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# LEAF ANATOMICAL STUDIES OF THE GENUS HIERACIUM (ASTERACEAE) IN IRAN AND ITS TAXONOMIC IMPLICATION

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Leaf anatomy of 17 taxa of the genus *Hieracium* belonging to two subgenera and six sections was examined using light microscopy in order to evaluate its systematic important. The studied anatomical characteristics show considerable variability among different species but are constant among different populations of one species. 28 characters were examined and among them, some characters have taxonomic value and can be used in delimitation of subgenera, sections, subsections and species. The most important characters are: presence or absence of lateral vascular bundles; main midrib shape; the thickness of epidermis, parenchyma, collenchyma and cuticle; the length of vascular bundles, xylem and phloem; lamina symmetry; the number of lateral vascular bundles and in few cases stomata size, the shape of epidermal cells and anticlinal walls. Moreover, an identification key to leaf anatomy of the species of *Hieracium* in Iran is given.

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**Key words:** Asteraceae; Cichorieae; *Hieracium*; leaf transection; epidermis; stomata; Iran

**مطالعه ساختار تشریحی برگ در جنس Hieracium (تیره آفتابگردان) در ایران و کاربرد تاکسونومیکی آن**

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در این تحقیق، ساختار تشریحی برگ ۱۷ آرایه از جنس Hieracium متعلق به دو زیر جنس و ۶ بخشه با استفاده از میکروسکوپ نوری ارائه می‌شود. صفات تشریحی مطالعه شده در گونه‌های مختلف، متفاوت بودند در حالی که در جمعیت‌های متفاوت از یک گونه ثابت بودند. ۲۸ صفت تشریحی مطالعه شد که از میان آنها برخی صفات دارای ارزش تاکسونومیکی بودند و در جداسازی زیرجنس‌ها، بخشه‌ها، زیربخشه‌ها و گونه‌ها اهمیت داشتند. مهمترین این صفات عبارتند از: وجود یا عدم وجود دستجات آوندی جانبی؛ شکل رگبرگ اصلی؛ ضخامت اپیدرم، پارانشیم، کلانشیم و کوتیکول؛ طول دستجات آوندی و دستجات چوب و آبکش؛ تقارن برگ؛ تعداد دستجات آوندی جانبی و در موارد کمی اندازه سلول‌های روزنه، شکل سلول‌های اپیدرمی و دیواره جداکننده سلول‌ها. علاوه بر این، برای تاکسون‌های مطالعه شده کلید شناسایی آناتومی برگ داده شده است.

## INTRODUCTION

*Hieracium* L. (Asteraceae Martynov; tribe Cichorieae Lam. & DC.; subtribe Hieracinae Cass. ex Dumort.) with approximately 5000 (Show 1973) or 10000 (Beaman 1990) apomictic species is mainly distributed in Asia, Africa, Europe and America (Strother 2007).

The genus was divided into four subgenera:

*Pilosella* (Hill) S. F. Gray, *Hieracium*, *Stenotheca* Fries and *Mandonia* Arv.-Touv. (Zahn 1921-1923). Sell (1987) reported the subgenus *Pilosella* as a distinct genus, while the morphologically based infrageneric classification was supported in molecular analyses of *Hieracium* by Gaskin & Wilson (2007).

*Hieracium* is present by 2 subgenera ( *Pilosella* and *Hieracium)* and 16 species in Iran, without considering sectional and subsectional levels (Rechinger 1977). Tavakkoli & Assadi (2005, 2006 and 2007) increased the number of species to 23, including five endemic species ( *H. hoppeanum* Schultes, *H. cheirifolium* Boiss. & Haussken., *H. macrotrichum* Boiss. *H. azerbaijanense* Lack and *H. piranshahricum* Tavakkoli & Assadi) and nine hybrids (*H. × kozlowskyanum* Zahn in Vestn; *H. × maschukense* Litw. & Zahn; *H. ×macrotrichum* Boiss.; *H. × sintenisii* (Freyn) Sojak; *H.× auriculoides* (A. F. Lang) Sell & West; *H. × pannoniciforme* Litw. & Zahn; *H. × matrense* N. P.; *H. × ruprechtii* Boiss.; *H. × hypeuryum* N. p.).

According to Flora of USSR (Juxip 1960), the genus has been divided into 7 sections, four of which (sect. *Echinina* N. P., sect. *Praealtina* N. P., sect. *pratensina* Asch. et Graebn and sect. *Pilosellina* N. P.) are included in subgenus *Pilosella*. and three sections in subgenus *Hieracium* (sect. *Umbellata* Fr.; sect. *Prenanthoidea* Koch and sect. *Vulgatum* Fries). Among the sections of the genus, sect. *Pr*aealtina and sect. *Pilosellina* were have been divided to 4 and 2 subsections respectively.

The chemical (Petrovic & al. 1999) and molecular (Shi & al. 1996) studies on the genus *Hieracium* strongly support the morphologically based classifications and delimitation of the closely related species.

In the genus *Hieracium*, leaves and achenes morphology are used as discriminative characters at the subgeneric level while the morphology, distribution and density of glandular trichomes are useful in distinguishing of taxa at sectional, subsectional and specific levels (Juxip 1960).

Talukdar (2015) used macro-micromorphology and anatomical features of the cypsela of *Hieracium* as specific delimiting factor.

Zarinkamar (2007) studied the leaf anatomy of *H. pilosella* C. H. & F. W. and *H. procerum* but did not discuss them as diagnostic characters to distinguish the taxa.

Four species of the genus *Hieracium* have been subjected to morphological, palynological and anatomical studies by Qureshi & al. (2009), but only two of them including *H. umbellatum* L. and *H. vulgatum* Fries occur in Iran. They indicated some characters (size, the type and density of stomata and the shape and size of epidermis and guard cells) provide significant data for species identification and a better knowledge of the family Asteraceae.

The leaf anatomy is generally a useful character for classification and delimitation of taxa. There are limited anatomical studies reports on the genus *Hieracium*

The present study is to investigate leaf anatomical characteristics of the genus *Hieracium* and to evaluate the systematic significance of it in the taxonomy of the genus.

## MATERIAL AND METHODS

Information on the taxa examined, collection data and the voucher specimens used are given in table 1. The specimens were obtained from TARI and FAR herbaria or their natural habitats.

Mostly two or three specimens of each species were subjected to anatomical studies. In this study, basal or lower leaves were fixed in alcohol-glycerin (1:1). The epidermis and cross sections samples were taken from middle part of leaves. The sections were cleared with 10% sodium hypochlorite, then transferred into 5% acetic acid for 5 min. and rinsed in water. The samples were stained with methyl blue and carmine-vest and after being mounted in glycerin jelly, they were photographed in magnification ×32, ×100 and ×400 by light microscopy. Measurements were made based ten sections for each species using micro-measurement ver. 1.04 software.

Mature leaves of each taxon were also macerated in 30% KOH solution for about 5-6 hours. The epidermis samples (from the mid-lamina regions of adaxial and abaxial surfaces) were stained in a solution of 0.1% Fuchsine for 1 min., and then fixed in glycerin. For each sample, nearly 60 measurements were made using a graded ocular on a Ziess standard 20 light microscope at a magnification 400 ×.

The terminology of Van Cotthem (1970), Zahur and Parveen (1982) were used for leaf epidermis morphology.

## RESULTS

The main anatomical characters of leaf transversal sections and epidermis characteristics of the studied species are summarized in tables 2 & 3. Selected LM micrographs of anatomical cross-sections are presented in figs. 1-34.

### Leaf transversal section

The shape of main midrib ranges from triangular (figs. 7 A, 9 A, 15 A), oval or nearly oval (figs. 2 A, 11 A, 16 A) to semi-orbicular (figs. 3 A, 5 A, 13 A, 14

A) and nearly orbicular (figs. 1 A, 4 A, 6 A, 10 A, 12 A) and its thickness (MMT) varies from 0.5 mm in *H. vulgatum* to 1.7 mm in *H. verruculatum* Link. The thickness of upper and lower cuticle (UCT & LCT) ranges from 4.87 µm and 6.37 µm in *H. pilosella* C. H. & F. W. Schultz to 15.99 µm and 16.29 µm in *H.* × *auriculoides*, respectively. The thickness of upper epidermis (UET) ranges from 9.48 µm in *H. vulgatum* to 22.49 µm in *H. pilosella* whereas lower epidermis thickness (LET) ranges from 8.98 µm in *H. prenanthoides* Vill. to 19.97 µm in *H. bauhini* Besser. A minimum thickness of upper and lower parenchyma is observed in *H. umbellatum* (106.48 µm and 139.47 µm, respectively) while the maximum one is found in *H. prenanthoides* (846.91 µm and 251.53 µm, respectively). The thickness of upper collenchyma (UCOT) ranges from 22.49 µm in *H. pilosella* to 72.65 µm in *H.* × *maschukense*. Lower collenchyma thickness (LCOT) is in the range of 53.26 µm in *H. hoppeanum* to 137.69 µm in *H. verruculatum*. Regarding, the length of vascular bundles (VBL), the smallest length (149.89 µm) is observed in *H.* × *matrense* N. P. and the largest one (545.35 µm) in *H. verruculatum* (table 2). Based on presence of fiber around the midrib phloem, the species of *Hieracium* are divided into two groups, group I, with fiber including *H. prenanthoides*, *H. verruculatum*, *H. cheirifolium*, *H.* × *maschukense*, *H. echioides* subsp. *echioides* and subsp. *procerum*, *H.* × *aurculoides* and *H. hoppeanum* (figs. 2 B, 4 B, 5 B, 6 B, 7 B, 12 B, 13 B, 15 B and 16 B) and group II, without fiber including *H. umbellatum*, *H. vulgatum*, *H. piloselloides* (Vill) Sojak, *H. bauhini*, *H.* × *sintenisii* (Freyn) Sojak, *H.* × *matrense* and *H. pilosella* (figs. 1 B, 3 B, 9 B, 10 B, 11 B, 14 B and 17 B). Except *H. umbellatum*, *H. prenanthoides* and *H. vulgatum*, remaining of species have 1-3 lateral vascular bundles around the main vascular bundle. Bilateral symmetry is only seen in the species of sect. *Echinina* and *H.* × *auriculoides* (sect. *Praealtina*) while leaf symmetry of other species is bifacial.

## Leaf epidermis

The smallest stomata size is observed in *H. hoppeanum*, whereas *H. cheirifolium* has the largest one on the adaxial and abaxial surfaces (table 3). All taxa have anomocytic and anisocytic stomatal types. Most of epidermal cells are strongly irregular in shape with strongly sinuate anticlinal walls. In *H. bauhini* (Ad., Ab., fig. 27 A, B), *H.* × *sintenisii* (Ab., fig. 28 B), *H.* × *auriculoides* (Ad., fig. 29 A), *H.* × *ruprechtii* Boiss. (Ad., Ab., fig.32 A, B), *H. hoppeanum* (Ab., fig. 33 B), the epidermal cells are irregular with sinuate anticlinal walls. *Hieracium umbellatum* (Ad., fig.18 A), *H. hoppeanum* (Ad., fig.33 A) and *H. pilosella* (Ad., fig. 34 A) have polygonal to slightly sinuate cells with straight to slightly sinuate anticlinal walls on the adaxial surface. Nearly irregular cells and curved to nearly sinuate walls are present in *H. vulgatum* (Ad., Fig.20 A), *H.* × *maschukense* (Ad., fig. 23 A) and *H. pilosella* (Ab., fig. 34 B ).

Stomatal density varies from 47.33–210.14 per square millimeter on the adaxial epidermis and 64.78–189.13 on the adaxial epidermis. The stomatal density of *H. hoppeanum* is high comparing with the species of the genus. *Hieracium prenanthoides* and *H. cheirifolium* have the minimum stomatal density on the adaxial and abaxial surfaces, respectively.

## Leaf anatomy identification key for the studied *Hieracium* species

1. Leaves without lateral vascular bundles ..... ..... subgenus ***Hieracium*** (2)
-Leaves with lateral vascular bundles ..... ..... subgenus ***Pilosella*** (4)
2. The shape of main midrib nearly orbicular, with a minimum thickness of parenchyma; leaf adaxial epidermis with polygonal-slightly irregular cells and straight to slightly sinuate anticlinal walls ..... ..... sect. ***Umbellata*** (***H. umbellatum***)
-The shape of main midrib and leaf adaxial epidermis not as above ..... 3
3. Main midrib shape oval, with a maximum thickness of parenchyma and xylem; leaf adaxial epidermis with strongly irregular cells and strongly sinuate anticlinal walls ..... sect. ***Prenanthoidea*** (***H. prenanthoides***)
- Main midrib shape semi-orbicular, with a less thickness of parenchyma and xylem; leaf adaxial epidermis with nearly irregular cells and curved-nearly sinuate anticlinal walls ..... ..... sect. ***Vulgata*** (***H. vulgatum***)
4. The thickness of upper collenchyma more than 60 µm ..... sect. ***Echinina*** (5)
-The thickness of upper collenchyma less than 60µm . 8
5. The shape of main midrib semi-orbicular, with a minimum thickness of lower epidermis..... ..... ***H. cheirifolium***
-The shape of main midrib and epidermis thickness not as above ..... 6
6. Main midrib shape nearly orbicular ..... 7
-Main midrib shape triangular ..... ***H. echioides***
7. The number of lateral vascular bundles 3; with a maximum thickness of lower parenchyma; the stomata length larger than 30 µm ..... ***H. verruculatum***
-The number of lateral vascular bundles 2; with a minimum thickness of lower parenchyma; The stomata length shorter than 30 µm .. ***H.* × *maschukense***

8. Upper cuticle thickness more than 6 µm and phloem length more than 30 µm .....sect. ***Praealtina*** (9)
-Upper cuticle thickness less than 6 µm and phloem length less than 30 µm ..... sect. ***Pilosellina*** (14)
9. Main midrib shape triangular .....10
-Main midrib shape not as above .....11
10. The number of lateral vascular bundles 3 ..... ..... ***H. × ruprechtii***
-The number of lateral vascular bundles 2..... ..... ***H. piloselloides***
11. The thickness of main midrib more than 1 mm; midrib cuticle thickness less than 10 µm .....12
-The thickness of main midrib less than 1 mm; midrib cuticle thickness more than 10 µm.....13
12. The shape of main midrib nearly orbicular ..... .....***H. bauhini***
-The shape of main midrib oval .....***H. × sintenisii***
13. Lamina symmetry bilateral ..... ***H. × auriculoides***
- Lamina symmetry bifacial ..... ***H. × pannoniciforme***
14. Main midrib shape nearly oval..... ***H. hoppeanum***
-Main midrib shape nearly orbicular.....***H. pilosella***

## DISCUSSION

Leaf anatomical characteristics are presented here to provide valuable characters in delimitation of taxa in the genus *Hieracium*. Morphologically, this genus has two subgenera in Iran: *Hieracium* and *Pilosella*. The presence or absence of lateral vascular bundles in *Hieracium* species is an important diagnostic character for distinguishing of these two subgenera. In the genus, leaf anatomical differences at sectional and species levels include the shape of main midrib and epidermal cells; the thickness of parenchyma, collenchyma, epidermis and cuticle; lamina symmetry and stomata size.

According to Metcalf and Chalk (1957), anomocytic stomata is the predominant type in the genus *Hieracium*.

***Hieracium* subgenus *Hieracium***

The main morphological features of this subgenus are the presence or absence of basal leaves; achenes length 3-4 mm and pappus in two rows. One remarkable character observed in the subgenus is the absence of lateral vascular bundles.

**Sect. *Umbellata*** Fr. (figs. 1, 18)

*Hieracium umbellatum* is the only member of this section in Iran. Numerous stem leaves, the absence of glandular trichomes on the peduncle and involucres are the most characteristic features of this section.

According to anatomical characters, besides the nearly orbicular shape of the main midrib, its parenchyma thickness is less than other species of this subgenus.

Epidermal cells of adaxial surface are polygonal to slightly irregular with straight, slightly sinuate anticlinal walls, which is obviously different from observed patterns in two other sections of the subgenus. Therefore, leaf anatomy supports the unique systematic position of this species.

**Sect. *Prenanthoidea*** Koch. (figs. 2, 19)

This section includes *H. prenanthoides* and *H. azerbaijanense* Lack in Iran of which *H. prenanthoides* was examined in this study. This species is recognized by glandular trichomes on the peduncle, involucres and stem and hairy ligules.

The shape of main midrib is oval. The thickest parenchyma and a minimum thickness of lower epidermis belong to this species (table 2). Moreover, this species shows strongly irregular epidermal cells on adaxial and abaxial surfaces and possesses strongly sinuate anticlinal walls.

The mentioned anatomic differences in this section is supported by morphological characters.

**Sect. *Vulgata*** Fr. (figs. 3, 20)

This section consists of *H. vulgatum* and *H. pollichiae* Schultz in Iran. The main morphological features of this section are the presence of basal leaves and 2-3 stem leaves.

*Hieracium vulgatum* shows the minimum thickness of cuticle and upper epidermis layer compared to the two other sections of subgenus *Hieracium*. In addition, larger stomatal size and nearly irregular epidermal cells on the adaxial surface support the separation of this section from the sections *Umbellata* and *Prenanthoidea*.

***H*ieracium subgenus *Pilosella*** Hill

The species of the subgenus are distinguished by having basal leaves, presence or absence of stolon and stem leaves; leaves with entire margin; achenes length 2-2.5 mm and pappus in a row. The members of the subgenus in contrast to the subgenus *Hieracium* have lateral vascular bundles around the main vascular bundle.

**Sect. *Echinina*** N. P. (figs. 4–8, 21–25)

This section comprises of six species in Iran of which *H. echiodes*, *H. cheirifolium*, *H. verruculatum*, *H. × maschukense* are considered here. Presence or absence of glandular trichomes and distribution of simple trichomes on the stem length or lower part characterize all species of this section.

*Hieracium echioides* is well characterized by having triangular midrib in comparison with other

species of the section. Two subspecies of *H. echioides*, subsp. *echiodes* and subsp. *procerum* show high anatomical similarities (tables 2, 3). The shape of main midrib is nearly orbicular in *H. verruculatum*, the character that is also observed in *H.* × *maschukense* (*H. echiodes* × *verruculatum*). *Hieracium verruculatum* possesses the thickest cuticle, main midrib, lower collenchyma, upper parenchyma and the maximum length of vascular bundles and phloem among the species of this section.

From morphological point of view, *H.* × *maschukense* is intermediate between *H. echiodes* and *H. verruculatum*. This taxon has simple trichomes on the stem length (similar to *H. echioides*) and glandular trichomes on the involucres and peduncle (similar to *H. verruculatum*). In spite of maximum thickness of upper collenchyma in *H.* × *maschukense*, some of its anatomical characters are different from its parents and some of them are intermediate between parents, whereas some features of *H. echiodes* such as the length of vascular bundles and phloem and cuticle thickness are predominant in this hybrid (table 2).

The occurrence of semi-orbicular midrib in *H. cheirifolium* can be used for its separation from other species of this section.

With the exception of *H.* × *maschukense*, the rest of the species examined here show similar stomatal size and strongly irregular epidermal cells with strongly sinuate anticlinal walls on the adaxial and abaxial surfaces (Table 3).

Therefore, leaf anatomical characters can be provided some supports for the delimitation of the species of this section.

**Sect. *Praealtina*** N. P.

The members of this section were divided into four subsections: subsect. *Florentina* Juxip, subsect. *Bauhinia* Juxip, subsect. *Praealtoechinina* Juxip and subsect. *Praealtopilosellina* Juxip. The absence of stellate hairs and stolon characterize *H. piloselloides* (Vill.) Sojak of subsect. *Florentina*. *Hieracium bauhini* Besser and *H.* × *sintenisii* (Freyn) Sojak (subsect. *Bauhinia*) are characterized by having stolon and sparse indumentum to glabrescens. The presence of stellate and yellow or black glandular hairs and the absence of stolon are characteristics of the members of subsect. *Praealtoechinina*. The species of subsect. *Praealtopilosellina* can be distinguished by bifurcate inflorescence and a few capitules.

According to anatomical characters in this section, lamina thickness and stomata size of *H. piloselloides* (subsect. *Florentina*) are more than other species.

The upper cuticle with less thickness; main midrib and upper parenchyma with more thickness and the largest vascular bundles and phloem are observed in the members of subsect. *Bauhinia* (including *H. bauhini* and *H.* × *sintenisii*). In *H.* × *sintenissi* (*H. piloselloides* × *H. verruculatum*), the thickness of upper collenchyma and parenchyma and vascular bundles length are intermediate between *H. piloselloides* and *H. verruculatum*, whereas most of the anatomical features are different from both parents.

In subsect. *Praealtoechinina*, the thickness of midrib lower collenchyma is less than other subsections. Moreover, the higher thickness of cuticle belongs to *H.*× *auriculoides* from this subsection. Dominance of anatomical characteristics of *H. piloselloides* parent is also observed in the hybrid *H.* × *auriculoides*.

*H.*× *pannoniciforme* has inherited anatomical features of each three parents. This hybrid inherits upper collenchyma thickness and the phloem length of *H. piloselloides* parent. The thickness of upper and lower epidermis of the hybrid is similar to *H. verruculatum* and *H. echiodes.* Upper cuticle thickness is also inherited from *H. echoides* parent. (Table 2).

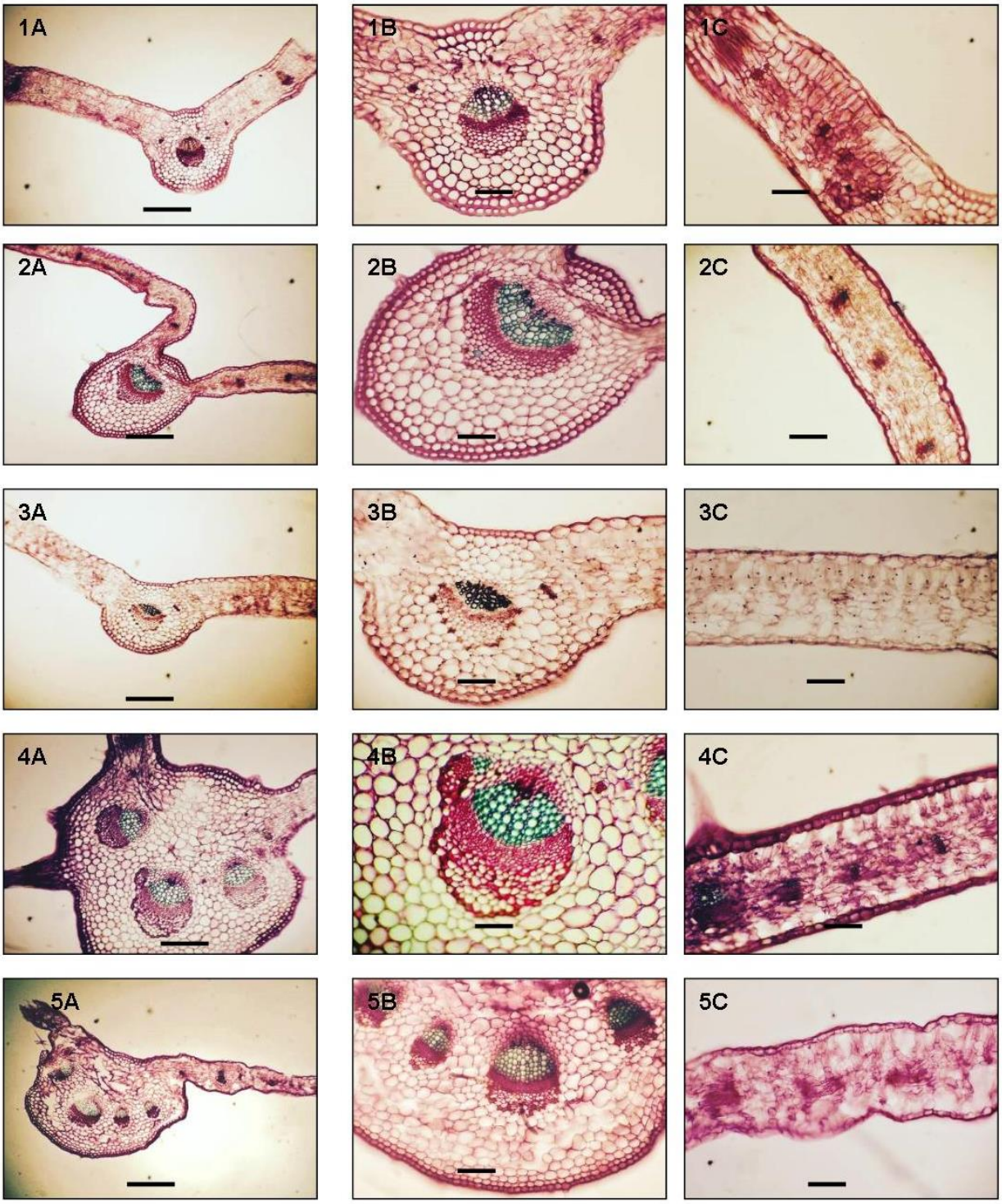
Although, the thickest lower collenchyma and the minimum thickness of upper collenchyma belong to *H.* × *ruprechtii* (subsect. *Praealtopilosellina*) but leaf anatomic characteristics do not provide reliable characters useful for the delimitation of the species within this subsection. Most of anatomical features of *H. piloselloides* are dominant in interspecific hybrid of *H.* × *matrense* (*H. pilosella* × *H. piloselloides*) whereas the hybrid *H.* × *ruprechtii* shows intermediate anatomical characters between its parents.

**Sect. *Pilosellina*** N. P.

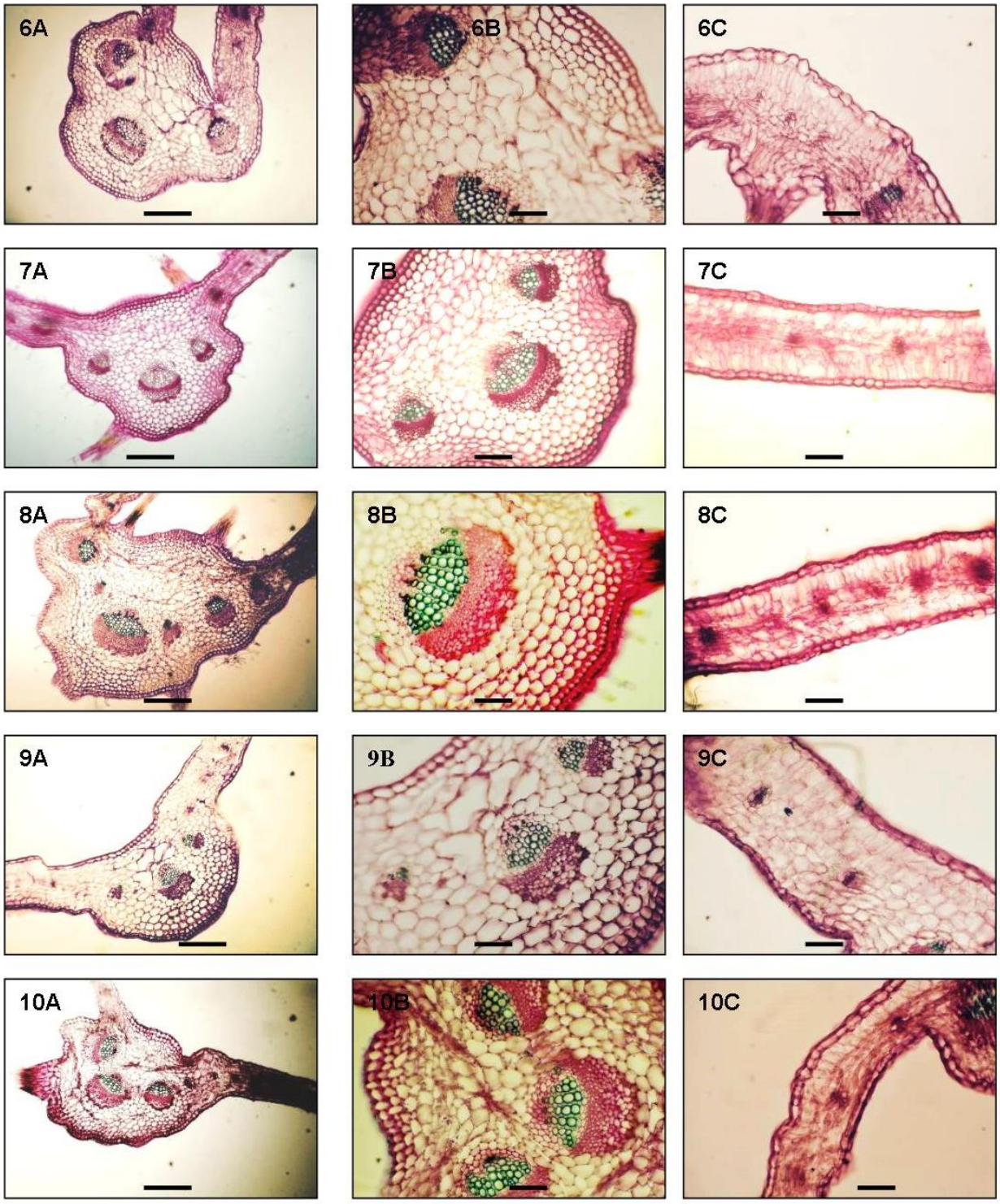
In this section, scapose stems with a single capitulum are characteristic. The members of this section were divided into two separate subsections: subsect. *Hoppeana* Juxip and subsect. *Pilosella* Juxip. In subsect. *Hoppeana*, the occurrence of short stolons with dense leaves can be used as an important character for its separation from subsect. *Pilosella.*

From the anatomical point of view, the members of this section have some important characters as: the minimum thickness of collenchyma and upper cuticle, shorter vascular bundles and phloem and stomatal size in comparison with other sections. Moreover, *H. pilosella* (subsect. *Pilosella*) differs from *H. hoppeanum* (subsect. *Hoppeana*) in main midrib shape, the thicker epidermis layer and upper parenchyma and larger stomatal size (tables 2, 3).

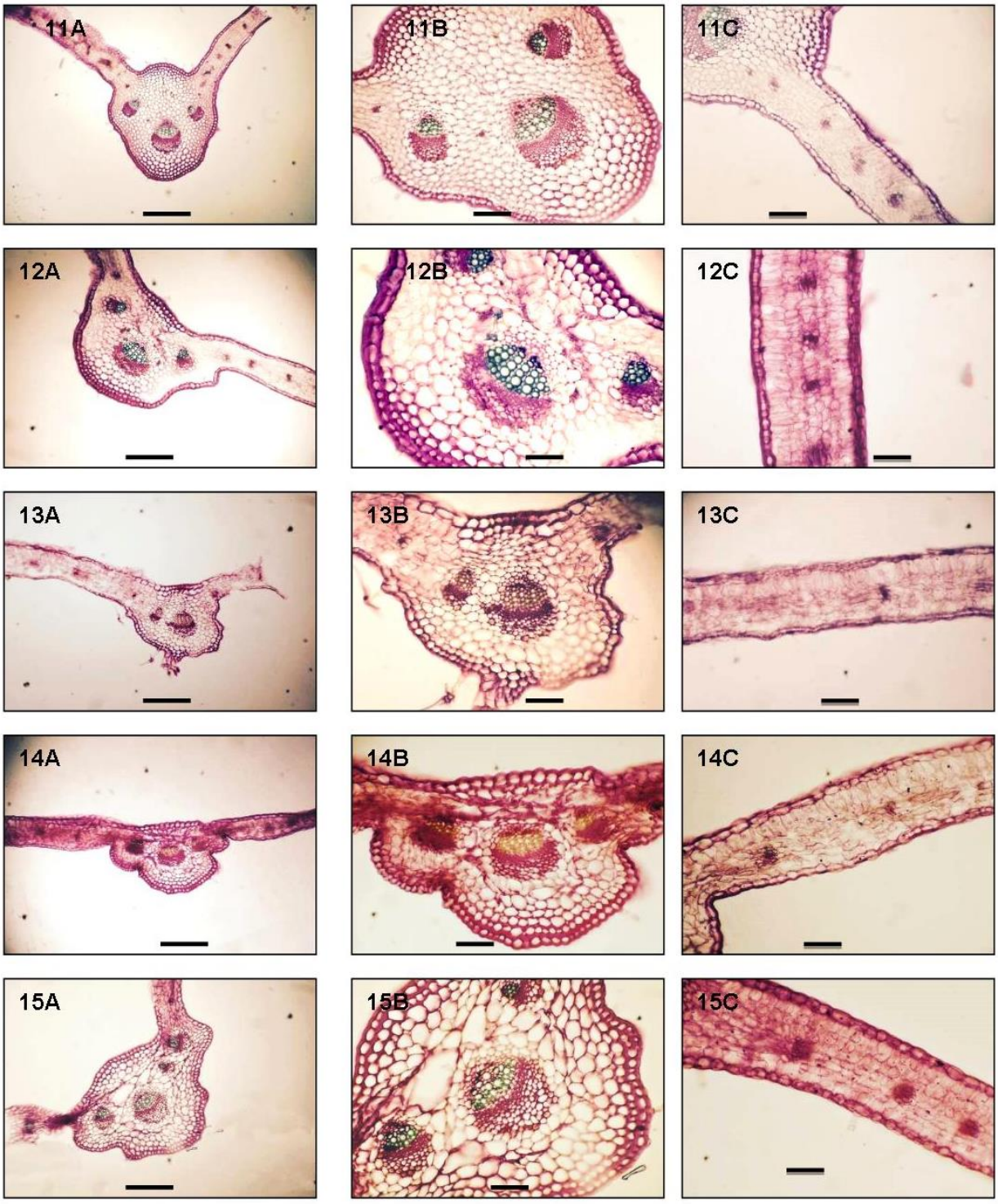
In conclusion, leaf anatomical features provide strong support for delimitation of taxa in the genus *Hieracium*.



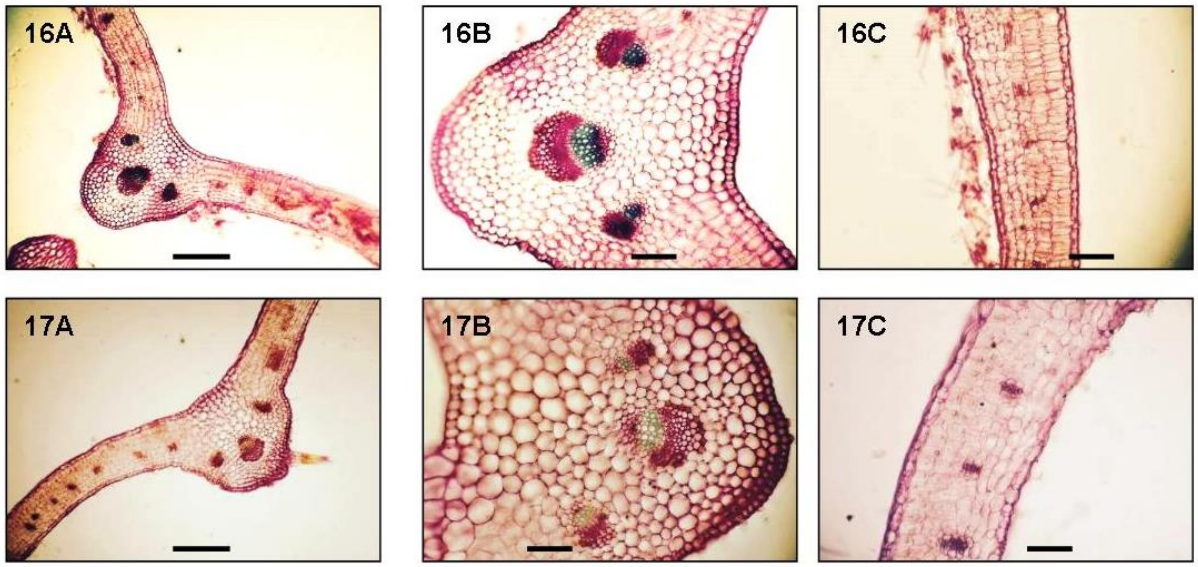

Figs. 1–5. LM micrographs of leaf transversal sections of *Hieracium* species. Midrib (figs. A, B) and blade (fig. C). 1 A-C, *H. umbellatum*; 2 A-C, *H. prenanthoides*; 3 A-C, *H. vulgatum*; 4 A- C, *H. verruculatum*; 5 A-C, *H. cheirifolium*. Scale bars: A = 300 µm, B & C = 100 µm.



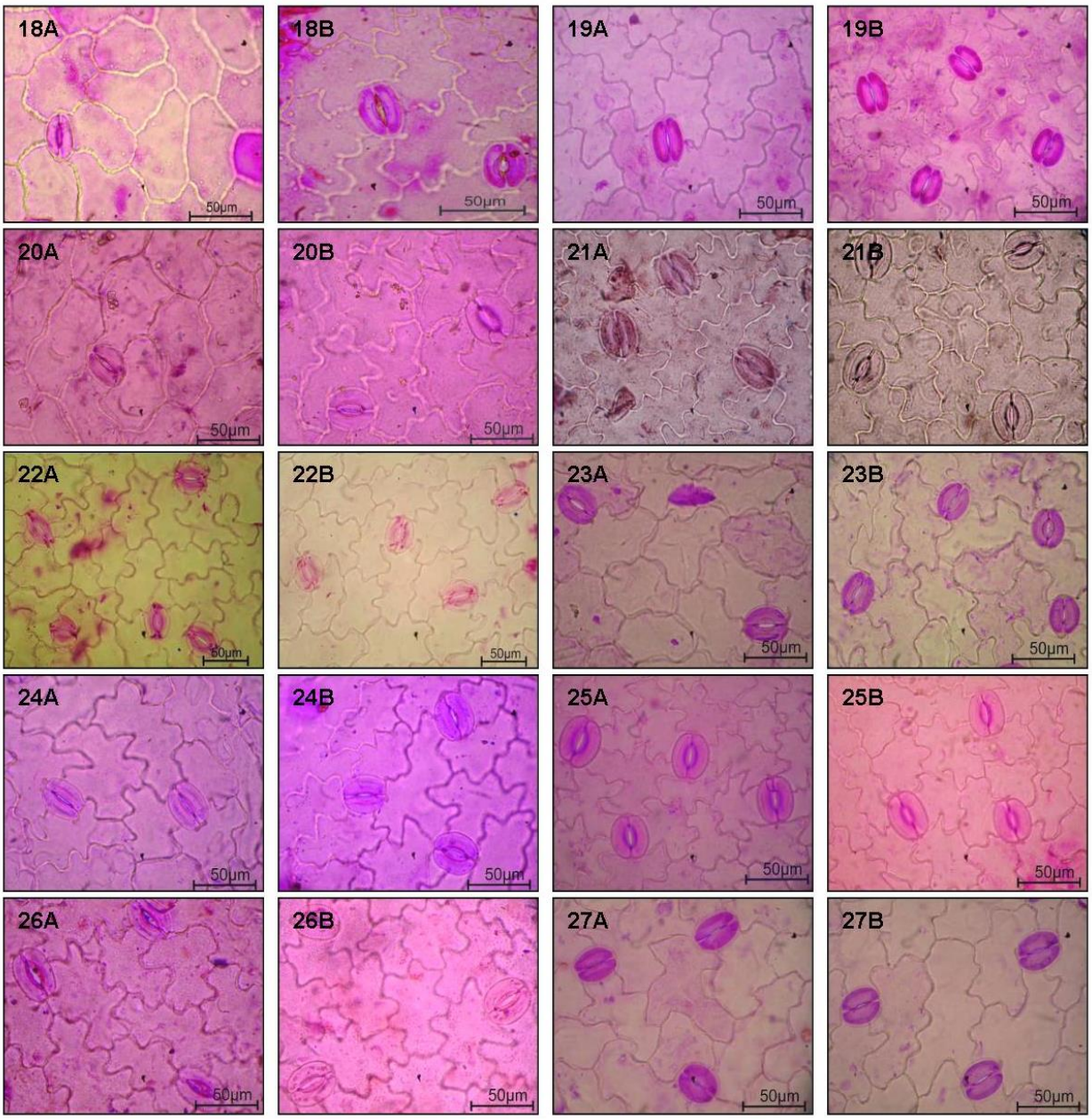

Figs. 6–10. LM micrographs of leaf transversal sections of *Hieracium* species. 6 A-C, *H.* × *maschukense*; 7 A-C, *H. echioides* subsp. *echioides*; 8 A-C, *H. echioides* subsp. *procerum*; 9 A-C, *H. piloselloides*; 10 A-C, *H. bauhini*. Scale bars: A = 300 µm, B & C = 100 µm.



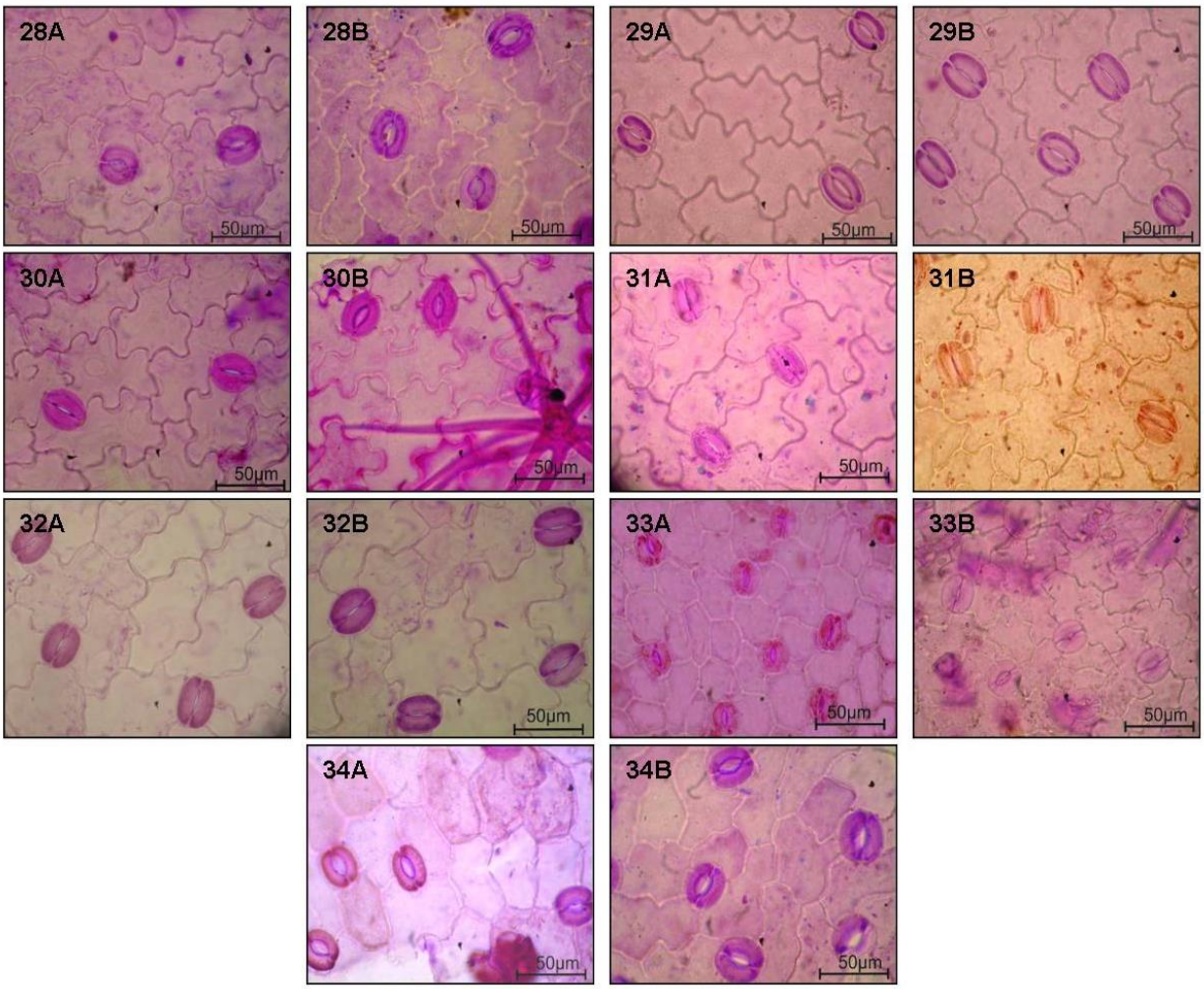

Figs. 11–15. LM micrographs of leaf transversal sections of *Hieracium* species. 11 A-C, *H.* × *sintenisii*; 12 A-C, *H.* × *auriculoides*; 13 A-C, *H.* × *pannoniciforme*; 14 A-C, *H.* × *matrense*; 15 A-C; *H.* × *ruprechtii*. Scale bars: A = 300 µm, B & C = 100 µm.




Figs. 16–17: LM micrographs of leaf transversal sections of *Hieracium* species. 16 A-C, *H. hoppeanum*; 17 A-B; *H. pilosella*. Scale bars: A = 300 µm, B & C = 100 µm.




Figs. 18–27. LM micrographs of the leaf epidermis of *Hieracium* species. 18 A-B, *H. umbellatum*; 19 A-B, *H. prenanthoides*; 20 A-B, *H. vulgatum*; 21A-B, *H. verruculatum*; 22 A-B, *H. cheirifolium*; 23 A-B, *H.* × *maschukense*; 24 A-B, *H. echioides* subsp. *echioides*; 25 A-B, *H. echioides* subsp. *procerum*; 26 A-B, *H. piloselloides*; 27 A-B, *H. bauhini*. 18 A –27 A = adaxial surface, 18 b –27 B = abaxial surface. Scale bars = 50 µm.




Figs. 28–34. LM micrographs of the leaf epidermis of *Hieracium* species. 28 A-B, *H.* × *sintenisii*; 29 A-B, *H.* × *auriculoides*; 30 A-B, *H.* × *pannoniciforme*; 31 A-B, *H.* × *matrense*; 32 A-B, *H.* × *ruprechtii*; 33 A-B, *H. hoppeanum*; 34 A-B, *H. pilosella*. 28 A–34 A = adaxial surface, 28 B –34 B = abaxial surface. Scale bars = 50 µm.

**Table 1. Collecting data of the specimens used in this study.**

| Taxa | Subgenera | Sections | Collection data |
|---|---|---|---|
| *Hieracium umbellatum* | *Hieracium* | *Umbellata* | Azarbayejan: Khoda afarin, Assem Varak, Arasbaran protected area, 8 km from Daran to Varzeghan, 1270 m, Ghahremani 6738.-Tehran: Karaj valley, Assem Varak, 2450 m, 25 August 1974, Wendelbo & al. 14476 (TARI). |
| *H. prenanthoides* | *Hieracium* | *Prenanthoidea* | Mazandaran: Nour, Chamestan, Lavij, Mirkhamand, 2200 m, 26 July 1989, Mozaffarian 67860( TARI); 80 km S. Sari, Dodangeh, Gatinu, 2800 m, 13 August 2008, Zare 7542.–Azarbayejan: Arasbaran protected area, Forests W. of Makidi, 1700 m, 27 August 1975, Runemark and Assadi 22050 (TARI). |
| *H. vulgatum* | *Hieracium* | *Vulgata* | Mazandaran: Polsefid, forest above the village Sangdeh, 1500–2500 m, 13 June 1995, Assadi 73464 (TARI). –Azarbayejan: Misho Dagh mountain, 1800–2400 m. Mohammady 6887 (FAR). |
| *H. verruculatum* | *Pilosella* | *Echinina* | Mazandaran: Lar valley, 2450 m, 2 July 1974, Wendelbo & Assadi 13389 (TARI).–Azerbaijan: ca. 50 km. W. of Khoy, near the village Pil-e Savar, close to the Turkish frontier, 2350 m, 25 July 1990, Olfat & Assadi 68859 (TARI).–Tehran: Ab–Ali road, 32 km to Polur, Lar valley, 2400 m, 18 July 1972, Babakhanlou & Amin 5574 (TARI). |
| *H. cheirifolium* | *Pilosella* | *Echinina* | Bakhtiari: Chelgerd, kuh-e Dol Kareh, 2750 m, 28 June 1998, Gholamian 2532 (TARI); Borujen, Dareh Riz Rangeland, 2555 m, 24 June 1996, Gholamian 430 (duplicate in TARI). |
| *H.* × *maschukense* | *Pilosella* | *Echinina* | Azerbaijan: Neur, Lisar protected area, 2600 m, 22 June 2007, Bidarlord 105 (FAR); Zanjan, Tarum, between Yahya abad and Parchineh village, 2000 m, Musavi 2511 (FAR).–Khorassan: Bojnurd, Darkesh , Nargesli Mountains, 1500 m, Tavakkoli 73013 (FAR).–Tehran: Gachsar, 1600–1800 m, Tavakkoli 37006 (FAR). |
| *H. echioides* subsp. *echioides* | *Pilosella* | *Echinina* | Azarbayejan: Urumieh, Silvana, Barde su, 1800–2200 m, 4 July 1974, Zehzad & Siami 2955 (TARI). |
| *H. echioides* subsp. *procerum* | *Pilosella* | *Echinina* | Mazandaran: Chalus, Pol-e Zangoleh, Ilka rivaer margin to Kuh-e Makloz, 2500–2800 m, 15 July 2008, Zare & Mashayechi 7535.–Azerbaijan: Arasbaran protected area, Doghrun and Kalan mountains, 2300–2500 m, 14 July 1977, Assadi & Sardabi 24117 (TARI). |
| *H. piloselloides* | *Pilosella* | *Praealtina* | Azerbaijan: ca. 70 km. NEE of Ahar, basins of kuh-e Kasabe, 2000 m, 21 June 1998, Assadi & Shahsavari 65907 (TARI); N. Kaleibar, 1500 m, 12 May 1998, Taheri 15317 (FAR). |
| *H. bauhini* | *Pilosella* | *Praealtina* | Gilan: near Damesh–East of Rudbar, 1700 m, 22 June 1975, Wendelbo & Ann Ala 18257 (TARI); Lahijan, Siahkal, Shah–Shahidan, 1500–2000m, 2 Agust 1992, Jamzad & Asri 71739 (TARI).–Azarbayejan: Arasbaran protected area, Saigran Dagh Mountain, 1300 m, 14 July 1977, Assadi & Sardabi 24251 (TARI). |
| *H.* × *sintenisii* | *Pilosella* | *Praealtina* | Azarbayejan: Bostan abad, Chinibulagh, 4 km Pischiclou, 2150–2350 m, Terme & Mousavi 30334 (IRAN). |
| *H.* × *auriculoides* | *Pilosella* | *Praealtina* | Mazandaran: Kandavan, 21 June 2003, Tavakkoli 37016 (FAR).–Azerbaijan: Arasbaran protected area, Doghrun Mountain, 2500–2800 m, 13 July 1977, Assadi & Sardabi 24054 (TARI); Subatan, Lisar protected area, 2045 m, 15 July 2007, Bidarlord 354 (FAR). |
| *H.* × *pannoniciforme* | *Pilosella* | *Praealtina* | Azerbaijan: Subatan, Lisar protected area, 2045 m, Bidarlord 592 (FAR); 6 km from Germi to Ani, W. of Easemar village, 900–1200 m, 23 June 1980, 34906 (TARI).–Tehran: 13 km from Kandavan to Haraz road, 2400 m, 4 June 1979, Assadi & Mozaffarian 32949 (TARI); karaj–Chalus pass, 3000 m, 18 August 1974, Dini 23762 (TARI). |
| *H.* × *matrense* | *Pilosella* | *Praealtina* | Mazandaran: Pol-e sefid, forest above the village Sangdeh, 1500–2500 m, 19 June 1995, Assadi 73458 (TARI); kojur, Shahkuh, 1500–2800 m, 24 June 2002, Zare & Mashayekh 7533. |
| *H.* ×*ruprechtii* | *Pilosella* | *Praealtina* | Gilan: Amarlou, Damash, kuh-e Angur–Chaleh,1750–1920 m, 5 July 1972, Terme & Daryadel 10321(IRAN). |
| *H. hoppeanum* | *Pilosella* | *Pilosellina* | Azerbaijan: Gardaneh Almas, highest pass on the road from Asalem to Khalkhal, 2350–2400 m, 16 June 1975, Wendelbo & Assadi, 18497(TARI). |
| *H. pilosella* | *Pilosella* | *Pilosellina* | Mazandaran: S. W. of Ramsar, E. of Javaherdeh, 26 June 1976, Runemark & Massoumi 20720 (TARI); Kojur, Shahkuh, 1500–2800 m, 1 July 2002, Zare & Mashayekh 7531.–Azerbaijan: Arasbaran protected area, 2400–2700 m, 15 July 1977, Assadi & Sardabi 24335 (TARI). |

Table 2. Anatomical measured characters in *Hieracium* species. Abbreviations: middle midrib Thickness (MMT); upper cuticle thickness (UCT); upper epidermis thickness (UET); upper collenchyma thickness (UCOT); lower collenchyma thickness (LCOT); upper parenchyma thickness (UPT); number of lateral vascular bundles (NLVB); vascular bundles length (VBL); xylem length(XL); phloem length (PhL); lower parenchyma thickness (LPT); lower epidermis thickness (LET); lower cuticle thickness (LCT); lamina symmetry (LS).

| **Taxa** | **Midrib shape** | **MMT** (mm) | **UCT** (µm) | **UET** (µm) | **UCOT** (µm) | **LCOT** (µm) | **UPT** (µm) | **NLVB** | **VBL** (µm) | **XL** (µm) | **PhL** (µm) | **LPT** (µm) | **LET** (µm) | **LCT** (µm) | **LS** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *H. umbellatum* | Nearly orbicular | 0.641 | 12.15 | 18.23 | 26.51 | 64.59 | 106.48 | 0 | 218.93 | 96.63 | 34.18 | 139.47 | 14.47 | 10.92 | bifacial |
| *H. prenanthoides* | Oval | 0.846 | 10.71 | 15.39 | 42.2 | 82.07 | 846.91 | 0 | 289.05 | 123.96 | 33.44 | 251.53 | 8.98 | 10.34 | bifacial |
| *H. vulgatum* | Semi-orbicular | 0.5 | 8.36 | 9.48 | 21.56 | 67.01 | 645.49 | 0 | 200.38 | 70.16 | 25.94 | 192.96 | 15 | 9.5 | bifacial |
| *H. verruculatum* | Nearly orbicular | 1.744 | 15.72 | 16.57 | 67.4 | 137.69 | 634.67 | 3 | 454.35 | 58.18 | 162.25 | 208.13 | 13.28 | 14.62 | bilateral |
| *H. cheirifolium* | Semi-orbicular | 1.019 | 10.96 | 16.8 | 64.01 | 105.24 | 530.74 | 3 | 341.54 | 45.42 | 146.42 | 218.76 | 10.65 | 9.46 | bilateral |
| *H.× maschukense* | Nearly orbicular | 1.180 | 10.02 | 13.34 | 72.65 | 80.61 | 505.91 | 2 | 328.1 | 50.79 | 135.73 | 179.46 | 14.93 | 10.28 | bilateral |
| *H. echioides* subsp. *echioides* | Triangular | 1.045 | 8.65 | 16.48 | 65.03 | 70.34 | 386.84 | 2 | 302.34 | 44.16 | 121.06 | 197.6 | 14.1 | 11.98 | bilateral |
| *H. echioides* subsp. *procerum* | Triangular | 1.34 | 10.83 | 15.78 | 69.96 | 75.88 | 448.3 | 2 | 360.29 | 52.3 | 133.88 | 184.82 | 13.7 | 10.08 | bilateral |
| *H. piloselloides* | Triangular | 0.849 | 11.05 | 19.49 | 41.47 | 70.71 | 214.79 | 2 | 253.51 | 94.61 | 35.49 | 210.57 | 17.81 | 12.47 | bifacial |
| *H. bauhini* | Nearly orbicular | 1.067 | 6.69 | 21.49 | 55.05 | 77.52 | 355.22 | 2 | 291.5 | 97.62 | 44.48 | 215.43 | 19.97 | 9.71 | bifacial |
| *H. × sintenisii* | Oval | 1.134 | 7.8 | 11.79 | 46.52 | 76.07 | 414.16 | 2 | 329.78 | 128.49 | 50.5 | 134.09 | 11.37 | 8.97 | bifacial |
| *H. × auriculoides* | Nearly orbicular | 0.834 | 15.99 | 21.55 | 59.54 | 69 | 232.47 | 2 | 278.71 | 107.68 | 37.25 | 121.42 | 13.62 | 16.29 | bilateral |
| *H. ×pannoniciforme* | Semi-orbicular | 0.678 | 10.18 | 16.79 | 40.73 | 62.37 | 195.74 | 1 | 208.23 | 67.68 | 31.18 | 95.77 | 12.88 | 6.66 | bifacial |
| *H. × matrense* | Semi-orbicular | 0.696 | 10.54 | 20.83 | 43.29 | 73.72 | 127.95 | 2 | 234.51 | 74.09 | 34.14 | 87.07 | 17.5 | 10.62 | bifacial |
| *H. × ruprechtii* | Triangular | 0.872 | 10.12 | 16.72 | 37.87 | 84.4 | 211.3 | 3 | 251.94 | 86.7 | 30.64 | 159.87 | 11.39 | 10.64 | bifacial |
| *H. hoppeanum* | Nearly oval | 0.743 | 5.98 | 13.98 | 37.04 | 53.26 | 212.2 | 2 | 195.42 | 61.07 | 28.7 | 133.31 | 11.64 | 6.7 | bifacial |
| *H. pilosella* | Nearly orbicular | 0.679 | 4.87 | 22.49 | 22.22 | 54.03 | 248 | 2 | 175.5 | 62.75 | 24.17 | 99.75 | 14.2 | 6.37 | bifacial |

Table 3. Characters of the leaf epidermis of *Hieracium* species examined. The numbers in parenthesis are the average of length and width of the stomata.

| Taxa | Adaxial epidermis | | | | | Abaxial epidermis | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Size of stomata | | Epidermal cells shape | Anticlinal wall pattern | Stomata density ($mm^{2)}$ | Size of stomata | | Epidermal cells shape | Anticlinal wall pattern | Stomata density ($mm^{2)}$ |
| | Stomata length (µm) | Stomata width (µm) | | | | Stomata length (µm) | Stomata width (µm) | | | |
| *H. umbellatum* | 26 (29.7) 32.6 | 19 (23.7) 28 | Polygonal-slightly irregular | Straight-curved, slightly sinuate | 58.91 | 26 (30.9) 35 | 22 (24.5) 29 | Strongly irregular | Strongly sinuate | 105.6 |
| *H. prenanthoides* | 24 (28.4) 31 | 23 (25.4) 28 | Strongly irregular | Strongly sinuate | 47.33 | 24 (29.5) 34 | 21 (24.9) 27 | Strongly irregular | Strongly sinuate | 135.3 |
| *H. vulgatum* | 29 (34.3) 39 | 27 (28.4) 31 | Nearly irregular | Curved-nearly sinuate | 55.2 | 31 (36.5) 42 | 24 (29.9) 31 | Strongly irregular | Strongly sinuate | 79.09 |
| *H. verruculatum* | 29 (33.1) 36 | 26 (29.4) 32 | Nearly irregular | Curved-nearly sinuate | 55.2 | 31 (36.5) 42 | 24 (29.9) 31 | Strongly irregular | Strongly sinuate | 79.09 |
| *H. cheirifolium* | 31 (35.7) 40 | 30 (33.7) 37 | Strongly irregular | Strongly sinuate | 58.3 | 32 (36.8) 40 | 29 (33.9) 36 | Strongly irregular | Strongly sinuate | 64.78 |
| *H. × maschukense* | 22 (28.48) 32 | 24 (26.28) 29 | Nearly irregular | Nearly sinuate | 79.91 | 22 (27.98) 29 | 22 (25.07) 29 | Strongly irregular | Strongly sinuate | 109.18 |
| *H. echioides* subsp. *echioides* | 29 (33.67) 36 | 25 (28.02) 31 | Strongly irregular | Strongly sinuate | 76.67 | 30 (33.17) 36 | 25 (28.44) 30 | Strongly irregular | Strongly sinuate | 124.65 |
| *H. echioides* subsp. *procerum* | 25 (30.4) 35 | 24 (28.9) 31 | Strongly irregular | Strongly sinuate | 75.07 | 30 (32.13) 38 | 27 (30.07) 33 | Strongly irregular | Strongly sinuate | 120.6 |
| *H. piloselloides* | 30 (33.2) 38 | 25 (28.29) 32 | Strongly irregular | Strongly sinuate | 83.89 | 29 (34.38) 39 | 24 (27.9) 32 | Strongly irregular | Strongly sinuate | 95 84 |
| *H. bauhini* | 27 (29.34) 33 | 24 (28.02) 32 | Irregular | Sinuate | 88.09 | 27 (32.22) 35 | 25 (28.13) 31 | Irregular | Sinuate | 103.1 |
| *H. × sintenisii* | 25 (29.41) 32 | 25 (28.32) 29 | Strongly irregular | Strongly sinuate | 87.01 | 26 (30.18) 33 | 25 (28.43) 31 | Strongly irregular | Strongly sinuate | 87.01 |
| *H. × auriculoides* | 26 (30.39) 34 | 22 (25.5) 29 | Irregular | Sinuate | 115.41 | 29 (32.9) 36 | 24 (27.2) 29.1 | Strongly irregular | Strongly sinuate | 113.7 |
| *H. × panonniciforme* | 26 (29.01) 32 | 23 (25.37) 27 | Strongly irregular | Strongly sinuate | 73.6 | 28 (31.69) 36 | 24 (27.26) 30 | Strongly irregular | Strongly sinuate | 71.56 |
| *H. × matrense* | 28 (30.34) 34 | 24 (27.36) 29 | Strongly irregular | Strongly sinuate | 89 | 29 (33.07) 35 | 24 (27.63) 30 | Strongly irregular | Strongly sinuate | 109.47 |
| *H. × ruprechtii* | 28 (30.07) 23 | 18 (20.47) 23 | Irregular | Sinuate | 97.48 | 18 (21.5) 24 | 20 (22.02) 24 | Irregular | Sinuate | 107.34 |
| *H. hoppeanum* | 16 (20.07) 23 | 18 (20.47) 23 | Polygonal-slightly irregular | Curved-slightly sinuate | 210.14 | 18 (21.5) 24 | 20 (22.02) 24 | Irregular | Sinuate | 189.13 |
| *H. pilosella* | 23 (26.99) 31 | 21 (24.71) 27 | Polygonal-slightly irregular | Curved-slightly sinuate | 175.25 | 25 (29.13) 32 | 23 (26.94) 31 | Nearly irregular | Curved-slightly sinuate | 166.21 |

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