Comparative Seed Morphology and Phylogenetics

Case Studies in Basal Angiosperms (ANITA) and Asterids (Lysimachia, Ericales).

IL-CHAN OH
Dissertation presented at Uppsala University to be publicly examined in Lindahlsalen, EBC, Norbyvägen 18A, Uppsala, Friday, May 15, 2009 at 10:00 for the degree of Doctor of Philosophy. The examination will be conducted in English.

Abstract

The first half of the thesis deals with the seed morphology of members of the ANITA grade at the base of the angiosperm phylogeny (Amborella, Nymphaeales, Illiciales, Trimeniaceae, and Austrobaileyaceae), especially Illiciaceae and Schisandraceae. Seed characters support a major division between the New World and Old World species of Illicium. Fossil seeds previously assigned to Illiciaceae were re-evaluated. In Schisandraceae, seed and leaf epidermal characters were added to a previously published morphological data matrix. Phylogenetic analysis using the extended data matrix shows that Kadsura and Schisandra appear to be supported as monophyletic sister taxa by a number of synapomorphies in reproductive and vegetative characters. Fossil seeds from the Eocene of North America show some similarities to the modern Schisandra glabra from North America, whereas fossils from Europe are more similar to modern Asian species.

In the second half of the thesis, seed morphology of Lysimachia and closely related taxa (Anagallis, Ardisiandra, Asterolimon, Glauk, Pelletiera, Trientalis) was investigated. The phylogenetic relationships among the endemic Hawaiian species of Lysimachia was also studied, using nuclear ribosomal DNA (ETS, ITS) and chloroplast DNA (rpl16, rpl20-rps12, rps16, trnH-psbA, trnS-G) sequence data. The seeds in Lysimachia and related taxa vary in, e.g., shape, seed coat structure and surface patterns. Seed surface patterns are mostly congruent with molecular phylogenetic relationships. A reticulate surface pattern is diagnostic for, e.g., the subgenus Palladia and the Hawaiian endemic subgenus Lysimachiods. Mapping seed characters onto a recent molecular-based phylogenetic tree, reveals that they provide potentially synapomorphic character states for various subclades of Lysimachia. The phylogenetic analysis based on the combined data set using nuclear ribosomal DNA and chloroplast DNA data provides new insights into the relationships within the Hawaiian subgenus Lysimachios. Here our results indicate that earlier taxonomic treatments of the group need to be partially revised.

Keywords: ANITA, basal angiosperms, cpDNA, ETS, fossil seeds, Hawaiian Lysimachia, Illiciaceae, ITS, Lysimachia, Myrsinaceae, nrDNA, phylogeny, rpl16, rpl20-rps12, rps16, Schisandraceae, seed morphology, trnH-psbA, trnS-G

Il-Chan Oh, Department of Systematic Biology, Evolutionary Biology Centre (EBC), Norbyvägen 18D, Uppsala University, SE-75236 Uppsala, Sweden and Department of Phanerogamic Botany, Swedish Museum of Natural History, P.O. Box 50007, SE-104 05 Stockholm, Sweden

© Il-Chan Oh 2009

ISSN 1651-6214
urn:nbn:se:uu:diva-100372 (http://urn.kb.se/resolve?urn=nbn:se:uu:diva-100372)
List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals:


Papers I–III have been reproduced with permission from the publisher.

In the papers included in this thesis II-Chan Oh has been responsible for the SEM studies of seeds, molecular lab work and the writing, with comments and suggestions given by the co-authors.
Cover photos: Seeds of ANITA members and *Lysimachia*

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Lysimachia mauritiana</em> (seed)</td>
<td><em>Lysimachia mauritiana</em> (surface)</td>
</tr>
<tr>
<td><em>Illicium floridanum</em> (seed)</td>
<td><em>Illicium floridanum</em> (hilar area)</td>
</tr>
<tr>
<td><em>Schisandra glabra</em> (seed)</td>
<td><em>Schisandra glabra</em> (hilar area)</td>
</tr>
</tbody>
</table>
Contents

Introduction .............................................................................................................................. 7
   Basal angiosperms ........................................................................................................... 9
   *Lysimachia* .................................................................................................................. 9
Aims ......................................................................................................................................... 11

Summary of papers .............................................................................................................. 12
   Paper I ............................................................................................................................ 12
   Paper II ........................................................................................................................... 14
   Evidence from the remaining ANITA members ......................................................... 16
   Discussion of papers I and II ....................................................................................... 22
   Paper III ......................................................................................................................... 24
   Paper IV ......................................................................................................................... 27

Sammanfattning (Swedish summary) .................................................................................. 29

Acknowledgements ............................................................................................................. 32

References ............................................................................................................................ 34
### Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>cpDNA</td>
<td>chloroplast DNA</td>
</tr>
<tr>
<td>ETS</td>
<td>external transcribed spacer</td>
</tr>
<tr>
<td>ITS</td>
<td>internal transcribed spacer</td>
</tr>
<tr>
<td>nrDNA</td>
<td>nuclear ribosomal DNA</td>
</tr>
<tr>
<td><em>rpl</em>16</td>
<td>ribosomal protein L16</td>
</tr>
<tr>
<td><em>rpl20-rps12</em></td>
<td>ribosomal protein L20-ribosomal protein S12</td>
</tr>
<tr>
<td><em>rps</em>16</td>
<td>ribosomal protein S16</td>
</tr>
<tr>
<td>SEM</td>
<td>scanning electron microscope</td>
</tr>
</tbody>
</table>
Introduction

The seed is one of the most remarkable innovations in the evolution of vascular plants. It is a significant unit for reproduction and dispersal providing protection to the embryo and supplying the growing embryo with nutrients. Seeds can tolerate harsh environments such as cold and dry conditions and survive during a dormant period in the soil for many years before germinating (Ingrouille 1992). Within the seed plants two major groups are recognized, gymnosperms with naked ovules and angiosperms with ovules enclosed in a carpellary tissue. In gymnosperms the embryo is nourished by the mother plant during its development and a nutritious tissue (secondary endosperm) surrounding the embryo is a further innovation in angiosperms that supports germination and early growth of the seedling and may be one of the factors that promoted the evolutionary success of the angiosperm. Today angiosperms are the most successful and highly diversified plant group comprising about 225,000-350,000 extant species (Kubitzki 1993).

The recognition of evolutionary signals using only seed characters is intricate because there are no congruent evolutionary trends indicating which type of seed characters are primitive or derived, and some morphological characters are hard to polarize without detailed ontogenetic investigation. However, seed morphology has previously been used as a source of information about the evolution of angiosperms (Bouman 1974, Corner 1976, Netolitzky 1926, Takhtajan 1988, Williams and Friedman 2002).

Ovules and seeds can be characterized in terms of their organization (e.g., orthotropous, anatropous, campylotropous), number of integuments (unitegmic, bitegmic), structure of seed wall (e.g., testal, exotestal, endotestal, tegmic) and a variety of morphological characters such as shape, size and surface pattern of seeds, hilum characters, micropyle, chalaza, raphe, and various subsidiary appendages (Fukuhara 1999, Werker 1997).

The ovule/seeds may be uncurved with the micropyle at one end and the attachment at the other (orthotropous, Fig. 1A). This type is rare in angiosperms and mainly found in one-seeded fruits, such as in Chloranthaceae, Piperaceae, Juglandaceae, Urticaceae, and Polygonaceae (Boesewinkel and Bouman 1984, Endress 1987, Endress 1994). Among the members of the ANITA grade (*Amborella*, Nymphaeales, Illiciales, Trimeniaceae, and Austrobaileyaceae) *Amborella* has almost orthotropous ovules (Endress 1994, Endress and Igersheim 1997, Endress and Igersheim 2000). From the orthotropous type various more complicated types can be derived, but this does
not necessarily mean that the orthotropous type is the ancestral one within the angiosperms (cf. Bocquet and Bersier 1960 versus Boesewinkel and Bouman 1984). The most common ovule type within the angiosperms is the anatropous type (about 80%; Boesewinkel and Bouman 1984), where the ovule is curved with the micropyle facing the placenta close to the funicle (Fig. 1B). This type is realized in the vast majority of angiosperms and occurs across the different orders of flowering plants. Among asterids anatropous ovules are the rule in, for example, Ericales, in families such as Myrsinaceae, Primulaceae, and Theophrastaceae (Johri et al. 1992). Among the ANITA members Nymphaeaceae (including Cabombaceae), Illiciaceae, Trimeniaceae, and Austrobaileyaceae have anatropous ovules and seeds. Schisandraceae also have anatropous ovules (Igersheim and Endress 1997), but seeds are campylotropous. In campylotropous ovules the ovule is also curved, but the funicle is attached midway between the chalaza and micropyle (Fig. 1C). This appears to be a rather derived seed type. It is found also in Papaveraceae, Capparaceae, Cactaceae, Fabaceae, Geraniaceae, and Malvaceae among others (Boesewinkel and Bouman 1984). In some cases, as in Schisandraceae, campylotropous seeds may develop from anatropous ovules.

Figure 1. Different ovule and seed types found in ANITA members. A. Orthotropous ovule. B. Anatropous ovule. C. Campylotropous ovule. D. Longitudinal section of Illicium ovule. E. Longitudinal section of Schisandra seed. A, B, C redrawn from Bocquet and Bersier (1960), D taken from Buxbaum (1961), E taken from Corner (1976).
Basal angiosperms

The recognition of a basalmost group of extant angiosperms has been one of the main issues in plant biology for more than a century (Arber and Parkin 1907, Cronquist 1981, Takhtajan 1997). Phylogenetically, basal angiosperms comprise more than 30 families including Amborellaceae, Austrobaileyaceae, Chloranthaceae, Illiciaceae, Lauraceae, Magnoliaceae, Nymphaeaceae, Piperaceae, Schisandraceae, Winteraceae and so on.

Several different groups have been suggested as sister to the rest of the angiosperms based on molecular phylogenetic analyses as well as morphological studies. Magnoliidae were regarded as a basal clade among angiosperms based on morphological analyses (Donoghue and Doyle 1989). Analysis of cpDNA rbcL sequences recognized the aquatic plant Ceratophyllum (Ceratophyllaceae) as the sister to all other extant angiosperms (Chase et al. 1993, Qiu et al. 1993). Nymphaeaceae, monocots, Piperales, and Aristolochiaceae were recognized as successive sister groups by combined analyses using both morphological and rRNA sequence data (Doyle 1994). Recent molecular and morphological phylogenetic studies suggested the so-called ANITA grade comprising Amborellaceae, Nymphaeaceae (including Cabombaceae), and Illiciaceae-Schisandraceae-Trimeniaceae-Austrobaileyaceae as the basalmost lineages within the angiosperms. The genus Amborella has been suggested as sister to all other extant angiosperms (Qiu et al. 1999, Qiu et al. 2000, Soltis et al. 2000, Soltis et al. 1999, Zanis et al. 2002, Zanis et al. 2003). While some of the ANITA members are monotypic or very species poor and restricted to small geographical areas at present (Amborella, New Caledonia; Austrobaileya, Queensland; Trimenia, Sulawesi, Australia, southwestern Pacific), others display a typical East Asian-North American disjunction and are moderately specious (Illiciaceae, Schisandraceae), and Nymphaeaceae are cosmopolitan consisting of about 60 to 70 herbaceous species.

Very recently the aquatic family Hydatellaceae, previously thought to be a monocotyledon and with the sole genus Trithuria comprising a dozen species in Australasia and India, has been shown to be a member of Nymphaeales (Friis and Crane 2007, Saarela et al. 2007, Sokoloff et al. 2008). The ovules in Hydatellaceae are anatropous, as in most other members of ANITA.

Lysimachia

Comprising more than 150 species, Lysimachia L. is a genus of perennial, biennial or annual herbs, or sometimes shrubs (Hawaiian spp.) with an almost worldwide distribution (Ståhl and Anderberg 2004), but with few species in Africa and its main center of diversity in south-western China, where about two-thirds of the species are found (Chen and Hu 1979, Hu and Kelso 1996).
Lysimachia has traditionally been regarded as a member of the family Primulaceae. However, based on phylogenetic analyses using molecular as well as morphological data Lysimachia, along with several other genera, is now often placed in Myrsinaceae (Anderberg and Ståhl 1995, Anderberg et al. 1998, 2002, Källersjö et al. 2000, Ståhl and Anderberg 2004). The monophyly of the genus and its infrageneric relationships have been studied by Hao et al (2004), Manns and Anderberg (2005), and Andeberg et al (2007).

One of the monophyletic groups within Lysimachia that is still not well understood from a phylogenetic point of view is subgenus Palladia, a group of species with white or sometimes pink or red flowers arranged in terminal racemes, and characteristic reticulate seeds. A close relationship between the species of subgenus Palladia and the endemic Hawaiian species of subgenus Lysimachiopsis has been suggested based on morphological and molecular data (Marr and Bohm 1997, Hao et al. 2004, Anderberg et al. 2007). However, it is still unclear whether they are sister groups or if one is part of the other (Anderberg et al. 2007).

Figure 2. Lysimachia clethroides, a member of L. subgen. Palladia. Photo by Arne Anderberg.
Aims

In papers I and II, I did comparative seed morphological studies in selected members of the ANITA grade. I wanted to know how character states are distributed within different members of ANITA, and whether or not seed characteristics in ANITA members are markedly ancestral. Using a (molecular) phylogenetic framework, I mapped morphological characters on phylogenetic trees in order to establish possible evolutionary or adaptive trends within the two closely related families Illiciaceae and Schisandraceae. In p. 16–21 in the thesis information is also provided on seed morphology of other members of ANITA. I used the literature and re-studied some fossils to see whether ANITA type seeds occur in the fossil record, mainly in the earliest records of angiosperms. Based on the results of my studies I discussed the usability of single morphological character complexes to infer plant phylogeny.

Paper III focuses on *Lysimachia* and closely related taxa. The aims are (1) to describe and document variation in seed morphology in a group of higher angiosperms, (2) to identify possible synapomorphies that are congruent with well-supported clades in the *Lysimachia* phylogeny, and (3) to reconstruct the evolution of seed characters.

Paper IV presents phylogenetic relationships among the endemic Hawaiian *Lysimachia* species using nrDNA (ETS, ITS) and cpDNA (*rpl*16, *rpl*20-*rps*12, *rps*16, *trnH-psbA, trnS-G) sequence data. The objectives of paper IV are (1) to elucidate phylogenetic relationships among the endemic Hawaiian *Lysimachia* species (subgenus *Lysimachiopsis*), (2) to compare and discuss our results with respect to previous classifications, biogeography, and morphology, particularly with respect to the *L. hillebrandii/remyui* complex, and (3) to provide a phylogenetic basis for an improved taxonomy of subgenus *Lysimachiopsis*.
Summary of papers

Paper I

In my first paper, I studied how seed morphological characters reflect relationships in Illiciaceae and how morphological characters are distributed when they are mapped on a molecular tree (Fig. 3). I also did a re-evaluation of the fossil record, i.e. seeds and fruits which previously have been ascribed to Illiciaceae, and established their affinities to modern species/species groups of *Illicium*.

Illiciaceae consists of only one genus, *Illicium*, with about 35 species, which are distributed in East Asia with about 30 species and southeastern North America, Mexico, and the West Indies with about 5 species (Keng 1993, Lin 1997, Smith 1947). Traditionally, Illiciaceae were included in Illiciales together with Schisandraceae and they have even been classified in Magnoliaceae (Rehder 1940). According to the updated APG classification, Illiciaceae is a member of Austrobaileyales (sensu APG II: Angiosperm Phylogeny Group, 2003) together with Austrobaileyaceae, Schisandraceae, and Trimeniaceae (APG 2003).

Investigation of morphological characters in basal angiosperms is particularly interesting and can provide useful data for a better understanding of phylogeny and character evolution in flowering plants, because they can be optimized on phylogenetic trees obtained from molecular studies. Recently, a nuclear rDNA ITS analysis of 15 species of *Illicium* suggested a major split between modern members of *Illicium* into an East Asian and North American clade (Fig. 1 in Hao et al. 2000).

Investigation of seed morphological characters in Illiciaceae in a molecular phylogenetic framework showed some significant characters in the structure of the hilar area. Most conspicuous is the hilar rim only found in North American species of *Illicium*, which would support a major division between East Asian and North American species.

Mapping morphological characters on the molecular tree showed that three synapomorphies from seed morphological characters support the North American clade but several other features are homoplastic.

In addition, a number of fossil seeds previously described as Illiciaceae were re-evaluated. *Illicium germanicum*, formerly described as very closely related to modern *Illicium*, differs from *Illicium* in many characters and should be excluded from this genus. Moreover, we discussed some seed
characters of modern and fossil species of Illiciaceae, morphological relationship within the ANITA taxa, and the modern disjunction of *Illicium* in a biogeographical context.

Figure 3. Morphological characters optimized on the molecular ITS tree of Hao et al. (2000): 1, 2a, 2b, and 3 correspond to clades referred to in the text. Black circles indicate apomorphies with a consistency index 1.0 and white circles characters with homoplasy (consistency index below 1.0). Bootstrap values from 100 replicates are indicated as bold numbers above branches.
In the second paper I studied seed morphology of around 20 species of Schisandraceae. At the same time leaf epidermal characters were studied mainly by the first author. The aim of this paper was to see how adding seed and leaf epidermal characters to a recently published data matrix of morphological characters would effect the phylogeny, resolution, and support of the phylogenetic trees obtained from the first analysis. Mainly, I was interested in whether or not additional morphological data would corroborate monophyly of the two currently recognized genera *Kadsura* and *Schisandra* within the Schisandraceae. Basically, the extremely elongated receptacle with widely spaced carpels is characteristic of *Schisandra*, whereas the receptacle in *Kadsura* is short with densely spaced carpels. Furthermore, the carpel in *Schisandra* is conspicuously constricted at the point of its attachment to the axis, whereas a pseudostigma occurs only in *Kadsura*. These features suggest the two genera to be monophyletic. Seed morphological data added to Hao et al.’s (2001) matrix did not provide further evidence (i.e. stronger support) for monophyly of the two genera. Instead, a number of characters appear to support the relationship of pairs of species, e.g. *K. coccinea* and *K. scandens*, both of which have conspicuously large seeds along with a multilayered mesotesta. Among leaf epidermal characteristics, species of *Kadsura* were found to be consistently amphistomatic, whereas species of *Schisandra* have stomata only on the abaxial leaf surface. In conclusion, *Kadsura* and *Schisandra* appear to be supported as monophyletic sister taxa by a number of synapomorphies in reproductive and vegetative organs (Fig. 4).

Fossils ascribed to Schisandraceae are rare in the Cretaceous. While leaf remains are difficult to ascribe to Schisandraceae because of the lack of synapomorphies for the family, pollen appear to prove the presence of the family in the Late Cretaceous. In the Early Cainozoic, leaf and seed remains from North America and Europe unambiguously belong to the family. Seeds from the Eocene of North America show some similarities to the modern *Schisandra glabra* from North America. By contrast, fossils from Europe show more similarities to modern Asian species.
Figure 4. One of 34 most parsimonious trees recovered from a branch-and-bound search. Morphological characters are mapped on the tree using the resolving option ACCTRAN. Black circles indicate apomorphies with a consistency index 1.0 and white circles characters with homoplasy (consistency index below 1.0). Autapomorphies are indicated following the species name. Numbers above circles refer to the character numbers, below circles to character states. Bootstrap support (%) from 1000 replicates is indicated above branches. Tree length is 107, CI = 0.664, HI = 0.561, RI = 0.700, RC = 0.464. Branches marked with a star collapse in the strict consensus tree. A = K. subgen. Cosbaea, B = K. subgen. Sarcocarpum, C = K. subgen. Kadsura, D = S. subgen. Pleiostema, E = S. subgen. Schisandra sect. Maximowiczia, F = S. subgen. Schisandra sect. Schisandra sect. Schisandra sect. Sphaerostema, H = S. subgen. Sinoschisandra (classification follows Saunders 1998, 2000)
Evidence from the remaining ANITA members

1. Amborellaceae (Pl. 1, Figs. 1-4)
Specimens examined: *Amborella trichopoda* Baill.; J. T. Johansson 62 (S), H. S. MacKee 5181 (K)
The gynoecia of Amborellaceae are apocarpous composed of 5-8 free, spirally arranged carpels (Endress 2001, Endress and Igersheim 2000). Each carpel contains a single, almost orthotropous (Pl. 1, Fig. 2), bitegmic and crassinucellate ovule (Endress 2001). The micropyle is formed by the lobed, annular inner integument (Endress and Igersheim 1997). The outer integument is annular and sometimes lobed (Endress and Igersheim 1997). The fruitlets are drupes, 6-9 mm long (including 0.5-1 mm stalk) and 3.5-4 mm broad. The epicarp of the drupe has a more or less smooth surface (Endress and Igersheim 2000) and the endocarp is sclerotic with a reticulate-rugose surface (Pl. 1, Fig. 1).

Seeds one per carpel, ovoid in shape with a beak-like remnant of the funicle (Pl. 1, Fig. 3), close to dark brown in colour. Dimensions of seed: length 3.5-3.6 mm; width (dorsiventrally) 1.4-1.6 mm; thickness (lateral width) 1.0-1.2 mm. Outer surface of testa is more or less rugose under LM and reticulate near micropylar area under SEM (Pl. 1, Fig. 4). The outer surface in the middle part of seed and funicular area is composed of many cells, which have space between cells. Testa is about 10 μm thick and membranous. It is 3-4 cell layers thick in the ovule (Endress and Igersheim 1997). Tegmen is two or three cell layers in the ovules (Endress and Igersheim 1997). In mature seeds the tegmen has two cell layers. Micropylar region is about 70 μm in diameter (Pl. 1, Fig. 4).

2-1. Nymphaeaceae (Pl. 1, Figs. 5-8)
Specimens examined: *Nuphar advena* (Aiton) W.T. Aiton; BGZ 537, BGZ 19963491
The gynoecia of Nymphaeaceae are partially fused to syncarpous ovaries composed of 1-47 carpels arranged in whorls (Endress 2001). The carpels contain three to 400 or more, anatropous (except for *Barclaya*, which has orthotropous (Schneider 1978)), bitegmic and crassinucellate ovules (Igersheim and Endress 1998). Most taxa have an aril except for *Nuphar* (“arilloid” in seed) and *Barclaya* (Hart and Cox 1995, Schneider and Williamson 1993). The micropyyle is formed by the annular, unlobed inner integument in *Barclaya, Nuphar, Nymphaea* or by both integuments in *Euryale, Victoria,* and *Barclaya* (Igersheim and Endress 1998, Winter 1993). The outer integument is unlobed, semiannular or sometimes annular (*Nuphar, Nymphaea*), or always annular (*Barclaya, Euryale, Victoria*) (Igersheim and Endress 1998). The fruits are fleshy berries, 3.5-4.2 cm long and 2.5-2.9 mm broad in examined species (*Nuphar advena*).
The following seed description is based on one species (*Nuphar advena*) from Nymphaeaceae.

Seeds are ovoid in shape, close to yellow brown in colour. Dimensions of seed: length 5.3-7.8 mm; width 3.8-4.7 mm; thickness 3.6-4.2 mm. Exotestal cells in surface view sinous, 52-81 μm x 18-31 μm (Pl. 1, Fig. 7). Outer surface of testa is smooth. Seeds are exotestal with seed coat formed mainly by the testa. Testa is about 200 μm thick, composed of one palisade outer layer and an inner sclerotic cell layer. The outer palisade cell layer is 85-90 μm high, with simple, minute pores in the anticlinal cell walls. The inner sclerotic cell layer is 105-115 μm thick, 3-5 cells deep consisting of more or less transversely elongated cells with lumens (Pl. 1, Fig. 8). The inner integument in the ovules consists of two cell layers (Igersheim and Endress 1998). In mature seeds the tegmen is crushed and the number of cell layers cannot be established. The circular cap, present at the apex, has micropyle in the center. Hilum scar extends between micropyle and end of raphe ridge (Pl. 1, Figs. 5-6).

Seeds of other Nymphaeaceae have the same principal organization and structure, but there is also a considerable diversity expressed, e.g., in the shape of the exotestal cells and the position of hilum and micropyle in relation to the germination cap (e.g., Collinson 1980). Exotestal cells in surface view of other taxa (e.g., *Nymphaea, Ondinea, Victoria*) in Nymphaeaceae have strongly undulate cells (Collinson 1980). Caps showing position of micropyle and hilum in different genera have morphological differences in Nymphaeaceae as well as testa structure and seed size (See Figs.1-5 in Collinson 1980).
Plate 1. Figs. 1-4. Fruits and seeds of Amborellaceae. Fig. 1. Fruit, pedicel at left. Fig. 2. Longitudinal section of fruit with intact seed inside. Fig. 3. Seed in lateral view. Arrow heads indicate micropylar area. Fig. 4. Micropylar area of seed. Figs. 5-8. Seeds of Nuphar advena (Nymphaeaceae). Fig. 5. Apical view of seed. Arrow heads indicate raphe ridge. Fig. 6. Circular cap showing micropylar area in center and hilum scar between micropylar area and end of raphe ridge. Fig. 7. Outer surface of testa showing sinous cells. Fig. 8. Longitudinal section of seed wall. Scale bars: Figs. 1-3, 5: 1 mm, Figs. 4, 8: 100 μm, Fig. 6: 300 μm, Fig. 7: 50 μm.
2-2. Cabombaceae
(The family is included in Nymphaeaceae according to APG 2003)
The gynoecia of Cabombaceae are composed of (1-) 2-18 (Cabomba (1-) 2-3 (-7) whorled and Brasenia 4-22 probably arranged in whorls) free carpels (Ito 1987, Raciborski 1894, Williamson and Schneider 1993). The carpels contain (1-) 3 (-5) ovules in Cabomba, 1 or 2 in Brasenia. The ovules are anatropous, bitegmic and crassinucellate (Igersheim and Endress 1998). An aril is not present. The micropyle is formed by the annular, unlobed inner integument. The outer integument is semiannular and unlobed (Igersheim and Endress 1998). The fruit is non-fleshy and aggregate; indehiscent or dehiscent with 2-3 seeds per follicle-like fruit in Cabomba and 1-2 seeds per achene-like fruit in Brasenia (Williamson and Schneider 1993).

The operculate seeds are endospermic, ovoid in shape, perisperm present with two cotyledons (Watson and Dallwitz 1992, Williamson and Schneider 1993). Collinson (1980) provided a useful survey of the seeds in Nymphaeaceae and Cabombaceae and the following description is mainly based on this work. Seeds are exotestal. Exotesta cells show distinct undulate anticlinal walls in surface view. In Brasenia the exotesta is developed as a distinct palisade layer, very similar to that of Illicium and some Schisandraceae. In Cabomba exotestal cells are generally lower and with tubercular protrusions. Inner layers of testa one (Cabomba) to three (Brasenia) cell layers deep of sclerotic cells. Tegmen is membranous and crushed. Both Cabomba and Brasenia has a distinct germination cap with micropyle and hilum placed more or less in the center.

3. Trimeniaceae (Pl. 2, Figs. 1-4)
Specimens examined: Trimenia weinmanniiifolia Seem.; A. C. Smith 8355 (S)
The gynoecia of Trimeniaceae are composed of one (rarely two) carpel(s) (Endress and Igersheim 1997, Philipson 1993). The carpels contain a single, anatropous, bitegmic and crassinucellate ovule (Endress 2001). The micropyle is formed by the lobed outer and inner integuments (Endress and Igersheim 1997). The fruit is berry, spherical in shape (Pl. 2, Fig. 1), 4.7-6 mm long and 2.7-3 mm broad. The pedicel is 1-2.2 mm long. The epicarp of the berry has a more or less smooth surface.

Seeds one per carpel, more or less ovoid in shape with micropylar area protruding (Pl. 2, Fig. 2). Dimensions of seed: length 3.0-3.5 mm; width 2.1-2.3 mm; thickness 1.9-2.1 mm. Outer surface of testa is reticulate with finely undulate cell walls (Pl. 2, Figs. 3-4). Testa is about 160-180 µm thick. Tegmen consists of three cell layers in the ovule (Endress and Igersheim 1997). In mature seeds the tegmen is crushed and the number of cell layers cannot be established.
4. Austrobaileyacea (Pl. 2, Figs. 5-8)
Specimens examined: *Austrobaileya scandens* C. T. White; B. Gray 1123 (L)
The gynoecia of Austrobaileyacea are apocarpous composed of 10-13 free, spirally arranged carpels (Endress 1993, Igersheim and Endress 1997). Each carpel contains (4-) 8-14 ovules arranged in two longitudinal series along the sutures (Bailey and Swamy 1949, Endress 1980, Takhtajan 1988). The ovules are anatropous, bitegmic, and crassinucellate. The micropyle is formed by the slightly lobed and annular inner integument. The outer integument is unlobed (Igersheim and Endress 1997). The fruits are ellipsoidal or globose fleshy berries, 4.2-7 cm long and 3.1-4 cm broad with a 1.3-2 cm long stalk (pedicel) (Pl. 2, Fig. 5). The epicarp of the berry is orange coloured and the fleshy endocarp is yellow (Endress 1980, Endress 1993).

Seeds three per fruit (Pl. 2, Fig. 6), broadly ovate, lenticular, and chestnut-like in shape (Pl. 2, Fig. 7), close to orange brown in colour. The seeds are ovate in transverse section. Dimensions of seed: length 2.3-2.5 cm; width 1.6-2.3 cm; thickness 1.3-2.0 cm. The outer surface of seed is ruminated. Seeds are sarcotestal with an outer soft layer and an inner sclerotic layer. Testa is about 850-1150 μm thick. The outer layer is composed of a thin cell layer, 20-40 μm high. The inner sclerotic cell layer is 810-1110 μm thick, 9-12 cells deep, with simple, minute pores in the anticlinal cell walls. The inner integument in the ovules consists of two (or three) cell layers (Igersheim and Endress 1997). In mature seeds the tegmen is crushed and the number of cell layers cannot be established. In lateral view the hilar area is obtuse and the chalazal area is rounded (Pl. 2, Fig. 7). A distinct raphe ridge extends from the hilar area to the chalaza and antiraphe ridge is also present.
Plate 2. Figs. 1-4. Fruits and seeds of *Trimenia weinmannii* (Trimeniaceae). Fig. 1. Fruit, pedicel at right. Fig. 2. Seed in lateral view, micropylar area at right. Fig. 3. Micropylar area of seed in lateral view. Fig. 4. Surface of seed showing reticulation with finely undulate cell walls. Figs. 5-8. Fruits and seeds of Austrobaileyaceae. Fig. 5. Fruit with seeds inside. Fig. 6. Three seeds in apical view. Arrow heads indicate hilar area. Fig. 7. Seed, hilar area at right. Fig. 8. Detail of hilar area. Arrow head indicates hilar area. Scale bars: Figs. 1-3: 1 mm, Fig. 4: 100 μm, Figs. 5-7: 1 cm, Fig. 8: 1.2 mm.
Discussion of papers I and II

*Fossil record*

There is no reliable fossil record of *Amborella*. Vesselless wood fossils described from India (Jurassic ?), Western North America (Cainozoic), and Greenland (Eocene) (Bailey and Swamy 1948a) are all problematic and have not been convincingly documented. Anatomical features of the secondary xylem (lacking vessels) obviously are indistinguishable from extant *Trochodendron* and *Tetracentron* (Bailey and Swamy 1948a). There is no fossil record of Austrobaileyaceae and Trimeniaceae. In contrast, Illiciaceae, Schisandraceae, and Nymphaeaceae have a fossil record that extends back to the Cretaceous (Jähnichen 1976; leaves, Manchester 1994, Frumin and Friis 1999, Friis et al. 2001; reproductive structures). While seeds from the Clarno Formation of the Eocene of Oregon are very similar to the modern North American *Schisandra glabra* in size, shape, seed surface, and hilar area, Late Cretaceous seeds from Central Asia with affinities to Illiciaceae differ from modern species by their extremely small size.

*Phylogenetic considerations*

Both seeds of Illiciaceae and Schisandraceae display characteristics that are useful for intrageneric classification (see the hilar rim in *Illicium*, paper 1, and the seed surface and number of cells forming the mesotesta in Schisandraceae, paper 2). The overall appearance of seeds belonging to these two families, however, does not provide any deeper insight into the phylogeny of basal angiosperms in general, i.e., there is no clear phylogenetic signal for a number of key-characters, such as the campylotropous versus anatropous seed organization in Schisandraceae and Illiciaceae, respectively. Another feature, the size of seeds in modern Illiciaceae and Schisandraceae does not correspond to the size classes of seeds reported for the Cretaceous (Tiffney 1984, Frumin and Friis 1999; Eriksson et al. 2000). The predominance of small seeds in the early fossil record of flowering plants also contradicts previous ideas about primitive characters in seeds, e.g. Goldberg (1986), who suggested medium-sized seeds to be primitive within angiosperms.

The nature of the ancestral seed type within angiosperms has been a matter of strong debate (Bocquet and Bersier 1960, Boesewinkel and Bouman 1984, Eames 1961). The presence of both anatropous and orthotropous seeds in early fossils of the angiosperms (Friis et al. 1997) and in extant ANITA members (see above), together with the lack of a solid phylogenetic framework for seed plants, make it impossible for the present to say whether one or the other type should be ancestral within angiosperms.

More interesting is the fact that basal angiosperm seeds are diverse in structure and organization. This is not surprising given the long history of
the ANITA grade and the enormous diversity in habit and reproductive biology exhibited by its various members.

**Biogeography**

Many extant basal angiosperms show disjunct distributions in both Asia and eastern North America, e.g., Illiciaceae and Schisandraceae. The fossil record supports the antiquity of ANITA lineages with unambiguous Early and mid-Cretaceous finds of Nymphaeales (Friis et al. 2001) and Illiciaceae (Frumin and Friis 1999). The fossil record of Illiciaceae and Schisandraceae further demonstrates that this ANITA family also had a more extensive geographical distribution in earlier geographical periods. Fruit, leaf and seed fossils of different periods during the Late Cretaceous and Early Cainozoic have been described from Europe, northwestern Asia, and North America (Frumin and Friis 1999, Mai 1970, Tiffney and Barghoorn 1979).
In this paper we investigated seed morphology in the genus *Lysimachia* and in six additional genera (*Anagallis*, *Ardisiandra*, *Asterolinon*, *Glaux*, *Pelletiera*, *Trientalis*), which are considered to be closely related to, or are placed within *Lysimachia* in previous molecular studies (Hao et al. 2004, Manns and Anderberg 2005, Anderberg et al. 2007).

The results in paper III show that three major types of seed shape can be recognized: 1) sectoroid, 2) polyhedral, and 3) coarsely rugose with concave hilar area. Most species correspond to the first type and have an obliquely sectoroid seed shape. The seeds are dorsiventrally and/or laterally flattened. Taxa with this type of seeds include the genus *Anagallis*, *Glaux maritima*, *Asterolinon adoeense* as well as most *Lysimachia* species. In addition, in sectoroid and polyhedral seeds the edges may be keeled or winged. The third seed shape type (coarsely rugose with concave hilar area) is only present in *Asterolinon linum-stellatum* and in the two species of genus *Pelletiera*.

In their seed coat structure, most species are characterized by a thin and continuous outer seed coat layer that is closely adhering to the inner seed coat. In a few species (*Trientalis europaea*, *Lysimachia vulgaris*, *L. thyrsiflora*, and *L. terrestris*), however, the outer layer of the seed coat is sponge-like.

Seed surface patterns can be divided into six main types: 1) reticulate, 2) tuberculate, 3) vesiculose, 4) colliculate, 5) undulate, and 6) poroid-alveolate. Seed surface patterns are mostly congruent with molecular phylogenetic relationships. A reticulate surface pattern is diagnostic of, e.g., *Lysimachia* subgenera *Palladia* and Hawaiian *Lysimachiopsis*.

Mapping seed characters onto a recent phylogenetic tree from Anderberg et al. (2007) (Fig. 5), reveals that they provide potentially synapomorphic character states for various subclades of *Lysimachia* (Fig. 6). Rugose seed shape and vesiculose seed coat surface are synapomorphic for the clade comprising the genus *Pelletiera* and *Asterolinon linum-stellatum* (clade III). A clade with *Lysimachia vulgaris*, *L. thyrsiflora*, and *L. terrestris* is characterized by a poroid-alveolate seed coat surface and a sponge-like outer seed coat layer (clade IV). Both of these character states have apparently evolved in parallel in *Trientalis europaea* (clade I) and along the branch leading to the clade with the three *Lysimachia* species. We also discuss possible habitat factors that may have favoured the independent evolution of particular seed types, such as winged seeds in various lineages.
Our conclusion is that seed morphology in *Lysimachia* and related taxa clearly is of systematic interest. In particular, seed shape and the structure of the seed coat provide potentially synapomorphic character states for various subclades of *Lysimachia*.

![Phylogenetic tree](image)

*Figure 5.* Simplified phylogenetic tree redrawn from the phylogenetic analysis of *ndhF* sequences by Anderberg et al. (2007). Note that this tree only includes species studied in the present paper, all others were pruned from the original tree. A clade (subg. *Lysimachia A*) including *T. europaea* is sister to all remaining *Lysimachia* species. Jackknife support values are given above branches.
Figure 6. Seed morphological characters mapped onto the simplified (only species included in the present study) phylogenetic tree from Anderberg et al.’s ndhF sequences (2007). Dimorphic character states are indicated with a shaded square (●). Major clades referred to in the text are numbered I–VI. A Seed shape. B Seed coat surface. C Sponge-like outer layer. D Wing
Paper IV

Paper IV focuses on the relationships of the endemic Hawaiian *Lysimachia* species constituting *L*. subgenus *Lysimachiopsis*. There are 16 endemic species of *Lysimachia* in Hawaii, which show considerable morphological variation, especially in leaf and perianth structure (Marr and Bohm 1997). However, previous taxonomic treatments indicate that the delimitation of taxa is problematic, especially for the four subspecies of *L. remyi* (ssp. *remyi*, *caliginis*, *kipahuluensis*, *subherbacea*) and the species of the *L. hillebrandii* complex (i.e. *L. hillebrandii*, *L. waianaeensis*, *L. ovoidea*), which were recognized as distinct by Marr and Bohm (1997).

We used nrDNA (ETS and ITS) and cpDNA (*rpl16*, *rpl20-rps12*, *rps16*, *trnH-psbA*, *trnS-G*) sequence data in order to reconstruct phylogenetic relationships. In general, nrDNA data sets contain more informative data and provide better resolution in phylogenetic trees than data sets from chloroplast markers. A phylogenetic analysis based on the combined data set using all nrDNA and cpDNA sequences provides new insights into the relationships within subgenus *Lysimachiopsis*, especially within the problematic *Lysimachia hillebrandii*/*L. remyi* complex (Fig. 7). The result from the molecular analysis provides support for the following clades: (1) *L. hillebrandii* and *L. waianaeensis* (clade D, 55% jackknife support: JS), (2) *L. remyi* ssp. *remyi*, *L. maxima*, and *L. remyi* ssp. *subherbacea* (clade E, 86% JS), (3) *L. remyi* ssp. *caliginis* and *L. remyi* ssp. *kipahuluensis* (clade G, 93% JS), and (4) *L. glutinosa*, *L. scopulensis*, and *L. kalalauensis* (clade H, 81% JS). The phylogenetic relationships among these taxa are largely congruent with the biogeographical distribution of the species in the Hawaiian Islands. Our results also indicate that earlier taxonomic treatments of the group need to be partially revised in order to reflect phylogenetic relationships.
Figure 7. Jackknife tree obtained from Xac analysis of combined nrDNA (ETS, ITS) and cpDNA (rpl16, rpl20-rps12, rps16, trnH-psbA, trnS-G) data sets. Jackknife support values (≥ 50%) are indicated above the nodes. Clades referred to in the text are designated by the letters A-H.
Sammanfattning (Swedish summary)

Fröväxter och frön

Fröväxterna är den största och mest differentierade gruppen bland växterna. De kännetecknas av att ha just frön, och uppkomsten av fröet innebar uppenbarligen en revolution i växternas utvecklingshistoria. Frön är reproduktions- och spridningsorgan, som också ger skydd åt och förser det växande embryot med näring. Frön kan tåla extrema förhållanden, både värme och kyla, och kan ligga många år i vila i jorden innan de gror.

Den största gruppen inom fröväxterna är blomväxterna, som funnits på jorden åtminstone sen tidig krita. De kännetecknas bland annat av att fröämnen, de unga frön, är inneslutna i ett fruktämne som utvecklas till en frukt. En ytterligare specialisering hos blomväxterna, jämfört med övriga fröväxter, är att frönas näringsvävnad, endospermet, endast bildas i fröämnen som blivit befruktade. Detta är en energibesparande mekanism och kanske en av faktorerna bakom blomväxternas framgång.

Frön är ett genomgående tema i den här avhandlingen, där frömorfologi studeras, i ett fylogenetiskt perspektiv, i två olika delar av blomväxtsystemet. Artikel I och II fokuserar på de basala grupper av angiospermer som brukar sammanfattas under beteckningen ANITA, medan artiklarna III och IV behandlar frömorfologi och fylogeni hos en grupp asterider, nämligen Lysimachia och närstående taxa i familjen Myrsinaceae.

ANITA

Det har länge spekulerats om vilken av de nulevande grupperna av blomväxter som sitter vid eller nära basen av blomväxternas släkträd. Genom fylogenetiska analyser baserade på molekylära data börjar nu bilden klara och några återkommande basala grupper i dessa analyser är Amborella, ett släkte med en enda art som utgör familjen Amborellaceae, Nymphaeaceae, näckrosväxterna, som bildar en grupp tillsammans med Cabombaceae, Illiciaceae, stjärnanisväxterna, som bildar en grupp tillsammans med Schisandraceae, Trimeniaceae och Austrobaileyaceae. De här grupperna av basala blomväxter brukar ofta betecknas ANITA efter de initialer som markerats ovan. ANITA är ingen naturlig (monofyletisk) grupp, utan består av en samling separata utvecklingslinjer nära blomväxternas rot.
Nyligen har ytterligare en grupp visat sig höra till ANITA, nämligen den lilla familjen Hydatellaceae, som tidigare trots vara monokotyledoner. Hydatellaceae består av små akvatiska gräslika örter som, mycket oväntat, tycks ha en fylogenetisk position nära näckrosväxterna.

Frömorfologi hos ANITA

Bland de fossil av växter som upptäckts och upptäcks finns många välbevarade frön, som kan ha mer eller mindre stora likheter med frön från nulevande grupper. Artiklarna I och II, liksom avsnitten på sid. 17–22 i avhandlingen, behandlar frömorfologi hos olika grupper inom ANITA. Även blad är ofta bevarade som fossil och i artikel I och II studeras, förutom frön, även epidermis från blad hos nulevande grupper inom ANITA.


I artikel II studeras frömorfologi och bladepidermis hos de två närmaststående släktena *Kadsura* (i Asien) och *Schisandra* (i Asien och Amerika) i familjen Schisandraceae. Här adderades våra data till en redan existerande datamatriks med morfologiska data. En tidigare fylogenetisk analys baserad på dessa data stödde den rådande uppfattningen att *Kadsura* och *Schisandra* är monofyletiska grupper, men våra nya data gav inget ökat stöd för detta. I stället kunde vi påvisa ett parvis släktsskap mellan vissa arter inom dessa grupper. Fossila frön av *Schisandra* från Nordamerika visar likheter med en nulevande nordamerikansk art, medan fossila frön från Europa överensstämmer bäst med nulevande arter i Asien.

Frömorfologi hos *Lysimachia*


delvis, också inkluderas. Det är därför viktigt att samtliga dessa släkten är med i den frömorfollogiska studien.

Våra resultat visar att frömorfollogiska karaktärer i flera fall stöder olika undergrupper inom Lysimachia i vid mening. Fröskal med retikulat ytmons- ter, till exempel, karaktäriserar både undersläktet Palladia och dess förmo- dade systergrupp, undersläktet Lysimachiopsis, en grupp arter som endast växer på Hawaii.

Fylogeni inom Lysimachia på Hawaii

Acknowledgements

The research work presented in the thesis has been carried out at the Departments of Palaeobotany and Phanerogamic Botany, Swedish Museum of Natural History and Department of Systematic Biology (previously Systematic Botany), Evolutionary Biology Centre (EBC), Uppsala University.

First of all, I would like to thank to my supervisor Professor Arne A. Andersberg, for his excellent academic guidance, endless encouragement, and trust. I have enjoyed very much working on the Lysimachia project!

Special thanks are also given to my co-supervisor Dr. Jürg Schönemberger for his kind help with manuscripts and many suggestions.

I am very grateful to my official supervisor Professor Mats Thulin in Uppsala University for reading manuscripts and help with so many things in the department.

I would like to thank Professor Sandra Baldauf, Professor Leif Tibell and Dr. Petra Korall for constructive comments on the manuscript of paper IV.

I am grateful to all members in Phanerogamic and Cryptogamic Botany Departments, Swedish Museum of Natural History. Special thanks to Ulf Swenson, Anna-Lena Anderberg, Johannes Lundberg, Jens Klackenberg, Mia Ehn, Lennart Stenberg, Thomas Karlsson, Dick Andersson, Maria Backlund, Anvar Sheikhamadi, Gunilla Dahlerus Lehman, Ulla-Karin Skärlund, and Erik Emanuelsson for their kind help and many suggestions.

Many thanks go to all former and present members in Molecular Systematics Laboratory, especially Mattias Myrenås, Pia Eldenäs, Ida Trift, Martin Irestedt, James S. Farris, and Mari Källersjö for their kind help and suggestions.

I would like to thank people in Botany Department, Stockholm University, special thanks to Ulrika Manns, Markus Englund for their kind help and suggestions.

I would like to express my sincere gratitude to my previous supervisor Professor Else Marie Friis for giving me an opportunity to do my PhD study (basal angiosperms project), initiating the idea of my PhD project, academic guidance, encouragement, patience, and positive and constructive discussions.

I would like to thank my previous co-supervisor Professor Birgitta Bremer for accepting me as a PhD student at Uppsala University, very fruitful discussion, and helpful comments on my manuscripts.
Thanks go to Dr. Thomas Denk as both my dear colleague and unofficial supervisor. He is a ‘super hero’ in the palaeobotany department and a lot of help to many people including me. I would like to express my deepest thank to Thomas for his encouragement, friendship, constructive criticism, unlimited ideas, and help with various other things.

I am very much grateful to Drs Jürg Schönberger and Maria von Balthazar, for their kindness, supplying material for my study, and many valuable suggestions.

Previous and present members of the Palaeobotany Department at the Swedish Museum of Natural History are thanked for their kindness and help. The more than warm atmosphere at the department has been a firm foundation for my life in Sweden. I would like to thank Kamlesh Khullar for help with many practical things. Special thanks to Dr. David Cantrill for English correction in my manuscripts; Catarina Rydin for helpful discussions and suggestions. Yvonne Arremo for help with SEM and photography, for delicious bakery for afternoon coffee breaks; Ove Johansson, Per-Olof Haglund, Lars Imby, Sandra May, Hervé Sauquet, and Caroline Strömberg for help with various things.

Thanks go to Dr. Ki Andersson and other members at the Department of Palaeozoology, Swedish Museum of Natural History for their kind help.

I am grateful to all people in Systematic Botany department, EBC, Uppsala University for their kind help and encouragement. Special thanks to Björn-Axel Beier, Per Kornhall, Magnus Popp, Jesper Kårehed, Annika Vinnersten, Per Erixon, Dick Andersson, Maria Backlund, Niklas Wikström, Elisabeth Långström, Bengt Oxelman, Sylvain Razafimandimbison, Katarina Andreasen, Hugo De Boer, Anneleen Kool, Magnus Lidén, Kristina Articus, Henrik Lantz, Inga Hedberg, Frida Eggens, Cajsa Anderson, Ulla Hedquist, Catarina Ekenäs, Anja Rautenberg, Sunniva Aagaard, and Johannes Lundberg for their kind help and many suggestions.

I would like to thank Professor Suk-Pyo Hong, who introduced me to botany and who has always encouraged me in various ways.

Thanks also to many Korean people and friends in Sweden for their concerns and support, especially Hyun-Duk Kim and Kyung-Sil Choo for their encouragement.

Last, but not least, I would like to thank my family for their endless love and support across the Eurasian continent. I hope this thesis would be a small gift to my mother in Korea and father in heaven. I always think of you and bear in mind your favorite motto, “Always be sincere and diligent”.

References


Acta Universitatis Upsaliensis

Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Science and Technology 634

Editor: The Dean of the Faculty of Science and Technology

A doctoral dissertation from the Faculty of Science and Technology, Uppsala University, is usually a summary of a number of papers. A few copies of the complete dissertation are kept at major Swedish research libraries, while the summary alone is distributed internationally through the series Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Science and Technology. (Prior to January, 2005, the series was published under the title “Comprehensive Summaries of Uppsala Dissertations from the Faculty of Science and Technology”.)