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# Stomatal variations and their position relative to leaf epidermal cells in ten Maple species

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**Abstract**

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In the present study, we investigated the structure of stomata in seven native species of Hyrcanian forests (*Acer hyrcanum, A. velutinum Boiss., A. campestre, A. platanoides L., A. cappadocicum, A. monspessulanum, A. amazandaranicum*), as well as non-native species that have fully adapted (*A. negundo, A. negundo variegatum, and A. palmatum*). We used light and electron microscopy to determine the form and position of the stomata in relation to the leaf epidermal cells. The length, width, shape, area, perimeter, and stomatal density were all measured. Our findings revealed that the stomata type of *A. negundo varengiayum, A. campestre, A. hyrcanum, A. mazandaranicum* and *A. monsspesulanum* is anomocytic, *A. platanoides* and *A. cappadocicum* have anomocytic stomata with wavy subsidiary cells, while *A. palmatum* has anisocytic stomata and *A. velutinum* has parasitic stomata. *A. negundo* has actinocytic stomata. Regarding the location of stomata relative to adjacent epidermal cells, we identified three types. In the first type, the stomata were flush with adjacent epidermal cells (*A. cappadocicum, A. negundo, A. platanoides*). In the second type, the stomata were higher (*A. negundo variegatum*), and in the third type, the stomata were lower (*A. velutinum, A. monspesulanom, A. campestre, A. mazandaranicum, A. hyrcanum*). The principal component analysis was used to determine the essential stomatal traits in differentiating between species. We also investigated the distribution of trees in the coordinate axis space based on two main components and performed cluster analysis based on stomatal characteristics. *A. platanoides, A. negundo, A. negundo variegatum* were in one cluster, while the other species were in separate clusters. The calculation of dissimilarity among the studied species revealed the lowest similarity between *A. negundo* and *A. hyrcanum* and the highest similarity between *A. campestre* and *A. mazandaranicum*. The results of the discriminant analysis identified stomatal density as the essential factor in differentiation between the studied species.

**Keywords**

*Acer*, form and position, principal component analysis, stomata

## Introduction

Stomatal characteristics are important for determining different species of a genus, as they are epidermal properties that can serve as suitable taxonomic criteria. The position of epidermal cells surrounding the guard cell is the most significant. Stomata are quickly affected by external environmental conditions. Many factors, including non-biological factors like temperature, humidity, air pollution, radiation, atmospheric carbon dioxide, moisture, and nutrients in the soil, as well as morphological and descriptive factors like leaf structure, position,

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photosynthesis, and herbal transpiration, play a role in modulating leaf stomata (Hong et al., 2018). Stomata are divided into different types based on their formation and communication with the adjacent epidermal cells, as well as the role they play (Gray et al., 2020). These types include parasytic (stomatal cells are closed by cells that are parallel to them); Diasytic (stomatal cells are closed by two cells of different sizes and the common wall of the accompanying cells is perpendicular to the central axis of the stomatal cells); Anisocytic (stomatal cells are closed by three cells of different sizes, one of which is smaller than the other two cells); Actinocytic (stomatal cells closed by a ring of radial cells); Anomocytic (the accompanying cells around the guard cells are uniform). The type of stomata has a systematic value and can be used to determine different species of a genus (Prabhakar, 2004). For example, the structure of the leaf surface and the type of stomata arrangement have been used to distinguish species of *Camellia* L. (Ao et al., 2007), and the type of stomata and associated cells have been used to separate different species of *Carpinus betulus* (Uzunova, 1999). Stomatal size and density have been reported as the best distinguishing traits of eight populations of *Pistacia atlantica*, and these characteristics may provide an initial screening method for classifying drought resistance (Belhadj et al., 2011). Stomatal size, sculpturing, and distribution have also shown taxonomic significance in identifying species in the genus *Premna* in the Lamiaceae family (Amran et al., 2023). Significant interpopulation differences in stomatal features have been found in *Lilium pumilum, L. brownii*, and *L. davidi*, offering potential opportunities for taxonomic discrimination (Ba et al., 2023). However, there are reports suggesting that the stoma has no taxonomic value. This is because they can be easily influenced by external environmental conditions. Stomatal index and morphological parameters, such as stomatal density, stomatal apparatus, and guard cell architecture, have been found to respond to environmental and physiological cues (Alushi and Veiz, 2020). In a study comparing London plane trees in highly urbanized and rural areas, it was observed that leaf size and stomate density were reduced in the urban environment. However, the area of palisade parenchyma cells and spongy parenchyma cells remained unchanged with urbanization (Pourkhabbaz et al., 2010). Kordalivand et al. (2015) reported that stomata were not efficient in differentiating between species of *Betula* spp. Similarly, Chapolagh Paridari et al. (2012) found that stomatal characteristics were not sufficient to distinguish between different species of the hornbeam genus (*Carpinus betulus, C. orientalis, and C. schuschaensis*). *Acer* L. belongs to the Sapindaceae family and consists of 126 species which are distributed in the temperate regions of the northern hemisphere. In Iran, there are 12 taxa, including *A. monspessulanum* with four subspecies and *A. velutinum* with two varieties (Asadi et al., 2019). The hybridization and diversity among *Acer* species make taxonomy identification challenging (Grimm et al., 2007; Grimm and Denk, 2014). In Iran, all species of *Acer* grow in the forests of the north of the country as well as in the west and south (Ghahreman, 1997) and belong to section Acer (*A. mazandaranicum, A. velutinum, A. monspessulanum, A. hyrcanum*) and section Platanoidea (*A. cappadocicum, A. platanoides, and A. campestre*) (Asadi et al., 2019). The maple species in this study belong to the Hyrcanian forests, which are one of the most valuable forests in the world and are considered a natural museum due to their estimated age of 25 to 50 million years and their 850 km length along the southern shores of the Caspian Sea.

## Materials and methods

Leaves were collected from the inner fertile shoots of the crown of 10 mature native species found in Hyrcanian forests on the southeastern shore of the Caspian Sea in areas away from pollution (Fig. 1). The species collected included *Acer hyrcanum, A. velutinum, A. campestre, A. platanoides* L., *A. cappadocicum, A. monspessulanum, A. mazandaranicum*, as well as non-native species *A. negundo, A. palmatum*, and subspecies *A. negundo variegatum* which are fully adapted to the area (Fig. 2). For each species, 5–10 trees were selected with a minimum distance of 100 meters between them, following the method used by Miles et al. (1995). From each tree, a total of 20–30 leaves were collected, with samples taken from both the north (shade exposure) and south (sun exposure) sides to ensure they were not directly exposed to sunlight, as described by Hatziskakis et al. (2011). Additionally, five leaves were randomly chosen from each species and dried for future use. The dried leaves were then boiled for 5 minutes and thin incisions were made on the dorsal side of each leaf. The samples were immersed in sodium hypochlorite for 5 minutes to remove the greenness of the plant and then rinsed with water. The morphological traits of the samples including Stomata Length, Stomata Width, the ratio of length to width, Area, Perimeter and Stomata Density were measured using digimizer ver 4.6.2.0 software under a light microscope at 40% magnification. To determine the stomatal position relative to leaf epidermal cells, an electron microscope (field emission


Fig. 1. The location of the studied habitats of the maple genus. D: Golestan, Kordkoy, Drazno. S: Golestan, Aliabad, Siamarzkoh. V: Golestan, Bandargaz, Vatana. SH: Golestan, Gorgan, Shastkalate. G: Golestan, Kalale, Golidagh. M: Golestan, Kalale, Maravetapeh. GNP: Golestan, Golestan nationalpark. SA: Mazandaran, Sari, Sangdeh. EG: Mazandaran, Nowshahr, Ecological garden.

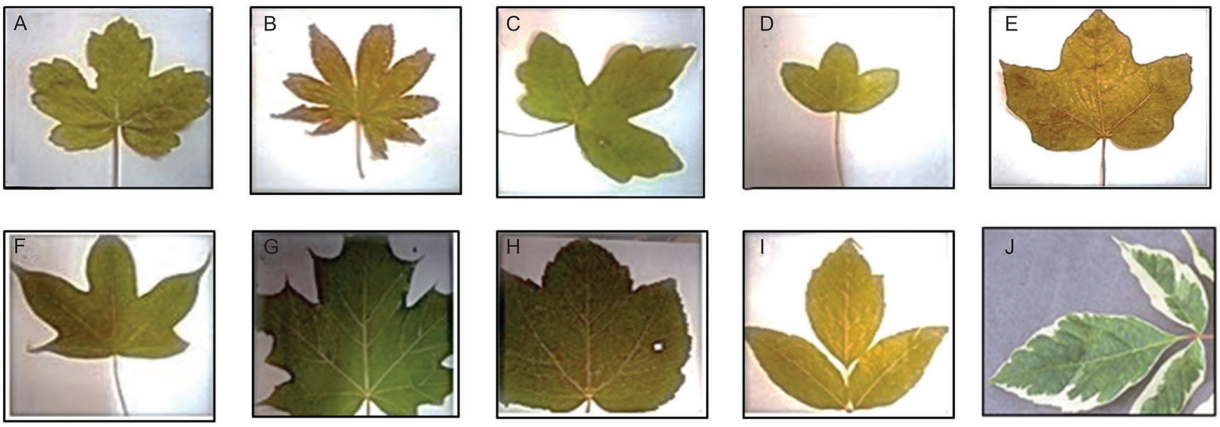


Fig. 2. The leaf of the studied species: A: *A. hyrcanum*, B: *A. palmatum*, C: *A. campestre*, D: *A. monsspesulanum*, E: *A. mazandaranicum*, F: *A. cappadocicum*, G: *A. platanoides*, H: *A. velutinum*, I: *A. negundo*, J: *A. negundo variegatum*

microscopy) was used. In order to photograph the specimens, they were placed in a Sputter Coater for 10–15 minutes and then a thin layer of gold was applied to the leaf surface using the Physical vapor deposition method. Photos were taken at 3 kV using FEM-SEM Mira 3-XMU to prevent sample degradation. The data obtained was analyzed using one-way analysis of variance (ANOVA) to compare the morphological traits between the species, and the Duncan test was used to compare the means between the species. Principal component analysis (PCA) was conducted to determine the most critical components that affect the separation of the species and extract the primary factors that explain the maximum variance. The distribution of trees in the coordinate axis space of the two primary axes, which justified the highest variance, was evaluated to assess the grouping. The studied species were separated into different clusters using Ward's cluster analysis. To confirm the validity of the grouping, discriminant analysis was used to determine the role of each trait in distinguishing the clusters from each other based on multivariate measurements.

## Results

Based on the obtained results, it was found that stomata are present on the dorsal surface of the leaf in all studied species. The upper surface of the leaf does not have stomata. Using light microscopy, five different types of stomata, were identified anomocytic, anomocytic with the wall of subsidiary wavy cells, actinocytic, parasitic, and anisocytic. The anomocytic stomatal type was found in the leaves of *A. negundo varengiayum, A. campestre, A. monsspesulanum, A. mazandaranicum*, and *A. hyrcanum* (Fig. 3A). The leaves of *A. platanoides* and *A. cappadocicum* have an anomocytic type with the wall of the subsidiary wavy cells (Fig. 3B) which is known as the Caryophyllaceous type. Anisocytic stomatal type (Fig. 3C) was only observed in *A. palmatum*, parasitic type (Fig. 3D) in *A. velutinum*, and actinocytic type (Fig. 3E) in *A. negundo*.

The types of stomata in maple species, based on the position of the stomatal guard cells relative to other epidermal cells, are shown in Figs 4, 5 and 6. The first type, called superficial air stomata, is characterized by stomata aligned with adjacent epidermal cells. This type is observed in species *A. cappadocicum*, *A. negundo*, and *A. platanoides*. The second type, found in *A. velutinum*, involves the deep development of stomata located under a mass of wax-shaped epidermal cells. Some plants also have depressions called caves or crypts in their epiderm, in which the stomas are located. Crypts can be filled with trichomes, as observed in *A. monspesulanom, A. campestre, A. hyrcanum,* and *A. mazandaranicum*. The third type is characterized by stomata located above the surface of the epidermis, and this type was observed in *A. negunda variegatum*.

## Evaluation of quantitative traits of stoma

The results of a one-way analysis of variance showed a significant difference among different species in terms of length, width, area, perimeter, and stomatal density at a 99% level of confidence ($P < 0.01$) (Table 1). Comparing the mean data using the Duncan test revealed that *A. hyrcanum* had the highest length and width of stomata,

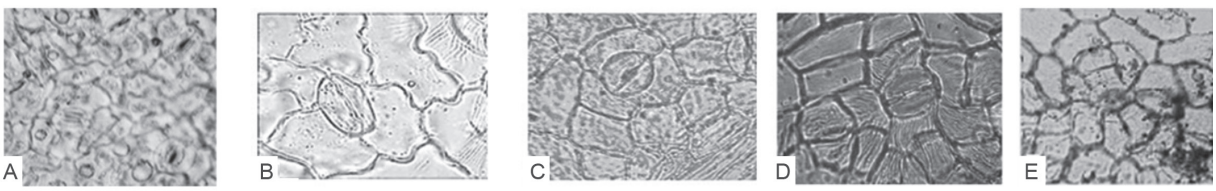


Fig. 3. Stomatal types in the studied species: A: anomocytic, B: anomocytic with wavy cell wall, C: Anisocytic, D: parasitic, E: actinocytic.

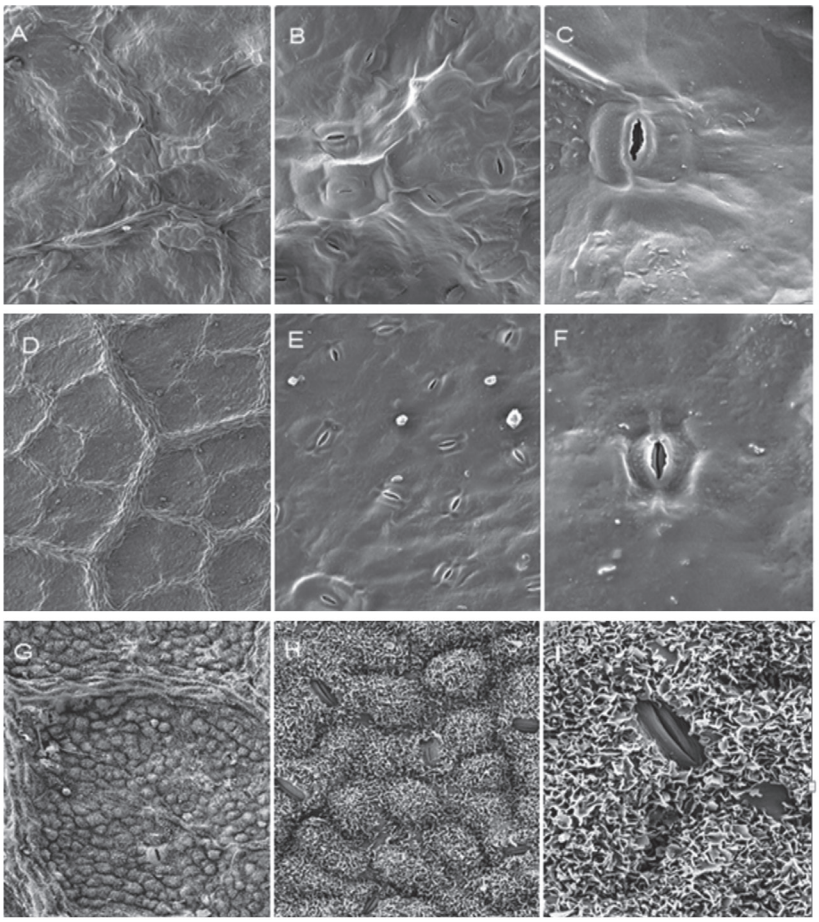


Fig. 4. Position of the stomata relative to the leaf epidermal cells. Longitudinal incision of the middle part of the leaf (A–C) *A. negundo variegatum*: (A) 500×, (B) 2,000×, (C) 5,000×, (D–F) A. platanoides: (D) 500×, (E) 2000×, (F) 5000×, (G–I) *A. hyrcanum*: (G) 500×, (H) 2000×, (I) 5000×.

resulting in the largest stomatal area, while *A. negundo* had the lowest (Table 2). Additionally, *A. negundo variegatum* and *A. platanoides* subspecies had the highest stomatal density, while *A. velutinum* had the lowest.

Principal component analysis was used, to determine the essential stomatal features for distinguishing between species. The results of the analysis showed that the first two components accounted for approximately 85.04% of the variance. The first component accounted for 66.74%, of the variance, while the second accounted for 18.2%. Stoma features such as area, perimeter, width, length, and density, had the largest contribution to the variances in the first component (Table 3).

Furthermore, the distribution diagram of trees in the coordinate axis space based on the two primary components could only categorize the listed species into two generic groups, with significant overlap among the species within each category (Fig. 7.B). To confirm the distribution of trees in the coordinate axis space, a cluster analysis was performed based on stomatal characteristics. The results corresponded to the separation of the species into different clusters based on their distribution in the coordinate axis space. *A. platanoides, A. negundo* and *A. negundo variegatum* were grouped together, while the other species formed a separate cluster (Fig. 7.A). Calculation of dissimilarity among the studied species indicated the highest distance (least similarity) between *A. negundo* and *A. hyrcanum* species and the lowest distance (highest similarity) between *A. campestre* and *A. mazandaranicum* species in the stomatal study (Table 4).

Discriminant analysis was utilized to assess the degree of similarity of stomatal features among different species. Discriminant analysis is a technique for categorizing variables into distinct categories. It finds an equation that can identify which group a person belongs to based on their characteristics. The results showed that the first two functions explained 98% of the variances (Table 5). Stomatal density, area, and perimeter were correlated with the first two functions. Interestingly, stoma width did not play a role in differentiating among species. In the first axis direction, which showed the highest correlation with stomatal density, *A. platanoides* and *A. negundo variegatum* were distinguished from other species. Stomatal density was identified as the most critical factor in species differentiation in this genus. Additionally, in line with the second function, perimeter attributes and the stomal area showed the highest correlation, *A. negundo* was distinguished from other species. The results showed that approximately 80% of the trees were correctly grouped into different categories (Fig. 8, Table 6).

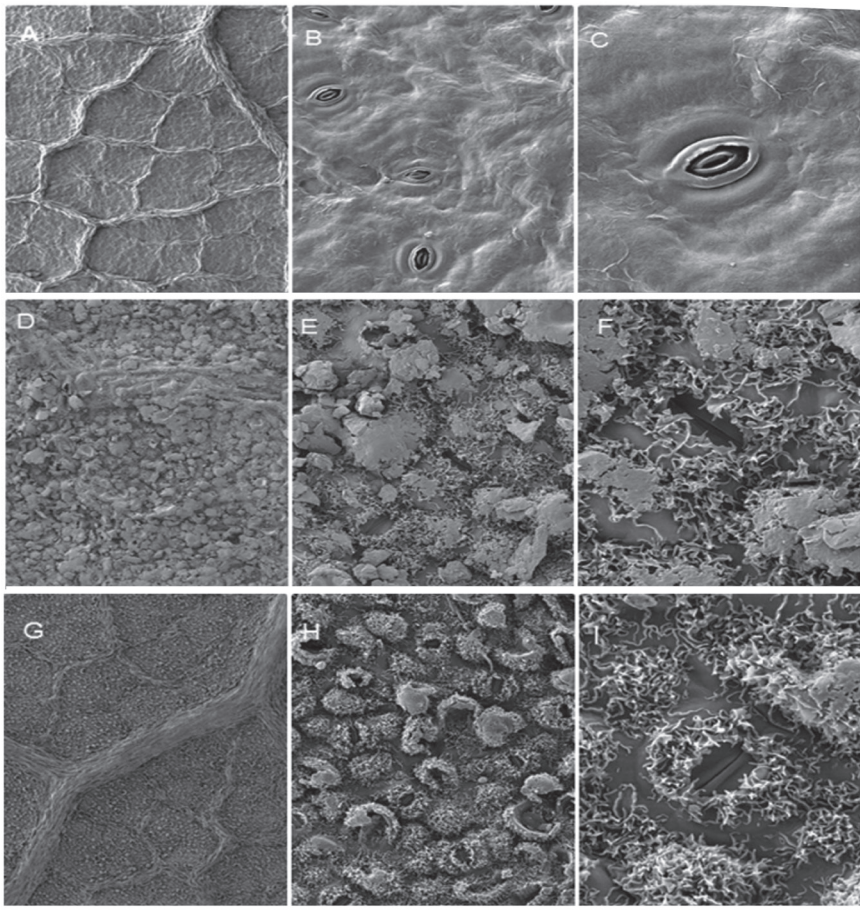


Fig. 5. Position of the stomata relative to the leaf epidermal cells. Longitudinal incision of the middle part of the leaf (A–C) *A. campestre*: (A) 500×, (B) 2,000×, (C) 5,000×, (D-F) *A. monsspesulanum*: (D) 500×, (E) 2,000×, (F) 5,000×, (G–I) *A. mazandaranicum*: (G) 500×, (H) 2,000×, (I) 5,000×.

**Discussion**

The micromorphological features of plant leaves serve as a promising tool for plant species identification and classification (Wang et al., 2020; Gang et al., 2021). One of these features in plant classification, is stomata Chapolagh Paridari et al., 2012). Stomata are classified based their formation, connection with the epidermal cell and role. We found that stoma only exists on the dorsal surface of the leaves of the studied species. We identified five different types of stomata, including anomocytic (*A. negundo varengiayum, A. campestre, A. monsspesulanum, A. mazandaranicum, A. hyrcanum*), anomocytic with the wall of subsidiary wavy cells (*A. platanoides, A. cappadocicum*), actinocytic (*A. negundo*), parasitic (*A. velutinum*), and anisocytic (*A. palmatum*). The stomata in plants of the same genus or different germplasm sources of the same species can also be different (Zhu et al., 2016). The existence of parastic stomata for the species of *A. monsspesulanum, A. campestre,* and *A. hyrcanum* was previously reported (Baranova et al., 1992), which this research confirms. Since a higher subsidiary cell density around the stomata increases the opening rate of the stomata (Oyeleke et al., 2004), plants with more companion cells (such as actinocytic and anomocytic type) play a more significant role in reducing greenhouse gases, especially carbon dioxide. Therefore, considering climate change in the present century, it is better to use species in forestry programs with more subsidiary cells around the stomata (Oyeleke et al., 2004). Thus, from this perspective, *A. negundo, A. mazandaranicum, A. campestre* and *A. hyrcanum* are probably more suitable for forestry than other maple species in Hyrcanian forests. There is an inverse relationship between stomatal density and size in some of the studied species. For example, there is low stomatal density in *A. velutinum, A. cappadocicum*, and *A. monsspesulanum* with high stoma area, and in *A. negundo variegatum* subspecies and *A. platanoides*, the stoma area is low, and its density is high (Guo et al., 2022). However, we cannot consider any special relationship in this regard for other species. For example, we can consider the stoma density in *A. hyrcanum* (maximum area) and *A. negundo* (lowest area), which do not differ much in terms of stoma density (563 and 600, respectively). The characteristics of plant epidermal stomata can play an essential role in studying the diversity of plant germplasm resources (Liu et al., 2014). Stomatal morphology in widely distributed species, such as Maple (which extends from intermediate altitudes to upper altitudes in the forests of northern Iran), is to be expected. However, accepting that the stomatal type changes in one species vary significantly with changing habitat conditions is

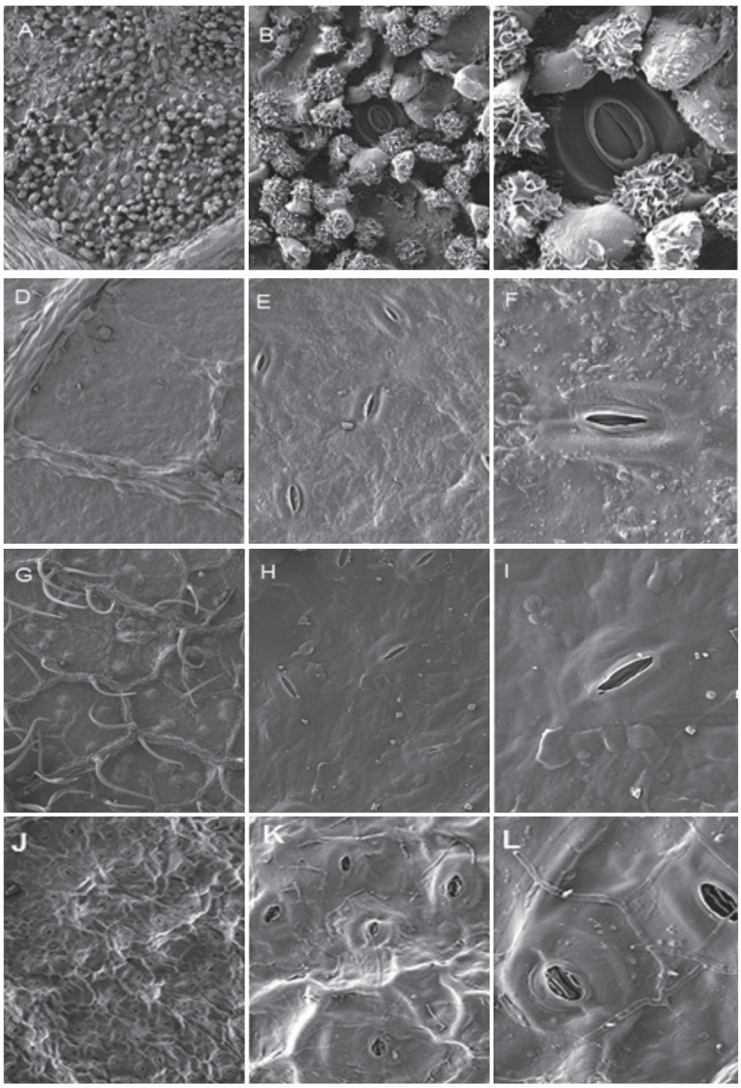


Fig. 6. Position of the stomata relative to the leaf epidermal cells. Longitudinal incision of the middle part of the leaf (A–C) *A. velutinum*: (A) 500×, (B) 2,000×, (C) 5,000×, (D–F) *A. cappadocicum*: (D) 500×, (E) 2,000×, (F) 5,000×, (G–I) *A. negundo*: (G) 500×, (H) 2,000×, (I) 5,000×, (J–L) *A. palmatum*: (J) 500×, (K) 2,000×, (L) 5,000×.

Table 1. The results of multivariate analysis of variance of stomatal traits

| Traits | Degree of freedom | Sum of squares | Mean squares | F | Sig |
|---|---|---|---|---|---|
| Stomata Length | Within groups | 116.186 | 1,045.672 | 26.288 | 0/00 |
| | Between groups | 4.420 | 928.144 | | |
| | Total | | 1,973.816 | | |
| Stomata Width | Within groups | 133.881 | 1,204.928 | 43.676 | 0.00 |
| | Between groups | 3.065 | 643.719 | | |
| | Total | | 1,848.647 | | |
| Area | Within groups | 90,562.663 | 815,063.966 | 35.534 | 0.00 |
| | Between groups | 2,548.642 | 535,214.886 | | |
| | Total | | 1,350278.852 | | |
| Perimeter | Within groups | 1,100.104 | 9,900.937 | 33.594 | 0.00 |
| | Between groups | 32.747 | 6,876.870 | | |
| | Total | | 16,777.807 | | |
| The ratio of length to width | Within groups | 0.013 | 0.119 | 5.408 | 0.00 |
| | Between groups | 0.002 | 0.513 | | |
| | Total | | 0.632 | | |
| Stomata Density | Within groups | 2,325,002.852 | 20,930,000 | 885.020 | 0.00 |
| | Between groups | 2,627.062 | 551,682.967 | | |
| | Total | | 21,481,682.967 | | |

Table 2. The results of multiple comparisons of studied leaf stoma traits using the Duncan test

| Trait / Species | Area | Perimeter | Stomata Length | Stomata Width | The ratio of length to width | Stomata Density |
|---|---|---|---|---|---|---|
| *A. velutinum* | 290.5$^{b}$±88 | 63.5$^{cd}$±10.28 | 21.37$^{bc}$±3.4 | 18$^{bc}$±2/9 | 0.89$^{c}$±0.075 | 300$^{g}$±83 |
| *A.cappadocicum* | 330$^{b}$±22.7 | 65.9$^{bc}$±3.08 | 23.46$^{a}$±1.1 | 14.17$^{cd}$±1 | 0.88$^{c}$±0.058 | 322$^{g}$±30 |
| *A.platanoides* | 217$^{c}$±32.4 | 55.6$^{e}$4±4.3 | 20.19$^{cd}$±1.71 | 14.07$^{e}$±1.1 | 0.87$^{c}$±0.046 | 1,067$^{b}$±48 |
| *A.hyrcanum* | 369.7$^{a}$±51 | 70.08$^{a}$±5.15 | 23.55$^{a}$±1.87 | 20.7$^{a}$±1.7 | 0.94$^{ab}$±0.032 | 563$^{c}$±50 |
| *A.campestre* | 293.2$^{b}$±65 | 63.2$^{cd}$±6.8 | 22.02$^{b}$±2.65 | 17.5$^{cd}$±1.8 | 0.91$^{abcd}$±0.038 | 466$^{f}$±25 |
| *A.monsspesulanum* | 342$^{a}$±39.9 | 68.44$^{ab}$±4.33 | 24.07$^{a}$±1.7 | 19.03±1.8 | 0.92$^{abc}$±0.04 | 306$^{g}$±75 |
| *A.negundo variegatum* | 178.9$^{d}$±5 | 48.75$^{f}$±0.61 | 16.92$^{e}$±2.6 | 13.9±0.7 | 0.9$^{a}$±0.02 | 1,400$^{a}$±10 |
| *A.mazandaranicum* | 275.4$^{e}$±42 | 61.54$^{d}$±4.2 | 20.5b$^{cd}$±1.82 | 18$^{bc}$±1.4 | 0.9$^{bcd}$±0.03 | 700$^{c}$±10 |
| *A.palmatum* | 234$^{c}$±5.4 | 57.5$^{e}$±2.32 | 19.64$^{d}$±0.9 | 16.3$^{d}$±0.9 | 0.89$^{cd}$±0.057 | 606$^{d}$±8 |
| *A.negundo* | 140.7$^{e}$±17 | 44.72$^{g}$±2.71 | 16.03$^{e}$±1.5 | 11.8$^{f}$±0.6 | 0.88$^{c}$±0.05 | 600$^{d}$±15 |

Lowercase letters indicate a statistically significant difference in the 99% probability level.

difficult because they have a systematic value (Haron and Moore, 1996) and can isolate plant species of one genus (Baranova, 1992). We cannot only consider the type of stomata in mature leaves as a discriminant trait, but in many cases, we can use it as an indicator of the taxonomic similarity of plant species (Van Cotthem, 1970). In *A. negundo variegatum, A. negundo,* and *A. palmatum*, which are non-native species, due to planting at low forest altitude and having relatively suitable ecological conditions such as sufficient humidity and a temperate climate, the stomata are flat and above the epidermal cells (Mohtashamian et al., 2017). In contrast, epidermal cells of species that grow and spread at high altitudes in Hyrcanian forests, such as *A. campestre, A. hyrcanum, A. mazandaranicum,* and *A. monsspesulanom* are located above the stoma (Karimi et al., 2021). The low level of stomata in comparison to the epidermal cells, as well as its enclosure by a cuticular layer, seem to be a mechanism for adapting to the climate and varying quality of sunlight in the habitat, reducing evapotranspiration (Holmes and Keiller, 2002). Meanwhile, in *A. velutinum* species, with a wide distribution from the altitude (from the plane to an altitude of 2,000 m) and favorable moisture conditions, the stomata are located below the surface of the epidermis. Similar results regarding the

Table 3. The hidden roots of stoma traits in the first two components

| Traits | First component | Second component |
|---|---|---|
| A | 0.971 | 0/175 |
| P | 0.982 | –0/060 |
| SW | 0.890 | 0/282 |
| R | 0.020 | 0/977 |
| SL | 0.925 | –0/012 |
| SD | –0.656 | 0/218 |
| Special amount | 4.001 | 1/098 |
| Explanatory variance | 66.74 | 18/27 |
| Cumulative variance | 66.74 | 85/04 |

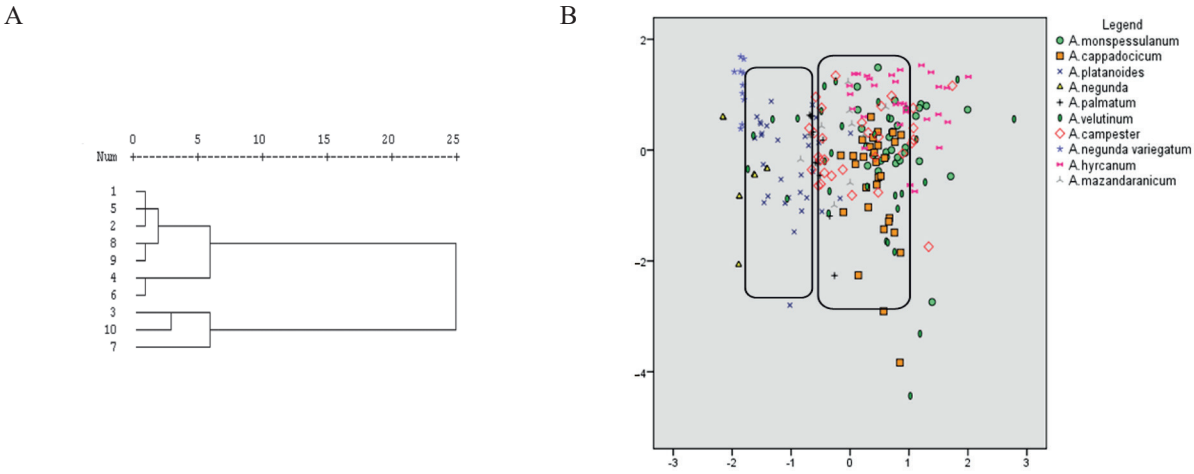


Fig. 7. A: Dendrogram obtained from cluster analysis based on the average of the studied stoma traits. 1 – *A. velutinum*, 2 – *A. cappadocicum*, 3– *A. platanoides*, 4 – *A. hyrcanum*, 5 – *A. campestre*, 6 – *A. monsspesulanum*, 7 – *A. negunda variegatum*, 8 – *A. mazandaranicum*, 9 – *A. palmatum*, 10 – *A. negunda*. B: The distribution diagram of studied trees in the coordinate axis space based on the first two components obtained from the analysis into principal components.

Table 4. The degree of dissimilarity resulting from cluster analysis based on stomatal traits

| Species | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.000 | 1.229 | 9.547 | 8.030 | 1.961 | 3.433 | 24.04 | 1/658 | 2/663 | 19/308 |
| 2 | | 0.000 | 11.229 | 8.573 | 2.276 | 3.252 | 30.69 | 4/013 | 5/606 | 25/167 |
| 3 | | | 0.000 | 24.877 | 9.448 | 19.361 | 11.16 | 6/256 | 3/167 | 7/751 |
| 4 | | | | 0.000 | 5.024 | 1.773 | 31.08 | 7/431 | 14/497 | 43/432 |
| 5 | | | | | 0.000 | 2.096 | 19.05 | 1/119 | 2/889 | 20/194 |
| 6 | | | | | | 0.000 | 31.13 | 5/175 | 9/751 | 34/894 |
| 7 | | | | | | | 0.000 | 15/020 | 12/474 | 12/172 |
| 8 | | | | | | | | 0/000 | 1/492 | 16/888 |
| 9 | | | | | | | | | 0/000 | 8/697 |
| 10 | | | | | | | | | | 0/000 |

1 – *A. velutinum*, 2 – *A. cappadocicum*, 3 – *A. platanoides*, 4 – *A. hyrcanum*, 5 – *A. campestre*, 6 – *A. monsspesulanum*, 7 – *A. negunda variegatum*, 8 – *A. mazandaranicum*, 9 – *A. palmatum*, 10 – *A. negunda*.

Table 5. Standardized coefficients of discriminant analysis based on stomatal traits

| Trait | First function | Second function | Third function | Fourth function | Fifth function |
|---|---|---|---|---|---|
| SD | 0.926* | 0.056 | –0.279 | 0.012 | 0.248 |
| SW | –0.130 | 0.713 | –0.421 | –0.267 | – 0.295 |
| A | –0.122 | 0.658* | –0.155 | –0.639 | – 0.347 |
| P | –0.124 | 0.627* | 0.021 | –0.466 | – 0.611 |
| SL | –0.104 | 0.523 | 0.256 | –0.748* | – 0.302 |
| R | 0.000 | 0.218 | –0.332 | –0.169 | 0.902* |
| Special amount | 44.155 | 1.786 | 0.901 | 0.169 | 0.052 |
| Explanatory variance | 93.8 | 3.8 | 1.9 | 0.4 | 0.1 |
| Cumulative variance | 93.8 | 97.6 | 99.5 | 99.9 | 100 |

*: Absolute value of maximum variance.

position of stomata relative to the cells of Linden leaf epidermal cells (*Tilla* spp.) at different habitat heights have been found (Yosefzadeh et al., 2010). Due to the close relationship with habitat characteristics, the size and density of the stomata are considerable at the genus, species, and varieties with different ecological ranges (Luo and Zhu 2001; Karimi, 2021). Since the increase in density in the species that are distributed at higher altitudes may be due to the increase in carbon dioxide efficiency (McElwain, 2004) or the plants strategy in water conservation (Schoettle and Rochelle, 2000), therefore, smaller stomata with higher density in *A. platanoides* which is distributed in the high altitudes in Hyrcanian forests are not far from expected (Salehi et al., 2016). The stomatal density in the subspecies *A. negundo variegatum*, is high due to planting in open forest and urban areas, direct light and ultraviolet rays, as well as a lack of humidity, compared to the species that are spread in dense and humid habitats (Cobanoglo et al., 2019). Also in this research, it was determined that stomatal density has a certain numerical range, so the distribution of species in different ecological conditions causes a change in the density range (Harrison et al., 2020). Therefore genetic factors may also influence its occurrence. *A. platanoides, A. negundo* and *A. negundo variegatum* based on the results of cluster analysis related to stomatal characteristics, were placed in one cluster, and the rest in a separate cluster. In terms of stomatal characteristics, the least similarity is between *A. negundo* and *A. hyrcanum* and the most similarity is between *A. campestre* and *A. mazandaranicum*. Discriminant function analysis is a way to separate variables into separate groups. In fact discriminant analysis is the diagnosis of an equation that predicts which group it belongs to by having the characteristics of each person from the society. This analysis was used to check the degree of similarity of stomatal traits based on the type of species. According to the results, the variation of stomatal density was found to be the most important factor in differentiation between species in this genus. However, we do not confirm the segregation of maple species in Hyrcanian forests only through stomata.

## Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Table 6. Grouping results of discriminant analysis based on stoma traits

| Species | Total | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | Total (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 30 | 23 | 0 | 0 | 0 | 2 | 5 | 0 | 0 | 0 | 0 | 76.4 |
| 2 | 30 | 2 | 24 | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 0 | 80 |
| 3 | 30 | 0 | 0 | 30 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100 |
| 4 | 30 | 0 | 0 | 0 | 28 | 1 | 0 | 0 | 0 | 1 | 0 | 93.3 |
| 5 | 30 | 0 | 0 | 0 | 2 | 28 | 0 | 0 | 0 | 0 | 0 | 93.3 |
| 6 | 30 | 1 | 4 | 0 | 0 | 3 | 22 | 0 | 0 | 0 | 0 | 73.3 |
| 7 | 10 | 0 | 0 | 0 | 0 | 0 | 0 | 10 | 0 | 0 | 0 | 100 |
| 8 | 10 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 9 | 1 | 0 | 90 |
| 9 | 10 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 9 | 0 | 90 |
| 10 | 10 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10 | 100 |
| Validity of the grouping | | | | | | | | | | | | 79.8 |

1 – *A. velutinum*, 2 – *A. cappadocicum*, 3 – *A. platanoides*, 4 – *A. hyrcanum*, 5 – *A. campestre*, 6 – *A. monsspesulanum*, 7 – *A. negunda variegatum*, 8 – *A. mazandaranicum*, 9 – *A. palmatum*, 10 – *A. negunda*.

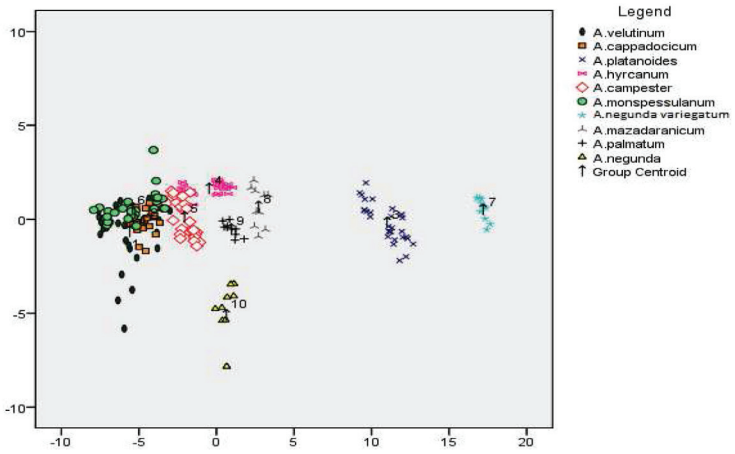


Fig. 8. Distribution of trees in the axis space of the discriminant function


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