

An Accurate Phenotypic Study of The Foliar Epidermis for Some Species of *Silene* L. From Caryophyllaceae Family in Iraq

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Abstracts: In the present research work, detailed micromorphological features study (investigated for foliar epidermal anatomy) for five species of the genus *Silene* L., belonging to the family Caryophyllaceae. One of the essential anatomical characteristics of the leaf in this genus observed two basic stomata types on both adaxial and abaxial epidermis, diacytic and anisocytic, from five species, three species have distinguished a diacytic type, exception two species *S. chlorifolia* and *S. longipetala* appeared to have both a diacytic and anisocytic, also has astrocytic crystals within epidermal cells. Also in the present work, 3 species out of 5 have trichomes on their both surfaces, observed having them multicellular uniseriate. All the taxa were the distinguished shape of the guard cells as reniform-like from a full short reniform in all species. So the general shape of ordinary epidermal cells, anticlinal wall of the epidermal cells, type of stomata cells, collar shape marginal of guard cells, and trichomes. Images enhanced the micromorphological characteristics under study. Information on the locations and dates of the collection was also confirmed. This study revealed that the micromorphological features of the family are taxonomically significant in the accurate identification of species.

Keywords: Caryophyllaceae, *Silene*, Species, Micromorphology, Epidermal Anatomy.

1. INTRODUCTION

Caryophyllaceae Juss., carnation or pink family contains about 2200 species belonging to 88 genera and three subfamilies Alsinoideae, Caryophylloideae, and Paronychioideae (Nyffeler and Egli, 2010; Ullah *et al.*, 2018a). The members of this family like *Silene* are cosmopolitan in distribution, which includes a number of ornamental and common plants (Rabeler and Hartman, 2005), it is mainly distributed in temperate regions of the northern hemisphere and has its main center of diversity in the Mediterranean and the Middle East (Chowdhuri 1957; McNeill, 1978; Greuter *et al.*, 1984; Greuter, 1995). Some species of Caryophyllaceae are growing in dry and exposed habitats. While other species are growing as a weed in different crops i.e. *Silene conoidea* (Ullah *et al.*, 2018,b). The species *Silene* shows their flexibility and ability in various environmental conditions, and this is indicated by the study of Zarrinkamar (2001), in the analysis of the different anatomical characteristics of leaves in 20 species belonging to 8 genera within the family, including *Silene* species. The variations in shape and size of the types of stomata, trichomes, and crystals of epidermal cells, which is one of the most important taxonomic characteristics in diagnosing the species, as it was shown through tests of 16 species of the genus *Silene* in Pakistan that the basic patterns of stomata in most of them were of the diacytic pattern, which is a distinguished characteristic of the Caryophyllaceae family, but in other species the anomocytic and anisocytic patterns were observed, while the *S. arenosa* type was easily distinguished by crystals of the cristarque type. As shown in the study by (Sahreem *et al.*, 2010).

Also, The comparative study was distinguished by (EL-Chamery *et al.*, 2021) depending on the anatomical structure of the stem and leaf in three species *S. leucophylla*, *S. oreosinacia*, and *S. schimperiana* endemic in South Sinai, where the results showed that The stomata were mostly of the diacytic type or of both diacytic and anomocytic types, the epidermal cells in the superficial view were straight or wavy, the presence of wax plates was also observed above the epicuticular wax platelets on both the epidermal cells and the guard cells, as the study showed presence of astrocytic or rosette crystals are abundant in both leaf and stem tissues of the studied species.

This research considers the first study in Iraq and scrutinizes both qualitative and quantitative characters of the foliar anatomical characteristics in species under investigation within the family (Caryophyllaceae) using light microscopy with many novel characters to taxonomical significance in the identification and species delimitation.

2. MATERIALS AND METHODS

28 samples were collected from different locations from the AL-Sulaimaniyah district from the mountains in northern Iraq, were collected from the Balkan Mountain in Penguin region between latitude 35° 62.38725' N and longitude 45° 94. 91482' E, Goby in Qara Dagh region between latitude 35° 16.5740' N and longitude 45° 21.2920' E, Azmar mountain between latitude 35° 37.6490' N and longitude 45° 28. 0780' E, Gweija Dagh between latitude 35° 37.1830' N and longitude 45° 28. 0160' E, and Haj-Omran from Erbil district between latitude 36° 40.9830' N and longitude 45° 0. 2030' E. during the period from March to May 2022 (Fig. 1).



Fig. 1. Photos of samples collection.

For the anatomical study of foliar anatomy, we followed the method of Foster (1977) with some modification according to Al-Hadeethi *et al.*, (2020 and 2021). Leaves from the collected specimens were cut and put in an ethanol concentration of 70% for about one and a half hours, to prevent them from drying. About five leaves of each plant specimen were kept in a test tube, It includes the midrib, part of the blade, and the margin of the leaf. Using a scraping method with a sharp blade to obtain the adaxial and abaxial of the epidermis. Then the samples were washed with distilled water to remove the remnants of tissues attached to the epidermis, then they were transferred to a Petri dish containing 5% sodium hypochlorite solution for 10 minutes to remove the chlorophyll pigment, then

they were placed with safranin pigment for 20 minutes after they washed two times in the ethanol solution concentration 70%. Finally, the sample was transferred to a glass slide and coated with a cover slide. Histological sections were tested using a light microscope type (Kruss optronic 22297Hamburg) and photographed by a digital camera type (AmScope Microscope MU1000).

The number of epidermal cells and stomata were counted on the same ocular and an average of ten was taken. For the determination of the stomatal index, the following formula of (Stace, 1965, Beerling and Kelly, 1997; Hasan *et al.*, 2018; El-Mahrouk *et al.*, 2016 and Khalaf and AL-Hadeethi, 2020).

$$\text{Stomatal index} = \frac{\text{number of stomata}}{\text{number of stomata} + \text{number of ordinary epidermal cells}} \times 100$$

3. RESULTS

In present research showed the important variations of micromorphological features of epidermal foliar anatomy between species under the study of the genus *Silene* L. observed two basic stomata types on both adaxial and abaxial epidermis, diacytic and anisocytic, from five species, three species have distinguished a diacytic type. (Fig. 2, 3, 4), exception two species *S. chlorifolia* and *S. longipetala* appeared to have both a diacytic and anisocytic and finally also has astrocytic crystals within epidermal cells. (Fig.2,4)

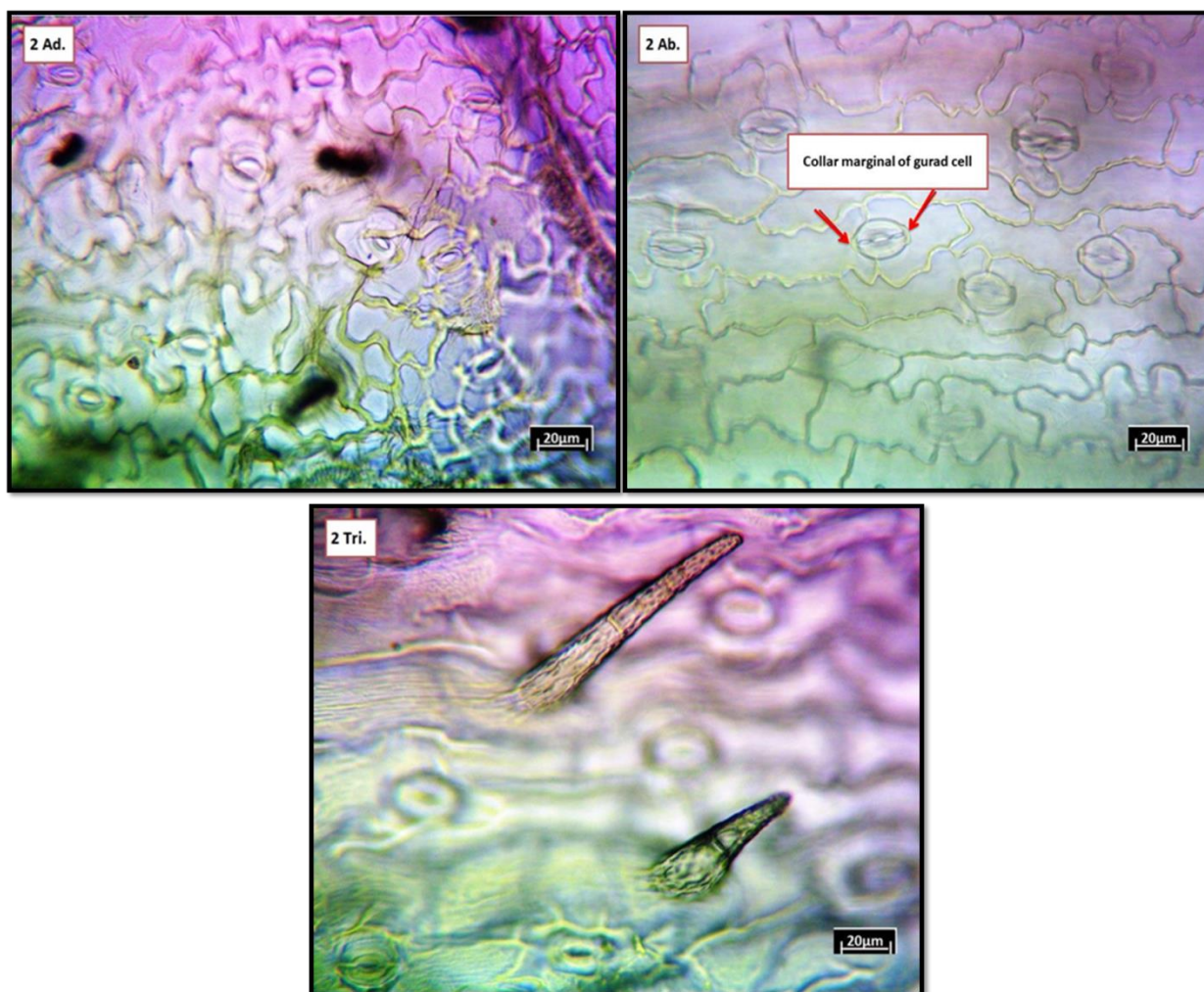


Figure 2: Variations in shapes , dimensions and stomatal complex on the both adaxial & abaxial of epidermal cells, 2. *S. ampullata* , 3. *S. chlorifolia*.

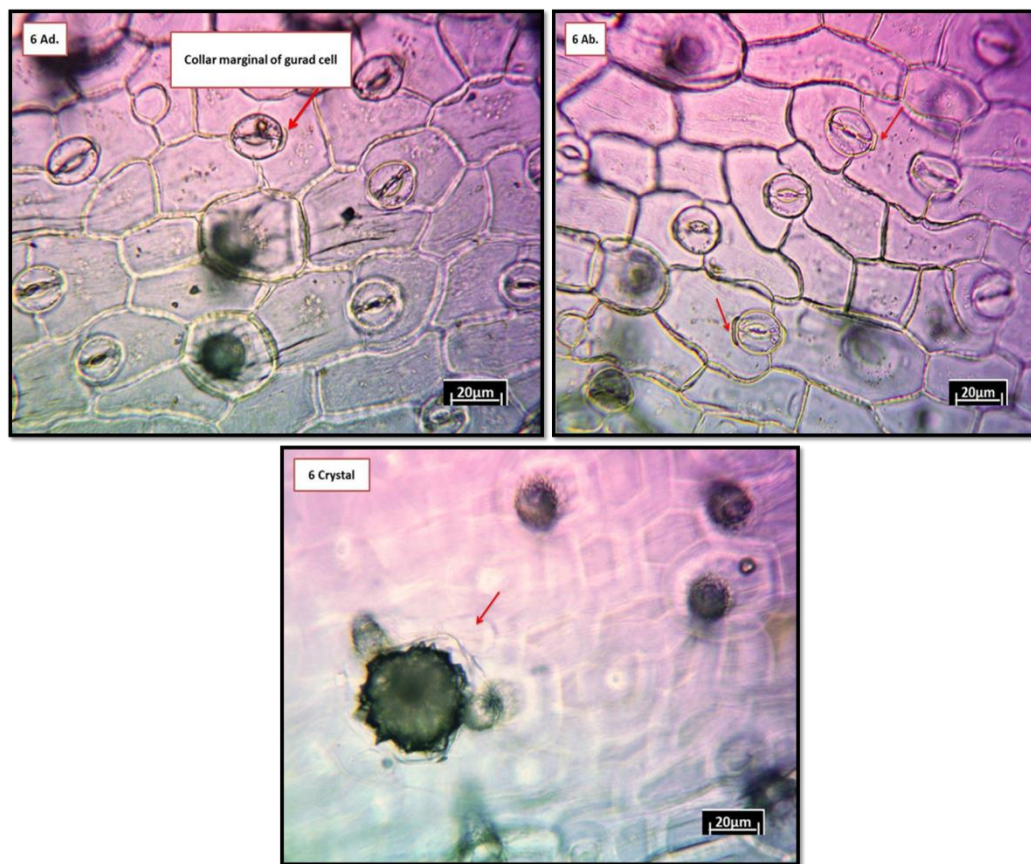


Figure 4: Variations in shapes, dimensions and stomatal complex on the both adaxial & abaxial of epidermal cells, 6. *S. longipetala*

Different shapes of epidermal cells have been observed in present finding irregular, wavy, tetragonal, and pentagonal. The anticlinal wall of the epidermal cells was also observed to differ among them i.e. straight, slightly undulated, and deeply undulated. As shown below in (Table 1).

Table (1) the variations micromorphological features for foliar anatomical epidermis in species of the genus *Silene* L.

Species		Type of stomata	Shape epidermal cells	Anticlinal wall of epidermal cells	Trichomes	Collar shape of guard cells
<i>S. ampulata</i> Boiss.	Ad.	Diacytic	Irregular	Deeply undulate	Multicellular uniseriate	Convex
	Ab.	Diacytic	Wavy	Deeply undulate		
<i>S. chlorifolia</i> Boiss.	Ad.	Diacytic, Anisocytic	Irregular	Slightly undulate	Absent	Convex
	Ab.	Diacytic, Anisocytic	Irregular	Slightly undulate		
<i>S. colorata</i> Poiret.	Ad.	Diacytic	Wavy	Deeply undulate	Multicellular uniseriate	Concave to coronal like
	Ab.	Diacytic	Wavy	Deeply undulate		
<i>S. conoidea</i> Boiss.	Ad.	Diacytic	Pentagonal irregular slightly	Slightly undulate	Multicellular uniseriate	Convex
	Ab.	Diacytic	Pentagonal palisade like	Slightly undulate		
<i>S. longipetala</i> Vent.	Ad.	Diacytic, Anisocytic	Tetragonal	Straight	Absent	Convex
	Ab.	Diacytic, Anisocytic	Tetragonal	Straight		

Also in the present work, 3 species out of 5 have trichomes on their both surfaces, observed having them multicellular uniseriate. (Fig. 2, 3).

These results showed different shapes for marginal walls of guard cells like collar pairs surrounding the outer margin of them; all species showed convex shapes except *S. colorata* appeared to be concave like a coronal shape. (Fig.3)

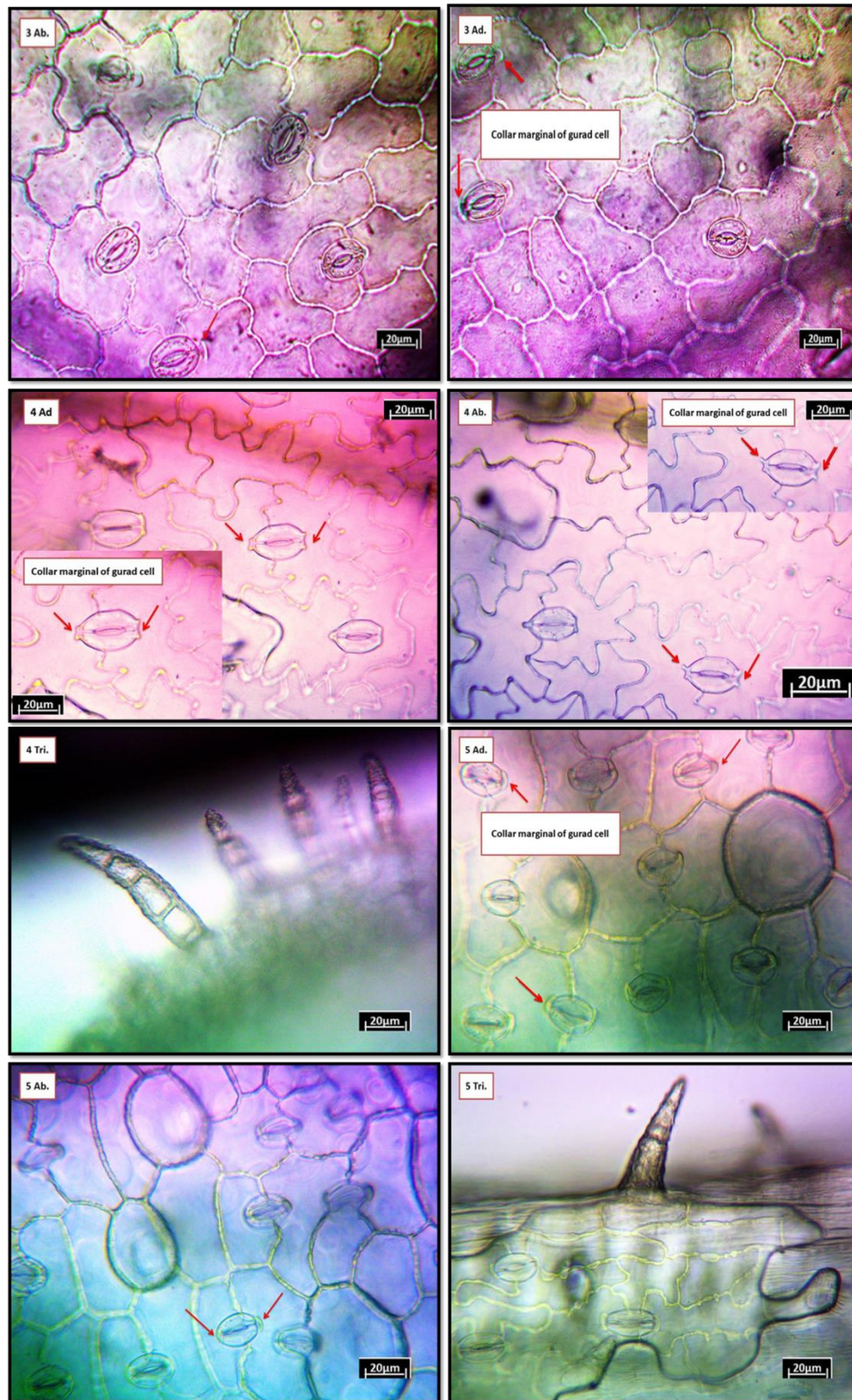


Figure 3: Variations in shapes, dimensions and stomatal complex on the both adaxial & abaxial of epidermal cells, 4. *S. colorata*, 5. *S. conoidea*.

Table (2) the variations of quantitative features and stomatal index for foliar anatomical epidermis in species of the genus *Silene* L. measurement in micrometer (μm).

Species	Adaxial			Abaxial		
	Length stoma	Width stoma	Stomatal index	Length stoma	Width stoma	Stomatal index
<i>S. ampulata</i> Boiss	29.2-33.2 (31.2)	26.1-29.2 (27.6)	16.6	35.6-37.2 (36.4)	22.8-25.2 (24)	14.2
<i>S. chlorifolia</i> Boiss	38.4-42.0 (40.2)	27.9-31.3 (29.6)	11.1	36.6-44.9 (40.6)	28.8-31.7 (30.2)	16.6
<i>S. colorata</i> Poiret.	42.8-45.6 (44.2)	26.3-29.2 (27.7)	14.2	44.8-49.3 (47)	25.8-32.4 (29.1)	16.6
<i>S. conoidea</i> Boiss.	42.2-46.8 (44.5)	24.9-25.7 (25.3)	20	35.6-40.6 (38.1)	24.7-26.3 (25.5)	20
<i>S. longipetala</i> Vent.	39.7-41.9 (40.8)	29.9-35.2 (32.5)	12.5	32.2-35.6 (33.9)	28.0-30.7 (29.3)	16.6

Table (2) showed different densities of stomata on the adaxial that little than the abaxial of the epidermis, observed minimum percentage of the stomatal index on the adaxial in the species *S. chlorifolia* that reaches (11.1), followed has *S. longipetala* that reaches (12.5), after that *S. colorata* (14.2) and finally *S. ampulata* (16.6). The species *S. conoidea* was distinguished that equal on the both adaxial and abaxial of epidermis that reaches (20).

While on the abaxial it has a minimum percentage in *S. ampulata* that reached (14.2), the stomatal index of other species was equal to that maximum reach (16.6).

While the dimensions of stomata, the minimum range for the length of a stoma on the adaxial in *S. ampulata* reaches (31.2) μm and (44.5) μm in *S. conoidea* as a maximum range. While other taxa showed approximately equal them. The range for the width of stomata on the same surface is a minimum range in *S. conoidea* (25.3) μm , and (32.5) μm in *S. longipetala* as a maximum range.

The dimensions of stomata on the abaxial, ranged the length of stoma in *S. longipetala* (33.9) μm as a minimum range and *S. colorata* (47) μm as a maximum range. While the range for the width stomata on the same surface, in *S. ampulata* (24) μm as a minimum range and (30.2) μm in *S. chlorifolia* as a maximum range.

All the taxa were distinguished in the shape of the guard cells as reniform-like from a full short reniform in all species. And the species *S. ampulata*, *S. chlorifolia*, *S. conoidea*, and *S. longipetala*, except *S. colorata* showed a narrow elongated reniform-like.

4. DISCUSSION

In this research work, both qualitative and quantitative characters for five taxa of the genus *Silene* L. (Caryophyllaceae) have been studied systematically. Qualitative features studied in this work include; the shape of epidermal cells, anticlinal wall, collar marginal of guard cells, types of stomata, and trichomes on both adaxial and abaxial surfaces. Quantitative features examined include; the length and width of stomata. Stomatal indexes were calculated for both adaxial and abaxial surfaces. The present results indicate certain facts about the taxonomic identification of plant species in the family (Caryophyllaceae). The foliar epidermal characters studied in this research carry significant information for the identification and species delimitation. Followed by (AL-Hadeethi *et al.*, 2016 and AL-Hadeethi *et al.*, 2016 and AL-Hadeethi *et al.*, 2022)

The present finding show that the species under study have mostly a diacytic type of stomata, which is similar to the previous work of Zarrinkamar, (2001); Ullah *et al.*, (2018, b) and EL-Chamery *et al.*, (2021). exception two species only *S. chlorifolia* and *S. longipetala* showed have them two stomata type diacytic and anisocytic on both adaxial and abaxial surfaces.

The anticlinal wall of epidermal cells mostly in the studied species was straight, slightly undulated, and deeply undulated. Which is also reported in a previous study by (Ullah *et al.*, 2018,b).

The shapes of epidermal cells were different between the taxa on both surfaces including, irregular on the adaxial, wavy on the abaxial in *S. ampullata*, tetragonal like a ribbed shape in *S. longipetala*, pentagonal irregular slightly on adaxial to pentagonal palisade like on abaxial in *S. conoidea*. These not compatible comparative to the previous work of (Ullah *et al.*, 2018,b).

One of the important anatomical features shown in this study and first time, collar shape marginal of guard cells, observed in all species, and distinguished in four species *S. ampullata*, *S. clorifolia*, *S. conoidea*, and *S. longipetala* as a convex shape, but in *S. colorata* as a concave to coronal like shape. This result didn't remind him of the work by (Ullah *et al.*, 2018,b).

These results characters appearing to be environmental changes basic to the statement of the plant host between populations countries (AL-Hadeethi *et al.*, 2020). As indicated by previous the study of Zarrinkamar, (2001), the species of this family show their flexibility and ability in various environmental conditions (Al-Zubaedy *et al.*, 2014). But not recorded their study to this result.

All the anatomical features that have been studied are very important and diagnostic to distinguish oneself among the studied species, such as the shapes and types of stomata, the presence or absence of trichomes and their shapes, and also the appendixes that are associated with guard cells. this is agreed with (AL-Hadeethi *et al.*, 2020; AL-Hadeethi *et al.*, 2020; AL-Hadeethi *et al.*, 2021 and Nadir *et al.*, 2020).

CONCLUSIONS

This study detected detailed micromorphological features study investigated for foliar epidermal anatomy for five species of the genus *Silene* L., samples were collected from various areas of northern Iraq. One of the essential anatomical characteristics of the leaf in this genus observed two basic stomata types on both adaxial and abaxial epidermis. So the general shape of ordinary epidermal cells, anticlinal wall of the epidermal cells, type of stomata cells, collar shape marginal of guard cells, and trichomes. This study revealed that the micromorphological features of the family are taxonomically significant in the accurate identification of species.

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