New insights in the long-debated evolutionary history of Triuridaceae (Pandanales)

Constantijn B. Mennes, Erik F. Smets, Sainge N. Moses, Vincent S.F.T. Merckx

Molecular Phylogenetics and Evolution 69 (2013) 994–1004

Contents lists available at SciVerse ScienceDirect

Molecular Phylogenetics and Evolution

journal homepage: www.elsevier.com/locate/ympev

New insights in the long-debated evolutionary history of Triuridaceae (Pandanales)

Constantijn B. Mennes [a,*], Erik F. Smets [a,b], Sainge N. Moses [c], Vincent S.F.T. Merckx [a]

[a] Naturalis Biodiversity Center, Leiden University, PO Box 9517, Leiden, The Netherlands
[b] Section Ecology, Evolution and Biodiversity Conservation, KU Leuven, Kasteelpark Arenberg 31, PO Box 2437, BE-3001 Leuven, Belgium
[c] Centre for Tropical Forest Sciences (CTFS), University of Buea, Department of Plant & Animal Sciences, P.O. Box 63, Buea, Cameroon

ARTICLE INFO

Article history:
Received 22 February 2013
Revised 29 May 2013
Accepted 31 May 2013
Available online 21 June 2013

Keywords:
Mycoheterotrophy
Pandanales
Triuridaceae

ABSTRACT

The mycoheterotrophic plant family Triuridaceae (Pandanales) is hypothesized to be an old family, mainly based on its pantropical distribution. The existence of fossils from the Upper Cretaceous, assigned to Triuridaceae may form additional support for a great age of the family, although the affinity of these fossils to Triuridaceae is questioned. Although the circumscription of Triuridaceae has never been problematic, probably due to its distinct morphological characters, its systematic relationship has been under debate since the family was described around 1840. The lack of synapomorphies suitable for resolving higher taxonomic relationships is a function of the family's reduced vegetative growth and the highly modified floral structures. Molecular studies have assigned Triuridaceae to Pandanales, but its exact phylogenetic position remains unknown. In the present study the phylogeny of the Pandanales was reconstructed using four molecular markers and the divergence age estimates were obtained with a relaxed molecular clock method. We found that Triuridaceae are monophyletic and most likely descent form the second major split in Pandanales. The relationships between the other Pandanales families (Cyclanthaceae, Pandanaceae, Stemonaceae and Velloziaceae) are otherwise in accordance with earlier studies. Velloziaceae are sister to the rest of the Pandanales, Stemonaceae are most likely sister to a clade consisting of Pandanaceae and Cyclanthaceae, and the latter two families are sister to each other. All currently recognized tribes within Triuridaceae are also monophyletic at current taxon sampling. We estimate that the family has a Cretaceous (or Lower Paleocene) stem age, which is in accordance with earlier predictions. This old age, along with elevated mutation rates indicated by long branch lengths and the family's mycoheterotrophic lifestyle, might account for the substantial morphological differences between Triuridaceae and its closest relatives.

1. Introduction

The mycoheterotrophic mode of life has allowed several plant lineages to grow in shaded conditions of the tropical forest understory, because uptake of carbon from their mycorrhizal partners enables them to grow without photosynthesis (Bidartondo, 2005; Leake, 1994). These plants are often specialized on a very narrow range of host fungi. Despite the intuitively vulnerable nature of extreme host specialization, some mycoheterotrophic lineages were recently found to be relatively old. The genus *Afrothismia* (Thismiaceae) for example, is estimated to have a crown age minimally in the Eocene and a stem age in the Cretaceous, implicating an apomorphy age for mycoheterotrophy of great antiquity (Merckx and Bidartondo, 2008), suggesting evolutionary stability is possible in mycoheterotrophic plants.

The family Triuridaceae Gardner (Pandanales Berchtold and J. Presl) is such a putatively ancient lineage. It consists of ~50 fully mycoheterotrophic species in nine genera (Maas-van de Kamer and Weustenfeld, 1998; Mabberley, 2008). According to Cheek (2003), the family can be subdivided in three tribes: the African Kupeaeae Cheek (*Kihansia* and *Kupea*), the pantropical Sciaphileae Miers (*Sciaphila*, *Seychellaria*, and *Soridium*) and the Neotropical Triurideae Miers (*Peltophyllum*, *Triuridopsis*, and *Triuris*). Triuridaceae have a pantropical distribution, reaching the subtropics in Argentina, Paraguay, and Japan (Maas-van de Kamer and Weustenfeld, 1998; Maas and Rübsamen, 1986; Van de Meerendonk, 1984). An old age of the family age was suggested by Leake (1994), on the basis of the pantropical distribution of *Sciaphila*. Ninety-million year old fossil flowers from the Turonian Raritan formation in New Jersey, USA were assigned to Triuridaceae (Gandolfo et al., 2002, 1998), suggesting an old age for the family as well. These fossils were classified as the sister group of Triurideae (Gandolfo et al., 2002). Despite morphological resemblance with extant Triuridaceae, the

* Corresponding author.
E-mail address: constantijn.mennes@naturalis.nl (C.B. Mennes).

1055-7903/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.ympev.2013.05.031

fossil flowers show a divergent pollen type, questioning their proposed affinity to Triuridaceae (Furness et al., 2002).

The phylogenetic relationships within Triuridaceae and its position in the Pandanales remain under debate (Davis et al., 2004; Rudall and Bateman, 2006). Like many mycoheterotrophic plants, Triuridaceae are characterized by reduced vegetative growth (i.e. lacking foliage leaves) and modified floral structures (Leake, 1994). Distinctive characters for resolving higher taxonomic relationships are thus mostly lacking, hampering the classification of the plants. The family was first described by Miers (1841) on the basis of *Triuris hyalina* and formalized into Triuraceae by Gardner (1843), later to be corrected to Triuridaceae by Lindley (1846). The latter name was validated and conserved in 1987 (Greuter et al., 1988). Although its circumscription has always been clear (Maas-van de Kamer, 1995) probably as a result of its distinct morphology, systematic relationships of Triuridaceae have long been rather obscure. The unclear affinities of *Sciaphila* (“affinitas plane obscura”) were pointed out by Endlicher as early as 1837, who assigned the genus to Artocarpeae (currently in Moraceae, Rosales). Champion (1847) positioned Triuridaceae also close to the Moraceae and Urticaceae. Heintze (1927) assigned it to Rosales, whereas Poulsen (1890) suggested affinities with Ranunculaceae. Thwaites (1861) also classified Triuridaceae as dicots. Lindley (1846) classified it in the Dictyogens, a “transition class” between monocots and dicots, whereas Miers (1850, 1841) treated Triuridaceae as endogens (monocots), suggesting a position between Fluviales (currently in Alismatales) and Burmanniaceae (1841). He later suggested that Fluviales are the closest relatives of Triuridaceae, whereas Le Maout and Decaisne (1868) included the family in Burmanniaceae. Mueller (1858) classified Triuridaceae in the Helobiae (currently in Alismatales), but Engler (1889) commented that it is not clear whether the family belongs to the monocots. However, he later decided that without evidence to the contrary, Triuridaceae must be assigned to the monocots (Engler, 1915). He furthermore assigned it to its own order Triuridales Engler (Cronquist, 1981; Engler, 1897), a move supported by many authors throughout the twentieth century (e.g. Schumann and Lauterbach, 1905; Hutchinson, 1934; Thorne, 1968; Cronquist, 1981; Dahlgren et al., 1985; Reveal, 1992). Thorne (1968) even added a superorder, Triuridiflorae. Cronquist (1988, 1981, 1968) considered both Triuridaceae and Petrosaviaceae to be assigned to the order Triuridales, which in itself was positioned in the subclass Alismatidae. Dahlgren et al. (1985) maintained the classification of Thorne (1968) in which Triuridaceae are classified as belonging to Triuridiflorae and points out that similarities with Alismatiflorae may well be the result of convergent evolution. Reveal (1992) placed Triuridiflorae (“Triuridanae”) in its own subclass Triurididae Takhtajan ex Reveal. Many authors mention the uncertainties of the proposed affinities. The existence of all these different classification schemes indicates that inference of the systematic relationships of Triuridaceae based on morphology only, is very difficult.

The development of molecular tools in systematics in the late 1990s resulted in remarkable new insights in the affinities of Triuridaceae. Chase et al. (2000) assigned the family to the order Pandanales, based on a nuclear 18S rDNA sequence of a *Sciaphila* species. Besides Triuridaceae and Pandanaceae, the morphologically divergent families Cyclanthaceae, Stemonaceae and Velloziaceae were also included in this newly established, highly diverse Pandanales. Additional studies conducted by Caddick et al. (2002) and Davis et al. (2004) provided further support for the placement of Triuridaceae in Pandanales based on plastid *rbcL* and *atpB* sequences (not obtained for Triuridaceae), nuclear 18S rDNA, mitochondrial *atpA*, and morphology. While this has resulted in well-resolved relationships between the chlorophyllous families (i.e. Cyclanthaceae, Pandanaceae, Stemonaceae, Velloziaceae), the phylogenetic position of the Triuridaceae remains problematic. Amplification of plastid DNA data from Triuridaceae is difficult (Chase et al., 2000) and no such data are available on GenBank, probably as a result of the loss of photosynthesis in Triuridaceae. Moreover, data from nuclear and mitochondrial genomes are scarce. The maximum parsimony (MP) analysis of Chase et al. (2000), points to a close relationship of Triuridaceae with Pandanaceae and Cyclanthaceae. MP analyses of mitochondrial *atpA* sequences of three species (*Sciaphila, Triuris* and *Lacandonia*) (Davis et al., 2004), yielded a number of trees, about half of which supported Triuridaceae forming a clade with Velloziaceae as the sister to all other Pandanales, and half supporting Velloziaceae to form the sister of the rest, with Triuridaceae as sister to Stemonaceae, Pandanaceae and Cyclanthaceae. Thus, these results show contradictory low-support topologies for the position of Triuridaceae in Pandanales. Rudall and Bateman (2006) conducted a phylogenetic analysis based on morphological data, in which the topology of the chlorophyllous families is in accordance with the conclusions of previous studies (Caddick et al., 2002; Davis et al., 2004). However, Triuridaceae were embedded in Stemonaceae. Shared characters between these families include the underground rhizome with seasonal aerial parts, scale leaves (although Stemonaceae also have foliage leaves, which Triuridaceae lack), inaperturate, spiny gemmate pollen and tepals with short filaments that are densely papillated. Since these different methods show contradictory solutions regarding the position of Triuridaceae, the family’s evolutionary history remains enigmatic.

Based on a more extensive molecular (nuclear and mitochondrial DNA) dataset, we aimed to resolve the phylogeny of the Pandanales including Triuridaceae. We extended the existing 18S rDNA and *atpA* datasets (Chase et al., 2000; Davis et al., 2004) by amplifying these regions for 15 Triuridaceae specimens (12 species). Furthermore, we extended the number of analyzed molecular markers by amplifying the mitochondrial regions *nad1b-c* and *matR*, because they have proved to be useful specifically for inferences in mycoheterotrophic plants (Merckx, 2008). We discuss the obtained phylogenetic hypothesis in reference to previous findings. Furthermore, we aimed to estimate divergence times in Pandanales, evaluating the claim that Triuridaceae are an old family.

2. Materials and methods

2.1. Taxon sampling

A total of 57 species were included in the four-gene analysis. Forty-nine of these are Pandanales ingroup taxa and eight are outgroup taxa, seven of which belong to Dioscoreales, the sister group of Pandanales (Chase et al., 2006; Soltis et al., 2011), and one of which belongs to the more distantly related Alismatales (Chase et al., 2006; Soltis et al., 2011). The ingroup consists of the Cyclanthaceae, Pandanaceae, Stemonaceae, Triuridaceae and Velloziaceae. Six of about 230 species of Cyclanthaceae (Harling et al., 1998) were sampled, 18 of about 800–900 species of Pandanaceae (Stone et al., 1998), four of about 35 species of Stemonaceae (Kubitzki, 1998a), and three of over 200 species of Velloziaceae (Kubitzki, 1998b). Twelve of $\sim$ 50 species of Triuridaceae (Maas-van de Kamer and Weustenfeld, 1998; Mabberley, 2008) were sampled. For Triuridaceae roughly 24% of the species were sampled including five of the nine genera. Our sampling includes all tribes of Triuridaceae and covers the pantropical distribution range of the family. All Triuridaceae taxa are represented by sequence data of at least three markers (see Appendix A). All photosynthesizing taxa are represented by data of all four DNA markers, except *Croomia* and *Acanthochlamys*, which are represented by three and two markers, respectively. In a few cases only partial sequences could be obtained for a particular marker. A second phylogenetic

analysis was conducted, based on all data of the four-gene analysis, supplemented by eight additional Triuridaceae taxa. For these additional taxa, data of at least two markers were available. However, only one *atpA* sequence for *Lacandonia schismatica* was available and included. For the molecular dating analysis, supplementary sequence data were obtained from GenBank, for taxa from all major lineages of monocots. See Appendix A for a complete list of the taxa and their GenBank accession numbers.

2.2. DNA extraction, PCR and sequencing

DNA was extracted from herbarium and silica dried material, using the Qiagen plant kit (Qiagen, Venlo, the Netherlands, 2006) according to the manufacturer's protocol. PCR was carried out using a Bio-Rad 96+ grad MyCycler thermocycler (Bio-Rad, the Netherlands). Each PCR reaction contained 17.3 μl MilliQ H_2O, 2.5 μl CoralLoad PCR buffer (Qiagen, the Netherlands), 1 μl dNTP (Qiagen, the Netherlands, 10 mM), 1 μl Bovine Serum Albumin (Promega, USA, 0.1 mg/ml), 1 μl forward primer (Eurofins, mwg operon, 10 mM), 1 μl reverse primer (Eurofins, mwg operon, 10 mM), 0.2 μl Taq polymerase (Qiagen, 5 units/μl) and 1 μl undiluted DNA template, to a total of 25 μl.

The nuclear 18S rDNA region was amplified using the primer combinations NS1 and NS2, NS3 and NS4, NS5 and NS6, and NS7 and NS8 (White et al., 1990), under the conditions described by Merckx et al. (2006). The mitochondrial F1-ATPase alpha subunit (*atpA*) region was amplified using the primer combination atpA-F and atpA-R (Eyre-Walker and Gaut, 1997). For some Triuridaceae samples the primer combination atpA-F-A1 and atpA-B-A1 (Davis et al., 2004) was used. PCR conditions were as follows, as adapted from Eyre-Walker and Gaut (1997): premelting of 4 min at 95 °C, followed by 35 cycles of denaturing of 1 min at 95 °C, annealing of 1 min at 50 °C and extension of 1 min 45 s at 72 °C, followed by a final extension step of 7 min at 72 °C. The mitochondrial maturase (*matR*) region was amplified using the primer combinations matR 26F and matR 1002, and matR 879F and matR 1858R (Meng et al., 2002; Zhu et al., 2007). PCR conditions were as follows, as adapted from Mower et al. (2010): premelting of 4 min at 94 °C, followed by 35 cycles of denaturing of 30 s at 94 °C, annealing of 45 s at 48 °C and extension of 2 min at 72 °C, followed by a final extension step of 7 min at 72 °C. The mitochondrial NADH dehydrogenase subunit 1 b-c intron (*nad1b-c*) was amplified using the primer combination nad1-B and nad1-C (Demesure et al., 1995) as well as the internal primer nad1-M (Freudenstein and Chase, 2001). PCR conditions were as follows, as adapted from Merckx et al. (2006): premelting of 5 min at 94 °C, followed by 35 cycles of denaturing of 30 s at 94 °C, annealing of 30 s at 44 °C and extension of 2 min at 72 °C, followed by a final extension step of 7 min at 72 °C. Sanger sequencing was conducted by the Macrogen sequencing facility (Macrogen, Amsterdam, the Netherlands).

2.3. Phylogenetic analyses

Sequence assembly and manual editing were done using Geneious Pro v6.1.4. (Drummond et al., 2013). Sequence alignments were generated using the default MUSCLE alignment settings (Edgar, 2004) as incorporated in Geneious Pro. Alignments were manually checked and ambiguities were adjusted. Phylogenetic relationships were inferred using maximum likelihood (ML) and Baysian Inference (BI) as optimality criteria. The software package jModelTest 2.1.1 (Darriba et al., 2012; Guindon and Gascuel, 2003) was used to find the best substitution model to fit the data under Akaike's Information Criterion (AIC). For the 18S rDNA dataset, the TIM2+G model was suggested as best fit, whereas for the *atpA* dataset TPM2uf+I+G was suggested, for the *matR* dataset SYM+G was suggested and for the *nad1b-c* dataset GTR+G was suggested. Since no TIM, TPM or SYM model is incorporated in any of the phylogenetic inference software packages used, the incorporated models with the lowest AIC value were selected; GTR+I+G for *atpA* and GTR+G for 18s rDNA and *matR*. The complete dataset was partitioned into four subsets for each of the analyzed regions with the corresponding substitution model. ML analyses were carried out using Garli v2.0 (Zwickl, 2006). Branch support in the ML trees was estimated using bootstrap support (BS; Felsenstein, 1985), with 500 replicates. BI analyses were carried out using MrBayes v3.2.1 (Ronquist et al., 2012). The Markov Chain Monte Carlo (MCMC; Geyer, 1991) analyses were conducted with 2 runs of 4 chains, 4,000,000 generations. One tree of every 1000 generations was sampled. The burn-in was set to 25%, and results were evaluated using Tracer v1.5 (Rambaut and Drummond, 2007) by checking the stability and distribution of the obtained likelihood values (a normal distribution was considered sufficient) and the ESS values for model parameters (over 200 was considered sufficient). Clades with over 85% bootstrap support were considered strongly supported and clades with over 75% bootstrap support were considered moderately supported. Lower support values were not interpreted as reliably supported clades at all. Clades with 95% or higher Bayesian posterior probability were considered to be significantly supported by the data. All shown trees resulted from ML analyses and were visualized using FigTree v1.3.1 (Rambaut, 2009).

2.4. Estimation of divergence time

Divergence times of the lineages within the Pandanales were estimated by conducting an uncorrelated lognormal relaxed clock analysis using BEAST v1.7.5 (Drummond et al., 2012). The analysis was performed assuming the Yule process of speciation (Gernhard, 2008) and using the GTR+I+G nucleotide substitution model, as selected by jModelTest 2.1.1 (Darriba et al., 2012; Guindon and Gascuel, 2003) based on Akaike's Information Criterion (AIC). Fossil calibration points were included using dated angiosperm fossils from literature. In order to be able to include a sufficient number of fossil calibration points, our sampling was increased by supplementing our 18S rDNA and *atpA* datasets with sequences for all monocot orders from GenBank. A total of four fossil calibration points were included (see Table 1), selected from Bremer (2000) and Merckx et al. (2008). Calibration point priors were modeled as a lognormal distribution, with the age of the fossil set as an offset value of the distribution. Log standard deviations of all modeled calibration point priors were set to the default 1.0, assuming a relatively broad width of the given probability distribution. The mean value was initially set to 1.0, providing a slightly older age optimum for the calibration points than the default 0.0, based on the assumption that the lineage to which the fossil is assigned is likely to be slightly older than the fossil. The following calibration points were used. (1) The stem node of Zingiberales was constrained to an offset age of 83 Ma on the basis of *Spirematospermum* fossils (fruits) (Rodríquez-de la Rosa and Cevallos-Ferriz, 1994). (2) The stem node of Arecaceae was constrained to an offset age of 89.5 Ma on the basis of *Spinizonocolpites* fossils (pollen) (Harley, 1996). (3) The crown node of the clade consisting of Poaceae/Flagellariaceae/Joinvilleaceae/Restionaceae was constrained to an offset age of 69.5 Ma on the basis of *Milfordia* fossils (pollen) (Lindler, 1987). (4) The crown node of Asparagales was constrained to an offset age of 93 Ma on the basis of *Liliacidites* fossils (pollen) (Ramirez et al., 2007). The root age of the tree was modeled as a lognormal distribution as well, with default initial value (28.0), log standard deviation and mean set to 1.0 and an offset of 134 Ma. This age was based on the split between the Acorales and the rest of the monocots from Bremer (2000), providing a minimum crown age of the monocots. The described

Table 1
Fossil calibration points.

Taxon	Material (reference)	Affinity	Node	Age estimate (offset) (Ma)	Prior distribution	Mean/ logSD
Spirematospermum	Fruits (Rodríquez-de la Rosa and Cevallos-Ferriz, 1994)	Stem Zingiberales	A	83	Lognormal	1.0/1.0
Spinizonocolpites	Pollen (Harley, 1996)	Stem Arecaceae	B	89.5	Lognormal	1.0/1.0
Milfordia	Pollen (Lindler, 1987)	Crown Flagellariaceae/ Joinvilleaceae	C	69.5	Lognormal	1.0/1.0
Liliacidites	Pollen (Ramirez et al., 2007)	Crown Asparagales	D	93	Lognormal	1.0/1.0

fossils of Triuridaceae from the Turonian (Gandolfo et al., 2002, 1998) were not used as calibration point, in order to obtain an independent estimate of the divergence time of Triuridaceae. All other priors were set to "uniform" (default). The topology was constrained in accordance with the most recent multi-gene monocot phylogenies available (Chase et al., 2006; Soltis et al., 2011), as well as the phylogeny of the Dioscoreales (Merckx et al., 2009) and the results of our ML and BI analyses for relationships between Pandanales families, starting from a random tree. Clades in the tree were constrained to the order level, but within Dioscoreales and Pandanales clades were constrained to the family level. In Triuridaceae clades were constrained according to the relationships obtained in this study (Fig. 1). MCMC (Geyer, 1991) analyses ran for 200,000,000 generations. One tree of every 10,000 generations was sampled. A maximum clade credibility tree was constructed with TreeAnnotator v1.7.1 (Drummond et al., 2012). Trees were evaluated using Tracer v1.5 (Rambaut and Drummond, 2007) by checking the stability and distribution of the obtained likelihood values and the ESS values for model parameters (see Section 3, two parameters did not reach an ESS value of 200 after 200,000,000 generations, see Section 4), and visualized using FigTree v.1.3.1 (Rambaut, 2009).

3. Results

3.1. Phylogenetic analysis

Separate analyses of the nuclear 18S rDNA (1970 bp) and the mitochondrial *atpA* (1181 bp), *matR* (1876 bp) and *nad1b-c* (1954 bp) regions do not show high-support (bootstrap or

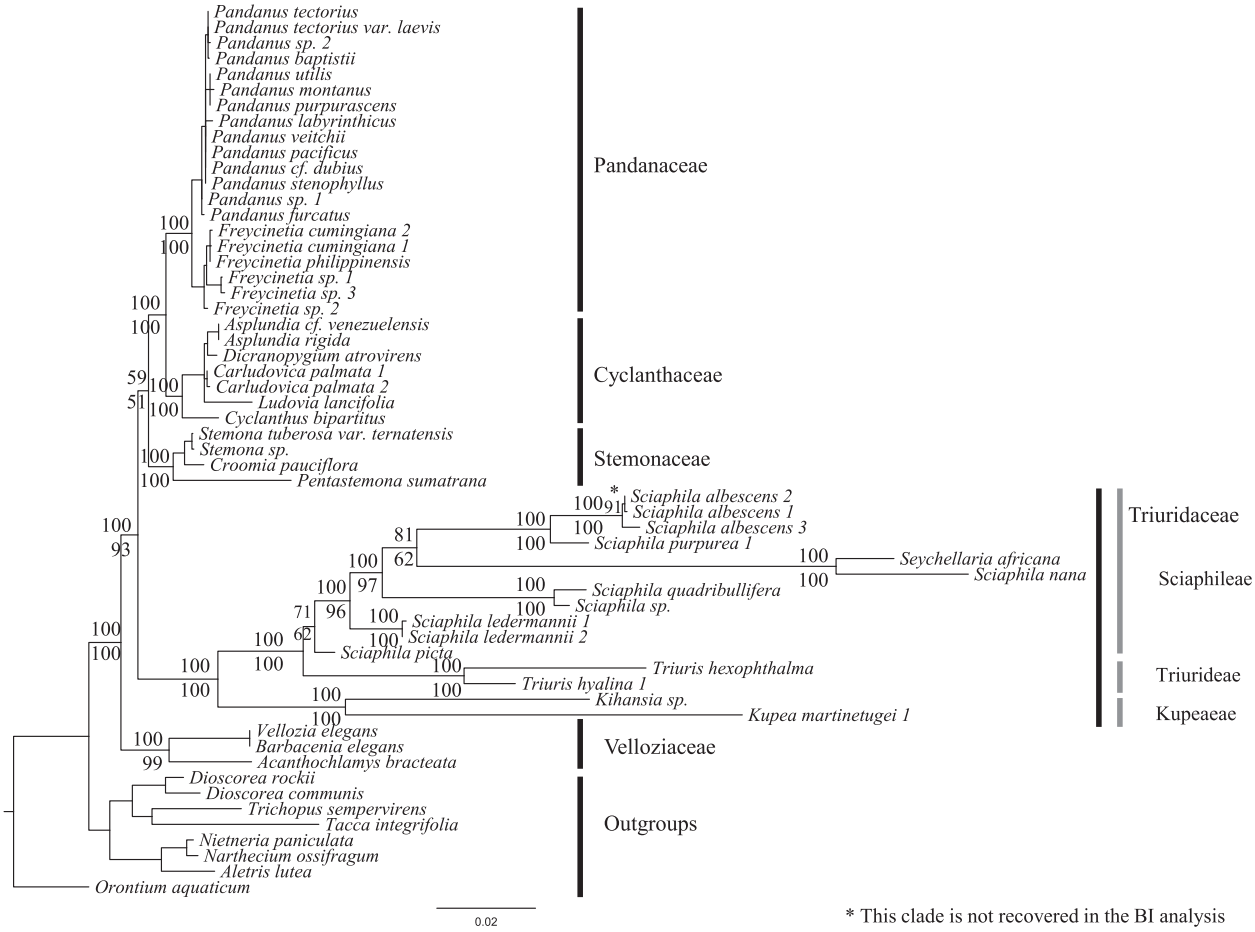

Fig. 1. Phylogeny of Pandanales based on a four-gene ML analysis (−ln L = 25439.89) combining 18S rDNA, *atpA*, *matR* and *nad1b-c* intron dataset. Tribe Sciaphileae contains all species of *Sciaphila* and *Seychellaria*, tribe Triurideae contains all species of *Triuris* and tribe Kupeaeae contains all species of *Kupea* and *Kihansia*. Values above branches are Bayesian posterior probabilities (expressed as percentages); those below branches are bootstrap support percentages. The scale bar shows the number of substitutions per site.

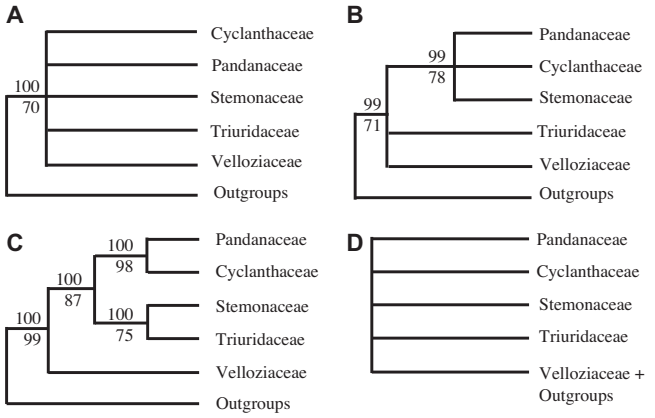

Fig. 2. Simplified topologies of Pandanales based on separate ML analyses of (a) 18S rDNA, (b) *atpA*, (c) *matR*, and (d) *nad1b-c*. Values above branches are Bayesian posterior probabilities (expressed as percentages); those below branches are bootstrap support percentages. Non-supported nodes above the family level were collapsed. The scale bar shows the number of substitutions per site.

posterior probability) conflicts (Fig. 2, also see SI Figs. 1–4), except between the results from the BI analyses of *atpA* and *matR* regarding the relative positions of Triuridaceae and Stemonaceae.

The highest likelihood tree of Pandanales, resulting from Maximum Likelihood analysis of our complete dataset of 57 taxa is shown in Fig. 1 ($-\ln L = 25439.89$). This phylogeny is in accordance with our results based on Bayesian Inference (not shown). All families are monophyletic with 100% posterior probability (PP) and at least 99% bootstrap support (BS). Our analyses indicate strong support (100% PP, 100% BS) for Velloziaceae as sister to the rest of Pandanales, but no (59% PP, 51% BS) support for the clade consisting of Stemonaceae, Pandanaceae and Cyclanthaceae as sister group of Triuridaceae. The relationships among the species in the families Cyclanthaceae and Pandanaceae are not well resolved. Furthermore, the Triuridaceae have longer branch lengths than the chlorophyllous taxa.

Within Triuridaceae, tribe Kupeaeae (*Kihansia* sp. and *Kupea martinetugei*) is sister to the rest (100% PP, 100% BS). Triurideae (*Triuris hexophthalma* and *Triuris hyalina*) and Sciaphileae (*Sciaphila* and *Seychellaria*) are sister to each other (100% PP, 100% BS). The monophyly of Sciaphileae is weakly supported (71% PP, 62% BS). The genus *Seychellaria* appears to be embedded in *Sciaphila*. Multiple accessions of the same species always group together (i.e. *Sciaphila ledermannii* and *Sciaphila albescens*).

The result of the second phylogenetic analysis, containing additional sequence data for eight Triuridaceae taxa (Fig. 3), resembles the result of the first phylogenetic analysis (Fig. 1), although the positions of *Sciaphila ledermannii*, *S. quadribullifera* and *S.* sp. are slightly different. Multiple inclusions of the same species appear together as clades (i.e. *Sciaphila albescens, S. purpurea, Triuris hyalina*). *Andruris* sp. and *Seychellaria madagascariensis* are grouping together with *Sciaphila nana* and *Seychellaria africana*, respectively, and appear to be embedded in *Sciaphila*. *Lacandonia schismatica* is positioned close to *Triuris*. *Sciaphila tenella* shows up as the sister group of the rest of the Sciaphileae and Triurideae, rendering Sciaphileae paraphyletic.

3.2. Divergence time estimation

The BEAST estimation of the divergence time of Triuridaceae (Fig. 4) shows a stem age with a 95% confidence interval between 103 and 62 Ma, and a crown age between 50 and 90 Ma. The age estimations of the major lineages in this monocot wide analysis are generally in accordance with previous dating studies, although slightly younger, see comparison in Table 2 (Janssen and Bremer, 2004; Merckx et al., 2008). Moreover, we found a substantial amount of rate variation (ucld.stdev = 0.96) in our dataset, indicating that there is considerable rate heterogeneity among the studied monocot lineages. Thus, the observed evolutionary rates deviate considerably from clock-like evolution.

4. Discussion

4.1. Phylogenetic position of Triuridaceae in Pandanales

The phylogeny of the Pandanales was reconstructed to evaluate the position of Triuridaceae. In general our combined nuclear (18S rDNA) and mitochondrial (*atpA*, *matR* and *nad1b-c*) datasets provide good resolution for this purpose. In the reconstructed phylogeny (Fig. 1), all included families (Cyclanthaceae, Pandanaceae, Stemonaceae, Triuridaceae and Velloziaceae) form monophyletic groups at current taxon sampling. In our separate analyses for each marker high support conflict was found only between *matR* and

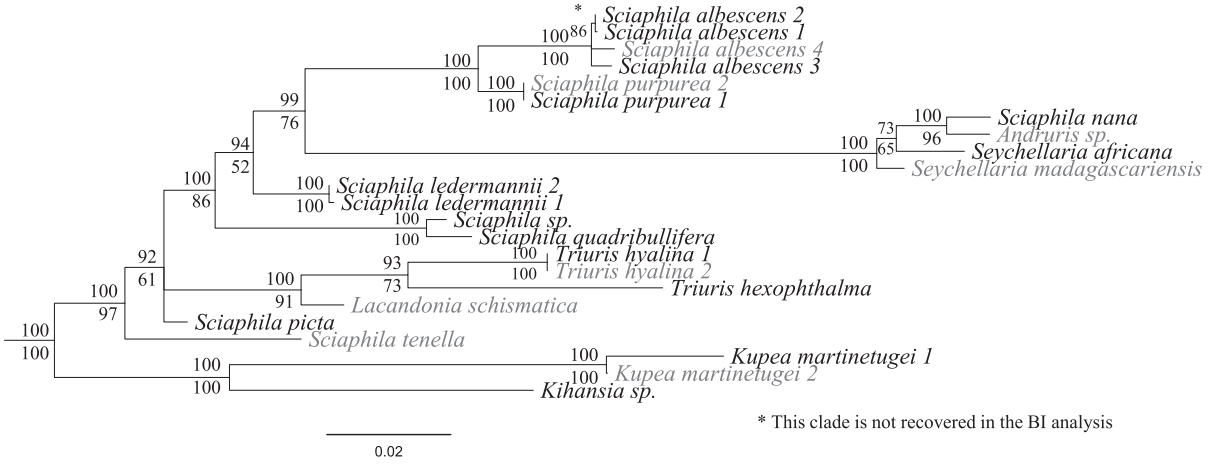

Fig. 3. A more detailed phylogeny of Triuridaceae that includes eight additional Triuridaceae taxa (in gray) for which we retrieved fewer than three genes. A more detailed version (including chlorophyllous Pandanales taxa) is shown in SI Fig. 5. Tribe Sciaphileae contains all species of *Sciaphila, Seychellaria* and *Andruris*, tribe Triurideae contains all species of *Triuris* and *Lacandonia* and tribe Kupeaeae contains all species of *Kupea* and *Kihansia*. Values above branches are Bayesian posterior probabilities (expressed as percentages); those below branches are bootstrap support percentages. The scale bar shows the number of substitutions per site.

atpA BI analyses. In the *matR* analysis, Triuridaceae and Stemonaceae form sister groups, as opposed to the result of *atpA*, in which Stemonaceae forms a clade with Pandanaceae and Cyclanthaceae. However, this conflict does not receive high bootstrap support, which suggests that it is based on a limited amount of characters in each marker. In the combined analysis, the reconstructed phylogenetic positions of the chlorophyllous families in Pandanales (Cyclanthaceae, Pandanaceae, Stemonaceae, Velloziaceae) are all in accordance with the conclusions of previous studies (Caddick et al., 2002; Chase et al., 2000; Davis et al., 2004; Rudall and Bateman, 2006). We found high support (100% PP, 93% BS) for Velloziaceae as the sister group of the rest of the Pandanales, indicating a rather robust placement of Triuridaceae in Pandanales. The topologies further suggest that Triuridaceae are sister to Stemonaceae, Cyclantheceae and Pandanaceae. However, this putative sister group relationship receives low branch support (59% PP, 51% BS), rendering the exact phylogenetic position of Triuridaceae somewhat enigmatic. However, both Triuridaceae and Stemonaceae are monophyletic with high branch support values (100% BS, 100% PP), contradicting the findings of Rudall and Bateman (2006), in which Triuridaceae appeared to be embedded in Stemonaceae based on morphology. However, a sister group relation between the two families cannot be rejected based on the presented data. Therefore, the uncertainties pointed out by earlier studies were not completely resolved (Chase et al., 2000; Davis et al., 2004; Rudall and Bateman, 2006). The only previous study in which Triuridaceae is found to be the descendent from the second major split in Pandanales is Davis et al. (2004). In roughly half of their obtained trees, Triuridaceae appears as sister to Stemonaceae, Cyclanthaceae and Pandanaceae. However, in this particular analysis the relationships among Pandanaceae, Cyclanthaceae and Stemonaceae are different from their general conclusions, and different from the findings of the present study. The long branch lengths of Triuridaceae as compared to the chlorophyllous Pandanales taxa, indicate elevated mutation rates in Triuridaceae.

Table 2
Divergence time estimations of monocots.

Order	Node	Age estimation (95% credibility interval in Ma)	Age estimation by Merckx et al. (2008) (95% credibility interval in Ma)	Age estimation by Janssen and Bremer (2004) (Ma)
Acorales	Stem	134–138	134	NA
	Crown	4–52	7–44	NA
Alismatales	Stem	116–137	123–133	131
	Crown	90–134	97–133	128
Petrosaviales	Stem	108–133	121–132	126
	Crown	8–96	87–102	123
Dioscoreales	Stem	96–123	119–130	124
	Crown	85–116	113–126	123
Pandanales	Stem	96–123	119–130	124
	Crown	69–110	116–130	114
Liliales	Stem	97–121	109–131	124
	Crown	43–105	78–131	117
Asparagales	Stem	94–116	98–126	122
	Crown	93–96	101–127	119
Arecales	Stem	90–93	94–122	120
	Crown	14–71	15–98	110
Commelinales	Stem	83–85	83–114	114
	Crown	47–84	50–104	110
Zingiberales	Stem	83–85	91–116	114
	Crown	30–75	52–96	88
Poales	Stem	84–105	89–120	117
	Crown	78–98	88–116	113

This consistent with findings in other mycoheterotrophic clades, such as Thismiaceae (Merckx et al., 2009).

4.2. Intrafamilial relationships in Triuridaceae

Within Triuridaceae, all tribes are resolved as monophyletic at current taxon sampling (Fig. 1), except in the analysis with addi-

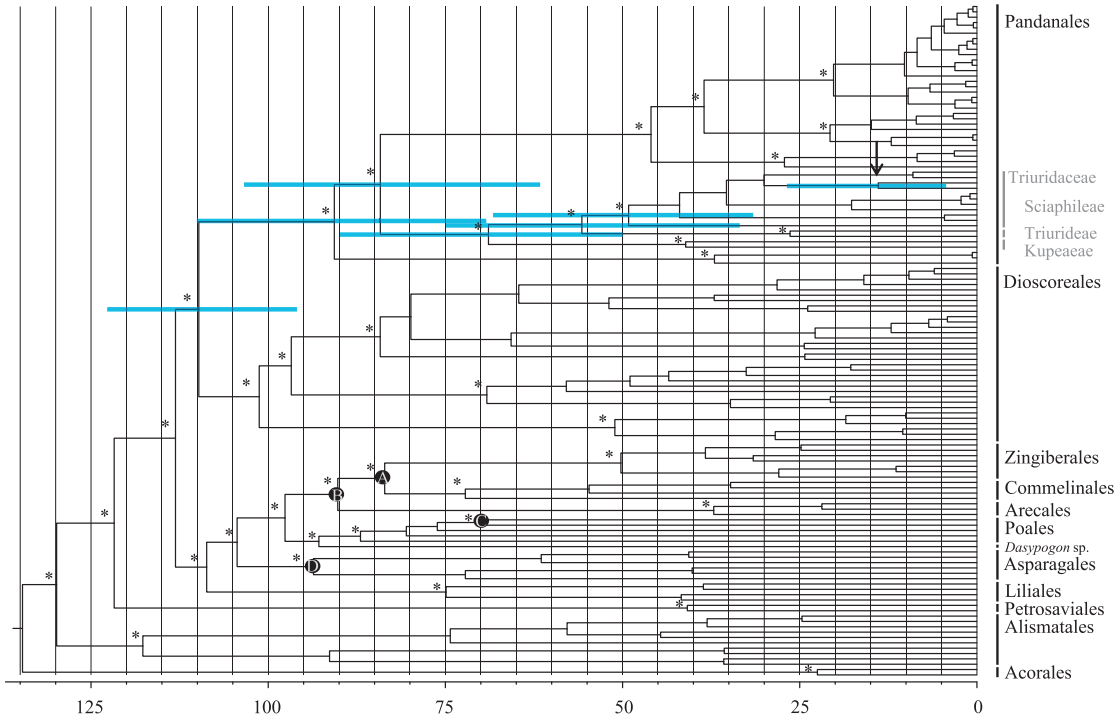

Fig. 4. Dated phylogeny of monocots, including Triuridaceae, inferred using the uncorrelated lognormal relaxed clock method. Based on 18S rDNA and *atpA* data, four fossil calibration points (Table 1) and assuming a Yule process of speciation. The scale bar represents time (million years ago; Ma), bars around nodes represent 95% confidence intervals. Contrained nodes are indicated with an asterisk (*).

tional taxa (Fig. 3). The basal position of the tribe Kupeaeae is in accordance with the findings of Rudall and Bateman (2006), who positioned this tribe as sister to the rest of Triuridaceae on the basis of floral morphology. The weak support for the monophyly of the tribe Sciaphileae is caused by the position of *Sciaphila picta*, since the rest of the tribe is strongly supported (100% PP, 96% BS). The paraphyly of the pantropical and speciose genus *Sciaphila* (due to the inclusion of *Seychellaria* and *Andruris*, Figs. 1 and 3) indicates that taxonomic revisions of the genera *Seychellaria* and *Andruris* are urgently needed, in order to retain a monophyletic *Sciaphila*. Alternative taxonomic solutions involving the maintenance of *Andruris* and *Seychellaria* would imply that *Sciaphila* is to be split in several genera. As opposed to *Sciaphila*, the genera *Seychellaria* and *Andruris* are mainly based on the presence of staminodes and appendages on the connectives, respectively, in male flowers (Hemsley, 1907; Schlechter, 1912; Giesen, 1938). Consequently new genera in *Sciaphila* are either likely to be based on similar minute floral characters, or synapomorphies may be lacking at all. Therefore, we consider further splitting of *Sciaphila* inappropriate. The close relationship of *Lacandonia schismatica* to *Triuris*, though based on very limited sequence information, provides further evidence that *Lacandonia* should be assigned to Triurideae (Maas-van de Kamer, 1995; Vergara-Silva et al., 2003). *Lacandonia* was formerly placed in its own family Lacandoniaceae, based on having its stamens surrounded by carpels, which is a unique feature in flowering plants (Martínez and Ramos, 1989). The position of *Sciaphila tenella* as sister to the clade containing Triurideae and Sciaphileae is taxonomically unexpected. This position is based on 18S rDNA and *matR* sequence data only, and may be explained by the limited amount of sequence data available, the long branch length leading to this taxon, or both. The different phylogenetic positions of *Sciaphila ledermannii, S. quadribullifera* and *S.* sp. that are observed in the analysis with additional taxa (Fig. 3) may be an artifact based on the presence of many taxa with missing data in this more detailed analysis.

4.3. Divergence time estimation

We found a substantial amount of rate variation in our dataset, indicated by a standard deviation of the rate parameter (ucld.stdev) close to 1. The BEAST package (Drummond et al., 2012) accommodates this by incorporating an uncorrelated lognormal relaxed molecular clock approach. However, two ESS values in this relaxed molecular clock analysis did not reach 200. The ESS values for the crown age of Kupeaeae and the ucld.stdev remained 57.5 and 119.9, respectively, after 200,000,000 generations. This is possibly caused by the extreme amount of rate variation in the dataset, indicated by the long branch lengths in Kupeaeae. The divergence time estimation indicates that the crown age of Triuridaceae is between 50 and 90 Ma (between Lower Eocene and Upper Cretaceous) and the stem age is between 62 and 103 Ma (between Lower Paleocene and Lower Cretaceous) (Fig. 4). These age estimates suggest an apomorphy age for mycoheterotrophy in Triuridaceae between 50 and 103 Ma. This consistent with age estimates for the origin of mycoheterotrophy in Thismiaceae (49–92 Ma) and *Afrothismia* (45–109 Ma) (Merckx et al., 2010). This observed great age, together with the high substitution rates and the mycoheterotrophic lifestyle, probably account for the substantial morphological differentiation of Triuridaceae, which has rendered distinctive characters ambiguous for their phylogenetic positioning. In a cladistic analysis Gandolfo et al. (2002) found that the fossil genera *Nuhliantha* and *Mabelia* (found in 90 million year old deposits in New Jersey) were placed as the sister group of Triurideae, However, our age estimate for the split between Sciaphileae and Triurideae is significantly younger (with a 95% confidence interval between 38 and 75 Ma). Thus our hypothesis rejects the possibility of 90 Ma Triurideae fossils, although these fossils would still fit in the overall Triuridaceae evolution as stem relatives.

4.4. Biogeographical implications

The reconstructed phylogenetic relations show considerable geographical structure. The Kupeaeae are exclusively known from Africa, whereas Triurideae occur exclusively in the Neotropics. Some sampled species of Sciaphileae occur in Africa or the Neotropics (*Sciaphila albescens*, *S. purpurea*, *S. ledermannii*, *S. picta*, and both *Seychellaria* species) while others are from South-East Asia (*Andruris* sp., *Sciaphila nana*, *S. quadribullifera*, *S.* sp., and *S. tenella*). Our combined analysis (Fig. 1) suggests that these Asian species of *Sciaphila* are embedded in African/Neotropical groups, tentatively placing the origin of the genus in Africa or the Neotropics rather than South East Asia. The split between Kupeaeae (all African species) and Triurideae–Sciaphileae (early diverging species all Neotropical) is estimated to have occurred between 50 and 90 Ma, suggesting that dispersal is most likely the mechanism behind this split, because the plate tectonic split between Africa and South America is estimated to have occurred earlier, circa 100 Ma (Raven and Axelrod, 1974). However, since the upper limit of the divergence time estimation (90 Ma) is only ten million years younger than the plate tectonic split (100 Ma), vicariance cannot be rejected as a possible driver of the split between these early lineages in Triuridaceae. *Seychellaria* however, diverged from ancestral *Sciaphila* species between 4 and 27 Ma (see arrow, Fig. 3), rejecting the possibility that its current disjunct East African-Malagasy distribution resulted from the split between Africa and Madagascar, circa 120 Ma (Rabinowitz et al., 1983). These results thus indicate that the breakup of West Gondwana and the subsequent split between Africa and South America may have had an impact on the early diversification events of Triuridaceae, similar to the reconstructed historical biogeography of Burmanniaceae (Merckx et al., 2008). However, the current pantropical distribution of *Sciaphila* probably results from dispersal events that postdate major plate tectonic splits in the past. More thorough sampling of especially Asian taxa, must be done to further clarify biogeographical trends in Triuridaceae.

5. Conclusion

Based on a nuclear and mitochondrial molecular dataset, the present study suggests that Triuridaceae has descended from the second major split in Pandanales. Triuridaceae is most likely sister to the clade consisting of Stemonaceae, Cyclanthaceae and Pandanaceae. Within Triuridaceae, all tribes are recovered as monophyletic groups. Furthermore, *Sciaphila* appears to be paraphyletic, due to inclusion of *Seychellaria* and *Andruris*. A Bayesian estimation of the divergence time in Triuridaceae results in a great age of the family, with a crown age between 50 and 90 Ma and a stem age between 62 and 103 Ma. This age estimate implies the the breakup of Gondwana may have had an influence on early Triuridaceae evolution, although the pantropical distribution of *Sciaphila* is probably the result of later dispersal events.

Acknowledgments

We would like to thank Paul Maas and Hiltje Maas-van de Kamer for their help and valuable comments on the manuscript. Furthermore, we would like to thank Marcel Eurlings for help and assistance with the molecular work. We would also like to thank Rogier van Vugt and Gerda van Uffelen of the Hortus Botani-

cus Leiden and Elke Bellefroid of the Nationale Plantentuin Meise for their help and cooperation in taxon sampling.

Appendix A. Taxon information

Ingroups

Four markers were amplified for each specimen, except if indicated otherwise. Collection numbers are indicated for newly extracted specimens. For Triuridaceae, country of origin is indicated as well.

Cyclanthaceae: *Asplundia* cf. *venezuelensis* (RBGB 19720037-; 18S: KF264476; *atpA*: KF258580, *matR*: KF258579; *nad1b-c*: KF258208), *Asplundia rigida* (RBGB 19790005-; 18S: KF264477; *atpA*: KF258578; *matR*: KF258199; *nad1b-c*: KF258207), *Carludovica palmata* 1 (HBL812119-22130; 18S: KF264478; *atpA*: KF258577; *matR*: KF258198; *nad1b-c*: KF258211), *Carludovica palmata* 2 (RBGB 19221361-; 18S: KF264479; *atpA*: KF258586; *matR*: KF258204; *nad1b-c*: KF258212), *Cyclanthus bipartitus* (RBGB 19510200-; 18S: KF264480; *atpA*: KF258587; *matR*: KF258205; *nad1b-c*: KF258210), *Dicranopygium atrovirens* (RBGB 19391773-; 18S: KF264481; *atpA*: KF258588; *matR*: KF258203; *nad1b-c*: KF258209), *Ludovia lancifolia* (RBGB 19810835-; 18S: KF264482; *atpA*: KF258589; *matR*: KF258202; *nad1b-c*: KF258206)

Pandanaceae: *Freycinetia cumingiana* 1 (HBL910022; 18S: KF298366; *atpA*: KF298286; *matR*: KF298292; *nad1b-c*: KF298298), *Freycinetia cumingiana* 2 (RBGB 20080173-09; 18S: KF298363; *atpA*: KF298287; *matR*: KF298293; *nad1b-c*: KF298299), *Freycinetia philippinensis* (HBL950608; 18S: KF298381; *atpA*: KF298288; *matR*: KF298294; *nad1b-c*: KF298300), *Freycinetia* sp. 1 (HBL981172; 18S: KF298367; *atpA*: KF298289; *matR*: KF298295; *nad1b-c*: KF298301), *Freycinetia* sp. 2 (RBGB 20080172-08; 18S: KF298365; *atpA*: KF298290; *matR*: KF298296; *nad1b-c*: KF298302), *Freycinetia* sp. 3 (RBGB 20080174-10; 18S: KF298362; *atpA*: KF298291; *matR*: KF298297; *nad1b-c*: KF298303), *Pandanus baptistii* (RBGB 19610454-; 18S: KF298360; *atpA*: KF298305; *matR*: KF298319; *nad1b-c*: KF298333), *Pandanus* cf. *dubius* (HBL910011; 18S: KF298359; *atpA*: KF298306; *matR*: KF298320; *nad1b-c*: KF298334), *Pandanus furcatus* (RBGB 10005462-; 18S: KF298358; *atpA*: KF298307; *matR*: KF298321; *nad1b-c*: KF298335), *Pandanus labyrinthicus* (RBGB 19381346-; 18S: KF298357; *atpA*: KF298308; *matR*: KF298322; *nad1b-c*: KF298336), *Pandanus montanus* (RBGB 2000134-92; 18S: KF298356; *atpA*: KF298309; *matR*: KF298323; *nad1b-c*: KF298337), *Pandanus pacificus* (RBGB 19630068-; 18S: KF298355; *atpA*: KF298310; *matR*: KF298324; *nad1b-c*: KF298338), *Pandanus purpurascens* (RBGB 19773283-; 18S: KF298354; *atpA*: KF298311; *matR*: KF298325; *nad1b-c*: KF298339), *Pandanus* sp. 1 (HBL920709/HBL920276; 18S: KF298361; *atpA*: KF298304; *matR*: KF298318; *nad1b-c*: KF298332), *Pandanus* sp. 2 (HBL990062; 18S: KF298364; *atpA*: KF298312; *matR*: KF298326; *nad1b-c*: KF298340), *Pandanus stenophyllus* (RBGB 19392017-; 18S: KF298353; *atpA*: KF298313; *matR*: KF298327; *nad1b-c*: KF298341), *Pandanus tectorius* (RBGB 20031134-52; 18S: KF298352; *atpA*: KF298314; *matR*: KF298328; *nad1b-c*: KF298342), *Pandanus tectorius* var. *laevis* (RBGB 19480610-; 18S: KF298351; *atpA*: KF298315; *matR*: KF298329; *nad1b-c*: KF298343), *Pandanus utilis* (RBGB 19074101; 18S: KF298350; *atpA*: KF298316; *matR*: KF298330; *nad1b-c*: KF298344), *Pandanus veitchii* (RBGB 19270660-; 18S: KF298349; *atpA*: KF298317; *matR*: KF298331; *nad1b-c*: KF298345)

Stemonaceae: *Croomia pauciflora* (18S: AF168835.1; *atpA*: AF197708.1; *matR*: AF197735.1; *nad1b-c*: na), *Pentastemona sumatrana* (HBL910375; 18S: KF264490 *atpA*: KF264506; *matR*: KF298369; *nad1b-c*: KF258576), *Stemona* sp. (HBL20081117; 18S: KF264491; *atpA*: KF264505; *matR*: KF258200; *nad1b-c*: KF258575), *Stemona tuberosa var. ternatensis* (HBL910374; 18S: KF298348; *atpA*: KF264504; *matR*: KF258201; *nad1b-c*: KF258574)

Triuridaceae: *Andruris* sp. (Sands, Pattison, Wood 2833, Papua New Guinea; 18S: KF197094; *atpA*: na; *matR*: KF197111; *nad1b-c*: na), *Kihansia* sp. (Sainge 1620, YA, Cameroon; 18S: KF197095; *atpA*: KF197072; *matR*: KF197113; *nad1b-c*: KF197102), *Kupea martinetugei* 1 (Merckx et al. 102, LV, Cameroon; 18S: KF197093; *atpA*: KF298373; *matR*: KF197112; *nad1b-c*: KF197103), *Kupea martinetugei* 2 (Sainge 1668, YA, Cameroon; 18S: na; *atpA*: KF298372; *matR*: KF197110; *nad1b-c*: na), *Lacandonia schismatica* (18S: na; *atpA*: AY299794.1; *matR*: na; *nad1b-c*: na), *Sciaphila albescens* 1 (O. Banki 108, Guiana; 18S: KF197092; *atpA*: KF197069; *matR*: KF298375; *nad1b-c*: KF197100), *Sciaphila albescens* 2 (Maas et al. 9650, U, French Guiana; 18S: KF197091; *atpA*: KF197071; *matR*: KF298376; *nad1b-c*: na), *Sciaphila albescens* 3 (Jansen-Jacobs 6567, Suriname; 18S: KF197090; *atpA*: KF197073; *matR*: KF298377; *nad1b-c*: KF197099), *Sciaphila albescens* 4 (Maas

(*continued on next page*)

et al.9667, Guiana; 18S: KF197089; *atpA*: na; *matR*: KF298378; *nad1b-c*: na), *Sciaphila ledermannii* 1 (Merckx et al. 128, LV, Cameroon; 18S: KF197088; *atpA*: KF197070; *matR*: KF197109; *nad1b-c*: KF197101), *Sciaphila ledermannii* 2 (Dessein 1850, BR, Gabon; 18S: KF197086; *atpA*: KF197077; *matR*: KF197108; *nad1b-c*: KF298346), *Sciaphila nana* (Poncrt s.n., 298, Vietnam; 18S: KF197085; *atpA*: KF197076; *matR*: KF197107; *nad1b-c*: KF298347), *Sciaphila picta* (Hammell 3105, Panama; 18S: KF197084; *atpA*: KF298368; *matR*: KF197106; *nad1b-c*: na), *Sciaphila purpurea* 1 (Alzatc 4151, 291, Colombia; 18S: KF197083; *atpA*: KF197079; *matR*: KF298379; *nad1b-c*: KF197096), *Sciaphila purpurea* 2 (Maas et al. 830, Venezuela; 18S: KF197087; *atpA*: na; *matR*: KF298380; *nad1b-c*: na), *Sciaphila quadribullifera* (Polak 1118, Papua New Guinea; 18S: KF197082; *atpA*: KF197078; *matR*: KF298371; *nad1b-c*: na), *Sciaphila* sp. (Prieditis s.n. (NHN-U 0253669), Borneo; 18S: KF264483; *atpA*: KF258583; *matR*: KF258193; *nad1b-c*: na), *Sciaphila tenella* (Polak 1117, Papua New Guinea; 18S: KF264484; *atpA*: na; *matR*: KF258194; *nad1b-c*: na), *Seychellaria africana* (Bridson 634 WAG, Tanzania; 18S: KF264485; *atpA*: KF258584; *matR*: KF258195; *nad1b-c*: na), *Seychellaria madagascariensis* (Dorr 4592 WAG, Madagascar; 18S: KF264486; *atpA*: na; *matR*: KF258196; *nad1b-c*: na), *Triuris hexophthalma* (Renz 14120, Guiana; 18S: KF264487; *atpA*: KF258585; *matR*: KF258197; *nad1b-c*: na), *Triuris hyalina* 1 (Sano & Laessoe 52195, Brazil; 18S: KF264488; *atpA*: KF258582; *matR*: KF298374; *nad1b-c*: na), *Triuris hyalina* 2 (Marquez-Guzman s.n. (d.d. 1990), Mexico; 18S: KF264489; *atpA*: KF258581; *matR*: na; *nad1b-c*: na)

Velloziaceae: *Acanthochlamys bracteata* (18s: AY952411.1; *atpA*: AY299698.1; *matR*: na; *nad1b-c*: na), *Barbacenia elegans* (RBGB 19084359-; 18S: KF197081; *atpA*: KF197075; *matR*: KF197105; *nad1b-c*: KF197098), *Vellozia elegans* (HBLA86167; 18S: KF197080 *atpA*: KF197074; *matR*: KF197104; *nad1b-c*: KF197097)

Outgroups phylogenetic analyses

Dioscoreales: *Aletris lutea* (18S: DQ786092.1; atpA: FJ215780.1; *matR*: KF264492; *nad1b-c*: DQ786160), *Dioscorea communis* (18S: EU186223.1; *atpA*: AY277804.1; *matR*: KF298370; *nad1b-c*: GQ469502), *Dioscorea rockii* (18S: DQ786090.1; *atpA*: EU421029.1; *matR*: KF264495; *nad1b-c*: DQ786157), *Narthecium ossifragum* (18S: AF309411.1; *atpA*: AY299809.1; *matR*: KF264496; *nad1b-c*: DQ786163.1), *Nietneria paniculata* (18S: EU186219.1; *atpA*: EU421041.1; *matR*: KF264497; *nad1b-c*: GQ469504), *Orontium aquaticum* (18S: AF293753.1; *atpA*: AY299816.1; *matR*: AF197745.1; *nad1b-c*: HM576829.1), *Tacca integrifolia* (18S: DQ786085.1; *atpA*: EU421045.1; *matR*: KF264494; *nad1b-c*: DQ786153), *Trichopus sempervirens* (18S: AF309395.1; *atpA*: JN850565.1; *matR*: KF264493; *nad1b-c*: KF264499)

Outgroups divergence time estimation

Acorales: *Acorus calamus* (18S: L24078.1; *atpA*: AF039256.1), *Acorus gramineus* (18S: AF197584.1; *atpA*: AY299699.1),

Alismatales: *Alisma plantago-aquatica* (18S: AF197585.1; *atpA*: AF197717.1), *Butomus umbellatus* (18S: AH003489.1; *atpA*: AY299733.1), *Gymnostachys anceps* (18S: AF069200.1; *atpA*: AF039244.1), *Orontium aquaticum* (18S: AF293753.1; *atpA*: AY299816.1), *Pleea tenuifolia* (18S: AF206995.1; *atpA*: AY299827.1), *Scheuchzeria palustris* (18S: AF069202.1; *atpA*: AY277803.1), *Syringodium filiforme* (18S: AF168876.1; *atpA*: DQ859116.1), *Tofieldia calyculata* (18S: AF207043.1; *atpA*: AY299851.1), *Triglochin maritimum* (18S: AF197586.1; *atpA*: AF197716.1), *Zostera marina* (18S: HQ445940.1; *atpA*: DQ859121.1)

Arecales: *Nypa fruticans* (18S: AY012357.1; *atpA*: U58833.1), *Phoenix* sp. (18S: AF206991.1; *atpA*: U58831.1), *Phytelephas aequatorialis* (18S: AY012398.1; *atpA*: AY299825.1)

Asparagales: *Cypripedium calceolus* (18S: AF069208.1; *atpA*: AY299755.1), *Isotria verticillata* (18S: AF135204.1; *atpA*: AY299788.1), *Ixiolirion tataricum* (18S: AF206940.1; *atpA*: AY299789.1), *Neuwiedia veratrifolia* (18S: AF168863.1; *atpA*: AY299813.1), *Tecophilaea cyanocrocus* (18S: AF207036.1; *atpA*: AY299848.1), *Xeronema callistemon* (18S: AF207056.1; *atpA*: AY299857.1)

Commelinales: *Anigozanthos flavidus* (18S: AF069214.1; *atpA*: AF039246.1), *Hanguana malayana* (18S: AF387604.1; *atpA*: AY299775.1), *Philydrella pygmaea* (18S: AF206990.1; *atpA*: AY299823.1), *Pontederia cordata* (18S: AF206998.1; *atpA*: AY299828.1)

Dioscoreales: *Afrothismia foertheriana* (18S: EU420988.1; *atpA*: EU421002.1), *Afrothismia hydra* (18S: EU420990.1; *atpA*: EU421004.1), *Aletris farinosa* (18S: AF309390.1; *atpA*: AY299706.1), *Aletris lutea* (18S: DQ786092.1; *atpA*: FJ215780.1), *Apteria aphylla* (18S: DQ786034.1; *atpA*: EU421007.1), *Burmannia alba* (18S: DQ786074.1; *atpA*: EU421008.1), *Burmannia itoana* (18S: DQ786078.1; *atpA*: EU421016.1), *Burmannia wallichii* (18S: DQ786069.1; *atpA*: EU421023.1), *Campylosiphon purpurascens* (18S: EU420996.1; *atpA*: EU421024.1), *Cymbocarpa refracta* (18S: DQ786038.1;

	atpA: EU421025.1), *Dictyostega orobanchoides* (18S: DQ786056.1; *atpA*: EU421026.1), *Dioscorea althaeoides* (18S: EU420997.1; *atpA*: EU421027.1), *Dioscorea communis* (18S: EU186223.1; *atpA*: AY277804.1), *Dioscorea prazeri* (18S: DQ786089.1; *atpA*: EU421028.1), *Dioscorea rockii* (18S: DQ786090.1; *atpA*: EU421029.1), *Geomitra clavigera* (18S: AF309405.1; *atpA*: EU421049.1), *Gymnosiphon bekensis* (18S: EU420998.1; *atpA*: EU421031.1), *Haplothismia exannulata* (18S: DQ786082.1; *atpA*: EU421037.1), *Hexapterella gentianoides* (18S: DQ786057.1; *atpA*: EU421038.1), *Lophiola aurea* (18S: DQ786091.1; *atpA*: EU421039.1), *Metanarthecium luteo-viride* (18S: AF309410.1; *atpA*: EU421040.1), *Narthecium ossifragum* (18S: AF309411.1; *atpA*: AY299809.1), *Nietneria paniculata* (18S: EU186219.1; *atpA*: EU421041.1), *Stenomeris dioscoreifolia* (18S: DQ786087.1; *atpA*: EU421042.1), *Tacca artocarpifolia* (18S: AF309397.1; *atpA*: EU421043.1), *Tacca chantieri* (18S: DQ786086.1; *atpA*: EU421044.1), *Tacca integrifolia* (18S: DQ786085.1; *atpA*: EU421045.1), *Tacca palmata* (18S: EU421000.1; *atpA*: EU421046.1), *Tacca plantaginea* (18S: U42063.1; *atpA*: EU421047.1), *Thismia panamensis* (18S: DQ786081.1; *atpA*: EU421050.1), *Thismia rodwayi* (18S: AF309403.1; *atpA*: AY299849.1), *Trichopus sempervirens* (18S: AF309395.1; *atpA*: AY299724.1), *Trichopus zeylanicus* (18S: AF309394.1; *atpA*: AY277805.1)
Liliales:	*Chamaelirium luteum* (18S: AF206884.1; *atpA*: AY299745.1), *Clintonia borealis* (18S: AF168833.1; *atpA*: AY299748.1), *Lilium superbum* (18S: AF206952.1; *atpA*: AY299797.1), *Trillium grandiflorum* (18S: AF168879.1; *atpA*: AF039253.1)
Petrosaviales:	*Japonolirion osense* (18S: AF206942.1; *atpA*: AY299790.1), *Petrosavia stellaris* (18S: AF206987.1; *atpA*: AY299821.1)
Poales:	*Ananas comosus* (18S: D29786.1; *atpA*: AY299710.1), *Cyperus alternifolius* (18S: AY952404.1; *atpA*: DQ401297.1), *Flagellaria indica* (18S: AF206913.1; *atpA*: AF039248.1), *Joinvillea ascendens* (18S: AF168855.1; *atpA*: AY124519.1), *Sparganium eurycarpum* (18S: AF069220.1; *atpA*: AY124509.1)
Dasypogonaceae:	*Dasypogon* sp. (18S: AJ417898.1; *atpA*: AY124503.1)
Zingiberales:	*Calathea loeseneri* (18S: AF069224.1; *atpA*: AY299735.1), *Canna indica* (18S: D29785.1; *atpA*: AY299741.1), *Heliconia rostrata* (18S: AF168850.1; *atpA*: AY299778.1), *Monocostus uniflorus* (18S: AF168861.1; *atpA*: AY299804.1), *Ravenala madagascariensis* (18S: AF069228.1; *atpA*: AY299830.1), *Strelitzia nicolai* (18S: AF069229.1; *atpA*: AY299843.1), *Tapeinochilos* sp. (18S: AH003649.1; *atpA*: AY299846.1)

Appendix B. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ympev.2013.05.031.

References

Bidartondo, M.I., 2005. The evolutionary ecology of myco-heterotrophy. New Phytol. 167 (2), 335–352.

Bremer, K., 2000. Early Cretaceous lineages of monocot flowering plants. Proc. Natl. Acad. Sci. USA 97 (9), 4707–4711.

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

Champion, J., 1847. On two new Ceylon plants related to *Sciaphila* of Blume. Calcutta J. Nat. Hist. 7, 463–469.

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

Chase, M.W., Fay, M.F., Devey, D.S., Maurin, O., Ronsted, N., Davies, T.J., Pillon, Y., Petersen, G., Seberg, O., Tamura, M.N., Asmussen-Lange, C.B., Kilu, K., Borsch, T., Davis, J., Stevenson, D.W., Pires, J.C., Givnish, T., Sytsma, K.J., McPherson, M.A., Graham, S.W., Rai, H.S., 2006. Multigene analyses of monocot relationships: a summary. Aliso 22, 63–75.

Cheek, M., 2003. Kupeaeae, a new tribe of Triuridaceae from Africa. Kew. Bull. 58, 939–949.

Cronquist, A., 1968. The Evolution and Classification of Flowering Plants. Houghton & Mifflin, Boston, Massachusetts.

Cronquist, A., 1981. An Integrated System of Classification of Flowering Plants. Columbia University Press, New York, New York.

Cronquist, A., 1988. The Evolution and Classification of Flowering Plants, second ed. New York Botanical Garden Press, New York, New York.

Dahlgren, R.M.T., Clifford, H.T., Yeo, P.F., 1985. The Families of the Monocotyledons. Structure, Evolution, and Taxonomy. Springer, Berlin.

Darriba, D., Taboada, G.L., Doallo, R., Posada, D., 2012. JModelTest 2: more models, new heuristics and parallel computing. Nat. Methods 9 (8), 772.

Davis, J.I., Stevenson, D.W., Peterson, G., Seberg, O., Campbell, L.M., Freudenstein, J.V., Goldman, D.H., Hardy, C.R., Michelangeli, F.A., Simmons, M.P., Specht, C.D., Vergara-Silva, F., Gandolfo, M., 2004. A phylogeny of the monocots, as inferred from *rbcL* and *atpA* sequence variation, and a comparison of methods for calculating Jackknife and Bootstrap values. Syst. Bot. 29 (3), 467–510.

Demesure, B., Sodzi, N., Petit, J., 1995. A set of universal primers for amplification of polymorphic non-coding regions of mitochondrial and chloroplast DNA in plants. Mol. Ecol. 4, 129–131.

Drummond, A.J., Suchard, M.A., Xie, D., Rambaut, A., 2012. Bayesian phylogenetics with BEAUti and the BEAST 1.7. Mol. Biol. Evol. 29 (8), 1969–1973.

Drummond, A.J., Ashton, B., Buxton, S., Cheung, M., Cooper, A., Duran, C., Field, M., et al., 2013. Geneious Pro v6.1.4. <http://www.geneious.com/> (21.02.13).

Edgar, R.C., 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res. 32 (5), 1792–1797.

Endlicher, S., 1837. Genera Plantarum Secundum Ordines Naturalis Disposita. Vindobonae, p. 282.

Engler, A., 1889. Triuridaceae. In: Engler, A., Prantl, K. (Eds.), Die Natürlichen Pflanzenfamiliën, ed. 1, II (1). Verlag von Wilhelm Engelmann, Leipzig, pp. 235–238.

Engler, A., 1897. Übersicht über die Unterabteilungen, Klassen, Reihen, Unterreihen und Familien der Embryophyta siphonogama. In: Engler, A., Prantl, K. (Eds.), Die Natürlichen Pflanzenfamiliën, Nachträge zum II.–IV. Teil. Verlag von Wilhelm Engelmann, Leipzig. p. 342.

Engler, A., 1915. Triuridaceae. In: Engler, A., Prantl, K. (Eds.), Die Natürlichen Pflanzenfamiliën, Nachträge IV zu II.1. Verlag von Wilhelm Engelmann, Leipzig. p. 8.

Eyre-Walker, A., Gaut, B.G., 1997. Correlated rates of synonymous site evolution across plant genomes. Mol. Biol. Evol. 14, 455–460.

Felsenstein, J., 1985. Confidence limits on phylogenetics: an approach using the Bootstrap. Evolution 39, 783–791.

Freudenstein, J.V., Chase, M.W., 2001. Analysis of mitochondrial *nad1b-c* intron sequences in Orchidaceae: utility and coding of length-change characters. Syst. Bot. 26 (3), 643–657.

Furness, C.A., Rudall, P.J., Eastman, A., 2002. Contribution of pollen and tapetal characters to the systematics of Triuridaceae. Plant Syst. Evol. 235, 209–218.

Gandolfo, M.A., Nixon, K.C., Crepet, W.L., Stevenson, D.W., Friis, E.M., 1998. Oldest known fossils of monocotyledons. Nature 394 (6693), 532–533.
Gandolfo, M.A., Nixon, K.C., Crepet, W.L., 2002. Triuridaceae fossil flowers from the Upper Cretaceous of New Jersey. Am. J. Bot. 89 (12), 1940–1957.
Gardner, G., 1843. Description of Peltophyllum, a new genus of plants allied to Triuris of Miers, with remarks on their affinities. Trans. Linn. Soc. London 19, 155–160 (1845).
Gernhard, T., 2008. The conditioned reconstructed process. J. Theor. Biol. 253 (4), 769–778.
Geyer, C.J., 1991. Markov Chain Monte Carlo maximum likelihood. In: Keramidas, E.M. (Ed.), Computing Science and Statistics: Proceedings of the 23rd Symposium on the Interface. Fairfax: Interface Foundation of North America Inc. Fairfax Station, Virginia. pp. 156–163.
Giesen, H., 1938. Triuridaceae. In: Engler, A. (Ed.), Das Pflanzenreich. Regni vegetabilis conspectus IV. 18. Verlag von Wilhelm Engelmann, Leipzig, pp. 1–84.
Greuter, W., and others, 1988. International Code of Botanical Nomenclature. Koelz Scientific, Königstein. Regnum vegetabile, p. 118.
Guindon, S., Gascuel, O., 2003. A simple, fast and accurate method to estimate large phylogenies by maximum-likelihood. Syst. Biol. 52 (5), 696–704.
Harley, M.M., 1996. Palm Pollen and the Fossil Record. Dissertation. Royal Botanic Gardens, Kew.
Harling, G., Wilder, G.J., Eriksson, R., 1998. Cyclanthaceae. In: Kubitzki, K. (Ed.), The Families and Genera of Vascular Plants. III Flowering Plants: Monocotyledons. Springer, Berlin, pp. 202–215.
Heintze, A., 1927. Cormofyternas Fylogeni (Phylogenie der Cormophyten). Hakan Ohlssons Boktryckeri, Lund, p. 77.
Hemsley, W.B., 1907. Two new Triuridaceae, with some remarks on the genus Sciaphila, Blume. Ann. Bot. – Lond. 21, 71–77.
Hutchinson, J., 1934. The Families of Flowering Plants. II. Monocotyledons. Macmillan and co. London, pp. 37–38.
Janssen, T., Bremer, K., 2004. The major monocot groups inferred from 800+ *rbcL* sequences. Bot. J. Linn. Soc. 146 (4), 385–398.
Kubitzki, K., 1998a. Stemonaceae. In: Kubitzki, K. (Ed.), The Families and Genera of Vascular Plants. III Flowering Plants: Monocotyledons. Springer, Berlin, pp. 422–425.
Kubitzki, K., 1998b. Velloziaceae. In: Kubitzki, K. (Ed.), The Families and Genera of Vascular Plants. III Flowering Plants: Monocotyledons. Springer, Berlin, pp. 459–467.
Le Maout, E., Decaisne, J., 1868. Traité general de botanique descriptive et analytique. Libairie de fermin didot frères, Paris, p. 553.
Leake, J.R., 1994. The biology of myco-heterotrophic ('saprophytic') plants. New Phytol. 127, 171–216.
Lindler, H.P., 1987. The evolutionary history of the Poales/Restionales – a hypothesis. Kew. Bull. 42, 297–318.
Lindley, J., 1846. The Vegetable Kingdom 213. Bradbury & Evans, London.
Maas, P.J.M., Rübsamen, T., 1986. Triuridaceae. Fl. Neotrop. Monogr. 40, 1–55.
Maas-van de Kamer, H., 1995. Triuridiflorae – Gardner's delight? In: Rudall, P.J., Cribb, P.J., Cutler, D.F., Humphries, C.J. (Eds.), Monocotyledons: Systematics and Evolution. Royal Botanic Gardens, Kew, pp. 287–301.
Maas-van de Kamer, H., Weustenfeld, T., 1998. Triuridaceae. In: Kubitzki, K. (Ed.), The Families and Genera of Vascular Plants. III Flowering Plants: Monocotyledons. Springer, Berlin, pp. 452–458.
Mabberley, D.J., 2008. Mabberley's Plant Book, third ed. Cambridge University Press, p. 876.
Martínez, E., Ramos, C.H., 1989. Lacandoniaceae (Triuridales): Una nueva familia de Mexico. Ann. Miss. Bot. Gard. 76 (1), 128–135.
Meng, S.W., Chen, Z.D., Li, D.Z., Liang, H.X., 2002. Phylogeny of Saururaceae based on mitochondrial *matR* gene sequence data. J. Plant. Res. 115 (1118), 71–76.
Merckx, V.S.F.T., 2008. Myco-heterotrophy in Dioscoreales: Systematics and Evolution. PhD Thesis, Katholieke Universiteit Leuven, Belgium.
Merckx, V.S.F.T., Bidartondo, M.I., 2008. Breakdown and delayed cospeciation in the arbuscular mycorrhizal mutualism. Proc. Roy. Soc. B 275 (1638), 1029–1035.
Merckx, V.S.F.T., Schols, P., Maas-van de Kamer, H., Maas, P., Huysmans, S., Smets, E.F., 2006. Phylogeny and evolution of Burmanniaceae (Dioscoreales) based on nuclear and mitochondrial data. Am. J. Bot. 93 (11), 1684–1698.
Merckx, V.S.F.T., Chatrou, L.W., Lemaire, B., Sainge, M.N., Huysmans, S., Smets, E.F., 2008. Diversification of myco-heterotrophic angiosperms: evidence from Burmanniaceae. BMC Evol. Biol. 8 (1), 178.
Merckx, V.S.F.T., Bakker, F.T., Huysmans, S., Smets, E.F., 2009. Bias and conflict in phylogenetic inference of myco-heterotrophic plants: a case study in Thismiaceae. Cladistics 25 (1), 64–77.
Merckx, V.S.F.T., Huysmans, S., Smets, E.F., 2010. Cretaceous origins of myco-heterotrophic lineages in Dioscoreales. In: Seberg, O., Petersen, G., Barfod, A.S., Davis, J. (Eds.), Diversity, Phylogeny, and Evolution in the Monocotyledons. Aarhus University Press, Denmark, pp. 39–53.
Miers, J., 1850. On the family of Triuriaceae. Proc. Linn. Soc. London 2, 72–77.
Miers, J., 1841. Description of a new genus of plants from Brazil. Trans. Linn. Soc. Lond. 19 tab. VII, 77–80 (1845).
Mower, J.P., Stefanović, S., Hao, W., Gummow, J.S., Jain, K., Ahmed, D., Palmer, J.D., 2010. Horizontal acquisition of multiple mitochondrial genes from a parasitic plant followed by gene conversion with host mitochondrial genes. BMC Biol. 8, 150.
Mueller, C. (1858). Triuriaceae. In: Walpers, G.G. (Ed.), Annales Botanices Systematicae, tomus V. Lipsiae Sumptibus Ambrosii Abel. pp. 915–919.
Poulsen, V.A., 1890. *Triuris major* sp. nov. Et Bidrag til Triuridaceernes Naturhistorie. Bot. Tidskr 17, 293–306.
Qiagen, 2006. DNEasy plant handbook, Plant Tissue (Mini Protocol). pp. 24–27.
Rabinowitz, P.D., Coffin, M.F., Falvey, D., 1983. The separation of Madagascar and Africa. Science 220 (4592), 67–69.
Rambaut, A., 2009. FigTree v1.3.1. <http://tree.bio.ed.ac.uk/software/figtree/> (21.02.13).
Rambaut, A., Drummond, A.J., 2007. Tracer v1.5. <http://beast.bio.ed.ac.uk/Tracer> (21.02.13).
Ramirez, S.R., Gravendeel, B., Singer, R.B., Marshall, C.R., Pierce, N.E., 2007. Dating the origin of the Orchidaceae from a fossil orchid with its pollinator. Nature 448 (7157), 1042–1045.
Raven, P.H., Axelrod, D.I., 1974. Angiosperm biogeography and past continental movements. Ann. Miss. Bot. Gard. 61 (3), 539–673.
Reveal, J.L., 1992. Validation of subclass and superordinal names in Magnoliophyta. Novon 2, 235–237.
Rodríquez-de la Rosa, R.A., Cevallos-Ferriz, S.R.S., 1994. Upper Cretaceous Zingiberalean fruits with in situ seeds from southeastern Coahuila, Mexico. Int. J. Plant Sci. 155 (6), 786–805.
Ronquist, F., Teslenko, M., Van der Mark, P., Ayres, D.L., Darling, A., Höhna, S., Larget, B., et al., 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Syst. Biol. 61, 539–542.
Rudall, P.J., Bateman, R.M., 2006. Morphological phylogenetic analysis of Pandanales: testing contrasting hypotheses of floral evolution. Syst. Bot. 31 (2), 223–238.
Schlechter, R., 1912. Neue Triuridaceae Papuasiens. Bot. Jahrbücher 49, 70–89.
Schumann, K., Lauterbach, C., 1905. Nachtr. Fl. Deut. Schutzgeb. Südsee, 54–56.
Soltis, D.E., Smith, S.A., Cellinese, N., Wurdack, K.J., Tank, D.C., Brockington, S.F., Refulio-Rodriguez, N.F., Walker, J.B., Moore, M.J., Carlsward, B.S., Bell, C.D., Latvis, M., Crawley, S., Black, C., Diouf, D., Xi, Z., Rushworth, C.A., Gitzendanner, M.A., Sytsma, K.J., Qui, Y.L., Hilu, K.W., Davis, C.C., Sanderson, M.J., Beaman, R.S., Olmstead, R.G., Judd, W.S., Donoghue, M.J., Soltis, P.S., 2011. Angiosperm phylogeny: 17 genes, 640 taxa. Am. J. Bot. 98 (4), 704–730.
Stone, B.C., Huynh, K.-L., Poppendieck, H.-H., 1998. Pandanaceae. In: Kubitzki, K. (Ed.), The Families and Genera of Vascular Plants. III Flowering Plants: Monocotyledons. Springer, Berlin, pp. 397–404.
Thorne, R.F., 1968. Synopsis of a putatively phylogenetic classification of the flowering plants. Aliso 6 (4), 57–66.
Thwaites, G.H.K., 1861. Enumerario Plantarum Zeylaniae. Dulau & Co. London, p. 294.
Van de Meerendonk, J.P.M., 1984. Triuridaceae. In: Van Steenis C.G.G.J. (Ed). , Flora Malesiana, Ser. I., 10, Martinus Nijhoff, The Hague, pp. 109–121.
Vergara-Silva, F., Espinosa-Matías, S., Ambrose, B.A., Vázquez-Santana, S., Martínez-Mena, A., Márquez-Guzmán, J., Martínez, E., Meyerowitz, E.M., Alvarez-Buylla, E.R., 2003. Inside-out flowers characteristic of *Lacandonia schismatica* evolved at least before its divergence from a closely related taxon, *Triuris brevistylis*. Int. J. Plant Sci. 164 (3), 345–357.
White, T.J., Bruns, T.D., Lee, S., Taylor, J.W., 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis, M.A., Gelfand, D.H., Sninsky, J.J., White, T.P. (Eds.), PCR Protocols: A Guide to Methods and Applications. Academic Press, Orlando, Florida, pp. 315–322.
Zhu, X.Y., Chase, M.W., Qiu, Y.L., Kong, H.Z., Dilcher, D.L., Li, J.H., Chen, Z.D., 2007. Mitochondrial *matR* sequences help to resolve deep phylogenetic relationships in rosids. BMC Evol. Biol. 7, 217.
Zwickl, D.J., 2006. Genetic Algorithm Approaches for the Phylogenetic Analysis of Large Biological Sequence Datasets under the Maximum Likelihood Criterion. Ph.D. Dissertation, The University of Texas at Austin.