

Molecular phylogeny of Philippine *Amorphophallus* (Araceae): evidence of polyphyly and multiple colonization events

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Abstract

Background and aims – The Philippines harbours 22 recognized species of *Amorphophallus*, with 20 endemic taxa; however, their phylogenetic relationships remain poorly understood owing to limited molecular data. This study explored the monophyly and phylogenetic placement of Philippine *Amorphophallus* using molecular markers.

Material and methods – Two plastid markers (*rbcl* and *matK*) and nuclear ribosomal DNA (ITS1) were sequenced for 26 Philippine *Amorphophallus* specimens. Maximum Likelihood and Bayesian inference analyses were performed on individual and combined datasets comprising 471 GenBank sequences representing 158 *Amorphophallus* species and three outgroups, and 63 Philippine *Amorphophallus* sequences generated in this study.

Key results – Philippine *Amorphophallus* is polyphyletic, with species distributed across different clades. A strongly supported Paeoniifolius-Manta clade was recovered, including several Philippine taxa (*A. paeoniifolius*, *A. rostratus*, *A. samarensis*, and *A. urceolatus*) characterized by sessile spadices and verruculate petioles. Two Palawan accessions (*A. natolii* and *A. cf. natolii*) grouped within *A.* subgenus *Metandrium*, distinctly separated from other Philippine species.

Conclusion – Results imply two independent colonization events from northern and southern biogeographical routes of Southeast Asia and Borneo. Unresolved polytomies indicate the need for phylogenomic studies. This first comprehensive molecular phylogeny of Philippine *Amorphophallus* provides a foundation for systematic revisions, biogeography, and recognition of latent diversity in this megadiverse archipelago.

Keywords

Bayesian inference, Borneo, endemism, ITS1, plastid markers

INTRODUCTION

Amorphophallus Blume ex Decne. is one of the most species-rich genera within the family Araceae, comprising over 200 described species, with the majority of its distribution found across tropical Asia and Africa (Ittenbach 2003; Sedayu et al. 2010; Fontarum-Bulawin et al. 2025). Members of this genus are characterized by thermogenic inflorescences, complex pollination

mechanisms, and reliance on corms for perennation (Zulfiqar et al. 2024). The Philippines is a megadiverse country and biodiversity hotspot, and is home to 22 recognized species, 20 of which are endemic (Fontarum-Bulawin et al. 2024; Miranda et al. 2025). Despite high rates of species endemism and continuous species discoveries, the phylogenetic relationships within Philippine *Amorphophallus* are poorly understood, largely due to a lack of molecular data and complex taxonomy.

Amorphophallus is centred in the Indo-Malayan biogeographic region, which accounts for approximately 56.65% of known species occurrences, with Southeast Asia representing the most species-rich subregion (Fontarum-Bulawin et al. 2025). The Philippines, situated at the eastern periphery of this diversity centre, is thus well positioned as a recipient of colonizing lineages from the broader Southeast Asian mainland and the Malesian archipelago.

Molecular phylogenetics continues to expand our knowledge of the evolutionary relationships of plants. Phylogenomic analyses in Araceae, beginning with the first whole-plastome study (Henriquez et al. 2014) and progressing to target capture and transcriptomic approaches, have advanced our understanding of diversification, whole-genome duplication, and evolutionary relationships (Haigh et al. 2023; Zhao et al. 2023). Studies on the cultivated species genomes of *Amorphophallus*, *A. albus* P.Y.Liu & J.F.Chen, and *A. konjac* K.Koch have been essential in understanding the evolution of organellar genomes, codon use, and adaptive evolution (Li et al. 2024).

Previous molecular phylogenetic studies have provided a foundational understanding of the relationships within *Amorphophallus*, identifying major subgenera and regional clades (Grob et al. 2004; Sedayu et al. 2010; Claudel et al. 2017; Wong et al. 2022). Within *Amorphophallus* subgenus *Amorphophallus*, Southeast Asian species are particularly diverse, characterized by morphological synapomorphies such as sessile spadices and verruculate petioles (Hettterscheid and Ittenbach 1996). However, the Philippine representatives have been poorly represented in these studies, as the majority have included only a few widespread species or species of economic importance. Phylogenetic work by Wong et al. (2022) demonstrated that Bornean taxa and Palawan-Philippine species are linked, inferring a connection in historical biogeography. Most recently, the continued exploration of endemic species in the Philippines has been conducted by Fontarum-Bulawin et al. (2024) who reported *A. samarensis* Bulawin, Medecilo & Alejandro from the Visayas.

This study presents the first molecular phylogenetic analysis of Philippine *Amorphophallus* within a global context, combining newly generated sequences from 26 Philippine specimens with 471 published sequences from 158 species representing the breadth of the genus in GenBank. Using plastid (*rbcl* and *matK*) and nuclear ribosomal (ITS1) DNA markers, maximum likelihood and Bayesian inference analyses were conducted on this global matrix to (1) determine the phylogenetic placement of Philippine *Amorphophallus* within the genus worldwide; (2) test whether Philippine species constitute a monophyletic or polyphyletic assemblage; and (3) elucidate the colonization routes and biogeographic origins of *Amorphophallus* in the Philippine archipelago.

MATERIAL AND METHODS

Taxon sampling and plant material

Prior to collection, we secured a permit from the Department of Environment and Natural Resources – Biodiversity Management Bureau (DENR GP Nos. 307 and 312). A total of 26 Philippine *Amorphophallus* specimens were collected across the archipelago from 2023 to 2024, following established protocols for field collection. Sampling was mostly from Luzon (17 specimens), followed by Visayas (5 specimens) and Palawan (4 specimens), representing approximately 50% of the recognized Philippine species. Specimens were identified to species based on diagnostic phenotypic characteristics using taxonomic keys and literature, as well as expert identification by regional experts. Figure 1 shows photographs of the collected species of *Amorphophallus* with available inflorescences. Specimens illustrated in Fig. 1A–J were collected in situ from various regions, while Fig. 1K was a cultivated specimen from Boracay. Supplementary material 1 lists all Philippine collections of *Amorphophallus* included in the study along with their corresponding voucher numbers deposited at the University of Santo Tomas Herbarium (USTH). Field-collected tissue was immediately preserved in silica gel for subsequent molecular analysis.

Of the 26 sampled collections, 12 were successfully identified to the species level, two of which represented the same species collected from different localities. However, not all identified collections included floral parts. Additionally, two collections bearing reproductive structures could not be identified to the species level because of their morphologically ambiguous floral characteristics, which deviated markedly from those of known species; therefore, recollection and further morphological analysis are needed to confirm their identities. The remaining 12 collections were sterile, further complicating identification, and the molecular markers employed in this study were insufficient to resolve their taxonomic placement.

DNA extraction, amplification, and sequencing

Total genomic DNA was extracted from silica-dried leaf tissue using the Qiagen DNeasy Plant Mini Kit (Qiagen, Hilden, Germany), following the manufacturer's protocol with minor modifications to optimize DNA yield and purity. DNA quality and concentration were assessed using nanodrop spectrophotometry and agarose gel electrophoresis. The extracted DNA was stored at -20°C until PCR amplification.

Three DNA regions were amplified using established primer sets and PCR protocols (Suppl. material 2). PCR reactions were performed in 25 µL volumes containing 12.5 µL GoTaq Green Master Mix (Promega), 1.0 µL each of forward and reverse primers (10 µM), 1.0 µL template DNA (10–50 ng/µL), and 9.5 µL nuclease-free water. PCR

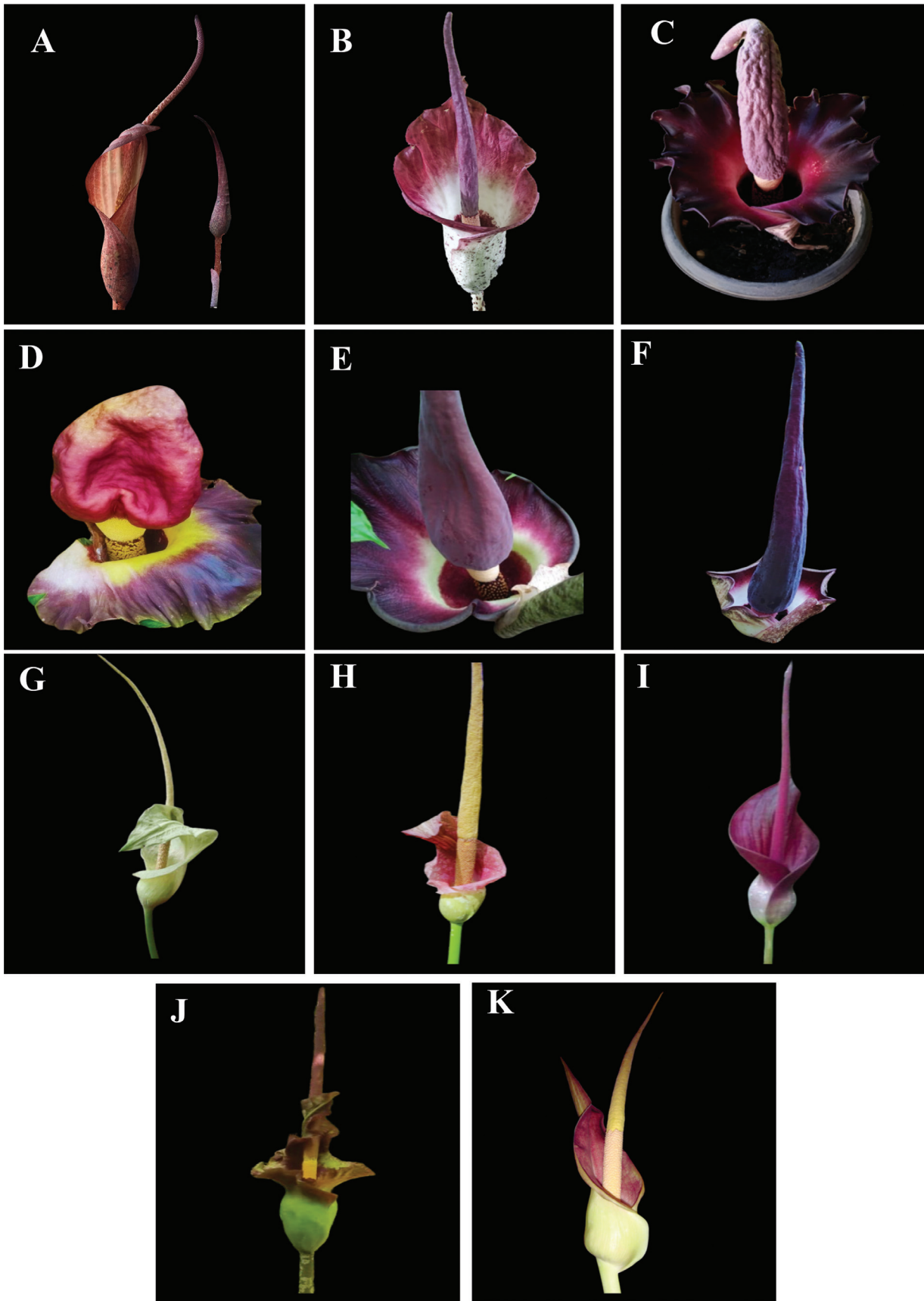


Figure 1. Inflorescences of *Amorphophallus* species collected for this study. **A.** *A. fontarumii* (Mamuyao, Tanay, Rizal). **B.** *A. samarensis* (Paranas, Samar). **C.** *A. flammeus* (Santa, Ilocos Norte). **D.** *A. paeoniifolius* (Yanaba, Bilar, Bohol). **E.** *A. urceolatus* (Ternate, Cavite). **F.** *A. yaoi* (Pandan, Antique). **G.** *A.* sp. ‘green flower’. **H.** *A.* sp. ‘maroon flower’ (G and H from Banton, Romblon). **I.** *A. adamsensis* (Adams, Ilocos Norte). **J.** *A. rostratus* (Mamuyao, Tanay, Rizal). **K.** *A. rayongii* (Boracay). Photos by Norilyn Bulawin.

products were visualized on 1.5% agarose gels stained with ethidium bromide under UV transillumination.

Amplified products were purified using the QIAquick PCR Purification Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The purified amplicons were sent to Macrogen Inc. (Seoul, South Korea) for bidirectional Sanger sequencing using the same primers employed in PCR amplification. Raw sequence chromatograms were manually inspected and edited using Geneious Prime 2024.0.5 (<https://www.geneious.com/>). Forward and reverse sequences were assembled into consensus sequences, and ambiguous bases were resolved by visually inspecting the chromatogram peaks. BLASTn searches were conducted against the NCBI GenBank database to verify sequence identity and to detect potential contamination.

Sequence alignment and phylogenetic analyses

In addition to the newly sequenced Philippine taxa (Suppl. material 3), we incorporated 158 *Amorphophallus* taxa with taxonomic coverage based on previous phylogenetic studies (Grob et al. 2004; Sedayu et al. 2010; Claudel et al. 2017; Wong et al. 2022). Three outgroup taxa were selected from related Araceae genera, *Anchomanes difformis* (Blume) Engl., *Gonatopus angustus* N.E.Br., and *Hapaline* sp., following standard practices in *Amorphophallus* phylogenetics.

Sequences for each marker were aligned separately using MAFFT v.7.490 (Kato and Standley 2013) with default parameters. Alignments were visually inspected and manually adjusted in Geneious Prime where necessary to optimize positional homology. For ITS1, ambiguously aligned regions were excluded from the phylogenetic analyses. Sequence characteristics, including alignment length, number of variable sites, parsimony informative characters, and conserved sites, were calculated using MEGA v.12 (Kumar et al. 2024).

Phylogenetic analyses were conducted for each marker independently, as well as for the combined plastid + nuclear dataset. For each dataset, optimal nucleotide substitution models were chosen using MrModeltest2 under the Akaike Information Criterion (AIC) (Nylander 2004). Maximum Likelihood (ML) analyses were conducted based on the GTR+ Γ +I model using RAxML-NG v.1.1 (Kozlov et al. 2019) with 1,000 bootstrap replicates. ML searches employed 20 random starting trees to ensure a thorough exploration of the tree space. Bayesian inference (BI) was performed using MrBayes v.3.2 under the GTR+I+ Γ model with four Markov Chain Monte Carlo (MCMC) chains (three heated, one cold) (Ronquist et al. 2012). Each run consisted of approximately 10 million generations, with sampling every 1,000th generation across two independent runs. Convergence was assessed by monitoring the standard deviation of split frequencies (< 0.01) and examining potential scale reduction factors (approaching 1.0). The first 25% of trees were discarded as burn-in, and the remaining trees were used to construct

50% majority-rule consensus trees with posterior probabilities (PP).

Prior to combining the datasets, individual gene trees were assessed through visual inspection for strongly supported topological conflicts (bootstrap support [BS] $\geq 70\%$, PP ≥ 0.95). No strongly supported incongruence was detected; therefore, the datasets were concatenated, and the combined matrix was partitioned by gene region, allowing for separate substitution models for each partition in both ML and BI analyses. Phylogenetic trees were visualized and edited using FigTree v.1.4.4 (Rambaut 2018) and Interactive Tree of Life (iTOL) (Letunic and Bork 2007). BS $\geq 70\%$ and PP ≥ 0.95 were considered strong indicators of phylogenetic relationships.

RESULTS

Sequencing success and sequence verification

The success of PCR amplification and sequencing varied for each of the markers. From the chloroplast markers, the *rbcl* region was successfully amplified for all 26 *Amorphophallus* specimens, while the *matK* marker yielded 18 specimens that produced high-quality sequences that were useful for phylogenetic analysis. The *matK* sequences of eight specimens were not obtained because of weak amplification and low-quality sequences, probably because of the degraded DNA template. For the ITS1 region, 19 specimens were successfully sequenced, but seven samples produced unreadable sequences because of suspected low DNA concentration or fungal contamination.

For *rbcl*, the closest matches were *A. albus*, *A. hottae* Bogner & Hett., *A. paeoniifolius* (Densst.) Nicolson, *A. sumawongii* (Bogner) Bogner & Mayo, and *A. titanum* (Becc.) Becc., indicating that the Philippine specimens are most likely affiliated with Southeast Asian species. For *matK*, the top matches were *A. angulatus* Hett. & A. Vogel, *A. eburneus* Bogner, *A. palawanensis* Bogner & Hett., *A. paeoniifolius*, and *A. titanum*. MNM05 collected from Palawan exhibited the highest similarity to *A. natolii* Hett., Wistuba, Amoroso, Medecilo & Claudel (99.89%), confirming a close affinity with this Palawan endemic.

Sequence characteristics and phylogenetic signal

The individual dataset consisting of ITS1 had 169 taxa and a total aligned length of 663 bp, with 333 variable sites (50.2%). Of these 333 variable sites, 208 were parsimony informative (31.4%). For the *rbcl* dataset, 187 taxa were included, consisting of 1,496 bp with 242 variable sites (16.2%) and 133 (8.9%) parsimony informative sites. The *matK* dataset contained 176 taxa and 1,850 bp, with 473 variable sites (25.6%) and 209 parsimony informative sites (11.2%). The combined plastid dataset (*rbcl* + *matK*) comprised 187 specimens and an aligned length of 3,355 bp, of which 699 bp (20.8%) were variable sites and 366 bp (10.9%) were parsimony-informative. The

combined plastid and nuclear dataset comprised the same 187 specimens—158 *Amorphophallus* species and three outgroups from which sequences were retrieved from GenBank, combined with 26 Philippine *Amorphophallus* specimens, for which new sequences were generated in this study—with an aligned length of 3,945 bp, of which 1,030 bp (26.1%) were variable sites and 546 bp (13.8%) were parsimony-informative (Suppl. material 4).

ITS1 had the highest number of parsimony informative characters (31.4 %). This is indicative of rapid changes and a greater phylogenetic signal at lower taxonomic levels. In contrast, *rbcL* was more conserved at 8.9% of informative sites, which is consistent with its slow rate of evolution, whereas *matK* was slightly higher at 11.2%. This is congruent with other molecular studies on Araceae that suggest combined plastid-nuclear datasets provide complementary phylogenetic signals (Claudel et al. 2017; Haigh et al. 2023). The GTR+ Γ +I model was selected as the optimal model for all datasets under AIC, indicating heterogeneous substitution rates with both among-site rate variation and a proportion of invariant sites.

Phylogenetic relationships and Philippine species placement

Single-marker phylogenetic analyses have provided preliminary resolution of *Amorphophallus* relationships. The *rbcL* tree, although highly conserved with limited resolution at the species level, successfully recovered major subgeneric clades with moderate support. Philippine taxa clustered with Southeast Asian species, suggesting regional phylogenetic affinity. Several Philippine accessions were grouped with Bornean and Sumatran species, supporting biogeographic connections between Palawan-Philippine assemblages and Sundaland flora. However, low bootstrap support (< 70%) at many nodes indicated insufficient phylogenetic signal for confident species-level resolution using *rbcL* alone.

Separate phylogenetic analyses of the plastid (cpDNA) and nuclear ribosomal DNA (nrDNA) markers were conducted to assess their individual topologies prior to the dataset combination. Although the overall bootstrap (BS) and posterior probability (PP) support was generally low across most nodes, both datasets supported the monophyly of *Amorphophallus*, with the exception of the Bayesian tree derived from the ITS region, in which the outgroup *Anchomanes difformis* failed to resolve as sister to the genus (Suppl. materials 5, 6). Notable topological discrepancies that are poorly supported were observed between the cpDNA and nrDNA trees (Suppl. materials 5, 6); the nrDNA tree exhibited weaker resolution, particularly within subgeneric groups, and Philippine species were less clearly resolved than the cpDNA tree (Suppl. materials 5B, 6B). The combined cpDNA (*rbcL* + *matK*) tree provided comparatively better resolution (Suppl. material 5A). These discrepancies likely reflect the distinct evolutionary histories captured by plastid

and nuclear genomes, which can yield incongruent phylogenetic signals owing to factors such as incomplete lineage sorting. Given these differences, the cpDNA and nrDNA datasets were combined to improve the resolution and provide a more comprehensive and robust phylogenetic framework for *Amorphophallus*.

The combined plastid and nuclear dataset provided substantially improved resolution and support compared with single-marker analyses. The resulting ML and BI trees were topologically congruent, with ML bootstrap (BS) values and BI posterior probabilities (PP) showing strong concordance. The combined phylogeny confirmed the monophyly of *Amorphophallus* (BS = 100%, PP = 1.00) and recovered major subgeneric divisions consistent with previous phylogenetic studies (Sedayu et al. 2010) (Fig. 2). However, Philippine taxa were distributed across multiple clades, confirming the polyphyly of Philippine *Amorphophallus* (Fig. 2).

Within *A.* subgenus *Amorphophallus*, a strongly supported Southeast Asian clade (BS = 89%, PP = 1.00) included most of the Philippine species (Fig. 2). Within this assemblage, the *Paeoniifolius*-*Manta* clade sensu Claudel et al. (2017) received strong support (BS = 95%, PP = 1.00) and encompassed several Philippine taxa including *A. urceolatus* Hett., *A. samarensis*, *A. rostratus* Hett., and multiple accessions of *A. paeoniifolius* from Bohol, Romblon, Nueva Ecija, and Bulacan, respectively (Fig. 2). These species share morphological synapomorphies, including sessile spadices, verruculate petiole ornamentation, and the absence of tuber offsets, corroborating phylogenetic grouping with morphological coherence. Interestingly, one specimen from Angat Watershed (originally identified as *A. rostratus*) was genetically indistinguishable from true *A. rostratus*, confirming species identity and extending its known distribution. Additionally, several accessions morphologically resembling *A. paeoniifolius* but from previously unreported localities (Nueva Ecija, San Miguel Bulacan) clustered within the *A. paeoniifolius* clade, suggesting potential cryptic diversity or intraspecific variation that warrants further investigation.

In comparison to most Philippine species that fall within *A.* subgenus *Amorphophallus*, two accessions from Palawan were assigned to *A.* subgenus *Metandrium*. *Amorphophallus natolii* and an unidentified specimen from Palawan (MNM05) formed a well-supported clade (BS = 96%, PP = 1.00) that was geographically isolated from all other Philippine members (Fig. 2). The isolation of *A.* subgenus *Metandrium* is supported by its morphology, characterized by stipitate spadices and distinctive tubers. The position of the Palawan clade indicates an independent colonization event in the Philippines, likely via southern routes connecting Palawan to Borneo, where *A.* subgenus *Metandrium* is more diverse. This result illustrates Palawan's unique biogeographic history and its position as a land bridge between Sundaland and the Philippine archipelago.



Figure 2. The majority-rule consensus tree from the Bayesian analysis of the combined cpDNA and nrDNA datasets for the genus *Amorphophallus*. Bayesian posterior probability (PP) values are given above the branches and bootstrap (BS) values from the RAxML analysis are given below the branches. Philippine collections of *Amorphophallus* (locality indicated in orange) are placed within the Southeast Asian (SEA) clade of subgenus *Amorphophallus*.

Unresolved relationships and phylogenetic challenges

Despite the increased resolution provided by the combined dataset, several polytomies remained unresolved, particularly within closely related taxa of the *Paeoniifolius*-Manta clade. Species-level relationships among *A. paeoniifolius* accessions from different islands showed limited resolution, suggesting rapid radiation or incomplete lineage sorting. Similarly, the placement of several Philippine endemics remained uncertain, with low statistical support for their sister-group relationships. These results highlight the limitations of three-marker approaches for fully resolving species-level phylogenies in rapidly diversifying lineages and underscore the need for phylogenomic studies using genome-scale datasets to resolve these relationships definitively.

DISCUSSION

Polyphyly and multiple colonization events

The polyphyletic nature of Philippine *Amorphophallus*, as revealed by our molecular phylogenetic analyses, provides compelling evidence for multiple independent colonization events in the Philippines. The distribution of Philippine taxa across at least two distinct clades—the *Paeoniifolius*-Manta clade within *A.* subgenus *Amorphophallus* and the Palawan endemic clade within *A.* subgenus *Metandrium*—suggests that the Philippine archipelago was colonized by different biogeographic sources at different times. This result is broadly consistent with the subgeneric framework established by Claudel et al. (2017), who showed that the four main clades of *Amorphophallus* largely correspond to distinct geographic and climatic regions, with *A.* subgenus *Amorphophallus* centred on mainland Southeast Asia and *A.* subgenus *Metandrium* distributed primarily across the Malesian region, including Borneo. More recently, the phylogenomic study of Pouchon et al. (2023), which analysed 71 plastid genes from 36 species using genome skimming, provided a revised divergence timeline for the genus and a biogeographic scenario linking the origins of the major clades to climate dynamics and long-distance dispersal events near the Oligocene–Miocene transition (~23 Ma). The placement of Philippine taxa in two phylogenetically and geographically distinct subgenera, as recovered by our analyses, is consistent with this broader phylogenomic framework and suggests that the Philippine archipelago has served as a secondary colonization target from distinct source regions over a protracted timeframe. This pattern aligns with broader biogeographic hypotheses for Philippine flora, which postulate that the archipelago has functioned as a biodiversity sink, receiving taxa from multiple source regions, including mainland Southeast Asia, Sundaland, and Wallacea (Jones and Kennedy 2008). Within this context, it is noteworthy that Southeast Asia represents the most species-rich subregion of the genus,

with Thailand harbouring the highest number of species owing to its favourable tropical and subtropical climatic conditions (Fontarum-Bulawin et al. 2025). The sporadic occurrence of *Amorphophallus* in some areas of its range further reflects the genus' annual cycle of growth and dormancy, which, combined with specific environmental requirements including suitable vegetation cover, altitude, light availability, humidity, and temperature, can result in localized absences even within otherwise suitable biogeographic corridors.

The two colonization events inferred for Philippine *Amorphophallus* likely correspond to distinct biogeographic routes. The *Paeoniifolius*-Manta clade, which includes the majority of Philippine species, shows strong phylogenetic affinity with Southeast Asian mainland taxa, consistent with the biogeographic signal for *A.* subgenus *Amorphophallus* documented by both Claudel et al. (2017) and Pouchon et al. (2023), suggesting a northern colonization route via Taiwan or the Ryukyu Islands. In contrast, the Palawan endemic clade within *A.* subgenus *Metandrium* exhibits closer relationships with Bornean taxa, indicating a southern colonization route via the Sunda Shelf, possibly facilitated by reduced sea levels during Pleistocene glacial periods when Palawan was in closer proximity to Borneo. This Borneo-to-Philippines dispersal route is well documented in other Araceae: Nauheimer et al. (2012) inferred that the Philippines were reached from Borneo four to five times in *Alocasia* (Schott) G.Don during the Late Miocene to Early Pliocene, underscoring the permeability of this corridor for tropical understory monocots. Similar patterns of multiple, temporally staggered colonisations have been documented in other Philippine plant groups: Hughes et al. (2015) showed that *Begonia* sect. *Baryandra* A.DC. colonized the archipelago from western Malesia in the late Miocene, with Palawan and Luzon serving as early entry points, while Atkins et al. (2001) inferred Bornean origins for Philippine *Cyrtandra* J.R.Forst. & G.Forst. during the Pleistocene. Taken together, these studies indicate that Borneo and the Sunda Shelf have repeatedly served as source regions for Philippine plant diversity via the Palawan corridor, while independent dispersal events from the Asian mainland via northern island chains have contributed additional lineages, producing the complex, polyphyletic distributional patterns now observed in *Amorphophallus* and other genera.

The *Paeoniifolius*-Manta clade and Philippine diversity

The strongly supported *Paeoniifolius*-Manta clade recovered in our analysis represents a major Southeast Asian lineage first formally defined by Claudel et al. (2017), characterized by distinctive morphological synapomorphies, including sessile spadices, verruculate petiole ornamentation, and the absence of tuber offsets (Hettterscheid and Ittenbach 1996; Sedayu et al. 2010). Our results are consistent with Sedayu et al. (2010) and

Claudel et al. (2017), who recognized a biogeographically distinct Southeast Asian clade spanning from India to the Philippines and encompassing Philippine endemics such as *A. palawanensis* and *A. paeoniifolius*. This affinity was further corroborated by Wong et al. (2022), who demonstrated that Philippine species, including *A. longispathaceus* Engl. & Gehrm., *A. declinatus* Hett., and *A. palawanensis*, cluster within the SEA clade alongside Bornean taxa, and by Pouchon et al. (2023), whose phylogenomic analysis placed *A. palawanensis* within this same clade, reinforcing the close evolutionary relationships between Philippine and broader Southeast Asian species of *Amorphophallus*. The inclusion of several Philippine taxa within this clade—*A. urceolatus*, *A. samarensis*, *A. rostratus*, and *A. paeoniifolius*—demonstrates that these species are phylogenetically nested within the Southeast Asian radiation (BS = 95%, PP = 1.00), a placement consistent with the regional affinities of this clade as established by Claudel et al. (2017) and further supported by Wong et al. (2022) for Bornean taxa. Although formal ancestral area reconstruction was beyond the scope of this study, this topological affinity suggests a Southeast Asian biogeographic origin for these Philippine lineages, a hypothesis that warrants explicit testing in future biogeographic analyses.

Within the *Paeoniifolius*-Manta clade, species-level relationships among Philippine taxa remain partially unresolved, with several polytomies indicating rapid radiation or insufficient phylogenetic signal from the three-marker dataset. This is not unexpected, given that similar polytomies have been reported in previous multi-locus studies of the genus (Sedayu et al. 2010; Claudel et al. 2017) and resolving them likely requires genome-scale data (Pouchon et al. 2023). The limited genetic differentiation among *A. paeoniifolius* accessions from different islands (Bohol, Romblon, Nueva Ecija, Bulacan) suggests either recent divergence or ongoing gene flow among island populations, consistent with the intraspecific morphological and palynological variation documented by Anil et al. (2023) across geographically disjunct morphotypes of this species. The wide distribution of *A. paeoniifolius* across island populations is further reflected in its status as the most frequently occurring species in the genus regionally (~12.31% of Southeast Asian occurrences), a pattern attributable in part to its widespread cultivation for its edible tuber and medicinal properties (Fontarum-Bulawin et al. 2025). However, the presence of morphologically distinct accessions within the *A. paeoniifolius* clade raises the possibility of cryptic species diversity, a concern further supported by the recent description of *A. minimus* R.Bustam., Claudel & M.N.Tamayo from Nueva Ecija (Bustamante et al. 2021) and *A. calcicola* M.N.Tamayo, Magtoto & Sumalinog from Bohol (Tamayo et al. 2021), both from localities represented in our sampling. These findings underscore the need for population genomic approaches and integrative taxonomic methods, combining

morphological, molecular, and ecological data, to fully resolve species boundaries within this morphologically variable clade (Hettterscheid et al. 2020; Zhao et al. 2020).

Palawan endemic clade and biogeographic distinctiveness

The phylogenetic isolation of Palawan *Amorphophallus* within *A.* subgenus *Metandrium* provides strong molecular evidence for the biogeographic distinctiveness of Palawan flora, in agreement with the subgeneric framework of Claudel et al. (2017), who placed *A.* subgenus *Metandrium* primarily within the Malesian region, with Borneo as a centre of diversity. The well-supported clade comprising *A. natolii* and an unidentified Palawan specimen (MNM05; BS = 96%, PP = 1.00) is sister to other species of *A.* subgenus *Metandrium* from Borneo, corroborating the phylogenetic affinity between Palawan and Sundaland taxa inferred by Wong et al. (2022). This result is consistent with geological evidence indicating that Palawan formed part of the Asian continental shelf during Pleistocene glacial maxima, when sea levels were approximately 120 m lower than present, facilitating land connections between Palawan and northern Borneo (Voris 2000). The subsequent post-glacial rise in sea levels effectively isolated Palawan from Borneo, likely promoting the in situ differentiation of endemic lineages, including *A. natolii*, which was described from limestone cliffs at El Nido by Hettterscheid et al. (2012). Parallel patterns of Pleistocene vicariance and post-isolation divergence along the Palawan–Borneo corridor have been documented in other plant groups, including *Alocasia* (Nauheimer et al. 2012) and *Cyrtandra* (Atkins et al. 2001), affirming that this route has been a recurrent conduit for biotic exchange between Sundaland and the Philippines (Jones and Kennedy 2008).

The morphological distinctiveness of *A.* subgenus *Metandrium*, characterized by stipitate spadices and distinctive tuber morphology (Hettterscheid and Ittenbach 1996; Claudel et al. 2017), further reinforces the phylogenetic separation of the Palawan clade from all other Philippine *Amorphophallus* species. These morphological differences likely reflect adaptations to Palawan's unique ecological conditions, including its extensive limestone substrates, seasonal rainfall regime, and distinct pollinator communities, conditions previously noted to promote localized endemism in *A. natolii* (Hettterscheid et al. 2012). The recognition of Palawan as a biogeographically distinct unit within the Philippines has important conservation implications, as the island harbours a disproportionately high proportion of endemic species relative to its area (Fontarum-Bulawin et al. 2025; Miranda et al. 2025). Our results reinforce the view that Palawan merits priority status in conservation planning for *Amorphophallus* and other aroid taxa, particularly given the ongoing threats to its limestone and lowland forest habitats.

Phylogenomic context and future directions

Although our three-marker approach provided valuable insights into the broad phylogenetic structure of Philippine *Amorphophallus*, several relationships remained unresolved, particularly at the species level within the *Paeoniifolius*-*Manta* clade. The limited phylogenetic signal from plastid markers (*rbcl*, *matK*) and nuclear ribosomal ITS1 is consistent with recent findings in Araceae phylogenomics demonstrating that genome-scale datasets are necessary for resolving relationships among closely related species (Henriquez et al. 2014; Haigh et al. 2023; Zhao et al. 2023). Within *Amorphophallus*, Pouchon et al. (2023) demonstrated that analysing multiple plastid genes from a broad species sampling via genome skimming substantially improved subgeneric resolution compared to single- or few-gene approaches; however, they acknowledged persistent uncertainties at the species level, underscoring the inherent limitations of plastid-only or lightly sampled nuclear data. Similarly, whole-chloroplast genome analyses of individual species, such as *A. konjac* and *A. albus*, have contributed to resolving shallow-level placement (Li et al. 2024); however, fine-scale resolution of intrageneric diversity across the archipelago will require substantially broader taxon sampling with genome-scale markers.

Future phylogenomic studies on Philippine *Amorphophallus* should employ next-generation sequencing approaches, such as target enrichment of hundreds of low-copy nuclear genes or whole-genome resequencing, following the methodological frameworks successfully applied across Araceae by Haigh et al. (2023) and Zhao et al. (2023). Such approaches would enable more robust tests of biogeographic hypotheses, including the precise timing and directionality of colonization events, the role of Pleistocene glacial cycling in shaping island distributions (Esselstyn et al. 2009), and the mechanisms driving rapid diversification in island systems. Levin (2003) highlighted the role of cytoplasmic factors and plastid–nuclear conflict in plant speciation, and given the topological discrepancies observed between our cpDNA and nrDNA trees, explicit tests of reticulate evolution and hybridization using genomic data, as advocated by Mallet et al. (2016), would be particularly informative for groups with complex island biogeographic histories. Additionally, population genomic studies targeting widespread species, such as *A. paeoniifolius*, are needed to elucidate patterns of gene flow among island populations and to identify cryptic diversity potentially masked by morphological plasticity, as highlighted by the intraspecific variation documented across its range (Anil et al. 2023).

Taxonomic implications and species delimitation

The phylogenetic results presented here have important implications for the taxonomy and species delimitation of Philippine *Amorphophallus*. The recovery of morphologically distinct accessions within the *A. paeoniifolius* clade suggests that current taxonomic

treatments may underestimate species diversity within this group. Several specimens from previously unreported localities (Nueva Ecija, San Miguel, Bulacan) clustered within the *A. paeoniifolius* clade but exhibited subtle morphological differences in leaf shape, petiole ornamentation, and inflorescence size. These differences, combined with genetic differentiation, suggest the possibility of cryptic species that warrant further investigation through integrative taxonomic approaches combining morphology, molecular phylogenetics, and population genetics. That unrecognized diversity persists even in well-collected localities is consistent with the broader pattern of ongoing species discovery in the Philippine archipelago: Hettterscheid et al. (2020) described five new species from various Philippine islands in a single paper, and subsequent work has continued to reveal novelties, including *A. minimus* from montane forest in Nueva Ecija, Luzon, the same province from which our morphologically divergent specimens were collected (Bustamante et al. 2021), and *A. calcicola* from karst forest in Bohol, Central Visayas, closely related to *A. longispathaceus* and distinguished by a suite of floral characters (Tamayo et al. 2021). Most recently, *A. samarensis* was described from Samar Island Natural Park, Eastern Visayas, resembling *A. calcicola* in several vegetative and floral characters but distinguishable by leaf rachis morphology, stigma shape, and anther structure (Fontarum-Bulawin et al. 2024). The description of these new species from Bohol, Nueva Ecija, and Samar demonstrates that Philippine *Amorphophallus* diversity remains incompletely documented and validates our inference that morphological differentiation observed among our specimens from novel localities may reflect genuine taxonomic novelty rather than intraspecific variation.

Similarly, the unidentified Palawan specimen (MNM05), which clustered with *A. natolii* but showed morphological differences, may represent an undescribed species or a distinct population of *A. natolii*. *Amorphophallus natolii*, described from limestone cliffs at El Nido, is among several *Amorphophallus* species endemic to Palawan (Hettterscheid et al. 2012), and the island's complex karst topography is known to harbour highly localized populations. These findings collectively highlight the need for continued field surveys, targeted collections from limestone and montane habitats, and integrative taxonomic revisions to document the full extent of *Amorphophallus* diversity in the Philippines.

CONCLUSION

This study provides the first comprehensive molecular phylogeny of Philippine *Amorphophallus*, revealing a polyphyletic assemblage resulting from at least two independent colonization events from Southeast Asia and Borneo. The recovery of a strongly supported *Paeoniifolius*-*Manta* clade and a distinct Palawan endemic clade within *A.* subgenus *Metandrium* provides

insights into the biogeographic history of the Philippine archipelago and the evolutionary processes shaping plant diversity in island systems. While our three-marker approach successfully resolved major relationships, several polytomies remain unresolved, highlighting the need for phylogenomic studies using genome-scale datasets. Our results have important implications for systematic revisions, conservation priorities, and the recognition of cryptic diversity within Philippine *Amorphophallus*. Future research should focus on phylogenomic analyses, population genetic studies, and integrative taxonomy to fully resolve species-level relationships and document the extent of *Amorphophallus* diversity in this megadiverse archipelago.

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SUPPLEMENTARY MATERIALS

Supplementary material 1

List of Philippine *Amorphophallus* species and specimens included in the study and their collection localities.

<https://doi.org/10.5091/plecevo.181704.suppl1>

Supplementary material 2

PCR primers and amplification protocols used for DNA barcoding of Philippine *Amorphophallus* species.

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Supplementary material 3

GenBank accession numbers of newly generated sequences for three molecular markers (*rbcl*, *matK*, and ITS1) from Philippine *Amorphophallus* collections.

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Supplementary material 4

Summary statistics of sequence characteristics for nuclear ribosomal DNA (nrDNA) and chloroplast DNA (cpDNA) markers derived from GenBank and the present study, used in the phylogenetic analyses of *Amorphophallus*.

<https://doi.org/10.5091/plecevo.181704.suppl4>

Supplementary material 5

Maximum likelihood trees inferred from the individual cpDNA (A) and nrDNA (B) datasets for the genus *Amorphophallus*. Philippine *Amorphophallus* collections are highlighted in red. Bootstrap (BS) values are given at supported nodes.

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Supplementary material 6

Bayesian inference trees inferred from the individual cpDNA (A) and nrDNA (B) datasets for the genus *Amorphophallus*. Philippine *Amorphophallus* collections are highlighted in red. Bayesian posterior probability (PP) values are given at supported nodes.

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