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SPECIALIZED STRUCTURES IN THE LEAF EPIDERMIS OF BASAL ANGIOSPERMS: MORPHOLOGY, DISTRIBUTION, AND HOMOLOGY[1]

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The morphology of specialized structures in the leaf epidermis of 32 species of basal (ANITA: *Amborella*, *N*ymphaeales, *I*lliciales, *T*rimeniaceae, and *A*ustrobaileyaceae) angiosperms, representing all seven families and 11 of 14 genera, was investigated using light and scanning electron microscopy. Distribution, density, and size of structures were also measured, and character evolution was analyzed. Hydropotes are a synapomorphy of Nymphaeales and ethereal oil cells are a synapomorphy of Austrobaileyales, but uniseriate nonglandular trichomes appear to have arisen independently several times. Specialized structures are frequently characterized by adjacent epidermal cells that have striking similarities in their form and arrangement (i.e., architecture) to subsidiary cells of certain types of stomatal complexes. Additionally, forms intermediate to oil cells and stomata, to trichomes and stomata, and to hydropotes and oil cells are present in some taxa. Thus, all of these specialized structures and their adjacent epidermal cells form complexes that may be homologous with, and evolutionarily derived from stomatal complexes, and the specialized structure, or portion thereof, may be homologous to the stoma or guard mother cell. Improved knowledge of the morphology and evolution of these structures in the earliest branching extant angiosperm lineages has a bearing on many diverse areas of botany.

Key words: Amborellaceae, Austrobaileyales, evolution, hydropotes, leaf epidermal anatomy, Nymphaeales, oil cells, trichomes.

Since 1999 and 2000, when several large-scale phylogenetic analyses (e.g., Mathews and Donoghue, 1999; Qiu et al., 1999, 2000; Graham and Olmstead, 2000) placed *Amborella trichopoda* Baill., Nymphaeales, and Austrobaileyales at the base of the extant angiosperm phylogenetic tree, the renewed interest in these groups resulted in numerous studies of various aspects of their biology (e.g., Endress and Igersheim, 2000; Carlquist and Schneider, 2001, 2002; Bernhardt et al., 2003; Feild et al., 2004; Carpenter, 2005). One aspect of ANITA (acronym of the families and orders within the first three clades: *Amborella*, *N*ymphaeales, *I*lliciales, *T*rimeniaceae, and *A*ustrobaileyaceae) angiosperms that has attracted relatively little attention is leaf epidermal anatomy. Carlquist (2001) and Baranova (2004) presented brief treatments of *Austrobaileya scandens* C. T. White, and *Amborella trichopoda* was summarized by Carlquist and Schneider (2001). I recently completed a comparative survey of stomatal architecture across all ANITA-grade families and Chloranthaceae (Carpenter, 2005). However, the morphology, distribution, and evolution of other specialized structures in the leaf epidermis of these plants (e.g., trichomes, ethereal oil cells, and hydropotes—specialized trichome-like structures in Nymphaeales) have been little examined or discussed.

Because plants communicate with their external environment and protect and maintain essential internal physiological and biochemical processes through such specialized epidermal structures, information on their morphology and evolution has bearing on a wide variety of issues. Aside from their proven value in the systematics and taxonomy of extant and fossil plants (e.g., Stace, 1965; Upchurch, 1984; Baranova, 1992a), specialized epidermal structures represent adaptations to a wide range of ecologies (cf. hydropotes and stomatodes of aquatic plants as in Kaul [1976] and Wilkinson [1979], and trichomes in xerophytic plants as in Ehleringer and Clark [1987]) and are of practical interest in agriculture because of their influence on the uptake of pesticides and fertilizers and their role in host–parasite interactions (e.g., Harr et al., 1991; Harr and Guggenheim, 1995). Recent workers interested in the molecular basis of plant development have been attracted to the leaf epidermis and its specialized structures such as trichomes, as a system offering many advantages and interesting questions for study (e.g., Ramsay and Glover, 2005).

The potential importance of specialized leaf epidermal structures in ANITA angiosperms in particular has been suggested by the few studies on such structures in these taxa prior to the formation of the ANITA hypothesis in 1999 and 2000. The presence of ethereal oil cells in leaves and other organs of *Austrobaileya* was considered by Bailey and Swamy (1949) to be a major line of evidence against a relationship with Dilleniaceae. Bailey and Swamy (1948) considered the lack of ethereal oil cells in *Amborella* to be an important character

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separating it from Monimiaceae. The presence of ethereal oil cells in the leaf epidermis in particular, led Baranova (1992a) to argue against a relationship between *Austrobaileya* and Annononaceae, or Myristicaceae, in favor of a relationship with Schisandraceae (as supported by Qiu et al., 1999, 2000). The increasing diversity of leaf epidermal secretory cells, hair bases, and other leaf characters seen through the Early Cretaceous, led Upchurch (1984) to conclude that angiosperms were undergoing a major adaptive radiation at that time. Yet despite these intriguing findings, these structures have been mentioned sporadically, and even less frequently illustrated in the literature on ANITA taxa. Some authors who mention ethereal oil cells in the leaf mesophyll of a given species or family fail to mention their occurrence in the leaf epidermis, as in Metcalfe's (1987) treatment of *Austrobaileya*, Philipson's (1993) treatment of Trimeniaceae, and Keng's (1993) treatment of Schisandraceae. With the current strongly supported and widely accepted rooting of the extant angiosperm tree, I believe that a comparative study of these structures from a large sample representing all ANITA families will yield data of taxonomic and systematic value for studies of living and fossil angiosperms, as well as hypotheses pertaining to many questions about their taxonomic distribution, evolution, and homology.

In this study, I examined the morphology of specialized leaf epidermal structures in mature leaves of 32 species of ANITA angiosperms, representing all seven families and 11 of the 14 genera. Structures in most of these species were examined and illustrated for the first time. I used the results to address several important questions including (1) What characters can be observed in the various structures, and how do these vary across the taxa? (2) What is the taxonomic distribution of the structures? Are previous reports concerning the lack of oil cells in *Amborella trichopoda* (e.g., Bailey and Nast, 1948) confirmed? (3) What is the ancestral state of angiosperms with regard to presence of structures and their character states? (4) How have these structures evolved within the ANITA taxa? (5) What does the evidence suggest about the homologies of the structures? Are leaf epidermal oil cells homologous with those in the leaf mesophyll and elsewhere in the plant? Is there evidence that some or all of these specialized epidermal structures might be homologous? (6) How do extant basal angiosperms compare to early Cretaceous angiosperm fossils in terms of these structures? What does this imply about ancestral character states and the taxonomic affinities of these fossils?

MATERIALS AND METHODS

Taxonomic sampling and specimen preparation—To maximize phylogenetic diversity sampled within ANITA taxa, sampling was guided by recent phylogenetic analyses of basal clades and other pertinent literature including Hao et al. (2000), Les et al. (1999), Saunders (1998, 2000), and Smith (1947). Material was obtained from herbaria, as well as from plants growing in botanic gardens and the wild. Collection and voucher data are given in the Appendix.

Leaf clearings were made by excising several sample pieces of roughly 0.25 cm^2 from near the midblade. A single mature leaf was sampled for all taxa examined except for *Amborella trichopoda* and *Austrobaileya scandens*, which were each represented by two leaves from each of two individuals (i.e., four leaves for each species). Samples were then immersed in two consecutive treatments of 5% KOH (12–24 h per treatment), rinsed in deionized water, treated for a few minutes in glacial acetic acid, and cleared in bleach (6% sodium hypochlorite). Following clearing, samples were dehydrated in an ethanol series, stained in 1% safranin O (in 100% ethanol) for a minimum of 3 d, and mounted onto microscope slides in Bioquip's Euparal (Rancho Dominguez, California, United States). Where possible, mesophyll tissue was removed under a dissecting microscope, leaving only the epidermes to be mounted. Specimens were examined and photographed with an Olympus BH-2 light microscope (Tokyo, Japan) and Microfire digital camera (Tokyo, Japan).

Leaf samples of Amborellaceae and Austrobaileyales were prepared for scanning electron microscopy by sampling pieces as described. Samples were placed in 1500-μL plastic reaction tubes, immersed in 100% chloroform, and placed in a water bath sonicator for 10 min. to remove epicuticular waxes and other debris. Samples were then rinsed several times in deionized water, and set to dry overnight on paper towels covered by inverted beakers. Leaf samples of *Victoria amazonica* were immersed in 10% Tween overnight, rinsed in deionized water, sonicated in chloroform as before, rinsed in deionized water, dehydrated in an ethanol series, and critical point dried in a Tousimis Sam dri-780A critical point dryer (Rockville, Maryland, United States). Samples were then mounted onto aluminum stubs with Ted Pella double stick adhesive (Redding, California, United States), sputter coated with gold with a Pelco Auto Sputter Coater SC-7 (Redding, California, United States), and examined and photographed at 10 kV in a Philips XL30 TMP scanning electron microscope (Eindhoven, Netherlands).

Character coding—For each sample (i.e., leaf), 20 of each type of specialized structure present on each surface (abaxial and adaxial) were coded for characters enumerated in the Results (*Characters*) section. While some of these characters have been previously mentioned in the literature (e.g., cuticular striations on ethereal oil cells and adjacent epidermal cells; Upchurch, 1984; Baranova 1992a), many have not, and I noted additional characters for each type of specialized structure after careful examination of all the taxa included here. Densities of structures on abaxial and adaxial surfaces were also calculated. A minority of specimens have a very low frequency of specialized structures, and for these, fewer than 20 structures were examined. Structures were measured in Adobe Photoshop Elements 2.0. Distances in pixels were obtained and converted to micrometers.

Analysis of character evolution—Character evolution was examined using MacClade 3.08 software (Maddison and Maddison, 1999) by mapping characters onto the basal portion of the most parsimonious tree obtained by Doyle and Endress (2000), which includes Chloranthaceae as the next lineage to diverge above the ANITA grade. Within this framework, topologies of Nymphaeales and Chloranthaceae were expanded according to the analyses of Les et al. (1999) and Qiu et al. (1999, 2000) respectively. Characters were assumed to be unordered, and the most parsimonious reconstruction/node option was used. I coded Chloranthaceae as lacking oil cells in the leaf epidermis (see Discussion, *Character evolution*).

For analysis of the evolution of trichomes, the species in the genera of Schisandraceae (i.e., *Kadsura* and *Schisandra*) that I examined are glabrous, but both genera are known to have species with pubescence (Saunders, 1998, 2000). I included these pubescent species in the analysis along with the ones I examined and ordered relationships according to the topologies obtained by Saunders (1998, 2000). Chloranthaceae are coded as questionable. Metcalfe (1987) mentioned that members of the family are glabrous, but Eklund et al. (2004) noted hairs in scattered species of the family. In Trimeniaceae, the specimen of *Trimenia weinmanniaefolia* that I examined had trichomes, but Metcalfe (1987) mentioned that some other species lack these; hence I placed *T. weinmanniaefolia* as sister to a clade of glabrous Trimeniaceae to depict this visually, although this had the same effect as coding the family as questionable.

RESULTS

Specialized structures were observed in the leaf epidermis of all seven ANITA grade families and in 11 of the 14 genera examined here (Table 1). Uniseriate, nonglandular trichomes and/or their associated abscission scars and foot cells were found in Amborellaceae (Figs. 5–12) and Trimeniaceae (Figs. 36–39). Ethereal oil cells were observed in the four families of Austrobaileyales (Austrobaileyaceae, Trimeniaceae, Schisandraceae, and Illiciaceae; Figs. 33–35, 40–52). Hydropotes were observed in Nymphaeaceae (Figs. 17–32). These consist of a unicellular or multicellular uniseriate hairlike portion that is abscised at maturity, leaving a base of three or four specialized epidermal cells set inside one another (Fig. 32). Mucilage hairs

TABLE 1. Distribution of specialized leaf epidermal structures in taxa of basal angiosperms. States: 0 = absent; 1 = present; ? = uncertain.

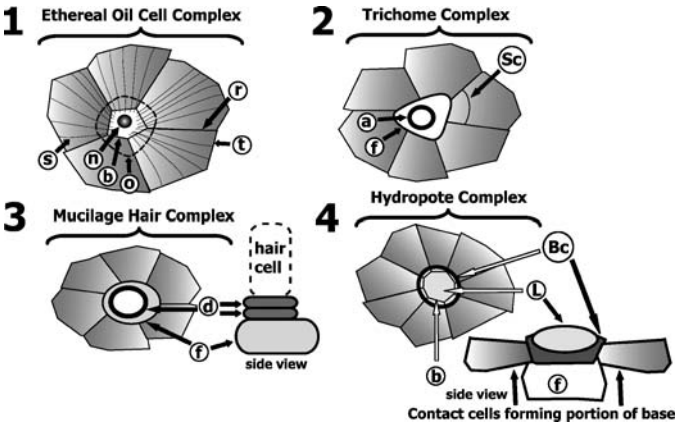
Taxon and specimen	Trichome complexes	Hydropote complexes	Oil cell complexes	Cuticular striations
Amborella trichopoda K.J. Carpenter 11, Leaf 1	0	0	0	0
Amborella trichopoda K.J. Carpenter 11, Leaf 2	0	0	0	0
Amborella trichopoda K.J. Carpenter 27, Leaf 1	1	0	0	0
Amborella trichopoda K.J. Carpenter 27, Leaf 2	1	0	0	0
Brasenia schreberi	0	1	0	0
Nuphar advena	0	1	0	0
Nuphar luteum	0	1	0	0
Nuphar polysepalum	0	1	0	0
Nymphaea caerulea	0	1	0	0
Nymphaea flava	0	1	0	0
Nymphaea nouchali	0	1	0	0
Euryale ferox	0	1	0	0
Victoria amazonica	0	1	0	0
Victoria cruziana	0	1	0	0
Austrobaileya scandens K.J. Carpenter 12, Leaf 1	0	0	1	1
Austrobaileya scandens K.J. Carpenter 12, Leaf 2	0	0	1	1
Austrobaileya scandens K.J. Carpenter 42, Leaf 1	0	0	1	1
Austrobaileya scandens K.J. Carpenter 42, Leaf 2	0	0	1	1
Trimenia weinmanniaefolia	1	0	1	1
Schisandra chinensis	0	0	1	1
Schisandra grandiflora	0	0	1	1
Schisandra incarnata	0	0	1	1
Schisandra longipes	0	0	1	0
Schisandra rubriflora	0	0	1	1
Schisandra sphenanthera	0	0	1	0
Kadsura borneensis	0	0	1	1
Kadsura coccinea	0	0	1	0
Kadsura heteroclita	0	0	1	1
Kadsura oblongifolia	0	0	1	1
Kadsura scandens	0	0	1	1
Illicium angustisepalum	0	0	1	0
Illicium dunnianum	0	0	1	1
Illicium floridanum	0	0	1	1
Illicium henryi	0	0	1	0
Illicium lanceolatum	0	0	1	0
Illicium parviflorum	0	0	1	1
Illicium simonsii	0	0	1	1
Illicium verum	0	0	1	1
Chloranthaceae[a]	?	0	0	1

[a] See Materials and Methods, *Analysis of character evolution* and Discussion, *Character evolution* for explanations of coding for Chloranthaceae.

(considered here as a type of hydropote—see Discussion) were found in Cabombaceae (Fig. 13–15).

Characters—The four specialized leaf epidermal structures examined here are similar in construction and six of the coded characters are common to all of them. Each type of specialized structure also has additional characters that are specific to it. Some of the observed character states are mentioned later in this section and in the Discussion, but a complete listing is given in Table 2.

Characters common to all specialized epidermal structures—(1) Complex type. The epidermal cells bordering the specialized structure frequently take the form of recognized stomatal complex types, so I used a subset of stomatal complex terminology to describe these patterns. Because a distinction between stomatal poles (i.e., the two areas where the guard cells contact) and the lateral face of the stoma (sides of the guard cells) underlies the definitions of many stomatal complex types (e.g., paracytic, diacytic, laterocytic), it is not possible to apply such terms to epidermal cells bordering specialized structures, because these have no definable polar or lateral regions. However, the definitions of some stomatal complex types do not entail such a distinction, and some of these terms are used here. Examples include the stephanocytic type, which comprises a rosette of subsidiary cells (Baranova, 1987), the similar actinocytic type, which has a rosette of subsidiary cells marked by radial elongation (Metcalfe and Chalk, 1950; Wilkinson, 1979), and the anomocytic type, which lacks subsidiary cells altogether (Metcalfe and Chalk, 1950). Stephanocytic, actinocytic, and other related forms (i.e., comprising rosettes of subsidiary cells) that I previously defined (Carpenter, 2005) are included in the *stephanocytic* category. Those complexes lacking subsidiaries are included in the *anomocytic* category. Complexes with one or more strongly specialized cells (i.e., *anatomically* specialized cells, which are notably different from adjacent nonspecialized cells in size, wall contour, or some other feature), even those that may resemble a paracytic or other such complex, are consigned to the *irregular* category. The complex types associated with specialized structures are compared to those of stomatal complexes within each taxon in Table 3, but it is important to note that stomatal complexes with strongly specialized subsidiary cells such as laterocytic and paracytic are considered here as irregular to facilitate comparison to complexes associated with specialized leaf epidermal structures where



Figs. 1–4. Illustrations of specialized leaf epidermal structures and their characters in surface view. **1.** Ethereal oil cell complex typical of those in Austrobaileyales comprising oil cell (o), depicted with a dashed line to indicate that the majority of the cell lies below the epidermis, its nucleus (n), base (b) formed by anticlinal contact cell walls, and cuticular striations (s). A radial wall (r) and tangential wall (t) are indicated. **2.** Trichome complex typical of Amborellaceae and Trimeniaceae showing abscission scar (a), foot cell to which the trichome was attached (f), and a strongly specialized contact cell (Sc). **3.** Mucilage hair complex typical of Cabombaceae with two disk-shaped cells (d) to which the mucilage hair is attached, and a foot cell (f), level with the epidermis, upon which the disk-shaped cells rest. **4.** Hydropote complex typical of Nymphaeaceae with base (b) formed by anticlinal contact cell walls, the lens-shaped cell (L), and the bowl-shaped cell (Bc). In surface view, the Bc often appears as a dark ring surrounding the L. A subepidermal foot cell (f) lies beneath the Bc and L.

terms such as paracytic and laterocytic cannot be applied. (2) Complex diameter. The diameter of the complex was measured at its widest point. (3) Number of contact cells. A contact cell is defined by Upchurch (1984) as any epidermal cell, whether specialized or not, that contacts the stoma. I used this term here similarly to denote any epidermal cell in contact with the specialized structure (or one of the cells—see Figs. 1–4). (4) Number of strongly specialized contact cells. Contact cells with strong anatomical specialization per complex were counted (Sc, in Fig. 2). Complexes with one or more strongly specialized cells are usually consigned to the *irregular* type (Character 1), but a minority of stephanocytic types also may have strongly specialized cells (e.g., Fig. 10). (5) Presence or absence of cuticular striations. Many taxa have striations radiating from the specialized cell and extending over adjacent contact cells (e.g., Figs. 33, 34, 42, 45, 47). (6) Presence or absence of a nucleus. Some specialized cells have a nucleus or its remnant (n in Fig. 1; Figs. 41, 45, 48, 52).

Characters specific to particular specialized epidermal structures—Because the first six characters are common to all specialized leaf epidermal structures, I continued the numbering of characters at seven for the additional characters specific to each type of structure. This was done for convenience and does not imply that characters numbered seven and higher are necessarily homologous across these different structures.

Specific characters for ethereal oil cells include (7) Base shape. The base is formed by anticlinal walls of abutting epidermal cells that surround the upper portion of the oil cell (b in Fig. 1). (8) Base diameter. The diameter of the base was measured at its widest point, which also corresponds to the diameter of the upper portion of the oil cell that emerges at the leaf surface. (9) Cell shape. The shape of the oil cell (o in Fig. 1) was recorded. (10) Cell diameter. The diameter of the oil cell was measured at its widest point, which lies below the epidermal surface (see Fig. 43).

For hydropotes, characters 7 and 8 are equivalent to the those for oil cells; hydropotes likewise have a "base" formed by the outline of the epidermal cells (b in Fig. 4) that surrounds a "bowl-shaped" cell (see Results, *Nymphaeales*), which is level with the epidermis. Characters 9 and 10 are the shape and diameter, respectively, of the "bowl-shaped" cell (Bc in Fig. 4).

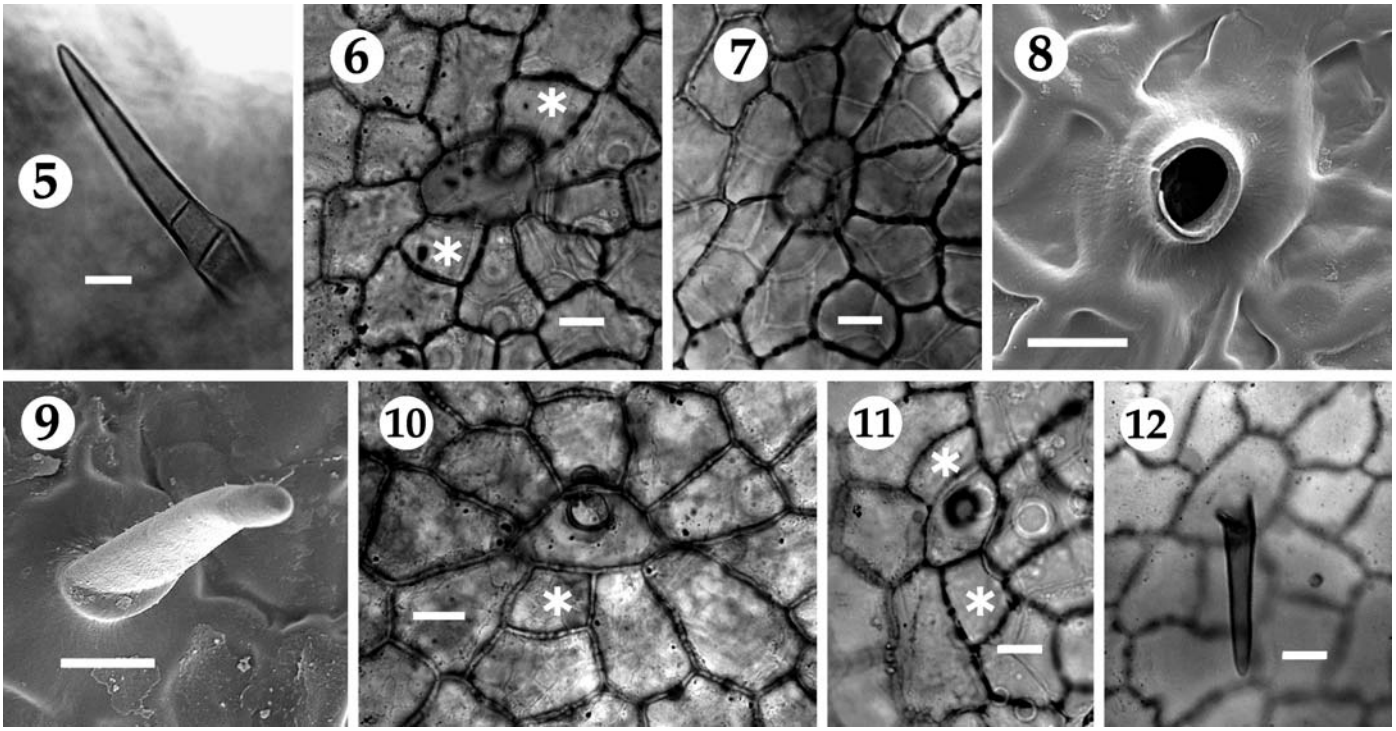
For the mucilage hairs of Cabombaceae, characters 7 and 8 are shape and diameter, respectively, of the foot cell (f in Fig. 3)—the specialized cell that is level with the epidermis and upon which the disk-shaped cells (see characters 9 and 10) rest. Characters 9 and 10 are shape and diameter, respectively, of the disk-shaped cell (d in Fig. 3)—the cell to which the hair was attached.

For the nonglandular hairs of Amborellaceae and Trimeniaceae, characters 7 and 8 are shape and diameter, respectively, of the foot cell, i.e., the cell to which the hair is attached—f in Fig. 2; Characters 9 and 10, shape and diameter, respectively, of the trichome abscission scar (a in Fig. 2), refer to the place where the trichome abscises and are more or less equivalent to the cross-sectional shape and diameter of the hair itself at or near its base.

Results by taxon—*Amborellaceae*—Small (less than 150 μm in length), uniseriate, nonglandular trichomes composed of one to three cells coated by a thick cuticle were observed on both leaves sampled of specimen *K.J. Carpenter 27* (Figs. 5–12). Both leaves had a sparse pubescence on the adaxial surface, while only one had a few trichomes on the abaxial surface. A minority of trichomes were fully intact but most were broken at various places, usually somewhere near the base (Fig. 8). The epidermal cells surrounding the foot cell often conform to stephanocytic (Figs. 7, 10) or irregular patterns (Figs. 6, 11), both of which are more common than anomocytic. Both leaves of the other individual, specimen *K.J. Carpenter 11*, were entirely glabrous. Ethereal oil cells were not observed in the leaf epidermis of either specimen.

Nymphaeales—Floating leaves of 10 species representing five of the eight genera of both families in this order were investigated. All had a dense covering of hydropotes on the abaxial surface (e.g., Figs. 25, 29). The hairlike portion of the hydropote was lacking in the majority of specimens; most had only the basal portions of these structures. In specimens with hairlike portions remaining, the number of cells ranged from 3–5 in *Euryale ferox* and *Victoria amazonica* (Fig. 25) to a dozen or more in the other genera. In leaf transverse sections, the hydropote bases of *V. amazonica* have an outermost cell shaped like a biconvex lens, set inside a larger bowl-shaped cell (Fig. 32), which completely encircles it in surface view (Fig. 29). This in turn is supported by a rectangular subepidermal "foot" cell (terminology from Lüttge and Krapf, 1969 and Wilkinson, 1979). The lens-shaped cell and bowl-shaped cell often stain more darkly than adjacent cells, and the former frequently appears to contain small structures, possibly vesicles or crystals.

Victoria amazonica and *E. ferox* had hydropote-like structures regularly distributed over their adaxial surfaces

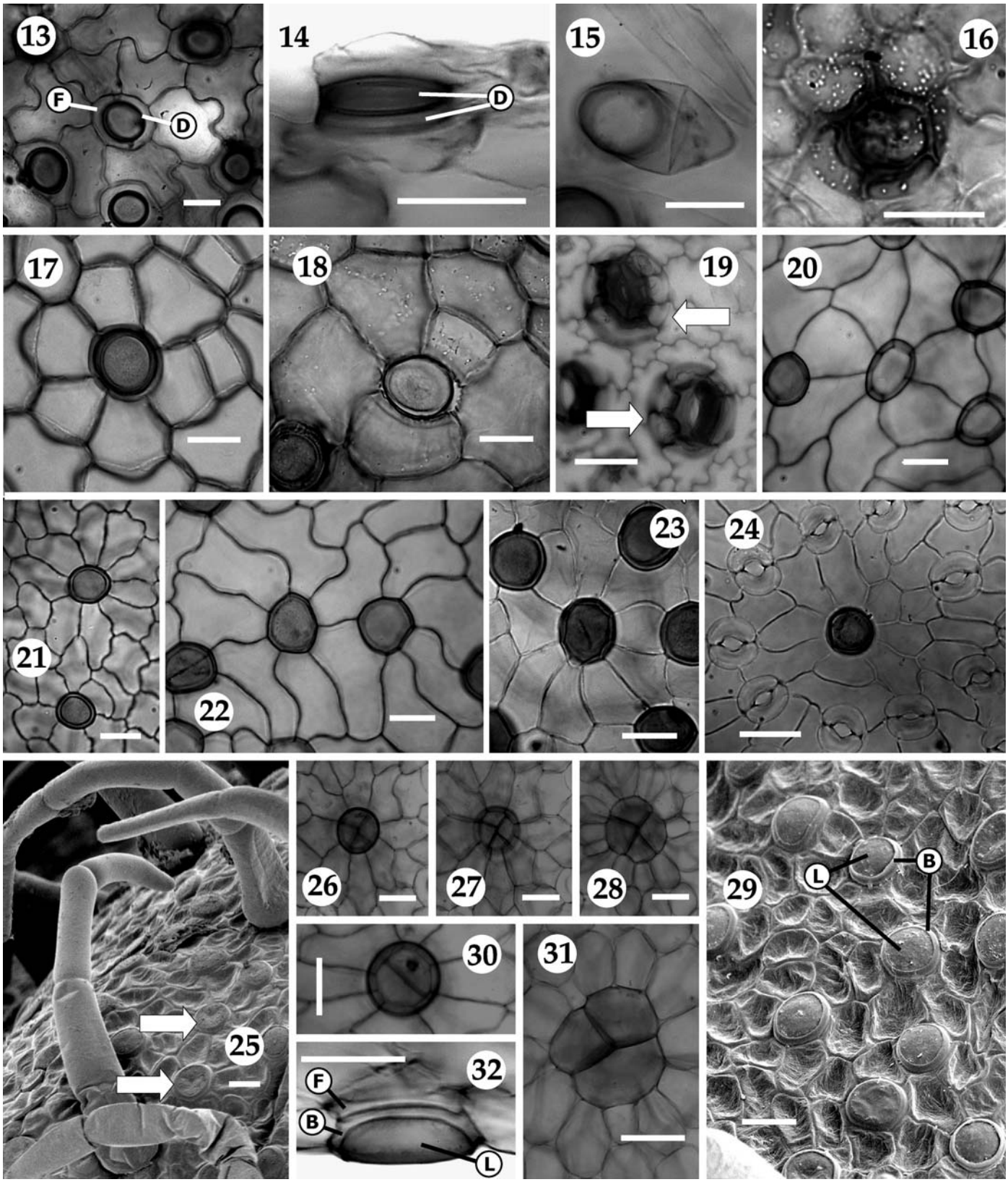


Figs. 5–12. Light (LM) and scanning electron micrographs (SEM) of uniseriate, nonglandular leaf trichomes and associated abscission scars, foot cells, and contact cells in *Amborella trichopoda, K.J. Carpenter 27*. LM are of cleared leaves. **5.** Abaxial, tricellular, uniseriate trichome, Leaf 1. **6.** Adaxial trichome complex with irregular architecture, Leaf 2. Asteriks denote two strongly specialized subsidiary cells. **7.** Adaxial trichome complex with actinocytic architecture, Leaf 1. **8.** SEM of adaxial trichome foot cell with trichome abscission scar, Leaf 3. **9.** SEM of adaxial trichome and portion of its base cell. Leaf 3. **10**. Trichome complex with actinocytic architecture with one strongly specialized subsidiary (asterik) formed by tangential division, Leaf 2. **11.** Adaxial trichome complex of irregular architecture, Leaf 2. Asteriks denote two strongly specialized subsidiary cells. **12.** Adaxial, unicellular trichome with its foot cell and the contact cells (Leaf 2). Bars = 20 μm.

(Figs. 24, 26–28, 30, 31), while *V. cruziana* had only a few such structures on the adaxial surface. In *Victoria*, these structures differ from abaxial hydropotes in that, in surface view, they show a highly conspicuous subepidermal complex of closely appressed cells that sometimes resembles a tetragonal pollen tetrad (Fig. 28). Other ones have three (Fig. 31) or two tightly appressed cells, while some have only one. Additionally, many of these structures appear to have a nucleus in the lens-shaped cell (Fig. 30). The adaxial structures in *Euryale* were not observed to have nuclei. *Nymphaea flava* had rare structures on the adaxial surface that appear to be intermediate between stomata and oil cells; in these, a large, spheroidal saclike object is attached at its base to a poorly developed pair of guard cells (Fig. 19). Unlike leaf epidermal ethereal oil cells, however, these saclike structures are positioned entirely above the leaf surface.

The mucilage hairs of *Brasenia schreberi* (Cabombaceae) are structurally more similar to hydropotes of Nymphaeaceae than to the trichomes of Amborellaceae or Trimeniaceae. They consist of a small unicellular or bicellular hair attached to two disk-shaped cells stacked one atop the other (Figs. 14, 15). These two disk-shaped cells in turn rest upon a larger foot cell that is level with the other epidermal cells (Fig. 13). The arrangement of these three cells as seen in leaf transverse section is similar to the three-cell arrangement of hydropotes in Nymphaeaceae (cf. Figs. 14 and 32). Also, *B. schreberi* has two spherical, thin-walled, oil cell-like structures above the adaxial epidermis. These have bases typical of oil cell complexes in Austrobaileyales and are filled with numerous, small prismatic crystals (Fig. 16).

Austrobaileyales—Representatives of all families and genera of this order were examined, and all were observed to have ethereal oil cells in varying densities on the abaxial leaf surface; however, the majority lack these on the adaxial epidermis. In Austrobaileyaceae, oil cells were found exclusively on the abaxial epidermis in the four leaf samples (Figs. 33–35). Trichomes are lacking. *Trimenia weinmannieafolia* Seem., (Trimeniaceae) had trichome foot cells, abscission scars, and oil cell complexes on both leaf surfaces. The oil cell complexes are typical for this order, but the trichome bases differ from those in other ANITA families in that their associated epidermal cells have a very high proportion of irregular architecture. In these, contact cells often appear much smaller than other epidermal cells (Fig. 37). The trichome abscission scars (Fig. 39), however, are similar to those in Amborellaceae. The two genera of Schisandraceae differed somewhat in distribution of oil cell complexes. Only one of the six species of *Schisandra* (*S. chinensis*) investigated had adaxial oil cell complexes, but these were found in four of five species of *Kadsura* (Fig. 41). Some species of *Kadsura* have very high densities, and *K. oblongifolia* Merrill is one of two ANITA-grade taxa with a higher density of oil cells on the adaxial surface than the abaxial surface. The species of Schisandraceae examined here were all glabrous. The eight species of Illiciaceae examined were highly variable in density



Figs. 13–32. LM (cleared leaves) and SEM of leaf hydropotes, mucilage hairs, and unidentified oil cell-like structures in Nymphaeales. **13.** Abaxial stephanocytic mucilage hair complex in *Brasenia scherberi* (Cabombaceae). Disk cells (d) and foot cell (f) are indicated. **14.** Abaxial mucilage hair of *B. schreberi* in transverse section, two disk cells (d) atop a foot cell. **15.** Abaxial bicellular mucilage hair, *B. schreberi*. **16.** Adaxial oil-cell-like structure, *B. schreberi*. Note the circular outline of the cell and numerous small prismatic crystals within; its base is similar to those in ethereal oil cell complexes in

of abaxial oil cell complexes. Only *Illicium parviflorum* had them on the adaxial surface (Fig. 50), where they occurred in greater density than on the abaxial surface. Illiciaceae have a much higher frequency of undulate bases (with a concavity in the walls of the epidermal cells forming the base; Fig. 49), and typically, larger oil cells than any other ANITA grade family. All investigated species of Illiciaceae are glabrous. In general, Illiciaceae have larger oil cells and bases than Schisandraceae, while those in both families generally exceed those in Trimeniaceae. Austrobaileyaceae have the smallest oil cells and bases in the order.

Character evolution—Analysis of character evolution indicated that leaf epidermal ethereal oil cells are a synapomorphy of Austrobaileyales (Fig. 60) and that hydropotes are a synapomorphy of Nymphaeales (Fig. 61). Trichomes are reconstructed as absent in the common ancestor of Nymphaelaes and in the common ancestor of Austrobaileyales, but are equivocal in the common ancestor of all angiosperms and a few other internal nodes (Fig. 62). The common ancestor of all angiosperms is thus reconstructed as lacking hydropotes and ethereal oil cells, although the presence of trichomes is unresolved. The evolution of most of the characters coded for these structures was not traced due to extensive variability within genera and for other reasons explained in the Discussion, *Character evolution*.

DISCUSSION

Morphology and distribution—Evidence presented here adds to that from a growing list of studies in which oil cells were found to be lacking in Amborellaceae (Bailey and Swamy, 1948; Money, et al., 1950; Carlquist and Schneider, 2001), counter to the earlier claim of their presence by Perkins (1898). The variability in distribution and density of trichomes in the four samples examined here also accords with Bailey and Swamy's (1948) observations that the presence and density of hairs and other leaf characters (e.g., size, form) often vary greatly, even among leaves of the same plant. The hairs observed here are similar to the ones noted by Bailey and Swamy (1948, p. 248), except that the "protuberant, multicellular pedestal[s]" they noted in some were not found here, nor were other specialized forms such as the "tufted, fan-shaped, stellate, or peltate forms" noted by Money et al. (1950, p. 374).

Oil cells have not been noted in Nymphaeales in any of a number of works that have addressed leaf anatomy (e.g., Metcalfe and Chalk, 1950; Goleniewska-Furmanowa, 1970; Kaul, 1976; Williamson et al., 1989; Schneider and Williamson, 1993; Williamson and Schneider, 1993; Les et al., 1999). While oil cell complexes of the type found in Austrobaileyales are absent in the nymphaealean taxa I examined, there are intriguing structures in a few species that do not conform to anything previously reported. In *V. amazonica*, Wilkinson (1979, p. 165) noted "a few" hydropotes on the adaxial surface, but in the sample I examined, the hydropote-like structures on the adaxial surface appear as a regular feature; they are more or less evenly spaced and are present in all four slides I prepared. These differ from abaxial hydropotes in their unusual subepidermal complexes (see Results, *Results by taxon*) and in the presence of nuclei in the lens-shaped cell of some, a character found in some oil cells of Austrobaileyales.

Perhaps even more intriguing are the rare adaxial structures of *Nymphaea flava* that appear intermediate between stomata and oil cells in having a large saclike structure attached to a pair of poorly developed guard cells (Fig. 19). *Brasenia schreberi* likewise has rare structures on the adaxial surface that bear some similarity to oil cell complexes in Austrobaileyales (Fig. 16). Goleniewska-Furmanowa (1970, p. 24) mentioned that the mucilage hairs of this species may vary widely and include "low glandular hairs with a bicellular stalk and unicellular head". She illustrated a range of hairs (depicted on the abaxial surface), including one with a globose, unicellular head supported by two flattened, disk-shaped cells sitting one atop the other. Despite this similarity in cell shape, the structures I observed differ in occurring on the adaxial surface and in containing numerous tiny prismatic crystals (as are common in Nymphaeaceae), which Goleniewska-Furmanowa (1970) commented were absent in Cabombaceae. I was not able to observe these structures in leaf transverse sections and thus was not able to confirm the presence of the two disk-shaped base cells, but in surface view, the base appears quite similar to the polygonal to elliptical bases seen in oil cell complexes of Austrobaileyales. Even supposing that these structures are rare adaxial, mucilage hairs, I find their similarity to oil cell complexes in Austrobaileyales to be nevertheless notable and intriguing.

In Austrobaileyales, morphology and distribution of ethereal oil cells largely conform to previously published descriptions and illustrations (e.g., Bailey and Nast, 1948; Bailey and Swamy, 1948; Upchurch, 1984; Baranova, 1992a).

Character evolution—Although the analysis of character evolution resolved the common ancestor of all extant angiosperms as lacking leaf epidermal ethereal oil cells and hydropotes, there are two points that must be considered before adopting this reconstruction. First, since *Amborella trichopoda* is likely the product of one of the oldest lineage-splitting events in extant angiosperms, is the sole representative of its family,

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Austrobaileyales. **17.** Abaxial hydropote complex with stephanocytic architecture, *Nuphar advena*. **18.** Abaxial hydropote complex with irregular architecture with one strongly specialized subsidiary, *N. polysepalum*. **19.** Adaxial oil-cell-like structures, *Nymphaea flava*. Note the elliptic outline of the cell (arrows), which is attached to a pair of guard cells. **20.** Abaxial actinocytic hydropote complex, *N. nouchali*. **21.** Abaxial hydropote complexes in *N. flava*; upper is actinocytic, lower has irregular architecture (one strongly specialized subsidiary). **22.** Two abaxial actinocytic hydropote complexes, *N. caerulea*. **23.** Abaxial actinocytic hydropote complex, *Euryale ferox*. **24.** Adaxial hydropote complex with irregular architecture and one strongly specialized subsidiary, *E. ferox*. Note the surrounding stomata. **25.** SEM of abaxial surface of *Victoria amazonica* showing many hydropotes without hairlike portions (arrows), and a few with these still attached. **26–28.** Different focal planes of one adaxial hydropote complex (weakly actinocytic architecture) of *V. amazonica* . **26.** Top of lens-shaped and bowl-shaped cells. **27.** Middle of the hydropote with cruciform pattern of subepidermal complex visible. **28.** Lower plane showing 4-celled subepidermal cell complex. **29.** SEM of abaxial surface of *V. amazonica* with detail of the basal portion of hydropote complexes. **30.** Adaxial hydropote of *V. amazonica* showing nucleus. **31.** A 3-celled subepidermal cell complex of an adaxial hydropote, *V. amazonica*. **32**. Abaxial hydropote (transverse section), *V. amazonica*. L = lens-shaped cell, B = bowl-shaped cell, F = foot cell. Bars = 20 μm.

TABLE 2. Observed character states and means, standard deviations, or ranges (in parentheses) for characters pertaining to specialized leaf epidermal structures in ANITA angiosperms. Standard deviations for density and characters 5 and 6 are not given when only one sample was examined. Characters 7–10 vary according to structure and are explained in the Results, *Characters* and in Figs. 1–4. Dashes indicate that structures are absent. Question marks indicate that the identity of the structure is uncertain. *Abbreviations*: A = anomocytic; Ab = Abaxial; Ad = Adaxial; C = circular; CCs = contact cells; E = elliptical; Ep = epidermis; I = irregular; O = ovate; P = polygonal; S = stephanocytic; Scs = strongly specialized contact cells; T = triangular; U = undulate. A dash between abbreviations indicates a range of morphologies grading between the two states. Character states indicated by abbreviations are listed in order of decreasing frequency. Rare indicates that five or fewer of the structure were observed throughout the sample.

Genus	Ep.	Structure (No. samples with structure)	Density (per mm^2)	1 and 2 Complex type and diameter (μm)	3 and 4: No. CCs / Scs per complex	5: Striae (%)[a]	6: Nucleus (%)[a]	7 and 8: Shape and diameter (μm)[b]	9 and 10: Shape and diameter (μm)[c]
Amborella	Ad	Trichomes (2 of 4)	1.2 ± 0.5	S, I, A	7.6 (6–10)	0 ± 0	0 ± 0	P–E, T	C–E
				152.4 ± 21.9	0.9 (0–7)			54.2 ± 3.8	21.2 ± 2.9
	Ab	Trichomes (1 of 4)	Rare	S, A	7.5 (7–8)	0	0	T	C
				123.1 ± 1.4	0 (0–0)			38.4 ± 7.5	16.7 ± 6.5
Brasenia	Ad	Oil cell-like structures? (1 of 1)	Rare	A, S	5.5 (5–6)	0	0	P–E	P–E
				64.7 ± 17.1	0 (0–0)			18.7 ± 5.8	30.4 ± 5.9
	Ab	Mucilage hairs (1 of 1)	347.4	A, S, I	6.4 (4–9)	0	0	P–E	C–E
				131.4 ± 21.2	0.1 (0–1)			39.9 ± 5.1	19.5 ± 2.7
Nuphar	Ad	—	—	—	—	—	—	—	—
	Ab	Hydropotes (3 of 3)	148.5 ± 66.6	A, I, S	4.8 (3–8)	0 ± 0	1.7 ± 2.9	C–E	P–E, C–E
				103.8 ± 9.9	0.3 (0–2)			17.9 ± 0.4	28.6 ± 2.0
Nymphaea	Ad	—	—	—	—	—	—	—	—
	Ab	Hydropotes (3 of 3)	188.3 ± 51.7	S, A, I	6.7 (5–9)	0 ± 0	0 ± 0	P–E, C–E, O	P–E, C–E, O, I
				108.3 ± 23.3	0.4 (0–6)			19.1 ± 2.6	24.3 ± 3.0
Euryale	Ad	Hydropotes (1 of 1)	0.76	S, A, I	7.6 (6–11)	0	0	P–E	P–E
				63.3 ± 7.5	0.2 (0–1)			12.4 ± 1.5	16.6 ± 1.2
	Ab	Hydropotes (1 of 1)	479.9	S, I, A	7.2 (4–10)	0	0	P–E	P–E
				78.3 ± 9.4	0.8 (0–2)			20.8 ± 1.8	24.8 ± 1.4
Victoria	Ad	Hydropotes (2 of 2)	2.3 [d]	S, I, A	8.7 (8–11)	0 ± 0	40 ± 56.6	P–E	P–E
				104.5 ± 19.3	0.2 (0–2)			21.4 ± 0.1	26.9 ± 2.1
	Ab	Hydropotes (2 of 2)	145.8 ± 58.1	S, A, I	7.2 (5–9)	0 ± 0	0 ± 0	P–E, C–E, O	P–E, C–E, O, I
				129.8 ± 16.0	0.1 (0–1)			26.0 ± 3.4	31.9 ± 3.8
Austrobaileya	Ad	—	—	—	—	—	—	—	—
	Ab	Oil cells (4 of 4)	0.4 ± 0.2	S, I, A	6.5 (5–8)	100 ± 0	15.0 ± 19.1	P–E	P–E, I
				162.8 ± 6.8	0.7 (0–4)			25.7 ± 1.5	34.2 ± 2.2
Trimenia	Ad	Oil cells (1 of 1)	1.76	A, I, S	4.9 (4–6)	95.0	30.0	P–E	P–E, O
				190.3 ± 28.9	0.5 (0–5)			27.9 ± 3.8	48.6 ± 4.2
	Ad	Trichomes (1 of 1)	1.76	I, S	5.7 (4–7)	70.0	0	P–E, O	C–E
				150.5 ± 25.0	3.5 (1–6)			55.4 ± 4.2	19.3 ± 1.3
	Ab	Oil cells (1 of 1)	6.03	I, A, S	5.7 (4–7)	5.0	15.0	P–E, U	P–E
				168.5 ± 22.0	0.7 (0–4)			30.4 ± 3.7	42.7 ± 3.4
	Ab	Trichomes (1 of 1)	4.02	I, A	6.6 (5–9)	20.0	0	C–E, P, O	C–E
				170.8 ± 38.6	1.6 (0–5)			47.2 ± 4.4	17.7 ± 2.2
Schisandra	Ad	Oil cells (1 of 6)	0.54	I, S	6.0 (5–7)	100.0	67.0	P–E, I	P–E
				191.6 ± 26.7	2.0 (0–4)			36.3 ± 4.2	44.1 ± 3
	Ab	Oil cells (6 of 6)	2.8 ± 1.9	A, I, S	6.3 (4–9)	49.2 ± 43.8	34.2 ± 29.7	P–E, I, U, O	P–E, I, O
				211.5 ± 39.7	0.5 (0–4)			37.3 ± 3.1	44.0 ± 2.0
Kadsura	Ad	Oil cells (4 of 5)	5.9 ± 5.4	I, S, A	5.5 (4–9)	58.3 ± 44.0	75.5 ±	P–E, U, I	P–E, O, I
				158.8 ± 18.9	1.1 (0–5)		39.2	35.5 ± 5.0	52.1 ± 8.3
	Ab	Oil cells (5 of 5)	5.9 ± 3.0	I, A, S	5.5 (3–10)	68.0 ± 41.5	60.0 ± 17.7	P–E, I, U, O	P–E, I, O
				165.2 ± 24.0	0.9 (0–6)			37.2 ± 6.0	49.6 ± 7.0
Illicium	Ad	Oil cells (1 of 8)	6.36	S, A, I	5.7 (4–7)	10.0	10.0	U, P–E	P–E
				134.8 ± 12.3	0.4 (0–2)			36.5 ± 4.9	86.0 ± 5.9
	Ab	Oil cells (8 of 8)	5.0 ± 6.0	A, S, I	6.2 (4–9)	45.6 ± 48.1	47.5 ± 37.0	P–E, U, I	P–E, O
				163.6 ± 40.7	0.5 (0–6)			37.9 ± 3.4	59.1 ± 12.1

[a] Percentage of structures with cuticular striations (striae) or with nucleus.

[b] Characters 7 and 8, respectively: for trichomes, shape and diameter of the foot cell; for mucilage hairs, shape and diameter of the foot cell; for hydropotes and oil cells, shape and diameter of the structure's base (see Results and Figs. 1–4).

[c] Characters 9 and 10, respectively: for trichomes, shape and diameter of the abscission scar; for mucilage hairs, shape and diameter of the disk cell; for oil cells, shape and diameter of the oil cell; for hydropotes, shape and diameter of the bowl-shaped cell (see Results and Figs. 1–4).

[d] Density is based only on *Victoria amazonica*; adaxial hydropotes are rare in *V. cruziana*.

and has a very restricted present-day distribution, it is difficult to believe that neither extinction nor phyletic evolution have occurred in Amborellaceae since its origin. Hence, the lack of leaf epidermal ethereal oil cells may represent a derived condition, just as I inferred that the predominantly paracytic stomatal architecture in *Amborella* is derived (Carpenter, 2005). Likewise, Nymphaeales have become highly modified for an aquatic existence, and a loss of epidermal oil cells may have occurred alongside, or as a result of, other modifications in the leaf epidermis. Second, examination of early fossil angiosperm leaves shows that taxa with leaf epidermal ethereal oil cells and/or trichomes appeared early in the history of the

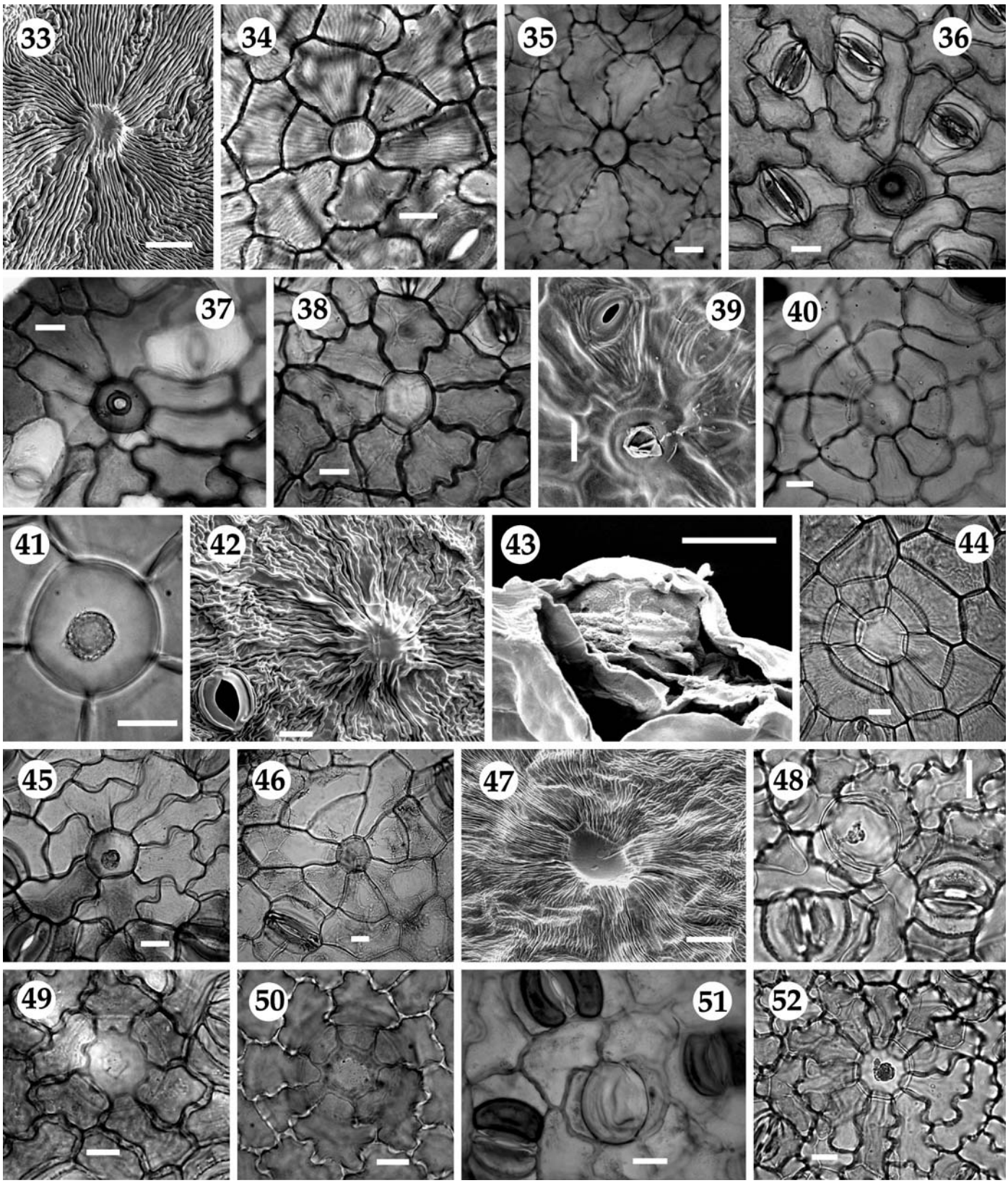
TABLE 3. Comparison of the architecture of specialized leaf epidermal complexes with that of stomatal complexes within each taxon. The most common type of architecture present in each structure is given. Data for stomatal architecture are taken from Carpenter (2005) and refer to abaxial stomatal complexes, except for taxa of Nymphaeales (*Brasenia*, *Nuphar*, *Nymphaea*, *Euryale*, and *Victoria*) in which they are adaxial. A dash indicates data are unavailable. A slash separating abbreviations indicates that both types are equally abundant. *Abbreviations*: A = anomocytic; ab = abaxial; ad = adaxial; I = irregular; S = stephanocytic. For purposes of comparison, stomatal types with strong specialization of subsidiary cells are considered "irregular" here so as to be compatible with criteria by which complexes of specialized stuctures were judged. The most common type of such "irregular" stomatal complexes is given in parentheses.

Taxon (Specimen)	Structure: Architecture	Stomatal architecture
Amborella trichopoda (*K.J. Carpenter 27*)	Trichomes (ad): I Trichomes (ab): A/S	I (paracytic)
Brasenia schreberi	Hydropotes (ab): A	S
Nuphar advena	Hydropotes (ab): A	S
Nuphar luteum	Hydropotes (ab): A	A
Nuphar polysepalum	Hydropotes (ab): A	S
Nymphaea caerulea	Hydropotes (ab): S	S
Nymphaea flava	Hydropotes (ab): S	S
Nymphaea nouchali	Hydropotes (ab): A/S	S
Euryale ferox	Hydropotes (ab): S	S
Victoria amazonica	Hydropotes (ad): S Hydropotes (ab): S	S
Victoria cruziana	Hydropotes (ad): S Hydropotes (ab): S	S
Austrobaileya scandens (*K.J. Carpenter 12*, Leaf 1)	Oil cells (ab): S	I (laterocytic)
Austrobaileya scandens (*K.J. Carpenter 12*, Leaf 2)	Oil cells (ab): S	I (laterocytic)
Austrobaileya scandens (*K.J. Carpenter 42*, Leaf 1)	Oil cells (ab): S	I (laterocytic)
Austrobaileya scandens (*K.J. Carpenter 42*, Leaf 2)	Oil cells (ab): S	I (laterocytic)
Trimenia weinmanniaefolia	Oil cells (ad): A Oil cells (ab): I Trichomes (ad): I Trichomes (ab): I	I (paracytic)
Schisandra chinensis	Oil cells (ad): I Oil cells (ab): A	I (laterocytic)
Schisandra grandiflora	Oil cells (ab): I	I (laterocytic)
Schisandra incarnata	Oil cells (ab): A	I (laterocytic)
Schisandra longipes	Oil cells (ab): A	—
Schisandra rubriflora	Oil cells (ab): A	I (laterocytic)
Schisandra sphenanthera	Oil cells (ab): A	I (laterocytic)
Kadsura borneensis	Oil cells (ad): I Oil cells (ab): I	I (paracytic)
Kadsura coccinea	Oil cells (ab): A	I (laterocytic)
Kadsura heteroclita	Oil cells (ad): S Oil cells (ab): A	I (laterocytic)
Kadsura oblongifolia	Oil cells (ad): I Oil cells (ab): I	—
Kadsura scandens	Oil cells (ad): I Oil cells (ab): A/S	I (laterocytic)
Illicium angustisepalum	Oil cells (ab): S	I (paracytic)
Illicium dunnianum	Oil cells (ab): S	I (paracytic)
Illicium floridanum	Oil cells (ab): A	I (paracytic)
Illicium henryi	Oil cells (ab): A	I (paracytic)
Illicium lanceolatum	Oil cells (ab): A	I (paracytic)
Illicium parviflorum	Oil cells (ad): S Oil cells (ab): A	I (paracytic)
Illicium simonsii	Oil cells (ab): A	I (paracytic)
Illicium verum	Oil cells (ab): I	I (paracytic)

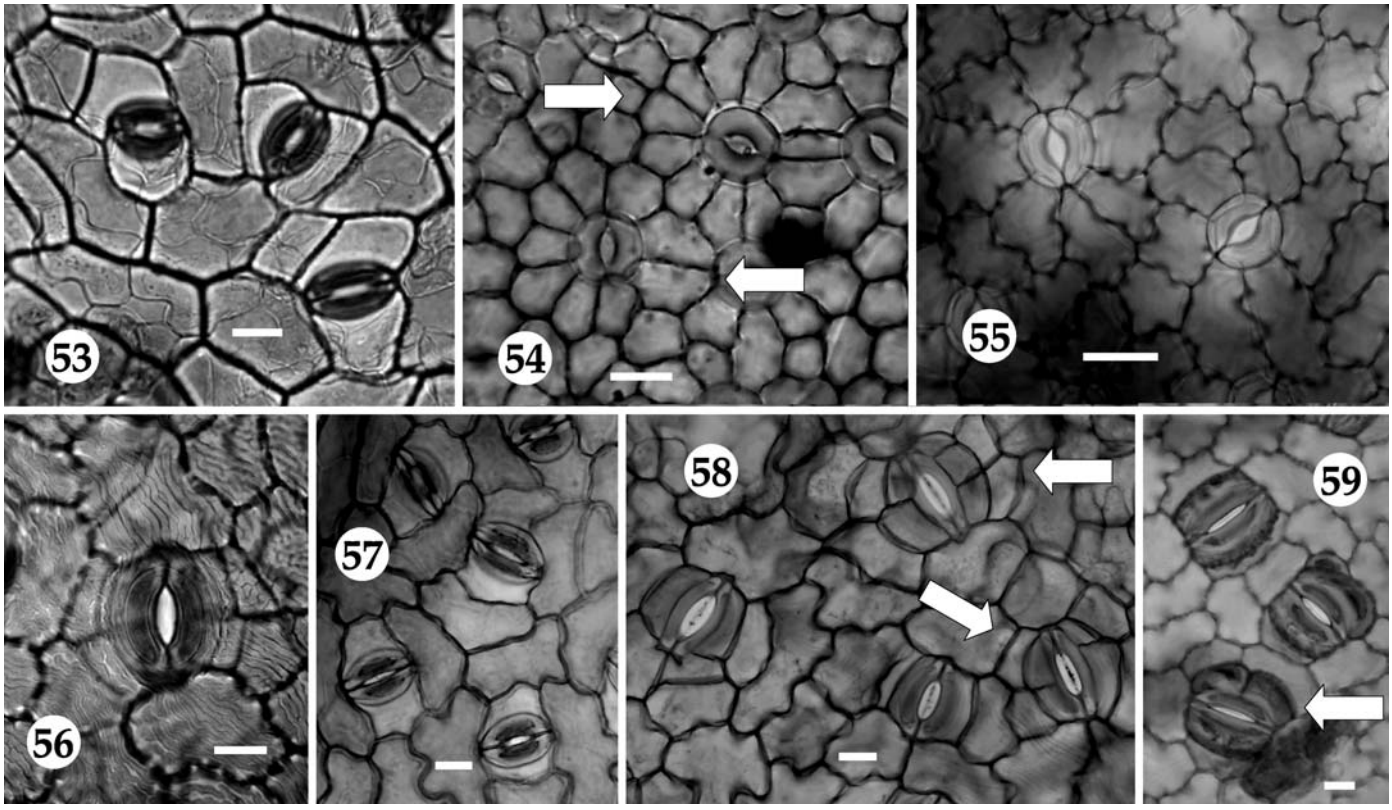
angiosperm clade, as discussed later (see also Upchurch, 1984). The occurrence of uniseriate trichomes in scattered species of woody ANITA taxa including *Amborella*, some Schisandraceae (Saunders, 1998, 2000) and some Trimeniaceae (Results, *Results by taxon*, and Metcalfe, 1987), as well as in early angiosperm fossil cuticles (Upchurch, 1984) may suggest that the presence of structural genes for such trichomes may be ancestral in angiosperms, or at least may have evolved early. The subsequent loss or suppression of trichomes in some extant ANITA taxa could possibly be explained through the MYB-bHLH-WD40 protein complex of transcription factors, which is thought to operate in all angiosperms and known to have the ability to quickly evolve to change expression of epidermal cell types (Larkin et al., 2003; Ramsay and Glover, 2005). As Ramsay and Glover (2005) point out, regulatory genes and their DNA-binding sequences are known to be able to evolve more quickly than the structural genes whose expression they mediate. The differing complement of trichomes in closely related species (e.g., as in Schisandraceae) may provide an intriguing system for further investigation of the role of this protein complex in angiosperms as a whole.

It is also notable that in this analysis (i.e., as based on Doyle and Endress' [2000] topology), leaf epidermal ethereal oil cells are a synapomorphy of Austrobaileyales, rather than Austrobaileyales + Other Angiosperms (Fig. 60). Although Chloranthaceae (the next lineage to branch above ANITA according to Doyle and Endress, 2000) are mentioned as having oil cells in the leaf mesophyll and other tissues (e.g., Swamy, 1953; Metcalfe, 1987; Eklund et al., 2004), none of these studies, nor a recent study of the leaf epidermis in this family (Kong, 2001), mention or illustrate oil cells in the leaf epidermis, so I coded Chloranthaceae as lacking these. Additionally, I have not observed leaf epidermal oil cells in the family either (personal observation). While oil cells are commonly held to be a symplesiomorphy of magnoliid plants (e.g., West, 1969), it appears that at least those in the leaf epidermis have originated (or were lost) independently a number of times. However, if the Doyle and Endress (2000) placement of Chloranthaceae is rejected, as in the APG II (2003) topology, then it is possible that this interpretation may change. However, with the APG II (2003) topology, until greater resolution is achieved, especially among Chloranthaceae, monocots, and "magnoliids" (Piperales, Canellales, Laurales, Magnoliales), which currently form a trichotomy sister to *Ceratophyllum* + Eudicots, it is not possible to draw any firm conclusions. One possible scenario that could support homology of leaf epidermal ethereal oil cells in Austrobaileyales and magnoliids (as circumscribed by APG II, 2003) would be if magnoliids are sister to Chloranthaceae + Monocots, all of which are sister to *Ceratophyllum* + Eudicots. Then, it could be hypothesized that leaf epidermal oil cells arose in the common ancestor of Austrobaileyales + Other Angiosperms, were retained in magnoliids, and lost in Chloranthaceae, monocots (although *Acorus* does have oil cells in the mesophyll (See Doyle and Endress, 2000) and *Ceratophyllum* + Eudicots.

Of the characters pertaining to the specialized leaf epidermal structures I coded, those showing systematic value include adaxial hydropotes, a synapomorphy of the *Victoria* + *Euryale* clade (see Les et al., 1999) and perhaps cuticular striations, which may represent a synapomorphy of Austrobaileyales + Other Angiosperms (although some Austrobaileyales lack these). A high frequency of undulate ethereal oil cell bases (exceeding all other types of bases) seems a potentially useful



Figs. 33–52. LM (cleared leaves) and SEM of leaf epidermal oil cell and trichome complexes in Austrobaileyales. **33.** SEM of abaxial ethereal oil cell complex, *Austrobaileya scandens, K.J. Carpenter 12*, Leaf 3. Note the raised outer portion of the oil cell with deep, radiating striations. **34.** Abaxial ethereal oil cell complex with irregular architecture (three strongly specialized subsidiaries), *A. scandens K.J. Carpenter 12*, Leaf 1. **35.** Abaxial actinocytic ethereal oil cell complex. Note the smooth contour of the arc described by the tangential walls of the subsidiary cells. *A. scandens, K.J. Carpenter 12*,



Figs. 53–59. LMs of the most common type(s) of stomatal complexes in the seven ANITA families. (Other, less common types are encountered in each family as well—see Carpenter [2005].) **53.** Paracytic complexes, *Amborella trichopoda* (Amborellaceae), *K.J. Carpenter 27*, Leaf 2; **54.** Actinocytic complexes (arrows), *Brasenia schreberi* (Cabombaceae); **55.** An actinocytic (left) and anomocytic (right) complex, *Nymphaea nouchali* (Nymphaeaceae); **56.** Laterocytic complex, *Austrobaileya scandens* (Austrobaileyaceae), *K.J. Carpenter 42*, Leaf 2; **57.** Paracytic complexes, *Trimenia weinmannieafoila* (Trimeniaceae); **58.** Laterocytic (arrows) and paracytic complexes, *Kadsura scandens* (Schisandraceae); **59.** Paracytic and laterocytic (arrows) complexes, *Illicium parviflorum* (Illiciaceae). Bars = 20 μm.

taxonomic character for identification of extant, and possibly fossil taxa, as these are restricted to certain species of Illiciaceae (*Illicium floridanum*, *I. lanceolatum*, and *I. parviflorum*). This is also true of oil cells exceeding 70 μm in diameter, which are restricted to *I. floridanum* and *I. parviflorum*—both of which are New World taxa. Densities and sizes of various structures vary considerably among the genera. In *Illicium* for example, density of abaxial oil cells varied from 1.03 cells/mm^2 in *I. dunnianum* to 16.7 cells/mm^2 in *I. floridanum*. For this reason, the evolution of these characters was not traced.

Homology—*Derivation from stomatal complexes*—The most notable unifying characteristic shared by the four specialized structures examined here is the similarity in the architecture (form and orientation) of their contact cells to that of certain types of stomatal complexes. The numerous examples of stephanocytic, actinocytic, and related types of architecture (Figs. 7, 10, 13, 17, 20–23, 35, 38, 40, 50, 52) and

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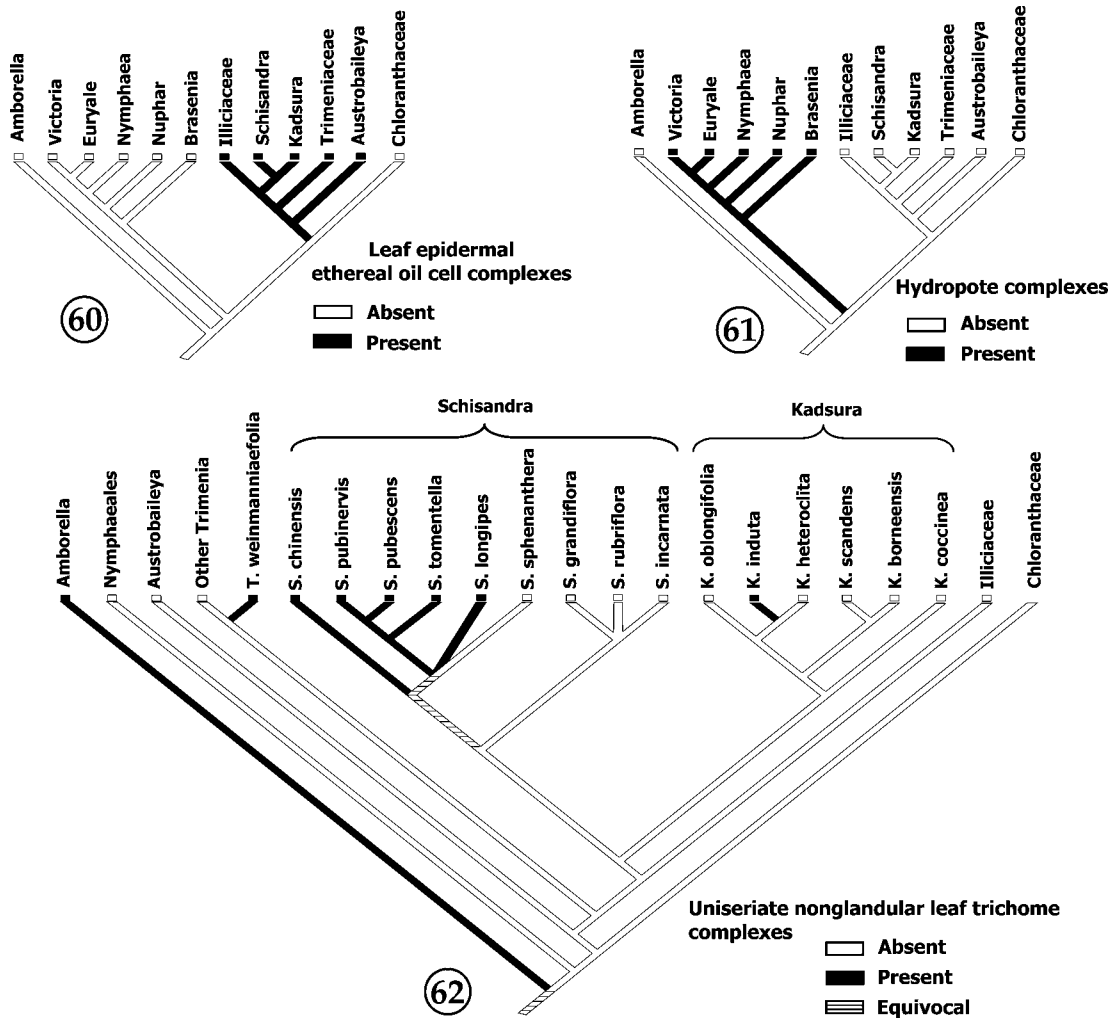
Leaf 1. **36**. Abaxial trichome complex of *Trimenia weinmaniaefolia* (Trimeniaceae) with "paracytic" (irregular) architecture very similar to that in the nearby stomata. **37.** Abaxial trichome complex of *T. weinmanniaefolia* with irregular architecture (1 strongly specialized subsidiary). **38.** Abaxial ethereal oil cell complex of *T. weinmanniaefolia* with actino-stephanocytic architecture. **39.** SEM of an abaxial trichome base cell with abscission scar adjacent to a stoma, *T. weinmanniaefolia*. **40.** Abaxial ethereal oil cell complex, *Kadsura heteroclita* (Schisandraceae). The close alignment of the subsidiaries with the adjacent cycle of cells suggests the former were derived from the latter by tangential division, as is common in stomatal complexes in Austrobaileyales (Carpenter, 2005). **41.** Adaxial ethereal oil cell with nucleus, *K. borneensis*. **42.** SEM of an abaxial ethereal oil cell complex, *K. borneensis*. Striations radiate from the oil cell outward over adjacent epidermal cells. **43.** SEM of a leaf transverse section with abaxial ethereal oil cell (*K. borneensis*). **44.** Abaxial stephanocytic ethereal oil cell complex with polygonal base and a polygonal/curved oil cell, *K. borneensis*. **45.** Abaxial anomocytic ethereal oil cell complex with polygonal base, *Schisandra rubriflora* (Schisandraceae). **46.** Abaxial ethereal oil cell complex with irregular architecture (4 strongly specialized subsidiaries), *S. sphenanthera*. Note the lack of striations. **47.** SEM of abaxial ethereal oil cell complex, *S. rubriflora*. **48.** Abaxial ethereal oil cell complex with "paracytic" architecture similar to that in the nearby stomata in *Illicium simonsii* (Illiciaceae). **49.** Abaxial staphanocytic ethereal oil cell complex in *I. floridanum* and an undulate base. **50.** Adaxial stephanocytic ethereal oil cell complex in *I. parviflorum* and large oil cell. **51.** Abaxial "paracytic"oil cell complex with architecture similar to that of the nearby stomata, *I. lanceolatum*. **52.** Abaxial ethereal oil cell complex with actinocytic architecture and oil cell with nucleus, *I. simonsii*. Bars = 20 μm.



Figs. 60–62. Reconstructions of the evolution of specialized leaf epidermal structures in basal angiosperms. The overall phylogeny is based on Doyle and Endress (2000). The phylogeny of Nymphaeales is based on Les et al. (1999). **60.** Leaf epidermal ethereal oil cell complexes. **61.** Hydropote complexes. **62.** Uniseriate nonglandular leaf trichome complexes. The phylogeny of Schisandraceae (*Schisandra* and *Kadsura*) is based on Saunders (1998, 2000). See Materials and Methods, *Analysis of character evolution*, for details of this analysis.

irregular architecture (Figs. 6, 10, 11, 18, 21, 34, 36, 37, 46, 48, 51), many of which bear a striking similarity to the stomatal architecture in certain ANITA-grade plants (see Carpenter, 2005), point to two important hypotheses. First, they suggest that the development of these specialized structures is at some point, linked to and coordinated with, the development of the surrounding epidermal cells. Accordingly, I propose that the term *complex* be added to the names of these specialized structures to refer to them plus their associated contact cells, as is the convention with stomata. Thus, as the term *stomatal complex* refers to the stoma (guard cell pair) plus its associated contact cells, so I propose, for example, that the term *ethereal oil cell complex* be used to refer to the oil cell plus its associated contact cells. Even without considering development, I believe this is justifiable on purely morphological grounds, because mature stomatal types are named without regard to their development (see Baranova, 1992b). A second hypothesis follows from this; specifically, each of these specialized structures, or a portion thereof, is homologous with the guard cell pair or its immediate meristematic precursor (i.e., the guard mother cell; see Pant, 1965), and complexes of all of these specialized structures are homologous with stomatal complexes. As such, the leaf epidermal ethereal oil cells in Austrobaileyales would not be homologous with the ethereal oil cells in the leaf mesophyll or elsewhere in these plants (e.g., ground tissues in roots and stems; see Bailey and Nast, 1948; Bailey and Swamy, 1949).

Previous studies have alluded to the stephanocytic patterns associated with specialized leaf epidermal structures that I noted earlier, although they were not explicitly named as such (The stephanocytic type was not recognized until Baranova, 1987.). Bailey and Nast (1948, p. 83), in describing Illiciaceae and Schisandraceae, called attention to the "radial pattern in the rosettes of cells that surround the secretory cells." Metcalfe (1987) also noted similar patterns in epidermal cells bordering oil cells of Trimeniaceae. Likewise, Baranova (1992a, p. 11) noted in Austrobaileyaceae and Illiciaceae, the presence of leaf epidermal ethereal oil cells surrounded by "unique rosettes, formed by radially arranged cells of the epidermis" (translated from Russian).

The other type of architecture I recognize as common to specialized leaf epidermal structures and stomata, i.e., irregular, has not to my knowledge been recognized previously in the literature. Most of the strongly anatomically specialized contact cells of these complexes appear to be the result of a tangential division (i.e., a division more or less parallel to the specialized structure, as opposed to a radial or perpendicular division; t and r, respectively, in Fig. 1) occurring in a contact cell. This is suggested by the notably thin tangential wall of the strongly specialized contact cell and the very close, if not exact alignment of the two cells (e.g., Figs. 6, 10, 11, 18, 21, 34, 40, 44, 46). This pattern is very common in stomatal complexes of Amborellaceae and Austrobaileyales and is seen occasionally in Nymphaeales as well (Carpenter, 2005).

Anomocytic architecture in specialized leaf epidermal complexes of these taxa is less common overall than stephanocytic and irregular (Tables 2 and 3). Because the anomocytic type has no subsidiary cells (and hence no recognizable order), then if all or the great majority of complexes were anomocytic, a major line of evidence would be lost in support of the two hypotheses I advanced previously. However, the presence of some anomocytic types is not unexpected, because anomocytic architecture is quite common in stomatal complexes of Nymphaeales (Carpenter, 2005) and, as explained later in this section, increased proportions of anomocytic complexes may be expected in the case of one hypothesis.

Two other lines of evidence also support the hypothesized homology of specialized leaf epidermal complexes and stomatal complexes. The first is the presence of structures intermediate in form between stomata and these other specialized structures, such as those of *Nymphaea flava*, in which spherical, oil cell-like structures are found attached to a base of two poorly formed guard cells (Fig. 19). Another intermediate form occurs in *Illicium*, in which oil cells assume a shape and size similar to a typical guard cell pair and are bounded by one subsidiary on either side (Figs. 48, 51), thus matching the appearance of the predominant paracytic stomatal type (also seen in Figs. 48, 51) in this family. (These are rare but occur in *Illicium henryi, I. simonsii,* and *I. lanceolatum*). This is also observed in trichome complexes in *Trimenia weinmanniaefolia* (Fig. 36). Another example of an intermediate form could be the adaxial hydropote complexes of *Victoria*, some of which have nuclei, as are found in some ethereal oil cells of Austrobaileyales. I also observed an abaxial hydropote complex in *Nuphar luteum* with a nucleus (apparently in the lens-shaped cell). Grüss (1926) illustrated abaxial hydropotes with nuclei in *Nuphar*, *Nymphaea*, and *Victoria*, and Lüttge and Krapf (1969) illustrated large nuclei in *Nymphaea,* mentioning that their presence may be related to the secretory functions of these cells (i.e., the lens-shaped cell). A second line of evidence, mentioned by Jalan (1965), is that oil cells and stomata develop simultaneously in the genus *Schisandra*.

Objections to the hypothesized homology between stomatal complexes and specialized leaf epidermal complexes may be raised on two different grounds that merit further discussion. First, Endress and Igersheim (2000) reported that, although they did not study development, they believed epidermal oil cells in carpels of Austrobaileyales developed in a manner similar to that described by Tucker (1976) for *Saururus cernuus* L. (Saururaceae). Tucker (1976) explained that oil cell initials first appear in the subprotodermal layer of this species. In the course of their development, they grow intrusively toward the leaf surface where they push protodermal cells aside, until finally their upper portion appears at the leaf surface. Judging from Tucker's (1976) photographs, the leaf epidermal oil cells in *S. cernuus* resemble those in Austrobaileyales and appear to be surrounded by similar rosettes of epidermal cells. If leaf epidermal oil cells in Austrobaileyales develop in a similar manner, some doubt may be cast on the hypothesis that the oil cell itself is homologous to the stoma or guard mother cell in this clade. This is due to the fact that all portions of stomatal complexes, including the stoma and surrounding subsidiary cells, are entirely epidermal, not subepidermal in origin. However, a few points must be considered when making inferences on the homology of epidermal oil cells in Austrobaileyales from development of those in *S. cernuus*. First, Saururaceae are not closely related to Austrobaileyales in any of a number of recent phylogenetic analyses or classifications of angiosperms (e.g., Qiu et al., 1999, 2000; Doyle and Endress, 2000; APG II, 2003; Hilu, et al., 2003), and their leaf epidermal oil cells probably represent a convergence (see previous section on character evolution). At the very least, the oil cells of *S. cernuus* differ from Austrobaileyales in that their reported maximum diameter of 20–26 μm is considerably smaller than any I measured for genera in Austrobaileyales (Table 2). Second, other studies indicate that leaf epidermal oil cells in Austrobaileyales do not share the *S. cernuus* mode of development. Bailey and Nast (1948) mentioned that oil cells in the lower epidermis of Illiciaceae and Schisandraceae originate in the epidermis and expand into subepidermal layers of leaf during development—the exact opposite of the *S. cernuus* type of development. Money et al. (1950) noted that oil cells sometimes originate in the leaf epidermis in genera (including *Austrobaileya* and *Trimenia*) that they believed were placed in or related to Monimiaceae. Third, even if leaf epidermal oil cells in Austrobaileyales do conform to the mode of development in *S. cernuus*, this would not necessarily rule out homology of the oil cell with the guard mother cell. It would, however, require the additional step of a guard mother cell becoming displaced downward by one cell layer. Even if no homology exists between the oil cell and the guard mother cell, the resemblance between the architecture of epidermal oil cell complexes and stomatal complexes seems great enough to suggest that these structures each represent manifestations of an underlying principle of organization inherent in the leaf epidermis of these plants and therefore may well be homologous at some level.

A second objection could also be voiced. Specifically, if specialized leaf epidermal complexes did arise through a modification of stomatal complex developmental mechanisms, then should not both types of complexes within any given taxon have similar architecture most, if not all of the time? Of the 45 pairwise comparisons between specialized epidermal complexes and stomatal complexes listed in Table 3, only 19 are in agreement (i.e., have the same type of architecture as their most common type: see Figs. 53–59 for most common stomatal types in ANITA families). Could this suggest that the similarities observed between the architecture of these two classes of complexes are merely a matter of coincidence? To counter this objection, I point out that the expectation that most or all of the pairwise comparisons should yield agreement is based upon the assumption that divergence from stomatal architecture is unlikely to occur. I contend that divergence may be expected because subsidiary/contact cells of

stomatal complexes, in playing an important role in regulation of guard cell turgor and hence in stomatal opening and closing, are likely to be under very different functional constraints and selection pressures than those of specialized leaf epidermal complexes. A shift toward less highly ordered types of architecture such as anomocytic and stephanocytic (which Baranova [1987] considered a modification of the anomocytic type) in specialized leaf epidermal complexes, especially in Austrobaileyales (Table 3), may have occurred as a result. Furthermore, analysis of character evolution (Figs. 60, 61) favors a single origin for hydropote complexes (which here also include mucilage hair complexes—discussed in the *Hydropote, mucilage hair, and trichome complexes* section) and ethereal oil cell complexes, specifically in the common ancestor of Nymphaeales and Austrobaileyales, respectively. Under this scenario, the origins of these structures predate the appearance of any of the extant taxa, thus allowing for a greater time in which divergence may have occurred.

Hydropote, mucilage hair, and trichome complexes—The hydropotes of Nymphaeaceae, the mucilage hairs of Cabombaceae, and the nonglandular, uniseriate hairs of Amborellaceae and Trimeniaceae could all be called trichomes, and have been treated differently by various authors. Goleniewska-Furmanowa (1970) discussed hydropotes of Nymphaeaceae and mucilage hairs of Cabombaceae as separate structures, while Wilkinson (1979) grouped these together as types of hydropotes. Eklund et al. (2004, p. 122), in reconstructing the phylogeny of Chloranthaceae, coded Nymphaeales as uncertain for their character "trichomes on stems, petioles or veins." I concur with Wilkinson (1979) that the mucilage hairs of Cabombaceae and the structures in Nymphaeales are similar enough to be grouped as hydropotes. Both of these have a three-celled base system (Figs. 14, 32) and deciduous hairlike portions that generally leave no abscission scar. The similarity in structure of the trichomes and their bases in Amborellaceae and Trimeniaceae (Figs. 6–8, 10, 11, 36, 37, 39), both of which comprise a nonglandular, uniseriate hair that abscises and leaves a distinct scar of a type not seen in Nymphaeales, argue in favor of considering these as the same structure for the study of character evolution. However, although I argue that hydropotes (including the mucilage hairs of Cabombaceae) and trichomes in ANITA taxa are homologous with stomata (i.e., evolutionarily derived from guard cell pairs or their meristematic precursor), I think they are distinct enough that they should be considered different structures and their evolution should be traced separately. In addition to the structural dissimilarities noted here, they also have different functions. Unlike the trichomes in the woody ANITA taxa, the hydropotes of Nymphaeales have secretory and absorptive functions (Lüttge and Krapf, 1969; Wilkinson, 1979) highly specialized to their aquatic habitats. Also, Kaul (1976) and Wilkinson (1979) both pointed out that hydropotes occur in many different, widely separated groups of aquatic angiosperms and in aquatic ferns as well. Kaul (1976) hypothesized that hydropotes had numerous different origins and are one example of the convergent morphologies of aquatic plants.

Paleobotany and evolution—Upchurch (1984) noted two types of secretory cells in Early Cretaceous (Aptian stage) angiosperm fossils. The "radiostriate" type, present in *Eucalyptophyllum* and Drewry's Bluff Leaf Type #1, is described as having an angular outline with radiating striations—a type he noted as similar to oil cells in Illiciaceae, Schisandraceae, Magnoliales, etc. A second type, also found in *Eucalyptophyllum*, and other fossils, is noted to have a smooth, thin outer cuticle lacking striations, similar to ones in extant Illiciaceae. The radiostriate type is comparable to ones in Austrobaileyales examined here, but a minority of taxa I examined have otherwise similar cells that lack striations (e.g., *Schisandra sphenanthera*, Fig. 46; Tables 1 and 2). The second, "smooth type," was found here only in Illiciaceae and is especially prominent in the two New World taxa examined: *I. floridanum* and *I. parviflorum*. These intergrade with the radiostriate types in terms of size, but many are considerably larger. Upchurch's (1984) photographs of both types in the later (Albian) *Sapindopsis* show a radiostriate type of about half the diameter of a smooth type. Such a size difference is common in the Illiciaceae examined here. A hair base from the Aptian Dispersed Cuticle Number 3 illustrated by Upchurch (1984) resembles the hair bases of *Amborella trichopoda*. A variety of other, putatively more derived hair bases and secretory cell complexes that Upchurch found in the later (Albian) fossils belonging to the *Sapindopsis*/Platanoid complex, were not observed in any of the ANITA taxa I examined.

As such, aside from the more specialized hair types observed in *Amborella* by Bailey and Swamy (1948) and Money, et al. (1950), the woody ANITA taxa appear to have a range of morphology of specialized leaf epidermal structures generally comparable to the Zone I (Aptian) fossils studied by Upchurch (1984). However, on the basis of these structures alone, it is probably not possible to rule out a relationship between Aptian fossils and other groups of putatively primitive angiosperms such as Magnoliales and Laurales. While the hair base of Dispersed Cuticle Number 3 is similar in appearance to those of *Amborella* examined here, uniseriate hairs with simple bases that might leave similar abscission scars are not unusual in "primitive" angiosperms and have been noted in a number of families in Magnoliales (e.g., Magnoliaceae, Annonaceae), Laurales (e.g., Lauraceae, Hernandiaceae) and basal eudicots (e.g., Ranunculaceae, Menispermaceae) (see Metcalfe and Chalk, 1950; Baranova, 1972; Metcalfe, 1987). "Radiostriate" secretory cells known from Aptian cuticles and also noted here in the majority of species of Austrobaileyales (i.e., as noted before, except in taxa such as *Schisandra sphenanthera* with otherwise similar complexes that lack striations) were also noted by Upchurch (1984) in some representatives of Magnoliales, Laurales, and Piperales. Likewise, the larger, smooth type of secretory cell I noted in some Illiciaceae was also noted by Upchurch (1984) in Aptian fossils and in extant "magnoliid" groups such as Calycanthaceae.

I have examined and illustrated specialized leaf epidermal structures in a large sample of ANITA taxa, many of which were never examined. The taxonomic distribution and density of these structures were recorded, new characters pertaining to their morphology were coded, and their evolution was examined in light of the ANITA hypothesis. Important conclusions of this work include (1) Hydropotes are a synapomorphy of Nymphaeales and leaf epidermal ethereal oil cells are a synapomorphy of Austrobaileyales, but uniseriate nonglandular trichomes appear to have arisen independently a number of times in the ANITA taxa. (2) Leaf epidermal ethereal oil cells in Austrobaileyales are not homologous with oil cells elsewhere in the plant (e.g., the leaf mesophyll), nor

are they homologous with leaf epidermal oil cells in magnoliid taxa. (3) Undulate ethereal oil cell bases and oil cell diameters exceeding 70 μm characterize certain Illiciaceae and may be taxonomically useful characters. (4) Adaxial hydropotes are a synapomorphy of the *Victoria* + *Euryale* clade. (5) Cuticular striations may represent a synapomorphy of Austrobaileyales + Other Angiosperms. (6) Hydropotes, trichomes, and ethereal oil cells in these taxa are characterized by adjacent epidermal cells with striking similarities in their form and arrangement to subsidiary cells of certain types of stomatal complexes common in ANITA taxa. Hence, it appears that these specialized structures form the center of complexes comparable to stomatal complexes, and I propose to refer to them as "ethereal oil cell complexes," "trichome complexes," etc. (7) Forms intermediate to oil cells and stomata, to trichomes and stomata, and to hydropotes and oil cells, are observed in some taxa. (8) Because of this, and the similarity in complex architecture, I hypothesize that all of these specialized leaf epidermal complexes are homologous with and evolutionarily derived from stomatal complexes, with a portion of the specialized structure itself (e.g., the oil cell and trichome foot cell) possibly homologous to the guard cell pair or guard mother cell.

Questions about the functional significance of the architecture of the various leaf epidermal structures, as compared to stomatal complexes in ANITA-grade and other plants provide interesting subjects for further investigation. They will require an integrated approach drawing on diverse specialties such as plant ecophysiology, molecular mechanisms of cellular differentiation, cell to cell signaling, as well as additional morphological, ultrastructural, and developmental data on leaf epidermal cells and cuticles.

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APPENDIX. Taxa examined in this study. A dash indicates missing information. Voucher specimens are deposited in the following herbaria: DAV = University of California, Davis; HKU = University of Hong Kong; IBSC = South China Institute of Botany; UC = University of California, Berkeley.

Taxon; *voucher specimen*, source, herbarium.

Amborella trichopoda Baill; *K.J. Carpenter 11*, University of California, Santa Cruz Arboretum, DAV.

Amborella trichopoda Baill; *K.J. Carpenter 27*, National Tropical Botanical Garden, Kalaeo, Hawaii, DAV.

Austrobaileya scandens C.T. White; *K.J. Carpenter 12*, University of California, Santa Cruz Arboretum, DAV.

Austrobaileya scandens C.T. White; *K.J. Carpenter 42*, University of California, Davis Botanical Conservatory, DAV.

Brasenia schreberi J.F. Gmel; *La Rea J. Dennis 2426*, near Corvallis, Oregon, DAV.

Euryale ferox Salisb.; *Xie & Li 17*, Guangdong, P.R. China, IBSC.

Illicium angustisepalum, A.C. Sm.; *Lin Qi 25*, —, HKU.

Illicium dunnianum Tutch.; *K.J. Carpenter 18*; Near Wu Kau Tang, New Territories, Hong Kong, DAV.

Illicium floridanum Ellis; *K.J. Carpenter 9*; University of California, Santa Cruz Arboretum, DAV.

Illicium henryi Diels; *Hao Gang 288*, Wuhan Botanical Garden, Hubei, P.R. China, DAV.

Illicium lanceolatum A.C. Sm.; *K.J. Carpenter 1*; University of California, Berkeley Botanic Garden, DAV.

Illicium parviflorum Michx. ex. Vent.; *K.J. Carpenter 10*, University of California, Santa Cruz Arboretum, DAV.

Illicium simonsii Maxim.; *K.J. Carpenter 3*, University of California, Berkeley, Botanic Garden, DAV.

Illicium verum Hook.; *K.J. Carpenter 24*, South China Botanic Garden, Guangzhou, P. R. China, DAV.

Kadsura borneensis A.C. Sm.; *K.J. Carpenter 32*, Royal Botanic Gardens, Kew, DAV.

Kadsura coccinea A.C. Sm.; *K.J. Carpenter 20*, Lamma Island, Hong Kong, DAV.

Kadsura heteroclita Craib; *P.X. Tan 62890*, —, HKU.

Kadsura oblongifolia Merrill.; *K.J. Carpenter 22*, South China Botanic Garden, Guangzhou, P.R. China, DAV.

Kadsura scandens Blume; —, —, Botanical Gardens, Bogor, HKU.

Nuphar advena Ait.; *K.J. Carpenter 35*, Royal Botanic Gardens, Kew, DAV.

Nuphar luteum (L.) Sm.; *K.J. Carpenter 25*, Texas Hill Country, south of Austin, Texas, DAV.

Nuphar polysepalum Engelm.; *K.J. Carpenter 33*, Royal Botanic Gardens, Kew, DAV.

Nymphaea caerulea Savigny.; *K.J. Carpenter 38*, Royal Botanic Gardens, Kew, DAV.

Nymphaea flava Leitn.; *K.J. Carpenter 40*, Royal Botanic Gardens, Kew, DAV.

Nymphaea nouchali Burm f.; *K.J. Carpenter 39*, Royal Botanic Gardens, Kew, DAV.

Schisandra chinensis Baill.; *K.J. Carpenter 4*, University of California, Berkeley Botanic Garden, DAV.

Schisandra grandiflora Hook. f. & Thomson; *K.J. Carpenter 29*, Royal Botanic Gardens, Kew, DAV.

Schisandra incarnata Stapf; 1980 *Sino American Expedition 382*; Hubei Province, P.R. China, UC.

Schisandra longipes (Merril & Chun) R. M. K. Saunders; —, HKU.

Schisandra rubriflora Rehder; *K.J. Carpenter 30*, Royal Botanic Gardens, Kew, DAV.

Schisandra sphenanthera Rehder & Wilson; *K.J. Carpenter 31*, Royal Botanic Gardens, Kew, DAV.

Trimenia weinmanniaefolia Seem. *George W. Gillett 2179*, Marquesas Islands, UC.

Victoria amazonica Sowerby; *Jim Henrich s.n.,* Conservatory of Flowers, San Francisco, California, DAV.

Victoria cruziana Orbign.; *Chrissie Prychid s.n.*, Royal Botanic Gardens, Kew, DAV.