COMPARATIVE LEAF ANATOMY OF HEISTERIA (OLACACEAE)

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CONTENTS

SUMMARY

The leaf anatomy of all 33 species of Heisteria is described, based on a study of 143 speci-mens. There is a considerable amount of diversity in stomatal type (anisocytic, anomocytic, cyclocytic, laterocytic or paracytic), in occurrence and type of mesophyll sclereids, and of fibre bundles along the leaf margin. Outline and thickness of anticlinal epidermal cell walls, cuticle thickness, crystal complement, and stomatal size also vary, but often below the species level. The leaf anatomical diversity can be used for recognising 8 groups of varying distinctness in Heisteria. H. asplundii and H. skutchii with laterocytic stomata, and H. pentandra and H. scandens with paracytic stomata constitute the two most distinct infrageneric groups; the other six groups appear mutually more closely related and are partly linked through intermediates. A tentative phylogenetic classification of Heisteria and a discussion of the position of Heisteria in the Olaca-ceae is given.

INTRODUCTION

Heisteria is a genus of trees, shrubs and rarely climbers, mainly confined to the substage of tropical lowland forests of the New World (30 species) and Africa (3 species). The present study is complementary to a leaf anatomical generic survey of the Olacaceae (Baas et al., 1982) and Dr. H. Sleumer's revision of the family for Flora Neotropica (1983). During the initial stages of these studies it appeared that the genus *Heisteria* shows a considerable leaf anatomical diversity in several characters. It also turned out that material identified in several herbaria as *H. scandens* Ducke, in fact included two species which were very distinct on leaf anatomical grounds: *H. scandens* Ducke proper with paracytic stomata, and *H. barbata* Cuatrecasas with anisocytic, cyclocytic and anomocytic stomata. These initial results, together with the desirability to find additional characters to aid specific delimitation and identification, prompted the present comprehensive study including many type specimens.

Earlier leaf anatomical literature on Olacaceae (including *Heisteria*) has been cited in Baas et al. (1982), and will only be referred to as far as relevant to the present results.

MATERIALS AND METHODS

All New World material was derived from herbarium specimens used by Sleumer for his treatment of the genus in Flora Neotropica and identified by him personally. For the location of herbarium specimens and duplicates see Sleumer (1983). In addition, material of the three African species represented in the Rijksherbarium was studied.

Cuticular macerations, transverse and paradermal sections, and leaf clearings were prepared as described elsewhere (Baas et al., 1982). Not all 143 specimens studied were subjected to the full range of microtechnical procedures: for some specimens only paradermal sections or macerations were prepared to test constancy of certain epidermal characters; in few cases this restriction was necessary in view of scarcity of the precious type material.

In the following list of specimens types (including those of reduced species) are indicated. For more detailed information on localities and ecology of the species see Sleumer (1983).

H. acuminata (Humboldt & Bonpland) Engler. Type specimens: Colombia, Bonpland 1903 (type); Peru, Poeppig 2133 (type of *H. cyanocarpa* Poeppig); Colombia, Triana s.n. (type of *H. celastrinea* Triana & Planchon); Peru, Spruce 4531 (type of *H. pallida* Engler); Panama, Pittier 4006 (type of *H. longipes* Standley). Other specimens: Costa Rica, Utley & Utley 1148; Colombia, Triana s.n. (Cundinamarca); Venezuela, Steyermark et al. 101447; Peru, Diaz & Jaramillo 296, Revilla 220, Schunke 1204; Brazil, Kuhlmann 1672, Prance et al. P 25440, Sastre & Sastre 120; Bolivia, Prance et al. 8353.
H. amazonica Sleum. Brazil, Ducke s.n. (RB 24970, isotype).
H. amphoricarpa (Ducke) Sleum. Brazil, Ducke 1664 (type).
H. asplundii Sleum. Ecuador, Gentry & Shupp 26359.
H. barbata Cuatrecasas. Colombia, Schultes & Cabrera 12870, 15465 and 16694; Venezuela, Berry & Chesney 2171, Blanco 1187; Peru, Klug 684 (type); Brazil, Berg & Steward P 19885,

Ducke 698 and 721, Krukoff 6840, 6862 and 8799, Loureiro et al. s.n. (INPA 37701), Oliveira 5431 and 5571, Pires & Black 1283, Prance et al. 3076, 17789 and 22231, Rodrigues & Monteiro 8321, Rosa 93, Sastre & Sastre 165, A. Silva 433, N.T. Silva 858, N.T. Silva & Santos 4745, J.F. da Silva 460.

H. blanchetiana (Engler) Sleum. Brazil, Blanchet 3581 (type).

H. cauliflora J.E. Smith. Guyane Française, Mélinon 24, Wachenheim 59; Brazil, Egler 1399.

H. citrifolia Engler. Brazil, Anderson et al. 36487, Pohl s.n. (type).

H. coccinea Jacquin. Martinique, Anon. s.n. (type of *H. guianensis* Engler), C. Richard s.n. (probably type duplicate of *H. guianensis* Engler), Hahn 142.

H. concinna Standley. Panama, Foster 1968, Pittier 5197 (type).

H. costaricensis Donell Smith. Costa Rica, Donell Smith 4760 (type), Léon 2481.

H. densifrons Engler. Type specimens: Brazil, Martius Obs. 2904 (type); Venezuela, Spruce 3306 (type of *H. microcarpa* Spruce ex Engler); Brazil, Ducke s.n. (RB 18151, type of *H. sessilis* Ducke), Krukoff 6051 (type of *H. parvicalyx* A.C. Smith). Other specimens: Guyane Française, Oldeman B–1795; Brazil, Prance et al. 7673.

H. duckei Sleum. Brazil, Ducke s.n. (RB 19562), Ducke s.n. (RB 24969), Krukoff 8506, Nelson & Lima P 21069.

H. huberiana Sleum. Brazil, Campbell et al. P 24422, Ducke s.n. (MG 8996, type).

H. insculpta Sleum. Peru, Gentry et al. 20980.

H. latifolia Standley. Type specimens: Panama, Pittier 4156 (type); Ecuador, Schultze Rhonhof 2468 (type of *H. megalophylla* Sleum.). Other specimens: Colombia, Lawrance 782; Venezuela, Funk & Schlim 119.

H. laxiflora Engler. Brazil, Spruce 1549 (type).

H. macrophylla Oersted. Costa Rica, Tonduz 17656.

H. maguirei Sleum. Guyana, Maguire & Fanshawe 22914 (type); Brazil, Pires et al. 51221.

H. maytenioides Spruce ex Engler. Venezuela, Spruce 3508 (type).

H. media Blake. Belize, Schipp 970 (type of *H. chippiana* Standley).

H. nitida Spruce ex Engler. Type specimens: Peru, Spruce 4148 (type), Tessmann 4419 (type of *H. caloneura* Sleum.). Other specimens: Peru, Revilla 2381; Brazil, Krukoff 4671 and 5108, Maguire et al. 56762; Bolivia, White 291.

H. ovata Bentham. Type specimens: Brazil, Martius s.n. (type of *H. flexuosa* Engler), Spencer Moore 292 (type of *H. rubricalyx* Sp. Moore), Ducke s.n. (MG 7219, type of *H. micrantha* Huber), Krukoff 5343 (type of *H. krukovii* A.C. Smith); Colombia, Vageler 97 (type of *H. vageleri* Burret). Other specimens: Venezuela, Williams 11383, Maguire & Politi 28612; Peru, Klug 3305; Brazil, Guedes 579, Hatschbach 32098, M. Silva 2678.

H. parvifolia Smith. Ivory Coast, Leeuwenberg 3153; Cameroon, Zenker 4308.

H. pentandra (Bentham ex Reisseck) Engler. Venezuela, Spruce 3301 (type), Williams 15236.

H. perianthomega (Vellozo) Sleum. Brazil, Raddi s.n. (type of *H. raddiana* Bentham ex Hooker), Glaziou 10390, Mori 11892, Poland 6672.

H. salicifolia Engler. Brazil, Gaudichaud 848bis (type).

H. scandens Ducke. Type specimens: Panama, Pittier 4244 (type of *H. fatoensis* Standley); Brazil, Ule 9367 (type of *H. uleana* Sleum.); Peru, Williams 2719 (type of *H. eurycarpa* Standley). Other specimens: Panama, Mori & Kallunki 2810, von Wedel 1401; Colombia, Archer 2131, Cuatrecasas 15767; Suriname, BW 6933, Lindeman 3529 and 5183, Maguire 24859; Guyane Française, de Granville B–4670; Peru, Asplund 14658, Gentry et al. 18599 and 19009, Kayap 439, Tessmann 4650, Woytkowski 5135; Brazil, Archer 8285, Cordeiro 646, Fróes 23881 and 33621, Krukoff 6484, Prance et al. 15779 and 22965; Bolivia, Krukoff 11266.

H. silvianii Schwacke. Brazil, Reitz & Klein 8477.

H. skutchii Sleum. Costa Rica, Skutch 4896 (type).

H. spruceana Engler. Peru, Croat 19754, Klug 2554; Brazil, Prance et al. 3994 and 14797, Spruce 1510 (type).

H. trillesiana Pierre. Gabun, Kleine 2227 (type).

H. zimmereri Engler. Cameroon, Zenker 3576.

SURVEY OF THE LEAF ANATOMICAL CHARACTERS WITH COMMENTS ON
VARIABILITY AND TAXONOMIC VALUE

The unspecialised epidermal cells and cuticle (figs. 1–4)

The anticlinal walls of the unspecialised epidermal cells vary from straight to
strongly undulating (figs. 1–4). In the 17 species of which more than a single speci-
men was studied 3 species showed the complete range of variation of the genus, an-
other 8 species showed a more limited variation (e.g., predominantly straight to
curved, but sometimes weakly undulate), 3 species (*H. citrifolia, H. duckei, H. huber-
iana*) were constant for predominantly straight anticlinal walls, and 4 species were
constant for weakly to strongly undulating anticlinal walls (*H. barbata, H. coccinea,
H. densifrons, H. pentandra* – most specimens of *H. scandens* also have undulating
walls). Thus the diagnostic value of outline of the anticlinal epidermal cell walls
varies with the species and is also limited by the considerable overlap between the
categories 'straight to curved' and 'curved to undulate', etc.

In some species, thick anticlinal walls occur (fig. 3) in the abaxial and adaxial epi-
dermis (*H. citrifolia, H. duckei, H. laxiflora, H. maytenioides, H. perianthomega*;
some specimens of *H. ovata* have a tendency for thick walls too). In *H. periantho-
mega* there is variability from normal (i.e., fairly thin) to thick walls. In *H. citrifolia*
and *H. duckei*, the only other species which were tested for variability, thick epider-
mal walls appeared fairly constant.

In about half the species the anticlinal cuticular flanges and cell walls are pitted,
but often this varies in conspicuousness and this character has not been further ex-
plored for taxonomic analysis. The same applies to the variation in cuticular texture
(caused by attributes of the cuticular layer) which varies from smooth to granular.
Cuticular striation (due to parallel ridges) has been found in two specimens only: one
of *H. citrifolia* and one of *H. duckei* and cannot be relied on for diagnostic purpose
because of its inconstancy in these species.

Cuticular thickness varies between wide limits from about 1–3 μm in some species
up to 20 μm (*H. citrifolia, H. maytenioides*). In most specimens the adaxial cuticle
is thicker than the abaxial one. As far as tested cuticular thickness is fairly constant
at the species level. In for instance *H. acuminata* and *H. scandens*, of which many
specimens were studied, adaxial cuticular thickness only ranges between 1–4 μm.
The more variable species still cover a relatively small part of the total variation, e.g.
H. nitida (4–9 μm), *H. ovata* (6–14 μm), and *H. spruceana* (8–12 μm). The thickest
cuticles are from species occurring in open vegetation ('campo', 'caatinga', 'cerrado',
'restinga'), but several species from the lowland or premontane rainforest also have
thick cuticles (10–15 μm). To some extent cuticular thickness is also fairly well cor-
related with other leaf anatomical variables and has some value for infrageneric classi-
fication in *Heisteria* (cf. table 2).

The stomatal complex (figs. 1—4)

Stomatal terminology follows definitions in Wilkinson (1979) and Den Hartog-Van Ter Tholen & Baas (1978; note that the original definition of laterocytic stomata here differs from the inaccurately cited one by Wilkinson l.c.). Within *Heisteria* no less than five stomatal types occur: anisocytic, anomocytic, cyclocytic, laterocytic and paracytic (figs. 1—4).

Paracytic stomata are restricted to two species, *H. pentandra* and *H. scandens.* Likewise, laterocytic stomata only occur in *H. asplundii* and *H. skutchii*, occasionally together and intergrading with cyclocytic stomata. Anisocytic, cyclocytic, and anomocytic stomata together with their intermediates occur in varying proportions in all other species. Usually anisocytic stomata predominate and anomocytic stomata are most infrequent or even absent, but sometimes all three stomatal types are about equally common in the same leaf. The variation in relative frequency of one of these three stomatal types below the species level is considerable. Consequently the diagnostic and taxonomic value of each of these three types separately is very small, but the combination of anisocytic with one or both other types is constant for the majority of *Heisteria* species. The diagnostic value of paracytic and laterocytic stomata is great, but allows the separation of only few species.

The stomata are almost entirely confined to the abaxial leaf surface, with the exception of very infrequent adaxial stomata in the midrib region of some specimens (e.g. of *H. barbata*). Stomatal size varies considerable with average values for individual specimens ranging from 19—34 × 18—32 μm (length × width of guard cell pairs). Infraspecific variation is much smaller, e.g. 20—24 × 18—24 μm for *H. acuminata.* The smallest guard cell pairs are found in *H. acuminata, H. barbata, H. coccinea, H. costaricensis* and *H. scandens* (c. 19—23 × 18—22 μm); the largest stomata occur in *H. citrifolia* and *H. duckei* (length over 30 μm). The length/width ratio is mostly c. 1.1 (total range 0.9—1.3). Due to the continuous range of variation within *Heisteria* as a whole and the infraspecific variability, the diagnostic value of stomatal size is rather low and can only be used as additional evidence for excluding doubtful specimens from a species if it is well beyond the stomatal size range for that species.

As seen in transverse section the stomatal complex is always in level with the epidermis, with the exception of *H. maytenioides* where the stomata are sunken. Typically the guard cells in *Heisteria* have well developed cuticular ledges and more or less central, fair-sized lumina. *H. pentandra* and *H. scandens* are exceptional, not only in having paracytic stomata, but also in having narrow lumina in a high position (cf. Baas et al., 1982) and very inconspicuous cuticular ledges.

Lignified guard cells are infrequently present in various species, but predominate in most specimens of *H. scandens.*

Hypodermis, mesophyll and lamina thickness

Heisteria species typically lack a hypodermis; only in few cases the subepidermal layer above the midrib vascular system is slightly modified and could be termed a
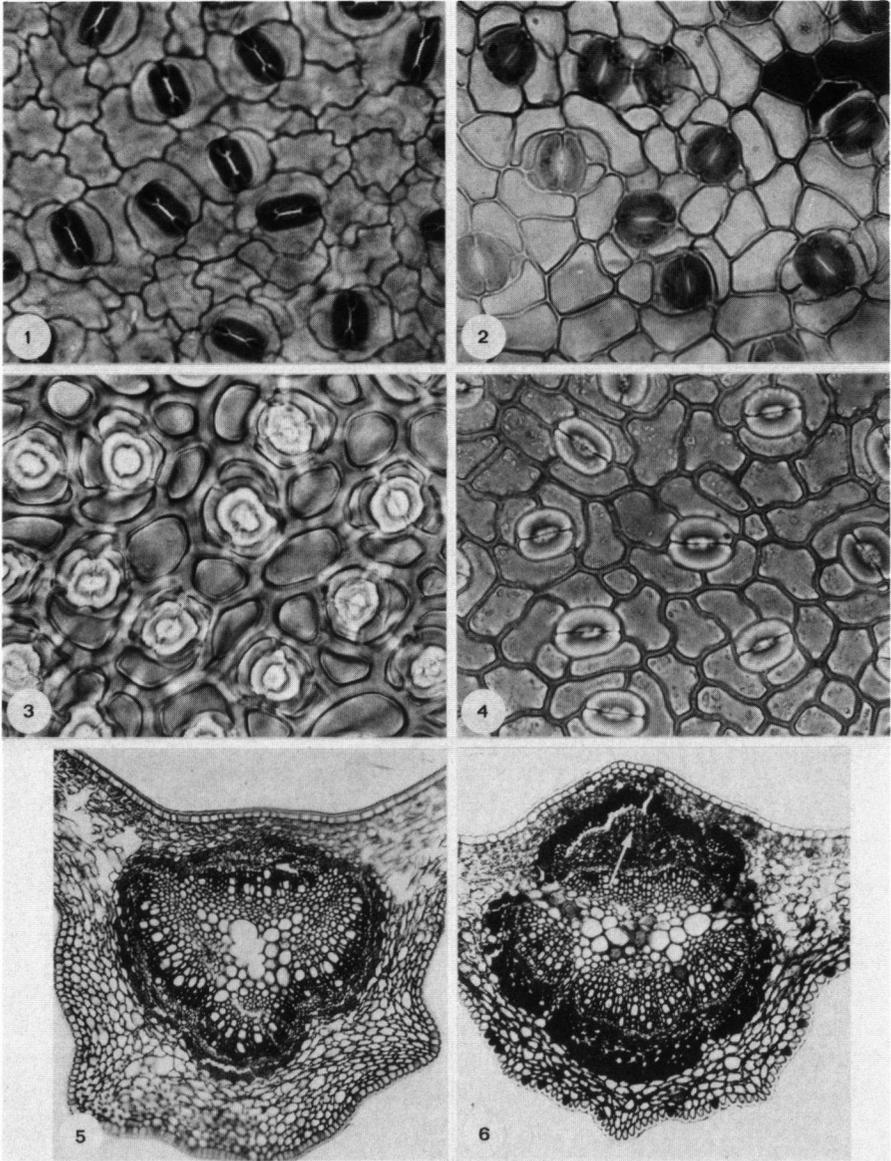
Fig. 1. *Heisteria scandens.* Paradermal section. Paracytic stomata and undulating anticlinal epidermal cell walls, x 340. – 2. *H. asplundii.* Paradermal section. Laterocytic stomata, x 340. – 3. *H. maytenioides.* Maceration. Thick anticlinal walls. Stomata (below level of leaf surface) mostly anisocytic, x 340. – 4. *H. silvianii.* Maceration. Stomata anomocytic, cyclocytic, aniso-cytic and intermediate, x 340. – 5. *H. zimmereri.* Transverse section of midrib with simple, closed vascular system, x 55. – 6. *H. cauliflora.* Ibid., complex vascular system with additional adaxial bundle (arrow), x 55.
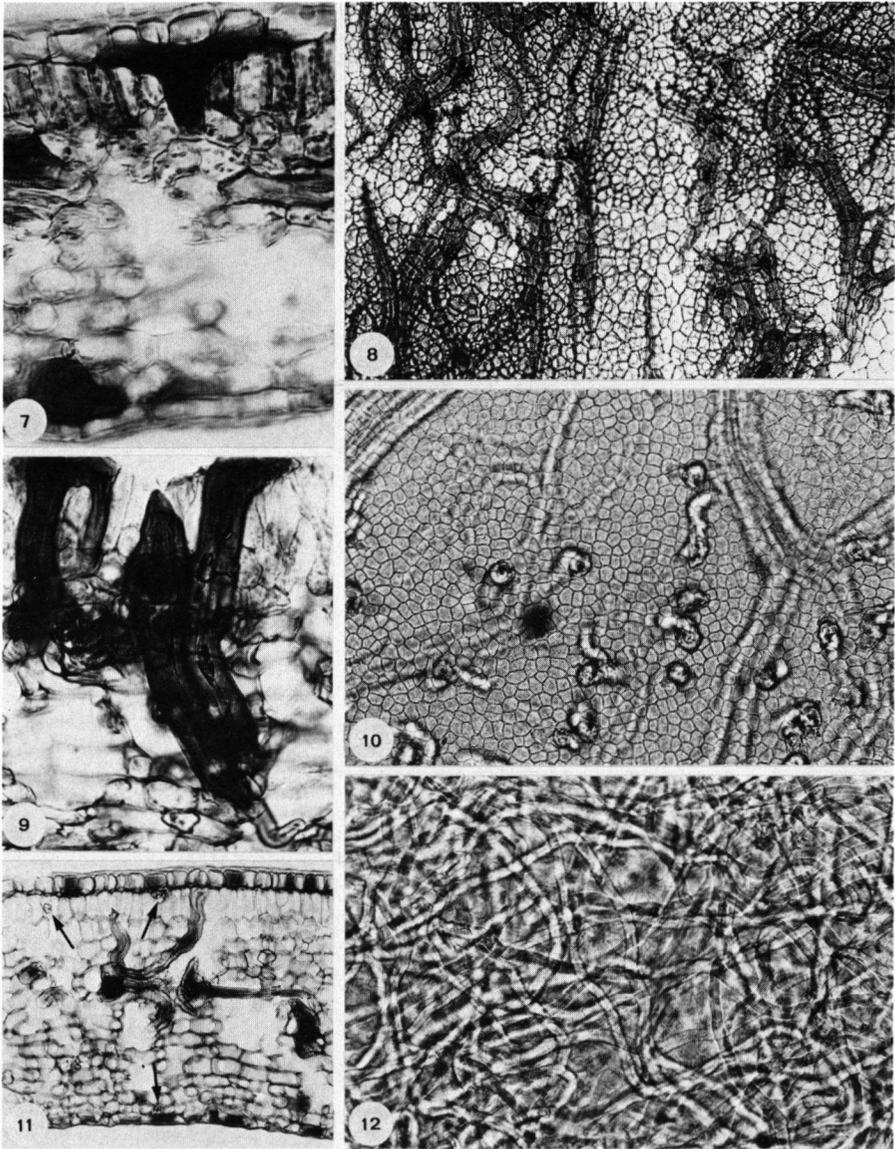
Fig. 7–12. Mesophyll sclereids. — 7. *Heisteria cauliflora (Wachenheim 59)*. Transverse section showing branched sclereids in subepidermal position, x 210. — 8. *H. ovata.* Paradermal section showing strongly branched subepidermal sclereids (as in fig. 7) in surface view, x 85. — 9 & 10. *H. duckei.* Columnar sclereids in transverse section and in leaf clearing, x 210 & x 85. — 11. *H. asplundii.* Intermediates between slender astrosclereids and filiform sclereids in transverse section; note also druses in mesophyll (arrows), x 85. — 12. *H. coccinea.* Leaf clearing with fibrous mesophyll sclereids, x 85.
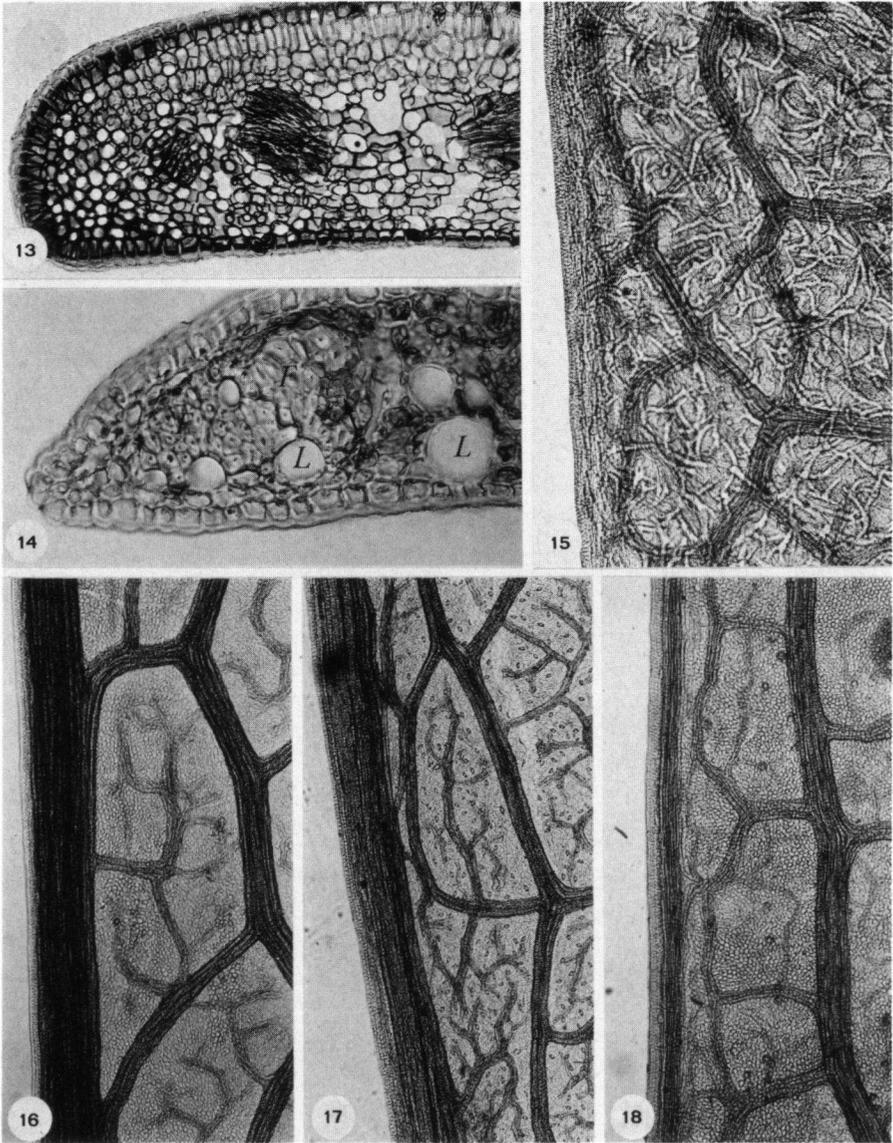
Fig. 13–18. Leaf margin. – 13. *Heisteria perianthomega.* Transverse section with unmodified vascular bundles, x 85. – 14. *H. scandens.* Transverse section (unstained) with marginal bundle largely composed of fibres (F); note laticifers (L) and vascular tissue (arrow), x 85. – 15–18. Leaf clearings, x 35. – 15. *H. perianthomega.* Marginal fibre bundle absent; note abundant astro-fili-form sclereids. – 16–18. Marginal fibre bundles of varying width; mesophyll sclereids absent. – 16. *H. pentandra.* – 17. *H. scandens.* – 18. *H. barbata.*

local hypodermis. In such cases the weak hypodermal differentiation is not constant at the species level.

The mesophyll of *Heisteria* is dorsiventral. The number of palisade layers varies from one to four and the shape of the palisade cells from tall and slender to almost square as seen in transverse section. The spongy tissue varies from compact to loose. These mesophyll characters vary strongly within individual species (e.g., *H. acuminata* covers the entire range for the genus), and are thus left out of the discussions on infrageneric classification. The same applies more or less to lamina thickness; for the whole genus it ranges from 100–400 μm, but individual species may cover considerable parts of this total variation.

Petiole and midrib (figs. 5 & 6)

The petiole and midrib in *Heisteria* are almost always provided with a simple closed cylinder of partly or wholly fused, collateral vascular bundles. In the midrib one can mostly distinguish an abaxial arc-shaped system and an adaxial, more or less plate-like system. Sheathing sclerenchyma fibres are always present in the midrib, and mostly also in the petiole.

In *H. asplundii, H. cauliflora, H. laxiflora,* and *H. parvifolia* (one of the two specimens studied) the midrib vascular system is more complex and includes one (rarely two) additional adaxial bundle(s) outside the cylinder and opposed to the adaxial plate. In *H. cauliflora* and *H. laxiflora* the additional bundles are quite conspicuous, in the other two species they are very small and sometimes almost only consist of phloem elements.

Rarely, and inconstant for the species, the midrib vascular system may be reduced to a single, incurved arc. We suspect this to be a common tendency towards the leaf tips (normally sections are from a central position in the lamina), but fairly commonly the vascular system in the distal part of the petiole also is not entirely closed.

The above described deviations from the standard vascularisation of midrib and petiole in *Heisteria* offer interesting transitions to the situation in other Olacaceae with more complex or more simple systems than in *Heisteria* respectively (cf. Baas et al., 1982).

Sclereid idioblasts (figs. 7–12 & 15)

In all *Heisteria* species the ground tissue of the petiole, and usually also of the midrib, is provided with solitary or clustered stone cells, showing a variable but always restricted degree of branching: astro-brachysclereids. In 23 species there are also idioblastic sclereids of various types throughout the mesophyll. Their frequency often varies considerably below the species level (see section on specific delimitation).

Four, partly intergrading, types can be distinguished:

1. Astrosclereids (i.e., sclereids branching more or less strongly in all directions).
2. Filiform sclereids (i.e., slender sclereids with little branching, but with branches in one predominant direction).

3. Columnar sclereids (unbranched or, if poorly branched, in transverse section most-
   ly T-shaped sclereids with the main body perpendicular to the leaf surface and the
   branches touching the epidermis).
4. Fibrous sclereids (i.e., very long, unbranched sclereid idioblasts).

Fibrous sclereids are restricted to a single species, *H. coccinea*, where they form a
major constituent of the mesophyll. In leaf clearings individual vascular bundles can-
not be observed because of their presence, and they produce an image of interwoven
fibres as in paper (fig. 12).

The other three types of sclereids all strongly intergrade with each other, often in
a single leaf or in different leaves of the same species. Size and cell wall thickness of
the sclereids can also vary from specimen to specimen. In view of the obvious links
and frequent transitions between astro-, filiform and columnar sclereids they have
been lumped for classification purposes.

Leaf margin (figs. 13–18)

In most *Heisteria* species the leaf margin contains a small vascular bundle with
rather small sclerenchyma caps or without any supporting sclerenchyma. In 8 species
the marginal bundle is modified through massive development of sclerenchyma fibres,
sometimes entirely replacing the vascular tissue (fig. 14); in this case the marginal
bundle is very close to the actual leaf margin (epidermis) and runs more or less con-
tinuously along its entire length (figs. 16–18). In the other species the marginal
bundles are small and, as seen in leaf clearings, are loops departing from a larger sub-
marginal bundle; normally these remain farther removed from the margin proper
than the fibrous bundles of the 8 exceptional species (fig. 15). The distinction be-
tween the two conditions is obscured by intermediate species with less strongly de-
veloped or discontinuous marginal, ± fibre bundles.

Together with presence or absence of mesophyll sclereids, the distribution of mar-
ginal fibre bundles provides a useful tool for infrageneric classification and identifica-
tion in *Heisteria*. With the exception of *H. densifrons*, all species with a distinct mar-
ginal fibre bundle are devoid of idioblastic mesophyll sclereids. Conversely, almost all
species which lack a marginal fibre strand possess mesophyll sclereids (*H. amphoricar-
pa, H. parvifolia* and *H. skutchii* excepted). This 'compensation' in sclerenchyma
development has an interesting parallel in two very closely related sections of the
genus *Ilex* (Aquifoliaceae), one of which is characterised by marginal sclerenchyma
strands and the other by mesophyll sclereids (Baas, 1975).

Laticifers (fig. 14)

In spite of a report in the older literature that laticifers may be absent in *Heisteria*
(Colozza, 1904) all samples studied by us showed laticifers in the mesophyll. Sleumer
(1935) had already remarked on their presence in *H. acuminata (H. rhaptostylum)*
for which Colozza reported them to be absent. In all specimens, except one (*Olde-
man B 1795* of *H. densifrons*) the laticifers are non-articulated. The exceptional arti-

culated laticifers have been pictured in Baas et al. (1982). The occurrence of both articulated and non-articulated laticifers within one genus, and even one species, is remarkable.

Abundance and diameter of the laticifers vary considerably within the genus as a whole (15–80 µm), but also within individual species, and is therefore not further considered.

Crystals (fig. 11)

Crystals, presumably of Ca-oxalate, are in *Heisteria* represented by solitary rhomboidal crystals and druses intergrading with clustered crystals. In almost all species rhomboidal crystals predominate, and are partly located in cristarque cells (with unilaterally thickened and lignified secondary walls) bordering the vascular bundles or bordering solitary or clustered sclereids in mesophyll or ground tissue of petiole and midrib. Cristarque cells have not been found in *H. amphoricarpa, H. coccinea* p.p. and *H. skutchii*. Usually the solitary crystals in 'ordinary cells' are also confined to bundle sheath cells, but in some species they also occur in ordinary mesophyll cells. Druses and clustered crystals as common types are confined to only part of the species; in *H. skutchii* they are the only crystal type found. Minute, cubical, spindle-shaped or rod-shaped crystals, possibly of another chemical composition, occasionally occur in epidermal cells; their presence appeared inconstant at the species level and is further not recorded.

Data on distribution and type of crystals are summarised in tables 1 and 2, not because of any claimed diagnostic or taxonomic value, but in order to give some additional circumstantial evidence for confirming specific identification.

GENERIC LEAF ANATOMICAL DESCRIPTION

Note: This description replaces a preliminary one (Baas et al., 1982). It is more comprehensive and contains some minor corrections.

In surface view: Indumentum absent. Epidermal cells with straight, curved, or undulating walls, occasionally with thin areas of cuticle in the loops of undulations. In some species with thick anticlinal walls. *Stomata* almost always restricted to the abaxial epidermis, anisocytic, anomocytic, cyclocytic, paracytic or laterocytic (or intermediate between the three former types; most frequently anisocytic and/to cyclocytic); guard cell pairs (18–)19–34(–40) µm long, (15–)18–32(–35) µm wide. Irregular *cork warts* (of traumatic origin) occasionally present.

In transverse section: *Lamina* 100–400 µm thick. Adaxial *cuticle* 1–20, abaxial cuticle 1–15 µm thick. *Stomata* mostly with fairly wide lumina in a central position and with well developed outer (and inner) cuticular ledges; in two species with narrow lumina in a high position and with very inconspicuous cuticular ledges. Stomata usually in level with epidermis, sunken in one species. *Hypodermis* typically

Table 1. Selected leaf anatomical characters of *Heisteria* species.

Species and number of specimens studied	average length of stomata (μm)	average thickness of adaxial cuticle (μm)	Stomatal type					marginal fibre bundle	mesophyll sclereids	crystal complement	outline of anticlinal epidermal cell walls	vascular system of midrib complex
			anisocytic	cyclocytic	anomocytic	paracytic	laterocytic					
acuminata (15)	20–24	1–3	+	±				+	–	r, d, mes (–)	s-u	–
amazonica (1)	26	15	+	+	±			–	col-astr	r	c-u	–
amphoricarpa (1)	24	4	+	+	+			–		r, d, c, mes	s	–
asplundii (1)	24	5					+	–	astr-fil	r, d, mes	s	±
barbata (27)	21–25	5–6	+	+	±/+			+		r	u	–
blanchetiana (1)	28	12	+	+				–	astr	r, (c)	s-c	–
cauliflora (3)	27–31	6–8	+	+				–	astr-fil(–)	r	(s-)c-u	+
citrifolia (2)	29–33	20	+	+	±			–	astr-fil	r	s(-c), T	–
coccinea (3)	19–20	2	+	+				–	fibres	r	(c-)u	–
concinna (2)	28–29	9	+	+	±			±	col-astr	r, (mes)	s-c(-u)	–
costaricensis (2)	21	1	+	±				+	–	r, d, (mes)	s-u	–
densifrons (6)	24–26	2	+	±	±			+	astr-fil	r, (d), mes	u	–
duckei (4)	33–34	14–15	+	+				–	col	r, (d), mes	s, T	–
huberiana (2)	26–28	6	+	±				–	astr(-fil)	r, d, c, (mes)	s(-c)	–
insculpta (1)	29	9	+	±				±	col(-astr)	r	s-c	–
latifolia (4)	24–26	4	+	±				+	–	r (d, c, mes)	c-u	–
laxiflora (1)	28	12	+	+				–	col (astr)	r	c, T	+
macrophylla (1)	24	2	+		±			+	–	r, d, c, mes	c-u	–

(Table 1 continued)

maguirei (2)	26–27	6–12	+	+			col	r	s	—
maytenioides (1)	24	20	+	±			col	r	s, T	—
media (1)	26	7	+	+			astr-col	r	s-c	—
nitida (7)	27–29	4–9	+/±	+	+		astr-brach (–)	r, (mes)	(s-)c-u	—
ovata (11)	25–27	6–14	+	+	+		astr-fil	r	s-c(-u)	—
parvifolia (2)	24–25	3–5	+	+			—	r	c-u	±
pentandra (2)	22–23	1–2	+	+		+	—	r	u	—
perianthomega (4)	26–27	7–15	+	+	±		astr(-fil)	r	s-c(-u), T(–)	—
salicifolia (1)	26	7	+	+	±		astr(-col)	r	c-u	—
scandens (26)	21–26	2–4	+	+		+	—	r (d, c)	(s-c)-u	—
silvianii (1)	25	10	+	+	+		(astr-col)	r, mes	s-c	—
skutchii (1)	28	4	±	+	±	+	—	d, mes	c-u	—
spruceana (5)	24–27	8–12	+	+	±		astr-col	r, (mes)	s-u	—
trillesiana (1)	26	7	+	±			col	r	c-u	—
zimmereri (1)	24	5	+	+			col-astr	r	c-u	—

Legend:

+ = character present and well developed or frequent; ± or ( ) = character poorly expressed or infrequent; – = character absent; (–) = character absent from one or some of the specimens studied.

astr = astrosclereids; fil = filiform sclereids; col = columnar sclereids; brach = brachysclereids; transitional sclereids types hyphenated.

crystal complement: r = solitary, rhomboidal crystals; d = druses; c = clustered crystals; mes = apart from present around vascular bundles, also present throughout mesophyll.

anticlinal walls: c = curved; s = straight; u = undulated; T = thick.

absent; only rarely very weakly differentiated in midrib region. *Mesophyll* dorsiventral, composed of 1−4 palisade layers of short to tall palisade cells and compact to loose spongy tissue. *Midrib* with a flat to raised adaxial surface and a prominently raised abaxial surface, provided with a closed or interrupted vascular cylinder sheathed by sclerenchyma fibres, rarely with additional adaxial bundles or reduced to a simple arc with incurved margins. *Veins* embedded in mesophyll, the major ones mostly with a complete sheath of sclerenchyma fibres, the smaller ones with adaxial and abaxial fibre caps. Minor veins terminating in slightly enlarged, weakly sclerified bundle sheath cells, or associated with mesophyll sclereids. *Leaf margin* in some species with a well developed marginal bundle, entirely or largely composed of fibres. *Petiole* supplied with a closed or variously interrupted vascular cylinder, mostly sheathed by sclerenchyma fibres. Secretory elements present as infrequent to numerous *laticifers* (diameter 15−80 μm), almost always unarticulated and typically situated in the spongy mesophyll or throughout the lamina. *Sclereids* always present as brachy-astrosclereids, in clusters or solitary in ground tissue of petiole and midrib, in many species also throughout the mesophyll as columnar, filiform, or fibrous idioblasts or astrosclereids or of intermediate types. *Crystals* present in varying frequency as solitary rhomboidal crystals (often in cristarque cells) and/or druses and clusters around vascular bundles or throughout mesophyll; infrequently as minute, cubical, spindle-shaped or rod-like crystals in epidermal cells. *Silicified cells* not observed.

INFRAGENERIC CLASSIFICATION OF HEISTERIA

The distribution of the most salient varying leaf anatomical characters over the individual species of *Heisteria* is given in table 1. This table makes individual species descriptions redundant. Using stomatal type, marginal fibre bundles, and mesophyll sclereids as the most important taxonomic markers, the species have been grouped in table 2. Here some subsidiary ('less important') characters have been listed as well for each species to enable further differentiation within the resulting groups, or to present reinforcing evidence of the coherence of the groups.

The procedure followed is rather arbitrary because it puts the characters used in an unargued hierarchical order. However, the present choice gives a practical means of quickly recognising groups on a combination of few characters; alternative character weighing would produce the same groups, but then partly in another relationship to each other. The leaf anatomical classification of table 2 is not intended as a formal taxonomic system. The salient macromorphological characters of *Heisteria* show such a complex pattern of variation that Sleumer (1983) refrained from any infrageneric classification beyond species delimitation and incidental notes on the mutual affinities of species pairs. Also his linear arrangement of species follows the sequence in which they are keyed out, and partly reflects similarities. Our leaf anatomical grouping may perhaps serve as a starting point for future attempts at a satisfactory infrageneric classification (see also Phylogenetic Speculation). In the subsequent discussion the groups of table 2 will also be compared with taxonomic notes by Sleumer (1983) and his linear species arrangement.

Group 1, *H. asplundii* and *H. skutchii*, combines laterocytic stomata (unique within the Olacaceae as a whole; Baas et al., 1982), presence of druses throughout the mesophyll, and similar cuticular thickness. The two species can be readily separated on presence (*H. asplundii*) or absence (*H. skutchii*) of mesophyll sclereids. Sleumer (1983) did not comment on possible affinities between these species, but in his key they are fairly close together (species 1 and 3, differing in number of stamens).

Group 2, *H. pentandra* and *H. scandens*, is very coherent. Both species are similar in all relevant leaf anatomical characters and are also next to each other in Sleumer's arrangement. Their paracytic stomata with reduced cuticular ledges and narrow lumina recall the situation in a large group of presumably only remotely related Olacaceae genera. Because of its leaf anatomical distinctness a formal status of subgenus or section could be advocated.

Group 3, *H. acuminata, H. barbata, H. costaricensis, H. latifolia* and *H. macrophylla* also form a rather coherent group. Apart from the shared stomatal type(s), marginal fibre bundle, and lack of mesophyll sclereids these species mostly contain druses and have part of their crystals scattered throughout the mesophyll (not in *H. barbata*); values for cuticular thickness (1–6 µm) and stomatal length (20–26 µm) are relatively low. In Sleumer's arrangement these species are more or less scattered. However, *H. barbata* is said to be close to *H. acuminata; H. costaricensis* seems connected by intermediates with *H. macrophylla*; and of *H. latifolia* it is stated that it might be a big-leafed form of *H. acuminata* with similarities in leaf characters to *H. macrophylla*. Thus, macromorphologically, affinities are also indicated between the 5 species of group 3. This in turn reinforces the taxonomic value of the combination of leaf anatomical characters on which this grouping is based. Some formal taxonomic status seems justified.

Group 4, *H. parvifolia* and *H. amphoricarpa* (from Africa and the Neotropics respectively), is largely defined by negative characters: absence of a marginal fibre strand and of mesophyll sclereids. Yet the species are also rather similar in other leaf anatomical characters so that true relationship cannot be excluded. The only difference lies in the presence or absence of druses, a character which is variable within some other species. Apart from its lack of mesophyll sclereids, *H. parvifolia* is also quite similar to the other African species (*H. trillesiana* and *H. zimmereri* of group 7). However, there is no compelling reason to assume closer phylogenetic ties between the three African species than between one of them with a Neotropical species (cf. Baas et al., 1982 on the conservatism of leaf anatomical features and geographical distribution in Olacaceae).

Group 5 is composed of the single species *H. densifrons*. It shares all characters with group 3 except its mesophyll sclereids and can be considered intermediate between group 3 and 6 & 7. In Sleumer's arrangement *H. densifrons* is close to *H. scandens* and *H. pentandra* (group 2) but these species differ strongly in their leaf anatomy (stomatal type, sclereids).

Group 6, composed of *H. concinna, H. insculpta* and *H. nitida* is poorly defined on account of the intermediate condition of the vascular bundles in the leaf margin; these tend to be predominantly composed of fibres, but are not so conspicuous and

Table 2.

STOMATAL TYPE		MESOPHYLL SCLEREIDS		Marginal fibre bundles present
anisocytic, cyclocytic, anomocytic	laterocytic		+/–	
	paracytic		absent	**Group 2** pentandra 23 / 2 / r / u scandens 24 / 3 / r(d,c) / u
			absent	**Group 3** acuminata 22 / 2 / r,d,mes / s-u barbata 23 / 6 / r / u costaricensis 21 / 1 / r,d,(mes) / s-u latifolia 25 / 4 / r,(d,c,mes) / c-u macrophylla 24 / 2 / r,d,c,mes / c-u
		astr - brach - col - fil (and/or)		**Group 5** densifrons 25 / 2 / r,(d),mes / u
		fibres		

Leaf anatomical species groups in *Heisteria*, primarily based on stomatal types, marginal fibre bundles and mesophyll sclereids. For each species additional information is given on stomatal length, thickness of adaxial cuticle (approximate averages for the species), crystal complement and most common outline of anticlinal epidermal cell walls. For group one presence or absence of mesophyll sclereids is also indicated.

Table 2 (continued).

Marginal fibre bundles intermediate	Marginal fibre bundles absent
	Group 1 asplundii 24 / 5 / r,d,mes / s / astr-fil skutchii 28 / 4 / d,mes / c-u / –
	Group 4 amphoricarpa 24 / 4 / r,d,c,mes / s parvifolia 25 / 4 / r / c-u
Group 6 concinna 29 / 9 / r,(mes) / s-c insculpta 29 / 9 / r / s-c nitida 28 / 7 / r,(mes) / c-u	**Group 7** amazonica 26 / 15 / r / c-u blanchetiana 28 / 12 / r,(c) / s-c cauliflora 29 / 7 / r / c-u citrifolia 31 / 20 / r / s,T duckei 34 / 15 / r,(d),mes / s,T huberiana 27 / 6 / r,d,c,(mes) / s laxiflora 28 / 12 / r / c,T maguirei 26 / 8 / r / s maytenioides 24 / 20 / r / s,T media 26 / 7 / r / s-c ovata 26 / 10 / r / s-c perianthomega 27 / 11 / r / s-c,T salicifolia 26 / 7 / r / c-u silvianii 25 / 10 / r,mes / s-c spruceana 26 / 10 / r,(mes) / s-u trillesiana 26 / 7 / r / c-u zimmereri 24 / 5 / r / c-u
	Group 8 coccinea 20 / 2 / r / u

Legend: astr = astrosclereids; fil = filiform sclereids; col = columnar sclereids; brach = brachy-sclereids; transitional sclereids types hyphenated.
crystal complement: r = solitary, rhomboidal crystals; d = druses; c = clustered crystals; mes = apart from present around vascular bundles, also present throughout mesophyll.
anticlinal walls: c = curved; s = straight; u = undulated; T = thick.

continuous along the entire leaf margin as in groups 2, 3 and 5. Through its meso-
phyll sclereids, relatively thick cuticle, and large stomata group 6 is closest to group 7.
Group 7 also contains the species which are ranked closest to *H. concinna, H. in-
sculpta* and *H. nitida* in Sleumer's arrangement (see below).

Group 7 with its 17 species (table 2) is the largest within *Heisteria*. Yet it is rather
coherent through its shared stomatal type, presence of mesophyll sclereids, lack of a
marginal fibre bundle, and tendencies for high values of cuticular thickness and sto-
matal size. Thick anticlinal walls are restricted to 4 (5) species of this group. Sleumer
gives very few taxonomic notes on the species of this group. Fruits of *H. periantho-
mega* are said to be difficult to distinguish from *H. ovata*; *H. salicifolia* is said to be
similar to *H. perianthomega*; and of *H. huberiana* it is suggested that it could be a
form of *H. cauliflora*. Leaf anatomy certainly does not contradict these statements,
but offers a few minor differences which might aid in identification (cf. table 1 and
2). In Sleumer's arrangement group 7 contains his species 9, 11, 12, 14, 15, 18, 19,
21, 22, 25, 26, 27, 28, 29, and 30. In the complete series from 8–30, species 8, 10
and 20 constitute the closely related group 6, but all the other missing numbers (13,
16, 17, 23, and 24) belong to group 3 which is leaf anatomically quite different. This
indicates artificiality of part of the linear arrangement.

Group 8 consists of the single species *H. coccinea*, which is very distinct on ac-
count of its dense network of unbranched fibres throughout the mesophyll. On ac-
count of its thin cuticle and small stomata leaf anatomical similarities are with groups
3 and 5 rather than with group 7. Sleumer puts *H. coccinea* next to *H. densifrons* and
H. nitida; leaf anatomy only supports the former affinity.

REMARKS ON SPECIFIC DELIMITATION AND INFRASPECIFIC VARIATION

In this study we had the unique opportunity to use a wealth of authentic material,
including many types, also of the species reduced in Sleumer's revision for Flora Neo-
tropica (1983). In all cases the leaf anatomical evidence could be used in support of
Sleumer's decisions (i.e., as far as leaf anatomy allows species discrimination). Yet
some species show a considerable amount of leaf anatomical variation, only partly
conveyed in table 1 which merits further discussion.

H. acuminata. This species is rather constant for most of its leaf anatomical char-
acters, with the exception of anticlinal wall outline and proportion of druses (absent
to abundant) in the crystal complement.

H. cauliflora. Of the three specimens studied, one (*Egler 1399*) lacks mesophyll
sclereids, which abound in the other specimens. Yet this specimen betrays its identity
as *H. cauliflora* through its complex midrib vascular system, which is very uncommon
in *Heisteria* as a whole. This represents an extreme case of infraspecific variation.
More moderate forms of variation from e.g. infrequent, small mesophyll sclereids to
abundant, massive sclereids occur in *H. densifrons*. Sclereid frequency also varies
strongly in *H. duckei* and *H. huberiana*.

H. nitida provides another example of extreme variation in sclereids: the type
(*Spruce 4148*) and two other specimens (*White 291* and *Revilla 2381*) lack meso-

phyll sclereids; the other 4 specimens studied have sclereids in very low to moderate frequency. In a way *H. nitida* thus provides a link between groups 3, 4 on the one hand and 5–7 on the other. Despite this variation there is no compelling reason to challenge the conspecificity of the *H. nitida* material. This is because of the good overall similarity in the other leaf anatomical features and the almost continuous variation from absence, via very sparse, to normal frequency of mesophyll sclereids.

H. spruceana also shows variation in sclereid differentiation but of a discontinuous nature: *Spruce 1510* (the type) and *Prance et al. 3994* have infrequent, virtually un-branched, columnar sclereids; *Croat 19754* and *Prance et al. 14797* have frequent, strongly branched, astro-filiform sclereids. This variation is suggestive of two taxonomic entities and merits further study correlating macromorphological and anatomical variation patterns within the material assigned to *H. spruceana* by Sleumer.

H. scandens, which was studied most extensively for infraspecific variation, appear-ed to be very constant leaf anatomically. There is only some variation in size (not continuity and position) of the marginal fibre strand, in outline of anticlinal walls, in relative frequency of crystal types, and in quantitative characters. The variation seems quite independent of whether the specimens were from climbers or erect trees – both habits being common in this species. *H. barbata*, earlier often confused with *H. scan-dens* differs consistently from *H. scandens*, and is in itself an even more constant spe-cies leaf anatomically (cf. table 1).

The above examples, especially of variation in mesophyll sclereids, weaken the ab-solute value of the characters used in the infrageneric classification. Possibly absence of sclereids can be interpreted in the same two ways as absence of crystals in wood: it may be genetically determined and inherent to the taxon or be an unexplained lack of expression of the genetic potential of an individual plant. In the latter case ab-sence of crystals, and by analogy of mesophyll sclereids, is of little taxonomic signifi-cance. However, in the vast majority of cases the characters used in table 2 provide reliable means to characterise species and species groups.

PHYLOGENETIC SPECULATION

In the paper on leaf anatomical variation in Olacaceae as a whole (Baas et al., 1982) a phylogenetic reconstruction was attempted on the presumedly derived (apo-morphic) or ancestral (plesiomorphic) nature of the different character states. In this analysis *Heisteria* appeared to have numerous plesiomorphic leaf anatomical charac-ters, viz. 1. lack of silicified cells; 2. closed, simple vascular system in midrib and petiole; 3. sclerenchyma support of midrib vascular system; 4. sclereids in the ground tissue of petiole and midrib; 5. laticifers; 6. some species with paracytic stomata; 7. mostly unlignified guard cells; 8. lack of a continuous hypodermis; 9. lack of epider-mal cells containing druses; 10. lack of bundles of minute, unlignified fibres; 11. most species with central lumina and well developed cuticular ledges; 12. lack of schizogenous secretory cavities. In other words, for all characters used in the cladistic analysis of the family at least a number of *Heisteria* species, but mostly all of them are plesiomorphic. Apomorphic states are restricted to stomatal types (all species

except *H. pentandra* and *H. scandens*); lignified guard cells (only common in *H. scandens*); and reduced ledges and narrow guard cell lumina (*H. pentandra* and *H. scandens*). There is no single derived leaf anatomical character that applies to all *Heisteria* species, and within the Olacaceae as a whole the genus is defined on the virtually constant occurrence of plesiomorphic characters – a capital sin in cladistic classification. One can only hope that the macromorphological generic characters contain synapomorphic traits, essential for the concept of *Heisteria* as a monophyletic genus.

The presumed sister group of *Heisteria* includes *Scorodocarpus, Brachynema* and the four genera of the *Strombosia* alliance (*Diogoa, Tetrastylidium, Strombosia,* and *Strombosiopsis*). This sister group differs mainly in its lack of laticifers. Taken together, *Heisteria* and this group of genera have the Couleae for sister group, which is different in schizogenous secretory cavities and in the constantly paracytic stomata. On the basis of outgroup comparison it is possible to suggest the plesiomorphic or apomorphic nature of the individual leaf anatomical characters that define the groups recognised within *Heisteria* (table 2). Three characters can be considered derived because they do not occur in the sister group or even in the rest of the family as a whole: 1. laterocytic stomata (group 1); 2. marginal fibre bundles (groups 2, 3 and 5 and group 6 as intermediate); 3. presence of fibrous sclereids throughout the mesophyll (group 8). Interpretation of the other characters is more complex. Mesophyll sclereids (other than unbranched fibres) are very common in the sister group of *Heisteria* (although not constant for all genera) and absent from the Couleae. They might represent a synapomorphy of *Heisteria* and its sister group, and have been subsequently lost in individual species or genera (groups 1–4 of *Heisteria*; *Brachynema, Diogoa* and *Strombosia* p.p. of the sister group) in parallel development. In this interpretation these mesophyll sclereids cannot be relied on heavily for phylogenetic classification of *Heisteria*. Stomatal types other than paracytic have been interpreted as derived for the family as a whole, but anisocytic and cyclocytic stomata and their intermediates are characteristic for the sister group of *Heisteria* and most species of *Heisteria* itself. This could imply that for these sister groups anisocytic and cyclocytic stomata represent another synapomorphy, thus rendering the character plesiomorphic for the individual genera and species. In this interpretation the paracytic stomata of *H. pentandra* and *H. scandens* are not relictual (plesiomorphic) but a new derivation in reversal of the more common trend from paracytic to aniso/cyclocytic stomata, i.e., a synapomorphy of *H. pentandra* and *H. scandens*. The narrow guard cell lumina and reduced cuticular ledges in these two species must also be interpreted as a synapomorphy. Their presence in a large group of Olacaceae genera, only remotely related to *Heisteria*, must then be the result of parallel development. The same applies to the lignified guard cells of *H. scandens* which have a parallel in a closely knit group of genera consisting of *Anacolosa, Cathedra* and *Phanerodiscus* (Baas et al., 1982).

For the occasional occurrence of complex vascular patterns in the midrib and of thick anticlinal epidermal cell walls, parallel development may also be assumed below the genus level. The same applies to distribution and types of crystals.

With the few more or less unambiguous apomorphic character states for individual
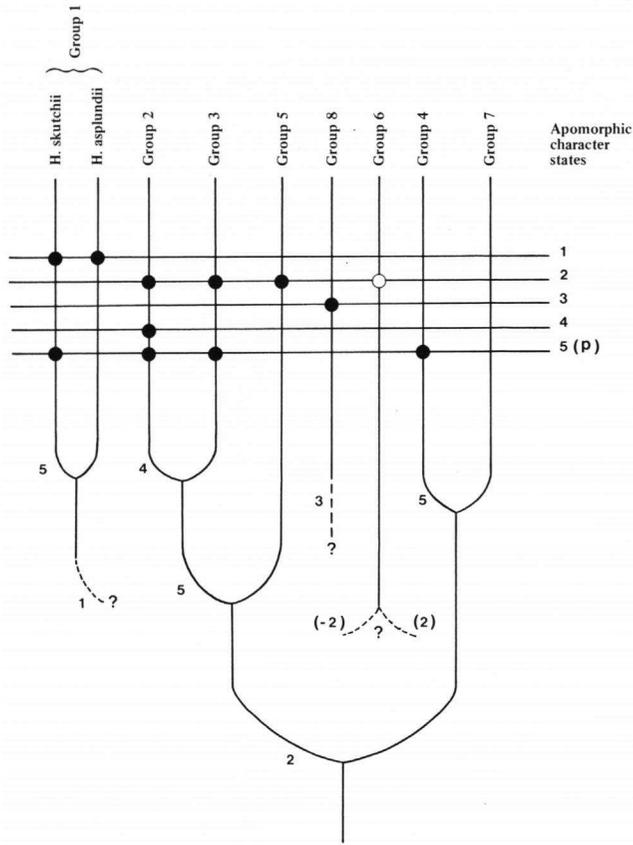
Fig. 19. Cladogram of *Heisteria*. Numbers for species groups as in table 2. Numbers at bifurcations represent evolutionary change to the following apomorphic character states:
1. laterocytic stomata.
2. marginal fibrous strand (intermediate in group 6).
3. mesophyll sclereids of long unbranched fibres.
4. paracytic stomata (with reduced ledges and high, narrow lumina).
5. loss of mesophyll sclereids (in parallel development).
? = unambiguous hypothetical derivation impossible on the basis of leaf anatomical data.

species groups, it has yet been attempted to construct a cladogram (fig. 19). It is fully realised that the number of characters available is too limited for a meaningful phylogenetic classification. This, together with the sometimes intergrading type of variation between alternative character states, makes the application of Hennigian cladistics virtually impermissible. The present attempt must only be regarded as a logical extension of the more successful cladistic analysis at the family level (Baas et al., 1982). In the cladogram it is clearly shown that group 7, containing the largest

number of species, is not characterised by any presumedly apomorphic leaf anatomical character state. This may thus be an unnatural assemblage. Perhaps further classification of this group will be possible on the basis of other morphological traits. In the cladogram groups 1, 8, and 6 have not been assigned links with other groups because no sister groups with shared leaf anatomical apomorphies could be indicated. Their position in the cladogram rests on phenetic similarity (see previous discussion of infrageneric classification of *Heisteria*). The intermediate position of group 6 would allow for two alternative derivations; it might also indicate hybridisation as a factor in the diversification of *Heisteria*. The position of group 1 near group 2 is based on the possible relationship between paracytic and laterocytic stomatal types (a subject inviting ontogenetic study), and on the fact that *H. asplundii* (group 1) shares its stamen number of 5 only with *H. pentandra* (group 2). The present cladogram implies that the reduction from 10 to 5 stamens in these two species is the result of parallel development.

The present, incomplete phylogenetic classification of *Heisteria*, with the recognition of group 7 as having retained all leaf anatomical attributes of '*Protoheisteria*' enables a more detailed and meaningful comparison of *Heisteria* with the other genera of the Olacaceae. In such a comparison the presumed sister group (see above) automatically emerges as the closest relative by circular argument. *Chaunochiton*, previously treated with *Heisteria* in the same tribe (e.g. Sleumer, 1935), differs strongly from '*Protoheisteria*' or group 7 in stomatal type, its accumulation of silica, simple open vascular system in petiole and midrib, and in lacking mesophyll sclereids. Our results reinforce the earlier suggestion (Baas et al., 1982) to treat *Chaunochiton* separately from *Heisteria*. The shared laticifers are probably a synplesiomorphy, and thus are not indicative of close mutual affinity.

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