

## 5 Leaf anatomy and foliar trichomes in Heliotropiaceae and their systematic relevance<sup>4</sup>

### Summary

The leaf anatomy and distribution of foliar trichome types of 65 species of Heliotropiaceae, has been investigated. The aim was to evaluate the systematic relevance of their diversity as compared to recent findings of systematic relationships within the family. The results of leaf anatomy patterns, especially venation, vascular system, various foliar trichome types, and localization of crystals are of surprisingly high systematic value and prove the actual proposed subdivision of this family based on molecular results. Each main clade identified in the molecular studies is well characterized on the basis of leaf anatomy.

### 5.1 Introduction

The Heliotropiaceae<sup>5</sup> are a family of nearly 450 species. They are easily recognized by their partial inflorescences of scorpioid cymes and by the morphology of the highly modified stigmatic head in the flower. The subdivision of the family is mainly based on generative characters, vegetative ones are scarcely considered. Heliotropiaceae are distributed worldwide

---

<sup>4</sup> Manuscript accepted as **DIANE N., JAKOB C. & HILGER H.H.** (in press): Leaf anatomy and foliar trichome in Heliotropiaceae and their systematic relevance. – *Flora* 198 (6).

Own contributions: anatomical investigations (partly in cooperation with C. JAKOB), drawings, photos (partly of C. JAKOB), writing manuscript.

<sup>5</sup> Due to the inclusion of Lennoaceae and Hydrophyllaceae into the old Boraginaceae (YATSKIEVYCH *et al.* 1986, FERGUSON 1999, GOTTSCHLING *et al.* 2001), we treat this taxon as “Boraginales”, and the former subfamilies of Boraginaceae as families of their own – a taxonomic status that has been discussed for nearly 200 years.

mainly in tropical and subtropical regions, and in dry, disturbed areas of warm-temperate to semi-arid regions. Four species are halophytes.

Although the indumentum is characteristic and namegiving for the Boraginales (“Asperifoliae”, in German: Rauhblattgewächse), little is known on the structural diversity of the trichomes and the leaf anatomy in general. Most descriptions have been published in the frame of general studies of the Boraginales, many of them decades or even more than a century age old (e.g., SCHIBLER 1887, SOLEREDER 1908, KRAGGE 1911, METCALFE & CHALK 1950). Actual investigations of leaf anatomy within Boraginales *s. str.* tribe *Boraginaea* are of SELVI & BIGAZZI (2001). Within Heliotropiaceae, only few detailed studies were made by BIDER (1935) of two species of *Heliotropium*, by FROHLICH (1978) of leaf morphology and anatomy of 17 Mexican species of *Heliotropium* section *Orthostachys*, by KUMAR & RAO (1994) of foliar epidermology of 6 species of *Heliotropium*, and by DiFULVIO (1982) of leaf anatomy of the monotypic Argentinian endemic *Ixorhea*.

A phylogenetic analysis of Heliotropiaceae based on molecular data has shown that the old generic limits and characters are no longer valid (DIANE *et al.* 2002a, HILGER & DIANE submitted). This is summarized in Table 5-1. While FÖRTHNER (1998), in his basic revision of the Heliotropiaceae, still recognized 8 genera (Table 5-1 column left side: *Argusia*, *Ceballosia*, *Heliotropium*, *Hilgeria*, *Ixorhea*, *Nogalia*, *Schleidenia*, *Tournefortia*), the most important results of the molecular studies was that *Heliotropium* and *Tournefortia*, in their old circumscription, are para- and polyphyletic, respectively (Table 5-1 columns right side). Therefore, *Heliotropium* sect. *Orthostachys* and *Tournefortia* sect. *Cyphocyema* should be recognized as genera of their own. The oldest available names are *Euploca* (NUTTALL 1837) for the former and *Myriopus* (SMALL 1933) for the latter taxon. *Schleidenia*, *Hilgeria*, *Argusia*, *Ceballosia*, and *Nogalia* fall into synonymy of either *Euploca* or *Heliotropium*. *Tournefortia* sect. *Tournefortia* is within *Heliotropium* *s.str.* in sistergroup relationship to a HELIOTROPIUM I<sup>6</sup> clade, and represents the woody (lianas, small trees) grade within herbaceous to subshrubby HELIOTROPIUM I species of the New World. The informal HELIOTROPIUM II clade represents the *Heliotropium* species of the Old World.

---

<sup>6</sup> Combinations have not yet been published. To avoid nomenclatural confusion, informal clades are written in capital letters.

The aim of the present study, covering 65 species, is a) to add more data of leaf anatomy and especially indumentum characters for the family, thus b) to evaluate the systematic relevance and/ or adaptive value of the morphological and anatomical diversity.

## 5.2 Materials and Methods

Representatives of 65 Heliotropiaceae of all genera and sections of *Tournefortia* and *Heliotropium* have been investigated, with only the exception of the small *Heliotropium* sections *Monimantha* and *Rutidotheca* of the HELIOTROPIUM II clade. They are listed in Table 5-2 (appendix). The species are arranged in alphabetical order of the clades identified according to DIANE et al. (2002a) and HILGER & DIANE (submitted), see also Table 5-1.

The leaves were fixed with AFE (acetic acid-formalin-ethanol). For light microscopy, the leaves were dehydrated with FDA (formaldehyde-dimethyl-acetal, GERSTBERGER & LEINS 1978) and, in most cases, embedded in paraplast via a graded ethanol-tertiary butanol series and microtome sectioned, or hand sections with a razor were made. Photographs of safranin-astra blue stained serial sections (10 µm), or sections bleached with chloral hydrate (saturated aqueous solution) and astra blue stained hand sections were taken with a Leitz Dialux 20 microscope and a Canon EOS D30 digital camera. For identification of crystal shapes, crossed polarising filters were used. Calcium oxalate and calcium carbonate were distinguished as described by HORNER & WAGNER (1992). For SEM studies, the material was dehydrated in a graded ethanol series, critical point-dried with CO<sub>2</sub>, sputter-coated with gold, and photographed with a LEO 430 Scanning Electron Microscope.

## 5.3 Results

The distribution of anatomical characters observed is shown in Table 5-3. The species are listed according to their clades in Table 5-1.

**General leaf morphology**—Generally, phyllotaxis is alternate, rarely pseudo-opposite, or pseudoternate. The leaves are simple and usually petiolate, rarely sessile, and estipulate. The lamina is linear, elliptic, or ovate to subcircular, the margin mostly entire, rarely sinuate to

pinnatifid, repand, crenate, or dentate, usually flat or sometimes revolute. The leaf apex is acute or acuminate, rarely rounded. The leaf base is cuneate or decurrent, rarely truncate to subcordate. The leaf surface is planar or bullate; a dense indumentum is usually present, and only a few species are glabrous.

Table 5-3. Leaf anatomy and distribution of foliar trichome types in the Heliotropiaceae. **H**, habitat (1=semi-arid with seasonal dry periods, 2=Andean scrub communities, 3=humid tropics, 4=halophytic, coastal and desert sands); **Lv**, leaf venation (P=hyphodromous venation, F= brochidodromous I, secondaries and tertiaries weakly prominent, S=brochidodromous II without prominent tertiaries, S+=brochidodromous II, with only a few prominent tertiaries, T=brochidodromous III, prominent tertiaries are opposite percurrent); **La**, leaf anatomy (B=bifacial, B+=subbifacial, I=isobilateral, one-layered, I2= isobilateral, two-layered, Ih=isobilateral, heterogenous, Im=isobilateral, multilayered, S=succulent); **Sd**, Stomata distribution (H=hypostomatous, A=amphistomatous, H+=hypostomatous, but with stomata present on the adaxial surface at strongly reduced density); **Ko**, Kranz-chlorenchyma organization in leaves (+=present, - =not present); **Gt**, glandular trichomes (+=present, - =not present); **Tt**, foliar trichome types (s=slender, sg=strigose, co=conical, ta= two-armed, ph=papillose-hispid, - =glabrous); **Ct**, lithocysts types (ac=multicellular lithocyst complexes, tc=trichome lithocysts, ic=unicellular lithocysts, trichome tip reduced, - =without lithocysts); **Cy**, idioblasts with crystals (s=crystalsand in mesophyll, d=crystal druses in mesophyll, d+= crystal tubes in mesophyll, n=crystals needles in epidermis, rarely additionally present in mesophyll or along the bundles, - = crystals lacking).

5 Comparative leaf anatomy in Heliotropiaceae

Clade	H	Lv	La	Sd	Ko	Gt	Tt	Ct	Cy			
<b>IXORHEA</b>												
<i>I. tschudiana</i>	2	F	lh	A	-	(+)	-	-	-	d	d+	
<b>MYRIOPUS</b>												
<i>T. psilostachya</i>	1	F	B	A	-	-	- s	ac	-	n	-	
<i>T. salzmannii</i>	1	F	B	A	-	-	- s	-	-	-	-	
<i>T. volubilis</i>	1	F	B	H	-	-	- s	ac	-	-	-	
<b>EUPLOCA</b>												
<i>Heliotropium</i> sect. <i>Orthostachys</i> (subsection)												
<i>H. ovalifolium</i> ( <i>Ebracteata</i> )	1	P	I	A	-	-	sg -	ac	-	-	-	
<i>H. procumbens</i> ( <i>Ebracteata</i> )	1	S	B	A	-	-	sg s	ac	-	-	-	
<i>H. campestre</i> ( <i>Ebracteata</i> )	1	P	B	A	+	-	sg -	-	-	-	-	
<i>H. chrysanthum</i> ( <i>Ebracteata</i> )	1	P	I	A	+	-	sg -	-	-	-	-	
<i>H. mendocinum</i> ( <i>Ebracteata</i> )	1	P	I	A	+	-	sg -	-	-	-	-	
<i>H. convolvulaceum</i> ( <i>Ebracteata</i> )	1	P	I	A	+	-	sg -	-	-	n	-	
<i>H. styotrichum</i> ( <i>Ebracteata</i> )	1	P	B	A	+	-	- s	-	-	-	-	
<i>H. tenuifolium</i> ( <i>Ebracteata</i> )	1	P	B	A	+	-	sg -	-	-	-	-	
<i>H. rariflorum</i> subsp. <i>hereroense</i> ( <i>Ebracteata</i> )	1	P	B+	A	+	-	sg -	ac	-	-	-	
<i>H. strigosum</i> ( <i>Ebracteata</i> )	1	P	B+	A	+	-	sg -	ac	-	-	-	
<i>H. bursiferum</i> ( <i>Ebracteata</i> )	1	P	B+	A	+	-	sg -	-	-	-	-	
<i>H. humifusum</i> ( <i>Ebracteata</i> )	1	P	I	A	+	-	sg -	-	-	-	-	
<i>Schleidenia</i>												
<i>H. antillanum</i>	1	P	B	A	+	-	sg -	-	-	n	-	
<i>Hilgeria</i>												
<i>H. hypogaea</i>	1	P	?	A	+	-	sg -	-	-	-	-	
<b>HELIOTHAMNUS</b>												
<i>H. mandonii</i>	2	T	B	H+	-	+	co -	-	tc	-	d	d+
<i>H. arborescens</i>	2	T	B	H+	-	+	co -	-	tc	-	d	-
<i>H. rufipilum</i>	2	T	B	H+	-	+	co s	-	tc	-	d	-
<i>H. incanum</i>	2	T	B	H+	-	+	co s	-	tc	-	d	d+
<i>H. submolle</i>	2	T	B	H+	-	+	co s	-	tc	-	d	d+
<i>H. adenogynum</i>	2	T	B	H+	-	+	co -	-	tc	-	d	-
<b>HELIOTROPIUM I</b> (section)												
<i>H. krauseanum</i> ( <i>Cochranea</i> )	1	T	B	H	-	+	co -	-	tc	-	d	-
<i>H. spec. nov.</i> ( <i>Cochranea</i> )	1	S	B	H	-	+	co -	-	tc	-	d	-
<i>H. nicotanaefolium</i> ( <i>Heliotrophytum</i> )	1	T	B+	A	-	+	co -	-	tc	-	d	-
<i>H. amplexicaule</i> ( <i>Heliotrophytum</i> )	1	S	l2	A	-	-	co -	-	tc	-	d	-
<i>H. paronychioides</i> ( <i>Plagiomeris</i> )	1	S	B+	A	-	-	co -	-	tc	-	-	-
<i>H. pinnatisectum</i> ( <i>Plagiomeris</i> )	1	S	l2	A	-	-	co -	-	tc	-	-	-
<i>H. transalpinum</i> ( <i>Coeloma</i> )	1	T	B	A	-	+	co ta	-	tc	-	d	-
<i>H. veronicifolium</i> ( <i>Coeloma</i> )	1	T	B+	A	-	+	co ta	-	tc	ic	-	-
<i>H. microstachyum</i> ( <i>Hypsogenia</i> )	1	S	lm	A	-	-	co -	-	tc	ic	-	-
<i>H. elongatum</i> ( <i>Tiaridium</i> )	1	T	B	A	-	-	co -	-	tc	ic	-	-
<i>H. indicum</i> ( <i>Tiaridium</i> )	1	T	B+	A	-	-	co -	-	tc	ic	-	-
<i>H. angiospermum</i> ( <i>Schobera</i> )	1	T	B	A	-	+	co -	-	tc	ic	-	-
<i>H. patagonicum</i> ( <i>Platygyne</i> )	5	P	S	A	-	-	-	-	-	ic	-	-
<i>H. curassavicum</i> ( <i>Platygyne</i> )	5	P	S	A	-	-	-	-	-	ic	-	d
<b>TOURNEFORTIA</b>												
<i>T. gnaphalodes</i>	5	P	S	A	-	+	- s	-	-	-	d	s
<i>T. argentea</i>	5	T	?	A	-	+	co -	-	tc	-	d	-
<i>T. rollotii</i>	3	T	B	H	-	+	co s	-	tc	ic	-	-
<i>T. glabra</i>	3	T	B	H	-	+	co -	-	tc	ic	-	-
<i>T. hirsutissima</i>	3	T	B	H	-	-	co -	-	tc	ic	-	-
<i>T. bicolor</i>	3	T	B	H	-	-	co -	-	tc	ic	-	-
<i>T. ternifolia</i>	3	T	B	H	-	+	co -	-	tc	ic	-	-
<i>T. microcalyx</i>	3	T	B	H	-	+	co -	-	tc	ic	-	-
<i>T. luzonica</i>	3	T	B	H+	-	-	co -	-	tc	ic	-	-
<b>CEBALLOSIA</b>												
<i>C. fruticosa</i> var. <i>angustifolia</i>	1	S	B+	A	-	+	co -	ac	tc	ic	-	d
<b>ARGUSIA</b>												
<i>A. sibirica</i>	1	S	B+	A	-	+	co -	-	tc	-	d	s
<i>A. sogdiana</i>	1	S	?	A	-	-	ph s	ac	-	-	d	d+
<b>HELIOTROPIUM II</b> (section)												
<i>H. erosum</i> ( <i>Pterotropium</i> )	1	S	B+	A	-	-	- s	ac	-	-	d	d+
<i>H. asperrimum</i> ( <i>Pterotropium</i> )	1	S	B+	A	-	+	ph s	ac	-	-	d	d+
<i>H. zeylanicum</i> ( <i>Zeylanica</i> )	1	S	B+	A	-	+	ph -	ac	-	-	d	s
<i>H. hirsutissimum</i> ( <i>Odontotropium</i> )	1	T	B+	A	-	+	ph -	ac	-	-	d	d+
<i>H. arbainense</i> ( <i>Odontotropium</i> )	1	T	B+	A	-	+	ph -	ac	-	-	d	d+
<i>H. supinum</i> ( <i>Chamaetropium</i> )	1	S	B	A	-	-	ph s	ac	-	-	-	-
<i>H. nelsonii</i> ( <i>Pseudocoeloma</i> )	1	S	B+	A	-	-	ph -	ac	-	-	d	s
<i>H. ciliatum</i> ( <i>Pseudocoeloma</i> )	1	S	I	A	-	+	ph -	-	-	-	d	d+
<i>H. aegyptiacum</i> ( <i>Heliotropium</i> )	1	S	I	A	-	-	ph s	-	-	-	d	d+
<i>H. oliverianum</i> ( <i>Heliotropium</i> )	1	S	I	A	-	-	ph s	-	-	-	d	-
<i>H. suaveolens</i> ( <i>Heliotropium</i> )	1	S+	?	A	-	-	ph -	-	-	-	-	-
<i>H. giessii</i> ( <i>Heliotropium</i> )	1	S+	I	A	-	+	ph -	-	-	-	d	-
<i>H. europaeum</i> ( <i>Heliotropium</i> )	1	S+	B+	A	-	+	ph -	-	-	-	-	-
<i>H. digynum</i> ( <i>Pleurolasia</i> )	1	S	B	A	-	+	ph -	-	-	-	d	-
<i>Nogalia</i>												
<i>N. drepanophyllum</i>	1	S	B+	A	-	+	ph -	-	-	-	-	-

**Venation** (Table 5-3 column 2 **Lv**; Figs. 5-1a to d)—In general, leaf venation is brochidodromous, differing in the prominence of secondary and tertiary veins on the abaxial surface, the orientation of the tertiary veins, or in the shape of the intercostal areas delimited by the secondary and tertiary veins.

Four types of venation are found. The description follows the terminology of HICKEY (1973) and ASH *et al.* (1999).

1 – *hyphodromous* (abbreviation in Table 5-3 and 5-4 : **P**, Fig. 5-1a): only one prominent midvein is visible on the abaxial leaf surface. All higher order veins are weakly developed and immersed in the mesophyll. This condition is found generally in EUPLOCA [with the only exception of *H. procumbens* (see type 3)], and in the succulent leaves of the halophytic *H. curassavicum*, and *H. patagonicum* of HELIOTROPIUM I, and *T. gnaphalodes* of the TOURNEFORTIA clade.

2 – *brochidodromous I* (**F**, Fig. 5-1b): several very fine and barely prominent secondary veins curve near the margin and join in a series of arches. The tertiaries are very fine, not prominent, randomly reticulate to weakly alternate percurrent, enclosing irregular four- to five-sided intercostal areas. This type of venation is found in MYRIOPUS and IXORHEA.

3 – *brochidodromous II* (**S**, Fig. 5-1c): several prominent secondary veins curve near the margin and join in a series of arches. Near the margin the secondaries are often immersed in the mesophyll (Fig. 5-1c left, **S**). Most or all tertiaries are not prominent. If elevated tertiaries are present they are opposite to additional alternate percurrent (Fig. 5-1c right, **S+**) and enclosing regular intercostal areas. This venation type is found in most species of HELIOTROPIUM II and several species of HELIOTROPIUM I, as well as in ARGUSIA and CEBALLOSIA, in EUPLOCA only in *H. procumbens*.

4 – *brochidodromous III* (**T**, Fig. 5-1d): like as type 3, with several prominent secondary veins curve near the margin and join in a series of arches. All tertiary veins are prominent and opposite percurrent, i.e., the tertiaries link neighboring secondaries in parallel rows without branching, in addition some tertiaries may alternate percurrent. Characteristic are the regular intercostal areas delimited by the tertiaries. Veins of higher order are also mostly elevated, leading to a characteristic rugose surface pattern of the lamina. This venation type is found in all species of HELIOTHAMNUS, many species of HELIOTROPIUM I, and all species of TOURNEFORTIA,

except the succulent *T. gnaphalodes* (see type 1). Within HELIOTROPIUM II this venation type is found in *H. hirsutissimum* and *H. arbainense*.

**Leaf anatomy** (Table 5-3 column 3 **La**; Figs. 5-2a to c)—In general, the leaves of Heliotropiaceae are bifacial, subbifacial or isobilateral with a one-layered palisade tissue. Four additional types are restricted to a few species.

1 – *bifacial* (**B**, Fig. 5-2a): this is the most common type, with a single adaxial layer of palisade parenchyma. Bifacial leaves are found in many species of EUPLOCA, HELIOTROPIUM I and II (species see Table 3). In MYRIOPUS, HELIOTHAMNUS, and TOURNEFORTIA it is the only leaf type with the exception of the halophytic *T. gnaphalodes*.

2 – *subbifacial* (**B+**, Figs. 5-2b, 5-4a, g): this differs from type 1 only by an additional, often discontinuous, one-layered palisade or palisade-like tissue on the abaxial side. The abaxial palisade cells are shorter than adaxial ones. This leaf type is found in EUPLOCA, HELIOTROPIUM I and II (species see Table 5-3), CEBALLOSIA and *Argusia sibirica*.

3 – *isobilateral, one-layered* (**I**, Fig. 5-2c): with a well developed and more or less uniform, one-layered palisade tissue on both sides. This type is found in EUPLOCA and in HELIOTROPIUM II (species see Table 5-3).

4 – *isobilateral, two-layered* (**I2**): an adaxial, two-layered palisade tissue, and a two-layered palisade-like tissue of shorter cells on the abaxial side is present in HELIOTROPIUM I (*H. pinnatisectum*, *H. amplexicaule*).

5 – *isobilateral, heterogenous* (**Ih**, Fig. 5-4f): restricted to IXORHEA and characterized by a two-layered adaxial and a one-layered abaxial palisade parenchyma.

In types 1 – 5 the spongy mesophyll is well developed and consists of isodiametric cells.

6 – *isobilateral, multilayered* (**Im**, Fig. 5-4c): characterized by a 6-layered palisade parenchyma, lacking spongy mesophyll, and found only in *H. microstachyum* (HELIOTROPIUM I).

7 – *succulent* (**S**, Fig. 5-4b): The succulent leaves are isobilateral without a differentiation in chlorenchyma and water-storage tissue (all mesophyll cells are able to store water). They are found in the halophytic species of HELIOTROPIUM I (*H. curassavicum*, *H. patagonicum*). A

differentiation in a two-layered palisade tissue each side and a water-storage tissue in the center parts of the leaves found is only in halophytic TOURNEFORTIA (*T. gnaphalodes*).

**Epidermis and stomata** (Table 5-3 column 4 **Sd**)—In all taxa investigated the leaf epidermis consists of a single cell layer. The cells are polygonal, and the adaxial epidermal cells are usually slightly larger than those on the abaxial side. The anticlinal cell walls are straight, irregular to sinuous, and of varying shape on both surfaces. The cuticle is thin (5 to 10  $\mu\text{m}$ ), in some EUPLOCA species thickened up to 20 or 25  $\mu\text{m}$ . Ornamentations are lacking.

Most of the species have amphistomatous leaves (**A**) with nearly homogenous stoma distribution on either side (species see Table 5-3). Hypostomatous leaves (**H**) are found in MYRIOPUS (*T. volubilis*), HELIOTROPIUM I (sect. *Cochranea*), and the non-halophytic TOURNEFORTIA. In HELIOTHAMNUS and *T. luzonica* (TOURNEFORTIA) there are some stomata present on the adaxial side (**H+**).

The stomata are always anomocytic (Ranunculaceous type), randomly orientated, oval in shape and variable in size, with (3-) 5 (-6) accessory cells (Fig. 5-4d). The stomata are on the same level of the epidermis except in IXORHEA and HELIOTROPIUM I (sect. *Cochranea*) where they are elevated (Figs. 5-4e, f) in a chimney-like form, and the accessory cells form a ring.

**Vascular system and Kranz-type leaf anatomy** (Table 5-3 column 5 **Ko**)—All species investigated exhibit collateral bundles with adaxial xylem. Collenchymatic tissue is frequently associated to the main veins. Veins of higher order are surrounded by parenchymateous single-layered bundle sheaths and their cells are mostly with, rarely without chloroplasts.

Kranz-type leaf anatomy is present in all species of EUPLOCA except *H. ovalifolium* and *H. procumbens*. The bundle sheath cells contain a large number of chloroplasts centripetally appressed to the cell wall (Figs. 5-2a, 5-4g). There are no additional radially arranged mesophyll cells around the veins (Figs. 5-2a, 5-4g).

**Foliar trichome types and lithocysts** (Figs. 5-3a to l; Table 5-3 columns 6-8, **Gt**, **Tt**, **Ct**)—In most taxa, both leaf surfaces are more or less densely covered with different types of uniseriate trichomes forming an indumentum of variable texture and density. Only the halophytic and succulent leaves of *H. patagonicum* and *H. curassavicum* (HELIOTROPIUM I) have glabrous leaves. Multiseriate trichomes lack completely.



Eight types of trichomes and lithocysts were identified. All are found on either side of the lamina, but with different density. Sometimes there are gradual transitions between trichome types especially on veins, leaf margins, and petioles. On veins the trichomes are often enlarged and the trichome type is indistinct.

For an unambiguous determination of the trichome type we present a key for all types found in the family (Figs. 5-3a to I). The terminology follows THEOBALD et al. (1979) and HARRIS & HARRIS (1994).

1 – *glandular, multicellular* (Fig. 5-3a): trichomes consist of two or three stalk cells, and a terminal more or less spherical gland cell (Fig. 5-4h). Glandular trichomes occur only in combination with other trichome types, and are thus always part of a heterotrichous indumentum. An exception is IXORHEA where it is the only trichome type. Usually they are much smaller than the eglandular, unicellular trichomes. They are found in HELIOTHAMNUS, some species of HELIOTROPIUM I and II, and TOURNEFORTIA, CEBALLOSIA, and *Argusia sibirica*. They are lacking in MYRIOPUS and EUPLOCA.

The isotrichous IXORHEA is characterized by having sessile, one-celled secretory glands (Figs. 5-4e, f).

2 – *unicellular, unbranched trichomes of slender shape* (s, Fig. 5-3f): the trichomes form a villous to lanate indumentum. They are usually long and slender, with unswollen base, smooth or sculptured with small papillae (Fig. 5-5a), and are not mineralized. The surrounding epidermis cells show no modification. This trichome type predominantly occurs as a part of a heterotrichous, rarely part of isotrichous indumentum in MYRIOPUS, EUPLOCA, HELIOTHAMNUS, TOURNEFORTIA, HELIOTROPIUM I, and *Argusia sogdiana*.

3 – *unicellular, unbranched trichomes on a pedestal of distinctly enlarged epidermis cells* (sg, Fig. 5-3e): the trichomes are strigose, uniform in length, thick-walled, with a slightly swollen base, and covered with blunt, conical papillae. The surrounding epidermis cells are enlarged and elevate the trichome base above the epidermis. These bases are strongly curved so that the trichomes are appressed to the epidermis. All trichome tips are pointing to the leaf apex (Figs. 5-5b, c). They are only found in EUPLOCA.

4 – *unicellular, unbranched, cystolith bearing trichomes* (co, tc, Figs. 5-3i to k): trichomes are strongly conical (co), born on a bulbous base, and smooth or covered with papillae of different

shape and density. The indumentum is anisotrichous, rarely uniform in length on either side of the lamina surface (Fig. 5-5d). Often the trichomes are reduced to small tips with strongly swollen bases (Figs. 5-3k, 5-5e). The surrounding epidermis cells are partly enlarged, but they never elevate the trichome base above the epidermis. Their swollen bases are only slightly curved so that the shafts are not appressed to the epidermis (Figs. 5-5d, e). The basal part of the enlarged bulbous bases is deeply embedded in the mesophyll. In most cases the trichomes of this type bear a cystolith of calcium carbonate of usually mussel- (Figs. 5-3k, 5-5h) or rarely bunch-shape (Figs. 5-5i, 5-6e) in their bulbous bases. The term trichome lithocyst (**tc**) is then applied. The cystolith is indistinctly stalked when the trichome is well developed (Figs. 5-3k, 5-4c, 5-5h), and distinctly stalked when the trichome apex is reduced to a tip (Figs. 5-3k, 5-5i).

This trichome type is found in species of HELIOTHAMNUS, HELIOTROPIUM I, TOURNEFORTIA, CEBALLOSIA, and *Argusia sibirica*. In HELIOTHAMNUS the trichomes are predominantly uniform in length and rarely contain cystoliths in the bases. In HELIOTROPIUM II, sporadically type 4-like trichomes occur on veins or leaf margins in addition to trichome of type 5. They are uniform in length and never bear a cystolith in their bases.

5 – *unicellular, unbranched trichomes with strongly swollen base* (**ph**, Figs. 5-3g, h): the spear-like trichomes form a papillose-hispid indumentum of uniform length. They are born on strongly swollen bases and have a smooth surface (Figs. 5-5f, g). The trichome tips and enlarged bases are sometimes mineralized. The deposits of calcium carbonate in the bulbous bases are sometimes cup-shaped (Figs. 5-3h, 5-5j), but never form a compact cystolith as described in type 4. The trichomes may be erect (Figs. 5-3h, 5-5g) to sharply curved above their bases, but they are not appressed to the epidermis (Figs. 5-3g, 5-5f). The surrounding epidermis cells are not modified.

This trichome type is restricted to HELIOTROPIUM II (with the single exception of *H. erosum* showing only trichomes of type 2), and it is intermediately developed, covered with papillae on surface, like trichomes of type 4, in *Argusia sogdiana* (Fig. 5-6a).

6 – *unicellular, two-armed* (**ta**, Fig. 5-3d): trichomes are regularly to irregularly two-armed, with a bulbous base and covered with conical papillae (Fig. 5-6b). The surrounding epidermis cells are not modified. This trichome type is only found in the heterotrichous indumentum of *H.*

*amplexicaule* and *H. veronicifolium* of HELIOTROPIUM I together with trichomes of type 4 (Figs. 5-3i to k).

7 – *unicellular lithocysts, with reduced trichome tip* (ic, Fig. 5-3I): these idioblasts can be recognized in surface view as cells with a small diameter in the center of radially arranged and enlarged epidermis cells (Fig. 5-6c). The main extension of the lithocyst lies deeply embedded in the mesophyll. The cystolith enclosed consists of calcium carbonate, is distinctly stalked, and mussel- (Figs. 5-3k, 5-6d) or bunch-shaped (Figs. 5-6e). Such lithocysts are restricted to HELIOTROPIUM I, TOURNEFORTIA, and CEBALLOSIA (species see Table 5-3).

8 – *multicellular lithocyst complex* (ac, Figs. 5-3b, c): trichome lithocysts of usually elongated shape, uniform in length, sculptured with small papillae, with one well developed, unstalked, mussel-shaped cystolith in its bulbous base. They are surrounded by radially arranged subsidiary lithocysts, each containing an unstalked mussel-shaped calcium carbonate cystoliths attached to the centripetal cell walls (Figs. 5-3b, c, 5-6f to h).

This lithocyst complex occurs only sporadically and sparsely as a part of a heterotrichous indumentum in some species of MYRIOPUS, EUPLOCA, many species of HELIOTROPIUM II (species see Table 5-3), CEBALLOSIA, and *Argusia sogdiana*. In the last species it is the dominant trichome type.

**Crystals and idioblasts** (Table 5-3 column 9, Cy; Figs. 5-6i to k)—Calcium oxalate crystals of various shape are found in different leaf tissues. Three types of crystal idioblasts were identified.

1 – *crystal druses or crystalsand* (d, s, Fig. 5-6i): crystal druses are situated in different densities only in the mesophyll, but never in the epidermis or along bundles. Only sporadically the crystal druses are accompanied by crystalsand. They are abundant in all HELIOTHAMNUS species, IXORHEA, and in most species of HELIOTROPIUM II, CEBALLOSIA, and ARGUSIA. Calcium oxalate crystals occur rarely in species of HELIOTROPIUM I (species see Table 5-3), while they are completely absent in the non-halophytic TOURNEFORTIA.

2 – *crystal tubes* (d+, Fig. 5-6i): the idioblasts form tubes in the palisade parenchyma and contain many crystal druses arranged in one line. They are present in IXORHEA, HELIOTHAMNUS, HELIOTROPIUM II (species see Table 5-3), and *Argusia sogdiana*. They are absent in HELIOTROPIUM I and TOURNEFORTIA.

3 – *crystal needles* (**n**, Figs. **5-6j, k**): bundles of calcium oxalate needles are found in low density in the epidermis, rarely additionally in the mesophyll or surround the vascular bundles. Frequently many adjacent epidermal cells containing these crystal needles form large complexes of dendritic shape. They are found in some species of MYRIOPUS and EUPLOCA (species see Table **5-3**).

## 5.4 Discussion

The results of leaf anatomy patterns, especially venation, vascular system, various foliar trichome types, and localization of crystals are of surprising high systematic value and prove the formerly proposed subdivision of this family based on molecular results (DIANE *et al.* 2002a, HILGER & DIANE submitted).

**General leaf morphology**—Size and shape of the leaves are of high variability within Heliotropiaceae and are in general not appropriate for systematic studies on higher taxonomic level (FÖRTHNER 1998). These characters may be interpreted under ecological points of view.

Semi-arid habitats promote small narrow- to linear-lanceolate leaves, sometimes with revolute leaf margins, and often a dense indumentum. This has evolved several times within most clades of Heliotropiaceae, especially within EUPLOCA, HELIOTROPIUM I, and also MYRIOPUS. On the other hand, humid tropical conditions or permanent water availability promote a broad-leaved shape. Species of HELIOTHAMNUS form a part of Andean scrub communities in rainless habitats. Their leaves are very often characterized by a dense indumentum, thus enabling fog condensation, which is the only reliable source of water in this regions.

**Venation**—While leaf size and shape may vary enormously within one group, leaf venation is usually constant. In general, the leaves of Heliotropiaceae show a brochidodromous venation. They differ in prominence of the secondary and tertiary veins, which may be immersed in the mesophyll, the orientation of the tertiaries, or in the shape of the intercostal areas. A brochidodromous venation with fine and barely developed veins of higher order and randomly reticulate to weakly alternate percurrent tertiaries enclosing irregularly four- to five-sided intercostals areas (type 2, **F**) is characteristic for MYRIOPUS and IXORHEA.

Starting from this venation type a reduction to a hypodromous venation (type 1, **P**) characterizes the leaves of EUPLOCA species. In addition to EUPLOCA only the halophytic species (*H. curassavicum*, *H. patagonicum*, *T. gnaphalodes*) show venation type 1 (**P**), which probably depends on the halophytic habitat conditions which promotes succulence with reduction of the venation.

Venation types 3 (**S**) and 4 (**T**) are variations, differing in the prominence of the tertiary veins, and depend strongly on leaf size. The secondaries are always elevated. When elevated tertiaries are present, they are opposite percurrent, delimiting regularly intercostals areas. These mixture of venation types characterize the leaves of the remaining clades of Heliotropiaceae. Only the generally broad leaved HELIOTHAMNUS and TOURNEFORTIA species are characterized by venation type 4 (**T**) exclusively, that means the larger the leaves are the stronger the veins of higher order may develop. Particularly the regular opposite position of the tertiaries may contribute to stabilizing the lamina and leads in addition to veins of higher order, to a characteristic rugose surface pattern.

**Vascular system and Kranz-type leaf anatomy**—Most important with regard to the vascular system is the arrangement of bundle sheaths in EUPLOCA. The sheath cells contain a large amount of chloroplasts centripetally appressed against the cell wall. This corresponds to the Kranz-chlorenchyma organization of HABERLANDT (1896).  $\delta^{13}\text{C}$ -measurements (*H. scabrum* Retz., *H. zeylanicum* Lam., DAS & RAGHAVENDRA 1973; *H. marifolium* Retz., *H. paniculatum* R.Br., SANKHLA *et al.* 1975) revealed that the C<sub>4</sub>-dicarboxylic acid pathway is indeed present. Comparable observations were made by FROHLICH (1978) for species of *Heliotropium* sect. *Orthostachys* of Mexico. Furthermore, he described the mesophyll cells as arranged more or less radially around the bundle sheath. We cannot confirm this observation. We did not find mesophyll cells arranged that way. Additionally, NAPP-ZINN (1984) pointed out that in dicotyledons the Kranz-type leaf anatomy is not developed with radially arranged mesophyll. C<sub>4</sub>-species are restricted to the EUPLOCA clade. Revising of *H. zeylanicum* (HELIOTROPIUM II) could not confirm the observations of C<sub>4</sub> anatomy by DAS & RAGHAVENDRA (1973).

The C<sub>4</sub> photosynthetic pathway developed only in the EUPLOCA lineage, and thus the genus “*Heliotropium*” must be substituted by *Euploca* in the list of genera in which both C<sub>3</sub> and C<sub>4</sub> photosynthesis evolved. All species of subsect. *Bracteata* investigated, and some species of subsect. *Ebracteata* are C<sub>4</sub>-plants, and thus Kranz-type leaf anatomy is not appropriate for the characterization of the two subsections of *Orthostachys*, as was suggested by FÖRTHNER (1998).

The presence of Kranz-anatomy in “*Hilgeria*” and “*Schleidenia*” corresponds to our molecular studies (DIANE *et al.* 2002a, HILGER & DIANE submitted) which showed that these two taxa are nested within EUPLOCA.

**Character combinations and systematic implications** (Table 5-4)—A summary of the results is shown in Table 5-4.

Clade	Taxon	H	Lv	La	Sd	Ko	Gt	Tt	Ct			Cy		
<b>IXORHEA</b>	<i>Ixorhea</i>	2	F	lh	A	-	(+)	-	-	-	-	-	d	d+
<b>MYRIOPUS</b>	<i>Tournefortia</i> sect. <i>Cyphocyema</i>	1	F	B	A H	-	-	-	s	ac	-	-	n	-
<b>EUPLOCA</b>	<i>Heliotropium</i> sect. <i>Orthostachys</i>	1	P	B B+ I	A	+	-	sg	s	ac	-	-	n	-
	<i>Schleidenia</i>	1	P	B	A	+	-	sg	-	-	-	-	n	-
	<i>Hilgeria</i>	1	P	?	A	+	-	sg	-	-	-	-	-	-
<b>HELIOTHAMNUS</b>	<i>Heliotropium</i> sect. <i>Heliothamnus</i>	2	T	B	H+	-	+	co	s	-	tc	-	-	d d+
<b>HELIOTROPIUM I</b>	sect. <i>Cochranea</i>	1	S T	B	H	-	+	co	-	-	tc	-	-	d -
	<i>Heliotropium</i> species of the New World	5	P	S	A	-	-	-	-	-	-	ic	-	d -
	remaining species	1	S T	B B+ I2 Im	A	-	+	co	ta	-	tc	ic	-	d -
<b>TOURNEFORTIA</b>	<i>Tournefortia</i> sect. <i>Tournefortia</i>	5	P T	S	A	-	+	co	s	-	tc	-	-	d s
	remaining species	3	T	B	H	-	+	co	s	-	tc	ic	-	-
<b>CEBALLOSIA</b>	<i>Ceballosia</i>	1	S	B+	A	-	+	co	-	ac	tc	ic	-	d -
<b>ARGUSIA</b>	<i>Argusia</i> <i>A. sibirica</i>	1	S	B+	A	-	+	co	-	-	tc	-	-	d s
	<i>A. sogdiana</i>	1	S	?	A	-	-	ph	s	ac	-	-	-	d d+
<b>HELIOTROPIUM II</b>	<i>Heliotropium</i> species of the Old World	1	S T	B B+ I	A	-	+	ph	-	ac	-	-	-	d d+ s
	<i>Nogalia</i>	1	S	B+	A	-	+	ph	-	-	-	-	-	-

Table 5-4. Summary of the results on leaf anatomy and distribution of foliar trichome types of the Heliotropiaceae. Abbreviations are as in Table 5-3. Highlighted are significant characters.

**EUPLOCA**—The EUPLOCA clade includes species of the current taxonomic circumscription of *Heliotropium* sect. *Orthostachys*, *Schleidenia* [which JOHNSTON (1928) already treated as subsection *Axillaria* of section *Orthostachys*], and *Hilgeria*. The leaves are characterized by a hypodromous venation (type 1, **P**—except *H. procumbens*), bilateral to isobilateral anatomy (**B**, **B+**, **I**), amphistomatous leaves (**A**), trichomes of type 3 (**sg**) and, sporadically, additional

lithocyst complexes of type 8 (**ac**) in Old World species. In two species crystal needles (**n**) are present in the epidermis and surrounding the bundles. Kranz-type leaf anatomy is restricted to species of this clade. Glandular trichomes, trichomes of the types 4 (**co**, **tc**), 5 (**ph**), 7 (**ic**), and calcium oxalate druses (**d**) in the mesophyll are lacking. Summarizing, strongly supported by leaf anatomical and molecular results (DIANE *et al.* 2002a, HILGER & DIANE submitted), we plead for a nomenclatural recombination within this clade to the genus *Euploca* Nutt. (NUTTALL 1837).

**HELIOTHAMNUS**—The species belonging to *Heliotropium* sect. *Heliothamnus* are characterized in the overall occurrence of brochidodromous venation type 4 (**T**), bilateral leaf anatomy (**B**), hypostomatous leaves with strongly reduced density of stomata on the adaxial leaf surface (**H+**), glandular trichomes and trichomes of type 4 (**co**, **tc**), and the occurrence of calcium oxalate druses (**d**) usually additional in the form of crystal tubes (**d+**) but lack of unicellular lithocysts (type 7, **ic**).

The occurrence of crystal tubes and the lacking of lithocysts separates these Andean species from the HELIOTROPIUM I clade.

**HELIOTROPIUM I and TOURNEFORTIA**—Molecular results (DIANE *et al.* 2002a, HILGER & DIANE submitted) show a close relationship between HELIOTROPIUM I and TOURNEFORTIA, which is also reflected in leaf anatomy. Both lineages are characterized by the presence of glandular trichomes, trichomes of type 4 (**co**, **tc**), and unicellular lithocysts of type 7 (**ic**). They lack calcium oxalate crystals in the form of crystal tubes (**d+**), multicellular lithocyst complexes (type 8, **ac**), trichomes of types 3 (**sg**) and 5 (**ph**), and calcium oxalate needles (**n**) in the epidermis.

The exclusive occurrence of venation type 4 (**T**), bilateral (**B**) and hypostomatous leaves (**H**), and the lack of calcium oxalate druses (**d**)—except the halophytes—separates TOURNEFORTIA from HELIOTROPIUM I. On the other hand, in HELIOTROPIUM I—except sect. *Cochranea*—, additional venation type 3 (**S**), and subbifacial to isobilateral (**B+**, **I2**, **Im**), always amphistomatous (**A**) leaves, and the occurrence of calcium oxalate druses (**d**) are characteristic.

**HELIOTROPIUM II**—This clade comprises *Heliotropium* species of the Old World including *Nogalia* (based on *Heliotropium drepanophyllum* Baker). With HELIOTROPIUM I they share brochidodromous venation types 3 (**S**) and 4 (**T**), bi- to isobilateral (**B**, **B+**, **I**) and amphistomatous (**A**) leaf anatomy, glandular trichomes and calcium oxalate druses in the

mesophyll (**d**). They differ from HELIOTROPIUM I and TOURNEFORTIA by the exclusive presence of trichomes of type 5 (**ph**), lithocyst complexes of type 8 (**ac**), and crystal tubes (**d+**). Unicellular lithocysts (type 7, **ic**) have not been found. With HELIOTHAMNUS they share crystal tubes and the lack of unicellular lithocysts.

**MYRIOPUS**—MYRIOPUS and TOURNEFORTIA have no close relationships. While the species of TOURNEFORTIA generally resemble HELIOTROPIUM I species and show nearly identical leaf anatomical features, the character set of MYRIOPUS species differs markedly. It is characterized by venation type 2 (**F**), bilateral hypo- and amphistomatous leaves, simple trichomes (type 2 **s**), lithocyst complexes (type 8, **ac**), and crystal needles (**n**) in the epidermis, while trichomes of type 4 (**co**, **tc**), unicellular lithocysts of type 7 (**ic**), and crystal druses (**d**) are lacking. Leaf anatomy thus strongly supports the molecular results of DIANE et al. (2002) and HILGER & DIANE (submitted). As in EUPLOCA, a re-establishment of the genus *Myriopus* Small (SMALL 1933) is inevitable.

**ARGUSIA, CEBALLOSIA, IXORHEA**—It is also possible to obtain systematic indications for CEBALLOSIA and ARGUSIA. Apparently the two species of *Argusia* do not constitute a natural group. Leaf anatomy of both *Argusia* species differs strongly, and *A. sibirica* seems to be more closely related to HELIOTROPIUM I species by sharing the same trichomes of type 4 (**co**, **tc**). *A. sogdiana* on the other hand seems to be connected with HELIOTROPIUM II by the presence of lithocyst complexes (type 8, **ac**), crystal tubes (**d+**), and the trichomes of type 5 (**ph**).

*Ceballosia* differs from HELIOTROPIUM I species only by having multicellular lithocyst complexes (type 8, **ac**) and seems to belong to this lineage.

*Ixorhea* is morphologically very aberrant and leaf anatomy does not at all contribute to its systematic position.

Combining all data, leaf anatomy of Heliotropiaceae is of unexpectedly high value with regard to their relationships and strongly supports the actual subdivision within this family based on molecular data (Table 5-1, DIANE et al. 2002a, HILGER & DIANE submitted).