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LEAF EPIDERMAL MICROMORPHOLOGY OF THE GENUS *HYPERICUM* (HYPERICACEAE) FROM IRAN

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Foliar micromorphological characters of 17 species of *Hypericum* L. belonging to five sections, *Androsaemum* (Duhamel) Godron, *Hypericum*, *Hirtella* Stef. (including 2 subsections: *Platyadenum* N. Robson et *Stenadenum* N. Robson), *Taeniocarpum* Jaub. et Spach. and *Drosanthe* (Spach) Endl. were examined using scanning electron microscopy (SEM). Based on the current result three types of epidermal cell shape (irregular polygonal, roughly regular rectangular and irregular elliptical); four types of papilla (triangular, with striate wall, finger and star shapes); two main types and three subtypes of epicuticular wax; seven type classes of outer stomatal and peristomatal rims/stomata ledge aperture; three types of wax distribution on the stomata rims/pore/epidermal cell and three types of inner stomatal rims variations (smooth, sinuolate and sinuolate-erose) were identified. The result revealed that leaf micromorphological evidences of *Hypericum* are taxonomically informative and can be used for separating different sections and species.

Key words: Hypericaceae, *Hypericum*, Iran, leaf, micromorphology

INTRODUCTION

The *Hypericum* comprises about 500 species of herbs, shrubs, and small trees (Robson 1985), taxonomically assigned to Hypericaceae (Crockett and Robson 2011), order Malpighiales (APG VI 2016, Wurdack and Davis 2009), mainly distributed in the temperate regions of the Northern hemisphere and especially in Eurasia (> 230 species) (Stevens 2007). Its elements show a preference for lowland and upland areas of cold temperate and high elevation mountain habitats of tropic and warm temperate regions (Nürk and Blattner 2010, Robson 1977, 1993, Stevens 2007). The genus name (Upereikon) was given by the ancient Greeks to ward off evil spirits (Robson 2003). *Hypericum* is one of few large genera with an almost complete taxonomic treatment (Meseguer *et al*. 2013). The genus was primarily described by Tournefort (1700) and the first taxonomic treatment of the whole genus (synaptic monograph of the Hypericineae) was published by Choisy (1821). This was followed by numerous changes resulted from the extensive studies of Robson, who provided

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a revision of the genus in 1977 and characters descriptions for 457 species, in a series of monographs and papers (Robson 1981, 1985, 1987, 1990, 1993, 1996, 2001, 2003, 2005, 2006, 2010*a*, *b*). In addition, Robson (1968) described 21 representatives of the genus for the area covered by Flora Iranica. Azadi (1999) reported 19 species and 5 sections of *Hypericum* for the flora of Iran. This includes 3 endemic (*Hypericum fursei* N. Robson, *H. dogonbadanicum* Assadi, and *H. asperulum* Jaub. et Spach) (Assadi 1984, Robson 1977) and one doubtful species (*H. heterophyllum*) (Azadi 1999). Among them, *H. perforatum*, *H. scabrum* and *H. helianthemoides* are the most abundant species of the genus in Iran (Azadi 1999, Robson 1968).

The representatives of the genus are well known for their use in traditional medicine (Barnes *et al*. 2001, Bertoli *et al*. 2003, Crockett and Robson 2011, Crockett *et al*. 2005, 2010, Curtis and Lersten 1990, Demirci *et al.* 2005, Jaimand *et al*. 2012, Sajjadi *et al*. 2001, Schwob *et al*. 2004); having great phytochemical potential (Maggi *et al*. 2010, Zobayed *et al*. 2006); anti-depressant, anti-retroviral (Ernst 2003, Wurglics and Schubert-Zsilavecz 2006) and antiviral properties (Sajjadi *et al*. 2001). The genus was subject of several other investigations, e.g. anatomical (Ciccarelli *et al*. 2001, Lotocka and Osińska 2010, Sirvent *et al.* 2003), karyological (Kogi 1984, Robson 1981), reproductive biological and apomixis (Schallau *et al.* 2010) and molecular studies (Nürk *et al*. 2012). However, foliar micromorphological features were poorly studied in this genus (Perrone *et al*. 2013). The current research supplements previous studies as a systematic survey of epidermis micromorphology of species *Hypericum* and related species in Iran, in order to recognise diagnostic and taxonomically important traits. The results can provide strong evidences for classification of *Hypericum*, especially Iranian representatives of the genus.

MATERIALS AND METHODS

Leaf samples used in this study were either collected from different parts of Iran (during 2013–2015) or obtained from Guilan (GUH), Tehran (TUH), Mazandaran Universities and other Iranian herbaria (Table 1). The voucher specimens are deposited in the Guilan University Herbarium. Flora Iranica (Robson 1968) and Flora of Iran (Azadi 1999) were the primary reference books for identification. The terminology used for micromorphology follows Barthlott *et al*. (1998) and Wilkinson (1979).

For scanning electron microscopy (SEM), well-developed leaves were selected, washed in water-detergent for about 20 min (in water bath at 45 °C), and then kept for drying. A portion of dried leaves were mounted on SEM stubs by double-sided adhesive tape of silver paint and finally coated with gold in a sputter coater. Scanning electron microscopy was carried out using a Vega Tescan Razi instrument.

Table 1

Localities and the site data of species used in this study. BH = Babolsar Herbarium, KH = Kurdistan Herbarium, TH = Tabriz Herbarium, TUH = Tehran University Herbarium and TARI = Research Institute of Forests and Rangelands.

Species	Collection site		Collectors	Alt. (m a.s.l.)	Accession No.
1. Section: *Androsaemum*	Guilan	Asalem to Khalkhal	Sabeti, Gheysari	1200	13556 (TUH)
1.1. *H. androsaemum*	Guilan	Talesh road, 5 km to Anzali		800	12798 (TUH)
2. Section: *Hypericum*	Mazandaran	Sange chal	Alinattajomrani	1300	(BH)
2.1. *H. tetrapterum*	Mazandaran	Ramsar to Javaherdeh	Azadi, Nikchehreh	1200	76919 (TUH)
2.2. *H. perforatum*	Mazandaran	Kelardasht, Mejel	Naqinezhad	2000	(BH)
	Mazandaran	Damavand, 5 km to Polur	Gholizadeh Azadi, Nikchehreh	2100	76922 (TUH)
2.3. *H. triquetrifolium*	Kurdistan	Marivan to Khav	Keshavarzi, Sardabi	1350 1300	18325 (TARI) 13595 (TUH)
		Zarivar Lake	Keshavarzi, Sardabi		
3. Section: *Hirtella*	Tehran	Haraz road, 5–8 km to Polur	Amin, Bazargan	2200	75718 (TARI)
3.1. Subsection: *Platyadenum*?	East Azerbaijan	Kordeh deh village	Kasebi, Imani	2400	5955 (TH)
3.1.1. *H. scabrum*					
3.1.2. *H. asperulum*	East Azerbaijan	From band uromieh to Ziveh	Ghahreman,	1500	13573 (TARI)
	Kurdistan	margavar region, Marivan	Aghustin, Maroofi	1320	7284 (KH)
3.1.3. *H. lysimachioides*	Kurdistan	18 km Sanandaj to Kamyaran	Maroofi	2100	8 (KH)
	Kermanshah	Kermanshah to Pareh	Hamzeh, Hatami	1615	22409 (TUH)
3.1.4. *H. hirtellum*	Tehran	Polur	Golkaryeh	2000	13566 (TUH)
		Lar, Gharehbulagh	Parsa	3300	13568 (TUH)
3.2. Subsection: *Stenadenum*	East Azerbaijan	Tabriz to Marand, Mishu-dagh mont. N slop, near Yam village, West of Tabriz	Azadi, Nikchehreh	1950	75614 (TUH)
3.2.1. *H. elongatum*	East Azerbaijan		Azadi, Nikchehreh	2000	75657 (TUH)

Table 1 (continued)

Species	Collection site		Collectors	Alt. (m a.s.l.)	Accession No.
3.2.2. *H. davisii*	East Azerbaijan	Kaleybar to Ahar. 20 km to	Asadi, Mozaffrian	1735	75666 (TARI)
	East Azerbaijan	Ahar, Sambran pass	Asadi, Sardabi	2000	75656 (TUH)
		Abbasabad protected area			
3.2.3. *H. apricum*	East Azerbaijan	Tabriz to Marand. Mishu-Dagh mont, N slope, near Yam village	Azadi, Nikchehreh	1950	75616 (TARI)
3.2.4. *H. helianthemoides*	Ardabil	Kivy	Azadi	1400	75761 (TARI)
	Kurdistan	Baneh, Taleh jar village	Kaffash	1550	4363 (KH)
3.2.5. *H. vermiculare*	Tehran	Damavand to Firuzkuh 27 km	Azadi	2050	75722 (TARI)
	Tehran	to Firuzkuh	Dini, Arazm	2000	169 (TUH)
		Tehran to Abali			
4. Section: *Taeniocarpium*	East Azerbaijan	Kaleybar	Attar, Dadjou	1600	17244 (TUH)
4.1. *H. hirsutum*	East Azerbaijan	Marand to Tabriz	Sabzi, Imani	1600	5081 (TH)
4.2. *H. linarioides*	Kurdistan	West of Sanandaj	Maroofi	1750	1259 (KH)
	East Azerbaijan	Tabriz, Ounebneali village	Ghahremani, Kasebi	1700	4809 (TH)
4.3. *H. fursei*	Guilan	Jirandeh region	Alinattajomrani	2150	(BH)
5. Section: *Drosanthe*	East Azerbaijan	West of Tabriz	Mozaffrian	1700	25859 (TARI)
5.1. *H. hyssopifolium*	East Azerbaijan	Bonab region	Kasebi	1825	4929 (TH)

RESULTS

In the current survey leaf epidermal structures of 17 species of *Hypericum* in Iran were examined (Table 2). Leaves of the all studied taxa are glabrous. However, four types of hair-like structure or papilla including: triangular in *H. hirsutum* (Fig. 1A), *H. hirtellum* (Fig. 1B), *H. scabrum* (Fig. 1C), *H. asperulum* (Fig. 1D); flat, with striate wall in *H. hirtellum* (Fig. 1B) and *H. elongatum* (Fig. 1F); finger shape in *H. scabrum* (Fig. 1C) and *H. elongatum* (Fig. 1F); and star shape papilla in *H. asperulum* (Fig. 1D); *H. scabrum* (Fig. 1E) and *H. elongatum* (Fig. 1F) were recorded.

Secretory cavities are observed in some species of the genus (Fig. 1G–L). They were especially recognised on the leaf adaxial surface of *H. fursei*, *H. davisii*, *H. vermiculare* and either of adaxial and abaxial sides of *H. perforatum*, *H. tetrapterum*, *H. asperulum* and *H. elongatum.* Their shape varies from oval to round in *H. perforatum* (Fig. 1G), *H. tetrapterum* (Fig. 1H), *H. davisii*, *H. elongatum* (Fig. 1I), and *H. fursei* (Fig. 1J); polygonal in *H. asperulum* (Fig. 1K) and sunken funnel shape in *H. vermiculare* (Fig. 1L).

Microscopic investigations displayed different shapes and patterns of epidermal cells forming the leaf surfaces. This includes: polygonal, irregular and isodiametric in *H. perforatum*, *H. tetrapterum*, *H. triquetrifolium* (lower side); *H. linarioides*, *H. hirsutum*, *H. vermiculare* and *H. elongatum*, rectangular roughly regular and isodiametric in *H. fursei*, *H. davisii*, *H. vermiculare* (upper side), *H. triquetrifolium* (upper side) and *H. helianthemoides* (upper side); elliptical, irregular and isodiametric in *H. hyssopifolium* and *H. helianthemoides* (lower side) in shape.

SEM observation revealed two main types and three subtypes of epicuticular wax patterns/crystals including (Fig. 2A–L).

Type I. Crystalloid: in this type epidermal cells carry only one type of epicuticular wax crystals (either platelets or rodlets). Two categories of platelets were recorded: parallel grouped platelet (including parallel stalked and parallel oriented platelet) and irregular platelet. Parallel stacked platelets were observed in 7 species: *H. tetrapterum* (Fig. 2A), *H. perforatum*, *H. hirsutum* (Fig. 2B), *H. linarioides* (Fig. 2C), *H. davisii*, *H. asperulum* and *H. elongatum* and parallel oriented platelets were present in *H. vermiculare* (Fig. 2D), irregular platelet in *H. triquetrifolium* (Fig. 2E) and rodlet is found in *H. scabrum* (Fig. 2F).

Type II. Syntopism: in this type the epidermal cell includes morphologically different crystalloids and three subtypes as followings:

- subtype I: this composed of two types of crystalloids in the same or different epidermal cells. The smooth layer and platelets in *H. helianthemoides* (Fig. 2G); crust and irregular platelets in *H. apricum* (Fig. 2H), *H. elongatum* and *H. fursei*; crust and parallel stacked platelets in *H. perforatum*, *H. linarioides* and *H. lysimachioides*; crust and parallel oriented platelets in *H. vermicu-*

Table 2
Leaf micro-morphological characters of Iranian species of *Hypericum*

Epidermal cell pattern (abaxial side/adaxial side)	Anticlinal / periclinal walls (adaxial side)	Anticlinal / periclinal walls (abaxial side)	Wax type of (adaxial side)	Wax type of (abaxial side)	Species
Reticulate / Reticulate	protruded / depressed-oblate	protruded / depressed-oblate	irregular platelets + crust + granules + aggregate + rodlets	irregular platelets + crust + granules	1. Section: *Androsaemum* 1.1. *H. androsaemum*
Reticulate / Reticulate	protruded / depressed	protruded + depressed / depressed	dense irregular platelet	dense irregular platelet	2. Section: *Hypericum* 2.1. *H. tetrapterum*
Reticulate / Reticulate	depressed / protruded	protruded / depressed	crust (at the centre) + platelets	dense irregular platelet	2.2. *H. perforatum*
Reticulate / Striate	protruded / depressed	depressed / protruded	dense irregular platelet	dense irregular platelet	2.3. *H. triquetrifolium*
Reticulate / Reticulate	depressed / protruded	protruded / depressed	rodlet platelets + cylindrical finger shape papillae	dense platelet + cuticular folding + triangular appressed papillae	3. Section: *Hirtella* 3.1. *H. scabrum*
Reticulate / Reticulate	depressed / protruded	protruded / depressed	irregular platelets + "cuticular" + "forked papilla"	irregular platelets + cuticular folding	3.2. *H. asperulum*
Regulate-Reticulate / Reticulate	oblate-depressed / protruded	depressed / protruded	irregular platelets + plates	crust + irregular platelets + plates	3.3. *H. lysimachioides*
Regulate-Reticulate / Regulate-Reticulate	protruded / protruded	protruded / oblate	irregular plates, crust and cylindrical papillae with striate wall	irregular plates + crust + cylindrical papillae with striate wall	3.4. *H. hirtellum*

Table 2 (continued)

Epidermal cell pattern (abaxial side/adaxial side)	Anticlinal / periclinal walls (adaxial side)	Anticlinal / periclinal walls (abaxial side)	Wax type of (adaxial side)	Wax type of (abaxial side)	Species
Regulate-Reticulate / Regulate-Reticulate	depressed / protruded	depressed / protruded	dense platelets (rodlet) + crust	irregular platelets + cuticular folding	4. Section: *Stenadenum* 4.1. *H. elongatum*
Striate-favularit / Striate-favularit	protruded / depressed	protruded / depressed	platelet + crust	dense platelet	4.2. *H. davisii*
Regulate-Reticulate / Regulate-Reticulate	depressed / protruded	depressed / protruded	irregular platelets + crust	irregular platelets + crust + cuticular folding	4.3. *H. apricum*
Regulate-Reticulate / Reticulate	depressed / oblate	protruded-ribbed / protruded	smooth layer + scattered platelets	irregular platelets + crust	4.4. *H. helianthemoides*
Regulate-Reticulate / Striate-favularit	protruded / depressed	protruded-depressed / depressed-oblate	crust + semi-orbicular platelets	irregular platelets	4.5. *H. vermiculare*
Regulate-Reticulate / Reticulate	ribbed-regulate / oblate	ribbed / protruded	platelet + crust	dense platelet	5. Section: *Taeniocarpium* 5.1. *H. hirsutum*
Reticulate / Reticulate	depressed / protruded	depressed / protruded	dense platelets	dense platelets + crust	5.2. *H. linarioides*
Reticulate / Reticulate	depressed / protruded	depressed / protruded	irregular platelets + crust + granules	dense irregular platelets + crust	5.3. *H. fursei*
Reticulate / Reticulate	protruded / depressed-oblate	depressed / protruded	membranous platelets + crust + cuticular folding	membranous platelet + crust + cuticular folding	6. Section: *Drosanthe* 6.1. *H. hyssopifolium*

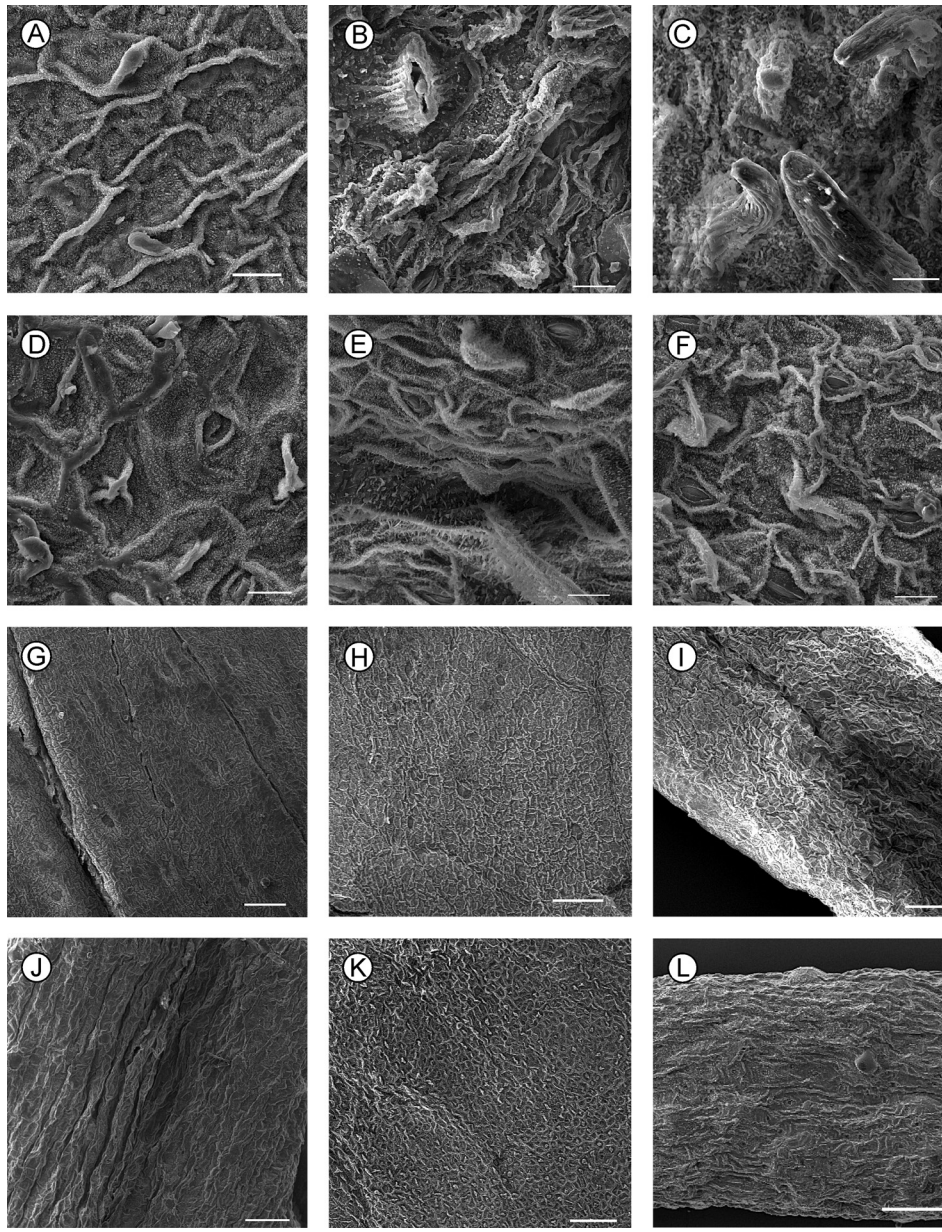

Fig. 1. A = *Hypericum hirsutum*; B = *H. hirtellum*; C, D = *H. scabrum*; E = *H. asperulum*; F = *H. elongatum*; G = *H. perforatum*; H = *H. tetrapterum*; I = *H. elongatum*; J = *H. fursei*; K = *H. asperulum*; L = *H. vermiculare* (scale bar = 20 μm for A–F; 200 μm for G–L)

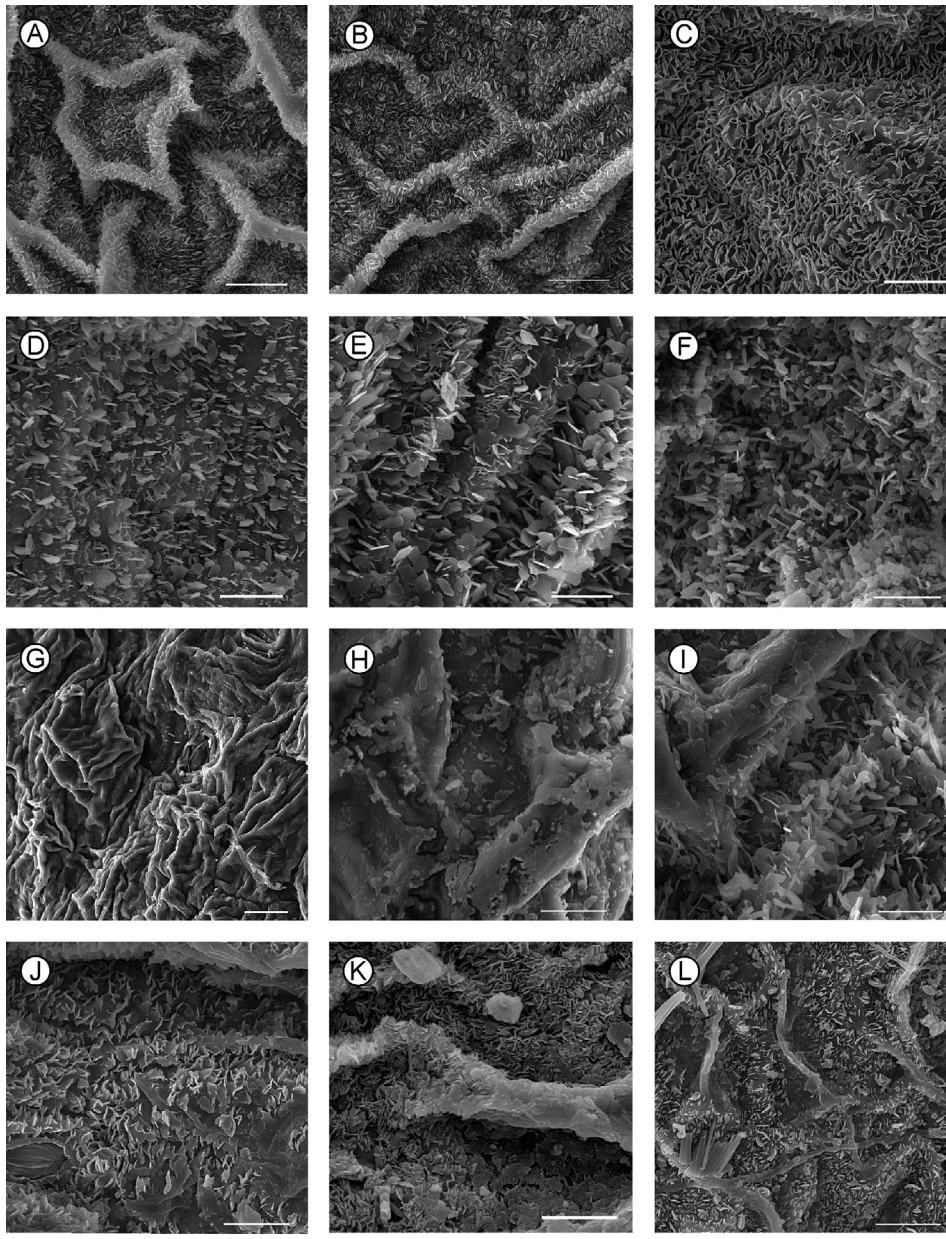

Fig. 2. A = *Hypericum tetrapterum*; B = *H. perforatum*; C = *H. vermiculare*; D, E = *H. triquetrifolium*; F = *H. scabrum*; G = *H. helianthemoides*; H, I = *H. apricum*; J = *H. hyssopifolium*; K = *H. lysimachioides*; L = *H. androsaemum* (scale bar = 20 μm)

lare; crust and membranous platelets in *H. fursei*, and crust and polyangular rodlet in *H. davisii* and *H. apricum* (Fig. 2I);
- subtype II: this composed of three different crystalloids (crust/platelets and plates) in the same or different epidermal cells. Plates are flat, often polygonal crystalloids, with distinct edges. This subtype was recorded in *H. hyssopifolium* (crust/membranous platelets and plates) (Fig. 2J) and *H. lysimachioides* (crust/parallel stacked platelets and plates) (Fig. 2K);
- subtype III: this includes four different crystalloids (crust/parallel stacked platelets/granules/longitudinally aggregated rodlets), recorded in *H. androsaemum* (Fig. 2L).

We observed cuticular folding in nine species: *H. elongatum, H. helianthemoides* (Fig. 2G), *H. lysmachioides*, *H. davisii*, *H. vermiculare*, *H. fursei*, *H. linarioides*, *H. hyssopifolium* and *H. apricum*.

The studied taxa were divided into two groups, regarding the position of stomata in relation to epidermal cells. The first group includes 12 species with stomata upper than epidermal cells and the second group contains 16 species with stomata lower than epidermal cells. Stomata position on adaxial and abaxial sides of a leaf, were identical in 5 species including *H. hirsutum* and *H. hyssopifolium* (stomata upper than epidermis); *H. asperulum*, *H. androsaemum* and *H. apricum* (stomata lower than epidermis).

Three types wax distribution on the stomata rims, pore and epidermal cells were identified:
- Type I. Stomata rim and guard cell covered by wax, pore free (includes 9 species);
- Type II. Stomata rims and pore free guard cell covered by wax (composed of 13 species);
- Type III. Stomata rims, guard cell and pore covered by wax (recorded in *H. scabrum* and *H. elongatum*).

The current SEM analysis also revealed 7 type classes of outer stomatal rims/peristomatal rims and stomata ledges aperture characters on both side of a leaf as followings:
- Type I. Raised/raised: this type was identified in *H. androsaemum* (Fig. 3A);
- Type II. Raised/overlapping: this type was recorded in *H. perforatum* (Fig. 3B), *H. apricum*, *H. hirsutum* and *H. helianthemoides* (Fig. 3C);
- Type III. Raised/overlapping-stout: this type was observed in *H. perforatum*, *H. elangatum* and *H. scabrum* (Fig. 3D);
- Type IV. Double raised/overlapping: this type was found in *H. vermiculare* (Fig. 3E);
- Type V. Overlapping/overlapping: this type was recognised in *H. helianthemoides* (Fig. 3F), *H. fursei* and *H. hyssopifolium* (lower surface);

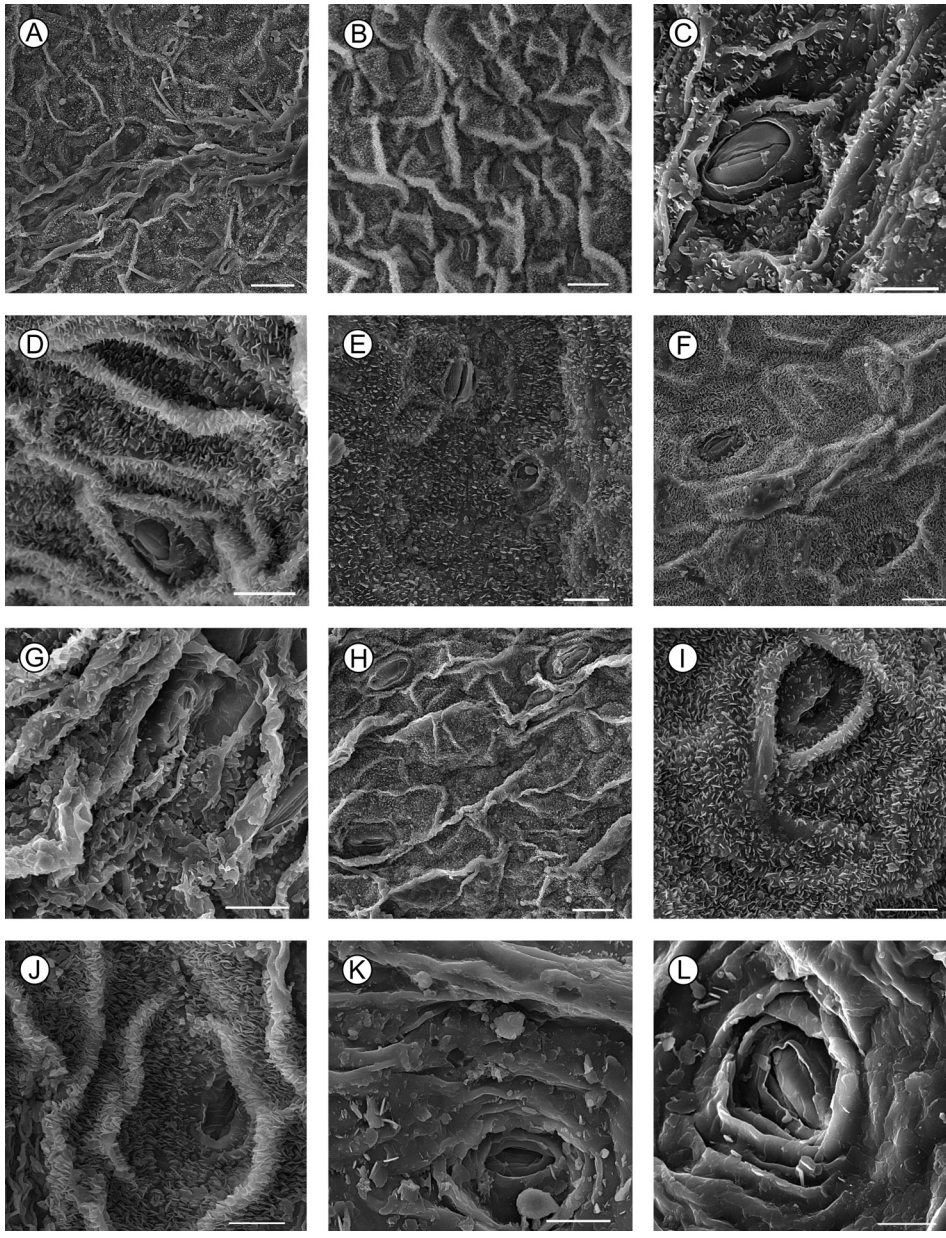

Fig. 3. A = *Hypericum androsaemum*; B = *H. hirsutum*; C = *H. helianthemoides*; D = *H. perforatum*; E = *H. scabrum*; F = *H. vermiculare*; G = *H. linarioides*; H = *H. fursei*; I = *H. hirtellum*; J = *H. asperulum*; K = *H. davisii* (scale bar = 10 μm)

- Type VI. Overlapping/overlapping-stout: this type was distinguished in *H. tetrapterum* and *H. hirtellum* (Fig. 3G);
- Type VII. Overlapping/stout: this type was observed in *H. asperulum* (Fig. 3H), *H. lysimachioides*, *H. davisii* (Fig. 3J), *H. vermiculare* (Fig. 3K) and *H. helianthemoides* (Fig. 3L).

Three types of inner stomatal rims variations were also recorded:

- Type I. Smooth inner stomatal rims: this type was observed in *H. androsaemum* (Fig. 3A);
- Type II. Sinuolate: this type was present in 7 species including: *H. triquetrifolium*, *H. hirtellum*, *H. helianthemoides* (Fig. 3L), *H. apricum*, *H. vermiculare* (Fig. 3K), *H. hirsutum* and *H. furseii*;
- Type III. Sinuolate-erose: this type was found in the 9 species: *H. tetrapterum*, *H. perforatum*, *H. scabrum* (Fig. 3I), *H. asperulum*, *H. lysimachioides*, *H. davisii* (Fig. 3J), *H. linarioides*, *H. hyssopifolium* (Fig. 3H) and *H. elongatum*.

DISCUSSION

This study demonstrates variation of micromorphological characters among the investigated taxa. Papilla, secretory cavities, epidermal cell shape and walls; epicuticular wax and cuticular folding; distribution of wax on the stomata rims, pore and epidermal cells; outer, inner and peristomatal rims type classes were the most important cells and surface sculptures (Koch *et al*. 2009) described in this study. Papillae or hair like structures are common to *Hypericum* (Metcalfe and Chalk 1957), especially in *H. androsacaeum*, *H. aegypticum*, *H. calycinum*, *H. cerastioides* and *H. olympicum* (Neinhuis and Barthlott 1997). They are small convex outgrowth, originated by expansion of the outer epidermis cell (Koch *et al*. 2009). In this research four morphologically distinct types of papilla were identified that can be used to separate different species (Wilkinson 1979).

In contrast to glandular hairs (that are unknown in this genus), secretory cavities (Metcalfe and Chalk 1957) or translucent secretory reservoirs (Perrone *et al*. 2013) have been reported in some species, e.g. *H. perforatum*, *H. tetrapterum*, *H. fursei* and *H. vermiculare*. Based on the findings their shape varied from species to species.

Epidermal cells shapes varied among the studied taxa and three types were identified.

In the previous study irregular isodiametric-elongated (in *H. perfoliatum*), irregular polyhedral convex cells (in *H. pubescens*), rectangular and elliptical isodiametric cells (in *H. hircinum*) epidermal cell shape were also recorded in different species of the genus (Perrone *et al*. 2013).

Based on the current result, leaf epidermal surface of the studied species benefit dense epicuticular wax covering (crystalloids and syntopism types).

Parallel stacked platelets were the most dominant patter among the examined species. This characteristic of family Hypericaceae was described as '*Hypericum*'-type by Ditsch and Barthlott (1994). In addition to wax crystals, epidermis of all examined taxa possess cuticular folds that are composed of medium (including smooth layer), 2 (crust) and 3 dimensional wax elements (different types of platelets, plates and polyangular rodlets) (Barthlott and Ehler 1977).

The studied taxa were divided into two main groups regarding the position of stomata. However, the extent to which stomata are raised above or sunken below the epidermis is of very limited importance in compared with their appearance in surface view (Metcalfe and Chalk 1957). In contrast, outer, inner and peristomatal rims configuration types, distribution of wax on the stomata rims, pore and guard cell patterns are considered as taxonomically informative traits (Akçin *et al.* 2013, Kumar and Murugan 2015).

Leaf micromorphological and structural diversity of the studied species may be attributed to different environmental conditions (Barthlott and Ehler 1977, Jetter and Riederer 1994, Koch *et al*. 2009). Iranian species of *Hypericum* are distributed in different phytological regions from mountain slopes; stony places, calcareous rocks, subalpine meadows; forest margins, floodplains and rivers habitats (Azadi 1999, Robson 1968). They are dominant in Irano-Turanian phytogeographical region characterised by low rainfall, long pride of dryness in summer, cold weather and frost in long winter time (Ghahreman and Attar 1999). This comprises several species of *Hirtella* section (with petals tapering to a claw and black capitate stipitate glands at apex) especially *H. scabrum* and *H. hirtellum* (from subsection *Platyandenum*) and *H. elongatum*, *H. helmanthoidae*, *H. vermiculare*, *H. apricum* and *H. davisii* (from subsection *Stenadeanum*) growing in dry steppe, gravelly mountain slopes and rocks of N, NW, E, C and S Iran at 800–3,400 m altitude (Azadi 1999, Robson 1968). All the above species have identical micromorphological characters (e.g. epicuticular wax types; star and triangular papilla; covered stomata rims and guard cell; raised outer stomata, overlapping-stout peristomata and sinuolate-erose stomata rims) that supports the current classification (Azadi 1999, Robson 1968).

However, *H. asperulum* and *H. lysimachioides* (from subsection *Platyandenum*) are two representatives of the same section, distributed in the Mediterranean region and Zagros phytogeographical subregion. This region borders the drier zones of central and southern Iran with annual rainfall of about 400 m (semidesert area) and xerophyte flora in subalpine belt, dry sunny, stony, mostly calcareous slopes (Ghahreman and Attar 1999). These species also show both micromorphological (e.g. types of cuticular folding; sunken stomata, overlapping/stout outer stomata, peristomatal and stomatal rims) and morphological affinities (ovate, oblong and lanceolate leaves; broad ovate and beaked capsule) (Azadi 1999, Robson 1968).

H. hirsutum, *H. fursei* (Iranian endemic) and *H. androsaemum* are growing in Hyrcanian, Caspian phytogeographical subregion (with high humidity and abundant rainfall of 700–1,500 mm). The two former species are placed in *Taeniocarpum* section along with *H. linarioides* (distributed in either of the Irano-Turanian and Hyrcanian regions). *H. hirsutum*, *H. fursei* have common morphological characters (soft, unicellular spreading, white hairs on stem, leaves and pedicels). While *H. linarioides*, separated from them by its glandular calyx margin, inflorescence with few flowers and cylindrical and distinct micromorphological trait especially absence of triangular papilla. *H. androsaemum* is a mesophyte (Peron *et al*. 2007) having distinct micromorphological (aggregate rodlets, raised stomata rims, peristomatal rims, smooth stomatal rims) and morphological traits (stamens in 5 bundles, 1 celled capsule, and keeled or winged seed) classified in *Androsaemum* section (Robson 1968, Shishkin 1949).

Despite of morphological similarities (e.g. stamens arranged in 3 bundles; leaves without or very few black glands, calyx margin and capsule surface with black gland), three species *Hypericum* including *H. tetrapterum* (mesohygrophyte), *H. perforatum* (mesophyte) and *H. triquetrifolium* (xerophyte), exhibit different ecological characterisation (Perrone *et al*. 2013). The two former species occur in the Hyrcanian and Mediterranean phytogeographical regions (with dry climate, 302 mm annual rainfall, including semidry forests and discontinuous limestone mountain) (Ghahreman and Attar 1999). While the third representative grows in the Irano-Turanian and Mediterranean phytogeographical regions. Based on the current result *H. tetrapterum* and *H. perforatum* can be separated from *H. triquetrifolium* (with non-entire platelet wax type) by some important micromorphological evidences, especially parallel stacked platelet sculpturing types.

CONCLUSION

Present results revealed a number of interesting epidermal micromorphological features in the genus *Hypericum*. They attributed to different environmental conditions and show ecological characterisation. These micromorphological data have diagnostic value and support the current classifications.

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REFERENCES

Akçin, E. Ö., Şenel, G. and Akcin, Y. (2013): Leaf epidermis morphology of some Onosma (Boraginaceae) species from Turkey. – *Turkish J. Bot.* **37**: 55–64.

APG IV (Angiosperm Phylogeny Group) (2016): An update of the angiosperm phylogeny group classification for the orders and families of flowering plants: APG IV. – *Bot. J. Linn. Soc.* **181**(1): 1–20. https://doi.org/10.1111/boj.12385

Assadi, M. (1984): New species and plant records from Iran. – *Iranian J. Bot.* **2**: 83–93.

Azadi, R. (1999): *Guttiferae*. – In: Assadi, M. *et al*. (eds): Flora of Iran. Vol. 27. R.I.F.R., Tehran, pp. 1–62.

Barnes, J., Anderson, L. A. and Phillipson, D. J. (2001): St. John's wort (Hypericum perforatum L.): a review of its chemistry, pharmacology and clinical properties. – *J. Pharm. Pharmacol*. **53**: 583–600. https://doi.org/10.1211/0022357011775910

Barthlott, W. and Ehler, N. (1977): Raster-Elektronenmikroskopie der Epidermisoberflächen von Spermatophyten. – *Trop. subtrop. Pflanzenwelt* **19**: 367–467.

Barthlott, W., Neinhuis, C., Cutler, D., Ditsch, F., Meusel, I., Theisen, I. and Wilhelmi, H. (1998): Classification and terminology of plant epicuticular waxes. – *Bot. J. Linn. Soc*. **126**: 237–260. https://doi.org/10.1111/j.1095-8339.1998.tb02529.x

Bertoli, A., Menichini, F., Mazzetti, M., Spinelli, G. and Morelli, I. (2003): Volatile constituents of the leaves and flowers of Hypericum triquetrifolium Turra. – *Flav. Frag. J.* **18**: 91–94. https://doi.org/10.1002/ffj.1161

Choisy, J. D. (1821): *Prodromus d'une monographie de la famille des Hypéricinées*. – J. J. Paschoud, Imprimeur-Libraire, Genève, Paris.

Ciccarelli, D., Andreucci, A. C. and Pagni, A. M. (2001): Translucent glands and secretory canals in Hypericum perforatum L. (Hypericaceae): morphological, anatomical and histochemical studies during the course of ontogenesis. – *Ann. Bot*. **88**: 637–644. https://doi.org/10.1006/anbo.2001.1514

Crockett, S. L. and Robson, N. K. (2011): Taxonomy and chemotaxonomy of the genus Hypericum. – *Med. Aromat. Plant Sci. Biotechnol*. **5**: 1–13.

Crockett, S. L., Schaneberg, B. and Khan, I. (2005): Phytochemical profiling of new and old world Hypericum (St. John's wort) species. – *Phytochem. Anal*. **16**: 479–485. https://doi.org/10.1002/pca.875

Crockett, S. L., Eberhardt, M., Kunert, O. and Schühly, W. (2010): Hypericum species in the paramos of central and south America: a special focus upon H. irazuense Kuntze ex N. Robson. – *Phytochem. Rev*. **9**: 255–269. https://doi.org/10.1007/s11101-009-9148-2

Curtis, J. and Lersten, N. (1990): Internal secretory structures in Hypericum (Clusiaceae): H. perforatum L. and H. balearicum L. – *New Phytol*. **114**: 571–580. https://doi.org/10.1111/j.1469-8137.1990.tb00427.x

Demirci, B., Baser, K. H. C., Crockett, S. L. and Khan, I. A. (2005): Analysis of the volatile constituents of Asian Hypericum L. (Clusiaceae, Hyperidoideae) species. – *J. Essent. Oil Res*. **17**: 659–663. https://doi.org/10.1080/10412905.2005.9699026

Ditsch, F. and Barthlott, W. (1994): Mikromorphologie der Epicuticularwachse und die Systematik der Dilleniales, Lecythidales, Malvales und Theales. – *Trop. subtrop. Pflanzenwelt* **88**: 7–74.

Ernst, E. (2003): *The genus Hypericum*. – Taylor and Francis, London, New York.

Ghahreman, A. and Attar, F. (1999): – *Biodiversity of plant species in Iran*. – Tehran University Press, Tehran.

Jaimand, K., Rezaee, M. B., Naderi, M., Mozaffrian, V., Azadi, R., Karimi, S. H. and Gholipoor, M. (2012): Chemical composition of the essential oils of six Hypericum species (Hypericaceae) from Iran. – *J. M. P. B.* **1**: 7–11.

Jetter, R. and Riederer, M. (1994): Epicuticular crystals of nonacosan-10-ol: in vitro reconstitution and factors influencing crystal habits. – *Planta* **195**: 257–270. https://doi.org/10.1007/bf00199686

Koch, K., Bohn, F. H. and Barthlott, W. (2009): Hierarchically sculptured plant surfaces and superhydrophobicity. – *Langmuir* **25**(24): 14116–14120. https://doi.org/10.1021/la9017322

Kogi, M. (1984): A karyomorphological study of the genus Hypericum (Hypericaceae) in Japan. – *J. Plant Res*. **97**: 333–343. https://doi.org/10.1007/bf02488666

Kumar, V. S. A. and Murugan, K. (2015): Taxonomic implications with special references to stomatal variations in Solanum species using light and scanning electron microscope. – *Int. J. Appl. Biol. Pharm*. **6**(2): 113–125.

Lotocka, B. and Osińska, E. (2010): Shoot anatomy and secretory structures in Hypericum species (Hypericaceae). – *Bot. J. Linn. Soc*. **163**: 70–86. https://doi.org/10.1111/j.1095-8339.2010.01046.x

Maggi, F., Cecchini, C., Cresci, A., Coman, M. M., Tirillini, B., Sagratini, G. and Papa, F. (2010): Chemical composition and antimicrobial activity of the essential oils from several Hypericum taxa (Guttiferae) growing in central Italy (Appennino Umbro-Marchigiano). – *Chem. Biodiv*. **7**: 447–466. https://doi.org/10.1002/cbdv.200900091

Meseguer, A. S., Aldasoro, J. J. and Sanmartín, I. (2013): Bayesian inference of phylogeny, morphology and range evolution reveals a complex evolutionary history in St. John's wort (Hypericum). – *Mol. Phyl. Evol*. **67**(2): 379–403. https://doi.org/10.1016/j.ympev.2013.02.007

Metcalfe, C. R. and Chalk, L. (1957): *Anatomy of Dicotyledons*. Vol. 1. – Clarendon Press, Oxford, 724 pp.

Neinhuis, C. and Barthlott, W. (1997): Characterization and distribution of water-repellent, self-cleaning plant surfaces. – *Ann. Bot.* **79**(6): 667–677. https://doi.org/10.1006/anbo.1997.0400

Nürk, N. M. and Blattner, F. R. (2010): Cladistic analysis of morphological characters in Hypericum (Hypericaceae). – *Taxon* **59**: 1495–1507. https://doi.org/10.2307/20774044

Nürk, N. M., Mandriñán, S., Carine, M. A., Chase, M. W. and Blattner, F. R. (2012): Molecular phylogenetic and morphological evolution of St. John's Wort (Hypericum; Hypericaceae). – *Mol. Phyl. Evol.* **66**: 1–16. https://doi.org/10.1016/j.ympev.2012.08.022

Perrone, R., Rosa, P. D., Castro, O. D. and Colombo, P. (2013): Leaf and stem anatomy in eight Hypericum (Clusiaceae). – *Acta Bot. Croat.* **72**: 269–286. https://doi.org/10.2478/botcro-2013-0008

Qu, L.-P., Widrlechner, M. P. and Rigby, S. M. (2010): Analysis of breeding systems, ploidy, and the role of hexaploids in three Hypericum perforatum L. populations. – *Ind. Crops. Prod*. **32**: 1–6. https://doi.org/10.1016/j.indcrop.2010.01.005

Robson, N. K. B. (1968): *Hypericum L.* – In: Rechinger, K. H. (ed.): Flora Iranica. Akad. Druck u. verlagsanstalt, Graz, Austria, pp. 2–20.

Robson, N. K. B. (1977): Studies in the genus Hypericum L. (Guttiferae): 1. Infrageneric classification. – *Bull. Brit. Mus. (Nat. Hist.) Bot.* **5**: 291–355.

Robson, N. K. B. (1981): Studies in the genus Hypericum L. (Guttiferae): 2. Characters of the genus. – *Bull. Brit. Mus. (Nat. Hist.) Bot.* **8**: 55–226.

Robson, N. K. B. (1985): Studies in the genus Hypericum L. (Guttiferae): 3. Sections 1. Campylosporus to 6a. Umbraculoides. – *Bull. Brit. Mus. (Nat. Hist.) Bot.* **12**: 1–325.
Robson, N. K. B. (1987): Studies in the genus Hypericum L. (Guttiferae): 7. Section 29. Brathys part 1. – *Bull. Brit. Mus. (Nat. Hist.) Bot.* **16**: 1–106.
Robson, N. K. B. (1990): Studies in the genus Hypericum L. (Guttiferae): 8. Sections 29. Brathys part 2. Trigynobrathys. – *Bull. Nat. Hist. Mus. London, Bot.* **20**: 1–151.
Robson, N. K. B. (1993): Parallel evolution in tropical montane Hypericum. – *Opera Bot.* **121**: 263–274.
Robson, N. K. B. (1996): Studies in the genus Hypericum L. (Guttiferae): 6. Sections 20. Myriandra to 28. Elodes. – *Bull. Nat. Hist. Mus. London, Bot.* **26**: 75–217.
Robson, N. K. B. (2001): Studies in the genus Hypericum L. (Guttiferae) 4 (1). Sections 7. Roscyna to 9. Hypericum sensu lato (part 1). – *Bull. Nat. Hist. Mus. London, Bot.* **31**: 37–88.
Robson, N. K. B. (2003): *Hypericum botany*. – In: Ernst, E. (ed.): The genus Hypericum. Taylor and Francis, London, New York, pp. 1–22.
Robson, N. K. B. (2005): Hypericum sect. Ascyreia (Clusiaceae): new species and subspecies from China. – *Acta Phytotax. Sinica* **43**: 271–277.
Robson, N. K. B. (2006): Studies in the genus Hypericum L. (Clusiaceae). Section 9. Hypericum sensu lato (part 3): subsection 1. Hypericum series 2. Senanensia, subsection 2. Erecta and section 9b. Graveolentia. – *Syst. Biodivers.* **4**: 19–98. https://doi.org/10.1017/s1477200005001842
Robson, N. K. B. (2010*a*): Studies in the genus Hypericum L. (Hypericaceae) 5 (2). Section 17. Hirtellato 19. Coridium. – *Phytotaxa* **4**: 127–258. https://doi.org/10.11646/phytotaxa.4.1.3
Robson, N. K. B. (2010*b*): Studies in the genus Hypericum L. (Hypericaceae) 5 (1). Section 10. Olympiato 15/16. Crossophyllum. – *Phytotaxa* **4**: 5–126. https://doi.org/10.11646/phytotaxa.4.1.2
Sajjadi, S. E., Rahiminezhad, M., Mehregan, I. and Poorasar, A. (2001): Constituents of essential oil of Hypericum dogonbadanicum Assadi. – *J. Essent. Oil Res.* **13**: 43–44. https://doi.org/10.1080/10412905.2001.9699600
Schallau, A., Arzenton, F., Johnston, A. J., Hähnel, U., Koszegi, D., Blattner, F. R., Altschmied, L., Haberer, G., Barcaccia, G. and Bäumlein, H. (2010): Identification and genetic analysis of the apospory locus in Hypericum perforatum L. – *Plant J.* **62**: 773–784. https://doi.org/10.1111/j.1365-313x.2010.04188.x
Schwob, I., Bessiere, J. M., Masotti, V. and Viano, J. (2004): Changes in essential oil composition in Saint John's wort (Hypericum perforatum L.) aerial parts during its phenological cycle. – *Biochem. Syst. Ecol.* **32**: 735–745. https://doi.org/10.1016/j.bse.2003.12.005
Shishkin, B. K. (1949): *Flora of the U.S.S.R. XV. Malvales, Parietales, Myrtiflorae*. – Izdatel'stvo Akademii Nauk SSSR, Moskva, Leningrad.
Sirvent, T. M., Krasnoff, S. B. and Gibson, D. M. (2003): Induction of hypericins and hyperforins in Hypericum perforatum in response to damage by herbivores. – *J. Chem. Ecol.* **29**: 2667–2681. https://doi.org/10.1023/b:joec.0000008011.77213.64
Stevens, P. F. (2007): *Hypericaceae*. – In: Kubitzki, K. (ed.): The families and genera of vascular plants. Springer Verlag, Berlin, Heidelberg, pp. 194–201.
Tournefort, J. P. (1700): *Institutiones rei herbariae*. – Typographia Regia J. Anisson, Paris.
Wilkinson, H. P. (1979): *The plant surface (mainly leaf). Part 1: Stomata*. – In: Metcalfe, C. R. and Chalk, L. (eds): Anatomy of the Dicotyledons. 2nd ed. Clarendon Press, Oxford, pp. 97–167.

Wurdack, K. J. and Davis, C. C. (2009): Malpighiales phylogenetics: gaining ground on one of the most recalcitrant clades in the angiosperm tree of life. – *Am. J. Bot*. **96**: 1551–1570. https://doi.org/10.3732/ajb.0800207

Wurglics, M. and Schubert-Zsilavecz, M. (2006): Hypericum perforatum: a 'modern' herbal antidepressant pharmacokinetics of active ingredients. – *Clinic. Pharmacokin.* **45**: 449–468. https://doi.org/10.2165/00003088-200645050-00002

Zobayed, S. M. A., Afreen, F., Goto, E. and Kozai, E. T. (2006): Plant environment interactions: accumulation of hypericin in dark glands of Hypericum perforatum. – *Ann. Bot*. **98**: 793–804. https://doi.org/10.1093/aob/mcl169