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Morphological and anatomical study of the genus *Hedera* in Iran

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Abstract

Hedera is a genus of evergreen climbers distributed throughout Europe, North Africa, Macaronesia, and Asia. In this study, the morphological and anatomical structures of 11 populations from two species of *Hedera* (*H. helix* and *H. pastuchovii*) have been considered to evaluate the relationships in this genus. In total, 19 quantitative and eight qualitative morphological features were evaluated and measured. Ward's dendrogram showed two main clusters. The first cluster is composed of populations of *H. helix* and the second cluster is composed of two subclusters containing populations of *H. pastuchovii*. Both species showed a bifacial leaf anatomical structure, so the mesophyll is differentiated into palisade and spongy tissues. Petiole cross sections showed rounded shape with wavy margin. Pedicel cross sections are similar in the general shape but the margins are different between two species. Both are rounded shape and the margin is quite wavy in *H. helix* and straight in *H. pastuchovii*. Number of vascular bundles of pedicel is different between two species. Two types of covering trichomes were identified including stellate trichomes in *H. helix* and scale-like trichomes in *H. pastuchovii*. Studied taxa were clearly separated by selected morphological and anatomical characters and these data provide additional evidence for species relationships between them.

Keywords: *Araliaceae*, evergreen, leaf, petiole, trichome

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خلاصه

Hedera L. (عشقه‌ایان) جنسی از بالاروندگان همیشه سبز است که در اروپا، آفریقای شمالی، ماکارونزی و آسیا پراکندگی دارد. در این مطالعه، ساختار ریخت‌شناختی و تشریحی ۱۱ جمعیت از دو گونه *Hedera* (*H. helix* و *H. pastuchovii*) مورد بررسی قرار گرفتند و روابط آن‌ها نیز در این جنس ارزیابی گردید. در مجموع، ۱۹ ویژگی کمی و هشت ویژگی کیفی ریخت‌شناختی ارزیابی و اندازه‌گیری شدند. دندروگرام ward دو خوشه اصلی را نشان داد. خوشه نخست متشکل از جمعیت‌های *H. helix* و خوشه دوم از دو زیرمجموعه تشکیل شده و شامل جمعیت‌های *H. pastuchovii* است. هر دو گونه یک ساختار تشریحی دووجهی را نشان می‌دهند، بنابراین مزوفیل با بافت‌های نردبانی و اسفنجی متمایز می‌شود. از سوی دیگر، سطح مقطع دمبرگ گرد با حاشیه موج‌دار است. شکل کلی سطح مقطع در دمگل مشابه اما حاشیه‌ها در هر دو گونه با هم متفاوت بوده، به طوری که این حالت در هر دو گونه گرد ولی حاشیه در *H. helix* تقریبا موج‌دار و در *H. pastuchovii* صاف می‌باشد. تعداد دستجات آوندی در دمگل بین دو گونه نیز متفاوت است. دو نوع کرک شامل کرک‌های ستاره‌ای در *H. helix* و کرک فلسی در *H. pastuchovii* مشخص شد. گونه‌های مورد مطالعه به وضوح توسط ویژگی‌های انتخابی ریختی و تشریحی از هم جدا شده و این داده‌ها شواهد زیادی برای روابط بین این دو گونه فراهم می‌کند.

واژه‌های کلیدی: برگ، دمبرگ، عشقه‌ایان، کرک، همیشه سبز

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Introduction

Hedera L. (*Araliaceae*) is a genus of evergreen climbers distributed throughout Europe (Stace 1997), North Africa (Rutherford *et al.* 1993), Macaronesia (Rutherford *et al.* 1993), and Asia (Tobler 1912). This genus is a considerable element in European and Asian woodlands, comprising a large component of the forest understory. *Hedera* is a liana with adventives fixing roots with two stem types: fertile and sterile. Leaves are alternate, dimorph, palmate-lobed at the sterile stems and ovate with smooth margins at the fertile stems. In all organs of plant, there are secretive ducts (Fahn 1979). This genus has certain anatomical features depending on the growing conditions, such as the type cells of the palisade tissue or secretive ducts (Esau 1965). The ivy is ornamental and medicinal plant and sometimes it is an invasive species.

This genus is well-established morphologically (Seeman 1868, Eyde & Tseng 1971), but the delimitation of species and recognition of taxa are still being discussed. Between nine and 15 species have been recognized in the last treatments (Pojarkova 1951, McAllister 1982, 1988, McAllister & Rutherford 1983, 1990, Rutherford 1984, 1989, Rutherford *et al.* 1993, Rose 1996, Ackerfield 2001, Ackerfield & Wen 2002). Despite15 species in the world, *Hedera* includes three species in the Flora Iranica region (Browicz 1973). The general lineage concept (de Queiroz 1998, 2007) was assumed using morphological diagnostic capability as the main operational criterion for evaluating species. The use of quantitative morphological characters in taxonomy is traditionally eventual on the existence of discrete diagnostic characters. When plant species are the result of recent divergence and gene flow and/or hybridization occur, the use of morphological characters may help in species identification and delimitation. Trichome (Seemann 1868, Hibberd 1893, Tobler 1912, Lawrence & Schultze1942, Mcallister 1981, Rose 1996) and juvenile leaf (Rutherford *et al.* 1993, Ackerfield & Wen 2002, Valcárcel 2008, Valcárcel & Vargas 2010) are important morphology characters in *Hedera*. Trichome morphology has been widely used to delimit taxa. Species of *Hedera* fall into two groups: those with stellate trichomes, and those with scale-like trichomes (Mcallister 1981, Ackerfield 2001). Juvenile leaf morphology from entire to 3–7-lobed may help in species identification and evaluation (Valcárcel & Vargas 2010).

Due to the relative stability of some anatomical traits related to environmental factors, the anatomical results are important in the systematic of *Hedera*. The anatomical structure of some *Hedera* species shows that, anatomical features can be valuable in species delimitation especially about similar taxa. Taxonomic value of epidermis anatomical features is well documented in botanical literature (Dilcher 1974, Metcalf 1985). The data on anatomy of the genus *Hedera* is relatively poor and only few works are available. Savulescu & Luchian (2009) studied comparative anatomy of the vegetative organs of the *H. helix* and found that, the leaves are hypostomatic type with tectorial hairs and the mesophyll is bifacial. Into the mesophyll are vascular bundles of collateral type with the xylem outside and phloem inside. Crystals of calcium oxalate are in some cells of mesophyll and secretive ducts are into the median nervure. Konarska (2014) investigated the flower nectaries of *H. helix* and found that, the gland is made up of single-layered epidermis covered with a massive cuticle and several layers of secretory parenchyma composed of tiny, compactly arranged cells containing chloroplasts and druses. Savulescu & Luchian (2009) studied the diagnostic value of *Hedera* epidermis and illustrated that epidermis is made up of only one cells layer with polygonal cells with thin lateral walls and corrugated. Amini *et al.* (2020) surveyed micro-morphology of epidermis and show that, there were two types of epidermal cells: puzzle-shaped cells and polygonal cells.

Despite its ecological impacts and economic importance, however, the taxonomy and relationships of the species of *Hedera* are unclear. This study examines the patterns of morphological variation among *Hedera* taxa by emphasizing juvenile phase of plant morphology,

leaf anatomy and trichome characters. In addition, *Hedera* species are not efficiently separated due to morphological similarities. Species relationship based on anatomical structure in *Hedera* species is evaluated for the first time in Iran. The specific goals of this study were as follows: (1) to examine morphological characters that could be useful to separate taxa, (2) to assess the value of anatomical characters and trichome morphology in two species of *Hedera* in Iran, and (3) to evaluate the affinities and relationships of taxa.

Materials and Methods

- Morphological methods

In the present study, 11 populations of two *Hedera* species were collected from different locations in Iran and preserved in the Gonbad Kavous University Herbarium (Iran). Identification of populations was carried out on the basis of Flora Iranica (Browicz 1973). A list of voucher specimens of the corresponding species is given in table 1.

Nineteen quantitative and eight qualitative morphological features were evaluated and measured (Table 2). These features were internode length, petiole length, blade length, blade width, sepal length, sepal width, petal length, petal width, anther length, anther width, stamen length, style length, fruit length, fruit width, seed number, seed length, seed width, peduncle length, pedicel length, leaf form, leaf base, trichome type, trichome color, petal color, seed form, seed color, fruit color.

To reveal the species relationships, Cluster Analysis (CA) and Principal Component Analysis (PCA) were applied (Ingrouille 1986). The average taxonomic distances and squared Euclidean distances were used as dissimilarity coefficient in the cluster analysis of morphological data. In order to determine the most variable morphological characters between the studied species, factor analysis based on principal components analysis was performed by PAST software Ver. 2.17 (Hammer *et al.* 2001).

- Anatomical methods

Fresh leaf materials of the second or third nodes of the stem were fixed in the field with formalin-acetic acid-alcohol (FAA). Four cross-sections were measured for each sample to assess the consistency of anatomical characters. All materials were boiled for 15 minutes and then fixed in Carnoy solution (alcohols to acetic acid in proportion 3:1). Handmade cross-sections were obtained from leaf blades, petioles and pedicels using commercial razor blades. The cross-sections were obtained using carmine and methylene green double staining method. Subsequently, the materials were washed in distilled water (1 minute) and dehydrated through an ethyl alcohol (70%) and were mounted on microscopic glass slides. Slide sections were studied and photographed.

Results

- Macro-morphological study

Our observations indicated that, the general shape of the plant is somehow similar in the species studied. Both species are evergreen climbing or ground-creeping woody plants with heterophylly leaves having alternate phyllotaxy. Main differences between these two species are in leaf shape and size (particularly of the juvenile leaves), the structure of the leaf trichomes and the color of the flowers and fruit. This climbing plant presents heteroblasty due to two different growth phases, the vegetative and reproductive phases. Heteroblasty in *Hedera* is apparent in leaf morphology; the leaves are generally lobate in the vegetative phase but entire in the reproductive phase. The typical lanceolate leaves with rounded and rarely oblong-elliptic lobes and cordate or cuneate at the base that characterize the vegetative phase in *H. pastuchovii* Woron. ex Grossh. are completely different from the typical heart-shaped leaves of *H. helix*, with the base of cordate and palmately five-lobed on stem. Two species are covered with diverse hairs. *Hedera helix* had more dense hair cover (stellate) and *H. pastuchovii* had scale-like trichomes. The flowers are greenish-yellow with five small petals and five sepals. The fruit color is another difference in these species

which in *H. helix* is black while *H. pastuchovii* shows brown color.

In order to define the diagnostic value of morphological characters in species delimitations in studied *Hedera* species, cluster analysis by Ward's method was performed on the base of 27 qualitative and quantitative features (Fig. 1). Ward's dendrogram showed two main clusters (Fig. 1). First cluster composed of populations of *H. helix*. Second cluster composed of two subclusters and contained populations of *H. pastuchovii*. Principal component analysis revealed that, there were two components providing more than 76% of total observed variation in studied morphological characters. Studying the component loading was evident that fruit color, trichome color and leaf form are most important features in the first factor and petal color, seed form and trichome type are most significant in the second factor. PCA ordination based on studied morphological characters separated species in to distinct groups (Fig. 2). PCA confirmed the results of cluster analysis by Ward's method based on qualitative and quantitative features of two studied species (Fig. 2).

Table 1. List of the Iranian species of *Hedera* used in the present study along with their localities and vouchers preserved in the Gonbad Kavous University Herbarium (Gonbad Kavous, Iran)

Taxa	Collection data
H. helix L. (cultivated)	Tehran prov.: Research Institute of Forests and Rangelands, 1250 m, Haji Moradkhani (803298)
H. helix (wild)	Golestan prov.: Gonbad Kavous, 900 m, Haji Moradkhani (803297)
H. helix (wild)	Fars prov.: Shiraz, Kamfiruz, Tang-e Bostanak, 1500 m, Haji Moradkhani (803299)
H. pastuchovii Woron. ex Grossh.	Golestan prov.: Gorgan, Naharkhoran forest, 1000 m, Haji Moradkhani (803270)
H. pastuchovii	Golestan prov.: Gorgan, Ziarat village, 950 m, Haji Moradkhani (803273)
H. pastuchovii	Gilan prov.: Gisum forest, 800 m, Haji Moradkhani (803289)
H. pastuchovii	Gilan prov.: Fuman forest, Mirmahaleh, 950 m, Haji Moradkhani (803292)
H. pastuchovii	Gilan prov.: Somee Sara, 1000 m, Haji Moradkhani (803290)
H. pastuchovii	Mazandaran prov.: Nur forest, 1050 m, Haji Moradkhani (803279)
H. pastuchovii	Mazandaran prov.: Najardeh, 980 m, Haji Moradkhani (803278)
H. pastuchovii	Mazandaran prov.: Sari, 800 m, Haji Moradkhani (803295)

Table 2. Quantitative and qualitative morphological characters in *Hedera* species

No.	Character	No.	Character
1	Internode length (mm)	15	Seed number (mm)
2	Petiole length (mm)	16	Sed length (mm)
3	Blade length (mm)	17	Seed width (mm)
4	Blade width (mm)	18	Peduncle length (mm)
5	Sepal length (mm)	19	Pedicel length (mm)
6	Sepal width (mm)	20	Leaf form (heart-shaped 1; lanceolate 2)
7	Petal length (mm)	21	Leaf base(cuneate 1; cordate 2)
8	Petal width (mm)	22	Trichome type (scale-like 1; stellate 2)
9	Fruit length (mm)	23	Trichome color (white 1; grey 2)
10	Fruit width (mm)	24	Petal color (pale green 1; green-yellow 2)
11	Anther length (mm)	25	Seed form (ovate 1; circular 2)
12	Anther width (mm)	26	Seed color (black 1; brown 2)
13	Stamen length (mm)	27	Fruit color (black 1; brown 2)
14	Style length (mm)	-	-

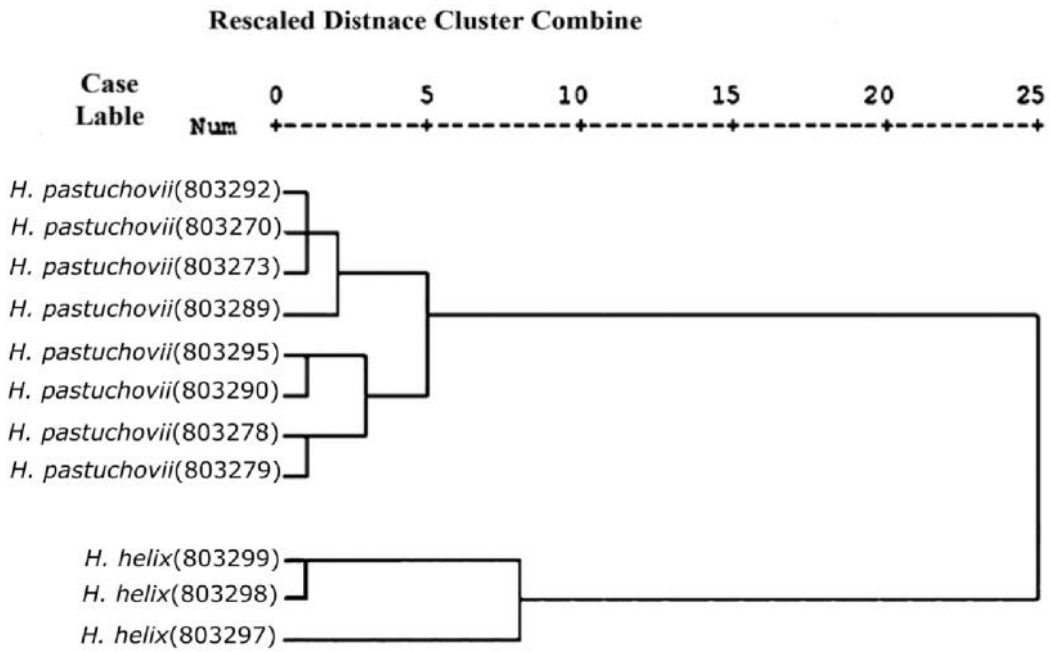

Fig. 1. Cluster analysis (Ward's method) based on the morphological features of *Hedera.*

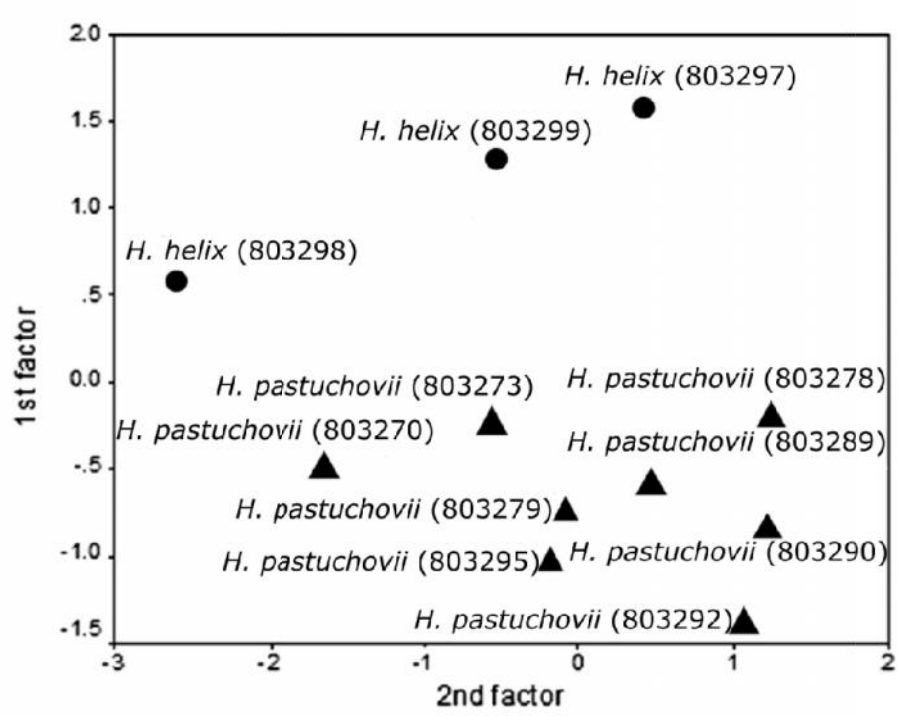

Fig. 2. PCA plot of *Hedera* species based on the studied morphological data.

- Anatomical study

Selected LM micrographs of cross-sections of leaf blades, petioles and pedicels as well as the type of indumentums are presented in figures 3–6. Most characters show significant variability between two species but were constant among different specimens of each species studied. Some of the most important anatomical characters of leaves, petioles and pedicels in these species are as follows: transverse section shape, type of mesophyll (dorsi-ventral vs. isobilateral), thickness of collenchyma and sclerenchyma, collenchyma and sclerenchyma layer number, thickness of phloem and xylem**,** thickness of cuticle, number of secretary canal, spongy parenchyma layer number, Palisade parenchyma layer number, number of vascular bundles, thickness of pith, density and distribution of druses, trichome type.

- Leaf section

Cross sections of the midrib and leaf blade presented the following features (Fig. 3): the upper and lower epidermis consist of a single cell layer with no significant difference in size. The outline of the leaf blades was homogeneously linear. Both species showed a bifacial leaf anatomical structure, so the mesophyll is differentiated into palisade and spongy tissues. In general, the cross-sections are shown according to their plane of dorsi-ventral symmetry: bifacial which is characterized by palisade parenchyma arranged in 2–3 layers on the upper surface (or ventral side) and the lower mesophyll filled by spongy parenchyma (Fig. 3D). The spongy parenchyma consists of 6–8 layers of irregular cells in most populations. The shape of vascular bundles varies considerably between two species of *Hedera* studied here from circular (*H. helix*, Fig. 3A) through continuous arc median bundle often with curved ends (*H. pastuchovii*, Fig. 3C). Two species possess inorganic deposits in their mesophyll most likely of calcium oxalate compounds. Druse crystals were mainly distributed in the mesophyll and around the vascular bundles of the leaf mostly in high density. They were densely distributed in the mesophyll of *H. pastuchovii* (Fig. 3H), but sparsely in *H. helix* (Fig. 3A).

Table 3. Midrib characteristics of *Hedera* species

Taxon	MBS	Ms	Cln	Sln	Xth	Pth	Ppln	Spln	Dd	Trt
H. helix	Circular	Bifacial	4	4	22.66±4.3	14.3±2.2	3	6	Sparse	Stellate
H. helix	Circular	Bifacial	4	3	21.34±1.8	14.3±2.1	2	6	Sparse	Stellate
H. helix	Circular	Bifacial	4	4	19.36±2.2	14.1±2.2	3	6	Sparse	Stellate
H. pastuchovii	Open U-shaped	Bifacial	6	5	41.56±2.3	23.3±4.2	3	8	Dense	Scale-like
H. pastuchovii	Open U-shaped	Bifacial	7	6	38.32±3.3	19.7±1.2	3	8	Dense	Scale-like
H. pastuchovii	Open U-shaped	Bifacial	6	6	40.26±1.3	25.6±1.2	2	8	Dense	Scale-like
H. pastuchovii	Open U-shaped	Bifacial	7	5	42.36±2.2	25.3±2.7	3	8	Dense	Scale-like
H. pastuchovii	Open U-shaped	Bifacial	6	6	50.43±5.3	21.2±1.2	3	7	Dense	Scale-like
H. pastuchovii	Open U-shaped	Bifacial	7	6	48.66±4.3	26.4±3.4	2	7	Dense	Scale-like
H. pastuchovii	Open U-shaped	Bifacial	6	6	47.53±6.3	24.3±2.2	3	8	Dense	Scale-like
H. pastuchovii	Open U-shaped	Bifacial	6	5	44.86±5.3	18.5±4.5	3	8	Dense	Scale-like

MBS = Median bundle shape; **Ms** = Mesophyll symmetry; **Cln** = Collenchyma layer number; **Sln** = Sclerenchyma layer number; **Xth** = Xylem thickness; **Pth** = Phloem thickness; **Ppln** = Palisade parenchyma layer number; **Spln** = Spongy parenchyma layer number; **Dd** = Druses density; **Trt** = Trichome type.

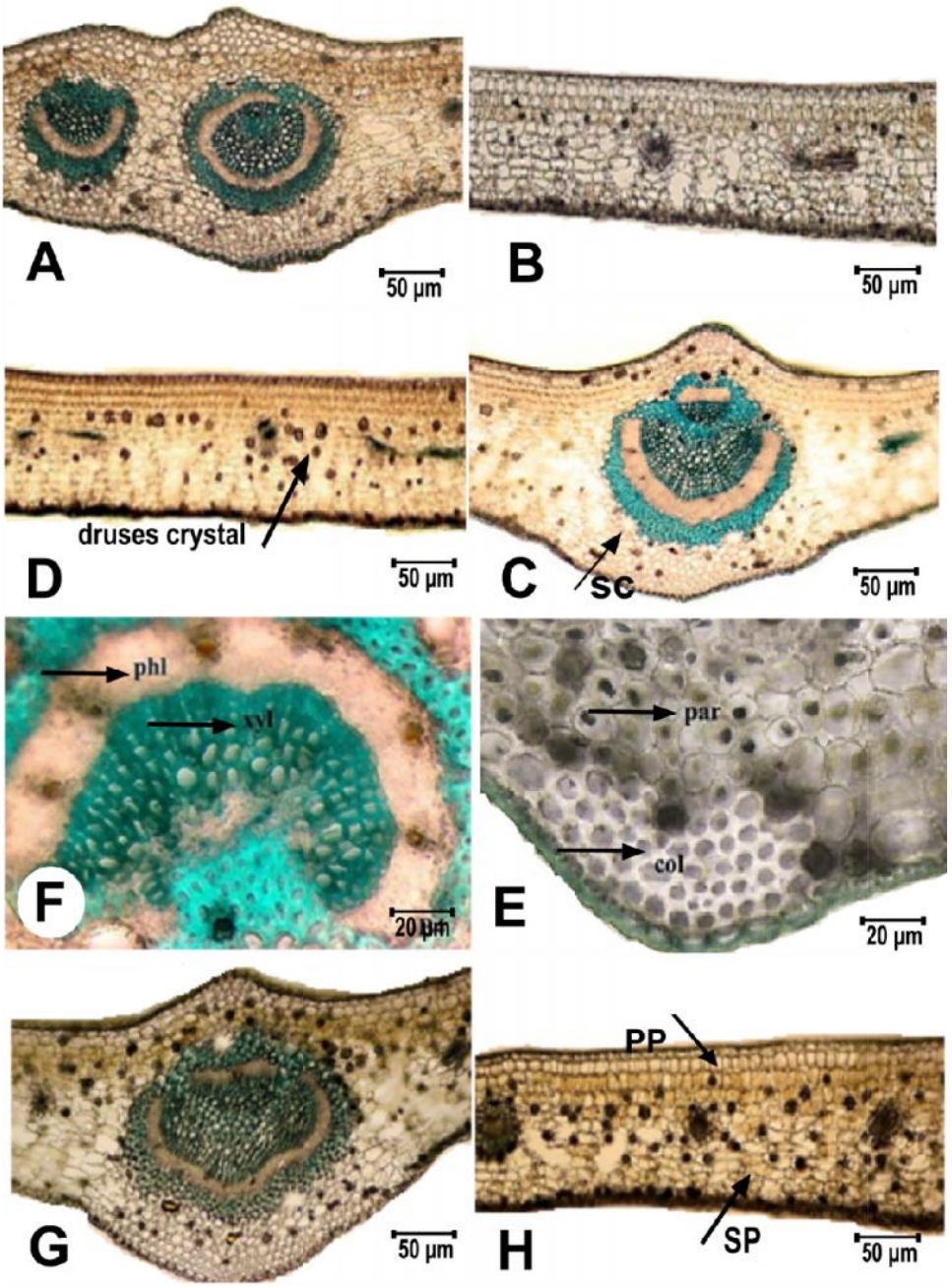

Fig. 3. Transverse sections of midrib in *Hedera*: A-B. *H. helix*, C-D. *H. pastuchovii*, E-F. *H. pastuchovii*, G-H. *H. pastuchovii*.

- Petiole section

Petiole cross sections showed rounded shape with wavy margin (Fig. 4). The epidermis is composed of single layered cells. The collenchyma tissue is located under the epidermis of the stem. They are 4–5-layered in both species. The cortex tissue is composed of 4–5-layered parenchyma cells. A ring of sclerenchyma is present in both studied species. Vascular bundles are arranged in a single circle. Shape and number of vascular bundles is different. Number of vascular bundles is 5–7 in two sizes with U-shaped outline. There are five vascular bundles in *H. pastuchovii* (Fig. 4C) and seven in *H. helix* (Fig. 4A). The xylem is surrounded by sclerenchymatous cells. Parenchyma cells in *H. helix*, comprises of the 9–2 secretary canals (Fig. 4A) but is 6–8 in *H. pastuchovii* (Fig. 4C). Pith thickness in *H. helix* (55 µm) is smaller than *H. pastuchovii* (123 µm). Druse crystals were mainly distributed in the mesophyll and were densely distributed in *H. pastuchovii* (Fig. 4C), but sparsely in *H. helix* (Fig. 4A).

- Pedicel section

Pedicel cross sections of both species of *Hedera* are studied. These two species are similar to each other in the general shape of cross section but the margins are different between two species. Both are rounded shape and the margin is quite wavy in *H. helix* (Fig. 5A) and straight in *H. pastuchovii* (Fig. 5E). Sclerenchyma tissue thickness in ranging from 6.5 µm in *H. helix* to 19.2 µm in *H. pastuchovii*. Sclerenchyma tissue thickness in *H. pastuchovii* is 19.2 µm and that is the maximum sclerenchyma thickness (Fig. 5D). Vascular bundles are arranged in a single circle. Number of vascular bundles is different. Vascular bundles in *H. helix* are 12 (Fig. 5A) and in *H. pastuchovii* are 24 (Fig. 5C). Pith thickness in *H. helix* (95 µm) is smaller than *H. pastuchovii* (153 µm).

Table 4. Petiole characteristics of *Hedera* species

Taxon	MBS	Cln	Sln	Xth	Pth	Vbn	Scn	Pt	Dd	Trt
H. helix	Open U-shaped	4	2	15.2±2.5	6.3±2.1	5	11	55±5	Sparse	Stellate
H. helix	Open U-shaped	4	2	18.2±3.3	5.5±2.2	5	9	58±3	Sparse	Stellate
H. helix	Open U-shaped	4	2	14.4±3.2	7.3±1.5	5	12	54±2	Sparse	Stellate
H. pastuchovii	Open U-shaped	5	5	39.5±4.4	17.3±2.2	7	8	124±12	Dense	Scale-like
H. pastuchovii	Open U-shaped	5	4	43.2±3.5	18.3±2.2	7	7	114±10	Dense	Scale-like
H. pastuchovii	Open U-shaped	5	4	36.3±2.2	15.6±4.1	7	8	123±9	Dense	Scale-like
H. pastuchovii	Open U-shaped	4	4	41.5±2.3	18.2±2.2	7	6	118±11	Dense	Scale-like
H. pastuchovii	Open U-shaped	4	5	39.3±4.2	15.1±2.4	7	6	116±14	Dense	Scale-like
H. pastuchovii	Open U-shaped	5	5	43.1±5.3	17.1±2.3	7	7	121±16	Dense	Scale-like
H. pastuchovii	Open U-shaped	5	4	41.2±3.6	16.5±2.5	7	8	117±13	Dense	Scale-like
H. pastuchovii	Open U-shaped	5	5	36.2±2.4	15.3±2.6	7	8	122±11	Dense	Scale-like

MBS = Median bundle shape; **Cln** = Collenchyma layer number; **Sln** = Sclerenchyma layer number; **Xth** = Xylem thickness; **Pth** = Phloem thickness; **Vbn** = Vascular bundles number; **Scn** = Secretary canal number; **Pt** = Pith thickness; **Dd** = Druses density; **Trt** = Trichome type.

Table 5. Pedicel characteristics of *Hedera* species

Taxon	MBS	Sth	Cln	Xth	Pth	Pt	Vbn	Scn	Trt
H. helix	Open V-shaped	6.9±0.4	6	25±3	17±2	95±11	12	24	Stellate
H. helix	Open V-shaped	8.2±1.5	7	27±3	18±3	93±7	14	22	Stellate
H. helix	Open V-shaped	6.5±0.7	6	24±2	17±4	95±1	12	22	Stellate
H. pastuchovii	Triangular	18.3±0.5	4	35±3	25±3	153±2	24	32	Scale-like
H. pastuchovii	Triangular	16.2±0.7	5	38±2	24±2	150±3	23	33	Scale-like
H. pastuchovii	Triangular	14.7±0.5	4	34±3	27±3	151±4	24	29	Scale-like
H. pastuchovii	Triangular	19.3±0.9	4	33±2	26±2	149±3	24	31	Scale-like
H. pastuchovii	Triangular	17.2±0.5	5	35±3	27±3	148±2	22	32	Scale-like
H. pastuchovii	Triangular	19.9±0.7	4	39±3	24±2	153±3	23	33	Scale-like
H. pastuchovii	Triangular	18.5±0.5	4	35±4	28±3	151±1	22	35	Scale-like

MBS = Median bundle shape; **Sth** = Sclerenchyma thickness; **Cln** = Collenchyma layer number; **Xth** = Xylem thickness; **Pth** = Phloem thickness; **Pt** = Pith thickness; **Vbn** = Vascular bundles number; **Scn** = Secretary canal number; **Trt** = Trichome type.

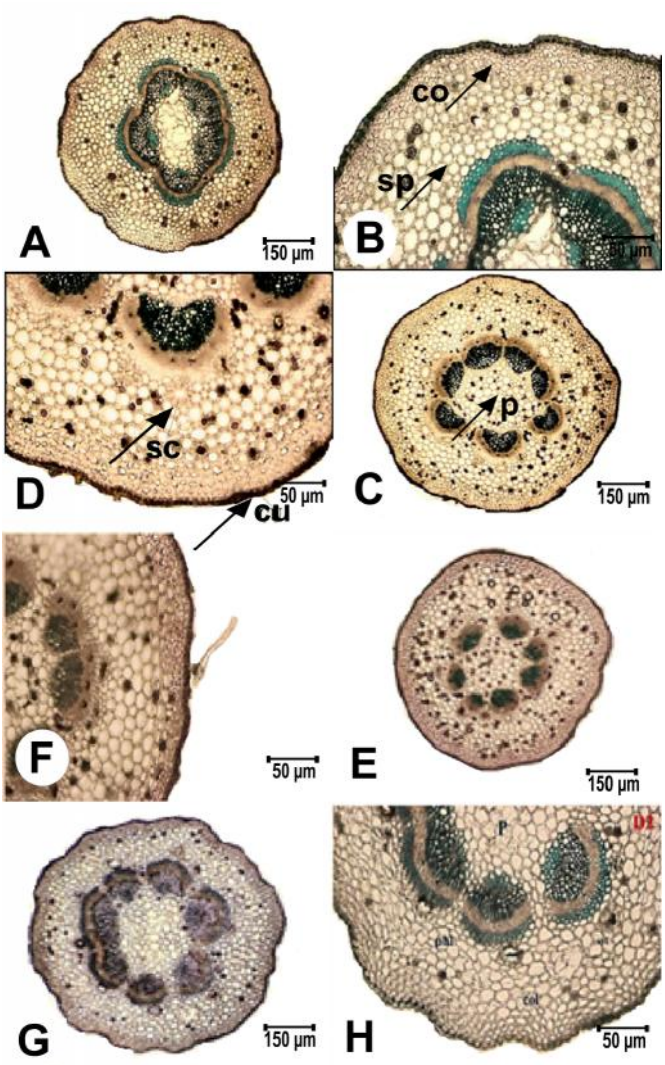

Fig. 4. Transverse sections of petiole in *Hedera*: A-B. *H. helix*, C-D. *H. pastuchovii*, E-F. *H. pastuchovii*, G-H. *H. pastuchovii*.

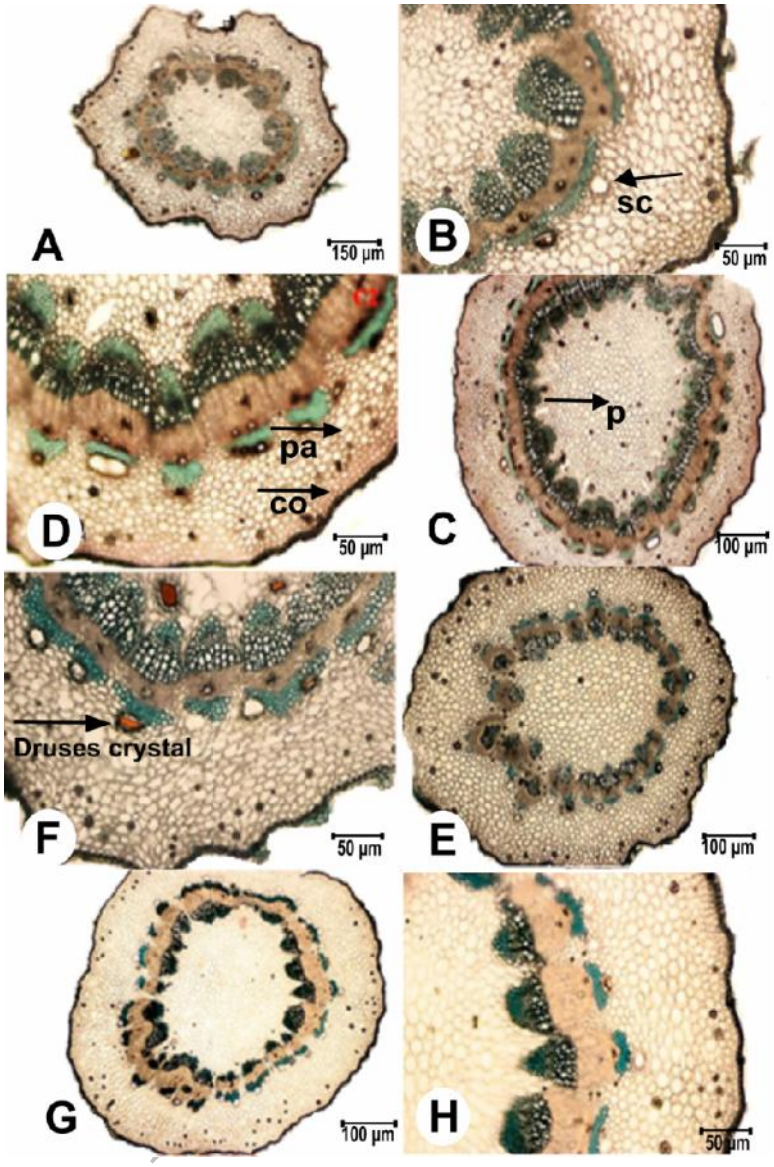

Fig. 5. Transverse sections of pedicel in *Hedera*: A-B. *H. helix*, C-D. *H. pastuchovii*, E-F. *H. pastuchovii*, G-H. *H. pastuchovii*.

- Trichomes

Trichomes were studied at light microscopy level. Two types of covering trichomes were identified:

1. Stellate trichomes

Unicellular and adpressed to the leaf surface, this type was seen on the adaxial and abaxial leaf side of *H. helix*, as well as on the petiole and pedicle of all examined population (Fig. 6D).

2. Scale-like trichomes

With a multicellular base, this trichome was branchy slightly higher than the stalk. It was observed on the abaxial and adaxial leaf side as well as on the petiole and pedicle of *H. pastuchovii* (Fig. 6A-C).

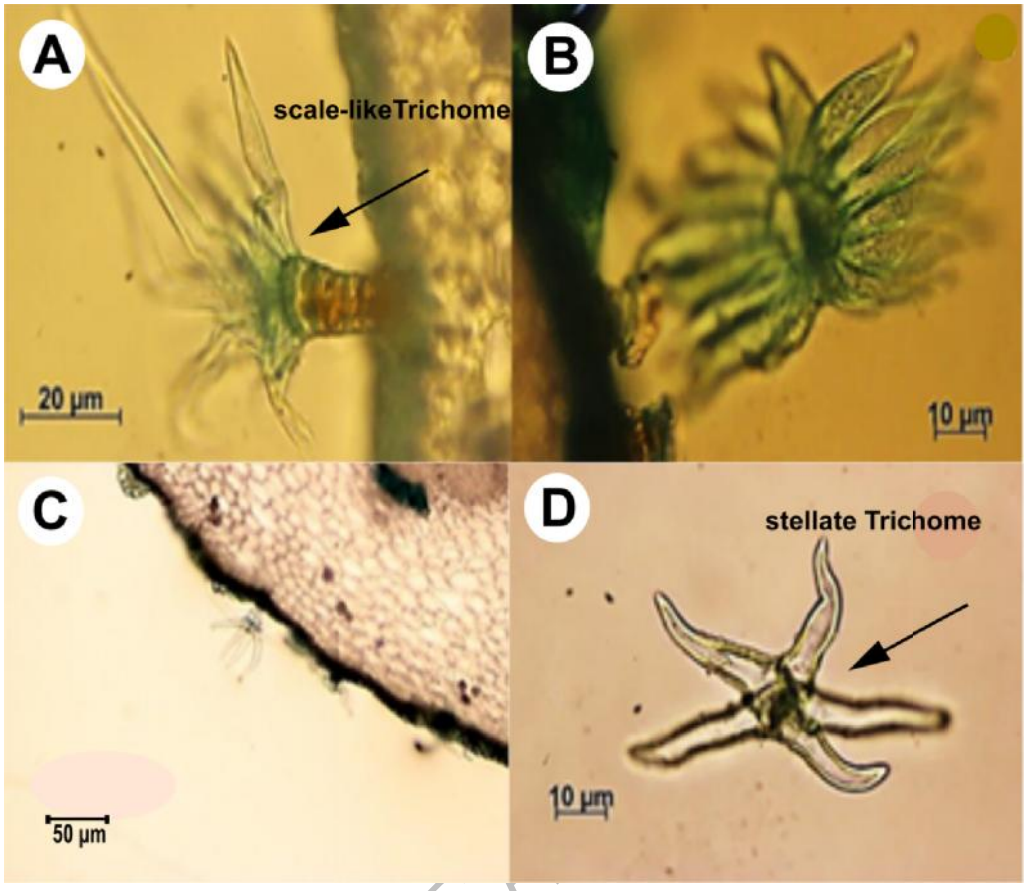

Fig. 6. Trichome types in *Hedera*: A-C. *H. pastuchovii*, D. *H. helix.*

Discussion

Hedera has gained little attention in previous morphological and anatomical studies; hence, this study presents the first comprehensive investigation of this genus in Iran. The distinguished morphological characters support the existence of two different species which are in agreement with earlier molecular studies (two distant lineages, Vargas *et al.* 1999, Ackerfield & Wen 2003, Valcárcel *et al.* 2003, Amini *et al.* 2020) and previous cytogenetic results (two ploidy levels; Vargas *et al.* 1999). The two species of *Hedera* which were studied here are mainly in agreement with McAllister & Rutherford's proposal. Furthermore, Valcárcel and Vargas demonstrated that, the leaf features primarily is diagnostic character, as lobate leaves from the vegetative phase and entire in the reproductive phase in two species of *Hedera* studied. Kost *et al.* (2003) revealed that, length of leaf, petiole and internode are the most vital characters for determination of *H. colchica* and *H. helix* from Russia and Ukraine which is in disagreement with our morphometric analyses.

In the present study, anatomical results is in line with Savulescu & Luchian's findings that, there are not any structural differences between the fertile and sterile leaves , the mesophyll is bifacial and vascular bundles are collateral with the external xylem and internal phloem.

In these two species, dense druse crystals were mainly distributed in the mesophyll (Konarska 2014), but detected near the epidermis scarcely. These species grow at an altitude between 800 and 1500 m. However, it is known that density of druse crystals is an environmentally influenced anatomical character and we cannot use it as a strong taxonomic character for grouping species (Kilic 2009).

Similar to Valcárcel & Vargas (2010), our findings provide statistical support for the identification of two species as well as the two major groups traditionally recognized in *Hedera* based on trichomes: the stellate group (*H. helix*) and the scale-like group (*H. pastuchovii*). The trichome future could be affected by such factors as drought and UV irradiation (Agrawal & Fishbein 2006). The investigations of trichomes in this study have shown that, scale-like and stellate covering trichomes with different density were observed on the abaxial and adaxial leaf side of all examined species. In the current research, dense trichomes were observed in *H. helix* and thricomes with low density were distributed in *H. pastuchovii*. The morphological characteristic of the leaves, such as size, stomata and trichomes, were related to habitat adaptation (Zhang & Marshall 1995). Stomata structure and trichomes in *Hedera* showed that, they were valuable taxonomical and ecological characters and could be used as distinguished characters in other studies.

The unusual distribution of *Hedera* is the result of rapid diversification driven by events of independent hybridization, and polyploidization was compared with the establishment of geographically isolated populations (Vargas *et al.* 1999, Ackerfield & Wen 2003). This process lead to a genus with similar species, often differing in chromosome number and distinguished by morphological characters of the leaf and stem hairs (McAllister & Rutherford 1983, Vargas *et al.* 1999, Valcárcel & Vargas 2010). Hybridization is an important evolutionary mechanism that brings about two genomes of divergent. It can produce new genetic and phenotypic traits that can help the species ecological adaptation (Freeland *et al.* 2011). Interspecific hybridization occurs frequently in various plants groups but it is under influence of different factors, for example the genetic structure of the species involved the fitness of the hybrid and genotype-environment interaction (Freeland *et al.* 2011). Natural distribution of *H. helix* is in Europe with 2n=2x= 48, whereas *H. pastuchovii* distributed mainly in Caucasus, Iran and Afghanistan with 2n=6x= 144 ploidy level. However, polyploidy, and to a lesser extent hybridization, are common phenomena within the genus. The low level of molecular divergence discovered in the extant species of *Hedera* show that, they are likely to be the result of recent speciation processes (Ackerfield & Wen 2003). The recent divergence of the species and the contention of allopolyploidization as the main force in this process may have greatly forbidden the foundation of clear limits and contributed to multiple taxonomic proposals. Hypothesis that any morphological change has a genetic basis can be concluded that, a complete lack of gene flow is required for two species to be distinguished by one or more discrete fixed characters. Morphological studies showed that, the varieties growing closer to each other and in the same ecological conditions become similar. It means that, ecological conditions have strong influence on the features of *Hedera*. Species differentiation is an important taxonomic task which can be achieved through a combination of various characteristics and approaches.

In conclusion, present study was carried out to provide additional evidence for taxonomists that, they could differentiate two species of *H. helix* and *H. pastuchovii* by using a combination of morphological and anatomical data. These taxa differ in important morphological and anatomical characteristics. Generally, statistical and bioinformatics tests revealed the great difference between two species. The genera such as *Hedera* seem to have extended their distribution recently. So there is a hypothesis that, the high similarities in the two species studied can be due to recent divergence of these species.

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References

Ackerfield, J. & Wen, J. 2002. A morphometric analysis of *Hedera* L. (the ivy genus, Araliaceae). Adansonia 324: 197–212.

Ackerfield, J. & Wen, J. 2003. Evolution of *Hedera* (the ivy genus, Araliaceae): insights from chloroplast DNA data. Intrnational Journal of Plant Sciences 164: 593–602.

Ackerfield, J. 2001. Trichome morphology in *Hedera* (Araliaceae). Edinburgh Journal of Botany 58: 259–267.

Agrawal, A.A. & Fishbein, M. 2006. Plant defence syndromes. Ecology 87: 132–149.

Amini, E., Nasrollahi, F., Sattarian, A, Haji Moradkhani, M., Boozarpour, S. & Habibi, M. 2020. Micromorphological, anatomical and molecular study of *Hedera* species (Araliaceae) in Iran. Acta Biologica Szegediensis (In Press).

Browicz, K. 1973. *Hedera. In*: Rechinger, K.H. (ed.). Flora Iranica. Akademische Druck-U. Verlagsanstalt, Graz, Austria. Vol. 102: 1–4.

de Queiroz, K. 1998. The general lineage concept of species, species criteria, and the process of speciation: A conceptual unifi cation and terminological recommendations, pp. 57–75. *In*: D.J. Howard & S.H. Berlocher (eds). Endless forms: Species and speciation. Oxford University Press, Oxford, UK.

de Queiroz, K. 2007. Species concepts and species delimitation. Systematic Biology 56: 879–886.

Dilcher, K.L. 1974. Approaches to the identification of Angiosperm leaf remains. Botanical Review 40: 2–157.

Esau, K. 1965. Plant anatomy. Ed. John Wiley and Sons, London, Sydney.

Eyde, R.H. & Tseng, C.C. 1971. What is the primitive floral structure of Araliaceae. Journal of the Arnold Arboretum 52: 205–239.

Fahn, A. 1979. Secretory Tissues in Plant. Academic Pess. London, New York, San Francisco.

Freeland, J.R., Kirk, H. & Peterson, S.D. 2011. Molecular Ecology. 2nd ed., Wiley-Blackwell, UK.

Hammer, O., Harper, D. & Ryan, P.D. 2001. PAST: Paleontological Statistics softwarepackage for education and data analysis. Palaeontologia Electronica 4: 1–9.

Hibberd, S. 1893. The ivy: a monograph. W.H. & L. Collingridge, London.

Ingrouille, M.J. 1986. The construction of cluster webs in numerical taxonomic investigations. Taxon 35: 541–545.

Kilic, S. 2009. Anatomical and pollen characters in the Genus *Silene* L. (Caryophyllaceae) from Turkey. Botany Research Journal 2: 34–44.

Konarska, A. 2014. Characteristics of flower nectaries of *Hedera helix* L. (Araliaceae). Acta Scientiarum Polonorum Hortorum Cultus 13: 109–122.

Kost, E.A., Rudakova, V.S., Volkova, S.A. & Shipunov, A.B. 2003. Morphological leaf-variability of West Caucasian *Hedera* L. (Araliaceae) species. Wulfenia 10: 51–59.

Lawrence, G.H.M. & Schulze, A.E. 1942. The cultivated Hederas. Gentes Herbariorum 6: 107–173.

Mcallister, H. 1981. New work on ivies. International Dendrology Society 81: 106–109.

Mcallister, H.A. 1988. Canary and Algerian ivies. The Plantsman 10: 27–29

Mcallister, H.A. & Rutherford, A. 1990. *Hedera helix* L. and *H. hibernica* (Kirchner) bean (Araliaceae) in the British Isles. Watsonia 18: 7–15.

McAllister, H.A. 1982. New work on ivies. International Dendrological Society 1981: 106–109.

McAllister, H.A. & Rutherford, A. 1983. The species of ivies. Ivy Journal 9: 45–54.

Metcalfe, C.R. & Chalk, L. 1985. Anatomy of Dicotyledons. Vols I and III. Oxford University Press.

Pojarkova, A.I. 1951. The Chinese species of ivy and their taxonomic and geographic connections. Natulaesystematicae ex herbariobotanici nomine

V.L. Komaroviiacademiaescientiarum URSS XIV: 224–264.

Rose, P.Q. 1996. The gardener's guide to growing ivies. David and Charles, London, UK.

Rutherford, A. 1984. The history of the Canary Islands ivy and its relatives. Ivy Journal 10: 13–18.

Rutherford, A. 1989. The ivies of Andalusia (Southern Spain). Ivy Journal 15: 7–17.

Rutherford, A., McAllister, H.A. & Mill, R.R. 1993. New ivies from the Mediterranean area and Macaronesia. The Plants Man 15: 115–128.

Savulescu, E. & Luchian, V. 2009. Comparative Anatomy of the vegetative organs of the *Hedera helix* L. (Araliaceae): Scientific Papers, Usamv Bucharest, Series A, Vol. LII, ISSN 1222-5339.

Seeman, B. 1868. Revision of the natural orders of Hederaceae L. Reeve & Co., London, UK.

Stace C.A. 1997. New flora of the British Isles, 2nd ed. Cambridge University Press, Cambridge.

Tobler, F. 1912. Die Gattung *Hedera.* Verlag Von Gustav Fischer, Jena.

Valca´rcel, V. 2008. Taxonomy, systematics and evolution of *Hedera* L. (Araliaceae). PhD dissertation, Universidad Pablo de Olavide, Sevilla, Spain (in English but two sections introduction and conclusions in Spanish).

Valca´rcel, V., Fiz, O. & Vargas, P. 2003. Chloroplast and nuclear evidence for multiple origins of polyploids and diploids of *Hedera* (Araliaceae) in the Mediterranean basin. Molecular Phylogenetics Evolution 27: 1–20.

Valca´rcel, V. & Vargas, P. 2010. Quantitative morphology and species delimitation under the general lineage concept: optimization for *Hedera* (Araliaceae). American Journal of Botany 97: 1555–1573.

Vargas, P., McAllister, H.A., Morton, C., Jury, S.L. & Wilkinson, M.J. 1999. Polyploid speciation in *Hedera* (Araliaceae): phylogenetic and biogeographic insights based on chromosome counts and ITS sequences. Plant Systematics and Evolution 219: 165–179.

Zhang, J.W. & Marshall, J.D. 1995. Variation in carbon isotope discrimination and photosynthetic gas exchange among populations of *Pseudotsugamenziesii* and *Pinus ponderosa* in different environments. Functional Ecology 9: 402–412.