



Universiteit
Leiden
The Netherlands

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

Mennes, C.B.

Citation

Mennes, C. B. (2015, October 6). *Antiquities of the rainforest: evolution of mycoheterotrophic angiosperms growing on Glomeromycota*. Retrieved from <https://hdl.handle.net/1887/35731>

Version: Not Applicable (or Unknown)

License: [Licence agreement concerning inclusion of doctoral thesis in the Institutional Repository of the University of Leiden](#)

Downloaded from: <https://hdl.handle.net/1887/35731>

Note: To cite this publication please use the final published version (if applicable).

Cover Page



Universiteit Leiden



The handle <http://hdl.handle.net/1887/35731> holds various files of this Leiden University dissertation

Author: Mennes, Constantijn

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

Issue Date: 2015-10-06

Chapter 6

Summary and conclusions

Summary and conclusions

Mycoheterotrophic plants growing on AM fungi have evolved many times independently. As presented in **Chapter 1**, the main aim of this thesis is to understand the evolutionary history of mycoheterotrophic angiosperms growing on AM fungi. New insights are presented in the evolutionary trajectories towards this peculiar lifestyle. For three fully mycoheterotrophic lineages (the families Triuridaceae and Corsiaceae, and the genus *Epirixanthes* [Polygalaceae]) phylogenetic reconstructions and historical biogeography are discussed in **Chapters 2-4**. Moreover, implications for the phylogeny and biogeography of mycoheterotrophic angiosperms in general are presented in **Chapter 5** based on all evidence presently available. Based on these insights, the following research questions are addressed.

Does a molecular dating analysis point towards a great antiquity for Triuridaceae, as was suggested previously?

Based on the pantropical distribution of the genus *Sciaphila*, the fully mycoheterotrophic family Triuridaceae (Pandanales) was suggested to be rather old (Leake, 1994). The existence of putative Triuridaceae fossils of Upper Cretaceous age from New Jersey (USA) further seemed to suggest a great age of the family. However, a detailed divergence time estimation of the family was hitherto lacking. The study presented in **Chapter 2** provides a phylogeny of Triuridaceae, which is needed to study the family's age of divergence. The relationships among the order Pandanales were reconstructed based on four molecular markers. We found that the monophyletic family Triuridaceae likely descended from the second major split in the order. The discovered relationships between the remaining (chlorophyllous) families in Pandanales (i.e. Cyclanthaceae, Pandanaceae, Stemonaceae and Velloziaceae) are identical to those published previously. Moreover, all tribes recognized in Triuridaceae are monophyletic at the current taxon sampling (although Sciaphileae becomes paraphyletic when taxa are included for which limited DNA sequence data are available). Based on this phylogeny, using a Bayesian relaxed molecular clock approach, we estimated a Cretaceous (or Lower Paleocene) stem age of Triuridaceae, which indeed points towards a great antiquity for the family.

What is the reason that systematic affinities of Triuridaceae have been uncertain for over 150 years?

The circumscription of Triuridaceae was always clear (Maas-van de Kamer, 1995). However, the classification of the family among the angiosperms has been debated since the family's description in 1841 (Miers, 1841). Triuridaceae were placed in dicots (Champion, 1847; Twaites, 1861; Poulsen, 1890; Heintze, 1927), monocots (e.g. Miers, 1841, 1850; Decaisne, 1868; Mueller, 1858; Engler, 1915), and "dictyogens", a transition class between monocots and dicots (Lindley, 1846). Subsequent authors followed the classification of Engler (1897), in which Triuridaceae were classified in

its own monocot order Triuridales (e.g. Schumann & Lauterbach, 1905; Hutchinson, 1934; Thorne, 1968; Cronquist, 1981; Dahlgren et al., 1985; Reveal, 1992). Based on DNA sequence data Chase et al. (2000) placed Triuridaceae in the order Pandanales, a move supported by subsequent findings of Caddick et al. (2002), Davis et al. (2004) and **Chapter 2** of this thesis. This implies that earlier classifications did not reflect evolutionary relationships, likely as a result of the lack of distinct morphological characters to resolve high-level taxonomic relations. The reconstructed old age and the elevated mutation rates indicated by long-branch lengths in the phylogeny as presented in **Chapter 2**, as well as the mycoheterotrophic lifestyle of Triuridaceae, most likely account for the strong morphological differences between Triuridaceae and its chlorophyllous relatives. This is probably the source of the systematic problems in Triuridaceae.

What are the inter- and intra-familial relationships of Corsiaceae?

Corsiaceae form a monocot family that has a clear floral synapomorphy: its outer median tepal is enlarged and forms a labellum, which is similar (but not homologous) to the orchid labellum (Rudall & Bateman, 2002). However, despite this synapomorphy the monophyly of the family is questioned on the basis of morphological differences and phylogenetic inference (Ibisch et al., 1996; Neyland & Hennigan, 2003). Moreover, lack of available material has prevented attempts to reliably place the family, although a close relationship to Liliales has been proposed based on limited DNA sequence data (Davis et al., 2004; Fay et al., 2006; Petersen et al., 2013). In **Chapter 3** a phylogenetic reconstruction of Corsiaceae is presented that is based on a dataset containing DNA sequences from most monocot orders. We found that the genera *Corsia* and *Arachnitis* form a strongly supported clade and each genus emerge at the same phylogenetic position independently. This strongly suggests the family is monophyletic. Moreover, additional DNA sequence data from all three genomic compartments (i.e. nuclear, mitochondrial and plastid) indicate a strongly supported sister-group relation with Campynemataceae (Liliales), corroborating earlier findings.

What explains the disjunct distribution of Corsiaceae?

The family Corsiaceae consists of three genera that have a disjunct distribution across the southern Pacific Ocean; *Corsia* is found in Australasia whereas *Arachnitis* is found in southern South America. The enigmatic genus *Corsiopsis* is exclusively known from a single locality in southern China. Although that genus was not included in this study, we estimated divergence times using an Bayesian uncorrelated lognormal relaxed-clock analysis in **Chapter 3**. We found a mean crown age of Corsiaceae is of 53 Ma (95% confidence interval 30–76 Ma). This crown age overlaps with the plate-tectonic split of South America, Antarctica and Australasia from Gondwana, indicating that the current disjunct distribution of *Corsia* and *Arachnitis* is explained

by ancient Gondwana vicariance.

What is the closest chlorophyllous relative of Epirixanthes?

Although a close relationship between *Epirixanthes* (Polygalaceae) and *Salomonina* (Polygalaceae) was suggested previously based on morphology (e.g. Bello et al., 2012), lack of DNA sequence data has prevented further testing this hypothesis. In **Chapter 4** a phylogenetic reconstruction of Polygalaceae is presented based on nuclear and plastid DNA sequence data. Our results indicate a strongly supported sister-group relation between *Epirixanthes* and *Salomonina*, supporting earlier findings. We furthermore estimated divergence times using a Bayesian uncorrelated lognormal relaxed-clock analysis. This points towards a Miocene-Oligocene origin, implying that *Epirixanthes* is a relatively young mycoheterotrophic lineage. This might explain the morphological similarity to *Salomonina*.

To which fungi are species of Epirixanthes symbiotically bound?

Using morphology, Imhof (2007) identified the fungal symbionts of *Epirixanthes* as arbuscular mycorrhizal (AM) fungi. However, the degree of specialization towards these fungi remained largely unknown. By sequencing partial nuclear 18S rDNA in **Chapter 4**, we found that species from *Epirixanthes* are exclusively linked to AM fungi of Glomeraceae. Species from the chlorophyllous relatives *Salomonina* and *Polygala* are linked to both Glomeraceae and Acaulosporaceae. This suggests a higher degree of specialization in *Epirixanthes* as compared to its closest relatives. However, as most AM fungi engaged in mycoheterotrophic interactions belong to Glomeraceae, only plants that are linked to rather narrow clades of Glomeraceae are interpreted as highly specialized. Thus, species of *Epirixanthes* can be regarded as more specialized towards AM fungi than their close relatives, but they are less specialized as compared with some other mycoheterotrophic species.

What general trends can be observed by comparing all lineages of mycoheterotrophic plants growing on AM fungi?

Based on the **Chapters 2-4**, as well as additional previous studies (e.g. Goldblatt et al., 2008; Merckx et al., 2008, 2010a, 2013c), we addressed the hypothesis that *different independently evolved lineages with similar ancestral areas have similar estimated divergence times* in **Chapter 5**. To study this, divergence times for all mycoheterotrophic lineages growing on AM fungi are estimated based on Bayesian uncorrelated lognormal relaxed-clock analyses. Moreover, broad ancestral areas were reconstructed for all lineages for which sufficient data were available. We observed a complex pattern in which different lineages have originated at different times on different continents. Mycoheterotrophic lineages growing on AM fungi have evolved in all tropical regions. However, Southeast Asian taxa tend to be younger than lineages that originated elsewhere (e.g. in the Neotropics and/or Africa). The results

furthermore indicate that all lineages have evolved in rainforest environments. This implies evolution of mycoheterotrophy is associated with rainforest habitats.

Suggestions for future studies

This thesis provides novel insights in the evolution of mycoheterotrophy, in particular in the phylogenetic relationships and biogeography of lineages growing in symbiosis with AM fungi. As our understanding of the deep evolutionary history of mycoheterotrophic plants increases, new exciting questions emerge and gaps in our knowledge becomes apparent. Based on the presented results, I propose the following avenues for future research.

Genome-wide phylogenetic inferences. For some lineages, particularly for Triuridaceae, problems stills exist regarding the exact phylogenetic placement. Since the presented results for Triuridaceae in **Chapter 2** are based on only four DNA loci, much resolution can probably be gained by analyzing more DNA sequence data. Next generation sequencing has already yielded promising results in Corsiaceae (**Chapter 3**), indicating its strong potential for reconstructing evolutionary history. Moreover, including more species of mycoheterotrophic plants (this thesis includes a third of all species) will also increase the resolution of our evolutionary inferences.

Fungal specificity. Though not the focus of this thesis, the ability of the studied plants to obtain carbon compounds from fungi is what sets these plants apart from other angiosperms. **Chapter 4**, and many other studies (e.g. Merckx & Bidartondo, 2008; Merckx et al., 2012; Hynson & Bruns, 2009), shed light on the ecological interactions between mycoheterotrophic plants and their fungal symbionts. These results indicate that most AM fungi involved in mycoheterotrophy belong to the family Glomeraceae, and that some mycoheterotrophic species are highly specialized, whereas others are not. However, we are only beginning to understand the evolutionary transition from mutualism to parasitism in mycorrhizal symbioses.

Pollination. Although insights on phylogeny, biogeography and fungal ecology in mycoheterotrophic plants growing on AM fungi have increased considerably in the past few years, pollination mechanisms remain largely unknown. Such insights on pollination biology would be a valuable contribution to the existing knowledge and will clarify why selection has favoured such mycoheterotrophic plants having complex flowers while they generally remain rather small - and some even barely emerge from the leaf litter.

Conservation. The studied mycoheterotrophic plants are almost exclusively found in rainforests and our findings in **Chapter 5** suggest evolution of these plants might be contingent on stable rainforests habitats. This implies mycoheterotrophic angiosperms linked to AM fungi are probably vulnerable to deforestation. As several newly described species (e.g. Cheek, 2003; Sainge, 2013) are documented

Antiquities of the rainforest

as threatened and other species might well be extinct already (e.g. Maas et al., 1986), attempts should be made to better understand how we can prevent these plants from going extinct. Exploration of the possibility to cultivate mycoheterotrophic plants might be promising, and may also yield important insights in the ecological interactions between the plants and their fungal symbionts.