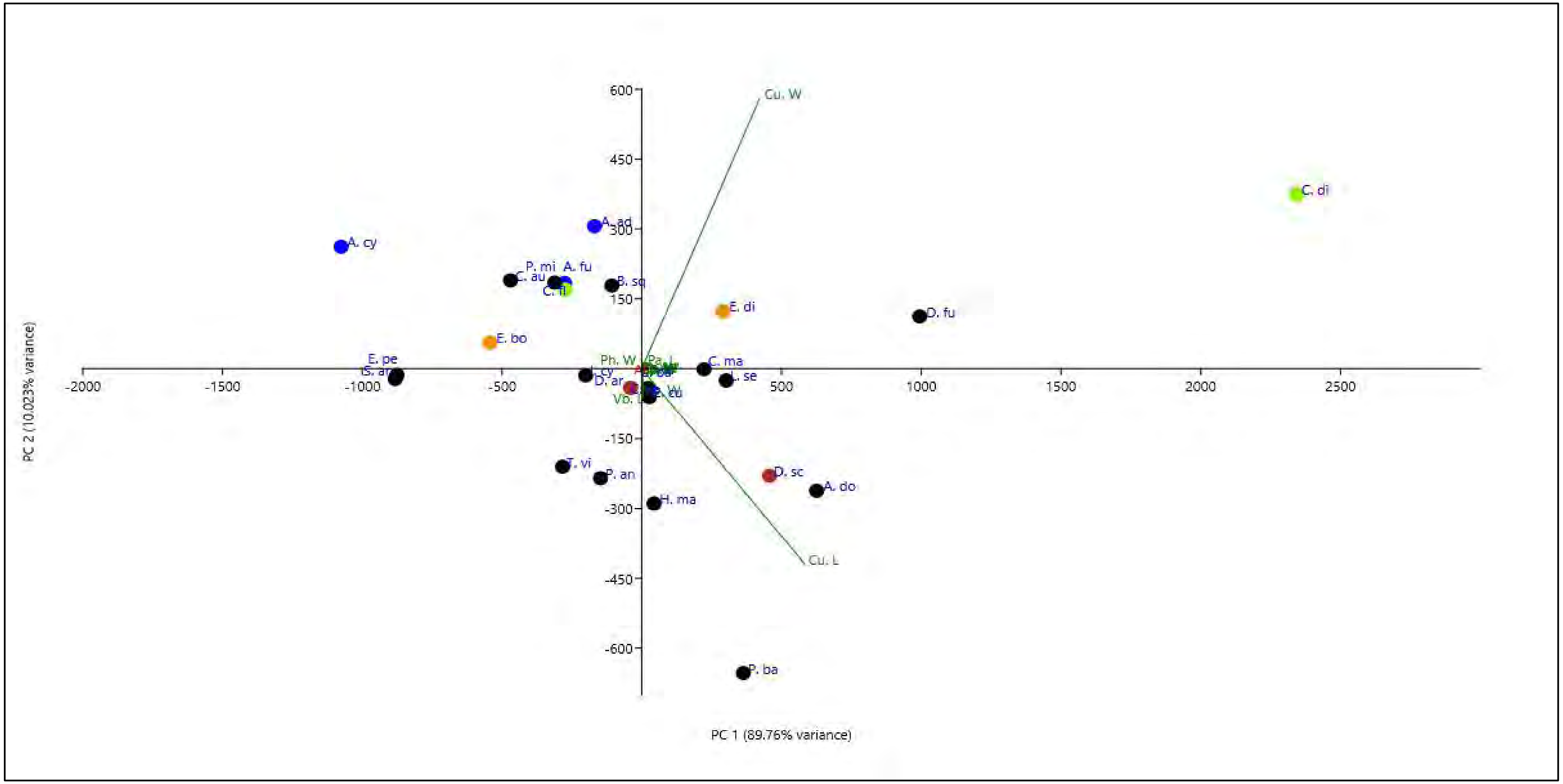

Figure 30. Utility of culm features in discriminating among species of grasses by PCA (length and width of epidermis, parenchyma, chlorenchyma, xylem, phloem, vascular bundles, culm, sclerenchyma)

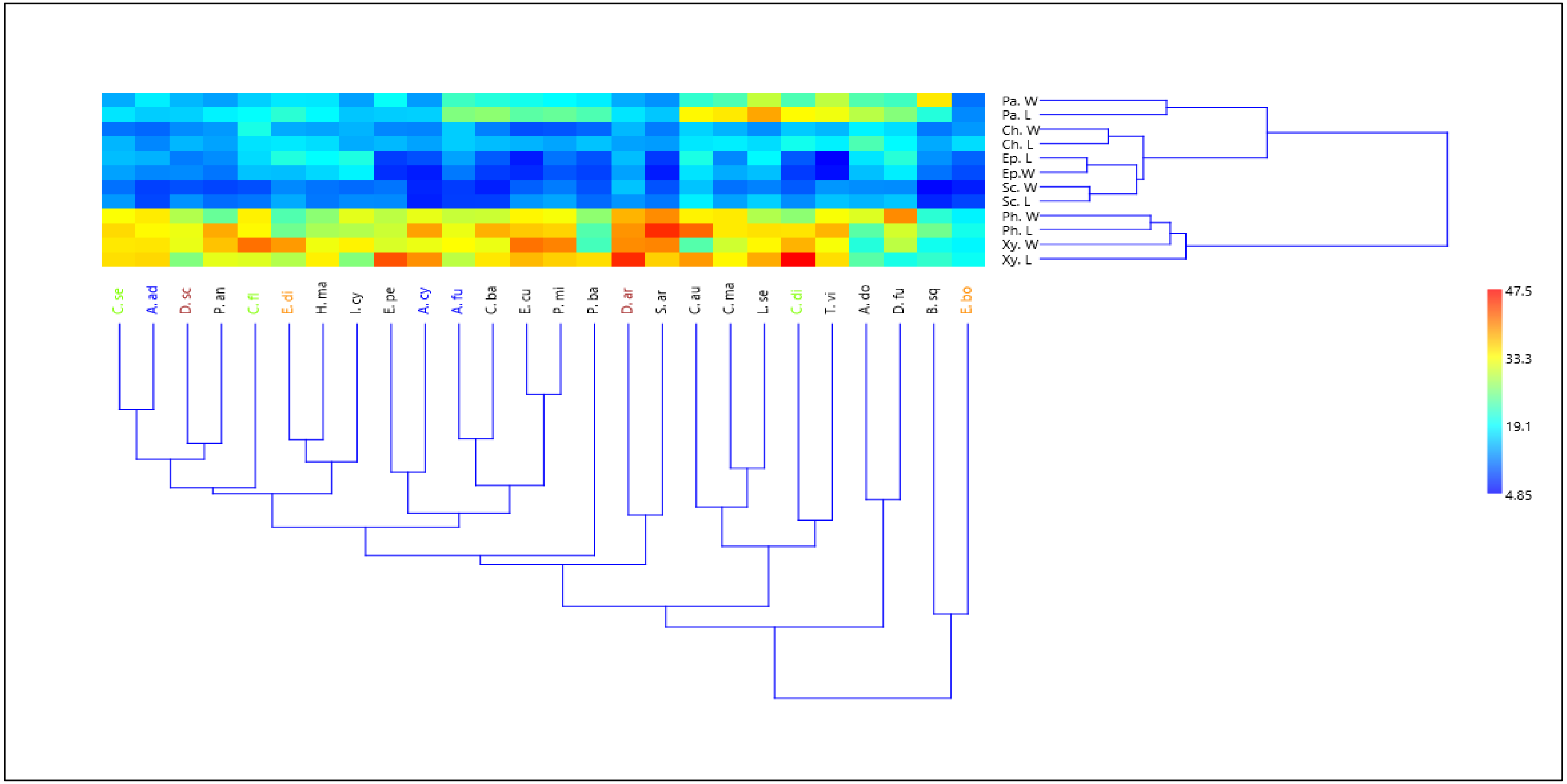

Figure 31. Dendrogram showing the similarity index of Poaceous taxa based on quantitative parameters of culm

Table 23. Qualitative characteristics based on culm anatomical features of selected Poaceous taxa

Plant Name	Ep. layers	No. of Major VB	No. peripheral VB	No. of Chlorenchyma layer	No of Sc layer	Bundle sheath made up of		Ground Pa	Central Cavity	Cortical /Marginal cavities	Scs Hy strands
						MS	PS				
Aristida adscensionis	1	8	11	2	3	+	+	++	-	-	+
Aristida cyanantha	1	16	22	7	7	+	+	+++	-	+	+
Aristida funiculate	2	19	23	4	5	+	+	+	+	+	+
Arundo donax	1	12	21	6	5	+	+	++	-	-	+
Boissiera squarrosa	2	10	17	5	4	+	+	+	+	+	+
Cenchrus setigerus	1	19	9	4	2	+	+	++	-	+	+
Chloris barbata	2	14	14	4	3	+	-	+	+	-	+
Chrysopogon aucheri	1	8	13	3	3	+	+	++	-	-	+
Cymbopogon martini	1	9	6	4	4	+	+	+	+	+	+
Dactyloctenium aristatum	1	20	10	9	2	+	-	++	-	-	+
Dactyloctenium scindicum	1	15	12	4	3	+	-	+	+	-	+
Diplachne fusca	1	15	22	9	1	+	+	+	+	+	+

Enneapogon persicus	1	12	7	4	4	+	+	+	+	-	+
Eragrostis curvula	2	8	12	3	10	+	-	+++	-	-	+
Eremopyrum bonaepartis	1	5	22	2	7	+	-	++	-	+	+
Eremopyrum distans	1	13	15	4	8	+	+	++	-	-	+
Hordeum marinum subsp. gussoneanum	1	11	12	4	8	+	+	++	-	-	+
Imperata cylindrica	2	12	9	4	2	+	+	++	-	+	+
Leptothrium senegalense	1	10	10	4	4	+	+	+++	-	-	+
Panicum antidotale	1	12	10	5	4	+	+	++	-	+	+
Cenchrus divisus	1	42	13	8	5	+	-	++	-	-	+
Cenchrus flaccidus	1	13	21	5	5	+	+	++	-	-	+
Phalaris minor	1	13	14	4	3	+	+	++	-	+	+
Piptatherum baluchistanicum	1	14	14	7	6	+	+	+	+	-	+
Schismus arabicus	1		12	5	5	+	-	+	+	-	+
Tetrapogon villosus	1	10	9	4	5	+	-	++	-	-	+

Ep: Epidermis, Ch: Chlorenchyma, Pa: Parenchyma, Sc: Sclerenchyma, Vb: Vascular bundle, Scs Hy: Sclerenchymatous hypodermis

Table 24. Qualitative characteristics based on culm anatomical features of selected Poaceous taxa

Species Name	Cu shape	Ep shape	Scs Hy shape	Pa shape	Ch shape	Vb Arrangements	Cut structure	Xy Shape	Ph Shape
Aristida adscensionis	Terete	Square to rectangular	Hexagonal	Hexagonal	Annular	Two Rows	Smooth	Oval	Angular
Aristida cyanantha	Elliptical	Square to Rectangular	Angular	Angular to Isodiametric	Isodiametric	Three rows	Smooth	Round	Angular
Aristida funiculate	Semiterete	Angular	Hexagonal	Irregular	Angular	Three rows	Smooth	Round to Oval(M) Isodiametric(P)	Tetra to hexagonal
Arundo donax	Terete	Angular	Tetra to Hexagonal	Round	Angular	Scattered	Smooth	Oval to Round(M) Isodiametric(P)	Angular
Boissiera squarrosa	Elliptical	Rectangular to Angular	Tetra to Hexagonal	Angular to Isodiametric	Lamellar to Annular	Two rows	Undulated	Round to Oval	Angular
Cenchrus setigerus	Terete	Angular	Angular	Isodiametric	Angular	Scattered	Smooth	Round(M) Isodiametric(P)	Angular
Chloris barbata	Quadrangular	Rectangular	Angular	Isodiametric	Lamellar	Two rows	Smooth	Round	Angular
Chrysopogon aucheri	Elliptical	Square	Tetra to Hexagonal	Hexagonal	Lamellar	Scattered	Smooth	Round(M) Oval(P)	Angular
Cymbopogon martini	Terete	Rectangular to Angular	Hexagonal	Round to Isodiametric	Angular	Single rows	Smooth	Round	Rectangular to Hexagonal
Dactyloctenium aristatum	Quadrangular	Rectangular	Tetra to Hexagonal	Irregular	Angular	Scattered	Smooth	Round to Oval	Angular
Dactyloctenium scindicum	Semiterete	Angular	Angular	Irregular	Angular	Two rows	Undulated	Oval(M) Round(P)	Rectangular to Hexagonal

Diplachne fusca	Terete	Angular	Triangular to Hexagonal	Angular	Angular	Four rows	Smooth	Round	Tetra to hexagonal
Enneapogon persicus	Elliptical	Round	Triangular to Hexagonal	Hexagonal	Angular	Two rows	Smooth	Round	Rectangular
Eragrostis curvula	Elliptical	Rectangular	Hexagonal	Round to Isodiametric	Angular	Two rows	Smooth	Round	Hexagonal
Eremopyrum bonaepartis	Terete	Round to Angular	Hexagonal	Tetra to Isodiametric	Angular	Scattered	Smooth	Round to Oval (M) Round to Isodiametric(P)	Tetra to Hexagonal
Eremopyrum distans	Terete	Rectangular	Tetra to Hexagonal	Irregular	Isodiametric	Three rows	Smooth	Round	Tetra to Hexagonal
Hordeum marinum subsp. gussoneanum	Terete	Square to Rectangular	Angular	Irregular	Lamellar	Two rows	Smooth	Round	Tetragonal
Imperata cylindrica	Elliptical	Angular	Angular	Irregular	Angular	Scattered	Undulated	Oval(M) Isodiametric(P)	Tetra to Hexagonal
Leptothrium senegalense	Elliptical	Rectangular to Angular	Hexagonal	Irregular	Lamellar	Two rows	Smooth	Round	Tetragonal
Panicum antidotale	Terete	Round	Hexagonal	Isodiametric	Lacunar	Scattered	Undulated	Round &Oval	Tetragonal
Cenchrus divisus	Terete	Square	Angular	Round to Angular	Hexagonal	Scattered	Smooth	Round	Tetragonal
Cenchrus flaccidus	Terete	Round	Angular	Round to Oval	Isodiametric	Scattered	Smooth	Round(M) Oval(P)	Triangular
Phalaris minor	Quadrangular	Square	Tetra to Hexagonal	Irregular	Angular	Three rows	Smooth	Round	Tetra to Hexagonal
Piptatherum baluchistanicum	Elliptical	Square	Hexagonal	Isodiametric	Angular	Three rows	Smooth	Round(M) Isodiametric(P)	Tetra to Hexagonal

Schismus arabicus	Elliptical	Rectangular	Angular	Irregular	Lamellar	Single row	Smooth	Round &Oval	Tetragonal
Tetrapogon villosus	Terete	Round	Hexagonal to Isodiametric	Isodiametric	Annular	Two rows	Smooth	Round(M) Isodiametric(P)	Angular

Ep: Epidermis, Ch: Chlorenchyma, Pa: Parenchyma, Sc: Sclerenchyma, Vb: Vascular bundle, Cu: Culm, Xy: Xylem, Ph: Phloem, Cu: Culm, Scs Hy: Sclerenchymatous hypodermis, Cut: Cuticle

Table 25. Quantitative data of histological properties of studied Poaceous species

Plant Species	L.W	Epidermal cell (µm)	Chlorenchyma (µm)	Parenchyma (µm)	Sclerenchyma (µm)	Vascular bundle (µm)	Xylem (µm)	Phloem (µm)	Culm (µm)
Min-max=Mean ± SE									
Aristida adscensionis	L	12-17=14.8±0.82689177	11.75-13=12.35±0.231840462	13.75-17.25=16.25±0.637377439	7.75-9.75=8.75±0.395284708	75.25-87=83.1±2.074548144	31.5-37.25=35.5±1.030776406	30.25-35.5=33.55±0.916515139	975-1001=985.6±4.445222154
	W	10.25-13.5=12.35±0.584166072	9.5-12=10.6±0.491172068	17.25-19.25=18.15±0.340954542	7.75-9=8.45±0.215058132	47-50.25=48.4±0.635413251	34-35.5=34.7±0.242383993	32.75-35.75=34.35±0.6254998	938-988=970.6±10.52425769
Aristida cyanantha	L	8.75-9.75=9.25±0.176776695	12.75-14.75=13.95±0.348209707	15.5-17.75=16.45=0.398434436	5.25-7.75=6.6±0.471699057	50.25-55.5=52.15±0.982980163	38.5-40.5=39.5±0.395284708	37.25-40.25=38.45±0.496235831	255-302=280.2±9.366963222
	W	5.25-7.75=6.4±0.465026881	11.75-12.75=12.2±0.16583124	13-14.75=13.55±0.310241841	6.5-7.25=6.85±0.15	25.25-32=29.25±1.535008143	31.25-33.5=32.2±0.413823634	29.75-33.5=32±0.702673466	393-408=401.2±2.853068524
Aristida funiculate	L	13.25-14.5=13.95±0.215058132	15.75-17=16.25±0.237170825	24.75-27.75=26.2±0.489897949	7-9=8.25±0.353553391	51.25-77=63.6±4.248234928	26.75-32=29.55±0.856592085	30.25-33=32.2±0.502493781	907-1003=972.8±18.81063529
	W	10.75-12.25=11.6±0.26925824	15.75-17=16.25±0.237170825	21.75-23.25=22.45±0.289395923	7-9=8.15=0.331662479	61-63.25=62.4±0.407737661	32.25-34.25=33.3±0.357071421	28.25-32.25=30.15±0.846315544	796-823=808.8±5.228766585
Arundo donax	L	15.75-19.5=17.7±0.629483916	21.75-24.5=23.3±±0.588430115	27.25-31.25=29.35±0.645174395	13.5-15.75=14.95±0.398434436	105.25-122=111.4±2.991237202	23.5-24.75=2±0.262202212	23.25-25.25=24.05±0.365718471	1902-1988=1962.6±15.64161117
	W	14.5-17.25=15.55±0.48347699	17.75-19.25=18.45±0.289395923	23-24.5=23.6±0.291547595	12-13.75=12.75±0.306186218	62.25-69.5=66.25±1.425219281	20.25-22=21.05	30.25-32.75=31.35±0.465026881	962-988=976.2±4.38634244

							±0.289395923		
Boissiera squarrosa	L	12.25-13.75=13±0.25	12.75-15.75=14.3±0.604152299	19.75-22=21.05±0.443001129	5.75-7.75=6.85±0.340954542	97.75-110.75=104.85±2.080564827	19.75-23=21.85±0.573367247	24.25-26.5=25.3±0.413823634	1008-1158=1109.4±26.88977501
	W	10.25-12.25=11.25±0.395284708	10.25-12.25=11.55±0.348209707	32.75-35.75=34.55±0.598957428	4.5-5.75=5.15±0.231840462	63.75-72.75=68.3±1.560048076	19.25-21.75=20±0.447213595	21-22=21.5±0.176776695	887-931=906.2±7.206941099
Cenchrus setigerus	L	18-19.5=18.8±0.289395923	16.25-18.75=17.15±0.437321392	36-39.75=38.25±0.617454452	15.25-19.75=16.45±0.834415963	90.25-97.75=93.85±1.391042774	37-38.75=38±0.370809924	31.75-38.75=34.9±1.25399362	1505-1607=1562.2±22.79122638
	W	14.5-17.25=15.55±0.48347699	12.25-13.25=12.8±0.16583124	25.5-38.75=29.95±2.276785014	11-13.25=12.1±0.451386752	54.75-63.75=59.2±1.560048076	31.25-36.5=33.65±0.944060379	27-33=28.95±1.044030651	966-988=978.2±4.079215611
Chloris barbata	L	8.75-11=9.95±0.413823634	12.75-17.25=15.35±1.014273139	25.75-29.75=26.95±0.73058196	7-9.5=8.3±0.508674749	83.25-99.75=92.55±2.934067143	33-37.25=34.6±0.792937576	36.75-38.5=37.65±0.340954542	1308-1388=1346.4±13.05603309
	W	7.25-9.5=8.15±0.375832409	11.5-12.75=12.05±0.215058132	19.75-22=21.05±0.398434436	5.25-7.75=6.55±0.496235831	54.5-64=58.25±1.57718103	30.75-33.25=32.2±0.421307489	28.75-32=30.4±0.545435606	788-821=801.8±5.8
Chrysopogon aucheri	L	19-22=20.55±0.577711	16.75-19.75=17.75±0.541987085	30.5-38.75=33.65±1.509552914	17.75-18.75=18.25±0.176776695	100.25-113=107.4±2.188892414	38.25-39.75=38.95±0.289395923	41.25-42.25=41.75±0.176776695	805-815=808.2±1.854723699
	W	16.7-19=17.55±0.398434436	16-17.25=16.65±0.231840462	19-24.5=21.75±0.974679434	15.25-16.25=15.75±0.176776695	75.75-85.5=80.35±2.00093728	23.25-24.25=23.75±0.176776695	33-34.75=33.95±0.348209707	695-709=703.2±2.973213749
Cymbopogon martini	L	11.5-13.75=12.45±0.382426464	17.25-19.5=18.15±0.375832409	32.25-37=34.5±0.832916562	12.75-14.75=13.9±0.331662479	99.5-108=102.8±1.709166464	30.75-35.5=33.7±0.9367497	32.25-35.75=34.4±0.696419414	1469-1505=1482.6±6.392182726

	W	13.75-15.25=14.45±0.266926956	14.25-15.5=14.85±0.231840462	21.75-25.5=23.3±0.73058196	9.75-12=11.15±0.407737661	66.75-72.75=69.5±1.204159458	29.75-31.5=30.6±0.291547595	32.75-35.75=34.4±0.556776436	921-988=952.4±12.51239386
Dactyloctenium aristatum	L	14.5-16.25=15.45±0.348209707	12.75-15.5=13.85±0.465026881	15.75-19.25=17.65±0.594768863	12.25-14=13.15±0.322102468	90.5-101.25=96.85±1.991544627	43.5-47=45.15±0.687386354	38.25-41.75=39.25±0.637377439	1276-1308=1293.8±5.877074102
	W	12.75-15.75=13.9±0.509901951	13.75-16=15.15±0.407737661	13.25-15.5=14.45±0.456891672	15.25-16.25=15.75±0.176776695	68.25-75.75=72.8±1.374772708	38.5-40.75=39.7±0.390512484	35.25-39.25=37.45±0.751664819	751-788=766±6.473020933
Dactyloctenium scindicum	L	10.25-13=11.75±0.575543222	13.5-18=15.05±0.80389676	15.25-18.25=16.5±0.670820393	12-13.75=13±0.306186218	80.25-88.75=84.4±1.372953022	23-28.5=26.25±1.051784198	30.5-33.75=32.25±0.575543222	1794-1824=1808±5.394441584
	W	11-13.75=12.6±0.471699057	12-13.25=12.65±0.231840462	14.5-15.5=15.1±0.20310096	8.75-9.75=9.25±0.176776695	101-112.75=106.8±1.893079502	30.25-33.25=32.1±0.509901951	25.25-31.75=29±1.112429773	882-922=904.4±6.682813779
Diplachne fusca	L	19.75-23=21.25±0.675462804	16.5-20.5=18.85±0.823862853	25.75-27=26.5±0.223606798	13.75-17.75=15.85±0.823862853	70.25-75.75=73.5±1.06653645	19-22.25=20.4±0.709753478	26-34.75=30.8±1.664707181	2001-2101=2043.6±23.06859337
	W	17-19.25=18.15±0.451386752	15.75-18.25=17.25±0.480884602	21.75-23.25=22.55±0.289395923	12-13.25=12.65±0.231840462	49.25-50.5=49.85±0.231840462	27.25-31=29.55±0.677310859	38.25-41.75=39.75±0.77055175	1478-1503=1493.8±4.726520919
Enneapogon persicus	L	7-9.75=8.25±0.575543222	14.5-15.75=15.15±0.231840462	15.25-17=16.25±0.32596012	12-13=12.55±0.183711731	75.75-87.75=82.9±2.180309611	39.5-47=43±1.459880132	25.75-32.25=30.35±1.20052072	583-618=601.4±5.626721959
	W	6.75-8=7.35±0.231840462	11.75-13.25=12.4±0.26925824	18.75-20.5=19.45±0.310241841	10.25-12.75=11.4±0.430116263	47-58.25=50.3±2.059126028	2.5-39.75=31.15±7.174869337	26-31=29.55±0.913099118	288-305=296.6±3.140063694
Eragrostis curvula	L	5.25-8.75=6.25±0.637377439	13.25-16.75=14.85±0.654790043	22.25-25.25=24.1±0.497493719	12.75-13.5=13.2±0.145773797	99.75-112=104.15±2.22289226	34-39.5=37.2±1.010569147	33.25-38.5=36.3±1.073545528	1352-1369=1360.8±3.168595904

	W	5.25-9=7.15±0.709753478	7.75-10.75=9.25±0.518411034	18.25-21.75=20.1±0.567890835	9.25-12.25=10.2±0.538516481	43.5-47.25=45.2±0.80389676	39.75-42.25=41.15±0.509901951	32-34.5=33.75±0.447213595	756-801=788±8.300602388
Eremopyrum bonaepartis	L	8.5-12=10.3±0.588430115	15.25-22=17.15±1.261447581	11.75-13.75=12.55±0.365718471	7.75-9.75=8.65±0.407737661	100.25-115.75=109.1±2.744995446	18.5-20.75=19.65±0.451386752	19.5-20.75=20.15±0.231840462	784-884=828.6±22.5756506
	W	8-9.75=9.05±0.310241841	12.25-14.75=13.35±0.430116263	10.25-12.25=11.2±0.339116499	5.25-7.25=6.45±0.365718471	44-50.5=47.15±1.27377392	17.75-19.75=18.65±0.340954542	17-19.75=18.45±0.566789202	521-578=550.2±10.70233619
Eremopyrum distans	L	19.75-22=20.85±0.375832409	15.75-19.25=17.9±0.640312424	19.75-22.25=21.45±0.443001129	13.25-15.5=14.35±0.437321392	88.75-97.75=91.45±1.672199151	22-34.25=29.05±2.280898946	23-29=25.3±1.044030651	1439-1488=1465.4±9.43716059
	W	14.25-17=15.5±0.474341649	12.75-15.5=14.35±0.527967802	17-19.25=17.9±0.4	11.75-13.75=12.5±0.353553391	63.5-68.5=65.35±0.896521054	37-41=38.9±0.678232998	22.25-24.5=23.55±0.40620192	1008-1124=1093±21.65871649
Hordeum marinum subsp. *Gussoneanum*	L	18.25-20.5=19.15±0.422788363	16.5-18=17.2±0.289395923	18.25-20.25=18.9±0.384057287	11-12=11.55±0.16583124	82-88.75=84.4±1.21346611	26-39.5=34.1±2.767670501	26.5-32=28.5±0.990580638	1488-1554=1509.4±11.48303096
	W	15.75-17.75=16.85±0.340954542	12.75-15.5=14±0.575543222	15.75-20.25=17.75±0.724568837	10.25-12.75=11.3±0.502493781	65.75-72=67.95±1.099431671	31-34.25=32.45±0.532681894	2.5-34.25=26.85±6.100512274	593-661=611.8±12.43945336
Imperata cylindrica	L	19.25-22.25=20.7±0.604152299	13.5-15.25=14.45±0.289395923	15.25-16.25=15.9±0.187082869	11.25-13.5=12.65±0.407737661	76.25-82.75=79.35±1.20052072	24.25-32=26.4±1.428723206	26.5-31.75=29.05±1.044030651	1105-1205=1148.4±16.95169608
	W	16.5-19.75=18.5±0.60724789	14-16=14.9±0.407737661	12.75-14.75=13.8±0.339116499	10.25-11.25±10.75±0.176776695	67-71.25=68.8±0.838152731	28.5-38.25=33.95±1.730967937	29.5-33.75=31.75±0.720243015	673-703=691.8±5.462600113
Leptothrium senegalense	L	14.5-16.25=15.45±0.348209707	13.75-15.75=15±0.353553391	15.75-19.25=17.65±0.594768863	12.25-14.25=13.4±0.384057287	91.25-101.25=96.7±1.984943324	33-38=35.1±0.816241386	34.75-35.75=35.35±0.16955825	1276-1308=1293.8±5.877074102

	W	12.75-15.75=13.9±0.509901951	10.25-12.25=11.25±0.379143772	13.25-15.5=14.45±0.456891672	10.25-11.75=11.15±0.257390754	68.25-75.75=72.8±1.374772708	33.5-35.5=34.55±0.398434436	30.25-35.25=33.1±0.9	751-788=766±6.473020933
Panicum antidotale	L	12-13.5=12.7±0.266926956	13-15.25=13.95±0.443001129	17.75-19.5=18.4±0.302076149	10.25-11.5=10.9±0.231840462	150.25-159=153.75±1.594913791	29.5-34=31.8±0.933407735	36.75-39.75=38±0.512347538	1305-1329=1316±4.847679857
	W	10.25-12.25=11.35±0.375832409	12.75-13.75=13.35±0.16955825	12.75-15.25=13.8±0.502493781	7.75-9.75=8.65±0.407737661	94.75-110.25=101.65±2.537961781	34.25-39=36.65±0.970180396	24-27.25=24.8±0.619475585	531-564=550±5.991660872
Cenchrus divisus	L	9-10.5=9.8±0.266926956	18-22=20.05±0.784219357	31.75-34.75=33.45±0.649037749	11.75-14.75=12.7±0.538516481	91.5-100.25=95.95±1.579952531	46-49.75=47.5±0.711512474	32.75-36.75=34.75±0.745821695	2899-3008=2982.2±20.86959511
	W	7-9.5=8.1±0.491172068	14-17.25=16.15±0.594768863	22-24.5=23.3±0.470372193	9-10.75=9.85±0.302076149	69-73=71.15±0.853668554	36-39.25=37.55±0.677310859	24.75-28.5=26.9±0.687386354	2491-2508=2500.6±2.942787794
Cenchrus flaccidus	L	16-18.25=17.1±0.430116263	16.75-18.5=17.5±0.32596012	18.25-20.25=19.25±0.32596012	10.75-12.75=11.45±0.348209707	72-75.75=73.95±0.739087275	26.25-38.75=31.4±2.111871208	29.5-36.75=33.6±1.343037602	966-1001=982.4±7.131619732
	W	14.75-16.5=15.7±0.320156212	19.75-21=20.45±0.215058132	15.75-17=16.45±0.215058132	8-10.25=9.2±0.413823634	54.75-63.75=58.25±1.693738469	38.75-44.25=41.25±0.977880361	31-37=34.05±1.162970335	793-808=798.6±2.731300057
Phalaris minor	L	8-10.5=9.35±0.515994186	14.25-17.75=15.7±0.743303437	22-24.5=23.35±0.515994186	10.25-11.25=10.75±0.176776695	207-230.75=223.6±4.375071428	33.75-36.5=35.15±0.451386752	22.75-24.75=23.65±0.375832409	1908-2008=1972.4±19.12223836
	W	7.75-9.75=8.5±0.353553391	9.25-12=10.55±0.496235831	15.75-19.75=18.2±0.743303437	8-10.25=9.35±0.375832409	126-135.75=129.85±1.789902232	21.25-24.5=22.85±0.645174395	25.5-28.75=27.05±0.604152299	504-533=514.8±5.462600113
Piptatherum baluchistanicum	L	10.5-12=11.3±0.289395923	12.75-15.5=14.05±0.555652769	24-25.75=24.75±0.32596012	12.75-15.25=14.1±0.444409721	100.25-113=107.8±2.314897406	34.25-39.5=35.9±0.950657667	33.25-37.75=35.7±0.860232527	901-988=940±15.85875153

	W	9-10.25=9.55±0.215058132	8.75-10.75=9.6±0.331662479	18-20.5=19.1±0.527967802	11.25-12.25=11.75±0.176776695	73.75-85.25=79.6±2.141261311	39.25-42.25=40.2±0.538516481	31.75-34.25=32.85±0.527967802	777-801=792.2±4.397726685
Schismus arabicus	L	7-9.5=8±0.454147553	12.75-14.75=13.55±0.348209707	15.25-16.5=15.95±0.215058132	10.25-12.75=11.5±0.425734659	96.25-100.75=98.7±0.923309266	33.75-37.25=35.85±0.718505393	43.75-47=45.1±0.615426681	577-614=600.2±6.319810124
	W	5.75-7.25=6.3±0.254950976	11.25-12.25=11.75±0.176776695	12-14.75=13±0.487339717	8.5-10.25=9.45±0.289395923	49.5-57=53.4±1.266392514	39.25-40.75=40.1±0.26925824	38.75-40.75=39.7±0.357071421	244-304=287.2±11.0516967
Tetrapogon villosus	L	4.25-5.5=4.85±0.231840462	17.25-19.5=18.3±0.390512484	30.25-34.75=32.5±0.911729126	15.25-16.25=15.75±0.176776695	63.75-75.25=68.9±1.896707674	33.25-37.25=35.15±0.796868873	36.75-38.25=37.4±0.26925824	1189-1203=1197.8±2.477902339
	W	5.25-6=5.55±0.145773797	14.5-15.75=15.15±0.231840462	28.25-30.5=29.65±0.392109679	12.75-13.75=13.25±0.176776695	87-91.5=88.8±0.834415963	30.25-37.25=32.9±1.218605761	30.25-34.5=32.95±0.755810823	466-501=484.6±6.021627687

L: Length, W: Width

Table 26. Dichotomous key based on the culm anatomical characters of Poaceous taxa

Link Character	Leads	Characters	Taxa/ Go to link character
1	+	Culm quadrangular	2
	-	Culm non-quadrangular	4
2	+	Epidermis cell square	*Phalaris minor*
	-	Epidermis cell rectangular	3
3	+	Angular sclerenchyma cell in hypodermis	*Chloris barbata*
	-	Tetra to hexagonal sclerenchyma cell in hypodermis	*Dactyloctenium aristatum*
4	+	Culm semiterete	5
	-	Culm non-semiterete	6
5	+	Three rows of vascular bundles	*Aristida funiculata*
	-	Two rows of vascular bundles	*Dactyloctenium scindicum*
6	+	Culm elliptical	7
	-	Culm terete	14
7	+	Vb rows	8
	-	Vb scattered	13
8	+	Vb single row	*Schismus arabicus*
	-	Vb two or three rows	9
9	+	Vb two rows	10
	-	Vb three rows	*Piptatherum baluchistanicum*
10	+	Epidermis cell round	*Enneapogon persicus*
	-	Epidermis cell not round	11
11	+	Cuticle undulated	*Boissiera squarrosa*
	-	Cuticle smooth	12
12	+	Angular chlorenchyma cell	*Eragrostis curvula*
	-	Lamellar chlorenchyma cell	*Leptothrium senegalense*
13	+	Hexagonal parenchyma cell	*Chrysopogon aucheri*

	-	Irregular parenchyma cell	*Imperata cylindrica*
14	+	Phloem angular	15
	-	Phloem not angular	17
15	+	Vb two rows	*Tetrapogon villosus*
	-	Vb scattered	16
16	+	Parenchyma cell round	*Arundo donax*
	-	Parenchyma cell isodiametric	*Cenchrus setigerus*
17	+	Phloem cell triangular	*Cenchrus flaccidus*
	-	Phloem cell not triangular	18
18	+	Chlorenchyma cell lamellar	*Hordeum marinum* subsp. gussoneanum
	-	Chlorenchyma cell non lamellar	19
19	+	Vb rows	20
	-	Vb scattered	22
20	+	Vb single row	*Cymbopogon martini*
	-	Vb more than one row	21
21	+	Vb three rows	*Eremopyrum distans*
	-	Vb four rows	*Diplachne fusca*
22	+	Parenchyma cell square	*Cenchrus divisus*
	-	Parenchyma cells not square	*23*
23	+	Cuticle smooth	*Eremopyrum bonaepartis*
	-	Cuticle undulated	*Panicum antidotale*

Conclusion

4. Conclusion

The present research is the opening systematics study of the angiospermic flora of Baluchistan concerning palynology and anatomy. A total of 109 angiosperms belonging to two dicots and one monocot family, were included. Asteraceae was the leading family with 52 members, followed by Poaceae with 38 members, and Boraginaceae with 17 species. The palynological and anatomical examination was found highly significant in the separation, discrimination, and identification of taxa.

a) Palynology

Pollen analysis utilizing light microscopy and scanning electron microscopy, of 109 angiosperms of Baluchistan revealed that the pollen morphology of the investigated species is important in the separation and delimitation of taxa at a genus and species level which was further reinforced by the distinctive documentation and visualization. For the taxonomic distinction of the species under investigation, the differences in polarity, shape class, number, and arrangement of apertures, Amb, NPC, polar and equatorial views, exine sculpturing, exine surface, aperture membrane, edges of apertures, and lacuna shape proved significant. The important diagnostic variations were within shape (prolate spheroidal, oblate spheroidal, suboblate, subprolate, and prolate), number of apertures (tricolpate, tricolporate, tetracolpate, tetracolporate, hexacolpate, hexacolporate), polar and equatorial views (from circular to triangular obtuse/truncate, convex, concave, and elliptic), exine (echinate, lophate, scabrate, verrucate, psilate, gemmate, foveolate, perforate or non-perforate), aperture membrane (smooth, granulate, operculate), Amb (goniotreme, peritreme, and ptychotreme). The taxonomic key ultimately establishes the species boundaries necessary for accurate taxonomic identification by accessing the heterogeneity in various pollen characteristics. The multivariate analysis such as PCA, dendrogram, ridge line plot, normal probability plot, and correlation plot, further provided in-depth insights into the characterization of species.

b) Anatomy

The anatomy of 79 angiospermic species from the arid to semiarid regions of Baluchistan was documented via microscopic imaging analysis. The petiole wings, outline, cuticle, groove in the upper surface, shapes and number of layers of epidermal cells, parenchyma cells, collenchyma cells, chlorenchyma cells, sclerenchyma cells, vascularization (number and arrangement of vascular bundles, shapes of xylem and phloem cells), air spaces/cavities, and trichomes were comprehensively examined. The petiole anatomy of 39 Asteraceous and 14 Boraginaceous species was determined by comprehensively examining the histology of each species comparatively. Some of the highly significant traits taxonomically were the shapes and size of cells (such as collenchyma was found as angular, lamellar, lacunar, and annular), vascular bundle arrangement (collateral closed, collateral open, amphicribral, and bicollateral), outline (sulcate, flat, oval, round or circular), trichomes (unicellular, uniseriate, and multiseriate). The quantitative data analysis determined important trends in the data set via constructing PCA, dendrogram, and correlation plot and investigated the positively and negatively correlated traits, phylogenetic lineage, and diagnostic traits for species identification.

The study of the culm anatomy of 26 Poaceous species determined that the anatomy of these organs could be of great taxonomic importance. The diagnostic characters were major and peripheral vascular bundles, central and marginal cavities, bundle sheath, sclarification, hypodermis, culm shape (terete, elliptical, semi terete, quadrangular), vascular bundles arrangement (one row, two rows, multiple rows, and scattered). The shapes of different cells possessed variations. All these differences were successfully used in the construction of the taxonomic key. Further, the alterations in the qualitative characters were analyzed via multivariate analysis. PCA, two-way dendrogram, and correlation plots demonstrated the diversity from species to species. Conclusively the variations in the examined palynological and anatomical traits hold great taxonomic value for the delimitation of angiosperms.

Future perspectives

5. Future Perspectives

- The systematics of the studied angiospermic flora can further be strengthened by carrying out DNA barcoding studies for the confirmation of the identification of species at the molecular level.
- Analysing the genes that control the examined palynological and anatomical traits, in the future will be a significant contribution to linking modern systematics with molecular taxonomy.
- The details to the current studies can be added by transmission electron microscopic (TEM) examination.
- In future the advanced phylogenetic studies on the taxonomic data utilizing recent bioinformatics tools for the delimitation of angiospermic flora will be highly beneficial.
- In Asteraceae, future research examining ektexiniuos bodies (formation on the pore surface) and mesoporia (distance between the pores) will support efforts to produce more precise taxonomic markers for Asteraceous species.
- The correct identification via palynological and anatomical atlas could be highly useful in the investigation of adulteration in herbal medicines.
- The unique flora of Baluchistan mostly has traditional medicinal values, chemical exploration via modern techniques such as high-performance liquid chromatography, and spectrophotometry will have significant importance for the pharmaceutical industry, local communities, and national revenue.

References

Abbasi, M., Attar, F. and Nejad, F. G. (2011). Anatomical studies on several species of Heliotropium L. in Iran. *Notulae Scientia Biologicae, 3*(4), 35.

Abid, R. and Qaiser, M. (2023). Pollen morphology of Lactuca L. (s. lat.) (Cichorieae: Asteraceae) from Hindukush, Western Himalayan and Karakorum ranges and its taxonomic significance. *Palynology, 47*, 1-13.

Abid, R. B. and Qaiser, M. (2022). Pollen Morphology of Melanoseris Decne. (Cichorieae-asteraceae) From Pakistan and Western Himalayas and Its Taxonomic Significance. *Research square,* 1-25.

Achakzai, K., Firdous, S., Bibi, A. and Khalid, S. (2016). Juniper (Juniperus excelsa M. BIEB) forest of Ziarat in danger of vanishing: a review. *American-Eurasian Journal of Agriculture and Environmental Sciences*, *16*(2), 320-325.

Adedeji, O. (2004) Leaf epidermal studies of the species Of *Emilia Cass.* (Senecioneae, Asteraceae) in Nigeria. *Botanica Lithuanica*, *10*(2),*1392-1665.*

Adedeji, O. and Jewoola, O. A (2008). Importance of leaf epidermal characters in the Asteraceae family. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 36*(2), 7-16.

Ahmad, F., Khan, M. A., Ahmad, M., Zafar, M., Nazir, A. and S. K. Marwat. (2009). Taxonomic studies of grasses and their indigenous uses in the salt range area of Pakistan. *African Journal of Biotechnology, 8*(2),231-249.

Ahmad, M., Bano, A., Zafar, M., Khan, M. A., Chaudhry, M. J. I. and Sultana, S. (2013). Pollen morphology of some species of the family Asteraceae from the alpine zone, Deosai Plateau, northern Pakistan. *Palynology, 37*, 189-195.

Ahmed, K., Shahid, S., Ismail, T., Nawaz, N. and Wang, X. J. (2018). Absolute homogeneity assessment of precipitation time series in an arid region of Pakistan. *Atmósfera*, *31*(3), 301-316.

Ahmed, K., Shahid, S., Nawaz, N. and Khan, N. (2019). Modeling climate change impacts on precipitation in arid regions of Pakistan: a non-local model output statistics downscaling approach. *Theoretical and Applied Climatology*, *137*(1), 1347-1364.

Akçin, Ö. E., Kandemir, N. and Akçin, Y. (2004). A morphological and anatomical study on a medicinal and edible plant Trachystemon orientalis (L.) G. Don (Boraginaceae) in the Black Sea Region. *Turkish Journal of Botany*, *28*(4), 435-442.

Akhtar, A., Ahmad, M., Mahmood, T., Khan, A. M., Arfan, M., Abbas, Q. and Khan, A. (2022). Microscopic characterization of petiole anatomy of Asteraceous taxa of Western Himalaya-Pakistan. *Microscopy Research and Technique*, *85*(2), 591-606.

Akinnubi, F. M., Akinloye, A. J. and Oladipo, O. T. (2013). Petiole anatomy of some species of Asteraceae in southwest Nigeria. *African Journal of Plant Science*, *7*(12), 608-612.

AL-Hadeethi, M. A., Al-Anbari, A. K. and AL-Aani, M. N. (2016). Anatomical study of vegetative parts and Powder microscopy of Cordia myxa L.(Boraginaceae) in Iraq. *International journal of advances in chemical engineering and biological sciences, 3*, 232-235.

Ali, A., Anjum, S., Shoukat, K., Masood, A., Baloch, A. S., Jamali, A. Z. and Ismail, T. (2023). Morphological traits characterizing environmental adaptation of some wild plants collected from the Sibi and Quetta Regions of Balochistan. *Pakistan's Multidisciplinary Journal for Arts & Science*, *4*(02), 33-52.

Ali, S. and Perveen, A. (2021). Pollen vitality and germination capacity in three taxa of the genus Brassica L. (BraL. (caceae). *Pakistan Journal of Botany*, *53*,1079-1082.

Ali, S. I. (2008). Significance of flora with special reference to Pakistan. *Pakistan Journal of Botany*, *40*(3), 967-971.

Ali, S. I. and Qaiser, M. (1986). A phytogeographical analysis of the phanerogams of Pakistan and Kashmir. *Proceedings of the Royal Society of Edinburgh, Section B: Biological Sciences*, *89*, 89-101.

Al-Khafaji, B. A. and Al-Bermani, A.K. (2014). Comparative Study of stem Anatomy for some Poa L. Species (Poaceae) in Iraq. *Kufa Journal for Agricultural Science*, 6(4),1-15.

Al-Shammary, K. I. A. (1991). Systematic studies of the Saxifragaceae sl, chiefly from the southern hemisphere. University of Leicester (United Kingdom).

Al-Suboh, K. I., Al-Khesraji, T. O. and Al-Assie, A. H. (2019). Comparative anatomical study of three Centaurea species (Asteraceae) from Iraq. *Plant Archives 19*(2), 3404-3410.

Anjum, P. and Muhammad, Q. (2012). Pollen flora of Pakistan-LXIX. Poaceae. *Pakistan Journal of Botany,44*(2), 747-756.

Apóstolo, N. M., Luna, A. L. and Yormann, G. E. (2022). Morpho-anatomy of Bambusa multiplex, B. tuldoides and B. vulgaris cv. vittata culm leaves (Poaceae–Bambusoideae-Bambuseae). *Flora.*, 297, 1-14.

Arnold, E. (1973). Peacock's Elementary Microtechnique. Pitman Press, Bath, Great Britain.

Aslam, S., Siddiqui, S., Ullah, U., Manzoor, U., Lateef, T., Samreen, N. and Ghalib, S. A. (2022). Vertebrate Wildlife of Pakistan: A Review.

Attar, F., Esfandani-Bozchaloyi, S., Mirtadzadini, M., Ullah, F. and Zaman, W. (2019). Foliar and stem epidermal anatomy of the tribe Cynoglosseae (Boraginaceae) and their taxonomic significance. *Microscopy Research and Technique*, X *82*(6), 786-802.

Baas, P. (1982). Systematic, phylogenetic, and ecological wood anatomy—history and perspectives. In *New perspectives in wood anatomy: published on the occasion of the 50th anniversary of the International Association of Wood Anatomists* Dordrecht: *Springer Netherlands* 23-58.

Bahadur, S., Ahmad, M., Zafar, M., Sultana, S., Begum, N., Ashfaq, S. and Ayaz, A. (2019). Palyno-anatomical studies of monocot taxa and its taxonomic implications using light and scanning electron microscopy. *Microscopy Research and Technique*, *82*(4), 373-393.

Bahadur, S., Rehman, S., Long, W., Ahmad, M., Ullah, F. and Butt, M. A. (2023). Foliar micromorphology with emphasis on the trichomes diversity and its taxonomic relevance in selected tribes of Asteraceae from Hainan Island. *Flora, 300*, 1-18.

Baloch, M. P., Marri, M. Y. and Qaisrani, M. A. (2000). Plants treasures, traditional knowledge and baloch society Botany. *Balochistan*.

Banan, S. A., Al-Watban, A. A., Doaigey, A. R., Alsahli, A. A. and El-Zaidy, M. (2019). Anatomical adaptations in species of Poaceae growing in Al-Hair region of Riyadh, Saudi Arabia. *African journal of plant science, 13*(7),201-208.

Bano, A., Zahid, M. A., Arsalan, A., Abdullah, M., Khan, A., Saleem, G. and Ahmed, N. (2015). A preliminary study of soil analysis of Sonmiani, Gadani and Kund Malir coasts of District Lasbela, Balochistan, Pakistan.

Baranova, M. A. (1992). The epidermal structures and systematic position of the Austrabaileyaceae. *Botaničeskij žurnal*, *77*(6), 1-17.

Barthlott, W. and Theisen, I. (1998). Epicuticular wax ultrastructure. In *Flowering Plants· Monocotyledons: Lilianae (except Orchidaceae)* Berlin, Springer Berlin Heidelberg. 20-22.

Baser, B., Özler, H., Cabi, E., Dogan, M. and Pehlİ, S. V. (2009). Pollen morphology of the genus Eremopyrum (Poaceae) in Turkey. *World Applied Sciences Journal*, *6*(12), 1655-1659.

Bercu, R. and Popoviciu, D. R. (2014). Anatomical study of *Ficus carica* L. leaf. *Annals of the Romanian Society for Cell Biology, 19*(1), 33-37.

Bibi, T., Ahmad, M., Tareen, N. M., Jabeen, R., Sultana, S., Zafar, M. and Zain-ul-Abidin, S. (2015). The endemic medicinal plants of Northern Balochistan, Pakistan and their uses in traditional medicine. *Journal of Ethnopharmacology, 173*, 1-10.

Binzet, R., Kandemir, I. and N. Orcan. (2018). Numerical taxonomic study of the genus Onosma L. (Boraginaceae) from eastern mediterranean region in Turkey. *Pakistan. Journal of. Botany*, 50,561-573.

Blackmore, S. (1986). The identification and taxonomic significance of lophate pollen in the Compositae. *Canadian Journal of Botany, 64,* 3101-3112.

Buys, M. H. and Hilger, H. H. (2003). Boraginaceae cymes are exclusively scorpioid and not helicoid. *Taxon*, 52,719-724.

Buzdar, M. A., Latif, R. A., Ahmed, I. and Uthal, D. L. (2023). Appraisal of Wastewater Generation at Nani Mandir of Hingol National Park and Community Based Holistic Conservation.

Cantino, P. D., Wagstaff, S. J. and Olmstead, R. G. (1998). Caryopteris (Lamiaceae) and the conflict between phylogenetic and pragmatic considerations in botanical nomenclature. *Systematic Botany*, 369-386.

Carlquist, S. (1970). Wood anatomy of insular species of Plantago and the problem of raylessness. *Bulletin of the Torrey Botanical Club*, 353-361.

Carlquist, S. (1977). Wood anatomy of Onagraceae: additional species and concepts. *Annals of the Missouri Botanical Garden*, 627-637.

Carlquist, S. (1981). Wood anatomy of Nepenthaceae. *Bulletin of the Torrey Botanical Club*, 324-330.

Carlquist, S. (1990). Wood anatomy and relationships of Lactoridaceae. *American Journal of Botany*, *77*(11), 1498-1505.

Chandio, T. A., Khan, M. N., Muhammad, M. T., Yalcinkaya, O. and Kayis, A. F. (2020). Co-exposure of neurotoxic contaminants (Pb and Mn) in drinking water of Zhob District, Baluchistan Pakistan. *Environmental nanotechnology, monitoring & management*, *14*, 1-11.

Chaudhary, A. H., Khokhar, S. N., Zafar, Y. and Hafeez, F. (1981). Actinomycetous root nodules in angiosperms of Pakistan. *Plant and Soil*, *60*, 341-348.

Chen, J. and Craven, L. (2007). Flora of China. Flora of China 13, 321-328.

Chen, J.-H., Sun, H. and Yang, Y. P. (2008). Comparative morphology of leaf epidermis of Salix (Salicaceae) with special emphasis on sections Lindleyanae and Retusae. *Botanical journal of the Linnean Society, 157*(2), 311-322.

Coutinho, A. P., Aguiar, C. F., Bandeira, D. S. D. and Dinis, A. M. (2011). Comparative pollen morphology of the Iberian species of Pulicaria (Asteraceae, Inuleae, Inulinae) and its taxonomic significance. *Plant Systematics and Evolution, 297*, 171-183.

Daironas, J. V., Serebryanaya, F. K. and Zilfikarov, I. N. (2014). Comparative morphological and anatomical study of Onosma caucasica Levin. ex M. Pop. and Onosma sericea Willd. (Boraginaceae Juss.). *Pharmacognosy Journal, 6*, 22-28.

Dajoz, I., Till-Bottraud, I. and Gouyon, P. H. (1991). *Evolution of pollen morphology. Science, 253*(5015), 66-68.

Dawar, R., Qaiser, M. and Perveen, A. (2002). Pollen morphology of Inula L. (s. str.). *Pakistan Journal of Botany, 36*, 719-724.

De las Mercedes Sosa, M., Via do Pico, G. M. and Dematteis, M. (2014). Comparative anatomy of leaves and stems in some species of the South American genus Chrysolaena (Vernonieae, Asteraceae) and taxonomic implications. *Nordic Journal of Botany, 32*(5), 611-619.

De Micco, V. and Aronne, G. (2012). Morpho-anatomical traits for plant adaptation to drought. Plant responses to drought stress: from morphological to molecular features. *Springer Berlin Heidelberg*, 37-61.

Díez, M. J. and Valdés, B. (1991). Pollen morphology of the tribes Eritrichieae and Cynoglosseae (Boraginaceae) in the Iberian Peninsula and its taxonomic significance. *Botanical Journal of the Linnean Society, 107*,49-66.

Ekeke, C. and C. A. Ogazie. (2020). Systematic Significance of Petiole Anatomical Characteristics in Some Members of Asteraceae from Some Parts of Nigeria. *Singapore Journal of Scientific Research,* 10, 387-399.

Elkiran, O. (2023). The comparative morphological, anatomical and palynological studies on the genus *Helleborus* (Ranunculaceae) growing in turkey. *Pakistan Journal of Botany,*55,539-547.

Ellis, R. P. (1979). A procedure for standardizing comparative leaf anatomy in the Poaceae. II. The epidermis as seen in surface view. *Bothalia*, 12(4),641-671.

Erdtman, G. (1963). Palynology. In *Advances in botanical research* (Vol. 1, pp. 149-208). Academic Press.

Erdtman, G. (1969). Handbook of palynology: morphology, taxonomy, ecology.

Erdtman, G. (1969). Handbook of palynology: morphology-taxonomy-ecology: *an introduction to the study of pollen grains and spores*.

Erwin, D. M. and Stockey, R. A. (1991). Silicified monocotyledons from the Middle Eocene Princeton chert (Allenby Formation) of British Columbia, Canada. *Review of Palaeobotany and Palynology,70*(1-2), 147-162.

Eymann, J., Degreef, J., Häuser, C., Monje, J. C., Samyn, Y. and VandenSpiegel, D. (2010). *Manual on field recording techniques and protocols for all taxa biodiversity inventories and monitoring*. Abc Taxa.

Ferguson, I. K. and Skvarla, J. J. (1982). Pollen morphology in relation to pollinators in Papilionoideae (Leguminosae). *Botanical Journal of the Linnean Society*, *84*(3), 183-193.

Ferrauto, G., Costa, R. and Pavone, P. (2017). Pollen diversity in the genus Ptilostemon (Asteraceae, Cardueae) from Italy and its taxonomic and palynoecological implications. *Plant Biosystems, 151*, 276-290.

Frodin, D. G., Lowry II, P. P. and Plunkett, G. M. (2010). Schefflera (Araliaceae): taxonomic history, overview, and progress. *Plant Diversity and Evolution*, *128*(3), 561.

García, D. L. Q., Chávez, R. P. and Sánchez, M. (1997). Morphology of pollen grains of the family Boraginaceae from the Chamela biology station, Jalisco, Mexico. *Polybotany*, 4,37-53.

Gasparino, E. C., Souza, C. N. D. and Cruz-Barros, M. A. V. D. (2014). Pollen flora of reserve of the state park of the fountains of Ipiranga (São Paulo, São Paulo state, Brazil). Families: 141-Boraginaceae and 149-Gesneriaceae. *Hoehnea, 41*, 423-430.

Gibson, A. C. (1994). Vascular tissues. In *Caryophyllales: evolution and systematics* (pp. 45-74). Berlin, Heidelberg: Springer Berlin Heidelberg.

Goodman, S. M. and Abdul Ghafoor, A. G. (1992). The ethnobotany of southern Balochistan, Pakistan, with particular reference to medicinal plants.

Grant-Downton, R. (2009). Pollen terminology. An illustrated handbook.

Guimarães, J. T. F., Carreira, L. M. M., Alves, R., Martins e Souza Filho, P. W., Giannini, T. C., Macambira, H. J. and Rodrigues, T. M. (2018). Pollen morphology of the Poaceae: implications of the palynological and paleoecological records of the southeastern Amazon in Brazil. *Palynology*, *42*(3), 311-323.

Gürdal, B. and Nath, E. O. (2022). A comparative anatomical study on genus *Pulicaria* gaertn. (Compositae) from Turkey and its taxonomic implication. *Pakistan Journal of Botany, 54*,1849-1858.

Halbritter, H., Ulrich, S., Grímsson, F., Weber, M., Zetter, R., Hesse, M. and Frosch-Radivo, A. (2018). Pollen morphology and ultrastructure. *Illustrated Pollen Terminology*, 37-65.

Hameed, A., Zafar, M., Ullah, R., Shahat, A. A., Ahmad, M., Cheema, S. I. and Majeed, S. (2020). Systematic significance of pollen morphology and foliar epidermal anatomy of medicinal plants using SEM and LM techniques. *Microscopy Research and Technique*, *83*, 1007-1022.

Hameed, M., T. Nawaz, M. Ashraf, A. Tufail, H. Kanwal, M. S. A. Ahmad, M. and Ahmad, I. (2012). Leaf anatomical adaptations of some halophytic and xerophytic sedges of the Punjab. *Pakistan Journal of Botany, 44*,159-164.

Hammer, Ø., Harper, D. A. and Ryan, P. D. (2001). PAST: Paleontological statistics software package for education and data analysis. *Palaeontol*, *4*(1),1- 9.

Harun, N., Shaheen, S., Ahmad, M., Abbas, Z., Bibi, F. and Arshad, F. (2022). Scanning electron microscopic imaging of palynological metaphors: A case study of family Poaceae taxa. *Microscopy Research and Technique*, *85*(5), 1703-1712.

Hasegawa, T. M., Itagaki, T. and Sakai, S. (2023). Pollen morphology for successful pollination dependent on pollinator taxa in a generalist plant: relationship with foraging behavior. Oecologia, 1-10.

Hassan, K., Amin, A., Ellmouni, F. and Abbas, H. (2022). Taxonomic revision and anatomical studies of the genus aegilops l.(poaceae) with sectional confirmation. *Fayoum Journal of Agricultural Research and Development, 36*(3),342-355.

Heneidak, S. and Shaheen, A.S.M. (2007). Characteristics of the proximal to distal regions of the petioles to identify 15 tree species of Papilionoideae-Fabaceae. *Bangladesh Journal of Plant Taxonomy, 14*(2), 101-115.

Hidalgo, O., Susanna, A., Garcia-Jacas, N. and Martin, J. (2008). From acaveate to caveate: evolution of pollen types in the Rhaponticum group (Asteraceae, Centaureinae) related to extreme conditions. *Botanical Journal of the Linnean Society*, *158*, 499-510.

Hooker, J. D. (1879). Flora of British India, Vol. II. *Flora of British India, Vol. II.*

Hussain, A., Potter, D., Hayat, M. Q., Sahreen, S. and Bokhari, S. A. I. (2019). Pollen morphology and its systematic implication on some species of Artemisia L. from Gilgit-Baltistan Pakistan. *Bangladesh Journal of Plant Taxonomy, 26,* 157-168.

Iamonico, D., Hussain, A. N., Sindhu, A., Saradamma, V. N., Kumar, A., Shaheen, S., Munir, M. and Fortini, P. (2023). Trying to Understand the Complicated Taxonomy in Amaranthus (Amaranthaceae): Insights on Seeds Micromorphology. *Plants, 12*, 987-1013.

Jamro, S., Channa, F. N., Dars, G. H., Ansari, K. and Krakauer, N. Y. (2020). Exploring the evolution of drought characteristics in Balochistan, *Pakistan. Applied Sciences, 10*(3), 913-927.

Jan, P. S., Sadia, B., Yousaf, A., Naz, N., Rehmat, N., Tahira, B. and Bazai, Z. A. (2021). Ethnobotanical study of flora of Gulistan, district Killa Abdullah, Balochistan, Pakistan. *Pure and Applied Biology, 5,* 361-368.

Janaćković, P., Gavrilović, M., Rančić, D., Dajić-Stevanović, Z., Giweli, A. A. and Marin, P. D. (2019). Comparative anatomical investigation of five Artemisia L. (Anthemideae, Asteraceae) species in view of taxonomy. *Revista Brasileira de Botanica, 42,* 135-147.

Janackovic, P., Gavrilovic, M., Rancic, D., Stesevic, D., Dajic-Stevanovic, Z. and Marin, P. D. (2021). Anatomical traits of Artemisia umbelliformis subsp.

eriantha (Asteraceae) alpine glacial relict from Mt. Durmitor (Montenegro). *Botanica Serbica*, *45*(1), 23-30.

Janaćković, P., Gavrilović, M., Rančić, D., Stešević, D., Dajić-Stevanović, Z., Marin,

Jansen, S., Baas, P. and Smets, E. (2001). Vestured pits: their occurrence and systematic importance in eudicots. *Taxon*, *50*(1), 135-167.

Jeffrey, C. and Kadereit, J. W. (2007). Families and Genera of Vascular Plants. Flowering Plants, Eudicots, Asterales. *Springer-Verlag*, 61-87.

Joujeh, R., Zaid, S. and Mona, S. (2019). Pollen morphology of some selected species of the genus Centaurea L. (Asteraceae) from Syria. *South African Journal of Botany, 125*, 196-201.

Judd, W.S., Campbell, C.S., Kellogg, E.A., Stevens, P.F. and Donoghue, M.J. (2002). Plant Systematics: A Phylogenetic Approach, 2nd edn (Sinauer Associates: Sunderland, MA).

Kamel, E. A. and Loutfy, M. H. A. (2001). The significance of cuticular features, petiole anatomy and SDS-PAGE in the taxonomy of the Lauraceae. *Pakistan Journal of Biological Sciences 4*(9), 1094-1100.

Kamel, M. A., El Hadidy, A. M., Hamed, S. T. and Hussein, N.R. (2018). A Palynological review for some species of family Boraginaceae Juss. From the Egyptian Flora. *Annual Research & Review in Biology, 30*,1-16.

Kandemir, N., Çelik, A., Shah, S. N. and Razzaq, A. (2020). Comparative micro-anatomical investigation of genus Heliotropium (Boraginaceae) found in Turkey. *Flora, 262*, 151495.

Kasem, W. T. (2015). Macro and micromorphological studies on seven species of Heliotropium L. (Boraginaceae Juss.) in southwest of Saudi Arabia. *American journal of plant sciences*, *6*,1370-1378.

Kasem, W. T. (2015). Macro and micromorphological studies on seven species of Heliotropium L.(Boraginaceae Juss.) in south west of Saudi Arabia. *American Journal of Plant Sciences*, *6*(09), 1370.

Kassi, A. M., Khan, S. D., Bayraktar, H. and Kasi, A. K. (2014). Newly discovered mud volcanoes in the Coastal Belt of Makran, Pakistan—tectonic implications. *Arabian Journal of Geosciences*, *7*, 4899-4909.

Kayani, S., Hussain, M., Ahmad, M., Zafar, M., Sultana, S., Butt, M. A. and Mir, S. (2019). Scanning electron microscopy (SEM) and light microscopy (LM)-based Palyno-morphological views of Solanaceae in Western Himalaya_*Microscopy Research and Technique*, 82, 63-74.

Kellogg, E. A.(2015). Flowering plants. Monocots*: Poaceae* (Vol. 13). Springer.

Khan, A., Ahmad, M., Sultan, A., Ullah, Z., Majeed, K., Ullah, T. and Zafar, M. (2023). Contribution to pollen morphology of Astragalus L. section Aegacantha Bunge (Galegeae, Fabaceae) and its systematic significance. *Palynology*,48(1) 1-14.

Khan, M. Z., Ghalib, S. A., Zehra, A. and Hussain, B. (2010). Bioecology and conservation of the birds of Hingol National Park, Balochistan. *Journal of Basic & Applied Sciences, 6*(2), 175-184.

Khan, N., Shahid, S., Chung, E. S., Behlil, F. and Darwish, M. S. (2020). Spatiotemporal changes in precipitation extremes in the arid province of Pakistan with removal of the influence of natural climate variability. *Theoretical and Applied Climatology*, *142*, 1447-1462.

Khan, S. A., Ahmed, B., Salehatahir, S., Rajput, M. T., Arshad, F. and Naz, H. (2017). Angiospermic Fossil pollen isolated from the Shele of Bara Formation, Rani Kot, Sindh, Pakistan. *Pakistan journal of botany*, (5), 1757-1761.

Khan, S., Shahab, S., Fani, M. I., Wahid, A. and Khan, A. (2021). Climate and Weather Condition of Balochistan Province, Pakistan. *International Journal of Economic and Environmental Geology*, *12*(2), 65-71.

Kim, K., Labbé, N., Warren, J. M., Elder, T. and Rials, T. G. (2015). Chemical and anatomical changes in Liquidambar styraciflua L. xylem after long term exposure to elevated CO2. *Environmental Pollution*, 198, 179-185.

Kocsis, M. and Borhidi, A. (2003). Petiole anatomy of some Rubiaceae genera. *Acta Botanica Hungarica*, *45*(3-4), 345-353.

Koyuncu, O., Yaylacı, O.K., Özgişi, K., Sezer, O. and Öztürk, D. (2013). A new Onosma (Boraginaceae) species from central Anatolia, Turkey. *Plant Systematics and Evolution*, 299,1839-1847.

Kumar, N. and Nautiyal, S. (2017). Leaf anatomy of two genera of tribe Eragrostideae (Poaceae) from Mandalforest of Kedarnath wildlife sanctuary, Uttarakhand, India. *International journal of botany studies, 2*(5),50-55.

Landi, L., Torrati-Guioti, P. G. and Gasparino, E. C. (2022). Pollen morphology of Boraginaceae sl from Brazilian forest fragments: aperture types and ornamentation on Cordiaceae and Heliotropiaceae. *Palynology*, *46*, 1-13.

Leandro, T. D., Manvailer, V., Arruda, R. D. C. and Scremin-Dias, E. (2022). Pantanal flood pulse reveals constitutive and plastic features of two wild rice species (Poaceae, Oryzoideae): implications for taxonomy, systematics, and phylogenetics. *Revista Brasileira de Botanica, 45*(4),1261-1278.

Lee, G. A., Davis, A. M., Smith, D. G. and McAndrews, J. H. (2004). Identifying fossil wild rice (Zizania) pollen from Cootes Paradise, Ontario: a new approach using scanning electron microscopy. *Journal of Archaeological Science*, *31*(4), 411-421.

Leporatti, M. L. and Lattanzi, E. (1999). Contribution to the knowledge of the Flora of Makran (Southern Pakistan). *Webbia*, *53*(2), 283-335.

Liu, Q., Zhao, N. X. and Hao, G. (2004). Pollen morphology of the Chloridoideae (Gramineae). *Grana*, *43*(4), 238-248.

Londoño, X., Camayo, G. C., Riaño, N. M. and López, Y. (2002). Characterization of the anatomy of Guadua angustifolia (Poaceae: Bambusoideae) culms. *Bamboo science and culture*, *16*(1), 18-31.

Łotocka, B. and Geszprych, A. (2004). Anatomy of the vegetative organs and secretory structures of *Rhaponticum carthamoides* (Asteraceae). *Botanical Journal of the Linnean Society,144*(2), 207-233.

Mabel, A. F., Johnson, A. A. and Temitope, O. O. (2013). Petiole anatomy of some species of Asteraceae in southwest Nigeria. *African journal of plant science., 7*, 608-612.

Magallón, S. and Castillo, A. (2009). Angiosperm diversification through time. *American journal of botany*, *96*(1), 349-365.

Maggi, F., Kolarčik, V. and Mártonfi, P. (2008). Palynological analysis of five selected Onosma taxa. *Biologia., 63*, 183-186.

Mahmud, S., Hamza, S., Irfan, M., Huda, S. N. U., Burke, F. and Qadir, A. (2022). Investigation of groundwater resources using electrical resistivity sounding and Dar Zarrouk parameters for Uthal Balochistan, Pakistan. *Groundwater for sustainable development*, *17*,1-13.

Majeed, S., Ahmad, M., Ozdemir, F. A., Demirpolat, A., Şahan, Z., Makhkamov, T. and Nabila. (2023). Micromorphological characterization of seeds of dicot angiosperms from the Thal desert (Pakistan). *Plant Biosystems-An International Journal Dealing with all Aspects of Plant Biology*, *157*(2), 392-418.

Majeed, S., Zafar, M., Althobaiti, A. T., Ramadan, M. F., Ahmad, M., Makhkamov, T. and Yaseen, G (2022). Comparative petiole histology using microscopic imaging visualization among Amaranthaceous taxa. *Flora, 297*, 152178.

Makesh-Kumar, B., Stephan, J. and Kumar, P. (2022). Comparative Anatomical Study of the Grasses in the Range Lands of Kovilpatti, Tamil Nadu of India.

Mander, L., Li, M., Mio, W., Fowlkes, C. C. and Punyasena, S. W. (2013). Classification of grass pollen through the quantitative analysis of surface ornamentation and texture. *Proceedings of the Royal Society B: Biological Sciences*, *280*(1770),1-7.

Mander, L., Parins-Fukuchi, C., Dick, C. W., Punyasena, S. W. and Jaramillo, C. (2021). Phylogenetic and ecological correlates of pollen morphological diversity in a Neotropical rainforest. *Biotropica, 53*(1), 74-85.

Manzoor, M., Ayesha, A. and Durrani, M. J. (2013). Uses of fruits, vegetables and herbs for the treatment of diabetes by the people of Quetta city. *Science Technology and Development*, *32*(1), 24-27.

Martin, J., Torrell, M. and Valles, J. (2001). Palynological features as a systematic marker in Artemisia L. and related genera (Asteraceae, Anthemideae). *Plant Biology, 3*, 372-378.

Mazari, P. and Q. R. Liu. (2019). Pollen morphology and systematic significance of some Onosma L. species (Boraginaceae) distributed in Pan Himalayan regions. *Pakistan Journal of Botany, 51*,2237-2250.

Mazari, P., Hao, J. C., Liu, Q. R. and Ahmad, L. (2018). Pollen morphology of genus Eritrichium Schrad. (Boraginaceae) from Pan Himalaya and China (Pollen morphology of Eritrichium Schrad. species). *Pakistan Journal of Botany, 50*,1539-1550.

McKim, S. M. (2019). How plants grow up. *Journal of Integrative Plant Biology*,61(3),257-277.

Melo-de-pinna, G. F. and Menezes, N. L (2002). Vegetative organ anatomy of *Ianthopappus corymbosus* Roque & Hind (Asteraceae-Mutisieae). *Revista Brasileira de Botanica, 25*, 505-514.

Meo, A. A. and Khan, M. A. (2006). Pollen morphology as an aid to the identification of Chrysanthemum species (Compositae-Anthemideae) from Pakistan. *Pakistan Journal of Botany, 38*(1), 29-41.

Meo, A. A. and Khan, M. A. (2009). Pollen Morphology of rare taxa Laggera alata and its related species Pluchea lanceolata of tribe Plucheeae (Asteraceae). *Pakistan Journal of Botany, 41*, 1539-1544.

Metcalfe, C. R. (1973). Metcalfe and Chalk's Anatomy of the dicotyledons and its revision. *Taxon*, *22*(5-6), 659-668.

Metcalfe, C. R. and Chalk, L. (1979). Anatomy of the dicotyledons. Vol I. Systematic anatomy of leaf and stem, with a brief history of the subject. Anatomy of the dicotyledons. Vol I. Systematic anatomy of leaf and stem, with a brief history of the subject., (Ed. 2).

Metcalfe, C. R. and Chalk, L. (1979). Anatomy of the dicotyledons. Oxford: Clarendon Press VI 276 p.

Mohtashamian M, Hajishirkia'ee, F. A., Fatehi, F. and Rastegar, A. (2022). Petiole anatomy: a contribution to the taxonomy of Acer L.(Sapindaceae) in Iran. *Nordic journal of botany,* (6),1-10.

Moore, P.D., Webb, J.A. and Collison, M.E. (1991). Pollen Analysis. 2nd Edition, Blackwell, *Oxford,* 1-216.

Mousa, M. O., Abood, N. M., Shahatha, S. S. and Alawadi, H. F. N. (2021). Anatomical Study of the Stems of Some Wild Species of Poaceae Family in the Western Desert. In *IOP Conference Series: Earth Environ Sci, 735* (1), 12-51.

Muller, J. (1981). Fossil pollen records of Angiosperm. *Botanical Review*

Nasir, E. and Ali, S. (1971). Flora of Pakistan. Department of Botany. University of Karachi, Karachi, Pakistan. 112-115.

Nazish, M. and Althobaiti, A. T. (2022). Palyno-Morphological Characteristics as a Systematic Approach in the Identification of Halophytic Poaceae Species from a Saline Environment. *Plants.*, *11*(19), 2-18.

Noor, W., Sadia, B., Saba, k., Asyia., Rehmat, Y., Ahmad, J. and Yasmeen. (2020). Comparative study of soil physical properties of higher and lower elevation of Loralai Baluchistan Pakistan. *International Journal of Biosciences. 16*(6), 90-97.

Noor, W., Zafar, M., Ahmad, M., Althobaiti, A. T., Ramadan, M. F., Makhkamov, T., Bibi, S. and Khan, A. (2023). Petiole micromorphology in Brassicaceous taxa and its potential for accurate taxonomic identification. *Flora 303*, 152280.

Noraini, T. and Cutler, D. F. (2009). Leaf anatomical and micromorphological characters of some Malaysian Parashorea (Dipterocarpaceae). ***Journal of tropical forest science,*** *2*, 156-167.

Noraini, T., Ruzi, A. R., Ismail, B. S., Hani, B. U., Salwa, S. and Azeyanty, J. A. (2016). Petiole vascular bundles and its taxonomic value in the tribe Dipterocarpeae (Dipterocarpaceae). *Sains Malays.*, *45,* 247-253.

Nurul-Aini, C. A. C., Noraini, T., Latiff, A., Chung, R. C. K., Nurhanim, M. N. and Ruzi, M. (2013). Systematic significance of petiole anatomical characteristics in Microcos L. (Malvaceae: Grewioideae). *Malayan Nature Journal, 65*(2&3), 145-170.

Okeke, C. U., Iroka, C. F., Izundu, A. I., Okereke, N. C., Onwuasoeze, C. I. and Nyananyo, B. L. (2015). Comparative systematic leaf and petiole anatomical studies of the genus Stachytarpheta found in Awka Nigeria. *Journal of Medicinal Plants*, *3*(4), 82-84.

Ola, H. A. E., Reham, E. F., Eisa, S. S. and Habib, S. A. (2012). Morpho-anatomical changes in salt stressed kallar grass (*Leptochloa fusca* L. Kunth). *Research journal of agriculture and biological sciences*, *8*(2), 158-166.

Osman, A. K. (2006). Contributions to the pollen morphology of the tribe Inuleae (subfamily Asteroideae-Compositae) in the flora of Egypt. *Feddes Repertorium: Zeitschrift für botanische Taxomonie und Geobotanik, 117,* 193-206.

Osman, A. K. (2009). Contributions to the pollen morphology of Tribe Cardueae (Cichorioideae-Compositae). *Feddes Repertorium, 120*, 145-157.

Ovchinnikova, S., Tajetdinova D. and K. Tojibaev. (2021). Taxonomic analysis of the family Boraginaceae in the "Flora of Uzbekistan". EDP Sciences. In *BIO Web of Conferences*, *38*, 95-100.

Ozcan, M., Ünver, M. C. and Eminağaoğlu, Ö. (2014). Comparative anatomical and ecological investigations on some *Centaurea* (Asteraceae) taxa from Turkey and their taxonomic significance. *Pakistan Journal of Botany, 46*(4), 1287-1301.

Ozler, H., Cabi, E., Us, E. and Pehlivan, S. (2009). Pollen morphology of Agropyron gaertner in Turkey. *Bangladesh Journal of Plant Taxonomy*, *16*(1), 21-28.

Padrón-Mederos, M. A. and La Serna-Ramos, I. E. (2011). Pollen morphology of genus Allagopappus Cass. (Asteraceae: Inuleae), endemic to the Canary Islands, Spain. Plant Biosystems-an International Journal Dealing with all Aspects of *Plant Biology, 145*, 809-817.

Paterson, A. H., Bowers, J. E., Chapman, B. A., Peterson, D. G., Rong, J. and Wicker, T. M. (2004). Comparative genome analysis of monocots and dicots, toward characterization of angiosperm diversity. *Current opinion in biotechnology*, *15*(2), 120-125.

Peng, Y. L., Gao, X. F. and Peng, L. (2013). Pollen morphology of Youngia and six related genera (Asteraceae: Cichorieae) and its systematic significance. *Phytotaxa, 139*, 39-62.

Pereira-Coutinho, A. and Dinis, A. M. (2007). A contribution to the ultrastructural knowledge of the pollen exine in subtribe Inulinae (Inuleae, Asteraceae). *Plant Systematics and Evolution, 269*, 159-170.

Perveen, A. (2000). Pollen Characters and Their EvolutionarySignificance with Special Reference to the Flora of Karachi. *Turkish Journal of Biology, 24*,365-378.

Perveen, A., and Qaiser, M. (2012). Pollen flora of Pakistan-LXIX. Poaceae. *Pakistan Journal of Botany*, *44*(2), 747-756.

Pornponggrungrueng, P. and Chantaranothai, P. (2002). Pollen morphology of the tribe Inuleae (Compositae) in Thailand. *Thai Forest Bulletin (Botany), 3*0, 116-123.

Punt, W., Hoen, P., Blackmore, S., Nilsson, S. and Le Thomas, A. (2007). Glossary of pollen and spore terminology. *Review of Palaeobotany and Palynology, 143*(1-2), 1-81.

Qureshi, A. L., Jamali, M. A., Hussain, S., Memon, F. A., Zaidi, A. Z., Zafar, S. and Ahmed, W. (2022). Subsurface depleting aquifers in the sedimentary terrain of Quetta Valley in Balochistan: a review. *Arabian Journal of Geosciences*, *15*(21), 1648.

Qureshi, R. (2012). Medicinal flora of hingol national park, Baluchistan, Pakistan. *Pakistan Journal of Botany*, *44*(2), 725-732.

Qureshi, S. J., Khan, M. A., Arshad, M., Rashid, A. and Ahmad, M. (2009). Pollen fertility (viability) status in Asteraceae species of Pakistan. *Trakia Journal of Sciences, 7,* 12-16.

Rabiae, E., and Elbadry, S. H. (2020). Morphology, anatomy, palynology and seed micromorphology of Libyan Endemic *Bellis sylvestrisvar.* Cyrenaica (Asteraceae). *International Journal of Pharmaceutical Compounding,* 46-50.

Rabizadeh, F. (2020). The first anatomical, morphological, and ecological study of the endemic Iranian *Moltkia gypsacea* from the Boraginaceae family. *Journal of Advanced Pharmacy Education and Research, 10*, 170-180.

Raees, K., Abidin, S. Z. U., Mumtaz, A. S., Jamsheed, S. and Ullah, H. (2017). Comparative leaf and pollen micromorphology on some Grasses taxa (Poaceae) distributed in Pakistan. *International Journal of Nature and Life Sciences*, *1*(2), 72-82.

Rafique, T., Hameed, M., Naseer, M., Rafique, R., Sadiq, R., Zikrea, A. and Sajjad, M. R. (2021). Comparative Leaf Anatomy of Grasses (Poaceae) in Faisalabad Region of Pakistan. *Polish Journal of Environmental Studies, 30*(6), 5701-5709.

Reitsma, T., (1969). Size modification of recent pollen grains under different treatments. *Review of Palaeobotany and Palynology, 9*(3-4), 175-202.

Salamah, A., Luthfikasari, R. and Dwiranti, A. (2019). Pollen morphology of eight tribes of asteraceae from universitas indonesia campus, depok, Indonesia. *Biodiversitas Journal of Biological Diversity, 20*, 152-159.

Saleem, M. (1990). Autecological characteristics of chrysopogon aucheri and cymbopogon jwarancusa, dominant rangeland grasses in Baluchistan. 1-103.

Salgado-Labouriau, M. L. and Rinaldi, M. (1990). Palynology of Gramineae of the Venezuelan mountains. *Grana*, *29*(2), 119-128.

Scheel, R., Ybert, J. P. and Barth, O. M. (1996). Pollen morphology of the Boraginaceae from Santa Catarina State (southern Brazil), with comments on the taxonomy of the family. *Grana*., *35*, 138-153.

Schneider, H., Schuettpelz, E., Pryer, K. M., Cranfill, R., Magallón, S. and Lupia, R. (2004). Ferns diversified in the shadow of angiosperms. *Nature*, *428*(6982), 553-557.

Shabestari, E. S. B., Attar, F., Riahi, H. and Sheidai, M. (2013). Pollen morphology of Centaurea L. (Asteraceae) in Iran. *Acta Botanica Brasilica, 27*, 669-679.

Shah, S. N., Ahmad, M., Zafar, M., Razzaq, A., Malik, K., Rashid, N. and Zaman, W. (2018). Foliar epidermal micromorphology and its taxonomic implications in some selected species of Athyriaceae. *Microscopy Research and Technique*, *81*(8), 902-913.

Shaheen, S., Khan, M. A., Shahid, M. N., Shamim, Z., Rasool, B., Hussain, K. and Khan, F. (2022). Morphological and palynological assessment of taxonomically problematic genus Paspalum based on light and scanning electron microscopy. *Microscopy Research and Technique*, *85*(2), 623-629.

Shahzad, S. M. (2022). Maritime Tourism Potential of Lasbela District (Pakistan): *The Course of Sustainability. Pakistan Journal of Medical Research*, *4*(1), 87-109.

Shamah, A. B., Ahlam, A. A. W., Abdullah, R. D., Abdulaziz, A. A. and Mohamed, E. Z. (2019). Anatomical adaptations in species of Poaceae growing in Al-Ha'ir region of Riyadh, Saudi Arabia. *African Journal of Plant Science 13*(7), 201-208.

Siddiqui, T. and Qaiser, M. (1988). A palynological study of the Family Gramineae from Karachi, Pakistan. *Pakistan Journal of Botany*, *20*(2), 161-176.

Sims, H. J. and McConway, K. J. (2003). Nonstochastic variation of species-level diversification rates within angiosperms. *Evolution*, *57*(3), 460-479.

Singh, A. P. and M. Parabia. (2003). The floral diversity of Gujarat State: A review. *Indian forester.*, *129*(12), 1461-1469.

Siqueiros-Delgado, M. (2007). Culm anatomy of Bouteloua and relatives (Gramineae: Chloridoideae: Boutelouinae). *Acta botanica Mexicana*, (78), 39-54.

Skvarla, J. J., Rowley, J. R., Hollowell, V. C. and Chissoe, W. F. (2003). Annulus–pore relationship in Gramineae (Poaceae) pollen: the pore margin of Pariana. *American Journal of Botany*, *90*(6), 924-930.

Souza, D. M. D., Sa, R. D., Araujo, E. L.and Randau, K. P. (2017). Anatomical, phytochemical and histochemical study of Solidago chilensis Meyen. *Analytical and Bioanalytical Chemistry, 90*, 2107-2120.

Spooner, B. (1988). Baluchistan: Geography, history, and ethnography.

Stephen, A., Nikita, S., Nidhi, T., Noorunnisa, B. and Ravikumar, K. (2017). Light microscopic study of pollen morphology on selected species of Jatropha L. *Journal of Phytological Research, 30*(1), 1-6.

Stewart, R.R., Ali, S. and Nasir, E., (1972). An annotated catalogue of the vascular plants of West Pakistan and Kashmir. Fakhri Printing Press, Karachi, Pakistan, 1-1028.

Sufyan, M., Badshah, I., Ahmad, M., Zafar, M., Bahadur, S. and Rashid, N. Identification of medicinally used Flora using pollen features imaged in the scanning electron microscopy in the lower Margalla Hills Islamabad Pakistan. *Microscopy and Microanalysis* 2018, *24*(3), 292-299.

Taia, W. K. (2005). "Modern trends in plant taxonomy." *Asian Journal of Plant Sciences,* 4(2)184-202.

Talebi, S. M., Sheidai, M., Atri, M., Sharifnia, F. and Noormohammadi, Z. (2012). Palynological study of the genus Linum in Iran (a taxonomic review). *Phytologia Balcanica*, *18*(3), 293-303.

Tareen, R. B., Bibi, T., Khan, M. A., Ahmad, M., Zafar, M. and Hina, S. (2010). Indigenous knowledge of folk medicine by the women of Kalat and Khuzdar regions of Balochistan, Pakistan. *Pakistan Journal of Botany*, *42*(3), 1465-1485.

Teke, H. I. and Binzet, R. (2017). Anatomical, morphological and palynological studies of some Onosma L. (Boraginaceae) taxa endemic to Anatolia. *Pakistan Journal of Botany*, 49, 579-588.

Tekin, M. and Kartal, C. (2016). Comparative anatomical investigations on six endemic Tanacetum (Asteraceae) taxa from Turkey. *Pakistan Journal of Botany*,*48*(4), 1501-1515.

Telleria, M. C. and Katinas, L. (2009). New insights into the pollen morphology of the genus Mutisia (Asteraceae, Mutisieae). *Plant systematics and evolution*, *280*(3), 229-241.

Torrey, J., and Gray, A. (1969). A flora of North America. Hafner Pub. Co.

Tropicos.org. Missouri Botanical Garden. 18 Mar2023. http://www.tropicos. org/ Name/42000357.

Uga, Y., Ebana, K., Abe, J., Morita, S., Okuno, K. and Yano, M. (2009). Variation in root morphology and anatomy among accessions of cultivated rice (*Oryza sativa* L.) with different genetic backgrounds. *Breed Science, 59*(1), 87-93.

Ullah, A. (2022). Folk benefits from the indigenous angiosperm's flora of Shiekh Buddin National Park Dera Ismail Khan, Pakistan. *Sarhad Journal of Agriculture*, *38*(3), 902-911.

Ullah, I., Ahmad, M., Jabeen, A., Yusuf, M. O., Arfan, M., Kilic, O. and Usma, A. (2021). Palyno-morphological characterization of selected allergenic taxa of family Poaceae from Islamabad-Pakistan using microscopic techniques. *Microscopy Research and Technique*, *84*(11), 2544-2558.

Umber, F., Zafar, M., Ullah, R., Bari, A., Khan, M. Y., Ahmad, M. and Sultana, S. (2022). Implication of light and scanning electron microscopy for pollen morphology of selected taxa of family Asteraceae and Brassicaceae. *Microscopy Research and Technique*, *85*(1), 373-384.

Urtubey, E. and Tellería, M. C. (1998). Pollen morphology of the subfamily Barnadesioideae (Asteraceae) and its phylogenetic and taxonomic significance. *Review of Palaeobotany and Palynology*, *104*(1), 19-37.

Vincent, P. L. D. and Norris, F. G. (1989). An SEM study of the external pollen morphology in Senecio and some related genera in the subtribe Senecioninae (Asteraceae: Senecioneae). *South African Journal of Botany, 55*, 304-309.

Volkova, O. A., Severova, E. E. and Polevova, S. V. (2013). Structural basis of harmomegathy: evidence from Boraginaceae pollen. *Plant Systematics and Evolution, 299*, 1769-1779.

Wang, H., Wortley, A. H. and Blackmore, S. (2009) Pollen morphology of Crepidinae and Lactucinae (Asteraceae: Cichorieae) and its systematic significance. *Grana, 48*, 160-178.

Wei, C. X., Jardine, P. E., Mao, L. M., Mander, L., Li, M., Gosling, W. D. and Hoorn, C. (2023). Grass pollen surface ornamentation is diverse across the phylogeny:

Evidence from northern South America and the global literature. *Journal of Systematics and Evolution*, 1-15.

Wells, C. L. and Pigliucci, M. (2000). Adaptive phenotypic plasticity: the case of heterophylly in aquatic plants. *Perspectives in Plant Ecology, Evolution and Systematics, 3*(1), 1-18.

Wilkinson, H. P. (1994). Leaf and twig anatomy of the Pterostemonaceae (Engl.) Small: ecological and systematic features. *Botanical Journal of the Linnean Society*, *115*(2), 115-131.

Wooller, M. J. (2002). Fossil grass cuticles from lacustrine sediments: a review of methods applicable to the analysis of tropical African lake cores. *Holocene.*, *12*(1), 97-105.

Yang, C., Zhang, X., Li, J., Bao, M., Ni, D. and Seago, J. L. (2014). Anatomy and Histochemistry of Roots and Shoots in Wild Rice (Zizania latifolia Griseb.). *Journal of Botany*, 1-9.

Yang, C., Zhang, X., Zhou, C. and Seago, J. L. (2011). Root and stem anatomy and histochemistry of four grasses from the Jianghan Floodplain along the Yangtze River, China. *Flora-Morphology, Distribution, Functional Ecology of Plants*, *206*(7),653-661.

Younas, M., Gul, S., Shaheen, U., Rehman, S. U., Nawaz, M., Ziad, T. and Ismail, T. (2022). Soil Quality of Agricultural Lands: A Study In Loralai District, Balochistan, Pakistan. *Plant Cell Biotechnology and Molecular Biology*, 42-53.

Yousaf, Z., Zafar, M., Ahmad, M., Sultana, S., Rozina, Ozdemir, F. A. and Abidin, S. Z. U. (2022). Palyno-anatomical microscopic characterization of selected species of Boraginaceae and Fabaceae. *Microscopy Research and Technique*, *85*(4), 1332-1354.

Zafar, M., Ahmad, M. and Khan, M. A. (2007). Palynology of family Asteraceae from flora of Rawalpindi-Pakistan. *International Journal of Agriculture and Biology*, *9*(1), 156-161.

Zarin, P., Ghahramaninezhad, F. and Masoumi, A. A. (2010). Comparative anatomy and pollen features of Amblyocarpum and Carpesium (Asteracerae: Inuleae) in Iran. *Iran Journal of Botany, 16*, 29-53.

Zavada, M. and de Villiers, S. (2000). Pollen of the Asteraceae from the Paleocene-Eocene of South Africa. *Grana, 39*, 39-45.

Zhao, Z., Skvarla, J. J., Jansen, R. K. and DeVore, M. L. (2000). Phylogenetic implications of pollen morphology and ultrastructure in the Barnadesioideae (Asteraceae). *Lundellia, 2000*, 26-40.

PUBLISHED PAPERS

QUAID-I-AZAM UNIVERSITY

DEPARTMENT OF PLANT SCIENCES

Subject: **Publication of W – Category Ms. Bibi Sadia (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. Bibi Sadia** has published research papers in W-Categories as given below:

S. No.	Paper Title	Year	Impact Factor
1.	Bibi Sadia, Mushtaq Ahmad, Fazal Ullah, Muhammad Zafar, Shazia Sultana, Abdulwahed Fahad Alrefaei, Wajia Noor, Asma Ayaz And Wajid Zaman.2023. Desert Blooms: Unraveling Palyno-Anatomical Diversity In Arid Boraginaceous Taxa. Pakistan Journal of Botany, *56*(5),1-16.	2024	0.9
2.	Sadia, B., Ahmad, M., Zafar, M., Noor, W., Manzoor, M., Gillani, S. W., & Sultana, S. (2024). Taxonomic implications of petiole microanatomical traits among Asteraceae tribes from arid–semiarid regions of Baluchistan, Pakistan. *Genetic Resources and Crop Evolution*, 1-23.	2024	2.33
3.	Sadia, B., Ahmad, M., Ramadan, M. F., Zafar, M., Sultana, S., Noor, W., & Pieroni, A. (2024). Systematic Implications of Palynomorph Diversity Using Microscopic Trends Among Asteraceous Flora From the Drylands of Baluchistan, Pakistan. *Microscopy and Microanalysis*, ozae039.	2024	2.9

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DESERT BLOOMS: UNRAVELING PALYNO-ANATOMICAL DIVERSITY IN ARID BORAGINACEOUS TAXA

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Abstract

The significance of palyno-anatomical features in characterizing Boraginaceous taxa from the arid regions is determined. The pollen micromorphology is carried out utilizing LM and SEM. For petiole anatomy, the sections were prepared using Shandon microtome and visualized under LM. The distinct significant palynological characters are hetropolarity, isocolpate, heterocolpate, porocolpate, polar, equatorial views, shape class, Amb, and exine ornamentation. Similarly, the petiole outline, cell shapes, number of layers, air spaces, and arrangement of vascular bundles are important distinguished anatomical features. Significant diagnostic variations were observed in the analyzed palyno-anatomical features, which efficiently differentiated the species within the same genera of *Heliotropium, Rochelia,* as well as the varieties of the single species *Lappula* spp., *L. spinocarpos*, *L. spinocarpos* subsp. *ceratophora, O. limitanea* var. *limitanea, O. limitanea* var. *major, P. intermedium* var. *intermedium, P. intermedium* var. *calathicarpum*. The quantitative data is compiled into a matrix, and subjected to statistical analysis via NCSS. The boxplot analysis identified the outliers in the data which assisted in taxa discrimination. The species of *Heliotropium* and *Paracaryum* were in the same cluster. In contrast, those of *Onosma, Rochelia,* and *Lappula* were in the different clusters in the hierarchal cluster analysis. The highest positive correlation existed between the polar axis with the equatorial diameter and mesocolpium with the polar length of the colpi. Meanwhile, exine thickness and equatorial width of colpi were negatively correlated. This research will help in the creation of pollen atlas and petiole anatomical documentation for the accurate identification of Boraginaceous taxa.

Key words: Pollen; Heterocolpate; Porocolpate; Anatomy; Petiole.

Introduction

The family Boraginaceae, also called the Borage or forget me not family, has some of the most valuable and extensive anatomical, morphological, ecological, and pharmacological traits of any family in the world (Rabizadeh, 2020; Yousaf *et al*., 2022). This family is worldwide in distribution, most common in temperate regions and is characterized by its vast diversity, with 2,300 species and approximately 130 genera (Buys & Hilger, 2003; Yousaf *et al*., 2022; Attar *et al*., 2019). In Pakistan, there are 32 genera and 135 species represented, including cultivated varieties such as *Anchusa* and *Cordia* (Nasir, 1989). Scorpioid inflorescences, a gynobasic style, and an ovary with two carpels separated into four nutlets are diagnostic traits of this family. Four subfamilies comprise the Boraginaceae family: Boraginoideae, Cordioideae, Ehretioideae, and Heliotropioideae – now treated as an independent family Heliotropiaceae APG (Rabizadeh, 2020). These species also have significant roles in cosmetology and pharmacology (Yousaf *et al*., 2022).

In plant taxonomy, plant anatomy is crucial. The idea is to create a system of classification for plants that will list all of their distinctions and similarities in chronological sequence (Okeke *et al*., 2015). Mabel *et al*., (2013) and Adedeji (2004) have all emphasized the taxonomic significance of anatomical traits, which, in addition to other characteristics, are useful for identifying and classifying plants. Taxonomists use various aspects and disciplines to classify taxa into relevant categories. One significant aspect is the anatomical characters of petiole, which are the key parameter used in identifying and classifying numerous plant families and have been employed to distinguish various genera (Metcalfe & Chalk, 1979). Recently, petiole anatomy is becoming more and more studied as a supplemental tool for plant taxonomy. The classification of plants using this line of evidence has advanced significantly. Additionally, some authors have concluded that the arrangement of vascular bundles in various petiole sections has taxonomic significance (Ekeke & Ogazie, 2020).

Noraini *et al*., (2016) highlighted the potential of petiole vascular patterns in distinguishing certain taxa. The anatomical features found in petiole include vascular tissue patterns, the existence/absence of sclerenchymatous cells encompassing the vascular bundles, medullary vascular bundles, and types of trichomes. Several studies demonstrated the utility of petiole anatomy in grouping genera and identifying species, as evidenced by Kocsis & Borhidi (2003), Noraini & Cutler (2009), and Gürdal & Nath (2022). While limited research exists on petiole anatomy within Boraginaceae. In Boraginaceae species, petiole anatomy variations could be linked to their habitat preferences, water availability, and overall growth

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RESEARCH ARTICLE

Taxonomic implications of petiole microanatomical traits among Asteraceae tribes from arid–semiarid regions of Baluchistan, Pakistan

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Abstract In the present study, the petiole anatomy of 39 species from tribes Cichorieae (18), Cardueae (10), Anthemideae (5), Inuleae (4), Gymnarrheneae (1), Astereae (1), and Gnaphalieae (1) was investigated to determine their taxonomic significance. Leica Light Microscope (Model 1000) embedded with the Infinity 1–5 C-MEL (Canada) digital camera was used to take the photomicrographs. Results revealed intra and interspecific variation. Among the examined traits, the significant systematic qualitative characteristics are petiole outline, arrangement and number of vascular bundles, shape and number of layers of different cells, and air spaces. The delimitation of taxa up to species level is carried via taxonomic key based on these qualitative traits. The quantitative data of length and width of the petiole, epidermal cells, chlorenchyma cells, parenchyma cells, vascular bundles, xylem vessels, and phloem cells, were statistically analyzed, collected into a matrix, and examined their significance in the species separation. Variations were observed in the petiole and vascular bundle size on the multivariate ordination of principal components. The highest positive correlation (0.87 – 0.98) is found in xylem vessels length and width with phloem cells length and width. The least positive correlation (0.07) was between vascular bundle length with xylem vessels and phloem cells. The two-way dendrogram on neighbor-joining clustering in multivariate analysis summarized that the selected quantitative traits are significant in distinguishing the examined species. Conclusively petiole anatomical traits are significant diagnostic tools that assist the separation of Asteraceous taxa.

Keywords Histology · Anatomy · Petiole · Arid–semiarid

Abbreviations

NARC	National Agricultural Research Centre
ISL	Islamabad
QAU	Quaid e Azam University
DPX	Dibutylphthalate Polystyrene Xylene
UPGMA	Un-weighted Pair Group Clustering Method
PCA	Principal Component Analysis
SEM	Scanning Electron Microscopy
PCs	Principal Components

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Systematic Implications of Palynomorph Diversity Using Microscopic Trends Among Asteraceous Flora From the Drylands of Baluchistan, Pakistan

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Abstract

Pollen micromorphological traits with taxonomic implications are first reported from the study area for 50 Asteraceous taxa belonging to nine tribes. Cichorieae (21 taxa), Cardueae (11 taxa), Inuleae (six taxa), and Anthemideae (four taxa) are the leading tribes. The research included *Cousinia haeckeliae*, *Himalaiella afghana, Pterachaenia stewartii* (endemic to Afghanistan and Pakistan), and *Xylanthemum macropodum* (endemic to Baluchistan). Light and scanning electron microscopy were employed for the visualization of pollen photomicrographs. The data was analyzed statistically via SPSS, PAST, and Origin. Significant diagnostic qualitative and quantitative palynological traits were explored for discrimination down to the species level within the tribes. All the investigated taxa possessed radial symmetry, isopolarity, and monad form (characters for distinction at the subdivision level). The aperture types were trizonocolporate, tetrazonocolporate, and tricolporate with number position and character (NPC) formulas $N_3P_4C_5$, $N_4P_4C_5$, and $N_3P_4C_3$. Goniotreme, peritreme, and ptychotreme types of amb were recognized. Echinate, echinate lophate, scabrate, and gemmate sculpturing were present with and without perforated surface patterns. Variations in the shapes in polar and equatorial views and lacuna shapes further assisted the separation of taxa. The observed shape classes were perprolate, prolate spheroidal, prolate, subprolate, oblate spheroidal, suboblate, and oblate. Principal component analysis, correlation, standard probability plots, and ridge line paired features plot for quantitative variables determined the positive correlation between the length and width of colpi in equatorial and polar view with polar axis and equatorial diameter and number of spines between colpi with the number of spines per pollen. The number of spines per pollen was negatively correlated with the width and length of colpi in the polar view. Multiple sample analysis of variance (ANOVA) concluded that a high statistically significant difference exists among the means of analyzed traits. The examined qualitative and quantitative palynological traits revealed noticeable variations, thus providing the source for species discrimination in Asteraceous tribes.

Key words: Asteraceae, exine sculpturing, lacuna, scanning electron microscopy

Introduction

Family Asteraceae is the second largest family, following Orchidaceae among angiosperms in terms of species, while first in terms of genera, consisting of 23,000 species and 1,500 genera (Zavada & de Villiers, 2000). It is one of the most influential families in industry, pharmacy, and food. Presented by 770 species and 188 genera, Asteraceae in Pakistan is the most prominent angiospermic family. Nineteen tribes have been recognized in Pakistan and distributed into five subfamilies (Jeffrey & Kadereit, 2007). The distribution of the species in tribes is still not in the placement, having a high possibility of refreshing the classification. This research gap in taxonomic studies such as palynology, anatomy, and seed morphology must be investigated for the correct species classification.

Pollen analysis of different Asteraceae members from other world regions has been investigated. Nineteen taxa of the genus *Centura* from Iran were examined by Shabestari et al. (2013), and eight tribes of Asteraceae from Indonesia (Salamah et al., 2019) have been investigated. Various features like the symmetry of different types such as radiosymmetrical (Shabestari et al., 2013), radioasymmetrical, tricolpate aperture, spheroidal, prolate to subprolate, microechinate to regulate, and scabrate sculpturing with dense acute and sparse spinules (Shabestari et al., 2013) have been studied in the members of Asteraceae. Several species of the *Mutisia* genus from Asteraceae were analyzed for pollens using transmission electron microscopy (TEM). Radiosymmetrical and radioasymmetrical types of pollens were recognized using light microscopy (LM) and scanning electron microscopy (SEM)

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