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PHYLOGENETICS

Morphological character evolution of *Amorphophallus* (Araceae) based on a combined phylogenetic analysis of *trn*L, *rbc*L and *LEAFY* second intron sequences

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ABSTRACT. Sequences of three different genes in 69 taxa of *Amorphophallus* were combined to reconstruct the molecular phylogeny of this species-rich Aroid genus. The data set was analyzed by three different methods, Maximum Parsimony, Maximum Likelihood and Bayesian analysis, producing slightly different tree topologies. Three major clades identified in all analyses reflect the biogeographical distribution of *Amorphophallus*. Some clades were supported by morphological characters such as sessile/nonsessile stigma, pollen opening mechanism, shape of the main segments of the lamina, growth cycle, and berry colour. When optimised, a nonsessile stigma may have evolved from a sessile one with several reversals. Pollen opening by connective rupturing evolved from pollen opening by pores. Unequally shaped segments of the lamina evolved from equally shaped segments. Simultaneously existing leaf and inflorescences evolved from alternating leaves and inflorescences. Blue, purple, green, and yellow berries evolved from red/orange/white ones.

Keywords: *Amorphophallus*; Araceae; Character optimization; *LEAFY*; Molecular phylogeny; *rbc*L; *trn*L.

INTRODUCTION

The species-rich Aroid genus *Amorphophallus* currently encompasses ca. 200 species distributed throughout the (paleo)tropics, from West Africa, the western border, eastward into Polynesia and southeastward to Australia (1 species). The eastern distribution border is due to the cultivated species *A. paeoniifolius*, which might not represent a natural distributional border. The northernmost border of the genus is situated in the tropical and subtropical areas of Central Asia, China, and South Japan (Hetterscheid and Ittenbach, 1996). A recent molecular study using a combination of *mat*K and *rbc*L sequences revealed the position of *Amorphophallus* and other Araceae as a monophyletic clade in a basal node of the order Alismatales (Tamura et al., 2004). Araceae, together with Arecaceae and Orchidaceae, were also found among the oldest families of monocot with crown node ages reaching back into the Early Cretaceous (Wikström et al., 2001; Jannsen and Bremer, 2004). The most recent study (Cabrera et al., 2008) of Araceae phylogeny, using a combination of *mat*K, *rbc*L, the *trn*K intron, the *trn*L intron, and the *trn*L-*trn*F spacer, shows the tribe Thomsoniae (*Amorphophallus* + *Pseudodracontium*) as a basal sister-clade to a clade consisting of the tribes Caladieae and Zomicarpeae.

Amorphophallus was first placed in the tribe Thomsoniae (Blume, 1835; Bogner et al., 1985). Tribe Thomsoniae consisted of two closely related genera, *Amorphophallus* and *Pseudodracontium*. Molecular evidence indicates that these two genera could be merged into a single genus, *Amorphophallus* (Grob et al., 2002, 2004).

Several attempts have been made to reveal the phylogenetic relationships within the genus *Amorphophallus* sensu lato (incl. *Pseudodracontium*). The latest studies (Grob et al., 2002, 2004) used molecular data for phylogenetic reconstruction. This paper attempts to interpret morphological character evolution in *Amorphophallus* based on a combined nuclear and plastid phylogeny that is more completely sampled than in previous studies. The species sampled were carefully selected to come up with a representative subset of the total morphological diversity present in *Amorphophallus*. The morphological characters by which these species differ were coded (for more details, see below) and then plotted on the most likely molecular phylogenetic tree reconstructed to trace their evolution.

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MATERIALS and METHODS

Materials

Of a total of 25 species of *Amorphophallus*, the *rbc*L gene, *trn*L intron, and *LEAFY* second intron were sequenced. These markers were chosen because they provided sufficient phylogenetic resolution in previous studies (Grob et al., 2002, 2004). Frohlich and Meyerowitz (1997) also pointed out that the second intron of *LEAFY* might have evolved at a high rate and might be useful for reconstructing phylogenies of closely related species. *LEAFY* was also shown to provide more phylogenetically informative characters than nrITS, *trn*L-*trn*F, *trn*D-*trn*T, or *mat*K-*trn*K by Oh and Potter (2003).

The sequences obtained were then combined with sequences of another 44 species of *Amorphophallus*, two of *Pseudodracontium* and one outgroup already previously published. Among the 25 species sequenced in this study, *A. bangkokensis* (= *A. paeoniifolius* var. *bangkokensis*) and *A. galbra* from Papua New Guinea were sampled to compare them with *A. paeoniifolius* and *A. galbra* from Australia already previously sequenced. *Hapaline* sp. representing Caladieae was chosen as an outgroup because of its close relationship with *Amorphophallus* (French et al., 1995; Cho and Palmer, 1999; Rothwell et al., 2004).

Total genomic DNA was obtained from fresh leaf tips of living specimens at the Hortus Botanicus Leiden and Wageningen University Botanical Garden using the DNeasy Plant Mini Kit (Qiagen) following the manufacturer's protocol. DNA sequences obtained were deposited in GenBank (Table 1).

Amplification

The *rbc*L gene was amplified and sequenced with primers 1F (ATGTCACAACAAACAGAAAC), 724R (GCGTTGGAGAGATCGTTTCT), 636F (TCGCATGTACCTGCAGTAGC) from Fay et al. (1997) and 1460R from Olmstead et al. (1992). The *trn*L intron was amplified using universal primers "c" (CGAAATCGGTAGACGCTACG) and "d" (GGGGATAGAGGGACTTGAAC) (Taberlet et al., 1991). The second intron of *LEAFY* was amplified with the primers *FLint2* F1 (CTTCCACCTCTACGACCAGTG) and *FLint2* R1 (TCTTGGGCTTGTTGATGTAGC) (Grob et al., 2004).

Amplifications were done with a Biometra Thermocyler T3. A 50 µL reaction mix was composed of 41.4 µL milliQ water, 5 µL PCR buffer, 2 µL dNTP's 0.2 µL (25 µM) primer forward and reverse, 0.2 µL Taq polymerase and 1 µL (10× diluted containing ca. 5 ng DNA) template for *rbc*L. The *trn*L mix consisted of 6 µL miliQ water, 5 µL PCR buffer, 2 µL dNTP's, 0.5 µL (25 µM) primer forward and reverse, 0.4 µL Taq polymerase and 1 µL (10× diluted) primer for a total of 50 µL mix. The *LEAFY* mix consisted of 36 µL milliQ water, 5 µL buffer, 2 µL dNTP's, 0.2 µL (25 µM) primer forward and reverse, 0.2 µL *Taq* polymerase, 1 µL (undiluted) primer and 5 µL $MgCl_2$. The thermal cycling protocol consisted of 35 cycles with an initial denaturation phase at 94ºC for 5 min, followed by a denaturation at 94ºC for 30 s, annealing at 52ºC for 30 s, an extension phase at 72ºC for 1 min and a final extension of 5 min at 72ºC. The PCR products were purified using QIAquick PCR purification columns (Qiagen) and eluded in 50 µL elution buffer. Cycle sequences for all three genes were performed using identical primers at a lower concentration (2.5 µM). Sequence products were cleaned by Sephadex G50 AutoSeq columns (Amersham-Pharmacia Biotech) and run on an ABI 377 Prism Automatic sequencer (PE Applied Biosystems).

Alignments

Raw sequences were examined, translated and corrected using Sequencher 4.0.1 (1998). Corrected sequences were directly aligned by eye using PAUP* 4.0b10 for Microsoft Windows and MacClade version 4.06 (Maddison and Maddison, 2003). When ambiguous bases were encountered, chromatograms were checked. BLAST searches were done to confirm gene identity. Shared indels were treated as single characters. Insertions were coded as 1 and the deletions as 0. Inapplicable character states (such as appendix diameter in case of an absent appendix) were coded as missing.

Phylogenetic analyses

Maximum Parsimony (MP) trees were reconstructed with PAUP* using unweighted maximum parsimony. Heuristic searches were performed with 100 replicates, random addition, and TBR swapping. Three different heuristic searches were performed, each with different MaxTree settings (10,000; 25,000; infinite). The tree file from the infinite MaxTree setting was used to compute Bootstrap Support (BS) values. Bootstrap analysis was performed using 2000 replicates, each with 100 heuristic searches with random additional sequences, TBR swapping and 10 trees saved per replicate. Nodes with over 70% BS were considered significantly supported (Soltis and Soltis, 2003). Maximum Likelihood (ML) analyses were carried out using PAUP* to calculate the tree with the highest likelihood score which was subsequently used for morphological character optimization. Bayesian analysis was done using MrBayes 3.0 (Ronquist and Huelsenbeck, 2003). Nucleotide substitution models were determined separately for each gene using MrModeltest 1.1b (Nylander, 2002) to determine the best substitution model. The Bayesian analysis was initiated with random starting trees and run for 2×10^6 generations. One tree was saved every 10 generations. After 250,000 generations, a stable probability was reached. All non-significant generations ($p < 0.5$) were discarded for the consensus tree.

A matrix of 70 morphological characters was used to reconstruct character evolution (Table 2). Morphology characters were plotted on the MP tree with the highest likelihood score (Figure 3) using the assumptions of MP with the trace character command (ACCTRAN optimisa-

Table 1. Specimens examined. Sections after Ittenbach (1997). Groups after Van der Ham et al. (1998). Asterisks indicate species examined by Grob et al., 2002; 2004. All vouchers are deposited at NHN-L.

Species	Voucher number	Origin	GenBank accession		
			*trn*L	*rbc*L	*LEAFY*
Amorphophallus abyssinicus (A. Rich.) N. E. Br.*	HAM 0066	Nigeria	AF387433	AF497060	AF497006
Amorphophallus amygdaloides Hett. & M. Sizemore	HAM 969	Thailand, Kanchanaburi Province	DQ012435	DQ012482	DQ012459
Amorphophallus angolensis (Welw. ex Schott) N. E. Br.*	HAM 0015	Gabon	AF387458	AF497061	AF497007
Amorphophallus angustispathus Hett.	HAM 1128	Burma (Myanmar), Mandalay Division	DQ012436	DQ012483	DQ012460
Amorphophallus ankarana Hett., Bogner & Ittenb.*	HAM 0048	Madagascar	AF387434	AF497062	AF497010
Amorphophallus bangkokensis Gagnep.	HAM 1334	Thailand, exact loc. unknown.	DQ012453	DQ012500	DQ012476
Amorphophallus baumannii (Engl.) N. E. Br.*	HAM 0667	Ghana, Brong-Ahafo	AF387436	AF497063	AF497013
Amorphophallus beccarii Engl.*	HAM 0525	Indonesia, Sumatra	AF387437	AF497064	AF497030
Amorphophallus borneensis Engl. & Gehrm.	HAM 158	Kalimantan	DQ012437	DQ012484	DQ012461
Amorphophallus brevispathus Gagnep.*	HAM 0674	Thailand, Muak Lek	AF387438	AF497065	AF497021
Amorphophallus canaliculatus Ittenb., Hett. & Lobin*	HAM 0014	Gabon	AF387439	AF497066	AF497008
Amorphophallus cirrifer Stapf.*	HAM 0450	Thailand, Saraburi	AF387440	AF497067	AF497026
Amorphophallus coaetaneus S.Y. Liu & S.J. Wei*	HAM 0338	China, Yunnan	AF387381	AF497068	AF497016
Amorphophallus commutatus (Schott) Engl.*	HAM 0218	India, Trichur	AF387441	AF497069	AF497036
Amorphophallus corrugatus N. E. Br.*	HAM 0082	Thailand, Chiang Mai	AF387442	AF497070	AF497045
Amorphophallus dactylifer Hett.	HAM 0226	Philipp. Luzon, near Baguio	DQ012438	DQ012485	DQ012462
Amorphophallus declinatus Hett.	HAM 1007	Philippines, Palawan	DQ012439	DQ012486	DQ012463
Amorphophallus decus-silvae Backer & Alderw.*	HAM 0549	Indonesia, Java	AF387443	AF497071	AF497031
Amorphophallus discophorus Backer & Alderw.	HAM 537	Indonesia, Java, Mt. Wilis, above Kediri	DQ012440	DQ012487	DQ012464
Amorphophallus dracontioides (Engl.) N. E. Br.*	HAM 0340	Ghana, Legon Accra	AF387444	AF497072	AF497011
Amorphophallus eburneus Bogner*	HAM 0299	Malaysia, Sarawak	AF387445	AF497073	AF497038
Amorphophallus eichleri (Engl.) Hook.*	HAM 0406	Africa, cult. (origin unknown)	AF387446	AF497074	AF497014
Amorphophallus galbra Bailey PNG	1851A	PNG	DQ012441	DQ012488	DQ012465
Amorphophallus galbra Bailey* AUS	HAM 0174	Australia	AF387447	AF497075	AF497036
Amorphophallus glossophyllus Hett.	HAM 0242	Central Vietnam, northern part of Tay Nguyen Plateau	DQ012442	DQ012489	DQ012466
Amorphophallus henryi N. E. Br.*	HAM 0271	Taiwan, Tainan Hsien	AF387448	AF497076	AF497022
Amorphophallus hewittii Alderw.	Boyce. *s.n.*	East Malaysia, Sarawak	DQ012443	DQ012490	DQ012467
Amorphophallus hirsutus Teijsm. & Binn.*	HAM 0567	Indonesia, Sumatra	AF387449	AF497077	AF497041
Amorphophallus hirtus N. E. Br.*	HAM 0132b	Taiwan, Tainan Hsien	AF387450	AF497078	AF497023

Table 1. (Continuation)

Species	Voucher number	Origin	GenBank accession		
			*trn*L	*rbc*L	*LEAFY*
Amorphophallus hohenackeri Engl. & Gehrm.	HAM 0011	India, Kerala State, Calicut University campus	DQ012444	DQ012491	DQ012468
Amorphophallus hottae Bogner & Hett.*	HAM 0915	Malaysia, Sarawak	AF387451	AF497079	AF497039
Amorphophallus impressus Ittenb.	HAM 1380	East Africa, Malawi (exact loc. unknown)	DQ012446	DQ012493	DQ012470
Amorphophallus interruptus Engl. & Gehrm.	HAM 522	Vietnam, Binh Dong, Ninh Binh	DQ012445	DQ012492	DQ012469
Amorphophallus johnsonii N. E. Br.	HAM 1076	West Africa, Benin (exact loc. unknown)	DQ012447	DQ012494	DQ012471
Amorphophallus konjac K. Koch*	HAM 0251	China	AF387452	AF497080	AF497049
Amorphophallus konkanensis Hett., S.R. Yadav & K.S. Patil	HAM 1134	India, Ratnagiri (Maharashtra State)	DQ012448	DQ012495	DQ012472
Amorphophallus krausei Engl.*	HAM 0768	Thailand, Khlong Lam Nai	Af387453	AF497081	AF497046
Amorphophallus lambii Mayo & Widjaja*	HAM 0834	Malaysia, Sabah	AF387454	AF497082	AF497035
Amorphophallus lanuginosus Hett.	HAM 1239	Vietnam, Hon Tre Island	DQ012449	DQ012496	DQ012473
Amorphophallus laoticus Hett.	HAM 1377	laos, near Houaysay	DQ012450	DQ012497	DQ012474
Amorphophallus lewallei Malaisse & Bamps*	HAM 0468	Burundi	AF387455	AF497083	AF497015
Amorphophallus longiconnectivus Bogner	HAM 1131	India, Madya Pradesh, Piparia	DQ012451	DQ012498	DQ012475
Amorphophallus longituberosus (Engl.) Engl. & Gehrmn.*	HAM 0289	Thailand, Kanchanaburi	AF387456	AF497084	AF497050
Amorphophallus margaritifer (Roxb.) Kunth*	HAM 0422	India, Bengal	AF387457	AF497085	AF497024
Amorphophallus maxwellii Hett.*	HAM 0361	Thailand, Kanchanaburi	AF387459	AF497086	AF497047
Amorphophallus mossambicensis Klotzsch ex Garcke	HAM 0448	Zambia, loc. unknown	DQ012452	DQ012499	-
Amorphophallus muelleri Blume*	HAM 0334	Thailand, Kanchanaburi	AF387460	AF497087	AF497025
Amorphophallus napalensis (Wall.) Bogner & Mayo*	HAM 0227	Nepal	AF387462	AF497088	AF497048
Amorphophallus ochroleucus Hett. & V. D. Nguyen*	HAM 0927	Vietnam, Ke Bang	AF387463	AF497090	AF497018
Amorphophallus paeoniifolius (Dennst.) Nicolson*	HAM 0378	Indonesia, Sumatra	AF387464	AF497091	AF497042
Amorphophallus palawanensis Bogner & Hett.*	HAM 0124	Philippines, Palawan	AF387465	AF497092	AF497040
Amorphophallus pendulus Bogner & S.J. Mayo	Atlanta Bot. Gard. *s.n.*	From tissue culture in Atlanta Bot. garden	DQ012454	DQ012501	DQ012477
Amorphophallus pingbianensis H. Li & L.C. Long*	HAM 0670	China, Yunnan	AF387466	AF497093	AF497017
Amorphophallus pusillus Hett. & Serebr.*	HAM 0247	Vietnam	AF387467	AF497094	AF497043
Amorphophallus pygmaeus Hett.*	HAM 0104	Thailand (s. loc.)	AF387468	AF497095	AF497029
Amorphophallus rhizomatosus Hett.*	HAM 0878	Vietnam Bach Ma	AF387469	AF497096	AF497054
Amorphophallus sagittarius Steenis*	HAM 0491	Indonesia, Java	AF387470	AF497097	AF497032
Amorphophallus salmoneus Hett.	HAM 0036	Philipp., Palawan, Langen Island	DQ012455	DQ012502	DQ012478
Amorphophallus scutatus Hett. & T.C. Chapman	HAM 1003	Thailand, Saraburi Province	DQ012456	DQ012503	DQ012479

Table 1. (Continuation)

Species	Voucher number	Origin	GenBank accession		
			*trn*L	*rbc*L	*LEAFY*
Amorphophallus smithsonianus Sivad.*	HAM 0216	India, Trivandrum	AF440738	AF497098	AF497053
Amorphophallus sumawongii (Bogner) Bogner*	HAM 0714	Thailand, Ban Boa Nan Ching	AF387471	AF497099	AF497044
Amorphophallus symonianus Hett. & M. Sizemore*	HAM 0728	Thailand, Udon Thani	AF387472	AF497100	AF497019
Amorphophallus taurostigma Ittenb., Hett. & Bogner*	HAM 0409	Madagascar, Tulear	AF387473	AF497101	AF497012
Amorphophallus thaiensis S.-Y. Hu	HAM 946	Thailand, Doi Chiang Dao, market	DQ012457	DQ012504	DQ012480
Amorphophallus tinekeae Hett. & A. Vogel	HAM 0477	Sabah, Gomantong Caves, Sunkau	DQ012458	DQ012505	DQ012481
Amorphophallus titanum (Becc.) Becc. ex Arcang.*	Bot. Gard. Bonn *s.n.*	Indonesia, Sumatra	AF387474	AF497102	AF497033
Amorphophallus variabilis Blume*	HAM 0467	Java, Kebun Raya	Af387475	AF497103	AF497034
Amorphophallus yumnanensis Engl.*	HAM 0157	Thailand, Mae Hong Song	AF387476	AF497104	AF497020
Amorphophallus zenkeri (Engl.) N. E. Br. *	HAM 0339	Cameroon	AF387477	AF497105	AF497009
Hapaline sp. Schott*	HAR 056	Thailand, Suratthani market	AF387483	AF497112	AF497057
Pseudracontium harmandii Engl.*	HAM 0420	Vietnam	AF387478	AF497106	AF497051
Pseudracontium lanceolatum Serebr.*	HAM 0179	Vietnam, Dong Nai	AF387479	AF497107	AF497052

tion) in MacClade version 4.06 (Maddison and Maddison, 2003).

Optimised morphological characters (Figure 4)

Five characters were used for optimisation. The first one was stigma morphology (Table 2, char. 2): styles in *Amorphophallus* vary from almost absent (stigma directly sessile on the ovary) to very long and thin (stigma nonsessile). The second was the opening mechanism of the pores (Table 2, char. 18): in most *Amorphophallus* species pores open independently from each other per anther. In a few species the connectival tissue is very thin and ruptures when the pores open. Adjacent pores then "fuse" into a larger pore, discharging pollen from two adjacent thecae. The third character was the lamina architecture (Table 2, char. 49): decompound lamina in *Amorphophallus* usually have three easily recognisable main segments. These are usually equal in shape and complexity, but in several species the single posterior segment is much less complexely divided and often shorter than the laterals. The fourth character was the growth cycle (Table 2, char. 58): most *Amorphophallus* species show sympodial cyclic growth with a resting period alternating with an active growing period after a shorter or longer seedling period of monopodial growth, terminated by the first occasion of flowering. During the resting period (identified as -r- in the states of character 58) no plant parts are above soil. Two out of four different sympodial cycle types are dominant. In one or these, a single inflorescence develops on a node, and afterwards the plant goes dormant again without leaf development for the rest of the season (char. 58, state 1). A single leaf emerges in the next season, but no inflorescence. The leaf devours the old node and builds a new one. This is the most common cycle type and is found only in Asian species. The other common type shows development of an inflorescence preceding that of a leaf in the same season, with the leaf again devouring the old node and building a new one. This cycle-type is found exclusively in all African species. The two other cycle types also show flowering and leaf development in the same season but with different timing of node-development relative to the African cycle-type (Table 2, char. 58, states 3 and 4). The last character optimised was berry colour (Table 2, char. 65): of all Araceae genera, *Amorphophallus* shows the highest diversity in colour of the mature berries with red/orange being dominant. Other colours are white, blue, purple, yellow, green, and brown.

RESULTS

DNA sequence variation

The *rbc*L sequence alignment consisted of 1512 characters, of which 1347 characters were constant, 79 were parsimony-uninformative, 86 were informative, and 7 were indels. The *trn*L alignment consisted of 963 characters and included 28 indels. A region of TA repeats with a total length of 207 bp in *A. hohenackeri*

Table 2. Morphological characters and character states used in morphological character optimization.

Female flowers

1. Ovary (number of locules): 1 one; 2 two; 3 three; 4 four.
2. Stigma: 1 nonsessile; 2 sessile.
3. Stylar length (relative to ovary): 1 much shorter (almost nil); 2 shorter (appr. 0.5×); 3 ± equal; 4 distinctly longer (2× and more).
4. Stigma diameter (relative to style-diam.): 1 ± equal; 2 larger.
5. Stigma: overall shape in longitudinal section: 1 depressed; 2 globose / hemispheric; 3 conical; 4 obconical.
6. Stigma: structure: 1 entire; 2 with central depression; 3 one-lobed; 4 two-lobed; 5 three-lobed; 6 four-lobed; 7 multi-lobed; 8 bilabiate (folded); 9 ± cup shaped.
7. Placentation: 1 basal; 2 axillary, halfway up the length of the placenta.

Male flowers

8. Average number of stamens per flower: 1 one; 2 (2-)3-5(-6); 3 more (indeterminate).
9. Filaments: 1 (near-)absent (anthers ± sessile); 2 present.
10. Filaments: shape: 1 slender; 2 thick.
11. Filaments (majority): fusion: 1 entirely free; 2 partly connate, sometimes forming a column; 3 entirely connate, forming a column or cushion.
12. Lower filaments: transforming to staminodes (1): 1 not transformed; 2 filaments swollen & fused; 3 filaments swollen & fused, anthers absent (flowers fully staminodial).
13. Lower male flowers: reduction transformation (to hairs): 1 not transformed; 2 transformed.
14. Anthers (majority): fusion: 1 free; 2 fused.
15. Anther-shape: 1 rectangular/cubic; 2 globose/subglobose/hemispheric.
16. Thecae: 1 bilocular; 2 unilocular.
17. Pores: position: 1 apical; 2 lateral; 3 subapical.
18. Pores; opening mechanism: 1 by own opening; 2 fusing with others, connective rupturing.
19. Connective: shape: 1 flat; 2 ridge-like; 3 elongate (vertically); 4 hemispheric.

Pollen

20. Pollen: exine ornamentation: 1 psilate; 2 striate; 3 verrucate; 4 echinate; 5 areolate; 6 fossulate; 7 reticulate; 8 striate/scabrate; 9 scabrate.
21. Polar caps: 1 present; 2 absent.

Spadix

22. Spadix: 1 (sub-)sessile; 2 distinctly stipitate.
23. Length relative to spathe: 1 distinctly shorter than spathe; 2 ± equalling spathe; 3 longer than spathe.
24. Female zone: length rel. to male zone: 1 much shorter than male zone (less than 0.2×); 2 shorter than male zone (0.2 - 0.9×); 3 ± equalling male zone; 4 longer than male zone (1.1 - 1.9×); 5 much longer than male zone (2.0× and more).
25. Female zone: fertility: 1 entirely fertile; 2 with staminodia.
26. Female to male zone: 1 adjacent (contiguous); 2 separated by sterile zone.
27. Sterile zone between male and female zone: 1 (largely) naked (sometimes with flower remains); 2 with sterile structures (staminodes or pistillodes).
28. Male zone: disposition stamens: 1 congested or slightly distant; 2 loosely arranged; 3 aligned/fused into vertical ridges; 4 aligned/fused into a lax spiral; 5 (sub)verticillate/dense spiral.
29. Upper part of male zone: fertility: 1 entirely fertile; 2 with interspersed sterile structures (hairs).
30. Male zone: diam. relative to female zone: 1 distinctly narrower than fem. Zone; 2 appr. equalling female zone; 3 distinctly exceeding fem. Zone.
31. Male zone: transition to appendix: 1 contiguous; 2 naked zone, distinct from appendix ("stipe").

Appendix

32. Presence: 1 present; 2 absent.
33. Appendix: length rel. to male ± fem. zone: 1 shorter; 2 ± equal; 3 longer.
34. Appendix: diameter (base) rel. to male zone: 1 less than in male zone; 2 ± equal or slightly broader; 3 exceeding.
35. Appendix: general shape (lateral view): 1 ovoid/globose (1:1 – ca. 1.5:1); 2 shortly conical (ca. 2:1); 3 elongate (2.5:1 or more).
36. Wall structure: 1 individual elements (staminodes) visible; 2 no elements visible, or only at the base or top.
37. Wall: elements (staminodes): 1 verrucae; 2 high ridges/columns (incl. papillae/short ridges); 3 shallow ridges/colums; 4 broad, flat colums; 5 hairs; 6 bristles.

Table 2. (Continuation)

38. Wall, high ridges: shape: 1 long (rugae); 2 short (papillae).

Spathe

39. Transition base-limb: 1 gradual, not or shallowly constricted; 2 distinctly constricted.
40. Spathe base: surface within: 1 smooth; 2 sculptured.
41. Spathe base: sculpture type: 1 small (simple) verrucae; 2 large verrucae (sometimes irregular); 3 papillae (sometimes hairlike); 4 hairs; 5 ridges; 6 verrucae-ridges (intermediates); 7 transverse ridges.
42. Spathe base: degree of sculpturing: 1 dense/numerous; 2 scattered/few.
43. Spathe base: 1 short and loosely convolute; 2 strongly convolute, forming a basal tube/chamber; 3 margins fused.
44. Limb: apex: 1 acuminate; 2 acute; 3 obtuse.
45. Limb: margin shape: 1 straight; 2 undulate; 3 plicate/strongly folded.

Peduncle

46. Length rel. to spathe: 1 shorter than or equalling spathe; 2 longer than spathe.
47. During fruiting: 1 elongating; 2 no substantial growth.

Leaf

48. Duration: 1 short lived (1 season, between two dormancy periods); 2 long lived (2 - 5 years).

Lamina

49. Main segments: 1 appr. equally shaped; 2 anterior segment distinctly less divided.
50. Main posterior segment: division: 1 moderately subdivided; 2 strongly subdivided.
51. Leaflets (all): base: 1 all/most sessile; 2 basal ones distinctly petiolulate.
52. Leaflets upper surface colour: 1 green/grey-green; 2 deep velvety green.
53. Leaflets variegation: 1 all green (no variegation); 2 heavily splashed with white dots; 3 white line along midrib (and occ. sec. veins); 4 greyish area along midrib; 5 scattered white spots; 6 checkered.
54. Leaflets margin: 1 green; 2 violet/lilac.
55. Rhachises: 1 winged throughout; 2 winged distal from the main basal branchings; 3 (largely) unwinged.
56. Foliar bulbils: 1 absent; 2 present.
57. Foliar bulbils: dislodging: 1 dislodging completely from rachis; 2 dislodging with parts of the rachis.
58. Growth cycle [] = node; () = season; r = rest: 1 -r-(infl.)]-r-[(leaf)-r-(infl.)]-r-[(leaf)-r- (1/2 node/s.); 2 -r-(infl.]+[leaf)-r-(infl.]+[leaf)-r- (2 x 1/2 node/s.); 3 -r-([leaf+infl.])-r-([leaf+infl.])-r- (1 node/s.); 4 -r-([leaf+infl.]+[leaf+infl.)-r- (2 or more nodes/s.)

Tuber/rhizome

59. Number of modules: 1 one; 2 more than one.
60. Orientation: 1 vertical; 2 horizontal.
61. Module shape: 1 saucer shaped (extremeley depressed); 2 globose/depressed-globose; 3 elongate (very narrow, growing apex with numerous cataphylls or -scars); 4 elongate (thick, no cataphylls or -scars).
62. Underground vegetative propagation: 1 only by division (break-up) of mature tuber/rhizome; 2 by development of subsidiary tubers (slowly separating); 3 by offset-development (usually seasonal).
63. Offsets: shape: 1 globose; 2 shortly elliptic/fusiform; 3 shortly cylindric; 4 distinctly elongate (rhizomatous); 5 obconic (= globose with short "stalk").
64. Root-scars: 1 with distinct, annular thickenings (abscission); 2 without annular thickenings.
65. Berries: colour: 1 red/orange; 2 blue; 3 white; 4 yellow (with our without white base); 5 green; 6 pale brownish (soil colour); 7 purple.
66. Berries: surface: 1 smooth; 2 ridged/verrucate.
67. Spathe at anthesis: 1 opening, separating from spadix; 2 clasping the spadix.
68. Spathe: overall colour intensity/-type inside: 1 pale (whitish, greenish, pinkish); 2 dark (purple, blackis-grey).
69. Spathe margin: 1 straight; 2 broadly rounded/curved inwards; 3 sharply curved inwards-channeled.
70. Number of inflorescences per flowering event: 1 one inflorescence; 2 more than one (in synflorescence).

was excluded from the analysis. Among the 756 included characters, 634 were constant, 74 were parsimony-uninformative, and 48 are parsimony-informative. The *LEAFY* alignment consisted of 591 characters and included 67 indels. Two regions of 70 bp in *A. angustispathus* and *A. konkanensis* and 29 bp in *A. hohenackeri*, respectively, were considered unalignable, and therefore excluded from the analysis. Of the 492 included characters, 257 were constant, 88 were parsimony-uninformative, and 147 were parsimony-informative. The combined matrix consisted of 3066 characters. A total of 306 characters were excluded, leaving 2760 characters in the analysis, of which 2238 were constant, 241 were parsimony-uninformative, and 281 are parsimony-informative. Among the three genes, *LEAFY* generated the most variation, followed by *trn*L. The *rbc*L gene was found to be a much more conserved region.

Maximum parsimony and maximum likelihood analyses

All three MaxTree settings produced the same parsimony scores with a tree length of 1030 steps, CI=0.590, RI=0.701, and RC=0.414. Compared to the MP strict consensus presented by Grob et al. 2002, 2004, the MP tree presented in this study has a better resolution both for basal and terminal clades (Figure 1). In total, 22 ingroup nodes are supported by >70% BS. The MP strict consensus contains three major clades, namely African, Southeast Asia (SEA) and Continental Asia clades. The African clade comprises 13 species and is supported by 56% BS. The SEA clade comprises 21 species from the Indo-Pacific archipelago, the Philippines, Papua New Guinea, and Australia and is supported by 70% BS. The biggest major clade (<50% BS) in the MP strict consensus is the Continental Asia clade, which covers the taxa distributed from

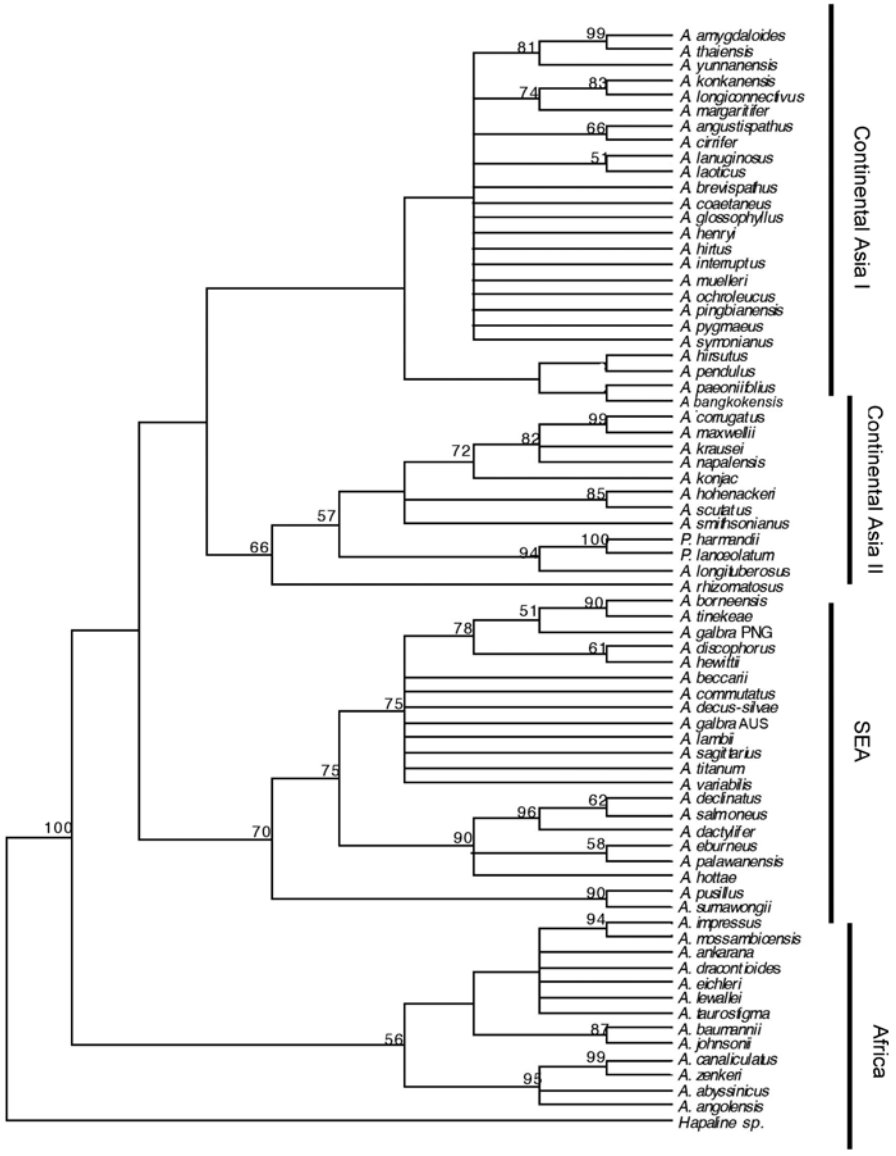

Figure 1. Maximum Parsimony strict consensus of 253,317 MPTs. BS values are given above the nodes.

India to China, Taiwan, and Thailand. This clade contains two species, *A. hirsutus* (Sumatra) and *A. pendulus* (Borneo) which do not occur on continental Asia. The clade is subdivided into two subclades, Continental Asia I (<50% BS) and II (66% BS), which reflect no biogeographical distinction. They are, however, supported by unique berry colours.

Bayesian analysis

With MrModeltest 1.1b (Nylander, 2002), two models of nucleotide substitution, GTR+G+I for *rbc*L and HKY+G for both *trn*L and *LEAFY* were selected. The Bayesian analysis resulted in a 50% majority consensus tree (Figure 2) with one major topological difference as compared to the MP strict consensus obtained. Along the backbone of the tree, three major clades collapsed into a polytomy. In the tips, however, the Bayesian consensus tree was better resolved than the strict consensus tree.

Well supported clades

Each method used has its own advantages and disadvantages. Maximum Parsimony is capable of analyzing large sequence datasets, but can perform poorly if there is substantial variation in branch lengths. Bayesian analysis is better suited for tackling such branch length variation, but the prior distributions for parameters must be specified, and it is difficult to decide whether MCMC has run long enough before reliable results are obtained (Holder and Lewis, 2003). All resulting cladograms from both analyses are congruent in several parts, though, and apart from their geographical coherence, the larger clades can be defined by some morphological synapomorphies. First of all, the

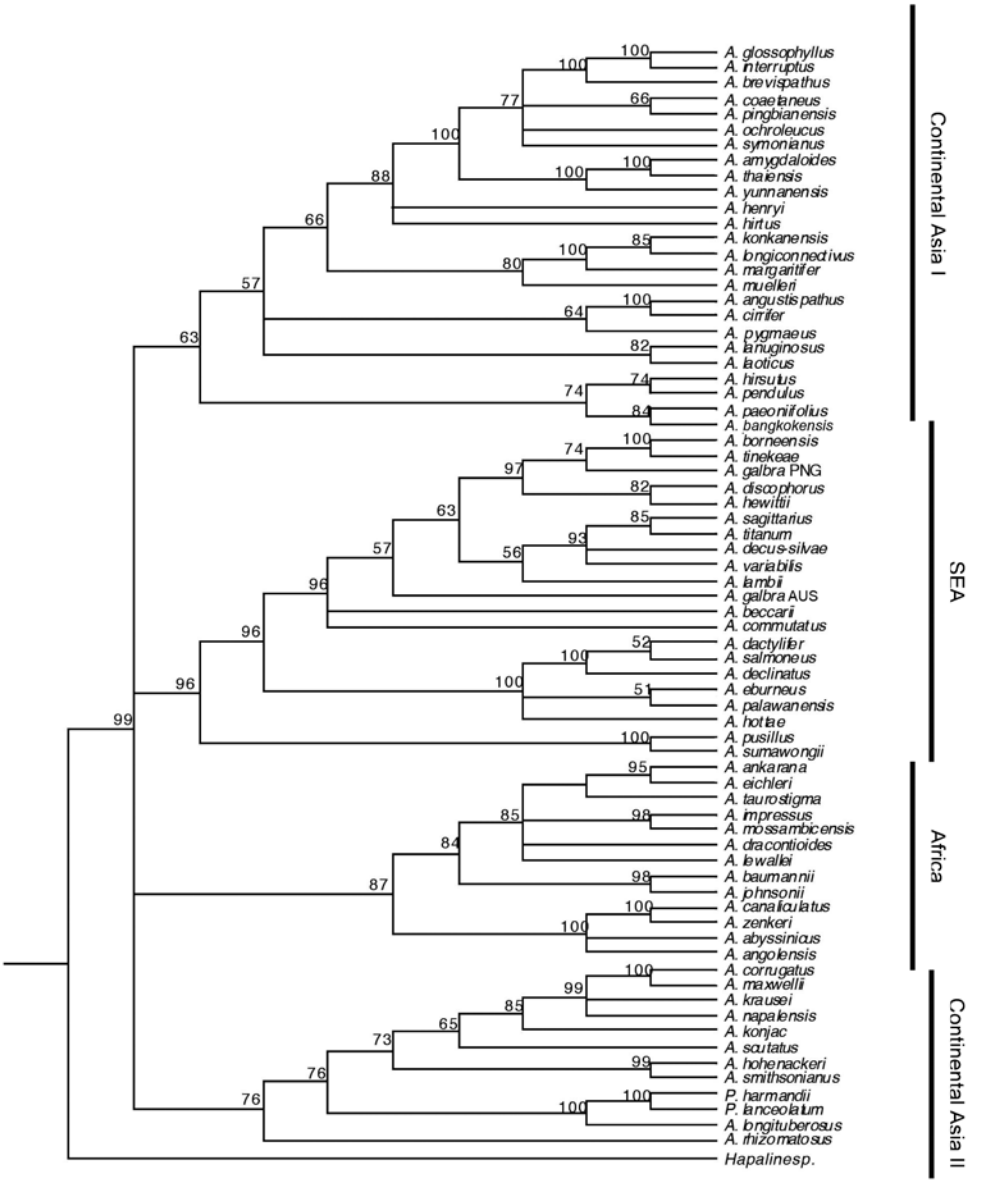

Figure 2. Bayesian 50% majority rule consensus tree. Posterior probability values are given above the nodes.

monophyly of African clade is supported by all analyses (Figures 1, 2, 3). The African species are characterized by a unique seasonal growth cycle in which two tuber nodes are active within a single season. These nodes have a differential development: the first produces an inflorescence and the second a leaf (Table 2, char. 58, state 2).

Secondly, in the MP and Bayesian analyses (Figures 1, 2), *A. galbra* from PNG is placed in a different clade as compared to *A. galbra* from Australia. The sequences are derived from plants with conspicuously different vegetative morphologies. The PNG specimen is a large plant up to two meters high. The petiole and peduncle bear crusty patches resembling lichens and are generally multicolourous. The spathe is multicolourous. The Australian specimen, on the other hand, is a small plant, not higher than 30 cm. The petiole and peduncle are entirely green and smooth. The spathe is entirely green. As both specimens have a very different morphology and end up in very different phylogenetic positions both in the MP and Bayesian trees, it suggests that *A. galbra* needs further taxonomic revision and perhaps a redefinition of its species boundaries.

Thirdly, *A. paeoniifolius* and *A. bangkokensis* always cluster together, despite their considerable vegetative morphological differences. *A. paeoniifolius* is a plant of which the tuber has thick annular root scars, the offsets are very short and thick, the petiole is usually strongly warty, rarely smooth, and the petiole is always blotched

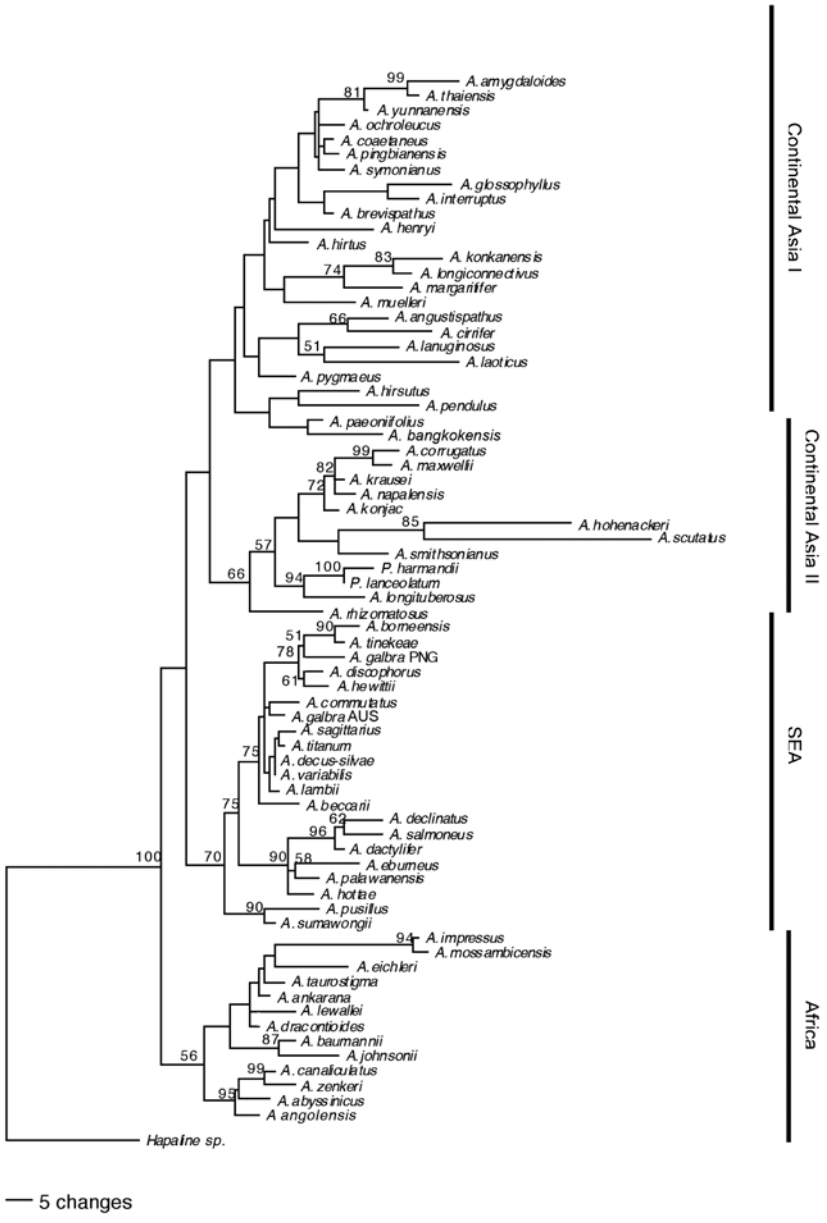

Figure 3. The Maximum Parsimony tree of 253,317 trees with the highest likelihood score (-ln L = 9144.6). Length=1030 steps, CI=0.590, RI= 0.701, RC=0.414. BS values are given above the nodes.

and never striped. In contrast, *A. bangkokensis* has a tuber lacking annular root scars, its rhizomatous offsets are long and thin, and the petiole is smooth with a stripe-like pattern. The inflorescences of both species are very much alike although that of *A. bangkokensis* is consistently small (spadix not longer than 15 cm) and that of *A. paeoniifolius* is hardly ever that short but may reach up to 50 cm.

Fourthly, the clade of *A. pusillus* (Vietnam) and *A. sumawongii* (Thailand), which was also found by Grob et al. (2002, 2004) was recovered in all analyses of this study as well. It is supported by several morphological characters.

DISCUSSION

Resolving the basal backbone of the MP strict consensus has shed more light on some aspects of evolution of the genus *Amorphophallus*. The African and Southeast Asian clades seem well defined, despite the presence of the Indian species *A. commutatus* in the latter which seems better fitted in the continental Asian clade. The continental Asian clade is more difficult to interpret since it covers both a wide geographical range and large morphological variation.

Morphological character evolution

With MacClade, character evolution was optimized most parsimoniously using ACCTRAN optimization.

When plotted on the MP tree with the highest likelihood score, some morphological characters were found to correlate well with molecular based clades. These characters include a stigma (either nonsessile or sessile), a pore opening mechanism, the shape of the main segments of the leaf lamina, the growth cycle, and a berry colour (Figure 4).

Style length

The African clade and outgroup seem to be characterized by a sessile stigma, with the exception of *A. impressus*, *A. taurostigma, A. dracontiodes, A. canaliculatus* and *A. angolensis* which have a nonsessile stigma. A nonsessile stigma can be found in all other species except for *A. cirrifer, A. angustispathus, A. pendulus*, and *A. sagittarius*. A nonsessile stigma seems to be evolved from a sessile one. The morphology of the stigma (either sessile or nonsessile) is phylogenetically informative for the entire Araceae family (Mayo et al., 1997), and it would be interesting to investigate whether nonsessile stigma's also developed in selected clades only during the evolution of *Amorphophallus* when a fully sampled and resolved phylogeny of the genus becomes available.

Pollen opening mechanism

All species sampled seem to be characterized by pollen being released through individual pores, with the exception of *A. glossophyllus, A. interruptus* and *A. brevispathus* which have pollen realeased after a rupturing of the connective, which has been transformed in these species into a very thin tissue layer partly covering the pores. Simultanously the two pores on a theca also merge into one because of the rupturing of the connective. It would be interesting to investigate whether these species also have a unique pollen dispersal method as compared with the remaining species sampled. Pollen release by connective rupturing seems to be derived from pollen released directly from the pores.

Leaf lamina segments

The leaf lamina in Amorphphallus is called "decompound" because it is split up in three main segements, each usually branching to various degree and carrying highest order segments shaped as individual small leaves ("leaflets"). In most species sampled, the main segments of the leaf lamina are more or less equally shaped. Distinctly less divided anterior main leaf segments evolved from equally shaped segments three times in the Asian clades. From an ontogenetic point of view, it has not been possible yet to determine if these segments have an arrested development. Evo-devo studies could shed further light on the homology of these structures. It is suggested that this phenomenon in the *Pseudodracontium* species may be the result of heterochronic development because other plant parts show similar phenomena (e.g. staminodial appendix, male flowers with long thin filaments and separated anthers, unilocular thecae; Hetterscheid and Claudel, in prep.)

Growth cycle

The African clade is well supported by a unique growth cycle where the season's growth starts with a developing inflorescence and the leaf appearing either after a short while or almost simulaneously. The appearing inflorescence terminates the sympodial shoot initiated the season before while the leaf developing alongside it in the same season develops from a lateral continuation shoot and initiates the next sympodial cycle. The dormancy period of an individual plant therefore occurs in the middle of the development of a sympodial module from leaf initiation to inflorescence termination. One season of growth is characterized by the termination of the previous year's module and the initiation of the next, which is not terminated that season by another inflorescence but undergoes a dormancy period and is later terminated by an inflorescence in the next season. Therefore inflorescence and leaf appearing in the same growing season do not belong to one and the same sympodial module but to two consecutive ones separated by a dormancy period.

A seemingly similar situation of simultaneously existing leaf and inflorescence in the same season is also found in a few continental Asian species like *A. ochroleucus*, *A. coaetaneus* and *A. rhizomatosus*. However, in these instances the leaf appears first, and several weeks or months later the inflorescence appears, and the latter is the termination of the same shoot from which the leaf has developed. This situation repeats itself the next season.

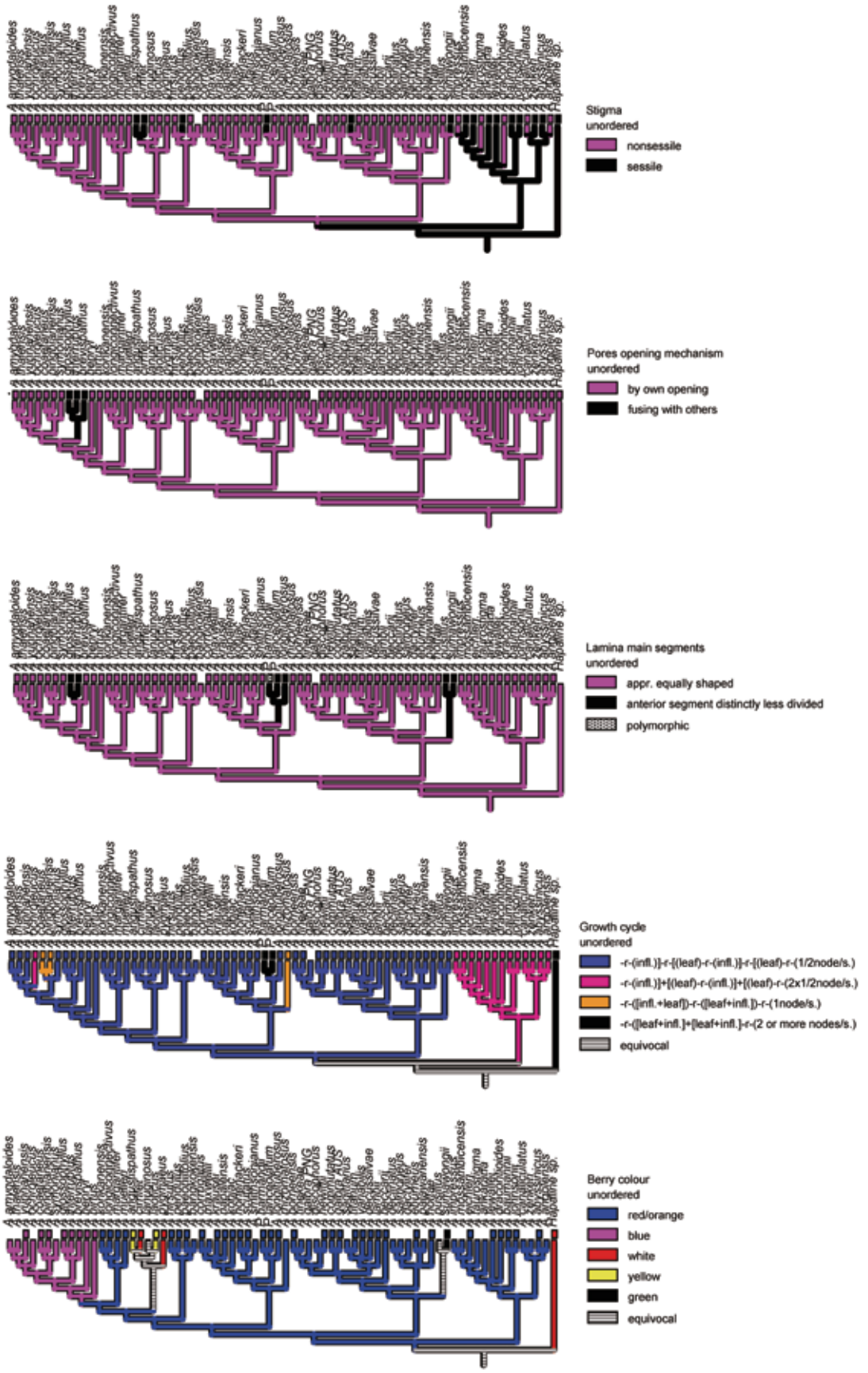

Figure 4. Reconstruction of character state evolution of several morphological characters optimized on the MP tree with the highest likelihood, using the trace character command in MacClade version 4.06 (Maddison and Maddison, 2003).

Therefore, leaf and inflorescence of one season's growth belong to the same sympodial module. Both these cycle types differ from the most common one (present in most Asian species), in which each season's growth sees either an inflorescence or a leaf developing, but never in the same season.

This latter type of growth cycle is reconstructed to be ancestral to the one in e.g. *A. ochroleucus*, whereas it cannot be decided if the dominant Asian cycle type is ancestral to the African species cycle or the reverse. The optimisation shows ambiguity at this point. A large variation in growth cycles exist within the Araceae family (Scribailo and Tomlinson, 1992; Mayo et al., 1997). It would be very interesting to study these different types of inflorescence, leaf, and shoot development in a phylogenetic context for the whole family.

Berry colour

A clade of 13 taxa in the Continental Asia I clade shows the possession of blue/purple berries, which do not occur in other clades. Blue berries occur in the taxa with the northernmost distribution of *Amorphophallus* and might have evolved as a response to birds that focus on blue berries for food in that particular geographical region (Hetterscheid and Ittenbach, 1996). Blue/purple, green, white, and yellow berries are all reconstructed as derived from red/orange or white berries. It is interesting to note how variable the inflorescence morphology is for species within the blue-berried clade, with species like *A. brevispathus*, *A. coaetaneus*, *A. kiusianus* and *A. yunnanensis* (see Plate 1). From a macromorphological point, this clade would not be retrievable, and this is proven in such an analysis (Hetterscheid and Hovenkamp, in prep.). Observations like this indicate the great morphological flexibility in *Amorphophallus*, which may well be due to a strong adaptibility to different pollination resources (Hetterscheid, in prep.). The clade containing the smallest species of *Amorphophallus—A. obscurus* (not analysed here), *A. polyanthus* (not analysed here), *A. pusillus*, *A. serrulatus* (not analysed here) and *A. sumawongii* (*A. pusillus* having an inflorescence of a mere 5 cm high—is further characterised by all species sharing an atypical leaflet-structure (rhombic - obovate) and verrucate berries with a very unorthodox colour (green in *A. sumawongii*; dirty pinkish-brownish in *A. polyanthus*). Ecologically, the members of this group seem to be adapted to a survival strategy suitable to forest floor conditions indicated by the type of pollination (fungus syndrome; Kite and Hetterscheid, 1997) and type of dispersal (berries without striking, bird-attracting colours and infructescence held close to the soil). The position of this small clade of purely Thai-Indochinese species in the large Southeast Asia clade is not supported by morphology, especially because of the presence of elongate tubers, a character very common in other Thai-Indochinese species but absent in all other Southeast Asian species.

With the three-gene-based phylogeny of *Amorphophallus* presented here, more insight could be obtained about the evolution of ecologically interesting features such as dwarfism, life cycle, and berry colour. A next step would be to unravel the genetics of these features by crossing species from sister groups with different habit sizes, life cycles, and berry colours for quantitative trait loci (QTL) analysis. Although nothing is known about the genetics of these features yet, it seems feasible that only a small number of genes is involved in their initiation and development because of the recurrent evolution of the same type of life cycle and berry colour in *Amorphophallus*.

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依據 *trn*L, *rbc*L 與 *LEAFY* 第二內插子序列之親緣分析推論天南星科魔芋屬植物之形態演化

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本研究使用 69 個分類群，結合三個不同的基因序列以重建富含眾多物種的天南星科魔芋屬植物的分子親緣關係。序列資料集以最儉約法、最大似然法與貝葉氏分析三種不同的方法產生了三個稍微不同的親緣樹。所有的分析顯示了反映魔芋屬的地理分布關係的三個主要枝系。有的枝系得到形態特徵上的支持，例如柱頭是否具柄、花粉釋出機制、葉身主裂片的形狀、生長周期與漿果顏色。在特徵演化的最優化之下顯示具柄的柱頭可能是演化自無柄柱頭物種，並經歷數次逆轉演化；花粉釋出機制為藥隔破裂演化自孔裂花藥；葉身形狀不對稱的裂片演化自對稱的裂片；葉與花序同時存在者是從葉與花序不同時出現者演化而來；具有藍色、紫色、綠色與黃色的漿果是由紅色、橙色或白色的漿果演化而來。

關鍵詞：魔芋屬；天南星科；特徵最佳化；*LEAFY* 基因；分子親緣；*trn*L 序列；*rbc*L 基因。

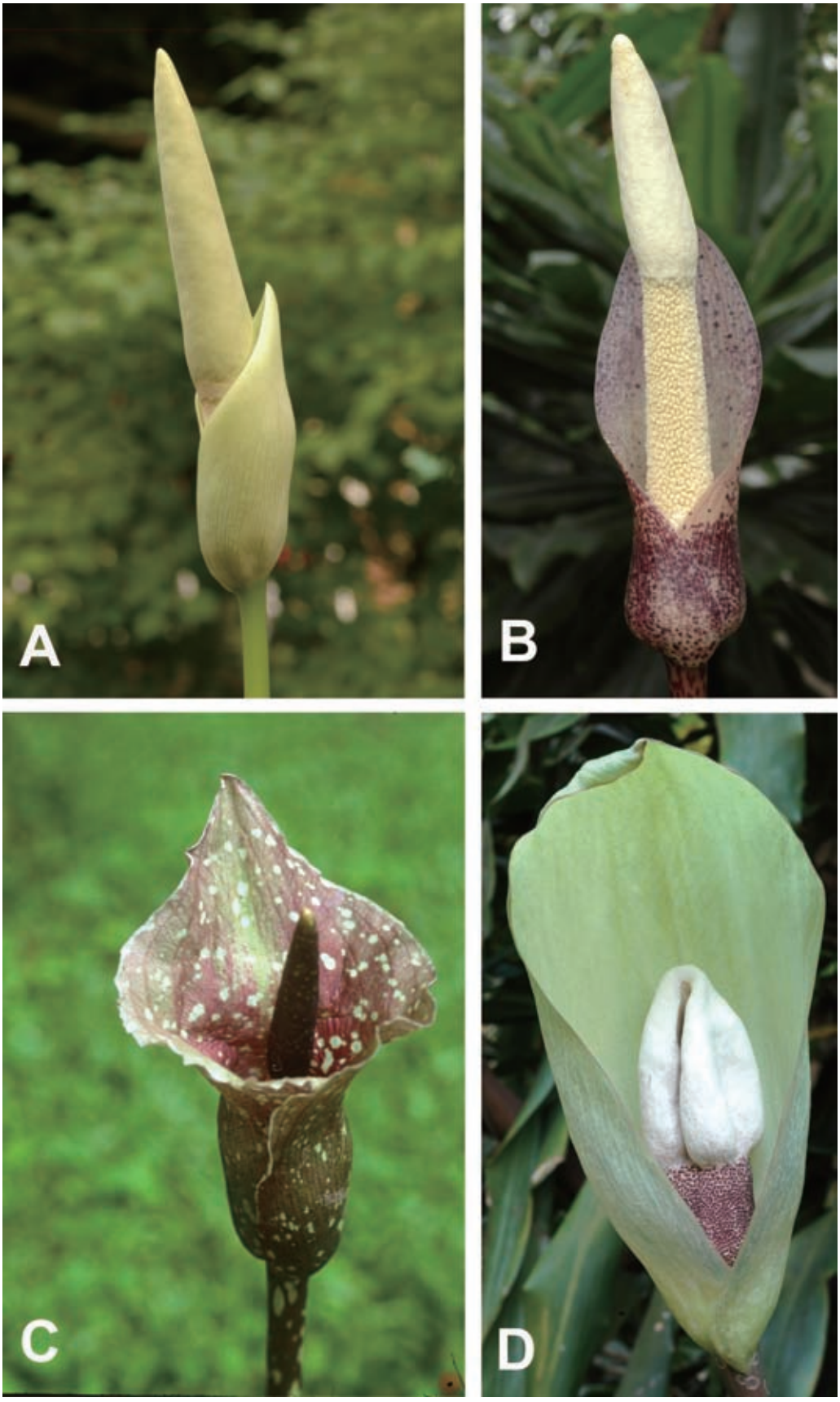

Plate 1. *Amorphophallus* species of the "blue berry"-clade: A, *A. brevispathus*; B, *A. ochroleucus*; C, *A. kiusianus*; D, *A. yunnanensis*. (A, B, D: photos by last author; C: photo by C.-I Peng).

Appendix 1. Data matrix of the morphological characters used for optimalisation. State numbers refer to Table 2. Unknown or inapplicable characters are marked with the symbol "-".

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35
A. abyssinicus	(12)	2	1	-	3	3	1	2	2	2	(12)	1	1	1	1	1	1	1	1	(36)	2	1	1	2	1	1	-	1	1	2	1	1	(23)	3	3
A. amygdaloides	2	1	2	2	2	4	1	2	2	2	(23)	3	1	1	1	1	1	1	1	2	2	2	1	2	1	1	-	1	1	2	1	1	3	3	3
A. angolensis	2	1	(123)	2	3	(13)	1	2	2	2	1	1	1	1	1	1	1	1	1	1	2	1	3	3	1	1	-	1	1	2	1	1	3	3	3
A. angustispathus	2	2	-	1	3	4	1	2	2	2	2	2	1	2	1	1	1	1	1	2	2	1	(23)	2	1	1	-	2	1	2	1	1	(23)	(23)	3
A. ankarana	1	2	-	-	2	(45)	1	2	1	-	-	-	1	1	(12)	1	1	1	1	1	2	1	3	2	1	1	-	1	1	2	1	1	3	2	3
A. bangkokensis	2	1	4	2	(34)	(45)	1	2	2	2	3	1	1	1	1	1	1	1	1	1	2	1	(12)	3	1	1	-	1	1	2	1	1	2	3	2
A. baumannii	2	2	-	-	3	4	1	2	2	2	3	1	1	1	1	1	1	1	1	6	2	2	3	(23)	1	(12)	1	1	1	2	1	1	3	2	3
A. beccarii	1	1	1	2	1	2	1	2	2	2	2	1	1	1	1	1	1	1	1	(12)	2	1	1	2	1	1	-	1	1	2	1	1	2	2	3
A. borneensis	2	1	4	2	3	4	1	2	2	2	2	1	1	1	1	1	1	1	1	1	2	1	3	3	1	1	-	1	1	2	1	1	3	2	3
A. brevispathus	1	1	2	2	2	(12)	1	2	2	2	3	1	1	1	1	1	1	2	1	1	2	1	3	2	1	1	-	1	1	3	1	1	3	2	3
A. canaliculatus	2	1	1	2	1	4	1	2	2	2	(23)	1	1	1	1	1	1	1	1	1	2	1	3	3	1	1	-	1	1	2	1	1	3	2	3
A. cirrifer	2	2	-	-	2	4	1	2	2	2	3	1	2	1	1	1	1	1	2	5	2	1	3	2	2	1	-	2	2	2	1	1	3	3	3
A. coaetaneus	2	1	3	2	1	(24)	1	2	2	2	(13)	1	1	1	1	1	1	1	1	2	2	1	3	2	1	1	-	1	1	2	1	1	3	(23)	3
A. commutatus	1	1	2	1	(12)	2	1	2	2	2	1	1	1	1	1	1	1	1	1	2	2	(12)	3	3	1	1	-	1	1	2	1	1	3	2	3
A. corrugatus	1	1	(34)	1	(34)	8	1	2	2	2	2	1	1	1	1	1	2	1	4	2	2	2	1	(34)	1	1	-	1	1	2	(12)	1	1	(23)	2
A. dactylifer	1	1	4	2	3	(45)	1	2	2	2	3	1	1	1	1	1	1	1	1	1	2	1	3	(34)	1	1	-	1	1	2	1	1	3	2	3
A. declinatus	1	1	2	2	2	4	1	(12)	2	2	(23)	1	1	1	1	1	1	1	1	1	2	1	3	3	1	1	-	1	1	2	1	1	3	2	3
A. decus-silvae	2	1	3	1	2	4	1	2	2	2	2	1	1	1	1	1	1	1	1	6	2	1	3	5	1	1	-	1	1	2	1	1	3	3	3
A. discophorus	2	1	3	1	3	4	1	2	2	2	3	1	1	1	1	1	1	1	1	2	2	1	2	4	1	1	-	1	1	2	1	1	3	3	3
A. dracontioides	1	1	3	1	(13)	1	1	2	2	2	1	1	1	1	1	1	1	1	1	4	2	2	1	2	1	1	-	1	1	3	1	1	(23)	2	1
A. eburneus	1	1	1	2	1	4	1	3	2	2	3	1	1	2	1	1	1	1	1	2	2	2	2	2	1	1	-	3	1	2	1	1	3	2	3
A. eichleri	(12)	2	-	-	3	4	1	2	2	2	2	1	1	1	1	1	1	1	1	1	2	1	(23)	3	1	1	-	1	1	2	1	1	(23)	(23)	(23)
A. galbra	1	1	2	2	1	(45)	1	2	2	2	3	1	1	1	1	1	1	1	1	9	2	1	1	2	1	1	-	1	1	2	1	1	1	2	3
A. galbra PNG	1	1	2	2	1	(45)	1	2	2	2	3	1	1	1	1	1	1	1	1	9	2	1	1	2	1	1	-	1	1	2	1	1	1	2	3
A. glossophyllus	1	1	2	2	1	(456)	1	2	2	2	2	1	1	1	1	1	1	2	1	2	2	1	3	2	1	1	-	1	1	2	1	1	3	3	3
A. henryi	2	1	3	1	3	(45)	1	2	2	2	2	1	2	1	1	1	1	1	1	1	2	1	3	3	1	1	-	1	1	1	1	1	3	2	3
A. hewittii	2	1	4	2	4	4	1	2	2	2	2	1	1	1	1	1	1	1	1	1	2	1	3	4	1	1	-	1	1	2	1	1	3	3	3
A. hirsutus	(23)	1	4	2	4	(45)	1	2	2	2	3	1	1	1	1	1	1	1	(13)	1	2	1	1	3	1	1	-	1	1	3	1	1	2	3	2
A. hirtus	2	1	2	2	3	4	1	2	2	2	3	1	1	1	1	1	1	1	1	1	2	2	3	2	1	1	-	1	2	3	1	1	3	2	3
A. hohenackeri	(23)	1	1	2	1	(45)	1	2	2	2	3	3	1	1	1	1	1	1	1	6	2	2	2	3	1	1	-	1	1	2	1	1	3	3	3

Appendix 1. (Continuation)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35
A. hottae	1	1	2	2	3	(45)	1	2	2	2	3	1	1	1	1	1	1	1	1	2	2	?	3	(23)	1	1	-	3	1	2	1	1	3	2	3
A. impressus	(23)	1	1	2	1	4	1	2	1	2	1	1	1	1	1	1	1	1	1	1	2	1	3	3	1	2	1	1	1	3	1	1	3	2	3
A. interruptus	1	1	3	2	1	(456)	1	2	2	2	3	3	1	1	1	1	1	2	1	2	2	1	3	2	1	1	-	4	1	2	1	1	3	2	3
A. johnsonii	2	2	-	-	3	4	1	2	1	2	?	1	1	1	1	1	1	1	1	1	2	1	3	3	1	2	1	1	1	2	1	1	3	3	3
A. konjac	(23)	1	(34)	2	1	(45)	1	2	2	2	(23)	1	1	1	1	1	1	1	1	6	2	1	3	3	1	(12)	1	1	1	2	1	1	3	2	3
A. konkanensis	(34)	1	2	2	1	(456)	1	2	2	2	3	3	1	1	1	1	1	1	1	1	2	2	3	2	1	1	-	1	1	1	1	1	3	2	3
A. krausei	1	1	(23)	(12)	(12)	(245)	1	2	2	2	3	3	1	1	1	1	1	1	1	2	2	1	(123)	2	1	(12)	2	1	1	2	1	1	(123)	(23)	3
A. lambii	2	1	4	2	4	4	1	2	2	2	2	1	1	1	1	1	1	1	1	1	2	1	3	3	1	1	-	1	1	3	1	1	3	3	3
A. lanuginosus	2	1	1	2	1	(126)	1	2	2	2	3	1	1	2	1	1	1	1	1	1	2	1	(23)	2	1	(12)	2	2	2	2	1	1	3	3	3
A. laoticus	2	1	4	2	1	4	1	2	(12)	2	3	1	1	1	1	1	1	1	1	1	2	1	3	(34)	1	1	-	1	(12)	2	1	1	3	2	3
A. lewallei	2	2	-	-	2	4	1	2	2	2	2	1	1	1	1	1	1	1	1	1	2	1	2	2	1	1	-	1	1	3	2	1	3	2	3
A. longiconnectivus	(23)	1	2	2	2	4	2	1	2	2	?	1	1	1	1	1	2	1	3	1	2	2	2	2	1	(12)	2	1	1	2	2	1	1	1	2
A. longituberosus	(234)	1	(23)	2	1	(456)	1	2	2	2	3	2	1	1	1	1	2	1	4	2	2	1	(12)	2	1	1	-	1	1	2	1	1	1	2	(23)
A. margaritifer	2	1	2	2	2	4	2	2	2	2	3	2	1	1	1	1	1	1	1	1	2	2	2	2	1	1	-	1	1	2	-	2	-	-	-
A. maxwellii	1	1	4	2	2	5	1	2	2	2	3	2	1	1	1	1	1	1	2	6	2	1	(23)	2	1	1	-	1	1	1	1	1	3	2	3
A. mossambicensis	1	2	-	-	1	(14)	1	2	2	2	1	1	1	1	1	1	1	1	1	(16)	2	1	2	2	1	1	-	1	1	2	1	1	3	2	3
A. muelleri	(23)	1	2	2	1	(456)	1	2	1	-	-	-	1	1	1	1	1	1	1	1	2	(12)	(23)	(234)	1	1	-	1	1	2	1	1	(123)	3	3
A. napalensis	1	1	3	2	1	5	1	2	2	2	2	1	1	1	1	1	1	1	1	2	2	1	1	2	1	1	-	1	1	2	1	1	2	2	3
A. ochroleucus	2	1	2	2	1	4	1	2	2	2	2	3	1	1	1	1	1	1	1	7	2	1	2	2	1	1	-	1	1	2	1	1	1	2	3
A. paeoniifolius	(23)	1	4	2	4	(45)	1	2	2	2	3	1	1	1	1	1	1	1	1	1	2	1	(123)	(245)	1	1	-	1	1	(123)	1	1	(123)	3	(12)
A. palawanensis	1	1	2	2	1	(12)	1	2	2	2	3	1	1	1	1	(12)	1	1	1	2	2	1	1	1	1	1	-	1	1	2	1	1	2	3	3
A. pendulus	(23)	2	1	2	3	(45)	1	2	2	2	2	1	1	1	1	1	1	1	1	2	2	1	3	2	1	1	-	3	1	2	1	1	3	(12)	3
A. pingbianensis	(12)	1	3	2	1	4	1	2	2	2	(13)	1	1	1	1	1	1	1	1	?	?	1	(23)	2	1	1	-	1	1	1	1	1	(23)	2	3
A. pusillus	1	1	4	2	4	(79)	1	1	2	2	1	1	1	1	1	1	1	1	1	2	2	1	3	2	1	2	2	2	1	1	1	1	3	2	3
A. pygmaeus	1	1	2	2	1	7	1	(23)	2	2	3	1	1	1	1	1	1	1	1	2	2	1	3	2	1	1	-	1	1	2	1	1	2	3	3
A. rhizomatosus	1	1	2	1	1	5	1	2	2	2	3	1	1	1	1	1	1	1	1	2	2	1	3	3	1	1	-	1	1	2	1	1	3	2	3
A. sagittarius	1	2	1	2	(13)	5	1	2	2	2	2	1	1	1	1	1	1	1	1	2	2	2	1	2	1	1	-	1	1	2	1	1	2	2	3
A. salmoneus	1	1	4	2	4	(68)	1	1	1	-	-	3	1	1	1	1	1	1	1	6	2	1	2	2	1	1	-	1	1	2	1	1	1	2	3
A. scutatus	(12)	1	4	2	3	1	1	2	2	2	3	3	1	1	1	1	1	1	2	5	2	1	(23)	2	1	1	-	1	1	1	1	1	3	2	3
A. smithsonianus	2	1	2	2	2	(456)	2	2	2	2	3	3	1	1	1	1	1	1	1	8	2	1	3	2	1	1	-	1	1	2	1	1	3	2	3

Appendix 1. (Continuation)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35
A. sumawongii	1	2	-	-	1	1	1	2	2	2	2	2	1	1	1	1	1	1	1	2	2	1	1	2	1	1	-	1	1	3	1	1	2	2	2
A. symonianus	2	1	2	2	1	2	1	2	2	2	1	1	1	1	1	1	1	1	1	1	2	1	1	2	1	1	-	1	1	3	1	1	1	2	2
A. taurostigma	2	1	2	2	3	(45)	1	2	2	2	2	1	1	1	1	1	1	1	1	6	2	1	3	3	1	1	-	1	1	2	1	1	3	2	3
A. thaiensis	1	1	3	2	4	8	1	2	2	2	2	1	1	1	1	1	1	1	1	2	2	2	1	(23)	1	1	-	1	1	2	1	1	(23)	3	2
A. tinekeae	2	1	4	2	3	3	1	2	2	2	2	1	1	1	1	1	1	1	1	1	2	1	3	3	1	1	-	1	1	1	1	1	3	2	3
A. titanum	(23)	1	4	1	2	(12)	1	2	2	2	2	1	1	1	1	1	1	1	1	1	2	1	3	2	1	1	-	1	1	3	1	1	3	3	3
A. variabilis	(23)	1	(23)	2	3	(456)	1	2	2	2	2	1	1	1	1	1	1	1	1	2	2	1	3	2	1	1	-	1	1	2	1	1	3	(13)	3
A. yunnanensis	2	1	3	2	(12)	(24)	1	2	2	2	2	1	1	1	1	1	1	1	(12)	2	2	2	1	2	1	1	-	1	1	2	1	1	3	3	2
A. zenkeri	2	2	1	2	1	(14)	1	2	2	2	(23)	1	1	1	1	1	1	1	1	1	2	1	3	(23)	1	1	-	1	1	3	1	1	3	3	3
P. harmandii	(12)	(12)	(12)	(12)	(12)	(12)	1	2	2	1	(23)	1	1	(12)	2	2	2	1	1	(12)	1	1	(12)	2	1	1	-	2	1	3	2	1	1	2	2
P. lanceolatum	(12)	(12)	(12)	(12)	(12)	(12)	1	2	2	1	(23)	1	1	(12)	2	2	2	1	1	(12)	1	1	(12)	2	1	1	-	2	1	3	2	1	1	2	2
Hapaline sp.	1	2	-	1	1	4	1	2	2	2	3	1	1	1	2	1	1	1	1	4	2	-	(12)	2	1	2	1	1	1	2	-	2	-	-	-