Antiquities of the rainforest: evolution of mycoheterotrophic angiosperms growing on Glomeromycota

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Chapter 1

General introduction

Next page: *Corsia* cf. *huonensis*, drawing by R.C. van der Stelt

Corsia cf. huonensis

General introduction

"Mr. Spruce's expedition has added much to our catalogue of these species. He found them particularly to abound in the forests of the Rio Uaupés, and other tributaries of the Rio Negro. They are there generally known to the Indians by the name of Juruparì-erenuana*, that is, "Devil's beard"; "but assuredly," adds Mr. Spruce, "the Devil is not so black as he is painted, if these pretty things resemble anything about his sable majesty."*

– George Bentham, "On the South American Triurideae and leafless Burmanniaceae from the collections of Mr. Spruce" (1855)

Plants are the main primary producers in terrestrial ecosystems, as they are able to assimilate carbon dioxide through photosynthesis into sugars. There are, however, exceptions to this autotrophic mode of life in plants. In a few lineages of land plants, most of which are flowering plants, selection has favoured characteristics leading to ways that obviate the need for photosynthesis, namely by obtaining carbohydrates from other organisms: they are heterotrophic. Heterotrophic plants can be either parasitic on other plants or parasitic on soil fungi. An example of the first group of parasites is the Malesian genus *Rafflesia*, which includes the species, *R. arnoldii* R. Br., with the world's largest flower. Apart from its spectacular flower, the vegetative parts of this plant are reduced to few-celled threads embedded in the tissues of its host plant (e.g. Nikolov et al., 2014). This exemplifies the potential of extreme evolutionary adaptation to the parasitic mode of life. The second group comprises the putative parasites on soil fungi: the mycoheterotrophic plants. This remarkable group of plants includes roughly 520 species that are able to obtain carbon compounds from fungi. Besides these fully mycoheterotrophic species, some plants are putatively "partially" mycoheterotrophic, capable of obtaining carbon through both mycoheterotrophy and photosynthesis. Although the term "mycoheterotrophy" was first used as recently as 1994 by Jonathan Leake (Leake, 1994), the phenomenon has been studied for some two centuries (Bidartondo, 2005). Mycoheterotrophic plants are often referred to as "saprophytes", although this term is misleading as it implies the plants are physiologically linked to dead organic material rather than to fungi (Leake, 1994). This thesis aims to shed light on some of the evolutionary trajectories that gave rise to mycoheterotrophic plants.

1.1. Mycoheterotrophy, mostly an extreme type of mycorrhizal symbiosis

Roughly 80% of all land plants, including the majority of the economically important crops, potentially engage in an intimate mutualistic symbiosis with soil fungi: the mycorrhizal symbiosis. This symbiosis occurs in the roots of the plants. In this widespread symbiosis, the plant provides photosynthetically synthesized carbohydrates to the fungi, and it obtains water and minerals from the fungi (Smith & Read, 2008; Merckx et al., 2013a). Such symbioses are believed to be extremely ancient and considered to have been important in the successful colonization of the land by plants during the Ordovician (Bidartondo et al., 2011). In some plants evolution has led to break-down of this mutualism and the obtaining of carbohydrates, water and minerals from their fungal symbionts (Bidartondo, 2005). This nutrient flow from autotrophic plant through fungus to mycoheterotrophic plant was first demonstrated by Björkman (1960) using radioactively labelled isotopes. Using these radioactive tracers, Björkman (1960) observed more transport of carbon and phosphorous from spruce trees (*Picea* sp.) to the mycoheterotrophic species *Hypopytis monotropa* than to surrounding chlorophyllous plants. He furthermore identified fungi that formed mycorrhizas with spruce roots from the roots of *H. monotropa* by cultivating them, and he observed that *H. monotropa* plants that were physically separated from the roots of spruce trees by metal sheets showed reduced growth (Björkman, 1960). This experimental evidence unravelled some of the physiological processes that underly mycoheterotrophy. These findings were later corroborated by stable isotope analyses of carbon and nitrogen signatures of chlorophyllous plants, ectomycorrhizal fungi and mycoheterotrophic plants (Hynson et al., 2013). The stable isotopes ^{13}C and ^{15}N are enriched in these mycoheterotrophic plants as compared to neighboring chlorophyllous plants. This enrichment reflects the natural abundance of these isotopes in ectomycorrhizal fungi. This demonstrates a mechanism for nutrition in mycoheterotrophic plants different from that in neighbouring chlorophyllous plants, implying that the carbon and nitrogen in these mycoheterotrophic plants most likely have a fungal source. This phenomenon is hypothesized to be a means by which plants evade heavy competition for light in tropical understorey habitats, and are able to grow and reproduce in the shade (Leake, 1994; Bidartondo, 2005). Although some mycoheterotrophic orchids (e.g. *Epipogium roseum*, some *Gastrodia* spp., *Wullschlaegelia aphylla* (Merckx et al., 2013b)) exploit saprophytic fungi, the vast majority of species are symbiotically linked to mycorrhizal fungi (Waterman et al., 2013). As a consequence of the mycoheterotrophic lifestyle, many mycoheterotrophic plants exhibit reduced vegetative morphology and modified floral structures, exemplified by the short stem, scale-like leaves, and fused and peculiarly shaped tepals in *Thismia neptunis* (Thismiaceae, Fig. 1). The vast majority of all mycorrhizal symbioses, as well as all mycoheterotrophic angiosperms except those in the species-rich families Orchidaceae and Ericaceae, involve plants linked to mycorrhiza from the arbuscular type (arbuscular mycorrhizal fungi, AMF). These fungi belong to the phylum Glomeromycota and penetrate the root cells of host plants (as opposed to “ectomycorrhizal” fungi which usually grow between root cells), in which they often form tree-like structures called “arbuscules” which can have different forms and shapes (Smith & Read, 2008). The number of mycoheterotrophic angiosperms growing in symbiosis with this type of fungus is approximately 240 (Merckx et al., 2013b). These plants form the focus of this thesis.

1.2. A brief history of the systematics of mycoheterotrophic plants

Since the discovery and description of plants that were later found to be mycoheterotrophic from the eighteenth century onwards (e.g. Linnaeus, 1753; Graves, 1819; Blume, 1825), biologists have wondered about their obscure and remarkable nature. Although the genus *Monotropa* (Ericaceae) played an important role in the early research on mycorrhizal symbiosis (and generally in our understanding of symbioses; see Bidartondo (2005) for a review), it was problematic to classify mycoheterotrophic plants and understand their systematic relationships. First, they led to problems in taxonomy because it was unclear how to classify these plants given their peculiar morphology (a detailed example of this is given for Triuridaceae in Chapter 2). As a consequence, mycoheterotrophic plant groups were often lumped together (e.g. Corsiaceae and Thismieae in Burmanniaceae (Bentham & Hooker, 1883)) or placed in isolated orders (e.g. Triuridales (Engler, 1897)). Later, when evolutionary relationships among land plants were revealed by DNA sequence data (e.g. Chase et al., 2000), phylogenetic placement of these plants proved to be problematic as their genomes were strongly divergent as a result of elevated substitution rates (Merckx et al., 2006; 2009a). The elevated substitution rates, as indicated by long branches in phylogenetic reconstructions, are likely the result of the loss of evolutionary constraints on photosynthesis-related genes, particularly in the plastid genome (Lemaire et al., 2011; Barrett & Davis, 2012). Therefore, it was rather difficult to identify the closest "green" relatives of these plants and to unravel their evolutionary history. In recent years, however, scientists have overcome this problem by focusing on DNA sequence data from the nuclear and mitochondrial genomic regions (e.g. Merckx et al., 2006, 2008, 2013c) or on full plastome datasets (e.g. Lochageva et al., 2014). Although most mycoheterotrophic plant families are now phylogenetically placed with reasonable support values in the Tree of Life (i.e. the relationships with their closest chlorophyllous relatives are known), a lack of vouchered plant collections has further hampered evolutionary studies, as only a limited percentage of the described species is generally available for study.

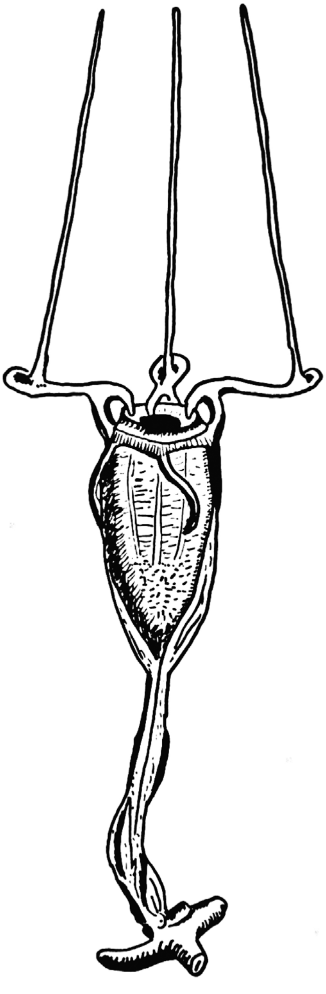

Fig. 1. An example of the strongly reduced habit and highly modified flowers in mycoheterotrophic plants. *Thismia neptunis* redrawn from Engler (1889a).

This is probably due to the plants' inconspicuous nature; they are indeed only rarely collected. Since these plants are mostly rather small and often slender and hidden in leaf litter, they are easily overlooked in the field. Despite these limitations, a growing number of studies has been made on the evolution of mycoheterotrophic plants since the publication of Leake (1994) and the molecular revolution in plant systematics (Chase et al., 1993). As it turns out, many lineages of mycoheterotrophic plants are highly informative for unravelling particular evolutionary processes, such as historical biogeography (Merckx et al., 2008, 2013c), specialization on mycorrhizal fungi (Merckx et al., 2012), or genomic evolution (Barrett & Davis, 2012). Moreover, the current distribution and reconstructed phylogenies of several mycoheterotrophic lineages (e.g. Burmanniaceae (Merckx et al., 2008); *Voyria* (Gentianaceae; Merckx et al., 2013c)) may provide new insights on ancient rainforest occurrence and help in reconstructing palaeoenvironments. At the same time scientists are increasing understanding of the physiology and ecological interactions of these plants (e.g. Hynson et al., 2013), deepening our understanding of the nature of parasitism and fungal specialization in general.

1.3. On the origin of mycoheterotrophic plants

Full mycoheterotrophy is estimated to have evolved at least 47 times during the evolution of land plants (Merckx et al., 2013a). A single liverwort, as well as several gametophytes of ferns and lycophytes are mycoheterotrophic (Merckx et al., 2013b). A curious case is the gymnosperm *Parasitaxus usta* (Podocarpaceae), which appears to be mycoheterotrophic, yet it differs largely (for example in being directly linked to another plant, but lacking a typical haustorium) from other mycoheterotrophic and parasitic plants (Feild & Brodribb, 2005). The majority of the mycoheterotrophic plants however, belongs to the angiosperms. Mycoheterotrophy occurs in a wide array of taxonomic groups in monocots and eudicots. However, most families containing mycoheterotrophic plants belong to the monocots (Fig. 2). Eight angiosperm families contain mycoheterotrophic species that are linked to AMF. In recent years, phylogenetic inference and divergence time estimations have resulted in the insights presented below, about the evolutionary history of these lineages (e.g. Janssen & Bremer, 2004; Goldblatt et al., 2008; Merckx et al., 2008, 2010a, 2013c). The evolutionary history of the families Triuridaceae, Corsiaceae and Polygalaceae is particularly interesting as these enigmatic groups contain several disjunctly distributed taxa (e.g. *Arachnitis, Corsia* and *Seychellaria*, see Merckx et al., (2013b)) and little is known about their phylogenetic relationships and biogeographic history. These families form the subjects of this thesis.

1.4. *Petrosavia* (Fig. 3G)

Petrosavia (Petrosaviaceae) is a fully mycoheterotrophic genus that comprises three species and is the sister group of the chlorophyllous monospecific genus *Japonolirion*

Fig. 2. Order-level phylogenetic reconstruction of angiosperms based on the APGIII system (APG, 2009). Families containing mycoheterotrophic lineages are indicated; red bars show mycoheterotrophic plants growing on AM fungi, blue bars show those growing on ectomycorrhizal fungi. The blue bar of Orchidaceae (Asparagales) also includes species growing on saprotrophic fungi. The scale bar indicates numbers of species. This representation is modified from Merckx et al. (2013a).

from Japan (Cameron et al., 2003). Its species occur in forests in Southeast Asia and southern Japan (Chen & Tamura, 2000; Ohashi, 2000; Merckx et al., 2013b). The stem age of the genus has been estimated to be 123 million years old (Ma) (Janssen & Bremer, 2004). *Petrosavia sakuraii* was found to grow in symbiosis with a narrow set of AM fungi (Yamato et al., 2011a); it is probably mainly self-pollinating (Takahashi et al., 1993). The method of seed dispersal in species of *Petrosavia* remains unknown (Merckx et al., 2013b).

1.5. Burmanniaceae (Fig. 3A)

Burmanniaceae (Dioscoreales) is a pantropical family of about 96 species of which 59 are fully mycoheterotrophic. All chlorophyllous species belong to *Burmannia*, though this genus also includes fully mycoheterotrophic species (37 of 56 spp. are chlorophyllous). The genus is widely distributed in all (sub)tropical regions. The remaining mycoheterotrophic species belong to seven other genera; *Campylosiphon* (2 spp. found in the Neotropics and West Africa, respectively), *Hexapterella* (2 spp. found in the Neotropics), *Dictyostega* (1 sp. found in the Neotropics), *Miersiella* (1 sp. found in the Neotropics), the pantropically distributed genus *Gymnosiphon* (32 spp.), *Apteria* (1 sp. occurring in the (sub)tropics of the Americas), and *Marthella* (1 sp. exclusively found in Trinidad) (Merckx et al., 2013b). The species mostly occur in rainforests, but *Apteria aphylla* and chlorophyllous species of *Burmannia* sometimes occur in wet savanna (Merckx et al., 2013b). The family has an estimated mean stem node age of 109 Ma (Merckx et al., 2010a). The family probably greatly diversified during the warm Eocene (Merckx et al., 2008). Furthermore, early speciation and migration events are hypothesized to be influenced by the tectonic split of South America and Africa (Merckx et al., 2008). Members of Burmanniaceae mostly grow in symbiosis with AM fungi of Glomeraceae, but sometimes (also) with Acaulosporaceae (Merckx et al., 2012). Little is known about the pollination of Burmanniaceae. Although floral morphology (coloured flowers, presence of septal nectaries) suggests insect pollination, self-pollination likely occurs in some species (e.g. Zhang & Saunders, 1999, 2000; Merckx et al., 2013b). The dust-like seeds are probably dispersed by either water or wind (Maas-van de Kamer, 1998).

1.6. Thismiaceae (Fig. 3D)

Thismiaceae (Dioscoreales) forms a paraphyletic family of about 63 species in four closely related genera (*Thismia* (~45 spp.; occurring in South America, Asia, Australia and New Zealand), *Tiputinia* (1 sp. found in Ecuador), *Haplothismia* (1 sp. found in India) and *Oxygyne* (4 or 5 spp.; found in tropical Africa and southern Japan)) and a single more distantly related genus (*Afrothismia*, 12 spp. found in tropical Africa). Thismiaceae mainly are found in the tropical regions of the world, although a few species occur in subtropical and temperate areas. All species occur in forest habitats (Maas et al., 1986; Merckx et al., 2013b), although a single species

(*Thismia americana*) was found in open prairie vegetation in North America (Merckx & Smets, 2014). The clade of Thismiaceae (i.e. all genera except *Afrothismia*) has an estimated mean stem age of 79 Ma. *Afrothismia* has an estimated mean stem age of 95 Ma (Merckx et al., 2010a). Remarkably disjunct distribution patterns are found in some genera. The widespread genus *Thismia* occurs in Southeast Asia and South America, but is absent from Africa. Moreover, *Thismia* also occurs in temperate regions of Australia and New Zealand and has a remarkable historical occurrence of a single species in North America (Merckx & Smets, 2014). The enigmatic genus *Oxygyne* is known from western and central Africa, as well as from southern Japan and the islands of Shikoku, Okinawa and Yakushima (Merckx et al., 2013b). Species of *Thismia* and *Afrothismia* grow in symbiosis with AM fungi of Glomeraceae (Merckx & Bidartondo, 2008; Merckx et al., 2012). Pollination of species of Thismiaceae remains poorly studied, but the wide array of colours and shapes in the family suggests primarily insect pollination (Merckx et al., 2013b). The dust-like seeds might be dispersed by either water or wind (Maas et al., 1986).

1.7. Triuridaceae (Fig. 3E)

Triuridaceae is a fully mycoheterotrophic family of about 50 species in 11 genera (*Andruris* (5 spp.; found in Asia and Australia), *Hyalisma* (1 sp. found in India and Sri Lanka), *Kupea* (2 spp. found in tropical Africa), *Kihansia* (1 or 2 spp. found in tropical Africa), *Lacandonia* (2 spp. found in the Neotropics), *Peltophyllum* (2 spp. found in the Neotropics), the pantropically distributed genus *Sciaphila* (~29 spp.), *Seychellaria* (3 spp.; occurring in tropical Africa, Madagascar, the Comores and the Seychelles), *Soridium* (1 sp. found in the Neotropics), *Triuris* (3 spp. found in the Neotropics) and *Triuridopsis* (2 spp. found in the Neotropics) in three tribes (Sciaphileae, Triurideae and Kupeaeae) (Mennes et al., 2013; Merckx et al., 2013b). The family has a pantropical distribution, reaching subtropical regions in Argentina, Paraguay and Japan (Maas-van de Kamer & Weustenfeld, 1998; Maas & Rübsamen, 1986; Van de Meerendonk, 1984). The species are found in humid forests (Merckx et al., 2013b). All genera except the pantropical genus *Sciaphila* are restricted to a single continent (Merckx et al., 2013b). The distribution of *Sciaphila* was suggested to be an indication of a great age of the family (Leake, 1994). This putative old age is further suggested by the existence of putative Triuridaceae fossils (*Mabelia* and *Nuhliantha*) (Gandolfo et al., 1998, 2002). AM fungi of Glomeraceae have been detected in the roots of species of *Schaphila* and *Kupea*, although a wider range of fungi from Glomeraceae, Gigasporaceae and Acaulosporaceae was found in the roots of *Sciaphila ledermannii* (Yamato et al., 2011b; Merckx et al., 2012). The genus *Lacandonia* has its stamens surrounded by carpels; an extremely rare "inside-out" configuration (Martínez & Ramos, 1989). Floral morphology in Triuridaceae strongly suggests that the plants are pollinated by insects, as indicated by the presence of scent and tepals with various types of appendages (e.g. Maas-van de Kamer, 1995; Maas-van de Kamer & Weustenfeld, 1998). Seeds are perhaps dispersed by animals, water and/or wind (Maas-van de Kamer, 1995; Maas-van de Kamer & Weustenfeld, 1998).

1.8. Corsiaceae (Fig. 3C)

Corsiaceae (Liliales) is a fully mycoheterotrophic family that consist of roughly 30 species in three genera (*Arachnitis* (1 or 2 spp.), *Corsia* (~27 spp.) and *Corsiopsis* (1 sp.)), which occur disjunctly in Australasia, South America and China (Neinhuis & Ibisch, 1998; Zhang et al., 1999; Jones & Gray, 2008). All species occur in temperate/subantarctic or tropical rainforests (Merckx et al., 2013b), although *Arachnitis* is also found in open habitats on the Falkland Islands (Cribb et al., 1995). *Arachnitis uniflora* was found to grow in symbiosis with a narrow range of AM fungi of Glomeraceae (Bidartondo et al., 2002). The fungal symbionts of species of *Corsia* and *Corsiopsis* remain unstudied (Merckx et al., 2013b). The presence of protandrous flowers suggests cross-pollination (Rudall & Eastman, 2002; Ibisch et al., 1996). Seed dispersal mechanisms are unknown (Merckx et al., 2013b).

1.9. *Geosiris* (Fig. 3F)

The family Iridaceae (Asparagales) contains a single fully mycoheterotrophic genus (*Geosiris*) with two species occurring in the rainforests of Madagascar and the Comores (Goldblatt et al., 2008; Goldblatt & Manning, 2010). The stem age of *Geosiris* was estimated to be roughly 49 Ma (Goldblatt et al., 2008). The common ancestor of *Geosiris* and its relatives is hypothesized to have reached Africa-Madagascar about 55 Mya via long distance dispersal across the proto-Indian Ocean (Goldblatt et al., 2008). Mycorrhizal symbionts of *Geosiris* are supposedly AM fungi similar to those in other Iridaceae, but this remains to be investigated (Merckx et al., 2013b). Likewise, little is known about pollination and seed dispersal in this genus (Merckx et al., 2013b).

1.10. *Epirixanthes* (Fig. 3B)

Epirixanthes (Polygalaceae, Fabales) is the only fully mycoheterotrophic genus in the rosids and comprises six species, all occurring in the tropical rainforests of Southeast Asia (Van der Meijden, 1988; Pendry, 2010). Based on morphology symbiotic fungi from the roots of *Epirixanthes* were identified as AM fungi (Imhof, 2007). Seed dispersal by ants was suggested, also based on morphology (Van der Meijden, 1988), and this might be corroborated by observations made of ants visiting flowers during fieldwork for this thesis (see Fig. 3B). Autogamy has been suggested to be the most likely pollination mechanism in species of *Epirixanthes* (Wirz, 1910), although cross-pollination may be possible at some stage during development (Van der Meijden, 1988). More studies on pollination and seed dispersal in *Epirixanthes* are needed.

1.11. Gentianaceae (Fig. 3H)

The family Gentianaceae (Gentianales) is a cosmopolitan family (absent from Antarctica) that consists of roughly 1650 species in 92 genera (Merckx et al., 2013b). Gentianaceae have several mycoheterotrophic representatives. The largest mycoheterotrophic genus *Voyria* has 19 species, all of which are mycoheterotrophic. *Voyriella* is a monospecific mycoheterotrophic genus. The mostly chlorophyllous genus *Exochaenium* has only a single mycoheterotrophic reresentative (*E. oliganthum*), whereas four of the 68 species of *Exacum* are mycoheterotrophic (Maas & Ruyters, 1986; Merckx et al., 2013b). Species of *Voyria* and *Voyriella* occur in the (sub)tropical areas of America, whereas a single mycoheterotrophic species of *Voyria* occurs in western and central Africa. The mycoheterotrophic species of *Exochaenium* occurs in Africa and those of *Exacum* occur in Asia (Merckx et al., 2013b). All mycoheterotrophic species of Gentianaceae grow in tropical rainforest habitats, although some species of *Voyria* have extended their range into savanna and/or subtropical habitats (Maas & Ruyters, 1986; Merckx et al. 2013b). The mean stem age of *Voyria* was estimated to be 47 Ma. The mycoheterotrophic species in the genera *Exacum*, *Exochaenium* and *Voyriella* have estimated stem ages of 6-16 Ma, 3-7 Ma and 15-42 Ma, respectively (based on a single mycoheterotrophic species represented for each genus). The trans-Atlantic distribution of *Voyria* is hypothesized to be the result of long distance dispersal or migration along a North Atlantic land bridge during the Miocene Climatic Optimum (Merckx et al., 2013c). Mycoheterotrophic species of Gentianaceae are symbiotically linked to AM fungi; fungi of Glomeraceae and Gigasporaceae were detected in the roots of species of *Voyria* and fungi of Glomeraceae were found in the roots of *Exochaenium oliganthum* (Merckx et al., 2012; 2013b). Many species of *Voyria* and mycoheterotrophic *Exacum* are probably cross-pollinated as is evidenced by floral morphology (bright colours, presence of nectar and scent) (Maas & Ruyters, 1986; Merckx et al., 2013b), although autogamy has been reported in *Voyriella* (Oehler, 1927).The presence of herkogamy in *Exochaenium oliganthum* (Kissling & Barrett, 2013) suggests cross-pollination, but cleistogamous flowers are also reported, suggesting selfing as pollination strategy as well (Raynal-Roques 1967, Merckx et al., 2013b). Seed dispersal mechanisms in mycoheterotrophic Gentianaceae remain poorly studied (Merckx et al., 2013b).

1.12. Towards a complete overview of the evolution of mycoheterotrophy

Despite the reduced fitness of albino individuals (i.e. mycoheterotrophic plants of otherwise chlorophyllous species) in some orchids (e.g. Roy et al., 2012) and the intuitive vulnerability of the extreme host specialization observed in some species (e.g. *Afrothismia* spp. (Merckx & Bidartondo, 2008) and *Arachnitis uniflora* (Bidartondo et al., 2002)), an important observation from the age estimations available for some of the mycoheterotrophic lineages described above, is that most lineages are rather old (i.e. some date back to the Cretaceous, others are at

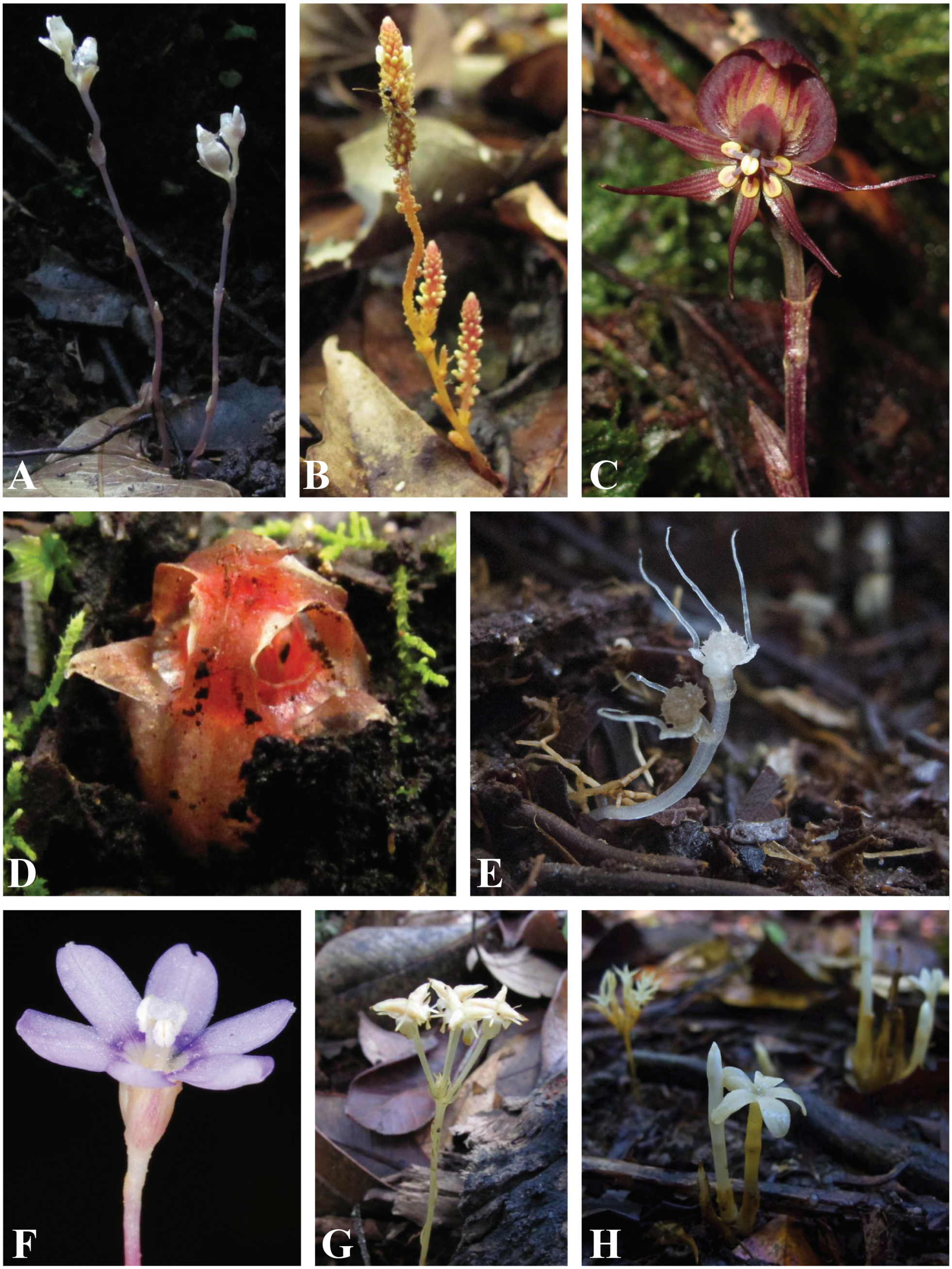

Fig. 3. Mycoheterotrophic representatives of each family containing AM mycoheterotrophic species. Photos by C.B. Mennes, except where noted. (A) *Gymnosiphon* cf. *aphyllus* pictured in Sabah, Borneo. (B) *Epirixanthes elongata* from Sabah, Borneo. (C) *Corsia* cf. *huonensis* from Huon Peninsula, Papua New Guinea (photo: S.P. Lyon). (D) *Thismia rodwayi* pictured near South Sister, Tasmania. (E) *Triuris hyalina* growing in French Guiana (photo: V.S.F.T. Merckx). (F) *Geosiris aphylla* from Madagascar (photo by Ehoarn Bidault, modified from Merckx et al., 2013b). (G) *Petrosavia stellaris* growing on Mount Kinabalu, Borneo. (H) *Voyria caerulea* pictured in French Guiana (photo: V.S.F.T. Merckx).

least several millions of years old). This was first hypothesized for some groups based on their pantropical distribution. For example, for Triuridaceae an ancient age of divergence was hypothesized by Leake (1994), on the basis of the widely distributed genus *Sciaphila*. Later, Cretaceous fossils were found and were assigned to Triuridaceae (Gandolfo et al., 1998, 2002), also suggesting a great age of the family. No divergence time estimation has been carried out for Triuridaceae thus far, and its phylogenetic position remains enigmatic (e.g. Rudall & Bateman, 2006). Similarly, the family Corsiaceae was found to be an early diverging lineage in Liliales, although its exact position remains unknown (e.g. Davis et al., 2004; Fay et al., 2006; Petersen et al., 2013, Kim et al., 2013). Moreover, a putative Gondwanan origin was suggested by Zhang et al. (1999) based on the remarkably disjunct distribution of the family. This might suggest an old age of the family, as well as a vicariance-based scenario explaining its distribution. The last mycoheterotrophic lineage to be studied in detail was *Epirixanthes* (Polygalaceae). However, for this sole mycoheterotrophic representative of the rosids little information was available about its evolutionary history prior to the present study due to the lack of DNA sequence data. Many unrelated lineages of mycoheterotrophic plants are often found growing together in the rainforest understorey (Leake, 1994), which raises the question whether common evolutionary trajectories towards mycoheterotrophy can be found in unrelated groups. Triuridaceae, Corsiaceae and *Epirixanthes* are among the last lineages for which the phylogenetic position is not yet confidently resolved. Thus, information on divergence times and historical biogeography of Triuridaceae, Corsiaceae and *Epirixanthes* is thus far incomplete. By obtaining this information, an overview of the evolutionary history of all mycoheterotrophic lineages will be available, allowing us to get new insights in the evolution of mycoheterotrophic plants growing on AM fungi in general.

1.13. Aims and outline of this thesis

This thesis aims at understanding the evolutionary history of mycoheterotrophic angiosperms living in symbiosis with (and probably parasitizing) arbuscular mycorrhizal fungi. It presents three studies of mycoheterotrophic lineages for which the evolutionary history has been unclear: Triuridaceae, Corsiaceae and *Epirixanthes*. A fourth study provides a biogeographical meta-analysis of all mycoheterotrophic plants growing on AM fungi. The latter study combines data from all studies mentioned above and includes over one third of the described species across all eight families and their mycoheterotrophic lineages.

Chapter 2 discusses new insights in the evolutionary history of the fully mycoheterotrophic family Triuridaceae (Pandanales). We present the first phylogenetic study encompassing all three tribes. We found that Triuridaceae and all its tribes form a monophyletic family and probably descend from the second major split in Pandanales. We furthermore estimate the stem age of the family. It is discussed whether or not the stem age, elevated mutation rates indicated by long branch lengths, as well as the mycoheterotrophic lifestyle, might account for the substantial morphological differences between Triuridaceae and its closest relatives.

A second study focuses on the phylogenetic relationships and divergence time estimation of Corsiaceae (Liliales) and is presented in **Chapter 3**. Corsiaceae forms a prime example of a family which has a disjunct distribution across the southern Pacific Ocean. This chapter presents a phylogenetic reconstruction of the family which is needed to understand its historical biogeography. We identify the closest chlorophyllous relatives of Corsiaceae. We then estimate divergence times of Corsiaceae, and discuss whether this age overlaps with the plate-tectonic split of Gondwana. We address the question of whether a vicariance-based scenario might explain the current distribution of the family.

In **Chapter 4** the evolution of mycoheterotrophy in Polygalaceae is discussed. During the Kinabalu – Crocker Range scientific expedition (Naturalis Biodiversity Center – Sabah Parks, 2012), material of four of the six species of *Epirixanthes* (Polygalaceae) was collected. We reconstruct the phylogenetic relationships of the genus. Divergence times are also inferred, and we discuss whether or not the evolutionary history of *Epirixanthes* was influenced by the complex environmental dynamics in Southeast Asia during this time. Additionally, we studied the fungal symbionts of *Epirixanthes* and compare the specificity towards fungal lineages with that of *Salomonia* and *Polygala.*

Chapter 5 provides a meta-analysis in which we analyze divergence times of all mycoheterotrophic lineages growing on AM fungi in a standardized way. Moreover, global historical biogeographic patterns are inferred for the families that are found on multiple continents. Evolutionary trends are discussed; we addressed the hypothesis that different lineages with similar ancestral areas originated simultaneously.