

## Reconstructing Phylogenies of *Alocasia* spp. (Araceae) Distributed in Indonesia for Conservation Prioritization

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### ABSTRACT

Phylogenetic analysis is beneficial to plant conservation prioritization. Conservation does not only deal with species but also their evolutionary potential. A phylogenetic tree is usually reconstructed by using the alignment of DNA sequences. However, with the availability of megatrees, subsetting with a list of species of concern is possible. Here we reconstructed a list of 53 *Alocasia* spp. (Araceae) from Indonesia and adjacent regions using available megatrees and publicly available DNA sequences in the GenBank. The result showed placed and unplaced *Alocasia* spp. on the terminal nodes of reconstructed phylogenetic trees. The unplaced species show that publicly available DNA sequences are not yet available, therefore should be prioritized for sequencing. The placed species in the phylogeny could be used for conservation prioritization of these *Alocasia* spp.

*Keywords:* *Alocasia*, Indonesia, Megatree, Phylogeny, Plant conservation

### Introduction

As a part of the Malesia region, Indonesia has a humid tropic climate and complex physiography which supports its rich biota [1]. One of them is *Alocasia* genus, whose main center of geographic expansion, diversity, and endemism is in Borneo [2, 3], and the second is New Guinea/Australia [2]. Therefore, the two provinces of Indonesia (Kalimantan and Papua) are two regions of *Alocasia* diversity centers. *Alocasia* is distributed in the tropic and subtropic of mainland Asia, the Malesia region, to Oceania and Australia [3]. This genus is estimated to contain 121 species, of which 78 species have been described [4].

The first summarized diversity of *Alocasia* in Indonesia was done in 1991 [5] and according to the study, out of the 13 species of *Alocasia* in Australia, seven were found in Indonesia. *Alocasia* in West Malesia and Sulawesi were subsequently summarized [2]. There were 31 species found in that area, 17 of which were found in Indonesia. In the same year, an expedition by the staff of Bogor

Botanical Gardens in Kolaka, Sulawesi, discovered a new species, *Alocasia suhirmaniana* Yuzammi & A. Hay [6]. Four years later, an expedition of Bogor Botanical Gardens in Toli-toli, Sulawesi, yielded the discovery of a new species, *A. megawatie* Yuzammi & A. Hay [7]. After nearly a decade, one of the Bali Botanical Gardens' staff discovered a new species circulating in the nursery, namely *A. baginda* A. Kurniawan & P. C. Boyce [8]. As a result of the summary and discovery of the new species, Indonesia now has 27 species of *Alocasia*, in which 20 species are endemic.

According to a phylogenetic tree constructed from chloroplast and mitochondrial DNA, *Alocasia* is a *Pistia* clade member, including the genera *Arisaema*, *Colocasia*, *Pinellia*, *Stuednera*, and *Typhonium* [9]. A molecular study was conducted and used the genus in the *Pistia* clade [3], where the *Colocasia* species were more numerous. This study discovered that *Colocasia gigantea* did not belong to the same clade as other *Colocasia*

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species, but instead formed a clade with *Alocasia*. Furthermore, the name *Colocasia gigantea* was changed to *Leucocasia gigantea*, which was previously assigned to this species. The discovery of the new genus *Vietnamocasia* in Vietnam altered the composition of the clade *Pistia* once more, with *Vietnamocasia* becoming the sister clade of *Alocasia*, which is strongly supported based on nuclear and chloroplast DNA [10].

Phylogeny of Malesian *Alocasia* in Indonesia has been conducted. Firstly, RAPD analysis was used to investigate the phylogenetic relationship of *Alocasia suhirmaniana* in the Longiloba group [11]. The study yielded two groups: the Longiloba group and the *Alocasia* sp. Southeast Sulawesi group. The Longiloba group produces three subgroups: *suhirmaniana*, *longiloba* and *watsoniana*. *A. suhirmaniana* is a distinct species. Then, the phytochrome C/phyC gene (nuclear DNA) and four plastid loci were used, namely the trnL intron, trnL-F intergenic spacer, rpl20-rps12 intergenic spacer, and trnK/matK region, to study the biogeography of the genus *Alocasia* based on its distribution, except for New Guinea (chloroplast) [3]. This work reported floristic exchange in Miocene, the origin of *Alocasia macrorrhizos* and *Alocasia cucullata* and revealed a new genus and species, *Englerarum hypnosum*, by plastid and nuclear DNA sequences [12].

Phylogeny refers to the relationship of evolutionary history between groups of organisms from the higher to the lower taxonomic level [13]. Phylogeny is not only beneficial in identifying and clarifying taxonomic ambiguities [13], but also a tool in biogeographic history [14], evolutionary and ecological [15], a priority of conservation [13], and conservation management [16].

Megatrees are available [17, 18] and a list of taxa can be plotted against them to construct phylogenetic trees. As a matter of fact, different tree topologies may arise from different loci: plastid or nuclear, but many types of megatrees do not state clearly what are the original sequences. This study aimed to check if a list of *Alocasia* spp. distributed in Indonesia and adjacent areas can be successfully plotted against a megatree, track sequence types for constructing the megatree, reconstruct phylogenetic trees based on those sequence data from GenBank or other public databases, and compare topologies of reconstructed phylogenetic trees. Additionally, this study's result will guide

prioritization for this genus of Araceae and further sequencing project of *Alocasia* spp.

## Material and Methods

### Compiling records and validating scientific names

We searched the list of *Alocasia* spp. distributed in Indonesia in virtual herbarium: Bioportal Naturalis, Kew, RBG Sydney, China Virtual Herbarium (CVH), and Atlas of Living Australia (ALA) by keywords: *Alocasia*, distribution, and Indonesia. Additionally, we consulted Herbarium Bogoriense (BO) to get the list of *Alocasia* spp. collections. Furthermore, we compiled publications related to *Alocasia* spp. from various authors [5, 8, 19]. The combined data was tabulated and screened with a spreadsheet (MS-Excel). We used the LCVP implemented in R [20] to validate accepted scientific names. We also checked the IUCN conservation status of species under the genus *Alocasia* in the list using taxize in R [21].

### Subsetting a megatree and analyzing the output

We used V.PhyloMaker implemented in R [17] to subset GBOTB.extended Megatree by a list of accepted names (LCVP output). To annotate extracted Alismatales.aln sequences, we downloaded all available *Alocasia* sequences (TaxID: 4455) from the GenBank using PhylotaR [22]. We performed command-line BLAST (query: seed sequences of PhylotaR's clusters, subject: extracted Alismatales.aln). BLAST hits showed sequence ids, therefore the identity of concatenated sequence in manually extracted Alismatales.aln could be determined. Additionally, we used MegaX to construct a phylogenetic tree using sequences of a nuclear locus (a cluster of PhylotaR downloaded sequences).

### Constructing maximum likelihood phylogenetic trees

We manually extracted corresponding sequences from Alismatales.aln [18] and constructed a maximum likelihood (ML) phylogenetic tree with 1000 bootstraps in MegaX [23]. Because GenBank also deposits sequences of nuclear loci, we constructed a phylogenetic tree of our species list by phyC as a comparison of previously generated trees. In this case, we also used MegaX to determine the best nucleotide substitution model prior to ML tree construction.

### Editing and characterizing trees

We used a number of software to edit generated trees. The generated tree (newick format) was edited in Phylo.io [24] for expanding and collating branches, saved in svg format, and edited in InkScape ver. 0.92 (<https://inkscape.org/>), then exported to PNG format with a resolution of 600 dpi. We used Phylomatic in Phylocom [25] for subsetting larger trees generated by MegaX. To count tips and internal nodes of the generated trees, we used an R-package “ape” [26, 27].

### Results and Discussion

The list of *Alocasia* spp. distributed in Indonesia and adjacent regions with LCVP accepted

names are shown in Table 1 (Appendix). *Alocasia macrorrhizos* was not found in LCVP. However, it is available in the GenBank, *A. esculenta* (BO-0104554) was not found in both databases and was later confirmed to be misidentified. None of the species listed is on the IUCN red list. We present only a phylogenetic tree generated by V.PhyloMaker (Figure 1) and describe the characteristics of other trees by a number of tips and internal nodes (Table 2). Tree names in Table 2 are started with the software used, sequences used (if any), and ended with the number of tips. Figure 1 shows the combination of V.PhyloMaker 53 and V.PhyloMaker\_36 tree in which 36 species plotted in tips and 17 species (*A. aequifolia* to *A. venusta*)

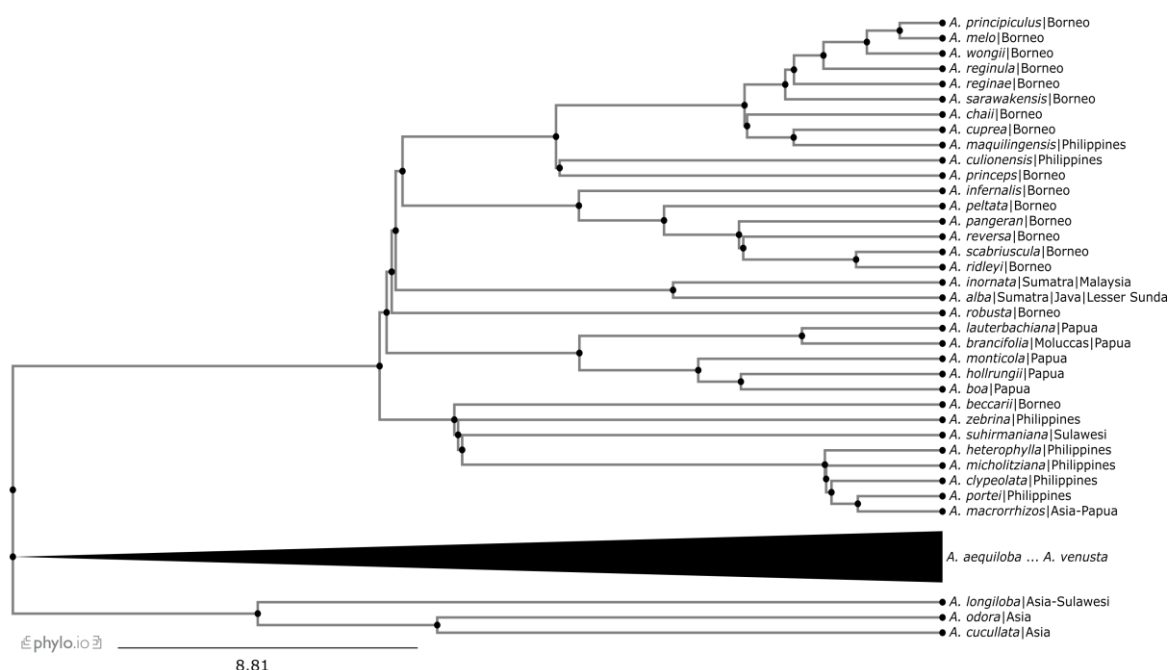


Figure 1. V.PhyloMaker generated phylogenetic tree of *Alocasia* spp. distributed in Indonesia and adjacent regions. This tree shows individual tips of 36 *Alocasia* spp. with available sequence in GenBank whereas other 17 without sequence are collated (*A. aequifolia* – *A. venusta*). Distribution is shown next to each species name.

Table 2. Five trees of *Alocasia* spp. reconstructed in this study.

Tree Name	Tips	Internal Nodes	Parsim./Seq. Length	Nucl. Subst. Model	Remarks
V.PhyloMaker_53	53	51	-	-	Unrooted, includes branch length
V.PhyloMaker_36	36	35	-	-	Rooted, includes branch length
MegaX_SmithBrown_36	36	34	41/10652	Tamura 3-parameter	Rooted, includes branch length
MegaX_phyC_65	65	63	36/1068	Kimura 3-parameter	Unrooted, includes branch length
MegaX_phyC_33	33	53	-	-	Unrooted, includes branch length

Parsim./Seq.Length: parsimony informative sites, Nucl. Subst.: Nucleotide substitution model.

collated. By the time we did this analysis, these 17 species were those without any available sequences in the GenBank. Table 2 shows that MegaX generated a tree by using extracted *Alismatales.aln* (MegaX\_SmithBrown\_36) showed a similar topology with that of generated by V.PhyloMaker (V\_PhyloMaker\_36). On the other hand, the tree that was generated using phyC sequences (MegaX\_phyC\_33) showed different topology. In this case, MegaX\_phyC\_33 is merely a subset of MegaX\_phyC\_65 by Phylomatic.

Our performed PhylotaR pipeline resulted 16 clusters, 361 sequences, and 74 source taxa. Seed sequences of six PhylotaR's clusters with BLAST hits extracted *Alismatales.aln* are presented in Table 3. The table shows that the original sequences of the phylogenetic tree generated by V.PhyloMaker (V.PhyloMaker\_53) were trnL-trnF intergenic spacer, tRNA-Leu (trnL) gene, rbcL gene, and matK gene (chloroplastic/plastid loci).

Considering the results, we believe that the tree presented in Figure 1 is the best among others listed in Table 2. The tree consists of 7 clades: *A. principiculus* – *A. maquilangensis*, *A. culionensis* & *A. priceps*, *A. infernalis* – *A. ridley*, *A. inornate* & *A. alba*, *A. robusta*, *A. lauterbachiana* – *A. boa*, *A. beccarii* – *A. macrorrhizos*, and *A. longiloba* – *A. cucullata*. It is important to note that the collated clades are those *Alocasia* spp. without sequence data in GenBank: *A. aequiloba*, *A. arifolia*, *A. baginda*, *A. balgooyi*, *A. celebica*, *A. flemingiana*, *A. kerinciensis*, *A. lancifolia*, *A. megawati*, *A. minuscula*, *A. nicolsonii*, *A. puber*, *A. puteri*, *A. pyrospatha*, *A. wentii*, and *A. venusta*. They should be prioritized in future DNA sequencing projects.

Thirty-six out of 53 *Alocasia* spp. were successfully plotted by V.PhyloMaker (Figure 1) and we showed that the original sequences were chloroplast loci: trnL-trnF intergenic spacer, tRNA-Leu (trnL) gene, rbcL gene, and matK gene. These loci are common in plant DNA barcoding [28-30]

Table 3. Six out of 16 GenBank seed sequence clusters downloaded by PhylotaR with BLAST hits extracted *Alismatales.aln*

PhylotaR's cluster	Seed Seq. GenBank Acc. Number	N_taxa	N_seqs	Med_sql	MAD	Seq. annotation
1	AB494512.1	71	78	407	0.975319	<i>Alocasia cucullata</i> , 3'trnL gene, trnL-trnF intergenic spacer, trnF gene, partial and complete sequence
8	JQ238783.1	14	14	406.5	0.992647	<i>Alocasia</i> sp. Bo011 tRNA-Leu (trnL) gene, partial sequence; trnL-trnF intergenic spacer, complete sequence; and tRNA-Phe (trnF) gene, partial sequence
2	JQ238645.1	68	73	518	0.525183	<i>Alocasia acuminata</i> tRNA-Leu (trnL) gene, partial sequence; chloroplast
6	JQ238697.1	68	73	518	0.525183	<i>Alocasia</i> sp. Bo011 tRNA-Leu (trnL) gene, partial sequence; chloroplast
5	JQ933213.1	6	19	710	0.562336	<i>Alocasia odora</i> voucher P.Boyce, s.n., (K) ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; plastid
9	JF828121.1	5	13	838	0.891231	<i>Alocasia macrorrhizos</i> maturase K (matK) gene, partial cds; chloroplast

N\_taxa: number of taxa, N\_seq: number of sequences, Med\_sql: median of sequence length, MAD: score of maximum alignment density (those with values less than 0.75 should be dropped).

therefore the universal PCR primers should be available and the PCR amplification procedures should already be established. Therefore, sequencing of the 16 *Alocasia* spp. without any molecular resources in the GenBank could be performed easily. However, the fact that a sequencing project has not been established for these species raised a new question. This might be related to many obstacles in DNA sequencing in developing countries with abundant biodiversity, as discussed in some research papers [31, 32]. In fact, the new molecular resources from this 16 *Alocasia* spp. will be useful to the evolutionary and related studies of this genus.

The topology of generated trees from chloroplast sequences differs from nuclear ones (Table 2). This kind of discordance is common in plants where chloroplast and nuclear genomes have different inheritance and recombination patterns [33]. In *Alocasia*, phylogenetic trees from nuclear sequences showed grouping based on morphological characters, whereas those from chloroplast sequences were based on geographical origins [3], and this trend is also shown in Figure 1. However, in this current work, we only focused on how to use publicly available resources for constructing a phylogenetic tree from a list of *Alocasia* spp. Therefore, future works should further test the hypothesis on affinities to the morphological and geographical origin of trees constructed from nuclear and plastid sequences. This kind of work will be more comprehensive if new sequences from species that currently has no molecular resources are already available.

One of the considerations in using phylogenies to guide conservation is the determination of unique lineages [34]. The phylogenetic tree presented in Figure 1 could be used as a conservation strategy framework based on the taxa's evolutionary history. A number of scenarios could be arranged to conserve as many as unique lineages by the available conservation resources. For example, for a given number of species to be conserved, those species from different clades in the phylogenetic tree are preferred rather than of the same number but from only a clade.

## Conclusion

We demonstrated reconstruction of phylogenetic trees of *Alocasia* spp. distributed in Indonesia by two methods: megatree subsetting and using publicly available DNA sequences. Thirty six out

of 53 *Alocasia* spp. could be placed in the tips of a GBOTB.extended subtree which could be used as a framework to arrange conservation scenarios. This work also guides future DNA sequencing projects, especially for those *Alocasia* spp. currently without molecular resources.

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Supplementary 1

Table 1. Compiled list of 53 *Alocasia* spp. (Araceae) distributed in Indonesia and adjacent regions

ID	Submitted_Name	LCVP_Accepted_Taxon	Distribution									Source		
			Sum	Jav	LSI	Bor	Slw	Mol	Pap	Phi	Mly		Asia	
1	<i>A. principiculus</i>	<i>A. principiculus</i> A.Hay	-	-	-	1	-	-	-	-	-	-	-	Naturalis
2	<i>A. melo</i>	<i>A. melo</i> A.Hay & al.	-	-	-	1	-	-	-	-	-	-	-	Hay
3	<i>A. wongii</i>	<i>A. wongii</i> A.Hay	-	-	-	1	-	-	-	-	-	-	-	Hay
4	<i>A. reginula</i>	<i>A. reginula</i> A.Hay	-	-	-	1	-	-	-	-	-	-	-	Hay
5	<i>A. reginae</i>	<i>A. reginae</i> N.E.Br.	-	-	-	1	-	-	-	-	-	-	-	Naturalis
6	<i>A. sarawakensis</i>	<i>A. sarawakensis</i> M.Hotta	-	-	-	1	-	-	-	-	-	-	-	Naturalis
7	<i>A. chaii</i>	<i>A. chaii</i> P.C.Boyce	-	-	-	1	-	-	-	-	-	-	-	Hay
8	<i>A. cuprea</i>	<i>A. cuprea</i> K.Koch	-	-	-	1	-	-	-	-	-	-	-	Hay
9	<i>A. maquilingsis</i>	<i>A. maquilingsis</i> Merr.	-	-	-	-	-	-	-	1	-	-	-	BO
10	<i>A. culionensis</i>	<i>A. culionensis</i> Engl.	-	-	-	-	-	-	-	1	-	-	-	BO
11	<i>A. princeps</i>	<i>A. princeps</i> W.Bull	-	-	-	1	-	-	-	-	-	-	-	Naturalis
12	<i>A. infernalis</i>	<i>A. infernalis</i> P.C.Boyce	-	-	-	1	-	-	-	-	-	-	-	Hay
13	<i>A. peltata</i>	<i>A. peltata</i> M.Hotta	-	-	-	1	-	-	-	-	-	-	-	BO
14	<i>A. pangeran</i>	<i>A. pangeran</i> A.Hay	-	-	-	1	-	-	-	-	-	-	-	Hay
15	<i>A. reversa</i>	<i>A. reversa</i> N.E.Br.	-	-	-	1	-	-	-	-	-	-	-	Naturalis
16	<i>A. scabriuscula</i>	<i>A. scabriuscula</i> N.E.Br.	-	-	-	1	-	-	-	-	-	-	-	Naturalis
17	<i>A. ridleyi</i>	<i>A. ridleyi</i> A.Hay	-	-	-	1	-	-	-	-	-	-	-	Hay
18	<i>A. inornata</i>	<i>A. inornata</i> Hallier.f.	1	-	-	-	-	-	-	-	1	-	-	Naturalis
19	<i>A. alba</i>	<i>A. alba</i> Schott	1	1	1	-	-	-	-	-	-	-	-	Naturalis, Kurniawan
20	<i>A. robusta</i>	<i>A. robusta</i> M.Hotta	-	-	-	1	-	-	-	-	-	-	-	Naturalis
21	<i>A. lauterbachiana</i>	<i>A. lauterbachiana</i> (Engl.) A.Hay	-	-	-	-	-	-	1	-	-	-	-	rbgsydney
22	<i>A. brancifolia</i>	<i>A. brancifolia</i> (Schott) A.Hay	-	-	-	-	-	1	1	-	-	-	-	Naturalis
23	<i>A. monticola</i>	<i>A. monticola</i> A.Hay	-	-	-	-	-	-	1	-	-	-	-	Naturalis
24	<i>A. holtrungii</i>	<i>A. holtrungii</i> Engl.	-	-	-	-	-	-	1	-	-	-	-	Naturalis
25	<i>A. boa</i>	<i>A. boa</i> A.Hay	-	-	-	-	-	-	1	-	-	-	-	Naturalis
26	<i>A. beccarii</i>	<i>A. beccarii</i> Engl.	-	-	-	1	-	-	-	-	-	-	-	Naturalis
27	<i>A. zebrina</i>	<i>A. zebrina</i> Schott ex VanHoutte	-	-	-	-	-	-	-	1	-	-	-	BO
28	<i>A. suhirmaniana</i>	<i>A. suhirmaniana</i> Yuzammi & A.Hay	-	-	-	-	1	-	-	-	-	-	-	Naturalis
29	<i>A. heterophylla</i>	<i>A. heterophylla</i> (C.Presl) Merr.	-	-	-	-	-	-	-	1	-	-	-	BO
30	<i>A. micholitziana</i>	<i>A. micholitziana</i> Sander	-	-	-	-	-	-	-	1	-	-	-	BO
31	<i>A. clypeolata</i>	<i>A. clypeolata</i> A.Hay	-	-	-	-	-	-	-	1	-	-	-	rbgsydney
32	<i>A. portei</i>	<i>A. portei</i> Schott	-	-	-	-	-	-	-	1	-	-	-	Naturalis
33	<i>A. macrorrhizos</i>	-	1	1	1	1	1	1	1	1	1	1	1	Naturalis
34	<i>A. longiloba</i>	<i>A. longiloba</i> Miq.	1	1	1	1	1	-	-	-	1	1	1	Naturalis, Hay
35	<i>A. odora</i>	<i>A. odora</i> (Lindl.) K.Koch	-	-	-	-	-	-	-	-	-	-	1	Naturalis
36	<i>A. cucullata</i>	<i>A. cucullata</i> (Lour.) G.Don	-	-	-	-	-	-	-	-	-	-	1	BO
37	<i>A. aequiloba</i>	<i>A. aequiloba</i> N.E.Br.	-	-	-	-	-	-	1	-	-	-	-	BO
38	<i>A. arifolia</i>	<i>A. arifolia</i> Hallier.f.	1	-	-	-	-	-	-	-	-	-	-	Naturalis
39	<i>A. baginda</i>	<i>A. baginda</i> Kurniawan & P.C.Boyce	-	-	-	1	-	-	-	-	-	-	-	BO
40	<i>A. balgooyi</i>	<i>A. balgooyi</i> A.Hay	-	-	-	-	1	-	-	-	-	-	-	Naturalis
41	<i>A. celebica</i>	<i>A. celebica</i> Engl. ex Koord.	-	-	-	-	1	-	-	-	-	-	-	Hay, BO
42	<i>A. esculenta</i>	-	-	-	-	-	-	-	-	-	-	-	-	BO
43	<i>A. flemingiana</i>	<i>A. flemingiana</i> Yuzammi & A.Hay	-	1	-	-	-	-	-	-	-	-	-	Naturalis
44	<i>A. kerinciensis</i>	<i>A. kerinciensis</i> A.Hay	1	-	-	-	-	-	-	-	-	-	-	Naturalis
45	<i>A. lancifolia</i>	<i>A. lancifolia</i> Engl.	-	-	-	-	-	-	1	-	-	-	-	Naturalis
46	<i>A. megawatae</i>	<i>A. megawatae</i> Yuzammi & A.Hay	-	-	-	-	1	-	-	-	-	-	-	BO
47	<i>A. minuscula</i>	<i>A. minuscula</i> A.Hay	-	-	-	1	-	-	-	-	-	-	-	Hay
48	<i>A. nicolsonii</i>	<i>A. nicolsonii</i> A.Hay	-	-	-	-	-	-	1	-	-	-	-	Naturalis
49	<i>A. puber</i>	<i>A. puber</i> (Hassk.) Schott	1	1	-	-	-	-	-	-	-	-	-	Naturalis, Hay
50	<i>A. puteri</i>	<i>A. puteri</i> A.Hay	-	-	-	1	-	-	-	-	-	-	-	Hay
51	<i>A. pyrospatha</i>	<i>A. pyrospatha</i> A.Hay	-	-	-	-	-	-	1	-	-	-	-	Naturalis
52	<i>A. venusta</i>	<i>A. venusta</i> A.Hay	-	-	-	1	-	-	-	-	-	-	-	Hay
53	<i>A. wentii</i>	<i>A. wentii</i> Engl. & K.Krause	-	-	-	-	-	-	1	-	-	-	-	Naturalis
Total			7	5	3	23	6	2	11	8	3	4		

Sum: Sumatra, Jav: Java, LSI: Lesser Sunda Islands, Bor: Borneo, Slw: Sulawesi, Mol: Mollucas, Pap: Papua, Phi: Philippines, Mly: Malaysia, Asia: Mainland Asia. Twenty-seven species distributed in Indonesia are in bold.

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