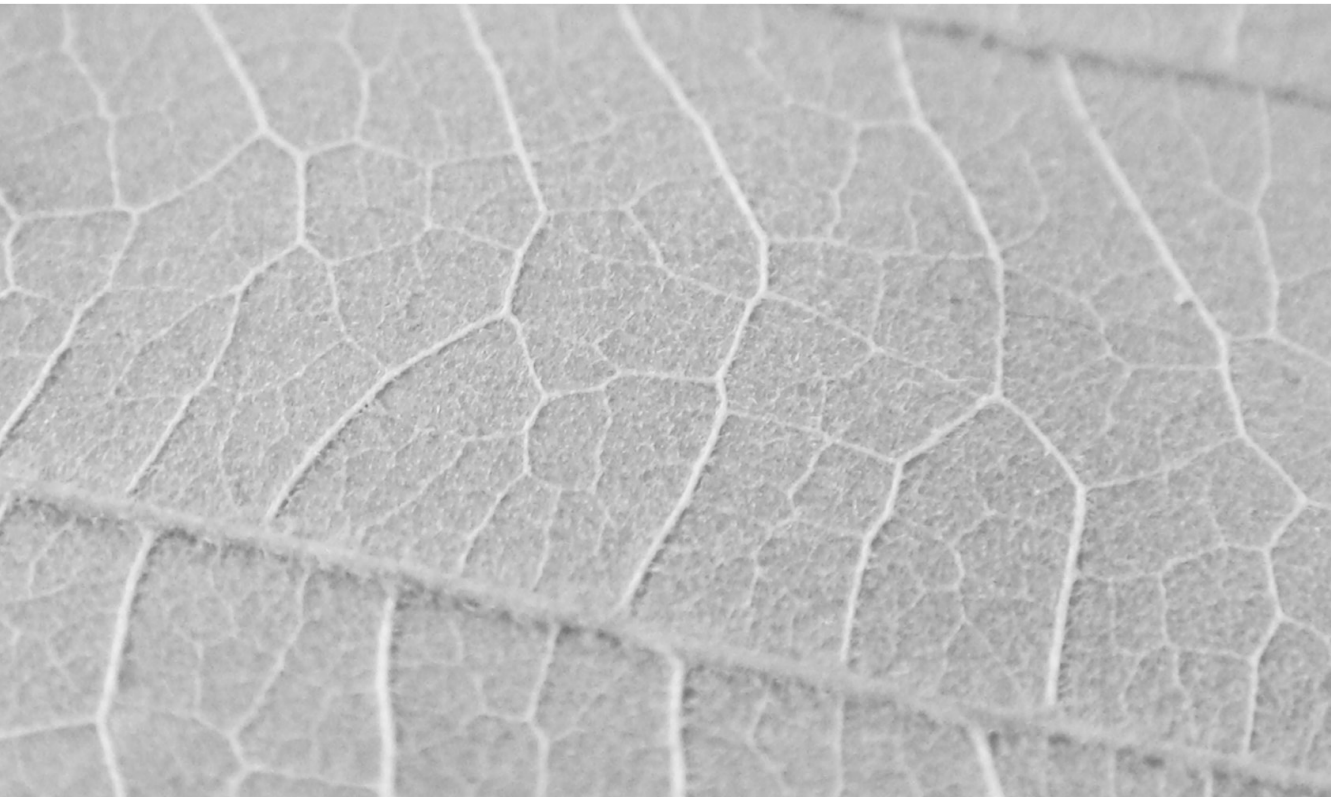
Chapter 1

General Introduction



General Introduction

One of the major problems left in the classification of tribe Millettieae of the Leguminosae (Fabaceae) concerns the *Derris*-like taxa. Up to now every researcher had different solutions, some would unite all taxa into a single genus, others divided them into several genera. The purpose of this thesis is to tackle the problems at various levels and from different viewpoints. The species will be defined first, after which their phylogeny based on molecular and morphological data will be inferred. The resulting phylogeny will form the basis for a new and less subjective classification. Finally, the biogeographic history of the taxa will be analysed. This introduction provides general information of palaeotropic *Derris*-like taxa.

General morphology, ecology and utility of the Asian *Derris*-like taxa

"*Derris*-like taxa" contain members of the tribe Millettieae (Fabaceae), characterized by their imparipinnate leaves with opposite leaflets and typical flat, usually winged, indehiscent pods. The plants have a pantropical distribution. According to the most recent generic circumscription proposed by Adema (2000), the Asian *Derris*-like taxa consist of the genera *Aganope* Miq., *Derris* Lour. [including *Brachypterum* (Wight & Arn.) Benth.] and *Paraderris* (Miq.) Geesink. *Derris*-like taxa are sometimes very similar because of their overlapping morphological features. Some species also show a high variation in morphological characters and a wide distribution. This makes the plants sometimes difficult to identify correctly to the species level. Most species of Asian *Derris* are lianas, which may climb over trees or other vegetation forming a thick cover (Fig. 1-1: A). However, the tree and tree-like habit can also be found (2 or 3 species which were previously placed in *Brachypterum* according to Geesink (1984), and a single species of Asian *Aganope*). *Derris* (not including *Brachypterum*) and *Paraderris* prefer sunlight and water. Many species grow naturally in fully exposed areas nearby or along waterways (Fig. 1-1: A), generally at low altitudes (not higher than 500 m). A few species are specialized in dryer habitats with a limited water supply such as limestone mountains (Fig. 1-1: B). Species of *Brachypterum* seem to be more drought tolerant than *Derris* s.s. and *Paraderris* as many of them usually occur in dry deciduous forest, along the roads or in dry, very disturbed areas (Sirichamorn, personal observation; Fig. 1-1: C, D). The species grow on a range of soil types varying from coarse sand to heavy clay (Hamid, 1999). Some species of the Asian *Derris*-like taxa are common weeds in forest plantations (Fig. 1-1: E).

Fig. 1-1. Habitat of *Derris*-like taxa: **A**. *Paraderris elliptica* (Wall.) Adema, typical for most of *Derris*-like taxa which usually grow in fully exposed areas nearby or along waterways, climbing over other trees and forming a thick cover. **B**. *Derris* sp., an example of the few *Derris* species inhabiting limestone mountains. **C**. *Derris scandens* (Roxb.) Benth., a species previously placed in *Brachypterum*, grows in a very disturbed, grass covered area. **D**. *Derris thorelii* (Gagnep.) Craib, a species possibly placed in *Brachypterum*, grows also in a disturbed area, roadside. **E**. *Derris amoena* Benth., a common weed in para-rubber plantations, covering the ground and climbing up a *Hevea* tree.

As mentioned earlier, they all have imparipinnate leaves with opposite leaflets. The number of leaflets per leaf varies from three to nine, which is most frequently found in species of *Derris* s.s. and *Aganope*. Up to 41 leaflets per leaf can be found in species previously placed in *Brachypterum* (Fig. 1-2: A). Reddish or golden-brownish young leaves (Fig. 1-2: B) can be found in many species of *Derris* and *Paraderris* but this is usually absent in *Aganope*. Leaflets vary in shape and size, but all have an entire margin. Stipellae are generally absent but usually present in species which have one-winged pods. Inflorescences are true panicles in *Aganope*. They are usually pseudoracemes or pseudopanicles in *Derris* and *Paraderris* (Fig. 1-2: C, D): flowers in fascicles on very short lateral axes called "brachyblasts"; panicles are rare. (Fig. 1-2: E, F); intermediate forms (Fig. 1-2: G) between true panicles and pseudoracemes/ pseudopanicles are also found, but uncommon. The calyx is usually reddish, or sometimes greenish with short lobes. The petals are typically white, pinkish or rarely deep purplish, with or without basal callosities on the standard petals. The stamens are diadelphous in *Aganope* but monadelphous with basal fenestrae in *Derris* and *Paraderris*. Pods are indehiscent, typically thin and leather-like (derris, Greek: skin, leather coat). The shape of the pods varies (Fig. 1-3: A to I). Seed chambers are found in some species (Fig. 1-3: G to I), which were previously included in *Brachypterum*. The wings of the pods can be found along the upper suture or along both sutures; wingless pods are rare. Seeds with a distinctly eccentric hilum are found in *Aganope*, a central or slightly eccentric hilum are found in *Derris* and *Paraderris*. The flowering time varies per species. Some species flower in the rainy season, while others prefer drier conditions. According to field observations, the species are mostly pollinated by bees.

Species of *Derris*-like taxa are well known as an important source of rotenone toxin, which occurs in every plant part, but in higher amounts especially in stems and roots. Rotenone is used as an effective, organic and unselective insecticide and piscicide (fish poison). It can thus be applied to control a large array of pests on a range of crops in various forms, i.e. in sprays, as dust, or dips or baits. Rotenone rapidly biodegrades when exposed to air and sunlight and leaves little or no residues.

Fig. 1-2. Some examples of morphological characters found in *Derris*-like taxa: **A**. Species of *Derris* to be transfered to *Brachypterum,* e.g., *D. microphylla* (Miq.) B.D. Jacks., usually have more leaflets than *Derris* or *Paraderris*. **B**. Reddish young leaves are often present in *Derris* or *Paraderris*, but absent in *Aganope*. **C**. *Derris trifoliata* Lour. **D**. *Derris scandens* (Roxb.) Benth. The inflorescence typical for *Derris* and *Paraderris* is a pseudoraceme/pseudopanicle type, characterized by the presence of the reduced, wart or knob-like lateral axes called "brachyblasts" (indicated by red arrows in **C** and **D**), on which the fascicles of flowers are born. **E**. *Derris* sp. and **F**. *Derris marginata* (Roxb.) Benth. have a true panicle, characterized by the absence of brachyblasts and thus the flowers are born solitarily on elongated lateral branches. This is rare in *Derris*, only two species shown in **E** and **F** have this type of inflorescence. **G**. *Derris alboruba* Hemsl., an example of an intermediate inflorescence which basally elongated lateral branches, resembling a true panicle, but higher up the rachis bears only brachyblasts and resembles a pseudoraceme.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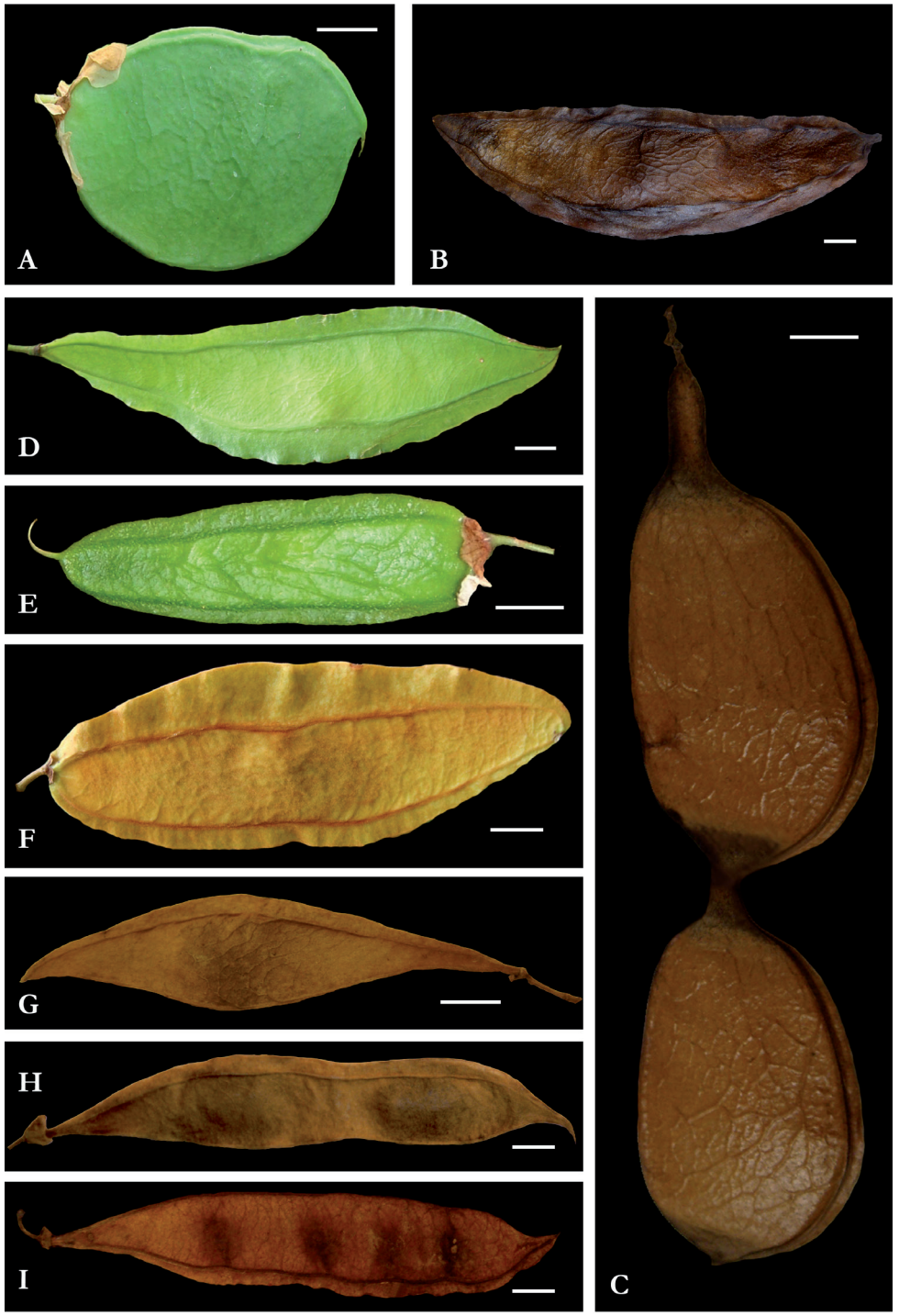
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The chemical is extremely toxic to insects and fish because it is easily absorbed through the trachea or gills and interferes with cellular respiration, but only slightly toxic to mammals (Hamid, 1999). Although the use of rotenone as an insecticide is being phased out and has been banned in some countries e.g. the Netherlands since about 1980 due to the introduction of synthetic insecticides and the lack of convincing data about determination of its toxicity, rotenone has been continuously used by ichthyologists for collecting fish in their studies of marine fish biodiversity, because only small quantities are necessary and have only minor side effects to the environment (Robertson and Smith-Vaniz, 2008). Traditionally, the tuba root [*Paraderris ellipica* (Wall.) Adema, see Fig. 1-1: A and 1-4: A] and tuba merah [*Paraderris montana* (Benth.) Adema] have been used as a fish poison throughout SE Asia and the Pacific. Several other species of *Derris* have also been reported as piscicide and are also locally used as an insecticide. However, *P. elliptica* is the main species that is widely cultivated as a major source of rotenone. Several cultivars of this species have been selected of which "Sarawak Creeping", "Changi No.3" and "Ngawi" are reported to be commercially superior (Hamid, 1999).

Because of the toxicity of *Derris*-like taxa, many species are also used traditionally as medicines in many countries. In Thailand the roots of *P. elliptica* itself are used as emmenagogue and the stems as a blood tonic. Stems of *D. scandens* (Roxb.) Benth. (Fig. 1-1: C, 1-2: D and 1-4: B) are used as a diuretic, laxative, expectorant, emmenagogue and in the treatment of osteoarthritis, common cold and backache. Roots of this species are also used in India to increase milk secretion after childbirth. The whole plant of *D. trifoliata* Lour. (Fig. 1-2: C and 1-4: C) is used in India and Indonesia as stimulant, antispasmodic, and counter-irritant, and against rheumatism, chronic paralysis, and dysmenorrhea. Thai traditional doctors used roots or stems as a laxative, carminative, and anti-arthritis treatment (Hamid, 1999). Stems of *D. reticulata* Craib (Fig. 1-4: D) contain some sweet chemical substance and are used as a sweetener in local medicine; they are also used as an expectorant or a laxative in Thailand. A solution of crushed leaves of *D. elegans* Graham ex Benth. is used to wash snake bites in Papua New Guinea.

Other uses of *Derris*-like taxa are also reported. For example, species which are trees, i.e., *D. robusta* (Roxb. ex DC.) Benth. (Fig. 1-4: E, F) and *D. microphylla* (Miq.) B.D. Jacks. (Fig. 1-2: A), are cultivated as timbers and shade trees and sometimes for

Fig. 1-3. Variation in pods of *Derris*-like taxa: **A**. *Derris trifoliata* Lour. **B**. *Aganope thyrsiflora* (Benth.) Polhill. **C**. *Aganope heptaphylla* (L.) Polhill. **D**. *Derris marginata* (Roxb.) Benth., **E**. *Derris reticulata* Craib. **F**. *Derris ferruginea* (Roxb.) Benth. **G**. *Derris scandens* (Roxb.) Benth. **H**. *Derris robusta* (Roxb. ex DC.) Benth. **I**. *Derris eriocarpa* F.C. How. Seed chambers can be seen in **G** to **I**. Scale bar = 1 cm.

ornamental purposes (Thothathri, 1982; Thothathri and Rugayah, 1997; Hamid, 1999; Du Puy et al., 2002) such as *D. scandens* which is full of white-pinkish blossoms during its flowering season (Fig. 1-4: B). Like other leguminous plants which usually contribute to nitrogen fixation, species of *Derris* can also be grown as green manure to improve soil (Hamid, 1999; Thothathri and Rugayah, 1997).

The Taxonomic History of the genus *Derris* and other related genera

The genus *Derris* was established by Joao de Loureiro in 1790 to accommodate two new species: *Derris pinnata* Lour. and *D. trifoliata*. As the genus has indehiscent pods, it was firstly placed in the tribe Dalbergieae by Bentham (1860). However, he already remarked that the *Derris* species have several characters that deviate from the other Dalbergieae, for example, the inflorescence, adhesion of wings and keel petals and upper filaments uniting with others. Before being described by Loureiro in 1790, Linnaeus (1747) gave it the name *Pterocarpus* but the name was later transferred to another group of Leguminosae. Adanson (1763) described *Salken* Adans. and *Solori* Adans., both based on plates in Hortus Malabaricus (van Rheede, 1686; 1688). In 1775, *Deguelia* was established by Aublet, later used by Taubert (1891) for *Derris* in a wide sense (s.l.). Wight and Arnott (1834) described subgenus *Brachypterum* of the genus *Dalbergia* L.f. Three years later the subgenus was raised to generic level (Bentham, 1837). In 1849, the genus *Ostryocarpus* Hook.f. was described (Hooker, 1849), followed by Miquel (1855), who established the genus *Aganope* to accommodate several *Derris*-like species which have true paniculate inflorescences. He also recognized *Derris* sect. *Paraderris* in the same year. Bentham (1860), in his "Synopsis of Dalbergieae", united all the old-world *Derris*-like genera, together with some American species, into a single genus called *Derris* s.l., which was divided into five sections: *Aganope*, *Brachypterum*, *Derris* (*Euderris*), *Dipteroderris* and *Paraderris*. The African genus *Leptoderris* Dunn was established in 1910. One year later Dunn also described another genus *Ostryoderris* Dunn (1911) for African species of *Derris*-like plants, which have inflorescences similar to those of *Ostryocarpus* (Hooker, 1849) but pods similar to those of *Derris*. Later, Roberty (1954) described the monotypic genus *Xeroderris* to accommodate a *Derris*-like tree species found in arid areas of Africa, *X. chevalieri* (Dunn) Roberty [≡ *X. stuhlmannii* (Taub.) Mendonça & Sousa].

Fig. 1-4. Species of Asian *Derris*-like taxa with various uses: **A**. *Paraderris elliptica* (Wall.) Adema, as toxin and medicine. **B**. *Derris scandens* (Roxb.) Benth., as medicine and ornamental plant. **C**. *Derris trifoliata* Lour., as medicine and occasionally toxin. **D**. *Derris reticulata* Craib, as medicine and sweetener in medicine recipes. **E**, **F**. *Derris robusta* (Roxb. ex DC.) Benth. and **G**. *Derris amoena* Benth., as toxin and stems ocessionally as rope.


A


B


C


D


E


F


G

Hutchinson (1964) united *Leptoderris* with *Derris* because of its typical *Derris*-like pods. In 1971 Polhill reinstated *Aganope* to generic level and also transferred species of *Derris* with panicles to it. Polhill also combined *Aganope* and *Ostryoderris* together into *Aganope*, but kept *Ostryocarpu*s and *Xeroderris* apart. Later Polhill (1981) and Geesink (1981, 1984) officially transferred many genera with indehiscent pods, including *Derris*, from tribe Dalbergieae to tribe Millettieae, because they showed a close morphological, anatomical, and chemical resemblance with *Millettia* Wight & Arn. and related genera. After his intensive study of the tribe Millettieae, Geesink (1984) reinstated many of Bentham's sections of *Derris* s.l. to generic level, i.e., *Brachypterum* (previously section *Brachypterum* Wight & Arn.), *Deguelia* (the new-world species of section *Euderris*, including section *Fasciculati* Benth. of the genus *Lonchocarpus* Kunth), *Derris* s.s. (old-world species of section *Euderris* and section *Dipteroderris*) and *Paraderris* (formerly section *Paraderris*). He also reinstated *Leptoderris* to generic level. Instead of raising section *Aganope* to genus as proposed by Polhill (1971), Geesink synonymized it with *Ostryoderris, Ostryocarpus* and *Xeroderris* under the oldest name *Ostryocarpus*. According to Geesink the name *Derris* should be conserved over the older name *Salken*, and similarly *Brachypterum* is to be preferred over *Solori*. Adema (2000) carried out a cladistic analysis based on morphological characters. The results suggested that the genus *Brachypterum* had to be reunited with *Derris*. He partially accepted Polhill's (1971) concept by considering *Aganope* as a distinct genus though closely related to *Ostryocarpus*. In contrast to Polhill, Adema proposed to synonymise *Xeroderris* with *Aganope*. In addition, Adema also concluded that the American *Derris* "*Deguelia*" was not closely related to Asian *Derris* and the inclusion of this genus with *Lonchocarpus* is possibly correct.

The neotropical *Derris*-like genus *Lonchocarpus* also shows a history of synonymisation with various genera. The genus was merged with either *Deguelia* (Bentham, 1860*;* Macbride, 1943), *Derris* (Macbride, 1943), *Muellera* L.f. (Geesink, 1984) and *Philenoptera* Fenzl. ex A. Rich. (Bentham, 1860). *Lonchocarpus* also shows morphological overlap with some other genera of Millettieae, e.g., *Dahlstedtia* Malme, *Margaritolobium* Harms and even *Millettia*. Accepting *Lonchocarpus* in a taxonomic broad sense (*Lonchocarpus* s.l.), thus including several genera, causes a problem comparable with *Derris*. The result will be a morphologically heterogeneous genus that, consequently, is difficult to diagnose. According to Geesink (1984), *Deguelia* and *Philenoptera* are distinct from *Lonchocarpus* and this generic circumscription is still widely accepted nowadays. However, the classification and phylogenies of these neotropical genera are not the main focus of this thesis.

Molecular phylogeny of *Derris*-like taxa

Systematics of living organisms are nowadays based on DNA sequence data as these reflect gene-level changes, which are believed to reflect true phylogenies. However, there are only few molecular phylogenetic studies directly related to the species of Asian *Derris*-like taxa. A few species (not more than three species for each genus) were included in the phylogenetic studies of the tribe Millettieae by Lavin et al. (1998) and Hu et al. (2000, 2002). Besides the small samples, taxa representing different infrageneric groups of *Derris* were not included in those studies. Apparently, the resulting phylogenies based on *trnK/matK* (Hu et al., 2000) and nuclear ITS/5.8S (Hu et al., 2002) revealed that *Derris* s.l. (sensu Bentham, 1860) was a polyphyletic taxon. The genus *Aganope* (treated as *Ostryocarpus* in those analyses) appeared to be a basal clade in the Millettieae phylogeny, far separate from the other *Derris*-like taxa. The neotropical *Lonchocarpus* and the African *Philenoptera* were also not closely related to Asian *Derris*. However, even in the sense of Geesink (1984a) and Adema (2000) *Derris* s.s. was probably also not monophyletic, because a representative of *Brachypterum*, *B. robusta* (Roxb.) Geesink [=*Derris robusta* (Roxb. ex DC.) Benth.], was always placed outside *Derris* (s.s.) in all phylogenies. Up to now, all phylogenies could not equivocally solve the generic circumscriptions among the *Derris*-like taxa because of insufficient sampling. Thus the problem of generic delimitation still needs an improved phylogenetic analysis.

Problems of a generic/infrageneric circumscription of *Derris*-like taxa

In conclusion, there are two competing generic/infrageneric circumscriptions of *Derris*-like taxa. The first one is *Derris* in a wide taxonomic sense including many genera and infrageneric taxa, which was already refuted by the results of the molecular phylogenies. The second is *Derris* in a narrow sense next to various genera, which is the preferred option. However, the phylogeny of the three phylogenetically and morphologically closely related genera *Brachypterum*, *Derris* s.s. and *Paraderris* remains far from solved. In addition, the relationships between the Asian-African genus *Aganope* and its closely related African genera *Ostryoderris*, *Ostryocarpus* and *Xeroderris* are also still obscure and make it impossible to draw final conclusions with regards to their generic circumscriptions. Many species of *Derris* s.s. also show morphological similarities among them, which sometimes makes it difficult to distinguish the taxa. Species of *Paraderris*, for example *P. elliptica*, are introduced, widely cultivated and then finally naturalised. The species consists of several cultivars or natural morphological forms which consequently leads to a possible infraspecific classification. More detailed phylogenetic studies based on various sources of phylogenetically informative data with sufficiently sampled species can provide an exact circumscription of Asian *Derris* and allies.

Species Diversity of *Derris*-like taxa in Thailand

Thailand is centrally located within the SE Asia mainland, a hot and humid climate zone where a variety of tropical ecosystems can be found and hence, are able to support a large variety of plant species, including *Derris*-like taxa. Within the Indo-Malayan realm, the country is situated between two major biogeographical regions, the Indochinese region in the north and the Sundaic region in the south (Mackinnon and Mackinnon, 1986; Mackinnon, 1997). Because Thailand lies at the crossroads between these regions of the lndo-Malayan Realm, the composition of the Thai flora closely resembles that of neighbouring countries. However, at species or genus level there are some endemics in Thailand. Thus, the country could be considered as a perfect collective center of botanical diversity of 3 major regional elements: Indo-Burmese, Indo-Chinese and Malesian (van Welzen et al., 2001). However, the study of plant diversity, especially of Fabaceae, in this area is lacking, slowly carried out or not completely finished. The first taxonomic account of Asian *Derris*-like taxa in Thailand was prepared by Craib (1928). At that time 18 species of Asian *Derris*-like taxa were recognised under *Derris* s.l. According to the most recent preliminary observation of Adema (2000), 16 species of *Derris*-like taxa occur in Thailand, 17 are found in Malesia and nine are common to both areas. As time goes by, more and more unidentified Thai specimens of *Derris*-like taxa have been collected, but the identification is still dependent on the literature of neighbouring countries such as the Flore du Cambodge du Laos et du Viêtnam (Phan and Vidal, 2001) and Flora of China (Wei et al., 2008). The study of these plants will provide more substantial and fundamental data for the study of the Fabaceae. It will contribute directly to the Flora of Thailand and Flora Malesiana and also provide the necessary information for sustainable management and conservation of Thai *Derris* and its related genera, especially the rare, endemic or endangered species.

Research Questions, aims and outline of the thesis

The following research questions are addressed:

1) How many and which species of *Derris*-like taxa occur in Thailand? How do they differ morphologically and ecologically? What are their diagnostic morphological characters?

2) Is the most recent generic concept of *Derris*, *Derris* sensu Adema, monophyletic? If not, how about the monophyly of *Derris* sensu Geesink or other previous generic concepts?

3) How are the three genera, *Brachypterum, Derris s.s.* and *Paraderris*, related to each other and to other genera of Millettieae, particularly *Derris*-like taxa such as *Aganope, Deguelia, Leptoderris, Lonchocarpus, Ostryocarpus and Philenoptera*?

4) How are the infrageneric groups previously proposed for the genus related to each other? Are those groups monophyletic and does the molecular phylogeny corroborate these or other infrageneric groups?

5) Can the clades be classified as genera or infrageneric taxa? Are they recognizable morphologically and how can we explain the trends in morphological evolution of *Derris*-like taxa?

6) Where and when did major diversification events occur in the *Derris*-like taxa? How can we explain the paleotropical intercontinental disjunctions (PIDs) found in some species of *Aganope*, *Brachypterum* and *Derris*?

The basis for all research are the herbarium collections and field observations. Without those we would still have no idea about the evolution in time and space of the *Derris*-like clades.

In ***chapter 2***, a revision of *Derris*-like taxa in Thailand is carried out, using Adema's concepts of *Aganope*, *Derris* s.s. (including *Brachypterum*) and *Paraderris*. Vegetative and reproductive morphological characters are carefully examined and used in the descriptions and a data matrix for future phylogenetic analyses. Geographical and ecological data, such as flowering period, are obtained from herbarium specimens and field observations. All literature related to this genus is reviewed again. All currently recognised species are enumerated. Two new species are described and illustrated. Keys to the genera and species are provided, together with descriptions and notes for all taxa.

In ***chapter 3***, the monophyly of *Derris* s.s. (Adema's generic concept) is tested using a molecular phylogenetic approach. In this chapter, especially the phylogeny of the old world *Derris*-like taxa is highlighted. Three chloroplast markers, *trnK-matK*, *psbA-trnH* and *trnL-F intergenic* spacer, and one nuclear marker, ITS/5.8S, were sequenced for all genera of palaeotropic (Asian and African) *Derris*-like taxa and also some neotropic genera (American *Derris*). Sequence data of other genera in the tribe Millettieae obtained from GenBank are also included in the analyses in order to understand the phylogenetic relationship between all taxa. Monophyly of each palaeotropic *Derris*-like taxon is reported, together with the non-monophyly of

Derris s.s. and the infrageneric classification previously proposed. Characters supporting each clade are briefly discussed.

Chapter 4 presents the integration of molecular and morphological data as total evidence for a phylogenetic reconstruction of *Derris*-like taxa. More insight is gained in the evolution of specific morphological traits. A new generic circumscription is proposed in order to maintain the monophyly of *Derris* s.s, by reinstating the genus *Brachypterum* and by synonymising *Paraderris* with *Derris*. Characters supporting each genus are presented in a taxonomic treatment and nomenclatural changes are made where necessary.

Chapter 5 is the proposal to conserve the name *Brachypterum* against *Solori*. The latter is the oldest available name and should be used, but it is long forgotten and never used, not even a single species was described in it.

Chapter 6 deals with the historical biogeography of three genera, namely *Aganope*, *Brachypterum* and *Derris* s.s (including *Paraderris* according to the results of the *Chapter* 3 and 4). Molecular dating were performed in a Bayesian framework with the aid of the program BEAST, and ancestral area reconstruction (AAR) analyses using parsimony-based S-DIVA and likelihood based analyses under the dispersal-extinction-cladogenesis model, implemented the programs RASP and Lagrange respectively. As the combinations proposed in *Chapter* 4 are not yet validly described, the new species names used in this Chapter are in between quotation marks "..." and without author name.