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Chapter 1  General Introduction

This dissertation studies four aspects of the plant genus Trigonostemon Blume (Euphorbiaceae): the taxonomy, pollen morphology, molecular phylogeny and historical biogeography. The background of the research, limitations in the previous studies and an outline of the dissertation are provided in this chapter.

Background: the family Euphorbiaceae and the genus Trigonostemon

The plant family Euphorbiaceae is one of the most morphologically diverse groups among the Angiosperms. The family (sensu APG IV 2016, see below) contains 218 genera and c. 6700 species (Stevens 2001 onwards). A diagnostic set of characters applying to all Euphorbiaceae is lacking. There are a few characters that in combination are more or less typical of the majority of genera and species, e.g., the presence of stipules, a superior ovary and a rhegma (an often explosively dehiscing fruit, where only the columella remains), but exceptions are rampant (except the superior ovary). The exact circumscription of the Euphorbiaceae varied enormously through the ages, many small families have been included or excluded, see Radcliffe-Smith (1987) for an overview (e.g., Buxaceae, Daphniphyllaceae, Stilaginaceae). With the advancement of APG, the order Malpighiales, in which the Euphorbiaceae are classified, proved to be difficult to solve phylogenetically. Presently (APG IV 2016; Figure 1), the Euphorbiaceae are split in various families. For the Pandaceae and Putranjivaceae, two morphologically rather different families, it is now clear that they are not closely related to the Euphorbiaceae. The former subfamilies with two ovules per locule now form the Phyllanthaceae and Picrodendraceae (sister clades in APG IV). The subfamilies with one ovule per locule (Acalyphoideae, Crotonoideae, Euphorbioideae) formed at first the Euphorbiaceae in the strict sense, till it became obvious that the Rafflesiaceae form a clade just above the basal clade of the Euphorbiaceae (Wurdack & Davis 2009). The parasitic Rafflesiaceae have inferior ovaries with many ovules per locule, uniting them with the Euphorbiaceae would not only mean that a well-recognisable family will disappear (Rafflesiaceae), but also that the Euphorbiaceae will lack any diagnostic characters. Therefore, the basal Peroideae clade (Wurdack et al. 2005) is now accepted as the separate family Peraceae (APG IV 2016), next to the Rafflesiaceae and Euphorbiaceae.

Trigonostemon was established by Blume in 1825. The genus name refers to the stamens that form a triangle when seen from above. It includes a total of 59 species (Chapter 4), ranging from S India and Sri Lanka to S China, throughout mainland SE Asia and Malesia to NE Australia and the W Pacific (Govaerts et al. 2000; Map 1). The genus has always been classified in the subfamily Crotonoideae. There are four clades in the Crotonoideae based on the molecular phylogeny of Wurdack et al. (2005), the upper two (marked as C1 and C2 in Wurdack et al. 2005) are characterised by inaperturate pollen grains. Trigonostemon is the largest genus in the C2 clade within the polytomy of 25 other genera including Ostodes Blume (Figure 2), which is often considered to be closely related to Trigonostemon. Dimorphocalyx Thwaites was not included in the phylogeny, although it was also considered to be a close relative of Trigonostemon and Ostodes. Among the various genera, Trigonostemon can be recognised mainly by having colourful petals in both staminate and pistillate flowers and 3 or 5 connate stamens (Figure 40).
Limitations in previous studies

*Trigonostemon* is a difficult group in taxonomy. In identification keys it is generally keyed out negatively, always at the end of blocks and often in various blocks (e.g., van Welzen 2005). Within the genus, more than 140 taxa have been described (Govaerts et al. 2000), but the species delimitation has always been blurred and problematic. On the one hand, there is a limitation in the use of the floral characters in species delimitation; on the other hand, massive variation exists in the vegetative characters (for the taxonomic history and a detailed discussion of characters see Chapter 2). It is often difficult for botanists to decide whether a character is useful without studying the whole genus. As a result, many species were described merely based on extreme forms in a continuous variation. A comprehensive revision was urgently needed, in which the variation in all characters would be carefully examined and evaluated and all species would be revised by the same criteria.

Pollen morphology often provides extra evidence for taxonomy (Erdtman 1952). *Trigonostemon* pollen has hardly been studied before, but in the only literature (Punt 1962) a unique type of exine ornamentation was found. The ‘croton pattern’ ornamentation is a key character in the
pollen of Crotonoideae and a few other taxa (Punt 1962; Nowicke 1994), but the pollen of some *Trigonostemon* species was shown to have a verrucose exine. This is possibly indicative of an evolutionary change, but before concrete conclusions could be made, a more extensive exploration of the pollen morphology of the whole genus was needed.

Although it is clear that *Trigonostemon* (one whorl of 3 or 5 stamens) should be classified in the subfamily Crotonoideae, opinions are divided as to its generic circumscription: Müller (1865, 1866) merged *Dimorphocalyx* (Thwaites 1861; two whorls of 7–20 stamens, Figure 40k) and *Tritaxis* Baill. (Baillon 1858; three whorls of c. 13 stamens, for illustration see Gagnepain 1925b) with *Trigonostemon* into a *Trigonostemon* sensu lato; other botanists, e.g., Bentham (1878, 1880) and Pax (1890), treated *Trigonostemon* strictly according to Blume’s concept (1825). Furthermore, the previous infrageneric classifications of *Trigonostemon* (for a detailed review of the infrageneric classifications see Chapter 2) are problematic. They only relied on morphological characters of a limited number of species, and contained several cases of misidentification and misplacement.

Phylogenetic systematics has the potential to solve these problems. Compared to classical taxonomy, the cladistic approach (Hennig 1965) incorporates evolutionary theory in its methodology by discriminating between primitive and derived homologous character states (though the discrimination of characters and their states can be subjective). Taxa are considered phylogenetically related because of shared derived characters. This provides a relatively more objective way to select useful characters compared to classical taxonomy. The selection of characters (and how much weight to put on each selected character) is also critical in classical taxonomy, but it is based on comparative morphology (van Steenis 1957) and depends heavily on one’s (subjective) experience. Nucleotide sequences in the plant genome provide a much larger amount of data (characters) to evaluate the similarity between taxa, and the subjectivity has changed to the alignment phase to establish which parts of the sequences are homologous. The results obtained by molecular data can also be used to analyse and interpret the changes in macromorphology and pollen morphology.
Trigonostemon has a typical Southeast Asian distribution. This area comprises six of the 25 biodiversity hotspots identified by Myers et al. (2000). Most species of *Trigonostemon* are found in the Flora Malesiana region (van Steenis 1950; Raes & van Welzen 2009), which contains one of the world’s richest floras (42,000 vascular plant species as estimated by Roos (1993), or even up to 45,000 species as estimated by Corlett & Primack (2011)). The migration routes (particularly those across the Wallace’s line; van Welzen et al. 2011 and references therein) of plants and animals in this area have always been a subject of great interest in biogeography, but the general migration patterns can only be discovered when a sufficient number of case studies are performed. *Trigonostemon* can provide a good addition to this research subject, because its distribution covers all SE Asian regions with the only exception of Sulawesi. Previous studies have shown that the Philippines often acted as a stepping stone for the migrations between West and East Malesia (e.g., Nauheimer et al. 2012, Thomas et al. 2012). The migration routes of *Trigonostemon* can be used to compare with the previous studies and evaluate this conclusion.
Outline of this dissertation

Chapters 2–4 are taxonomic revisions of *Trigonostemon*. These chapters only deal with the species within *Trigonostemon sensu stricto* (Blume 1825), aiming to correct the previous nomenclatural errors, and to provide a reliable delimitation between the species. These chapters serve as the basis of the following chapters. Species boundaries were determined by morphological discontinuities (i.e., the ‘morphological species concept’). Expressions of this concept can be found in e.g., Linnaeus 1736, 1754, Lindley 1832, Bentham 1875, Gray 1887, Du Rietz 1930, Davis & Heywood, 1963 and Cronquist 1978. In practice, both the differences and similarities (the two phases of ‘analysis’ and ‘synthesis’ as argued by van Steenis 1957) among specimens were always taken into account. Chapter 2 does not include data obtained from the field work in the Philippines. This field trip could continue somewhat unexpectedly, and resulted in many new data for various species that were poorly understood from herbarium material. This necessitated a new article (Chapter 3), in which the descriptions of the relevant species were updated. Time, normally limited, permitted the final revision of the non-Malesian species of the genus, mainly found in the Indian subcontinent, Indochina and South China. The results can be found in Chapter 4.

Chapter 5 is an exploration of the pollen morphology of *Trigonostemon* and its close relatives, aiming to gain insight in the delimitation and infrageneric classification of *Trigonostemon*. The pollen of various species often has a different degree of resistance to acetolysis. Whether the anther is mature or not also affects the pollen morphology. Pollen of young flowers tends to have closed lumina. When assessing the variation, deformed or too young pollen grains were not used to avoid bias. *Trigonostemon* appeared to have two main types of pollen (one type that could be subdivided into two). The next chapter explains the correlations between these pollen types and the molecular phylogeny.

Chapter 6 reconstructs the phylogenies of *Trigonostemon* and its close relatives using five molecular markers (the nuclear ITS and chloroplast *trnK* intron, *trnT*-L, *trnL*-F and *rbcL* sequences). This is the core chapter, aiming to (1) confirm the systematic position of *Trigonostemon* in the Euphorbiaceae, (2) clarify the generic delimitation of *Trigonostemon* and its relatives/look-alikes (i.e., *Dimorphocalyx*, *Tritaxis* and *Ostodes*), (3) interpret the evolutionary trends in *Trigonostemon* in reference to the pollen morphology and (4) revise the infrageneric classification of the genus. About two third of the species of *Trigonostemon* were represented in the analyses. The molecular phylogenies were inferred by maximum parsimony, maximum likelihood and Bayesian methods. Characters in morphology and pollen morphology were mapped on the phylogeny to visualize their evolutionary trends. Because the phylogeny will clarify the generic boundaries, one might argue that it would have been better to have the phylogenetic analyses before the taxonomic revisions, but the single species are the actual entities in the analyses, and it was necessary to have a clear species concept before reconstructing the phylogenies.

Chapter 7 builds on the previous chapter. Historical biogeographic analyses of the genera *Trigonostemon* and *Dimorphocalyx* are made, in order to investigate their geographical origin and dispersal routes, and to provide an explanation for the rapid diversification in sect. *Trigonostemon* from a historical biogeographic angle. A Bayesian dating analysis was performed to infer the divergence times of the nodes, and the ancestral areas were optimised using a likelihood analysis under the Dispersal-Extinction-Cladogenesis (DEC) model and a Statistical Dispersal-Vicariance analysis (S-DIVA). The dispersal/vicariance events were discussed in light of the tectonic history of SE Asia, and the historical biogeography was reconstructed.