



Giant taro and its relatives: A phylogeny of the large genus *Alocasia* (Araceae) sheds light on Miocene floristic exchange in the Malesian region

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ABSTRACT

Alocasia comprises over 113 species of rainforest understorey plants in Southeast Asia, the Malesian region, and Australia. Several species, including giant taro, *Alocasia macrorrhizos*, and Chinese taro, *Alocasia cucullata*, are important food plants or ornamentals. We investigated the biogeography of this genus using plastid and nuclear DNA sequences (5200 nucleotides) from 78 accessions representing 71 species, plus 25 species representing 16 genera of the *Pistia* clade to which *Alocasia* belongs. Divergence times were inferred under strict and relaxed clock models, and ancestral areas with Bayesian and maximum likelihood approaches. *Alocasia* is monophyletic and sister to *Colocasia gigantea* from the SE Asian mainland, whereas the type species of *Colocasia* groups with *Stuednera* and *Remusatia*, requiring taxonomic realignments. Nuclear and plastid trees show topological conflict, with the nuclear tree reflecting morphological similarities, the plastid tree species' geographic proximity, suggesting chloroplast capture. The ancestor of *Alocasia* diverged from its mainland sister group c. 24 million years ago, and Borneo then played a central role in the expansion of *Alocasia*: 11–13 of 18–19 inferred dispersal events originated on Borneo. The Philippines were reached from Borneo 4–5 times in the Late Miocene and Early Pliocene, and the Asian mainland 6–7 times in the Pliocene. Domesticated giant taro originated on the Philippines, Chinese taro on the Asian mainland.

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1. Introduction

The Malay Archipelago has long attracted the attention of biogeographers because of its outstanding biodiversity, endemism, and complex geological history (Wallace, 1869; Morley, 1998; Lohman et al., 2011). Of particular interest has been to understand the intermixing of ancestrally Laurasian and Gondwanan lineages in this region, and molecular phylogenies combined with molecular clocks now allow a more precise understanding of the direction and timing of such intermixing (Lohman et al., 2011; Richardson et al., 2012). Biogeographic studies of Malesian plant clades utilizing these methods are available for seven groups. These are the stone oak genus, *Lithocarpus* (Cannon and Manos, 2003), the myrmecophytic Euphorbiaceae genus *Macaranga* (Bänfer et al., 2006), the Meliaceae tribe Aglaieae (Muellner et al., 2008), the Annonaceae genus *Pseuduvaria* (Su and Saunders, 2009), the large genera *Cyrtandra* (Gesneriaceae; Clark et al., 2008) and *Begonia* (Begoniaceae; Thomas et al., 2011), and the palm genus *Livistona* (Arecaceae; Crisp et al., 2010). These studies revealed predominant

west to east dispersal and diversification after the late Miocene. This general pattern may be explained by the large source region of wet forest west of the Wallace line and a later emergence of landmasses east of the Wallace line, leading to a more frequent colonization from west to east (Richardson et al., 2012).

A plant group that is extremely species-rich in the Malesian region is the Araceae, a family with a relatively extensive fossil record going back to the Upper Early Cretaceous, Paleocene, and Eocene (Friis et al., 2004, 2010; Wilde et al., 2005; Herrera et al., 2008). Among the largest genera in the family is *Alocasia*, a member of the relatively derived *Pistia* clade (Renner and Zhang, 2004; Cabrera et al., 2008; Cusimano et al., 2011). *Alocasia* currently comprises 113 species, with 27 more awaiting description (Nicolson, 1968, 1987; Hay and Wise, 1991; Hay, 1998, 1999; Boyce, 2008; PB unpublished data). The natural range of *Alocasia* extends from India and Sri Lanka through Indochina to China and southern Japan, the Malay Archipelago and Oceania; a single species is indigenous in Australia (Fig. 1). Several species are commercially important indoor plants, others are cultivated outdoors, such as *Alocasia cucullata* (Chinese taro), an ethnobotanically important plant throughout Asia, and *Alocasia macrorrhizos* (giant taro) a tropical ornamental cultivated for its tubers and leaves, used as animal fodder (Weightman, 1989; Mayo et al., 1997). The wild origin of these two species is unknown (Hay, 1999; Boyce, 2008).

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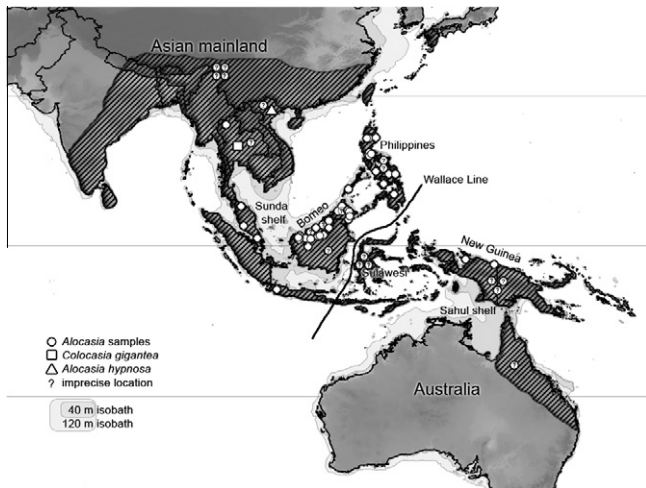


Fig. 1. Map showing the global distribution of *Alocasia* (shaded area) and origin of samples included in this study. Circles refer to *Alocasia* samples; circles with a question mark indicate locations without GPS data. The square shows the collection location of the *Colocasia gigantea* sample, the triangle that of the *Alocasia hypnosa* sample. The 40 m and 120 m isobaths are shown as pale grey outlines.

The typical *Alocasia* habitat is the understory of perhumid lowland forest; only a few species grow >1000 m altitude or in light-gaps, clearings, or secondary vegetation (Hay and Wise, 1991; Hay, 1998; Boyce, 2008). Growth forms range from small herbaceous to thick-stemmed massive plants with huge leaves (Fig. 2). Seed dispersal is by birds and pollination by drosophilid flies (genus *Colocasiomyia*) that use the spadices as breeding sites (Ivancic et al., 2005, 2008; Sultana et al., 2006). Little is known about the specificity of *Alocasia* pollinators or hybridization in nature, but morphologically polymorphic species or species 'complexes' have been suspected as involving hybridization (Hay, 1998).

Molecularly, hybridization can be detected when plastid and nuclear sequences yield different tree topologies, which can point to the maternally inherited plastid genome coming from a different source than an individual's nuclear genome. Given the evidence for widespread hybridization and chloroplast capture in plants (Bänfer et al., 2006; Cristina Acosta and Premoli, 2010; Manen et al., 2010)



Fig. 2. Representative species of *Alocasia*. Habit of *A. robusta* at disturbed forest edge, Sarawak, Malaysia (left); habit of *A. reversa* on limestone rocks, Sarawak, Malaysia (upper middle), inflorescence of *A. longiloba* 'denudata' in rainforest understory in Singapore (right), colocasioid venation on lower leaf surface of *A. sarawakensis* in Sabah, Malaysia (lower middle).

it is surprising that only two studies of Southeast Asian plant groups have compared plastid and nuclear histories. In stone oaks, *Lithocarpus*, Cannon and Manos (2003) found that the nuclear DNA data contained less geographical structure than the plastid data, indicating that gene flow mediated through pollen is less restricted than purely seed-mediated (chloroplast) gene flow, and in *Macaranga*, Bänfer et al. (2006) documented plastid haplotype sharing by up to seven species and as many as six co-existing haplotypes in a single species.

Here we use both nuclear and plastid sequences and broad geographic sampling of species to address the following questions: (i) Is *Alocasia* monophyletic and which clade or species in the *Pistia* group is it most closely related to? (ii) Where do the cultivated species giant taro, *A. macrorrhizos*, and Chinese taro, *A. cucullata*, come from? And (iii) Do nuclear and plastid data yield congruent topologies or is there evidence of hybridization? Because of its species diversity and wide distribution range, understanding the biogeography of *Alocasia* also sheds light on floristic links across the Malesian regions.

2. Materials and methods

2.1. Taxon sampling and number of species

Of currently 113 *Alocasia* species, we here sample 71, represented by 78 plants. Table S1 provides a list of the species with author names, geographic origin of material, herbarium vouchers, and GenBank accession numbers for all sequences. Of these species, 32 were named in the past 20 years, and the discovery of new species continues (e.g., Kurniawan and Boyce, 2011). At the moment, 27 species await description once complete flowering and fruiting material is available (PB, personal collections). Our sampling covers the geographic range and morphological diversity of *Alocasia*, except for New Guinea, which is underrepresented (Hay and Wise, 1991 recognized five groups there of which our sample includes one). Of the taxonomically problematic species *Alocasia longiloba* we included 13 accessions (with nine different species names) and of *Alocasia robusta* (Fig. 2, left photo) three. Leaf material came from herbarium specimens or silica-dried leaf samples (Table S1). Determination of plant material relied on comparison with herbarium material carried out by PB and LN. As outgroups, we included 25 species representing 16 genera of the *Pistia* clade (Renner and Zhang, 2004; Cabrera et al., 2008; Cusimano et al., 2011), usually the type species of the genus name.

2.2. Isolation of DNA, amplification, and sequencing

DNA isolation followed standard protocols. To deduce phylogenetic relationships, we relied on the nuclear phytochrome C gene (*phyC*), and four plastid loci, the *trnL* intron, the *trnL-F* intergenic spacer, the *rpl20-rps12* intergenic spacer, and the *trnK/matK* region. Total DNA from silica-dried leaves was extracted with the NucleoSpin plant kit according to the manufacturer's protocol (Macherey–Nagel, Düren, Germany). Sequencing of the >2500 nucleotide (nt)-long *trnK* marker, amplified in one piece with the primer pair *trnK*-3914F (dicot) – *trnK*-16R (Johnson and Soltis, 1994), was problematic. Consequently, we designed new internal primers and amplified the section in four pieces: *trnK*-3914F – *trnK*-AR-alo, *trnK*-19F – *trnK*-RM-ara, *trnK*-FM-ara – *trnK*-1760R-alo, and *trnK*-1640F-alo – *trnK*-R1-mono. The new primer sequences are as follows: *trnK*-AR-alo 5'-CTC TTG AAA GAG AAG CCG ATA TAG-3', *trnK*-19F 5'-TGT TCT GGC CAT ATC GCA CTA TG-3', *trnK*-RM-ara 5'-AAG ATG TTG ATC GTA AAT AAG AGG-3', *trnK*-FM-ara 5'-GTT TTG CTG TCA TTA TGG AAA TTC-3', *trnK*-1760R-alo 5'-TAC CGC TGA AGG ATT TAT TAG GAC-3', *trnK*-1640F-alo 5'-GGG ACT CAT CTT

CTG ATG AAG AAA-3', *trnK*-R1-mono 5'-CAT TTT TCA TTG CAC ACG RC-3'. *PhyC* was also amplified in two pieces with the newly designed primers: A20F – 748R and 430F – AR: A20F: 5'-CAC TCA ATC CTA CAA ACT GGC-3', 748R: 5'-ACA AGA TCC ATG ACA TTA GGT GAT T-3', 430F: 5' CTC GTG ATG TCT GTC ACA ATA AG-3' and AR: 5'-GAA TAG CAT CCA TTT CAA CAT C-3'. The *rpl20-rps12* intergenic spacer was amplified using the primers and PCR conditions described in Renner and Zhang (2004). The *trnL* intron and *trnL-F* intergenic spacer was amplified according to Taberlet et al. (1991) with the primer pairs c–d and e–f, respectively. PCR products were cleaned using the NucleoSpin® Extract II kit (Macherey–Nagel, Dueren, Germany), and sequencing reactions were run on an ABI 3130 capillary sequencer (Applied Biosystems), following the manufacturers' protocols. Sequence editing and alignment were carried out in Sequencher 4.7 (Gene Codes, Ann Arbor, Michigan, USA) and McCade 4.0 (Maddison and Maddison, 2000). New sequences generated for this study have been deposited in GenBank (accession numbers in Table S1), and alignments for this study in TreeBASE (submission ID 12182).

2.3. Phylogenetic analysis

Individual and combined phylogenetic analyses were performed under likelihood (ML) optimization and the GTR + Γ substitution model, using RAxML 7.0.4 (Stamatakis, 2006) and the RAxML-GUI 0.93 (Silvestro and Michalak, 2010). Statistical support was assessed via 100 bootstrap replicates. Throughout this paper, 70–84% bootstrap support is considered moderate and 85–100% strong support.

2.4. Divergence dating

Dating relied on Bayesian divergence time estimation as implemented in BEAST 1.61 (Drummond et al., 2006; Drummond and Rambaut, 2007). Identical sequences and sequences differing only in nucleotide ambiguities were removed, yielding a matrix of 81 species (56 *Alocasia*). For the dating runs, we used the HKY + Γ model of substitution, which is an extension of the K80 + Γ model identified as the best fit in jModelTest (Posada, 2008), and a Yule tree prior as the tree model. Runs were repeated under either a strict clock model or a relaxed clock model, the latter with rate variation across branches uncorrelated and lognormally distributed. MCMC chains were run for 16 million in the strict clock model, and for 50 million generations in the relaxed clock model, with parameters sampled every 1600th and 5000th generation, respectively. Tracer (1.50) was used to assess effective sample sizes (ESS) for all estimated parameters and to judge the percentage of burn-in for tree constructions. Trees were combined in TreeAnnotator (1.6.1), and maximum clade credibility trees with mean node heights were visualized in R (www.r-project.org) with the package phyloch (www.christophheibl.de/Rpackages.html).

For calibration, we used the age of the leaf fossil *Caladiosoma messelense* from the Messel Formation near Frankfurt, Germany (47–47.5 Ma), which resembles species of *Alocasia* and *Colocasia* in its colcasoid leaf venation (Fig. 2). This venation consists of fine veins that branch almost at right angles from the primary lateral veins and arch towards the leaf margin, sometimes fusing to an interprimary collective vein. It occurs in *Alocasia*, *Colocasia*, *Ariopsis*, *Remusatia*, and *Steudnera*, but also in the South American genera *Caladium* and *Xanthosoma*. The Eurasian origin of the fossil, however, fits better with a placement as a relative of Southeast Asian Araceae. Other members of the *Pistia* clade, such as the *Areae*, do not possess colcasoid venation, either due to a loss of the character or because of multiple gains in *Alocasia*, and the *Colocasia* clade. To account for the uncertain placement of *C. messelense* in the *Pistia* clade, we explored the effects of different fossil-

cum-topology combinations on the resulting age estimates (*Results*), always using a normally distributed prior age distribution that had a mean of 47.25 Ma and a standard deviation (SD) of 0.15 Ma. This narrow SD is justified by the well-constrained dating of the Messel Formation (V. Wilde, Senckenberg Museum, Frankfurt, Germany, pers. comm., 2009). Additionally, one run was performed without constraining the topology. We also ran an analysis with an empty alignment (the “prior-only” option in BEAST) to compare the posterior parameter values without and with the data. This allows evaluating the influence of prior distributions on the outcome of Bayesian analyses. For absolute ages we relied on the geologic time scale of Walker et al. (2009).

2.5. Ancestral area optimization

To infer the geographic history of *Alocasia*, we used two approaches: Dispersal vicariance analysis (DIVA) in a Bayesian framework, using the S-DIVA (Statistical Dispersal-Vicariance) program in RASP 1.1 (Yu et al., 2010, 2011), and a dispersal-extinction-cladogenesis model (DEC) in a likelihood framework, using Lagrange (Ree et al., 2005; Ree and Smith, 2008). S-DIVA applies the DIVA method of Ronquist (1997) to the multiple trees from a Bayesian analysis, which has the advantage that uncertainties in phylogenetic inference can be taken into account. In contrast, Lagrange uses a likelihood approach that takes into account the branch length of a given tree. As input for S-DIVA we used the BEAST MCMC tree chain obtained under the strict clock model applied to the nuclear data (minus the burn-in of the divergence dating analysis) and the maximum clade credibility tree to plot the results. As input tree for Lagrange we used the same BEAST maximum clade credibility tree. A Python script was created using the online Lagrange configurator. We did not constrain the dispersal probabilities to avoid over-parameterization.

Species distributions were categorized into eight areas: (A) Asian mainland (including Sumatra and Java), (B) Borneo (including the Philippine island Palawan, which geologically belongs to the Sunda shelf), (C) The Philippines, (D) Sulawesi, (E) New Guinea, (F) Australia, (G) Mediterranean area, and (H) Seychelles. In S-DIVA, as combined ancestral areas we allowed three combinations: Asia with the Mediterranean area (AG), the Asian mainland with Borneo (AB), and New Guinea with Australia (EF). Lagrange requires connectivity between areas, which we provided by allowing combined ancestral areas of adjacent regions: AB, AG, AH, BC, BD, CD, CE, DE, and EF. *Alocasia* spec. nov. BO07, from the Botanical Garden in Bogor has identical nuclear sequences as *Alocasia portei* and *A. macrorrhizos*. Its reported origin (Sulawesi) could not be verified, and it was therefore excluded from this analysis.

3. Results

3.1. Sequence data

Four hundred twenty-one sequences were newly generated for this study. The aligned *trnL* sequences comprised 731 positions, of which we excluded 260 of one poly-T run, two poly-A runs, and a TA tandem array region. The *trnL-F* alignment comprised 439 nucleotides of which one poly-T run with five nucleotides was excluded. The length of the *rpl20-rps12* alignment was 808 nucleotides of which we excluded 22 nucleotides of two poly-A runs and one poly-T run. The *trnK/matK* alignment included no ambiguously aligned sections and was used in its entirety for a length of 2504 aligned positions. The alignment of the nuclear low copy gene *phyC* comprised 1074 nucleotides, including four that showed double peaks. These sites were excluded from phylogenetic analyses.

3.2. Phylogenetic relationships in the plastid and nuclear trees

Plastid trees from the four data partitions revealed no statistically supported discrepancies and the sequences were therefore concatenated. Comparison of the combined plastid tree with the nuclear *phyC* tree, however, revealed topological contradictions. In both trees, species of *Alocasia* form a strongly supported clade with the exception of *Alocasia hypnosa*, which in the plastid tree (Fig. 3a) is sister to most of the remaining *Pistia* clade, but in the nuclear tree is placed more centrally in the *Pistia* clade (Fig. 3b). For the remaining taxa, both trees reveal the same main lineages: (a) *Alocasia* plus *Colocasia gigantea* (indicated by yellow branches in Fig. 3), (b) the Areae clade (*Arisaema*, *Arum*, *Biarum*, *Dracunculus*, *Eminium*, *Helicodicerus*, *Lazarum*, *Pinellia*, *Sauromatum*, *Theriophorum*, and *Typhonium*; blue branches in Fig. 3), and (c) the *Colocasia* clade (*Colocasia* as to its type species, *Ariopsis*, *Remusatia*, and *Stuednera*; green branches in Fig. 3).

Within core *Alocasia* (excluding *A. hypnosa*), the plastid phylogeny shows six geographic clades, numbered and color-coded in Fig. 3a. An Asian mainland clade (clade i shown in green) is sister to a polytomy that comprises a Philippine clade (clade ii shown in purple), a New Guinean clade (iii, yellow), a Bornean clade (iv, blue), another Asian mainland clade (v, green), and a poorly supported clade of Bornean and Philippine species (vi, blue). The position of the sole Australian species of *Alocasia*, *A. brisbanensis*, remains unresolved.

The nuclear phylogeny revealed four main clades (labeled A to D in Fig. 3b), which often include species with similar morphological traits. Clade A contains most species of the *longiloba* group. All have strongly rhythmic growth, thinly membranous cataphylls, and solitary or rather few leaves with often peltate, pendulous blades; however, two Sulawesi species that morphologically are convincingly part of this group are in Clade C. Clade A further contains a group of massive species with syleptic renewal, bi-modular inflorescences, long petiolar sheath, and large, short-lived leaves that all occur in the Indochinese region. Clade B comprises all species with a massive caulescent habit, including species with proleptic renewal growth (e.g., *A. robusta*) and sylleptic renewal growth (e.g., *A. macrorrhizos*). Clade B also includes the edible *A. macrorrhizos* and three accessions of *A. robusta*. Clade C contains only species with short-lived leaves and proportionately long petiolar sheaths, including the New Guinean *Xenophya* species group (Nicolson, 1968), which has hemianatropous or anatropous ovules and a usually persisting spathe limb (Hay and Wise, 1991). Clade D contains small or robust often lithophytic species usually referred to as the *princeps* group (*Alocasia princeps*, *Alocasia pangeran*, *Alocasia principulus*, *Alocasia wongii*, *Alocasia ridleyi*, *Alocasia "inopinata"* (P. Boyce, ined.), and *Alocasia reversa*), characterized by long erect petioles, narrowly triangular leaf blades, the lower spathe constriction occurring well above the sterile interstice between the pistillate and staminate flowers, relatively elongated inflorescences with tapering appendices (Hay, 1998), and the similar *scabriuscula* group (*Alocasia scabriuscula*, *Alocasia melo*, *Alocasia reginula*, *Alocasia kulat*, *Alocasia reginae*, *Alocasia chaili*, *Alocasia infernalis*, and *Alocasia nebula*), characterized by leathery to subsucculent leaves, several to many inflorescences not interspersed by foliage leaves, and the spathe constriction often above the sterile interstice of the spadix (Hay, 1998).

Topological differences between the plastid and nuclear trees were also found in the taxonomically problematic *A. robusta* and *A. longiloba* of which our sampling included, respectively, three and 13 accessions (the last with nine species names). In the nuclear tree, 11 of the 13 accessions of the *A. longiloba* species complex group together (yellow bar in Fig. 3b), while in the plastid tree (3a) they are partly found in clade I, partly in clade iv, and partly in clade v. The three accessions of *A. robusta* group together in

the nuclear tree (clade B in Fig. 3b), although the *phyC* sequences of the two Sabah plants differ from the Sarawak plant in two substitutions. In the plastid tree (Fig. 3a), the Sabah plants have identical sequences, but the plant from Sarawak differs substantially.

3.3. Divergence time estimation

Divergence time estimation (Fig. 4) relied on the nuclear dataset because it better reflects bi-parental inheritance. Since the relationships of *A. hypnosa* to the other groups (*Alocasia*/*C. gigantea*; the *Colocasia* clade; and the Areae clade; color-coded in Fig. 3) were not confidently resolved by our data, calibration with the *Caladiosoma messelense* leaf fossil (Section 2) was applied to 15 possible topologies for these clades. The results (Table S2) show that ages in the *Alocasia* crown node hardly differed among the 15 trees. The best ESS values were reached with the topology in which the Areae were sister to the remaining taxa, and the topology obtained from an unconstrained run also showed this placement of the Areae. The strict clock model and the relaxed clock model gave estimates for the *Alocasia* crown that on average differed by 6.16 ± 0.9 Ma, with the relaxed clock model yielding older ages. Mean ages from the strict clock model generally fall inside the 95% highest posterior density (HPD) intervals of the relaxed model, but the converse is not the case (Table S2). The mean *phyC* substitution rate was 0.00060 ± 0.00001 per site/million years under the strict clock and 0.000055 ± 0.00003 under the relaxed clock.

According to the strict clock model, the split between *Alocasia* and *C. gigantea* occurred at 24.1 Ma, i.e., the transition of the Oligocene to the Miocene, and *Alocasia* began diversifying in the Mid-Miocene (13.5 Ma). Dates are ~ 1.47 times older in the relaxed clock analysis, which places the stem of *Alocasia* in the Mid-Oligocene (29.1 Ma) and its crown in the Early Miocene (19.3 Ma). Exact dates with HPD for biogeographically important nodes are shown in Table S2, and for all nodes in the preferred tree in Table S3.

3.4. Ancestral area optimization

Bayesian (S-DIVA) and likelihood-based (Lagrange) ancestral area reconstruction on the nuclear tree yielded mostly congruent ancestral areas except for nodes 24, 26, and 41 (marked in bold in Fig. 4 and Table S3). Node 24 involves the sparsely sampled outgroups *Sauromatum* and *Lazarum*. Nodes 26 and 41 are the deepest splits in core *Alocasia* (i.e., excluding *A. hypnosa*, which clearly is not a member of a monophyletic genus *Alocasia*). S-DIVA and DEC both inferred that the split between *Alocasia* and *C. gigantea* occurred on the Asian mainland, but they differed in the inferred distribution of most recent common ancestor of the *Alocasia* crown group (node 26): S-DIVA inferred an origin in Asia, Lagrange an origin on Borneo. Both approaches inferred the combined area Sundaland as the second-most probable ancestral region for this node. For node 41, Lagrange inferred an origin on Borneo, while S-DIVA inferred an origin on the Asian mainland. S-DIVA also inferred combined ancestral areas more often than did Lagrange, resulting in the inference of fewer dispersal events by S-DIVA. Of the 54 divergence events in the tree, 18 or 19 involve dispersal events, with the majority dispersals out of Borneo (13 inferred by Lagrange, 11 by S-DIVA), in contrast to only three or four dispersal into Borneo. The Philippines were colonized 4–5 times and are the likely region from which New Guinea and Australia were colonized (node 42 in Fig. 4). Sulawesi was reached once from Borneo and once from the Philippines. Dispersal and divergence events are more or less evenly distributed over time (Fig. 4).

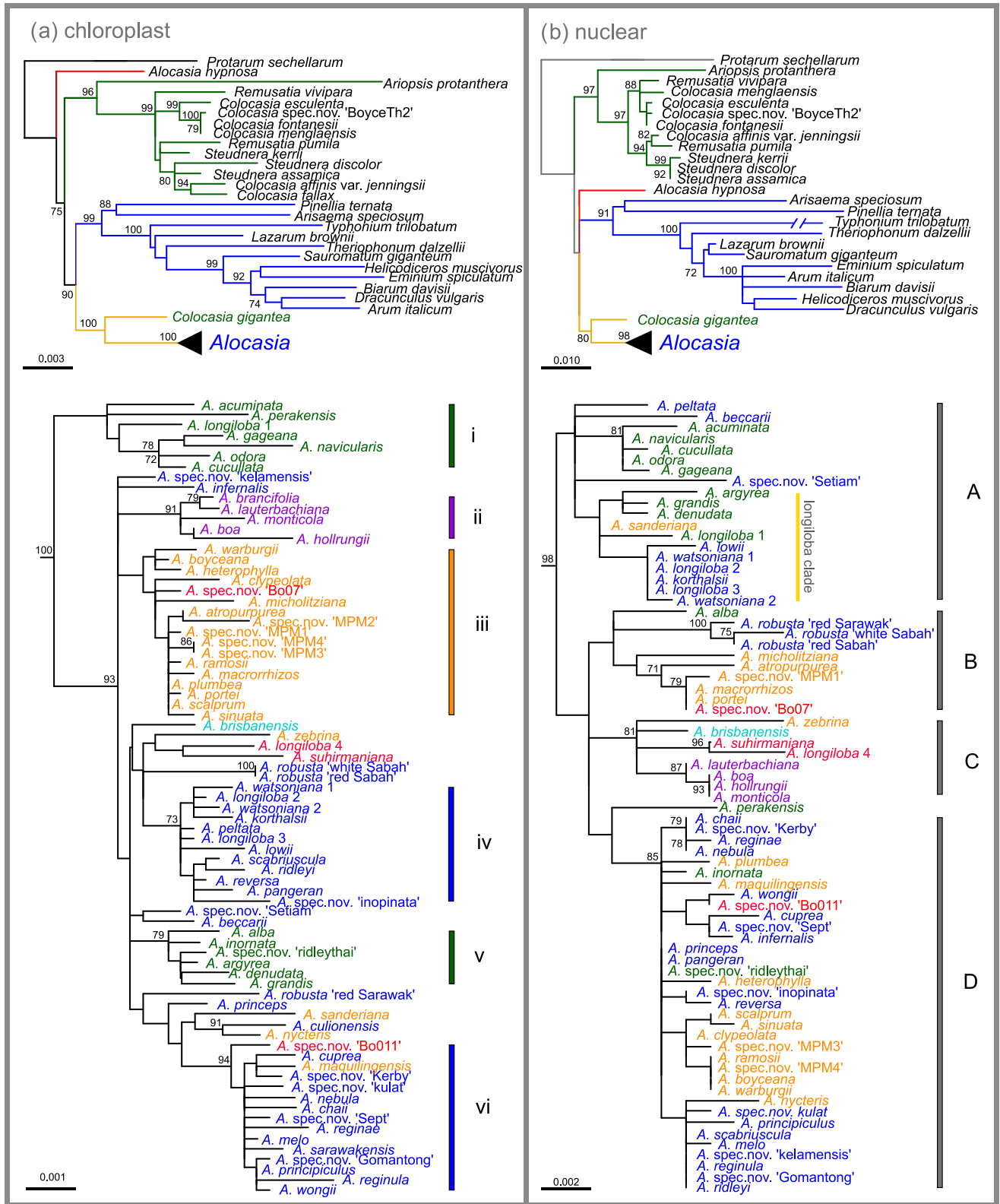


Fig. 3. Maximum likelihood (ML) phylogenies for *Alocasia* and its outgroups obtained from the plastid DNA data (a) and nuclear DNA data (b). The upper section of each tree shows the outgroups, the lower section the ingroup. Numbers at nodes indicate ML bootstrap support from 100 replicates. Species names are colored according to the origin of the samples; green: Asian mainland, blue: Borneo, orange: Philippines, red: Sulawesi, purple: New Guinea, cyan: Australia. Branch colors indicate the main clades among the outgroups; green: *Colocasia* clade, blue: *Areae* clade, red: *Alocasia hypnosa*. Clades discussed in the text are marked by letters.

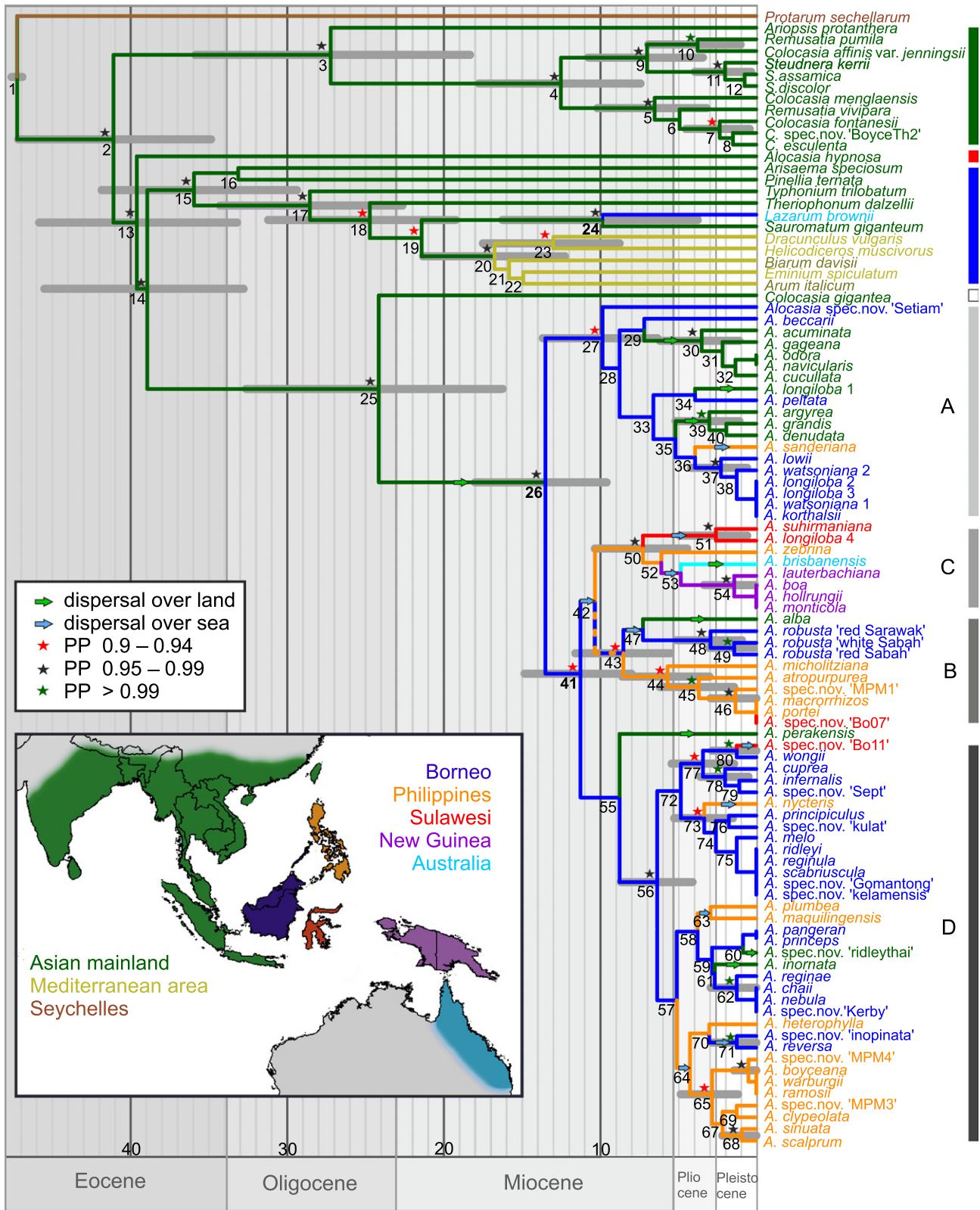


Fig. 4. Chronogram for *Alocasia* and its outgroups obtained from nuclear DNA data modeled under a global clock. Node heights represent mean ages and bars the 95% highest posterior density intervals. Species names are colored according to the geographic origin of the sample, with branch colors indicating the most likely area inferred with the DEC approach. Green arrows indicate dispersal over land and blue arrows over sea. Ancestral areas at nodes 24, 26, and 41 (in bold) were reconstructed differently in S-DIVA and DEC. Stars at nodes indicate levels of support; black: PP > 0.99, green PP > 0.95, red: PP > 0.9. Divergence times and posterior probability values for each node are listed in Table S3. Clades discussed in the text are marked by letters. An asterisk (*) marks accessions with near-identical sequences, that were excluded from dating analyses.

4. Discussion

4.1. Monophyly and phylogenetic relationships

The least expected findings of this study probably are that a monophyletic circumscription of *Alocasia* requires the removal of *A. hypnosa* and that the closest relative of *Alocasia* is *Colocasia gigantea*, while the type species of *Colocasia* (*C. esculenta*) groups elsewhere in the phylogeny of the *Pistia* clade (Fig. 3). *Colocasia gigantea* occurs naturally from Southwest China, Indochina, and Thailand through Peninsular Malaysia and Sumatra to Java. This large-flowered species was originally described as *Caladium giganteum* (Blume, 1823), and Schott (1857) then made it the basis of a new genus, *Leucocasia*, recognizing that it did not fit in *Caladium* because of its unilocular ovaries with sub-orthotropous ovules. Later, Hooker (1893) transferred the species to *Colocasia* where it indeed fit well in terms of its placement and leaf thickness. A few species of *Alocasia*, for example, *A. macrorrhizos*, however, also have such leaves. Since the name *Colocasia* must stay with *C. esculenta*, *C. gigantea* could now either be reinstated as *Leucocasia gigantea* (as yet another monospecific genus of the Araceae) or it could be transferred into *Alocasia*.

Alocasia hypnosa, the surprising non-*Alocasia* discovered here, was described in 2005 from plants collected in southern Yunnan, China, and then cultivated at the Xishuangbanna Botanical Garden (Wang et al., 2005). It has recently also been collected in northern Vietnam. The species is characterized by a purple spathe, numerous long stolons, and seasonal deciduousness and was placed in *Alocasia* because of its few basal ovules, red colored fruits, and its overall similarity to *Alocasia odora*. Our data reveal that it is a genetically highly distinct lineage of the *Pistia* clade, but more loci will need to be sequenced for a secure placement of this species.

4.2. Incongruence of nuclear and plastid phylogenies, and relationships in the *A. robusta* and *A. longiloba* species groups

Most *Alocasia* clades in the plastid phylogeny (Fig. 3a) are restricted to a particular geographic region. By contrast, most major clades in the nuclear phylogeny comprise morphologically similar species from the Philippines, Asia, and Borneo (Fig. 3b). The morphological similarities were specified in section 3.2 and mainly concern species of the *longiloba* group, the *scabriuscula* group, the *princeps* group, and the *Xenophya* group (Nicolson, 1968; Hay and Wise, 1991; Hay, 1998).

The observation that relationships seen in plastid data, which reflect maternal inheritance (and local seed dispersal), correlate with geographic proximity, while those seen in nuclear data correlate more with morphological species boundaries has been made in many other studies (e.g., Soltis and Kuzoff, 1995; Cristina Acosta and Premoli, 2010; Manen et al., 2010; Rautenberg et al., 2010). For South-East Asia, this is the case in stone oaks (Cannon and Manos, 2003) and *Macaranga* (Bänfer et al., 2006). Where plant species hybridize (even just occasionally), the combination of mostly local seed dispersal and obligate out-breeding (via inter-plant pollen transport) may lead to the long persistence of the genetic signal left by the hybridization. Such conditions would seem to apply in *Alocasia*, in which complete temporal separation of individuals' male and female function enforces outcrossing and in which seed dispersal is by understory birds. The *Colocasiomyia* flies that pollinate *Alocasia* sometimes visit co-flowering *Alocasia* species although it is not known whether they can successfully oviposit in more than one species (Toda and Lakim, 2011). Many cultivated species of *Alocasia* have been crossed, including phylogenetically distant ones (Garner, 2010; L. Garner, Aroidia research, Florida City, Florida, pers. comm., February 2011; *A. odora* × *A. nyc-*

teris, *A. odora* × *A. portei*, *A. macrorrhizos* × *A. sinuata*), suggesting that hybridization may also occur in nature.

The morphologically polymorphic species *A. robusta* and *A. longiloba* of which we included several accessions both revealed nuclear/plastid tree discrepancies. The accessions of *A. robusta* from Sabah and Sarawak group together in the nuclear tree (Fig. 3b; clade B), even though *phyC* sequences of the two Sabah plants differ from the Sarawak plant in two substitutions. In the plastid tree (Fig. 3a), the Sabah plants have identical sequences, but the plant from Sarawak differs substantially and placed apart from the other two. Typical *A. robusta* (Fig. 2, left photo) has dark red spathe limbs, and mature inflorescences that smell of decomposing meat, suggesting sapromyophilous pollination. A form with white spathe limbs and sweet odor exists in Sabah, where it co-occurs with the red spathe form (Hay, 1998), and our two accessions represented these color morphs.

The *A. longiloba* species complex comprises seven species from Sundaland and Sulawesi that share unifoliar growth modules and a non-persistent lower spathe during fruit ripening, viz. *Alocasia denudata*, *Alocasia korthalsii*, *A. longiloba*, *Alocasia lowii*, *Alocasia putzeysii*, *Alocasia thibautiana*, and *Alocasia watsoniana* (Hay, 1998; our Fig. 3). Hay saw them as closely related to *Alocasia sanderriana* (Philippines), *Alocasia boyceana* (Philippines), and *Alocasia suhirmaniana* (Sulawesi). We sampled all these species except *A. putzeysii* and *A. thibautiana*. The nuclear phylogeny (Fig. 3b) shows that the *longiloba* group sensu Hay indeed clusters with *A. sanderriana* as he suggested, but also with *Alocasia grandis* and *Alocasia agyrea*, which he did not consider part of the *longiloba* complex. The estimated divergence dates of the relevant species fall in the Pliocene (Fig. 4) and are thus relatively old. *Alocasia lowii* from Borneo is distinguished by two substitutions in its nuclear *phyC*-gene from *A. watsoniana* and *A. korthalsii*, perhaps reflecting beginning speciation. In Sarawak, *A. lowii* is restricted to limestone hills, where *A. korthalsii* and *A. watsoniana* have never been found.

4.3. Divergence date estimation and the role of dispersal in *Alocasia*

The inferred stem age of *Alocasia* and other ages inside the genus are robust to topological changes in the *Pistia* clade (Table S2). In the following discussion, we focus on the *Alocasia* ages obtained under the strict clock model, which on average were younger than those obtained with the relaxed clock. We prefer this model because it uses fewer parameters than the relaxed clock model, reducing the risk of over-parameterization. Diversification in *Alocasia* apparently began in the Middle Miocene and intensified in the Late Miocene and Pliocene (Fig. 4). Spread from the Asian mainland to the Malesian region can be inferred from the distribution of the relevant outgroups, including the closest living relative of *Alocasia*, *Colocasia gigantea* (Fig. 1).

Borneo has played a central role in the geographic expansion of *Alocasia*. Although only reached 3–4 times, 11–13 of the 18–19 inferred dispersal events originated on Borneo. The Philippines were reached from Borneo 4–5 times in the Late Miocene and Early Pliocene, and the Asian mainland was “recolonized” 6–7 times in the Pliocene. Even though Borneo is the clear center of *Alocasia* dispersal in the Sunda region, the eastern part of the Malesian Archipelago was colonized from the Philippines. At least one dispersal event occurred from the Philippines to Sulawesi in the late Miocene and a second to New Guinea and Australia at the Miocene/Pliocene boundary, followed by a single recent dispersal from Borneo to Sulawesi. The west to east dispersal of *Alocasia* with several crossings of the Wallace line started at approximately 5–7 Ma as also found in Aglaieae (Muellner et al., 2008), *Pseuderuvaria* (Su and Saunders, 2009), and *Begonia* (Thomas et al., 2011). Overall, we inferred at least 10 dispersal events across ocean expanses (light-blue arrows in Fig. 4). *Alocasia* fruits are red berries,

0.3–1 cm in diameter and dispersed by understory birds, but we know of no field observations on the ecology of dispersal, germination, and seedling establishment. The closest living relative revealed in this study, *Colocasia gigantea*, produces white fruits that measure only 5 mm in diameter and are densely packed along an infructescence.

New Guinea is the only region underrepresented in our sampling (Hay and Wise, 1991; cf. Section 2.1). The five New Guinean species included here belong to a morphologically homogeneous group, called *Xenophya* group (Nicolson, 1968). Of the eight unsampled New Guinean species, two belong to the *Xenophya* group and six do not (Hay and Wise, 1991). This suggests that New Guinean *Alocasia* may comprise more independent arrivals than just the one inferred here.

Overall the inferred node ages match the tectonic and climate history of the Malesian region. Collision between the Australian and Eurasian plates started in the Late Oligocene, about 25 Ma ago (Hall, 2002, 2009) and led to island emergence. Mid-Miocene pollen records indicate a warm, moist climate and rainforest expansion on these newly forming islands (Morley, 1998), and groups adapted to rainforest understorey, such as *Alocasia*, could therefore plausibly spread and diversify. Land bridges repeatedly connected some areas, including New Guinea and Australia on the Sahul shelf, and Indochina, Sumatra, Java, and Borneo on the Sunda shelf. During the last glacial maximum (LGM), sea levels were approximately 120 m lower than today, resulting in the complete exposure of the Sunda shelf and allowing overland migration between the Asian mainland and Borneo, and even sea level reduction by just 40 m already connected these islands (Fig. 1, 40 m isobath). Such sea level lowering occurred several times, mainly in the Pleistocene, but probably also during the Pliocene and the late Miocene (Miller et al., 2005). Whether *Alocasia* could take advantage of such newly exposed land bridges would have depended on the new biota; savanna vegetation during the LGM on much of the exposed shelf area (as suggested by Bird et al., 2005 and Cannon et al., 2009) would have hindered migration of rainforest-associated *Alocasia* species. Indeed, of the 24 *Alocasia* lineage splits that fall into the Pleistocene (Fig. 4), only one involves a range expansion on the Sunda shelf (from Borneo to Asia).

4.4. Geographic origin of the cultivated species *A. cucullata* and *A. macrorrhizos*

The ornamental *Alocasia cucullata* (Chinese taro) and the tuber crop *A. macrorrhizos* (giant taro) have been cultivated for a long time (Boyce, 2008). Starch grains of *A. macrorrhizos* have been found on Solomon Island stone tools dated to 27,000 years before present (Loy et al., 1992). Today, *A. cucullata* is found in gardens throughout tropical Asia, and *A. macrorrhizos* throughout the tropics. Neither species has been found far from human settlements (Hay, 1999; Boyce, 2008). Our plastid and nuclear trees place *A. cucullata* close to *A. odora* and *Alocasia navicularis* inside an Asian mainland clade (Fig. 3a and b). The inferred recent divergence of these three species (1.31 Ma, 0–2.9 95% HPD; Fig. 4) and their low sequence divergence (6 substitutions in plastid sequences of *A. cucullata* compared to *A. odora*, 11 to *A. navicularis* and one in the nuclear sequences) point to *A. cucullata* being a domesticated form of *A. odora* or *A. navicularis*, which both range from India through Indochina to South China.

Alocasia macrorrhizos groups with Philippine species and is identical in its nuclear sequences with *A. portei* and a plant of unknown origin cultivated in the Bogor Botanical Garden (*Alocasia* spec. nov. Bo7). Hay (1998) already suspected a Philippine origin of *A. macrorrhizos* and suggested hybridization with *A. portei*.

5. Conclusions

The diversification of *Alocasia* started in the Miocene (at c. 13.5 Ma under a strict clock model or c. 19.3 Ma under a relaxed clock), when the climate changed to warm and humid conditions, which led to the expansion of rainforest in the Malesian region. Exposed land bridges and smaller inter-island distances due to lower sea levels permitted repeated clade expansion from and to Borneo and the Philippines, as well as occasional crossing of the Wallace line to Sulawesi, New Guinea, and Australia. That the plastid tree topology reflects geographic proximity while the nuclear tree more closely matches morphological resemblance, suggests occasional hybridization and local seed dispersal (reflected in the maternal plastid tree) as documented in many other plant genera (Soltis and Kuzoff, 1995; Cannon and Manos, 2003; Bänfer et al., 2006; Cristina Acosta and Premoli, 2010; Manen et al., 2010; Rautenberg et al., 2010). Giant taro, *A. macrorrhizos*, which is now cultivated in gardens worldwide, originated on the Philippines, while the Chinese taro, *A. cucullata*, originated on the Asian mainland. More generally, our findings underline the great mobility of plants, the increased recognition of which from dated molecular phylogenies is among the main paradigm shifts in the field of biogeography in recent years.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ympev.2011.12.011.

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