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Leaf and stem anatomy in eight *Hypericum* species (Clusiaceae)

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Abstract – Foliar micromorphology, epicuticular wax morphology and anatomical features of leaves and stem, particularly secondary xylem, were examined with light microscopy, general and histochemical staining and scanning electron microscopy in eight *Hypericum* species. Outer tegument tissue and type of secondary xylem are determining characteristics. Secondary xylem is ring-porous in *H. perforatum, H. perfoliatum, H. tetrapterum, H. triquetrifolium, H. androsaemum* and *H. hircinum.* In *H. aegypticum* and *H. pubescens* xylem is diffuse-porous, which is considered to be a more primitive type. These characteristics may be considered an additional criterion for species identification.

Keywords: Anatomy, epiderma, epicuticular wax, *Hypericum***,** morphology, SEM, stem, xylem

Introduction

The genus *Hypericum* L. includes about 460 species of tree, shrub and herb and is distributed worldwide. It has great phytochemical potential, with its secretory structures specialized in the synthesis and accumulation of biologically active substances (BLENK 1884; GREEN 1884; WEILL 1903; SIERSCH 1927; METCALFE et al. 1950; CURTIS and LERSTEN 1990; NAHRSTED and BUTTERWECK 1997; BARONI FORNASIERO et al. 1998; BARONI FORNASIERO et al. 2000; CICCARELLI et al. 2001a, b; ONELLI et al. 2002) in both vegetative and reproductive organs. This phytochemical diversity has been investigated in taxonomic and morphological studies (CROCKETT and ROBSON 2011, NURK and CROCKETT 2011). Only METCALFE et al.(1950) have made specifically anatomical studies, and all the most recent work has investigated the secretory structures (LOTOCKA and OSINSKA 2010, GITEA et al. 2011). This study contributes to knowledge of vegetative organ anatomy in *Hypericum* species from heteroge-

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neous environments. Our aim is thus to provide a detailed description of the micro-morphological and anatomical characteristics of the leaf and stem in *Hypericum* species.

Materials and methods

Plant sampling

The samples were collected directly from the sites in the literature (GIARDINA et al. 2007) during the flowering period. The following were examined (TUTIN et al. 1972, PIGNATTI 1982):

Hemicryptophyte scapose (H scap)

Mesophytes

Hypericum perforatum L. – present throughout Europe except in the far north, present throughout Italy up to an altitude of 1400 m;

H. perfoliatum L. – grows in Mediterranean regions, present throughout Italy up to an altitude of 1400 m;

H. pubescens Boiss. – colonizes humid environments, salty habitats at times, and is present in Portugal and southern Spain, Sicily and Malta;

Meso-hygrophyte

Hypericum tetrapterum Fr. – colonizes marshes and cane-brakes, present in south-central Europe, extends into Sweden and grows throughout Italy;

Xerophyte

Hypericum triquetrifolium Turra – grows in the eastern Mediterranean, in Italy present only in the southernmost regions.

Nanophanerophytes (NP)

Mesophytes

Hypericum androsaemum L. – present in western Europe, locally in southern Europe, and is found throughout Italy up to an altitude of 1400 m;

H. hircinum L. – in moist, shady localities, present in the Mediterranean region and found throughout central and southern Italy.

Chamaephyte fruticose (Ch frut)

Xero-halophyte

Hypericum aegypticum L. – colonizes maritime cliffs and is present on central-eastern Mediterranean islands from Sardinia to Crete.

Tissue analysis

Some samples were sectioned when fresh and stained with phloroglucinol in alcoholic solution and 2% hydrochloric acid for lignified components of the cell wall, with chlor-zinc iodide for the presence of cellulose in plant tissue, Sudan III to ethyl alcohol at 80% saturated solution for cutin and suberin (CATALANO 1925), iodine iodide solution (Lugol) for starch (JOHANSEN, 1940), Ruthenium Red for pectic-like substances (JENSEN 1962) and potassium bichromate for tannins (FAURE 1914). Other samples were fixed with FAA (90%

ethanol, 5% formalin, 5% acetic acid) (SASS 1958) and after dehydration in graded ethanol were embedded in paraffin, following the protocol of BECCARI and MAZZI (1966). As a counterstain, 1% safranin in alcoholic solution was used in addition to the more specific reagents in plant microtechnique. Transverse sections of fresh stem were made with bare--handed microtome (A.M.G. Diagnostici). The sections, about 10 μm each, were made with Jung-R 2050 – Supercut microtome (Reichert-Jung/Leica). The basal and cauline leaves and the stem were cut to about half the maximum vegetative development for each species. The foliar morphological parameters (FMP) were measured for each species i.e. average thickness of the foliar lamina in correspondence with the median vein, average thickness of the lamina in the section between the margin and the median vein, of the adaxial and abaxial surfaces (cuticular and epidermal), of the mesophyll, of the palisade and spongy tissue, as well as the Foliar Epidermal Parameters (FEP) i.e. length, width, and thickness of the epidermal cells, the number of cells and stomatal density × mm^2, stomatal polar and equatorial axis, stomatal complex. Epicuticular wax morphology was investigated and compared using three different methods:

1.epidermal epoxy replicas according to the LAROCHE (1982) protocol;

2.extraction of the cuticle according to the CHRISTOPHEL et al. (1996) protocol;

3.SEM (Scanning Electron Microscope) observations.

Fresh and permanent preparations were photographed using LM Leica DMLS, while digital images were obtained using a NIKON DS camera Head DS-Fi1. The S.E.M. images were obtained using a LEO 420 Cambridge.

Data analysis

The dimensions of the epidermal cells, the stomatal polar and equatorial axes, and cell and stomatal density × mm^2 were measured using an image analyzer program integrated with a DS camera head DS-Fi1. Plants were collected on reaching their maximum vegetative development. On each sampling date, ten individuals were collected randomly. All morphometric data were statistically analysed to obtain mean, median and mode as well as standard deviation and variance. Epicuticular wax morphology was classified according to WILKINSON (1979). The anatomical terminology used is that according to ESAU (1965).

Results

Foliar micromorphology

Hypericum perforatum and ***H. perfoliatum.*** The epidermis is glabrous on both leaf surfaces. The adaxial surface (Figs. 1A, C) shows very convex cells range, the longitudinal and radial walls are slightly wavy. These surfaces have no stomata. There are very evident veins consisting of long, narrow cells in rows, the lower the order of the vein the more rows. The abaxial surface (Figs. 1B, D) is composed of cells from irregularly isodiametric to more or less elongate, but all with slightly wavy longitudinal and radial walls.The stomata are at the same level as the other epidermal cells and are numerous and arranged irregularly. The abaxial surface is covered with numerous depressions corresponding to the location of translucent secretory reservoirs and the stomata found in the vicinity of these structures are arranged in a circular pattern around them, while those between the depressions have an

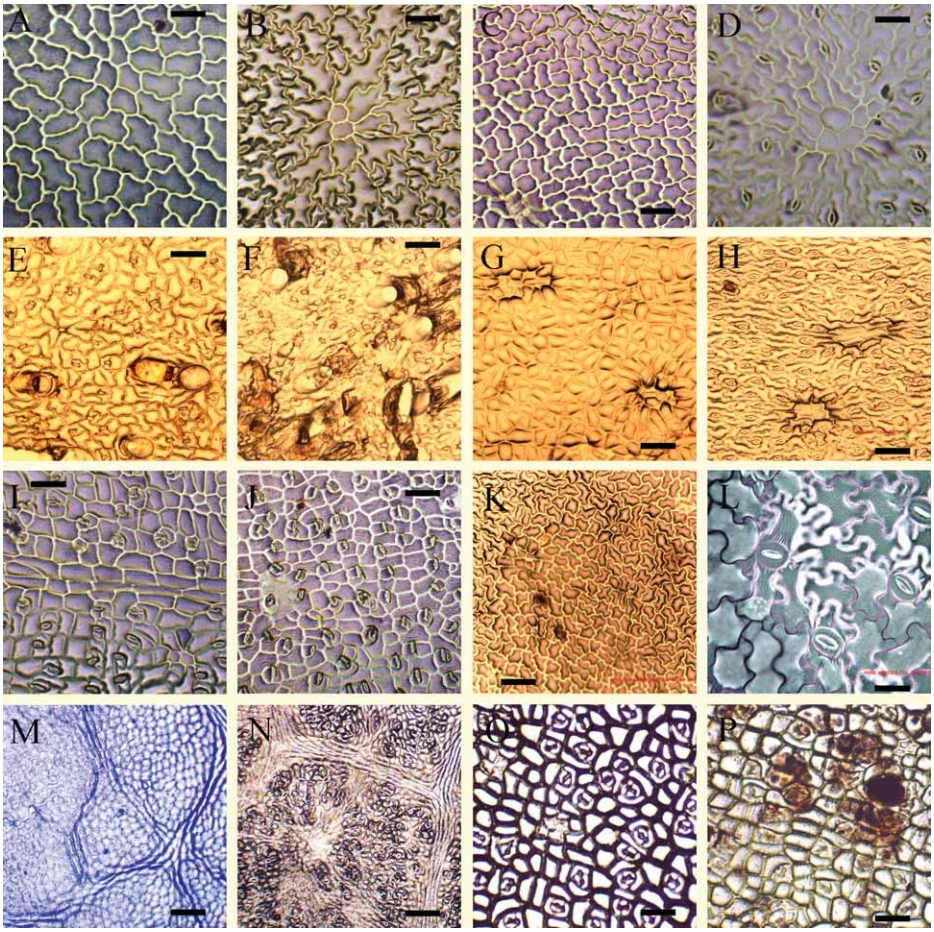

Fig. 1. General characteristics of the adaxial and abaxial epidermis in frontal views (LM). *H. perforatum*; A – adaxial surface showing slightly wavy wall cells; B – abaxial surface with circular depressions consisting of 2 or more slightly larger epidermal cells and stomata (scale bars 50 μm). *H. perfoliatum*; C – adaxial surface with slightly wavy wall small cells; D – abaxial surface with depressions corresponding to the translucent glands (scale bars 50 μm). *H. pubescens*; E – adaxial surface showing irregular polyhedral convex cells mixed with small anisocytic stomata and vestiges of trichomes; F – abaxial surface with stomata and vestiges of trichomes (scale bars 100 μm). *H. tetrapterum;* G – adaxial surface with isodiametric cells and wide elongated depressions of various shapes; H – abaxial surface with depressions and several anisocytic stomatal complexes (scale bars 100 μm). *H. triquetrifolium,* I – adaxial surface showing veins formed by numerous rows of rectangular elongated cells and stomata; J – abaxial surface with stomata and depressions (scale bars 50 μm). *H. androsaemum*; K – adaxial surface (scale bars 100 μm); L – rectangular and elliptical cells with highly corrugated walls, abaxial surface (scale bars 25 μm), a polarized light, showing enlarged detail of anisocytic stomatal types and cuticular thickening of stomatal rims. *H. hircinum*; M – adaxial surface (scale bars 100 μm) showing a large number of small isodiametric cells contained by ribs; N – abaxial surface (scale bars 50 μm) showing areolas with internal stomata end depressions. *H. aegypticum*; O – adaxial surface with isodiametric cells with linear walls; P – cells with rounded corners, stomata and abaxial surface (scale bars 50 μm)

open pattern. In *H. perfoliatum*, the stomata are very numerous and are confined inside the areolae demarcated by the veins found in great numbers on the abaxial surface; the stomata are anisocytic and slightly sunken, strongly elliptic with the polar axis oriented parallel to the major leaf axis. In *H. perforatum*, these depressions are circular, far from perforated, consisting of two or more slightly larger epidermal cells, with less undulating, less convex walls, even concave in some cases. For this reason the whole structure appears depressed in relation to the average level of the epidermis. Also in *H. perfoliatum* there are depressions corresponding to the translucent glands, which penetrate to various depths throughout the mesophyll. SEM observations confirm those from the light microscope. In *H. perforatum* (Fig. 2A), cuticular ornamentation on the adaxial surface presents type »b« granules, while the abaxial surface is characterized by cells with cuticular ornamentation of a cross-linked mixed type with waxy laminar flake deposits (Fig. 2B). In *H. perfoliatum* (Fig. 2C) the adaxial surface presents rod and filament type »e« ornamentation, dense, very small and in tiny isolated outgrowths or in groups. The abaxial surface (Fig. 2D) is morphologically very similar to the adaxial but with much less obvious cuticular ornamentation mostly confined to the grooves between cells. The epicuticular wax is more abundant. Once again, there are depressions, commonly found on the adaxial surface, corresponding to the translucent glands located in the mesophyll.

Hypericum pubescens. Trichomes are present on all leaf surfaces, the veins are mostly long, the pubescence »marked and felted« (PIGNATTI 1982). The trichomes are simple with a widened and stubby base inserted on the epidermis and surrounded by 8–9 epidermal cells arranged in a rosette. The elongated body is made up of 5–6 sloping uniseriate items, of which the distal one appears narrow and pointed. Both adaxial (Fig. 1E) and abaxial (Fig. 1F) surfaces are composed of irregular polyhedral convex cells, mixed with small anisocytic stomata deeply embedded in the epidermis. All adaxial and abaxial surface cells are covered with conspicuous cuticular ornamentation. SEM confirms observations obtained with epoxy replicas but focuses on the very dense cuticular ornamentation on both epidermises (Figs. 2E, F), including the basal items which constitute the foot of all the hairs; even the body of the hairs is scattered with the same type »j« plate and scale ornamentation, miniscule scales arranged more or less vertically. On the adaxial epidermis we also find the characteristic depressions which indicate the presence of translucent glands in the mesophyll, the base of which is enclosed by two adjacent cells, similarly ornamented.

Hypericum tetrapterum and ***H. triquetrifolium***. Trichomes are absent on both leaf surfaces. The adaxial surface (Figs. 1G, I) is composed of vaguely polygonal isodiametric cells with slightly corrugated longitudinal and radial walls in *H. tetrapterum*, a rectangular-sinuous pattern in *H. triquetrifolium*, but strongly convex in both species. On both leaf surfaces the veins are characterized by numerous rows of rectangular cells, more or less elongated and paired, but offset by about half a cell. The anisocytic stomata are randomly arranged on both epidermal surfaces, except around the characteristic depressions, which are fairly numerous and complex; they indicate the presence of translucent glands in the mesophyll and are arranged in a circle. These depressions have one to five cells at the base, from which others radiate out, larger, elongated and arranged in a star shape. The abaxial surface (Figs. 1H, J) is characteristic for its epidermal cells, which are always very convex, wider and more regular in *H. tetrapterum* and with strongly undulating longitudinal and radial walls in *H. triquetrifolium*. SEM shows the epidermal morphology of the adaxial and abaxial surface to be rich in cuticular ornamentation. In *H. tetrapterum* this ornamentation is mainly confined

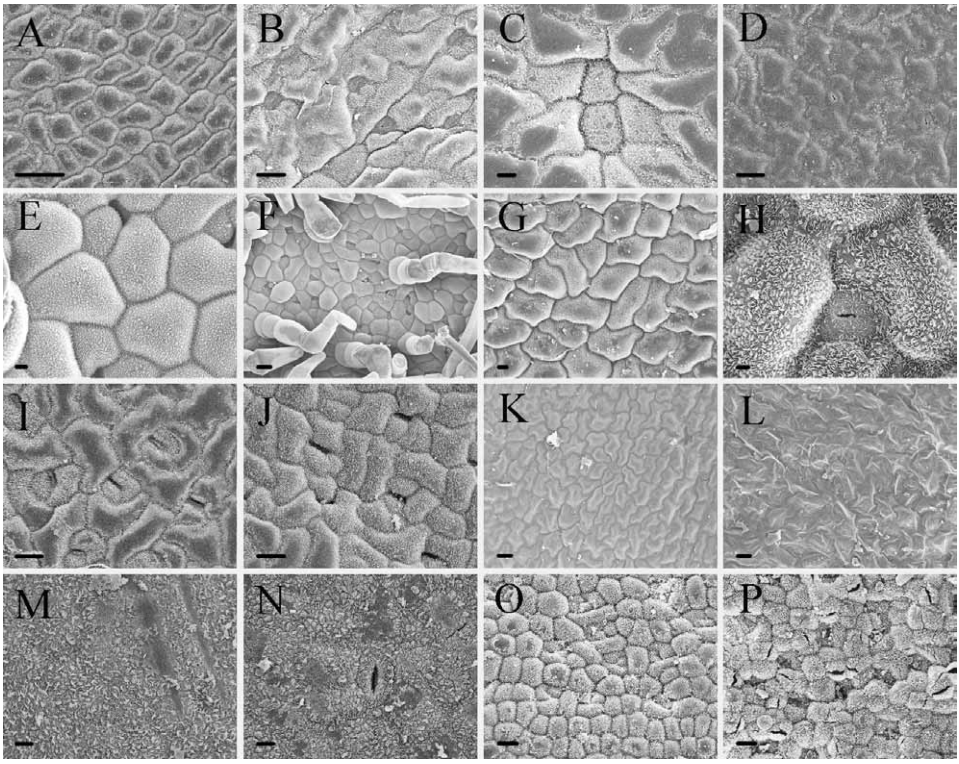

Fig. 2. Leaf blade epidermis scanning electron microscopy (SEM) images. *H. perforatum*; A – adaxial surface showing convex isodiametric cells with a waxy coating (scale bars 60 μm); B – abaxial surface with depressed stomata characterized by ornamented guard cells (scale bars 20 μm). *H. perfoliatum*; C – adaxial surface (scale bars 6 μm) detail of cells at the base of depression; D – abaxial surface (scale bars 20 μm). *H. pubescens*; E – adaxial surface (scale bars 4 μm) detail of the epidermis with »plates and scales« ornamentation and depressed stomata; F – abaxial surface (scale bars 20 μm) with trichomes. *H. tetrapterum;* G – adaxial surface (scale bars 6 μm) showing isodiametric cells with marked waxy coatings; H – abaxial surface (scale bars 2 μm) detail of ornamentation and slightly sunken stomata. *H. triquetrifolium;* I – adaxial surface showing cells with waxy body and anisocytic stomata, (J) abaxial surface (scale bars 20 μm). *H. androsaemum*; K – adaxial surface; L – abaxial surface (scale bars 20 μm) with several stomata elevated compared to level of epidermis. *H. hircinum*; M – adaxial surface (scale bars 60 μm) with marked epicuticular ornamentation; N – abaxial surface (scale bars 20 μm) showing stomata with highly cutinized rims. *H. aegypticum;* O – adaxial surface (scale bars 20 μm) showing cuticles with thick ornamentation; P – abaxial surface (scale bars 60 μm) with depressed stomata

towards the margins between adjacent cell walls and is of plate and scale type »j« according to the classification of WILKINSON (1979). Thus, the epidermal cells present »flake« ornamentation arranged vertically, very densely on the body of the epidermal cells and on the guard cells in *H. tetrapterum* (Figs. 2G, H), while in *H. triquetrifolium* these are strongly waxy, with type »b« granules on both surfaces (Figs. 2I, J).

Hypericum androsaemum and ***H. hircinum***. Trichomes are absent on both leaf surfaces. The adaxial surface is composed of rectangular and elliptical isodiametric cells which are strongly convex but with highly undulating longitudinal and radial walls; there is no sign of areolae that delimit these cells, nor stomata in *H. androsaemum*. Veins are present rarely, with a pattern from undulating to linear; the cells are small and numerous but convex (Figs. 1K, L). In *H. hircinum*, the adaxial leaf surface (Fig. 1M) shows a mosaic of small areolae of various dimensions. These areolae are constituted by veins that run through the mesophyll. Within each areola there are elliptical or polygonal cells with slightly wavy walls, the stomata are absent. The abaxial surface of *H. hircinum* also presents a strong mosaic pattern, with the presence of more or less irregular areolae (Fig. 1N). Anisocytic stomata are present on the abaxial surface of both species and are slightly sunken, especially compared to the convex character of the surrounding epidermal cells. SEM shows that the cuticular ornamentations in *H. androsaemum* are scarce and barely detected and are type »k« crusts and layers (Figs. 2K, L), while in *H. hircinum* these ornamentations are fairly minute granules of type »c« and »b« on the adaxial and abaxial surface respectively, and are also present on the stomatal guard cells (Figs. 2M, N). The surfaces of these two species present depressions of various shapes which correspond to the translucent glands present in the mesophyll at various depths.

***Hypericum aegypticum*.** Trichomes are absent on both leaf surfaces. The adaxial surface (Fig. 1O) of both basal and cauline leaves presents an epidermis of isodiametric cells (generally circular, elliptical, rarely rectangular) with linear walls and rounded corners, strongly convex and smooth. The leaves are amphistomatic, with few anisocytic stomata. The abaxial surface (Fig. 1P) is made up of cells with the same morphological structure as those on the adaxial surface; the stomata are more numerous and also anisocytic. Under SEM (Fig. 2O), the micromorphology of the tangential walls of adaxial epidermal cells is richer in detail. These walls are composed of cuticle with thick ornamentation which, according to WILKINSON (1979), is of type »d« rods and filaments, and extends over the entire epidermal surface, even where very convex. Even the guard cells have this ornamentation, and are thus barely distinguishable. The abaxial surface (Fig. 2P) is characterized by the presence of densely ornamented epicuticular wax. Thus, the surface appears encrusted by these deposits which increase the impermeability. On neither surface were found the characteristic depressions indicating the presence of translucent glands. Even when present, these are found only deep into the spongy tissue and thus far from leaf surfaces.

The foliar epidermal parameters (FEP) are shown in table 1, the characteristics of the stomatal complex in table 2.

Leaf anatomy

Dorsiventral laminae are present in *H. perforatum* (Fig. 3A), *H. perfoliatum* (Fig. 3B), *H. tetrapterum* (Fig. 3D), *H. androsaemum* (Fig. 3F) and *H. hircinum* (Fig. 3G), isobilateral in *H. pubescens* (Fig. 3C), *H. triquetrifolium* (Fig. 3E) and *H. aegypticum* (Fig. 3H). When the leaves are dorsoventral, they present a protrusion corresponding with the median veins, while the two halves of the lamina are generally more or less convex on the adaxial side. Both the adaxial and abaxial epidermis are mono-stratified. The adaxial chlorenchyma is also mono-stratified, with the exception of *H. hircinum*, which is bi-stratified, and the spongy tissue is generally thin (Tab. 3).

Tab. 1. Foliar Epidermal Parameters (F.E.P.) of *Hypericum* L.

Species	Surface	Cell lenght µm					Cell width µm					Cell thickness µm					Cells freq./mm^2				Morphology of epicuticular waxes
		Measures of central tendency			Measures of dispersion		Measures of central tendency			Measures of dispersion		Measures of central tendency			Measures of dispersion		Measures of central tendency		Measures of dispersion		
		Mean	Mode	Median	σ Standard deviation	σ^2 Variance	Mean	Mode	Median	σ Standard deviation	σ^2 Variance	Mean	Mode	Median	σ Standard deviation	σ^2 Variance	Mean	Median	σ Standard deviation	σ^2 Variance	
Hypericum perforatum	AD	72.0	72.0	72.0	3.59	12.89	28.0	30.0	28.0	3.16	10.00	17.0	16.0	17.0	1.83	3.33	546	546	20.78	432	granules type »b«
	AB	60.0	60.0	60.0	4.69	22.00	25.0	24.0	25.0	1.33	1.78	13.0	12.0	13.0	1.49	2.22	1010	1008	32.89	1082	
H. perfoliatum	AD	61.0	62.0	61.5	6.68	44.67	24.0	24.0	24.0	2.26	5.11	12.0	12.0	12.0	1.49	2.22	1263	1266	72.19	5211	rods and filaments type »e«
	AB	48.0	43.0	47.5	5.60	31.33	26.0	26.0	26.0	2.49	6.22	8.0	8.0	8.0	1.83	3.33	1317	1324	51.72	2675	
H. pubescens	AD	72.1	70.0	71.5	4.31	18.54	42.0	40.0	42.5	4.29	18.44	21.0	20.0	20.5	2.05	4.22	480	483	43.97	1933	plates and scales type »j«
	AB	46.0	46.0	46.0	3.02	9.11	26.0	26.0	26.5	2.91	8.44	17.0	16.0	17.0	1.25	1.56	571	568	50.33	2533	
H. tetrapterum	AD	57.0	56.0	56.0	5.62	31.56	40.0	41.0	40.5	6.31	39.78	13.0	12.0	13.0	1.56	2.44	457	471	42.10	1772	plates and scales type »j
	AB	80.0	78.0	79.0	8.03	64.44	44.0	47.0	46.0	7.79	60.67	17.0	16.0	17.0	1.56	2.44	684	700	61.13	3737	
H. triquetrifolium	AD	64.0	60.0	63.5	7.48	56.00	28.0	29.0	28.0	3.16	10.00	21.0	24.0	21.0	2.26	5.11	691	710	61.25	3752	granules type »b«
	AB	54.1	54.0	54.0	5.82	33.88	24.0	27.0	24.5	3.13	9.78	13.0	13.0	13.0	1.41	2.00	1124	1122	68.38	4676	
H. androsaemum	AD	50.1	49.0	49.5	3.03	9.21	27.0	26.0	26.5	2.45	6.00	14.0	15.0	14.5	1.70	2.89	1404	1411	76.05	5784	layers and crusts type »k«
	AB	41.0	40.0	40.5	3.71	13.78	50.1	49.0	49.5	4.70	22.10	16.1	19.0	16.0	1.97	3.88	821	836	65.09	4237	
H. hircinum	AD	34.1	37.0	34.0	3.18	10.10	20.0	19.0	19.5	1.41	2.00	8.1	8.0	8.0	0.99	0.99	2568	2587	93.35	8713	granules type »c« AD /»b«AB
	AB	29.0	30.0	29.0	1.83	3.33	17.1	18.0	17.5	1.85	3.43	4.0	4.0	4.0	0.67	0.44	3538	3561	80.70	6513	
H. aegypticum	AD	34.1	35.0	34.0	2.51	6.32	22.1	21.0	21.5	1.60	2.54	15.1	15.0	15.0	1.45	2.10	2283	2305	90.97	8275	rods and filaments type »d«
	AB	35.0	34.0	34.5	1.76	3.11	22.1	21.0	21.5	1.85	3.43	13.0	13.0	13.0	1.15	1.33	2321	2343	75.95	5769	

Symbols: AD =Adaxial surface; AB = Abaxial surface.

Tab. 2. Characteristics of the stomatal complex in *Hypericum*

Species	stomatal complex types		Middle dimensions			Arrangement of stomata on the leaf blade
	Paracytic	Anisocytic	Polar diameter µm	Equatorial diameter µm	Density (× mm^2)	
Hypericum perforatum	–	*	22AB	12AB	358AB	hypostomatic
H. perfoliatum	–	*	23AB	6AB	275AB	hypostomatic
H. pubescens	–	*	19AD/15AB	8AD/7AB	115AD/ 420AB	amphistomatic
H. tetrapterum	–	*	8AD/9AB	6AD/5AB	23AD/ 457AB	amphistomatic
H. triquetrifolium	+	*	27AD/23AB	13AD/11AB	168AD/345AB	amphistomatic
H. androsaemum	–	*	21AB	16AB	228AB	hypostomatic
H. hircinum	–	*	21AB	16AB	211AB	hypostomatic
H. aegypticum	–	*	25AD/20AB	20AD/20AB	380AD/263AB	amphistomatic

+ – present; ***** – predominant; **– –** absent; AD – adaxial surface; AB – abaxial surface.

Tab. 3. Leaf typologies and Foliar Morphological Parameters (FMP) in *Hypericum*

Species	Mesophyll structure		Foliar Morphological Parameters (FMP)						
	Dorsiventral	Isobilateral	Middle thickness of leaf blade µm	Middle thickness in proximity to midrib µm	Middle thickness epid. AD µm	Middle thickness epid. AB µm	Mesophyll µm	Palisade µm	Spongy tissue µm
Hypericum perforatum	+	–	170	243	19	15	112	48	64
H. perfoliatum	+	–	78	230	15	10	40	23	17
H. pubescens	–	+	142	250	17	21	103	60AD/32AB	39
H. tetrapterum	+	–	105	156	13	17	74	30	44
H. triquetrifolium	–	+	165	325	22	14	132	50AD/48AB	33
H. androsaemum	+	–	116	158	14	15	116	55	61
H. hircinum	+	–	126	315	9	5	90	40	50
H. aegypticum	–	+	450	690	25	30	400	120AD/91AB	112

+ – present; **– –** absent; AD – adaxial surface; AB – abaxial surface

On the isobilateral lamina, the epidermises are mono-stratified, the mesophyll appears homogeneous and not distinguishable in the palisade and spongy tissue. Veins of each order pass through the mesophyll and in particular the spongy tissue. The median veins are particularly large and protrude from the abaxial surface. They have a parenchymatous sheath and

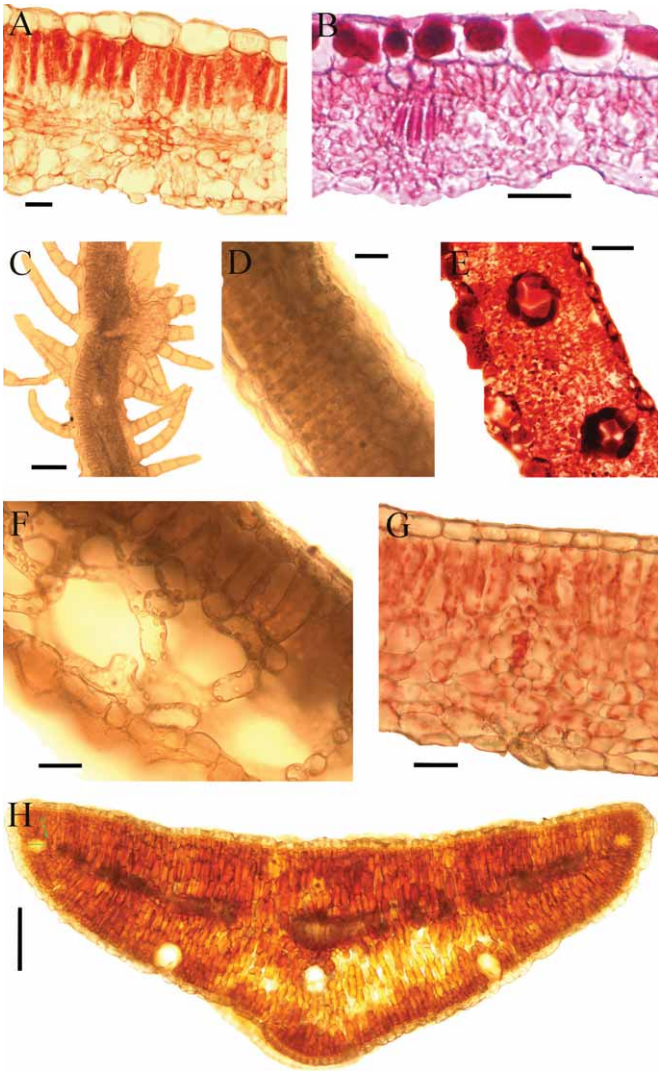

Fig. 3. Transversal sections of the leaf middle portion. *H. perforatum*; A – dorsiventral leaf, thick adaxial surface, large palisade chlorenchyma, thin abaxial surface (scale bars 25 μm). *H. perfoliatum;* B – dorsiventral leaf, highly thickened adaxial surface with tanniferous cells, undifferentiated mesophyll (scale bars 50 μm). *H. pubescens;* C – isobilateral leaf, thin adaxial surface with many large multicellular trichomes, thin abaxial surface with very pronounced vein and numerous trichomes (scale bars 100 μm). *H. tetrapterum;* D – dorsiventral leaf, thin adaxial and abaxial surface, mesophyll with large chlorenchyma (scale bars 100 μm). *H. triquetrifolium;* E – isobilateral leaf, thin epidermis with tanniferous cells, mesophyll with translucent glands and type A secretory canals (scale bars 50 μm). *H. androsaemum;* F – dorsiventral leaf, extremely thin epidermis, spongy tissue with translucent glands (scale bars 25 μm). *H. hircinum;* G – dorsiventral leaf, large mesophyll with veins in the spongy tissue and type B secretory canals associated with the phloem (scale bars 25 μm). *H. aegypticum;* H – plano-convex isobilateral leaf, coriaceous and thin epidermis, wide and mucilaginous chlorenchyma with several translucent glands (scale bars 50 μm)

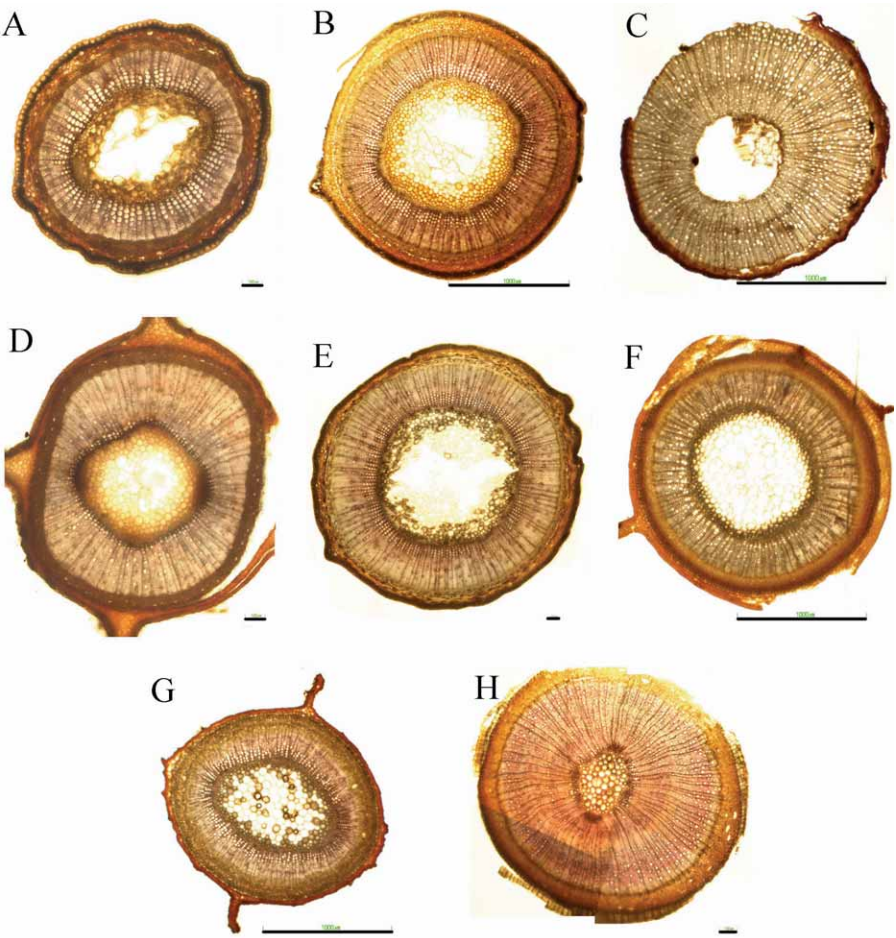

Fig. 4. Transverse section of fresh stem of mature plant made with bare-handed microtome. *H. perforatum*; A – lignified stem with elliptical cross-section and two small slightly prominent wings (scale bars 100 μm). *H. perfoliatum*; B – lignified stem with slightly wavy circular profile and two small evident wings (scale bars 1000 μm). *H. pubescens*; C – very lignified stem with almost circular profile (scale bars 1000 μm). *H. tetrapterum*; D – lignified stem with rectangular profile and four evident longitudinal triangular wings (scale bars 100 μm). *H. triquetrifolium*; E – lignified stem with very wavy elliptical profile in correspondence with two small wings (scale bars 100 μm). *H. androsaemum;* F – slightly lignified stem with circular profile and two small evident wings (scale bars 1000 μm). *H. hircinum;* G – lignified stem with wavy elliptical profile and two long, thin wings (scale bars 1000 μm). *H. aegypticum;* H – very lignified stem with circular suberous profile (scale bars 100 μm)

a collenchyma cap, which make the vein even more prominent and conspicuous. The chlorenchyma is interrupted in this particular section of the lamina. Translucent glands are found in the mesophyll of all the *taxa*, always very numerous and of various sizes, floating within the thickness; when present below the adaxial epidermis these glands are larger than those found above abaxial epidermis, while those at the center of the mesophyll are the largest of

all and even partially invade the palisade. In some cases (*H. perforatum, H. perfoliatum*) these are at a tangent to the two epidermises.

Stem anatomy

From a morphological point of view the stem presents: in hemicryptophyte scapose species, lignified and prostrate at the base, briefly creeping, in *H. perforatum* and *H. perfoliatum* respectively; lignified creeping with ascending branches in *H. pubescens*; prostrate then erect, ligneous in *H. tetrapterum* and *H. triquetrifolium*, in the latter strongly branching (PIGNATTI 1982). An evergreen shrub in the two nano-phanerophytes, *H. hircinum* and *H. androsaemum*; a ligneous shrub with contorted branches in *H. aegypticum*, a chamaephyte fruticose. In cross-section, the hemicryptophyte scapose stem has a profile from circular to elliptical with two small wings, along which are found black nodules, respectively, in *H. perforatum* (Fig. 4A), *H. perfoliatum* (Fig. 4B), and *H. triquetrifolium* (Fig. 4E). In *H. perforatum* and *H. triquetrifolium* the outline also appears slightly wavy, particularly in correspondence with the two small wings; *H. tetrapterum* presents four triangular wings (Fig. 4D). Wings are absent in *H. pubescens*. In *H. hircinum* and *H. androsaemum* two wings are evident (Figs. 4G, F), in *H. aegypticum* the stem profile is circular without wings (Fig. 4H). Anatomically, independently of the presence of more or less prominent wings, including the longitudinal triangular wings of *H. tetrapterum*, the young stem presents a mono-stratified epidermis with thin cuticle followed, in a centripetal direction, by a multilayered chlorophyll parenchyma (3–4 layers), a reserve parenchyma with rounded cells and, finally, by a mono-stratified endodermis with lenticular thickenings on the radial walls. The stele begins with a pericycle which is also mono-stratified, followed centripetally by concentric rings of phloem and xylem; in the phloem at this juvenile stage, type A and B secretory canals present in the cortex at various depths cannot yet be distinguished; the xylem is composed predominantly of protoxylem and a few metaxylematic elements in radial position, separated by numerous medullary rays.

In the mature stem of *H. perforatum*, *H. perfoliatum*, *H. tetrapterum* and *H. triquetrifolium* we always find an outer mono-stratified epidermis where, however, the constituent cells become smaller and the thickness of the outer tangential walls increases through enrichment with cutin. In *H. pubescens*, the stem trichomes dry out and are partly lost because the outermost layers tend to become enriched with suberin. The sub-epidermal chlorenchyma thins, but persists, while the cortex is enriched with a reserve parenchyma mixed with tanniferous cells. Centripetally, the cortex is delimited by a closed endodermal sheath, followed by a pericycle with some sclerenchymatous elements. The stele is composed of phloem and numerous, scattered type A secretory canals in a ring. In *H. pubescens* in particular, black nodules are found in the outer cortex, in the phloem, at the border between xylem and phloem and also at the end of the xylematic ring tangential to the medulla. *H. tetrapterum* has a rectangular profile, with rounded edges on each side, there is a triangular wing expansion; a mono-stratified epidermis with large cells and black nodules underlies the entire stem, including the wings. Centripetally there follows a chlorenchyma and, finally, more internally, a reserve parenchyma, which thins and surrounds the stem, giving rise to a thin cortex containing a few type B secretory canals. We then find an endodermoide, a pericycle with numerous sclerenchymatous elements and finally, the phloem, where many irregularly distributed type B secretory canals are highlighted externally, and

more regularly arranged type A canals in the centripetal direction. *H. perforatum* has a slightly elliptical profile, a wider medulla, and is rich in reserve parenchyma. Externally there is a mono-stratified epidermis consisting of more or less large rounded cells, a thin cortical parenchyma and a wide cortex with numerous lacunae, which are more numerous below the two small wings. The phloem underneath contains type A and B secretory canals, interspersed with the lacunae. The xylem is generally robust and often ring-porous, except in *H. pubescens* (Fig. 5C) and *H. aegypticum* (Fig. 5H), where it is diffuse-porous. The dif-

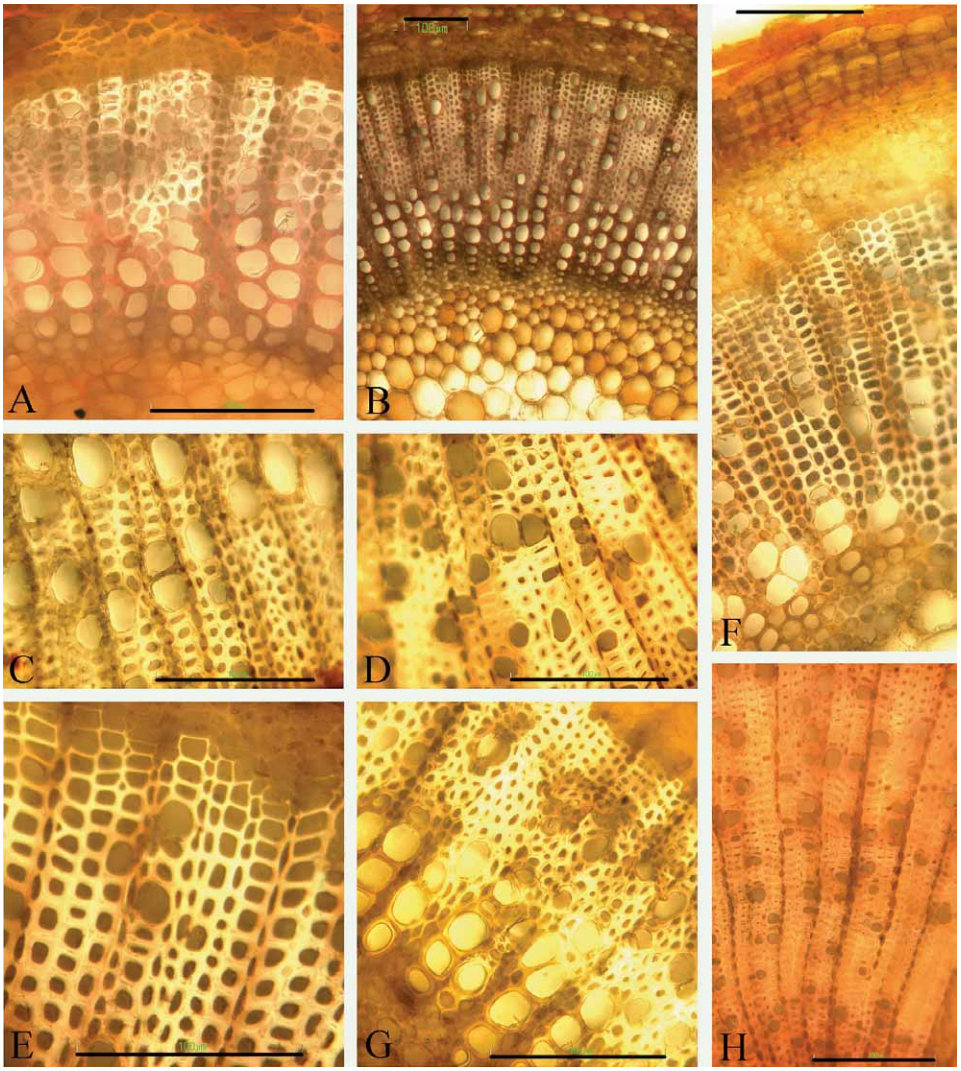

Fig. 5. Details of secondary xylem: diffuse-porous xylem (A, B, D, E, F, G) and ring-porous xylem (C, H). *Hypericum perforatum* (A), *H. perfoliatum* (B), *H. pubescens* (C), *H. tetrapterum* (D), *H. triquetrifolium* (E), *H. androsaemum* (F), *H. hircinum* (G), *H. aegypticum* (H). Scale bars 100 μm.

Tab. 4. Stem morpho-anatomy in *Hypericum*

taxa	n. middle vessels × mm^2	∅ middle vessels µm	secondary xylem	habitus	environment	secretory structures	wings/stem cross-section
Hypericum perforatum	4	20	ring-porous	creeping ascending lignified	dry meadows scrubland. uncultivated land	type A and B secretory canals in chlorenchyma and phloem. type B secretory canals in cortex	two small / slightly elliptical
H. perfoliatum	6	21	ring-porous	creeping ascending lignified	scrubland. woods. hedges	type A secretory canals in phloem. type B secretory canals in cortex	two small / circular
H. pubescens	8	27	diffuse-porous	creeping ascending branches lignified	wet and sometimes salty habitats	type A secretory canals in phloem (small and rare). type B secretory canals in cortex (few). black nodules in outer cortex. between xylem and phloem and between xylem and medulla	absent / circular
H. tetrapterum	3	19	ring-porous	prostrate lignified	marshes and cane-brakes	type A and B secretory canals in phloem. type B secretory canals in cortex (few). black nodules on wings	four triangular / rectangular
H. triquetrifolium	6	20	ring-porous	prostrate very branching lignified	uncultivated ground	type A secretory canals in phloem (small). type B secretory canals in outer cortex	two small / slightly wavy elliptical
H. androsaemum	3	19	ring-porous	erected shrub	woods. wet and shady localities	type A secretory canals in phloem (few). type B secretory canals in outer cortex	two small evident / circular
H. hircinum	3	19	ring-porous	erected shrub	ravines. wet and shady localities	type A secretory canals with rhomboidal and circular lumen (2:1) and large type B secretory canals in phloem	two long. thin / wavy elliptical
H. aegypticum	2	15	diffuse-porous	depressed shrub with contorted branches	maritime cliffs	type A secretory canals in phloem (small). type B secretory canals in cortex (large and numerous)	absent / circular

fuse-porous xylem is characterized by two extreme types: that of *H. pubescens*, where the number of vessels per mm^2 is generally high, with a peak of 8 per mm^2, and large diameter of 27 μm; and that of *H. aegypticum*, which presents the lowest number of vessels (2 per mm^2) and at the same time a significantly lower average vessel diameter of 15 μm. In ring-porous xylem in *H. perforatum*, *H. perfoliatum*, *H. tetrapterum* and *H. triquetrifolium* (Tab. 4) the medullary rays are uniseriate, while the vessels that make up most of the ring xylem are well lignified with a small lumen in *H. tetrapterum* (Fig. 5D). In *H. perforatum* (Fig. 5A), *H. perfoliatum* (Fig. 5B) and *H. triquetrifolium* (Fig. 5E) these vessels are less lignified, with wider lumens. The medullary rays of *H. pubescens* are always uniseriate, while the vessels are numerous and well-lignified. There are also black nodules on the outer cortex, in the phloem, in the border between the xylem and phloem as well as at the end of the xylematic ring tangential to the medulla. Further details are reported in table 4.

Discussion

The species investigated colonize diverse environments, from mesic to wet conditions, from marsh and reed to sea cliffs, and are therefore characterized by a remarkable eco-morphological plasticity. However, all are adapted to withstand prolonged drought for the duration of the dry season, which in the Mediterranean climate extends from April to October, with a variable thermopluviometric performance that has been more marked in recent years. The species are mostly mesophytes (*H. perforatum*, *H. perfoliatum*, *H. pubescens*, *H. androsaemum*, *H. hircinum*), but there are also meso-hygrophytes such as *H. tetrapterum*, and xerophytes and xero-halophytes such as *H. triquetrifolium* and *H. aegypticum* respectively. With the arrival of spring and summer, the sudden increase in temperature and drastic reduction in rainfall means all the species suffer thermal water stress, even those in wetlands where the regime is temporary or fluctuating. All this is reflected in the micromorphological features of the epidermal cells, which are constant and genetically controlled (Carr et al. 1971, Faggetter 1987), although influenced by environmental conditions (Bartholott 1981), together with the overall structure of the stomatal complex. The mesophyte can face moderate water deficiency due to its cuticular thickening, waxy coating and variable cell and stomatal frequency. The adaxial and abaxial epidermal cells mainly show convex surfaces, with various types of ornamentation (Tab. 1), even at the level of the stomatal guard cells. There are thin pruinose coatings with a water-repellent function in taxa from humid or marshy environments and thick coatings in those growing in sunny, arid or salty habitats. All stomatal complexes are anisocytic, with high density per mm^2 in species with hypostomatic leaves (*H. perforatum*, *H. perfoliatum*, *H. androsaemum*, *H. hircinum*) as some grow at altitude, where they are subject to temperature fluctuations, others in very sunny and arid habitats. The leaves, with their limited surface area, present thick, waxy cuticular coatings that distinguish one individual from another (Tab. 1). The presence of epicuticular waxes, regardless of type, is of great importance for the prevention of water loss. These waxes increase the capacity of the leaves to bind water and exchange gases through their water-repellency (Polací et al. 2004) and reduce the interception of solar rays in exposed environments. A further property of the wax is to minimize mechanical damage and inhibit fungal and insect attack (Eglinton and Hamilton 1967). The arrival of summer drastically reduces the grassy mantle, and leaves that are still green at this point are a good resource for insects. Consequently, the wax layer tends to vary with season and age of species (Eglinton

and HAMILTON 1967). Species with hypostomatic leaves, as well as having a high stomatal frequency, present a similarly elevated frequency of epidermal cells on both leaf surfaces; these cells are also long, narrow and fairly thin (Tab. 1). Species with amphistomatic leaves show lower stomatal frequency on the adaxial surface and a high frequency on the abaxial surface with reduced leaf surfaces (*H. pubescens*, *H. tetrapterum*). They also possess, like the stomata, a low frequency of epidermal cells which are short, wide and thick, in agreement with their growth habitat. *H. triquetrifolium*, a xerophyte with amphistomatic leaves and anisocytic stomatal complexes, is characterised by a high frequency of cells and stomata, especially on the abaxial surface of the leaf. Moreover, the particular morphology of the epicuticular waxes of type »b« granules (Tab. 1) ensures the efficient reflection of light and heat. As a xero-halophyte exposed to sea spray, *H. aegypticum* is moderately crassulent and has a high stomatal frequency on both leaf surfaces; the stomata also have polar and equatorial axes similar to those of *H. triquetrifolium*, a species that grows in arid and uncultivated environments.

The presence of trichomes on all foliar surfaces in *H. pubescens* slows down transpiration, reduces thermal load and photo-induced lesions as well as repelling small insects and pathogenic agents. Considering that these taxa grow in open, bright environments, their trichomes may fulfil most of the potential roles mentioned above. Comparison of stem anatomy is useful for characterization, both for the distribution of type A secretory structures in the entire thickness of the cortex, and the type B secretory structures present in the phloem at different depths, and even more so for the xylem type, which lends itself to phylogenetic interpretation and can be either ring-porous or diffuse-porous. Ring-porous xylem, not very common and typical of temperate zone species, can be interpreted as evidence of greater evolutionary specialization, influenced also by environmental characteristics. Diffuse-porous xylem, present in *H. aegypticum* and *H. pubescens*, possessing a parenchyma mixed with mechanical and conduction elements, is apotracheal, a typology considered to be more primitive than the paratracheal type. The »apotracheal diffuse« type is even more primitive than other typologies. Being shorter, the vessels of diffuse-porous xylem show a less efficient flow velocity. In the case of *H. pubescens*, this eco-physiological feature is subordinate to the average diameter and the number of vessels × mm^2, which are the highest of all the taxa examined.

In conclusion, interspecific differences can be seen in density, type and position of the stomatal complex, the amount and morphology of epicuticular wax, secondary wood features such as ring-porous or diffuse-porous xylem as well as the number and average diameter of vessels. Hence, foliar and stem anatomy can be considered an additional tool for taxonomic and evolutionary studies in *Hypericum*.

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