DIVERSITY, PHYLOGENY, AND EVOLUTION IN THE MONOCOTYLEDONS
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Cretaceous Origins of Myco-Heterotrophic Lineages in Dioscoreales

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Abstract—Shifts from autotrophy to myco-heterotrophy occurred in many clades of arbuscular mycorrhizal angiosperms. In the monocot order Dioscoreales, in which 14 of 23 genera include achlorophyllous myco-heterotrophic species, there is evidence for multiple independent origins of a myco-heterotrophic lifestyle. To estimate the divergence times of these myco-heterotrophic lineages, a Bayesian relaxed clock analysis was applied to a three-gene (18S rDNA, *atpA*, *nad1 b-c*) dataset of Dioscoreales using previously published estimates of crown and stem node ages of Dioscoreales as secondary calibration points. Our results indicate that extant myco-heterotrophic lineages of Dioscoreales are relatively ancient and at least two clades are estimated to have Late Cretaceous origins. Although the timing of the origin of the myco-heterotrophic mode of life remains speculative, the crown groups of these two myco-heterotrophic clades are estimated to be at least 45 million years old. This suggests that myco-heterotrophs can emerge, persist, and diversify over a considerable amount of time.

Keywords—Achlorophyllous plants, Burmanniaceae, diversification, molecular dating, Thismiaceae.

The roots of about 80% of all land plants form mutualistic associations with Glomeromycota fungi known as arbuscular mycorrhizas. This symbiosis improves the nutrition uptake of the plant due to the easy penetration of the rhizosphere by the hyphae, which in turn receive plant carbohydrates that are

needed for the completion of the fungal life cycle (Smith and Read 2008). Fossil evidence has shown that arbuscular mycorrhizas were present at least 400 million years ago, and it has been suggested that they have assisted the invasion of plants into terrestrial ecosystems (Pirozynski and Malloch 1975; Remy et al. 1994; Bonfante and Genre 2008).

Within several clades of arbuscular mycorrhizal flowering plants the arbuscular mycorrhizal mutualism evolved into parasitism leading to non-photosynthetic plants that 'steal' carbohydrates and nutrients from mycorrhizal networks without any apparent reward for the fungi (Selosse et al. 2006). This strategy allows the plants – which are described as myco-heterotrophic (Leake 1994) – to grow in the shaded conditions of the forest understory habitats (Bidartondo et al. 2004). There are more than 400 species of myco-heterotrophic plants in 87 angiosperm genera (Leake 2004). Despite this relatively large number, the timing of the origin of myco-heterotrophic clades in various angiosperm lineages remains speculative. Many myco-heterotrophic plant genera have pantropical distributions and are therefore presumed to be relatively old (Leake 1994). However, there is only one series of fossils that may be assigned to an extant myco-heterotrophic lineage. These fossils are from the Upper Cretaceous (± 90 million year old [my]) and show affinities with extant Triuridaceae, a family that exclusively contains achlorophyllous myco-heterotrophic plants (Gandolfo et al. 1998, 2002). However, it remains speculative whether the fossilized plants in question were in fact myco-heterotrophic (Gandolfo et al. 2002) and belong to Triuridaceae (Furness et al. 2002).

The increasing availability of sequence data from myco-heterotrophic plants now allows us to estimate the date of the split between myco-heterotrophic lineages and their autotrophic relatives with molecular clock methods. With a Bayesian uncorrelated relaxed clock method and nuclear and mitochondrial sequence data we construct a hypothesis for the phylogenetic relationships in Dioscoreales and simultaneously estimate the divergence times of the myco-heterotrophic lineages within the order. Dioscoreales (sens. Caddick et al. 2002b) have approximately 655 species, and are one of the smaller orders of monocotyledons (Govaerts et al. 2007). Of the 23 genera of Dioscoreales, *Dioscorea* L. ('yam') comprising ca. 450 species is by far the most species-rich, geographically widespread, and economically important genus of the order. In contrast, the second largest genus, *Burmannia* L., contains only ca. 60 species and more than half of the genera of Dioscoreales consist of only one or two species (Maas-van de Kamer 1998, Govaerts et al. 2007). The current circumscription of the order Dioscoreales by Caddick et al. (2002b) recognizes three families: Nartheciaceae, Burmanniaceae, and Dioscoreaceae. In this classification all myco-heterotrophic Dioscoreales species are part of Burmanniaceae. However subsequent molecular studies have provided ample evidence that the myco-heterotrophic lineage Burmanniaceae tribe Thismieae – often regarded as a separate family Thismiaceae (Schlechter 1921; Hutchinson 1934, 1959; Dahlgren et al. 1985; Takhtajan 1997) – is

not directly related to the remaining Burmanniaceae. Instead Thismiaceae were found to be the sister group of *Tacca* Forst. (Dioscoreaceae) (Merckx et al. 2006; Yokoyama et al. 2008). More recently analyses using 18S rDNA and *atpA* data revealed the paraphyly of Thismiaceae, as the African genus *Afrothismia* Schltr. did not form a clade with the other genera of Thismiaceae (Merckx and Bidartondo 2008; Merckx et al. 2009). According to these results myco-heterotrophy evolved independently in at least three major clades within Dioscoreales: Burmanniaceae, Thismiaceae, and *Afrothismia*. These studies also indicate that the earlier five-family classification by the Angiosperm Phylogeny Group (APG 1998) reflects the phylogenetic relationships in Dioscoreales better than the three-family classification by Caddick et al. (2002b). Hence we use the APG classification (including Nartheciaceae, which were still 'unplaced' in APG) to denote clades throughout this study.

Materials and Methods

Taxon Sampling—According to Caddick et al. (2002b) Dioscoreales comprises 23 genera. However, these authors recognized *Geomitra* Becc. as a separate genus from *Thismia* Griff. based on their analyses, which place *Geomitra clavigera* Becc. within Burmanniaceae tribe Burmannieae (Caddick et al. 2002a). We speculate that his odd position is the result of a long-branch attraction artifact due to the use of parsimony inference methods and plastid nucleotide data (*rbcL* and *atpB*), which was shown to have higher rates of nucleotide substitutions in achlorophyllous taxa of Dioscoreales (Caddick et al. 2002a). Previous studies have provided ample evidence that this genus should be reduced to *Thismia* (Stone, 1980; Rübsamen 1986; Maas-van de Kamer 1998; Merckx et al. 2006; Merckx et al. 2009) and we included this species as *Thismia clavigera* (Becc.) F. Muell. in our study. A new genus of Thismiaceae, *Tiputinia* P.E. Berry & C.L. Woodw., was recently described from Ecuador leveling the number of Dioscoreales genera again to 23 (Woodward et al. 2007). With data of *Marthella* Urb., *Miersiella* Urb. (Burmanniaceae), and *Oxygyne* Schltr. (Thismiaceae) missing, our study samples 20 genera. Our sampling includes only nine species of *Dioscorea*. Although it would be desirable to increase this number, most previous studies supported *Dioscorea* as a monophyletic group (Caddick et al. 2000b; Wilkin et al. 2005). Sequences of *Pandanus tectorius* Parkinson ex J.P. du Roi (Pandanaceae) and *Kupea martinetugei* Cheek (Triuridaceae) were used as outgroups.

Data—Available Dioscoreales accessions (*atpA*: Davis et al. 2004; Merckx and Bidartondo 2008; Merckx et al. 2009; *nad1 b-c*: Merckx et al. 2006, 2008; 18S rDNA: Caddick et al. 2002b; Merckx et al. 2006, 2008, 2009; Merckx and Bidartondo 2008) were taken from GenBank. Additional 18S rDNA and *nad1 b-c* sequences were obtained following the methods described by Merckx et al. (2006). A number of *atpA* sequences were obtained with the methods described by Merckx and Bidartondo (2008). All data sets were manually aligned in Mac-

Clade 4.04 (Maddison and Maddison, 2001). The 18S rDNA data set consisted of 80 taxa and 1673 characters. Because we were unable to obtain *nad1 b-c* data for some *Afrothismia* species and *atpA* data for some *Burmannia* species the *nad1 b-c* data set of 1686 characters consisted of 75 taxa and the *atpA* data included 1140 characters and 76 taxa. All GenBank accessions are listed in the Appendix.

Phylogenetic Analysis and Molecular Dating—Phylogenetic relationships and divergence times were simultaneously estimated with a Bayesian uncorrelated relaxed clock analysis using BEAST v1.4.7 (Drummond and Rambaut 2007). Phylogenetic analyses have shown the presence of considerable substitution rate heterogeneity in 18S rDNA and *atpA* of Dioscoreales (Merckx et al. 2009). This extreme rate heterogeneity may challenge the accuracy of relaxed clock methods. Most relaxed clock approaches consider closely related branches to have more similar rates of evolution than distant branches, a property termed 'autocorrelation' (Pybus 2006). However, if rates of adaptive substitution are not tied to inherited factors this assumption is questionable (Ho 2009). BEAST does not assume autocorrelated rate change *a priori* and thus we speculate it may be able to model the observed rate heterogeneity more accurately (Drummond et al. 2006). Moreover, it is currently the only application that can infer phylogenies under a relaxed clock model (Renner 2005; Pybus 2006).

The GTR+I+G model of evolution was identified as the best-fit model for each data set separately using ModelTest v3.06 under both AIC and LRT (Posada and Crandall 1998) and this model of nucleotide evolution was used for each partition separately during the BEAST analysis following the 'BEAST partitioning' manual (http: //tlpcouvreur.googlepages.com/beastpartitioning). Other BEAST analysis settings were specified using BEAUti v1.4.7 (Drummond and Rambaut 2007): the uncorrelated lognormal clock model (Drummond et al. 2006) was chosen and two normally distributed secondary calibration points were selected: a prior of 121 ± 2.1 my was set for the root of the tree (split between Pandanales and Dioscoreales) and a prior of 116 ± 2.6 my for the crown node of Dioscoreales. These age estimation were taken from a previous study by Merckx et al. (2008), who used Penalized Likelihood rate smoothing on a comprehensive 18S rDNA dataset of monocots with six calibration points to infer the divergence times within Burmanniaceae. The secondary calibration points are consistent with previous estimates of stem and crown age estimates of Dioscoreales based on different datasets and different relaxed molecular clock methods (Janssen and Bremer 2004; Merckx and Bidartondo 2008). Secondary calibration points may cause major inconsistencies and their use for molecular dating analyses is not favored (e.g.,. Shaul and Graur 2002). However, no fossils of Dioscoreales are currently known, prohibiting the use of primary calibration points for this study. Yet, by transferring the error associated with the age estimations to the new analysis – using normally distributed calibration points – the use of secondary calibration points becomes much more sound. Also, Merckx et al. (2008) did not detect large differences in age estimations of Burmanniaceae between

primary and secondary calibration strategies. Apart from the calibration points the distribution of all other analysis priors was set to uniform. A starting tree for the BEAST analysis was constructed with GARLI v0.951 (Zwickl 2006) and subsequently dated using non-parametric rate smoothing (NPRS) in r8s v1.71 (Sanderson 2003) to meet the priors set for the BEAST analysis. This was done to overcome a bug in BEAST v1.4.7. Without specifying a tree as a starting point for the analysis the program will construct an UPGMA tree. When this tree does not meet the specified priors it will prevent the analysis from starting ('zero likelihood error'). Although our starting tree is sub-optimal it is only used as a starting point for the MCMC analysis and when the analysis runs for a sufficient amount of time this tree will not influence the results.

Using BEAST posterior distributions of parameters were approximated using two independent Markov chain Monte Carlo analyses of $3x10^7$ generations following a discarded burnin of 3,000,000 generations (10%). Convergence of the chains was checked using TRACER 1.4 (Rambaut and Drummond, 2007) and the effective sampling size (ESS) parameter was found to exceed 100 for all parameters, which suggests acceptable mixing and sufficient sampling. The XML BEAST input file is available from the first author on request.

Results

Phylogenetic Relationships—The Bayesian relaxed clock analysis yielded a robust phylogeny (Fig. 1): most deep relationships are significantly supported (Bayesian posterior probability ≥95%). Only the position of Trichopodaceae does not receive support. Seven well-supported major clades within Dioscoreales can be distinguished. These account for the six families in Dioscoreales and *Afrothismia*. Nartheciaceae are the first diverging lineage in Dioscoreales, followed by Burmanniaceae. The sister group of Burmanniaceae is maximally supported and contains five strongly supported clades. All taxa of *Dioscorea* form a highly supported clade with *Stenomeris* nested within it. *Trichopus*, *Tacca* and *Afrothismia* are each strongly supported monophyletic groups, and also comprise a clade with the remaining Thismiaceae (*Thismia*, *Haplothismia* Airy Shaw, and *Tiputinia*). The inferred phylogeny provides evidence for at least six independent origins for a myco-heterotrophic mode of life in Dioscoreales. A minimum of four shifts to myco-heterotrophy occurred in Burmanniaceae. The Thismiaceae and *Afrothismia* account for the two other shifts.

Divergence Time Estimates—The mean divergence time estimates and corresponding confidence intervals of the seven main clades of Dioscoreales are listed in Table 1. All seven major clades originated before the end of the Cretaceous (stem node significantly older than 65.5 my). According to these estimates two myco-heterotrophic clades originated before the end of the Cretaceous: Thismiaceae and *Afrothismia.* The stem node of a clade within Burmanniaceae with myco-heterotrophic species of *Burmannia*, *Campylosiphon* Benth., *Dictyostega*

Fig. 1. Maximum clade credibility chronogram of Dioscoreales obtained by Bayesian uncorrelated relaxed clock analysis of 18S rDNA, *atpA*, and *nadı b-c* data. Nodes that were constrained (secondary calibration points) are indicated with open circles. Bayesian posterior probabilities of major clades are shown above branches. Achlorophyllous myco-heterotrophic branches are shown in grey. Mean stem node ages of these clades are shown in boxes (in my). Grey bars denote 95% confidence interval on these estimates. Vertical dashed line delimits the Cretaceous-Tertiary boundary.

Miers, *Apteria* Nutt., *Hexapterella* Urb., and *Gymnosiphon* Blume is estimated to have a mean age of 74.9 my (Cretaceous) but the upper limited of the 95% confidence interval is 52.2 my (Eocene). The stem nodes of three other myco-heterotrophic lineages within Burmanniaceae are significantly younger than the K/T boundary. These clades are estimated to have diverged during a period spanning the Paleocene and the Miocene.

Discussion

Phylogenetic Relationships in Dioscoreales—The results of the phylogenetic analyses corroborate previous studies (Merckx and Bidartondo 2008; Merckx et al. 2009) and are in disagreement with the current classification of Dioscoreales (Caddick et al. 2002b). The topology suggests that both Burmanniaceae and Dioscoreaceae sens. Caddick et al. (2002b) are not monophyletic. The surprising position of *Afrothismia*, outside Thismiaceae, as found by Merckx and Bidartondo (2008) and Merckx et al. (2009), is supported by this analysis. Hence molecular data support the hypothesis that *Afrothismia* and Thismiaceae diverged independently, acquiring a myco-heterotrphic mode of life and a highly similar habit through convergent evolution. Our topology and previous phylogenetic analyses (Merckx et al. 2006, 2009; Merckx and Bidartondo 2008; Yokoyama et al. 2008) underline the need for a revised Dioscoreales classification. Future work will focus on establishing an updated classification that reflects the phylogenetic relationships in this order.

Divergence Time Estimates in Dioscoreales—We find evidence for a Cretaceous origin of major Dioscoreales lineages. This observation supports the results of Bremer (2000) and Janssen and Bremer (2004) that major Dioscoreales clades, like most extant monocot families, were already present at the Cretaceous-Tertiary boundary. Moreover the crown nodes of Burmanniaceae, Thismiaceae and *Afrothismia* date back to the Late Cretaceous, although for Burmanniaceae a Paleocene or Eocene crown node age cannot be rejected. Diversification in Thismiaceae and *Afrothismia* started notably earlier than in related autotrophic clades and as a result Thismiaceae and *Afrothismia* species are remarkably old. In addition, since the age estimations were obtained through an analysis incorporating secondary calibration points and accompanying errors from a study that used fossil calibration points as minimum age constraints, all resulting age estimates should be treated as minimum ages (Sanderson et al. 2004). Therefore the actual divergence times may be considerably older. On the other hand, it is possible that the elevated substitution rates of 18S rDNA, *nad1 b-c* and *atpA* in these taxa (Merckx et al. 2006, 2009) may cause artifacts, and DNA regions without this extreme rate heterogeneity should be used to verify these estimates. However, an ancient origin of Thismiaceae and *Afrothismia* is not unexpected. Thismiaceae have a widespread distribution with *Thismia* occurring in the Neotropics, North America, South East Asia, Australia, and New

Zealand. Yet their absence from volcanic islands with suitable habitats (e.g., West Indies, Hawaii, the Comoros, Vanuatu, etc.) indicates that long-distance dispersal is difficult for these taxa. Moreover, most Thismiaceae and particularly species of *Afrothismia* are restricted to areas that are hypothesized to have served as rainforest refugia during various periods of global drought (e.g., Guineo-Congolian rainforest and Eastern Arc mountains for *Afrothismia* (Franke 2004; Cheek and Jannerup 2005) and the Mata Atlantica forest for neotropical species of *Thismia* (Franke 2007)). These observations seem to support an ancient origin for Thismiaceae and species of *Afrothismia* and require further investigation.

Origin of Myco-Heterotrophy—Our analyses show that several different Dioscoreales lineages – at least six according to the presented topology – acquired a fully myco-heterotrophic habit independently. Because many achlorophyllous *Burmannia* species are not represented in our sampling, the number of independent origins for strict myco-heterotrophy in Dioscoreales is likely to be higher. Although there is no direct evidence that the shift from autotrophy to myco-heterotrophy occurred in the common ancestor of strictly achlorophyllous clades, such an assumption seems reasonable. The alternative, that all extant species of these clades (e.g., *Thismia*, *Afrothismia*, *Gymnosiphon* and relatives) became myco-heterotrophic independently, would require that all the various morphological and physiological adaptations associated with a myco-heterotrophic mode of life evolved independently in every single species and that all green-leaved ancestors became extinct. According to our molecular dating results, at least two achlorophyllous myco-heterotrophic lineages within Dioscoreales originated as early as the Late Cretaceous. Whether the shift from autotrophy towards myco-heterotrophy occurred directly after or even simultaneously with the origin of these lineages is speculative. It may well

Clade	Stem node age	Crown node age
Nartheciaceae	116 (111-120)	41 (10-92)
Burmanniaceae	109 (98-118)	75 (52-99)
Dioscoreaceae	100 (86-113)	26 (9-54)
Afrothismia	95 (79-109)	63 (45-83)
Trichopodaceae	90*	19 (2-42)
Thismiaceae	79 (68-92)	68 (49-85)
Taccaceae	79 (68-92)	35 (15-60)

Table 1. Crown and stem node age estimates in my for the seven major Dioscoreales clades recovered with a Bayesian uncorrelated relaxed clock analysis. 95% confidence intervals are listed in parentheses. Clades containing myco-heterotrophic species are indicated in bold. * No confidence interval for the stem node of Trichopodaceae could be calculated because this node is present in less than 50% of the sampled trees.

be possible that such shifts occurred a considerable time after the origins of the lineages in question. However, due to the reasons mentioned above a shift towards myco-heterotrophy in Thismiaceae and *Afrothismia* probably occurred before the diversification within these lineages and thus before the estimated crown node ages of Thismiaceae and *Afrothismia* (Table 1). The upper limits of the credibility intervals for both crown node ages are placed in the Eocene. Crown node estimates are extremely vulnerable to sampling (Pirie et al. 2005) and because our sampling is far from complete our estimates should be regarded as minimum ages. Despite the impossibility of accurately pinpointing the emergence of myco-heterotrophy these results show that at least in Dioscoreales a myco-heterotrophic mode of life is in some cases ancient, particularly when compared to the origin of the extant diversity of the autotrophic species. This demonstrates that myco-heterotrophic angiosperm lineages were able to emerge, persist, and diverge over a considerable amount of time and thus are remarkably evolutionarily stable. An ancient origin of a myco-heterotrophic mode of life is probably not restricted to Dioscoreales. Given the phylogenetic position of myco-heterotrophic lineages such as Triuridaceae (Pandanales), Corsiaceae (Liliales), and *Petrosavia* Becc. (Petrosaviaceae) and age estimates for these groups (Janssen and Bremer 2004; Merckx et al. 2008) a pre-Tertiary origin of these clades is highly plausible.

Extant species of myco-heterotrophic Dioscoreales are exceptionally well adapted to a life in the understory of moist, dense primary rain forests, hence they are almost exclusively found in such habitats (Maas et al. 1986). However, fossil evidence indicates that tropical rain forest as we know it today only appeared after the K/T boundary and that Late Cretaceous forests were apparently more open and drought adapted (Tiffney 1984; Wing and Boucher 1998; Morley 2000; Johnson and Ellis 2002). Therefore if ancestral species of Thismiaceae and *Afrothismia* were already fully myco-heterotrophic in the late Cretaceous they would have lived in the most shaded local habitats in open sub-humid forests. A shift to myco-heterotrophy can best be explained as an escape from the intensive competition for sunlight (Bidartondo et al. 2004) and thus seems much more likely when shaded habitats are available. A recent study on Malpighiales diversification opposed the absence of closed-canopy rain forest in the Cretaceous and provided evidence for a Mid-Cretaceous origin of modern tropical rain forests (Davis et al. 2005). These forests may have been an ideal shaded habitat for pre-Tertiary myco-heterotrophic species.

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Appendix 1

Voucher information and GenBank accessions of taxa used in this study. *Taxon*—18S rDNA, *atpA*, *nad1 b-c*; Voucher, Country; — = missing sequence.

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Burmanniaceae: *Apteria aphylla* (Nutt.) Barnh. ex Small—DQ786035, EU421007, DQ786094; Chase 156, NCU; USA. *Burmannia alba* Mart.—DQ786074, EU421008, DQ768133; Nakajima et al. 323, U; Brazil. *Burmannia bicolor* Mart.—DQ786073, GQ469514, DQ786132; Ruysschaert 0636, GENT; Surinam. *Burmannia biflora* L.—DQ786070, GQ469515, DQ786142; Chase 157, NCU; USA. *Burmannia capitata* (Walt. ex Gmel.) Mart.—DQ786066, GQ469516, DQ786128; Neyland 958, MCN; USA. *Burmannia capitata* (Walt. ex Gmel.) Mart.—DQ786065, EU421009, DQ786129; Maas et al. 9606, U; French Guiana. *Burmannia coelestis* Don—DQ786068, EU421010, DQ786123; Cameron s.n., NCU; Malaysia. *Burmannia congesta* (Wright) Jonk.—DQ786061, EU421011, DQ786120; Jongkind 5463, WAG; Liberia. *Burmannia congesta* (Wright) Jonk.—EU816712, EU421012, EU816757; Merckx et al. 119, LV; Cameroon. *Burmannia damazii* Beauverd—DQ786071, EU421013, DQ786127; da Silva et al. 2195, U; Brazil. *Burmannia disticha* L.—U59947, —, DQ786124; Wilkin 1017, K; Thailand. *Burmannia flava* Mart.—DQ786076, GQ469517, DQ786131; Jansen-Jacobs et al. 5379, U; Guyana. *Burmannia flava* Mart.—DQ786077, EU421014, DQ786130; daSilva et al. 2087, U; Brazil. *Burmannia hexaptera* Schltr.—EU420994, EU421015, EU816758; Merckx 101, LV, Cameroon. *Burmannia itoana* Mak.—DQ786078, EU421016, DQ786145; Kun-Ping Lo 821, PPI; Taiwan. *Burmannia juncea* Sol. ex R.Br.—DQ786063, GQ469518, DQ786143; Harwood 1499, BR; Australia. *Burmannia latialata* Pobég.—DQ786062, EU421017, DQ786125; Jongkind 5923, WAG; Gabon. *Burmannia ledermannii* Jonk.—DQ786079, —, DQ786135; van Royen 4478, L; New Guinea. *Burmannia longifolia* Becc.—AF309398, EU421018, DQ786138; Cameron s.n., NCU; Malaysia. *Burmannia lutescens* Becc.—AF309401, AY299732, DQ786144; Caddick 352, K; Malaysia. *Burmannia madagascariensis* Mart.—AF309399, EU421019, DQ786126; Caddick 312, K; Madagascar. *Burmannia madagascariensis* Mart.—EU420995, EU421020, EU816759; De Block 1978, BR; Madagascar. *Burmannia oblonga* Ridl.—DQ786064, EU421021, DQ786140; Wilkin 866, K; Thailand. *Burmannia oblonga* Ridl.—GQ469513, GQ469519, GQ469501; Ruyters s.n., LV; Thailand. *Burmannia pusilla* (Wall. ex Miers) Thw.—DQ786075, —, DQ786136; Madhusoodanan s.n., U; India. *Burmannia* sp.—EU816702, —, DQ887985; Dessein 1021, BR; Zambia. *Burmannia stuebelii* Hieron. and Schltr.—DQ786067, EU421022, DQ786139; Weigend 98/420, K; Peru. *Burmannia wallichii* (Miers) Hook.f.—DQ786069, EU421023, DQ786141; Zhang s.n., K; Hong Kong.

Campylosiphon purpurascens Benth.—EU420996, EU421024, EU816760; Banki 1257, U; Guyana. *Cymbocarpa refracta* Miers—DQ786038, EU421025, DQ786095; Kress s.n., US; Costa Rica. *Dictyostega orobanchoides* (Hook.) Miers—DQ786056, EU421026, DQ786119; Maas et al. 9620, U; French Guiana. *Gymnosiphon aphyllus* Bl.—AF309402, EU421030, DQ786102; Caddick 353, K; Malaysia. *Gymnosiphon bekensis* Letouzey—EU420998, EU421031, EU816761; Merckx et al. 117, LV; Cameroon. *Gymnosiphon breviflorus* Gleason—DQ786041, EU421032, DQ786098; Ek 1577, U; French Guiana. *Gymnosiphon capitatus* (Benth.) Urb.—DQ786054, EU421033, DQ786114; Maas et al. 9616, U; Guyana. *Gymnosiphon divaricatus* (Benth.) Benth. and Hook.f.—DQ786044, EU421034, DQ786107; Maas et al. 9657, U; Guyana. *Gymnosiphon longistylus* (Benth.) Hutch. and Dalziel—DQ786051, EU421035, DQ786103; Breteler et al. 9705, WAG; Gabon. *Gymnosiphon minutus* Snelders and Maas—DQ786048, EU421036, DQ786109; Maas et al. 9651, U; French Guiana. *Hexapterella gentianoides* Urb.—DQ786057, EU421038, DQ786118; Maas et al. 9614, U; French Guiana. **Dioscoreaceae:** *Dioscorea althaeoides* R. Knuth—EU420997, EU421027, —; RBGE 19940649; China. *Dioscorea bulbifera* L.—AF069203, FJ215775, —; RBGE 19821960; Cultivated. *Dioscorea caucasica* Lipsky—FJ215769, FJ215779, —; RBGE 19110024; Cultivated. *Dioscorea communis* (L.) Caddick & Wilkin—EU186223, AY277804, GQ469502; V. Merckx 2, LV; cultivated. *Dioscorea elephantipes* (L'Hér.) Engl.—FJ215767, FJ215777, GQ469503; RBGE 19280228; South Africa. *Dioscorea prazeri* Prain & Burkill—DQ786089, EU421028, DQ786156; Wilkin 878, K; Thailand. *Dioscorea rockii* Prain & Burkill—DQ786090, EU421029, DQ786157; Chase 21052, K; Sri Lanka. *Dioscorea sylvatica* (Knuth) Eckl.—FJ25768, FJ215778, —; RBGE 19803437; South Africa. *Dioscorea tokoro* Makino ex Myabe—DQ786088, FJ215776, DQ786158; Merckx 01, LV; Cultivated. *Stenomeris dioscoreifolia* Planch.—DQ786087, EU421042, DQ786159; Risdale 550, ISU; Philippines. **Nartheciaceae:** *Aletris lutea* Small— DQ786092, FJ215780, DQ786160; Anderson 36, LV; USA. *Lophiola aurea* Ker Gawl.—DQ786091, EU421039, DQ786161; Newell 23/8, K; USA. *Metanarthecium luteo-viride* Maxim.—AF309410, EU421040, DQ786162; Inoue s.n., K; Japan. *Narthecium ossifragum* Huds.—AF309411, AY299809, DQ786163; Jaquemart 46-9, LV; Belgium. *Nietneria paniculata* Steyerm.— EU186219, EU421041, GQ469504; O. Hokche & P.J.M. Maas 849, U; Venezuela. **Pandanaceae (outgroup):** *Pandanus tectorius* Parkinson ex Du Roi—AY952391, EU421052, GQ469505; Merckx 201, LV; Cultivated. **Taccaceae:** *Tacca artocarpifolia* Seem.—AF309397, EU421043, DQ786155; Caddick 305, K; Madagascar. *Tacca chantrieri* André—DQ786086, EU421044, DQ786152; Chase 175, NCU; Cultivated. *Tacca integrifolia* Ker-Gawl.—DQ786085, EU421045, DQ786153,; Boyce 1074, K; Malaysia. *Tacca leontopetaloides* (L.) Kuntze—EU420999, AF039252, GQ469506; Wilkin 817, K; Thailand. *Tacca palmata* Blume—EU421000, EU421046, —; Chase 6201, K; Cultivated. *Tacca palmatifida* Baker—DQ786084, FJ215774, DQ786084; Chase 1377, K; Indonesia. *Tacca parkeri* Seem.—EU421001, AY299849, —; Berry 5620, MO; Venezuela. *Tacca plantaginea* (Hance) Drenth—U42063, FJ215773, GQ469507; BG Leiden 520520, Cultivated. **Thismiaceae:** *Afrothismia foertheriana* T. Franke,

Sainge & Agerer—EU420988, EU421002, —; Merckx et al. 126, LV; Cameroon. *Afrothismia gabonensis* Dauby & Stévart—FJ215766, FJ215772, —; Dauby 167, BRLU; Gabon. *Afrothismia gesnerioides* H. Maas—EU420989, EU421003, GQ469508; Merckx et al. 110, LV; Cameroon. *Afrothismia hydra* Sainge and Franke—EU420990, EU421004, —; Merckx et al. 115, LV; Cameroon. *Afrothismia hydra* Sainge and Franke—FJ215765, FJ215771,—; Merckx et al. 113, LV; Cameroon. *Afrothismia korupensis* Sainge & T. Franke—EU420991, EU421005, GQ469509; Merckx et al. 114, LV; Cameroon. *Afrothismia winkleri* Schltr.—EU420992, EU421006, —; Merckx et al. 106, LV; Cameroon. *Haplothismia exannulata* Airy Shaw—DQ786082, EU421037, DQ786146; Sasidharan and Sujanapal 30476, KFRI; India. *Thismia aseroe* Becc. —AF309404, EU421048, DQ786149; Caddick 349, K; Malaysia. *Thismia clavigera* (Becc.) F. Muell.—AF309405, EU421049, DQ786150; Caddick 354, K; Malaysia. *Thismia panamensis* (Standley) Jonk.—DQ786081, EU421050, DQ786151; Aizprua 2946, LV; Panama. *Thismia rodwayi* F. Muell.—AF309403, AY299849, DQ786148; Wapstra s.n., HO; Australia. *Thismia taiwanensis* Yang, Saunders and Hsu—DQ786080, EU421051, DQ786147; Yang et al. 28981, PPI; Taiwan. *Tiputinia foetida* P. Berry & C.L. Woodward—FJ215764, FJ215770, GQ469510; Alvaro Javier Perez Castaneda s.n., LV; Ecuador. **Trichopodaceae:** *Trichopus sempervirens* (H. Perrier) Caddick & Wilkin—AF309395, AY299724, —; Wilkin et al. 948, K; Madagascar. *Trichopus zeylanicus* Gaertn.—AF309394, AY277805, GQ469511; Chase 16354, K; Sri Lanka. **Triuridaceae (outgroup):** *Kupea martinetugei* Cheek—EU816706, GQ469512, GQ469520; Merckx et al. 102, LV; Cameroon.