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Chapter 1

General Introduction

General Introduction

Ficus L. (Moraceae) is an important plant genus for various and diverse reasons. In Buddhist religion it is prominent, because the Lord Buddha attained enlightenment while meditating under a tree belonging to *Ficus religiosa* L. (Berg & Corner, 2005). Hindus conduct various meditative paces around fig trees, while the sadhus (hindu ascetics) also meditate beneath sacred fig trees (Murty, 2014).

In pollination biology *Ficus* is one of three taxa with an obligatory, symbiotic relationship with its pollinators, whereby the larvae of the pollinators, while feeding on a part of the seeds, are protected by the fruits (or figs). In *Ficus* pollination is carried out by the fig wasps. The other two groups are *Yucca* (Pellmyr et al., 1996; Proctor et al., 1996; Pellmyr, 2003) and Phyllanthaceae (Kawakita and Kato, 2004), both pollinated and the seed predated upon by moths. Pollen loaded female fig wasps enter the fig via the ostiole, loosing their wings in the process due to the scales in the ostiole. They pollinate the pistillate flowers, of which there are two types, those with a long and those with a short stigma. The wasps can only deposit eggs in the flowers with short stigmas. The developing larvae eat the seeds. The male wasps hatch first and gather around the ostiole, where staminate flowers shed their pollen. Once the females hatch from the pupae, they are inseminated by the males and gather pollen while leaving the fig passing the withered scales in the ostiole. The male wasps die in the fig (Ramírez, 1969; Wiebes, 1979; Anstett et al., 1997; Kjellberg et al., 2001).

Ecologically, *Ficus* is important for two reasons. Many species start as slow growing epiphytes in high trees, which, at one stage, send out a root to the soil, after which growth becomes vigorous, making more and more (interconnecting) roots around the host tree. The host tree dies, either because of the competition for food or light or because it is strangled (hence the name strangler figs). In the ecosystem the figs are keystone species to uphold the food chain by producing plentiful food for various wild animals, especially during the dry season when other trees are only present vegetatively or even shed their leaves (Berg & Corner, 2005; Berg et al., 2011; Shanahan et al., 2001, Harrison et al., 2012).

In this thesis the focus is on a group within *Ficus, F.* subsect. *Urostigma* (Gasp.) Berg. The species are found from West Africa and Madagascar through the Asian mainland to Japan and via southern Malesia to Australia and the Pacific. The species have highly variable morphological characters and can exhibit a wide distribution range (Berg & Corner, 2005). The systematic circumscription of the subsection and its classificatory relationships with other subsections are problematic. Furthermore, morphologically some species of subsect. *Urostigma* are very variable and show character combinations that make it difficult to distinguish the species from others. These problems will be addressed, discussed and (partly) solved in this thesis. Also presented will be a hypothesis of the evolution of the group (phylogeny) and its historical biogeography.

General morphology, leaf anatomy, and pollen morphology of *Ficus* subsect. *Urostigma*

The species of subsect. *Urostigma* can be recognised by a combination of various traits: They are mainly hemi-epiphytic trees or shrubs (stranglers; Fig. 1-1A, B). All of them show intermittent growth, whereby long internodes without leaves alternate with short shoots with leaves. The species are often deciduous, and the leaves are clearly articulate or subarticulate in most Asian species. There are two forms of stipules, long ones (more than 2 cm long) that are thin and caducous and which appear on the long shoots (Fig. 1-1C) and short ones (usually less than 2 cm), that are thicker and more persistent and found on the short shoots (Fig. 1-1D).

The figs are often borne below the leaves, sometimes only in the leaf axils (Fig. 1-1E), and in some species on spurs (brachyblasts) on the older wood (Fig. 1-1F). The figs occur solitary, in pairs, or up to eight together on the spurs. The number of basal bracts is usually three. The syconium (fig) is subglobose to subpyriform and varies in size. The syconium changes colour during maturation, from greenish to finally black, but with various pathways via white, pink and purple. Staminate flowers occur near the ostiole (opening of the fig) or they are dispersed among the pistillate flowers, but sometimes they can be abundant around the ostiole with a few dispersed. The staminate flowers are mostly sessile, rarely shortly pedicellate (Fig. 1-1G). Pistillate flowers are sessile or pedicellate. The ovary is white or red brown. The styles differ in length, long-styled flowers are mostly sessile; short-styled ones are generally pedicellate and their ovaries tend to be longer than those of the long-styled flowers. There is only one stigma, which entangles with those of adjacent flowers, thus forming a syn-stigmatic layer.

Leaf anatomy shows the presence of glandular hairs and simple hairs. The glandular hairs are elongate or cylindrical with 1- or 2-celled heads (Fig. 1-2A), or ellipsoid with 4-celled heads. Simple hairs are mostly single-celled with a pointed tip (Fig. 1-2B). Many species have translucent hairs on the inner surface of the syconium among the flowers, these are called "internal hairs". The epidermis is mostly single-layered and generally thicker at the adaxial side of the leaf (Fig. 1-2C), although it may have proliferated to form a multiple epidermis in some species (Fig. 1-2D). In surface view, the epidermis shows a pattern of 5-16 radiating cells, forming a rosette around the base of every lithocyst (enlarged epidermal cell with a cystolith in it, see below; Fig. 1-2E). The lithocysts are generally cells containing a hanging, short, club-shaped crystal (cystolith), which resembles an abortive hair (Berg and Corner, 2005). The lithocysts come in two forms. "Enlarged lithocysts" consist of very large cells, which deeply intrude into the palisade or spongy mesophyll and which are

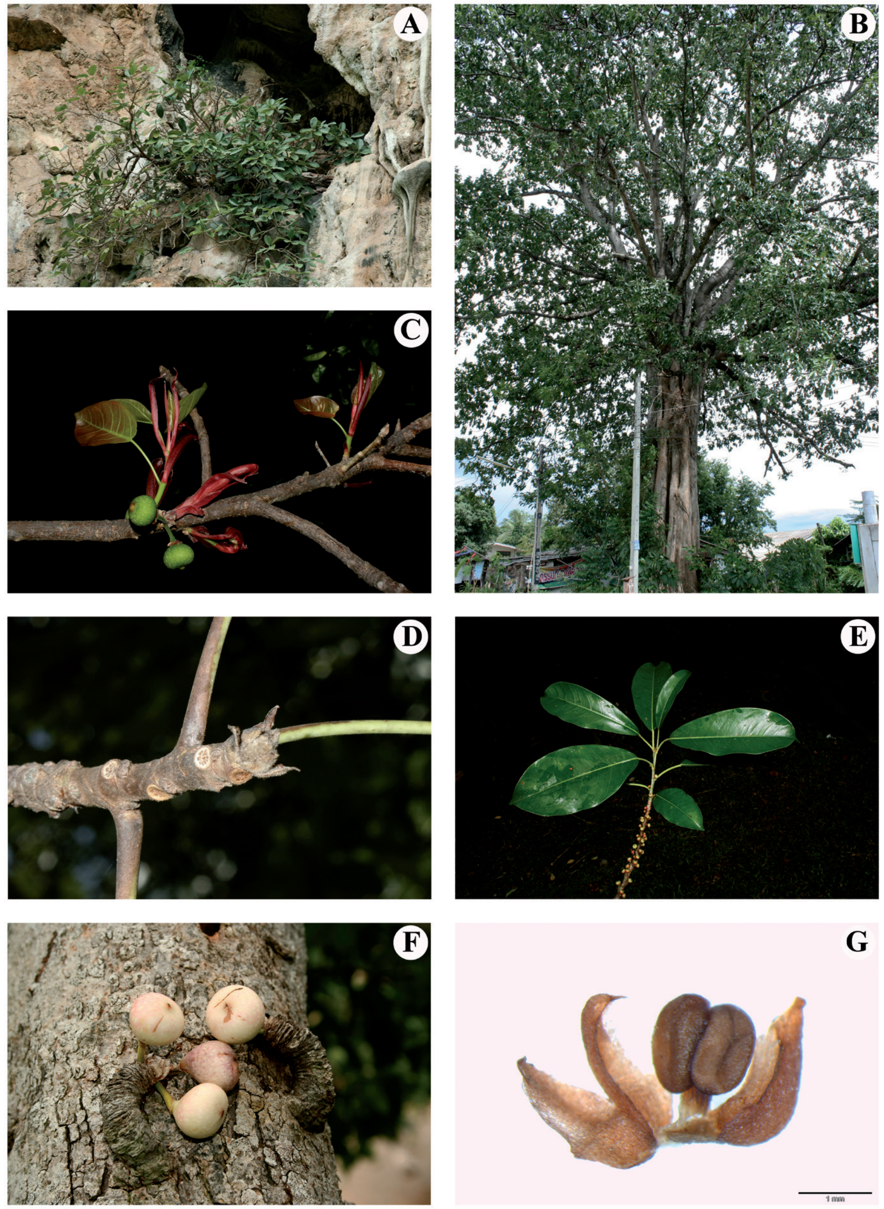

FIGURE 1-1: A. *Ficus orthoneura* in habit. B. *Ficus superba* in habit. C. Long stipulate form of *Ficus subpisocarpa* subsp. *pubipoda*. D. Short stipulate form of *Ficus subpisocarpa* subsp. *pubipoda*. E. Living twig of *Ficus glabella* shows the figs in leaf axils of fallen leaves. F. Figs on the spur of *Ficus superba*. G. Staminate flower of *Ficus saxophila* showing one stamen (*Kostermans 335*). All photographs by B. Chantarasuwan.

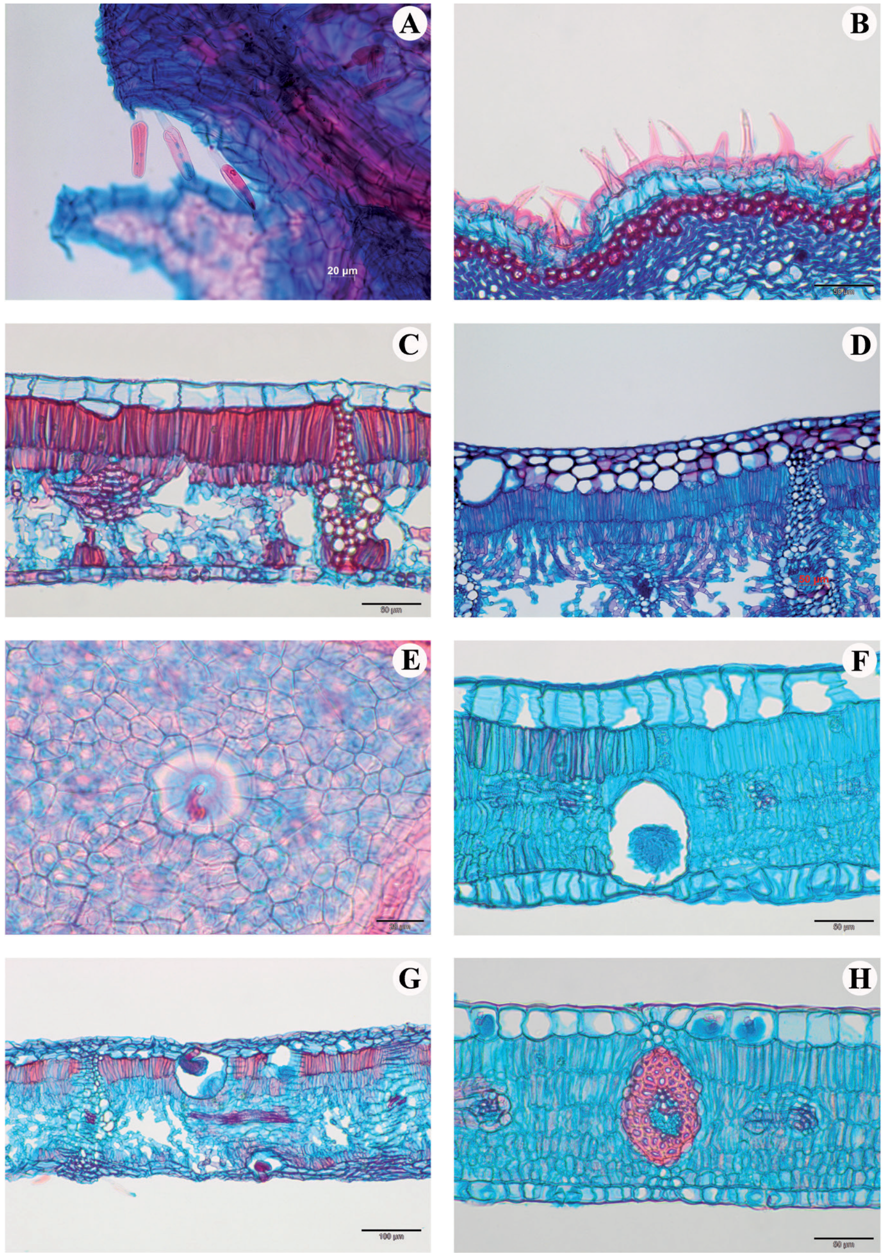

FIGURE 1-2: A. Glandular hairs with two head cells of *Ficus arnottiana* (*Haines 3546*). B. Simple hairs of *Ficus prolixa* (*Fosberg 31278*). C. The single layered epidermal cells of *Ficus caulocarpa* (*Chantarasuwan 071010–2*). D. The multiple layered epidermal cell of *Ficus arnottiana* (*Haines 3546*). E. The rosette-like epidermal cells surrounding the base of a lithocyst of *Ficus caulocarpa* (*Chantarasuwan 071010–2*). F. Enlarged lithocyst abaxially in the epidermis of *Ficus cordata* (*Seydel 3186*). G. Enlarged lithocysts in both abaxial and adaxial epidermis of *Ficus arnottiana* (*Haines 3546*). H. Epidermal lithocysts of *Ficus religiosa* (*Chantarasuwan 150910–2*). All photographs by B. Chantarasuwan.

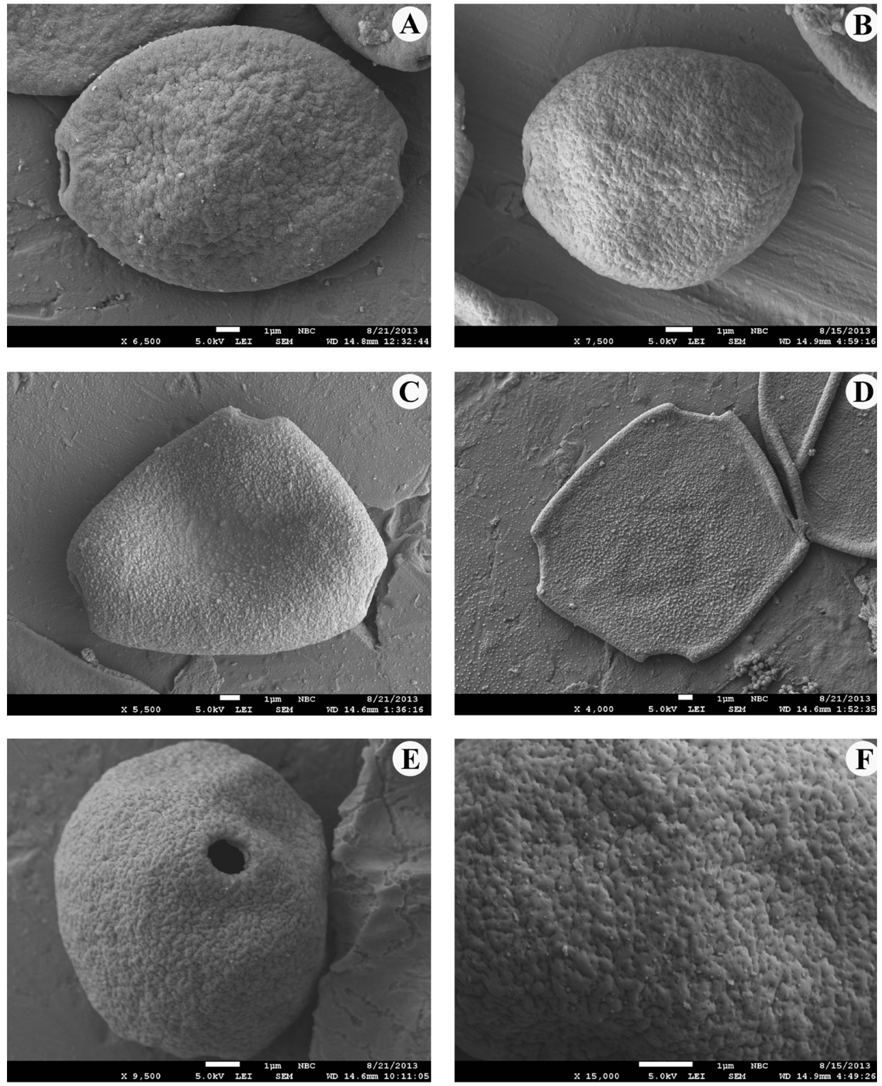

FIGURE 1-3: A. An ellipsoid 2–porate grain of *Ficus religiosa* (*Koelz 4030*). B. A gibbous 2–porate grain of *Ficus densifolia* (*Etienne 5156*). C. In polar view a triangular 3–porate grain of *Ficus salicifolia* (*Léonard 4959*). D. A quadrangular 4–porate grain of *Ficus salicifolia* (*Léonard 4959*) in polar view. E. The circular pore of *Ficus orthoneura* (*Cavalerie & Fortunat 2050*). F. The microrugulate pollen surface of *Ficus densifolia* (*Etienne 5156*). All photographs by B. Chantarasuwan.

surrounded by radiating epidermal cells in surface view. They mostly appear on the abaxial side of the lamina (Fig. 1-2F), except in *F. arnottiana* (Miq.) Miq., which shows abundant enlarged lithocysts adaxially and very few abaxially (Fig. 1-2G). The smaller lithocysts are adaxial epidermal cells of normal size, but they are not always consistently present in all species (Fig. 1-2H).

The pollen grains are very small to small, and they are 2–porate and ellipsoid or asymmetrically ellipsoid ('gibbous') (Fig. 1-3A,B) or sometimes 3–porate with a triangular polar view (Fig. 1-3C), rarely 4–porate monads quadrangular in polar view (Fig. 1-3D). The pores are circular (Fig. 1-3E). The exine is less than 1 μm thick, and the ornamentation is nearly always scabrate (elements < 1 μm high: finely punctate, microrugulate or microverrucate) (Fig. 1-3F)

Ecological and economic importance of *Ficus* subsect. *Urostigma*

All species of *Ficus* subsect. *Urostigma* occur mainly in tropical regions, though *F. subpisocarpa* Gagnep. can extend into the subtropics. The habitat of most species is generally a dry type of vegetation and/or there are seasonal conditions with a water deficit during a period of the year (Berg & Corner, 2005) such as found in savannah or on limestone. Only a few species live under everwet conditions, like *F. caulocarpa* (Miq.) Miq., which is commonly found in rain forests, and *F. verruculosa* Warb. that is abundant along streams or in swamps in Africa. *Ficus superba* (Miq.) Miq., though living under everwet conditions, is found along the coast in a saline habitat, which also causes physiological drought.

The species of the subsection are important food plants, not only in the wild, but also cultivated as the leaves are used as forage for cattle in Africa and Asia. Young shoots and young figs are eaten by humans. The shoots of at least five species in northern Thailand are eaten (Chantarasuwan & van Welzen, 2012), while some species are utilised in traditional medicine (de Padua et al., 1999) and/or they are sacred trees. Often species are used as ornamental plants and they can even be treated as bonsai, fetching high commercial prices.

The taxonomic history of *Ficus* subsect. *Urostigma*

The history of *Ficus* subsection *Urostigma* can be divided into five periods. Since Linneaus founded the genus *Ficus* in1753, his *F. religiosa* was the first species of the subsection that was described, thus forming the type of the subsection. In this period many species were described and an infrageneric classification was lacking. *Ficus virens* was described by Aiton (1789) from a cultivated plant growing in Kew gardens, but introduced from the West Indies by James Gordon around 1762. *Ficus tsjakela* Burm.f. was based on the vernacular name Tsjakela (Burman, 1768). Thunberg described the first African species, *F. cordata*, in 1786, the same year that Forster described *F. prolixa* from the Society Islands in the W Pacific. The first period ended with the publication of *F. saxophila* and *F. glabella* by Blume (1825).

The second period began when botanists (Gasparrini, 1844; Miquel, 1847) started to break up *Ficus* into several genera. Many species were first published under the generic name "Urostigma" in that time.

In the third period Miquel (1867) reversed the idea of breaking up *Ficus* and he re-united the genera again. Instead he introduced an infrageneric subdivision of six subgenera, *Urostigma* being one of them. Miquel further subdivided subgen. *Urostigma* according to distributions and morphological characters into series, whereby he recognised six series for Asia and Australia, three series for Africa, and five series for America. In 1887 King divided *Ficus* into seven sections based on the morphology of the flowers, which equalled the subgenera of Miquel. King divided his section *Urostigma* into series and subseries based on leaf characters. In 1960, Corner re-used the rank "subgenus". He recognized three subgenera, one of them subg. *Urostigma* with seven sections, among which sect. *Urostigma*, which contained four series. However, the species of subsect. *Urostigma*, as they are recognised today, were still spread over several sections or series and not yet members of a single taxon.

Berg started the fourth period with the recognition of subsection *Urostigma* in 2004. He united Corner's sections *Leucogyne*, *Urostigma*, and *Conosycea* into sect. *Urostigma*, which he subdivided into subsect. *Urostigma* (containing Corner's sect. *Leucogyne* and *Urostigma*) and subsect. *Conosycea* (containing Corner's sect. *Conosycea*). All African species were included in subsect. *Urostigma* in this period. The revision work of *Ficus* subsect. *Urostigma* by Chantarasuwan et al. (2013) was inspired by Berg's classification.

The fifth, the cladistic period, ran parallel with the fourth one. Classifications reflected monoplyletic groups based on phylogenies of large, mainly molecular data sets analysed with Bayesian and likelihood methods. For *Ficus* this period started when Weiblen (2000) published the first phylogeny based on DNA sequences and morphology. He was followed by Jousselin et al. (2003), who combined ITS and ETS data to construct the core phylogenetic relationships among 41 species of *Ficus*. For subsect. *Urostigma* real phylogenetic analyses began when Rønsted et al. (2005) combined ITS and ETS in their phylogenetic work, which included nine species of subsect. *Urostigma*. Rønsted et al. (2008) extended the phylogeny by adding the *G3pdh* marker for nearly half of the species of subsect. *Urostigma*. Most recently, Chantararasuwan et al. (2014) used four genes (ITS, ETS, *G3pdh*, and *ncpGS*) in combination with morphology and leaf anatomy to construct a phylogeny. The results showed subsect. *Urostigma* to be monophyletic and this new classification of *Ficus* subsect. *Urostigma* is presented here.

Problems

Morphologically, many species of subsect. *Urostigma* are very variable, which makes it difficult to distinguish species such as *F. virens*, which overlaps in some characters with *F. geniculata* Kurz (Berg, 2007). Another problem was caused by *Ficus rumphii* and *F. amplissima*, previously placed in section *Leucogyne* by Corner (1959), but transferred to subsect. *Urostigma* by Berg and Corner (2005). However, molecular phylogenetic research by Rønsted et al. (2005) showed that *F. rumphii* is embedded in subsect. *Conosycea*. Thus, the systematic position of both species is still doubtful. To resolve these problems, extra morphological studies (Chantarasuwan et al., 2013) and leaf anatomical studies (Chantarasuwan et al., 2014) were applied to find more characters. The combined results of both studies, elaborated with new phylogenetic analyses (Rønsted et al., 2005, 2008; Chantarasuwan et al., submitted) resolved the problem of the circumscription of the species and their classification. *Ficus amplissima* and *F. rumphii* will be part of subsect. *Conosycea* and no longer belong to subsect. *Urostigma*. This decision is corroborated by the pollinators. *Eupristina* wasps pollinate figs of subsect. *Conosycea* figs, while subsect. *Urostigma* is pollinated by species of *Platyscapa* (Wiebes, 1979; Berg and Wiebes 1992; Berg and Corner 2005; Cruaud et al. 2009). The leaf anatomy of *F. arnottiana* (subsect. *Urostigma*) presented yet another problem. *Ficus arnottiana* shows morphological similarity with species of subsect. *Conosycea*, but the molecular phylogeny of Chantarasuwan et al. (in press) shows *F. arnottiana* to be firmly embedded within subsect. *Urostigma*.

Research Questions

Based on the taxonomic problems encountered, the following research questions are addressed:

1) Which species can we morphologically distinguish in *Ficus* subsect. *Urostigma*? What are their diagnostic morphological characters? What is the extent of morphological overlap between the species?

2) Do the species of *Ficus* subsect. *Urostigma* differ in leaf anatomy? Does the leaf anatomy provide proper diagnostics for the recognition of species? Will leaf anatomy strengthen or improve morphological species circumscriptions?

3) Does pollen morphology show the same functionality as leaf anatomy in the characterisation of species?

4) What is the most likely phylogeny of *F.* subsect. *Urostigma*? How do the two species in section *Leucogyne*, *F. amplissima* and *F. rumphii*, fit in? How is *Ficus* subsect. *Urostigma* related to other subsections and sections within *Ficus* subgenus *Urostigma*?

5) How can the phylogenetic results be translated into a classification? Are clades recognisable with the aid of morphology, leaf anatomy, and/or pollen morphology? How can we explain the evolutionary trends in morphology, leaf anatomy, and pollen morphology?

6) Where and when did the major diversification events occur in the *Ficus* subsect. *Urostigma*? Which scenario results from the historical biogeography of the species? How can we explain the disjunction between the African and Asian-Australian species?

7) Which species of *Ficus* subsect. *Urostigma* are used by man and for which purposes?

Thesis goal and outline

The goal of this Ph.D. research is to focus on the systematics of *Ficus* subsect. *Urostigma* and related subsections or sections, as well as to resolve the phylogenetic relationships with other subsections or sections.

Chapter 2 contains a revision of *Ficus* subsect. *Urostigma* based on a morphological species concept. Vegetative and reproductive characters are carefully examined and used in species circumscriptions and descriptions and as characters in a data matrix for phylogenetic analyses. All data, including geography, uses, ecological data, are obtained from herbarium specimens and from field trips in Thailand. All literature related to the subsection is reviewed. Three new species and two new varieties are described. An identification key to the species is provided, together with descriptions and notes per taxon.

Chapter 3 deals with the leaf anatomy of *Ficus* subsect. *Urostigma*. Anatomical characters of the leaves are carefully examined and described per species.
A key to the species based on leaf anatomical characters is provided.

In **Chapter 4** the pollen morphology of *Ficus* subsect. *Urostigma* is studied. Pollen of the different species is described and compared.

Chapter 5 presents the integration of molecular, morphological and leaf anatomical data in a total evidence approach of the phylogenetic reconstruction of *Ficus* subsect. *Urostigma*. More insight is gained in the evolution of specific morphological and leaf anatomical traits. A new circumscription of the subsection is proposed, a taxonomic treatment is provided and nomenclatoral changes are made, even new entities are described.

In **Chapter 6** *Ficus cornelisiana* is described as a new species within *Ficus* subsect. *Urostigma* from the Sino-Himalayan region based on a new combination of morphological and anatomical characters.

Chapter 7 deals with the historical biogeography of *Ficus* subsect.*Urostigma*. Molecular dating was performed in a Bayesian framework with the program BEAST, and ancestral area reconstructions were made with S-DIVA in the program RASP.

Chapter 8 presents the utilization of *Ficus* subsect. *Urostigma* in Thailand. Species are used as food, ornamental plants or sacred trees.