Miscellaneous Contributions to the Taxonomy and Mycorrhiza of AMF-exploiting Myco-heterotrophic Plants

Dissertation
zur Erlangung des Doktorsgrades der Naturwissenschaften
(Dr. rer. nat.)
der Fakultät für Biologie der Ludwig-Maximilians-Universität
München

vorgelegt von

Thassilo Franke

München

15. Januar 2007

Datum der mündlichen Prüfung: 16. Mai 2007
Erstgutachter: Prof. Dr. Reinhard Agerer
Zweitgutachter: Prof. Dr. Hans-Jürgen Tillich

Acknowledgements

First and foremost I would like to thank my wife Paras for her willingness to sacrifice a normal private life for the sake of research, and her never-ending patience during my field trips, when the two of us were separated for weeks and sometimes months.

I am deeply grateful to my supervisor Reinhard Agerer, who didn't just allow me, but eagerly encouraged me, from the very beginning of our collaboration, to freely cross the borders between mycology and the biological disciplines representing the other branches of the tree of life, botany in particular. No wonder that much of the content of this thesis is a rather unusual output for a member of a mycological research team.

While working on this thesis, I also profited from conversations with many colleagues. Special thanks are due to Philomena Bodensteiner, Rita Verma, Eva Facher, Harald Förther, Alexander Kocyan, Stefan Raidl and my dear friend and travel companion Ludwig Beenken.

I'm very much indebted to Gabriele Chrupalla of the Cameroonian Baptist Mission, who helped me out of loads of trouble during my first trip to Cameroon and offered me so much help and hospitality whenever I came to Buea or Douala. She also introduced me to Karin von Löbenstein of WWF, who in turn introduced me to Moses Nsanyi Sainge, thus sowing the seeds of a long-standing friendship. Moses is by far the most talented field-botanist I have ever met. Without his infallible nose for the highly concealed myco-heterotrophic plants this thesis wouldn't have been possible. I truly miss his healthy sense of humor and the wonderful time we spent together in the field.

These acknowledgements wouldn't be honest, if I forgot to mention all the people who helped me in the field, all the herbarium workers, conservation-biologists, field-botanists, camp-managers, field-guides, porters and bush taxi drivers many of whose names I can't remember. Just to mention a few: Mbella, Maurice, Paul, Julie, Comfort, Theophilus, Frieda, Nkogge, Hoffman, Ri, Stanley, Mambo, Idi, Paulo, Vincent, Emanuel, Eva, …

I would particularly like to thank my friend and mentor Günter Gerlach, who filled me with enthusiasm for pollination biology and the various kinds of interactions among unrelated organisms. It is thanks to him that I studied biology.

Last but not least I want to thank my parents Linda and Erich Franke for their endless love and support. To them this thesis is dedicated.

The diversity of species cannot make sense unless we also understand the diversity of interactions among them.

John N. Thompson 1994

Contents

Von dieser Familie (Burmanniaceae incl. Thismiaceae), deren Arten zumeist in Regenwäldern an dicht beschatteten, locker humösen Stellen wachsen, kannte man lange Zeit nur wenige Arten. Es sind aber in neuester Zeit mehr, namentlich aus Kamerun bekannt geworden, und es ist anzunehmen, dass bei eingehender Erforschung der Regenwälder noch mehr aufgefunden werden.

Adolf Engler 1908

1. Introduction

1.1. Mycorrhizal symbiosis

Green plants gain access to carbon by reducing atmospheric carbon dioxide through a water-dependent, solar-powered and chlorophyll-catalyzed process referred to as photosynthesis. The final product of this complex chain of interlinked chemical reactions is sugar, which in turn is the initial substance for the plant's energy supply, as well as for the accretion of biomass (Sitte et al. 2002).

Moreover the vast majority of land plants use part of their self-synthesized carbohydrates as a 'currency' to purchase minerals, nitrogen and phosphorus in particular, which they obtain from soil-inhabiting fungi (Smith and Read 1997). This plant-fungus interaction, for which Frank (1885) introduced the term "mycorrhiza" (Greek: mýkes = fungus, rhíza = root), is a classical example for mutual symbiosis in terms of inter-specific bidirectional exchange of valuable compounds (Smith and Read 1997). Under normal conditions a mycorrhiza-forming plant benefits from its associated fungus' superior ability to extract minerals from the soil and to scavenge useful compounds from organic matter, whereas the fungus benefits from its associated plant's ability to synthesize energy rich carbohydrates through photosynthesis (Chapin 1995; Northup et al. 1995; Bending and Read 1996; Jongmans et al. 1997; Smith and Read 1997). Just like the alga-fungus association in lichens, or the nitrogen fixing bacteria in the root-nodules of legumes and alders, mycorrhizae are consequently regarded as a mutually beneficial association of dissimilar organisms (Peterson et al. 2004). However according to de Bary (1884) a bidirectional exchange of nutrients between two dissimilar organisms is not always balanced and can easily shift to parasitism, especially if one partner faces inconvenient environmental conditions.

1.2. Mycorrhizal networks

Forest ecosystems, e.g. show a very uneven distribution of light (Larcher 1994; Aussenac 2000). Whereas large canopy trees have unhindered access to light, understorey trees, tree seedlings, shrubs and ground-dwelling herbs have to cope with the deep shade of the forest floor (Whitmore 1990). Apart from sunflecks, heavily shaded understoreys sometimes receive less than 1% of the above-canopy solar radiation (Chazdon and Fetcher 1984; Lusk et al. 2006). The forest's soil is colonized by a patchwork of fungal mycelia, each of which being connected with several unrelated mycorrhizal plants, which in turn are linked to several mycelia at the same time (Read 1989; Molina 1992; Horton and Bruns 1998; Onguene and Kuyper 2002; Giovannetti et al. 2004). Hence the roots of these plants are interconnected

by common mycorrhizal networks. Laboratory and field experiments showed that, besides regulating plant-fungal nutrient fluxes, artificially established mycorrhizal networks also enabled inter-plant transfer of carbon via fungal hyphae (Simard et al 1997, 2002 [see references therein]). This implies that plants that grow under unfavourable light conditions, but "share" the same mycorrhizal fungus with tall trees, might indirectly benefit from the photosynthesis performed high up in the canopy (Kennedy et al. 2003). If regarding canopy trees as carbon-sufficient donor plants and their seedlings as carbon-deficient receiver plants, common mycorrhizal networks can undoubtedly contribute to the mutualistic nature of the mycorrhizal association (Read 1989).

But does this also apply to mycorrhizal plants that never reach the canopy? Plants, such as understorey trees, shrubs and herbs of the forest floor, are constantly confronted with conditions obstructing effective photosynthesis (Chazdon and Fetcher 1984; Whitmore 1990; Aussenac 2000; Lusk et al. 2006). By using stable isotope analysis Gebauer and Meyer (2003) could demonstrate that several forest floor dwelling orchids gain considerable amounts of carbon from mycorrhizal fungi. The existence of vital achlorophyllous mutants (albinos) of *Cephalanthera damasonium* (MILLER) DRUCE, which necessarily depend on 100% fungal carbon supply, enabled Julou et al. (2005) to quantify the amount of fungal carbon in green ("healthy") individuals of this species. They found that green individuals of *C. damasonium* derive 48.9 % of their carbon from mycorrhizal fungi (mainly Thelephoraceae and Cortinariaceae). Hence it is very likely that the amount of carbon *C. damasonium* extracts from its common mycorrhizal network exceeds the amount of carbon it feeds into it.

Although this hypothesis is still a matter of dispute, as far as other green plants are concerned, there is conclusive evidence that leaf-less chlorophyll-deficient respectively permanently achlorophyllous mycorrhizal plants such as *Corallorhiza trifida* CHATELAIN (Orchidaceae), *Monotropa hypopitys* L. (Ericaceae) or *Cryptothallus mirabilis* MALMBORG (Aneuraceae) indeed "cheat" common mycorrhizal networks out of carbon (Björkman 1960; McKendrick et al. 2000; Bidartondo et al. 2003). Due to their incapability to perform photosynthesis, these plants must receive all carbon from a fungal host; thus the plant-fungal association undoubtedly shifts from mutualism to parasitism (Leake 1994; Raynal-Roques and Paré 1998). According to Brundrett (2004) these mycorrhizas are the reverse of relationships between higher plants and parasitic fungi. Consequently achlorophyllous mycorrhizal plants are most appropriately referred to as mycoparasites or myco-heterotrophs, the latter term now being well established among researchers studying these plants (Cronquist 1981; Leake 1994, 2005; Raynal-Roques and Paré 1998).

1.3. Saprophytes versus plants parasitizing fungi

However, until recently myco-heterotrophic plants were principally known as 'saprophytes' (greek: sapros = rotten; phyton = plant; Leake 2005). Due to the paradox that these plants lack both chlorophyll and haustorial connections to adjacent green plants, it was widely believed that they feed upon dead organic material such as decaying wood and leaf litter (Johow 1885; Groom 1895). Kamienski (1881, 1882) was the first who realized that all essential nutrients required by the myco-heterotrophic *M. hypopitys* must be provided by the associated mycorrhizal fungus. He further argued that the fungus might enable *M. hypopitys* to indirectly parasitize the adjacent autotrophic plants via joint hyphal bridges – a hypothesis that meets with increasing approval these days (see below). In the years following Kamienski's investigation, the number of researchers appreciating the real trophic relationships of myco-heterotrophic plants slowly increased, although occasional attempts to introduce more appropriate names, such as 'fungus-feeding', 'fungus-digesting' or 'mycoparasitic' plants, didn't succeed within the scientific community (Frank 1891; Camp 1940; Malims-

Smith 1952). Consequently the use of the term 'saprophyte' was maintained by many authors who knew very well that these plants in fact received nutrients from fungal hosts (MacDougal 1899; Burgeff 1932; Maas et al. 1986b [see 'General Introduction']). In a landmark comprehensive review covering the past 150 years of 'saprophytic' plants research, Leake (1994) invented the term 'myco-heterotrophy'. Eleven years later he followed up with an updated review article, headed with the provocative title: "Plants parasitic on fungi: unearthing the fungi in myco-heterotrophs and debunking the 'saprophytic' plant myth", intending to definitely blow the idea of 'compost eating' plants out of peoples' minds (Leake 2005). Finally it is worth mentioning that several researchers, including Leake, refer to the mycorrhizal relationship of myco-heterotrophic plants as 'epiparasitism', emphasizing that myco-heterotrophic plants indirectly parasitize adjacent green plants by tapping common mycorrhizal networks (Björkman 1960, Cullings et al. 1996; Bidartondo et al. 2002; Leake 2005). However, this rather 'phytocentric' term doesn't apply to several myco-heterotrophic orchids, which proved to exclusively parasitize non-mycorrhizal wood- and litter decaying fungi (Burgeff 1932; Lan et al. 1994; Yamato et al. 2005). Perhaps the most accurate term to describe the nature of this association from both plant and fungus perspectives was introduced by Brundrett (2004), who defined a mycorrhizal association where only the plant receives substantial benefit from nutrient exchange as 'exploitative mycorrhiza' ('exploitative' plant versus 'exploited' fungus).

1.4. Myco-heterotrophy

According to Leake (2005) over 400 achlorophyllous plant species in 87 genera lead a myco-heterotrophic mode of life throughout their entire lifespan. In addition to this, there are about 30,000 green plant species, which have achlorophyllous early developmental stages, exclusively depending on fungal carbon supply (Leake 2005). The great majority of these temporary myco-heterotrophic plants are orchids, but a shift from myco-heterotrophy to phototrophy during ontogeny does also occur in Ericaceae (subfam. Pyroloideae), Lycopodiaceae, Psilotaceae and Ophioglossaceae, in the latter three families in combination with an alternation in nuclear phase (Bruchmann 1898; Velenovsky 1905; Holloway 1917; Schmid and Oberwinkler 1994; Rasmussen 1995).

Myco-heterotrophy has evolved several times independently within the plant-kingdom and is presently known to occur in two species of liverworts, several ophioglossoid fern, whisk fern, and clubmoss gametophytes, the prothallia of few leptosporangiate ferns, one gymnosperm and numerous monocotyledonous as well as dicotyledonous angiosperms (Bruchmann 1898; Holloway 1917; Leake 1994; Schmid and Oberwinkler 1994, 1995;. Franke 2002 [**paper II**]; Bidartondo et al. 2003; Imhof 2003; Feild and Brodribb 2005).

Due to a common evolutionary trend even unrelated species of myco-heterotrophic plants share numerous similar features (Leake 1994; compare **Fig. 1A** in Franke et al. 2000 [**paper I**]; **Fig. 1a** in Franke 2002 [**paper II**] and **Fig. 1A** in Sainge et al. 2005 [**paper VI**]). Without the need for assimilatory tissue, their leaves are reduced to vestigial scales, which are often restricted to the generative shoot (**Fig. 1a** in Franke 2002 [**paper II**]). Compared to photosynthesizing plants, the number of stomata is highly reduced and in many myco-heterotrophic plants they lack completely (Johow 1889; Oehler 1927; Maas and Ruyters 1986). Although chloroplasts are still present in the aerial shoots of several species (e.g. *Corallorhiza trifida*), it is very unlikely that these organelles contribute considerably to the plant's carbon supply, in particular if taking into account that myco-heterotrophic plants spend most of their life-cycle subterranean (Montfort 1940; Montfort and Küsters 1940). The function of the aerial shoot is exclusively restricted to reproduction. Some myco-heterotrophic plants are autogamous and thus may flower subterranean (Bernard 1909; Fuchs and Ziegenspeck 1926),

but the vast majority must at least expose their fruits to the above-ground environment in order to guarantee effective seed (spore) dispersal. One of the few known exceptions is represented by the orchid genus *Rhizantella* whose two species spend their entire life cycle underground – including fructification (Hunt 1953; George 1980).

1.5. Adapting to the myco-heterotrophic mode of life

Several genera, such as *Burmannia*, *Sebaea*, *Cephalanthera* and *Epipactis* comprise an assembly of species, each of which being situated at a different station along the evolutionary road towards myco-heterotrophy (Leake 1994; Rasmussen 1995; Bateman et al. 2005; Franke et al. 2006 [**paper VII**]). If brought into the appropriate order, these assemblies are referred to as 'progressive series'. The genus *Sebaea* e.g., shows a 'transition' from comparatively large leaved autotrophic species, such as *S. spathulata* STEUD. to species with tiny, yet green scale leaves, like *Sebaea filiformis* SCHINZ and finally to completely myco-heterotrophic forms like the achlorophyllous *Sebaea oligantha* SCHINZ (Franke 2002 [**paper II**], Franke et al. 2006 [**paper VII**]). However the term 'transition' must be used with reservation, as each of these species, like any biological species, represents a preliminary terminal stage of its own evolutionary history and not the predecessor of a contemporaneously existing 'more advanced' species (Henning 1982).

The root systems of myco-heterotrophic plants are highly modified as well and exhibit different adaptations to the myco-heterotrophic mode of live. Several genera, such as *Voyria* (Gentianaceae), *Sciaphila* (Triuridaceae), *Zeuxine* (Orchidaceae) or *Burmannia* (Burmanniaceae) display 'progressive series' from species with fairly extended, frequently branched root systems resembling those of green plants, to species with extremely condensed subterranean organs, difficult to assign to either root or shoot homologues (Burgeff 1932; Imhof 1997, 1999b, 2004; Imhof and Weber 1997, 2000; Stadler 2006).

The subterranean organs of myco-heterotrophic plants are so variable in shape that only a few examples will be mentioned here. In many species the roots accumulate in 'bird nest'-like clumps, or more or less dense coralloid clusters; as in *Neottia nidus-avis* (L.) L.C.M. RICHARD (Orchidaceae), *Monotropa hypopitys* (Ericaceae), *Afrothismia saingei* TH. FRANKE (Thismiaceae) or *Thismia* spp. sect. Sarcosiphon (Thismiaceae) (Kamienski 1882; Smith 1911; Rasmussen 1986; Franke 2004 [**paper III**, see **Fig. 1A**]). In contrast to this, the root systems of *Voyria flavescens* GRISEBACH (Gentianaceae), *Kupea martinetugei* CHEEK & S.A. WILLIAMS (Triuridaceae), *Burmannia tenella* Benth. (Burmanniaceae), or *Arachnitis uniflora* PHIL. (Corsiaceae), are stellate structures consisting of only a few exogenously arising, short and unbranched succulent roots (Imhof 1999c; Franke 2002 [**paper II**, see **Fig. 1a**]; Cheek et al. 2003; Domínguez and Sérsic 2004). This type of root arrangement is commonly referred to as 'morgensternartig' (German: spiked mace-like; Johow 1885; Imhof et al. 1994). Perhaps the highest extent of surface reduction is represented in the genera *Corsiopsis* (Corsiaceae), *Oxygyne*, and *Tiputinia*, (both Thismiaceae), whose aerial shoot derive from a single unbranched rapaceous organ (Abe and Akasawa 1989; Zhang et al. 1999; Woodward et al. in prep.). In view of the highly reduced surface area, it seems obvious that the subterranean organs of most myco-heterotrophic plants are ill-adapted for the conventional tasks of a root system, namely the uptake and long-distance translocation of water and nutrients. Hence all of these essential requirements have to be provided by the fungal host's extraradical mycelium.

1.6. AMF-exploiting myco-heterotrophic plants

Besides several achlorophyllous orchids (tribes Epipogieae; Gastrodieae; Vanilleae), which parasitize, wood- and litter decaying fungi ((Burgeff 1932; Lan et al. 1994; Yamato et al. 2005), the vast majority of myco-heterotrophic plants is specialized on mycorrhizal host fungi (Leake 2005). Most myco-heterotrophic plants from temperate regions, including achlorophyllous Ericaceae (subfamily Monotropoideae), several orchids (e.g. *Corallorhiza maculata* (RAFINESQUE) RAFINESQUE) and the subterranean liverworth *Cryptothallus mirabilis*, are associated with regionally prevalent ectomycorrhizal fungi (EMF; mainly Basidiomycota; Taylor et al. 2002), whereas the majority of tropical species (e.g. Burmanniaceae, Triuridaceae, Gentianaceae) parasitize arbuscular mycorrhizal fungi (AMF, Glomeromycota; Bidartondo et al. 2002; Franke et al. 2006 [**paper VII**]).

Exploitative arbuscular mycorrhiza has evolved independently in numerous di- and monocotyledonous angiosperms which represent about half of all lifelong myco-heterotrophic plant species (Leake 2004). Their distribution range covers the humid tropical and subtropical regions of five continents, including many remote oceanic islands, e.g. the Seychelles, the Palau archipelago or New Caledonia. Two exceptional species even occur in boreal, respectively subantarctic environments (Pfeiffer 1914; Domínguez and Sérsic 2004).

Knöbel & Weber (1988) were the first who assigned the fungal hosts of a myco-heterotrophic plant to AMF. Their study is based on anatomical investigations of the fungal structures within the roots of the achlorophyllous *Voyria truncata* (STANDLEY) STANDLEY & STEYERMARK (Gentianaceae). After this ground-breaking, but little noticed publication (written in German), AMF were anatomically detected in numerous other mainly tropical myco-heterotrophic plants within the Burmanniaceae, Gentianaceae, Corsiaceae, Thismiaceae and Triuridaceae (Domínguez & Sérsic 2004; Franke 2002 [**paper II**, see **Fig. 3; Fig. 4**]; Imhof 1997; 1999a, b, c; 2001; 2003; Imhof & Weber 1997; 2000; Imhof et al. 1994). Although mycorrhizal structures provide enough diagnostic characters, to unambiguously assign the concerned fungus to AMF, there is no getting away from the fact that anatomical identification techniques soon come up against limiting factors, if attempting to go beyond the phylum level (Redecker et al. 1997).

In recent years molecular methods have proved to be a convenient way to identify AMF associated with myco-heterotrophic plants, even above genus level. For the first time Yamato (2001) isolated and sequenced the DNA of an AMF of the genus *Glomus* from the roots of *Sciaphila tosaensis* MAKINO (Triuridaceae). Bidartondo et al. (2002) detected AMFs in the roots of *Arachnitis uniflora* PHIL. (Corsiaceae), *Voyriella parviflora* (MIQUEL) MIQUEL. (Gentianaceae) and five *Voyria* species, by analyzing fungal ITS, as well as 18S rDNA sequences. Moreover they discovered that all species are highly specialized to a few narrow lineages within a clade of *Glomus* (*Glomus*-group A as defined by Schwarzott et al. 2001). Franke et al (2006 [**paper VII**]) detected representatives of the *Glomus*-group A lineage in the roots of 11 AMF-exploiting myco-heterotrophic plants from four different families (Burmanniaceae, Thismiaceae, Triuridaeae and Gentianaceae).

1.7. The secret life of AMF-exploiting myco-heterotrophic plants.

A remarkable common feature of most AMF-exploiting myco-heterotrophic plants is their apparent scarcity (Stone 1980; Franke 2004 [**paper III**]). Many species are known exclusively from the type collection and had never been found again (Maas et al. 1986a). However this doesn't necessarily imply that these plants are always scarce in numbers, but rather that – due to their cryptic mode of life – they are difficult to find (Franke et al. 2000 [**paper**

I]). During the vegetative phase, which occupies the largest part of an AMF-exploiting myco-heterotrophic plant's life cycle, nothing indicates its whereabouts; only during the short generative phase, when aerial shoots are produced, do AMF-exploiting myco-heterotrophic plants expose themselves to the collector's eye. The vast majority of species produce flowers exclusively during the rainy season, when the little available light is diffused by the glittery surface of the wet forest floor, causing the plants' outline to perfectly fuse with the surrounding leaf litter (Stone 1980; Sainge 2003). Hence, the rare occasions when AMF-exploiting myco-heterotrophic plants are discovered by chance usually happen during "[…] trip stops, either for felling or climbing trees or for culinary or sanitary purposes […]", as pointed out by van de Meerendonk (1986). The circumstance that these plants are difficult to find however is accompanied by one major problem: AMF-exploiting myco-heterotrophic plants aren't necessarily rare, but if they are nobody will notice.

Many AMF-exploiting myco-heterotrophic plants are considered to represent local endemics, because they were collected only once at a single spot (Cheek et al. 2003). However, it repeatedly occurred that new populations of an apparent endemic were located long after the species' original discovery and often far away from its type locality (Stone 1980). The most likely reason for this phenomenon is the fact that these cryptic plants are usually discovered coincidently (see above). Since the coincidental discovery of a well concealed plant is always a matter of chance, the coincidental rediscovery of the same species shortly after its first sighting, requires even more chance. The best examples to illustrate this paradoxon are given by representatives of the genus *Thismia* (Thismiaceae). The Southeast Asian *Thismia clavigera* (BECC.) F. V. MUELL., e.g. was discovered by Odoardo Beccari in 1866 in Sarawak and never found again, until more than a century later, it was almost simultaneously rediscovered at two distant places – North Sumatra and Pulau Langkawi – both over a thousand kilometers away from the species' type locality and separated from it by the South China Sea (Beccari 1877; Stone 1980). The neotropical species *Thismia glaziovii* Pouls. *Thismia luezelburgii* GOEBEL & SÜSSENGUTH and *Thismia hyalina* (MIERS) F. VON MÜLLER experienced a similar fate. All three species seemed to be endemic to the Mata Atlantica (costal rain forest of SE-Brazil), until, several decades after their discovery, each species was found again on the opposite side of the Amazon basin, respectively in Panama (*T. luezelburgii*), thousands of kilometers away from their type localities (Maas et al. 1986a; Maas and Maas-van de Kamer 1988). Although these are not the only examples of transamazonian disjunction in the neotropical flora (Lima 1953; Mori et al. 1981), there is no doubt that the Mata Atlantica has one of the highest levels of endemism in South America. In view of the fact that only about 6% of the Atlantic costal rain forest of Brazil remains intact, it is almost certain that a considerable amount of the locally described species are now extinct. Unfortunately this pessimistic prediction also concerns AMF-exploiting myco-heterotrophic plants, if taking into account that the Mata Atlantica is the primary diversity hotspot of the neotropical representatives of the genus *Thismia* (Maas et al. 1986a). Whereas the three *Thismia* species mentioned above obviously escaped extinction thanks to a disjunct distribution pattern, the destiny of three other *Thismia* species of the Mata Atlantica area is very uncertain: *Thismia caudata* MAAS & MAAS, *Thismia fungiformis* (TAUBERT EX WARMING) MAAS & MAAS and *Thismia macahensis* (MIERS) F. V. MÜLLER were only collected once, way over 100 years ago and never seen since (Maas et al. 1986a). The fact of the matter however is that 94 % of their original habitat was replaced by farmland (Prance et al. 2000), thus the chances to rediscover these elusive little plants is near to nothing, unless they'll prove to have disjunct distribution patterns as well. These examples illustrate in a drastic way how fragmentary the knowledge about the diversity and distribution of AMF-exploiting myco-heterotrophic plants actually is, and how many additional requirements concerning their exploration and conservation still exist.

1.8. The diversity and ecology of AMF-exploiting myco-heterotrophic plants from tropical Africa – aims of the project

If compared to tropical Asia and the Neotropics, very little is known about AMF-exploiting myco-heterotrophic plants in tropical Africa. The first descriptions of African AMF-exploiting myco-heterotrophic plants were brief notes in scope of miscellaneous floristic treatments (Bentham 1849; Engler 1894; Wright 1897, 1898). These were succeeded by three more comprehensive papers by Engler (1905, 1910) and Schlechter (1906). Within these early milestone contributions the genera *Afrothismia* and *Oxygyne* (Thismiaceae) were described and the family Triuridaceae was firstly recorded for Africa. With regard to the Burmanniaceae Engler (1905) proposed to intensify the research on African myco-heterotrophic plants by saying: "Alles dies zeigt, wie viel Interessantes noch bei Burmanniaceen zu finden sein dürfte, wenn dieselben in ihrer Heimat längere Zeit beobachtet werden könnten." and Schlechter (1906) added "Es ist daher nur zu wünschen, dass in Zukunft die botanischen Sammler, die Gelegenheit haben werden, die afrikanischen Wälder weiter zu erforschen, auch diesen, allerdings zwischen dem abgefallenen Laub im Humus nicht leicht aufzufindenden, Thismieen mehr Aufmerksamkeit schenken werden." However, their appeals apparently didn't gain a hearing, as only nine new taxa of African AMF-exploiting myco-heterotrophic plants were described in the succeeding 90 years, in other words only a single new species per decade. Besides scientific disregard, the only other possible explanation for this phenomenon could be a mysterious impoverishment of the African Flora (incl. Madagascar) in AMF-exploiting myco-heterotrophic plants if compared to other tropical regions.

Encouraged by Engler and Schlechter's appeals I decided to write a doctoral thesis about the diversity and ecology of AMF-exploiting myco-heterotrophic plants, putting the main emphasis on tropical Africa. The core project was carried out in Southwest Cameroon between October 1999 and October 2003 financed by the BIOLOG (Biodiversity and Global Change) programme of the BMBF (German Ministry of Education and Research). The thesis also includes two publications, dealing with South American AMF-exploiting myco-heterotrophic plants.

The aims of the project were:

1. to inventory the AMF-exploiting myco-heterotrophic plants of Cameroon's Southwest Province and to describe new taxa,

2. to analyze the root anatomy and mycorrhiza of selected AMF-exploiting myco-heterotrophic plants and to structurally identify their associated fungi,

3. to identify the fungal hosts of a large sampling of Cameroonian AMF-exploiting myco-heterotrophic plants, applying molecular techniques.

2. Discussion

The seven publications this thesis is based on, exclusively deal with the investigation of AMF-exploiting myco-heterotrophic plants, covering three main topics:

1. **paper II** is about root (procaulome) anatomy of the achlorophyllous *Voyria flavescens* Grisebach (Gentianaceae) and the structure-based identification of its fungal hosts,

2. **paper VII** deals with the molecular identification of fungal hosts of eleven species of AMF-exploiting myco-heterotrophic plants from tropical Africa,

3. **papers I**, **III**, **IV**, **V** and **VI** introduce five taxonomic novelties, one of which is a member of the genus *Triuridopsis* (Triuridaceae; **paper I**), whereas the remaining four belong to the genus *Afrothismia* (Thismiaceae; **papers III - VI**). All new species are achlorophyllous AMF-exploiting myco-heterotrophic herbs.

2.1. Root (procaulome) anatomy and structural identification of fungal hosts

On the first glance the subterranean structures of *Voyria flavescens* (Gentianaceae) resemble a condensed rhizomatous axis with a stellate arrangement of short, unbranched roots (**Fig 1a** in Franke 2002 [**paper II**]). However a careful structural investigation reveals that this highly condensed spiked mace-like organ cannot be assigned to a root-system in the conventional sense. The root-like elongated lateral projections[1] have no root cap and display a unique process of apical growth. They arise exogenously from a vertical axis ('rhizoferous axis' in Franke 2002 [**paper II**]), which lacks scales and is not subdivided into nodes and internodes (**Fig 1a**; **Fig 2a** in Franke 2002 [**paper II**]). Due to the lack of a pericycle all lateral projections are generated at the central axis' apical meristem, or occasionally at the meristematic tip of a single projection, resulting in the formation of plantlets. Velenovsky (1905), who studied the myco-heterotrophic seedlings of the One-sided Wintergreen (*Pyrola secunda* L., Ericaceae, subfamily Pyroloideae), referred to such structures as 'Prokaulome' (procaulomes), whereas Rasmussen (1995) introduced the term 'mycorhizome' to characterize an early developmental stage in orchid ontogenesis, which derives from the protocorm and is neither clearly assignable to root nor rhizome. In both, *Pyrola secunda* and green orchids the procaulome respectively mycorhizome generates a photosynthesizing aerial shoot, which in turn produces true adventitious roots in the course of further development. This step is omitted by *V. flavescens*, as the plant dies off after the formation of the achlorophyllous areal shoot. Consequently the occurrence of procaulomes[2] in mature myco-heterotrophic plants such as *V. flavescens* raises the assumption that these plants are paedomorphs, which are sexually reproductive yet juvenile in morphology – thus representing a case of neoteny.

Several authors share the opinion that myco-heterotrophic plants like *V. flavescens* are indirect plant parasites, thus being linked to green host plants by 'hyphal bridges' instead of haustorial connections (Bidartondo et al. 2002, 2003; Leake 2004, 2005). In 1996 Cullings et al. resurrected the term 'epiparasitism', which was introduced by Björkmann (1960) to describe the trophic relationship between *Monotropa hypopitys* L. and adjacent mycorrhizal trees, but didn't gain acceptance among contemporary scientists (Wallace 1975). In his 'glossary of terms' Bidartondo (2005) redefined 'epiparasitism' as a tripartite symbiosis between a

[1] referred to as 'roots' in Franke (2002 [**paper II**])

[2] being unaware of Velenovsky's (1905) invention of the word 'Prokaulom', Franke (2002) used the younger term 'mycorrhizome', which must be considered as a synonym of the former, because it signifies the same developmental stage as 'Prokaulom', though in a different plant family.

parasitic lineage (myco-heterotrophic plant), an intermediate host lineage (mycorrhizal fungus) and an ultimate host lineage (mycorrhizal green plant).

The anatomical analysis of the subterranean structures of *V. flavescens* however revealed the destructive forces fungal hyphae are confronted with when attempting to colonize the procaulome, thus questioning the appropriateness of the terms 'hyphal bridge' or 'intermediate host lineage' (**Fig 3b**; **Fig 5e,f** in Franke 2002 [**paper II**]). A longitudinal section through one of the procaulome's root-like projections reveals that the cortical cells of the proximal section are tightly packed with mortal remains of disintegrated fungal hyphae, whereas the hyphae within the distal section exhibit all stages of successive enzymatic degeneration (**Fig 3b** in Franke 2002 [**paper II**]). Soil-borne hyphae exclusively enter the root-like projection at a conspicuous collar-like structure (dark banded zone) just behind the tip (**Fig 2a,b; Fig 5b** in Franke 2002 [**paper II**]), whereas – due to the procaulome's inability to excrete solid compounds – the indigestible remains are deposited within the root-like projection's proximal part. This implies that each root-like projection functionally corresponds to a digestive tract, with the only difference that the 'food' is not manoeuvred through the tract, but remains at the very place of its enzymatic breakdown, while new digestive tissue is constantly regenerated. As soon as the procaulome is cramped by too much accumulated waste, it develops a generative shoot and finally dies off after fructification, thus circumventing the necessity of defecation. Seen from this perspective, there remains little doubt that *V. flavescens* is the 'exploiter' and the associated fungus is the 'exploited', no matter were the exchanged nutrient originally come from. Hence, Brundrett's (2004) term 'exploitative mycorrhiza' far more accurately reflects the nature of this association than 'epiparasitism'.

Although arbuscules were lacking, the fungal associates of *V. flavescens* were assigned to the arbuscular mycorrhizal fungi (AMF; Glomeromycota) on the basis of structural features, such as thick-walled aseptate hyphae considerably varying in diameter, as well as typical growth habits of hyphal appressoria and the presence of soil-borne auxiliary cells emerging from root invading hyphae (**Fig. 4**; **Fig. 5 c,d** in Franke 2002 [**paper II**]).

2.2. Molecular identification of fungal hosts

Nowadays the fastest, most convenient and most precise way to identify root colonizing fungi is without doubt selective fungal DNA-sequence analysis (Husband 2004). Franke et al (2006 [**paper VII**]) were the first to employ molecular techniques (partial 18s rDNA sequencing) to identify the fungal hosts of myco-heterotrophic plants from tropical Africa. Their investigation revealed that all analysed specimens, comprising eleven species from four different families (Burmanniaceae, Thismiaceae, Triuridaeae and Gentianaceae), equally favoured AMFs of the family-ranking *Glomus*-group A lineage as fungal hosts (**Fig. 2** in Franke et al. 2006 [**paper VII**]). The result is perfectly congruent with Bidartondo et al.'s (2002) analysis of the fungal hosts of the South American *Arachnitis uniflora* PHIL. (Corsiaceae), *Voyriella parviflora* (MIQUEL) MIQUEL (Gentianaceae) and five *Voyria* species, thus providing conclusive evidence for a transatlantic distribution of the same host preferences. A possible reason for this phenomenon may be found in the different root colonization strategies of AMFs (Franke et al. 2006 [**paper VII**]). Hart and Reader (2002) could show that both, root colonisation extent and root fungal biomass, are significantly higher in members of the *Glomus*-group A lineage than in any other lineage of arbuscular mycorrhizal fungi, such as *Glomus*-group B, Acaulosporaceae or Gigasporaceae. Considering the parasitic nature of myco-heterptrophic plants, it seems obvious that a fungus, which extensively colonizes the root tissue and provides great amounts of exploitable biomass, is a much more lucrative host than a fungus, which is only sparsely distributed within the root tissue (Franke et al. 2006 [**paper VII**]). However this idea still requires experimental confirmation.

2.3. Diversity of AMF-exploiting myco-heterotrophic plants

2.3.1. Taxonomic novelties

It seems to suggest itself that mycologists are used to screen the forest floor more carefully for small and inconspicuous things than most botanists do. For this reason it is little surprising that mycologists repeatedly brought to light new myco-heterotrophic plants (Maas and Maas 1987, De la Sota 1960). One of the new species described in this thesis – *Triuridopsis intermedia* TH. FRANKE, BEENKEN & CH. HAHN – e.g. was collected as a by-product of a mycological field trip to Bolivia's Alto Beni region (Franke et al. 2000 [**paper I**]). The inconspicuous little plants grew in large numbers amidst one of three long-term observation plots of the "Proyecto de Investigationes Agroécologicas y Forestales en el Alto Beni" (PIAF), but stayed unnoticed during a botanical inventory three years before (Seidel 1995). The new species, which is the first record of the family Triuridaceae for Bolivia, represents the second species of the genus *Triuridopsis*. The genus was established by Maas-van de Kamer and Maas (1994) with the description of *Triuridopsis peruviana* MAAS-VAN DE KAMER & MAAS from Amazonian Perú. It belongs to the neotropical tribe Triurideae, which also encompasses the genera *Lacandonia*, *Peltophyllum* and *Triuris*. Except of *Lacandonia*, which has hermaphroditic flowers, Triurideae are dioecious (Ambrose et al. 2006). As the name indicates, *Triuridopsis* (Greek: ópsis = resemble) shares many common features with the genus *Triuris*. According to Maas-van de Kamer and Maas (1994) the two genera can only be distinguished by a few differences in the staminate flowers. Whereas the (apparently) sessile anthers of *Triuris* are inserted into a cone-shaped androphore, those of *Triuridopsis* are distinctly filamented and surround a centrally inserted sterile projection (**Fig. 1C,D** in Franke et al. 2000 [**paper I**]).

The new species was named *Triuridopsis intermedia* (Lat.: intermedius = intermediate, between two things) because its well developed filaments and the central projection are generic key characters of *Triuridopsis*, whereas its long thread-like tepal appendages (several times extending the length of the tepals), as well as the longitudinal anther dehiscence slits (stomia) are typical features of all representatives of the genus *Triuris*, yet lacking in *Triuridopsis peruviana* (Maas and Rübsamen 1986; Maas-van de Kamer and Maas 1994).

However in a recent investigation of the floral development of Mexican Triuridaceae Ambrose et al. (2006), contrary to previous reports (e.g. Maas-van de Kamer and Maas 1994), stress that *Triuris brevistylis* J.D. SMITH in fact does have filamented stamens. The filaments are clearly visible in ontogenetic sequences prior to the expansion of the androphore. Moreover does the unexpanded androphore of a young staminate flower of *T. brevistylis* (Fig. 77 in Ambrose et al. 2006) strikingly resemble the sterile projection of the staminate flower of *T. intermedia*, thus implying that the androphore in *Triuris* and the sterile projection in *Triuridopsis* are homologue structures. As these new insights invalidate the main distinguishing characters between the two genera, it seems questionable if the generic status of *Triuridopsis* is still legitimate. Consequently it should be considered to reject the genus *Triuridopsis* in favour of *Triuris*.

The other four taxonomic novelties presented in this thesis, which are all representatives of the genus *Afrothismia*, were brough to light during a BMBF (German Ministry of Education and Research) financed project to investigate the diversity and ecology of African myco-heterotrophic plants (Franke 2004, Franke et al. 2004, Sainge & Franke 2005, Sainge et al. 2005 [**papers III - IV**]).

The genus *Afrothismia* was established by R. Schlechter in 1906 basing on *Afrothismia winkleri* (ENGL.) SCHLTR. from Cameroon, which Engler (1905) described as *Thismia winkleri* ENGL. (Engler 1905; Schlechter 1906). In the same paper Schlechter described a second species, *Afrothismia pachyantha* SCHLTR. Engler (1908) however insisted in his taxonomic concept, which regards *Afrothismia* as an African section of the pantropically distributed genus *Thismia*. Consequently he restored *Thismia winkleri* ENGL. and changed *Afrothismia pachyantha* SCHLTR. into *Thismia pachyantha* (SCHLR.) ENGL. In his revision of the Thismieae Schlechter (1921) again upgraded *Afrothismia* to genus level by arguing: "Ich bin fest davon überzeugt, daß diese Gattung, welche Engler ebenfalls mit *Thismia* vereinigen will, unter allen Umständen getrennt gehalten werden muss." Finally Jonker (1938), by adopting Schlechter's taxonomic concept in his monograph of the Burmanniaceae (incl. Thismiaceae), ended this taxonomic argy-bargy for the time being. A recently published molecular analysis however revealed that the genus *Thismia* sensu Jonker (including the neotropical genus *Ophiomeris*), indeed is paraphyletic to *Afrothismia* (Merckx et al. 2006). A final decision of whether *Afrothismia* will be better maintained or rejected as a generic name is still outstanding. After Schlechter's discovery of *A. pachyantha* in 1906 not a single new species was added for more than 60 years. Only 1988, while revising the Burmanniacae for the Flora of Tropical East Africa, E. Cowley came across a herbarium specimen of a yet undescribed Tanzanian *Afrothismia* species (Cowley 1988). The specimen, which was collected in 1961 wasn't recognized for 27 years, as it was erroneously assigned to the Aristolochiaceae. The new species, which was named *Afrothismia insignis* COWLEY, as well as *Afrothismia winkleri* (ENGL.) SCHLTR. var. *budongensis* COWLEY a dubious taxon from Uganda (Sainge and Franke 2005), were the first records of the genus for East Africa (Cowley 1988). After another 15 years of stagnation things changed dramatically from 2003 until 2006, when seven new species of *Afrothismia* were discovered, thus more than tripling the species number in only three years. The start of this "boom" in descriptive taxonomy was made by H. Maas-van de Kamer with the description of *Afrothismia gesnerioides* MAAS-VAN DE KAMER from South Cameroon (Maas-van de Kamer 2003). It was followed in the same year by M. Cheek's *Afrothismia baerae* CHEEK from Kenia (Cheek 2003). The next four new species published were those, which are subject to this thesis, *Afrothismia saingei* TH. FRANKE, *Afrothismia foertheriana* TH. FRANKE, SAINGE & AGERER, *Afrothismia hydra* SAINGE & TH. FRANKE and *Afrothismia korupensis* SAINGE & TH. FRANKE – all from Cameroon's Southwest Province (Franke 2004, Franke et al. 2004, Sainge & Franke 2005, Sainge et al. 2005 [**papers III - IV**]). The so far last species to be added was *Afrothismia mhoroana* CHEEK from Tanzania (Cheek & Jannerup 2005).

However, another four *Afrothismia* species still await description; one species (*Afrothismia* 'Kupe' spec. nov. in Franke et al. 2006 [**paper VII**]) was discovered at Mt. Kupe, the other three came across while investigating herbarium specimens. The due descriptions of these species will be subject to a monographic treatment of the genus (Franke, Merckx & Sainge, in prep.).

There are two major reasons for the delay of the envisaged generic revision: On the one hand there is uncertainty concerning the legitimacy of the generic name *Afrothismia* (see above), while on the other hand there is an ongoing dispute about the correct phylogenetic placement of *Afrothismia* and three related genera (*Thismia*, *Oxygyne* and *Haplothismia*). As pointed out by Franke (2004 [**paper III**]) and Sainge et al. (2005 [**paper VI**]) the leading view always oscillated between treating them as a distinct family – the Thismiaceae, or as a tribe within the Burmanniaceae – the Thismieae. According to the Angiosperm Phylogenetic Group (APG 2003) they are currently considered to be part of the Burmanniaceae. However, this notion is refuted by Merckx et al. (2006), revealing that *Afrothismia* and related genera (except *Oxygyne*, which was not sampled) together with the genus *Tacca* (Dioscoreaceae)

form a monophyletic lineage, which in turn is the sister-group to a clade comprising all Burmanniaceae sensu stricto as well as the Dioscoreaceae sensu stricto. Hence, both families, the Burmanniaceae and the Dioscoreaceae, proved to be polyphyletic. These results confirm earlier authors who treated the Thismiaceae as a distinct family on the basis of morphological features (Franke 2004 [**paper III**, see references therein]). Consequently *Afrothismia* spp. are referred to as Thismiaceae in Franke et al. (2006 [**paper VII**]) as well as in this treatise. However in the preceding papers they were still treated as Thismieae, giving way to a reviewer's critique of the earliest draft.

Another problematic issue of the envisaged monographic treatment concerns an objective species delimitation, which requires the examination of herbarium specimens in good quantities and if possible from a wide range. Unfortunately these preconditions are not given in the case of *Afrothismia*, as most species were collected only once, at a single spot and comprise only few individuals. A highly problematic case in terms of species delimitation e.g. is represented by *Afrothismia gesnerioides*, which was described after a single collection from the Campo area in South Cameroon (Maas-van de Kamer 2003). Another 'morpho-type' of this species, which is referred to as *Afrothismia* aff. *gesnerioides* in Franke et al. (2006 [**paper VII**]) however, occurs at Mt. Kupe in Cameroon's Southwest Province. Although being strikingly similar in external floral characters, both species differ considerably in view of the internal architecture of the perianth tube. Both species inhabit different so called glacial rain forest refuges – the Southern Cameroon Plateau, respectively the Western Cameroon Mountains (Sosef 1994). Glacial rain forest refuges are areas, where rain forests persisted during the major glacial periods of the Pleistocene (starting 2.5 Myr B.P.), when the overall climate of the African continent was significantly dryer than today (Hamilton and Taylor 1991; Sosef 1994; Plana 2004). This implies that the populations of *A. gesnerioides* and *A.* aff. *gesnerioides* must have been repeatedly separated by arid savannah corridors for thousands of years. During these times of geographic isolation divergent evolution may have lead to major differences in the floral architecture, which in turn may have resulted in reproductive isolation due to an adaptation to different pollinators. If this was the case, *A.* aff. *gesnerioides* and *A. gesnerioides* would unambiguously represent different species. However, two collections – one of each type – don't provide enough clues to support this hypothesis, since there is no evidence that the differences in floral morphology are really consistent.

Of the ten *Afrothismia* species described so far, seven were discovered in Southwest- respectively South Cameroon – if taking into account the yet undescribed *Afrothismia* species from Mt Kupe (*Afrothismia* 'Kupe' spec. nov. in Franke et al. 2006 [**paper VII**]) the number rises to eight. This clearly indicates that the Cameroonian glacial rain forest refuges represent a diversity hotspot of the genus *Afrothismia*. Thus it is almost certain that the palaeoclimatic events of the past 2.5 million years played a major role in the evolution of *Afrothismia*.

2.3.2. Conservation

Compared to most other AMF-exploiting myco-heterotrophic plants, *Afrothismia* species have conspicuous, often very colorful flowers, which can get quite sizable in some species. *Afrothismia saingei* e.g. has flowers that measure up to 9 cm in length and are provided with bright yellow tepals as well as red arrow-shaped markings at the base of the white perianth tube (**Fig. 2** in Franke 2004 [**paper III**]). If seen from this perspective it is almost a miracle that these spectacular plants persistently escaped attention for such a long time. The reason for this paradox may not be found in the flowers themselves, but rather in their point of attachment. If assuming that flowers like these would dangle from the tip of a branch, there is no doubt that they would have been noticed much earlier. People who search for something,

automatically focus their attention to places where they would expect the searched-after most likely to be – and if the searched-after is a flowering plant, the decomposing foliage and rotten wood of the forest floor is definitely not the first choice to look at. Keeping this in mind, a careful screening of the forest floor at the right time and the right area can reveal hitherto undescribed species of *Afrothismia* and other AMF-exploiting myco-heterotrophic plants even at places where one wouldn't expect to find anything new.

One of these places is the Korup Forest Dynamic Plot (KFDP), which is a 50 hectare transect of evergreen Guineo-Congolean rain forest in Cameroon's Korup National Park and the type locality of the recently described *Afrothismia hydra* and *Afrothismia korupensis* (Sainge & Franke 2005 [**paper V**]; Sainge et al. 2005 [**paper VI**]). The 500 x 2000 m plot was established in 1994 by the Center for Tropical Forest Science (CTFS), in order to monitor the composition and demography of woody plants in a tropical forest (Thomas & al. 2003). Although the plot's vegetation was tagged, mapped and constantly observed, both new species had escaped attention for almost a decade. *Afrothismia hydra* has small but colourful red and yellow flowers and occurs at the plot in abundance (Sainge & Franke 2005 [**paper V**]). Its fruits contain hundreds of sticky seeds attached to a distinct column-like structure (Sainge & Franke 2005 [**paper V**]). A frequently travelled footpath running directly through a large population of *A. hydra* was found to be neatly lined up with dozens of individuals for several meters outside the core population (Franke, pers. observ.). Metaphorically speaking this observation shows that human feet can be an effective means of seed dispersal for *A. hydra*, on the other hand it illustrates that AMF-exploiting myco-heterotrophic plants rather end up getting squashed beneath botanists' shoes, than between paper sheets in a botanist's plant press, thus resulting in their poor representation in herbaria.

This situation makes it difficult to correctly evaluate the conservational status of AMF-exploiting myco-heterotrophic plants, as it is impossible to distinguish between persistently overlooked common species and those which are really rare. In 1995 Cheek and collaborators discovered a new genus and species of Triuridaeae – *Kupea martinetugei* CHEEK & S.A. WILLIAMS – at Mt Kupe in Southwest Cameroon (Cheek et al. 2003). According to the Red List categories and criteria of the IUCN (2001), the authors considered the species to be critically endangered, because they counted only 250 individuals at two close-by locations. However, one year before Cheek et al.'s paper was released, Franke and collaborators discovered two additional population sites of this elusive little plant during a myco-heterotrophic plant inventory at the peripheral zone of the Onge Forest Reserve, ca. 100 km SW of the type locality (see 'Habitat & Ecology' in Franke et al. 2004 [**paper IV**]). Three years later M. Sainge found *K. martinetugei* in Southeast Cameroon, ca. 350 km SE of the type locality, thus downgrading its conservational status from being "critically endangered" to "vulnerable" (Sainge & Franke, in prep.). Unfortunately the positive alteration of a rare species' conservational status is rather the exception than the rule. Franke (2004 [**paper III**]) described *Afrothismia saingei* from a single location at Mt Kupe, close to the place where Cheek discovered *K. martinetugei*. Despite extensive screenings for myco-heterotrophic plants in adequate habitats throughout Cameroon's Southwest Province, *A. saingei* had never been seen again. The same fate experienced *Afrothismia foertheriana*, which was described after four flowering individuals from a single population in the peripheral zone of the Onge Forest Reserve, the area where *K. martinetugei* was found for the second time (Franke et al. 2004 [**paper IV**]). Just like *A. saingei*, the species was never seen elsewhere and is currently considered to be critically endangered (Franke et al. 2004 [**paper IV**]). In an essay, titled "The tropical flora remains undercollected" Prance et al. (2000) propose that "More detailed studies of selected small areas in the tropics are likely to yield many more new species as well as most useful demographic data about those that are already described. The more complete the inventory is the better the data we will have to provide the rationale for conservation […]"

Although applying to biological exploration in the tropics as a whole, do Prance at al.'s general suggestions highlight some of the most urgent additional requirements of AMF-exploiting myco-heterotrophic plant research.

The BIOLOG project represents one of these detailed studies of selected small areas in the tropics, which were proposed by Prance et al. (2000). The main goal was the inventory of all myco-heterotrophic species in Cameroon's Southwest Province. Altogether 17 species of AMF-exploiting myco-heterotrophic plants, as well as two achlorophyllous orchids, were recorded at ten different localities, covering most of the previously described taxa of this region (tab. 1). In fact the only species that didn't come across, despite extensive screenings of suitable habitats, were *Oxygyne triandra* SCHLTR. (Thismiaceae) and *Afrothismia pachyantha* (Franke et al. 2004 [**paper IV**]). Both species are exclusively known from the type specimens, which had been collected in 1905 at the southeastern foothills of Mt. Cameroon – an area where most natural vegetation had been replaced by oil palm and rubber plantations (Franke et al. 2004 [**paper IV**]). As they were not found anywhere else in the province, their fate is still shrouded in uncertainty. The apparent rediscovery of *A. pachyantha* by Cheek and collaborators (Cheek and Williams 1999) turned out to be a mistake, as they failed to notice that the plant they took for *A. pachyantha* represented a new species, which is closely related or conspecific with the recently described *Afrothismia gesnerioides* (see above).

However, plans to increase the efforts to rediscover *O. triandra* and *A. pachyantha* and to develop regional management plans for the conservation of Cameroonian myco-heterotrophic plants were choked by the end of 2003. In scope of the preparation of the second phase of the BIOLOG programme (2004-2008), it was decided to amalgamate all West Africa-based BIOLOG projects and concentrate them in Benin (BIOTA West). As Benin doesn't have any suitable habitats for AMF-exploiting myco-heterotrophic plants, this decision made a successful continuation of the project impossible.

Table 1. Myco-heterotrophic plant taxa recorded for Southwest Cameroon

Nr.	**Art:**	**Familie:**
1	*Sebaea oligantha* (GILG) SCHINZ	Gentianaceae
2	*Kupea martinetugei* CHEEK & S.A. WILLIAMS	Triuridaceae
3	*Sciaphila ledermannii* ENGL.	Triuridaceae
4	*Afrothismia foertheriana* T. FRANKE, SAINGE & AGERER	Thismiaceae
5	*Afrothismia* aff. *gesnerioides* MAAS-VAN DE KAMER	Thismiaceae
6	*Afrothismia hydra* SAINGE & T. FRANKE	Thismiaceae
7	*Afrothismia korupensis* SAINGE & T. FRANKE	Thismiaceae
8	*Afrothismia* 'Kupe' spec. nov	Thismiaceae
9	*Afrothismia saingei* T. FRANKE	Thismiaceae
10	*Afrothismia winkleri* (ENGL.) SCHLTR.	Thismiaceae
11	*Burmannia congesta* (WRIGHT) JONKER	Burmanniaceae
12	*Burmannia hexaptera* SCHLTR.	Burmanniaceae
13	*Gymnosiphon* cf. *longistylus* (BENTH.) HUTCH. ET DALZ.	Burmanniaceae
14	*Gymnosiphon squamatus* WRIGHT	Burmanniaceae
15	*Gymnosiphon* cf. *usambaricus* ENGL.	Burmanniaceae
16	*Auxopus macranthus* SUMMERH.	Orchidaceae
17	*Epipogium roseum* (D. DON.) LINDL.	Orchidaceae
18	*Gymnosiphon* spec. 1	Burmanniaceae
19	*Gymnosiphon* spec. 2	Burmanniaceae

3. Summary

AMF-exploiting myco-heterotrophic plants don't perform photosynthesis, but receive all essential nutrients, including carbon from root (procaulome)-colonizing arbuscular-mycorrhizal fungi (AMF; Glomeromycota). Due to the plants' parasitic nature contrivances enabling phototrophic metabolism are either reduced or lack completely.

Anatomical analyses provide an insight of how myco-heterotrophic plants interact with their fungal hosts. *Voyria flavescens* is an AMF-exploiting myco-heterotrophic Gentianaceae from Amazonian Perú. The subterranean organs of the mature plant correspond to early developmental stages in the ontogeny of wintergreens and orchids, which are referred to as procaulomes, respectively mycorhizomes. This gives rise to the assumption that *Voyria flavescens* represents a case of neoteny. The colonization of the procaulomes' cortical tissue by fungal hyphae is regulated by a temporarily penetrable circular zone just behind the tip of its root-like projections. The hyphae within the cortical tissue are successively digested. Although lacking both, arbuscules and vesicles, the fungal host displays other unambiguous anatomical features of AMF.

Molecular methods provide a convenient and very precise way of host identification. The fungal endophytes of 11 AMF-exploiting myco-heterotrophic plants from tropical Africa were identified by partial 18S rDNA sequencing. All investigated plants proved to be associated with AMF of the family-ranking *Glomus*-group A lineage. These fungi, which are also known to be parasitized by South American AMF-exploiting myco-heterotrophic plants, seem to be more lucrative hosts than other members of the Glomeromycota.

Due to their concealed nature, AMF-exploiting myco-heterotrophic plants are difficult to find. Thus it is not surprising that a considerable number is still undescribed. Repeatedly new species were brought to light as by-products of mycological field trips. This was the case in *Triuridopsis intermedia*, which, besides being a new species, also represents the first record of the family Triuridaceae for Bolivia. Recent ontogenetic investigations of the closely related genus *Triuris* however question the legitimacy of *Triuridopsis* as a distinct genus.

Another four taxonomic novelties were discovered during an inventory of the AMF-exploiting myco-heterotrophic plants of Cameroon's Southwest Province. All four new species belong to the genus *Afrothismia*. The overall phylogenetic position of *Afrothismia* and three allied genera is still unclear and oscillates between treating them as a distinct family – the Thismiaceae or a tribe of the Burmanniacae – the Thismieae. The four original descriptions compiled in this thesis stand for a recent upswing in descriptive taxonomy during which the number of *Afrothismia* species was more than tripled in only three years. The four new taxa are *Afrothismia hydra*, *Afrothismia korupensis*, *Afrothismia saingei* and *Afrothismia foertheriana*. The correct phylogenetic placement of *Afrothismia* within the Dioscoreales is still a matter of dispute.

In 2001 respectively 2002 large flocks of *Afrothismia hydra* and *Afrothismia korupensis* were found at a permanent observation plot in Korup National Park, where both new species escaped attention during the past decade. *Afrothismia saingei* was discovered in 2002 at a single spot at Mt Kupe. It was described after three individual plants and never seen since. *Afrothismia foertheriana* was discovered the same year in the peripheral zone of the Onge forest reserve and never found elsewhere. Extensive screenings of suitable habitats in the region were without success, thus leading to the assumption that *Afrothismia saingei* and *Afrothismia foertheriana* might be critically endangered. However reliable estimations of the

conservational status of AMF adapted myco-heterotrophic plants are extremely difficult due to the plants' cryptic mode of life.

References

Abe C, Akasawa Y (1989) A new species of *Oxygyne* (Burmanniaceae) found in Shikoku, Japan. Journal of Japanese Botany 64: 161-164.

Ambrose BA, Espinosa-Matías S, Vásquez-Santana S, Vergara-Silva F, Martínez E, Márquez-Guzmán J, Alvarez-Buylla ER (2006) Comparative developmental series of the Mexican Triurids support a euanthial interpretation for the unusual reproductive axes of *Lacandonia schismatica* (Triuridaceae). American Journal of Botany 93: 15-35.

APG **(**2003) An update of the angiosperm phylogeny group classification for the orders and families of flowering plants: APG II. Botanical Journal of the Linnéan Society 141: 399-436.

Aussenac G (2000) Interactions between forest stands and microclimate: Ecophysiological aspects and consequences for silviculture. Annals of Forest Sciences 57: 287-301.

Bateman RM, Hollingsworth PM, Squirell J, Hollingsworth ML (2005) Phylogenetics: Neottieae. In: Pridgeon AM, Cribb PL, Chase MW, Rasmussen FN (eds.) Genera Orchidacearum (4): Epidendroideae 1. Oxford University Press, Oxford, UK.

Beccari O (1877) Burmanniaceae. Malesia 1: 240-254.

Bending GD, Read DJ (1996) Nitrogen mobilization from protein-polyphenol complex by ericoid and ectomycorrhizal fungi. Soil Biology and Biochemistry 28: 1603-1612.

Bentham G (1849) Burmanniaceae. In: Hooker WJ (ed.) Niger Flora: p. 528. London, UK.

Bernard N (1909) L'evolution dans la symbiose des orchidees et leurs chamignons commensaux. Annales des Sciences Naturelles Paris, 9. sér., 9: 1-196.

Bidartondo MI (2005) The evolutionary ecology of myco-heterotrophy. New Phytologist 167: 335-352.

Bidartondo MI, Redecker D, Hijri I, Wiemken A, Bruns TD, Domínguez L, Sérsic A, Leake JR, Read DJ (2002) Epiparasitic plants specialized on arbuscular mycorrhizal fungi. Nature 419: 389-392.

Bidartondo MI, Bruns TD, Weiß M, Sérgio S, Read DJ (2003) Specialized cheating of the ectomycorrhizal symbiosis by an epiparasitic liverwort. Proceedings of the Royal Society of London. Series B, Biological Sciences 270: 835-842.

Björkman E (1960) *Monotropa hypopitys* L. – an epiparasite on tree roots. Physiologia Plantarum 13: 308-327.

Bruchmann H (1898) Über die Prothallien und die Keimpflanzen mehrerer europäischer Lycopodien. Friedrich Andreas Perthes, Gotha, Germany.

Brundrett M (2004) Diversity and classification of mycorrhizal associations. Biological Reviews 79: 473-495.

Burgeff H (1932) Saprophytismus und Symbiose. Gustav Fischer, Jena.

Camp WH (1940) Aphyllous forms in *Pyrola*. Bulletin of the Torrey Botanical Club 67: 453-465.

Chapin FS (1995) New cog in the nitrogen cycle. Nature 377: 199-200.

Chazdon RL, Fetcher N (1984) Photosynthetic light environments in a lowland tropical rainforest in Costa Rica. Journal of Ecology 72: 553-564.

Cheek M (2003) A new species of *Afrothismia* (Burmanniaceae) from Kenya. Kew Bulletin 58: 951-955.

Cheek M, Jannerup P (2005) A new species of *Afrothismia* (Burmanniaceae) from Tanzania. Kew Bulletin 60: 593-596.

Cheek M, Williams S (1999) A review of African saprophytic flowering plants. In: Timberlake J, Kativu S (eds.) African plants: biodiversity, taxonomy & uses. Proceedings of the 1997 AETFAT congress, Harare, Zimbabwe: pp. 39-49. Royal Botanic Gardens, Kew, UK.

Cheek M, Williams S, Etuge M (2003) *Kupea martinetugei*, a new genus and species of Triuridaceae from western Cameroon. Kew Bulletin 58: 225-228.

Cowley, EJ (1988) Burmanniaceae. In: Polhill RM (ed.), Flora of Tropical East Africa. Balkema, Rotterdam, The Netherlands.

Cronquist A (1981) An integrated system of classification of flowering plants. Columbia University Press, New York, USA.

Cullings KW, Szaro TM, Bruns TD (1996) Evolution of extreme specialization within a lineage of ectomycorrhizal epiparasites. Nature 379: 63-66.

De Bary A (1884) Vergleichende Morphologie und Biologie der Pilze, Mycetozoa und Bacterien. Engelmann, Leipzig, Germany.

De la Sota ER (1960) *Mamorea singeri*, un nuevo género y especie de Burmanniaceae. Darwiniana 12: 43-47.

Domínguez LS, Sérsic A (2004) The southernmost myco-heterotrophic plant, *Arachnitis uniflora*: root morphology and anatomy. Mycologia 96: 1143-1151.

Engler A (1894) Burmanniaceae africanae. Botanische Jahrbücher für Systematik, Pflanzengeschichte und Pflanzengeographie 20: 138.

Engler A (1905) *Thismia winkleri* Engl., eine neue afrikanische Burmanniaceae. Botanische Jahrbücher für Systematik, Pflanzengeschichte und Pflanzengeographie 38: 89-91.

Engler A (1908) Burmanniaceae. Die Pflanzenwelt Afrikas II: pp. 400-403. Leipzig, Germany.

Engler A (1910) Eine bisher in Afrika nicht nachgewiesene Pflanzenfamilie, Triuridaceae. In: Engler A (ed.) Beiträge zur Flora von Afrika. XXXV. Botanische Jahrbücher für Systematik, Pflanzengeschichte und Pflanzengeographie: 303-307.

Feild TS, Brodribb TJ (2005) A unique mode of parasitism in the conifer coral tree *Parasitaxus ustus* (Podocarpaceae). Plant, Cell and Environment 28: 1316-1325.

Frank B (1885) Über die auf Wurzelsymbiose beruhende Ernährung gewisser Bäume durch unterirdische Pilze. Berichte der deutschen botanischen Gesellschaft 3: 128-145.

Frank B (1891) Über die auf Verdauung von Pilzen abzielende Symbiose der mit endotropher Mykorrhiza begabten Pflanzen, sowie der Legominosen und Erlen. Berichte der Deutschen Botanischen Gesellschaft 9: 244-253.

Franke T (2002) The myco-heterotrophic *Voyria flavescens* (Gentianaceae) and its associated fungus. Mycological Progress 1: 367-376.

Franke T (2004) *Afrothismia saingei* (Burmanniaceae), a new myco-heterotrophic plant from Cameroon. Systematics and Geography of Plants 74: 27-33.

Franke T, Beenken L, Hahn C (2000) *Triuridopsis intermedia* spec. nov. (Triuridaceae), a new myco-hetrotrophic plant from Bolivia. Plant Systematics and Evolution 225: 141-144.

Franke T, Merckx V, Sainge MN (in prep.) A monographic revision of *Afrothismia* (Thismiaceae) OR: A monographic revision of *Thismia*, subgen. *Afrothismia* (Thismiaceae).

Franke T, Sainge MN, Agerer R (2004) A new species of *Afrothismia* (Burmanniaceae; tribe Thismieae) from the western foothills of Mt. Cameroon. Blumea 49: 451-456.

Franke T, Beenken L, Döring M, Kocyan A, Agerer R (2006) Arbuscular mycorrhizal fungi of the *Glomus*-group A lineage (Glomerales; Glomeromycota) detected in myco-heterotrophic plants from tropical Africa. Mycological Progress 5: 24-31.

Fuchs A, Ziegenspeck H (1926) Entwicklungsgeschichte der Achsen der einheimischen Orchideen und ihre Physiologie und Biologie. I. *Cypripedium*, *Helleborine*, *Limodorum*, *Cephalanthera*. Botanisches Archiv 14: 165-260.

Gebauer G, Meyer M (2003) ^{15}N and ^{13}C natural abundance of autotrophic and mycoheterotrophic orchids provides insight into nitrogen and carbon gain from fungal association. New Phytologist 160: 209-223.

George AS (1980) *Rhizanthella gardneri* R.S. Rogers the underground orchid of Western Australia. American Orchid Society Bulletin 49: 631-646.

Giovannetti M, Sbrana C, Avio L, Strani P (2004) Patterns of below-ground plant interconnections established by means of arbuscular mycorrhizal networks. New Phytologist 164: 175-181.

Groom P (1895) Contributions to the knowledge of monocotyledonous saprophytes Journal of the Linnéan Society of London 31: 149-215.

Hamilton AC, Taylor D (1991) History of climate and forests in tropical Africa during the last 8 million years. In: Myers N (ed.) Tropical forests and climate: pp. 65-78. Kluwer, Dordrecht, The Netherlands.

Hart MM, Reader RJ (2002) Taxonomic basis for variation in the colonization strategy of arbuscular mycorrhizal fungi. New Phytologist 153: 335-344.

Henning W (1982) Phylogenetische Systematik. Paul Parey, Berlin, Germany.

Holloway JE (1917) The prothallus and young plant of *Tmesipteris*.Transactions of the Royal Society of New Zealand 50: 1-44.

Horton TR, Bruns TD (1998) Multiple-host fungi are the most frequent and abundant ectomycorrhizal types in a mixed stand of Douglas-fir (*Pseudotsuga menziesii*) and bishop pine (*Pinus muricata*). New Phytologist 139: 331-339.

Hunt TE (1953) Australia's subterranean orchids. Orchid Journal 2: 303-305.

Husband R (2004) The diversity, ecology and molecular detection of arbuscular mycorrhizal fungi. In Gillings M, Holmes A (eds.), Plant Microbiology: pp. 1-17. Garland Science/BIOS Scientific Publishers, Abingdon, UK.

Imhof S (1997) Root anatomy and mycotrophy of the achlorophyllous *Voyria tenella* Hooker (Gentianaceae). Botanica Acta 110: 298-305.

Imhof S (1999a) Anatomy and mycotrophy of the achlorophyllous *Afrothismia winkleri*. New Phytologist 144: 533-540.

Imhof S (1999b) Root morphology, anatomy and mycotrophy of the achlorophyllous *Voyria aphylla* (Jaqu.) Pers. (Gentianaceae). Mycorrhiza 9: 33-39.

Imhof S (1999c) Subterranean structures and mycorrhiza of the achlorophyllous *Burmannia tenella* Benth. (Burmanniaceae). Canadian Journal Botany 77: 637-643.

Imhof S (2001) Subterranean structures and mycotrophy of the achlorophyllous *Dictyostega orobanchoides* (Hook.) Miers (Burmanniaceae). Revista de Biologia Tropical 49: 239-247.

Imhof S (2003) A dorsiventral mycorrhhizal root in the achlorophyllous *Sciaphila polygyna* (Triuridaceae). Mycorrhiza 13: 327-332.

Imhof S (2004) Morphology and development of the subterranean organs of the achlorophyllous *Sciaphila polygyna* (Triuridaceae). Botanical Journal of the Linnean Society 146: 295-301.

Imhof S, Weber HC (1997) Root anatomy and mycotrophy (AM) of the achlorophyllous *Voyria truncata* (Standley) Standley and Steyermark (Gentianaceae). Botanica Acta 110: 127-134.

Imhof S, Weber HC (2000) Root structures and mycorrhiza of the achlorophyllous *Voyria obconica* Progel (Gentianaceae). Symbiosis 29: 201-211.

Imhof S, Weber HC, Gomez LD (1994) Ein Beitrag zur Biologie von *Voyria tenella* Hook. und *Voyria truncata* (Standley) Standley and Steyermark (Gentianaceae). Beiträge zur Biologie der Pflanzen 68: 113-123.

IUCN (2001) IUCN Red List Categories and Criteria: Version 3.1. IUCN Species Survival Comisson. IUCN, Gland, Switzerland and Cambridge, UK.

Johow F (1885) Die chlorophyllfreien Humusbewohner West-Indiens, biologisch-morphologisch dargestellt. Jahrbücher für Wissenschaftliche Botanik 16: 415-449.

Johow F (1889) Die chlorophyllfreien Humuspflanzen nach ihren biologischen und anatomisch-entwicklungsgeschichtlichen Verhältnissen. Jahrbücher für Wissenschaftliche Botanik 20: 475-524.

Jonker FP (1938) A monograph of the Burmanniaceae. Mededeelingen van het Botanisch Museum en Herbarium van de Rijksuniversiteit te Utrecht 51: 1-279.

Jongmans A, Van Breemen N, Lundström U, Van Hees PAW, Finlay RD, Srinivasan M, Unestam T, Giesler R, Melkerud PA, Olsson M (1997) Rockeating fungi. Nature 389: 682-683.

Julou T, Burghardt B, Gebauer G, Berveiller D, Damesin C, Selosse M-A (2005) Evolution of mixotrophy in orchids: insight from a comparative study of green and achlorophyllous *Cephalanthera damasonium*. New Phytologist 166: 639-653.

Kamienski F (1881) Die Vegetationsorgane der *Monotropa hypopytis* L. Vorläufige Mittheilung. Botanische Zeitung 29: 458-461.

Kamienski F (1982) Les organes végétativs du *Monotropa hypopithys* L. Mémoires de la Sociéte National des Sciences Naturelles e Mathématiques de Cherbourg 26: 1-40.

Kennedy PG, Izzo, AI, Bruns TD (2003) High potential for common mycorrhizal networks between understorey and canopy trees in a mixed evergreen forest. Journal of Ecology 91, 1071-1080.

Knöbel M, Weber HC (1988) Vergleichende Untersuchungen zur Mycotrophie bei *Gentiana verna* L. und *Voyria truncata* (Stand.) Stand. and Stey. (Gentianaceae). Beiträge zur Biologie der Pflanzen 63: 463-477.

Lan J, Xu JT, Li JS (1994) Study on symbiotic relation between *Gastrodia elata* and *Armillariella mellea* by autoradiography. Acta Mycologica Sinica 13: 219-222.

Larcher W (1994) Ökophysiologie der Pflanzen. Verlag Eugen Ulmer, Stuttgart, Germany.

Leake JR (1994) The biology of myco-heterotrophic ('saprophytic') plants. Tansley Review No. 69, New Phytologist 127: 171-216.

Leake JR (2004) Myco-heterotroph/epiparasitic plant interactions with ectomycorrhizal and arbuscular mycorrhizal fungi. Current Opinion in Plant Biology 7: 422-428.

Leake JR (2005) Plants parasitic on fungi: unearthing the fungi in myco-heterotrophs and debunking the 'saprophytic' plant myth. Mycologist 19: 113-122.

Lima D (1953) Notas sôbre a dispersão de algunas espécies vegetais no Brasil. Anais de la Sociedad Biologica de Pernambuco 11: 25-49.

Lusk CH, Chazdon RL, Hofmann G (2006) A bounded null model explains juvenile tree community structure along light availability gradients in a temperate rain forest. Oikos 112: 131-137.

Maas PJM, Maas H (1987) Ecuadorian saprophytes, a preliminary review. Opera Botanica 92: 131-145.

Maas PJM, Maas-van de Kamer H (1988) Burmanniaceae. In: Pinto P, Lozano G (eds.) Flora de Colombia. Universidad Nacional de Colombia, Colciencias, Bogota, Colombia.

Maas PJM, Rübsamen T (1986) Triuridaceae. Flora Neotropica Monograph 40. The New York Botanical Garden, Bronx, New York, USA.

Maas PJM, Ruyters P (1986) *Voyria* and *Voyriella* (Saprophytic Gentianaceae). Flora Neotropica Monograph 41. The New York Botanical Garden, Bronx, New York, USA.

Maas PJM, Maas-van de Kamer H, van Benthem J, Snelders HCM, Rübsamen T (1986a) Burmanniaceae. Flora Neotropica Monograph 42. The New York Botanical Garden, Bronx, New York, USA.

Maas PJM and Collaborators (1986b) Saprophytes pro parte. General Introduction. Flora Neotropica Monographs 40, 41, 42. The New York Botanical Garden, Bronx, New York, USA.

Maas-van de Kamer H (2003) *Afrothismia gesneriodes*, another new species of *Afrothismia* (Burmanniaceae) from tropical Africa. Blumea 48: 475-478.

Maas-van de Kamer H, Maas PJM (1994) *Triuridopsis*, a new monotypic genus in Triuridaceae. Plant Systematics and Evolution 192: 257-262.

MacDougal DT (1899) Symbiotic Saprophytism. Annals of Botany 13: 1-47.

Malims-Smith A. (1952) The so-called saprophytic orchids. The Naturalist: 159-163.

McKendrick SL, Leake JR, Read DJ (2000) Symbiotic germination and development of myco-heterotrophic plants in nature: transfer of carbon from ectomycorrhizal *Salix repens* and *Betula pendula* to the orchid *Corallorhiza trifida* through shared hyphal connections. New Phytologist 145: 539-548.

Merckx V, Schols P, Maas-van de Kamer H, Maas PJM, Huysmans S, Smets E (2006) Phylogeny and evolution of Burmanniaceae (Dioscoreales) based on nuclear and mitochondrial data. American Journal of Botany 93: 1684-1698.

Meerendonk JPM van de (1984) Triuridaceae. In: Steenis CGGJ van (ed.) Flora Malesiana, Ser. 1., 10: pp. 109-121. Martinus Nijhoff, The Hague, The Netherlands.

Molina R, Massicotte H, Trappe JM (1992) Specificity phenomena in mycorrhizal symbiosis: community-ecological consequences and practical implications. In: Allen MF (ed.) Mycorrhizal functioning: an integrative plant-fungal process: pp. 357-423. Chapman and Hall, New York, USA.

Montfort C (1940) Beziehungen zwischen morphologischen und physiologischen Reduktionserscheinungen im Bereich der Licht-Ernährung bei saprophytischen Orchideen. Berichte der Deutschen Botanischen Gesellschaft 58: 41-49.

Montfort C, Küsters G (1940) Saprophytismus und Photosynthese. I. Biochemische und physiologische Studien an Humus-Orchideen. Botanisches Archiv 40: 571-633.

Mori SA, Boom BM, Prance GT (1981) Distribution patterns and conservation of eastern Brazilian costal forest tree species. Brittonia 33: 233-245.

Northup RR, Yu Z, Dahlgren RA, Vogt KA (1995) Polyphenol control of nitrogen release-from pine litter. Nature 377: 227-229.

Oehler E (1927) Entwicklungsgeschichtlich-zytologische Untersuchungen an einigen saprophytischen Gentiananceen. Planta 3: 641-729.

Onguene NA, Kuyper TW (2002) Importance of the ectomycorrhizal network for seedling survival and ectomycorrhizal formation in rain forests of South Cameroon. Mycorrhiza 12: 13-17.

Peterson RL, Massicotte HB, Melville LH (2004) Mycorrhizas: Anatomy and Cell Biology. NRC Research Press, Ottawa, Canada.

Pfeiffer NE (1914) Morphology of *Thismia americana*. Botanical Gazette 66: 122-135.

Plana V (2004) Mechanisms and tempo of evolution in the African Guineo-Congolean rainforest. Philosophical Transactions of the Royal Society of London, Series B 359: 1585-1594.

Prance GT, Beentje H, Dransfield J, Johns R (2000) The tropical flora remains undercollected. Annals of the Missouri Botanical Garden 87: 67-71.

Rasmussen HN (1986) The vegetative architecture of orchids. Lindleyana 1: 42-50.

Rasmussen HN (1995) Terrestrial orchids from seed to mycotrophic plant. Cambridge University Press, Cambridge, UK.

Raynal-Roques A, Paré J (1998) Biodiversité des Phanérogames parasites: leur place dans la classificacion systématique. Adansonia, sér. 3, 20: 313-322.

Read DJ (1989) Ecological integration by mycorrhizal fungi. In: Nardon P, Gianinazzi-Pearson V, Grenier AM, Margulis L, Smith DC (eds.) Endocytobiology IV: pp. 100-106. INSA, Villeurbanne, France.

Redecker D, Thierfelder H, Walker C, Werner D (1997) Restriction analysis of PCR amplified internal transcribed spacers of ribosomal DNA as a tool for species identification in different genera of the order Glomales. Applied and Environmental Microbiology 63: 1756-1761.

Sainge MN (2003) Studies on the phenology of African myco-heterotrophic plants (Abstract). Tagungsband der 16. Jahrestagung der Gesellschaft für Tropenökologie: p. 66 Rostock, Germany.

Sainge MN, Franke T (2005) A new species of *Afrothismia* (Burmanniaceae) from Cameroon. Nordic Journal of Botany 23: 299-303.

Sainge MN, Franke T (in prep.) Recent new records of myco-heterotrophic plants in Cameroon.

Sainge MN, Franke T, Agerer R (2005) A new species of *Afrothismia* (Burmanniaceae; tribe Thismieae) from Korup National Park, Cameroon. Wildenowia 35: 287-291.

Schlechter R (1906) Burmanniaceae africanae. Botanische Jahrbücher für Systematik, Pflanzengeschichte und Pflanzengeographie 38: 137-143.

Schlechter R (1921) Die Thismieae. Notizblätter des Botanischen Gartens und Museums zu Berlin-Dahlem 8: 31-45.

Schmid E, Oberwinkler F (1994) Light and electron microscopy of the host-fungus interaction in the achlorophyllous gametophyte of *Botrychium lunaria*. Canadian Journal of Botany 72: 182-188.

Schmid E, Oberwinkler F (1995) A light- and electronmicroscope study on a vesicular-arbuscular host-fungus interaction in gametophytes and young sporophytes of the Gleicheniaceae (Filicales). New Phytologist 129: 317-324.

Schwarzott D, Walker C, Schüssler A (2001) *Glomus* the largest genus of the arbuscular mycorrhizal fungi (Glomales), is nonmonophyletic. Molecular Phylogenetics and Evolution 21: 190-197.

Seidel R (1995) Inventario de los árboles en tres parcelas de bosques primario en la Serranía de Marimonos, Alto Beni. Ecología de Bolivia 25: 1-35.

Simard SW, Perry DA, Jones MD, Myrold DD, Durall DM, Molina R (1997) Net transfer of carbon between ectomycorrhizal tree species in the field. Nature 388: 579-582.

Simard SW, Jones MD, Durall DM (2002) Carbon and nutrient fluxes within and between mycorrhizal plants. In: van der Heijden MGA & Sanders IR (eds.) Mycorrhizal Ecology: pp. 33-74. Springer, Germany.

Sitte P, Weiler EW, Kadereit JW, Bresinsky A, Körner C (2002) Lehrbuch der Botanik für Hochschulen / begr. von Strasburger E, Noll F, Schenk H, Schimper AFW. 35. Auflage. Spektrum Akademischer Verlag, Heidelberg, Germany.

Smith JJ (1911) Zur Systematik von *Thismia clandestina* Miq. und *Thismia versteegii* J.J. Smith. In: Ernst A, Bernard CH (eds.) Beiträge zur Kenntnis der Saprophyten Javas. Annales du Jardin Botanique de Buitenzorg 9: 55-60.

Smith SE, Read DJ (1997) Mycorrhizal symbiosis. 2nd ed. Academic Press, London, UK.

Sosef MSM (1994) Refuge Begonias. Taxonomy, phylogeny and historical biogeography of *Begonia* sect. *Loasibegonia* and sect. *Scutibegonia* in relation to glacial rain forest refuges in Africa. PhD thesis. Wageningen, The Netherlands.

Stadler M (2006) Funktionsanatomische Untersuchungen der unterirdischen Strukturen und Mykorrhizierung anhand ausgewählter Vertreter der Burmanniaceae (Dioscoreales, Monocotyledonae) Zulassungsarbeit im Fachbereich Biologie an der Ludwig-Maximilians-Universität München, Germany.

Stone B (1980) Rediscovery of *Thismia clavigera* (Becc.) F. v. M. (Burmanniaceae). Blumea 26: 419-425.

Thomas DW, Kenfack D, Chuyong GB, Sainge MN, Losos EC, Condit RS, Songwe N (2003) Tree species of Southwestern Cameroon: Tree distribution maps, diameter tables, and species documentation of the 50-hectare Korup Forest Dynamic Plot. Center for Tropical Forest Science of the Smithsonian Tropical Research Institute and Bioresources Development and Conservation Programme-Cameroon, Washington, D.C., USA.

Taylor DL, Bruns TD, Leake JR, Read DJ (2002) Mycorrhizal specificity and function in myco-heterotrophic plants. In: van der Heijden MGA & Sanders IR (eds.) Mycorrhizal Ecology: pp. 375-413. Springer, Germany.

Velenovsky J (1905) Über die Keimpflanzen der Pirolaceen. Bulletin international de l'Académie des Sciences de Bohème 10: 1-6.

Wallace GD (1975) Studies of the Monotropoideae (Ericaceae): Taxonomy and distribution. Wassman Journal of Biology, 33: 1–88.

Whitmore TC (1990) An Introduction to Tropical Rain Forests. Oxford University Press, Oxford, UK.

Woodward C, Berry P, Maas H, Swing K (in press). *Tiputinia foetida*, a new mycoheterotrophic genus of Burmanniaceae (subfamily Thismioideae) from Amazonian Ecuador, and a likely case of deceit pollination. Taxon.

Wright CH (1897) 546. *Gymnosiphon squamatum*. Bulletin of miscellaneous information, Royal Gardens Kew: 281.

Wright CH (1898) Burmanniaceae. In: Thiselton-Dyer W (ed.) Flora of tropical Africa: pp. 10-12. London, UK.

Yamato M (2001) Identification of a mycorrhizal fungus in the roots of achlorophyllous *Sciaphila tosaensis* Makino (Triuridaceae). Mycorrhiza 11: 83-88.

Yamato M, Yagame T, Suzuki A, Iwase K (2005) Isolation and identification of mycorrhizal fungi associating with an achlorophyllous plant, *Epipogium roseum* (Orchidaceae). Mycoscience 46: 73-77.

Zhang D, Saunders RMK, Hu C-M (1999) *Corsiopsis chinensis* gen. et sp. nov. (Corsiaceae): First record of the family in Asia. Systematic Botany 24: 311-314.

5. Appendix

This thesis is based on the following articles, which are referred to in the text by their Roman numerals (I-VII, in a chronological order):

Franke T, Beenken L, Hahn C (2000) *Triuridopsis intermedia* spec. nov. (Triuridaceae), a new myco-hetrotrophic plant from Bolivia. Plant Systematics and Evolution 225: 141-144. …….……. I

Franke T (2002) The myco-heterotrophic *Voyria flavescents* (Gentianaceae) and its associated fungus. Mycological Progress 1: 367-376. …….……. II

Franke T (2004) *Afrothismia saingei* (Burmanniaceae), a new myco-heterotrophic plant from Cameroon. Systematics and Geography of Plants 74: 27-33. ……..…… III

Franke T, Sainge MN, Agerer R (2004) A new species of *Afrothismia* (Burmanniaceae; tribe Thismieae) from the western foothills of Mt. Cameroon. Blumea 49: 451-456. ……..……... IV

Sainge MN, Franke T (2005) A new species of *Afrothismia* (Burmanniaceae) from Cameroon. Nordic Journal of Botany 23: 299-303. ……..…… V

Sainge MN, Franke T, Agerer R (2005) A new species of *Afrothismia* (Burmanniaceae; tribe Thismieae) from Korup National Park, Cameroon. Wildenowia 35: 287-291. ……..……...……... VI

Franke T, Beenken L, Döring M, Kocyan A, Agerer R (2006) Arbuscular mycorrhizal fungi of the *Glomus*-group A lineage (Glomerales; Glomeromycota) detected in myco-heterotrophic plants from tropical Africa. Mycological Progress 5: 24-31. …….…… VII

I

Plant Syst. Evol. 225: 141–144 (2000)

Plant Systematics and Evolution

Triuridopsis intermedia spec. nov. (Triuridaceae), a new myco-heterotrophic plant from Bolivia

T. Franke, L. Beenken, and **C. Hahn**

Institut für Systematische Botanik der Ludwig-Maximilians-Universität, München, Germany

Received April 14, 2000
Accepted September 24, 2000

Abstract. A new species of *Triuridopsis* (Triuridaceae) is described. It shows a number of similarities to the genus *Triuris*. This is the first record of the family for Bolivia.

Key words: Triuridaceae, *Triuridopsis intermedia*, Taxonomy, myco-heterotrophic plants, Bolivia.

The Triuridaceae represent a pantropically distributed family with a doubtful phylogenetic position within the Angiospermae (Maas- van de Kamer 1995, Chase et al. 2000). All species are myco-heterotrophic, i.e. they parasitize on endomycorrhizal fungi (Leake 1994). As a result of this biological strategy they lack chlorophyll and have reduced photosynthetic organs.

Most Triuridaceae are very inconspicuous and it is extremely hard to discover them in the deep shade of the forest floor. This could be the reason why all members of this family are severely undercollected and distribution data are scarce. Most Triuridaceae require permanently humid conditions and grow in undisturbed lowland and submontane rain forests (Maas and Rübsamen 1986, personal observations).

Referring to the monographic treatment of neotropical Triuridaceae by Maas and Rübsamen (1986), there were no records of Triuridaceae occurring in Bolivia by that time, although many regions of the country offer ideal ecological conditions. For this reason the discovery of a new species of this family in Bolivia was not very surprising.

Triuridopsis intermedia[1] Th. Franke, Beenken and Ch. Hahn, spec. nov. Fig. 1

Herba myco-heterotrophica, pusilla, alba, translucens, subaphylla. Plantae dioicae. Inflorescentiae racemosae, multiflorae. Bracteae squamiformes. Flores trimeri. Tepala tria, basaliter connata, convexa, triangulata, apice cum appende flagelliforme. Flos masculus cum projectura centrali, brevi. Stamina tria, alterna, filamentis crassis. Antherae bithecales. Thecae bisporangiatae, longitudinaliter, ventraliter dehiscentes. Flos femineus receptaculo hemisphaerico, carpellis liberis monospermis numerosis. Semina ovoidea. Rhizoma subterranea, internodiis longis, nodis cum squamulis singulis. Radices binae, oppositae, ex nodis orientes.

Typus: Bolivia. Dpto. La Paz, Prov. Nor Yungas, Serranía de Marimonos, Alto Beni region, Sapecho, road from Coroico, Caranavi to Palos Blancos, approx. 5.5 km NO of the

[1] Etymology: the specific epithet is derived from the assumed mediatory position of this species between *Triuridopsis* and *Triuris* (lat: intermedius = intermediate).

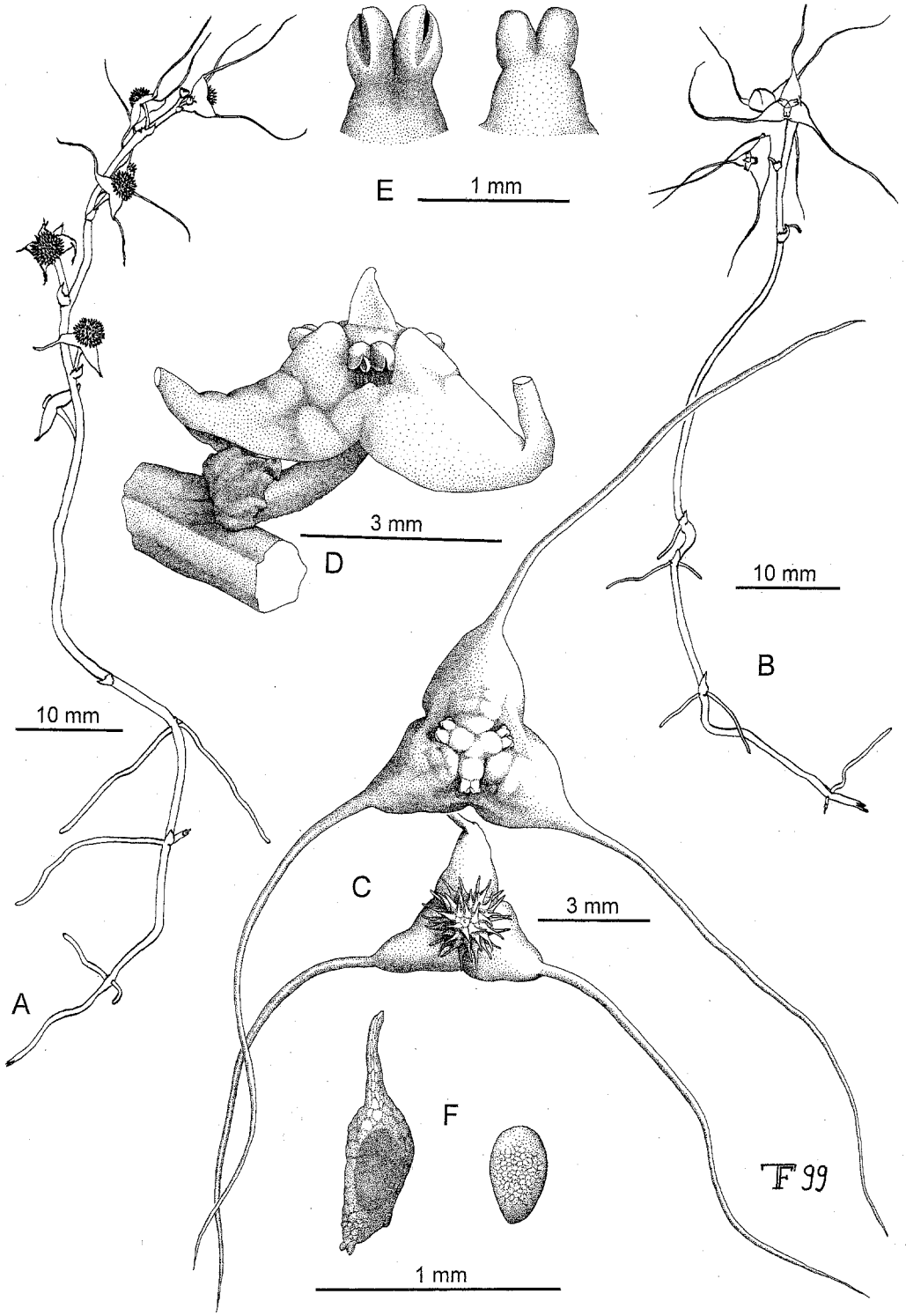


Fig. 1. *Triuridopsis intermedia* (Beenken 1051). **A** Habit of pistillate plant, **B** Habit of staminate plant, **C** Staminate and pistillate flower, **D** Staminate flower, showing sterile projection, **E** Stamen shown from below and above, **F** Carpel and seed

village Sapecho, plot[2] P3, 15°32′S, 67°21′W, alt. approx. 600 m a.s.l., leg. L. Beenken and Ch. Hahn, 1051, 07.05.1998, dried and in alcohol (holotype: M; isotypes: LPB; U).

Myco-heterotrophic herbs, dioecious, whitish hyaline, 4–12 cm high, with distinctly elongate subterraneous rhizome.

Internodes of the rhizome 4–36 mm long. Each node provided with one scale-like leaf and two opposing roots. Scale-like leaves triangular-ovate, acute, 1.4–2.7 mm long, 1.0–1.7 mm wide. Roots glabrous, originating endogenously, up to 55 mm long, 0.4–1.2 mm in diameter, partially darkened by endomycorrhizal hyphae. Stem erect, 20–60 mm long, sometimes provided with a single scale-like leaf. The latter triangular-ovate, acute, 1.7–2.3 mm long, 0.8–1.2 mm wide. Inflorescence a terminal 2–16-flowered raceme 10–70 mm long. Bracts triangular, 1.0–4.0 mm long, 0.7–1.7 mm wide, acute, apex curved inward. Pedicels 2.3–6.0 mm long.

Staminate flowers trimerous, with long-caudate tepals, center provided with a short pyramidal projection. Tepals three, triangular, 2.0–4.0 × 2.0–4.0 mm, basally connate and convex, forming a small cavity below each anther. Apical appendages flagelliform, 8.0–17.0 mm long, rolled inwards in bud. Stamens three, alternating with tepals. Anthers bithecal, thecae bisporangiate, 0.7–0.9 × 0.3–0.5 mm, dehiscing ventrally by a longitudinal slit. Filaments fleshy, 0.6–0.8 mm long, overtopped by the highly arched bases of the tepals. Central, sterile projection 0.2–0.9 mm long, occasionally scarcely developed.

Pistillate flowers trimerous, with long-caudate tepals, apocarpous. Tepals three, triangular, 2.0–4.0 × 2.0–4.0 mm, with recurved margins, basally connate. Apical appendages flagelliform, 7.0–19.0 mm long, rolled inwards in bud. Receptacle hemispherical, provided with 45–160 free carpels. Ovaries ovoid, 0.8–0.9 × 0.5–0.6 mm. Style apical, 0.5–0.6 mm long, persistent for a long time, stigmatic zone indistinct. Seeds ovoid, 0.4–0.6 × 0.2–0.4 mm.

[2] See Seidel (1995).

This delicate myco-heterotrophic plant species, was found exclusively in the less disturbed core region of a small plot of lowland rain forest within a vastly deforested area. It was growing in deeply shaded, humid locations in rich soil, forming large flocks of individuals. For more detailed ecological and geological information about this region see Seidel (1995) and Elbers (1995).

The genus *Triuridopsis* was introduced by Maas-van de Kamer and Maas (1994) with the description of *Triuridopsis peruviana* H. Maas and Maas from the Amazonian lowland rain forest near Iquitos/Perú. *Triuridopsis* is very closely allied to the genus *Triuris*. As Maas-van de Kamer and Maas (1994) pointed out, the most characteristic differences with *Triuris* are the distinctly filamented stamens and a sterile projection in the center of the male flower. In contrast to this, the male flowers of all *Triuris* species are provided with a cone-shaped androphore, which encloses the sessile anthers.

Taking into account the characters mentioned above, our new species can be definitely placed into the genus *Triuridopsis*, although it shows a couple of striking differences to *Triuridopsis peruviana* (Table 1). Some characters, e.g. the form of the tepal appendages or the position of the anther dehiscence slits, lead to the assumption that this species holds a linking position between *Triuridopsis* and *Triuris* (Table 1). For this reason we name it *Triuridopsis intermedia*.

The most obvious difference between the two species of *Triuridopsis* refers to the number of stamens. In contrast to *T. peruviana*, which has six monothecal stamens, *T. intermedia* has three bithecal stamens. Maas-van de Kamer and Weustenfeld (1998) assumed, that the monothecal stamens of *T. peruviana* might be equivalent to six half-stamens. The presence of three bithecal stamens in *T. intermedia* supports this hypothesis. Within the genus *Triuris* there are also species with three bithecal and – as in *Triuris hexophthalma* Maas and Rübsamen – species with six monothecal stamens (Maas and Rübsamen 1986).

Table 1. Principal features of the two *Triuridopsis* species and the genus *Triuris*

	Triuris spp.[a]	*Triuridopsis intermedia*	*Triuridopsis peruviana*[b]
Androphore	+	–	–
Sterile projection of male flowers	–	Short	Long
Stamina	3 (6)	3	6
Thecae per stamen	2 (1)	2	1
Filaments	–	3	6
Position of dehiscence slit of thecae	Longitudinal, ventral	Longitudinal, ventral	Horizontal
Position of anthers	Inside a cavity formed by the androphore	Above a cavity formed by the tepals	Free, bent upwards
Tepal appendages	Long, flagelliform	Long, flagelliform	Short
Rhizome	Short, contracted[c]	Elongated	Elongated
Roots per node	∞[c]	2	2

[a] Data taken from Maas and Rübsamen (1986)
[b] Data taken from Maas-van de Kamer and Maas (1994)
[c] According to Imhof (1998), *Triuris hyalina* Miers has heteromorphic rhizomes, consisting of starlike clumps of several roots, as well as elongated parts with only two roots per node

The authors would like to thank Dr. Mónica Moraes R. (La Paz) for organizing a collection permit and for her help and hospitality. Thanks also due to Dr. Stephan Beck (La Paz) for his hospitality and the organization of field trips, as well as to the staff members of the "Proyecto de Investigaciones Agroecólogicas y Forestales en el Alto Beni" (PIAF) at Sapecho, for guiding us to their project areas and the permission to collect plants.

References

Chase M. W., Soltis D. E., Soltis P. S., Rudall P. J., Fay M. F., Hahn W. H., Sullivan S., Joseph J., Molvray M., Kores P. J., Givnish T. J., Systema K. J., Pires J. C. (2000) Higher-level systematics of the monocotyledons: an assessment of current knowledge and a new classification. In: Wilson K. L., Morrison D. A. (eds.) Monocots. Systematics and Evolution, CSIRO Publishing, Collingwood, pp. 3–16.

Elbers J. (1995) Estudio de suelos en la zona de colonizacíon Alto Beni, La Paz, Bolivia. Ecol. Bolivia 25: 37–69.

Imhof S. (1998) Subterranean structures and mycotrophy of the achlorophyllous *Triuris hyalina* (Triuridaceae). Can. J. Bot. 76: 2011–2019.

Leake J. R. (1994) The biology of myco-heterotrophic plants. New Phytol. 127: 171–216.

Maas P. J. M., Rübsamen T. (1986) Triuridaceae. Fl. Neotrop. Monogr. 40: 1–55.

Maas-van de Kamer H. (1995) Triuridiflorae – Gardener's delight? In: Rudall P. J., Cribb P. J., Culter D. F., Humphries C. J. (eds.) Monocotyledons: systematics and evolution. Royal Botanic Gardens, Kew, 287–301.

Maas-van de Kamer H., Maas P. J. M. (1994) *Triuridopsis*, a new monotypic genus in Triuridaceae. Plant Syst. Evol. 192: 257–262.

Maas-van de Kamer H., Weustenfeld T. (1998) Triuridaceae. In: Kubitzki K. (ed.) The Families and Genera of Vascular Plants, Vol. 3, pp. 452–458.

Seidel R. (1995) Inventario de los árboles en tres parcelas de bosques primario en la Serranía de Marimonos, Alto Beni. Ecol. Bolivia 25: 1–35.

Addresses of the authors: Th. Franke, (e-mail: thafra@botanik.biologie.uni-muenchen.de), L. Beenken (e-mail: lbeenken@botanik.biologie.uni-muenchen.de) and Ch. Hahn (e-mail: ch.hahn@botanik.biologie.uni-muenchen.de), Institut für Systematische Botanik der Ludwig-Maximilians-Universität München; Sektion Mykologie; Menzinger Str. 67; D-80638 München, Germany.

II

The myco-heterotrophic *Voyria flavescens* (Gentianaceae) and its associated fungus

Thassilo FRANKE*

The myco-heterotrophic Gentianaceae *Voyria flavescens* from the Peruvian Amazon was investigated morphologically and anatomically. Most attention was paid to the subterranean structures and the mycorrhiza. The architecture of the underground organs and the process of root development in particular indicates neoteny. The stellately arranged roots are lacking a pericycle, an endodermis in the conventional meaning, a rhizodermis and a root-cap. All roots emerge exogenously from a so-called 'rhizoferous axis'. The root-tips show a capacity of exogenous shoot-regeneration. Further it was found that fungal penetration is restricted to dark banded zones of the root's surface. These zones are the result of a unique mode of apical growth. The associated fungi colonize the entire cortex intracellularly. In the innermost cortical layers the hyphae are longitudinally spreading, whereas they successively degenerate in the outer cortex. In the final phase of disintegration the hyphal coils almost entirely consist of a highly refractive crystalline substance. The mycorrhizal pattern corresponds to a specialized *Paris*-type vesicular-arbuscular mycorrhiza, lacking both, arbuscles and vesicles. Due to auxiliary cells, being connected to root invading hyphae, an associated fungus could be identified as most likely being a member of Gigasporaceae (Glomeromycota).

In contrast to green plants, that fix atmospheric carbon dioxide photosynthetically, a variety of angiosperms, pteridophytes and liverworts get access to carbon, partially or even exclusively, by 'digestion' of root colonizing fungi (see LEAKE 1994 and refs. therein). LEAKE (1994) described this type of metabolism as 'myco-heterotrophy', while others used the term 'mycoparasitism' (CRONQUIST 1981, RAYNAL-ROQUES & PARÉ 1998).

It is very likely that within Gentianaceae myco-heterotrophy has evolved several times independently (LEAKE 1994, OEHLER 1927, RAYNAL 1967b, NILSSON & SKVARLA 1969). The genus *Sebaea* e.g., shows a transition from comparatively large-leafed autotrophic species, such as *Sebaea spathulata* Steud. to species with vestigial scale leaves, like *Sebaea filiformis* Schinz and finally to completely myco-heterotrophic forms like the achlorophyllous *S. oligantha* Schinz (RAYNAL-ROQUES & PARÉ 1998). The 'semi-chlorophyllous' genera *Bartonia* and *Obolaria* are considered to be myco-heterotrophic as well, as their areal shoots evidently do not contain sufficient amounts of chlorophyll for an effective photosynthetic carbon supply (GILLETT 1959, LEAKE 1994, NILSSON & SKVARLA 1969). HOLM (1906) described these genera as being 'hemisaprophytes', which is in this context synonymous with hemimyco-heterotrophs. In contrast to this, all species of *Cotylanthera*, *Voyria* and *Voyriella* apparently completely lack chlorophyll and the function of their areal shoots is exclusively restricted to sexual reproduction (FIGDOR 1897, JOHOW 1885, 1889, LEAKE 1994, MAAS & RUYTERS 1986, RAYNAL 1967a, SVEDELIUS 1902).

Within the Gentianaceae, *Voyria* is the most studied myco-heterotrophic genus. It comprises 19 widely distributed species throughout tropical and subtropical America, *Voyria primuloides* Baker being the only exception, due to its occurrence in tropical West- and Central Africa (MAAS & RUYTERS 1986, RAYNAL 1967a). JOHOW (1885) was the first to realize, that the architecture of the root system varies considerably within the genus. He interpreted this variation as an adaptation to different substrate compositions. Concerning the mycorrhiza, he was thoroughly convinced that all plants presently known as myco-heterotrophs are saprophytes and interpreted the numerous hyphae within the root tissue of *Voyria* as belonging to a parasitic fungus. In a second publication JOHOW (1889) critically reviewed the studies of KAMIENSKI (1882) and FRANK (1885, 1887, 1888) and cautiously supported the hypothesis that the presence of the fungus could be of advantage for the plants. In recent years IMHOF (1997, 1999, see also IMHOF & WEBER 1997, 2000 and IMHOF, WEBER & GOMEZ 1994) undertook systematic morphological and anatomical investigations of subterranean parts of the genus *Voyria*. In contrast to JOHOW (1885, 1889), IMHOF (1997) explained the interspecific variation of the root system's structure as a result of increasing specialisation towards myco-heterotrophy. Regarding the four species he examined, IMHOF (1997) con-

* Thassilo Franke, Department Biology I, Biodiversity Research, Systematic Mycology, Menzinger Str. 67, D–80638 München
e-mail: thafra@botanik.biologie.uni-muenchen.de

cluded at a phylogenetic progression from *V. truncata* (Standley) Standley & Steyermark with an 'extended, branched and runner-like' root system, to a presumably more advanced, so-called 'morning-star type' of rootsystem, as in *V. tenella* W.J. Hooker and *V. obconica* Progel. He pointed out that *Voyria aphylla* (Jaquin) Persoon holds an intermediate position between these two types of root system. According to IMHOF (1999) the differences in the mycorrhizal patterns reflect this progression, too. In all four species mentioned above, the mycorrhizas are differently specialized forms of the *Paris*-type vesicular-arbuscular mycorrhiza, that is characteristic for Gentianaceae and several related families (DEMUTH & WEBER 1990, KNÖBEL & WEBER 1988, IMHOF 1997, 1999, IMHOF & WEBER 1997, 2000).

In the present study morphology, anatomy and the mycorrhiza of *Voyria flavescens* Grisebach are investigated and compared to species already examined by IMHOF (see references above). In addition to this it is attempted to identify the associated fungus. According to SMITH & READ (1997), the term 'vesicular-arbuscular mycorrhiza' (VAM) is used instead of 'arbuscular mycorrhiza' (AM), to describe those mycorrhizal associations formed by members of the recently established phylum Glomeromycota (SCHÜßLER, SCHWARZOTT & WALKER 2001).

Material and methods

The material was collected between July and September 1998, in the surrounding area of the biological research station "Estación Biológica Quebrada Blanco (EBQB)" in north-eastern Peruvian Amazonia (4°21'S, 73°09'W). The site is characterized by lowland tropical rain forest of the "bosque de altura" type (ENCARNACIÓN 1985) and annual rainfall is around 3000 mm (FELDMANN, VERHAAGH & HEYMANN 2000). With one exception, all plants of *Voyria flavescens* were growing under humid and shady conditions within the uppermost soil layer of the forest floor. A single specimens was found on top of a termite mound of *Embiratermes neothenicus* Holm (Isoptera, Nasutitermitinae). Altogether six specimens were carefully removed from the substrate and immediately fixed in formalin-acetic acid-alcohol (FAA) (RUZIN 1999). One specimen was later transferred into 70 % ethanol for storage. After surveying the anatomical structures, the remaining five plants were dried and mounted on herbarium sheets. All specimens were deposited in the following herbaria (collection numbers in brackets): M (98/100a, b), USM (98/100c)

A complete root system and some solitary roots were cleared in 10 % KOH for 1h at 90 °C and after acidifying with 1 % HCL for 3 min. at room temperature stained with 0.05 % trypan blue for 10 min. at 90 °C (RAJAPAKSE & MILLER 1992). The root system was transferred into lacto-phenol for storage (RAJAPAKSE & MILLER 1992). After removing surplus stain in lacto-phenol (1h at room temperature) an entire solitary root was mounted on a microscope slide, using polyvinyl alcohol lacto-glycerol (PVLG) as a coverslip mounting medium (KOSKE & TESSIER 1983). Following the instructions given by RUZIN (1999) some roots were gradually dehydrated in ethanol and embedded in glycol methacrylate (Leica Hisoresin®). The hardened samples were cut in 5, 10, 12, 20 and 30μm sections, using a Microm HM-S 340 rotary microtome (Microm, Heidelberg, Germany). One part of the cut sections was mounted unstained on microscope slides with Roth Entellan® coverslip mounting medium and an equal part was stained with 1% toluidine blue $O_{(aq.)}$ solution at pH 5,6 in phosphate buffer (1 - 5 min. at room temperature) before mounting (RUZIN 1999). In order to trace suberized and lipoid structures, other Historesin®-sections were stained with Sudan III (KREISEL & SCHAUER 1987).

The sections were examined under a Leitz Dialux 22 compound microscope (Leitz, Wetzlar, Germany) with brightfield illumination and Nomarski Differential Interference Contrast.

For the examination of the root surface, a LEO 438 VP scanning electron microscope (LEO Electron Microscopy Inc., USA) was used. After gradual dehydration in acetone and critical point drying (ANDERSON 1951), the material was sputtered with platinum (60 sec. at 20°C and 20 mA), using a BAL-TEC SCD 050 sputter coater (BAL-TEC AG, Balzers, CH).

Results

The specimens of *V. flavescens* show a considerable variation in stem-size (not shown) as well as in number and shape of the petals. The stem is 8–24 cm high and the petals of the tetra-, penta- or hexamerous corolla are obtuse-elliptic to acute-lanceolate (Fig. 1b-d). All aerial parts are yellow with an increasing intensity towards the flower. The remaining morphological characters, as far as observed, agree with the description given by MAAS & RUYTERS (1986).

The root system has a star-like appearance (Fig. 1a, Fig. 2a). Numerous unbranched roots arise exogenously from a vertical, slightly elongated axis. This rhizoferous axis is neither subdivided into nodes and internodes nor does it bear any scales. From it's apical pole arise 1–5 aerial shoots (plant with a single aerial shoot shown in Fig. 1a). Each shoot is provided with only four concentrical vascular bundles (Typ B.1., according to OEHLER 1927). Transverse sections of the rhizoferous axis show a distinct medullar tissue consisting of large, isodiametrical cells often elongated longitudinally (Fig. 3c). Their non-lignified walls are comparatively thick. The medullar tissue is surrounded by up to eight bicollateral bundles, that are embedded in a multilayered cortex (Fig. 3c). Staining with Sudan III reveals a very faint suberin lamella running through the periclinal walls of the innermost cortical cellular layer (Fig. 3c). In centrifugal direction up to 20 layers of thin walled, isodiametrical cortical cells follow (Fig. 3c). Many cortical cells contain remains of digested fungal hyphae (Fig. 3c). *V. flavescens* is capable of producing new plantlets from its root- tips. The plantlets arise exogenously and

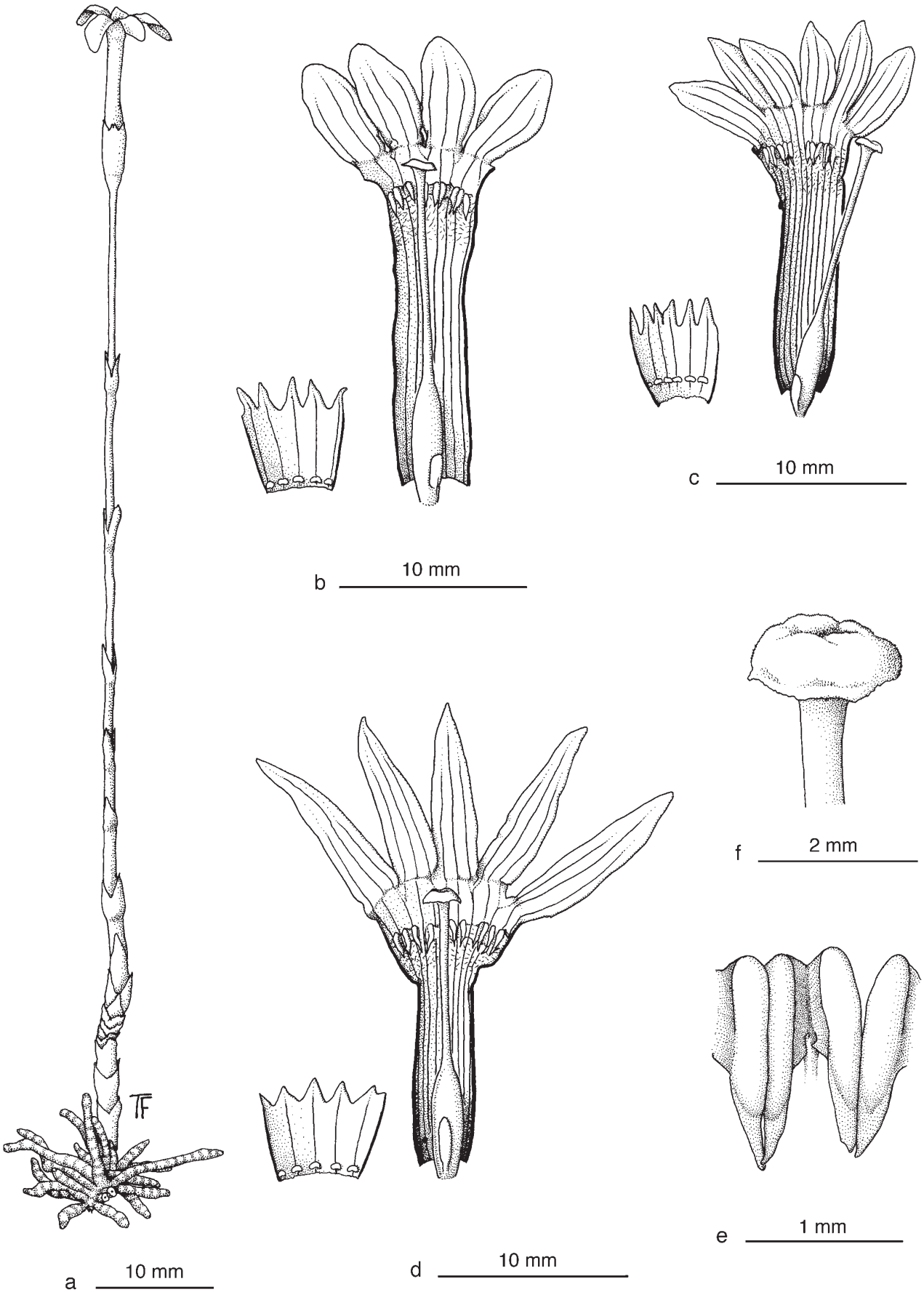


Fig. 1. *Voyria flavescens*. – **a**. Habit. – **b**. - **d**. Dissected flowers with calyx shown on the left. – **e**. Stamina. – **f**. Stigma. (a. drawn from 98 / 100 a, b. & c. from 98 / 100 c, d. from 98 / 100 b).

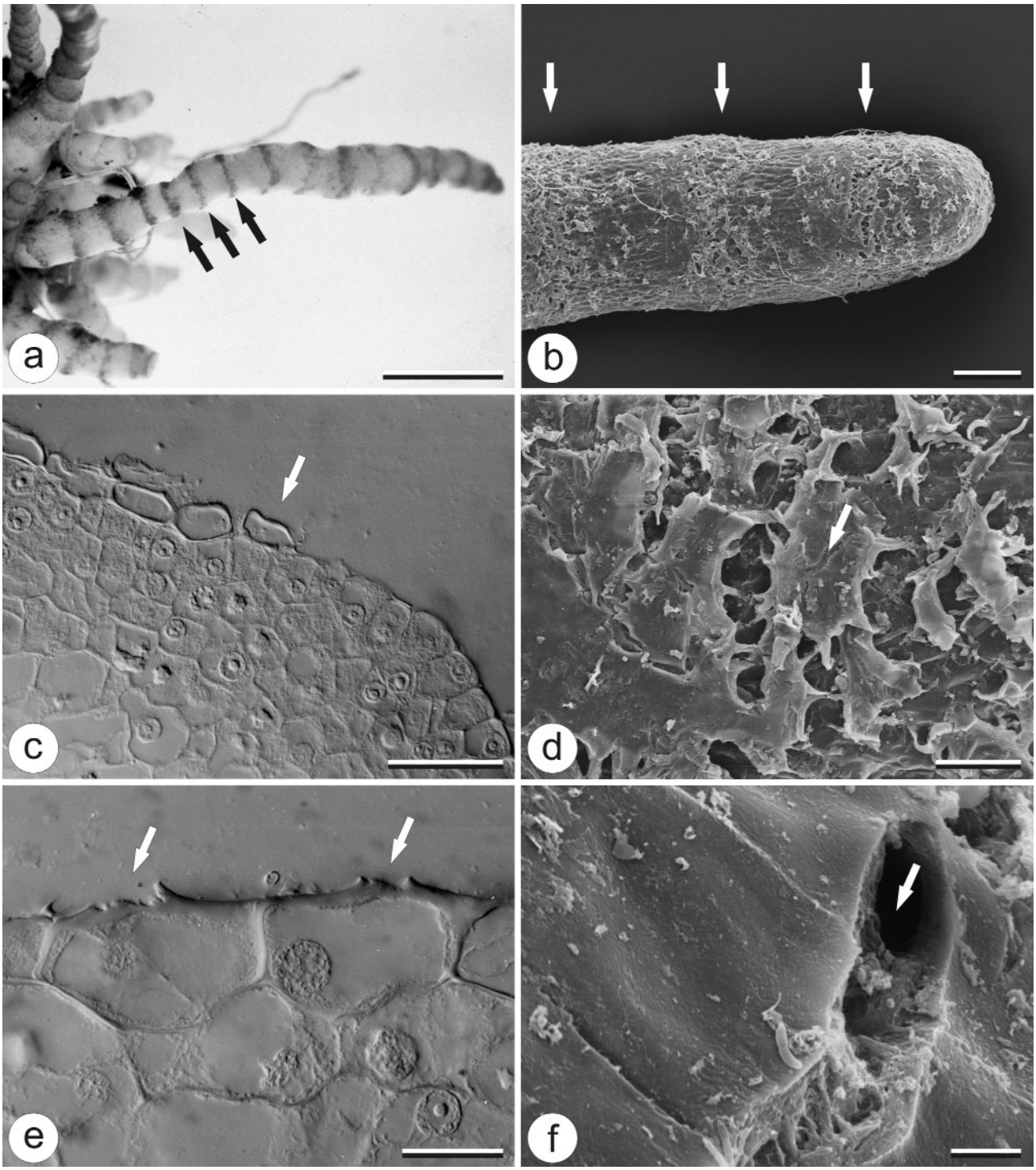


Fig. 2. Root morphology and anatomy of *Voyria flavescens*. – **a**. Roots with dark banded zones (arrows), bar = 3 mm. – **b**. SEM-picture of root tip, showing surface structure of dark banded zones (arrows), bar = 200 μm. – **c**. Longitudinal section through root tip showing off-scaling dead dermal cell (arrow), bar = 50 μm. – **d**. SEM-picture showing aggregates of dead dermal cells within dark banded zone (arrow), bar = 40 μm. – **e**. Transversal section through dark banded zone with bursting outer periclinal walls of dermal cells (arrows), bar = 15μm. – **f**. SEM-picture showing deep crack running through the root surface within dark banded zone (arrow), bar = 5 μm.

their roots emerge before the apical shoot primordium becomes visible.

The cylindrical, round-tipped roots of *V. flavescens* have an average length of 5–15 mm and are 1–2 mm thick. The older roots in particular have a somewhat knobby irregular shape (Fig. 2a). The root tips lack a root-cap (Fig. 2b). All roots are distinctly striped with alternating light and dark bands (Fig. 1a, Fig. 2a). This segmentation is caused by a telescope-like overlapping of the root's dermal cellular layers (Fig. 2b). The whitish surface of each dermal segment changes distally into a narrow brown zone (Fig. 2a), overlapping the proximal white zone of the subsequent segment (Fig. 2b, c). This process is repeated several times, resulting in the formation of up to 20 such dermal segments per root. The overlapping brown zone is caused by withering dermal cells (Fig. 2b-f, Fig. 3a). The periclinal walls of these cells increase in thickness towards the segment's distal margin (Fig. 2c), where deep cracks start running in all directions through the surface (Fig. 2e, f). Finally the dermal layer collapses into scattered aggregates of dead cells that gradually scale off (Fig. 2b-d). Beneath these off-scaling dead cells emerges a light colored cellular layer that forms the surface of the subsequent dermal segment(Fig. 2b-d). The periclinal walls of the dermal cells are coated with a sudanIII-positive suberin lamella, partially withered within the brown zone.

The cells of the cortex are arranged in 8–12 irregular layers (Fig. 3a, b). Their walls are very thin, intercellular spaces do not occur. The great majority are densely colonized by fungal hyphae and have a more or less isodiametrical shape, while the cells that are located close to the central cylinder are increasingly longitudinally elongated and synchronously declining in transversal diameter (Fig. 3a, b).

The transition from cortical to vascular tissue is not very pronounced (Fig. 3a, b). Staining with Sudan III reveals a faint suberin lamella coating the slightly thickened inner tangential walls of the innermost cortical layer. Since these cells are very inhomogeneous in size and very unevenly arranged, the use of the term endodermis seems rather inappropriate. A pericycle is also difficult to define, as sieve elements and their companion cells are frequently directly adjoining the innermost cortical layer (Fig. 3a). The vascular tissue consists of a penta- to heptarch leptome and a central hadrome, which is formed by up to 9 tracheidal xylem elements (Fig. 3a).

Whereas the aerial shoot never gets infected by the associated fungus, the root system of all investigated plants shows a uniform pattern of fungal colonization and disintegration (Fig. 3b, Fig. 5a). The extraradical hyphae of the associated fungus are amber-colored to light-brown, frequently branching and vary considerably in diameter (2 μm–12 μm). Occasionally off-branching hyphae are bearing bundles of more or less globose, warty auxiliary cells (Fig. 4a). Each auxiliary cell contains a clump of a highly refractive substance (Fig. 4b). Auxilliary cells were detected in three plant individuals, that were collected at two distant locations. Microscopic slides that clearly show extraradical hyphae with auxilliary cells penetrating the root surface are added to voucher specimen 98/100a.

Most of the hyphae are aseptate and very thick-walled. Septa do only occasionally occur, especially within delicate thin-walled branches. The hyphae are running in all directions across the root's surface, where they repeatedly form knobby appressoria-like swellings. However a successful penetration of the dermal cell walls and the tissue beneath could exclusively be observed within the root's dark banded zones (Fig. 5b)

Individual intraradical hyphae are very difficult to trace within cells that are tightly packed with hyphal coils in different stages of disintegration (Fig. 5c). The withering dermal cells as well as the cellular layer designated to replace them are never colonized by the fungus. Some of the invading hyphae seem to grow straight in a centripetal direction until they reach the 1–3 layers of longitudinally elongated cortical cells that are adjoining the endodermis. Here they grow mainly in longitudinal direction while off-branching hyphae run in centrifugal direction, colonizing the outer cortical tissue (Fig. 3b, Fig. 5d). It was also frequently observed that, especially close to the root-tip, colonization occurred directly after penetrating the root surface. Neither vesicles nor arbuscle-like structures could be observed. Within the more or less isodiametrical cortical cells, the hyphae form dense coils and undergo a process of gradual disintegration (Fig. 3b, Fig. 5e, f). The hyphae are constantly inflating, while synchronously the consistency of the cytoplasm becomes more and more inhomogeneous (Fig. 5e, f). An increasing amount of highly refractive crystalline inclusions finally replaces the entire fungal cytoplasm while the fungal cell-wall completely disappears (Fig. 3b, Fig. 5c, e, f). If heated to 60 °C, the crystalline substance melts, forming spherical droplets that stain bright red with Sudan III. On recooling the substance recrystallizes. The crystalline inclusions are absent from the cortical cells of the rhizoferous axis and the proximal part of older roots, that exclusively contain the crumpled remains of completely disintegrated hyphae (Fig. 3c).

Discussion

The stellate root-system of *V. flavescens* shows many unusual features that are very difficult to relate to established morphological and anatomical structures. The term 'rhizome' cannot be used to describe the rhizoferous axis, as the latter is neither subdivided into nodes and internodes nor does it bear any scales. A morphologically similar architecture is represented in the fungus-colonized mycorrhizome* of *Neottia nidus-avis* Rich. (Orchidaceae), that is provided with densely clustered exogenous roots (BERNARD 1902; RAUH 1937). In *V. flavescens* even the term 'root' has to be used with reservation, as

* The term "mycorrhizome" was introduced by RASMUSSEN (1995) to characterize a transitional rhizoferous structure exclusively found in orchids, that derives from the protocorm and changes into a mature rhizome in the course of further development.

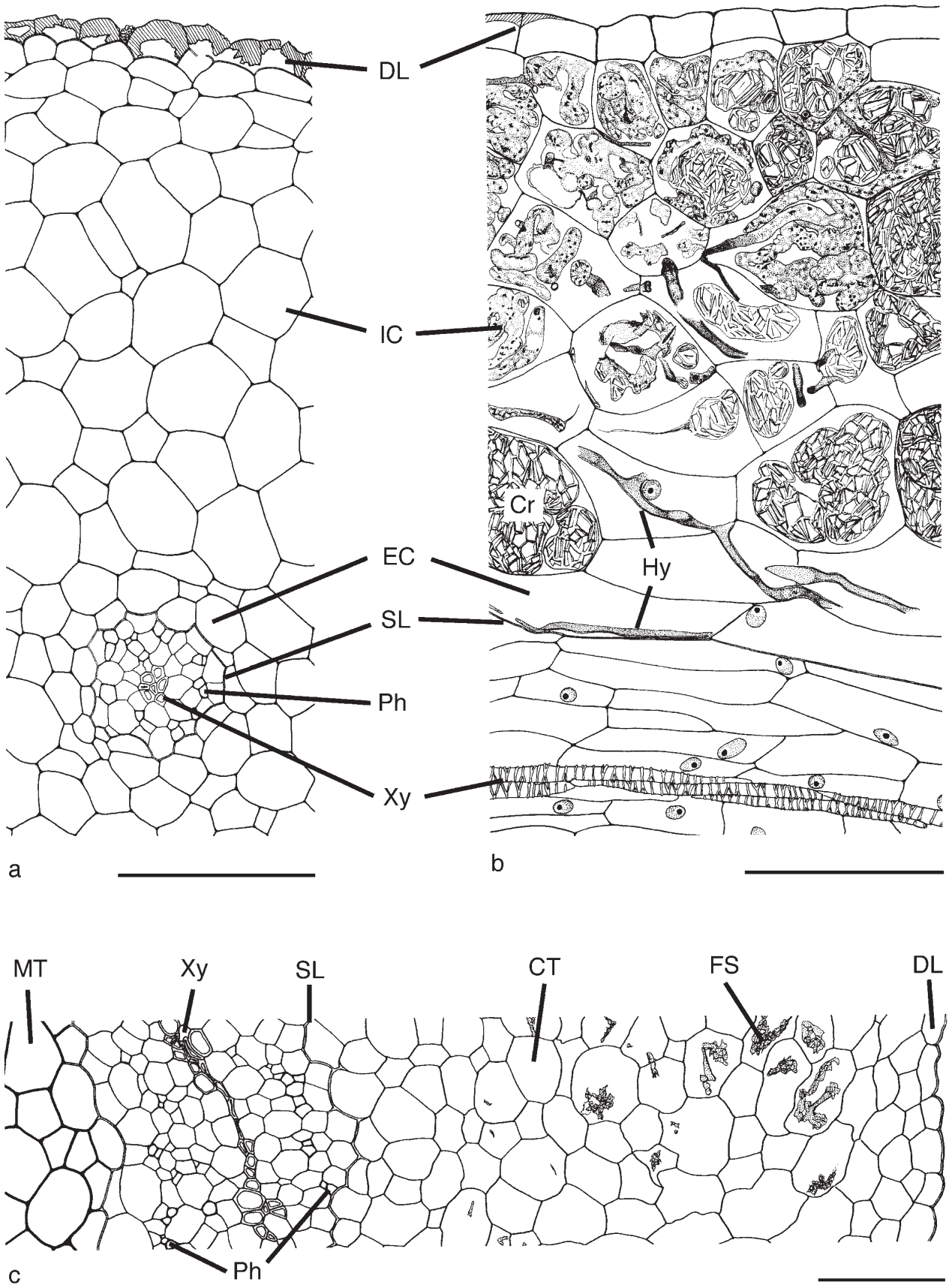


Fig. 3. Root anatomy and mycorrhiza of *Voyria flavescens*. – **a**. Transversal section through the root's dark banded zone (fungal structures not shown!). – **b**. Longitudinal section through the root's light banded zone, showing mycorrhizal pattern. – **c**. Transversal section through the rhizoferous axis. Cr = crystalline substance, CT = cortical tissue, DL = dermal layer, EC = elongated cortical cells, FS = fungal structures, Hy = hyphae, IC = isodiametrical cortical cells, MT = medullar tissue, Ph = phloem, SL = suberin lamella, Xy = xylem. Bar = 10 μm.

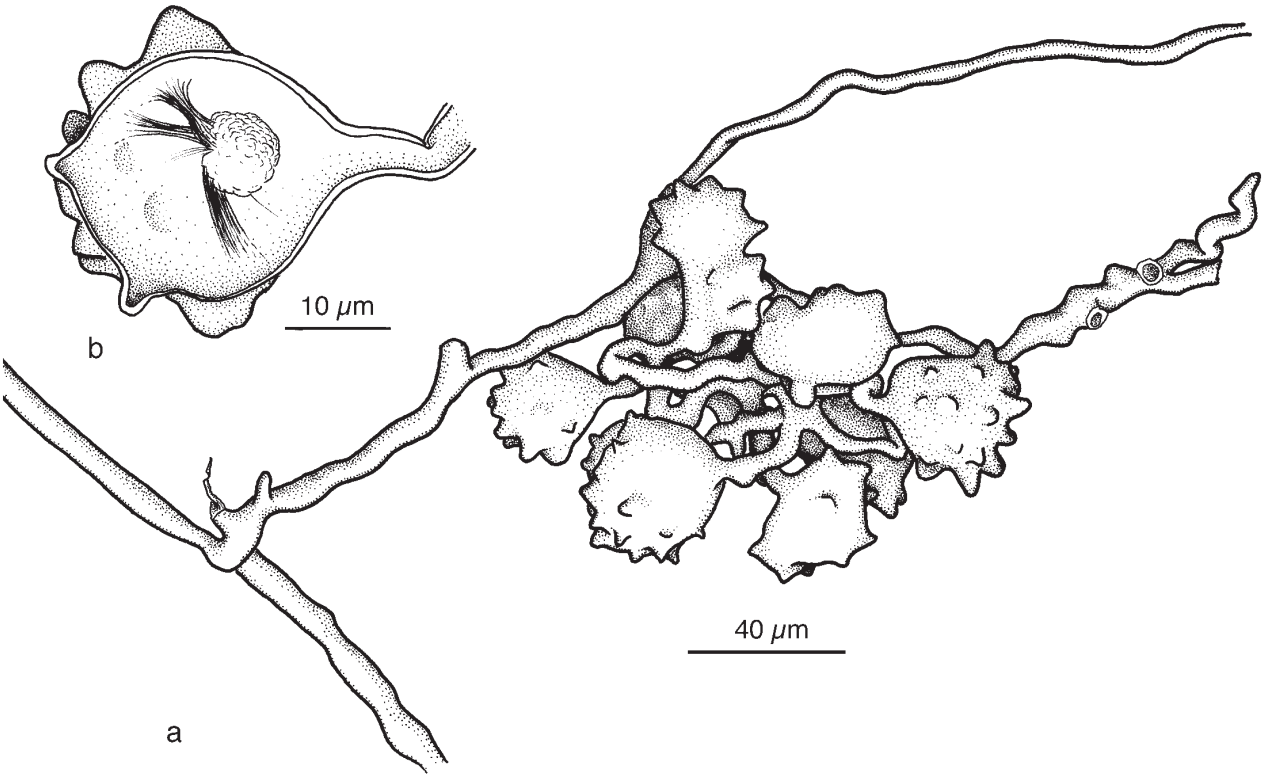


Fig. 4. Auxilliary cells. – **a**. Extraradical hyphae with bundle of auxilliary cells. – **b**. Optical section through auxilliary cell, showing a highly refractive inclusion.

the process of apical growth is completely unique (see explanation below) and many crucial anatomical root-characters like e.g. the root-cap and the pericycle are simply lacking. Since these differences give reason for doubting that the 'root' of *V. flavescens* is homologous to the root in the conventional sense, root-specific terms like 'rhizodermis' and 'exodermis' are not used in this context. However the term 'root' itself is maintained to avoid confusion with earlier publications.

A similar arrangement of the underground organs was also described for *Voyria tenella* Hook. and *Voyria obconica* Progel and is to be expected in related species within *Voyria* subgen. *Leiphaimos* (Schltdl. & Cham.) V.A. Albert & L. Struwe (JOHOW 1885; SVEDELIUS 1902; VIGODSKY-DE PHILIPPIS 1938; IMHOF, WEBER & GOMEZ 1994; IMHOF 1997, IMHOF & WEBER 2000).

In contrast to this, some other species of *V.* subgen. *Leiphaimos* (e.g. *V. aphylla* (Jacq.) Pers.) and probably all species of *V.* subgen. *Voyria* Aubl. (e.g. *V. truncata* (Stand.) Stand. & Stey.) have a comparatively large, repeatedly branched root-system (KNÖBEL & WEBER 1988; IMHOF, WEBER & GOMEZ 1994; IMHOF & WEBER 1997; IMHOF 1999). A transverse section of the root of e.g. *V. truncata* shows a clear anatomical subdivision into central cylinder (with well developed pericycle), endodermis, cortex, exodermis and epidermis (= rhizodermis) (IMHOF & WEBER 1997). The roots are provided with numerous shoot primordia that arise endogenously in the axiles of likewise endogenously formed lateral roots, a phenomenon that is also known to occur in autotrophic Gentianaceae, namely in *Gentianella fimbriata* (L.) Borkh. (RAUH 1937; KNÖBEL & WEBER 1988; IMHOF, WEBER & GOMEZ 1994).

Compared to this, it seems to be very likely that the mycorhizome-like vegetative structures of *V. flavescens*, *V. tenella* and *V. obconica* represent a case of neoteny. This consideration is supported by the observations made by IMHOF, WEBER & GOMEZ (1994), who collected plants of *V. tenella* at different stages of development. The youngest seedling they found, was a 2 mm long fleshy structure, apparently exclusively consisting of roots. Neither scale-leaves nor shoot primordia could be detected. The further developmental sequences showed an increasing number of roots leading to the formation of a stellate root-system, finally producing 3-4 successively emerging aerial shoots. The formation of a mature root system, as it is represented in *V. truncata* and apparently most, if not all, autotrophic Gentianaceae, seems to be completely omitted from the developmental sequence. Since the tendency of extreme reduction is a general character of neoteny, it is not surprising that the vascular system of the aerial shoots of *V. flavescens*, *V. tenella* and *V. obconica* comprises only four concentrical bundles. In contrast to this, the aerial shoots of all other anatomically investigated species of *Voyria* have a more complex vascular system (SOLEREDER 1908, OEHLER 1927, TER WELLE 1986).

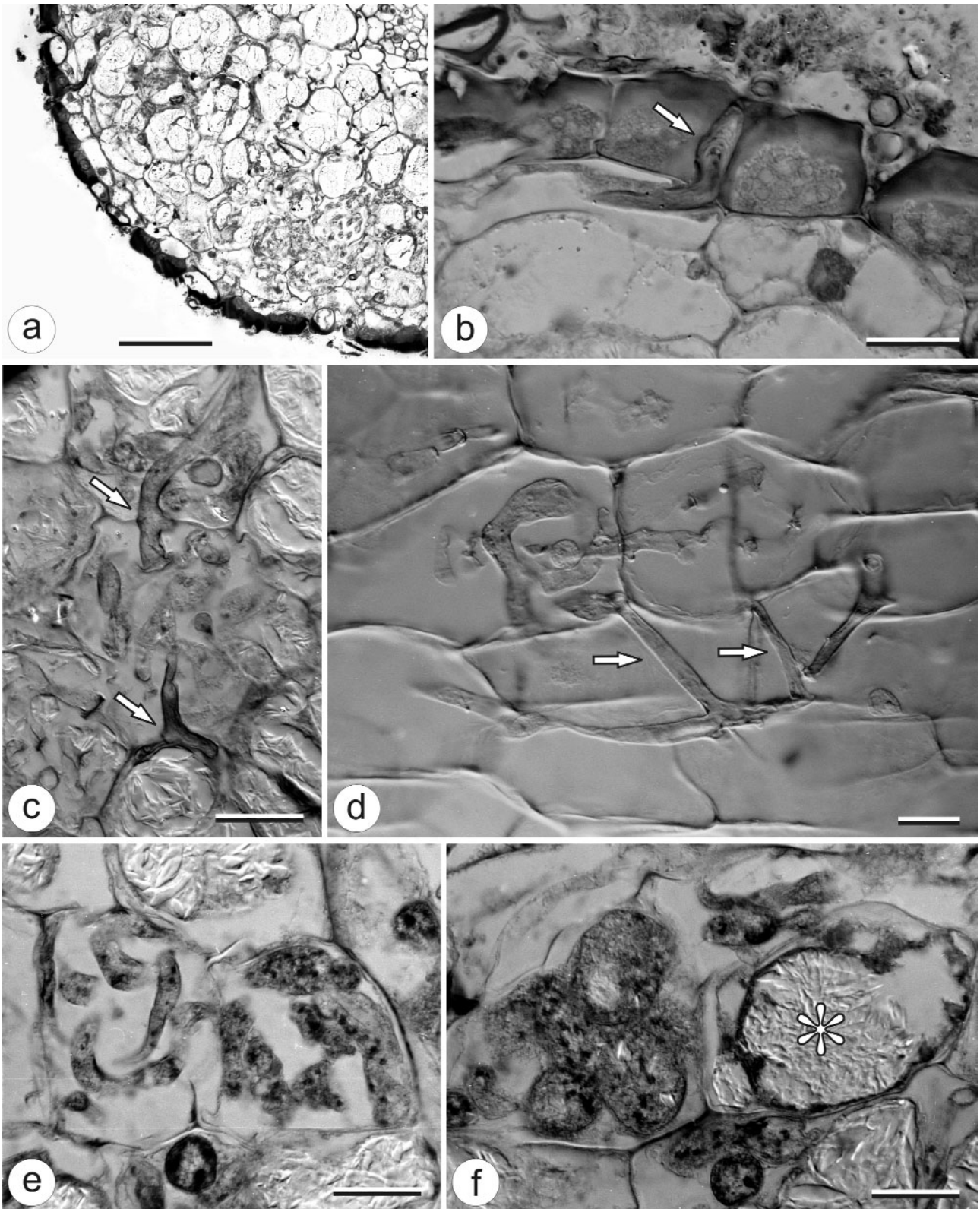


Fig. 5. Mycorrhiza of *Voyria flavescens*. – **a**. Transverse section of root showing mycorrhiza, bar = 100 μm. – **b**. Invading hypha (arrow), penetrating through anticlinal spaces between fading dermal cells, bar = 20 μm. – **c**. Intraradical hypha (arrows), finding it's way through the cortex, bar = 20 μm. – **d**. Branching intraradical hyphae (arrows) recolonizing outer cortical cells from the inside, bar = 20 μm. – **e**. - **f**. Coiling hyphae, showing different phases of disintegration and the highly refractive crystalline substance (asterisk), bar = 20 μm.

According to IMHOF, WEBER & GOMEZ (1994) *V. tenella* undergoes an annual life-cycle. This is apparently also the case in *V. flavescens*, since no remains of old aerial shoots could be detected. However, since there is no evidence that the plants grow from seed to flowering in one year, the use of the term ´hapaxanth' seems to be more appropriate than 'annual'. Presumably the ability to produce rootborne plantlets, as it was observed in *V. flavescens* and also *V. tenella* (IMHOF, WEBER & GOMEZ 1994), should guarantee permanent access to the fungal mycelium that once fed the fading mother-plant. This phenomenon is known to occur in the hapaxanth myco-heterotrophic orchid *Neottia nidus-avis* (WARMING 1874; BERNARD 1902; CHAMPAGNAT 1971).

A unique feature of *Voyria flavescens* are the distinctly striped roots (Fig. 1a, Fig. 2a). Due to the lack of a protecting root-cap, the dermal cells of the root tip are directly exposed to mechanical stress caused by the soil particles, resulting in a massive reinforcement of their outer periclinal walls. After a certain period of time the frontmost cellular layer bursts, laying bare the cellular layer underneath that soon looses it's meristematic activity and faces the same destiny again. The remaining cells of the bursted layer form a collar-like structure, that remains visible as a dark band. The alternation of these dark collars with whitish, non reinforced lateral dermal layers is responsible for the root's telescope-like segmentation. In *V. tenella* the frontmost cells of the uniformly whitish roots don't produce armor-like reinforcements (own obs.), resulting in a process of constant off-scaling during apical growth (IMHOF 1997). According to IMHOF & WEBER (2000) the dermal cells of *V. obconica* scale off as well.

A successful fungal penetration was exclusively observed within the dark banded zones (Fig. 5b). Since most of the dermal cells of these sections are either damaged or dead, penetration is doubtlessly facilitated here. This results in the formation of ring-like passage zones.

According to IMHOF & WEBER (2000), this mycorrhizal pattern is accurately characterized by using the term 'specialized *Paris*-type vesicular-arbuscular mycorrhiza' and agrees in most aspects with the observations made by IMHOF (1997) and IMHOF & WEBER (2000) in *Voyria tenella* and *V. obconica* (Fig. 3b, Fig. 5a).

The longitudinal spreading of living hyphae within the inner cortex, as well as the centrifugal recolonization of cortical cells by off-branching hyphae can frequently be observed (Fig. 5d). The same colonization strategy was reported to occur in *V. obconica* and *V. tenella* by IMHOF (1997) and IMHOF & WEBER (2000) who interpreted this phenomenon as a way of sustainable use of the host fungus by the plant. Due to the elongated shape of the innermost cortical cells, the number of anticlinal walls, that have to be penetrated by spreading hyphae is distinctly reduced. This guarantees a fast accessibility of all cortical zones. The disintegration process of the hyphal coils mainly agrees with the observations made by IMHOF (1997) and IMHOF & WEBER (2000) in *V. tenella* and *V. obconica*. The only difference concerns the intra-hyphal crystalline substance, since it was not mentioned by the authors cited above (Fig. 3b, Fig. 5f). JOHOW (1889) however reported a substance of that kind to occur in intraradical hyphae colonizing the roots of *V. aurantiaca* Splitgerber and *V.aphylla* (Jacq.) Pers. The substance apparently is a lipoid or chemically related material, since the crystals, that melted at about 60°C, proved to be sudanIII-positive in the liquid phase. The apparent presence of the same substance in the auxiliary cells indicates that it might serve as a reserve material (Fig. 4b). This leads to the assumption, that *V. flavescens* might be able to stimulate the intra-radical hyphae to produce this reserve material in enormous amounts, making it accessible for the plant. Since the fungus seems to transform almost its entire biomass into this substance, the process is lethal for the concerned hyphal coil.

YAMAMOTO (2001) was the first to identify a vesicular-arbuscular mycorrhical fungus of the genus *Glomus* (Glomeraceae, Glomeromycota), being associated with a myco-heterotrophic plant (*Sciaphila tosaensis* Makino (Triuridaceae)), by using molecular techniques. The presence of soil-borne auxiliary cells, emerging from root invading hyphae, makes it very likely that *V. flavescens* is able to form mycorrhizae with Gigasporaceae (Glomeromycota) (HALL 1984, MORTON & BENNY 1990, MORTON & REDECKER 2001 [Table I.]).

Acknowledgements

I thank Dr. Eckhard Heymann, for making it possible to carry out field studies at the "Estacion Biologica Quebrada Blanco". Further, I am very grateful to Camilo Flores, Emérita Tirado Herrera and Jürgen Skrabal for their help during field work and to Dr. Asunción Cano for his administrative support. Finally I express my thanks to Dr. Eva Facher for technical assistance with SEM.

References

ANDERSON TF (1951) Techniques for the preservation of three-dimensional structure in preparing specimens for the electron microscope – Transactions of the New York Academy of Science, Series II 12: 130-134.

BERNARD N (1902) Études sur la tubérisation. – Revue Générale de Botanique **14**: 1-92.

CHAMPAGNAT M (1971) Recherches sur la multiplication végétative de *Neottia nidus-avis* Rich. – Annales des Sciences Naturelles Botanique et Biologie Végetale, 12 sér., **12**: 209-248.

CRONQUIST A (1981) An integrated system of classification of flowering plants. Columbia University Press, New York, USA.

DEMUTH K, WEBER HC (1990) Strukturelle Inkompatibilität der vesiculär-arbusculären Mycorrhizapilze in Enzianen (Gentianaceae). – Angewandte Botanik **64**: 247-252.

ENCARNACIÓN F (1985) Introducción a la flora y vegetación de la Amazonía peruana: estado actual de los estudios, medio natural y ensayo de una clave de determinación de las formaciones vegetales en la llanura amazónica. – Candollea **40**: 237-252.

FELDMANN M, VERHAAGH M, HEYMANN E (2000) *Sericomyrmex* ants as seed predators. – Ecotropica **6**: 207-209.

FIGDOR W (1897) Über *Cotylanthera* Bl.. Ein Beitrag zur Kenntnis tropischer Saprophyten. – Annales du Jardin Botanique de Buitenzorg **14**: 213-239.

FRANK B (1885) Ueber die auf Wurzelsymbiose beruhende Ernährung gewisser Bäume durch unterirdische Pilze. – Berichte der Deutschen Botanischen Gesellschaft 3: 128 – 145.

FRANK B (1887) Ueber neue Mycorhiza-Formen. – Berichte der Deutschen Botanischen Gesellschaft **5**: 395-408.

FRANK B (1888) Ueber die physiologische Bedeutung der Mycorhiza. – Berichte der Deutschen Botanischen Gesellschaft **6**: 248-269.

GILLETT JM (1959) A revision of *Bartonia* and *Obolaria* (Gentianaceae). – Rhodora Vol. 61, No. **722**: 43-63.

HALL IR(1984) Taxonomy of VA mycorrhizal fungi. – In: POWELL CL, BAGYARAJ DJ (eds) VA Mycorrhizae, pp. 57-94, CRC Press, Boca Raton, Florida, USA.

HOLM T (1906) *Bartonia*, Muehl. An Anatomical Study. – Annals of Botany Vol. 20, No. **80**: 441-447.

IMHOF S (1997) Root anatomy and mycotrophy of the achlorophyllous *Voyria tenella* Hooker (Gentianaceae). – Botanica Acta **110**: 298-305.

IMHOF S (1999) Root morphology, anatomy and mycotrophy of the achlorophyllous *Voyria aphylla* (Jaqu.) Pers. (Gentianaceae). – Mycorrhiza **9**: 33-39.

IMHOF S, WEBER HC (1997) Root anatomy and mycotrophy (AM) of the achlorophyllous *Voyria truncata* (Standley) Standley and Steyermark (Gentianaceae). – Botanica Acta **110**: 127-134.

IMHOF S,WEBER HC (2000) Root structures and mycorrhiza of the achlorophyllous *Voyria obconica* Progel (Gentianaceae). – Symbiosis **29**: 201-211.

IMHOF S, WEBER HC, GOMEZ LD (1994) Ein Beitrag zur Biologie von *Voyria tenella* Hook. und *Voyria truncata* (Standley) Standley and Steyermark (Gentianaceae). – Beiträge zur Biologie der Pflanzen **68**: 113 - 123

JOHOW F (1885) Die chlorophyllfreien Humusbewohner West-Indiens, biologisch-morphologisch dargestellt. – Jahrbücher für Wissenschafltiche Botanik **16**: 415 – 449.

JOHOW F (1889) Die chlorophyllfreien Humuspflanzen nach ihren biologischen und anatomisch – entwicklungsgeschichtlichen Verhältnissen. – Jahrbücher für Wissenschafltiche Botanik **20**: 475 – 524.

KAMIENSKI F (1982) Les organes végétativs du *Monotropa hypopithys* L.. – Mémoires de la Sociéte National des Sciences Naturelles e Mathématiques de Cherbourg **26**: 1 – 40.

KNÖBEL M, WEBER HC (1988) Vergleichende Untersuchungen zur Mycotrophie bei *Gentiana verna* L. und *Voyria truncata* (Standley) Standley and Steyermark (Gentianaceae). – Beiträge zur Biologie der Pflanzen **63**: 463 – 477.

KOSKE RE, TESSIER B (1983) A convenient, permanent slide mounting medium. – Mycological Society of America Newsletter **34** (2): 59.

KREISEL H, SCHAUER F(1987) Methoden des mykologischen Laboratoriums. VEB Gustav Fischer Verlag, Jena.

LEAKE JJ (1994) The biology of myco-heterotrophic ("saprophytic") plants. Tansley Review No. 69. – New Phytologist **127**: 171-216.

MAAS PJM, RUYTERS P (1986) *Voyria* and *Voyriella* (Saprophytic Gentianaceae). – Flora Neotropica Monograph **41**: 1-93.

MORTON JB, BENNY GL (1990) Revised classification of arbuscular mycorrhizal fungi (Zygomycetes): a new order, Glomales, two new suborders, Glomineae and Gigasporineae, and two new families, Acaulosporaceae and Gigasporaceae, with an emendation of Glomaceae. – Mycotaxon **37**: 471-491.

MORTON JB, REDECKER D (2001) Two new families of Glomales, Archaesporaceae and Paraglomaceae, with two new genera Archaespora and Paraglomus, based on concordant molecular and morphological characters. – Mycologia **93**: 181-195.

NILSSON S, SKVARLA JJ (1969) Pollen Morphology of Saprophytic Taxa in the Gentianaceae. – Annals of the Missouri Botanical Garden **56**: 420-438.

OEHLER E (1927) Entwicklungsgeschichtlich-zytologische Untersuchungen an einigen saprophytischen Gentiananceen. – Planta **3**: 641-729.

RAJAPAKSE S, MILLER JC Jr (1992) Methods for studying vesicular-arbuscular mycorrhizal root colonization and related root physical properties. – In: NORRIS JR, READ DJ, VARMA AK (eds) Methods in Microbiology Vol. 24, Techniques for the study of mycorrhizae, pp. 302-316, Academic Press, London.

RASMUSSEN H (1995) Terrestrial orchids from seed to mycotrophic plant. Cambridge University Press, Cambridge.

RAUH W (1937) Die Bildung von Hypokotyl- und Wurzelsprossen und ihre Bedeutung für die Wuchsformen der Pflanzen. – Nova Acta Leopoldina **4**: 395-553.

RAYNAL A (1967a) Étude critique des genres *Voyria* et *Leiphaimos* (Gentianaceae) et Revision des *Voyria* d'Afrique. – Adansonia, sér **2**, 7: 53-71.

RAYNAL A (1967b) Sur un *Sebaea* Africain Saprophyte (Gentianaceae). – Adansonia, sér **2**, 7: 207-219.

RAYNAL-ROQUES A, PARÉ J (1998) Biodiversité des Phanérogames parasites: leur place dans la classificacion systématique. – Adansonia, sér. **3**, 20: 313-322.

RUZIN S (1999) Plant microtechnique and microscopy. Oxford University Press, New York, Oxford.

SCHÜßLER A, SCHWARZOTT D, WALKER C (2001) A new fungal phylum, *Glomeromycota*: phylogeny and evolution. – Mycological Research **105** (12): 1413-1421.

SVEDELIUS N (1902) Zur Kenntnis der saprophytischen Gentianaceen. – Bihang til konglia svenska vetenskaps-akadeniens handlingar **28** (Afd. III, No. 4): 3-16.

VIGODSKY-DE PHILIPPIS A (1938) Studio morfologico ed anatomico di "*Leiphaimos brachyloba*" (Grieseb.) Urb. var. "*cumbrensis*" Urb. et Ekm. – Nuovo giornale botanico italiano **45**: 190-194.

WALKER C, SANDERS FE (1986) Taxonomic concepts in the Endogonaceae: III. The seperation of *Scutellospora* gen. nov. from Gigaspora Gerd. & Trappe. – Mycotaxon **27**: 169-182

WARMING E (1874) Om rødderne hos *Neottia nidus-avis* Lin. – Videnskabelige Meddelelser fra den Naturhistoriske Forening i Kjøbenhavn pp. 26-32.

TER WELLE BJM (1986) Anatomy – In: Maas PJM, Ruyters P (eds.) *Voyria* and *Voyriella* (Saprophytic Gentianaceae). – Flora Neotropica Monograph **41**: 1-93.

YAMAMOTO M (2001) Identification of a mycorrhizal fungus in the roots of achlorophyllous *Sciaphila tosaensis* Makina (Triuridaceae). – Mycorrhiza **11**: 83-88.

Accepted: 12.09.2002

III

Syst. Geogr. Pl. **74**: 27-33 (2004)

Afrothismia saingei (Burmanniaceae, Thismieae), a new myco-heterotrophic plant from Cameroon

Thassilo Franke

Department Biology I and GeoBio – Center^{LMU}, Biodiversity Research: Systematic Mycology,
Menzinger Str. 67, D-80638 München, Germany
[thafra@web.de]

Abstract. – Afrothismia saingei, *a new species of Burmanniaceae (tribe Thismieae) from Mt. Kupe in South-West Cameroon is described and illustrated. It differs from the other species of the genus* Afrothismia *by the size of its flower, the long, distinctly heteromorphic tepals with fringed basal extensions, the dorsal invagination of the perianth tube and the unique shape of the internal flange.*

Introduction

In his monographic treatment of the Burmanniaceae, Jonker (1938) proposed a subdivision of the family into two tribes: the Burmannieae and the Thismieae. While many present day authors still maintain Jonker's tribal concept (Stevenson & Laconte 1995; Maas – van de Kamer 1998; Cheek & Williams 1999; Kiew 1999; Caddick & al. 2002a; Yang & al. 2002; APG 2003; Sainge & Franke in press), others have well-founded arguments to follow Agardh (1853), who was the first to consider the Thismieae to represent a distinct family (Chase & al. 1995; Takhtajan 1997; APG 1998; Caddick & al. 2000a, 2000b; Chase & al. 2000; Caddick & al. 2002b; Neyland 2002; Thiele & Jordan 2002). Since Schlechter (1921), who still distinguished ten genera within the Thismieae, the number of genera was subsequently declining, finally settling down to four (Maas – van de Kamer 1998). The genera accepted by Maas – van de Kamer (1998) are *Thismia* Griff., *Haplothismia* Airy Shaw, *Afrothismia* (Engl.) Schltr. and *Oxygyne* Schltr. All remaining generic names were absorbed by the largest genus *Thismia*.

A remarkable common feature of most Thismieae is their extreme scarcity. The majority of species are known exclusively from the type collection (Jonker 1938; Maas & al. 1986; Stone 1980). The reason for this situation seems to be their myco-heterotrophic mode of life. Most species grow in the leaf litter of tropical lowland and submontane rain forests, where they digest fungal hyphae within their roots (Groom 1895; Leake 1994; Cheek & Williams 1999; Imhof 1999). Due to this adaptation the plants do not perform photosythesis and the function of their leafless, achlorophyllous aerial shoots is restricted to sexual reproduction (Leake 1994). Since Thismieae generally produce flowers and fruits during the rainy season, the plants spend most of their life cycle subterraneously and are well hidden from the collector's eye (Stone 1980; Cheek & Williams 1999; Sainge 2003).

Surprisingly, during the last five years several new taxa of Thismieae, including a new species of the African genus *Afrothismia*, have been discovered (Kiew 1999; Thiele & Jordan 2002; Yang

 ISSN 1374-7886

& al. 2002; Sainge & Franke in press). While investigating the ecology of myco-heterotrophic plants on the western slopes of Mt. Kupe / SW-Cameroon, another new species of *Afrothismia* was discovered, which is described below as *Afrothismia saingei*.

Afrothismia saingei Th.Franke, **sp. nov.** – Differt ab omnibus speciebus generis longitudine florium; tepalis heteromorphis; appendice tepalorum longo ciliato-inciso; tubo perigonii apice, dorso invaginato et perigonii intra limbo lamellato quinquelobato provisa. – *Type*: Cameroon; South-West Province; Meme Division; Western slopes of Mt. Kupe, above Mbulle (small village between Tombel and Nyasoso); 4° 47' 56'' N, 9° 40' 26'' E (GPS reading was received in open field ca. 1km SW of type locality); ca. 970 m a.s.l.; *Sainge M.* 1053, 7.10.2002 (YA holo-; B, BR iso-).

Myco-heterotrophic herb. Stem whitish to yellowish, with clusters of proximally inflated roots and few scale-like leaves, sometimes branching, distinctly ribbed, up to 38 mm long, 2-2.4 mm in diam. Proximal inflated part of root yellowish, tubercle-like, ellipsoid to ovoid, 1.4 × 1 mm; distally elongate part of root whitish, up to 56 mm long, 0.2-0.3 mm wide. Leaves scale-like, triangular-ovate, adaxially concave, 2.3-5.3 × 1.9-3.2 mm. Inflorescence one-flowered. Bracts whitish translucent, faintly red, deltate, acuminate, adaxially deeply concave, with rectangular, often revolute basal lobes, 7-7.5 × 7.8-8.3 mm. Flowers sessile, epigynous, zygomorphic; perianth basally fused, forming a distinctly inflated tube with six heteromorphic free tepals that are unequal in length. Lower part of perianth tube obovoid ventricose, increasingly rugose in distal direction, whitish-translucent; the lowest third with crimson nerves alternating with likewise crimson arrow-head-like marks; length from base of tube to opposite perianth wall 13-15.8 mm long, width at widest point 8.9-10.4 mm. Upper part of perianth tube yellowish, cylindric, sharply bent to an angle of ca. 55°, dorsally invaginated into a semicircular plication resulting in a dorsal position of the mouth, 2.6-5 × 4.8-6 mm; base of upper part of perianth tube provided with an undulate, densely papillose internal flange, divided into six unequal lobes, width of widest (dorsal) lobe 2.5-2.6 mm. Mouth circular, surrounded by a fleshy, slightly recurved annulus, bright yellow, 5.1-6.3 mm in diameter. Tepals yellow merging to orange towards the tip, distinctly heteromorphic, arranged in dorsal, lateral, respectively ventral pairs, arising from a collar-like structure below the annulus. Each tepal provided with two basally inserted, ± distinctly fringed, whitish extensions. Dorsal tepals linear-triangular with recurved margins, bent over the mouth, 12.8-22.1 × 2.2-3 mm (width mesuared at the base); length of basal extensions 3.4-5.7 mm. Lateral tepals of similar shape, but generally longer, in young flowers rather straight, 20.3-35 × 2-2.8 mm; length of basal extensions 5.0-7.5 mm. Ventral tepals bent upwards, linear-triangular with long caudate apices, tips distinctly clavate, 42.5-73 × 2.8-3.0 mm, length of basal extensions 5.7-8 mm. Stamens six, inserted at the base of perianth tube; basal part of filaments over 4.4-4.5 mm adnate to perianth tube, upper part of filaments free, club-shaped, reflexed, 2.1 × 0.6 mm; anthers 1.3-1.4 × 0.8-1 mm, touching the stigma with an apical lobate connective appendage; thecae dehiscing longitudinally, half-way sunken into the fleshy connective, facing towards the perianth wall; connective appendage obcordate, densely papillose, 0.6 × 0.7 mm. Ovary unilocular, creamy-white, subspherical, partly enclosed by clasping

→

Figure 1. Afrothismia saingei. *A, habit; B, lateral view of flower; C, longitudinally dissected flower, showing internal structures of perianth tube; D, transversally dissected flower, showing arrangement of stamens; E, dorsal and lateral view of stigma and style; F, dorsal, ventral and lateral view of anther and free section of filament. Drawn by T. Franke from* Sainge M. *1053 (YA).*

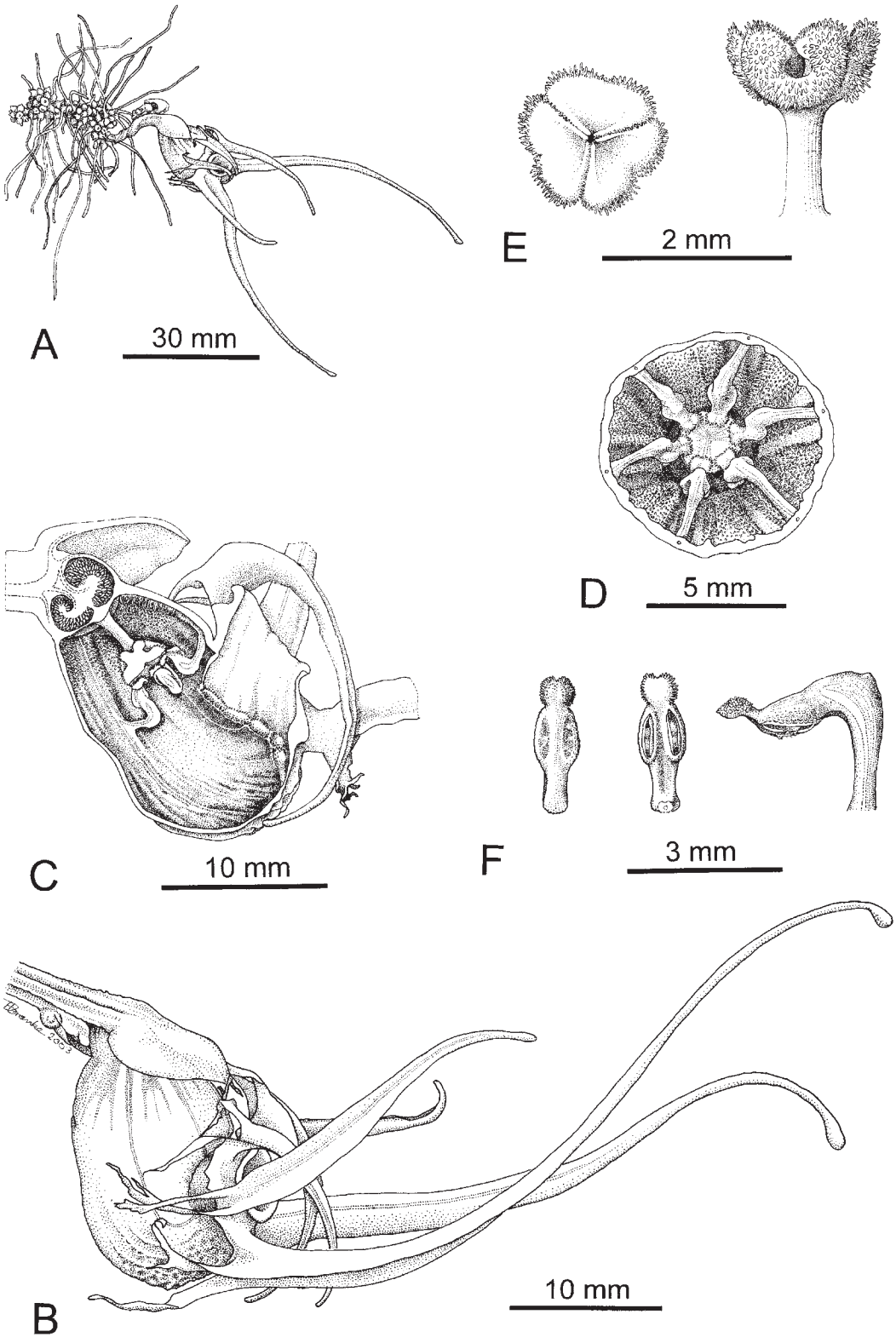

A
30 mm
E
2 mm
D
5 mm
C
10 mm
F
3 mm
B
10 mm

Figure 2. Afrothismia saingei. *Phot. T. Franke*.

bract, 2.1-3.3 × 4.1-5 mm; placentas 3, in their basal part connate into a central sterile column, in the upper part free and bearing numerous ovules. Style glabrous, 1.6 × 0.6 mm; stigma subspherical, densely papillose, 1.2 × 2.3 mm, consisting of three stigmatic lobes fused to each other. Fruits not seen. – Fig 1, 2.

Distribution. Afrothismia saingei is known exclusively from one locality on the western slopes of Mt. Kupe, the tallest of the Bakossi Mountains in Cameroon's South-West-Province. The area belongs to the Lower Guinean regional subcentre of endemism and is part of the Western Cameroon mountain glacial refuge sub-area, which is one of the richest plant diversity centres in Africa (Morton 1972, White 1979, Beentje & al.1994, Sosef 1994, Mutke & al. 2001). Hence, it is not surprising that Mt. Kupe is place of origin of several recently discovered botanic novelties, including a new myco-heterotrophic genus of Triuridaceae (Cheek & Cable 1997; Stoffelen & al. 1997; Cheek & al. 2003). The discovery of *A. saingei* supports Cheek & Cable's (1997) statement, that Mt. Kupe is the richest site for myco-heterotrophic plants in Africa.

Ecology. The type locality is situated within primary submontane evergreen rain forest on a SSE-running ridge at 970 m above sea level. The plants were growing in a thick layer of organic matter, densely interwoven by roots of neighbouring plants like *Cola cauliflora, C. verticellata, Garcinia*

sp., *Hugonia obtusifolia*, *Penianthus* cf. *camerounensis*, *Rothmannia* sp., *Tabernaemontana brachyantha* and *Trichilia* sp. After screening the whole area only three individuals of *A. saingei* were found growing approximately 50 m apart from each other. In contrast to most other myco-heterotrophic plants, that usually occur in company with different likewise myco-heterotrophic species, *A. saingei* was growing solitarily. The only other myco-heterotrophic plants that were found in the more distant surroundings were *Epipogium roseum* (common), *Afrothismia winkleri* (fairly common) and *Burmannia congesta* (rare).

Notes. *Afrothismia saingei* can easily be distinguished from the other species of *Afrothismia* by the size of its flower (the largest within the genus; fig. 1A, B), the distinctly heteromorphic tepals (fig. 1A, B; 2), the long, fringed basal tepal extensions (fig. 1B, C; 2), the dorsal invagination of the perianth tube (fig. 1C) and the unique shape of the internal flange (fig. 1C).

A remarkable feature that all species of *Afrothismia* have in common, are the reflexed stamens, that are partly fused to the perianth wall and touch the stigma with an apical connective appendage. This feature is also found in the flowers of the southeast Asian genus *Stenomeris* (Dioscoreaceae) (Burkill 1960, Caddick & Wilkin 1998, Caddick & al. 2000a). According to Burkill (1960), who examined the floral morphology of *Stenomeris dioscoreifolia*, this type of staminal arrangement seems to be the result of an adaptation to a complex pollination process. Burkill's assumption is based exclusively on morphological features and for this reason it remains hypothetical. In contrast, there do exist a few observations of flower-visiting insects in *Afrothismia*: Engler (1905) mentioned small dipterans that he found in the lower part of the perianth tube of *Afrothismia winkleri*. Cheek & Williams (1999) reported two dipterans of the same species, that left the perianth tube of *A. pachyantha* after a stay of several seconds. In October 2002 a drosophilid fly was observed and photographed when it carefully inspected the tepals of a still undescribed species of *Afrothismia* for several minutes (pers. observation). All these observations strongly suggest myophily; however the function of the internal floral structures remains unknown. Hence, specific investigations of the reproductive biology of this genus might provide an interesting area for further study.

Etymology. The specific epithet honours field botanist Sainge Nsanyi Moses, who discovered this plant.

Acknowledgements. – This study was financially supported by the BIOLOG (Biodiversity and Global Change) programme of the German Ministry of Education and Research (BMBF) and carried out under the supervision of R. Agerer / LMU München, to whom the author wants to express his special thanks. L. Beenken and D. Podlech / LMU München are thanked for constructive discussions and the latin diagnosis. Moreover E. Robbrecht, H. Maas – van de Kamer, P. Rudall and M. Cheek are thanked for their comments and suggestions. Further the author is greatly indebted to the staff of WWF / Nyasoso, the people of Mbulle and the field guides Stanley and Ri for their warm hospitality and constant support in the field.

References

Agardh J.G. (1853) Theoria systematis plantarum. Lund, Gleerup.

APG (1998) An ordinary classification for the families of flowering plants. *Ann. Missouri Bot. Gard.* **85**: 531-553.

APG (2003) An update of the angisperm phylogeny group classification for the orders and families of flowering plants: APG II. *Bot. J. Linn. Soc.* **141**: 399-436.

Beentje H. J., Adams B. & Davies S.D. (1994) Regional Overview: Africa. – In: Davies S.D., Heywood V.H. & Hamilton A.C. (eds.), Centres of Plant Diversity, Vol. 1: 101-148. WWF & IUCN.

Burkill I.H. (1960) The organography and the evolution of Dioscoreaceae, the family of the yams. *J. Linn. Soc., Bot.* **56**: 319-412.

Caddick L.E. & Wilkin P.(1998) A revision of the genus Stenomeris (Dioscoreaceae). *Kew Bull.* **53**: 703-712.

Caddick L.E., Rudall P.J., Wilkin P. & Chase M.W. (2000a) Yams and their allies: systematics of Dioscoreales. In: Wilson K.L., Morrison D.L. (eds.) Monocotyledons: systematics and evolution: 475-487. Melbourne, CSIRO.

Caddick L.E., Rudall P.J. & Wilkin P. (2000b) Floral morphology and development in Dioscoreales. *Feddes Repert.* **111** (3-4): 189-230.

Caddick L.R., Wilkin P., Rudall P.J., Hedderson T.A.J. & Chase M.W. (2002a) Phylogenetics of Dioscoreales based on combined analyses of morphological and molecular data. *Bot. J. Linn. Soc.* **138**: 123-144.

Caddick L.R., Wilkin P., Rudall P.J., Hedderson T.A.J. & Chase M.W. (2002b) Yams reclassified: A recircumscription of Discoreaceae and Dioscoreales. *Taxon* **51**: 103-114.

Chase M.W., Stevenson D.W., Wilkin P. & Rudall P.J. (1995) Monocot systematics: a combined analysis. In: Rudall P.J., Cribb P.J., Cutler D.F., Humphries C.J. (eds.), Monocotyledons: systematics and evolution: 685 - 735. Kew, Royal Botanic Gardens.

Chase M.W., Soltis D.E., Soltis P.S., Rudall P.J., Fay M.F., Hahn W.H., Sullivan S., Joseph J., Molvray M., Kores P.J., Givnish T.J., Sytsma K.J. & Pires J.C. (2000) Higher-level systematics of Monocotyledons: an assesment of current knowledge and a new classification. In: Wilson K.L., Morrison D.L. (eds.) Monocotyledons: systematics and evolution: 475-487. Melbourne, CSIRO.

Cheek M. & Cable S. (1997) Plant inventory for conservation management: the Kew-earthwatch programme in Western Cameroon, 1993-1996. In: Doolan S. (ed.), African rainforest and the conservation of biodiversity, Proceedings of the Limbe Conference, 17-24 January 1997: 29-38. Limbe Botanic Garden, Cameroon.

Cheek M. & Williams S. (1999) A review of African saprophytic flowering plants. In: Timberlake J., Kativu S. (eds.), African plants: biodiversity, taxonomy & uses. Proceedings of the 1997 AETFAT congress, Harare, Zimbabwe: 39-49. Kew, Royal Botanic Gardens.

Cheek M., Williams S. & Etuge M. (2003) Kupea martinetugei, a new genus and species of Triuridaceae from western Cameroon. *Kew Bull.* **58**: 225-228.

Engler A. (1905) Thismia winkleri Engl., eine neue afrikanische Burmanniacee. *Bot. Jahrb. Syst.* **38**: 89-91.

Groom P. (1895) On Thismia aseroe (Beccari) and its mycorrhiza. *Ann. Bot.* **9**: 327-361.

Imhof S. (1999) Anatomy and mycotrophy of the achlorophyllous Afrothismia winkleri. *New Phytol.* **144**: 533-540.

Jonker F.P. (1938) A monograph of the Burmanniaceae. *Meded. Bot. Mus. Herb. Rijksuniv. Utrecht* **51**: 1-279.

Kiew R. (1999) Thismia goodii (Burmanniaceae), the blue-capped Thismia, a new species from Borneo. *Gard. Bull. Singapore* **51**: 179-182.

Leake J. J. (1994). The biology of myco-heterotrophic ("saprophytic") plants. – Tansley Review No. 69. *New Phytol.* **127**: 171-216.

Maas P.J.M., Maas – van de Kamer H., van Benthem J., Snelders H.C.M. & Rübsamen T. (1986) Burmanniaceae, *Flora Neotropica* **42**: 1-189.

Maas – van de Kamer H. (1998) Burmanniaceae. In: Kubitzki K. (ed.), Families and genera of vascular plants, Monocotyledons, Lilianae (except Orchidaceae): 154-164. Berlin, Springer.

Morton J.K. (1972) Phytogeography of the West African Mountains. In: Valentine D.H. (ed.) Taxonomy, Phytogeography and Evolution: 221-236. London, Academic Press.

Mutke J., Kier G., Braun G., Schulz Chr & Barthlott W. (2001) Patterns of African vascular plant diversity – a GIS based analysis. *Syst. Geogr. Pl.* **71**: 1125-1136.

Neyland R. (2002) A phylogeny inferred from large-subunit (26S) ribosomal DNA sequences suggests that Burmanniales are polyphyletic. *Austral. Syst. Bot.* **15**: 19-28.

Sainge M.N. (2003) Studies on the phenology of African myco-heterotrophic plants (Abstract). Tagungsband der 16. Jahrestagung der Gesellschaft für Tropenökologie: 66 Rostock, Germany.

Sainge M.N. & Franke T. (in press) A new species of Afrothismia (Burmanniaceae) from Cameroon. *Nordic J. Bot.*

Schlechter R. (1921) Die Thismieae. *Notizbl. Bot. Gart. Berlin-Dahlem* **8**: 31-45.

Sosef M.S.M. (1994) Refuge Begonias. Taxonomy, phylogeny and historical biogeography of Begonia sect. Loasibegonia and sect. Scutibegonia in relation to glacial rain forest refuges in Africa. PhD thesis. Wageningen.

Stevenson D.W. & Laconte H. (1995) Cladistic analysis of monocot families. In: Rudall P.J., Cribb P.J., Cutler D.F., Humphries C.J. (eds.), Monocotyledons: systematics and evolution: 685 - 735. Kew, Royal Botanic Garden.

Stoffelen P., Cheek M., Bridson D. & Robbrecht E. (1997) A new species of Coffea (Rubiaceae) and notes on Mt. Kupe (Cameroon). *Kew Bull.* **52**: 989-994.

Stone B. (1980) Rediscovery of Thismia clavigera (Becc.) F. v. M. (Burmanniaceae). *Blumea* **26**: 419-425.

Takhtajan A. (1997) Diversity and classification of flowering plants. New York, Columbia University Press.

Thiele K.R. & Jordan P. (2002) Thismia clavarioides (Thismiaceae), a new species of fairy lantern from New South Wales. *Telopea* **9**: 765-771.

White F. (1979) The Guineo-Congolian Region and its relationships to other phytochoria. *Bull. Jard. Bot. Nat. Belg.* **49**: 11-45.

Yang S.Z., Saunders R.M.K. & Hsu, C.J. (2002) Thismia taiwanensis sp. nov. (Burmanniaceae tribe Thismieae): First record of the tribe in China. *Syst. Bot.* **27**: 485-488.

Manuscript received August 2003; accepted in revised version October 2003.

IV

BLUMEA 49 (2004) 451–456

A NEW SPECIES OF AFROTHISMIA (BURMANNIACEAE; TRIBE: THISMIEAE) FROM THE WESTERN FOOTHILLS OF MOUNT CAMEROON

TH. FRANKE[1], M.N. SAINGE[2] & R. AGERER[1]

SUMMARY

Afrothismia foertheriana, a new species of Burmanniaceae (tribe: Thismieae) from the peripheral zone of the Onge Forest Reserve in Cameroon's Southwest Province is described and illustrated. The papillose, multicellular floral trichomes, the tepal's erose margins, the small, zygomorphic perianth mouth and the dull purplish brown coloration give *A. foertheriana* a distinctive appearance within the genus. The species is here assessed as being critically endangered.

Key words: Burmanniaceae, Thismieae, *Afrothismia foertheriana*, Cameroon, conservation, taxonomy.

INTRODUCTION

All species of the small genus *Afrothismia* (Burmanniaceae; tribe: Thismieae) are achlorophyllous myco-heterotrophic herbs, receiving all essential nutrients from root colonizing fungi (Leake, 1994; Cheek & Williams, 1999; Imhof, 1999). Due to this adaptation the plants do not perform photosynthesis and the function of their aerial shoots is restricted to sexual reproduction. Since species of *Afrothismia* generally produce flowers and fruits during the rainy season, the plants spend most of their life cycle subterraneously and are well hidden from the collector's eye (Cheek & Williams, 1999; Sainge, 2003).

Afrothismia winkleri (Engl.) Schltr. and *A. pachyantha* Schltr., the two first described species of this genus, were discovered almost at the same time in Cameroon's Southwest Province (Engler, 1905; Schlechter, 1906). Both type localities are situated along the eastern, respectively south-eastern foothills of Mt Cameroon (4095 m), representing one of the country's most densely populated regions. Recent efforts to find both species in this area failed and brought to light an alarming situation. The natural vegetation around the village of Muea (formerly 'Neu Tegel'), which is the place where H. Winkler collected *A. winkleri* in 1904 (Engler, 1905), has been completely replaced by banana plantations. The hills around the village of Moliwe, where Schlechter discovered *A. pachyantha* and another population of *A. winkleri* in 1905 (Schlechter, 1906), experienced

1) Department Biology I and GeoBio-Center[LMU], Biodiversity Research, Systematic Mycology, Menzinger Str. 67, D-80638 München, Germany; thafra@web.de (author for correspondence); myrrhmyk@lrz.uni-muenchen.de

2) CTFS / BDCPC / Korup Forest Dynamic Plot, Korup National Park, P.O. Box 36, Mundemba, Cameroon; sainge2001@yahoo.com

the same fate, since most of the area is covered by oil palm and rubber plantations at the present time. The small patches of rain forest still existing on some steep slopes between Moliwe and the village of Saxenhof, as well as the southerly situated Bimbia-Bonadikombo community forest, were vainly scoured by us for *Afrothismia* during the rainy seasons of 2000, 2001 and 2002; and by others during 1991–1993 (Cheek & Ndam 1996; Cable & Cheek 1997).

Since there remains little hope to find *Afrothismia* on the east side of Mount Cameroon, another attempt was made on the western foothills in October 2002. The screening was carried out in the peripheral zone of the Onge Forest Reserve close to the village of Diongo. Although neither *Afrothismia winkleri* nor *A. pachyantha* could be registered, two different species of *Afrothismia* were found. One species was identified as the recently described *Afrothismia hydra* Sainge & Th. Franke, which also occurs further west in the nearby Korup National Park and Southern Nigeria (Sainge & Franke, in press). The second species matches none of the seven known species of *Afrothismia* and will be described below (Engler, 1905; Schlechter, 1906; Cowley, 1988; Maas-van de Kamer, 2003; Cheek, 2003; Franke, 2004; Sainge & Franke, in press).

Afrothismia foertheriana Th. Franke, Sainge & Agerer, *spec. nov.* — Fig. 1, 2

Differt ab specibus ceteris generis floribus obscuri-coloratis (obscure brunnei-purpureis), marginibus tepalorum irregulariter dentatis, tubo perigonii trichomalibus papillosis obtecto, apertura zygomorpha minuta. — Typus: *Th. Franke & M. Sainge 02/030* (holo YA; iso B, WAG), Cameroon, Southwest Province, Ndian Division, Diongo Community Forest, peripheral zone of the Onge Forest Reserve, c. 8 km NE of Diongo village, alt. c. 220 m, 7 Oct. 2002.

Myco-heterotrophic herb. Stem whitish to yellowish, with clusters of proximally inflated roots and few scale-like leaves, sometimes branching, distinctly ribbed, up to 37 mm long, 1.7 mm diameter. Proximally inflated part of root yellowish, tubercle-like, ellipsoid to ovoid, 1.6–1.9 by 1–1.2 mm; distally elongate part of root white, up to 48 mm long, 0.3 mm diameter. Leaves scale-like, narrowly triangular, 2.7–5 by 1.1–2.5 mm. Inflorescence 1- or 2-flowered. Bracts whitish translucent, faintly purplish brown, broadly triangular, acuminate, 6.7–7.5 by 5.8–6.6 mm. Flowers sessile, epigynous, zygomorphic; perianth basally fused, forming an inflated tube with six free tepals. Lower part of perianth tube obovoid, ventrally rugose to plicate, with prominent, longitudinally running ribs on the inner wall, whitish translucent, with dark purplish brown stripes alternating with dark purplish brown arrow-head-like marks, converging into a likewise coloured radial band just below the base of the tepals; length of perianth tube 13–15.8 mm, width at widest point 8.3–8.8 mm. Upper part of perianth tube forming an elliptic scutiform plate girded by the tepal bases, glossy, whitish translucent, speckled with purplish brown dots and lines, 8.3–8.8 by 7.3–7.5 mm, marginally set with multicellular, papillose trichomes, 0.3–1.6 mm in length. Mouth strongly zygomorphic, inserted within the upper third of the perianth plate, obovate surrounded by a velvety rim with roof-like projecting upper half, 1.8 mm at widest point; upper part of perianth tube provided with an internal, marginally densely papillose flange, up to 2.3 mm wide, ventrally declining in width. Tepals pointing straight forwards, distally approaching each other, similar in shape and length, each tepal with erose membranaceous, whitish translucent margins basally converging into distinctly fringed extensions, dark purplish

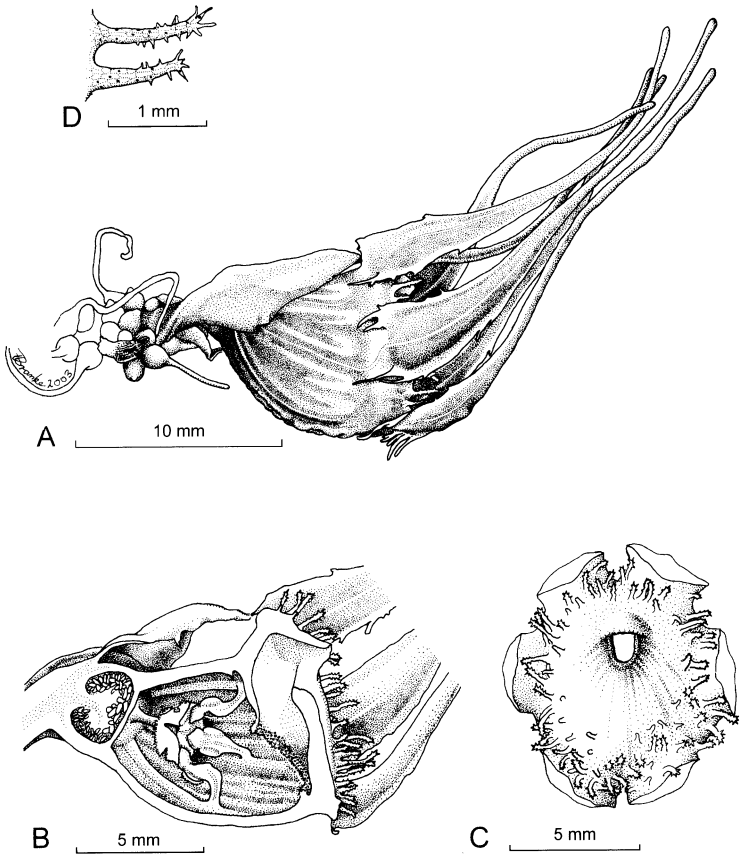


Fig. 1. *Afrothismia foertheriana* Th. Franke, Sainge & Agerer. A. Lateral view of flower; B. longitudinally dissected flower, showing internal structures of perianth tube; C. frontal view of flower (tepals removed); D. multicellular, papillose trichomes (all: *Th. Franke & M. Sainge 02/030*). Drawn by Th. Franke.

brown, 20–22.7 by 3.3–4 mm (width measured at the base), basal extensions 2.4–2.6 mm long. Stamens 6, inserted at the base of perianth tube; basal part of filaments 4 mm adnate to perianth tube, upper part of filaments free, club-shaped, reflexed, 1.8 by 0.4 mm; anthers 1.2 by 1.3 mm, touching the stigma with an apical lobate connective appendage; thecae dehiscing longitudinally, half-way sunken into the fleshy connective, facing towards the perianth wall; connective appendage deltoid, densely papillose, 0.7 by 0.8 mm. Ovary unilocular, white, with a band of 6 purplish brown dots, subspherical, partly enclosed by clasping bract, 1.8–2.6 by 3.3–3.8 mm; placentas 3, in their basal part connate into a central sterile column, in the upper part free and bearing numerous ovules. Style glabrous, 1.4 by 0.5 mm; stigma subspherical, densely papillose, 1.2 by 2.9 mm, consisting of three stigmatic lobes fused to each other. Fruit a pyxidium, 3.8 by 5.8 mm, sterile column enormously distending, 10.8–14.6 by 2.2–2.7 mm, pushing aside the lid-like receptacle, lifting the seeds above the fruit bottom. Seeds long-ellipsoid with loose reticulate testa, 0.5–0.6 by 0.3 mm.

Distribution — Cameroon: western foothills of Mt Cameroon.

Habitat & Ecology — The new species is known only from a single locality, which is situated on a narrow terrace, nestled into a steep slope, covered by open canopy forest. The vegetation was composed of small trees and treelets, tall monocotyledonous herbs and scattered larger trees. A brief plant inventory yielded the following woody plant species: *Antidesma vogelianum* Müll. Arg., *Cola flavo-velutina* K. Schum., *Craterispermum aristatum* Wernham, *Dicranolepis disticha* Planch., *Dicranolepis* spec., *Diospyros cinnabarina* (Gürke) F. White, *Diospyros preussii* Gürke, *Diospyros suaveolens* Gürke, *Diospyros* spec. 1 and spec. 2, *Jollydora duparquetiana* (Baill.) Pierre, *Lasianthera africana* P. Beauv., *Maesobotrya dusenii* (Pax) Pax, *Massularia acuminata* (G. Don) Bullock ex Hoyle, *Microdesmis puberula* Hook.f. ex Planch., *Octoknema affinis* Pierre, *Pycnanthus angolensis* (Welw.) Warb., *Rhabdophyllum* cf. *calophyllum* (Hook.f.) Tiegh., *Rinorea subintegrifolia* (P. Beauv.) Kuntze, *Scyphocephalium mannii* (Benth.) Warb., *Strombosia grandiflora* Hook.f. ex Benth., *Strombosia pustulata* Oliv., *Tapura africana* Oliv., *Trichoscypha* spec. In contrast to most other myco-heterotrophic plants, which usually occur in company with different likewise myco-heterotrophic species, *A. foertheriana* was growing solitarily. The only other myco-heterotrophic plants that were found in the more distant surroundings were *Afrothismia hydra*, *Burmannia congesta* (Wright) Jonker, *Burmannia hexaptera* Schltr., *Gymnosiphon* cf. *longistylus* (Benth.) Hutch. & Dalziel; *Kupea martinetugei* Cheek & S.A. Williams, *Sciaphila ledermannii* Engl. and *Sebaea oligantha* (Gilg) Schinz.

Fig. 2. *Afrothismia foertheriana* Th. Franke, Sainge & Agerer. Habit. Photograph by Th. Franke (*Th. Franke & M. Sainge 02/030*).

Afrothismia foertheriana was growing in groups of scattered individuals, showing an obvious preference for raised microhabitats, e.g. horizontally running tree roots (Fig. 2). In some cases, the rhizomes and most roots were exposed to the air with only the distal parts of the filiform roots entering the uppermost soil strata (Fig. 2). Since the great majority of individuals were in the fruiting phase when discovered in October, it seems likely that the peak of the flowering season is around August/September.

Etymology — The specific epithet honours my (T.F.) colleague and friend Harald Förther, to whom I am much indebted for his constant readiness to help and advise me during my educational career.

Note — The type locality of *Afrothismia foertheriana* belongs to the Lower Guinean regional subcentre of endemism and is part of the Western Cameroon mountain glacial refuge sub-area, which is one of the richest plant diversity centres in Africa (Morton, 1972; White, 1979; Beentje et al., 1994; Sosef, 1994; Mutke et al., 2001). Many of the species in this region are highly endemic and for this reason seriously threatened by human impact. Hence, the conservation status of *Afrothismia foertheriana* is assessed as CR B1 [a, b (iii)] following the guidelines of the IUCN (2001). In other words, the species is believed to be critically endangered because its geographic range is estimated to less than 100 km^2 and because it is known only from a single location, where it is severely threatened by illegal logging and the extent of agricultural activities (mainly oil palm and cocoa cultivation).

ACKNOWLEDGEMENTS

This study was financially supported by the BIOLOG (Biodiversity and Global Change) programme (Grant 01LC0007) of the German Ministry of Education and Research (BMBF). L. Beenken and D. Podlech / LMU München are thanked for constructive discussions and the Latin diagnosis. Furthermore, the authors are greatly indebted to the people of Diongo and the guides Mambo and Robert for their warm hospitality and constant support in the field.

REFERENCES

Beentje, H.J., B. Adams & S.D. Davies. 1994. Regional Overview: Africa. In: S.D. Davies, V.H. Heywood & A.C. Hamilton (eds), Centres of Plant Diversity, Vol. 1: 101–148. WWF & IUCN.

Cable, S. & M. Cheek. 1997. The plants of Mount Cameroon, a conservation checklist. Royal Botanic Gardens, Kew.

Cheek, M. 2003. A new species of Afrothismia (Burmanniaceae) from Kenya. Kew Bulletin 58: 951–955.

Cheek, M. & N. Ndam. 1996. Saprophytic flowering plants of Mount Cameroon. In: L.J.G. van der Maesen et al. (eds), The Biodiversity of African Plants: 612–617. Kluwer Academic Publishers, The Netherlands.

Cheek, M. & S. Williams. 1999. A review of African saprophytic flowering plants. In: J. Timberlake & S. Kativu (eds), African plants: biodiversity, taxonomy and uses. Proceedings of the 1997 AETFAT congress, Harare, Zimbabwe: 39–49. Royal Botanic Gardens, Kew.

Cowley, E.J. 1988. Burmanniaceae. In: R.M. Polhill (ed.), Flora of Tropical East Africa: 1–9. Balkema, Rotterdam, Brookfield.

Engler, A. 1905. Thismia winkleri Engl., eine neue afrikanische Burmanniaceae. Bot. Jahrb. Syst. 38: 89–91.

Franke, Th. 2004. Afrothismia saingei (Burmanniaceae), a new myco-heterotrophic plant from Cameroon. Syst. Geogr. Pl. 74: 27–33.

Imhof, S. 1999. Anatomy and mycotrophy of the achlorophyllous Afrothismia winkleri. New Phytol. 144: 533–540.

IUCN. 2001. IUCN Red List Categories and Criteria: Version 3.1. IUCN Species Survival Commission. IUCN, Gland, Switzerland & Cambridge, UK.

Leake, J.J. 1994. The biology of myco-heterotrophic ('saprophytic') plants. Tansley Review No. 69. New Phytol. 127: 171–216.

Maas-van de Kamer, H. 2003. Afrothismia gesneriodes, another new species of Afrothismia (Burmanniaceae) from tropical Africa. Blumea 48: 475–478.

Morton, J.K. 1972. Phytogeography of the West African Mountains. In: D.H. Valentine (ed.), Taxonomy, Phytogeography and Evolution: 221–236. Academic Press, London.

Mutke, J., G. Kier, G. Braun, Chr. Schulz & W. Barthlott. 2001. Patterns of African vascular plant diversity – a GIS based analysis. Syst. Geogr. Pl. 71: 1125–1136.

Sainge, M.N. 2003. Studies on the phenology of African myco-heterotrophic plants (abstract). In: U. Karlowski, P. Kiehl, T. Lemke, T. Pitsch & R. Paschen (eds), Tagungsband der 16. Jahrestagung der Gesellschaft für Tropenökologie: 66. Rostock, Germany.

Sainge, M.N. & Th. Franke. In press. A new species of Afrothismia (Burmanniaceae) from Cameroon. Nordic J. Bot.

Schlechter, R. 1906. Burmanniaceae africanae. Bot. Jahrb. Syst. 38: 137–143.

Sosef, M.S.M. 1994. Refuge Begonias. Taxonomy, phylogeny and historical biogeography of Begonia sect. Loasibegonia and sect. Scutibegonia in relation to glacial rain forest refuges in Africa. PhD thesis. Wageningen.

White, F. 1979. The Guineo–Congolean Region and its relationships to other phytochoria. Bull. Jard. Bot. Belg. 49: 11–45.

V

Nordic Journal of Botany

A new species of Afrothismia (Burmanniaceae) from Cameroon

M. N. Sainge and T. Franke

Sainge, M. N. & Franke, T. 2005. A new species of *Afrothismia* (Burmanniaceae) from Cameroon. – Nord. J. Bot. 23: 299-303. Copenhagen. ISSN 0107-055X.

Afrothismia hydra, a new species of Burmanniaceae (tribe Thismieae) from South west Cameroon is decribed, illustrated and compared with related taxa.

M. N. Sainge, CTFS / BDCPC / Korup Forest Dynamic Plot; Korup National Park, P.O. Box 36, Mundemba, Cameroon. E-mail: sainge2001@yahoo.com. – T. Franke, Department Biology I and GeoBio – Center^{LMU}, Biodiversity Research: Systematic Mycology, Menzinger Str. 67, D-80638 München, Germany. E-mail: thafra@web.de.

Introduction

The genus *Afrothismia* was established by R. Schlechter in 1906 and based on *Afrothismia winkleri* (Engl.) Schltr., formerly described as *Thismia winkleri* Engl. (Engler 1905, Schlechter 1906). It comprises three species: *A. winkleri*, occuring in Cameroon with one variety known from Uganda, *A. pachyantha* Schltr. also found in Cameroon and *A. insignis* Cowley from Tanzania (Schlechter 1906, Hepper 1968, Cowley 1988). All species are myco-heterotrophic, i.e. they parasitise on root colonizing fungi (Leake 1994, Raynal-Roques & Paré 1998). As a result of this ecological strategy they lack chlorophyll and have reduced photosynthetic organs.

During a study of the biology of African myco-heterotrophic plants, a new species of *Afrothismia* was discovered in Korup National Park, Cameroon.

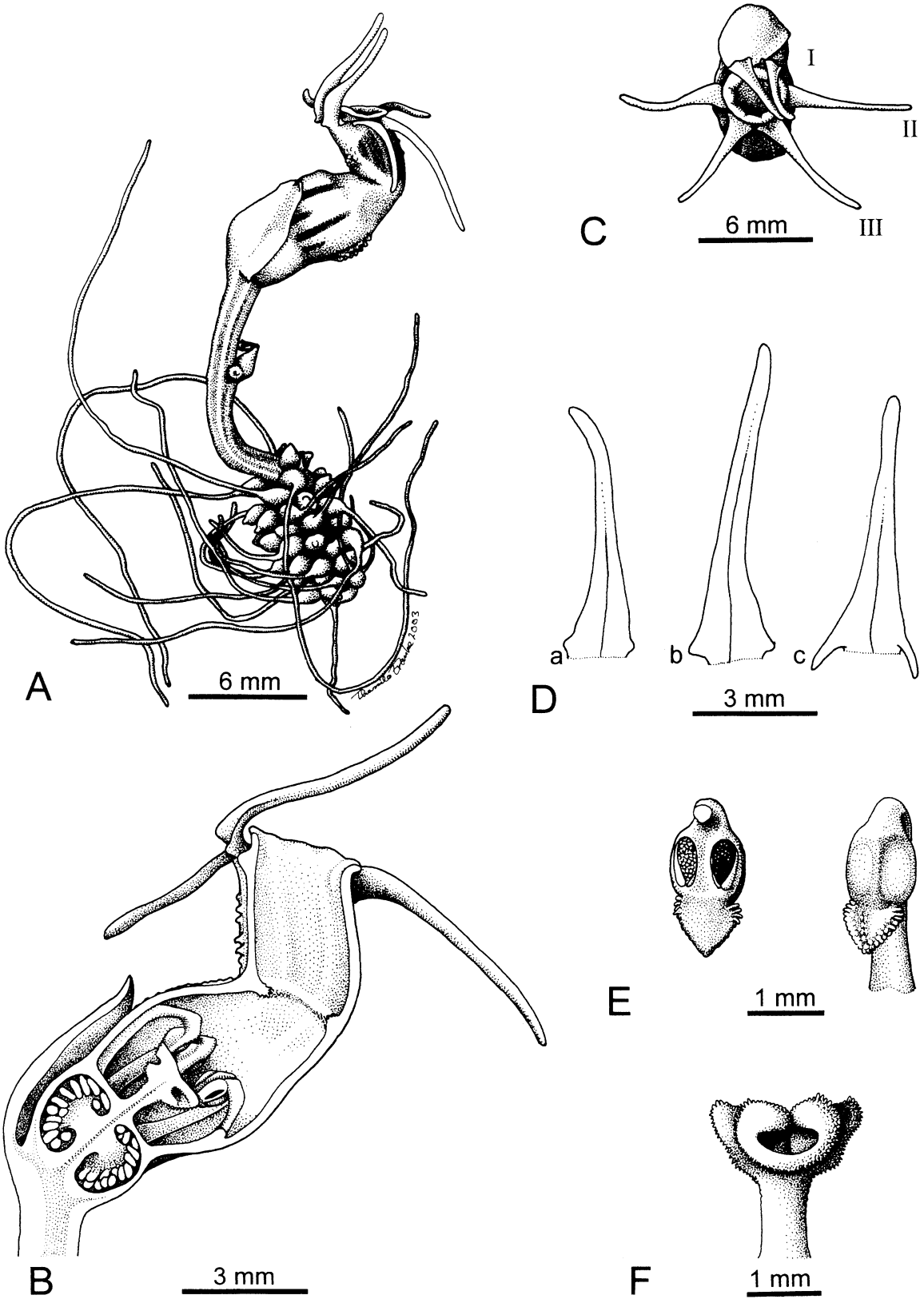
Afrothismia hydra Sainge & Th. Franke, sp. nov.

Type: Cameroon, South west Province, Ndian Division, Southern Korup National Park, close to Chimpanzee Camp, ca. 230 m, 21st October, 2001, Sainge M. 910 (Holotype: YA; Isotype: B, K).

Differt ab *Afrothismia winkleri* (Engl.) Schltr. habitu minore, latere ventrali tubus perigonii leviter curvato nec geniculato, ovario erecto nec oblique recurvato, insuper costis interstaminalibus et lobis deltoideis intus tubo perigonii deficientibus. – Figg. 1-2.

Myco-heterotrophic herb. Stem whitish to yellowish, with clusters of proximally inflated roots and few scale-like leaves, sometimes branching, distinctly ribbed, 17 – 70 mm long, 1.2 – 2 mm wide. Proximal inflated part of root yellow, tubercle-like, ellipsoid to ovoid, 1.3 – 1.6 × 0.9 – 1.1 mm. Distal elongated part of root whitish, up to 50 mm long, 0.2 – 0.3 mm wide. Scale-like leaves triangular lanceolate, adaxially concave, 3 – 4.3 × 2 – 2.1 mm. Inflorescence a 1 – 3 (6) – flowered scorpioid cyme. Bracts reddish, turning whitish translucent towards the margin, deltoid, acute to acuminate, adaxially deeply concave, with small free basal lobes, 4.3 – 5.3 × 4 – 4.7 mm. Flowers epigynous, zygomorphic, tepals partly fused, forming a perigone-tube with 6 free tepal sections. Perigone-tube bent to an angle of (90) 105 to 130° near the middle, where it is divided into two parts by an internal flange; lower part of perigone-tube translucent, urceolate, rugose, increa-

Accepted 15-7-2003


A
6 mm
B
3 mm
C
I
II
III
6 mm
D
a
b
c
3 mm
E
1 mm
F
1 mm

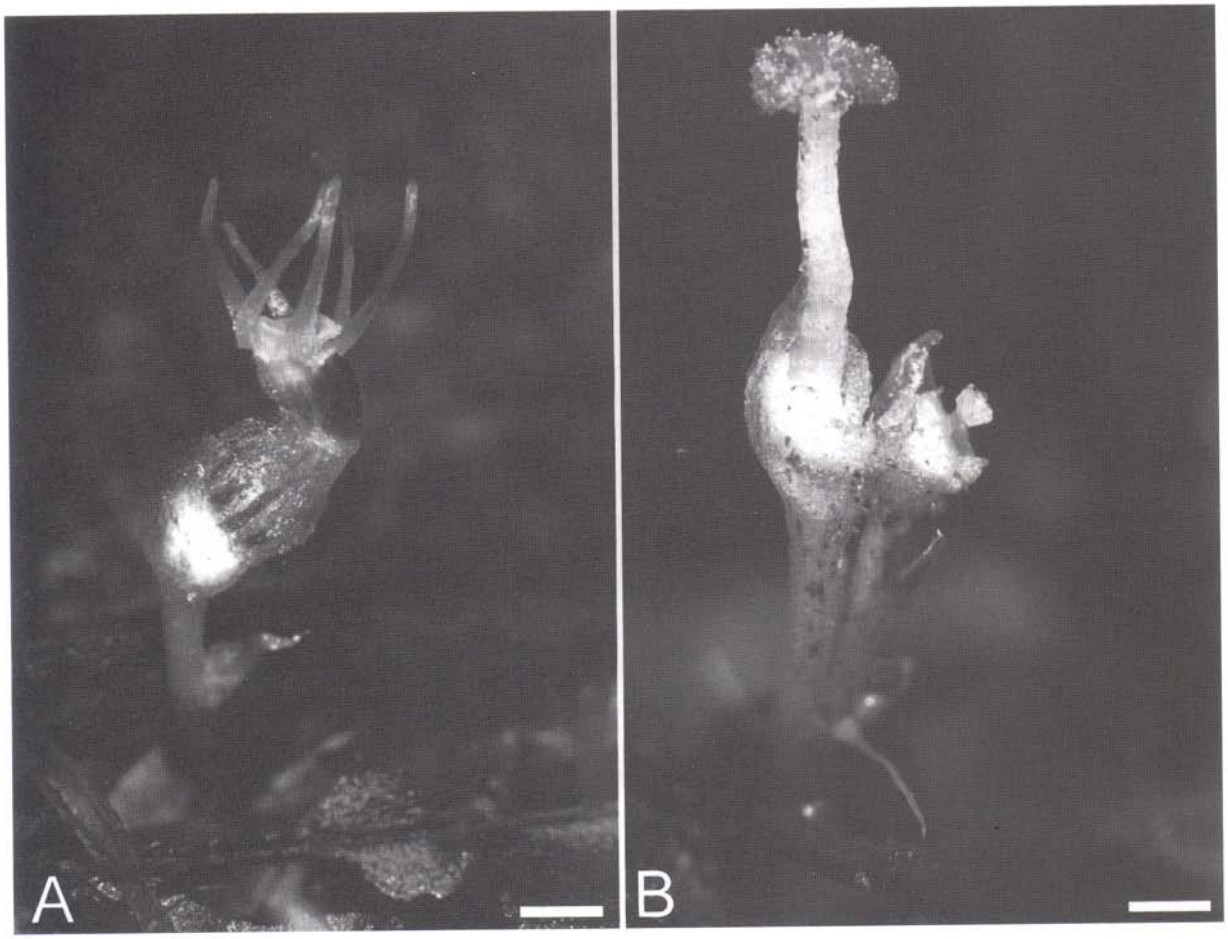


Fig. 2. *Afrothismia hydra* (Sainge M. 910). A: Flower in natural position; B: Mature fruit exposing seeds on column-like structure, closed immature fruit on the right. Scale = 3 mm.

singly papillose towards the ventral side, six veined, proximal part of veins mostly with crimson shorter and longer lines alternating each other, 4.3 – 4.8 × 4 – 4.8 mm; upper part of perigone-tube reddish-white with a distinct crimson ventral mark, dorsal side papillose, 3.1 – 4.3 × 2 – 3.5 mm (flowers of Franke Th. 02/013 entirely yellow, crimson pigment apparently lacking). Internal flange densely papillose, broadest at the dorsal side, up to 0.5 mm wide; mouth bright yellow, 2.8 – 3.2 mm in diameter, with a fleshy, glossy, slightly recurved rim, 0.5 – 0.9 mm high; free sections of tepals yellow, dorsiventrally flattened, narrow-lanceolate 5.3 – 7 × 0.8 – 1.2 mm (width measured above the mouth), the two dorsal free sections often bent above the mouth, each free section with two small, downward directed, blunt to acute extensions at the base, 0.2 – 0.7 × 0.2 – 0.3 mm. Stamens 6, inserted at base of perigone-tube; proximal half of filaments adnate to perigone-tube for 2 – 2.2 mm, free parts of filaments club-shaped, distally incurved, 1 × 0.3 mm (width measured at the middle); anthers hanging, 0.7 – 0.8 × 0.7 – 0.8 mm, touching the stigma with a lobe-shaped connective appendage; thecae dehiscing longitudinally, half-way merged into the fleshy connective, facing towards the perigone-wall; connective appendage, deltoid, acute, densely papillose, 0.5 – 0.6 × 0.5 – 0.7 mm. Ovary unilocular with basal placentation, cream-white, hemispherical, facing diagonally upwards, partly enclosed by

Fig. 1. *Afrothismia hydra* (all from Sainge M. 910, except D/b, D/c). A: Habit; B: Dissected flower, showing internal structures of perigone tube; C: Frontal view of flower, showing dorsal (I), lateral (II) and ventral (III) free tepal segments; D: Dorsal free tepal segments of three different specimens (D/a = Sainge M. 910, D/b = Franke Th. 02/013, D/c = Sainge M. 1068); E: Ventral and dorsal view of anther; F: Style and stigma.

Table 1. Principal morphological differences between *A. winkleri* and *A. hydra. (Afrothismia winkleri* (Engl.) Schltr. Cameroon, South west Province, Kupe – Manengouba Division, submontane forest on western slopes of Mt. Kupe above the village of Mbulle, ca. 6 km N. of Tombel, 4°48'N 9°41' E, ca. 900 m, 23th October, 2002, Franke Th.. 02/034 (B)).

Characters	*A. winkleri*[1]	*A. hydra*
Total length of perigone-tube (length of lower part + length of upper part)	ca. 16 mm	ca. 8.5 mm
Max width of lower part of perigone-tube	ca. 7.5 mm	ca. 4.5 mm
Angle between lower and upper part of perigone-tube	ca. 65°	ca. 115°
Ventral side of perigone-tube distinctly geniculate	+	–
Spaces between adnate filaments with 3 prominent ribs each	+	–
Ventral inner perigone wall with a row of deltoid acuminate lobes	+	–
Position of the ovary	facing diagonally downward	facing diagonally upward

clasping bract, 2 – 2.3 × 2.7 – 4 mm; style laxely papillose at distal half, 0.8 – 0.9 mm; stigma hemispherical, short papillose, 0.7 – 0.9 × 1.6 – 1.8 mm, consisting of three stigmatic lobes fused to each other, each lobe conduplicate, dorsiventrally compressed, with the lateral margins bent upward covering the adaxial surface. Fruit a pyxidium with a distending column-like structure lifting the placenta 9 – 12 mm above the receptacle, exposing the seeds. Seeds long-ellipsoid with loose reticulate testa, 0.7 – 0.8 × 0.2 – 0.3 mm. – Figs 1-2.

Additional specimens studied: Nigeria, Ondo Province, Akure District, Akure F. R., Aponmu, E. N. E. of the Pilot Sawmill, in Observation Plot square A.3 (iv), 2nd November 1949, Keay R. W. J. 25540 (K, B).

Cameroon, South west Province, Ndian Division, Southern Korup National Park, along Chimpanzee trail, ca. 230 m, 11th October, 2002, Franke Th.. 02/013 (B). Cameroon, South west Province, Ndian Division, Diongo Community Forest, ca. 8 km S.E. of Bamuso, 4°25'N 8°57' E, ca. 200 m, 18th October, 2002, Sainge M. 1068 (YA), Franke Th.. 02/027 (B).

Flowering and fruiting: Anthesis from May to December, with peak in September. Fruiting from September to December, with peak in late October.

Distribution and ecology: Known from four localities. Three localities in South west Cameroon and one locality in southern Nigeria. All of these sites are situated within the Guineo-Congolean regional center of endemism at 150 – 300 m above sea level (Beentje et al 1994, Gartlan 1994). Rainfall in this area is high, reaching 2500 – 5500 mm per year.The type locality is situated in a primary forest on poor sandy soil. The vegetation is composed of the following woody plant species: *Piptostigma* sp., *Polyceratocarpus parviflorus*, *Uvariodendron giganteum*, *Uvariopsis korupensis*, *Tabernemontana brachyantha*, *Garcinia conrauana*, *Diospyros preussii*, *Diospyros pseudomespilus*, *Dichostemma glaucescens*, *Protomegabaria stapfiana*, *Hymenostegia afzelii*, *Phyllobotryon spathulatum*, *Carapa* sp., *Strombosia pustulata*, *Craterispermum aristatum*, *Oubanguia alata*, *Rhaptopetalum* sp., *Cola cauliflora*, *Cola chlamydantha*, *Cola rostrata*, *Cola semecarpophylla*, *Rinorea lepidobotrys*, *Rinorea oblongifolia*.

Afrothismia hydra is growing in groups of scattered individuals within leaf litter, often in raised microhabitats, e.g. on horizontally running tree roots. In some cases, the rhizomes and most roots are exposed to the air with only the distal parts of the filiform roots entering the uppermost soil strata.

Etymology: The specific epithet was chosen since

the tentacle-like free tepal segments make the flower to look like a polyp of the genus *Hydra* (Cnidaria).

Note: *Afrothismia hydra* seems to be closely related to *Afrothismia winkleri* (Engl.) Schltr., from which it mainly differs by the size, the form and inner structure of the perigone tube, as well as the position of the ovary (Tab. 1). It is similar to *Afrothismia winkleri* (Engl.) var. *budongensis* Cowley from Uganda, which is generally bigger in size and has much wider and longer free tepal segments. Due to the very bad condition of the type, consisting of a single pickled plant with an incomplete, violently lacerated flower, further comparison was impossible.

Acknowledgements – This study (FK 01LC0007) was financially supported by the BIOLOG (Biodiversity and Global Change) programme of the German Ministry of Education and Research (BMBF) and carried out under the supervision of Prof. Dr Reinhard Agerer / LMU München, to whom we want to express our special thanks. Moreover we want to thank Ludwig Beenken and Prof. Dr Dietrich Podlech / LMU München for constructive discussions and the latin diagnosis.

References

Beentje, H. J. et al. 1994. Regional Overview: Africa. – In: Davies, S. D., Heywood, V. H. & Hamilton, A. C. (eds), Centres of Plant Diversity,Vol. 1. The World Wide Fund For Nature (WWF) & IUCN. The World Conservation Union, pp. 101-148.

Cowley, E. J. 1988. Burmanniaceae. – In: Polhill, R. M. (ed.), Flora of Tropical East Africa. A. A. Balkema / Rotterdam / Brookfield, pp. 1-9.

Engler, A. 1905. *Thismia winkleri* Engl., eine neue afrikanische Burmanniacae. – Bot. Jahrb. Syst. 38: 89-91.

Gartlan, S. 1994. Korup National Park Cameroon. – In: Davies, S. D., Heywood, V. H. & Hamilton, A. C. (eds), Centres of Plant Diversity, Vol. 1. The World Wide Fund For Nature (WWF) & IUCN. The World Conservation Union, pp. 158-162.

Hepper, F. N. 1968. Burmanniaceae. – In: Hepper, F. N. (ed.), Flora of West Tropical Africa. 2nd edn., Vol. 3., London, UK: The Crown Agents for Overseas Governments and Administration, pp. 176-180.

Leake J. J. 1994. The biology of myco-heterotrophic ("saprophytic") plants. – Tansley Review No. 69. New Phytol. 127: 171-216.

Raynal-Roques, A. & Paré, J. 1998. Biodiversité des Phanérogames parasites: leur place dans la classificacion systématique. – Adansonia, sér. 3, 20: 313-322.

Schlechter, R. 1906. Burmanniaceae africanae. – Bot. Jahrb. Syst. 38: 137-143.

VI

MOSES N. SAINGE, THASSILO FRANKE & REINHARD AGERER

A new species of *Afrothismia (Burmanniaceae,* tribe *Thismieae)* from Korup National Park, Cameroon

Abstract

Sainge, M. N., Franke, T. & Agerer, R.: A new species of *Afrothismia (Burmanniaceae,* tribe *Thismieae)* from Korup National Park, Cameroon. – Willdenowia 35: 287-291. – ISSN 0511-9618; © 2005 BGBM Berlin-Dahlem.

Afrothismia korupensis, a new species of this genus of achlorophyllous, myco-heterotrophic herbs is described from evergreen Guineo-Congolean rain forest in the Korup National Park, Cameroon, and illustrated. It differs from all other species of the genus in the internal structure of its perianth tube with six radially arranged cuneate partitions at the base, the absence of an internal flange and the laterally winged staminal filaments, each with a conical projection.

Introduction

All species of the small African genus *Afrothismia* Schltr. are achlorophyllous, myco-heterotrophic herbs, receiving essential nutrients from root colonizing fungi (Leake 1994, Cheek & Williams 1999, Imhof 1999). *Afrothismia* and the related genera *Thismia* Griff., *Haplothismia* Airy Shaw and *Oxygyne* Schltr. form a natural unit, which holds a still unclear phylogenetic position within the *Dioscoreales* (Caddick & al. 2000a-b, Caddick & al. 2002a-b, Angiosperm Phylogeny Group 1998, 2003). Since Agardh (1853), the leading view oscillates between treating them as a separate family *(Thismiaceae)* or as a tribe within the *Burmanniaceae (Thismieae).* According to the Angiosperm Phylogeny Group (2003), they are currently considered to be part of the *Burmanniaceae*.

Afrothismia is known to occur in at least six countries of tropical Africa, with the highest diversity in southwestern Cameroon.

While performing phenological research at the Korup Forest Dynamic Plot (KFDP), a new species of *Afrothismia* was discovered. KFDP is a 50 hectare transect of evergreen Guineo-Congolean rain forest, which was established by the Center for Tropical Forest Science (CTFS), in order to monitor the composition and demography of woody plants in a tropical forest (Thomas & al. 2003). The plot is located in the southern Korup National Park in Cameroon's South West Province, which shelters one of Africa's highest plant diversities and is also the type locality of the recently described *Afrothismia hydra* Sainge & T. Franke (Sainge & Franke 2005).

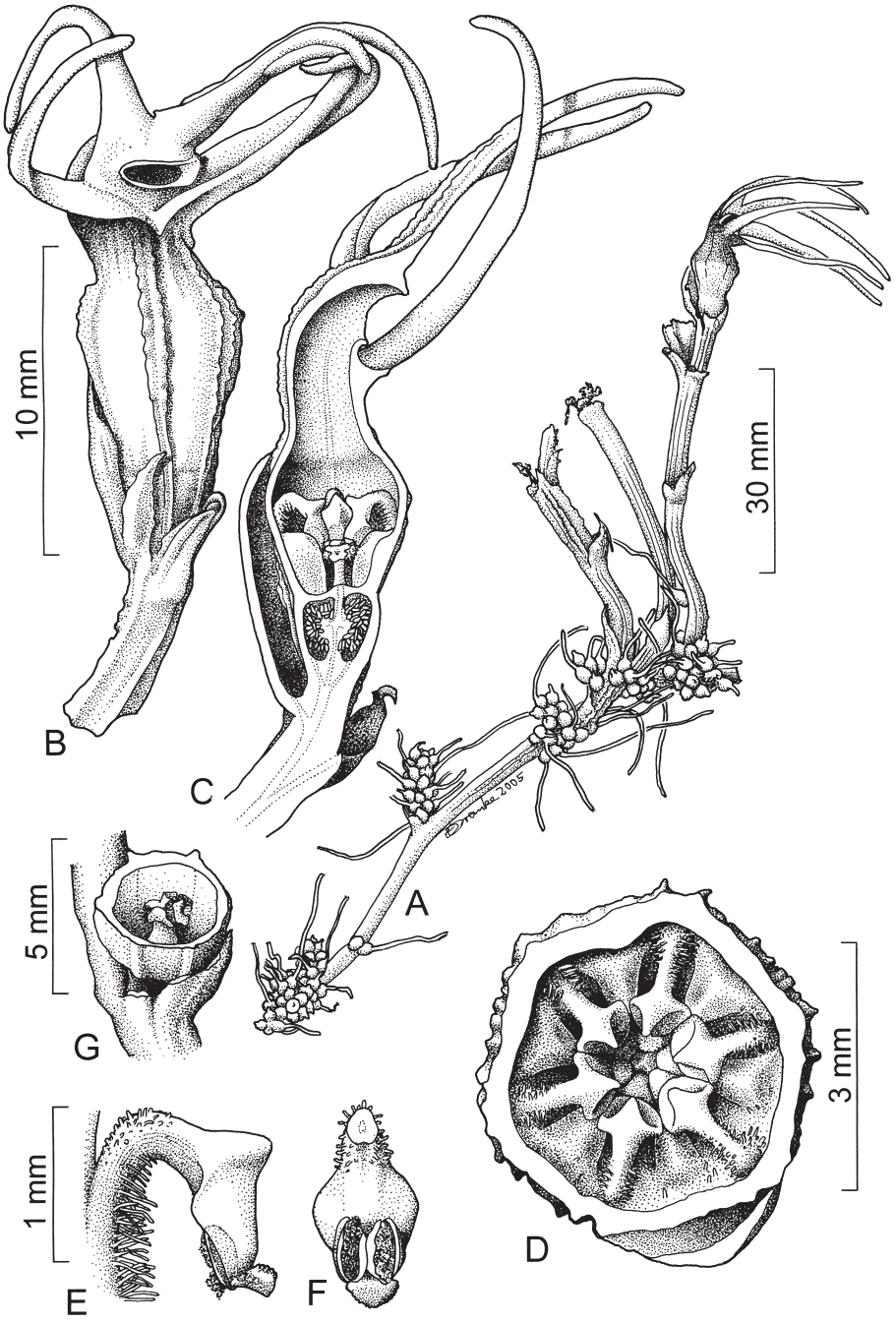


Fig. 1. *Afrothismia korupensis* – A: habit; B: flower; C: longitudinally sectioned flower, showing internal structures of perianth tube; D: transversely sectioned flower, showing arrangement of stamens; E: lateral view of stamen; F: ventral view of distal part of filament, anther and connective appendage; G: open fruit with stalked placentas (seeds lacking). – Drawn by T. Franke.

Until 2003 only three species and one variety of *Afrothismia* were published (Engler 1905, Schlechter 1906, Cowley 1988). In the following three years five new species were described and with the present contribution the number of species is tripled (Maas-van de Kamer 2003, Cheek 2003, Franke 2004, Franke & al. 2004, Sainge & Franke 2005).

Afrothismia korupensis Sainge & T. Franke, **sp. nov.**
Holotypus: Cameroon, South West Province, Ndian Division, Southern Korup National Park, close to Chimpanzee Camp, Korup Forest Dynamic Plot (KFDP), 5°04'04"N, 8°51'21"E, c. 230 m, 9.7. 2002, *Sainge M. 991* (YA; isotypus: B) – Fig. 1.

Differt ab speciebus omnibus generis in structura interiore tubi perianthii cum 6 volvis longitudinalibus centrum tubi veines prominentibus, tubo sine limbo interio filamentis apiceus veines distincte dilatata, dorso processo conico provisis.

Dull white myco-heterotrophic herb. *Shoot* with clusters of proximally inflated roots and few scale-like leaves, epiterranean part distinctly ribbed and often branching, 25-120 mm long, 1-2 mm in diam.; *roots* with their proximally inflated part beige-coloured, tubercle-like, ellipsoid to ovoid, 1.2-1.7 mm long and 1-1.2 mm in diam.; distal elongated part whitish, up to 16 mm long and c. 0.3 mm in diam. *Scales* deltoid to triangular-lanceolate, 1.7-2.9(-4.4) × 2-3.3 mm (width measured at base). *Inflorescence* 1-2(-3)-flowered, flowers opening subsequently. *Floral bracts* deltoid, with two deep fissures at each side, therefore seemingly 3-lobed, 5.8-10 × 3.3-5.8 mm (width measured at base); floral bract and opposite scale forming an involucre-like structure. *Flowers* zygomorphic. *Perianth* basally fused, forming an upright, obpyriform tube with six free tepals; lower half of perianth tube and tepals white, upper half of perianth tube and area around mouth deep crimson. *Perianth tube* 10-12 mm long and 4.5-6.3 mm in diam. (at widest point), with six protruding veins, each crested with one or two knobbly ribs running from the base of the ovary to the proximal third of the tepals; lower part of tube obovoid, ± rugose to plicate, inner wall with six radially arranged, cuneate, fleshy processes, dividing the base into six cavities, each covered by a stamen; upper part of tube distinctly hood-shaped, inner wall smooth, without an internal flange; mouth circular to elliptic, oblique, with a white, protruding rim, 2.5-3.3 mm in diam. *Tepals* patent to slightly incurved, similar in shape and length, dorsiventrally flattened, narrow-lanceolate, 16-28 mm × 1.4-2 mm (width measured at the base); margins of adjacent tepals fused to each other c. 0.3 mm above point of insertion. *Stamens* six, inserted at the base of the perianth tube; proximal part of filaments c. 2.5 mm long, adnate to the perianth tube, ventrally densely pubescent; distal part of filaments free, c.1.2 mm long and 0.4 mm in diam., reflexed, ventrally decreasingly pubescent to glabrous, dorsally papillose, distally provided with lateral, broadly deltoid wings and a dorsal fleshy, conical projection; anthers glabrous, c. 0.5 × 0.4 mm, distal connective appendage deltoid, dorsally papillose, firmly adnate to the stigmatic surface; thecae dehiscing longitudinally, half-way sunken into the fleshy connective, facing the perianth wall. *Ovary* unilocular, obovoid, 3.3-4.2 mm high and 3-3.3 mm in diam.; placentas 3, basally connate into a sterile central column; *style* glabrous, c. 0.7 mm long; stigma subspherical, densely papillose, c. 0.9 mm in diam., consisting of three stigmatic lobes fused to each other. *Fruit* a pyxidium, subspherical, 3.8-4.2 mm high and 3.3-3.8 mm in diam., sterile placental column not distended.

Etymology. – *Afrothismia korupensis* is named after the Korup National Park, where it was discovered.

Distribution and ecology. – *Afrothismia korupensis* is only known from the Korup National Park, where a small population of 12 individuals was discovered in 2002, growing in stony, very nutrient-poor soil on the floor of the evergreen Guineo-Congolean rain forest (for a comprehensive description of the vegetation see Thomas & al. 2003). The type locality is situated on a hill slope in the northeastern part of the Korup Forest Dynamic Plot (KFDP) at an altitude of c. 230 m. Rainfall in the area is high, exceeding an annual average of 5000 mm (Thomas & al. 2003). A

plant inventory of the type locality yielded the following woody plant species: *Amphimas ferrugineus* Pierre ex Pellegr., *Angylocalyx oligophyllus* (Bak.) Bak.f., *Araliopsis soyauxii* Engl., *Cola praeacuta* Brenan & Keay, *Deinbollia unijuga* D. W. Thomas, *Desbordesia glaucescens* (Engl.) Tiegh., *Diospyros preussii* Gürke, *Diospyros zenkeri* (Gürke) F. White, *Diogoa zenkeri* (Engl.) Exell & Mendonça, *Drypetes staudtii* (Pax) Hutch., *Hymenostegia afzelii* (Oliv.) Harms, *Klaineanthus gaboniae* Pierre ex Prain, *Phyllanthus polyanthus* Pax, *Pierreodendron africanum* (Hook.f.) Little, *Strombosia pustulata* Oliv., *Symphonia globulifera* Linn.f., *Tapura africana* Oliv., *Trichoscypha patens* Engl., *Uapaca staudtii* Pax, *Xylopia aethiopica* (Dunal) A. Rich., *Zanthoxylum gilletii* (D. Wild.) P. G. Waterman.

Three specimens of *A. korupensis* were removed from the population in July 2002, almost at the peak of the rainy season. In June 2003 20 individuals were counted at the same spot. Other myco-heterotrophic herbs growing close by, were *Sciaphila ledermannii* Engl., *Burmannia hexaptera* Schltr. and *Gymnosiphon* cf. *longistylus* (Benth.) Hutch. & Dalziel.

Acknowledgements

This study was financially supported by the BIOLOG (Biodiversity and Global Change) programme (Grant 01LC0007) of the German Ministry of Education and Research (BMBF). The discovery of this remarkable new species would have been impossible without the support of the Center for Tropical Forest Science (CTFS) of the Smithsonian Tropical Research Institute and D. W. Thomas, G. B. Chuyong and D. Kenfack in particular. The authors are grateful for D. Podlech (Ludwig-Maximilians-Universität München) for preparing the Latin diagnosis and M. Cheek for reviewing a draft of the paper.

References

Agardh, J. G. 1853: Theoria systematis plantarum. – Lund, etc.

Angiosperm Phylogeny Group (APG) 1998: An ordinal classification for the families of flowering plants. – Ann. Missouri Bot. Gard. **85:** 531-553.

— 2003: An update of the angiosperm phylogeny group classification for the orders and families of flowering plants: APG II. – Bot. J. Linn. Soc. **141:** 399-436.

Caddick, L. E. & Rudall, P. J. 2000a: Floral morphology and development in *Dioscoreales*. – Feddes Repert. **111:** 189-230.

— , —, Wilkin, P. & Chase, M. W. 2000b: Yams and their allies: systematics of *Dioscoreales*. – Pp. 475-487 in: Wilson, K. L. & Morrison, D. L. (ed.), Monocotyledons: systematics and evolution. – Melbourne.

— , Rudall, P. J., Wilkin, P., Hedderson, T. A. J. & Chase, M. W. 2002a: Phylogenetics of *Dioscoreales* based on combined analyses of morphological and molecular data. – Bot. J. Linn. Soc. **138:** 123-144.

— , Wilkin, P., Rudall, P. J., Hedderson, T. A. J. & Chase, M. W. 2002b: Yams reclassified: A recircumscription of *Dioscoreaceae* and *Dioscoreales*. – Taxon **51:** 103-114.

Cheek, M. 2003: A new species of *Afrothismia (Burmanniaceae)* from Kenya. – Kew Bull. **58:** 951-955.

— & Williams, S. 1999: A review of African saprophytic flowering plants. – Pp 39-49 in: Timberlake, J. & Kativu, S. (ed.), African plants: biodiversity, taxonomy & uses. Proceedings of the 1997 AETFAT congress, Harare, Zimbabwe. – Kew.

Cowley, E. J. 1988: *Burmanniaceae*. – Pp 1-9 in: Polhill, R. M. (ed.), Flora of Tropical East Africa. – Rotterdam & Brookfield.

Engler, A. 1905: *Thismia winkleri* Engl., eine neue afrikanische *Burmanniaceae*. – Bot. Jahrb. Syst. **38:** 89-91.

Franke, T. 2004: *Afrothismia saingei (Burmanniaceae),* a new myco-heterotrophic plant from Cameroon. – Syst. Geogr. Pl. **74:** 27-33.

— , Sainge, M. N. & Agerer, R. 2004: A new species of *Afrothismia (Burmanniaceae;* tribe *Thismieae)* from the western foothills of Mt. Cameroon. – Blumea **49:** 451-456.

Imhof, S. 1999: Anatomy and mycotrophy of the achlorophyllous *Afrothismia winkleri*. – New Phytol. **144:** 533-540.

Leake, J. J. 1994: The biology of myco-heterotrophic ("saprophytic") plants. – New Phytol. **127:** 171-216.

Maas-van de Kamer, H. 2003: *Afrothismia gesnerioides,* another new species of *Afrothismia (Burmanniaceae)* from tropical Africa. – Blumea **48:** 475-478.

Sainge, M. N. & Franke, T. 2005: A new species of *Afrothismia (Burmanniaceae)* from Cameroon. – Nordic J. Bot. **23:** 299-303.

Schlechter, R. 1906: *Burmanniaceae* africanae. – Bot. Jahrb. Syst. **38:** 137-143.

Thomas, D. W., Kenfack, D., Chuyong, G. B., Sainge, M. N., Losos, E. C., Condit, R. S. & Songwe, N. 2003: Tree species of southwestern Cameroon: Tree distribution maps, diameter tables, and species documentation of the 50-hectare Korup Forest Dynamic Plot. – Washington, D.C.

Addresses of the authors:

Moses N. Sainge, Korup Forest Dynamic Program, Korup National Park, P.O. Box 36, Mundemba, South West Province, Republic of Cameroon.

Thassilo Franke & Reinhard Agerer, Department Biology I and GeoBio-Center, Ludwig-Maximilians-University, Biodiversity Research: Systematic Mycology, Menzinger Str. 67, D-80638 München, Germany; e-mail: thassilo.franke@web.de

VII

Mycol Progress (2006) 5: 24–31
DOI 10.1007/s11557-006-0500-2

ORIGINAL ARTICLE

Thassilo Franke · Ludwig Beenken · Matthias Döring · Alexander Kocyan · Reinhard Agerer

Arbuscular mycorrhizal fungi of the *Glomus*-group A lineage (Glomerales; Glomeromycota) detected in myco-heterotrophic plants from tropical Africa

Received: 2 January 2006 / Revised: 26 January 2006 / Accepted: 6 February 2006 / Published online: 6 April 2006

Abstract We amplified and sequenced partial 18S rDNA of fungi in the roots of 11 African myco-heterotrophic plants out of four angiosperm families (Burmanniaceae, Thismiaceae, Triuridaceae, and Gentianaceae). The sequences were cladistically analyzed with published sequences of arbuscular mycorrhizal fungi. We show that all investigated African myco-heterotrophic plants are associated with arbuscular mycorrhizal fungi within a clade of *Glomus* (*Glomus*-group A). We reveal a fine-level mycorrhizal specificity for a particular set of arbuscular mycorrhizal fungi within *Glomus*-group A by *Afrothismia hydra* (Thismiaceae). Furthermore, we show that the roots of two myco-heterotrophic plant individuals, besides being colonized by representatives of *Glomus*-group A, also contain DNA of *Acaulospora* sp. Consequently, *Acaulospora* is interpreted as a facultative mycorrhizal associate.

Keywords Africa · Myco-heterotrophic plants · Arbuscular mycorrhiza · Glomeromycota · Partial 18S rDNA

Introduction

In contrast to green plants that fix atmospheric carbon dioxide photosynthetically, a variety of angiosperms, pteridophytes, and liverworts get access to carbon partly, or even exclusively, by the digestion of fungal hyphae (see Leake 1994 and references therein). As a result of this ecological strategy, photosynthetic organs and organelles like, e.g., leaves, stomata, and chloroplasts are highly reduced or lacking completely. Leake (1994) described this type of metabolism as "myco-heterotrophy," while others used the term "mycoparasitism" (Cronquist 1981; Raynal-Roques and Paré 1998).

Regarding the source of carbon, myco-heterotrophic plants can be divided into two groups. The species of the first group benefit from their associated saprotrophic fungi's ability to extract carbon from dead organic material. This form of myco-heterotrophy applies to several achlorophyllous orchids (Burgeff 1932, Terashita and Chuman 1987). The second group is associated with ecto- or arbuscular mycorrhizal fungi (AMF) (Bidartondo et al. 2002; Björkman 1960; Knöbel and Weber 1988; Kretzer et al. 2000). Here, the plants hook themselves into mycorrhizal networks and benefit from carbon that is fed in via the photosynthetic activity of interlinked green plants (McKendrick et al. 2000). Several authors use the term "epiparasitism" to describe this kind of myco-heterotrophy (Bidartondo et al. 2002; Björkman 1960; Leake 2004).

Since Knöbel and Weber's (1988) anatomical investigation of the achlorophyllous *Voyria truncata* (Standley) Standley & Steyermark (Gentianaceae), it has been known that AMF (Glomeromycota) are exploited by myco-heterotrophic plants. In the years following Knöbel and Weber's investigation, AMF were anatomically detected in four additional *Voyria* species and numerous other tropical myco-heterotrophic plants within the Burmanniaceae, Corsiaceae, Thismiaceae and Triuridaceae families (Domínguez and Sérsic 2004; Franke 2002; Imhof 1997, 1999a–c, 2001, 2003; Imhof and Weber 1997, 2000; Imhof et al. 1994).

In recent years, molecular methods have proved to be a convenient way to identify AMFs associated with myco-heterotrophic plants, even above the genus level. For the first time, Yamato (2001) isolated and sequenced the DNA of an AMF of the genus *Glomus* from the roots of *Sciaphila tosaensis* Makino (Triuridaceae). Bidartondo et al. (2002) detected AMFs in the roots of *Arachnitis uniflora* Phil. (Corsiaceae), *Voyriella parviflora* (Miq.) Miq. (Gentianaceae),

T. Franke (✉) · L. Beenken · A. Kocyan · R. Agerer
Department Biology and GeoBio-Center LMU,
Biodiversity Research,
Ludwig-Maximilians-Universität München,
Menzinger Str. 67,
D-80638 München, Germany
e-mail: thafra@web.de
Tel.: +49-89-17861196
Fax: +49-89-17861195

M. Döring
Fachbereich Biologie, Spezielle Botanik und Mykologie,
Philipps-Universität Marburg,
35032 Marburg, Germany

Table 1 Myco-heterotrophic plant taxa investigated in this study

Plant taxon/specimen number	Family	Collection site	Herbarium voucher number	Mycobiont clone	GenBank accession number
Afrothismia foertheriana	Thismiaceae	Diongo Community Forest	Franke, Th. & Sainge, M. 02/030 (WAG)	α	DQ371658
				β	DQ371659
Afrothismia aff. *gesnerioides*	Thismiaceae	Mt. Kupe (Kupe village)	Sainge M 1002 (B)	α	DQ371669
				β	DQ371670
				γ	DQ371671
				δ	DQ371667
				ε	DQ371668
Afrothismia hydra/1	Thismiaceae	Korup National Park (Chimpanzee trail)	Franke Th. 02/013 (B)	α	DQ371660
				β	DQ371661
Afrothismia hydra/2	Thismiaceae	Diongo Community Forest	Franke Th. 02/027 (B)		DQ371662
Afrothismia hydra/3	Thismiaceae	Korup National Park	Sainge M. 910 (B)	α	DQ371663
				β	DQ371664
Afrothismia korupensis	Thismiaceae	Korup National Park	Sainge M. 991 (B)	α	DQ371665
				β	DQ371666
Afrothismia saingei	Thismiaceae	Mt. Kupe (Mbulle)	Sainge M. 1053 (BR)		DQ371677
Afrothismia winkleri	Thismiaceae	Mt. Kupe (Mbulle)	Franke Th. 02/034 (B)		DQ371678
Afrothismia "Kupe" sp. nov.	Thismiaceae	Mt. Kupe (Kupe village)	Sainge M. 1003 (WAG)	α	DQ371672
				β	DQ371674
				γ	DQ371673
				δ	DQ371675
				ε	DQ371676
Burmannia congesta/1	Burmanniaceae	Bimbia-Bonadikombo Community Forest	Franke Th. 02/061 (M)	α	DQ371679
				β	DQ371680
				γ	DQ371681
Burmannia congesta/2	Burmanniaceae	Bimbia-Bonadikombo Community Forest	Franke Th. 02/062 (M)	α	DQ371684
				β	DQ371682
				γ	DQ371683
Burmannia hexaptera/1	Burmanniaceae	Mt. Kupe	Franke Th. 01/016 (M)		DQ371685
Burmannia hexaptera/2	Burmanniaceae	Diongo Community Forest	Franke Th. 02/025 (M)	α	DQ371687
				β	DQ371688
				γ	DQ371686
Sciaphila ledermannii	Triuridaceae	Mt. Kupe	Franke Th. 01/014 (M)	α	DQ371691
				β	DQ371692
Kupea martinetugei	Triuridaceae	Diongo Community Forest	Franke Th. 02/023 (M)	α	DQ371689
				β	DQ371690
Sebaea oligantha	Gentianaceae	Bakossi Mountains	Franke Th. 01/011 (M)	α	DQ371693
				β	DQ371694
				γ	DQ371696
				δ	DQ371695
				ε	DQ371697

and five *Voyria* species by analyzing fungal ITS, as well as 18S rDNA sequences. Moreover, they discovered that all species are highly specialized to a few narrow lineages within a clade of *Glomus* (*Glomus*-group A as defined by Schwarzott et al. 2001).

Whereas most knowledge about arbuscular mycorrhiza (AM)-adapted myco-heterotrophic plants was gathered in the neotropics, comparatively little is known about myco-heterotrophic plants in tropical Africa. The only extensive examination of an African myco-heterotrophic plant was performed by Imhof (1999a), who investigated the anatomy and mycorrhiza of a species of the genus *Afrothismia* (Thismiaceae) from Gabon.

The main goal of this study was to determine the fungal endophytes (mycobionts) of several African myco-heterotrophic plants. For this reason, we used the universal eukaryotic primer NS31 in combination with the fungus-specific primer AM1 to amplify a 550-bp fragment of the mycobionts' 18S rRNA gene (Helgason et al. 1998; Simon et al. 1992). This molecular technique has been frequently employed to determine the mycorrhizal associates of green plants and the AMF diversity from different ecosystems (Daniell et al. 2001; Helgason et al. 1998, 1999, 2002; Husband et al. 2002a,b; Kowalchuk et al. 2002; Lee et al. 2001–2003; Vandenkoornhuyse et al. 2002). In the present study we were able to identify the mycobionts of 11 myco-heterotrophic plants out of four families (Burmanniaceae, Thismiaceae, Triuridaceae, and Gentianaceae) from the evergreen rain forest of southwestern Cameroon.

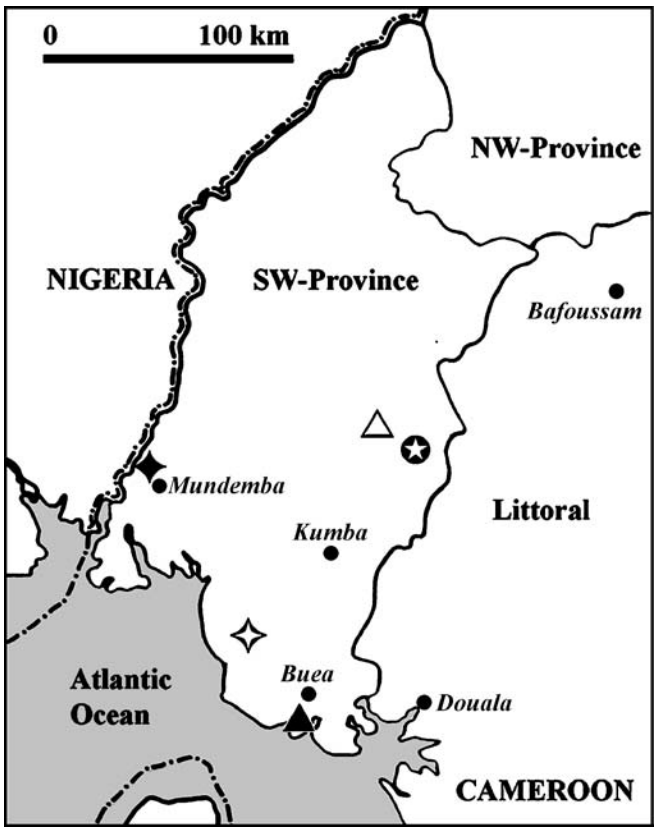


Fig. 1 Map of Cameroon's Southwest Province showing the five sampled localities: ✦=Korup National Park, ✧=Diongo Community Forest, ▲=Bimbia-Bonadikombo Community Forest, △=Bakossi Mountains, ✪=Mount Kupe

Materials and methods

Sample collection

The samples were obtained from five different collection sites in Cameroon's Southwest Province during the rainy seasons (August–October) of 2001 and 2002 (Table 1, Fig. 1). All sites represent evergreen Guineo-Congolean rainforest, located within the western Cameroon mountain glacial refuge subarea (Beentje et al. 1994; Morton 1972; Mutke et al. 2001; Sosef 1994; White 1979).

Molecular analysis

Altogether, 16 single plant individuals were analyzed (Table 1). The root samples were fixed and stored in 2% CTAB lysis buffer. DNA was extracted from 100 mg (wet weight) root tissue of each sample using a Qiagen DNeasy Plant Mini Kit (Qiagen, Basel, Switzerland), according to the manufacturer's instructions, and eluted in 100 µl of the supplied elution buffer. Partial small subunit (SSU) DNA fragments (~550 bp) were amplified through PCR using Taq DNA polymerase (Fermentas, Vilnius, Lithuania), a universal eukaryotic primer NS31 (Simon et al. 1992), and a general fungal primer AM1 (Helgason et al. 1998) designed to exclude plant DNA sequences.

Reactions were performed in a final volume of 50 µl containing 2 µl of the extracted DNA solution, 500 µM of each dNTP, 10 pmol of each primer, 25 mM MgCl2, 0.005% milk powder, 5 units of Taq DNA polymerase, and the supplied 10× buffer.

The PCR program was as follows: 94°C for 3 min, 60°C for 1 min, 72°C for 1 min and 30 s (1 cycle), 94°C for 1 min, 60°C for 1 min, 72°C for 1 min and 30 s (28 cycles), 94°C 1 min, 60°C 1 min, and 72°C 10 min (1 cycle).

The amplified products were selected by gel electrophoresis (agarose 1%) and purified using a Qiagen MiniElute PCR Purification Kit and directly cloned into pDrive cloning vector using a Qiagen PCR Cloning plus Kit. Qiagen EZ Competent cells were transformed and plated on selective medium following the manufacturer's instructions. Per sample, ten colonies of putative positive transformants were picked and directly transferred to a new PCR (NS31/AM1) with the following program: 94°C for 3 min, 60°C for 1 min, 72°C for 1 min and 30 s (1 cycle), 94°C for 1 min, 60°C for 1 min, 72°C for 1 min and 30 s (25 cycles), 94°C for 1 min, 60°C for 1 min, and 72°C for 5 min (1 cycle). The amplification products were tested for RFLP by digestion with Hinf I (Fermentas) according to the manufacturer's instructions. Different RFLP types were selected by gel electrophoresis (agarose 3%) and the corresponding DNA purified using the Qiaquick~PCR Purification Kit. For cycle sequencing, BigDye Terminator

Table 2 Fungal taxa included in the DNA matrix for phylogenetic analysis

Fungal taxon	GenBank accession number
Acaulospora laevis	Y17633
Acaulospora rugosa	Z14005
Acaulospora scrobiculata	AJ306442
Acaulospora spinosa	Z14004
Archaeospora leptoticha	AJ301861
Archaeospora trappei	Y17634
Diversispora spurcum	AJ276077
Entrophospora colombiana	Z14006
Entrophospora contigua	Z14011
Geosiphon pyriformis	Y15904
Gigaspora albida	Z140099
Gigaspora decipiens	U96146
Gigaspora gigantea	Z14010
Glomus caledonium	Y17635
Glomus claroideum	AJ276075
Glomus clarum	AJ276084
Glomus coremioides	AJ249715
Glomus coronatum	AJ276086
Glomus "*etunicatum*-like"	Y17644
Glomus fasciculatum	Y17640
Glomus fragilistratum	AJ276085
Glomus geosporum	AJ245637
Glomus hoi	AF485888
Glomus intraradices	AJ536822
Glomus lamellosum	AJ276087
Glomus luteum	AJ276089
Glomus manihotis	Y17648
Glomus microaggregatum	U96144
Glomus mosseae	U96141
Glomus proliferum	AF213462
Glomus sinuosum	AJ133706
Glomus versiforme	AJ276088
Glomus verruculosum	AJ301858
Glomus vesiculiferum	L20824
Glomus viscosum	Y17652
Paraglomus brasilianum	AJ301862
Paraglomus occultum	AJ276081
Scutellospora cerradensis	AB041344
Scutellospora projecturata	AJ242729
Outgroup	
Mortierella polycephala	X89436
Endogone pisiformis	X58724

Ready Reaction Kit v3.1 (Applied Biosystems, Foster City, CA, USA) was used. Purified sequencing reactions with the primers NS31 and AM1 were run on an ABI Prism 3100 automated sequencer (Applied Biosystems).

Data analysis

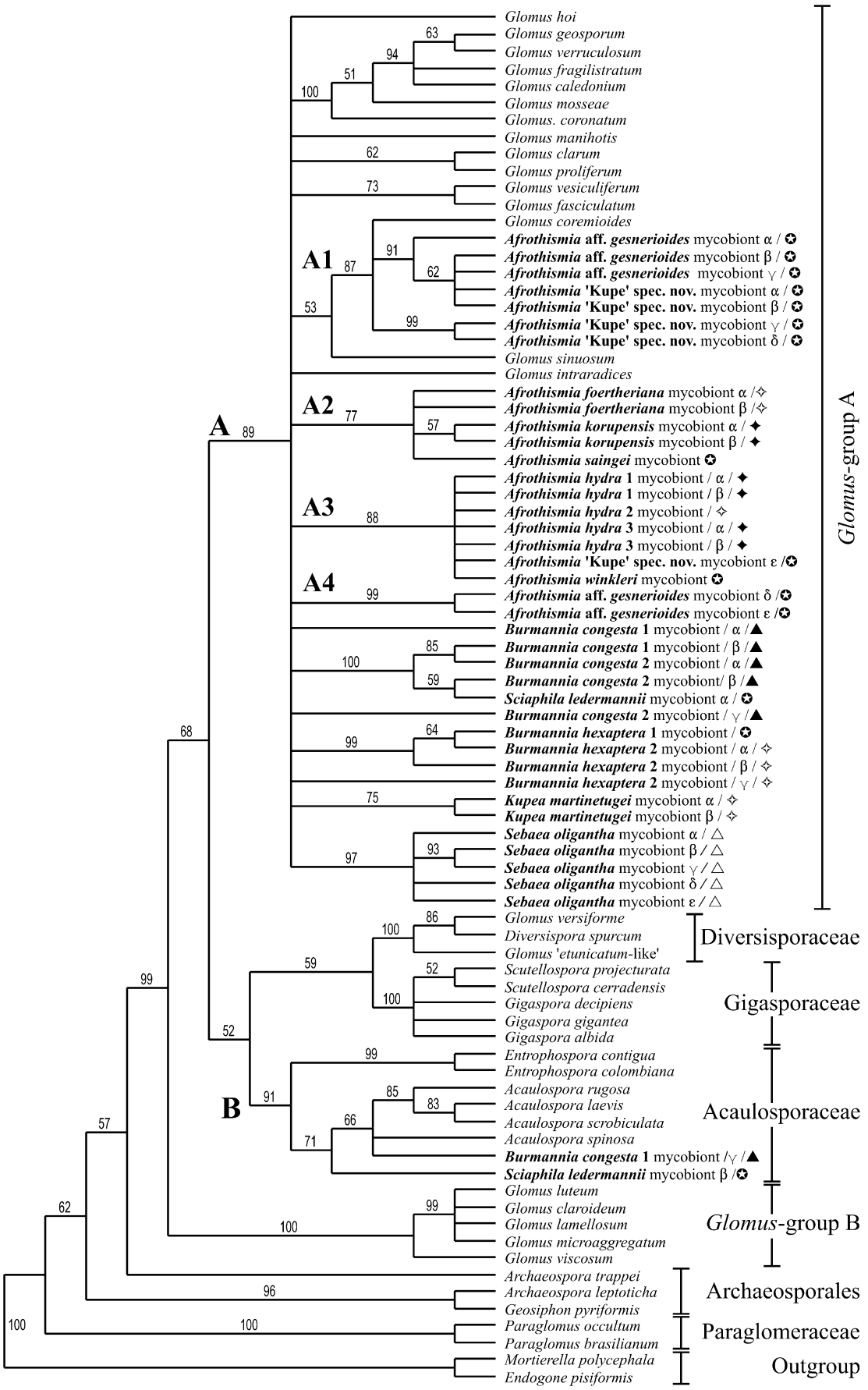
The resulting forward and reverse nucleotide sequences were edited with Sequencher 4.06 (Gene Codes, Ann Arbor, MI, USA) and submitted to a Blast search in GenBank as a first step to check if and to which extent they match with known SSU sequences of AMF. All sequences were manually aligned using MacClade 4.0 PPC (Sinauer, Sunderland, MA, USA). The fungal endophytes' approximate phylogenetic position was then determined by comparison with phylogenetic treatments, which represent the basis of the new AMF taxonomy (Schüβler et al. 2001b; Schwarzott et al. 2001). For this reason, corresponding sequences of several representatives of each phylogenetic unit, as defined by Schwarzott et al. (2001), were obtained from GenBank and included in the alignment (Table 2).

Phylogenetic analyses were performed with PAUP version 4.0b10 (Swofford 2002) with the heuristic search option activated, ten replicates of random-taxon entry, and tree bisection reconnection (TBR) swapping with MulTrees and steepest descent. Gaps were treated as missing values. MaxTrees was set to 5,000. Bootstrap analysis was performed with 1,000 replicates under the heuristic search (Felsenstein 1985). All molecular characters were assessed as independent, unordered, and equally weighted using Fitch parsimony (Fitch 1971). Uninformative characters were excluded from the analysis. For this analysis, *Mortierella* Coem. and *Endogone* Link were used as outgroup taxa.

Results

Clones were preselected by RFLP analysis, to separate multiple fungal associations and to avoid repeatedly sequencing the same mycobiont strains. For this reason, one representative clone of each RFLP pattern found in each myco-heterotrophic plant sample was sequenced. The number of different RFLP patterns, and sequenced clones, varied between one and five per plant sample (Table 1). Altogether, a total of 40 selected clones were sequenced. Blast search revealed that all obtained mycobiont sequences correspond to SSU sequences of members of the Glomeromycota. Hence, no DNA of unrelated fungal taxa was coamplified under the applied PCR conditions.

The cladistic analysis resulted in 5,000 most parsimonious trees (tree length 766, CI 0.435, RI 9.796). The mycobionts are distributed in two clades (Fig. 2, clades A


Glomus hoi
Glomus geosporum
Glomus verruculosum
Glomus fragilistratum
Glomus caledonium
Glomus mosseae
Glomus. coronatum
Glomus manihotis
Glomus clarum
Glomus proliferum
Glomus vesiculiferum
Glomus fasciculatum
Glomus coremioides
Afrothismia aff. gesnerioides mycobiont α / ✪
Afrothismia aff. gesnerioides mycobiont β / ✪
Afrothismia aff. gesnerioides mycobiont γ / ✪
Afrothismia 'Kupe' spec. nov. mycobiont α / ✪
Afrothismia 'Kupe' spec. nov. mycobiont β / ✪
Afrothismia 'Kupe' spec. nov. mycobiont γ / ✪
Afrothismia 'Kupe' spec. nov. mycobiont δ / ✪
Glomus sinuosum
Glomus intraradices
Afrothismia foertheriana mycobiont α /✧
Afrothismia foertheriana mycobiont β /✧
Afrothismia korupensis mycobiont α / ✦
Afrothismia korupensis mycobiont β / ✦
Afrothismia saingei mycobiont ✪
Afrothismia hydra 1 mycobiont / α / ✦
Afrothismia hydra 1 mycobiont / β / ✦
Afrothismia hydra 2 mycobiont / ✧
Afrothismia hydra 3 mycobiont / α / ✦
Afrothismia hydra 3 mycobiont / β / ✦
Afrothismia 'Kupe' spec. nov. mycobiont ε /✪
Afrothismia winkleri mycobiont ✪
Afrothismia aff. gesnerioides mycobiont δ /✪
Afrothismia aff. gesnerioides mycobiont ε /✪
Burmannia congesta 1 mycobiont / α /▲
Burmannia congesta 1 mycobiont / β /▲
Burmannia congesta 2 mycobiont / α /▲
Burmannia congesta 2 mycobiont/ β /▲
Sciaphila ledermannii mycobiont α / ✪
Burmannia congesta 2 mycobiont / γ /▲
Burmannia hexaptera 1 mycobiont / ✪
Burmannia hexaptera 2 mycobiont / α / ✧
Burmannia hexaptera 2 mycobiont / β / ✧
Burmannia hexaptera 2 mycobiont / γ / ✧
Kupea martinetugei mycobiont α / ✧
Kupea martinetugei mycobiont β / ✧
Sebaea oligantha mycobiont α / △
Sebaea oligantha mycobiont β / △
Sebaea oligantha mycobiont γ / △
Sebaea oligantha mycobiont δ / △
Sebaea oligantha mycobiont ε / △
Glomus versiforme
Diversispora spurcum
Glomus 'etunicatum-like'
Scutellospora projecturata
Scutellospora cerradensis
Gigaspora decipiens
Gigaspora gigantea
Gigaspora albida
Entrophospora contigua
Entrophospora colombiana
Acaulospora rugosa
Acaulospora laevis
Acaulospora scrobiculata
Acaulospora spinosa
Burmannia congesta 1 mycobiont /γ /▲
Sciaphila ledermannii mycobiont β /✪
Glomus luteum
Glomus claroideum
Glomus lamellosum
Glomus microaggregatum
Glomus viscosum
Archaeospora trappei
Archaeospora leptoticha
Geosiphon pyriformis
Paraglomus occultum
Paraglomus brasilianum
Mortierella polycephala
Endogone pisiformis
A
A1
A2
A3
A4
B
Glomus-group A
Diversisporaceae
Gigasporaceae
Acaulosporaceae
Glomus-group B
Archaeosporales
Paraglomeraceae
Outgroup

◀ **Fig. 2** Strict consensus of 5,000 equally most parsimonious trees with a length of 766 steps. Bootstrap support values are shown above *branches*. Samples consisting of more than one specimen are *numbered*; *Greek letters* indicate mycobiont clones isolated from individual plant specimens; *symbols* indicate sampling sites: ✦=Korup National Park, ✧=Diongo Community Forest, ▲=Bimbia-Bonadikombo Community Forest, △=Bakossi Mountains, ✪=Mount Kupe

and B). Clade A (bootstrap support=89) comprises all selected representatives of *Glomus*-group A and 38 mycobiont clones. The 21 *Afrothismia* mycobiont clones are distributed in four subordinate clades (Fig. 2, clades A1, A2, A3, and A4) within clade A. Clade B (bootstrap support=91) comprises all selected representatives of the Acaulosporaceae and two mycobiont clones.

Discussion

Taxonomy of AMF is presently changing dramatically, and as a consequence, several new phylogenetic units have been established (Helgason et al. 2003; Schüβler et al. 2001b; Schwarzott et al. 2001). For this reason we determined the approximate phylogenetic position of the myco-heterotrophic plant mycobionts by comparison with phylogenetic treatments, representing the basis of the new AMF taxonomy (Schüβler et al. 2001b; Schwarzott et al. 2001). With the exception of an apparent polyphyly of *Glomus* s.s. which is also shown in other published N31/AM1 amplified SSU rDNA cladograms (e.g., Douhan et al. 2005; Helgason et al. 2002, 2003), our cladistic analysis is in accordance with most major groupings reported in the neighbor-joining tree by Schwarzott et al. (2001). Because our phylogenetic trees were computed from 550-bp 18S rDNA fragments, instead of sequencing nearly the full-length 18S rRNA gene as performed by Schwarzott et al. (2001), only about one third of the characters were taken into account. For this reason, differences in our trees are considered as artifacts (Fig. 2). Although a high degree of resolution is always desirable in phylogenetic analyses, our intention was not to study detailed relationships of the Glomeromycota but rather to identify mycorrhizal fungi associated with African myco-heterotrophic plants. Therefore, we employed a frequently used and well-established technique to determine AMF diversity in root samples collected in the field (Daniell et al. 2001; Helgason et al. 1998, 1999, 2002; Husband et al. 2002a,b; Kowalchuk et al. 2002; Lee et al. 2001–2003; Vandenkoornhuyse et al. 2002). In this context, it should be mentioned that the presence of Archaeosporaceae and Paraglomeraceae in the roots of the investigated myco-heterotrophic plants remains uncertain because the applied primer AM1 is not well conserved in the SSU region of these taxa (Daniell et al. 2001; Douhan et al. 2005; Helgason et al. 2002; Husband 2004; Schüβler et al. 2001a).

In the present study we provide evidence that all investigated African myco-heterotrophic plants are associated with AMFs of the family-ranking *Glomus*-group A clade (Glomerales). This supports previous molecular investigations by Bidartondo et al. (2002) who found narrow lineages within *Glomus*-group A to be equally favored by several South American myco-heterotrophic plants (Corsiaceae, Gentianaceae) as well. The transatlantic distribution of this mycorrhizal association provides strong evidence that fungi within *Glomus*-group A are preferably exploited by AM-adapted myco-heterotrophic plants, regardless of their geographic origin. Yamato (2001) detected the DNA of a *Glomus* species in the roots of the Japanese *Sciaphila tosaensis*, but did not assign it any further. Regarding that the two African Triuridaceae of our sampling (*Sciaphila ledermannii* Engl., *Kupea martinetugei* Cheek & S. Williams) are both colonized by fungi of the *Glomus*-group A clade, it is very likely that the mycobiont of *S. tosaensis* is related to this lineage as well. Hence, we predict that future examinations will reveal that most if not all AM-adapted myco-heterotrophic plants of tropical Asia are associated with fungi within *Glomus*-group A.

In our phylogenetic analysis we found clues indicating a high degree of mycorrhizal specificity of *Afrothismia hydra* Sainge & T. Franke (Thismiaceae) to a particular lineage within *Glomus*-group A (Fig. 2). In subclade A3 the mycobiont sequences of all three *A. hydra* specimens (Fig. 2, *A. hydra* 1–3) are almost identical, even though the plants were obtained from three different populations at two distant sites (Table 1, Fig. 1). In contrast to *A. hydra*, the fungal sequences of the remaining *Afrothismia* species were each obtained from single individuals (Table 1). Although there is no conclusive evidence for fine-level mycorrhizal specificity of these species, it should be noted that the AMF of subclade A2 seem to be equally favored by three different *Afrothismia* species (*A. foertheriana* T. Franke, Sainge & Agerer; *A. korupensis* Sainge & T. Franke; and *A. saingei* T. Franke), all obtained from different locations (Table 1, Fig. 1). Bidartondo et al. (2002) showed that the mycobionts of eight specimens of the achlorophyllous *Arachnitis uniflora* (Corsiaceae), which were collected at three different sites, were markedly similar as well.

It is remarkable that only two out of 40 mycobiont clones fell within a lineage outside of *Glomus*-group A (Fig. 2, *Burmannia congesta* 1/γ and *S. ledermannii* β within clade B). However, these fungal sequences were isolated from the roots of two individual plants, which in turn also harbored mycobionts of the *Glomus*-group A clade (Fig. 2, *B. congesta* 1/α and 1/β, *S. ledermannii* α). Thus, it is very likely that *B. congesta* (Wright) Jonker (Burmanniaceae) and *S. ledermannii* obligatorily depend on representatives of *Glomus*-group A, although partly colonized by *Acaulospora*. In this case, *Acaulospora* would be a facultative mycobiont. This interpretation is strongly supported by Hart and Reader (2002), who carried out a comparative analysis of the colonization strategy of 21 AMF isolates, representing the three traditional families Glomaceae (including *Glomus*-group A, -group B and Diversisporaceae as defined by Schüβler et al. 2001b), Acaulosporaceae, and Gigasporaceae. They found that the Acaulosporaceae established a much less extensive mycelium in either roots or soil than members of the other two

families. On the other hand, members of *Glomus*-group A (not distinguished as such in the text) were leading the score in terms of spatial root colonization extent and root fungal biomass. Considering the parasitic nature of myco-heterotrophic plants, it seems obvious that a fungus, which extensively colonizes the root tissue and provides great amounts of exploitable biomass, is a much more lucrative mycobiont than a fungus, which is only sparsely distributed within the root tissue. As a consequence, a fungus of the *Glomus*-group A clade seems to be a far better catch for a myco-heterotrophic plant than a representative of the Acaulosporaceae. Morton and Redecker (2001) reported that members of Archaeosporaceae and Paraglomeraceae exhibit an even patchier mycorrhizal distribution than the Acaulosporaceae. For this reason it seems very unlikely that members of these families play a major role in the nutrition of myco-heterotrophic plants.

According to Hart and Reader (2002) Gigasporaceae establish extensive mycelia in the soil, whereas the extent of root colonization is low. Because large external mycelia pose considerable carbon sinks themselves, it remains doubtful if Gigasporaceae can transfer sufficient amounts of carbon to achlorophyllous myco-heterotrophic plants. This idea might explain why in the present study no sequences of Gigasporaceae were detected. However, in earlier investigations, members of the Gigasporaceae were found twice in the roots of *Voyria* (Bidartondo et al. 2002; Franke 2002). Thus, they might represent facultative mycobionts of myco-heterotrophic plants as well. This assumption is supported by Bidartondo et al. (2002), who identified a single representative of the genus *Gigaspora* Gerd. & Trappe in the roots of *Voyria tenuifolia* Grieseb., which were likewise colonized by two *Glomus*-group A mycobionts.

In the present study, members of the family-ranking *Glomus*-group B clade were apparently absent from the roots of the investigated myco-heterotrophic plants. Husband et al. (2002a), who studied the AMF diversity in a tropical forest using the primer pair NS31/AM1, did not detect *Glomus*-group-B-related SSU sequences in their root samples either. They assumed that two mismatches of the primer AM1 for *Glomus*-group-B-related SSU sequences might be responsible for the apparent absence of these fungi from their study site. In contrast to this, Douhan et al. (2005) were able to amplify the SSU sequence of a member of *Glomus*-group B with NS31 and AM1 under variable PCR conditions. Moreover, they checked the priming site homology of various fungal taxa with AM1 and did not find any mismatches for *Glomus etunicatum* W.N. Becker & Gerd., which falls within the *Glomus*-group B clade (not to be confused with *Glomus* "*etunicatum*-like" Y17644, which belongs to the Diversisporaceae). Since Bidartondo et al. (2002), who used different primer combinations, could not assign any of their mycobiont sequences to representatives of *Glomus*-group B either, technical inadequacy might not be the reason why these fungi were not detected in the roots of myco-heterotrophic plants.

Besides making attempts to find conclusive reasons why certain phylogenetic units within the Glomeromycota might be unsuitable mycobionts, one should, on the other hand, also ask why members of the *Glomus*-group A clade are obviously favored by all AM-adapted myco-heterotrophic plants so far investigated. Answers to this important question are most likely to be found in those plant genera which contain both photoautotrophic and AM-adapted myco-heterotrophic species, such as *Burmannia* L. (Burmanniaceae), *Exacum* L., or *Sebaea* Sol. ex R.Br. (both Gentianaceae). The large genus *Sebaea* (60–100 species according to Struwe et al. 2002) displays a transition from comparatively large-leafed autotrophic species, such as *Sebaea spathulata* Steud., to species with vestigal scale leaves, like *Sebaea filiformis* Schinz, and finally, to completely myco-heterotrophic forms like the achlorophyllous *Sebaea oligantha* Schinz, which proved to be associated with representatives of Glomus-group A (Fig. 2). This suggests that the identification of mycorrhizal associates, other than *Glomus*-group A in the roots of green *Sebaea* species, might provide further clues of how extreme mycorrhizal specificity could be correlated to the evolution of a myco-heterotrophic mode of life. Investigations to find out the facts about this idea are on the way.

Acknowledgements This study was financially supported by the BIOLOG (Biodiversity and Global Change) program (Grant 01LC0007) of the German Ministry of Education and Research. The authors want to express their special thanks to Moses Sainge, without whom the investigation of so many rare myco-heterotrophic plants would have been impossible and to Susanne Renner who gratefully allowed us to use the technical equipment of her lab.

References

Beentje HJ, Adams B, Davies SD (1994) Regional overview: Africa. In: Davies SD, Heywood VH, Hamilton AC (eds) Centres of plant diversity, vol 1. WWF & IUCN, pp 101–148

Bidartondo MI, Redecker D, Hijri I, Wiemken A, Bruns TD, Domínguez L, Sérsic A, Leake JR, Read DJ (2002) Epiparasitic plants specialized on arbuscular mycorrhizal fungi. Nature 419:389–392

Björkman E (1960) *Monotropa hypopitys* L.—an epiparasite on tree roots. Physiol Plant 13:308–327

Burgeff H (1932) Saprophytismus und Symbiose. Gustav Fischer, Jena

Cronquist A (1981) An integrated system of classification of flowering plants. Columbia University Press, New York

Daniell TJ, Husband R, Fitter AH, Young JPW (2001) Molecular diversity of arbuscular mycorrhizal fungi colonizing arable crops. FEMS Microbiol Ecol 36:203–209

Domínguez LS, Sérsic A (2004) The southernmost myco-heterotrophic plant, *Arachnitis uniflora*: root morphology and anatomy. Mycologia 96:1143–1151

Douhan GW, Petersen C, Bledsoe CS, Rizzo DM (2005) Contrasting root associated fungi of three common oak-woodland plant species based on molecular identification: host specificity or non specific amplification? Mycorrhiza 15:365–372

Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39:783–791

Fitch WM (1971) Toward defining the course of evolution: minimum change for a specified tree topology. Syst Zool 20:406–416

Franke T (2002) The myco-heterotrophic *Voyria flavescens* (Gentianaceae) and its associated fungus. Mycol Prog 1:367–376

Hart MM, Reader RJ (2002) Taxonomic basis for variation in the colonization strategy of arbuscular mycorrhizal fungi. New Phytol 153:335–344

Helgason T, Daniell TJ, Husband R, Fitter AH, Young JPW (1998) Ploughing up the wood-wide web? Nature 394:431

Helgason T, Fitter AH, Young JPW (1999) Molecular diversity of arbuscular mycorrhizal fungi colonizing *Hyacinthoides non-scripta* (bluebell) in a seminatural woodland. Mol Ecol 8:659–666

Helgason T, Merryweather JW, Denison J, Wilson P, Young JPW, Fitter AH (2002) Selectivity and functional diversity in arbuscular mycorrhizas of co-occurring fungi and plants from a temperate deciduous woodland. J Ecol 90:371–384

Helgason T, Watson IJ, Young JPW (2003) Phylogeny of the Glomerales and Diversisporales (Fungi: Glomeromycota) from actin and elongation factor 1-alpha sequences. FEMS Microbiol Lett 229:13–127

Husband R, Herre EA, Turner SL, Gallery R, Young JPW (2002a) Molecular diversity of arbuscular mycorrhizal fungi and patterns of host association over time and space in a tropical forest. Mol Ecol 11:2669–2678

Husband R, Herre EA, Young JPW (2002b) Temporal variation in the arbuscular mycorrhizal communities colonising seedlings in a tropical forest. FEMS Microbiol Ecol 42:371–384

Husband R (2004) The diversity, ecology and molecular detection of arbuscular mycorrhizal fungi. In: Gillings M, Holmes A (eds) Plant microbiology. Garland Science/BIOS Scientific, Abingdon, pp 1–17

Imhof S (1997) Root anatomy and mycotrophy of the achlorophyllous *Voyria tenella* Hooker (Gentianaceae). Bot Acta 110:298–305

Imhof S (1999a) Anatomy and mycotrophy of the achlorophyllous *Afrothismia winkleri*. New Phytol 144:533–540

Imhof S (1999b) Root morphology, anatomy and mycotrophy of the achlorophyllous *Voyria aphylla* (Jaqu.) Pers. (Gentianaceae). Mycorrhiza 9:33–39

Imhof S (1999c) Subterranean structures and mycorrhiza of the achlorophyllous *Burmannia tenella* Benth. (Burmanniaceae). Can J Bot 77:637–643

Imhof S (2001) Subterranean structures and mycotrophy of the achlorophyllous *Dictyostega orobanchoides* (Hook.) Miers (Burmanniaceae). Rev Biol Trop 49:239–247

Imhof S (2003) A dorsiventral mycorrhhizal root in the achlorophyllous *Sciaphila polygyna* (Triuridaceae). Mycorrhiza 13:327–332

Imhof S, Weber HC (1997) Root anatomy and mycotrophy (AM) of the achlorophyllous *Voyria truncata* (Standley) Standley and Steyermark (Gentianaceae). Bot Acta 110:127–134

Imhof S, Weber HC (2000) Root structures and mycorrhiza of the achlorophyllous *Voyria obconica* Progel (Gentianaceae). Symbiosis 29:201–211

Imhof S, Weber HC, Gomez LD (1994) Ein Beitrag zur Biologie von *Voyria tenella* Hook. und *Voyria truncata* (Standley) Standley and Steyermark (Gentianaceae). Beitr Biol Pflanz 68:113–123

Knöbel M, Weber HC (1988) Vergleichende Untersuchungen zur Mycotrophie bei *Gentiana verna* L. und *Voyria truncata* (Stand.) Stand. and Stey. (Gentianaceae). Beitr Biol Pflanz 63:463–477

Kowalchuk GA, De Souza FA, Van Veen JA (2002) Community analysis of arbuscular mycorrhizal fungi associated with *Ammophila arenaria* in Dutch costal sand dunes. Mol Ecol 11:571–581

Kretzer AM, Bidartondo MI, Grubisha LC, Spatafora JW, Szaro TM, Bruns TD (2000) Regional specialization of *Sarcodes sanguinea* (Ericaceae) on a single fungal symbiont *Rhizopogon ellenae* (Rhizopogonaceae) species complex. Am J Bot 57:1778–1782

Leake JR (1994) The biology of myco-heterotrophic ('saprophytic') plants. Tansley review no. 69. New Phytol 127:171–216

Leake JR (2004) Myco-heterotroph/epiparasitic plant interactions with ectomycorrhizal and arbuscular mycorrhizal fungi. Curr Opin Plant Biol 7:422–428

Lee JK, Eom AH, Lee SS, Lee HC (2001) Mycorrhizal symbioses found in roots of fern and its relatives in Korea. J Plant Biol 44:81–86

Lee JK, Eom AH, Lee SS (2002) Multiple symbiotic associations found in the roots of *Botrychium ternatum*. Mycobiology 30:146–153

Lee JK, Tae MS, Eom AH, Lee SS (2003) Restriction analyses of PCR amplified partial SSU ribosomal DNA to distinguish arbuscular mycorrhizal fungi from other fungi colonizing plant roots. Mycobiology 31:68–73

McKendrick SL, Leake JR, Read DJ (2000) Symbiotic germination and development of myco-heterotrophic plants in nature: transfer of carbon from ectomycorrhizal *Salix repens* and *Betula pendula* to the orchid *Corallorhiza trifida* through shared hyphal connections. New Phytol 145:539–548

Morton JK (1972) Phytogeography of the West African Mountains. In: Valentine DH (ed) Taxonomy, phytogeography and evolution. Academic, London, pp 221–236

Morton JB, Redecker D (2001) Two new families of Glomales, Archaeosporaceae and Paraglomaceae, with two new genera *Archaeospora* and *Paraglomus*, based on concordant molecular and morphological characters. Mycologia 93:181–195

Mutke J, Kier G, Braun G, Schulz C, Barthlott W (2001) Patterns of African vascular plant diversity—a GIS based analysis. Syst Geogr Plants 71:1125–1136

Raynal-Roques A, Paré J (1998) Biodiversité des Phanérogames parasites: leur place dans la classificacion systématique. Adansonia 20:313–322

Schüβler A, Gehrig H, Schwarzott D, Walker C (2001a) Analysis of partial Glomales SSU rRNA gene sequences: implications for primer design and phylogeny. Mycol Res 105:5–15

Schüβler A, Schwarzott D, Walker C (2001b) A new fungal phylum, the Glomeromycota: phylogeny and evolution. Mycol Res 105:1413–1421

Schwarzott D, Walker C, Schüssler A (2001) *Glomus* the largest genus of the arbuscular mycorrhizal fungi (Glomales), is nonmonophyletic. Mol Phylogenet Evol 21:190–197

Simon L, Lalonde M, Bruns T (1992) Specific amplification of 18S fungal ribosomal genes from vesicular-arbuscular endomycorrhizal fungi colonizing roots. Appl Environ Microbiol 58:291–295

Sosef MSM (1994) Refuge Begonias. Taxonomy, phylogeny and historical biogeography of *Begonia* sect. *Loasibegonia* and sect. *Scutobegonia* in relation to glacial rain forest refuges in Africa. Ph.D. thesis, Wageningen University, Wageningen

Struwe L, Kadereit JW, Klackenberg J, Nielsson S, Thiv M, von Hagen KB, Albert VA (2002) Systematics, character evolution, and biogeography of Gentianaceae including a new tribal and subtribal classification. In: Struwe L, Albert VA (eds) Gentianaceae, systematics and natural history. Cambridge University Press, Cambridge, pp 21–309

Swofford DL (2002) PAUP*. Phylogenetic analysis using parsimony (*and other methods). Version 4. Sinauer Associates, Sunderland

Terashita T, Chuman S (1987) Fungi inhabiting wild orchids in Japan (IV). *Armillariella tabescens*, a new symbiont of *Galeola septentrionalis*. Nippon Kingakkai Kaiho 28:145–154

Vanderkoornhuyse P, Husband R, Daniell TJ, Watson LJ, Duck JM, Fitter AH, Young JPW (2002) Arbuscular mycorrhizal community composition associated with two plant species in a grassland ecosystem. Mol Ecol 11:1555–1564

White F (1979) The Guineo-Congolean region and its relationships to other phytochoria. Bull Jard Bot Natl Belg 49:11–55

Yamato M (2001) Identification of a mycorrhizal fungus in the roots of achlorophyllous *Sciaphila tosaensis* Makino (Triuridaceae). Mycorrhiza 11:83–88

Lebenslauf

Name:	Franke
Vorname:	Thassilo, Silvester
Geboren am:	18. 09. 1972 in München

Schulbildung:

1978 – 1982	Volksschule, München
1982 – 1992	Gymnasium, München
	Abschluss: allgemeine Hochschulreife
1992 – 1993	Wehrdienst, München

Universität:

1993 – 1999:	Studium der Biologie an der Ludwig-Maximilians-Universität München
	Abschluss: Diplom (Hauptfach: Systematische Botanik; Nebenfächer: Physiologische Botanik, Systematische Zoologie, Ökologie); Diplomarbeit in systematischer Botanik bei Prof. Dr. Hans-Jürgen Tillich mit dem Titel: „Untersuchungen zur Biologie von *Sciaphila purpurea* BENTH. (1855) (Triuridaceae, Monocotyledonae)“
September 1999:	Beginn der Dissertation unter Anleitung von Prof. Dr. Reinhard Agerer
2004 – 2005:	Einjährige Unterbrechung der Dissertation. Aufbaustudium im Fach „Wissenschaftskommunikation mit Schwerpunkt Naturfilm“ an der University of Otago Dunedin, Neuseeland
	Abschluss: Postgraduate Diploma in Natural History Filmmaking and Communication; Diplomarbeit: 24 minütiger Dokumentarfilm mit dem Titel: „Exhuming Adams“

Ehrenwörtliche Versicherung und Erklärung

Ich, Thassilo Franke, versichere hiermit ehrenwörtlich, dass die Dissertation von mir selbstständig, ohne unerlaubte Beihilfe angefertigt ist.

Hiermit erkläre ich, Thassilo Franke, dass ich mich anderweitig einer Doktorprüfung nicht unterzogen habe.

München, den 07. Dezember 2006