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

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Chapter 5

Did mycoheterotrophic plant lineages with similar ancestral distribution ranges originate simultaneously?

Collecting *Thismia rodwayi* and *T. hillii* in Australia and New Zealand. Photos: C.B. Mennes/ V.S.F.T. Merckx.

Did mycoheterotrophic plant lineages with similar ancestral distribution ranges originate simultaneously?

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5.1. Abstract

Mycoheterotrophy is a strategy in which plants obtain carbon from their mycorrhizal symbionts. This remarkable lifestyle is probably an adaptation to life in dark rainforest understories and has evolved many times independently in flowering plants. Many unrelated mycoheterotrophic species often grow together and have similar distribution ranges. Based on these observations we hypothesize that non-related lineages of mycoheterotrophic plants with similar ancestral distribution ranges originated contemporaneously. We addressed this hypothesis by constructing time-calibrated phylogenies for all lineages of arbuscular mycorrhizal (AM) mycoheterotrophic plants in a standardized way. In addition, we estimated the ancestral distribution range of the most recent common ancestor of each lineage. We subsequently used this information to compare the age and biogeographic origins of the groups. We furthermore assessed whether these lineages evolved in rainforest environments. We defined six broad ancestral areas in which mycoheterotrophic plants putatively originated based on the plants' extant distribution. Although we observe old and relatively young lineages in each of these areas, we conclude that lineages with similar ancestral ranges did not originate contemporaneously. Furthermore, Southeast Asian lineages tend to be younger than their Neotropical and African relatives. Moreover, all reconstructed ancestral areas were covered in rainforest at the onset of diversification in each lineage. This implies evolution of mycoheterotrophy is strongly associated with rainforests.

5.2. Introduction

Convergent evolution is very common in angiosperms. For example, water pollinated angiosperms are hypothesized to have evolved seven times independently in former subclass Alismatidae (now Alismatales) (Les et al., 1997; Stevens, 2001 onwards). Likewise, carnivorous lineages have evolved at least six times independently across five orders of angiosperms, resulting in different unrelated carnivorous plant taxa with similar mechanisms that obtain nutrients from animal tissues (Ellison & Gotelli, 2009). Another, even more pronounced example is C_4 photosynthesis. The combination of modifications in the photosynthetic apparatus leading to an increased efficiency under extreme light conditions, has evolved more than 45 times independently across 19 angiosperm families (Sage, 2003). At a smaller taxonomic scale, in the family Brassicaceae it is reported that the morphological characters used to delimit taxonomic groups (i.e. fruit morphology) caused problems in inferring phylogenetic relationships as a result of convergent evolution (Bailey et al., 2006)). Thus as a result of convergent evolution, characters that were originally interpreted as predictive for systematic relationships, were later identified as less informative as a result of homoplasy in some groups. Another good example of convergent evolution causing problems in reconstructing evolutionary relationships is mycoheterotrophy (Leake, 1994). Mycoheterotrophic plants consist of many, mostly unrelated lineages which were formerly often classified together based on their reduced vegetative structures and modified flowers (e.g. Leake, 1994; Mennes et al., 2013; Mennes et al., 2015a).

Fully mycoheterotrophic plants do not produce carbohydrates by photosynthesis, but rather rely on their fungal symbionts for the uptake of carbohydrates (Leake, 1994). By obtaining carbon compounds from fungi, plants are able to evade competition for scarce light in the forest understorey, particularly in tropical rainforests (Bidartondo, 2005). Fully mycoheterotrophic plants evolved from photosynthetic ancestors and this break-down of an originally mutualistic into an antagonistic mycorrhizal interaction with root-associated fungi has evolved at least 45 times in angiosperms (Bidartondo, 2005; Merckx et al., 2013a). Apart from the wide range of plants that engage in this symbiosis, the fungi involved are also highly diverse and are referrable to Basidiomycetes, Ascomycetes and Glomeromycetes (Bidartondo, 2005; Hynson & Bruns, 2010). The latter includes arbuscular mycorrhizal (AM) fungi, by far the most common mycorrhizal partners of land plants (Smith & Read, 2008). All mycoheterotrophic angiosperm lineages except those in the species-rich Orchidaceae and Ericaceae are linked to this type of mycorrhizal fungus (Bidartondo, 2005; Merckx et al., 2012; 2013a). Recent evolutionary studies have shown that many lineages of mycoheterotrophic plants growing in symbiosis with AM fungi are relatively old. For example, the stem age of Burmanniaceae has been estimated to exceed 100 million years (Merckx et al., 2010). Likewise, Triuridaceae have an estimated stem age dating back at least 62 million years, likely dating back to the

Cretaceous (Mennes et al., 2013). Although the genus *Voyria* (Gentianaceae) is estimated to be younger than these families, its stem age probably still exceeds 40 million years (Merckx et al., 2013c). The vast majority of mycoheterotrophic plants growing on AM fungi are tightly restricted to wet, mostly tropical closed-canopy forest habitats. Often, these plants co-occur in such habitats (Leake, 1994). Moreover, unrelated species are often growing together, implying they are adapted to similar habitats (Leake, 1994). There are only a few species that are known to occasionally occur in more open environments (i.e. *Apteria aphylla* (Burmanniaceae, Maas et al., 1986), *Voyria aphylla* (Gentianaceae, Maas & Ruyters, 1986), *Arachnitis uniflora* (Cribb et al., 1995) and *Thismia americana* (Merckx & Smets, 2014)).

Due to their almost exclusive occurrence in rainforest habitats and their similarities in distribution patterns some studies suggest that the diversification of AM mycoheterotrophic plants may be related to the expansion of rainforest habitats (Merckx et al., 2008, 2013c; Mennes et al. 2015b). The family Burmanniaceae is hypothesized to have diversified rapidly during the Eocene, when global temperatures peaked and rainforest coverage was much higher than at the present (Merckx et al., 2008). Moreover, the transatlantic distribution of *Voyria* was probably established during a similar climatic optimum in the Miocene (Merckx et al., 2013c). The diversification of the fully mycoheterotrophic genus *Epirixanthes* (Polygalaceae) is also possibly linked to rainforest expansion and other environmental dynamics, as suggested by Mennes et al. (2015b).

Insights into the evolutionary history of taxa strongly depend on the availability of well-resolved and dated phylogenies. Divergence time estimations have been reconstructed for all mycoheterotrophic angiosperm lineages growing on AM fungi (Janssen & Bremer, 2004; Goldblatt et al., 2008; Merckx et al., 2010, 2013c; Mennes et al., 2013, 2015a, b). Additionally, for some groups (e.g. Corsiaceae, Burmanniaceae, Gentianaceae) there are available ancestral area reconstructions based on these divergence time estimations (Merckx et al., 2008, 2013c; Mennes et al., 2015a). For example, the family Burmanniaceae was estimated to have a stem age of over 100 Ma and probably originated in the Neotropics or Africa, whereas for Corsiaceae a stem age of about 70 Ma was inferred, implying a southern Gondwana origin (Mennes et al., 2015a). The genus *Voyria* has an inferred a stem age of 54 Ma and a putative Neotropical origin. These results raise the question of whether similar patterns of ancestral range and divergence times can be found in the evolutionary history of unrelated lineages.

The present study aims to address the hypothesis that independently evolved AM mycoheterotrophic lineages with similar ancestral ranges have similar estimated stem and crown ages. First, we run Bayesian phylogenetic divergence time estimations for each lineage in a standardized way including some newly sampled taxa. Using this approach, we aim to maximize the reliability and the comparability of the divergence

time estimations. Secondly, we infer the ancestral distribution range of the most recent common ancestor for each lineage. The time of origin of lineages with similar ancestral ranges are compared and the occurrence of rainforests in the ancestral areas at the time of the stem age of each lineage assessed using palaeoenvironmental information from the literature. As all mycoheterotrophic species occur in the rainforest (only a few species are not restricted to this habitat), we expect all mycoheterotrophic lineages to have evolved in a rainforest environment, implying all ancestral areas were covered in rainforests. Mycoheterotrophic species from Burmanniaceae, Corsiaceae, Polygalaceae, Gentianaceae, Iridaceae, Petrosaviaceae, Thismiaceae and Triuridaceae are studied. With a total of 85 fully mycoheterotrophic species included, this study samples over one third of all mycoheterotrophic angiosperms linked to AM fungi (Merckx et al., 2013b). We compare our novel inferences with previously published biogeographic inferences (e.g. Merckx et al., 2008; Mennes et al., 2015a).

5.3. Materials and Methods

5.3.1. Taxon sampling and molecular methods

DNA sequence data for a total of 85 AM mycoheterotrophic species were included. Additionally, data for closely related chlorophyllous species were included based on the latest phylogenetic insights (Stevens, 2001 onwards). The majority of the sequence data was obtained from GenBank, but sequence information for 12 additional mycoheterotrophic taxa was newly acquired following the method by Mennes et al. (2013). Moreover, for several taxa for which sequence data are available, additional sequence data were obtained. See Appendix A.1 for a complete list of included taxa. Mycoheterotrophic lineages were analyzed independently, although a few closely related taxa were simultaneously analyzed in a single dataset. Selection of the loci on which the analyses were based follows the previously published procedures for each lineage. Taxa for which at least two loci were available were included.

The dataset of Corsiaceae (containing four mycoheterotrophic species and 22 chlorophyllous relatives of Liliales) was based on Mennes et al. (2015a) and included nuclear 18S rDNA and mitochondrial *atpA*, *matR*, *cob* and *nad5*.

The dataset of Dioscoreales (containing 42 mycoheterotrophic species, two of which were not sequenced previously, and 47 chlorophyllous relatives of Dioscoreales and Pandanales) was based on Merckx et al. (2010) and included 18S rDNA and *atpA* and *matR*. The mitochondrial locus *nad1* was omitted due to alignment difficulties in our dataset. These difficulties were caused by highly different sequences in the dataset; particularly sequences from some mycoheterotrophic taxa tended to be strongly deviating from others.

The dataset of *Geosiris* (Iridaceae) (containing a single mycoheterotrophic species

and 59 chlorophyllous representatives of Asparagales, Liliales and Pandanales) was based on Goldblatt et al. (2008) and included the plastid regions *matK, rbcL*, *rps4* and *trnL-F.* The locus *rps16* was omitted due to alignment difficulties in our dataset. The nuclear 18S rDNA region and the mitochondrial *atpA* and *cob* loci were added due to their usefulness in mycoheterotrophic taxa (Mennes et al., 2013, 2015a).

The dataset of Gentianaceae (containing 15 mycoheterotrophic species, one of which was not sequenced previously, as well as 85 chlorophyllous representatives of Gentianales (most of which are Gentianaceae)) was based on Merckx et al. (2013c) and included nuclear ITS. This dataset was supplemented by the plastid regions *matK* and *trnL*, which were available for many taxa. For the remaining taxa, only ITS was analyzed (see Appendix A.1).

The dataset of *Epirixanthes* (Polygalaceae) (containing four mycoheterotrophic species, and 27 chlorophyllous relatives from Polygalaceae) was based on Mennes et al. (2015b) and included ITS and *matK*.

The dataset of Pandanales (Triuridaceae) (containing 18 mycoheterotrophic species, four of which were not sequenced previously, and 98 additional monocot taxa) was based on Mennes et al. (2013) and included 18S rDNA, *atpA* and *matR*. As Petrosaviales comprise only two genera and the order is relatively closely related to Pandanales, it is also included in this analysis (a single mycoheterotrophic species). Only 18S rDNA and *atp*A were analyzed. The *rbcL* region used in Janssen & Bremer (2004) was not included in our analysis, because this region was not amplified for Triuridaceae and other Pandanales taxa.

5.3.2. Divergence time estimations

Estimations of divergence times for each lineage follow Mennes et al. (2015a), except the procedure for *Epirixanthes*, which is described in Mennes et al. (2015b). The analyzed dataset of *Epirixanthes* was considerably smaller than the other analyzed datasets, and therefore a shorter running time (50 million generations vs. 200 million, see below) and a single clock model were used. Six analyses were conducted, three of which correspond to the lineages Corsiaceae, *Epirixanthes* and *Geosiris* and their chlorophyllous relatives. The fourth analysis consists of Gentianaceae (including some outgroup taxa from Rubiaceae) with all mycoheterotrophic lineages (i.e. the fully mycoheterotrophic *Voyria* and *Voyriella* as well as the mycoheterotrophic representatives of *Exacum* and *Exochaenium*). The fifth analysis consists of the mycoheterotrophic lineages in Dioscoreales, i.e. those in Burmanniaceae, Thismiaceae and *Afrothismia* (including outgroups from Pandanales). The final analysis focusses on Triuridaceae (Pandanales) and *Petrosavia* (Petrosaviales) and consists of a relatively large taxon sampling including sequence data of representatives from all monocot orders. Each analysis has its own fossil calibration strategy, listed in Table 7. The selection of fossils for the monocot taxa (i.e. everything except Gentianaceae

Table 7. Fossil calibration point priors.

Analysis	Node	Age (Ma)	Fossil taxon	Reference
Corsiaceae	Stem Zingiberales	72.1	*Spirematospermum chandlerae*	Friis, 1988; Sohl & Owens, 1991
	Crown Arecaceae	83.6	*Sabalites carolinensis*	Berry, 1914; Gohn et al., 1992
	Crown Poaceae	66	*Changii indicum*	Courtillot & Renne, 2003; Prasad et al., 2011
	Stem Asparagales	93	*Liliacidites*	Ramirez et al., 2007; Gustafsson et al., 2010
	Stem Ripogonaceae	51	*Ripogonum tasmanicum*	Carpenter et al., 2007; Conran et al., 2009a
	Stem Luzuriaga	23.2	*Luzuriaga*	Lindqvist & Lee, 2009; Conran et al., 2014
Epirixanthes	Crown Polygalaceae	60.4	*	Forest et al., 2007
Geosiris	Stem Pandanales	109.5	*	Mennes et al., 2013
	Stem Astelia	23.2	*Astelia antiquua*	Lindqvist & Lee, 2009; Maciunas et al., 2011
	Stem Cordyline	22	*Paracordyline kerguelensis*	Giret et al., 1989; Conran, 1997
	Stem Dendrobium	23.2	*Dendrobium winikaphyllum*	Lindqvist & Lee, 2009; Conran et al., 2009b
	Stem Hemerocallidoideae	38	*Dianellophyllon eocenicum*	Alley et al., 1996; Conran et al., 2003
	Stem Yucca	14.5	*Protoyucca shadishii*	Tidwell & Parker, 1990; Perkins et al., 1998
Mycoheterotrophic Gentianaceae	Crown Gentianales	79	*	Bremer et al., 2004; Janssens et al., 2009
	Crown Potalieae	37.2	*Lisianthus*	Graham, 1984; Yuan et al., 2005; Favre et al., 2010
	Crown Swertiinae	15	*	Von Hagen & Kadereit, 2002; Yuan et al., 2005; Favre et al., 2010
Mycoheterotrophic Dioscoreales	Stem Pandanales	109.5	*	Mennes et al., 2013
	Stem Triuridaceae	86.3	*Mabelia connatifila*	Christopher, 1979; Gandolfo et al., 2002; Friis et al., 2011
Triuridaceae and *Petrosavia*	Crown monocots	134	*	Bremer, 2000
	Stem Luzuriaga	23.2	*Luzuriaga*	Lindqvist & Lee, 2009; Conran et al., 2014
	Crown Arecaceae	83.6	*Sabalites carolinensis*	Berry, 1914; Gohn et al., 1992
	Stem Zingiberales	72.1	*Spirematospermum chandlerae*	Friis, 1988; Sohl & Owens, 1991
	Crown Poaceae	66	*Changii indicum*	Courtillot & Renne, 2003; Prasad et al., 2011
	Stem Ripogonaceae	51	*Ripogonum tasmanicum*	Carpenter et al., 2007; Conran et al., 2009a
	Stem Caldesia	20	*Caldesia*	Tiffney, 1994; Traverse, 1994; Haggard & Tiffney, 1997
	Stem Triuridaceae	86.3	*Mabelia connatifila*	Christopher, 1979; Gandolfo et al., 2002; Friis et al., 2011

* These calibrations are not based on fossils, but instead are based on inferred ages from previous studies.

and *Epirixanthes*) followed Iles et al. (2015). All fossil calibrations were modeled as lognormal distributions with the minimum age of the fossil as offset value (mean = 5, sd = 1.25). All secondary calibrations were modelled as broad normal distributions with the estimated age as mean. Gamma-distributed priors (default settings) for substitutions (rate = 1.0) were used. Exponentially distributed priors were defined for the gamma shape of the substitution model, as well as for the standard deviation and the mean of the clock models. The latter was set to 10.0; all other prior values were kept at default. The Yule model (Gernhard, 2008) of speciation was set as prior on the birth rate. Clades were constrained according to previous phylogenetic studies in cases where our analyses showed deviating results (Chase et al., 2006; Goldblatt et al., 2008; Merckx et al., 2010, 2013c; Soltis et al., 2011; Barrett et al., 2013; Mennes et al., 2013, 2015a, b). Parameter settings for each prior were kept to their default values. The Markov Chain Monte Carlo (MCMC, Geyer, 1991) procedure was ran for 200 million generations, and one tree of every 10.000 was sampled. A burn-in of 10% was discarded.

5.3.3. Ancestral range estimation

We defined six broad ancestral areas of mycoheterotrophic plants growing on arbuscular mycorrhizal fungi following the description of the world-wide distribution of mycoheterotrophic plants by Merckx et al. (2013d). These areas are based on the main tropical regions of the world: 1) Central and South America. 2) Africa and Madagascar. 3) Southwest India and Sri Lanka. 4) Southeast Asia. 5) Australasia (i.e. tropical Australia, New Caledonia, Vanuatu and Malesia east of Indonesia). 6) Pacific Islands (i.e. the mostly volcanic islands northeast of Australasia). However, no mycoheterotrophic plants from the Pacific Islands were included, although a single chlorophyllous species of *Burmannia* was collected from there, and the range of *Sciaphila dolichostyla* includes this region (the included specimen was collected from Vanuatu). In addition, the temperate regions Australia/New Zealand and Southern South America were included for lineages of which some included species occur in there (i.e. Burmanniaceae, Corsiaceae and Thismiaceae). For *Voyria*, several fine-scale Neotropical ancestral areas were defined based on Merckx et al. (2013c,d): 1) Central America, 2) West Indies, 3) Guianas and Amazonia, 4) Atlantic Forest. Ancestral areas are inferred for each lineage that occurs in more than one area and of which more than a single specimen was included. Therefore, all lineages except *Afrothismia, Exacum, Exochaenium* and *Geosiris* were analyzed. Distribution information for each species in our dataset was obtained from the literature (Burmanniaceae: Maas et al., 1986; Corsiaceae: Van Royen, 1972; *Epirixanthes*: Van der Meijden, 1988; Gentianaceae: Maas & Ruyters, 1986, Merckx, 2013c; *Geosiris*: Goldblatt et al., 2008; Thismiaceae: Maas et al., 1986; Triuridaceae: Maas & Rübsamen, 1986; Van de Meerendonk, 1984), supplemented by information from the Global Biodiversity Information Facility (GBIF). This information is listed in Table 8. For most lineages, chlorophyllous sister groups consist of a wide range of

Table 8. Coding schemes used for each taxon per ancestral area reconstruction.

Taxon	Analysis	Coding	Interpretation
Epirixanthes kinabaluensis	"Epirixanthes"	10	Southeast Asia
Epirixanthes papuana	"Epirixanthes"	11	Southeast Asia and Australasia
Epirixanthes pallida	"Epirixanthes"	10	Southeast Asia
Epirixanthes elongata	"Epirixanthes"	10	Southeast Asia
Voyria aurantiaca	"Voyria"	01110	Central America, West Indies, Guianas/Amazonia
Voyria tenuiflora	"Voyria"	00010	Guianas/Amazonia
Voyria aphylla	"Voyria"	01111	Central America, West Indies, Guianas/Amazonia, Atlantic forest
Voyria primuloides	"Voyria"	10000	Africa
Voyria rosea	"Voyria"	00010	Guianas/ Amazonia
Voyria corymbosa	"Voyria"	01010	Central America, Guianas/ Amazonia
Voyria parasitica	"Voyria"	01100	Central America, West Indies
Voyria obconica	"Voyria"	00001	Atlantic forest
Voyria tenella	"Voyria"	01111	Central America, West Indies, Guianas/ Amazonia, Atlantic forest
Voyria flavescens	"Voyria"	01111	Central America, West Indies, Guianas/ Amazonia, Atlantic forest
Voyria caerula	"Voyria"	00011	Guianas/ Amazonia, Atlantic forest
Voyria clavata	"Voyria"	00010	Guianas/ Amazonia
Thismia megalongensis	"Thismia"	0001	Temperate Australasia
Thismia hillii	"Thismia"	0001	Temperate Australasia
Thismia clavarioides	"Thismia"	0001	Temperate Australasia
Thismia rodwayi	"Thismia"	0001	Temperate Australasia
Thismia aseroe	"Thismia"	0100	Southeast Asia
Thismia clavigera	"Thismia"	0100	Southeast Asia
Thismia huangii	"Thismia"	0100	Southeast Asia
Thismia taiwanensis	"Thismia"	0100	Southeast Asia
Haplothismia exannulata	"Thismia"	0010	India
Tiputinia foetida	"Thismia"	1000	Central/ South America
Thismia panamensis	"Thismia"	1000	Central/ South America
Burmannia flava	"Burmanniaceae"	1010000	Central/ South America, Temperate South America
Burmannia stuebelii	"Burmanniaceae"	1000000	Central/ South America
Burmannia biflora	"Burmanniaceae"	1000000	Central/ South America
Burmannia bicolor	"Burmanniaceae"	1000000	Central/ South America
Burmannia alba	"Burmanniaceae"	1000000	Central/ South America
Burmannia pusilla	"Burmanniaceae"	0000001	Sri Lanka
Burmannia latialata	"Burmanniaceae"	0100000	Africa/ Madagascar
Burmannia madagascariensis	"Burmanniaceae"	0100000	Africa/ Madagascar
Burmannia lutescens	"Burmanniaceae"	0001100	Southeast Asia , Australasia
Burmannia oblonga	"Burmanniaceae"	0001000	Southeast Asia
Burmannia ledermannii	"Burmanniaceae"	0000010	Pacific Islands
Burmannia hexaptera	"Burmanniaceae"	0100000	Africa/ Madagascar
Burmannia kalbreyeri	"Burmanniaceae"	1000000	Central/ South America
Burmannia longifolia	"Burmanniaceae"	0001100	Southeast Asia, Australasia
Burmannia disticha	"Burmanniaceae"	0001101	Southeast Asia, Australasia, Sri Lanka
Burmannia juncea	"Burmanniaceae"	0000100	Australasia

Burmannia itoana	"Burmanniaceae"	0001000	Southeast Asia
Burmannia wallichii	"Burmanniaceae"	0001000	Southeast Asia
Burmannia damazii	"Burmanniaceae"	1000000	Central/ South America
Burmannia capitata	"Burmanniaceae"	1000000	Central/ South America
Gymnosiphon cf. *cymosus*	"Burmanniaceae"	1000000	Central/ South America
Gymnosiphon suaveolens	"Burmanniaceae"	1000000	Central/ South America
Gymnosiphon divaricatus	"Burmanniaceae"	1000000	Central/ South America
Gymnosiphon longistylus	"Burmanniaceae"	0100000	Africa/ Madagascar
Gymnosiphon refractus	"Burmanniaceae"	1000000	Central/ South America
Gymnosiphon bekensis	"Burmanniaceae"	0100000	Africa/ Madagascar
Gymnosiphon brachycephalus	"Burmanniaceae"	1000000	Central/ South America
Gymnosiphon minutus	"Burmanniaceae"	1000000	Central/ South America
Gymnosiphon aphyllus	"Burmanniaceae"	0001100	Southeast Asia , Australasia
Gymnosiphon capitatus	"Burmanniaceae"	1000000	Central/ South America
Gymnosiphon recurvatus	"Burmanniaceae"	1000000	Central/ South America
Gymnosiphon breviflorus	"Burmanniaceae"	1000000	Central/ South America
Hexapterella gentianoides	"Burmanniaceae"	1000000	Central/ South America
Dictyostega orobanchoides	"Burmanniaceae"	1000000	Central/ South America
Campylosiphon purpurascens	"Burmanniaceae"	1000000	Central/ South America
Apteria aphylla	"Burmanniaceae"	1000000	Central/ South America
Burmannia congesta	"Burmanniaceae"	0100000	Africa/ Madagascar
Arachnitis uniflora	"Corsiaceae"	100	Temperate South America
Corsia cf. *brassii*	"Corsiaceae"	010	Australasia
Corsia cf. *huonensis*	"Corsiaceae"	010	Australasia
Corsia cf. *boridiensis*	"Corsiaceae"	010	Australasia
Campynema lineare	"Corsiaceae"	001	Temperate Australasia
Campynemanthe viridiflora	"Corsiaceae"	010	Australasia
Andruris sp.	"Triuridaceae"	0010	Australasia
Sciaphila nana	"Triuridaceae"	0001	Southeast Asia
Sciaphila cf. *dolichostyla*	"Triuridaceae"	0010	Australasia
Seychellaria africana	"Triuridaceae"	0100	Africa/ Madagascar
Seychellaria madagascariensis	"Triuridaceae"	0100	Africa/ Madagascar
Sciaphila polygyna	"Triuridaceae"	1000	Central/ South America
Sciaphila purpurea	"Triuridaceae"	1000	Central/ South America
Sciaphila albescens	"Triuridaceae"	1000	Central/ South America
Sciaphila quadribullifera	"Triuridaceae"	0010	Australasia
Sciaphila sp. 1	"Triuridaceae"	0001	Southeast Asia
Sciaphila sp. 2	"Triuridaceae"	0010	Australasia
Sciaphila sp. 3	"Triuridaceae"	0010	Australasia
Sciaphila ledermannii	"Triuridaceae"	0100	Africa/ Madagascar
Triuris hyalina	"Triuridaceae"	1000	Central/ South America
Triuris hexophthalma	"Triuridaceae"	1000	Central/ South America
Sciaphila picta	"Triuridaceae"	1000	Central/ South America
Kupea martinetugei	"Triuridaceae"	0100	Africa/ Madagascar
Kihansia sp.	"Triuridaceae"	0100	Africa/ Madagascar

pantropically distributed taxa (e.g. *Tacca* for Thismiaceae (Merckx et al., 2010); the families Stemonaceae, Pandanaceae and Cyclanthaceae for Triuridaceae (Mennes et al., 2013) and the majority of Gentianaceae for *Voyria* (Merckx et al., 2013c)). Therefore, we assumed little information could be obtained from including these taxa in the analyses, and thus the analyses were carried out without the chlorophyllous relatives of the mycoheterotrophic lineages. The only exception was Corsiaceae. This family consists of only three disjunctly distributed genera (two of which are included in this study) with a clearly defined and small outgroup (Campynemataceae; Mennes et al., 2015a). This renders ancestral area reconstruction including the outgroup more informative than an analysis without the outgroup. Ancestral areas were reconstructed for lineages that occur in more than one area as defined above. Therefore, *Afrothismia* was not analyzed as this genus is exclusively found in tropical Africa. We assume that Africa is the ancestral area of this genus. Likewise, for the monospecific mycoheterotrophic genus *Voyriella* a Neotropical origin was assumed. Moreover, *Petrosavia, Geosiris* and the mycoheterotrophic species of *Exacum* and *Exochaenium* were not analyzed, as only a single specimen was included for each of these generic lineages. Reconstruction of ancestral areas was carried out using the package BioGeoBEARS (Matzke, 2013) as incorporated in R (R Core Team, 2014). Each lineage was analyzed using the Dispersal-Extinction-Cladogenesis (DEC) model (Ree & Smith, 2008) and the DEC+j model (Matzke, 2014), which includes a parameter for a founder speciation event ("j"). The maximum range size was set to the maximum number of areas occupied by the species in a lineage. The remaining parameters were kept at their default settings. For each analysis we analyzed which model best suited the data using the Akaike Information Criterion (Akaike, 1973, 1974).

5.4. Results

Figures 17-22 show the phylogenetic trees resulting from the divergence time estimations for all lineages. Table 9 lists the estimated stem and crown ages for all mycoheterotrophic lineages growing on AM fungi, as well as the reconstructed 95% confidence intervals of the stem ages from previous studies for comparison.

Inferred ancestral areas for each lineage are shown in Figs. 23-28. A Dispersal-Extinction-Cladogenesis including founder speciation (DEC+j) model was suggested as best fit by the Akaike Information Criterion for the Triuridaceae, Burmanniaceae and Corsiaceae datasets. A DEC model was suggested as best fit for the remaining datasets, i.e. Thismiaceae excl. *Afrothismia*, *Voyria* and *Epirixanthes*. These analyses inferred ancestral areas in extant continents as defined above, although most continents have undergone major changes due to plate-tectonic processes since the inferred stem ages of the studied lineages. For Burmanniaceae and Triuridaceae, the results suggest a Central/ South American or African origin (Figs. 23 and 24). For Thismiaceae, all areas from which taxa were included are equally possible as

ancestral areas, indicated by the white-coloured pie in Fig. 25. Thus, the results are indecisive about the ancestral area of this lineage. An Australasian origin is most likely for Corsiaceae (Fig. 26). The genus *Voyria* is assumed to have originated in the Central or South America, as assumed from its current distribution. Our results furthermore suggest that the genus started to diversify in the Guianas and Amazonia (Fig. 27). Likewise, the genus *Epirixanthes* has probably evolved in Southeast Asia with subsequent range expansion to Australasia (Fig. 28).

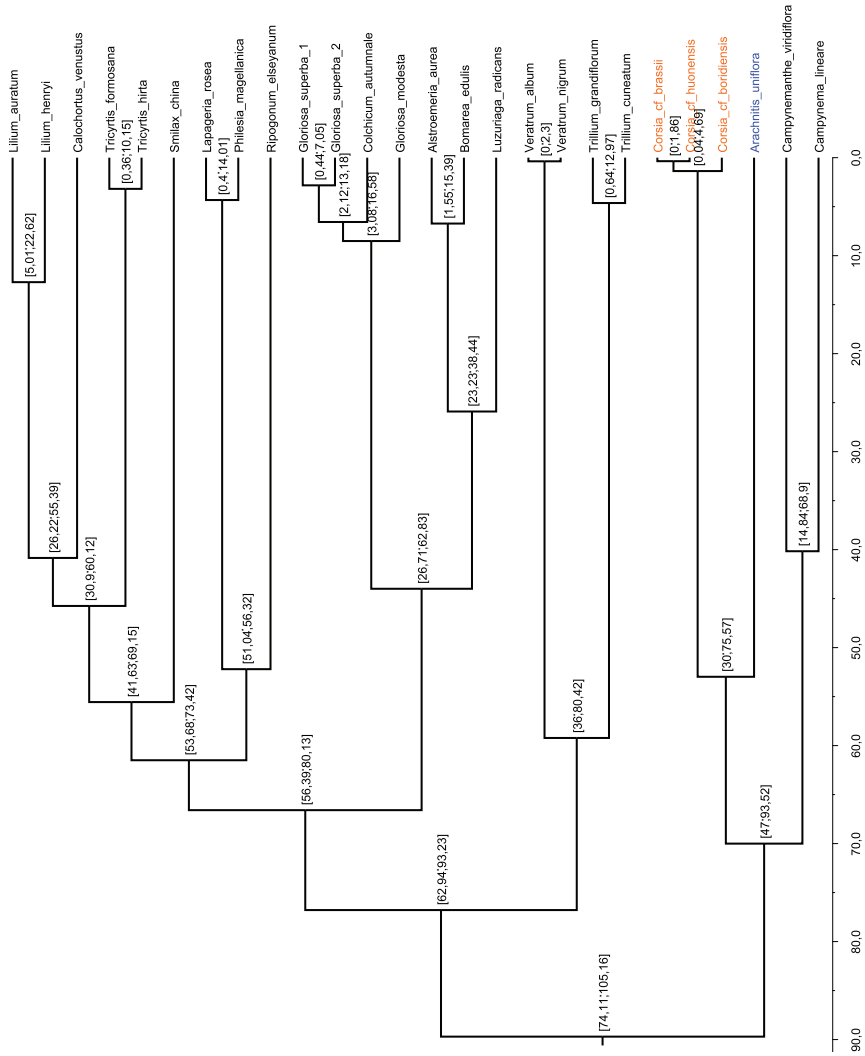

Fig. 17. Maximum clade credibility tree of Corsiaceae (Liliales) inferred using an uncorrelated lognormal relaxed clock method, based on a three-locus molecular dataset (nuclear 18S rDNA and mitochondrial *atpA* and *matR*). Coloured taxa show mycoheterotrophic species growing on AM fungi; orange taxa were collected from Australasia, those indicated in blue were collected from southern South America. Table 7 lists fossil calibration information. The scale bar shows time (million years ago; Ma), values between square brackets indicate upper and lower limits of the 95% confidence interval.

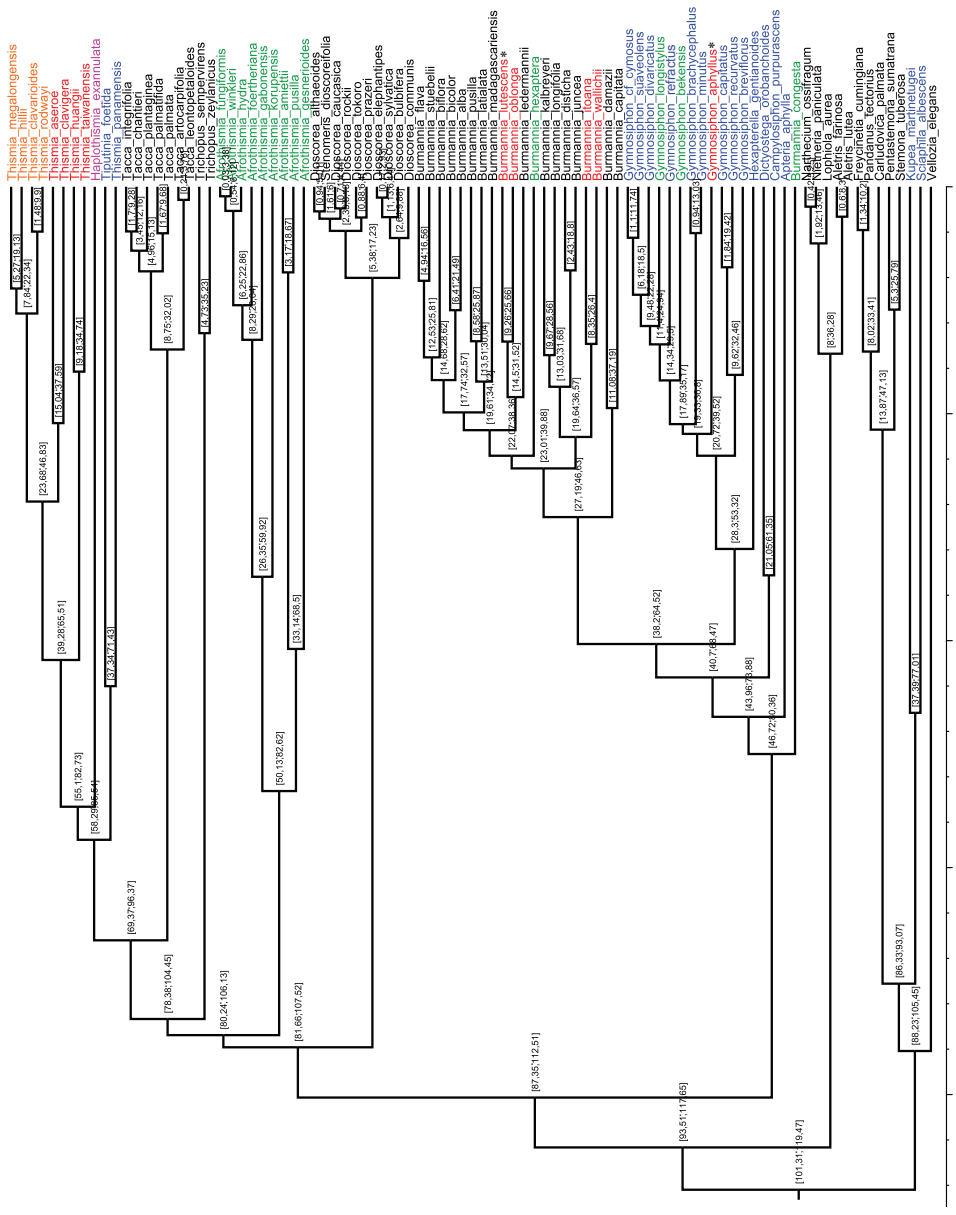

Fig. 18. Maximum clade credibility tree of Dioscoreales inferred using an uncorrelated lognormal relaxed clock method, based on a three-locus molecular dataset (nuclear 18S rDNA and mitochondrial *atpA* and *matR*). Coloured taxa show mycoheterotrophic species growing on AM fungi; red taxa were collected from Southeast Asia, orange taxa were collected from Australasia (including temperate regions), blue taxa were collected from the Americas, green taxa were collected from Africa or Madagascar and pink taxa were collected from India. Table 7 lists fossil calibration information. The scale bar shows time (million years ago; Ma), values between square brackets indicate upper and lower limits of the 95% confidence interval. Species occurring in both Southeast Asia and Australasia are indicated with an asterisk (*).

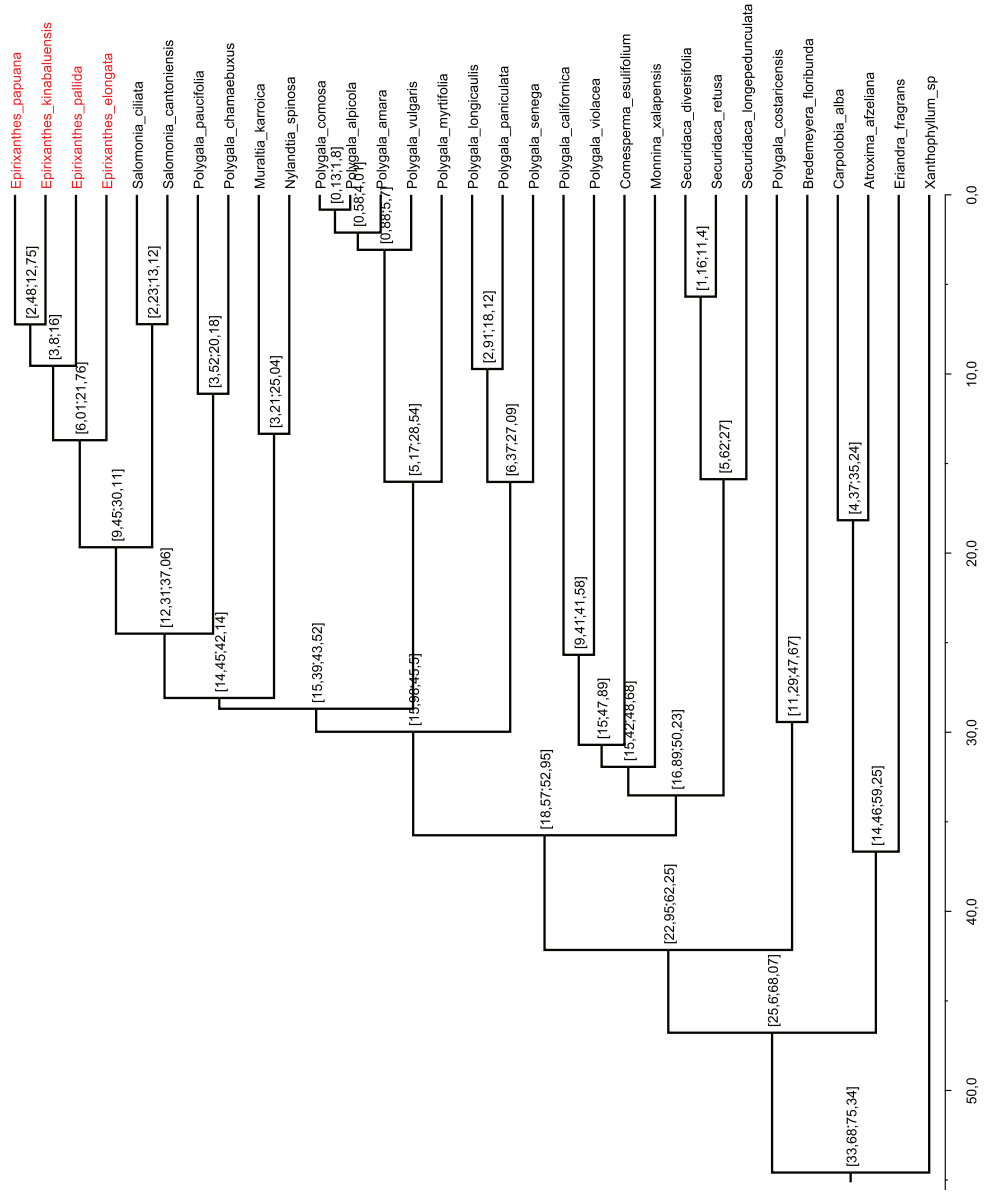

Fig. 19. Maximum clade credibility tree of *Epirixanthes* (Polygalaceae) inferred using an uncorrelated lognormal relaxed clock method, based on a two-locus molecular dataset (nuclear ITS and plastid *matK*). Coloured taxa show mycoheterotrophic species growing on AM fungi; red taxa were collected from Southeast Asia. Epirixanthes papuana also occurs in Papua New Guinea (Australasia). Table 7 lists fossil calibration information. The scale bar shows time (million years ago; Ma), values between square brackets indicate upper and lower limits of the 95% confidence interval.

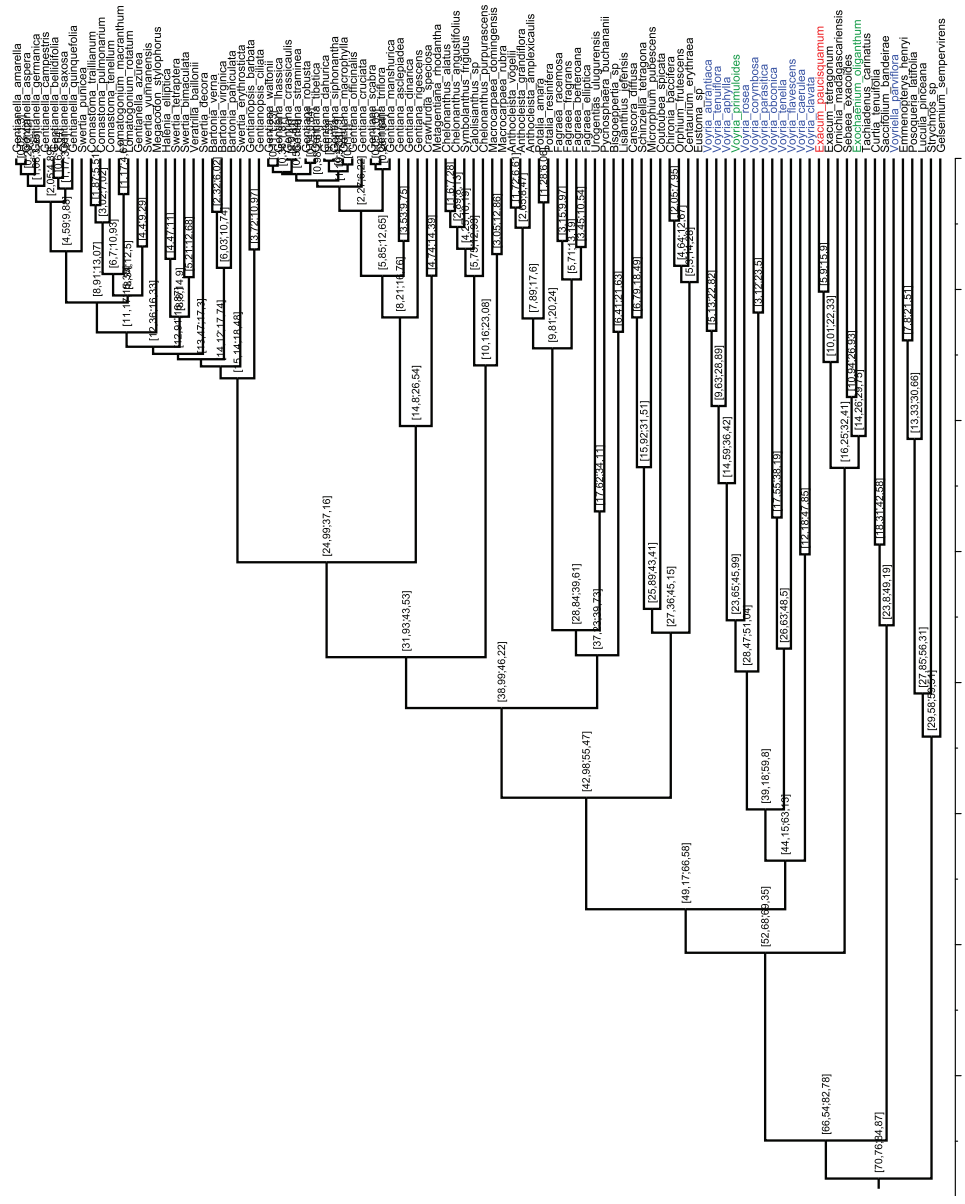

Fig. 20. Maximum clade credibility tree of Gentianaceae inferred using an uncorrelated lognormal relaxed clock method, based on a three-locus molecular dataset (nuclear ITS and plastid *matK* and *trnL-F*). Coloured taxa show mycoheterotrophic species growing on AM fungi; red taxa were collected from Southeast Asia, blue taxa were collected from the Americas and green taxa were collected from Africa or Madagascar. Table 7 lists fossil calibration information. The scale bar shows time (million years ago; Ma), values between square brackets indicate upper and lower limits of the 95% confidence interval.

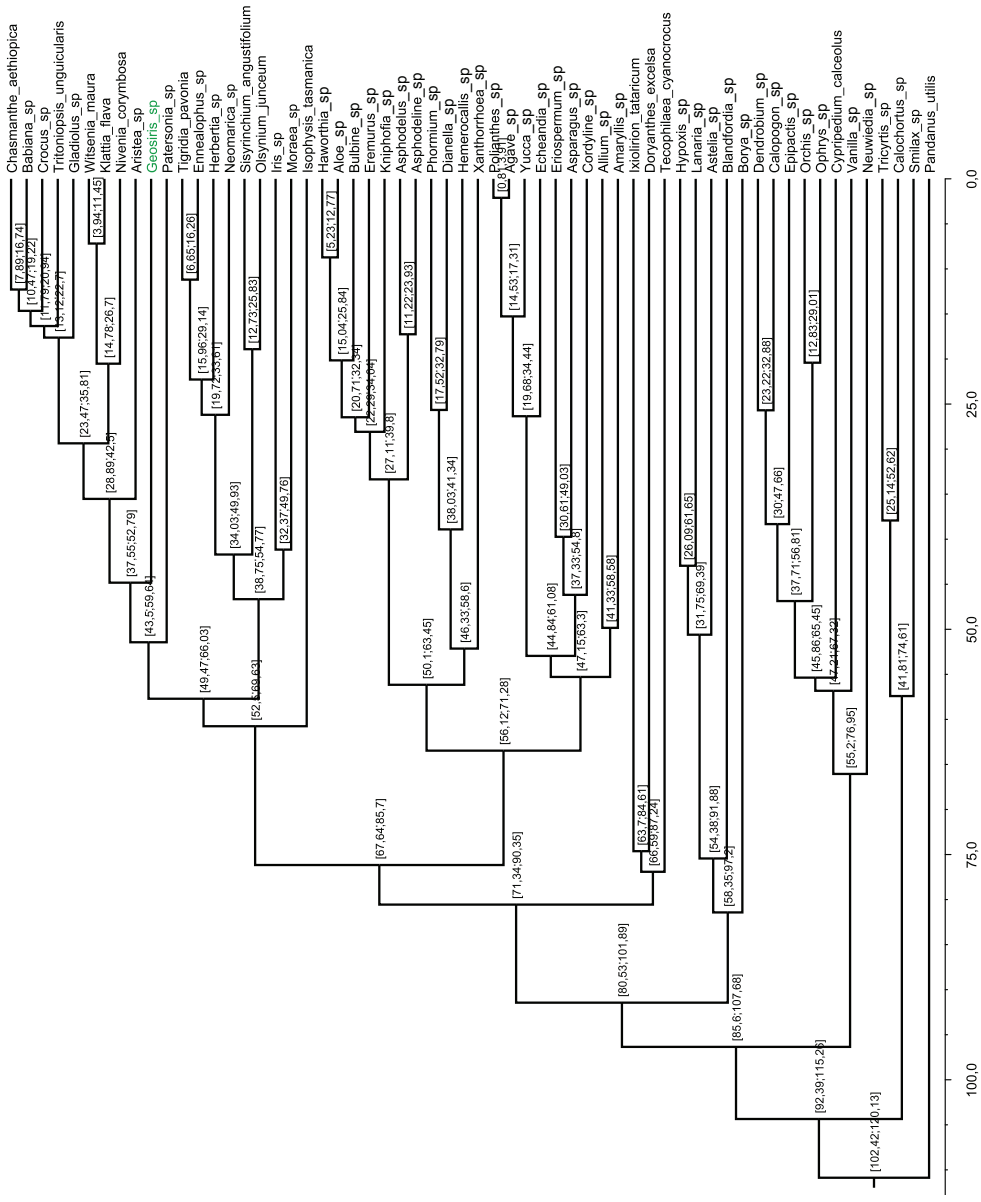

Fig. 21. Maximum clade credibility tree of *Geosiris* (Iridaceae) inferred using an uncorrelated lognormal relaxed clock method, based on a seven-locus molecular dataset (nuclear 18S rDNA and mitochondrial *atpA* and *cob*, and plastid *matK*, *rbcL*, *rps4* and *trnL-F*). Coloured taxa show mycoheterotrophic species growing on AM fungi; green taxa were collected from Madagascar. Table 7 lists fossil calibration information. The scale bar shows time (million years ago; Ma), values between square brackets indicate upper and lower limits of the 95% confidence interval.

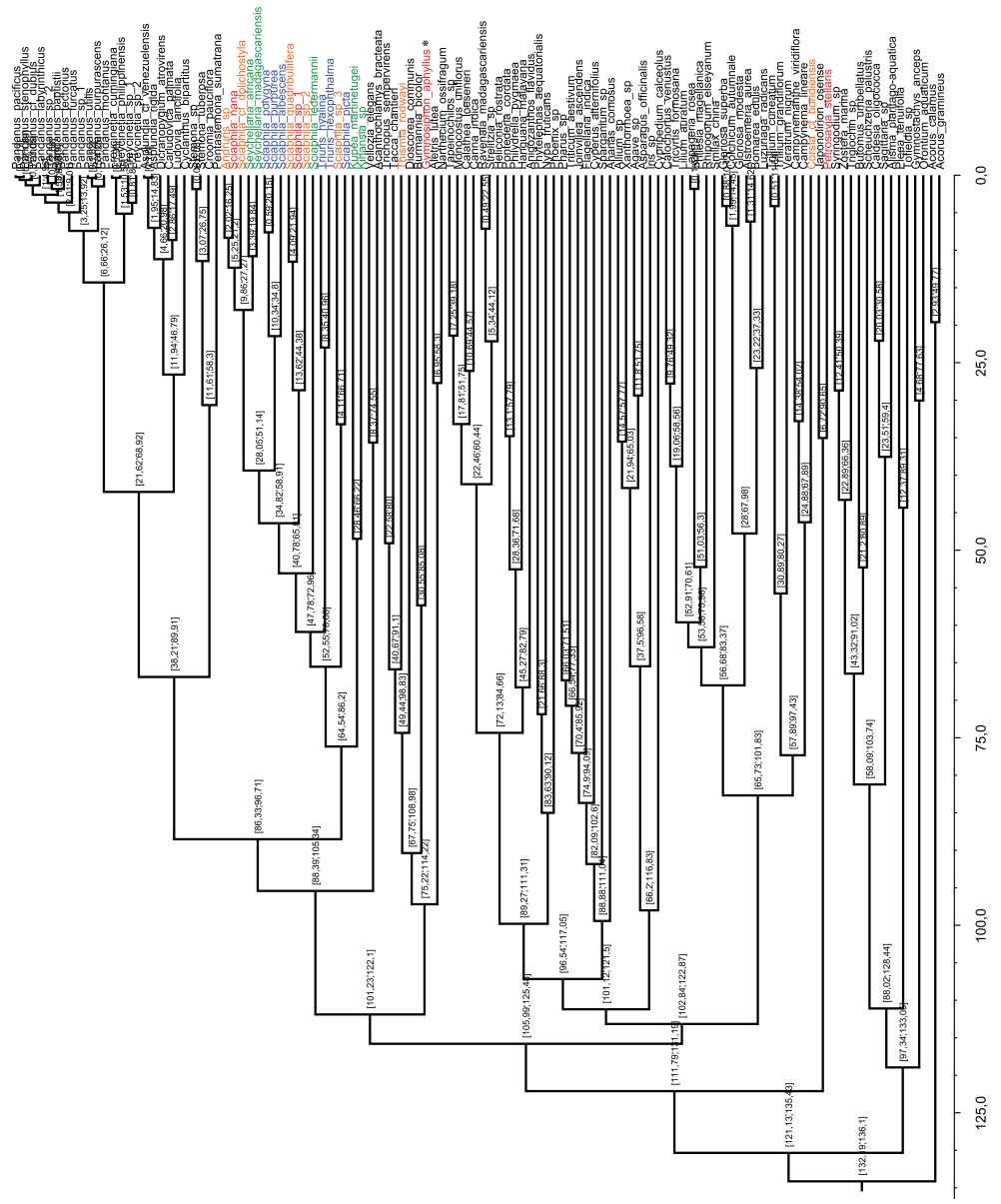

Fig. 22. Maximum clade credibility tree of Triuridaceae and *Petrosavia* inferred using an uncorrelated lognormal relaxed clock method, based on a four-locus molecular dataset (nuclear 18S rDNA and mitochondrial *atpA*, *matR* and *nad1b-c*). Coloured taxa show mycoheterotrophic species growing on AM fungi; red taxa were collected from Southeast Asia, orange taxa were collected from Australasia (including temperate regions), blue taxa were collected from the Americas and green taxa were collected from Africa or Madagascar. Table 7 lists fossil calibration information. The scale bar shows time (million years ago; Ma), values between square brackets indicate upper and lower limits of the 95% confidence interval. Species occurring in both Southeast Asia and Australasia are indicated with an asterisk (*).

5.5. Discussion

5.5.1. Divergence time estimations

Our study confirms earlier findings regarding estimates of stem and crown ages of mycoheterotrophic lineages growing on AM fungi (see Table 9). The estimated ages for the mycoheterotrophic lineages in Dioscoreales, Triuridaceae and *Georiris* are roughly in accordance with the published ages. *Petrosavia* shows a rather wide confidence interval (7-91 Ma), conflicting with the estimated stem age of 123 Ma in Janssen & Bremer (2004) (although the confidence intervals might partly overlap). For the mycoheterotrophic representatives of Gentianaceae we find slightly older estimated ages than those obtained by Merckx et al. (2013c), although with overlapping confidence intervals. The differences in estimated ages of the mycoheterotrophic lineages in Gentianaceae and *Petrosavia* are probably due to different datasets used, i.e. the addition of the plastid loci *trnL* and *matK* in the former and analyzing nuclear 18S and mitochondrial *atpA* instead of plastid *rbc*L in the latter. For the mycoheterotrophic representatives of Gentianaceae we find overlapping 95% confidence intervals with the estimates obtained by Merckx et al. (2013c). For *Petrosavia* it is unknown whether the confidence intervals overlap, but as *rbcL* is probably still functional in this genus (Logacheva et al., 2014), we cannot assume the old age found by Janssen & Bremer (2004) is an artifact of pseudogenization. More data is needed to elucidate the evolutionary history of this lineage. The results of this study provide comparable divergence times estimations between lineages and support the observation that mycoheterotrophic lineages have evolved independently at different times.

5.5.2. Ancestral range estimations

The presented ancestral range estimations for Burmanniaceae, Corsiaceae, *Epirixanthes*, Thismiaceae, Triuridaceae and *Voyria* complement previous biogeographic inferences in mycoheterotrophic lineages (e.g. Albert & Struwe, 1997; Mennes et al., 2015a; Merckx et al., 2008, 2013c). We addressed the hypothesis that different independent lineages with similar ancestral areas have similar ages. We found that this is probably not the case, as many lineages with similar ancestral areas (e.g. Burmanniaceae, Triuridaceae, *Voyria* and *Voyriella* from the Neotropics or Africa) originated at different times (100, 90, 54 and 36 Ma, respectively). Although we cannot rule out the possibility that some lineages might have originated contamporaneously in the same ancestral area as a result of overlapping confidence intervals (Table 9), we surprisingly observed a more complex pattern than the hypothesized scenario.

Lineages with a Central/ South American or African origin for example, range from a mean stem age of 100 Ma in Burmanniaceae (the oldest lineage), to 36 Ma in *Voyriella*. The former lineage however, does not exclusively consist of

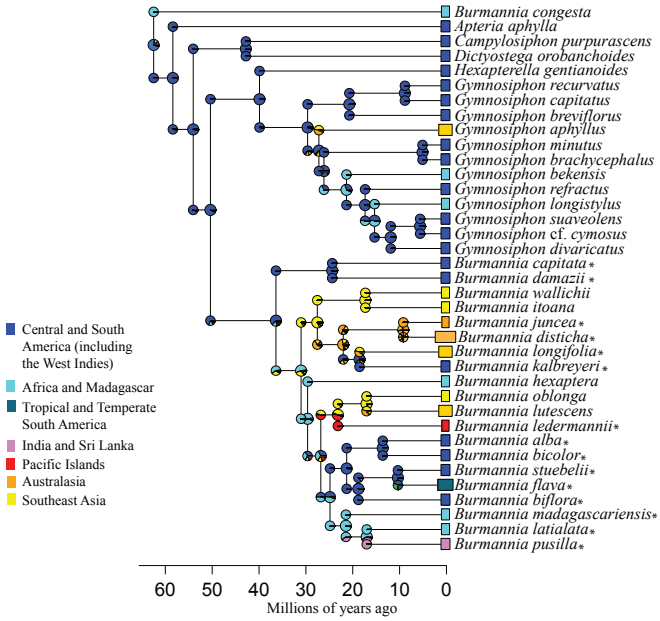

Fig. 23. Ancestral range estimation of Burmanniaceae inferred using BioGeoBEARS with a DEC+j model (see text). Parameter values for optimization of ancestral states: maximum number of areas = 7, d (dispersal) = 0.0011, e (extinction) = 0, j (founder effect speciation) = 0.0375 and LnL (log-likelihood) = -73.99. Colour interpretations are listed in the legend; different shades indicate a combination of areas. Some taxa have broader terminal squares, these indicate multiple areas in which the taxon occurs (see Table 8). Pie charts indicate relative chances of all possible ancestral areas. The scale bar indicates time (million years ago; Ma), chlorophyllous species are indicated with an asterisk (*).

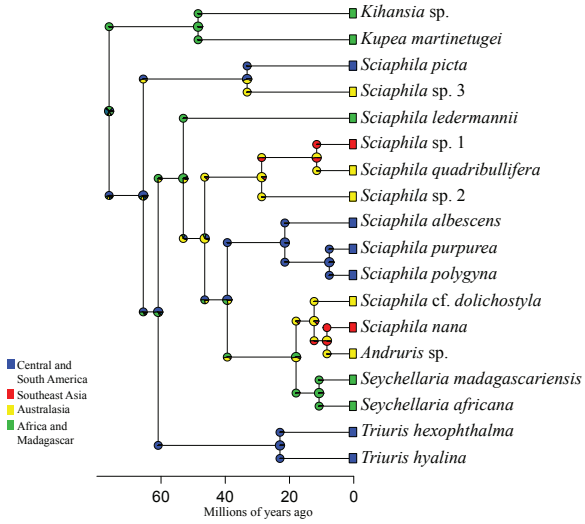

Fig. 24. Ancestral range estimation of Triuridaceae inferred using BioGeoBEARS with a DEC+j model (see text). Parameter values for optimization of ancestral states: maximum number of areas = 4, d (dispersal) = 0, e (extinction) = 0, j (founder effect speciation) = 0.1646 and LnL (log-likelihood) = -21.33. Colour interpretations are listed in the legend; different shades indicate a combination of areas. Pie charts indicate relative chances of all possible ancestral areas. The scale bar indicates time (million years ago; Ma).

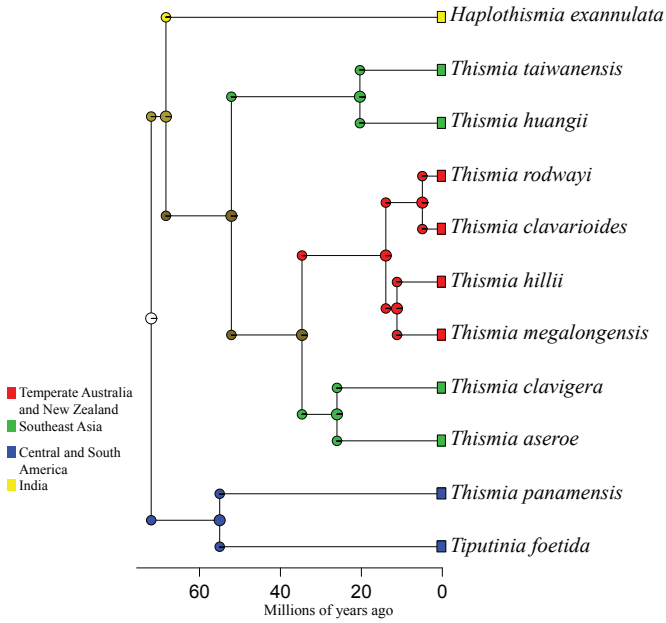

Fig. 25. Ancestral range estimation of Thismiaceae inferred using BioGeoBEARS with a DEC model (see text). Parameter values for optimization of ancestral states: maximum number of areas = 4, d (dispersal) = 0, e (extinction) = 0 and LnL (log-likelihood) = -8.84. Colour interpretations are listed in the legend; different shades indicate a combination of areas, white indicates all areas. Pie charts indicate relative chances of all possible ancestral areas. The scale bar indicates time (million years ago; Ma).

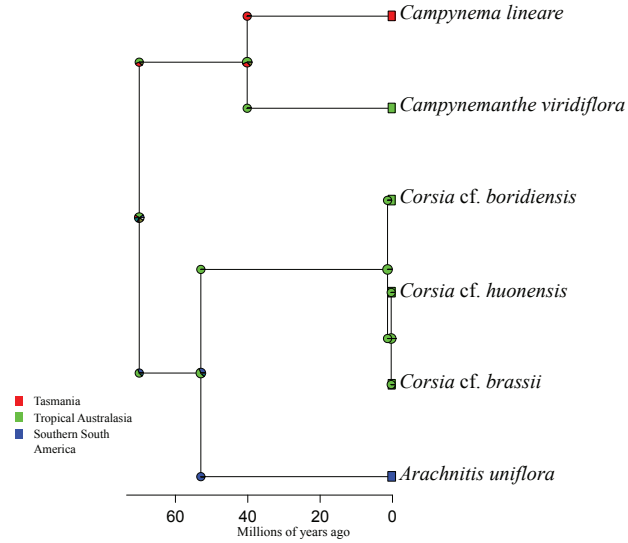

Fig. 26. Ancestral range estimation of Corsiaceae inferred using BioGeoBEARS with a DEC+j model (see text). Parameter values for optimization of ancestral states: maximum number of areas = 3, d (dispersal) = 0, e (extinction) = 0, j (founder effect speciation) = 0.2072 and LnL (log-likelihood) = -4.67. Colour interpretations are listed in the legend; different shades indicate a combination of areas. Pie charts indicate relative chances of all possible ancestral areas. The scale bar indicates time (million years ago; Ma).

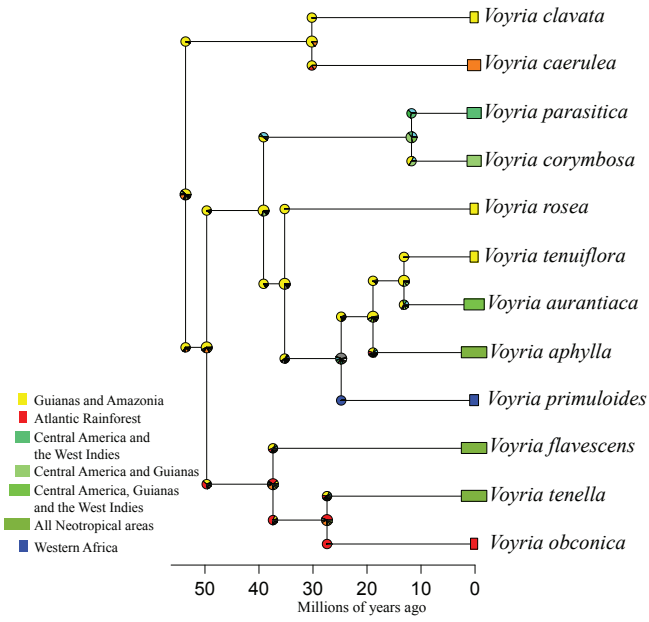

Fig. 27. Ancestral range estimation of *Voyria* inferred using BioGeoBEARS with a DEC model (see text). Parameter values for optimization of ancestral states: maximum number of areas = 5, d (dispersal) = 0.0085, e (extinction) = 0 and LnL (log-likelihood) = -36.13. Colour interpretations are listed in the legend; different shades indicate a combination of areas. Some taxa have broader terminal squares, these indicate multiple areas in which the taxon occurs (see Table 8). Pie charts indicate relative chances of all possible ancestral areas. The scale bar indicates time (million years ago; Ma).

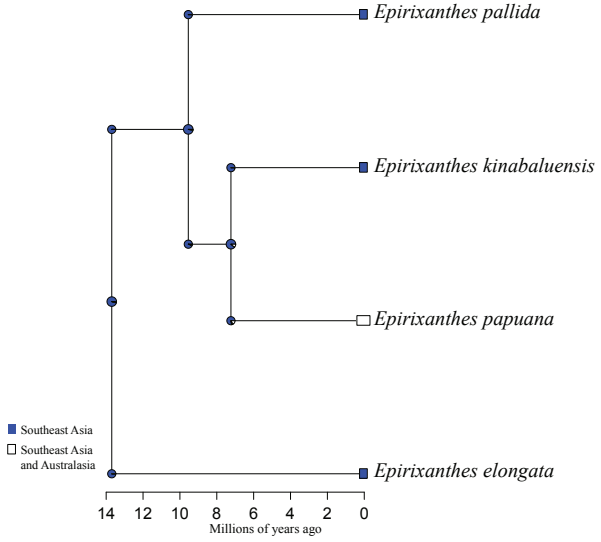

Fig. 28. Ancestral range estimation of *Epirixanthes* inferred using BioGeoBEARS with a DEC model (see text). Parameter values for optimization of ancestral states: maximum number of areas = 2, d (dispersal) = 0.0248, e (extinction) = 0 and LnL (log-likelihood) = -2.59. Colour interpretations are listed in the legend; a single taxon has a broader terminal square, this indicates multiple areas in which the taxon occurs (see Table 8). Pie charts indicate relative chances of all possible ancestral areas. The scale bar indicates time (million years ago; Ma).

mycoheterotrophic plants so that it is possible that this age estimate is not pertinent for the first full mycoheterotrophs in Burmanniaceae. However, the stem age of the fully mycoheterotrophic clade consisting of the genera *Gymnosiphon* and *Hexapterella* was estimated to be 51 Ma, still implying a great age of mycoheterotrophy in this family. The lineages Triuridaceae (mean stem age 90 Ma) and *Voyria* (mean stem age 54 Ma) probably originated in this region as well. As the lineages diversified when South America and Africa were closer together (e.g. see Sanmartín & Ronquist, 2004) and some genera occur on both continents, the exact ancestral area is unknown (Merckx et al., 2008; Mennes et al., 2013). However, for *Voyria* we found a strong indication that the area of origin of the genus lies in the Guianas and Amazonia (Fig. 27), which is in accordance with the findings of Albert and Struwe (1997) and Struwe et al. (2002). Moreover, the single African species (*V. primuloides*) probably resulted from a long-distance dispersal event (Merckx et al., 2013c). The ancestral area of Thismiaceae is ambiguous, probably due to limited taxon sampling, but the descendants from the first major split in this family (i.e. the Central and South American *Thismia panamensis* and *Tiputinia foetida*) suggest a Central/ South American origin for this ancient lineage at the current taxon sampling excluding the disjunctly distributed *Oxygyne*.

Similarly, lineages that we assume to have an exclusively African origin range from 93 Ma (*Afrothismia*) to 19 Ma (*Exochaenium oliganthum*). *Geosiris* (49 Ma) has a distribution that is restricted to Madagascar and the Comores. The common ancestor of *Geosiris* and its close relatives was hypothesized to have reached Africa and Madagascar from the proto Indian Ocean (Goldblatt et al., 2008). Although we did not reconstruct the ancestral area of *Exochaenium oliganthum*, we assume this species has originated in Africa based on its restricted distribution in this continent. This is in accordance with the findings of Kissling et al. (2009) and Kissling (2012).

A striking new result is that the Southeast Asian lineages have much younger estimated mean stem ages as compared to those from other tropical areas, i.e. 11 Ma in *Exacum paucisquamum*, 20 Ma in *Epirixanthes* and 41 Ma in *Petrosavia* (which might have originated elsewhere in Asia based on its wide Asian distribution). No fully mycoheterotrophic families putatively originated in Asia; all pantropical/widespread and relatively species rich lineages originated on other continents (Burmanniaceae, Triuridaceae, Corsiaceae). We assume that mycoheterotrophic *Exacum* species have originated in Southeast Asia based on their extant distribution. This is in accordance with the study of Yuan et al. (2003, 2005) that hypothesizes that mycoheterotrophic species in *Exacum* diverged from chlorophyllous ancestors in Southeast Asia. A Southeast Asian origin was furthermore hypothesized for *Epirixanthes*. A single species included in this study (*E. papuana*) has probably dispersed to New Guinea (Australasia) (Fig. 28). Possibly, the widespread *E. cylindrica* (which is not sampled) also reached the eastern limits of its distribution in New Guinea by dispersal. Moreover, for *Petrosavia* an Asian origin is highly plausible based on its current

distribution, although we cannot rule out the possibility of an ancestral area outside Southeast Asia, as some species of *Petrosavia* are found outside this region (Chen & Tamura, 2000; Ohashi, 2000; Merckx et al., 2013b). Additionally, Southeast Asian clades of otherwise widespread genera (e.g. in *Sciaphila* (Triuridaceae), *Thismia* (Thismiaceae), *Burmannia* and *Gymnosiphon* (both Burmanniaceae)) also tend to be younger than their Central/ South American or African relatives (see Figs. 17-22). Some Southeast Asian species have close relatives occurring in Australasia (e.g. *Burmannia*, *Epirixanthes*, *Sciaphila*), suggesting multiple dispersal events between these regions. Corsiaceae has an Australasian origin, which corresponds to the inferred southern Gondwana origin as found by Mennes et al. (2015a), because Australasia was part of Gondwana at the time of the mean stem age of Corsiaceae (70 Ma).

Only a single species from the Pacific Islands was included (chlorophyllous *Burmannia ledermannii*), which is embedded in a clade of species from other regions. As mostly widespread species of Triuridaceae and Burmanniaceae are hypothesized to have reached these islands, we expect that these mostly volcanic islands are either less suitable habitats for mycoheterotrophic plants, or, perhaps more likely, simply not (yet) colonized by mycoheterotrophic plants. However, more specimens are necessary to elucidate the role in the evolution of these islands in the historical biogeography of mycoheterotrophic plants growing on AM fungi.

All discussed patterns are only based on one third of all mycoheterotrophic species growing on AM fungi, implying that we cannot rule out the possibility of deviating patterns in the historical biogeography of other species (e.g. more ancient Southeast Asian lineages).

5.5.3. Relation to rainforest habitats

The vast majority of mycoheterotrophic plants symbiotically linked to AM fungi are restricted to rainforest. Estimates of the age of tropical rainforest habitats range from the Palaeocene (56-66 Ma) to the mid-Cretaceous (roughly 100 Ma) (Davis et al., 2005; Jaramillo et al., 2006; Couvreur et al., 2011). This may support the rainforest confinement of the ancestors of the studied lineages, particularly the ancient Neotropical or African clades in Triuridaceae, Burmanniaceae, Gentianaceae (and possibly Thismiaceae). Tropical Africa and the Neotropics have been covered with rainforest since the Late Cretaceous or Palaeocene (Morley, 2000), supporting the hypothesis that these mycoheterotrophic lineages evolved in rainforest environments. The terminal Eocene cooling event and the glacial cycles have probably had a stronger effect on the species composition in Africa as compared to South America (Morley, 2000). This might explain the particularly high number of mycoheterotrophic plants at proposed glacial refugia sites in Africa (Linder, 2001; Plana, 2004; Merckx et al., 2013d). The relative young ages of the Asian taxa can

be explained by the extreme environmental and climatological dynamics in this region during much of its geological history (Hall, 2009; De Bruyn et al., 2014). These dynamics have probably caused replacement of seasonal monsoon forests by the modern dipterocarp dominated forests in the Miocene (~5-23 Ma) (Morley, 2000). These changes in vegetation suggest environmental instability in Southeast Asia, which possibly had a restrictive effect on the origin of mycoheterotrophic lineages growing on AM fungi, as compared to lineages that originated elsewhere. Additionally, this environmental instability might have led to increased extinction rates among Southeast Asian AM mycoheterotrophic lineages. The remaining lineages originated in Eocene Madagascar (*Geosiris*) and Late Cretaceous Australasia (then part of Gondwana) (Corsiaceae). As these areas also contained rainforest habitats (Morley, 2000), we conclude that all studied mycoheterotrophic lineages most likely evolved in the rainforest. However, it is unknown whether this conclusion is true for mycoheterotrophic plants growing on other types of fungi (i.e. ectomycorrhizal and saprotrophic fungal interactions in Orchidaceae and Ericaceae) (Smith & Read, 2008). In Ericaceae, some mycoheterotrophic species were suggested to have evolved from chlorophyllous forest inhabitants (Cullings et al., 1996; Bidartondo, 2005), implying a putative forest origin for these taxa as well. However, more research is needed to assess whether the biogeographic history of these taxa is similar to the patterns found for mycoheterotrophic plants growing on AM fungi. Similarly, it is unknown whether Orchidaceae show a similar pattern. Orchidaceae is the family with the largest number of mycoheterotrophic species, which can be either fully, partially or initially mycoheterotrophic (Freudenstein & Barrett, 2010; Merckx et al., 2013b). Within Orchidaceae over 30 shifts from autotrophy to heterotrophy were hypothesized, which has resulted in roughly 210 mycoheterotrophic species in 43 genera across three subfamilies (Vanilloideae, Orchidoideae and Epidendroideae) (Freudenstein & Barrett, 2010). Additionally, all orchids are probably initially mycoheterotrophic (Leake, 1994), and many genera contain both autotrophic and mycoheterotrophic species (e.g. *Cephalanthera*, *Corallorhiza* and *Neottia*) (Freudenstein & Barrett, 2010; Merckx et al., 2013b). This implies that the evolutionary pathways to mycoheterotrophy in Orchids are different from those found in most other mycoheterotrophic lineages. As a consequence, we cannot assume that the biogeographic patterns found for mycoheterotrophic plants growing on AM fungi, are applicable to mycoheterotrophic orchids.

5.6. Conclusions and implications for conservation

Mycoheterotrophic plants linked to AM fungi occur in all three major tropical areas (i.e. Central/ South America, Africa/Madagascar and tropical Asia). Additionally, some lineages have extended their range to wet forest areas in subtropical and temperate regions (Merckx et al., 2013b). This study suggests that mycoheterotrophic lineages from Southeast Asian origin are younger than those from Africa and the Neotropics at the current taxon sampling. More ancient lineages of mycoheterotrophic plants

in Southeast Asia either did not originate or became extinct, but these scenarios remain speculative due to the lack of information available. The latter scenario might imply that mycoheterotrophic plants are vulnerable to the climatic changes that were more pronounced in Southeast Asia as compared to the other major tropical regions (e.g. Morley, 2000). Their possible vulnerability, the putative origin of mycoheterotrophic plants in rainforests and the previously reported link between mycoheterotrophic plants and rainforest habitats (e.g. Maas et al., 1986; Leake, 1994) have implications for conservation; protection of mycoheterotrophic plants is strongly dependent on conservation of rainforest habitats. The presented evidence strongly suggests that mycoheterotrophic species growing on AM fungi are vulnerable to extinction when rainforests are under threat. This is exemplified by several species of *Thismia* (Thismiaceae). *Thismia macahensis*, *T. caudata* and *T. fungiformis* are all likely extinct, as they are exclusively known from single collection sites which are now probably deforested (Maas et al., 1986). Additionally, some recently described species in Triuridaceae and *Afrothismia* are reported as being critically endangered due to their limited range and deforestation risk of their habitat (Cheek, 2003; Sainge, 2013). Conservation of rainforests is therefore highly important for mycoheterotrophic species growing on AM fungi.

Table 9. Summary of divergence time estimations (in Ma) and inferred ancestral areas for each lineage.

Independent lineage	Stem age (Ma)*	Crown age (Ma)*	Stem age (Ma) from literature	Ancestral area	Source
Afrothismia (Thismiaceae)	93 (80-106)	66 (50-83)	79-109	Possibly Africa^	Merckx et al. (2010a)
Burmanniaceae	100 (87-113)	63 (47-80)	98-118	Neotropics or Africa	Merckx et al. (2010a)
Corsiaceae	70 (47-93)†	53 (30-76)†	47-93	Australasia/ Gondwana	Mennes et al. (2015a
Epirixanthes (Polygalaceae)	20 (9-30)†	14 (6-22)†	9-30	Southeast Asia	Mennes et al. (2015b)
Exacum paucisquamum (Gentianaceae)	11 (6-16)	na	6-16	Southeast Asia^	Merckx et al. (2013c)
Exochaenium oliganthum (Gentianaceae)	19 (11-27)	na	3-7	Africa^	Merckx et al. (2013c)
Geosiris (Iridaceae)	45 (38-53)	na	49	Madagascar^	Goldblatt et al. (2008)
Petrosavia (Petrosaviaceae)	41 (7-91)	na	123	Possibly Asia^	Janssen & Bremer (2004)
Thismiaceae	83 (69-96)	72 (58-86)	68-92	unknown	Merckx et al. (2010a)
Triuridaceae	90 (86-97)	76 (65-86)	62-103	Neotropics or Africa	Mennes et al. (2013)
Voyria (Gentianaceae)	54 (44-63)	57 (49-67)	40-55	Neotropics	Merckx et al. (2013c)
Voyriella (Gentianaceae)	36 (24-49)	na	15-42	Neotropics^	Merckx et al. (2013c)

* Resulting from the present study.

^ Not analyzed using BioGeoBEARS, but inferred from the current distribution of the lineage.

† These inferred ages were obtained from Mennes et al. (2015a) (Corsiaceae) and Mennes et al. (2015b) (Epirixanthes).