Foliar and stem epidermal anatomy of the tribe Cynoglosseae (Boraginaceae) and their taxonomic significance

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Foliar and stem epidermal anatomical features of the tribe Cynoglosseae have been studied in detail for the taxonomic identification using light microscopy (LM) and scanning electron microscopic (SEM) techniques. A comparative study was conducted on different plant parts (leaf and stem epidermal anatomy) of 25 species belonging to eight genera of the tribe Cynoglosseae (Boraginaceae) collected from different phytogeographical regions of Iran for the first time. Different qualitative and quantitative characteristics were observed in detail using LM and SEM. Results showed that although generally the stem and leaf anatomical traits were similar, but some diagnostic features were examined for distinguishing the closely related genera in the tribe. The ratio of cortex/diameter of stem and phloem/xylem, the average row number of collateral, palisade and spongy cells, structure of trichomes, type of indumentum and palisade arrangement were found taxonomically important. The anatomical characters were statistically analyzed using cluster analysis and principal component analysis. The study found that stem and leaf eccentrics are variable in the genus but constant within species of the same genus. Most species had typical isobilateral leaves, but some showed an incipient dorsoventrally symmetry with a layer of abaxial palisade tissue. Eglandular trichomes were observed found in all the studied species, which were recognized based on structure and function. In present study some novel characters have been observed which are of great interest to the taxonomist for the correct identification some genera delimitations. The characters studied here are of less taxonomic value and delimitating at species level.

KEYWORDS
anatomy, microscope, tribe Cynoglosseae, trichome morphology

1 | INTRODUCTION

Boraginaceae s. str. is a sub-cosmopolitan family with central diversity in the temperate regions of the world (Weigend, Luebert, Selvi, Brokamp, & Hilger, 2013). Family (Boraginaceae s.str.) or—classically—as the typical subfamily Boraginaeaeaeaeae distributed throughout the world with about 130 genera and 2,300 species (Buys & Hilger, 2003). Subfamily Cynoglossoideae Weigend, is a broad subfamily having more than 50 genera and 900 species. Tribe Cynoglossoideae W. D. Koch, Subtr. Cynoglossoideae Weigend, is a broad subfamily considered restricted to the Old World, which having center of diversity in western Asia and the Mediterranean. Species of the family is mainly annual, bi-annual, or perennial herbs and shrubs, some trees and few lianes, distributed throughout the temperate and subtropical regions of the world (Retief & Vanwyk, 1997), with a rich diversity in Iran (Willis, 1973). Economically the family is of considerable importance (Willis, 1973). Members of the family is easily identified by their vegetative and flower characteristics (Khatamsaz, 2001; Nasir, 1987; Popov, 1953; Riedl, 1967). However, they form a very heterogeneous group with a wide range of variation, particularly in their floral and fruit characters. For this reason, the family has been variously divided into groups, whose number and limits are not very clearly defined. Many researchers work on the systematics and classification of the borage family (Cohen, 2014; Luebert et al., 2016; Refulio-Rodriguez &
Olmstead, 2014; Weigend et al., 2013; Weigend et al., 2014). Recently, many molecular researches revealed that a wide range of identified tribes falls into this clade (Weigend, Luebert, Selvi, Brokamp, & Hilger, 2016). Morphologically Subtribe are characterized having four nutlets, and share ovary, ovate-acuminate, dorsoventrally compressed, usually with a concave to slightly convex, or rarely flat central disc, and distinct margin. Central disc glochidiate, spiny, and rarely smooth. Margin winged or unwinged; marginal wing flat such as Mattiastrum Boiss. brand or incurved such as Paracaryum Boiss., usually simple but sometimes double structure.

Some morphological, palynological and molecular studies have been carried out on for the classification and identification of the family Boraginaceae. Previously some researchers delimited the species and clarify the relationship among species based on palynological characters to understand the taxonomy of the family Boraginaceae (Diez & Valdes, 1994; Liu, Li, Zhang, & Ning, 2010; Ning, Xi, & Zhang, 1992; Retief & Vanwyk, 1997; Scheel, Ybert, & Barth, 1996). Pollen morphology has been used as a valuable tool in delimiting genera within Cynoglosseae (Barbier & Mathez, 1973; Bigazzi, Nardi, & Selvi, 2006; Clarke, Chanda, & Sahay, 1979) studied several evolutionary hypotheses of the family. Fruit morphology has been used as the most important study for the taxonomy of Boraginaceae. Fruits possess several characteristics that offer valuable taxonomic character for the species identification in the tribe of Boraginaceae, like for example, straight or incurved nutlet, specialized form of emergence, position of attachment scar, distinctive form of prickles or glochids (Al-Shehbaz, 1991; Baillon, 1888; Gurke, 1893; Hilger, 2014; Riedl, 1997). Many studies including some recent work could not well define vegetative anatomical features of Boraginaceae (Jodin, 1903; Metcalfe & Chalk, 1950). Trichome diversity have been recorded by several researchers and have been used for grouping taxa in the family, such as Macronesian, Echium species, but the results do not correspond well with DNA phylogenies. Selvi and Bigazzi (2001) studied the foliar epidermal anatomy of about 50 species and sub species belong to 14 genera of the tribe Boragineae. In the study, they observed seven trichome types, majority of trichomes were like covering which almost found in

<table>
<thead>
<tr>
<th>Taxa</th>
<th>Locality</th>
<th>Collector &amp; Voucher</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cynoglossum teheranicum Riedl</td>
<td>Gilan, Langerud, chaff</td>
<td>Naghinezhad, 27,894-TUH</td>
</tr>
<tr>
<td>Cynoglossum creticum Mill.</td>
<td>Gilan, east-Azarbaijan, Kaleybar</td>
<td>Saeedi, 18,714-TUH</td>
</tr>
<tr>
<td>Cynoglossum officinale L.</td>
<td>Hariz, near Chorteh</td>
<td>Ghahreman, 43,255-TUH</td>
</tr>
<tr>
<td>Solenanthus circinatus Ledeb.</td>
<td>Tehran, Damavand</td>
<td>Talebi, 43,264-TUH</td>
</tr>
<tr>
<td>Solenanthus stamineus (Dest.) Wettst.</td>
<td>Tehran, Damavand Mazandaran, Karaj-Chalus Mazandaran, Kandovan-Siahbisheh</td>
<td>Talebi, 43,263-TUH</td>
</tr>
<tr>
<td>Rindera albida (Wettst.) Kusn.</td>
<td>Kordestan, Sanandaj</td>
<td>Attar, TUH-2465</td>
</tr>
<tr>
<td>Rindera buregi (Boiss.) Gürke</td>
<td>Razavi Khorasan, Kashmar, Kuhsorkh District</td>
<td>Joharchi, 45,219-FUMH</td>
</tr>
<tr>
<td>Rindera lanata (lam.) Bunge</td>
<td>Kordestan, Sanandaj</td>
<td>Attar, 14,292-TUH</td>
</tr>
<tr>
<td>Lindelofia kandavanensis Bornm. &amp; Gauba</td>
<td>Gilan, Bandar-e Anzali Mazandaran, Nowshahr Mazandaran, 40 km Tonekabon to Janat abad</td>
<td>Mozaffarian, 6,796-TARIMoradi, 40,051, 8,464-TUH</td>
</tr>
<tr>
<td>Trachelanthus cerinthoides (Boiss.) Kunze</td>
<td>Tehran, Desin</td>
<td>Attar, TUH-45739</td>
</tr>
<tr>
<td>Paracaryum cyclhymenium (Boiss.) H. Riedl</td>
<td>Kerman, 50 km w of Ravar, Khajeh mtTehran, road of Firozkuh</td>
<td>Assadi, 56,237-TARI</td>
</tr>
<tr>
<td>Paracaryum rugulosum (DC.) Boiss.</td>
<td>Esfahan, 50 km delijan</td>
<td>Assadi, 19,085-TARI</td>
</tr>
<tr>
<td>Paracaryum persicum subsp persicum (Boiss.) Boiss.</td>
<td>Esfahan, Ardestan on road to Taleghan</td>
<td>Assadi, 11,504-TARI</td>
</tr>
<tr>
<td>Paracaryum platycalyx Riedl</td>
<td>80 km from Zahedan on the road to Khash</td>
<td>Assadi, 22,793-TARI</td>
</tr>
<tr>
<td>Microparacaryum salsum (Boiss.) H.H. Hilger &amp; D. Podlech</td>
<td>Shahrud –Bastan:Tur</td>
<td>Assadi &amp; Sardabi, 19,513, 50,616-TARI</td>
</tr>
<tr>
<td>Paracaryum sintensisii Hausskn. ex Bornm.</td>
<td>Fars, 7 km from Evaj to Lar</td>
<td>Assadi &amp; Sardabi, 41,683-TARI</td>
</tr>
<tr>
<td>Paracaryum undulatum Boiss.</td>
<td>Tehran, Darakeh</td>
<td>Attar, 29,069-TUH</td>
</tr>
<tr>
<td>Paracaryum hirsutum Boiss.</td>
<td>Zanjan, Abhar</td>
<td>Attar, 4,239 -TUH</td>
</tr>
<tr>
<td>Mattiastrum cristatum Brand</td>
<td>Hamedan, 20 km s of Nahavand</td>
<td>Assadi &amp; Mozaffarian, 36,999-TARI</td>
</tr>
<tr>
<td>Microparacaryum intermedium (Fresen.) Hilger &amp; Podl.</td>
<td>Khorassan, Kashmar-Darvaneh</td>
<td>Dini &amp; Bazargan, 33,035-TARI</td>
</tr>
<tr>
<td>Mattiastrum honigbergeri Rech. f.</td>
<td>Afghanistan</td>
<td>Mozaffarian, 3,342-TARI</td>
</tr>
<tr>
<td>Mattiastrum luristicum (Náb.) H. Riedl</td>
<td>Khorassan, Tang-e Malavi</td>
<td>Forooghi, 3,289-TARI</td>
</tr>
<tr>
<td>Mattiastrum turcomanicum (Bormm. &amp; Sint.)</td>
<td>Gorgan, 22 km Marave tappeh on the road</td>
<td>Assadi &amp; Mozaffarian, 55,450-TARI</td>
</tr>
<tr>
<td>Mattiastrum modestum (Boiss. &amp; Hausskn.) Brand</td>
<td>Kerman, Lalzarhar, Baghabad; 9 km to Behbahan</td>
<td>Forooghi &amp; Assadi, 17,908, 38,731-TARI</td>
</tr>
<tr>
<td>Mattiastrum dielsii Bomm.</td>
<td>Afghanistan, Kabul</td>
<td>Assadi &amp; Mozaffarian, 31,241-TARI</td>
</tr>
</tbody>
</table>
Stem anatomical characters has been examined only in few taxa (Park, 1982) and shows no striking peculiarities. Besides, more studies focused mostly on the woody Macaronesian, Echium taxa (Aldridge, 1981; Carlquist, 1970), which have broad pith in pachycaulous. The foliar anatomy of the family is not well studied, but apparently foliar are mostly bifacial with 2–3, layers of parenchyma with palisade, on the upper surface and spongiosa parenchyma on lower surface. However, some species have isobilateral leaves. The mesophyll contains different types of calcium crystals that is, raphides, druses or solitary-prismatic crystals. Stomata found mostly in the family hypostomatic, amphistomatic, anomocytic, and rarely anisocytic, but many other types of stomata have also been found (Dasti, Bokhari, Malik, & Akhtar, 2003). Metcalfe and Chalk (1979) defined the general anatomical features of Boraginaceae. The remarkable structural diversity of the trichomes of the Boraginaceae has been the subject of several early surveys mostly based on light microscopy (LM; Lems & Holzapfel, 1968).

Many researchers have proven that taxonomic characters are of great interest for the correct identification of different plant groups (Esfandani-Bozchaloyi & Zaman, 2018; Shah, et al., 2018a; Shah, et al., 2018b; Shah, et al., 2018c; Ullah, et al., 2018a; Ullah, et al., 2018; Ullah, Zafar, et al., 2018b; Ullah, et al., 2018; Ullah, et al., 2018c; Zaman et al., 2018). From Iran palynomorphological characteristics of 31 species belonging to eight genera (Paracaryum, Mattiastrum, Microparacaryum, Rindera, Cynoglossum, Solenanthis, Trachelanthus, and Lindelofia) of the tribe Cynoglosseae (Boraginaceae) was examined through light microscopy (LM) and scanning electron microscopy (SEM) using the previous sample of Attar, et al. (2018). There is no detailed study on the anatomy of foliar and stem epidermal of Tr. Cynoglosseae from Iran. Furthermore, the taxonomic values of these characters are rather ambiguous. The objective of the present study is to clarify the taxonomic values of mentioned characters and specific emphasis was on their role in classification and determination of Tr. Cynoglosseae at infrageneric and intergeneric levels. In this study, we describe the morphology of different types of trichomes present on both surfaces (adaxial and abaxial) of the leaves of the tribe Cynoglosseae (Boraginaceae) using scanning electron microscopy. We also studied the stem morphology of the tribe in details. Different qualitative characters have been studied in this study to distinguish among the closely related genera and species of the tribe.
MATERIAL AND METHODS

2.1 | Plant material

Plant sample of the tribe Cynoglosseae were collected from different phytogeographical areas of Iran. A total of 25 species belonging to eight genera (Lindelofia, Mattiastrium, Microparacaryum, Paracaryum, cynoglossum, Rindera, Solenanthus, and Trachelanthus) of the tribe Cynoglosseae (Boraginaceae) were studied in detail for the foliar and stem surface anatomy (Table 1 and Figure 1). The studied specimens were collected from different habitats and some specimens were taken from herbarium of TUH, FUMH, and TARI. Voucher specimens were identified, mounted and deposited to TUH. For the foliar and stem epidermal anatomical analyses at least five specimens for each taxon were studied using SEM.

2.2 | Electron microscopy

To study the samples under SEM, dry leaves were kept on metallic stub using double adhesive tape in addition with gold coating for a period of 6 min in sputtering chamber (BAL-TEC, SCDOOS, Switzerland). Coating with gold by the physical vapor deposition method using restricted to 100 Å. The anatomical features of leaf and stem of the studied species were observed through TESCAN S8000 microscope. Micrographs were taken using the attached camera to the TESCAN microscope.

2.3 | Anatomical study

Cross sections were taken from the middle part of the leaf blades and stems, which were fixed in formalin-acetic acid solution (60 ml 97% ethanol, 5 ml glacial acetic acid, 10 ml 20% formaldehyde, and 25 ml distilled water) and keep for 48 hr for fixation. Samples were taken from the middle part of the leaf blade and lower part of second internode of stems to have the identical parts to be compared. Cross sections were hand-made and double colored by methyl green 0.1% and carmine 1% for 30 s and 15 min, respectively. Images were taken by a light microscope attached with Olympus Optical Co. (Europa) GMBH, Postfach 10 49 08, 20034, Hamburg, Germany digital camera. The slides of each of the studied plant parts were repeatedly examined under the microscope; the eye piece lens was on (×10) whereas the objective lenses were (×4 and ×20). Anatomical investigations of the leaves and stem were carried out of the 25 species belong to eight genera Lindelofia, Mattiastrium, Microparacaryum, Paracaryum, Cynoglossum, Rindera, Solenanthus, and Trachelanthus of the tribe Cynoglosseae (Boraginaceae) using the light microscope. The quantitative characters were analyzed through UTHSCSA Image tool, version 3.0 (2002). To elaborate the general outline of leaf cross section terminology of Cullen (1978) and Stearn (1983) was followed. Different qualitative and quantitative characters were observed for delimiting the genera and species in the tribe (Tables 2 and 3).

2.4 | Multivariate analysis

To observe significant differences such as delimitation and similarity, in the studied species Variance analysis (ANOVA) was performed. Cluster analysis and principal component analysis (PCA) were determined to check the species relationship (Podani, 2000). Furthermore, to check the multivariate analysis mean values of qualitative and quantitative characters were used as binary/multi-state characters (Tables 2 and 3). Multivariate statistical analysis was used for standardized variables. In the Cluster analyses the taxonomic distances and squared Euclidean distances were observed for the species delimitations coefficient while using cluster analysis of anatomical characters. For studying the most variable foliar epidermal anatomical characters PCA was carried out. PAST software was used for the statistical analysis of the data.

<table>
<thead>
<tr>
<th>No.</th>
<th>Characters</th>
<th>State of characters and their codes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Stem hairs</td>
<td>1 – unilateral hair, 2 – multilateral hair</td>
</tr>
<tr>
<td>2</td>
<td>Stem hairs status</td>
<td>0 – absence, 1 – presence</td>
</tr>
<tr>
<td>3</td>
<td>Collenchyma under the stem epidermis</td>
<td>0 – absence, 1 – presence</td>
</tr>
<tr>
<td>4</td>
<td>Midrib shape of dorsal surface</td>
<td>1 – dome-shaped, 2 – arc-shaped</td>
</tr>
<tr>
<td>5</td>
<td>Midrib collenchyma</td>
<td>0 – absence, 1 – presence</td>
</tr>
<tr>
<td>6</td>
<td>Hairs of midrib</td>
<td>0 – absence, 1 – presence</td>
</tr>
<tr>
<td>7</td>
<td>Status of palisade cells</td>
<td>1 – single-layered, 2 – multi-layered</td>
</tr>
<tr>
<td>8</td>
<td>Hair of dorsal leaf surface</td>
<td>0 – absence, 1 – presence</td>
</tr>
<tr>
<td>9</td>
<td>Hair of ventral leaf surface</td>
<td>0 – absence, 1 – presence</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>No.</th>
<th>Characters</th>
<th>State of characters and their codes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Thickness of stem vascular bundle</td>
<td>16 Average width of vascular bundles at midrib</td>
</tr>
<tr>
<td>2</td>
<td>Thickness of stem cortex</td>
<td>17 Lamina thickness of upper epidermis</td>
</tr>
<tr>
<td>3</td>
<td>Length of stem vascular bundles</td>
<td>18 Lamina lower cuticle diameter</td>
</tr>
<tr>
<td>4</td>
<td>Average width of stem vascular bundles</td>
<td>19 Lamina upper cuticle diameter</td>
</tr>
<tr>
<td>5</td>
<td>1st diameter of stem cross-section</td>
<td>20 Lamina lower epidermis diameter</td>
</tr>
<tr>
<td>6</td>
<td>2nd diameter of stem cross-section</td>
<td>21 Lamina length of vascular bundles</td>
</tr>
<tr>
<td>7</td>
<td>Stem sclerenchyma diameter</td>
<td>22 Lamina average width of vascular bundles</td>
</tr>
<tr>
<td>8</td>
<td>Stem epidermis diameter</td>
<td>23 Lamina thickness of spongy parenchyma</td>
</tr>
<tr>
<td>9</td>
<td>Stem cuticle thickness</td>
<td>24 Lamina thickness of palisade parenchyma</td>
</tr>
<tr>
<td>10</td>
<td>Number of stem vascular bundles</td>
<td>25 Total thickness of lamina</td>
</tr>
<tr>
<td>11</td>
<td>Stem collenchyma diameter</td>
<td>26 Total thickness of the lamina excluding epidermis</td>
</tr>
<tr>
<td>12</td>
<td>Midrib diameter</td>
<td>27 Upper cuticle thickness of midrib</td>
</tr>
<tr>
<td>13</td>
<td>Midrib diameter excluding epidermis</td>
<td>28 Thickness of cortex parenchyma of midrib</td>
</tr>
<tr>
<td>14</td>
<td>Lower epidermis diameter of midrib</td>
<td>29 Length of vascular bundles of midrib</td>
</tr>
<tr>
<td>15</td>
<td>Upper epidermis diameter of midrib</td>
<td>30 Thickness of lower cuticle of midrib</td>
</tr>
</tbody>
</table>
3 | RESULTS

In the present investigation a comparative anatomical study was conducted on leaf and stem of the tribe Cynoglosseae (Boraginaceae). Different features examined in this study were helpful in the taxonomic identification of some genera. The investigated specimens are given in Table 1. The anatomical characters are summarized in Tables 2 and 3.

3.1 | Infrageneric variation

Cluster analysis and PCoA ordination of the studied species were carried out based on quantitative and qualitative anatomical characteristics (Figures 2 and 3). These features were of great taxonomic value for the identification of some genera in the tribe. The figure shows that all investigated taxa fall into two clusters. The first cluster, consists of Cynoglossum creticum, C. teheranicum, and C. officinale were placed close to each other due to anatomical similarities. These species were characterized having: leaves dorsiventral and have not colenchyma layer in midrib (Figures 6g–i and 7g–i).

The second cluster comprised two sub-clusters: In the first sub-cluster, the Solananthus circinatus and S. stamineus were located close to each other (Figure 2). However, species in some genera such as Lindelofia kandavanensis, Rindera albida, R. lanata, Paracaryum persicum P. undulatum, P. sintenisi, P. hirsutum, Mattiastrum turcomanicum, M. modestum, and M. cristatum were placed intermixed and placed close to each other due to anatomical similarities (Figure 2). These species were characterized by having leaves isobilateral (Figures 7d–fa and 8a–h); that comprised of two layers of palisade parenchyma cells on the upper epidermis as well as two layers on the lower epidermises. Spongy parenchymatic cells with large intercellular cavities were 2–3 layered. However, these differences were not of diagnostic importance at species level. The phenogram resulted from the analysis does not clarify the relationships among taxa (Figure 2).

To determine the most dissimilar features of the studied taxa, PCA were performed, showing that first three factors comprised over 78.81% of the total variable. In the first factor with about 45% of total variation, such characters as width of spongy tissue/width of mesophyll tissue (μm/μm) and average row number of spongy cells and palisade cells have shown the highest correlation (>0.7). In the second factor with over 24% of total variation, width of phloem/width of xylem on midrib (μm/μm) has shown the highest correlation. These are the most variable anatomical characters among the studied taxa. PCoA plot based on qualitative and quantitative anatomical characters (Figure 3) confirms the results of cluster analysis by WARD method.

3.2 | Transverse sections of stem

The anatomical features of 18 studied taxa of the tribe Cynoglosseae based on transverse section of the leaf and stem anatomy. The cross
section taken from the stem of the examined taxa revealed the following elements (Figures 4 and 5). Stem cross section shape were found rounded in all taxa and the species *Mattiastrum modestum* has the smallest stem cross section (1,300 μm width), while *Paracaryum hirsutum* has the largest size (3,000 μm width). There was a single layer epidermis with rectangular cells, covered with simple hairs in some taxa. The collenchyma thin-walled cell layers were developed in all taxa and located close to the epidermis with 2–4 rows (20–74 μm width). The cells of the general cortex consist of parenchymatous cells in 4–5 layers in all the species. The width of cortex varies among the examined taxa (56–172 μm width). The vascular bundles were continuous along the stem. Phloem consists of thin-layered parenchymatous cells (29–71 μm width). Xylem has dense scleranchymatous cells (116–380 μm width). Phloem/xylem ratio varies among the examined taxa (0.16–0.3 μm width). Pith consists of large and cylindrical parenchymatous cells (700–1,758 μm width).
3.3 Transverse sections of leaves

Transverse and surface preparations of the leaves were investigated (Figures 6–8). Both upper and lower surfaces consisting of single-layered oval or rectangular epidermal cells and show the presence of an external cuticular layer. Both epidermises were covered with glandular trichomes.

Midrib consists of collenchyma cells located close to the lower and upper epidermis with 1–3 rows in all studied species (Figure 6); except Cynoglossum creticum, C. teheranicum and C. officinale, which have not collenchyma layer (Figures 6g–i). Vascular bundle surrounded by thin-walled, orbicular parenchymatous cells was with 4–10 rows occurs in the center of midrib. Phloem and xylem were

**FIGURE 5** Stem cross section in: (a) Cynoglossum creticum, (b) Mattiastrum cristatum, (c) Mattiastrum modestum, (d) Mattiastrum turcomanicum, (e) Microparacaryum intermedium, (f) Microparacaryum salsum, (g) Paracaryum hirsutum, (h) Paracaryum persicum, (i) Paracaryum sintenisii, and (j) Paracaryum undulatum [Color figure can be viewed at wileyonlinelibrary.com]
clear in the observed species. Mesophyll was isobilateral in all studied species; that comprised of two layers of palisade parenchyma cells on the upper epidermis as well as two layers on the lower epidermises (Figures 7 and 8). Spongy parenchymatic cells with large intercellular cavities were 2–3 layered. Vascular bundles were surrounded by a parenchymatic sheet. Dorsiventral type of mesophyll was present in Cynoglossum creticum, C. teheranicum, and C. officinale (Figure 7g–i). Width of palisade (141–316 μm width)
and spongy parenchyma (44–115 μm width) vary among the examined taxa.

### 3.4 | Structure and mineralization of trichomes

Leaf surfaces of all the studied taxa were covered densely by various types of uniseriate trichomes, developing an indumentum of variable density and texture, except *Trachelanthus cerinthoides*, which have not trichomes (Figure 12f). Two types of eglandular trichomes could be recognized based on their structural and functional features. For trichomes terminology we followed (Metcalfe & Chalk, 1989).

**Type 1 trichomes.** Eglandular with a smaller basal tubercle formed by 10–20 cells was found in *Cynoglossum creticum*, *Rindera bungei*, *Rindera lanata*, *Microparacaryum salsum*, *Solenanthus circinatus*, *Paracaryum*
cyclhymenium, P. persicum, P. platycalyx, P. rugulosum, P. undulatum, Mattiastrum dielsii, M. honigbergeri, M. luristanicum, M. turcomanicum, Cynoglossum officinale (Figures 9–12).

**Type 2 trichomes.** E glandular with unicellular hairs, without a distinct base was observed in Lindelofia kandavanensis, Rindera albida, S. stamineus, P. sintenisii, P. hirsutum, M. modestum, C. teheranicum, and M. cristatum (Figures 9b–d, 10b,d, 11b,e, and 12a,d,e).

### 4 | DISCUSSION

In the present study, the anatomical and surface foliar epidermal characters of the leaves and stem of 25 species belong to eight genera Lindelofia, Mattiastrum, Microparacaryum, Paracaryum, cynoglossum, Rindera, Solenanthus, and Trachelanthus of the tribe Cynoglosseae (Boraginaceae) were examined using LM and SEM. The SEM has
proven invaluable for observations of the surface morphology of plant material primarily due to the improved depth of field and high resolution, which are inaccessible with the LM (Saqib et al., 2018). The aim of present study was to find diagnostic features to separate genera and species of the tribe Cynoglosseae species. Cluster analysis and PCoA ordination of studied species examined in this study based on both quantitative and qualitative anatomical characteristics showed little taxonomic significant (Figures 2 and 3). However, these differences were of little diagnostic importance at species level. Our anatomical characteristics of the present study were incongruent with the result of Akcin (2008) and Attar et al. (2018). Various researcher used microscopic techniques for the taxonomic identification and species delimitations in various plant groups (Shah et al. 2018; Ullah, et al., 2018; Ullah, et al., 2018d).
Morphological characters are considered as a useful tool for species identification, as indicated previously (Akcin, 2008). Also fruits and seeds are known to be useful characters in the identification of Cynoglossum creticum, C. officinale, C. montanum, and C. glochidiatum (Akcin, 2008). In previous studies, the micromorphology of seed and fruit was performed in several species and their importance in plant taxonomy was emphasized (Khalik, El-Ghani, & El-Kordy, 2008).

Fruits possess a number of characteristics that offer valuable taxonomic character for the species identification of tribe in the Boraginaceae, like for example, straight or incurved nutlet, specialized form of emergence, position of attachment scar, distinctive form of prickles.
or glochids (Hilger, 2014). These characters were also valuable for the definition of genera, species and sub species (Selvi & Bigazzi, 2003; Selvi, Bigazzi, Hilger, & Papini, 2006). According to Attar et al. (2018) palynomorphological characteristics provide significant uses of pollen for the taxonomic identification of species in the tribe. They recovered two pollen types: (1) *Rindera tetraspis* type and (2) *Cynoglossum officinale* type based on aperture characters and surface ornamentation. They found that both genera and species were delimited in the tribe Cynoglosseae that incongruent with our present findings. According to Chacón, Luebert, Hilger, Ovchinnikova, and Selvi (2016) the phylogenetic analyses based on sequences from three cpDNA regions successfully resolved some major issues about the monophyly of the main tribes of Boraginaceae and provided more detailed insights into the evolution of the Cynoglosseae.

**FIGURE 11** Scanning electron micrographs of surfaces of leaves. (a) *Paracaryum rugulosum*, (b) *Paracaryum sintenisii*, (c) *Paracaryum undulatum*, (d) *Paracaryum undulatum*, (e) *Mattiastrum cristatum*, (f) *Mattiastrum dielsii*, (g) *Mattiastrum honigbergeri*, and (h) *Mattiastrum luristanicum*
The taxonomic treatment of the subtribe Cynoglossinae was suggested by Chacón et al., (2016) for their correct identification. However, there is a whole range of segregated genera that have been proposed for Cynoglossum and their phylogenetic relationships is not well studied in detail. Some species of the subtribe is monophyletic, but at present all of them appear to be nested in genus Cynoglossum (Chacón et al., 2016).

Omphalodes and Cynoglossum were retrieved as either poly or paraphyletic, show that the morphological characters used in traditional taxonomic classifications were highly homoplasious (Weigend et al., 2013). Although the polytomies obtained in Weigend et al. (2013) were here largely resolved. Most nodes unsupported in the species Lindelofia, Mattiastrum, Microparacaryum, Paracaryum, Pardoglossum, Rindera, Solenanthus, and Trachelanthus retrieved as either
para- or polyphyletic and/or nested in Cynoglossum as already suggested in Selvi, Coppi, and Cecchi (2011).

All recent studies show that there is still a lot of confusion with regards to infra-familial classification of Boraginaceae and that the various partial classifications published in the last decades have largely created unnatural units. However, the present study, indicates that the anatomical and surface foliar epidermal characters have little taxonomic uses to classify the species of the complex genus of tribe Cynoglosseae. It is evident in dendrogram (Figure 2) species in some genera such as Lindelofia kandavanesensis, Rinderia albida, R. lanata, Paracaryum persicum P. undulatum, P. sintensis, P. hirsutum, Mattiastrium turcomanicum, M. modestum, and M. cristatum were placed intermixed and this finding is in concordant with Chacón et al. (2016).

Anatomical and morphological characters to indicate phylogenetic relationships among taxa (Metcalfe & Chalk, 1979). Also, it is well known that anatomical traits provide taxonomically significant data in the numerous angiosperms including Boraginaceae family (Akçın & Binzet, 2010; Beyazoglu, Türkmen, Coşkunçelebi, Makbul, & Kodal, 2008; Binzet & Akçın, 2012; Coşkunçelebi, Makbul, Türkmen, & Beyazoglu, 2008; Makbul, Türkmen, Kandemir, & Beyazoglu, 2008). Metcalfe and Chalk (1979) pointed out that family Boraginaceae has both bifacial and isobilateral leaves. In our study, leaves were dorsiventral in Cynoglossum ceticum, C. teheranicum and C. officinale and isobilateral in the other species.

Selvi and Bigazzi (2001) studied about 50 species belonging to 14 genera of Borginaceae in this study no taxonomic significance has been observed. Different types of trichomes were reported in the study in which five types were eglandular and two were glandular. These trichomes diversity were taxonomically significant. The present study provides sufficient information about delimiting the species of the tribes at genus level, while little helpful at species level. Only some species as Cynoglossum ceticum, C. teheranicum and C. officinale were clearly separated, while some other species showed very closely related relationship.

5 | CONCLUSION

In the present study, the anatomical and foliar epidermal characters of the leaves and stem of 25 species of the tribe Cynoglosseae (Boraginaceae) were examined using LM and SEM. The aim of present study was to find diagnostic features to separate genera and species of the tribe Cynoglosseae. Cluster analysis of the studied species examined, based on both quantitative and qualitative anatomical characteristics showed little taxonomic significant. The present study provides sufficient information about delimiting the species of the tribes at genus level, while little helpful at species level. Only some species as Cynoglossum ceticum, C. teheranicum and C. officinale were clearly separated, while some other species showed very closely related relationship.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this article.

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