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# A SEM STUDY OF LEAF TRICHOMES IN HELIOTROPIACEAE

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Leaf trichomes are essential for plant physiology, defense mechanisms, and ecological interactions. In this research, we used scanning electron microscopy to examine the leaf trichomes of 11 species in the family Heliotropiaceae, including the representative species of the genera *Euploca*, *Heliotropium*, *Ixorhea*, and *Myriopus*. Six species from *Heliotropium*, three from *Euploca*, one from *Ixorhea*, and one from *Myriopus* were examined. Our detailed results revealed various trichome types, both glandular and non-glandular, across the studied species. The results demonstrated differences in trichome morphology, density, and distribution within and across the genera. The distribution of trichomes on the leaf surface of some species is dense and sparse in others. Also, leaf trichomes vary in size and both short and long types of trichomes were observed in some species. Most trichomes have decorations on their surface and variation can be seen in their shape. Furthermore, the distribution maps of the studied species were obtained and inferred with the trichome type. This study enhances our understanding of the anatomical diversity and ecological importance of leaf trichomes in the family, providing a basis for future studies in plant biology and ecology. Furthermore, the findings underscore trichomes' diversity and potential applications in taxonomic treatments.

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**Keywords**: Boraginales; Heliotropiaceae; Micromorphology; SEM; trichome

## مطالعه میکروسکوپ الکترونی کرک برگ در تیره آفتاب‌پرست

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کرک‌های برگ برای فیزیولوژی گیاه، مکانیسم‌های دفاعی و برهم‌کنش‌های بوم‌شناختی اساسی هستند. در این تحقیق از میکروسکوپ الکترونی روبشی (SEM) برای بررسی کرک برگ 11 گونه از تیره Heliotropiaceae که شامل سرده‌های Euploca، Heliotropium، Ixorhea و Myriopus هستند، استفاده شد. شش گونه از Heliotropium، سه گونه از Euploca، یک گونه از Ixorhea و یک گونه از Myriopus بررسی شدند. تجزیه و تحلیل دقیق ما انواع مختلفی از کرک‌های غده‌ای و غیرغده‌ای را در گونه‌های مورد مطالعه نشان داد. نتایج تفاوت‌هایی را در ریخت‌شناسی، تراکم و توزیع کرک در داخل و بین سرده‌ها نشان داد. توزیع کرک‌ها در سطح برگ برخی از گونه‌ها متراکم و در برخی دیگر کم است. همچنین، کرک‌های برگ از نظر اندازه متفاوت هستند و برخی از گونه‌ها دارای انواع کوتاه و بلند هستند. بیشتر کرک‌ها روی سطح خود تزئیناتی دارند و تنوع در شکل آنها به چشم می‌خورد. همچنین نقشه پراکنش گونه‌های مورد مطالعه به دست آمد و با نوع کرک مقایسه شد. این مطالعه درک ما از تنوع تشریحی و اهمیت اکولوژیکی کرک‌های برگ در این تیره را افزایش می‌دهد و پایه‌ای برای تحقیقات آینده در زیست‌شناسی و بوم‌شناسی گیاهی فراهم می‌کند. علاوه بر این، یافته‌ها تنوع کرک‌ها و استفاده آنها را به عنوان یک صفت بالقوه طبقه‌بندی برجسته می‌کند.

## INTRODUCTION

Heliotropiaceae (Boraginales) comprises four genera, including *Euploca* Nutt., *Heliotropium* Tourn. ex L., *Ixorhea* Fenzl., and *Myriopus* Small, with a total of 449 accepted species (Luebert & al. 2016; POWO 2024), which are distributed in tropical, subtropical, and temperate regions and their diversity center is in the Neotropics (Mabberley 2017). The species of this family include herbaceous, shrubs and trees that are often distinguished by having distinctly scorpioid cymose inflorescences but are unique in possession of conical stigmatic heads, which is a synapomorphy for this family (Luebert & al. 2016).

*Heliotropium* Tourn. ex L. is the largest genus in Heliotropiaceae with 255 accepted species (POWO 2024). This genus is cosmopolitan and its species are spread all over the world. Some species of this genus have medicinal properties (Akçin & al. 2007) and some are edible (Mutie & al. 2023). *Euploca* is the second largest genus of this family with 168 accepted species and its species are distributed in the tropical and subtropical biomes. The species of this genus show significant diversity in morphological, physiological, reproductive, and chemical characteristics, which makes it a potentially valuable system for evolutionary studies (Frohlich & al. 2022). *Myriopus*, the third genus of this family, has 25 accepted species distributed from Florida to Central and South America. The genus is exclusively a Neotropical element and Brazil is its center of biodiversity (De Melo & Gonçalves 2020). Genus *Ixorhea* has only one shrubby species (*I. tschudiana* Fenzl) native to northwestern Argentina. *Ixorhea* possesses the key synapomorphy for Heliotropiaceae in Boraginales, which supports its inclusion in the family (Luebert & al. 2011).

In many cases, trichome characteristics as apomorphic characters have helped botanists to solve complex taxonomic problems (Zarre 2003; El Beyrouthy & al. 2008; Ghahremaninejad 2004; Steyn & Vyk 2021). Considering the changes in the position of the species and genera of this family and the classification problems that still exist, due to the limited amount of work that has been done on the leaves and trichomes in some species of this family (Kandemir & al. 2020; Kasem 2015), there is a need for a more comprehensive study of trichomes as a classification tool in this family, like to the similar work performed on some species of *Heliotropium* (Perveen 2009). Additionally, trichomes can provide insights into varying ecological conditions across different regions and habitats (Abassi & al. 2012).

The scanning electron microscope (SEM) is a highly effective magnification instrument with significant scientific and industrial applications. The images produced by SEM can address a wide range of scientific challenges (Choudhary & Priyanka 2017). In botanical research, electron microscopy has frequently resolved taxonomic and evolutionary issues and revealed new features and characteristics (Norouzi & al. 2021). In this study, we aim to evaluate the significance of SEM images in providing data on the overall condition of trichomes in the Heliotropiaceae to be used in the taxonomic treatment of the genera in this family (Abassi & al. 2012).

## MATERIALS AND METHODS

### Preparation of specimens and SEM images

Six herbarium specimens of *Heliotropium* species, three of *Euploca*, one of *Ixorhea*, and one of *Myriopus* species were selected for analysis.

Based on the aim of the study no specific population was examined; instead, herbarium specimens of selected genera were examined. The specimens were obtained from the Naturhistorisches Museum (W), the Herbarium of the National Museum of Natural History: Smithsonian Institution (US), and the Museum National d'Histoire Naturelle (P). The SEM analysis was conducted at the Natural History Museum (NHM), Vienna. Herbarium specimens were prepared using standard procedures before imaging. Leaf samples with trichomes were carefully excised from the dried herbarium sheets and attached to SEM stubs with conductive adhesive tabs. Both adaxial and abaxial surfaces of the leaves were prepared for imaging. The samples were then coated with a gold-palladium alloy to improve conductivity and reduce charging effects during imaging. The preparation of the specimens involved gold sputtering using a HUMMER V apparatus and imaging was conducted using a JEOL JXA 6610LV scanning electron microscope (SEM). High-resolution SEM analysis was conducted to examine the trichomes at various magnifications, capturing details such as shape, size (Short: less than 50 μm; Long: more than 50 μm), and surface texture. Multiple digital images were taken from different regions of each sample to ensure comprehensive coverage. Trichome characteristics including type (glandular or non-glandular), size, density, and distribution patterns, were documented for each species. The voucher information of the studied taxa of Heliotropiaceae is given in Table 1. All herbarium acronyms used in this article comply with the authoritative online Index Herbariorum (Thiers 2024).

### Distribution map

Distribution maps were created using data from the GBIF (Accessed on June 20, 2024: https://www.gbif.org/) and POWO (Accessed on August 06, 2024: https://powo.science.kew.org/)

databases, highlighting the importance of geographic location of species. The geographical areas of the species have been randomly selected according to the extent of the family coverage. For the distribution map of the studied species, data points from GBIF were extracted and stored in Excel files. R version 4.4.0 packages including readxl (to read Excel files into R), dplyr (for data manipulation and transformation), leaflet (for creating interactive maps in R), and htmlwidgets (to create interactive web visualizations that can be embedded in R Markdown documents) were used to create a map for each species.

Table 1. The voucher information of the studied taxa of Heliotropiaceae.

| No. | Species | Country | Collector and number | QR code | Herbarium acronym |
|---|---|---|---|---|---|
| 1 | *Ixorhea tschudiana* Fenzl | Argentine | Borsini O.E. s.n. | P04527901 | P |
| 2 | *Euploca axillaris* (Greenm.) J.I.M.Melo | Mexico | s.l. 5036 | US02899492 | US |
| 3 | *Euploca humilis* (L.) Feuillet | Brazil | Hatschbach & Barbosa 78696 | W0254159 | W |
| 4 | *Euploca strigosa* (Willd.) Diane & Hilger | Pakistan | Arshad s.n. | W0254160 | W |
| 5 | *Heliotropium arborescens* L. | Austria (Cultivated) | s.l. s.n. | W0254133 | W |
| 6 | *Heliotropium bacciferum* Forssk. | Iran | sBornmuller 532 | W0254134 | W |
| 7 | *Heliotropium crispum* Desf. | Morocco | Staudinger 4721 | W0254135 | W |
| 8 | *Heliotropium ellipticum* Ledeb. | Armenia | Vitek & al. 04-0405 | W0254136 | W |
| 9 | *Heliotropium longiflorum* (A.DC.) Jaub. & Spach | Argentine | Forther 2 | W0254137 | W |
| 10 | *Heliotropium paronychioides* DC. | Argentine | Weigend & al. 6819 | W0254138 | W |
| 11 | *Myriopus salzmannii* (A.DC.) Diane & Hilger | Paraguay | Octolier 2398 | W0254158 | W |

## RESULTS

Examination of SEM on the selected specimens from 11 species of Heliotropiaceae shows a significant variation in the shape, size, distribution, and ornamentation of the trichome surface. Trichomes include glandular and non-glandular types and have a high diversity in size. Based on the distribution of trichomes on the leaf surface, sparse, dense, and very dense types were seen in these species. Ornamentation of the trichome surface, on most non-glandular trichomes, were round or oval protrusions, which are sparse, dense, or very dense in terms of density. Information about the trichome characteristics of the studied species is shown in Table 2.

**The trichome types**

**Glandular trichomes**

The glandular trichomes observed on the leaves of the studied species include unicellular glands in *Ixorhea tschudiana*, the only type of trichome in this species. Additionally, trichomes with short stalks were observed on the leaves of *Heliotropium ellipticum* and *H. arborescens* (Fig. 1).

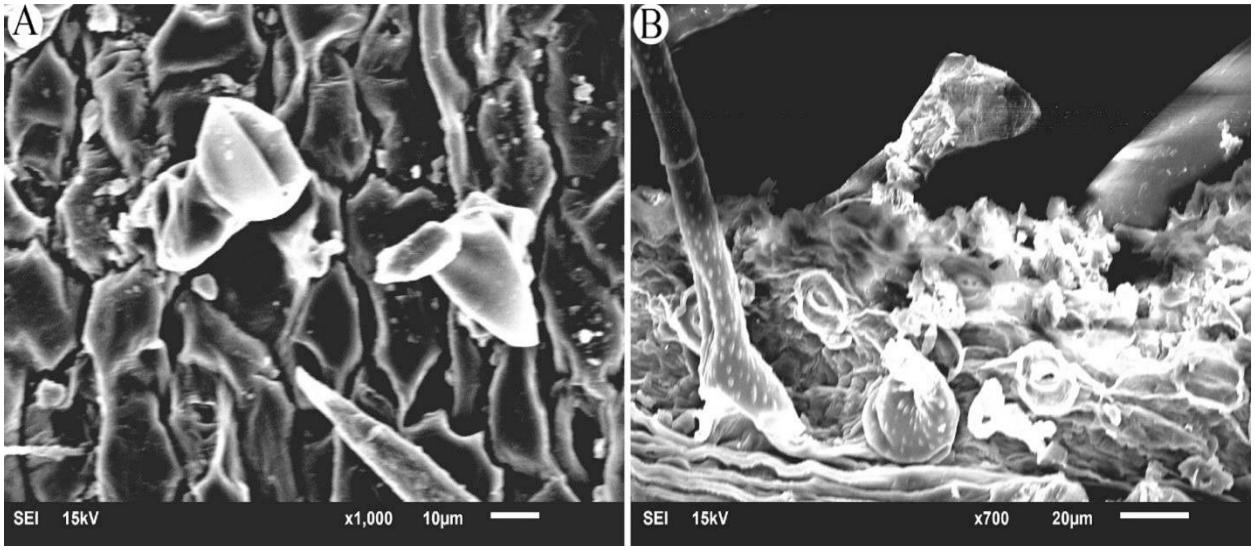


Fig. 1. Glandular trichomes in *Heliotropium* species. A, *H. ellipticum*; B, *H. arborescens*.

**Non-glandular trichomes**

Non-glandular trichomes show a much higher diversity than glandular trichomes. *Heliotropium arborescens* has curved horn-shaped hairs and short trichomes with a swollen base and a narrow tip (Fig. 2. A); *H. bacciferum* and *H. crispum* (Figs. 2. B-C) have curved horn-shaped trichomes, the base of some of them is multicellular and raised; *H. ellipticum*, *H. longiflorum*, and *H. paronychioides* (Fig. 2. D-F) have short trichomes with swollen bases and narrow tips. However, the trichome is slightly longer and the base is narrower on the veins. All three *Euploca* species have a special type of short and long bent trichomes that cover the entire leaf surface (Figs. 3. A-C). *Myriopus salzmannii* (Fig. 3.D) has short trichomes with a swollen base and narrow tip, horn-shaped trichomes of medium size, and long and simple trichomes.

**Bifurcate trichomes**

In addition to short non-glandular trichomes with a swollen base, *Heliotropium longiflorum* leaves also have bifurcate trichomes (Fig. 4). Furthermore, some trichomes have an intermediate state and seem to be at the beginning of the path becoming bifurcated trichomes, which are unique and are mentioned here for the first time.

**Trichomes distribution**

In addition to trichome traits, trichome distribution can be used as a diagnostic character in the species of this family. Figure 5 shows the variation of trichome distribution on the leaf surface of the studied species in the full view of the leaf.

In the microscopic examination, the trichome density ranged from sparse to very dense (Fig. 6).

**Trichome type and geographical distribution**

The distribution map of the studied *Heliotropium* species (Fig. 7) indicates that the types of trichomes vary across different geographical regions. *Heliotropium arborescens* is distributed in America, Africa, and Australia. Its leaves feature short and long non-glandular trichomes in various shapes, as well as glandular trichomes.

In *H. bacciferum*, which is found exclusively in Africa and the Arabian Peninsula, the trichomes are primarily thick and curved. These features likely help the plant adapt to drier environments by reducing water loss. In *H. crispum*, the trichomes resemble those of *H. bacciferum*, but the trichomes of this species do not have swollen bases, likely due to their growth in more humid environments. *Heliotropium ellipticum* is found from the Mediterranean region to East Asia, and is characterized by both glandular and non-glandular trichomes. It appears that *H. longiflorum*, found in a more restricted geographical area, is undergoing a notable change, with some of its trichomes exhibiting bifurcation. In contrast, *H. paronychioides*, native to a region in South America, displays only a single type of trichome-short trichomes with swollen bases.

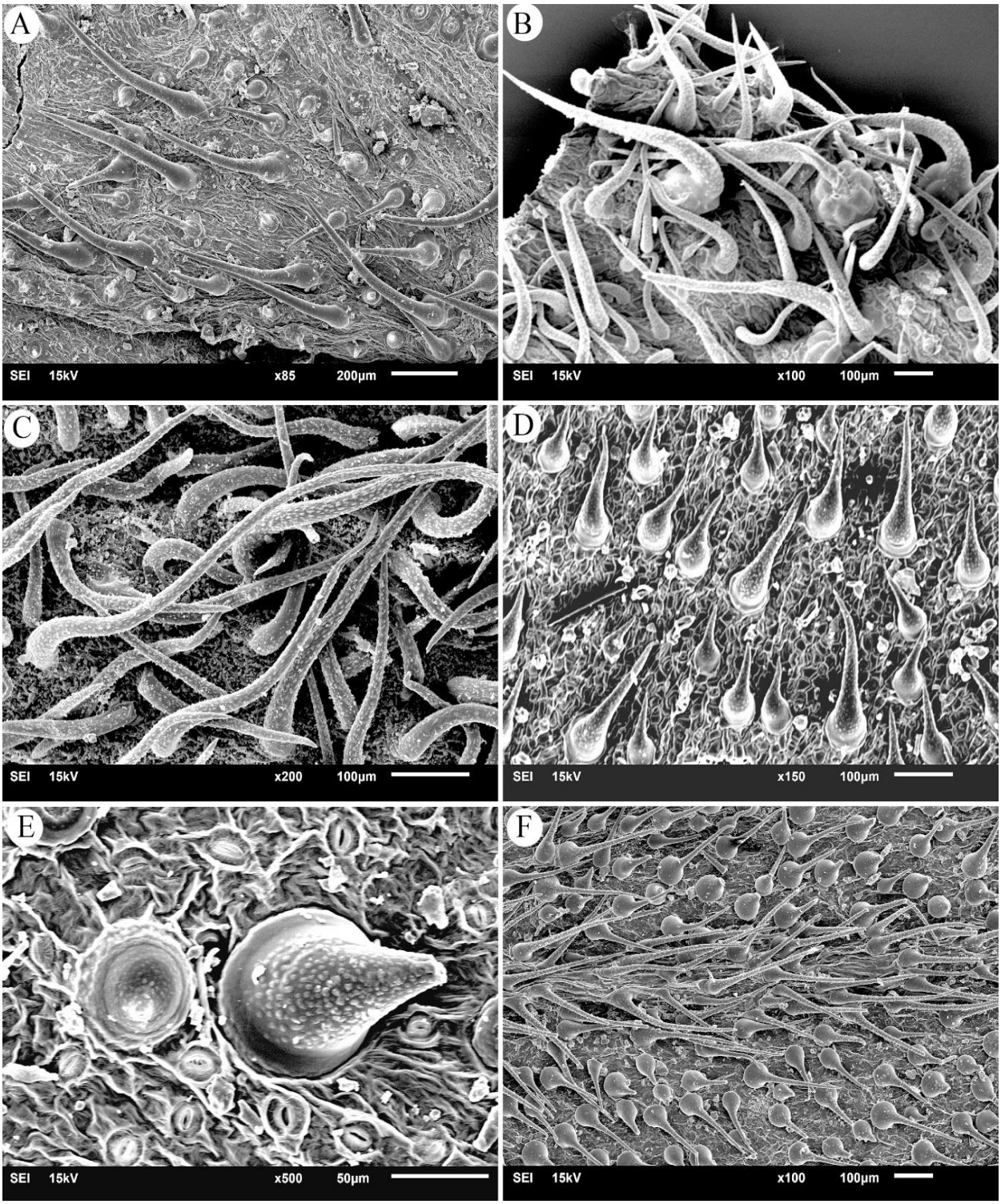


Fig. 2. Non-glandular trichomes. A, *Heliotropium arborescens*; B, *H. bacciferum*; C, *H. crispum*; D, *H. ellipticum*; E, *H. longiflorum*; F, *H. paronychioides*.

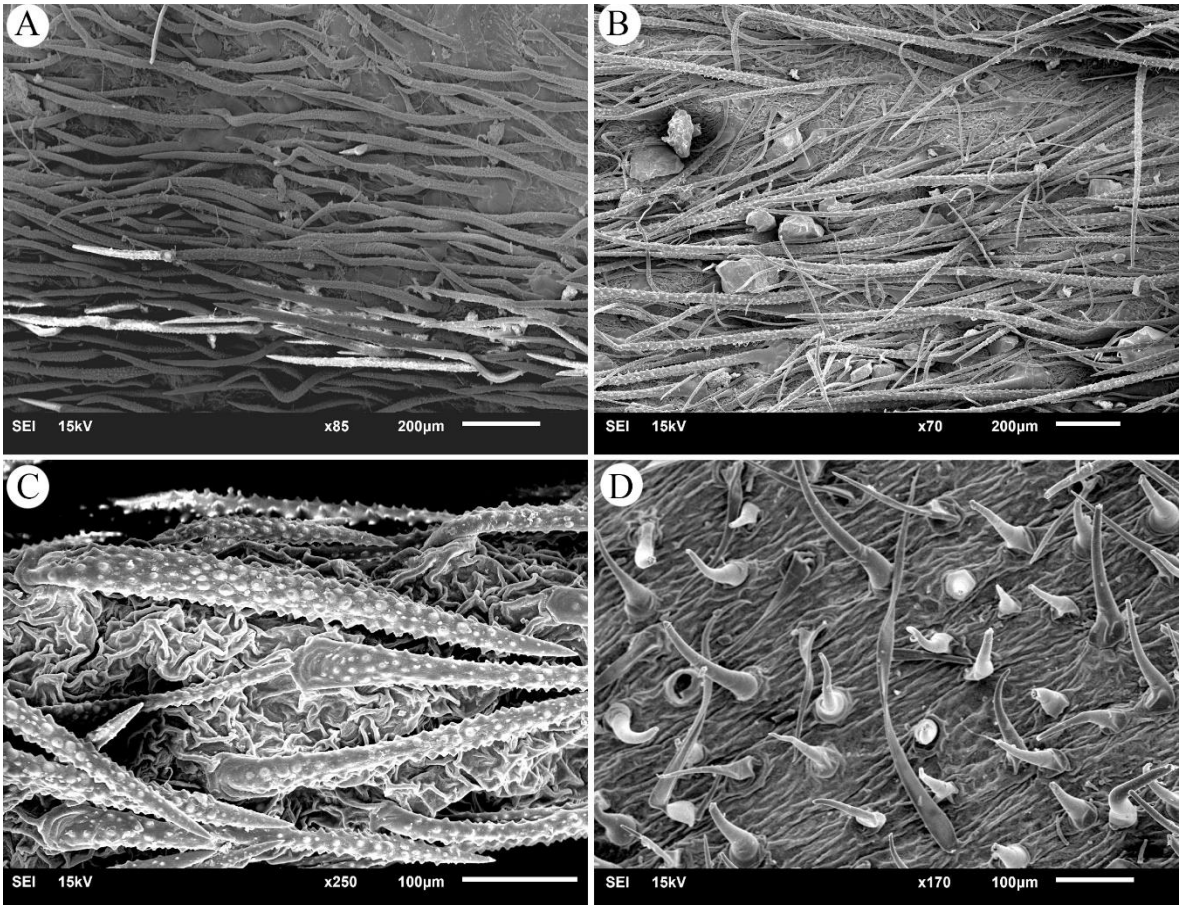


Fig. 3. None-glandular trichomes. A, *Euploca axillaris*; B, *E. humilis*; C, *E. strigosa*; D, *Myriopus salzmannii*.

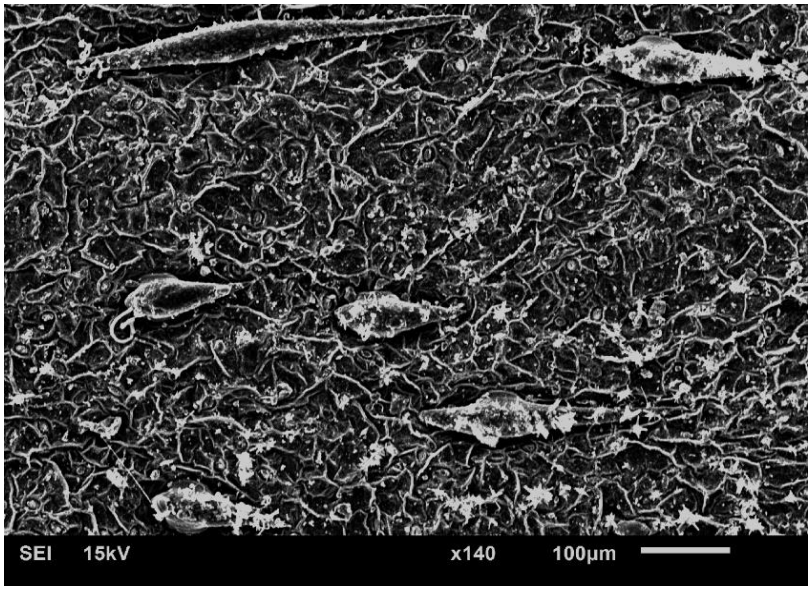


Fig. 4. Bifurcate trichomes in *Heliotropium longiflorum*.

Table 2. Morphological characteristics of trichomes in studied species of Heliotropiaceae.

| **Species** | **Types of Trichomes** | **Size** | **Distribution** | **Decoration shape** | **Decoration distribution** |
|---|---|---|---|---|---|
| *Ixorhea tschudiana* | Unicellular glands | Short | Sparse to dense | ------ | ----- |
| *Euploca axillaris* | Non-glandular | Long | Very dense | Cone-shaped | Dense |
| *Euploca humilis* | Non-glandular | Short or long | Very dense | Cone-shaped | Dense |
| *Euploca strigose* | Non-glandular | Short | Very dense | Cone-shaped | Dense |
| *Heliotropium arborescens* | Glandular and non-glandular | Short or long | Dense | Oval | Dense |
| *Heliotropium bacciferum* | Non-glandular | Short or long | Dense | Round | Dense |
| *Heliotropium crispum* | Non-glandular | Short or long | Very dense | Oval and Round | Very dense |
| *Heliotropium ellipticum* | Glandular and non-glandular | Short | Very dense | Round | Dense |
| *Heliotropium longiflorum* | Bifurcate and non-glandular | Short | Dense to very dense | Round | Sparse to dense |
| *Heliotropium paronychioides* | Non-glandular | Short | Very dense | Round | Sparse |
| *Myriopus salzmannii* | Non-glandular | Short or long | Sparse to dense | Oval | Rare to sparse |

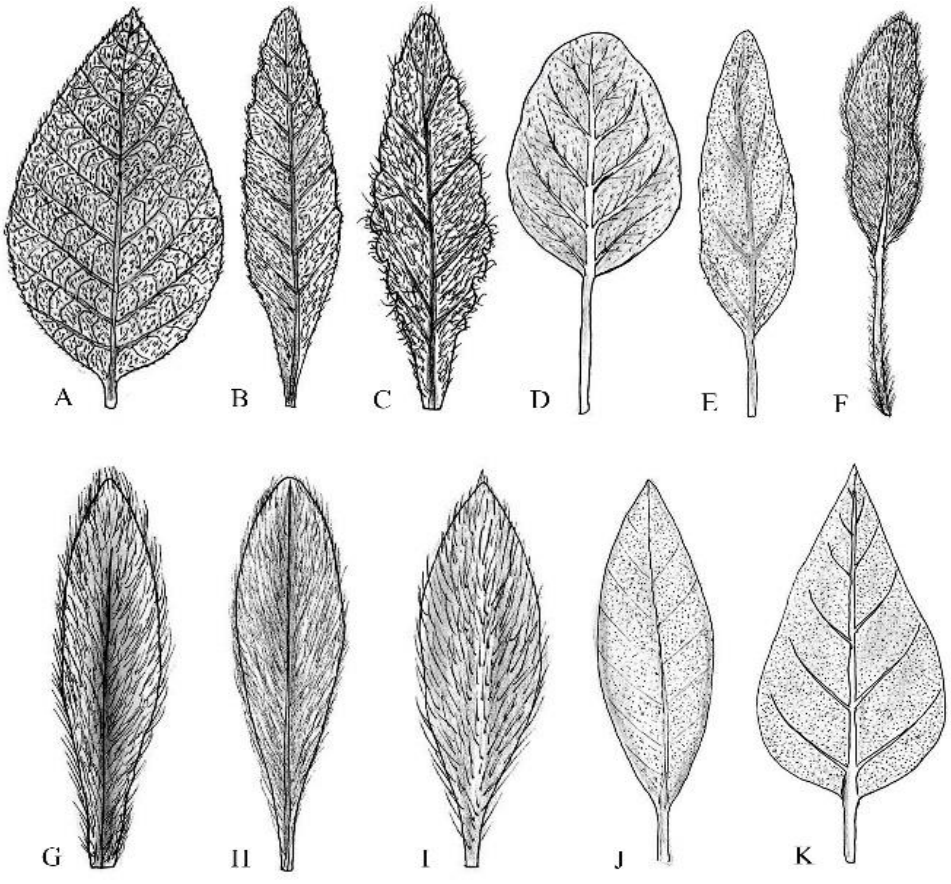


Fig. 5. Trichome distribution on the leaf surface of the studied species. A, *Heliotropium arborescens*; B, *H. bacciferum*; C, *H. crispum*, D, *H. ellipticum*; E, *H. longiflorum*, F, *H. paronychioides*; G, *Euploca axillaris*; H, *E. humilis*; I, *E. strigosa*; J, *Ixorhea tschudiana*; K, *Myriopus salzmannii* (drawing by the first author).

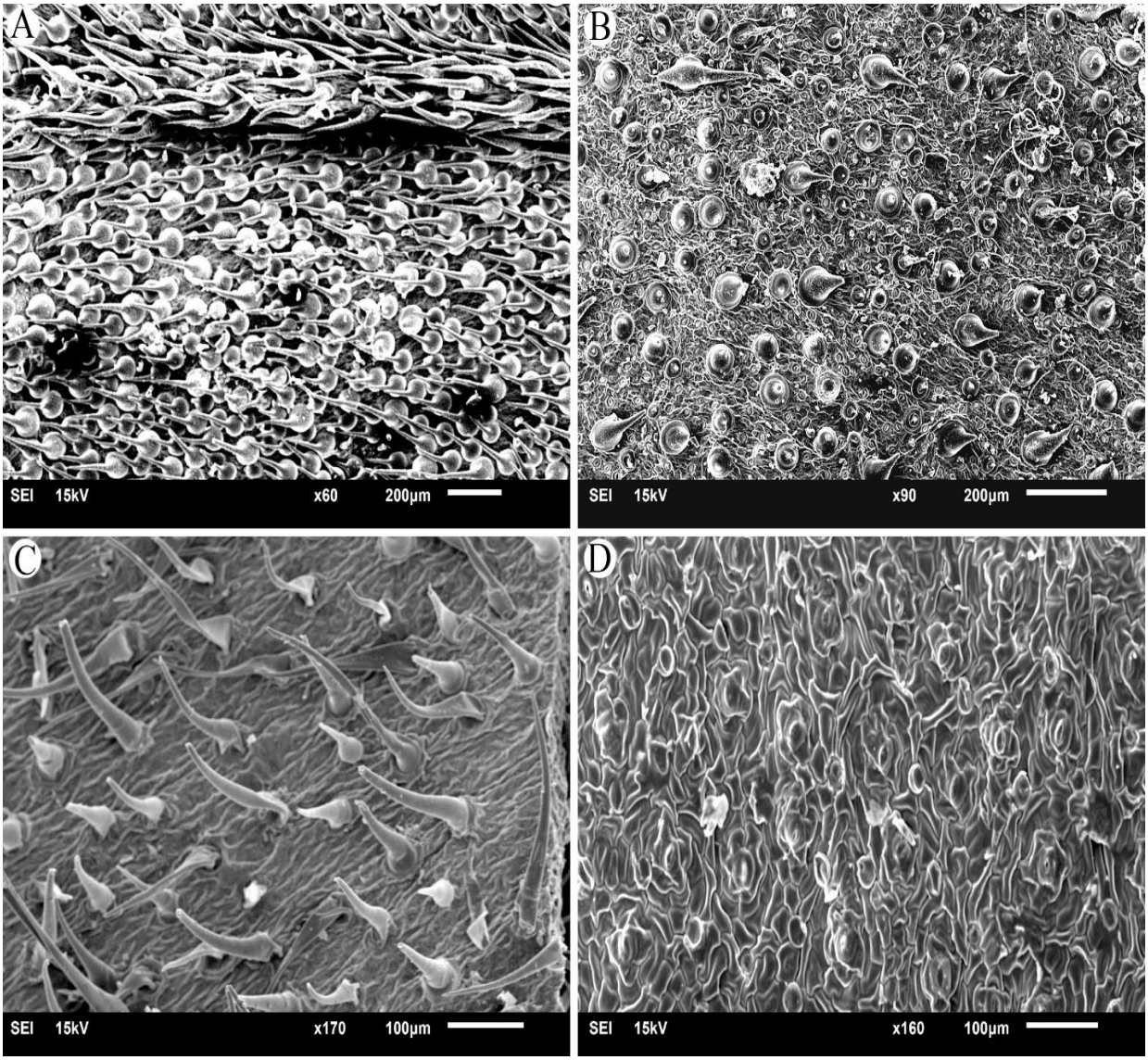


Fig. 6. Trichome distribution. A, very dense in *Heliotropium ellipticum*; B, Dense in *H. longiflorum*; C, Sparse to dens in *Myriopus salzmannii*; D, Sparse in *Ixorhea tschudiana*.

*Myriopus salzmannii* is distributed both in the vicinity of open waters in northeastern Brazil and in the center of South America (Fig. 8). It can be assumed that the range of its distribution and the effects of humidity and other ecological factors have caused the variety of trichomes to be observed on the leaves of this species. *Ixorhea tschudiana* is a species with limited distribution in Central America and has only unicellular glands. *Euploca*, *E .axillaris*, and *E. humilis* are distributed only in South America. Their trichomes are thin and mostly long, and their surface decorations do not have secondary protrusions. In *E. strigosa*, which is spread in Asia and especially in Africa, the trichomes are thicker, and secondary protrusions are observed on the decorations of the trichome surface. The traits mentioned appear to be effective in reducing water wastage and enhancing drought resistance in regions such as Africa.

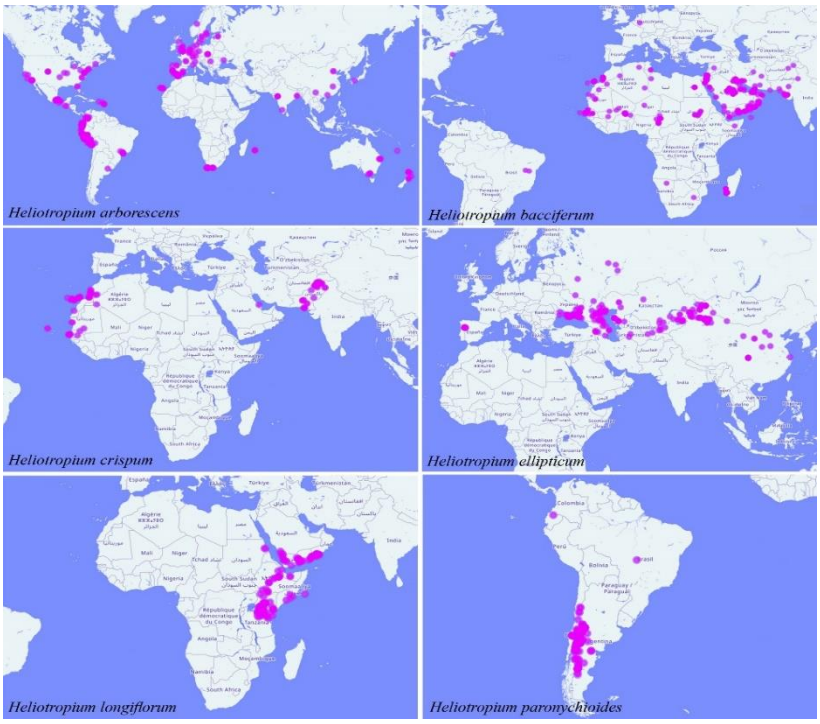


Fig. 7. The distribution map of the studied species of *Heliotropium*.

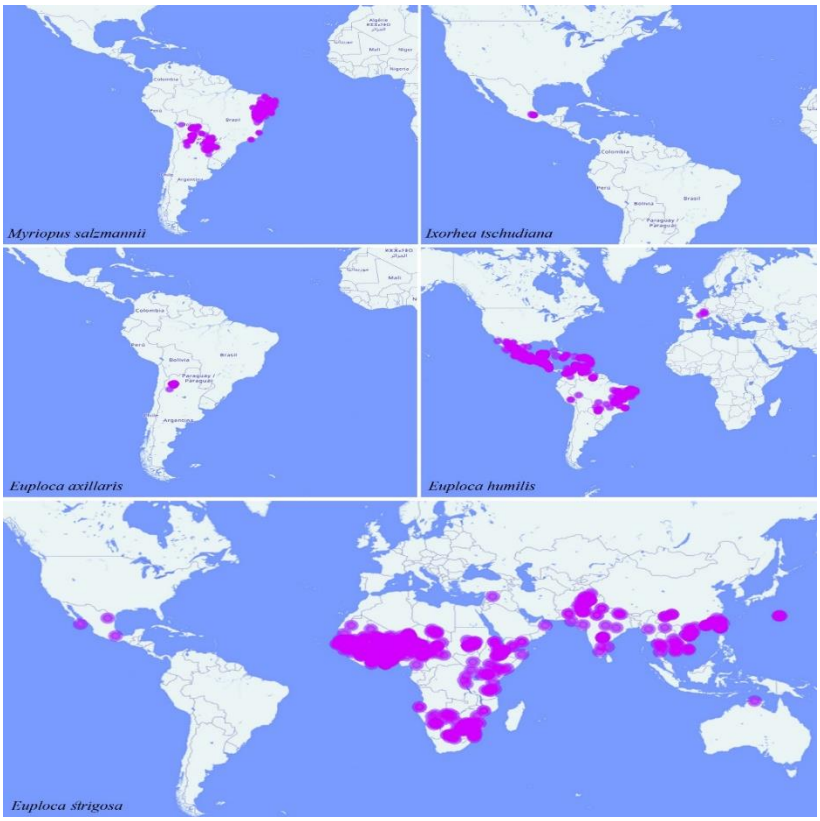


Fig. 8. The distribution map of the studied species of *Euploca*, *Ixorhea*, and *Myriopus*.

## DISCUSSION

One of the main characteristics of Heliotropiaceae taxa (and Boraginales) is their trichomes. However, despite the taxonomic problems in this group of plants, trichome characteristics have been used less to solve them. This work emphasizes the importance of using trichomes and their traits as a powerful taxonomic tool in the plants of this family and the entire Boraginales order. In a similar study, Mehrabian & al. (2014) examined the leaf trichomes of 32 species of *Onosma* using SEM showing the diversity of trichomes, they discussed the importance of trichomes as a classification tool in this genus. In the mentioned study, the type of trichomes and their surface decorations have been well investigated and the results are similar to the present work. Additionally, in a similar study that was conducted on 7 species of *Heliotropium* in Turkey (Kandemir & al. 2019), the trichome structure and its related traits were shown as a useful classification tool that can be used in solving taxonomic problems in this group of plants.

Phenotypic changes in plants are significantly influenced by climatic conditions and geographical regions, leading to adaptations that enhance survival and reproduction. For instance, studies show that rising temperatures and altered precipitation patterns can cause shifts in leaf morphology (Menzel & al. 2006). These adaptations are crucial for maintaining ecological balance and ensuring plant viability in facing climate change. In this study, by showing the distribution map of the studied species, an attempt has been made to determine the relationship between the geographical regions and the type of leaf trichome. This method can be applied to more species within this family, potentially providing a more comprehensive model of their relationships.

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