

THE PHYLOGENETIC SIGNIFICANCE OF FRUIT STRUCTURAL VARIATION IN THE TRIBE HETEROMORPHEAE (APIACEAE)

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Abstract

Fruit structure of Apiaceae was studied in 19 species representing the 10 genera of the tribe Heteromorphae. Our results indicate this group has a woody habit, simple leaves, heteromorphic mericarps with lateral wings. Fruits with bottle-shaped or bulging epidermal cells which have thickened and cutinized outer wall, regular vittae (one in furrow and two in commissure) and irregular vittae (short, dwarf, or branching and anatosmosing), and dispersed druse crystals. However, lateral winged mericarps, bottle-shaped epidermal cells, and branching and anatosmosing vittae are peculiar in the tribe Heteromorphae of Apioideae sub family. Although many features share with other early-diverging groups of Apiaceae, including *Annesorhiza* clade, Saniculoideae *sensu lato*, Azorelloideae, Mackinlayoideae, as well as with Araliaceae. Our study shows that fruit anatomy can be used to define the tribe by molecular phylogenetic studies and support that Heteromorphae are close to *Annesorhiza* clade and both are placed in the basal position of Apioideae.

Key words: Apiaceae, Heteromorphae, Fruit anatomy, Wing, Phylogeny.

Introduction

Several recent molecular data (Plunkett *et al.*, 1996a, b; Downie & Katz-Downie, 1999; Downie *et al.*, 1998, 2000, 2001) indicated that 5 endemic African genera of Apiaceae comprise a well-supported monophyletic group (named as Heteromorpha clade) that is sister to the rest of subfamily Apioideae. These genera were previously placed in tribe Apieae Drude (Pimenov & Leonov, 1993) and formally described by Downie *et al.* (1998, 2000, 2001) as a new tribe, Heteromorphae Watson & Downie, included *Anginon* Raf., *Dracosciadium* Hilliard & B.L. Burtt, *Glia* Sond., *Heteromorpha* Cham. & Schltld. and *Polemannia* Eckl. & Zeyh. Heteromorphae clearly diverged early in the evolutionary history of Apioideae, and are closely related to *Annesorhiza* Cham. & Schltld. and *Bupleurum* L. Four of its five genera are woody (*Anginon*, *Glia*, *Heteromorpha* and *Polemannia*), whereas *Dracosciadium* is a perennial herb (Van Why, 2000, 2001). Cerceau-Larrival (1974). Hilliard & Burtt (1986) reported that *Anginon*, *Dracosciadium* and *Heteromorpha* had oval pollen.

The further molecular study (Calviño *et al.*, 2006) shows that tribe Heteromorphae is expanded to include the eastern African and Madagascan genus *Pseudocarum* C. Norman and 5 genera endemic to Madagascar *Andriana* B-E. van Wyk, *Anisopoda* Baker, *Cannaboides* B-E. van Wyk, *Pseudocannaboides* B-E. van Wyk, and *Tana* B-E. van Wyk. In which woody *Pseudocarum*, *Andriana*, *Cannaboides* and herbaceous

Pseudocannaboides and *Tana* were excluded from tribe *Heteromorpha* by Van Wyk *et al.* (1999) on the basis of significant fruit morphological evidence. In a cladistic analysis using morphological data, Van Wyk (2001) suggested the all these genera are very closely related to the five genera comprising Heteromorphae.

The detailed fruit anatomy of African genera remains poorly known. Some fruit characters have been studied in *Andriana*, *Anginon*, *Cannaboides*, *Heteromorpha*, *Pseudocannaboides*, and *Tana* (Allison & Van Wyk, 1997; Winter *et al.*, 1993; Winter & Van Wyk, 1996; Van Wyk *et al.*, 1999), but important details essential for a rigorous comparisons are not available. In this paper, fruits of all genera currently placed in Heteromorphae (except *Anisopoda* because of fruit samples could not be obtained) were examined in detail to explore the potential value and importance of fruit features among the genera of the tribe, to identify structural characters that can be used to define the tribe by molecular phylogenetic studies, and to understand the relationships between Heteromorphae and other groups of the Apiaceae.

Materials and Methods

Sampling: The fruit structure of 19 species representing 10 genera (*Andriana*, *Anginon*, *Cannaboides*, *Dracosciadium*, *Glia*, *Heteromorpha*, *Polemannia*, *Pseudocannaboides*, *Pseudocarum*, and *Tana*) was studied in detail. Where possible, at least two mature fruits of each species were examined. Sample names and voucher information are provided in Table 1.

Table 1. Taxa of tribe Heteromorphae examined for fruit structure, together with voucher specimen details and localities.

Species	Voucher specimens	Locality
<i>Andriana tsaratananensis</i> (Humbert) B-E. van Wyk	Humbert 18374 (P)	Madagascar
<i>Anginon difforme</i> (L.) B.L. Burtt	Van Wyk 2744 (JRAU)	South Africa
<i>A. fruticosum</i> I.Allison & B.-E.van Wyk	Pahistrana 2397 (NBG)	South Africa
<i>A. jaarsveldii</i> B.L.Burtt	IA 157 (JRAU)	South Africa
<i>A. paniculatum</i> (Thunb.) B.L. Burtt	Taylor 11771 (PRE)	South Africa
<i>A. swellendamense</i> (Eckl. & Zeyh.) B.L. Burtt	Van Wyk 2963 (JRAU)	South Africa
<i>A. verticillatum</i> (Sond.) B.L. Burtt	IA 159 (JRAU)	South Africa
<i>Cannaboides betsileensis</i> (Humbert) B-E. van Wyk	Perrier de la Bâthie 6815 (P)	Madagascar
<i>Dracosciadium italaie</i> Hilliard & Burtt	Hilliard & Burtt 17732 (PRE)	South Africa
<i>D. saniculifolium</i> Hilliard & B.L.Burtt	Hilliard & Burtt 17692 (PRE)	South Africa
<i>Glia prolifera</i> (Burm. f) B. L. Burtt	Gan 7313 (PRE)	South Africa
<i>Heteromorpha arborescens</i> Cham. & Schltld.	Vlox 623 (PRE)	South Africa
<i>H. involucreta</i> Conrath	Winter 61 (JRAU)	South Africa
<i>H. pubescens</i> Burtt Davy	Winter 2837 (UNIN)	South Africa
<i>H. transvaalensis</i> H. Wolff	Strey 3651 (JRAU)	South Africa
<i>Polemanna montana</i> Schlechter & H. Wolff	Halliwooll 5088 (JRAU)	South Africa
<i>Pseudocannaboides andringitrensis</i> (Humbert) B-E. van Wyk	Perrier de la Bâthie 13741 (P)	Madagascar
<i>Pseudocarum laxiflorum</i> (Baker) B-E van Wyk	Humbert & Capuron 25427 (P)	Madagascar
<i>Tana bojeriana</i> (Baker) B.-E. van Wyk	Baron 5185 (P)	Madagascar

Anatomical studies: (1) All fruits were rehydrated and placed in FAA (37% formalin–glacial acetic acid–95% alcohol = 5 ml: 5 ml: 90 ml) for a minimum of 24 h and then treated largely following the method of Feder & O'Brien (1968) for embedding in glycol methacrylate (GMA). However, a minimum of 24 h was used for the first two infiltrations and a minimum of 5 days for the third infiltration. Transverse sections, about 3–4 µm thick, were cut using a Porter-Blum ultramicrotome. Samples were stained using the periodic acid-Schiff/toluidine blue staining method (Feder & O'Brien, 1968). Anatomical features were recorded using a camera lucida and also photographed. (2) Additional fruit samples were rehydrated and the exocarps removed. The three dimensional structure of the vittae and crystals was observed and photographed using an Olympus BX51 microscope and an Olympus DP26 digital camera.

Cladistic analysis: Fruit anatomical characters with the taxonomic importance (Table 2) and plant habit and leaf type were considered and logically polarized for cladistic analyses (Table 3). Araliaceae was chosen as outgroup in the cladistic analysis. The data were repeatedly analysed using the computer program PAUP* (Swofford, 2002). The search for most parsimonious tree by heuristic search and bisection-reconnection (TBR) branch swapping were used to examine support for the branches of the tree, bootstrap analysis based on 1000 replication was performed.

Results

A summary of the morphological and anatomical characters studied is provided in Table 2. Variation in fruit structure is illustrated in Figs. 1–3 and phylogenetic relationship in Fig. 4.

Fruit shape: The fruits of most taxa are homomorphic (Fig. 1A–K, P, Q–S), follower all species of *Heteromorpha* (*H. arborescens*, *H. involucreta*, *H. pubescens*, *H. transvaalensis* – Fig. 1L–O) have heteromorphic mericarps, in which one mericarp has three wings (one median and two marginal wings), and the other has two (lateral) wings (e.g. Fig. 1O). A lateral wing may be present in the mericarp with median and marginal wings in *H. pubescens* (Fig. 1N). The wings are long (wings are more than twice the mericarp thickness) in *H. involucreta*, *H. pubescens* and *H. transvaalensis* (Fig. 1M–O) and short (wings are less than twice the mericarp thickness) in *H. arborescens* (Fig. 1L). The species of the other genera have 5-angular mericarps (Fig. 1A–K, Q–S) except for *Polemanna montana* (Fig. 1P), in which the two mericarps are compressed dorsally and the marginal ribs winged. The marginal ribs are of *Anginon difforme* and *A. fruticosum* are also slightly winged (Fig. 1B, C).

Mericarp surface, epidermis, mesocarp and endocarp: The mericarp surface has unicellular hairs in *Heteromorpha pubescens* (Fig. 2F), unicellular and uniseriate multicellular hairs in *Cannaboides betsileensis* (Fig. 2G), and smooth in the other taxa examined. The epidermal cells are bottle-shaped in *Anginon difforme*, *A. fruticosum* and *Pseudocannaboides andringitrensis* (Fig. 2A). The outer wall of the epidermal cells is flattened in *Dracosciadium italaie* and *D. saniculifolium* (Fig. 2C) and bulging in the other species (Fig. 2B). The outer walls of the fruit epidermal cells in all taxa (except two species of *Dracosciadium*) are thickened and cutinized (Fig. 2A, B). A slightly lignified mesocarp occurs in the commissure of all species of *Anginon* and *Polemanna montana* (Fig. 2I), and in the ribs (or wings) of all species of *Heteromorpha* (Fig. 1G). In *Anginon paniculatum*, *A. swellendamense*, *A. verticillatum* and *Glia prolifera* a single layer of endocarp cells is slightly lignified (e.g. Fig. 2D).

Table 2. Summary of taxonomically important characters of habit, leaf and fruit structure of the taxa in tribe Heteromorpheae.

Species	Habit	Leaf type	Heteromorphic mericarps	Epidermal cells	Trichome cell	Lignified mesocarp commissure	Lignified endocarp	More than five vascular bundles in a mericarp	Rib ducts	Regular vittae	Short and dwarf vittae	Endosperm concave at furrow	Ventral endosperm	Commissure width (%)
<i>Andriana tsaratananensis</i>	woody	compound	-	bulging	-	-	-	-	+	furrow, commissure	-	+	flat	30
<i>Anginon difforme</i>	woody	simple or compound	-	bottle shaped	-	+	-	+	-	commissure	+	-	flat	60
<i>A. fruticosum</i>	woody	simple or compound	-	bottle shaped	-	+	-	-	-	commissure	+	-	flat	60
<i>A. jaarsveldii</i>	woody	compound	-	bulging	-	+	-	-	+	furrow, commissure	-	-	flat	60
<i>A. paniculatum</i>	woody	compound	-	bulging	-	+	+	-	-	furrow, commissure	+	-	concave	30
<i>A. wellendamense</i>	woody	simple or compound	-	bulging	-	+	+	-	-	furrow, commissure	+	-	flat	30
<i>A. verticillatum</i>	woody	compound	-	bulging	-	+	+	+	-	furrow, commissure	+	-	flat	50
<i>Cannaboides betsileensis</i>	woody	compound	-	bulging	1 or 2	-	-	-	+	furrow, commissure	-	+	convex	50
<i>Dracoxictadium itatae</i>	herbaceous	palmate	-	flattened	-	-	-	-	+	furrow, commissure	+	-	flat	40
<i>D. saniculifolium</i>	herbaceous	palmate	-	flattened	-	-	-	-	+	furrow, commissure	+	-	flat	40
<i>Glia prolifera</i>	woody	compound	-	bulging	-	-	+	-	-	furrow, commissure	+	+	concave	40
<i>Heteromorpha arborescens</i>	woody	simple or compound	+	bulging	-	-	-	+	-	furrow, commissure	+	+	flat	20
<i>H. involuacrata</i>	woody	compound	+	bulging	-	-	-	+	+	furrow, commissure	+	-	flat	40
<i>H. pubescens</i>	woody	simple	+	bulging	1	-	-	+	+	furrow, commissure	+	-	flat	30
<i>H. transvaalensis</i>	woody	simple	+	bulging	-	-	-	+	+	furrow, commissure	+	-	flat	80
<i>Polemammia montana</i>	woody	simple	-	bulging	-	+	-	-	+	furrow, commissure	-	-	flat	80
<i>Pseudocannaboides andringitrensis</i>	herbaceous	compound	-	bottle shaped	-	-	-	-	+	furrow, commissure	-	+	concave	10
<i>Pseudocarum laxiflorum</i>	woody	simple or compound	-	bulging	-	-	-	-	+	furrow, commissure	-	-	flat	60
<i>Tana bojerian</i>	herbaceous	compound	-	bulging	-	-	-	-	+	furrow, commissure	-	+	flat	20

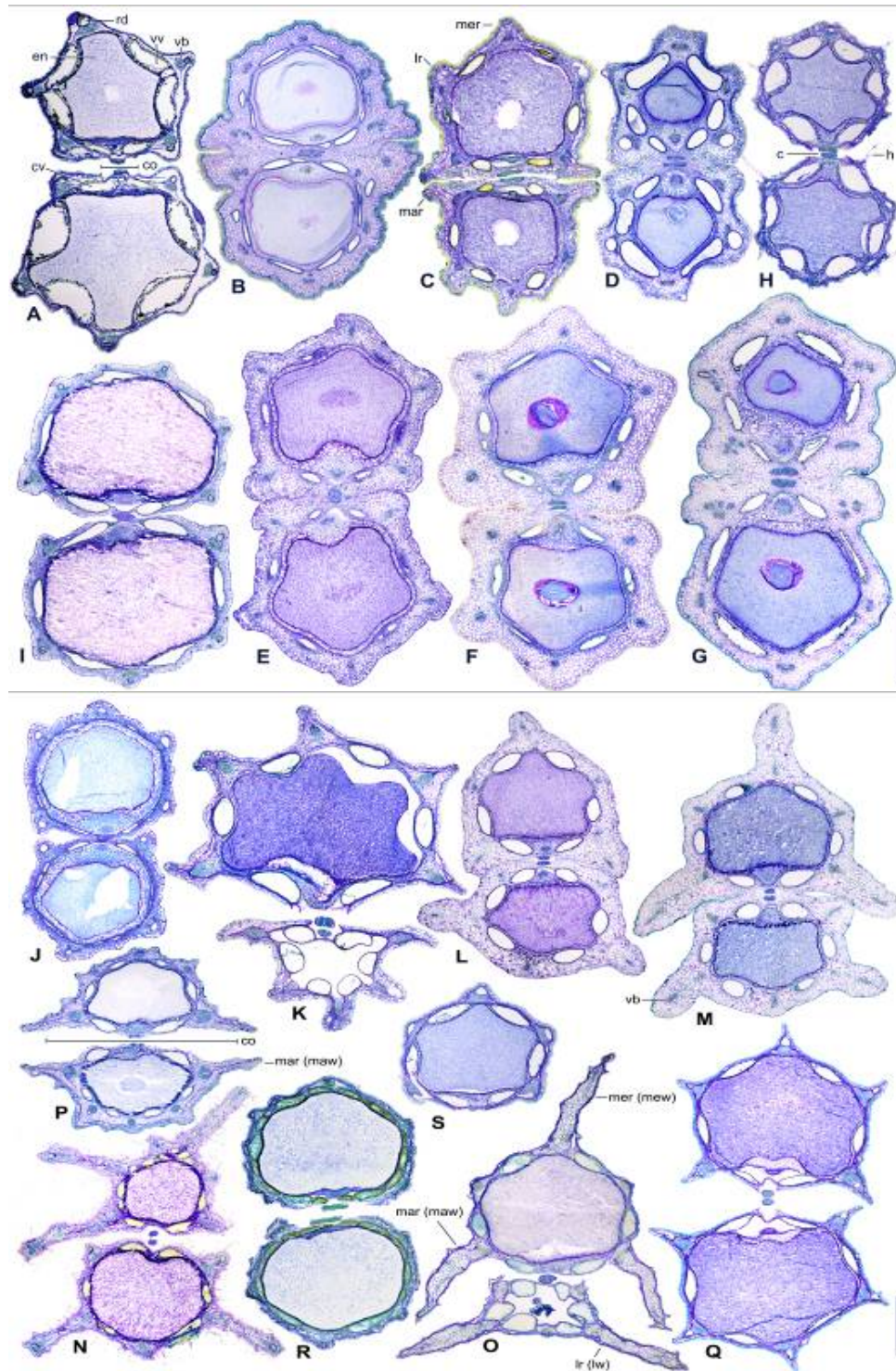


Fig. 1. Transverse sections of the fruits of Heteromorphae. The variation in mericarp shape, trichomes, secretory ducts, vascular bundles, lignification of the mesocarp, endosperm shape, commissural width and carpophore. (A) *Andriana tsaratananensis*. (B) *Anginon difforme*. (C) *A. fruticosum* (D) *Anginon jaarsveldii*. (E) *A. paniculatum*. (F) *Anginon swellendamense* (G) *A. verticillatum*. (H) *Cannaboides betsileensis*. (I) *Dracosciadium itala*. Abbreviations: c- carpophore; co - commissure; cv - commissural vitta; en - endosperm; h - hair; lr - lateral rib; mar - marginal rib; mer - median rib; rd - rib duct; vb - vascular bundle; vv - vallecular vitta. Scale bar = 0.5 mm. Transverse sections of the fruits of Heteromorphae. The variation in mericarp shape, trichomes, secretory ducts, vascular bundles, lignification of the mesocarp, endosperm shape, commissural width and carpophore. (J) *Dracosciadium saniculifolium*. (K) *Glia prolifera*. (L) *Heteromorpha arborescens*. (M) *H. involocrata*. (N) *H. pubescens*. (O) *H. transvaalensis*. (P) *Polemannia montana*. (Q) *Pseudocannaboides andringitrensis*. (R) *Pseudocarum laxiflorum*. (S) *Tana bojeriana*. Abbreviations: co = commissure; lr (lw) = lateral rib (lateral wing); mer (mew) = mar (maw) = marginal rib (marginal wing); median rib (median wing); vb = vascular bundle. Scale bar = 0.5 mm.

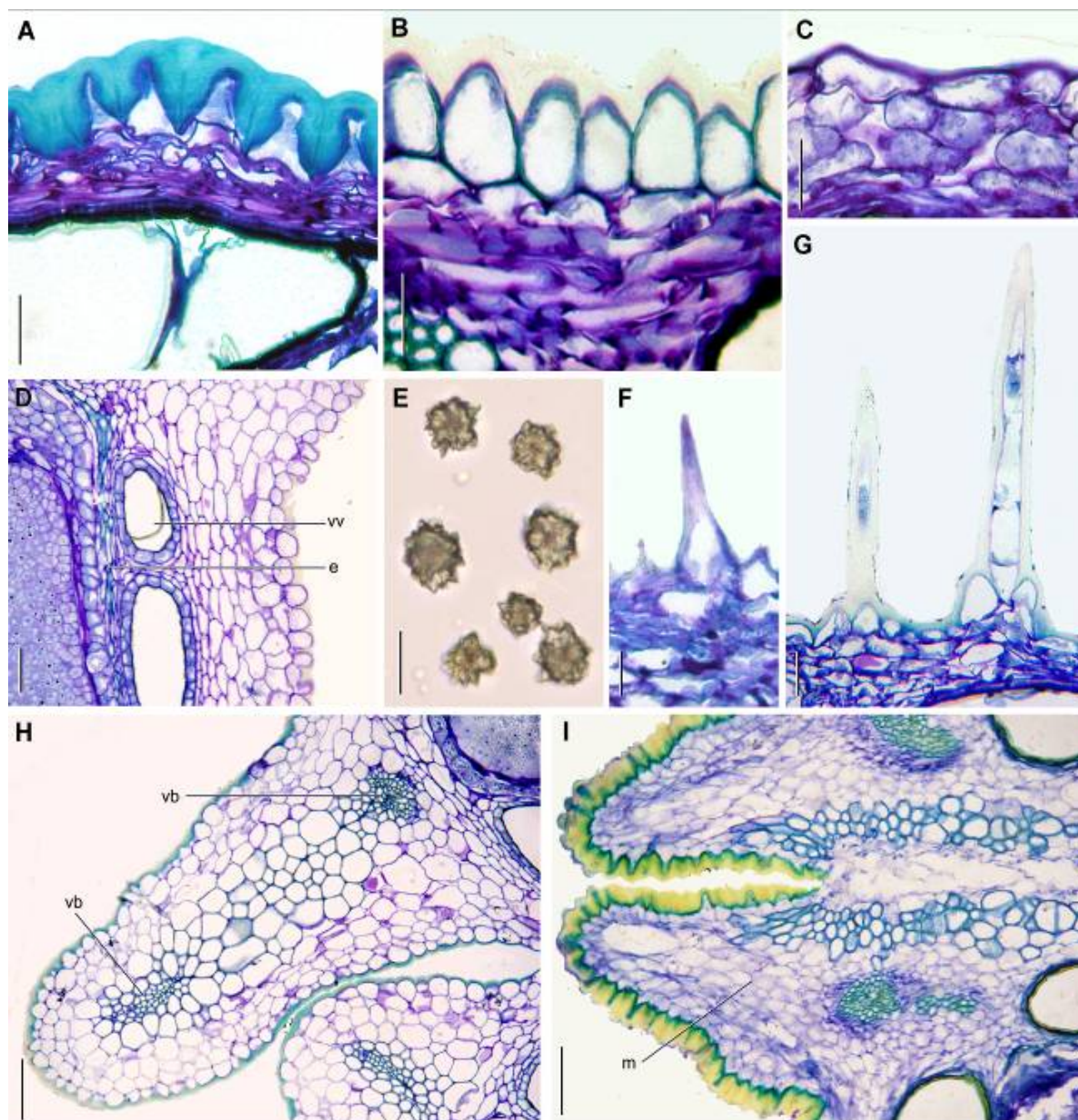


Fig. 2. Transverse sections of selected fruits of members of the Heteromorpeae to show the range of epidermal types with highly cutinized outer walls (from flattened periclinally, to bulging, bottle-shaped), elongated hairs of single cells and multicellular hairs, vallecular vittae, vascular bundle, lignified endocarp and mesocarp cells in commissure, and crystals. (A) *Pseudocannaboides andringitrensis*. (B) *Glia prolifera*. (C) *Dracosciadium italae*. (D) *Anginon swellendamense*. (E) *Polemammia montana*. (F) *Heteromorpha pubescens*. (G) *Cannaboides betsileensis*. (H) *Heteromorpha pubescens*. (I) *Anginon difforme*. Abbreviations: e = endocarp; m = mesocarp; vb = vascular bundle; vv = vallecular vitta. Scale bars = 0.1 mm in D, H, I; 0.05 mm in the others.

Vascular bundles in mericarps: Each mericarp has five vascular bundles in almost all taxa studied (e.g. Fig. 1A), although in *Anginon difforme* and *A. verticillatum* (Fig. 1B, G) each rib in the commissure may have more than one bundles. More than one bundles in each rib (or wings) are characteristics of all species of *Heteromorpha* (Fig. 2H).

Vittae and rib ducts: The three different types of vittae are present. Type 1: regular vittae, six regular vittae (vittae are as long as the mericarp length) in each mericarp, one vitta in each furrow and two vittae in the

commissure (e.g. *Pseudocannaboides andringitrensis*, *Heteromorpha pubescens*, *Anginon verticillatum* – Fig. 3A, B, E), or only two regular vittae in the commissure in *Anginon difforme* and *A. fruticosum* (e.g. Fig. 3D2); Type 2: short or dwarf vittae (almost as long as wide) alongside one or more of the regular vittae in many species (e.g. *Heteromorpha pubescens* and *Anginon verticillatum* - Fig. 3B, E), or without a fixed position in dorsal side in *Anginon difforme* and *A. fruticosum* (e.g. Fig. 3D1); Type 3: branching and anastomosing vittae in *Pseudocarpum laxiflorum* (Fig. 3C).

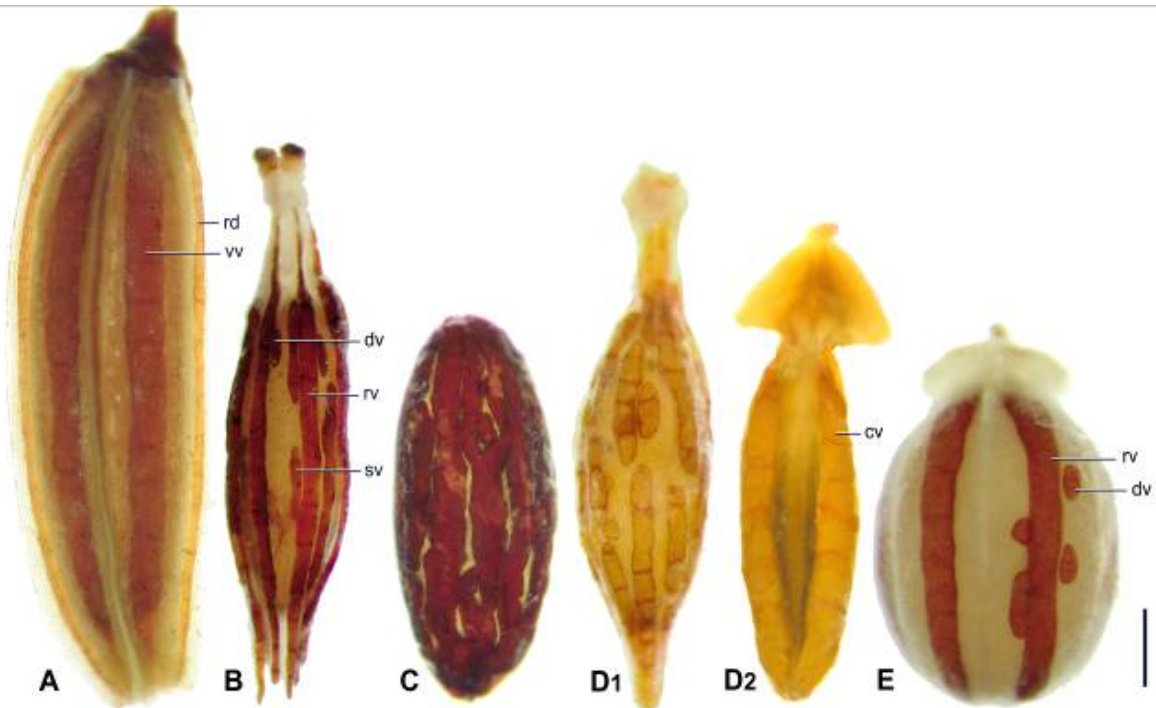


Fig. 3. Whole fruits of genera and species of the Heteromorphae with the exocarp removed or intact to show the variation in vittae (regular and irregular vittae) and rib ducts. (A) regular vittae and rib ducts in *Pseudocannaboides andringitrensis*. (B) regular, short and dwarf vittae in *Heteromorpha pubescens*. (C) branching and anastomosing vittae in *Pseudocarum laxiflorum*. (D1, 2) short and dwarf vittae in dorsal side (D1) and regular vittae in commissure (D2) of *Anginon difforme*. (E) regular and dwarf vittae in *Anginon verticillatum*. Abbreviations: cv = commissural vitta; dv = dwarf vitta; rd = rib duct; rv = regular vitta; sv = short vitta; vv = valecular vitta. Scale bar = 0.5 mm.

Table 3. Polarization of characters and character states* for groups of Apiaceae. Araliaceae was chosen as outgroup.

Groups	1	2	3	4	5	6	7	8	9	10	11	12	13
Araliaceae	0	0	0	0	0	0	0	0	0	0	0	0	0
Mackinlayoideae	0	0	1	1	0	0	0	0	1	1	0	0	0
Azorelloideae	0	0	2	1	0	0	0	0	1	1	0	0	0
Hermas clade	1	0	2	1	0	0	0	0	1	1	0	0	1
Steganotaenia & Polemanniopsis	0	1	2	1	1	0	1	0	2	1	1	1	0
Saniculoideae	1	1	2	1	0	0	1	0	2	1	0	1	0
Lichtenstenia clade	1	1	2	1	0	0	1	0	2	1	1	1	0
Annesorhiza clade	1	1	2	1	1	0	0	1	2	1	1	1	0
Heteromorphae	0	0	2	1	1	1	0	1	2	1	1	1	0
Bupleurum	1	0	2	1	0	0	0	1	2	1	1	1	1
Remaining Apioideae	1	1	2	1	0	0	0	1	2	1	1	1	1

*1. Plant habit: all or some woody (0); predominantly non woody (1), 2. Leaf: simple in some taxa (0); non simple (1), 3. Fruit: most fleshy (0); some fleshy (1); schizocarps only (2), 4. Fruit carpel: more than two carpels in most taxa (0); invariably bicarpellate (1), 5. Fruit heteromorphic, with wings: absent (0); present (1), 6. Bottle shaped epidermal cells with very thick cuticle: absent (0); present (1), 7. Rib duct: small (0); very large (1), 8. Regular vittae (vallecular and commissural vittae): absent (0); present (1), 9. Endocarp of more than one layer of cells: woody and thick (0); woody and not very thick (1); non woody (2), 10. Endosperm ruminant: present (0); absent (1), 11. Carpophore: absent or predominantly single (0); predominantly two (1), 12. Rhomboidal crystal: present (0); absent (1), 13. Druse crystals in mesocarp: dispersed (0); absent or commissural area only (1)

Rib oil ducts are vittae-like canals associated with the vascular bundles in the ribs (visible in Fig. 3A). If rib ducts are present, they are typically located to the outside of the vascular bundles (e.g. Fig. 1A, S). Rib oil ducts are absent in all species of *Anginon* (Fig. 1B-G), *Heteromorpha arborescens*, *H. involucreta* (Fig. 1L, M, 3G) and *Glia prolifera* (Fig. 1K).

Endosperm: The endosperm along the commissural face is concave in *Anginon paniculatum* (Fig. 1E), *Glia prolifera* (Fig. 1K), and *Pseudocannaboides andringitrensis* (Fig. 1Q) is slightly convex in *Cannaboides betsileensis* (Fig. 1H), and

more or less flat in all other species studied. The endosperm is obviously concave at the furrow in *Andriana tsaratananensis*, *Cannaboides betsileensis*, *Glia prolifera*, *Heteromorpha arborescens*, *Pseudocannaboides andringitrensis*, and *Tana bojeriana* (Fig. 1A, H, K, L, Q, S), but not in the others.

Commissure: The commissure is the region of the fruit where the two mericarps from each fruit are attached (e.g. Fig. 1A, P). In *Anginon difforme*, *A. fruticosum*, *Anginon jaarsveldii* (Fig. 1B-D), *Cannaboides betsileensis* (Fig. 1H), *Heteromorpha transvaalensis* (Fig. 1O), *Polemannia montana* (Fig. 1P), *Pseudocarum laxiflorum* (Fig. 1R) the

commissure occupies 50-80% of the width of the fruit, but in all other species it is relatively narrow.

Carpophores: The ventral bundles are opposite and form two carpophores (Fig. 1H) in all the taxa except in *Glia prolifera*, *Polemanna montana* (Fig. 1K, P), the bundles may be incompletely fused completely and therefore three or four bundles are still visible in the two carpophores of the mature fruits.

Crystals: Druse crystals (e.g. Fig. 2E) are present in all the taxa and they are dispersed in the mesocarp around the endocarp.

Cladistic analysis: The parsimonious trees with length 22, a consistency index (CI) of 0.6818 and a retention index (RI) of 0.75 were obtained (Fig. 4). Heteromorpheae is closely related to *Annesorhiza* and sister to the rest of subfamily Apioideae.

Discussion

A summary of character state distributions in the major lineages of Apiaceae is presented in Fig. 4. Two additional characters, generally accepted to be phylogenetically informative, are added here, namely habit (woodyness) and leaf type (simple vs compound). Woody plants are a feature of the family Araliaceae but are present in some genera of Apiaceae, especially in the African and Madagascar members of the tribe Heteromorpheae studied, and many of them have simple leaves – see Table 2. A woody habit and simple leaves are also found in the Mackinlayoideae, some Azorelloideae but rarely in “remaining Apioideae” e.g.

Nirarathamnos asarifolius Balf.f. Calviño *et al.* (2006) suggested ancestors of the southern African were herbaceous, but the common occurrence of woodiness and simple leaves in taxa studied are interpreted in the paper as the plesiomorphic characters as both occur in Azorelloideae, Mackinlayoideae of Apiaceae and Araliaceae (Fig. 4).

Heteromorphic mericarps with lateral wings: Among the taxa studied, *Heteromorpha* is characterized by heteromorphic mericarps with lateral wings as are *Polemanniopsis* (Van Wyk, 2000; Liu *et al.*, 2004) and *Steganotaenia* is heteromorphic in the floral stage (Liu *et al.*, 2004) and is therefore indicated as such in Figure 4. *Polemanniopsis* and *Steganotaenia* lack regular vallicular and commissural vittae, features shared with the subfamily Saniculoideae (Liu *et al.*, 2003, 2004). The heteromorphic mericarps of *Annesorhiza* have both lateral wings and marginal wings in one mericarp which differ from those of *Heteromorpha* (Liu, 2004). In other genera of Apioideae, lateral-winged mericarps were not observed except in *Molopospermum* W.D.J. Koch., *Dasispermum* Neck. ex Raf. The two genera sporadically show heteromorphy in their fruits, the wing number of *Molopospermum* may vary in different populations (pers. obs.) and *Dasispermum* often have ten unequal wings and show great variability within and between populations (Tilney & Van Wyk, 1995). The taxa with heteromorphic fruits are all from Africa and they are placed in a basal position in the Apioideae (Plunkett, 2001; Plunkett *et al.*, 2004; Calviño *et al.*, 2006). Lateral-winged mericarps are present in many genera of Azorelloideae (*Asteriscium* Cham. & Schldl., *Gymnophyton* (Hook f.) Gay, *Mulinum* Pers. and *Diposis* DC.), but these taxa are all homomorphic with woody endocarps.

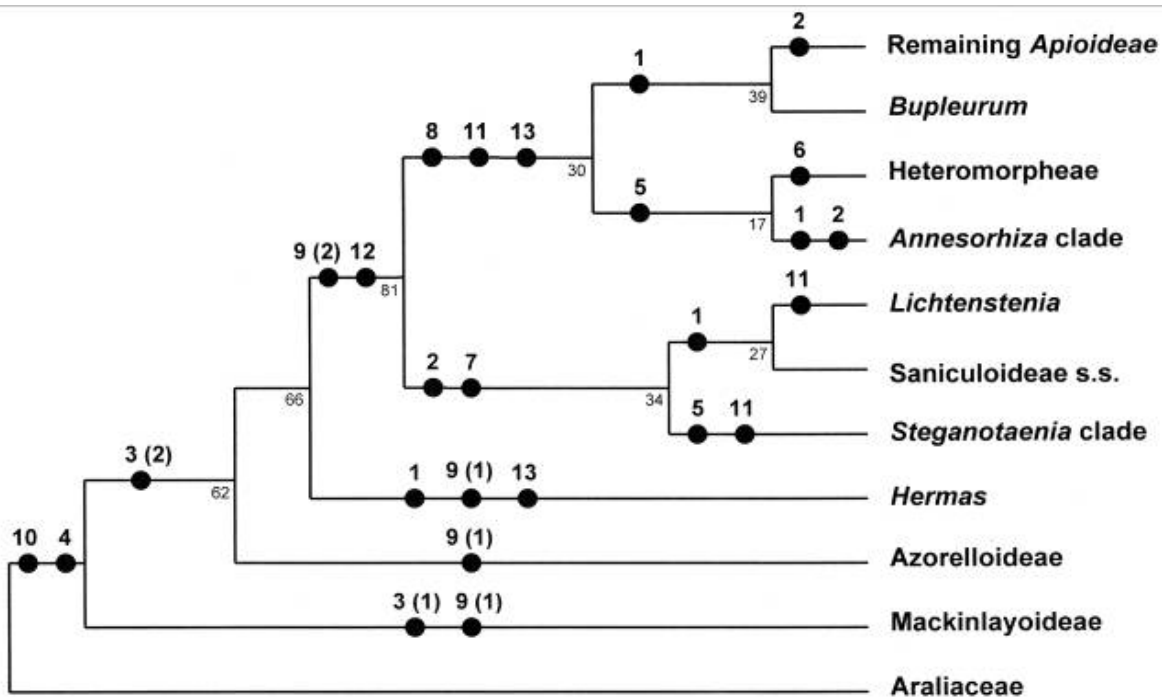


Fig. 4. The parsimonious tree (showing the relationships amongst major lineages of the Apiales) based on morphological characters on PAUP4.0b10 using Heuristic search strategy. Bootstrap values are presented below the branches and morphological data are presented above the branches.

Bottle-shaped epidermal cells and hairs: The bulging epidermal cells may occur in the other groups of the family. The bulging outer wall occur in some taxa of Saniculoideae (e.g. *Actinolema macrolema* Boiss., *Astrantia maxima* Pall., *Eryngium armatum* J.M. Coult. & Rose, and *Sanicula lamelligera* Hance) and Mackinlayoideae (e.g. *Micropleura flabellifolia* Mathias). Bottle-shaped cells similar to the taxa studied are not observed in other taxa of the family. Epidermal cells in *Polemanniopsis* and *Steganotaenia* and other “basal Apioideae” (*Annesorhiza* and *Chamarea*) are flattened periclinally, a characteristic of most “remaining Apioideae”. The very thick cuticle as in the Heteromorphae is not found in the other taxa of the family. The hair occurred in *Cannaboides betsileensis* (Fig. 2G) and *Heteromorpha pubescens* (Fig. 2F) are also observed in the “remaining Apioideae”, (e.g. unicellular hairs are present in *Peucedanum* L. and *Tordylium* Tourn. ex L. and uniseriate multicellular hairs occur in *Angelica* L.). Various types of multicellular structures (e.g. stellate, equisetiform, and digitiform) are present in Araliaceae, Mackinlayoideae, Azorelloideae and *Herma*, but trichomes seem to be simplified in Apioideae.

Endocarp lignifications: An endocarp composed of one layer of lignified cells (e.g. *Anginon swellendamense* - Fig. 2D) may also occur in *Polemanniopsis* and *Steganotaenia* (Liu *et al.*, 2003, 2004) and *Bupleurum rigidum* L. which is placed in the basal lineage of the genus – see Neves & Watson, 2004). This type of endocarp, here reported for the first time, is not homologous to the woody, multilayered endocarp interpreted as the plesiomorphic character (Fig. 4) is typically found in the Araliaceae, Mackinlayoideae and Azorelloideae. In subfamily Apioideae, fruits have a soft, parenchymatous endocarp or become hardened by subepidermal fibrous layers (Drude, 1897-1898).

Rib ducts and vittae: Secretory canals have a prominent place in the phylogenetic speculations Baumann (1946), Tikhomitov (1961) and Eyde & Tseng (1971). In the taxa studied, rib ducts are present in some species (e.g. *Andriana tsaratananensis*, *Pseudocannaboides andringitrensis* – Fig. 1A, Q), but absent in others (e.g. *Anginon difforme* and *Glia prolifera* – Fig. 1B, K), a characteristic feature of most Apioideae. In the subfamilies Mackinlayoideae and Azorelloideae, rib ducts are present in practically all genera. In the Saniculoideae *sensu stricto*, *Polemanniopsis* and *Steganotaenia*, the rib ducts are typically very large (Liu *et al.*, 2003, 2004). Large rib ducts are also present in *Lichtensteinia* Cham. & Schltld. and *Marlothiella* W. Wolff (Liu *et al.*, 2003, 2007), both genera were suggested be placed within an expanded Saniculoideae (Liu *et al.*, 2003), and molecular studies (Calviño *et al.*, 2006; Magee *et al.*, 2010) showed they were sister groups to the other Apioideae. Rib ducts may be present or absent in Araliaceae, (e.g. present in *Panax ginseng* C.A. Mey. and absent in *Acanthopanax brachypus* Harms). Regular vittae (one in furrow and two in commissure) are present in almost all the taxa studied as well as in genera placed in the basal position of Apioideae, such as *Annesorhiza* and *Chamarea*. This is

also true for some taxa of *Bupleurum* such as *B. condensatum* Shan & Yin Li and *B. densiflorum* Rupr.), but in some taxa of *Bupleurum* regular vittae are more than one in the furrow and commissure (e.g. *B. euphorbioides* Nakai and *B. gracillimum* Klotzsch). A lignified hypodermis was observed in some species of *Annesorhiza* and *Chamarea* (Tilney & Van Wyk, 2002; Liu, 2004), but not observed in the other Apiaceae. Another feature shared by these two genera is the development of leaves usually after flowering (Burtt, 1991; Van Wyk, 2000, 2001). This indicates their close relationship (Tilney & Van Wyk, 2002). Short vittae occurred in *Anginon*, *Dracosciadium*, *Glia*, *Heteromorpha* (Fig. 3E) are observed in *Bupleurum* (e.g. *commelynoideum* H. Boissieu and *B. euphorbioides*), but not found in *Annesorhiza* and *Chamarea*, and remain unknown in the “remaining Apioideae”.

Eyde & Tseng (1971) indicated “that the gynoecea of ancestral Araliaceae were well supplied with scattered secretory canals, that the canals have been lost in a few of the derived taxa, and that they have been localized in others, including the Umbelliferae”. The branching and anastomosing vittae as in *Pseudocarum laxiflorum* (Fig. 3C) are not observed in the “remaining Apioideae”, but occur in a few species of *Bupleurum* (e.g. *B. komarovianum* Lincz. and *B. scorzonifolium* Willd.), Saniculoideae (e.g. *Eryngium campestre* L., *Sanicula europaea* L.) and *Steganotaenia araliacea* Hochst., Azorelloideae (e.g. *Dickinsia* Jacq., *Klotzschia* Cham.) , and *Hermas* (e.g. *H. villosa* Thunb.), Mackinlayoideae (*Apiopetalum* Baill.) and Araliaceae (e.g. *Dendropanax* Decne. & Planch. and *Schefflera* J.R. Forst. & G. Forst.). Since branching and anastomosing vittae are the only type found in the Araliaceae, this character is here considered to be a plesiomorphic state.

Vascular bundles and carpophores: In most genera of Apiaceae and some taxa of Araliaceae such as *Kalopanax septemlobus* (Thunb.) Koidz., five vascular bundles are found in each carpel. Carpels with more than five bundles as in *Anginon* (Fig. 1G) and *Heteromorpha* (Fig. 2H), are relatively rare but are found in many groups of the Apiaceae, such as *Annesorhiza*, *Polemanniopsis*, Mackinlayoideae (e.g. *Apiopetalum*, *Mackinlaya* Hook. f., *Centella* L.), and also Araliaceae (e.g. *Macropanax oreophilus* Miq. and *Panax ginseng*). Such a broad distribution suggests that this feature is ancestral, but some taxa from the “remaining Apioideae” clade also have more than five vascular bundles per carpel, such as *Dasispermum* and *Ferulago* W.D.J. Koch, suggesting possible reversals among these more derived lineages

Ventral bundles are the vascular tissue associated with the commissure. They are opposite one another and form two carpophores in most genera of Apioideae (*Annesorhiza*, *Chamarea* *Bupleurum* and “remaining Apioideae”) as in Heteromorphae (Fig. 4). This condition also occurs in *Polemanniopsis*, *Steganotaenia*, *Lichtensteinia*. However the carpophore, if present, is predominantly single in Azorelloideae (e.g. *Astericum* Cham. & Schltld. and *Spananthe* Jacq. and Araliaceae (e.g. *Trachymene* Rudge).

Crystal type and distribution: Druse crystals that occur in all parts of the mesocarp (all around the endocarp) are found in all the genera of the Heteromorpheae and other basal groups of Apiaceae, *Lichtensteinia*, *Marlothiella*, *Annesorhiza* and *Chamarea*, Saniculoideae, *Polemanniopsis* and *Steganotaenia*, some taxa of Mackinlayoideae (e.g. *Pentapeltis* (Endl.) Bunge), Azorelloideae (e.g. *Huanaca* Cav.), and also Araliaceae (e.g. *Acanthopanax* Miq.) (Fig. 4). According to Burt (1991) crystals are usually absent in Apioideae, but if present, are only found on the commissural side. Rhomboidal crystals occurring in a single layer in the outermost cells of the endocarp or the innermost cells of the mesocarp are present in Azorelloideae, *Hermas*, Mackinlayoideae and also in some Araliaceae (e.g. *Astrotricha* DC. and *Trachymene*). The absence of Rhomboidal crystals is a convincing synapomorphy for the combined Saniculoideae and Apioideae.

Cladistics analysis: The characters with taxonomic importance, which could be logically polarized, were selected for cladistic analysis, using Araliaceae as outgroup. The 13 characters chosen and the polarization of character states are given in Table 3. The parsimonious tree with length of 22, a consistency index (CI) of 0.6818 and a retention index (RI) of 0.5114 were obtained.

Conclusions

Many of the unusual features that appear to characterize Heteromorpheae are in fact shared with other groups. These include woodiness, simple leaves, heteromorphic mericarps, bulging epidermal cells, regular and irregular vittae, lignified endocarp, carpophores with two bundles each and dispersed druse crystals. But Lateral winged mericarps, the bottle-shaped epidermal cells, and branching and anatosmosing vittae are peculiar to the Heteromorpheae. Our study show that the fruit anatomy is useful for understand the relationships between Heteromorpheae and other groups of the family.

In the Heteromorpheae, it is interesting to note that diversity in one character does not imply diversity in another. One character and its states may be tremendously variable, while another is fixed and totally invariable. Examples are the extreme diversity in the leaves of *Heteromorpha* combined with regular vittae (despite the diversity in this trait in other genera of the tribe). At the other extreme, there is *Bupleurum*, where all species have simple leaves but the vittae show almost the entire range of diversity of the whole subfamily. There is a lack of logical correlation between characters and their presumed plesiomorphic and apomorphic states. It therefore seems that diversification in the Heteromorpheae (and indeed in the whole of the Apiaceae) is not stepwise and predictable, but random; furthermore, it is not gradual but abrupt. Divergence in Apiaceae also does not necessarily means an increase in complexity, as elaboration and reduction seems equally likely (as is demonstrated in the Heteromorpheae). The ground plan for the extreme morphological diversity of the entire subfamily seems to have been laid down early on in the phylogenetic history, as can be seen in this small African tribe.

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