Phylogenomics, genome size evolution and repeat dynamics in the genus *Amomum* Roxb. (Zingiberaceae)

Fylogenomika, evoluce velikosti genomu a dynamika repetitivních sekvencí v rodu *Amomum* Roxb. (Zingiberaceae)

Mgr. Kristýna Hlavatá

Supervisor: Mgr. Tomáš Fér, PhD.

Consultant: Mgr. Eliška Záveská, PhD.

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Charles University, Faculty of Science

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Declaration

I hereby declare that I have composed this thesis by myself using the listed references. The thesis has not been submitted elsewhere, in whole or in part, for the same or any other academic degree.

Prohlášení

Prohlašuji, že jsem tuto práci zpracovala samostatně a že jsem uvedla všechny použité informační zdroje a literaturu. Tato práce ani její podstatná část nebyla předložena k získání tohoto nebo jiného akademického titulu.

V Praze, 19. 1. 2024

Kristýna Hlavatá

Original Papers

This thesis is based on the following four papers:

Paper I.

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Author contribution statement

I declare that I have contributed to all papers included in the thesis. My contributions to the particular papers are as follows:

Paper I.

Laboratory and taxonomic work – total contribution 30%.

Paper II.

Taxonomic work – total contribution 15%.

Paper III.

Field sampling, laboratory work, data processing and interpretation, manuscript writing – total contribution 80%.

Paper IV.

Data processing and interpretation, manuscript writing, review and editing – total contribution 70%.

On behalf of all the co-authors, I declare the above mentioned contribution of Kristýna Hlavatá in completing research and writing papers.

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Abstract

Amomum Roxb. s.l. (black cardamom) is a complicated genus in the ginger family Zingiberaceae (subfamily Alpinioideae) that according to some definitions includes groups of species recognized as separate genera, e.g. genus *Elettariopsis* Baker. Phylogenetic studies have so far failed to elucidate both the position of *Amomum* within Alpinioideae and the relationship between *Amomum* and other genera like *Elettariopsis*. In this work, *Amomum* was subjected to a detailed morphological analysis of the largest possible sampling, together with a phylogenetic analysis. *Amomum* was recircumscribed, *Amomum* s.s. and three new genera were established, three genera were resurrected, and genus *Elettariopsis* was merged into *Amomum*. Meanwhile, new species were described and others epitypified.

A well-resolved nuclear-gene phylogeny of *Amomum* s.s. was obtained using next-generation sequencing (Hyb-Seq) and showed the existence of four clades (A, B, C, D) within the genus. Clade D, the former genus *Elettariopsis*, was further divided into three subclades (D1–D3). A chloroplast DNA-based phylogeny supported this structure, but additional phylogenies based on ribosomal DNA were incongruent with it, indicating that the frequently used ITS marker and other rDNA markers may not be suitable for reconstructing deeper phylogenetic nodes.

Genome size is an intensely studied feature of plants with a fundamental impact on their growth, adaptation and speciation. Due to this, it can drive plant diversification and has been successfully used in plant systematics, as it may carry a phylogenetic signal. Genome size is less studied in tropical plants, but *Amomum* as a tropical genus distributed throughout much of the Indomalayan realm and growing in a variety of environments offered itself as a good model for examining genome size dynamics in tropical conditions. The evolution of absolute (2C) genome size in *Amomum* was found to be congruent with *Amomum*'s phylogeny, with 2C genome size gradually rising from the early diverging clade A to the most derived subclade D3 and an overall 4.5-fold 2C genome size range. Interestingly, the gradual increase of GS corresponds with the biogeographical history of the genus, which dispersed from its original location in southern China and northern Indochina (clades A, B and C) southeast over the Isthmus of Kra to Sundaland (subclades D1–D2) and then back north (subclade D3). Chromosomes were counted in selected accessions and two tetraploid species with 2n = 96 chromosomes were found. These were the first polyploids to be found in *Amomum*; the species *A. cinnamomeum* (subclade D3) had the largest 2C genome size at 15.66 pg, which is also the largest detected genome size in the order Zingiberales to date. The remaining diploids had 2n = 48 chromosomes.

Within diploids, genome size varied 2.7-fold, which prompted a repeatomic analysis of the genus (i.e. the quantification of the main groups of repetitive sequences in the genome). A subset of species was analysed using the RepeatExplorer pipeline to quantify and compare their repetitive sequences (repeats). Genome size and repeat content were significantly correlated, revealing that repeats were driving the genome size increase; the overall repeat content also carried phylogenetic signal. Repeat lineages of the *Ty1-Copia* superfamily (particularly SIRE) strongly contributed to the genome size increase and were prevalent in *Amomum*'s repeatomes. Most repeat lineages present in *Amomum* were significantly amplified in clade D. Two ancient hybridization events were revealed in *Amomum*: the hybrid origin of clade D, which may have caused repeat proliferation in this clade, and a hybridogenous group of species within this clade. A repeat-based

phylogenetic network showed congruency with the nuclear gene phylogeny. Finally, *Amomum* with a repeatome proportion reaching 88% was found to belong among monocot genera with largest repeatome proportions.

Keywords: *Amomum*, Zingiberaceae, genome size, genome evolution, classification, morphology, repetitive DNA, repeatome, phylogeny, next-generation sequencing, ancient hybridization, chromosome count

Abstrakt

Amomum Roxb. s.l. (černý kardamom) je složitý rod v čeledi zázvorovitých (Zingiberaceae) v podčeledi Alpinioideae, který podle některých vymezení zahrnuje i skupiny druhů považované za samostatné rody, jako je např. rod *Elettariopsis* Baker. Dosavadní fylogenetické výzkumy nedokázaly objasnit ani pozici rodu *Amomum* v podčeledi Alpinioideae, ani vztah mezi rodem *Amomum* a ostatními rody, jako je *Elettariopsis*. V této práci bylo *Amomum* podrobeno detailní morfologické analýze s použitím co největšího počtu vzorků, spolu s fylogenetickou analýzou. *Amomum* bylo nově vymezeno, bylo ustanoveno *Amomum* s.s. a tři nové rody, tři rody byly obnoveny a *Elettariopsis* byl včleněn do rodu *Amomum*. Mezitím bylo popsáno několik nových druhů a dva byly epitypifikovány.

S použitím sekvenování nové generace (next-generation sequencing; Hyb-Seq) byla získána dobře podpořená fylogeneze založená na jaderných genech, která prokázala v rodu existenci čtyř cladů (A, B, C, D); clade D, původní rod *Elettariopsis*, se dále dělí na tři subclady (D1 – D3). Fylogeneze založená na chloroplastové DNA tuto strukturu podpořila, ale doplňující fylogeneze založené na ribosomálním cistronu (rDNA) se od té založené na nukleárních genech odlišovaly; to poukazuje na potenciální nevhodnost často používaného markeru ITS při rekonstrukci hlubších fylogenetických nodů.

Velikost genomu je intenzivně zkoumaná vlastnost rostlin, která má zásadní vliv na jejich růst, adaptaci a speciaci. Jako taková může být rozhodujícím faktorem při diverzifikaci skupin a byla již úspěšně použita i v systematice rostlin, protože může nést fylogenetický signál. Velikost genomu tropických rostlin je méně prozkoumána, než v rostlinách mírného pásma. *Amomum*, jakožto tropický rod rozšířený napříč většinou indomalajské oblasti a rostoucí v různých typech prostředí, se ukázalo být dobrým modelem pro zkoumání velikosti genomu v tropických podmínkách. Bylo zjištěno, že se evoluce absolutní (2C) velikosti genomu v rodu *Amomum* shoduje s jeho fylogenezí; absolutní velikost genomu zde postupně stoupá od nejraněji divergujícího cladu A po nejvíce odvozený clade D (subclade D3) a její rozpětí je 4,5násobné. Zajímavostí je, že postupný růst velikosti genomu odpovídá biogeografickému vývoji rodu, který se rozšířil z původní oblasti jižní Číny a severní Indočíny (clady A, B a C) jihovýchodním směrem přes šíji Kra do Sundalandu (subclady D1 – D2) a poté zpět na sever (subclade D3). Zjištění počtu chromozomů vybraných jedinců vedlo k odhalení dvou tetraploidních druhů s počtem chromozomů 2n = 96. Tyto druhy byly prvními polyploidy objevenými v rodu *Amomum*. Druh *A. cinnamomeum* (subclade D3) měl největší 2C velikost genomu v rodu (15.66 pg), která je zároveň největší doposud zjištěnou velikostí genomu v řádu Zingiberales. Zbylé diploidní vzorky měly 2n = 48 chromozomů.

V rámci diploidů velikost genomu v rodu *Amomum* vykazovala 2,7násobné rozpětí, což bylo podnětem pro analýzu repeatomu v rodu (tj. kvantifikaci hlavních skupin repetitivních sekvencí v genomu). Vybraná podskupina druhů byla analyzována s použitím pipeline RepeatExplorer za účelem kvantifikace a porovnání jejich repetitivních sekvencí (repetic). Velikost genomu byla signifikantně korelována s obsahem repetic (repeatomu), který také nesl fylogenetický signál. Linie repetic ze superfamily *Ty1-Copia* (zejména SIRE) silně přispěly ke zvýšení velikosti

genomu v rodu *Amomum* a zároveň převládaly v jeho repeatomu. V clade D bylo zjištěno zmnožení většiny linií repetic. Další zkoumání odhalilo v *Amomum* dvě starobylé hybridizační události: hybridní původ cladu D, který mohl být příčinou zmnožení repetic v tomto cladu, a další hybridogenní skupiny druhů v tomto cladu. Fylogenetická síť založená na repeticích se shodovala s fylogenezí založenou na jaderných genech. Proporce repeatomu v rodu *Amomum* dosahovaly až 88 % a rod se tak umístil mezi jednoděložné rostliny s nejvyšší proporcí repetic.

Klíčová slova: *Amomum*, Zingiberaceae, velikost genomu, evoluce genomu, klasiikace, morfologie, repetitivní DNA, repeatom, fylogeneze, sekvenování nové generace, starobylá hybridizace, chromozomy

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1. General Chapters

INTRODUCTION

The family Zingiberaceae

Zingiberaceae (gingers) is a family of rhizomatous herbs (counting about 58 genera and over 1800 species) distributed throughout the world's tropics (Fig. 1), with the centre of their distribution in South-East Asia. They are plants of varying height, often with a pseudostem formed by leaf sheaths which can reach a length of several metres (some species of *Alpinia* Roxb. and *Etlingera* Giseke). They produce inflorescences, sometimes on tall stalks, with often brightly coloured bracts supporting cincinni of short-lasting flowers. Gingers are found both in lowlands and in mountainous areas, some inhabiting areas disturbed by human activity. Most grow terrestrially in the understory of tropical forests, with only few epiphytic species. Their life histories are adapted to local conditions and while in humid conditions they are evergreen, species in seasonal locations such as deciduous forests go dormant during the dry season, surviving as an underground rhizome (such as the most well-known *Zingiber officinale* Roscoe).

Fig. 1 Distribution of the Zingiberaceae (provided by the Angiosperm Phylogeny Website, www.mobot.org).

All members of the ginger family contain essential oils and are used both in their areas of origin (especially in Asia) and worldwide as spices, food, dyes, medicinal, ornamental and sometimes ritual plants. All parts of the plants are used, but rhizomes and fruits are often preferred as spices. The main and most famous spices include the above-mentioned *Zingiber officinale* (root ginger), *Curcuma longa* L. (turmeric), *Elettaria cardamomum* (L.) Maton (green cardamom) and *Amomum subulatum* Roxb. (black cardamom).

The family is further divided into several subfamilies: Siphonochiloideae, Tamijioideae, Zingiberoideae (mostly species with a period of dormancy, including *Zingiber officinale* and *Curcuma longa*) and Alpinioideae (evergreen species, including green cardamom, *Elettaria cardamomum* or galangal, *Alpinia galanga* (L.) Willd.). The most comprehensive phylogeny of Zingiberaceae so far, published in 2002 by Kress *et al.* (Fig. 2) raised many taxonomic questions and the polyphyletic character of the genus *Amomum* was one of them.

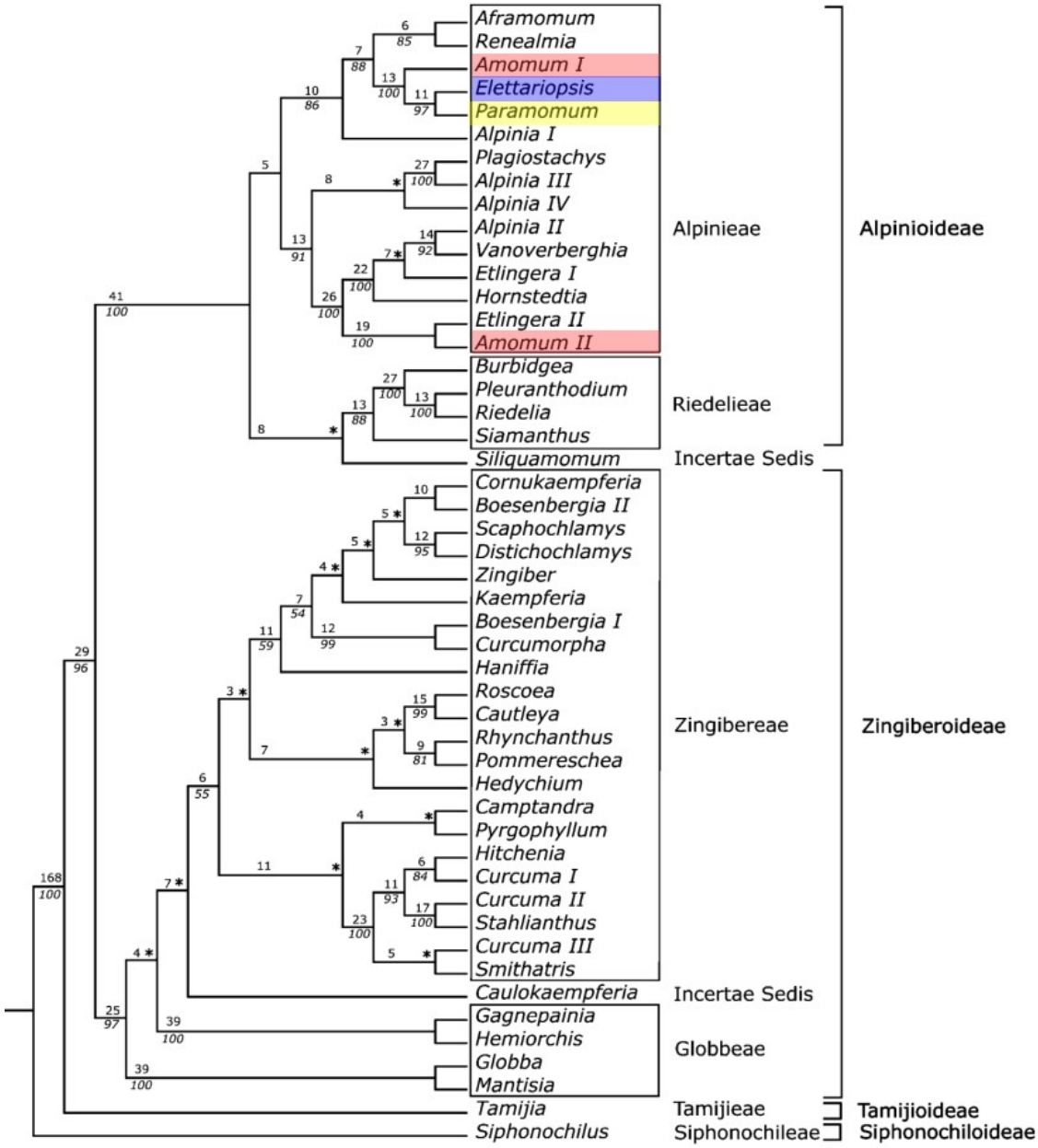

Fig. 2 Phylogeny of the family Zingiberaceae, adapted from Kress *et al.* 2002, showing the polyphyly of *Amomum* (highlighted in red) together with the closely related genera *Elettariopsis* Baker (blue) and the monotypic *Paramomum* S.Q.Tong (yellow). *Paramomum* was included in *Amomum* by T.L Wu (1997), but here it is supported as a separate genus. *Elettariopsis* was described by Kress as often indistinguishable from *Amomum* based on floral characteristics, but at the time of the study it was considered a separate genus. The sampling was, however, limited to 4 accessions of *Amomum* (one undetermined) and 3 *Elettariopsis* so the relationship of these two genera remained inconclusive.

Pitfalls of ginger taxonomy

Taxonomic work is often complicated in this group, as their main discriminatory characters are

found predominantly in the flowers, which are often difficult to obtain both in the field and in cultivation. The high water content of the plants, especially their inflorescences, does not lend itself well to drying for herbarium preservation, and the flowers, when available, lose most of their important characteristics in herbarium specimens. Furthermore, the flowers are quite ephemeral (most opening only for one day or night), which reduces the time frame for their collection and preservation. In other genera, important characters lie on underground parts such as rhizomes, which are equally difficult to preserve. In addition to the usual preservation of the plants, it is therefore necessary to provide detailed field notes and ideally a series of high-quality photographs including flower dissections, which is time-consuming and often difficult to achieve in field conditions.

These factors have always complicated taxonomic work and hindered the description of new species from living collections as well as from field specimens. Although modern molecular methods can aid in identification of taxa through DNA sequencing, studies of herbarium specimens remain a key part of taxonomic revisions and species determinations. Where sufficient protologues and materials are missing, as is the case of many older works, identification of plants is often unfeasible, and some species described in the early days of ginger taxonomy remain unidentified to this day.

The genus *Amomum* s.l.

Amomum Roxb. species are evergreen, rhizomatous plants with either loosely clasping distichous leaves or a pseudostem formed by leaf petioles. *Amomum* plants may grow in clumps or send individual pseudostems along a creeping rhizome. The inflorescences arise from the rhizome on a short peduncle and do not have an involucre of sterile bracts unlike e.g. *Etlingera* Giseke. *Amomum* flowers are trimerous, often white with a yellow stripe on the labellum which is sometimes flanked by red stripes. The single anther holds the style and has an anther crest of a varying shape. Fruits are fleshy hesperidia or capsules that may be smooth, grooved, winged or echinate. Like other representatives of the Zingiberaceae, *Amomum* (and *Elettariopsis*) plants contain various essential oils and often have a specific scent, which some have considered useful for their determination (Lim, 2003).

Like other gingers, *Amomum* is important not just as a part of tropical biodiversity but also for its species that are used as culinary spices and/or traditional medicinal plants. The type species *A. subulatum* Roxb., known as black cardamom or large cardamom, is cultivated mainly in Nepal and India (e.g. Ghanashyam Sharma *et al.*, 2016) and besides its long history as a spice and traditional medicine, modern studies revealed its antimicrobial and even anticarcinogenic properties (Ali *et al.*, 2023; Sharma *et al.*, 2023). The genus name *Amomum* originated from the Ancient Greek *ámōmon* (ἄμωμον; Liddell and Scott, 1940), a loanword from an unidentified Oriental language which also appears in Classical Syriac as ܗܡܡܐ (ḥəmāmā; Löw, 1881) hinting at the possible trade route of this spice and that it was already in use in ancient times. (However, it may have been a descriptor for the closely related green cardamom rather than other species currently recognized as *Amomum*; see below.)

Amomum s. l. is distributed from Sri Lanka and India over SE Asia to New Guinea, the Bismarck

Archipelago and as far as Australia (Mabberley, 2008). Due to its importance in culinary and medicinal use, it is possible that some of the most peripheral distribution is of anthropogenic origin.

Fig. 3 *Amomum subulatum* Roxb., a drawing by R.B. Peake in Roxburgh's *Plants of the coast of Coromandel* (Roxburgh *et al.*, 1819). (http://www.plantillustrations.org/illustration.php?id_illustration=61488)

A chaotic genus within the ginger family

The name *Amomum* was first established by Linnaeus (Linné and Dassow, 1747); however, the five species he included were later transferred to different genera and *Amomum* Roxb. was conserved by Burtt and Smith (1968) from the description of Roxburgh in *Flora Indica* (Roxburgh and Wallich, 1820), which was based on its flower and fruit morphology. The type species, *Amomum subulatum* Roxb. (black cardamom or large cardamom; Fig. 3) was first described and illustrated by Roxburgh in *Plants of the coast of Coromandel* (Roxburgh *et al.*, 1819) and later established as the conserved type (McVaugh, 1970).

Since then, *Amomum* s.l. had undergone numerous taxonomic rearrangements and has been divided into between two and five sub-sections by various authors (Baker, 1892; Hooker, 1894; Schumann, 1904; Loesener, 1930; Smith, 1985), sometimes based only on a collections from limited area of occurrence (Holttum, 1950; Smith, 1985; Sakai and Nagamasu, 1998).

For a long time, *Amomum*, similarly as the other similarly large genus in the Alpinioideae, *Alpinia*, served as a large „trash bin" genus for various species of uncertain placement, which were later transferred to other Zingiberaceae genera (such as e. g. *Elettaria cardamomum* or *Zingiber mioga* (Thunb.) Roscoe). The last comprehensive revision of *Amomum* s.l. was written by Schumann (1904). Phylogenetic studies investigating the Alpiniodeae and including *Amomum* s.l. had been carried out in the past by Kress *et al.* (2002), who placed *Amomum* s.l. into the tribe Alpinieae within the subfamily Alpinioideae and identified it as polyphyletic, since two distantly related clades of *Amomum* s.l. species (*Amomum* I and *Amomum* II in Fig. 2) were resolved based on ITS and matK phylogenetic trees. This was later confirmed by Xia *et al.* (2004), Pedersen (2004) and most recently by Droop (2012). However, since the sampling of these phylogenetic studies was often limited and the type species *A. subulatum* was not included, they could not provide a taxonomic solution for this complicated genus.

One of the long-term issues was the relationship of the genera *Amomum* s.l. and *Elettariopsis* Baker. Their morphology was very similar and in the phylogenetic study by Kress *et al.* (2002) one of the *Amomum* s.l. clades (*Amomum* I) was resolved as a paraphyletic with *Elettariopsis* and *Paramomum* (Fig. 2). The type species of neither *Elettariopsis* nor *Amomum* were included in phylogenetic analyses (Kress et al., 2002; Xia et al., 2004; Pedersen et al. 2004; Droop, 2012), although this would have helped to elucidate their taxonomy and nomenclature. *Elettariopsis* was originally distinguished from *Amomum* s.l. by its loosely clasping leafy shoots and open bracteoles (Baker, 1892), but this distinction was not absolute (Holttum, 1950; Kam, 1982). Xia *et al.* (2004) used fruit and anther characteristics in their study, where *Elettariopsis* was nested within their *Amomum maximum* clade with weak bootstrap support. Later, Droop (2012) defined *Elettariopsis* as distinguished from *Amomum* s.l. by "a tufted pseudostem, open bracteoles and a large, petaloid anther crest", noting that these characters, while found in *Amomum* s.l. species individually, were never found there in combination. However, she also noted that while *Elettariopsis* was, at the moment, recognized as a genus (based on its morphology), further sampling was needed to establish whether it would stand alone or become part of Xia's *A. maximum* clade (*Amomum* I clade in Kress, 2002; Droop, 2012). She suggested fruit morphology as a useful character for the delimitation of *Amomum* clades. Given its problematic delimitation and *Amomum*'s paraphyly, *Elettariopsis* was one of the last unrevised genera of the Alpinioideae.

Kress et al. (2002) also recovered a paraphyletic "Amomum II" clade (Fig. 2), which formed a group with accessions of *Alpinia*, *Etlingera*, *Vanoverberghia* Merr., *Hornstedtia* Retz. and *Plagiostachys* Ridl. Later studies investigating *Alpinia* and *Amomum* s.l. (Kress, 2005, 2007; Droop, 2012) eventually recovered up to nine *Amomum* clades in total, dispersed across the Alpinieae and intermixed with clades of other genera.

Despite having been included into *Amomum* s.l. in 1997 on the basis of morphology (Wu, 1997), the monotypic *Paramomum* was supported as a separate genus sister to *Elettariopsis* in the phylogenetic analyses of the Alpinioideae (Kress *et al.*, 2002; Pedersen, 2004; Xia *et al.*, 2004;

Droop, 2012). However, Lamxay and Newman (2012) considered it as the species *Amomum petaloideum*, and it is currently recognized as such (POWO, 2023).

As evidenced by the above, *Amomum* s.l. was in dire need of taxonomic treatment, which would not only elucidate the delimitation of this genus and resolve its paraphyly but would also clarify the position of *Elettariopsis* and reveal the status of other closely related genera. This would facilitate the understanding of relationships within Alpinioideae and could perhaps aid in the future treatment of the other complex genus, *Alpinia*.

Genome size and repeatome: factors correlating with Amomum's ecology

For decades, genome size (GS; nuclear DNA content) has been studied in the Zingiberaceae as a useful characteristic for taxonomy and study of evolutionary history (Leong-Škorničková et al., 2007; Záveská et al., 2011, 2023). While in some ginger groups changes in GS are well correlated with changes in ploidy levels (Leong-Škorničková *et al.*, 2007), in other related groups the variation in GS is influenced by other mechanisms, for example by the amplification of repetitive elements (repeats). These play an important role in the adaptation and evolution of plants (Jansz, 2019; Kumar and Mohapatra, 2021) and also in the diversification of new lineages (Gaiero *et al.*, 2019; Hloušková *et al.*, 2019). Repeat dynamics in the Zingiberaceae had not been investigated before, although they had been studied in the closely related family Musaceae (Novák *et al.*, 2014).

Many studies focused on temperate species have shown correlation of GS with ecology (e.g. habitat seasonality, latitude or temperature) and other factors influencing plant growth (Knight *et al.*, 2005; Pellicer *et al.*, 2018; Souza *et al.*, 2019; Cacho *et al.*, 2021), but it is not known what such correlations would be in tropical plant groups. To understand genome evolution and its correspondence to ecology in the tropics, GS variation, occurrence of polyploidy as well as dynamics of repetitive elements should be investigated. In plants, genome expansion can contribute to important evolutionary changes (e.g. Colnaghi *et al.*, 2020) and offers many advantages, such as morphological innovations and mutational robustness which may lead to speciation and prevent extinction (Crow and Wagner, 2006; Qiao *et al.*, 2022); however, genome size seems to be limited to a maximum of 150 Gb (Hidalgo *et al.*, 2017). Plants with larger genomes are reported to be less resilient in unstable environmental conditions due to their increased cell size and its consequences, such as slower growth or less effective water management (e.g. Mueller, 2015; Roddy *et al.*, 2020; Veselý *et al.*, 2020), and are therefore often selected against (Knight *et al.*, 2005). These constraints influence Zingiberaceae as well, although some seem to have developed a strategy where they circumvent the selection against large genomes by growing in favourable conditions. *Amomum* is one such genus, whose members with large GS (in fact, some of the largest GS in the Zingiberales) grow in shaded, moist areas in a seasonal environment, which allows them to effectively avoid the need to adapt to seasonal drought conditions (Záveská et al., 2023).

AIMS

As evidenced above, *Amomum* s.l. needed a taxonomic treatment, since its current status

complicated further research within the subfamily. A phylogeny based on more than a few phylogenetic markers was needed, with a broad sampling covering all known lineages.

Amomum also appeared as a good model group for studies on genome size evolution and repetitive element dynamics in the tropics, thanks to its i) wide geographic distribution spanning the seasonal as well as evergreen regions of the tropics and ii) highest infrageneric variation of GS within the entire family Zingiberaceae.

Based on the above, the following goals were set for this thesis:

i) to resolve the phylogenetic relationships between all lineages and genera belonging in the previously described *Amomum* s.l. within the subfamily Alpinioideae and suggest a new taxonomical concept of the group including identification of determining morphological characters

- Can ITS and *mat*K sequences be used to elucidate the phylogeny?
- What is the delimitation of *Amomum* s.s. based on molecular data?
- Can the remaining monophyletic lineages, originally belonging in *Amomum* s.l., be defined and named as new genera?
- Which morphological characters can be used together with the phylogeny for a description and recognition of these genera?

ii) to provide a robust phylogeny and an overview of biogeography of the correctly determined genus *Amomum* s.s.

- Can a phylogeny based on several hundred nuclear genes, obtained by next generation sequencing, provide a robust explanation of relationships in the group?
- Is there a difference between the nuclear gene, cpDNA and rDNA phylogenies?
- What are the differences between traditional and next-generation phylogenies?
- How can its biogeography be interpreted in the context of phylogeny?

iii) to examine the relationship of genome size and repeatome within *Amomum* s.s..

- Is evolution of absolute genome size explained by phylogeny?
- Are there any polyploid species?
- Is genome size variation caused by repeat dynamics?
- How do repeat composition and dynamics reflect phylogeny?

METHODS

Plant material

For this study a maximum possible range of sampling was attempted, including both *Amomum* and *Elettariopsis*, covering as much of their distribution areas as possible (from the Sub-

Himalayan region and southern China through Borneo and Southeast Asia to New Guinea and Queensland). Dried and spirit herbarium material, as well as living accessions from collections and the field, were used. To determine the specimens, identification keys, protologues and type material were used (for details see Paper I). Living specimens cultivated in various botanical institutions were used for DNA extraction, genome size measurement and root tip collection for chromosome counting. For details, see Papers I–IV. Paper I included the highest number of *Amomum* s.l. accessions, and Paper III the highest number of *Elettariopsis* accessions used in any phylogenetic analyses up to date. Due to restrictive costs, both the HybSeq analysis in Paper III and the repeatome analysis in Paper IV were carried out on limited subsets of *Amomum* accessions, which however spanned all major clades of the genus.

Morphology

As previously mentioned, most determining morphological characters in Zingiberaceae are found in flowers and fruits, which has its drawbacks also when working with *Amomum*. Like in other tropical groups, new species of *Amomum* had been (and are still) discovered continuously (e.g. *A. nagamiense* V.P.Thomas & M.Sabu, Thomas *et al.*, 2019), often remaining undescribed due to the difficulties of their cultivation and frequent reluctance to flower even if successfully cultivated. Collecting material also proved a challenge, as newly discovered localities in the wild as well as species habitat were often lost due to land management, while localities described in old protologues were often unclear and encompassed large areas such as Tonkin or Cochinchina (e.g. Gagnepain, 1908).

In Paper I, morphological characters were observed in *Amomum* to examine their correlation with phylogeny and find determining characters for groups defined by molecular analyses. Characters present on flowers and fruits were used, combined with other characters such as the habit or petiole presence. Dried vouchers, spirit specimens and detailed high-resolution photographs (including flower dissections) were also used for species with unavailable living specimens or in addition to them. Descriptions published in protologues, floras and monographs also served as a source of morphological characters.

The anther crest and fruit type have been successfully used as a determining character within the Zingiberaceae (Záveská *et al.*, 2012; Sangvirotjanapat *et al.*, 2022) and had been part of the morphological determination in *Amomum* s.l. since Schumann's time. When present, the anther crest in *Amomum* s.l. could be distinguished as either petaloid or non-petaloid and its shape varied from entire to variously trilobed (Droop, 2012). The fruit has been described by Lamxay and Newman (2012) as "either a smooth, fleshy berry or a dehiscent or indehiscent capsule which may be prickly, winged, ridged, or lobed, globose to ovoid, often with a persistent bract and calyx; the prickles, if present, are simple or branched, and the wings straight or wavy". Indeed, anther crest and fruit type proved to be the most informative characters in Paper I, and their mapping onto a Bayesian phylogeny showed that each of the groups determined by molecular analyses had its own distinct type/combination of these characters.

When designating the epitypes of *A. trilobum* and *A. unifolium* (Paper II), living collections that regularly flower in cultivation were specifically chosen to facilitate the clarity of determination.

Flow cytometry

Flow cytometry following the protocol of Doležel *et al.* (2007) was used to determine the absolute (2C) genome size (henceforth referred to as GS) of most *Amomum* accessions used in these studies. *Bellis perennis* was used as a standard. The resulting values were used to observe the dynamics of GS in *Amomum*, where this had not been examined before, and its relationship to phylogeny (Paper III). An observed wide range of GS values in diploid *Amomum* accessions was one of the factors that led to the examination of the repeatome in Paper IV.

Comparative phylogenetic methods

Briefly, the absolute GS (2C-values, Paper III, IV) and monoploid GS (1Cx-values, Paper IV) were mapped onto a robust nuclear-gene phylogeny using the *phytools* package implemented in R (Revell, 2012) to model the evolution of GS in *Amomum*. Additionally, the scaling parameters *lambda*, *kappa* and *delta* (Pagel, 1997, 1999) were estimated in Paper III to determine whether GS was associated with phylogeny (*lambda*), whether its evolution was punctual or gradual (*kappa*) and how fast it evolved (*delta*).

Chromosome counting

According to most studies in the Alpinioideae, chromosome count in the subfamily was stable at 2n = 48 (Mahanty, 1970; Beltran and Kam., 1984; Eksomtramage *et al.*, 2002). In order to identify polyploid specimens and examine the link between phylogeny, absolute GS, and chromosome number in *Amomum*, chromosome counting was carried out as part of the analyses in Paper III. For this purpose, root tips of a subsampling representing the main clades of the genus were used; however, although the sampling was limited, this may contribute to a deeper scientific understanding of the family as chromosome counts in the Zingiberaceae are still rather rare. Chromosomes were counted from actively growing root tips collected mainly from cultivated living specimens. For details on root tips treatment and chromosome slide preparation see Paper III.

Sequence data and its processing

DNA was extracted from collected plant material in order to obtain ITS, cpDNA, rDNA, Hyb-Seq, and genome skimming data. Sequence data of several accessions from previous studies was downloaded from NCBI GenBank or the Sequence Read Archive (SRA).

After assembling the largest number of *Amomum* samples so far (including the highest number of accessions belonging originally to *Elettariopsis*, and both type species), next-generation sequencing (NGS) target-enriched data was prepared using custom Zingiberaceae probes (design described in Carlsen *et al.*, 2018). The Hyb-Seq method (Weitemier et al., 2014; Schmickl et al., 2016) was used. The resulting sequence reads were processed in the HybPhyloMaker pipeline (Fér and Schmickl, 2018) to construct a phylogeny based on 449 nuclear genes. For comparison, a phylogeny based on the traditionally used ITS marker was also constructed, with the benefit of much larger sampling than in the case of Hyb-Seq due to the less prohibitive cost of this analysis.

Finally, the full chloroplast genome and the rDNA cistron, obtained as a part of the Hyb-Seq data, were used to construct two more phylogenies.

Since cytonuclear incongruence was observed in *Amomum* in the nuclear gene and chloroplast DNA analyses, a hypothesis arose that interspecific hybridization may have occurred in the genus. Hybridization events had been previously documented in the Zingiberaceae (Leong-Škorničková et al., 2007; Lim, 2008; Záveská et al., 2016; Skopalíková et al., 2023) and the great range of absolute GS in diploid *Amomum* species could also point to a ancient hybridization event, as hybridization is known to influence genome size and repeat dynamics (e.g. Heyduk et al., 2021; Wei et al., 2021). To investigate whether this was the case, a species network reconstruction was carried out in Paper IV using gene trees obtained by Hyb-Seq and the maximum pseudo-likelihood method in PhyloNet 3.6.1 (Than et al., 2008; Skopalíková et al., 2023). Two analyses, both allowing for two reticulations, were conducted to examine possible hybridization events in the whole genus and subsequently also in clade D (former *Elettariopsis*).

For the repeatome analyses in Paper IV, unenriched genome skimming (low-coverage sequencing) data was procured for a subset of species which included representatives of the main clades of *Amomum* and two outgroup species. Sequence reads obtained from this genome skimming were analysed using the RepeatExplorer pipeline (Novák *et al.*, 2020; see below).

Sequence data from Papers I, III and IV was uploaded to NCBI GenBank and SRA.

Repetitive elements (repeats)

Repeats, originally considered "ballast" DNA (Ohno, 1972), can occupy as much as 91 % of a plant's genome (*Allium sativum*; Sun *et al.*, 2020). Since their first discovery by Barbara McClintock in 1985, they have been found to play various roles in plant adaptation and evolution. Repeat dynamics (Pulido and Casacuberta, 2023) can significantly influence a plant's genome size (GS) and thus its life history. Selection favours plants with smaller genomes, as proposed by the large genome constraint hypothesis (Knight *et al.*, 2005). Repeats are also known to play a role in plant diversification or changing of life histories (Harkess *et al.*, 2016; Gaiero *et al.*, 2019; Hloušková *et al.*, 2019).

Furthermore, repeats can affect gene expression (Garrido-Ramos, 2012; Bennetzen and Wang, 2014) and thanks to their fast evolutionary rates, some may even exonify to become new genes (e.g. Mehrotra and Goyal, 2014; Kuo *et al.*, 2021). In the genome they are regulated mostly by DNA methylation and their amplifications and reductions are often driven by environmental factors (Jansz, 2019; Kumar and Mohapatra, 2021; Schley *et al.*, 2022), although not much research specifying those factors is currently available.

Repeats are classified into several groups based on their composition and mechanisms of reproduction (Wicker *et al.*, 2007). Besides a group of tandem repeats (including satellite DNA, tandem paralogues and rDNA sequences), a larger group of dispersed repeats includes the most abundant transposable elements (transposons) created by transposition, or "jumping" between locations. Transposons are further divided into Class I (retrotransposons, containing LTR-retrotransposons, autonomous LINEs and non-autonomous SINEs) and Class II (DNA transposons) (Wicker *et al.*, 2007; Richard *et al.*, 2008).

Neumann *et al.* (2019) established the newest classification of plant repeats to date (used in Paper IV – Table 1), as well as a retrotransposon protein domain database REXdb, which is implemented in the RepeatExplorer pipeline (Novák *et al.*, 2020) that was used in Paper IV.

The repeatome of *Amomum* was examined in Paper IV to determine whether it played a role in its genome size evolution, considering the wide range of *Amomum*'s diploid genome sizes (2.7-fold). To assess the repeatome content, a graph-based clustering method (RepeatExplorer; Novák *et al.*, 2020) was used to classify and quantify the repetitive elements in the genomes of selected species representing the four main clades. The RepeatExplorer pipeline clusters repetitive elements based on read similarity, where individual sequence reads are represented as vertices in a graph (Novák *et al.*, 2020). The clusters (represented as diagrams) are sorted by size and classified by a similarity search against custom-made databases such as REXdb (Neumann *et al.*, 2019); then they are grouped into superclusters based on their prevailing repeat type. RepeatExplorer provides a summary of read quantities in each cluster, enabling a quantification of repeat types in the specified genome.

Furthermore, a comparative analysis was conducted in RepeatExplorer, showing a visual representation of clusters of various repeat types present in *Amomum* and outgroup (*Renealmia* L.f. and *Aframomum* K.Schum.) species and thus facilitating a comparison of repeatomes in the group.

Table 1. A classification of the main types of repeats which appear in Paper IV, based on REXdb (RepeatExplorer output).

Class	Order	Superfamily		Lineage
Class I	LTR-retrotransposons	*Ty1-Copia*		Ale
				Angela
				Ikeros
				Ivana
				SIRE
				TAR
				Tork
		Ty3-Gypsy	Chromovirus	Athila
				CRM
				Retand
				Tekay
	Pararetrovirus			
	LINE			
Class II	Subclass I	TIR		EnSpm/CACTA
				hAT
				MuDR-Mutator
Satellite (tandem repeats)				
rDNA				

In addition, genome-skimming data of a subset of *Amomum* accessions was analyzed using TAREAN (Tandem Repeat Analyzer; Novák et al., 2017) to observe the structure of 5S rDNA graphs. The number of loops in these graphs can indicate hybridization in plant taxa, as previously described by Garcia *et al.* (2020); non-hybridogenous species tend to show one-looped graphs while species of hybrid origin display more loops obtained from their parent species.

RESULTS AND DISCUSSION

From Chaos to Order: Recircumscription and new species in *Amomum*

Though used and well-known locally, many species of *Amomum* s.l. and *Elettariopsis* had been found only on a small area of land and were often endangered by habitat loss by logging or similar activities, as was often documented in their protologues (e.g. Lamxay and Newman, 2012). This made the need for a taxonomic study more urgent - an improved classification could facilitate conservation efforts to preserve those species for the future.

A comprehensive study of *Amomum* s.l. based on both molecular and morphological characters was conducted in Paper I. From a total of 15 examined morphological characters found on the plants, the two most informative, anther crest and fruit type, were chosen and plotted on a Bayesian phylogeny based on nrITS (internal transcribed spacer) and *mat*K (maturase K) markers, which were found to be variable and were widely used in phylogenetic studies on the Zingiberaceae, e.g. by Kress *et al.* (2002, 2005, 2007), Pedersen *et al.* (2004) Xia *et al.* (2004). The study encompassed 293 accessions of various genera, including 105 accessions of *Amomum* s.l. and 188 of other members of all known lineages within Alpinioideae. Furthermore, a distribution map of the recovered clades was prepared using the type locations of all included species.

This phylogeny recovered nine *Amomum* s.l. clades in total, three of them previously described by Xia *et al.* (2004). The type species *A. subulatum*, as well as *Elettariopsis curtisii*, the type of *Elettariopsis*, were both contained in Clade I together with other accessions of *Amomum* s.l. and *Elettariopsis*. Therefore, *Amomum* clade I was established as *Amomum* s.s. including *Elettariopsis*, three genera were resurrected (*Conamomum*, *Meistera* and *Wurfbainia*) and other original *Amomum* s.l. lineages were circumscribed as new genera *Epiamomum*, *Lanxangia* and *Sunamomum* (Fig. 4). Twenty *Amomum* species, for which the materials were insufficient for their correct placement, have remained in the genus as *incertae sedis* and will require additional reclassification in the future. (For more detail on *Amomum* recircumscription, consult Paper I.)

Members of *Amomum* s.s. have predominantly fan-shaped anther crests and usually winged fruits, although angled and grooved fruits are also present. Its distribution encompasses much of the distribution of the previous polyphyletic genus, with the centre of diversity in northeast India and Indochina and extending as far as Sundaland but no further than Wallace's Line (Fig. 5). Some species are found in New Guinea and Queensland, but these are hypothesised to have arrived there via anthropogenic transport due to their culinary or medicinal utility.

During the process of rercircumscription of *Amomum*, new undescribed species, well delimited by molecular and morphological characters, were recognized. Four of these (*A. cinnamomeum*, *A. corrugatum*, *A. lutescens* and *A. miriflorum*) were newly described after the recircumscription. All

these species were found in southern and central Vietnam and belonged to the former genus *Elettariopsis*. Furthermore, a need arose to epitypify previously known species *A. trilobum* and *A. unifolium*, whose types were described by Gagnepain in 1904 with unclear collection localities (*A. trilobum* being located in French Indochina and *A. unifolium* in Cochinchina or Tonkin by Gagnepain). Both epitypes were collected in southern Vietnam (Đồng Nai Province) and their living specimens are cultivated in Royal Botanic Garden Edinburgh to this date. This work (Paper II) contributed to better species delimitation, which brought clarification for further treatments of *Amomum*.

Further papers of the thesis (II to IV) focus only on the newly delimited *Amomum* s.s.

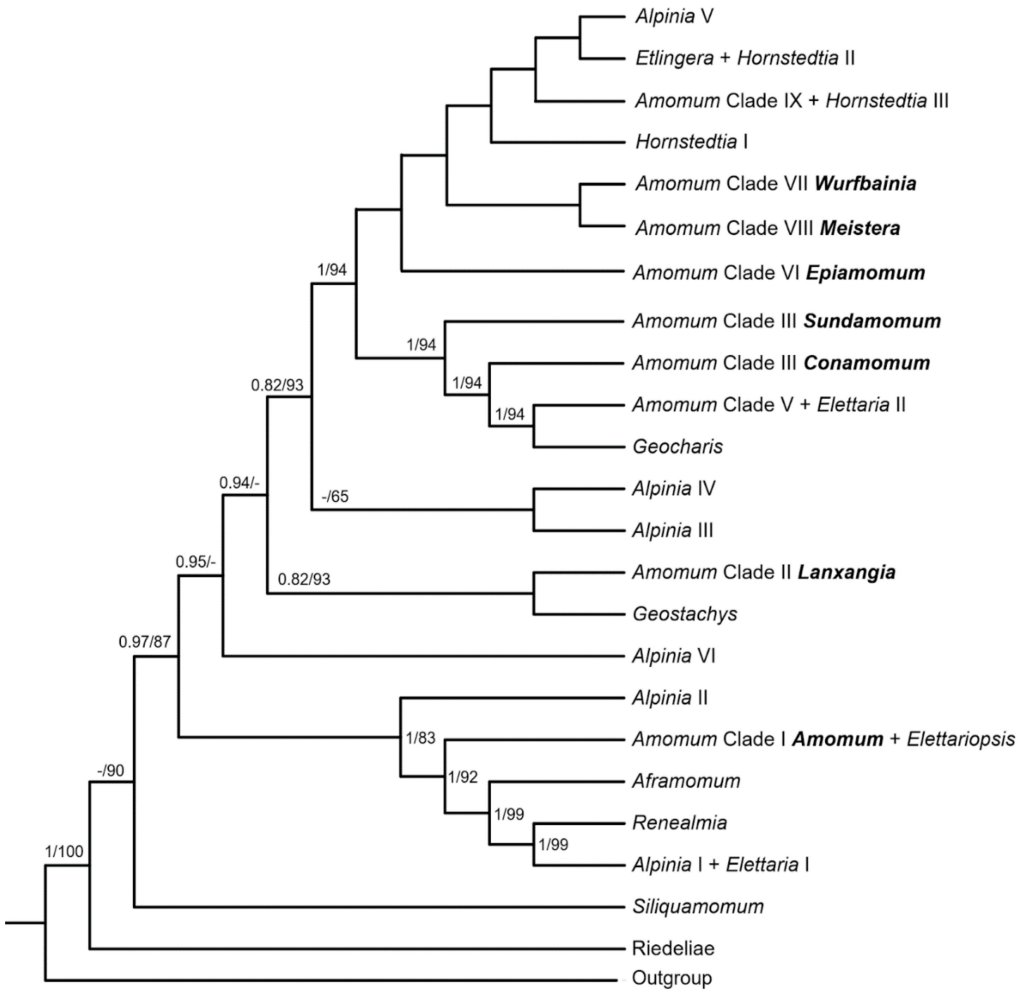

Fig. 4 The newly recircumscribed *Amomum* s.s. and new genera within the Alpinieae (adapted from Paper I, tree visualised in Interactive Tree of Life, Letunic and Bork 2021).

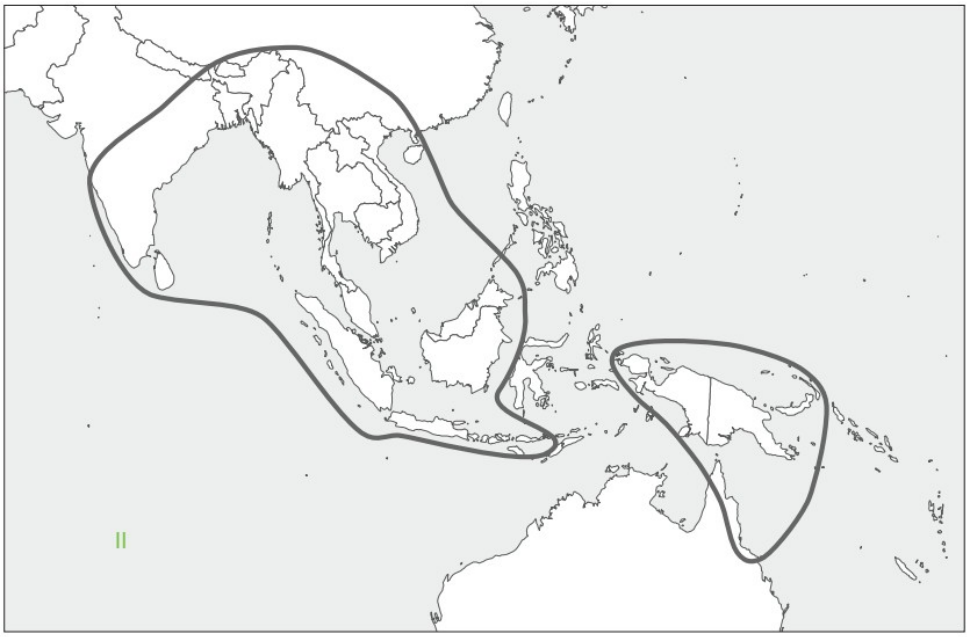

Fig. 5 The distribution of *Amomum* s.s. (adapted from Paper I).

Phylogeny and genome size in *Amomum* s.s.

After the establishment of *Amomum* s.s., a more robust phylogeny was needed. Previous infrageneric phylogenies in the Zingiberaceae were based only on several markers and had failed to reconstruct the internal relationships with adequate support (Ngamriabsakul, et al., 2003; Williams et al., 2004; Záveská et al., 2011). Recently developed next-generation sequences (NGS) methods enabled the use of several hundred genes to reconstruct a much more robust phylogeny.

The topologies of four phylogenies based on a) 449 nuclear genes, b) cpDNA, c) rDNA and d) ITS were compared. While all of them recovered four clades (A, B, C, and D) of identical species composition, the relationships between these clades differed between analyses. The ITS- and rDNA-based phylogenies differed most from the most robust nuclear gene phylogeny, indicating that analyses based solely on rDNA markers may retrieve well-supported, but misleading results in the deeper nodes due to the differing evolutionary rate and concerted evolution of these markers' multiple copies.

A mapping of absolute (2C) genome size on the nuclear gene phylogeny showed that absolute genome size increased throughout *Amomum* from the most basal clade A to the most derived D3. The highest absolute genome size (2C = 15.66 pg) was found in an accession of *A. cinnamomeum* within clade D3, which is the largest known 2C genome size in Zingiberales to date. Two tetraploids (*A. cinnamomeum* and *A*. aff. *biphyllum*; 2n = 96 chromosomes) were found; they are among the first polyploids to be found in the subfamily Alpinioideae. However, polyploidy studies in this subfamily are still scarce and other polyploid taxa may be eventually discovered in the future. The overall 2C GS range in *Amomum* was quite large: 4.5-fold including tetraploids and 2.7-fold in diploid accessions only. Based on the knowledge that such differences in diploid GS are often caused by repetitive element dynamics, these findings provided an impetus for the investigation of *Amomum*'s repeatome in Paper IV.

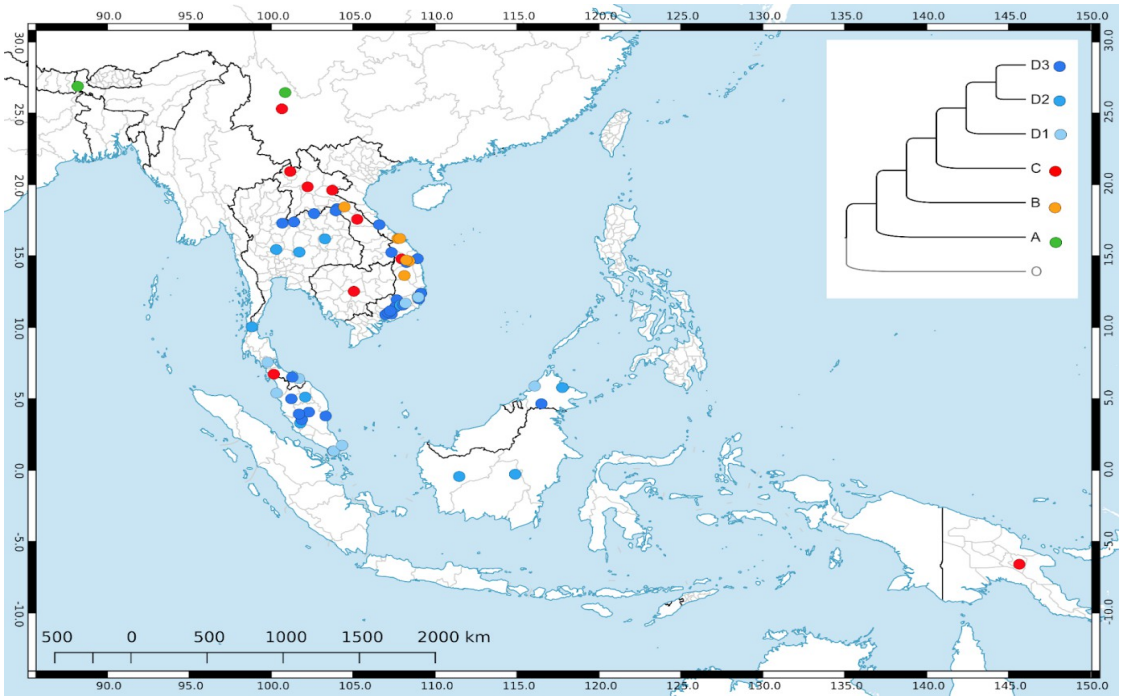

Fig. 6 Geographical distribution of *Amomum* accessions used in Paper III. The four main *Amomum* clades are delimited by circle colours (A – green, B – orange, C – red, D – blue). Nuclear gene phylogeny in the upper right corner shows the relationship between clades and the corresponding circle colours.

The visualisation of *Amomum* accessions on a map showed a trend where clades A–C with lower GS stayed above the Isthmus of Kra and had limited areas of dispersal while Clade D, especially subclade D3, seemed to have dispersed further south as far as Borneo, and then back north above the Isthmus of Kra (Fig. 6). This return to the north was surprising, considering that few plant species had migrated back north over the Isthmus of Kra in the past; however, it was not unfeasible, as the dispersal could take place through Sundaland, which was exposed in the Pleistocene. Older models suggested that the whole area was covered by a dipterocarp rainforest (Cannon et al., 2009; Raes et al., 2014), but newer studies conclude that a savannah corridor and a strip of rainforest were present (Bird et al., 2005; Wurster et al., 2019; Cheng et al., 2023), which would facilitate *Amomum*'s dispersal north. The exact mechanism of dispersal in *Amomum* is unclear. Its fleshy, basally placed fruits are presumed to be dispersed by mammals (Howe and Smallwood, 1982) but dispersal in this group is little studied (García-Robledo and Kuprewicz, 2009; Zou et al., 2016) and considering the creeping habit of some *Amomum* species and their reluctance to set flowers, it is possible that the dispersal may have at least partly occurred via vegetative reproduction (spreading by rhizomes).

Genome size in *Amomum* evolved in congruence with its phylogeny, which was confirmed by an estimation of Pagel's scaling parameters. These values suggested that the GS in *Amomum* evolved gradually from smaller to larger throughout the genus from clade A to clade D3. This evolution was possibly connected to the dispersal of the genus to the more stable conditions of Sundaland; upon their return north, some clade D3 species may have found a way to keep their large genomes by settling in shady, humid niches where the environmental pressures were not as high (Záveská et al., 2023).

In clade D, GS dynamics also reflected its pattern of dispersal. Clades D1 and D2 with lower GS stayed below the Isthmus of Kra (in an area of evergreen rainforest), while clade D3 with largest genome sizes was also dispersed north of the Isthmus (dry dipterocarp forests). This was incongruent with the large genome constraint hypothesis (Knight et al., 2005) but in agreement with the work of Záveská *et al.* (2023), who found that in Alpinioideae, plants find a way to avoid this constraint by growing in suitable niches within less suitable environments. Indeed, it was impossible to find a clear trend in *Amomum* accessions, as some plants with the largest genomes (including the tetraploid *A. cinnamomeum* from clade D3) were found growing in dry conditions, while others with smaller genomes were found in evergreen rainforest (such as *A. curtisii* from clade D1).

The repeatome of *Amomum* s.s.

A quantitative and comparative repeatome analysis showed that the composition of *Amomum*'s repeatome differed greatly from that of the outgroup, but it was relatively constant within the genus; however, the amounts of repeat sub-lineages (represented as read clusters in the analysis) differed across the group. In clade D, many of these sub-lineages were significantly amplified when compared to clades A-C and new unidentified LTR sub-lineages appeared in this clade, supporting its phylogenetic delimitation. Repeat composition within this clade was less varied, but it seemed that the amounts of repeat sub-lineages increased further from clade D1 to D3 (D2 representatives were unfortunately absent from the analysis). In some species, new types of LTR and other repeats seemed to contribute to their large GS, suggesting a possible development of new repeat (sub-)lineages within *Amomum*, likely from existing ones (as suggested in the review of Zattera and Bruschi, 2022). Similarly as in the related family Musaceae (Novák et al., 2014), *Ty1-Copia* repeats, particularly the SIRE lineage, also formed the majority of the repeatome in *Amomum*. This superfamily was shown to be prevalent in the whole ginger family.

The overall repeat content carried phylogenetic signal in the genus, but interestingly, further investigation revealed opposing phylogenetic signals in various clusters (sub-lineages) within repeat families, revealing that rather than at lineage level, repeat dynamics in *Amomum* occurred at the level of sub-lineages. This was prominent especially in the abundant SIRE lineage of the Ty1-Copia superfamily. Repeatome analyses on such a detailed level are rare in plants (most studies limiting themselves to quantifying the main superfamilies or families), but Suguiyama *et al.* (2019) described such dynamics in *Setaria italica*, showing sub-lineage admixture in some species. These findings may offer a new way of looking at transposable repeats and their evolution in plant genomes.

Since the previous study (Paper III) also discovered cyto-nuclear discordance in *Amomum*, possible ancient hybridization events were hypothesised. A PhyloNet analysis confirmed this hypothesis as it revealed two hybridization events within the genus: one in Clade D of Amomum (formerly classified as the genus *Elettariopsis*) and one in another group of hybridogenous species within this clade. An additional analysis of satellites (tandem repeats), which were found in low numbers in the RepeatExplorer analysis, showed that very low amounts of satellites are indeed present in *Amomum* compared to some other plant groups (e.g. in orchids; Chumová et al., 2021).

This analysis also procured 5S rDNA clusters, whose structure was examined to find out possible hybridization (as described in Garcia *et al.*, 2020). The increasing complexity (number of loops; Fig. 7) of 5S rDNA cluster graphs from clade A to clade C confirmed the hybrid origin of clade D and the existence of a hybrid group within this clade, revealing also the allotetraploidy of *A. cinnamomeum* and the possible hybridogenous origin of *A. miriflorum*.

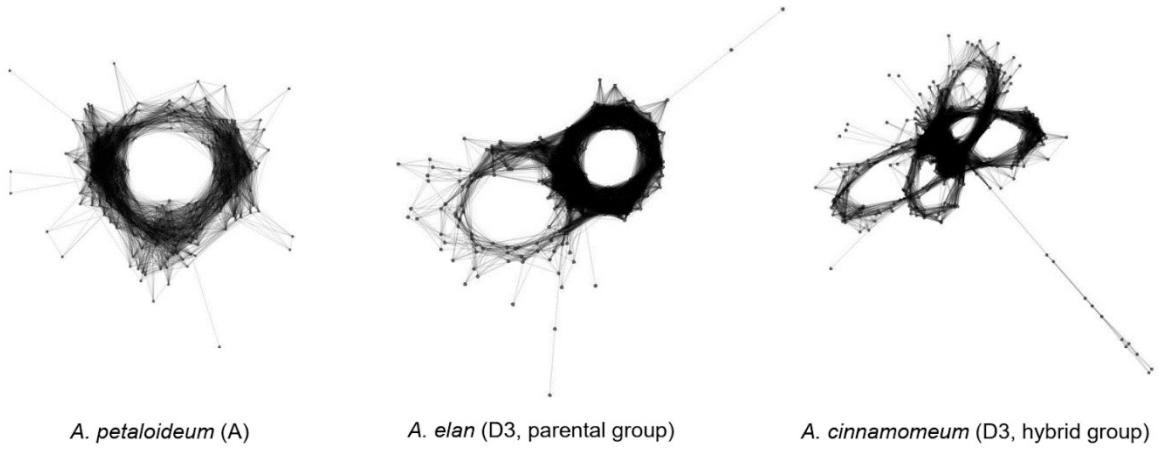

Fig. 7 An illustration of 5S rDNA cluster complexity in *Amomum*. One-looped cluster of *A. petaloideum* of the basal clade A, two loops in *A. elan* of the hybridogenous clade D, and four loops in the allotetraploid *A. cinnamomeum* of the hybrid group within clade D.

One of the aims of Paper IV was to examine whether an amplification of repetitive elements (repeats) was what led to a 2.7-fold GS variation at a constant chromosome number. Indeed, a significant correlation was observed between the total repeat content and GS in *Amomum*, as well as between GS and the content of the majority of repeat families, particularly those of the *Ty1-Copia* superfamily. Repeat dynamics, and subsequently GS, in *Amomum* seemed to have been influenced by the ancient hybridization event. As opposed to other noted examples in different plant species, where a burst of a specific repeat lineage caused a GS increase after hybridization (Renny-Byfield et al., 2013; Giraud et al., 2021; Kuo et al., 2021), multiple repeat lineages had apparently amplified in clade D of *Amomum* following hybridization. A similar amplification of multiple lineages was observed also in the closely related family Musaceae (Novák et al., 2014). An alternative explanation of this amplification could be the southward migration of this clade and its subsequent long-term spatial isolation (especially D3), which would cause an independent evolution of its repeatome including an amplification of repeats and subsequent increase in GS. Large GS would not be a hindrance in the more stable environments where these species settled (see Paper III), and therefore genome downsizing and repeat regulation would not have been necessary.

To examine the repeatome of *Amomum* in a broader context, a comparative analysis of repeatomes across monocots was carried out using a selection of whole-genome studies to acquire comprehensive repeatomic data. This selection narrowed the scope of the comparison, but still showed some notable trends in monocot repeatomes. *Amomum* with its repeatome occupying as much as 88% of the genome was shown to belong among monocots with the highest repeatome

proportions, as well as having the largest known repeatome proportion within the order Zingiberales. The highest monocot repeatome proportion was found to be 91% in *Allium sativum* (Sun et al., 2020). The *Ty1-Copia* superfamily dominated the genomes of groups within the order Zingiberales, while in other groups with large repeatome propotions (Amaryllidaceae, Asparagales and Poales) the *Ty3-Gypsy* superfamily was the most abundant. Few studies examine the reasons for the amplification of certain repeat types, but a recent study by Schley *et al.* (2022) note that in palms, *Ty1-Copia* elements amplified as part of a stress response, being located close to stress-response genes, and Chen *et al.* (2016) noted an activation of transposable elements in heat-stressed young seeds of rice. It would therefore seem that stress (or lack thereof) is one of the driving factors of repeat dynamics in plants; however, more data is needed to assess this with confidence.

Finally, based on previous works demonstrating that repeats may serve as phylogenetic markers (Dodsworth et al., 2015; Vitales et al., 2020), *Amomum*'s repeatome was used to conduct a phylogenetic analysis. Similarity matrices from the RepeatExplorer analysis were used to construct a consensus network which was indeed congruent with the nuclear gene-based phylogeny. In addition, the network structure further supported the hypothesis of clade D3's hybrid origin. This analysis proved the usefulness of repeatome data as supporting evidence in phylogenetic studies.

CONCLUSION AND FUTURE POSSIBILITIES

The insight into *Amomum*'s genome, repeatome and biogeography shown here is by no means exhaustive but can (and the author hopes it will) serve as a stepping stone for more comprehensive studies of this intriguing ginger genus in the future, and perhaps even aid in the cultivation and use of its culinary and medicinal members.

The recircumscription and further taxonomic treatment of *Amomum* has clarified the relationships within the genus as well as within the whole subfamily Alpinioideae and provided a robust phylogenetic framework for further studies in this genus. Genome size has been shown to correlate with phylogeny in *Amomum* s.s., and chromosome counting revealed the presence of polyploids in the group. Investigating the evolution of genome size in *Amomum* s.s. led to an examination of its biogeography and although the data available for this research was limited, it still showed a trend where the genus dispersed southeast from its original, seasonal area of dispersal (Indochina) to a wetter and warmer area of tropical rainforest (Sundaland) and then back north.

Like in many other plant groups, the Isthmus of Kra seems to be the dividing feature which the genus had to cross there and back again during its evolution. *Amomum*'s genome size seems to reflect this pattern, gradually increasing from the most basal clade A to the most derived subclade D3; however, the largest genome sizes are found in this subclade, which is dispersed both south and north of the Isthmus. Therefore, *Amomum* s.s. does not follow the large genome constraint hypothesis and seems to have found other strategies to keep a large genome in both stable (tropical rainforest) and less stable (seasonal dipterocarp forest) conditions. The existence of this strategy may prove useful to the genus in the current unstable climate, as well as aid in the conservation of *Amomum* and similar plant taxa.

The ancient hybridization found in *Amomum* s.s. is among the first documented in the Alpinioideae and seems to coincide with its repeatome pattern, where multiple repeat lineages were amplified in the hybridogenous clade D. This amplification has led to some members of clade D3 having repeatome proportions up to 88% of their genomes, which is among the highest amounts documented in monocots to date. Overall, the total amount of repeats was correlated with genome size, which suggests that hybridization was a driving factor of genome size evolution (mediated by repeatome dynamics) in this genus.

What are the possible steps to further broaden the knowledge of this tropical genus? Firstly, more specimens need to be collected in the field, especially in those areas of distribution where collections are scant, such as Laos and Cambodia. This will not only facilitate taxonomic research, but it may also aid in conserving or at least recording *Amomum*'s diversity in its endangered or disappearing habitats.

In order to further explore *Amomum*'s intricate genomic makeup, a greater sampling is needed to conduct a thorough repeatome analysis across the genus; ideally, a whole-genome sequencing of some of the species may shed more light on the repeats in *Amomum* in a genomic context. If more biogeographic and ecological data is available, an investigation of the relationship of repeat dynamics in *Amomum* to its ecology and life history could be enlightening not just for tropical botany but potentially also for its commercial growers, seeing as repeats may influence plant adaptation capacity and life histories.

SCIENTIFIC CONTRIBUTION OF THIS THESIS

This study is the first comprehensive work on *Amomum* s.l. since the times of Schumann. The genus has been recircumscribed and *Amomum* s.s. has been established as well as three resurrected (*Conamomum*, *Meistera* and *Wurfbainia*) and three new genera (*Epiamomum*, *Lanxangia* and *Sundamomum*) within the Alpinioideae. This helps the understanding of relationships within the whole subfamily and sets the ground for further studies on its genera, such as the similarly taxonomically complicated *Alpinia* s.l. Certain morphological characters (anther crest and fruit type) have been described as reliable determining characters to distinguish between *Amomum* s.s. and other morphologically similar genera, which will aid in determination of specimens in the field. This concept of *Amomum* s.s. and the new generic names have since been widely accepted and used in tens of both local and international studies on Asian Zingiberaceae (currently 62 references on WoS).

Next-generation sequencing (and the Hyb-Seq method) has been found useful in obtaining several hundred nuclear genes to reconstruct a robust phylogeny in *Amomum*. This study is the first application of Hyb-Seq on the genus level in the family Zingiberaceae. In addition, the traditionally used ITS marker and other rDNA phylogenetic markers procured a phylogenetic structure conflicting with the Hyb-Seq phylogeny, which suggests that previous studies based solely on ITS may need revision. A detailed inspection of Hyb-Seq results may also aid in finding the reason for these incongruences and possibly also in revealing hybridization events. These have not been well documented in the Zingiberaceae and are probably more common in this family (and possibly in other plant groups) than originally presumed.

For the first time, genome size and chromosome counts have been investigated in *Amomum*, and two polyploids have been found; until now, no polyploid species were known in the genus (nor in *Amomum* s.l.). Furthermore, the highest absolute genome size known in the order Zingiberales so far has been found in tetraploid *A. cinnamomeum*. The findings of *Amomum's* genome size dynamics show that although the absolute genome size correlates with its phylogeny, this tropical group does not seem to follow the large genome constraint hypothesis and species with large genomes are found also in its seasonal (less stable) area of dispersal. This suggests that conclusions regarding genome size drawn mostly from the observations of temperate areas may not apply to tropical plant groups. Additionally, *Amomum* seems to have followed a rarely documented route of migration in the past, where its most derived group (subclade D3) migrated northwards through Sundaland to the north of the Isthmus of Kra. These findings would further support the hypothesis that Sundaland was partially covered with a strip of rainforest at the time of its emergence.

This is the very first study on repeat dynamics in the Zingiberaceae, and the first comparative study of repeatomes within the whole monocot group. The repeatome structure in *Amomum* evolved and repeats amplified over time in congruence with ancient hybridization events within the genus, and the repeat-based phylogeny was congruent with the nuclear gene phylogeny from Hyb-Seq. These results suggest that hybridization may drive repeat proliferation and therefore genome size increase in (tropical) plants, and that repeats can be utilised as (supporting) phylogenetic evidence. The evidence of hybridization in *Amomum* is supported by the structure of 5S rDNA clusters; this is one of the few existing studies using this tool for hybrid detection, and it shows that it can be reliable as supporting evidence.

In conclusion, this study contributes not only to the knowledge of *Amomum* and the subfamily Alpinioideae where it belongs, but also provides insight into genome size, repeat dynamics and hybridization in tropical plant groups and connects all of these aspects into a multifaceted view. It also underlines the paramount importance of alpha taxonomy and fieldwork in the study of tropical plants and demonstrates an overview of tools which can be successfully used in the study of gingers and related plant groups.

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2. Original Papers

Paper I

Convergent morphology in Alpinieae (Zingiberaceae): Recircumscribing *Amomum* as a monophyletic genus

Hugo de Boer, Mark Newman, Axel Dalberg Poulsen, A. Jane Droop, Tomáš Fér, Lê Thị Thu Hiền, Kristýna Hlavatá, Vichith Lamxay, James E. Richardson, Karin Steffen & Jana Leong-Škorničková

Taxon 67(1), February 2018:6–36

Paper I

Convergent morphology in Alpinieae (Zingiberaceae): Recircumscribing *Amomum* as a monophyletic genus

Hugo de Boer,[1>2] Mark Newman,[3] Axel Dalberg Poulsen,[1>3] A. Jane Droop,[3] Tomáš Fér,[4] Lê Thị Thu Hiền,[5] Kristýna Hlavatá,[4] Vichith Lamxay,[6] James E. Richardson, [3>7]Karin Steffen[8] & Jana Leong-Škorničková[9]

[1]Natural History Museum, University of Oslo, P.O. Box 1172 Blindern, 0318 Oslo, Norway
[2]Department of Organismal Biology, Uppsala University, Norbyvägen 18D, 75236 Uppsala, Sweden
[3]Royal Botanic Garden Edinburgh, Inverleith Row, Edinburgh EH3 5LR, Scotland, U.K.
[4]Department of Botany, Faculty of Science, Charles University, Benátská 2, 128 00 Prague, Czech Republic
[5]Institute of Genome Research, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet, Hanoi, Vietnam
[6]Department of Biology, Faculty of Natural Science, National University of Laos, Dong Dok, Vientiane, Lao P.D.R.
[7]Programa de Biología, Universidad del Rosario, Carrera 26 No. 63B – 48, Bogotá, Colombia
[8]Division of Pharmacognosy, Department of Medicinal Chemistry, Uppsala University, Husargatan 3, 75123 Uppsala, Sweden
[9]Herbarium, Singapore Botanic Gardens, National Parks Board, 1 Cluny Road, 259569 Singapore

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Abstract

The tropical ginger genus *Amomum* (Zingiberaceae) has always posed challenges for classification based on morphological characters. Previous molecular phylogenetic studies showed *Amomum* to be paraphyletic but limited sampling and absence of the data of the type *Amomum subulatum* made it impossible to resolve the paraphyly and make nomenclatural changes. Here, *Amomum* is further investigated in a multi-marker phylogenetic framework using *matK* and nrITS including multiple accessions of the type, the genus *Elettaria* and additional accessions of *Amomum*, *Alpinia*, *Elettariopsis*, *Geocharis*, *Geostachys* and *Hornstedtia*. *Amomum* is shown to consist of nine clades and Alpinia of six. The genera *Elettaria*, *Elettariopsis*, *Plagiostachys*, and species in *Hornstedtia* are nested within these clades. Morphological studies of species previously subsumed in *Amomum* support recognition of new genera that correspond to well-delimited clades in the phylogenetic framework presented here. Recircumscription of the paraphyletic genus *Amomum* facilitates identification and creates nomenclatural stability. Three genera, *Conamomum*, *Meistera* and *Wurfbainia*, are resurrected, and three new genera *Epiamomum*, *Lanxangia* and *Sundamomum* are described, together with a key to the genera and a nomenclatural synopsis placing 384 specific names (incl. all synonyms) into the new generic framework. Of these 129 represent new combinations and 3 are replacement names. Types of *Geocharis* and *Geostachys* are designated. Further studies and specific sampling will be needed to resolve other branches of Alpinioideae containing other polyphyletic genera.

Keywords: Alpinieae; Alpinioideae; classification; *Conamomum*; *Epiamomum*; *Meistera*; internal transcribed spacer; *Lanxangia*; maturase K; morphology; phylogeny; *Sundamomum*; *Wurfbainia*

INTRODUCTION

Amomum Roxb. (Zingiberaceae: Alpinieae) as currently circumscribed is a plant genus of some 150 (Mabberley, 2008) to 188 (Govaerts, 2015) species. In the past the name has been applied more broadly and Index Kewensis lists 440 names in the genus (IPNI, 2017). *Amomum* is usually characterised by leafy shoots with close-clasping sheaths, blades usually more than six with a distinct plane of distichy, and inflorescences arising on leaf less shoots from the rhizome, although the same set of characters is also shared by several other Alpinioideae genera. The genus as currently circumscribed is distributed from Sri Lanka and India through SE Asia to New Guinea, the Bismarck Archipelago and Australia (Mabberley, 2008). A number of species are of economic importance, such as *Amomum compactum* Sol. ex Maton (Javanese cardamom), *A. subulatum* Roxb. (black cardamom), and *A. verum* Blackw. (cardamom from Cambodia and Thailand).

Within Zingiberaceae, *Amomum* belongs in the subfamily Alpinioideae (characterised by a plane of leaf distichy that is perpendicular to the direction of growth of the rhizome, and reduced or absent lateral staminodes) and tribe Alpinieae (characterised by fleshy indehiscent fruits and traditionally by the absence of extrafloral nectaries [Kress *et al.*, 2002], although the latter character has since been found to occur in various genera of this tribe as reported by Benedict *et al.*, 2015). The Alpinieae currently consist of 15 genera of which *Alpinia* K.Schum. and *Amomum* are the two most species-rich.

Classification of *Amomum* using morphological characters has long been a challenge. The generic name was first used by Linnaeus (1753) but, as explained by Burtt & Smith (1968), none of the species Linnaeus included is now in *Amomum*. The name now used is *Amomum* Roxb. which is a conserved name (Burtt & Smith, 1968; McVaugh, 1970). Roxburgh (1810) defined *Amomum* by its labellum, anther and fruits. In a group of genera belonging to the Monandria Monogynia with double anthers (i.e., anthers with two fertile thecae to each anther, rather than one, as in Cannaceae and Marantaceae), he described *Amomum* as "*Corolla* with interior border unilabiate. *Anther* with entire or lobate crest. *Capsule* 3-celled, many-seeded". A few years later, Roxburgh described and illustrated *Amomum subulatum* in his *Plants of the coast of Coromandel* (1819). This species is the conserved type of *Amomum* (McVaugh, 1970).

Most botanists in the 19th century had a broad concept of *Amomum* that included many species now classified in other genera, such as *Aframomum* K.Schum., *Alpinia*, *Etlingera* Giseke, *Hornstedtia* Retz. and *Renealmia* L.f. Bentham & Hooker (1883) classified *Amomum* into three sections, *A.* sect. *Amomum* ["*Euamomum*"], *A.* sect. *Cenolophon* (Blume) Benth. & Hook.f., and *A.* sect. *Geanthus* (Reinw.) Benth. & Hook.f., while Baker (1892) recognised five, *A.* sect. *Achasma* (Griff.) Baker, *A.* sect. *Amomum*, *A.* sect. *Cenolophon*, *A.* sect. *Geanthus*, and *A.* sect. *Hornstedtia* (Retz.) Baker. This broad generic concept persisted into the early 20th century with Gagnepain (1908) including in *Amomum* species that are now placed in *Elettariopsis* Baker and *Etlingera*.

Schumann was among the first to circumscribe *Amomum* more narrowly and his concept has been followed with minor variations since his monograph of the Zingiberaceae in Engler's *Das Pflanzenreich* (Schumann, 1904). In preparation for this monumental work, Schumann had published a study of the Zingiberaceae of Malaysia and Papuasia (1899) in which he followed the

broad concept of *Amomum* common to the 19th century. By 1904, however, he had concluded that *Aframomum*, *Hornstedtia* and *Phaeomeria* Lindl. ex K.Schum. should be recognised as separate genera.

Schumann (1904) recognised two sections, each with two series. *Amomum* sect. *Amomum* with anther appendages present was subdivided into *A.* ser. *Integrae* K.Schum. with entire anther appendages and *A.* ser. *Lobulatae* K.Schum. with bior trilobed anther appendages. The other section, *A.* sect. *Geanthus*, was characterised by the absence of anther appendages, and all but six of the species included in it are now classified in *Etlingera* or *Alpinia* sect. *Fax* R.M.Sm. Loesener (1930) maintained Schumann's *A.* sect. *Amomum* ser. *Integrae* and *A.* sect. *Amomum* ser. *Lobulatae* but excluded *A.* sect. *Geanthus*. The only attempt to classify the species based on morphology since then was a revision of *Amomum* in Borneo where five groups were recognised but not given formal names (Smith, 1985). For a full overview of historical classification see Table S1 (Electr. Suppl.).

One of the long-standing issues related to *Amomum* has been its delimitation from the morphologically similar *Elettariopsis* that usually has loosely clasping leafy shoots and open bracteoles. This distinction is not absolute however, and no single character will unambiguously distinguish *Amomum* from related genera as already noted by Holttum (1950). There are species of *Elettariopsis* with leafy shoots like those of *Amomum* and species of *Amomum* with open or absent bracteoles (Holttum, 1950). Kam (1982), in a revision of *Elettariopsis*, argued that *Amomum* must be revised before the limits of *Elettariopsis* could be truly delineated and its relationships revealed.

Regional revisions of *Amomum* covering China (Tsai *et al.*, 1981), Borneo (Smith, 1985, 1988; Sakai & Nagamasu, 1998), Peninsular Malaysia and Singapore (Holttum, 1950), Java (Backer & Bakhuizen van den Brink, 1968), Sumatra (Droop & Newman, 2014), and Cambodia, Laos and Vietnam (Lamxay & Newman, 2012) have been made, but a comprehensive revision has not been attempted recently, due in part to the large number of species, the lack of significant collections, and the complexity of morphological characters (Xia *et al.*, 2004) many of which are not well preserved in herbarium material.

Phylogenetic studies on relationships within the Zingiberaceae have focused on the genera *Aframomum* (Harris *et al.*, 2000), *Alpinia* (Rangsiruji *et al.*, 2000; Kress *et al.*, 2005), *Amomum* (Xia *et al.*, 2004), *Curcuma* L. (Záveská *et al.*, 2012, 2016; Leong-Škorničková *et al.*, 2015), *Etlingera* (Pedersen, 2004), *Globba* L. (Williams *et al.*, 2004), *Hedychium* J.Koenig (Searle & Hedderson, 2000), and *Renealmia* (Särkinen *et al.*, 2007), and used sequence data from the nuclear ribosomal ITS region (nrITS) (Harris *et al.*, 2000; Rangsiruji *et al.*, 2000; Searle & Hedderson, 2000; Wood *et al.*, 2000; Kress *et al.*, 2002, 2005; Pedersen, 2004; Williams *et al.*, 2004; Xia *et al.*, 2004; Särkinen *et al.*, 2007; Záveská *et al.*, 2012), and the chloroplast regions *trnL-F* intergenic spacer (Rangsiruji *et al.*, 2000; Särkinen *et al.*, 2007; Záveská *et al.*, 2012), *rps16* (Pedersen, 2004), *matK* (Kress *et al.*, 2002, 2005; Williams *et al.*, 2004; Xia *et al.*, 2004; Záveská *et al.*, 2012), and more recently also single-copy nuclear markers (Záveská *et al.*, 2016). The first molecular phylogeny of the family suggested that some morphological traits are homoplasious, and recircumscribed three paraphyletic tribes in a new classification of the family that recognises four subfamilies and six tribes: Siphonochiloideae (Siphonochileae), Tamijioideae

(Tamijieae), Alpinioideae (Alpinieae, Riedelieae), and Zingiberoideae (Zingibereae, Globbeae) (Kress *et al.*, 2002).

This molecular phylogeny (Kress *et al.*, 2002) found the Alpinieae tribe to consist of *Aframomum*, *Alpinia*, *Amomum*, *Elettariopsis*, *Etlingera*, *Hornstedtia*, *Paramomum* S.Q.Tong, *Plagiostachys* Ridl., *Renealmia* and *Vanoverberghia* Merr.; in which *Alpinia* and *Amomum* were found to be paraphyletic (Kress *et al.*, 2002). The genera *Aulotandra* Gagnep., *Cyphostigma* Benth., *Elettaria* Maton, *Leptosolena* C.Presl, *Geocharis* (K.Schum.) Ridl., and *Geostachys* (Baker) Ridl. were included based on morphology only (Kress *et al.*, 2002). The paraphyletic genera *Alpinia* and *Amomum* were further investigated in later studies (Xia *et al.*, 2004; Kress *et al.*, 2005, 2007). Xia *et al.* (2004) using molecular data based mostly on Chinese species confirmed *Amomum* to be paraphyletic and consisting of three groups, a Tsaoko group, a Villosum group and a Maximum group. The authors refrained from making nomenclatural changes and argued that increased sampling was necessary to include the type, *Amomum subulatum*, and to resolve the latter two grades. Kress *et al.* (2005, 2007) investigated the paraphyly of *Alpinia* and found it to consist of six clades with several other genera nested within these clades, *Leptosolena Plagiostachys* an *Vanoverberghia*, and the clades themselves intermixed with clades of *Aframomum*, *Amomum*, *Etlingera*, *Elettariopsis*, *Geocharis*, *Geostachys*, *Hornstedtia*, *Paramomum* and *Renealmia*. Droop (2012), who included samples from a much wider geographical area, retrieved an additional five clades and placed them into a larger framework of Alpinieae.

In this study, which builds on and further extends the work of Droop (2012), we investigate *Amomum* in a phylogenetic framework using expanded taxon sampling. We include multiple accessions of its type, *A. subulatum*, as well as that of *Elettaria*, in addition to more accessions of *Amomum*, *Alpinia*, *Elettariopsis*, *Geocharis*, *Geostachys* and *Hornstedtia*, with the aim of finding support for all clades consisting mainly of species currently classified in *Amomum* and resolving the current paraphyly of the genus. The ultimate objective is to circumscribe the clades supported by the molecular phylogeny with morphological characters enabling their identification. We refrain from addressing nomenclature in *Alpinia* and related genera as that would require similarly comprehensive sampling across the morphological and distributional variation of those genera.

MATERIALS AND METHODS

Plant material, DNA extraction and loci

Material for DNA extraction was obtained mainly through our collections supported by vouchers deposited at AAU, ANDA, ASSAM, BO, E, HAW, NLS, P, SAN, SING, UH and VNMN, or from existing specimens in these herbaria. Determinations were verified using identification keys (Baker, 1892; Schumann, 1904; Ridley, 1909; Holttum, 1950; Smith, 1985, 1986a; Sakai & Nagamasu, 1998; Wu & Larsen, 2000; Lamxay & Newman, 2012; Droop & Newman, 2014), and identities re-confirmed with protologues and type material. Additional data from previous studies (Rangsiruji *et al.*, 2000; Kress *et al.*, 2002, 2005, 2007; Pedersen, 2004; Xia *et al.*, 2004) was downloaded from NCBI GenBank. Species were selected to cover maximum morphological variability so far known in *Amomum* s.l. with maximum geographical coverage available to us. We have also aimed to cover all currently known genetic diversity in terms of all major clades recovered in previous studies (Xia *et al.*, 2004; Kress *et al.*, 2007; Droop, 2012) by inclusion of

multiple species from each of these clades. Types of genera previously synonymised with *Amomum*, i.e., *Conamomum* Ridl. (*C. utriculosum* Ridl.), *Meistera* Giseke (*M. koenigii* (J.F.Gmel.) Škorničk. & M.F.Newman) and *Wurfbainia* Giseke (*W. uliginosa* (J.Koenig) Giseke) were included as well as types of following Alpinioideae genera: *Aframomum* (*A. angustifolium* (Sonn.) K.Schum.), *Amomum* (*A. subulatum*), *Alpinia* (*A. galanga* (L.) Willd.), *Elettaria* (*E. cardamomum* (L.) Maton), *Elettariopsis* (*E. curtisii* Baker), *Geocharis* (*G. macrostemon* (K.Schum.) Holttum), *Hornstedtia* (*H. scyphifera* (J.Koenig) Steud.), and *Siamanthus* (*S. siliquosus* K.Larsen & Mood). Six loci were investigated, four plastid DNA regions, namely *matK*, *rps16*, *ndhF*, and *trnL-F* intron and spacer, and the nuclear ribosomal ITS region (ITS1-5.8S-ITS2) and *at103*. Since the other markers were difficult to amplify for most of the accessions, the analyses are based on nrITS and *matK* only. The nrITS and *matK* data matrix included a total of 293 sequences from 2 accessions of *Aframomum*, 105 of *Amomum* s.l., 23 of *Alpinia*, 3 of *Elettaria*, 7 of *Elettariopsis*, 3 of *Etlingera*, 3 of *Geocharis*, 2 of *Geostachys*, 6 of *Hornstedtia*, 1 of *Plagiostachys*, 2 of *Pleuranthodium* (K.Schum.) R.M.Smith, 4 of *Renealmia*, 1 of *Riedelia* Oliv., 1 of *Siamanthus* K.Larsen & Mood, 2 of *Siliquamomum* Baill. and 1 of *Siphonochilus* J.M. Wood & Franks. Species names and their authors, specimen voucher information, and GenBank accession numbers per molecular marker (including 184 new sequences, 105 nrITS and 79 *matK*) are summarised in Appendix 1 with accessions newly generated for this study marked by an asterisk.

DNA extraction and amplification

Total DNA was extracted using the Carlson/Yoon CTAB DNA isolation procedure (Doyle & Doyle, 1987; Yoon *et al.*, 1991) and a Mini-Beadbeater (BioSpec Products, Bartlesville, Oklahoma, U.S.A.) to pulverise the plant material. Extracts were purified using the GE Illustra GFXTM PCR DNA and Gel Band Purification Kit (GE Healthcare Life Sciences, Marlborough, Massachusetts, U.S.A.) following the standard protocol. Polymerase chain reaction (PCR) amplification of purified total DNA was performed in 200 µl reaction tubes with a total volume of 25 µl. Each tube contained a mixture of 2.5 µl reaction buffer (ABgene, Waltham, Massachusetts, U.S.A., 10×), 1.5 µl MgCl2 (25 mM), 1 µl dNTP's (20 µM), 0.125 µl Taq-polymerase (ABgene; 5 U/µl), 0.125 µl BSA (Roche Diagnostics, Basel, Switzerland), 1.25 µl of each primer (10 mM), 16.25 µl Milli-Q water and 1 µl template DNA. The following primer pairs were used for amplification and sequencing: ITS using the primer pair ITSP17 and ITS-26 S-82R (Popp & Oxelman, 2001), *ndhF* with pair ndhF-803F and ndhF-1603R (Olmstead & Sweere, 1994), *rps16* with pair rps16F and rps16R2R (Oxelman *et al.*, 1997), *trnL-F* spacer with trnL-BOCF and trnL-BOCR (Bolmgren & Oxelman, 2004), *at103* with pair at103F and at103R (Li *et al.*, 2008), and *matK* with *matK*_2.1aF (RBG-K, 2007) and *matK*_1440R (Fior *et al.*, 2006). ITS, *rps16* and *ndhF* were amplified with the following PCR protocol 95°C 5 min, (95°C 30 s, 58°C 1 min, 72°C 1 min) × 35, 72°C 5 min, 4°C ∞; *trnL-F* with 95°C 5 min, (95°C 30 s, 56°C 1 min, 72°C 1 min) × 35, 72°C 5 min, 4°C ∞; *matK* with 95°C 5 min, (95°C 30 s, 52°C 1 min, 72°C 1 min) × 35, 72°C 5 min, 4°C ∞; and *at103* with touchdown protocol 95°C 5 min, (95°C 30 s, 62°C–56°C 45 s, 72°C 45 s) × 35, 72°C 10 min, 4°C ∞. Sequencing was performed by Macrogen (Seoul, Korea and Amsterdam, the Netherlands) on ABI3730XL automated sequencers (Applied Biosystems, Foster City, California, U.S.A.).

Sequence alignment and phylogenetic analyses

Trace files were compiled into sequences with the program PreGap4 v.1.6 and edited using Gap v.4.11.2 (Bonfield *et al.*, 1995), both modules in the Staden package (Staden, 1996). Sequences were aligned automatically using MAFFT v.7 (Katoh *et al.*, 2002) as implemented in AliView v. 1.17.1 (Larsson, 2014). The final matrix of 162 accessions included ITS (95.1% of taxa) and *matK* (78.4%). The ndhF, trnL-F, at103 and rps16 were excluded due to high levels of missing data. Matrices were gap-coded using the Simmons and Ochoterena simple method (Simmons & Ochoterena, 2000) implemented in SeqState v.1.37 (Müller, 2005). Selection of best-fit models of nucleotide substitution for each data partition used in a Bayesian or maximum likelihood analysis was based on the Akaike information criterion (AIC) and AIC corrected for small sample size (AICc) as implemented in jModelTest v.0.1.1 (Guindon & Gascuel, 2003; Posada, 2008), and the model GTR+Γ+I was selected for all markers. Maximum likelihood tree searches and bootstrapping of the combined data (using 1000 replicates) used RAxML-HPC v.8.2.10 on XSEDE (Stamatakis *et al.*, 2008), and Bayesian tree searches used MrBayes v.3.2.6 (Huelsenbeck & Ronquist, 2001), both on the CIPRES cluster (Miller *et al.*, 2010). For the Bayesian analysis, the combined data were analysed using three partitions (nuclear, plastid, gap data), allowing partition models to vary by unlinking gamma shapes, transition matrices, and proportions of invariable sites. Markov chain Monte Carlo (MCMC) runs started from independent random trees, were repeated twice, and extended for ten million generations, with trees sampled every 1000th generation. We used the default priors in MrBayes, namely a flat Dirichlet prior for the relative nucleotide frequencies and rate parameters, a discrete uniform prior for topologies, and an exponential distribution (mean 1.0) for the gamma-shape parameter and branch lengths. Convergence was assessed by checking that the standard deviations of split frequencies were < 0.01; that the log probabilities of the data given the parameter values fluctuated within narrow limits; that the convergence diagnostic (the potential scale reduction factor given by MrBayes) approached one; and by examining the plot provided by MrBayes of the generation number versus the log probability of the data. Trees saved prior to convergence were discarded as burn-in (10,000 trees) and a consensus tree was constructed from the remaining trees. Independent MrBayes analyses per marker with the coded indels (ten million generations, two partitions sequence and gaps) were tested for topological congruence using the de Vienne congruence index (de Vienne *et al.*, 2007) and all were found to be highly congruent (*I*cong = 2.94, P-value = 4.44e−21). The data matrix and trees have been deposited in DRYAD (https:// datadryad.org; https://doi.org/10.5061/dryad.hq228).

Morphology, taxonomic treatment and synopsis

All species from *Amomum* s.l. involved in the study were scrutinised for critical morphological characters (habit, type of leafy shoot, presence of petiole, number of flowers supported by fertile bract, presence and shape of bracteole, flower type, calyx, shape and colouration of the labellum, presence and shape of staminodes, presence of staminal tube, anther crest shape and fruit type). The two most informative characters, anther crest and fruit type, were plotted on the Bayesian phylogenetic consensus tree of the combined dataset. Species originating from our own collections were examined from dried and spirit material and photographs, mostly including

detailed flower dissections. Species for which the data originated from GenBank were scored either from vouchers linked to these accessions (when available and showing the relevant characters) or from the protologues (see Taxonomic treatment section) and/or descriptions of these species in monographs and floras (Bentham & Hooker, 1883; Schumann & Hollrung, 1889; Baker, 1892; Schumann, 1899, 1904; Gagnepain, 1908; Valeton, 1918; Loesener, 1930; Holttum, 1950; Backer & Bakhuizen van den Brink, 1968; Kam, 1982; Burtt & Smith, 1983; Smith, 1985, 1986a, 1987; Larsen *et al.*, 1999; Wu & Larsen, 2000; Gao *et al.*, 2006; Larsen & Larsen, 2006; Poulsen, 2006; Sabu, 2006; Lamxay & Newman, 2012; Lau & Lim, 2012; Lamb *et al.*, 2013; Droop & Newman, 2014; Leong-Škorničková & Newman, 2015), as well as from photographs and vouchers of these taxa collected by us. Species not included in the study were scored as much as possible from the information given in the protologues and the cited literature resources above, as well as our own collections of these taxa. To build a comprehensive taxonomic synopsis, all names relevant to *Amomum* were gathered from the World Checklist of Selected Plant Families (WCSP, 2017) and the Zingiberaceae Resource Centre (Newman, 2017). Species originally described in *Amomum* but later combined in other genera and currently accepted there (e.g., *Aframomum*, *Alpinia*, *Costus* L., *Curcuma*, *Cyphostigma*, *Etlingera*, *Hornstedtia*, *Plagiostachys*, *Renealmia* and *Zingiber* Mill.) were excluded, and are not listed in the synopsis. Protologues and all available original material of relevant basionyms connected to *Amomum* in the broad sense: regional floras, revisions and other available material including specimens deposited in numerous herbaria (BM, C, CAL, E, K, NLS, P, SING), were consulted to establish the new generic placement of taxa not sampled in this study. Type locations of all species and their currently known distribution ranges were mapped to derive distribution ranges of the clades recognised at generic level. Currently accepted heterotypic synonyms were accepted except in rare cases when we had first-hand knowledge of taxa and could reinstate them. All heterotypic synonyms should undergo critical re-examination preferably from living material gathered in type localities or nearby. Names listed as *incertae sedis* include those for which the protologue and original material do not provide sufficient information to allow a new generic placement. Some of these names may even belong in other genera of Alpinioideae. Names to be accepted are in bold font; basionyms, homotypic synonyms and heterotypic synonyms follow in chronological order in normal font; and species that were included in the molecular analyses are marked with an asterisk.

RESULTS

Phylogenetic analyses and taxonomy

Phylogenies obtained under Bayesian or maximum likelihood (ML) optimisation revealed no statistically supported incongruences, defined as conflicting nodes with Bayesian posterior probabilities (PP) >0.95 or maximum likelihood bootstrap support (BS) >75, and in this paper we only present the trees from the combined analyses. Figure 1 shows the combined Bayesian consensus phylogeny with PP > 0.80 and BS values >60 indicated at the nodes. Figures S1–S4 (Electr. Suppl.) are the Bayesian and ML phylogenies for nrITS and *matK* respectively. Morphology of the anther crest and fruit type is plotted on the phylogeny per taxon. The phylogenies show the six *Alpinia* clades recovered by Kress *et al.* (2005) and the three *Amomum* clades recovered by Xia *et al.* (2004), and an additional six *Amomum* clades based on the

extended sampling done in this study (Fig. 1). Each of the nine *Amomum* clades plus one *Alpinia* clade including an *Amomum* species is described below.

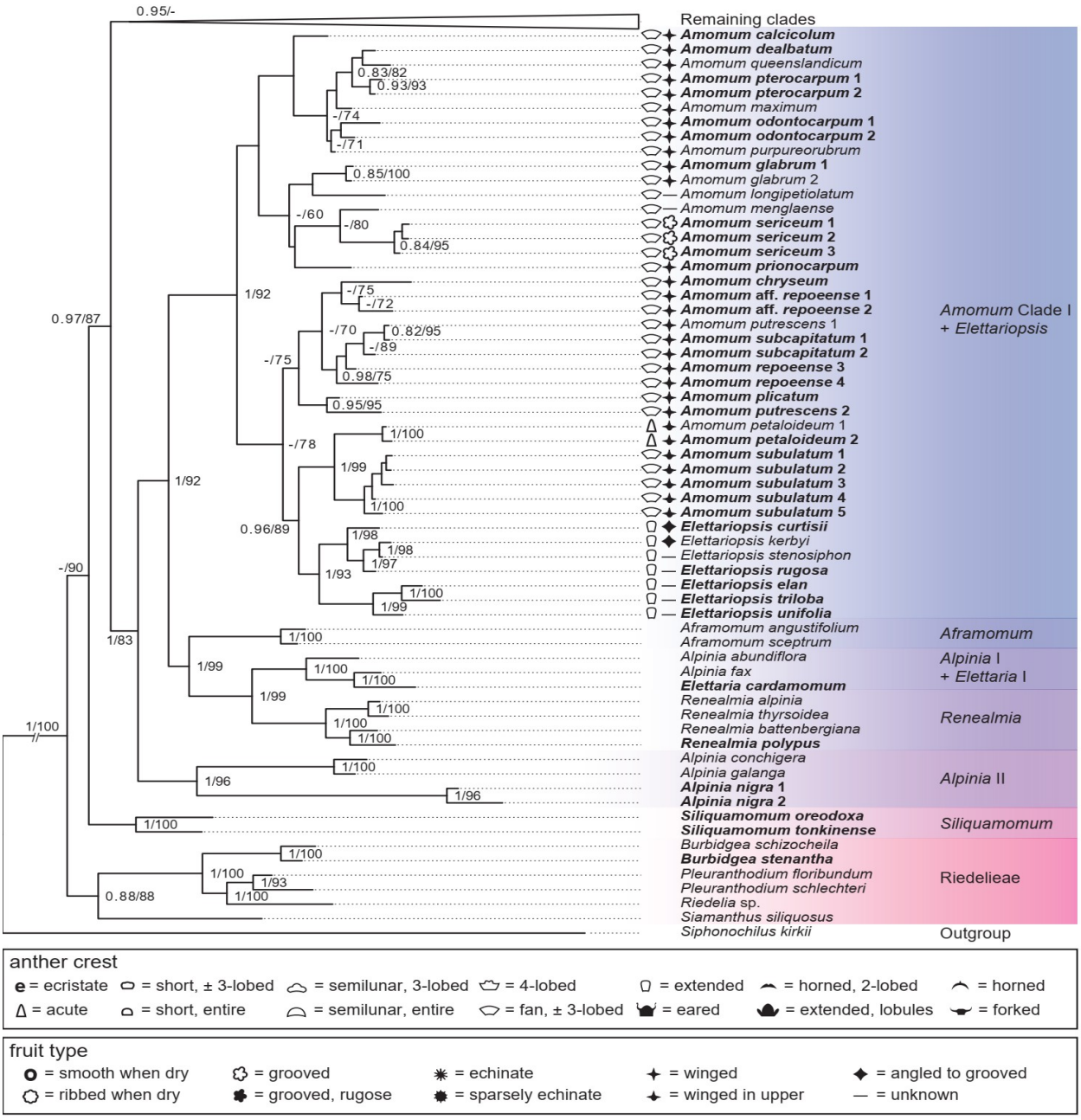
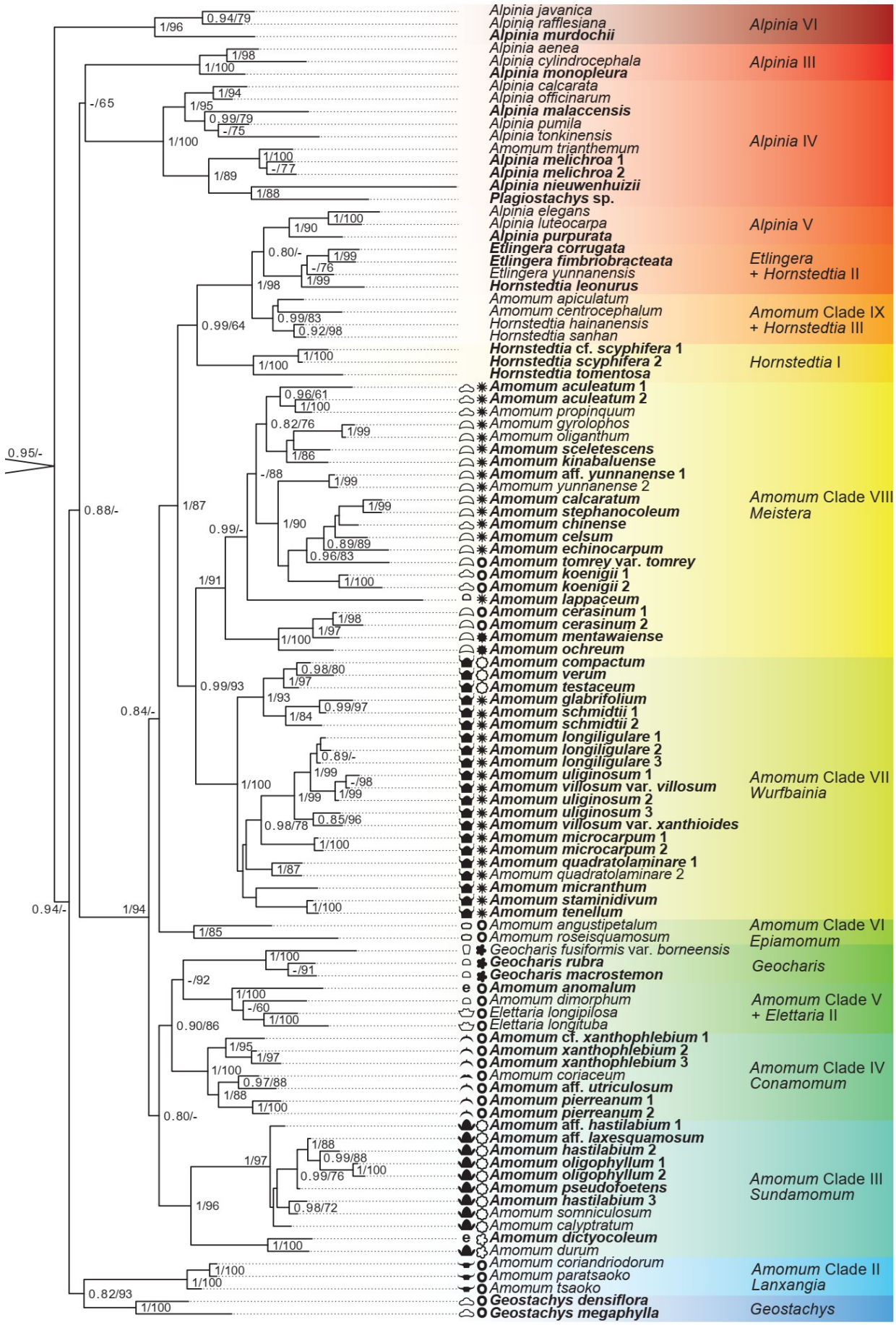


Fig. 1. Bayesian consensus phylogeny of the combined dataset of nrITS and *matK* with PP > 0.80 and BS >60 indicated at the nodes. Morphology of the anther crest and fruit type is plotted on the phylogeny. Material included in this study for the first time in bold, additional material downloaded from NCBI GenBank in normal font.

(Figure continued on next page)


Alpinia javanica
Alpinia rafflesiana
Alpinia murdochii
Alpinia aenea
Alpinia cylindrocephala
Alpinia monopleura
Alpinia calcarata
Alpinia officinarum
Alpinia malaccensis
Alpinia pumila
Alpinia tonkinensis
Amomum trianthemum
Alpinia melichroa 1
Alpinia melichroa 2
Alpinia nieuwenhuizii
Plagiostachys sp.
Alpinia elegans
Alpinia luteocarpa
Alpinia purpurata
Etlingera corrugata
Etlingera fimbriobracteata
Etlingera yunnanensis
Hornstedtia leonurus
Amomum apiculatum
Amomum centrocephalum
Hornstedtia hainanensis
Hornstedtia sanhan
Hornstedtia cf. scyphifera 1
Hornstedtia scyphifera 2
Hornstedtia tomentosa
Amomum aculeatum 1
Amomum aculeatum 2
Amomum propinquum
Amomum gyrolophos
Amomum oliganthum
Amomum sceletescens
Amomum kinabaluense
Amomum aff. yunnanense 1
Amomum yunnanense 2
Amomum calcaratum
Amomum stephanocoleum
Amomum chinense
Amomum celsum
Amomum echinocarpum
Amomum tomrey var. tomrey
Amomum koenigii 1
Amomum koenigii 2
Amomum lappaceum
Amomum cerasinum 1
Amomum cerasinum 2
Amomum mentawaiense
Amomum ochreum
Amomum compactum
Amomum verum
Amomum testaceum
Amomum glabrifolium
Amomum schmidtii 1
Amomum schmidtii 2
Amomum longiligulare 1
Amomum longiligulare 2
Amomum longiligulare 3
Amomum uliginosum 1
Amomum villosum var. villosum
Amomum uliginosum 2
Amomum uliginosum 3
Amomum villosum var. xanthioides
Amomum microcarpum 1
Amomum microcarpum 2
Amomum quadratolaminare 1
Amomum quadratolaminare 2
Amomum micranthum
Amomum staminidivum
Amomum tenellum
Amomum angustipetalum
Amomum roseisquamosum
Geocharis fusiformis var. borneensis
Geocharis rubra
Geocharis macrostemon
Amomum anomalum
Amomum dimorphum
Elettaria longipilosa
Elettaria longituba
Amomum cf. xanthophlebium 1
Amomum xanthophlebium 2
Amomum xanthophlebium 3
Amomum coriaceum
Amomum aff. utriculosum
Amomum pierreanum 1
Amomum pierreanum 2
Amomum aff. hastilabium 1
Amomum aff. laxesquamosum
Amomum hastilabium 2
Amomum oligophyllum 1
Amomum oligophyllum 2
Amomum pseudofoetens
Amomum hastilabium 3
Amomum somniculosum
Amomum calyptratum
Amomum dictyocoleum
Amomum durum
Amomum coriandriodorum
Amomum paratsaoko
Amomum tsaoko
Geostachys densiflora
Geostachys megaphylla
Alpinia VI
Alpinia III
Alpinia IV
Alpinia V
Etlingera + Hornstedtia II
Amomum Clade IX + Hornstedtia III
Hornstedtia I
Amomum Clade VIII Meistera
Amomum Clade VII Wurfbainia
Amomum Clade VI Epiamomum
Geocharis
Amomum Clade V + Elettaria II
Amomum Clade IV Conamomum
Amomum Clade III Sundamomum
Amomum Clade II Lanxangia
Geostachys

Clade I. Amomum. – This clade (1.00 PP/92 BS) contains *Amomum subulatum*, the type of *Amomum*, and *Elettariopsis curtisii*, the type of *Elettariopsis*. Twenty-six species in this clade are included in this study, 19 in *Amomum* and 7 in *Elettariopsis*. The five accessions of A. subulatum form a well-supported monophyletic clade (1.00 PP/100 BS). The multiple accessions of *A. pterocarpum* Thwaites, *A. odontocarpum* D.Fang, *A. glabrum* S.Q.Tong, *A. sericeum* Roxb. and *A. petaloideum* (S.Q.Tong) T.L.Wu, each also form clades with varying degrees of support. The multiple accessions of *A. repoeense* Pierre ex Gagnep., *A. subcapitatum* Y.M.Xia, and *A. putrescens* D.Fang do not form monophyletic clades and further investigation of these collections and taxa might elucidate these unexpected results. Two subclades are retrieved, one containing accessions from 12 species of *Amomum* and a second (– PP/78 BS) containing the type *A. subulatum*, accessions from 6 species of *Amomum* and accessions from all 7 included species of *Elettariopsis*.

Clade Geostachys. – This clade (1.00 PP/100 BS) consists of two species, *G. densiflora* Ridl. and *G. megaphylla* Holttum, and is a sister clade (0.82 PP/93 BS) to *Lanxangia* (1.00 PP/100 BS).

Clade II. Lanxangia. – This clade (1.00 PP/100 BS) contains *Amomum tsaoko* Crevost & Lemarié, *A. paratsaoko* S.Q. Tong & Y.M.Xia and *A. coriandriodorum* S.Q.Tong & Y.M. Xia, and is a well-supported sister clade (0.82 PP/93 BS) to *Geostachys* (1.00 PP/100 BS).

Clade III. Sundamomum. – This clade (1.00 PP/96 BS) contains eight species of *Amomum*. The two accessions of *A. oligophyllum* A.J.Droop form a well-supported clade (1.00 PP/100 BS), whereas the three accessions of *A. hastilabium* Ridl. are spread across clade III.

Clade IV. Conamomum. – This clade (1.00 PP/100 BS) contains four species of *Amomum*, *A. pierreanum* Gagnep., *A. coriaceum* R.M.Sm., *A. aff. utriculosum* (Ridl.) Holttum and *A. xanthophlebium* Baker, and is sister (0.90 PP/86 BS) to clade V and *Geocharis*. The two accessions of *A. pierreanum* and three of *A. xanthophlebium* form well-supported clades (resp. 1.00 PP/100 BS, and 1.00 PP/95 BS).

Clade V. – This clade (1.00 PP/100 BS) contains accessions of two species of *Amomum*, and two species of *Elettaria* that are part of ongoing research by A.D. Poulsen, M. Newman, C.S. Bjorå and M. Ardiyani. The two *Amomum* species are *A. dimorphum* M.F.Newman and *A. anomalum* R.M.Sm. The type of *Elettaria*, *E. cardamomum*, is found more basally branching in the tree together with *Renealmia* and *Alpinia fax* B.L.Burtt & R.M.Sm. and *A. abundiflora* B.L.Burtt & R.M.Sm.

Clade VI. Epiamomum. – This small clade (1.00 PP/85 BS) contains two species of *Amomum*, *A. angustipetalum* S.Sakai & Nagam. and *A. roseisquamosum* S.Sakai & Nagam., both endemic to Borneo.

Clade VII. Wurfbainia. – This clade (1.00 PP/100 BS) includes accessions from 13 species of *Amomum*, and is sister (0.99 PP/93 BS) to clade VIII (1.00 PP/91 BS). The three accessions of *A. longiligulare* T.L.Wu, two accessions of *A. microcarpum* C.F.Liang & D.Fang, and two accessions of *A. quadratolaminare* S.Q.Tong form well-supported clades (resp. 0.89 PP/–, 1.00 PP/100 BS, 1.00 PP/87 BS). The two accessions of *A. schmidtii* (K.Schum.) Gagnep. and three accessions of *A. uliginosum* J.Koenig each end up in different places within clade VII, and further morphological studies might shed light on this unexpected topology.

Clade VIII. Meistera. – This clade (1.00 PP/91 BS) includes accessions from 18 species of *Amomum*, and is sister (0.99 PP/93 BS) to clade VII (1.00 PP/100 BS). The two accessions of *A. yunnanense* S.Q.Tong, two accessions of *A. koenigii* J.F.Gmel, and two accessions of *A. cerasinum* Ridl. form well-supported clades (resp. 1.00 PP/99 BS, 1.00 PP/100 BS, 1.00 PP/98 BS). The two accessions of *A. aculeatum* Roxb. are not sisters, but form a well-supported clade with *A. propinquum* Ridl. (0.96 PP/61 BS).

Clade Hornstedtia I. – This clade (1.00 PP/100 BS) includes two species of *Hornstedtia*, *H. tomentosa* (Blume) Bakh.f. and *H. scyphifera* (J.Koenig) J.Koenig ex Steud., the type of the genus. *Hornstedtia* I is sister (0.99 PP/64 BS) to *Alpinia* V, *Etlingera*+*Hornstedtia* II and *Amomum* IX+*Hornstedtia* III.

Clade IX. – This clade (0.99 PP/82 BS) includes accessions of four species, *Amomum apiculatum* K.Schum., *A. centrocephalum* A.D.Poulsen, *Hornstedtia sanhan* M.F.Newman, and *H. hainanensis* T.L.Wu & S.J.Chen. Combined molecular phylogenetic and morphological studies of the remaining two clades of *Hornstedtia* are under way by A.D. Poulsen and Nurainas.

Clade Etlingera + Hornstedtia II. – This clade (1.00 PP/99 BS) includes three species of *Etlingera* and one of *Hornstedtia*, *H. leonurus* (J.Koenig) Retz. and is retrieved in this analysis as sister to Alpinia V, in a well-supported clade (1.00 PP/98 BS) together with *Amomum* IX+ *Hornstedtia* III.

Clade Alpinia V. – This clade (1.00 PP/90 BS) includes three species of *Alpinia* and is retrieved in this analysis as sister to the clade above.

Clade Alpinia IV. – This clade includes *Amomum trianthemum* K.Schum. Combined molecular phylogenetic and morphological studies of the species in this clade are under way by N. Sharp, A.D. Poulsen, M. Newman and M. Ardiyani.

Clade Alpinia III. – This clade (1.00 PP/100 BS) includes three species of *Alpinia* and is weakly supported sister clade (–/65 BS) to *Alpinia* IV.

Clade Alpinia VI. – This clade (1.00 PP/96 BS) includes three species of *Alpinia*, and is sister (0.94 PP/– BS) to the clade containing *Amomum* II–IX, *Alpinia* III–V, *Geostachys*, *Geocharis*, *Etlingera* and *Hornstedtia* I–III.

DISCUSSION

Phylogeny in light of recent studies

As in all previous phylogenetic studies, our results confirm the paraphyly of *Amomum* and *Alpinia* in tribe Alpinieae. In addition to the six *Alpinia* clades recovered by Kress *et al.* (2005) and the three *Amomum* clades recovered by Xia *et al.* (2004), we found six further *Amomum* clades. Each of these nine *Amomum* clades plus the one *Alpinia* clade including an *Amomum* species is discussed below.

Clade I. Amomum. – The clade corresponds with the Maximum group of Xia *et al.* (2004), but that study did not include *A. subulatum* nor the type of *Elettariopsis*. We retrieved two subclades, as did Xia *et al.* (2004), one containing accessions from 12 species of *Amomum* and a second containing the type *A. subulatum*, accessions from 6 species of *Amomum* and accessions from all 7

included species of *Elettariopsis*. The circumscription of *Elettariopsis* (10 spp.) has been uncertain and controversial since it was first described in 1892 (Baker, 1892). Kam (1982) considered it closely related to *Amomum*, and Xia *et al.* (2004) confirmed both its monophyly and close relationship to *Amomum*. Our molecular analyses reconfirm the finding that *Elettariopsis* is monophyletic (1.00 PP/93 BS), but also that it is nested within *Amomum* clade I. To maintain monophyly of *Amomum*, *Elettariopsis* in its entirety needs to be merged into *Amomum*. Furthermore, all eight other clades of *Amomum* need to be excluded and recircumscribed if we want to avoid merging *Alpinia*, *Amomum*, *Elettaria*, *Elettariopsis*, *Etlingera*, *Geocharis*, *Geostachyis*, *Hornstedtia* and *Plagiostachys* into one genus.

Clade Geostachys. – This clade was also retrieved by Kress *et al.* (2007). See the next paragraph for a discussion of the morphological differences between *Geostachys* and *Lanxangia*.

Clade II. Lanxangia. – This clade was also retrieved by Xia *et al.* (2004) and referred to as the Tsaoko group. Xia *et al.* (2004) did not include *Geostachys*, but a later study by Kress *et al.* (2007) did and also resolved its position in the Alpinieae in congruence with our results. Species in this clade have either bilobed or trilobed, forked anther appendages, and are easily recognised by their smooth fruit. The leaves when crushed have a rather pleasant odour, and the tip of the labellum is entire with very thin tissue (Xia *et al.*, 2004). Species in this clade have no stilt roots and the fertile bracts support a single flower, whereas species in *Geostachys* are stilt-rooted, the inflorescences are lax with visible rachis and the fertile bracts support cincinni of two to five flowers (Holttum, 1950) except four recently described species in which bracts supporting a single flower were reported. In addition, most *Geostachys* species have secund inflorescences.

Clade III. Sundamomum. – Xia *et al.* (2004) included only *Amomum laxesquamosum* K.Schum. from this group, and found it to be sister to *Hornstedtia*, *Etlingera*, *Vanoverberghia*, and the Villosum group (cf. clade VII below). Several of the species in this clade (III) and the succeeding clades IV, VII and VIII were placed in a single group by Smith (1985, 1986a), including from clade III *A. laxesquamosum* and *A. dictyocoleum* K.Schum. Sakai & Nagamasu (1998) later added several species to this group, of which *A. calyptratum* S.Sakai & Nagam., *A. durum* S.Sakai & Nagam. and *A. somniculosum* S.Sakai & Nagam. are included in this study. Xia *et al.* (2004) noted that the unique turbinate bracteoles of the Bornean *A. laxesquamosum* were concordant with its distinctive placement. Smith (1985) noted a clearly defined alliance around *A. laxesquamosum*, but also mentioned that some species were less easily placed.

Clade IV. Conamomum. – Smith (1985, 1986a) included *A. coriaceum* and *A. xanthophlebium* from this clade in her group IV. Importantly this clade includes the type of *Conamomum* (*C. utriculosum* Ridl.), a genus described by Ridley (1899) and later merged into *Amomum* by Holttum (1950).

Clade V. – This clade contains accessions of both *Amomum* and *Elettaria*, but the type of *Elettaria*, *E. cardamomum*, is found more basally branching in the tree together with *Renealmia* and *Alpinia fax* B.L.Burtt & R.M.Sm. and *A. abundiflora* B.L.Burtt & R.M.Sm. Species in this clade are part of ongoing research by A.D. Poulsen, M. Newman, C.S. Bjorå and M. Ardiyani, and here we refrain from describing a new genus for this clade as ongoing research including wider sampling will shed more light on which species of *Elettaria* need to be included.

Clade VI. Epiamomum. – This clade includes only two species, *Amomum angustipetalum* and

A. roseisquamosum. The former was added by Sakai & Nagamasu (1998) to Smith's group II which, until then, had only the two species placed there by Smith, *A. pungens* R.M.Sm. and *A. hansenii* R.M.Sm. (Smith, 1985). The grouping was based on their lateral petals that are centrally connate to each other and to the labellum in the lower part. The other species, *A. roseisquamosum*, described by Sakai & Nagamasu (1998), was treated as incertae sedis as it did not fit the groups defined by Smith (1985). However, they note that the aberrant flower morphology might be due to its rare spider-hunter bird pollination syndrome (Sakai & Nagamasu, 1998).

Clade VII. Wurfbainia. – The most prominent character of the species in this clade is the typical anther structure with a crest composed of three small lobes, of which the side lobes usually point upwards and the mid lobe is positioned behind the stigma, giving it an eared appearance. In the phylogenetic study of Xia *et al.* (2004), these taxa were placed in the tentative Villosum group with echinate fruit, but together with species from clade VIII below and an accession misidentified as *Etlingera littoralis* (J.Koenig) Giseke as explained by Pedersen (2004). Most species in this clade VII have echinate fruit, except for *A. compactum*, *A. testaceum* Ridl. and *A. verum* that have a finely ribbed fruit when dry.

Clade VIII. Meistera. – Taxa in this clade were also found to be monophyletic by Xia *et al.* (2004) and were placed in the tentative Villosum group together with taxa in clade VII. Most members of this clade have clearly echinate fruit, except *A. cerasinum*, *A. koenigii* and *A. tomrey* Gagnep. var. *tomrey* that have fruit that is smooth both when fresh and dry, and *A. mentawaiense* A.J.Droop and *A. ochreum* Ridl. that have only sparsely echinate fruit. Xia *et al.* (2004) also noted that the smooth fruit of *A. koenigiii* were at odds with the rest of the Villosum group, and likened the appearance of the capsule shape to that of the Tsaoko group type (clade II).

Clade Hornstedtia I. – This clade comprises two species that were recognised as distinct from *Amomum* by Schumann (1904). They both have radical, fusiform inflorescences, which distinguish them from all clades of *Amomum*.

Clade IX. – This clade includes accessions of four species, two in *Amomum* and two in *Hornstedtia*. The two *Amomum* species in this clade, *A. apiculatum* and *A. centrocephalum*, are endemic to Sumatra and it has been noted that their morphology is ambiguous within *Amomum* (Droop & Newman, 2014). *Hornstedtia* is polyphyletic and the last common ancestor of the species described as *Hornstedtia* includes *Etlingera*, *Amomum* clade VIII, and *Alpinia* clade V sensu Kress *et al.* (2005). Combined molecular phylogenetic and morphological studies of the remaining two clades of *Hornstedtia* are under way by A.D. Poulsen and Nurainas.

Clade Etlingera+Hornstedtia II. – This clade contains species that may be distinguished from all *Amomum* clades by the presence of a pronounced staminal tube. The relationship between *Etlingera* and *Hornstedtia leonurus* requires further investigation.

Clades Alpinia V, Alpinia III and Alpinia VI. – These clades, which are the *Alpinia eubractea* clade, the *Alpinia carolinensis* clade, and the *Alpinia rafflesiana* clade respectively of Kress *et al.* (2007), consist of species that can be clearly distinguished from the *Amomum* clades by their terminal inflorescences.

Clade Alpinia IV. – *Amomum trianthemum* K.Schum. is included in clade *Alpinia* IV, and

combined molecular phylogenetic and morphological studies of the species in this clade are under way by N. Sharp, A.D. Poulsen, M. Newman and M. Ardiyani.

Historical classifications in light of the phylogeny

A detailed overview of the historical classifications (incl. diagnostic characters and included species), as well as indication of placement of the species as supported by the present study is given in Table S1 (Suppl.).

• Schumann (1899) applied *Amomum* in a very broad sense. Of the 47 names he treated, only 8 were recognised as *Amomum* s.l. prior to our study (11 fall into *Amomum* s.l. clades in our study), while the vast majority are now classified in other genera of Alpinieae, mainly *Alpinia*, *Etlingera* and *Hornstedtia*. The infrageneric classification he proposed divided *Amomum* into five subgenera (*A.* subg. *Amomum* [*Autamomum*], *A.* subg. *Botryamomum* K.Schum., *A.* subg. *Hornstedtia* (Retz.) K.Schum., *A.* subg. *Mastigamomum* K.Schum., *A.* subg. *Nicolaia* (Horan.) K.Schum.). With the possible exception of *A.* subg. *Nicolaia* (consisting only of species now in *Etlingera*), none of the proposed subgenera is monophyletic. *Amomum* subg. *Hornstedtia* comprises mainly species currently placed in *Hornstedtia*, and two species in *Etlingera*. *Amomum* subg. *Amomum* [*Autamomum*] was further divided into four series (*A.* ser. *Densiflorae* K.Schum., *A.* ser. *Multiflorae* K.Schum., *A.* ser. *Laxiflorae* K.Schum., *A.* ser. *Pauciflorae* K.Schum.) and included four species of *Amomum* from our clade III *Sundamomum*, and one species each from clade I *Amomum* and clade VII *Wurfbainia*. The remaining species are now recognised in other genera or are unplaced here. *Amomum* subg. *Botryamomum* was based on three species now recognised as *Alpinia*, *Etlingera* and *A. villosum* (clade VII *Wurfbainia*). *Amomum* subg. *Mastigamomum* is composed of three species currently placed in *Elettaria* and falling into our clade V, and *A. gracile* Blume (clade VII *Wurfbainia*).

• Schumann (1904), after revising *Amomum* over its entire geographical range, practically abandoned his previous classification of 1899 that was based on Malaysian and Papuan species only. He removed the four subgenera *Hornstedtia*, *Nicolaia*, *Botryamomum* and *Mastigamomum* from *Amomum*. *Hornstedtia* was raised to generic rank, while species in the remaining subgenera were transferred to other genera (*Phaeomeria*, *Alpinia* and *Cyphostigma* respectively), with the exception of two species (*A. gracile*, *A. villosum*), which were transferred to *A.* sect. *Amomum* ser. *Lobulatae*. His new concept of *Amomum* included 86 species, of which 39 are still currently recognised in other genera or remain unplaced. In his new attempt, Schumann recognised two sections, each further subdivided into two series, and this classification represents the latest formal attempt at an infrageneric classification. As seen in Table S1 (Suppl.), neither of the two sections is monophyletic, and three of the four series are also clearly polyphyletic. *Amomum* sect. *Geanthus*, characterised by a lack of anther appendages, is composed of *A.* ser. *Oliganthae* K.Schum. and *A.* ser. *Polyanthae* K.Schum. Of these, *A.* ser. *Oliganthae* consisted of four species, now all placed in *Etlingera* and of similar morphology, and thus possibly monophyletic. *Amomum* ser. *Polyanthae* also mainly consists of species now recognised in *Etlingera*, but includes also species from our clades III *Sundamomum* and VII *Wurfbainia*, as well as two *Alpinia* species (retrieved in clade *Alpinia* I). *Amomum* sect. *Amomum* (with anther appendages) was subdivided into *A.* ser. *Integrae* (anther appendage entire) and *A.* ser. *Lobulatae* (anther bi- or trilobed). These

two series contain 44 species retrieved in our *Amomum* clades. Although both series are heavily polyphyletic with species from clades III, VII and VIII, it is notable that species in clades I and VI occur only in *A.* ser. *Integrae* (see Suppl. Table S1). Schumann also placed species now accepted in other genera (mainly *Hornstedtia* and *Etlingera*) in both series of *A.* sect. *Amomum*.

• Loesener (1930) adopted Schumann's classification of 1904 with certain modifications, and proposed placement of several species described since then. The most significant change in Loesener's classification was to accept *Achasma* Griff. and *Geanthus* Reinw. at generic rank leaving a more narrowly circumscribed *Amomum* comprising Schumann's *A.* ser. *Integrae* and *A.* ser. *Lobulatae*.

• Smith (1985) proposed an informal grouping of *Amomum* species in Borneo which, she noted, displayed considerable diversity of form and because several species deviate from what may be termed "typical" *Amomum*. She proposed at first four groups, but later added a fifth (Smith, 1989). These groups exclude species of *Achasma* and *Geanthus* that Smith (1986b) placed in synonymy under *Etlingera*. All five species included in Group I (characterised by cincinnate flowers) were retrieved in clade V in our study. Groups II and V consisted of a few species each, and all were retrieved or designated based on morphology in our clade VI *Epiamomum*. Group III was created for a specimen *Anderson S30713* (E), which Smith identified as *Amomum sarawacense* K.Schum. However, the type of *A. sarawacense* was never located and it remains doubtful whether the above specimen and *A. sarawacense*, which Schumann transferred into *Hornstedtia*, are the same. Our examination of *Anderson S30713* indicates that this collection represents a species belonging to our clade VI *Epiamomum*. Group IV contained the majority of Bornean species and, based on our studies, is highly polyphyletic, consisting of species retrieved in clades III *Sundamomum*, IV *Conamomum*, VII *Wurfbainia* and VIII *Meistera*.

• Sakai & Nagamasu (1998) added only a few newly described species to the framework built by Smith, but were unable to place the two species, *Amomum roseisquamosum* and *A. bilabiatum* into any of the groups. In our phylogeny, *A. roseisquamosum* was retrieved in clade VI *Epiamomum*, but *A. bilabiatum* S.Sakai & Nagam. was not included and remains unplaced.

Informative morphological characters

Of the various morphological characters, we have investigated (habit, type of leafy shoot, presence of petiole, number of flowers supported by fertile bract, presence and shape of bracteole, flower type, calyx, shape and colouration of the labellum, presence and shape of staminodes, presence of staminal tube, anther crest shape, fruit type), the two most informative across all clades were the shape of the anther crest and the fruit type. Anther morphology has been utilised extensively in Zingibereae classification. The presence and shape of anther appendages have proven useful in the infrageneric classification of *Globba* (Williams *et al.*, 2004). Similarly, the overall shape of anther, anther crest and, in particular, anther spurs are considered informative in *Curcuma* (Záveská *et al.*, 2012). Although the anther morphology was not studied in Alpinieae in great detail before, the presence and absence of anther crest and number of crest lobes had already been utilised in the infrageneric classification of Schumman (1904). Our work shows that further refinement of the anther crest shape enables better circumscription of the clades (see below). The value of fruit characters in Alpinieae was overlooked by early authors, but has been highlighted

by all recent studies dealing with *Amomum* and related genera (Xia *et al.*, 2004; Kress *et al.*, 2005, 2007; Poulsen, 2006, 2012).

Of the other characters we have examined, some proved to be partially informative, being consistent in certain clades, but variable in others. Cincinnate inflorescences appear consistently in *Geostachys*, where they provide an additional diagnostic character to distinguish it from clade II *Lanxangia*, as well as in clade V *Sundamomum* and *Geocharis*. In clade I *Amomum*, however, one subclade has cincinnate inflorescences while the others have inflorescences composed of bracts supporting single flowers, so this character may be taxonomically informative only at subgeneric rank. The presence and shape of the bracteole, a character which has been traditionally utilised to distinguish *Amomum* and *Elettariopsis*, is consistent only in some clades (tubular in clades *Geostachys*, II *Lanxangia*, *Geocharis*, clade III *Sundamomum* and VII *Wurfbainia* and VIII *Meistera*), but can be variously tubular or open to the base in all other clades including clade I, where it can even be missing. The presence and absence of staminodes is also consistent only in some clades (missing in *Geostachys*, clade II *Lanxangia* and clade III *Sundamomum*), always present and adnate to the filament in *Geocharis*, but variously present or absent in all other clades.

Although certain characters are consistent only in some clades, they may be used in combination to permit morphological circumscription of the clades as genera in this study.

Stigma morphology has been suggested as a potentially informative character though the respective authors examined geographically restricted ranges of species from Thailand (Kaewsri & Paisooksantivatana, 2007) and Sumatra (Droop & Newman, 2014), and we have been unable to apply their results to all the taxa we have studied. Low magnification SEM of critical point dried stigmas proved useful revealing infrageneric variation of *Etlingera* (Poulsen, 2006, 2012). The stigma does not preserve well in dry herbarium material and its morphology has not been described or illustrated in protologues in a sufficiently standardised manner to allow precise scoring in most taxa. Preliminary observations of taxa represented in our own collections are not a sufficient sample to allow sound and valid conclusions for all taxa in each clade but do indicate that a detailed investigation of *Amomum* s.l./Alpinioideae would be worth pursuing. Such a study would have to involve broad sampling and careful imaging from various angles of the stigma from living and spirit material, and would also have to take into account the occurrence of flexistyly (stylar movement) in order to assess the precise position of the ostiole on the stigma.

Fruit types and anther crests

Fruit type variation in Alpinieae is probably related to seed dispersal but this is poorly studied in Zingiberaceae (Zhou *et al.*, 2007; García-Robledo & Kuprewicz, 2009). Fleshy fruits in *Amomum* are, however, indicative of an adaptation to vertebrate seed dispersal (Howe & Smallwood, 1982). Fleshy fruits range from smooth, grooved, winged to echinate. The function of the fruit wings (clade I *Amomum*) is unclear but, in species that develop their fruit underground, the wings are reduced and the fruit is angled to grooved, rarely almost smooth. The function of the spines of echinate fruits is also unknown but, in our opinion, they are likely to prevent consumption of the fruits before they are ripe and split easily. A reduction of the spines leads to almost smooth fruit, and occurs in both clades with echinate fruits (clades VII *Wurfbainia* and VIII *Meistera*), but here the fruits appear well above ground, unlike those of winged species so the occurrence of smooth

fruits in these unrelated clades might be for a different purpose.

Classifications of the Alpinieae have been based on different morphological characters. The latest molecular phylogenies (Xia *et al.*, 2004; Kress *et al.*, 2005, 2007) have recognised the importance of fruit morphology and introduced a tentative new classification based on the fruit types in the Maximum, Villosum and Tsaoko groups. Morphogical character scoring and plotting of the fruit type and anther crest morphology on the phylogenies show the relevance of the combination of these two characters for classification in Alpinieae (Fig. 1). Fruit types in *Amomum* clade I (i.e., the Maximum group of Xia *et al.*, 2004 with the addition of *Elettariopsis*) range from grooved to angled to winged, and anther crests are largely fan-shaped with some taxa having extended or acute crests. All taxa sampled in *Amomum* clade II, which corresponds to the Tsaoko group of Xia *et al.* (2004), have smooth fruit when dry and forked 2- to 3-lobed anther crests. These characters are shared with *Geostachys*, but the single flower per bract distinguishes the former from the latter. Fruit types in *Amomum* clade III are grooved to ribbed, and anther crests extended with lobules, except in *A. dictyocoleum* which has ecristate anthers. All taxa sampled in *Amomum* clade IV have smooth fruit and typical, horned anther crests. Smooth fruit when dry characterises *Amomum* clade V, but anther crests vary from ecristate in *A. anomalum* to short and entire in *A. dimorphum*, to 4-lobed in the *Elettaria* species of this clade. *Amomum* clade VI taxa also has smooth fruit when dry, but the anther crest is short and more or less obscurely three-lobed. *Amomum* clade VII is highly consistent and characterised by echinate fruit and eared or crown-shaped anther crests. The only notable exceptions are *A. compactum*, *A. testaceum* and *A. verum* that have finely ribbed fruit when dry, but the same typical anther crests. Most taxa of *Amomum* clade VIII have echinate fruit but certain species show variation in fruit type from lesser echinate to smooth. The anther crests are mainly semilunar-entire or semilunar 3-lobed, only very rarely is the crest reduced, short and entire.

Distribution and biogeography

Amomum and allied genera of Alpinieae range from Sri Lanka, southern and eastern India through southern China and Southeast Asia to New Guinea and Queensland (Fig. 2). The highest diversity of *Amomum* clades is found in Borneo where all except *Amomum* clade II *Lanxangia* occur sympatrically. Species diversity and clade diversity do not overlap; the highest diversity of species is found in mainland southeast Asia among species in *Amomum* clades I, VII and VIII (cf. Fig. 1). Most clades are widespread across the Sunda shelf, except for *Amomum* clade II *Lanxangia*, which is restricted to southern China, northern Thailand, northern Laos and northern Vietnam, and clade VI *Epiamomum*, which is restricted to Borneo. The greatest diversity of *Amomum* clade I *Amomum* is found in NE India and the Indochinese floristic region, with several species in the *A. maximum* Roxb. alliance extending to Sundaland. No species occurs across Wallace's Line (Wallace, 1869; Mayr, 1944) in Sulawesi and the Philippines but a few species in the *A. maximum* alliance occur further east still, in New Guinea and wet tropical Australia (Queensland). This apparent disjunction could be due to long-distance dispersal but, as these taxa have disjunct distributions, it may be more likely to have occurred by anthropogenic transport across Wallace's Line to the Sahul shelf, New Guinea and onwards to northern Queensland. *Amomum* clade II *Lanxangia* is a small group of species that is geographically restricted to

southern China (Yunnan), northern Laos and Vietnam where all are montane species reported to occur between 1100 and 1800 m asl. Their adaptation to montane habitats would have limited natural dispersal through continuous lowland forests of the Sunda shelf during Pleistocene climate oscillations, as this would have been outside their niche. *Amomum* clade III *Sundamomum* includes 14 species that are mainly distributed in Borneo, Sumatra and West Java, with *Amomum* (*Sundamomum*) *hastilabium* extending to Peninsular Malaysia and southern Thailand, almost reaching the Isthmus of Kra. *Amomum* clade IV *Conamomum* consists of about 10 species distributed in primary evergreen lowland and montane forests from Indochina to the Malay Peninsula and Singapore, Borneo and Sumatra. Some of the species have disjunct distributions including both mainland southeast Asia and the Malay archipelago (such as *Amomum xanthophlebium*). *Amomum* clade VI *Epiamomum* is a small group of six Bornean species that are

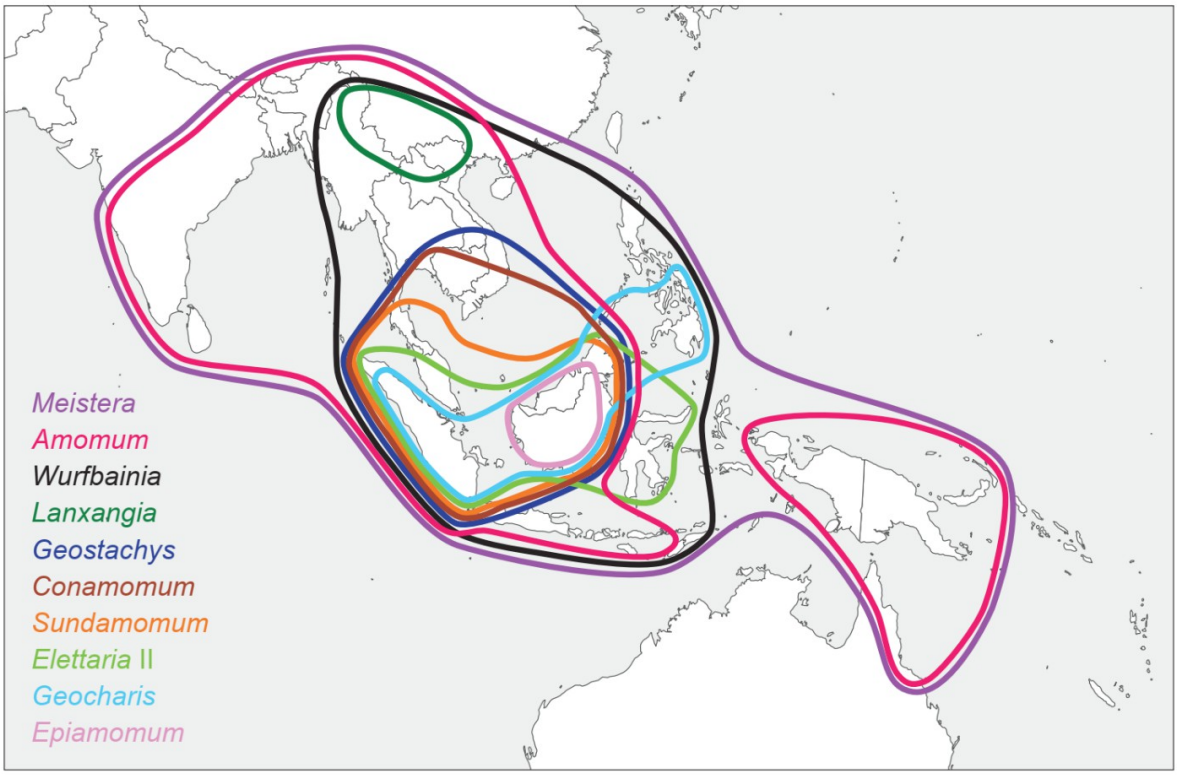


Fig. 2. Distribution ranges of *Amomum* and allied genera.

mostly known only from Sarawak, except *E. angustipetalum* that extends to Brunei and *E. borneense* that is known to occur also in Kalimantan. Several species in this clade have been described only recently, and it is conceivable that more undescribed species of this clade exist in Borneo. *Amomum* clade VII *Wurfbainia* is a large group that is most diverse in the Indochinese floristic region with only a few, often cultivated, species extending into Sundaland, the Philippines and one or two species across Wallace's Line to Sulawesi. *Amomum* clade VIII *Meistera* is both species-rich and the most widespread genus of *Amomum* s.l. It is distributed from India and Sri Lanka, throughout the Indochinese region to Sundaland, with *A. aculeatum* extending further across Wallace's Line to Sulawesi, New Guinea and Australia. *Amomum aculeatum* has a disjunct distribution across Wallace's Line to the Sahul shelf, New Guinea and

onwards to northern Queensland, and this distribution is more likely due to anthropogenic transport than to long-distance dispersal. However, the cause of these disjunctions remains to be tested.

TAXONOMIC TREATMENT

Key to *Amomum* and allied genera in Alpinieae

1. Bracts subtending single flowers.....2
(*Amomum* p.p., *Conamomum*, *Epiamomum*, *Geocharis*, *Lanxangia*, *Meistera*, S*undamomum*, *Wurfbainia*)

1. Bracts subtending 2 or more flowers (or at least in the lowermost bracts, i.e., *Geocharis*)...12
(*Amomum* p.p. – *chryseum*-to-*plicatum* clade, *Elettaria II*, *Geocharis*, *Geostachys*)

2. Fruit echinate or winged or angled.....3
(*Amomum* p.p., *Meistera*, *Wurfbainia*)

2. Fruit otherwise (smooth, ribbed when dry, grooved or grooved-rugose).....5
(*Amomum* p.p., *Conamomum*, *Epiamomum*, *Lanxangia*, *Meistera*, *Sundamomum*, *Wurfbainia*)

3. Fruit echinate.....4
(*Meistera*, *Wurfbainia*)

3. Fruit winged (at least partly), or angled to grooved..... ***Amomum*** p.p.
(all inclusive *Elettariopsis*, excl. *A. sericeum*)

4. Anther crest semilunar entire to semilunar 3-lobed..... ***Meistera***

4. Anther crest eared/crown-shaped .....***Wurfbainia*** p.p.

5. Anther crest 3-lobed with narrowly acute and down facing side lobes (horned).....***Conamomum***

5. Anther crest other than above 6
(*Amomum* p.p., *Epiamomum*, *Lanxangia*, *Meistera*, *Sundamomum*, *Wurfbainia*)

6. Anther crest eared/crown-shaped.....***Wurfbainia*** p.p.

6. Anther crest other than above 7
(*Amomum* p.p., *Epiamomum*, *Lanxangia*, *Meistera*, *Sundamomum*)

7. Anther crest obscurely 3-lobed with side lobes presented as thickened margins of the midlobe (extended with lobules), rarely anther ecristat.....***Sundamomum***

7. Anther crest other than above 8
(*Amomum* p.p., *Epiamomum*, *Lanxangia*, *Meistera*)

8. Bracteole tubular 9
(*Amomum* p.p., *Lanxangia*, *Meistera*)

8. Bracteole open (occurs only in Borneo)..... ***Epiamomum***

9. Fruit subglobose, grooved with irregular shoulders, apex somewhat depressed.....***Amomum*** p.p. (*Amomum sericeum* complex)

9. Fruit smooth, globose or ellipsoid.....10
(*Amomum* p.p., *Lanxangia*, *Meistera*)

10. Stilt roots present ..... ***Geostachys*** p.p.

10. Stilt roots absent 11
(*Lanxangia*, *Meistera* p.p.)

11. Staminodes present, small (2–7 mm) triangular or elongate.....*Meistera* (spp. with smooth fruits)

11. Staminodes absent (occurs only in S China, N Laos and N Vietnam).....***Lanxangia***

12. Staminodes connate to filament; staminodial tube always present).....***Geocharis***

12. Staminodes small triangulate or oblong, or absent, but never connate to filament; staminodial tube absent or present.....13
(*Amomum* p.p. – *chryseum*-to-*plicatum* clade, *Elettaria* II, *Geostachys*)

13. Fruit winged.....***Amomum*** p.p.

13. Fruit smooth.....14
(*Elettaria* II, *Geostachys*)

14. Inflorescence creeping, plants never stilt-rooted.....***Elettaria*** II p.p.

14. Inflorescence erect or decurved.....15
(*Elettaria* II p.p. – *A. dimorphum* and *A. anomalum*; *Geostachys*)

15. Anther crest well-developed, semilunar with more or less prominent two to three lobes.....***Geostachys***

15. Anther ecristate or with minute crest reduced to a small ridge.....***Elettaria*** II p.p.

Taxonomic recircumscription of *Amomum* and allied genera in Alpinieae

Amomum Roxb., Pl. Coromandel 3: 75. 1820, nom. cons. – Type: *A. subulatum* Roxb.

= *Geocallis* Horan., Prodr. Monogr. Scitam.: 33. 1862 – Type: *G. fasciculata* Horan.

= *Elettariopsis* Baker in Hooker, Fl. Brit. India 6: 251. 1892 – Type (designated by Holttum in Gard. Bull. Singapore 13: 215. 1950): *E. curtisii* J.G.Baker.

= *Paramomum* S.Q.Tong in Acta Bot. Yunnan. 7(3): 309–310. 1985 – Type: *P. petaloideum* S.Q.Tong. Fig. 1, clade I; Fig. 3.

Description. – Small to large-sized herbs, clump-forming to loosely clump-forming, rarely creeping. Leafy shoots composed of fewer than 10 leaves, almost always arranged in "palmate" rather than distichous fashion; leaves mostly petiolate (petioles up to 25 cm long). Peduncles usually short, creeping or ascending, rarely longer and erect (e.g., *A. putrescens*). Flowering heads few- to many-flowered, but almost always compact. Fertile bracts supporting either a single flower or a cincinnus of up to five flowers, soon decaying with age (not persisting to the fruiting stage). Bracteoles mainly open, but tubular in several species and rarely completely missing. Labellum white with a yellow patch in the centre and red marking, or yellow with or without red markings. Flowers mainly of the exposed type (for flower types see Leong-Skornickova & Newman, 2015), rarely approaching the gullet type. Small staminodes present but, in species previously classified in *Elettariopsis,* almost always absent. Anthers with a well-developed fan-shaped and more or less obscurely trilobed crest usually broader than long or extended, longer than wide and often bluntly rectangular (in most species previously classified as *Elettariopsis)* (Fig. 3). Fruit of most species more or less winged, at least in upper half, rarely also grooved *(Amomum sericeum)* or angled to grooved with a smooth to rugose surface (mainly species previously classified as *Elettariopsis).* The loss of wings seems to be connected to the fact that fruits of these species develop underground.

Distribution. – *Amomum*, as recircumscribed here now consists of approximately 64 species of which almost 30 were previously recognised as *Elettariopsis*. The greatest diversity of *Amomum* is found in NE India and the Indochinese floristic region, with several species in the *A. maximum* alliance extending to Sundaland. There seems to be a disjunction with no species occurring in Sulawesi and the Philippines, although a few species from the *A. maximum* alliance occur again in New Guinea and wet tropical Australia (northern Queensland).

Etymology. – Greek, *amomon*, an Indian spice.

Note. – Lamxay & Newman (2012) interpreted *Geocallis fasciculata* as probably conspecific with *A. aromaticum* Roxb. The shape of the rhizome, long ligules with sharp apices, flower shape, and gregarious flowering in masses depicted in the drawing (which is the only original element in existence), lead us to believe that the plant represents *A. maximum*.

Amomum andamanicum V.P.Thomas, Dan & M.Sabu in Blumea 55(3): 295, fig. 1, pl. 1, map. 2010.
Amomum argyrophyllum Ridl. in J. Fed. Malay States Mus. 10: 119. 1920.
Amomum billburttii Škorničk. & Hlavatá, **nom. nov.** ≡ *Elettariopsis burttiana* Y.K.Kam in Notes Roy. Bot. Gard. Edinburgh 40(1): 144. 1982.
Amomum biphyllum (Saensouk & P.Saensouk) Škorničk. & Hlavatá, **comb. nov.** ≡ *Elettariopsis biphylla* Saensouk & P.Saensouk in Phytotaxa 159(1): 23. 2014.
****Amomum calcicolum*** Lamxay & M.F.Newman in Edinburgh J. Bot. 69(1): 113–116, fig. 3. 2012.
Amomum carnosum V.P.Thomas & M.Sabu in Kew Bull. 67: 549. 2012.
Amomum chayanianum (Yupparach) Škorničk. & Hlavatá, **comb. nov.** ≡ *Elettariopsis chayaniana* Yupparach in Acta Bot. Yunnan. 30(5): 525–527, fig. 1–5. 2008.
Amomum chevalieri Gagnep. ex Lamxay in Edinburgh J. Bot. 69(1): 119–121. 2012.

Amomum chongeui (C.K.Lim) Škorničk. & Hlavatá, **comb. nov.** ≡ *Elettariopsis chong-eui* C.K.Lim in Fol. Malaysiana 14(2): 11. 2014 (“2013”).
****Amomum chryseum*** Lamxay & M.F.Newman in Edinburgh J. Bot. 69(1): 124–125, fig. 8. 2012.
Amomum fragile S.Q.Tong in Acta Phytotax. Sin. 27(4): 277. 1989.
Amomum fragrans Škorničk. & Hlavatá, **nom. nov.** ≡ *Elettariopsis perakensis* C.K.Lim in Fol. Malaysiana 14(2): 8. 2014 (“2013”).
Amomum garoense S.Tripathi & V.Prakash in Rheedea 9(2): 177. 1999.
****Amomum glabrum*** S.Q.Tong in Acta Phytotax. Sin. 27(4): 282. 1989.
Amomum hochreutineri Valeton in Boerlage, Icon. Bogor. 2: 311, t. 195. 1906.
Amomum latiflorum (Ridl.) Škorničk. & Hlavatá, **comb. nov.** ≡ *Elettariopsis latiflora* Ridl. in J. Straits Branch Roy. Asiat. Soc. 32: 154. 1863.
Amomum limianum (Picheans. & Yupparach) Škorničk. & Hlavatá, **comb. nov.** ≡ *Elettariopsis limiana* Picheans. & Yupparach in J. Jap. Bot. 87(2): 87. 2012.
****Amomum longipetiolatum*** Merr. in Lingnan Sci. J. 11: 40.
1932 ≡ *Elettariopsis longipetiolata* (Merr.) D.Fang in Guihaia 10(4): 293. 1990.
****Amomum maximum*** Roxb. in Asiat. Res. 11: 344. 1810.
Amomum meghalayense V.P.Thomas, M.Sabu & Sanoj in Phytotaxa 245(2): 178. 2016.
Amomum menglaense S.Q.Tong in Acta Bot. Yunnan. 13(3): 277. 1991.
Amomum mengtzense H.T.Tsai & P.S.Chen in Acta Phytotax. Sin. 17(4): 91. 1979.
Amomum mizanianum (C.K.Lim) Škorničk. & Hlavatá, **comb. nov.** ≡ *Elettariopsis mizaniana* C.K.Lim in Fol. Malaysiana 14(2): 13. 2014 (“2013”).
Amomum monophyllum Gagnep. in Bull. Soc. Bot. France 54: 163. 1907 ≡ *Elettariopsis monophylla* (Gagnep.) Loes. in Engler & Prantl, Nat. Pflanzenfam., ed. 2, 15a: 603. 1930.
****Amomum odontocarpum*** D.Fang in Acta Phytotax. Sin. 18(2): 224. 1980.
Amomum pauciflorum Baker in Hooker, Fl. Brit. India 6: 238. 1892.
Amomum petaloideum (S.Q.Tong) T.L.Wu in Novon 7: 411. 1998 (“1997”) ≡ *Paramomum petaloideum* S.Q.Tong in Acta Bot. Yunnan. 7(4): 309. 1985.
****Amomum plicatum*** Lamxay & M.F.Newman in Edinburgh J. Bot. 69(1): 157–160, fig. 21, 22. 2012.
Amomum poonsakianum (Picheans. & Yupparach) Škorničk. & Hlavatá, **comb. nov.** ≡ *Elettariopsis poonsakiana* Picheans. & Yupparach in J. Jap. Bot. 87(2): 87. 2012.
****Amomum prionocarpum*** Lamxay & M.F.Newman in Edinburgh J. Bot. 69(1): 160–163, fig. 22, 23. 2012.
****Amomum pterocarpum*** Thwaites, Enum. Pl. Zeyl.: 317. 1861.
= *Amomum microstephanum* Baker in Hooker, Fl. Brit. India 6: 239. 1892.
Amomum puberulum (Ridl.) Škorničk. & Hlavatá, **comb. nov.** ≡ *Elettariopsis puberula* Ridl. in Bull. Misc. Inform. Kew 1926(2): 88. 1926.
****Amomum purpureorubrum*** S.Q.Tong & Y.M.Xia in Acta Bot. Yunnan. 10(2): 207. 1988.
****Amomum putrescens*** D.Fang in Acta Phytotax. Sin. 16(3): 51. 1978.
****Amomum queenslandicum*** R.M.Sm. in Notes Roy. Bot. Gard. Edinburgh 38(3): 521. 1980.
Amomum ranongense (Picheans. & Yupparach) Škorničk. & Hlavatá, **comb. nov.** ≡ *Elettariopsis ranongensis* Picheans. & Yupparach in J. Jap. Bot. 87(2): 91. 2012.
Amomum repoeense Pierre ex Gagnep. in Bull. Soc. Bot. France 53: 144. 1906.
Amomum robertsonii Craib in Bull. Misc. Inform. Kew 1913(3): 117. 1913.

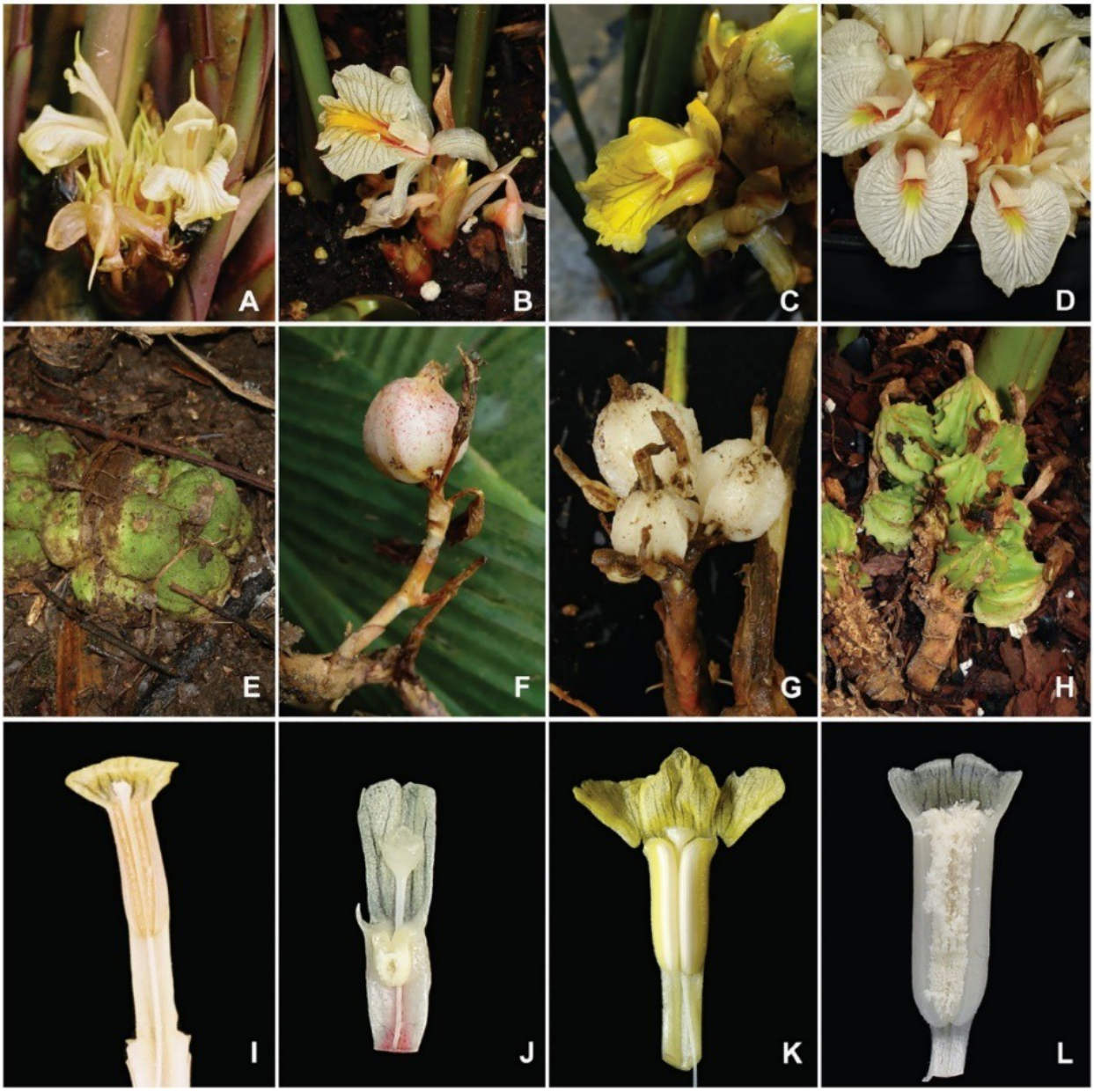


Fig. 3. *Amomum.* **A,** *Amomum subulatum* (flower); **B,** *Amomum trilobum* (flower); **C,** *Amomum putrescens* (flower); **D,** *Amomum pterocarpum* (flower); **E,** *Amomum sericeum* (grooved fruit); **F,** *Amomum curtisii* (angled to grooved fruit); **G,** *Amomum* aff. *repoeense* (winged fruit); **H,** *Amomum pterocarpum* (winged fruit); **I,** *Amomum subulatum* (stamen with a fan-shaped obscurely 3-lobed anther crest); **J,** *Amomum trilobum* (stamen with extended anther crest); **K,** *Amomum putrescens* (stamen with a broadly fan-shaped obscurely 3-lobed anther crest); **L,** *Amomum pterocarpum* (stamen with a fan-shaped obscurely 3-lobed anther crest). Photographs: A-L, Jana Leong-Skornickova.

***Amomum rugosum** (Y.K.Kam) Škorničk. & Hlavatá, **comb. nov.** ≡ *Elettariopsis smithiae* var. *rugosa* Y.K.Kam in Notes Roy. Bot. Gard. Edinburgh 40(1): 150. 1982 ≡ *E. rugosa* (Y.K.Kam) C.K.Lim in Fol. Malaysiana 4(3–4): 217. 2003.

***Amomum sericeum** Roxb., Fl. Ind. 1: 45. 1820 ≡ *A. dealbatum* var. *sericeum* (Roxb.) Baker in Hooker, Fl. Brit. India 6: 239. 1892.

Amomum siamense Craib in Bull. Misc. Inform. Kew 1912(10): 402. 1912.

Amomum slahmong (C.K.Lim) Škorničk. & Hlavatá, **comb. nov.** ≡ *Elettariopsis slahmong*

C.K.Lim in Fol. Malaysiana 4(3–4): 223. 2003.
Amomum smithiae (Y.K.Kam) Škorničk. & Hlavatá, **comb. nov.** ≡ *Elettariopsis smithiae* Y.K.Kam in Notes Roy. Bot. Gard. Edinburgh 40(1): 148. 1982.
****Amomum stenosiphon*** K.Schum. in Bot. Jahrb. Syst. 27(3): 320. 1899 ≡ *Elettariopsis stenosiphon* (K.Schum.) B.L. Burtt & R.M.Sm. in Notes Roy. Bot. Gard. Edinburgh 31(2): 312. 1972.
****Amomum subcapitatum*** Y.M.Xia in Acta Phytotax. Sin. 35(3): 259. 1997.
****Amomum subulatum*** Roxb., Fl. Ind. 1: 43. 1820.
Amomum sumatranum (Valeton) Škorničk. & Hlavatá, **comb. nov.** ≡ *Elettariopsis sumatrana* Valeton in Bull. Jard. Bot. Buitenzorg, sér. 3, 3: 148. 1921.
****Amomum trilobum*** Gagnep. in Bull. Soc. Bot. France 51: 453. 1904 ≡ *Elettariopsis triloba* (Gagnep.) Loes. in Engler & Prantl, Nat. Pflanzenfam., ed. 2, 15a: 603. 1930.
****Amomum unifolium*** Gagnep. in Bull. Soc. Bot. France 54: 403. 1907 ≡ *Elettariopsis unifolia* (Gagnep.) M.F.Newman in Edinburgh J. Bot. 54(1): 111. 1997.
Amomum wandokthong (Picheans. & Yupparach) Škorničk.& Hlavatá, **comb. nov.** ≡ *Elettariopsis wandokthong* Picheans. & Yupparach in Taiwania 55(4): 244. 2010.
Amomum yingjiangense S.Q.Tong & Y.M.Xia in Acta Bot. Yunnan. 10(2): 210. 1988.

Conamomum Ridl. in J. Straits Branch Roy. Asiat. Soc. 32: 121. 1899 – Type (designated by Turner in Asian J. Trop. Biol. 4: 7. 2000): *C. utriculosum* Ridl. Fig. 1, clade IV; Fig. 4.

Description. – Medium-sized to large, clump-forming plants with distichous leafy shoot consisting of sessile leaf blades. Young leaves often with a pink or red tinge underneath, fading with age. Bracts coriaceous, becoming brown and papery with age and persisting until fruiting (unlike those of *Lanxangia*), supporting a single flower; bracteoles tubular or split to base (when open, bracteoles broad and overlapping, enclosing base of the flower entirely). Flowers of gullet type or almost so, labellum yellow or yellow with red markings and small staminodes. Anther crest distinctly 3-lobed, lateral narrow lobes pointing downwards, and usually a small mid lobe positioned just behind the stigma, rarely reduced (e.g., in Bornean *A. coriaceum*). Fruits smooth, with appressed hair.

Distribution. – About 10 species distributed in primary evergreen lowland and montane forests from Indochina to the Malay Peninsula and Singapore, Borneo and Sumatra.

Etymology. – Ridley did not explain this name. Perhaps it refers to the shape of the inflorescence. Greek, *konos*, a pinecone or a geometrical cone, plus *amomon*.

Conamomum citrinum Ridl. in J. Straits Branch Roy. Asiat. Soc. 32: 121. 1899 ≡ *Amomum citrinum* (Ridl.) Holttum in Gard. Bull. Singapore 13(1): 207. 1950. = *Amomum cylindrostachys* Ridl. in J. Straits Branch Roy. Asiat. Soc. 61: 42. 1912.
Conamomum cylindraceum (Ridl.) Škorničk. & A.D.Poulsen, **comb. nov.** ≡ *Amomum cylindraceum* Ridl. in J. Straits Branch Roy. Asiat. Soc. 32: 136. 1899.
****Conamomum cylindrostachys*** (K.Schum.) Škorničk. & A.D. Poulsen, **comb. nov.** ≡ *Alpinia cylindrostachys* K.Schum. in Bot. Jahrb. Syst. 27(3): 299. 1899 ≡ *Languas cylindrostachys* (K.Schum.) Merr. in Univ. Calif. Publ. Bot. 15: 34. 1929 ≡ *Amomum coriaceum* R.M.Sm. in Bot.

J. Linn. Soc. 85(1): 61, fig 15a. 1982.

Conamomum flavidulum (Ridl.) Škorničk. & A.D.Poulsen, **comb. nov.** ≡ *Amomum flavidulum* Ridl. in J. Straits Branch Roy. Asiat. Soc. 46: 236. 1906.

****Conamomum pierreanum*** (Gagnep.) Škorničk. & A.D. Poulsen, **comb. nov.** ≡ *Amomum pierreanum* Gagnep. in Bull. Soc. Bot. France 53: 143. 1906.

Conamomum rubidum (Lamxay & N.S.Lý) Škorničk. & A.D.Poulsen, **comb. nov.** ≡ *Amomum rubidum* Lamxay & N.S.Lý in Edinburgh J. Bot. 69(1): 166, fig. 25, 26. 2012.

Conamomum spiceum (Ridl.) Skornick. & A.D.Poulsen, **comb. nov.** *Amomum spiceum* Ridl. in J. Straits Branch Roy. Asiat. Soc. 86: 309. 1922.

Conamomum squarrosum (Ridl.) Skornick. & A.D.Poulsen, **comb. nov.** *Amomum squarrosum* Ridl. in J. Straits Branch Roy. Asiat. Soc. 57: 104. 1910.

****Conamomum utriculosum*** Ridl. in J. Straits Branch Roy. Asiat. Soc. 32: 122. 1899

= *Amomum utriculosum* (Ridl.) Holttum in Gard. Bull. Singapore 13(1): 208. 1950.

****Conamomum xanthophlebium*** (Baker) Skornick. & A.D. Poulsen, **comb. nov.**

= *Amomum xanthophlebium* Baker in Hooker, Fl. Brit. India 6: 241. 1892.

= *Amomum stenoglossum* Baker in Hooker, Fl. Brit. India 6: 234. 1892.

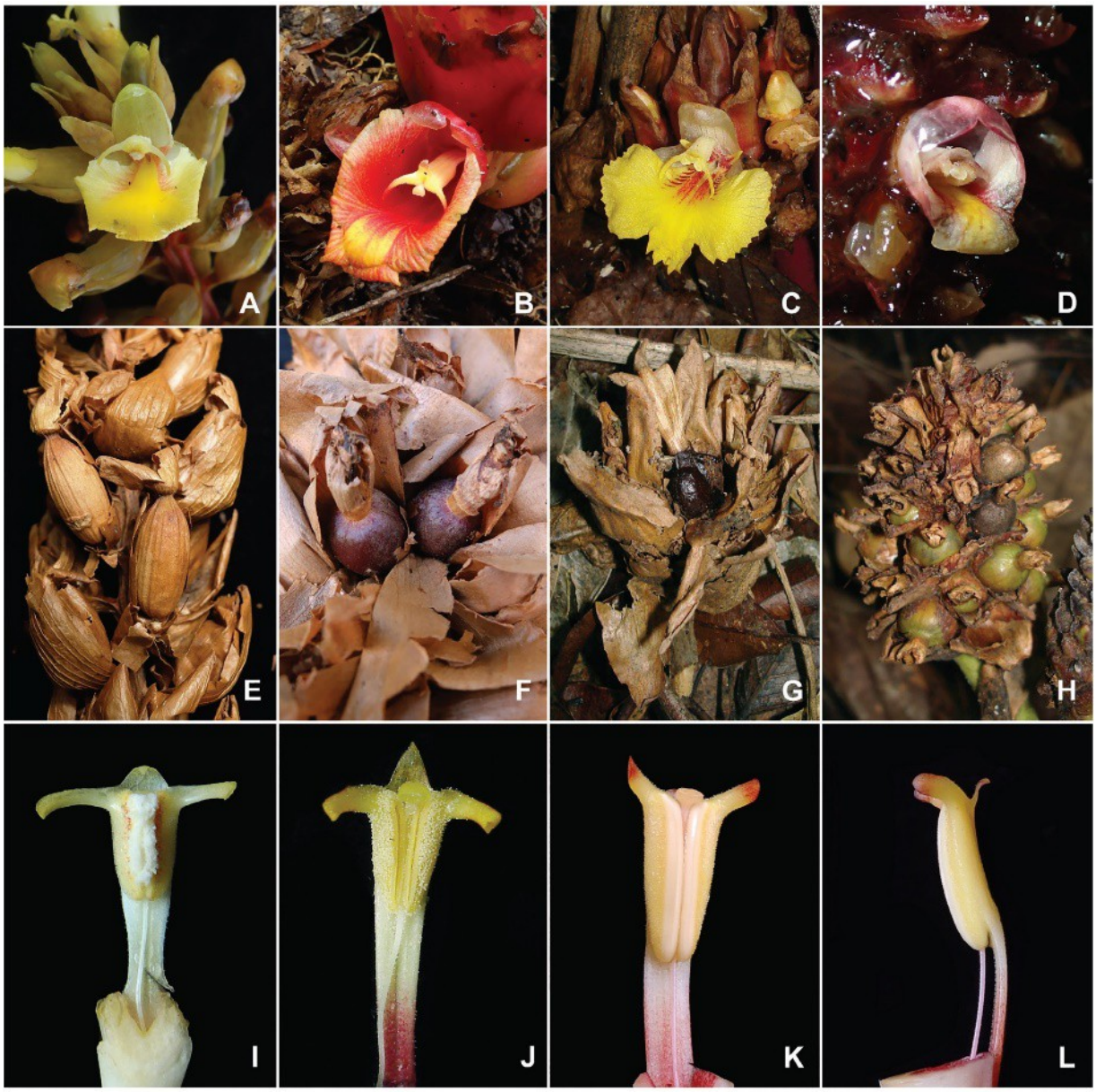


Fig. 4. *Conamomum.* **A,** *Conamomum* aff. *utriculosum* (flower); **B,** *Conamomum xanthophlebium* (flower); **C,** *Conamomum rubidum* (flower); **D,** *Conamomum coriaceum* (flower); **E,** *Conamomum* aff. *utriculosum* (smooth fruit); **F,** *Conamomum xanthophlebium* (smooth fruit); **G,** *Conamomum rubidum* (smooth fruit); **H,** *Conamomum coriaceum* (smooth fruit); **I,** *Conamomum* aff. *utriculosum* (stamen with horned anther crest); **J,** *Conamomum rubidum* (stamen with horned anther crest); **K,** *Conamomum xanthophlebium* (stamen with horned anther crest, front view); **L,** *Conamomum xanthophlebium* (stamen with horned anther crest, side view). Photos: A-L, Jana Leong-Skornickova.

Epiamomum A.D.Poulsen & Škorničk., **gen. nov.** – Type: *E. angustipetalum* (S.Sakai & Nagam.) A.D.Poulsen & Škorničk. (≡ *Amomum angustipetalum* S.Sakai & Nagam.). Fig. 1, clade VI; Fig. 5.

Diagnosis. – *Epiamomum* species are well supported as a distinct clade and characterised by the following combination of characters: coriaceous bracts supporting a single flower and persisting to fruiting stage, bracteoles open to base (or almost so), narrowly elongate and tubular flowers

with sessile or subsessile anthers and smooth, glabrous, unilaterally compressed fruits. The species are often epiphytic and so far geographically restricted to Borneo.

Description. – Small to medium-sized clump-forming plants, sometimes epiphytic, with distichous leafy shoot, leaf blades mostly sessile or subsessile, rarely with petiole up to 7 cm (e.g., *E. roseisquamosum*). Bracts coriaceous, persisting until fruiting stage, always subtending a single flower; bracteoles almost always open to base (bracteole shortly tubular at base in *E. hansenii*). Flowers narrowly elongate, tubular, with short and recurved labellum (not exceeding 1.5 cm), lateral staminodes mostly absent, if present, small and subulate. Flower colour pale to rich yellow, or white or white with pink (in *E. roseisquamosum*). Anther often sessile, if filament present, it is shorter than anther. Anther crest usually very short, not exceeding 2 mm long, truncate, more or less undulate. Fruits smooth, glabrous, usually somewhat unilaterally compressed.

Distribution. – Six species in Borneo. Most are known from Sarawak only, but *E. angustipetalum* extends to Brunei and *E. borneense* is known to occur also in Kalimantan. We believe that more species will be found in Borneo as exploration progresses.

Etymology. – The generic name denotes its affinity to *Amomum* and the epiphytic habit of some of these species.

Note. – Species of this genus were previously classified in informal Group II (*Amomum hansenii*, *A. pungens*) and Group V (*A. borneense*, *A. epiphyticum*) of Smith (1985, 1988). Later Nagamasu & Sakai (1996) described *A. roseisquamosum*, and noted that it did not closely match Group II or V, but indicated that it was closest to group Group V. Two years later Sakai & Nagamasu (1998) described *A. angustipetalum* and placed it in Group II. Our sampling included both *A. roseisquamosum* (cf. Group V) and *A. angustipetalum* (Group II).

Epiamomum angustipetalum (S.Sakai & Nagam.) A.D. Poulsen & Škorničk., **comb. nov.** ≡ *Amomum angustipetalum* S.Sakai & Nagam. in Edinburgh J. Bot. 55(1): 49. 1998. ***Epiamomum borneense*** (K.Schum.) A.D.Poulsen & Škorničk., **comb. nov.** ≡ *Zingiber borneense* K.Schum. in Engler, Pflanzenr. IV. 46 (Heft 20): 178. 1904 ≡ *Amomum borneense* (K.Schum.) R.M.Sm. in Notes Roy. Bot. Gard. Edinburgh 45(2): 337. 1989 ("1988").
Epiamomum epiphyticum (R.M.Sm.) A.D.Poulsen & Škorničk., **comb. nov.** ≡ *Amomum epiphyticum* R.M.Sm. in Notes Roy. Bot. Gard. Edinburgh 45(2): 338. 1989 ("1988").
Epiamomum hansenii (R.M.Sm.) A.D.Poulsen & Škorničk., **comb. nov.** ≡ *Amomum hansenii* R.M.Sm. in Notes Roy. Bot. Gard. Edinburgh 42(2): 303. 1985.
Epiamomum pungens (R.M.Sm.) A.D.Poulsen & Škorničk., **comb. nov.** ≡ *Amomum pungens* R.M.Sm. in Notes Roy. Bot. Gard. Edinburgh 42(2): 301. 1985.
Epiamomum roseisquamosum (S.Sakai & Nagam.) A.D. Poulsen & Škorničk., **comb. nov.** ≡ *Amomum roseisquamosum* S.Sakai & Nagam. in Edinburgh J. Bot. 53(1): 39. 1996

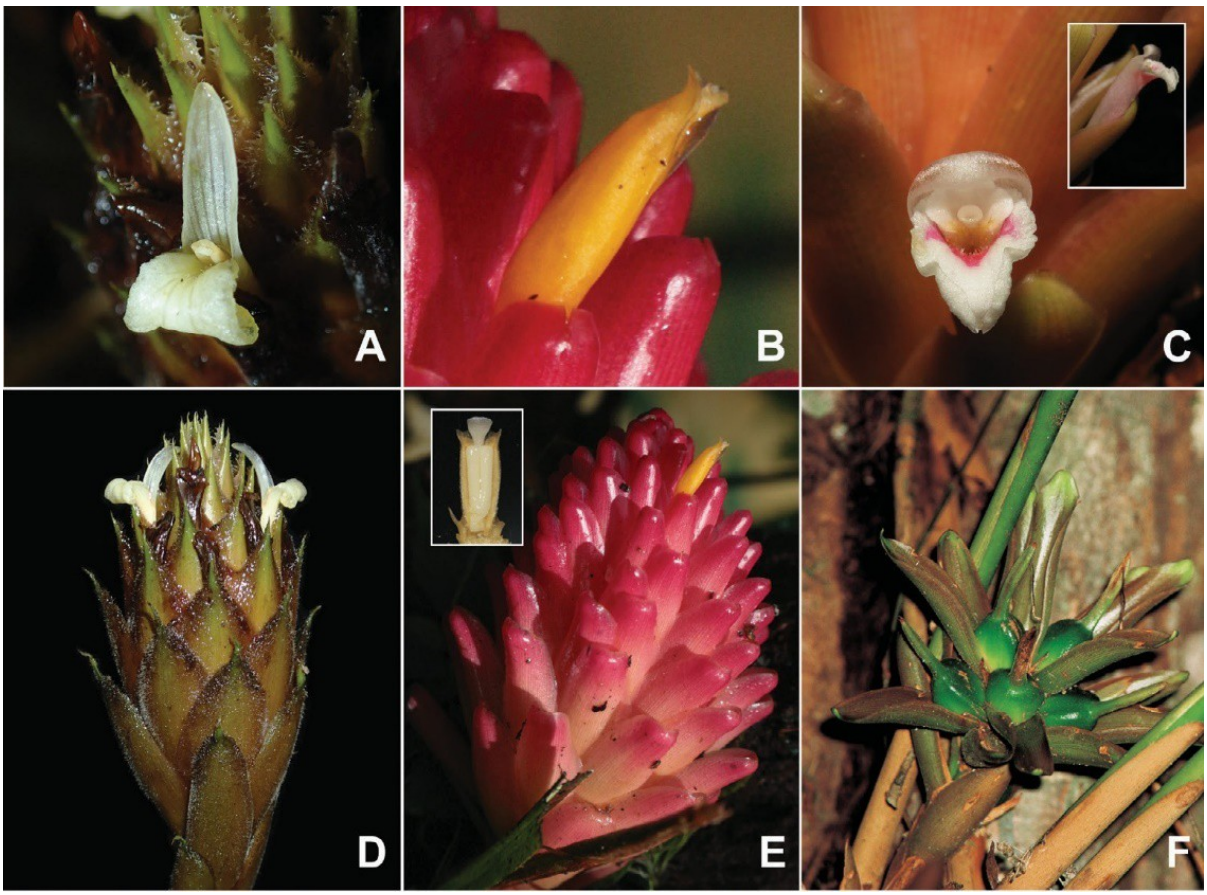


Fig. 5. *Epiamomum*. **A,** *Epiamomum angustipetalum* (flower); **B,** *Epiamomum epiphyticum* (flower); **C,** *Epiamomum roseisquamosum* (flower; side view in inset); **D,** *Epiamomum angustipetalum* (inflorescence); **E,** *Epiamomum epiphyticum* (inflorescence; stamen in front view in inset); **F,** *Epiamomum roseisquamosum* (infructescence with smooth fruits). Photos: A & D, Axel D. Poulsen; B & E, Michele Rodda; C, Chea Yiing Ling; F, Anthony Lamb.

Lanxangia M.F.Newman & Škorničk., **gen. nov.** – Type: *L. tsaoko* (Crevost & Lemarié) M.F.Newman & Škorničk. (≡ *Amomum tsaoko* Crevost & Lemarié). Fig. 1, clade II; Fig. 6.

Diagnosis. – *Lanxangia* species are characterised by the following combination of characters: bracts supporting a single flower, perishing by the time fruits develop, tubular bracteoles, absent staminodes, forked anther crest with 2–3 lobes and smooth fruits. *Geostachys*, the closest relative of *Lanxangia* according to molecular evidence, shares with it smooth fruits, but differs from *Lanxangia* by the presence of stilt-roots, prominent and inf lated sheathing bracts covering the peduncle, inflorescences and infructescences lax with visible rachis, the cincinni (rarely single flowers) are borne on long pedicels, and the anther crest is semilunar usually with obscure or prominent 2–3 lobes. The bracts of all but four species of *Geostachys* support more than one flower, and the inflorescences are often secund. The recognition of these two genera is also supported by the geographical disjunction between them.

Description. – Large clump-forming herbs with distichous leafy shoots composed of leaves with sessile blades. Inflorescences arising close to base of pseudostem, many-flowered. Bracts always subtending a single flower, bracteoles tubular. Flowers of open type with ovate to elliptic labellum and entire thin crisp margin. Staminodes absent. Anthers with well-developed, narrowly

semilunar anther crest, with 2–3 obscure lobes and crisp, often denticulate or frilly margin. Infructescences dense, fruits globose or sub-globose, smooth, usually dark red, bracts decayed when in fruit.

Distribution. – Fewer than 10 species occurring from 1100 to 1800 m asl in southern China (Yunnan), northern Thailand, northern Laos and northern Vietnam. They are locally cultivated for their fruits.

Etymology. – Lan Xang (One Million Elephants), a kingdom of Southeast Asia, 1354–1707, occupying much of central and northern Laos.

Notes. – Lau & Lim (2012) reported bracts supporting single flowers in 10 species of *Geostachys*, most of which contradicted earlier descriptions by Holttum (1950). We have re-examined most of these species and concur with Holttum. Bracts supporting single flowers have been reported in the protologues of four recently described species (*G. belumensis* C.K.Lim & K.H.Lau, *G. chayanii* Mayoe, *G. smitinandii* K.Larsen, *G. tratensis* Picheans. & Mayoe) but we have been unable to check the type specimens to verify these statements. We have made provision in the key to allow for this possibility.

Lanxangia capsiciformis (S.Q.Tong) M.F.Newman & Škorničk., **comb. nov.** ≡ *Amomum capsiciforme* S.Q.Tong in Acta Phytotax. Sin. 27(4): 282. 1989.

****Lanxangia coriandriodora*** (S.Q.Tong & Y.M.Xia) M.F. Newman & Škorničk., **comb. nov.** ≡ *Amomum coriandriodorum* S.Q.Tong & Y.M.Xia in Acta Bot. Yunnan. 10(2): 208. 1988.
= *Amomum inthanonense* Chaveer. & Tanee in Taiwania 53(1): 7–9, fig. 1–3. 2008.

Lanxangia jingxiensis (D.Fang & D.H.Qin) M.F.Newman & Škorničk., **comb. nov.** ≡ *Amomum jingxiense* D.Fang & D.H.Qin in Acta Phytotax. Sin. 27(6): 461. 1989.

****Lanxangia paratsaoko*** (S.Q.Tong & Y.M.Xia) M.F.Newman & Škorničk., **comb. nov.** ≡ *Amomum paratsaoko* S.Q.Tong & Y.M.Xia in Acta Bot. Yunnan. 10(2): 207. 1988.

Lanxangia scarlatina (H.T.Tsai & P.S.Chen) M.F.Newman & Škorničk., **comb. nov.** ≡ *Amomum scarlatinum* H.T.Tsai & P.S.Chen in Acta Phytotax. Sin. 17(4): 90. 1979.

Lanxangia thysanochilila (S.Q.Tong & Y.M.Xia) M.F.Newman & Škorničk., **comb. nov.** ≡ *Amomum thysanochililum* S.Q.Tong & Y.M.Xia in Acta Bot. Yunnan. 10(2): 205. 1988.

****Lanxangia tsaoko*** (Crevost & Lemarié) M.F.Newman & Škorničk., **comb. nov.** ≡ *Amomum tsaoko* Crevost & Lemarié, Cat. Prod. Indochine 1: 300. 1917.

Lanxangia tuberculata (D.Fang) M.F.Newman & Škorničk., **comb. nov.** ≡ *Amomum tuberculatum* D.Fang in Acta Phytotax. Sin. 16(3): 47. 1978.
= *Amomum hongtsaoko* C.F.Liang & D.Fang in Acta Phytotax. Sin. 16(3): 50. 1978.

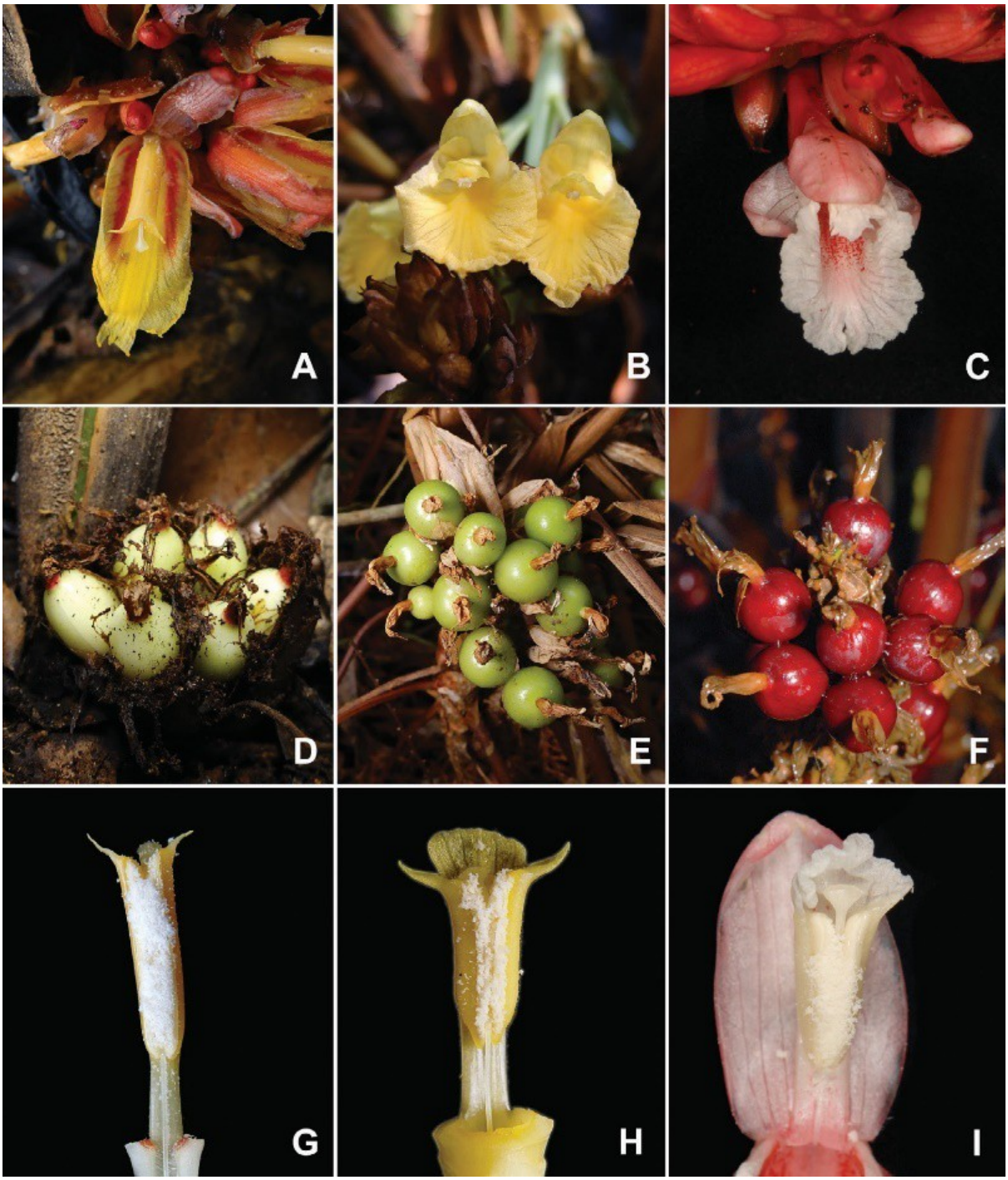


Fig. 6. *Lanxangia* and *Geostachys*. **A,** *Lanxangia* aff. *coriandriodora* (flower); **B,** *Geostachys densiflora* (flower); **C,** *Geostachys megaphylla* (flower); **D,** *Lanxangia* aff. *coriandriodora* (smooth fruit); **E,** *Geostachys densiflora* (smooth fruit); **F,** *Geostachys megaphylla* (smooth fruit); **G,** *Lanxangia* aff. *coriandriodora* (stamen with 2-lobed forked anther crest); **H,** *Geostachys densiflora* (stamen with 3-lobed semilunar anther crest); **I,** *Geostachys megaphylla* (stamen with obscurely 3-lobed semilunar anther crest). — Photos: A, B, D, E, G & H, Jana Leong-Škorničková; C, F & I, Otakar Šída.

Meistera Giseke, Prael. Ord. Nat. Pl.: 199. 1792 – Type: *M. koenigii* (J.F.Gmel.) Škorničk. & M.F.Newman (≡ *Amomum koenigii* J.F.Gmel.). Fig. 1, clade VIII; Fig. 7.

Diagnosis. – *Meistera* species are characterised by the following combination of characters: anther crest semilunar (entire, obscurely bilobed or 3-lobed), echinate fruits (rarely glabrous) and fertile bracts supporting a single flower. Species in the closely related *Wurfbainia*, which also have echinate fruits (rarely ribbed when dry, but never smooth), can be distinguished by their 3-lobed, crown-shaped anther crest, usually creeping habit and often clawed and more or less spoon-shaped labellum. *Meistera* includes species formerly placed in *Amomum* (characterised by winged angled or grooved fruits, and fan-shaped, extended or acute anther crest) from which they are genetically and morphologically clearly distinct.

Description. – Mostly medium-sized to large herbs, clump-forming to loosely clump-forming, rarely creeping. Leafy shoots distichous, with sessile or subsessile leaf blades, the petiole rarely developed (to 3(–5) cm). Peduncles usually short, creeping or ascending, rarely longer and erect (e.g., *M. sceletescens*). Flowering heads mostly many-flowered, compact. Fertile bracts always subtending a single flower, soon decaying with age, not persisting until fruiting; bracteoles tubular. Labellum white with a yellow patch and red markings (most common in Indochinese species) or, less commonly, plain rich yellow to orange, or red with yellow or cream spots (some species in Sundaland). Flowers always of exposed type in species of the Indochinese floristic region, but of gullet type with sides of labellum curved upwards and forming a chamber in Sundaland. Staminodes present in most species, usually small, subulate, to 3 mm long, rarely longer or absent. Anther crest well-developed, semilunar, either entire or broadly 3-lobed. Fruit manifestly echinate in the vast majority of species. In the Indochinese region, smooth fruits are encountered only rarely, e.g., in the type, *M. koenigii* and in *M. tomrey.* In Sundaland, species with partially (*M. ochrea*) or fully reduced spines (*M. cerasina*) are encountered; these fruits are conspicuous by their large size (more than 3 cm in diameter).

Distribution. – *Meistera* with 42 species and 3 varieties listed below is the most widespread genus of the former *Amomum* s.l. It is distributed from Sri Lanka and India, throughout the Indochinese region to Sundaland with a few species in the *Amomum aculeatum* alliance extending across Wallace's Line to Sulawesi, New Guinea and Australia.

Etymology. – George (or Georg) Meister (1653–1713), Saxon (German) gardener and botanist to the Elector of Saxony in Dresden, and then gardener for the Netherlands' Vereenigde Oost-Indische Compagnie, stationed in Java 1677–1688, from where he also visited the Dutch trading colony at Dejima, Japan.

Notes. – *Meistera* Giseke 1792 differs by one letter from the illegitimate *Meisteria* Scopoli (Olacaceae), which was published in 1777 as a superfluous name of *Pacourina* Aubl. (Compositae). *Meisteria* Siebold & Zucc. 1846 (Ericaceae) is a later homonym of Scopoli's name and therefore also illegitimate. None of these historical names has been in recent use. There are several such cases of pairs of generic names differing by the endings -a versus -ia. When called upon to rule whether these names are confusingly similar and to be treated as homonyms, the Committee on Nomenclature of Vascular Plants varies in its decisions. For example, *Eschweilera* DC. 1828 and *Eschweileria* Boerl. 1887 are treated as homonyms, while *Coluria* R.Br. 1823 and

Colura (Dumort.) Dumort. 1835 are not. It is in a situation like this, however, in which one of the names has not been in use for an extremely long time and can never be used in the future, that rulings on non-confusability tend to be made (J. McNeill, pers. comm.). The other relevant fact is that when *Amomum* Roxb. was being considered for conservation, neither the proposers (Burtt & Smith, 1968) nor the Committee for Spermatophyta (McVaugh, 1970) made any suggestion that *Meistera* might not need rejection because of its similarity to *Meisteria*. Established custom (*ICN*, cf. Pre. 13, McNeill *et al.*, 2012) is commonly a factor in a recommendation on confusability or not. We therefore conclude that *Meistera* Giseke can be safely used in Zingiberaceae without risk of confusion. We have corrected Thwaites's epithet *masticatorium* to *masticatorum*, following Kuntze who made the combination *Cardamomum masticatorum* (Thwaites) Kuntze. The majority of 3rd declension nouns form their genitive plural in -um. The genitive plural ending -ium is rather rare and would be incorrect in the case of the Late Latin noun *masticator* (P. Oswald, pers. comm.).

Meistera aculeata (Roxb.) Škorničk. & M.F.Newman, **comb. nov.** ≡ *Amomum aculeatum* Roxb. in Asiat. Res. 11: 344. 1810.
= *Amomum hatuanum* Náves in Fernandez-Villar & Naves, Nov. App.: 224. 1880.
= *Amomum ciliatum* Blume, Enum. Pl. Javae: 49. 1827.
= *Amomum flavum* Ridl. in J. Straits Branch Roy. Asiat. Soc. 32: 133. 1899.
= *Amomum aurantiacum* Ridl. in J. Fed. Malay States Mus. 10: 153. 1920.
Meistera aculeata var. ***gymnocarpa*** (Valeton) Škorničk. & M.F.Newman, **comb. nov.** ≡ *Amomum aculeatum* var. *gymnocarpum* Valeton in Nova Guinea 8: 926. 1913.
Meistera aculeata var. ***macrocarpa*** (Valeton) Škorničk. & M.F. Newman, **comb. nov.** ≡ *Amomum aculeatum* var. *macrocarpum* Valeton in Nova Guinea 8: 927. 1913.
Meistera acuminata (Thwaites) Škorničk. & M.F.Newman, **comb. nov.** ≡ *Amomum acuminatum* Thwaites, Enum. Pl. Zeyl.: 317. 1861.
= *Amomum acuminatum* var. *induta* K.Schum. in Engler, Pflanzenr. IV. 46 (Heft 20): 249. 1904.
Meistera agastyamalayana (V.P.Thomas & M.Sabu) Škorničk. & M.F.Newman, **comb. nov.** ≡ *Amomum agastyamalayanum* V.P.Thomas & M.Sabu in Edinburgh J. Bot. 69(2): 313. 2012.
Meistera benthamiana (Trimen) Škorničk. & M.F.Newman, **comb. nov.** ≡ *Amomum benthamianum* Trimen in J. Bot. 23: 265. 1885.
Meistera botryoidea (Cowley) Škorničk. & M.F.Newman, **comb. nov.** ≡ *Amomum botryoideum* Cowley in Kew Bull. 55(3): 674. 2000.
Meistera calcarata (Lamxay & M.F.Newman) Škorničk. & M.F.Newman, **comb. nov.** ≡ *Amomum calcaratum* Lamxay & M.F.Newman in Edinburgh J. Bot. 69(1): 110–113, fig. 1, 2. 2012.
Meistera cannicarpa (Wight) Škorničk. & M.F.Newman, **comb. nov.** ≡ *Elettaria cannicarpa* Wight, Icon. Pl. Ind. Orient. 6: 17, t. 2007. 1853 ≡ *Cardamomum cannicarpum* (Wight) Kuntze, Revis. Gen. Pl. 2: 686. 1891 ≡ *Amomum cannicarpum* (Wight) Benth. ex Baker in Hooker, Fl. Brit. India 6: 240. 1892.
Meistera celsa (Lamxay & M.F.Newman) Škorničk. & M.F. Newman, **comb. nov.** ≡ *Amomum celsum* Lamxay & M.F.Newman in Edinburgh J. Bot. 69(1): 117–119, fig. 4, 5. 2012.
Meistera cerasina (Ridl.) Škorničk. & M.F.Newman, **comb. nov.** ≡ *Amomum cerasinum* Ridl. in

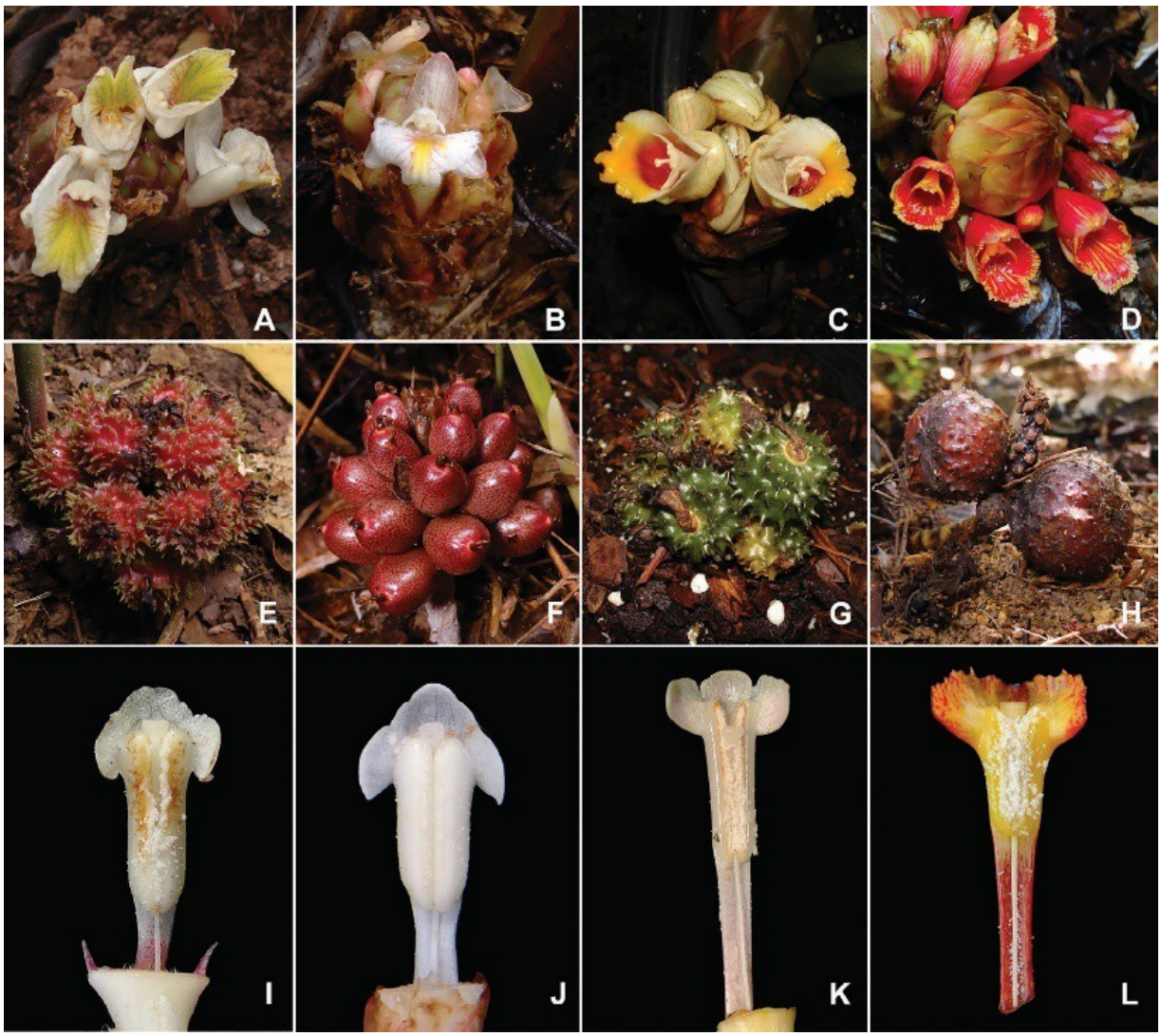


Fig. 7. *Meistera*. **A,** *Meistera chinensis* (flower); **B,** *Meistera koenigii* (flower); **C,** *Meistera aculeata* (flower); **D,** *Meistera ochrea* (flower); **E,** *Meistera chinensis* (echinate fruit); **F,** *Meistera koenigii* (smooth fruit); **G,** *Meistera aculeata* (echinate fruit); **H,** *Meistera ochrea* (sparsely echinate fruit); **I,** *Meistera chinensis* (stamen with semilunar 3-lobed anther crest); **J,** *Meistera koenigii* (stamen with semilunar 3-lobed anther crest); **K,** *Meistera aculeata* (stamen with semilunar 3-lobed anther crest); **L,** *Meistera ochrea* (stamen with semilunar entire to obscurely 2-lobed anther crest). — Photos: A–L, Jana Leong-Škorničková.J. Straits Branch Roy. Asiat. Soc. 46: 237. 1906.

Meistera chinensis (Chun ex T.L.Wu) Škorničk. & M.F. Newman, **comb. nov.** ≡ *Amomum chinense* Chun ex T.L. Wu in Chun, Fl. Hainan. 4: 533. 1977.
Meistera dallachyi (F.Muell.) Škorničk. & M.F.Newman, **comb. nov.** ≡ *Amomum dallachyi* F.Muell., Fragm. 8: 25. 1873.
Meistera deoriana (D.P.Dam & N.Dam) Škorničk. & M.F. Newman, **comb. nov.** ≡ *Amomum deorianum* D.P.Dam & N.Dam in Bull. Bot. Surv. India 34(1–4): 212. 1997 (“1992”).
Meistera echinocarpa (Alston) Škorničk. & M.F.Newman, **comb. nov.** ≡ *Amomum echinatum* Thwaites, Enum. Pl. Zeyl.: 316. 1861, nom. illeg. ≡ *Amomum echinocarpum* Alston in Trimen, Handb. Fl. Ceylon 6(Suppl.): 283. 1931. ***Meistera elephantorum*** (Pierre ex Gagnep.) Škorničk. &

M.F. Newman, **comb. nov.** ≡ *Amomum elephantorum* Pierre ex Gagnep. in Bull. Soc. Bot. France 53: 137. 1906.

Meistera fulviceps (Thwaites) Škorničk. & M.F.Newman, **comb. nov.** ≡ *Amomum fulviceps* Thwaites, Enum. Pl. Zeyl.: 317. 1861.

Meistera gagnepainii (T.L.Wu, K.Larsen & Turland) Škorničk. & M.F.Newman, **comb. nov.** ≡ *Amomum thyrsoideum* Gagnep. in Bull. Soc. Bot. France 49: 256. 1903, nom. illeg. ≡ *Amomum gagnepainii* T.L.Wu, K.Larsen & Turland in Novon 10(1): 90. 2000.

Meistera ghatica (K.G.Bhat) Škorničk. & M.F.Newman, **comb. nov.** ≡ *Amomum ghaticum* K.G.Bhat in Indian J. Forest. 11(4): 322. 1989 ("1988").

Meistera graminifolia (Thwaites) Škorničk. & M.F.Newman, **comb. nov.** ≡ *Amomum graminifolium* Thwaites, Enum. Pl. Zeyl.: 430. 1864.

****Meistera gyrolophos*** (R.M.Sm.) Škorničk. & M.F.Newman, **comb. nov.** ≡ *Amomum gyrolophos* R.M.Sm. in Notes Roy. Bot. Gard. Edinburgh 42(2): 305. 1985.

****Meistera kinabaluensis*** (R.M.Sm.) Škorničk. & M.F.Newman, **comb. nov.** ≡ *Amomum kinabaluense* R.M.Sm. in Notes Roy. Bot. Gard. Edinburgh 44(2): 233. 1987.

****Meistera koenigii*** (J.F.Gmel.) Škorničk. & M.F.Newman, **comb. nov.** ≡ *Amomum koenigii* J.F.Gmel., Syst. Nat., ed. 1791: 6. 1791.

= *Amomum corynostachyum* Wall., Pl. Asiat. Rar. 1: 48. 1830.

****Meistera lappacea*** (Ridl.) Škorničk. & M.F.Newman, **comb. nov.** ≡ *Amomum lappaceum* Ridl. in J. Straits Branch Roy. Asiat. Soc. 32: 134. 1899.

= *Amomum perakense* Ridl. in J. Straits Branch Roy. Asiat. Soc. 32: 135. 1899.

Meistera loheri (K.Schum.) Škorničk. & M.F.Newman, **comb. nov.** ≡ *Amomum loheri* K.Schum. in Engler, Pflanzenr. IV. 46 (Heft 20): 247. 1904.

Meistera masticatorum (Thwaites) Škorničk. & M.F.Newman, **comb. nov.** ≡ *Amomum masticatorum* Thwaites, Enum. Pl. Zeyl.: 317. 1861 ("*masticatorium*").

****Meistera mentawaiensis*** (A.J.Droop) Škorničk. & M.F. Newman, **comb. nov.** ≡ *Amomum mentawaiense* A.J.Droop in Edinburgh J. Bot. 71(2): 233. 2014.

Meistera mizoramensis (M.Sabu, V.P.Thomas & Vanchh.) Škorničk. & M.F.Newman, **comb. nov.** ≡ *Amomum mizoramense* M.Sabu, V.P.Thomas & Vanchh. in Nordic J. Bot. 31(5): 565. 2013.

Meistera muricarpa (Elmer) Škorničk. & M.F.Newman, **comb. nov.** ≡ *Amomum muricarpum* Elmer in Leaf l. Philipp. Bot. 8: 2896. 1915.

Meistera muricata (Bedd.) Škorničk. & M.F.Newman, **comb. nov.** ≡ *Amomum muricatum* Bedd. in Madras J. Lit. Sci., ser. 3, 1: 59. 1864.

= *Amomum holmesii* K.Schum. in Engler, Pflanzenr. IV. 46 (Heft 20): 256. 1904.

Meistera newmanii (M.Sabu & V.P.Thomas) Škorničk. & M.F. Newman, **comb. nov.** ≡ *Amomum newmanii* M.Sabu & V.P.Thomas in Edinburgh J. Bot. 69(2): 319. 2012.

Meistera nilgirica (V.P.Thomas & M.Sabu) Škorničk. & M.F. Newman, **comb. nov.** ≡ *Amomum nilgiricum* V.P.Thomas & M.Sabu in PhytoKeys 8: 100–104, fig. 1, 2. 2012.

****Meistera ochrea*** (Ridl.) Škorničk. & M.F.Newman, **comb. nov.** ≡ *Amomum ochreum* Ridl. in J. Straits Branch Roy. Asiat. Soc. 32: 135. 1899.

****Meistera oligantha*** (K.Schum.) Škorničk. & M.F.Newman, **comb. nov.** ≡ *Amomum oliganthum* K.Schum. in Bot. Jahrb. Syst. 27(3): 321. 1899.

= *Amomum gracilipes* K.Schum. in Engler, Pflanzenr. IV. 46 (Heft 20): 252. 1904.

= *Amomum hewittii* Ridl. in J. Straits Branch Roy. Asiat. Soc. 46: 238. 1906.

Meistera propinqua (Ridl.) Škorničk. & M.F.Newman, **comb. nov.** ≡ *Amomum propinquum* Ridl. in Publ. Bur. Sci. Gov. Lab. 35: 84. 1905.
Meistera sahyadrica (V.P.Thomas & M.Sabu) Škorničk. & M.F. Newman, **comb. nov.**
≡ *Amomum sahyadricum* V.P.Thomas & M.Sabu in Novon 22(3): 321. 2013.
****Meistera sceletescens*** (R.M.Sm.) Škorničk. & M.F.Newman, **comb. nov.** ≡ *Amomum sceletescens* R.M.Sm. in Edinburgh J. Bot. 47(3): 367. 1990.
****Meistera stephanocolea*** (Lamxay & M.F.Newman) Škorničk. & M.F.Newman, **comb. nov.** ≡ *Amomum stephanocoleum* Lamxay & M.F.Newman in Edinburgh J. Bot. 69(1): 173–176, fig. 27, 28. 2012.
****Meistera tomrey*** (Gagnep.) Škorničk. & M.F.Newman, **comb. nov.** ≡ *Amomum tomrey* Gagnep. in Bull. Soc. Bot. France 53: 145. 1906
Meistera tomrey var. ***stenophylla*** (Gagnep.) Škorničk. & M.F. Newman, **comb. nov.** ≡ *Amomum tomrey* var. *stenophyllum* Gagnep. in Bull. Soc. Bot. France 53: 146. 1906.
Meistera trichostachya (Alston) Škorničk. & M.F.Newman, **comb. nov.** ≡ *Amomum ciliatum* Baker in Hooker, Fl. Brit. India 6: 238. 1892, nom. illeg. ≡ *Amomum trichostachyum* Alston in Trimen, Handb. Fl. Ceylon 6(Suppl.): 283. 1931.
Meistera vermana (S.Tripathi & V.Prakash) Škorničk. & M.F. Newman, **comb. nov.** ≡ *Amomum vermanum* S.Tripathi & V.Prakash in Edinburgh J. Bot. 57(2): 257. 2000.
Meistera verrucosa (S.Q.Tong) Škorničk. & M.F.Newman, **comb. nov.** ≡ *Amomum verrucosum* S.Q.Tong in Acta Phytotax. Sin. 27(4): 280. 1989.
Meistera vespertilio (Gagnep.) Škorničk. & M.F.Newman, **comb. nov.** ≡ *Amomum vespertilio* Gagnep. in Bull. Soc. Bot. France 49: 255. 1903.
****Meistera yunannensis*** (S.Q.Tong) Škorničk. & M.F.Newman, **comb. nov.** ≡ *Amomum yunnanense* S.Q.Tong in Acta Bot. Yunnan. 12(2): 151. 1990.

Sundamomum A.D.Poulsen & M.F.Newman, **gen. nov.** – Type: *S. hastilabium* (Ridl.) A.D.Poulsen & M.F.Newman (≡ *Amomum hastilabium* Ridl.). Fig. 1, clade III; Fig. 8.

Diagnosis. – The species of *Sundamomum* are characterised by the following combination of characters: coriaceous bracts supporting a single flower and persisting to fructescence, flowers open to gullet-shaped, anther crest obscurely trilobed with sidelobes often reduced to a thickened angled margin, and fruits ribbed or grooved. The genus is so far only found in Sundaland.

Description. – Medium to large clump-forming plants with distichous leafy shoot consisting of sessile or shortly petiolate (up to 2.5 cm) leaf blades. Bracts coriaceous, turning brown and papery with age and, as in closely related *Conamomum*, often persisting until fruiting stage. Bracts always subtending a single flower, bracteoles tubular or split to base. Flowers of gullet to open type. Calyx truncate, often calyptrate, labellum obovate, mostly yellow or orange, less often white with yellow centre (rarely with red marking in centre). Staminodes almost always present, linear (strongly reduced in *S. dictyocoleum*). Anther crest obscurely trilobed, sidelobes as a thickened angled margin, midlobe thinner, sometimes somewhat ref lexed. Fruits globular to ellipsoid, shallowly/bluntly ribbed or almost smooth when fresh, always ribbed when dry.

Distribution. – Fourteen species, mainly distributed in Borneo, Sumatra and West Java, with *Sundamomum hastilabium* extending to Peninsular Malaysia and southern Thailand, almost reaching the Isthmus of Kra.

Etymology. – Sunda, the western part of Java, plus *Amomum*. Sundaland or the Sundaic Region is the area including Peninsular Malaysia, Sumatra, Java and Borneo, which is the centre of diversity of this genus.

Sundamomum borealiborneense (I.M.Turner) A.D.Poulsen & M.F.Newman, **comb. nov.** ≡ *Amomum ridleyi* R.M.Sm. in Notes Roy. Bot. Gard. Edinburgh 42(2): 311. 1985, nom. illeg. ≡ *A. sylvestre* Ridl. in J. Straits Branch Roy. Asiat. Soc. 46: 236. 1906, nom. illeg. ≡ *A. borealiborneense* I.M.Turner in Sandakania 12: 25. 1998.

****Sundamomum calyptratum*** (S.Sakai & Nagam.) A.D.Poulsen & M.F.Newman, **comb. nov.** ≡ *Amomum calyptratum* S.Sakai & Nagam. in Edinburgh J. Bot. 55(1): 52. 1998.

****Sundamomum dictyocoleum*** (K.Schum.) A.D.Poulsen & M.F. Newman, **comb. nov.** ≡ *Amomum dictyocoleum* K.Schum. in Bot. Jahrb. Syst. 27(3): 312. 1899.

****Sundamomum durum*** (S.Sakai & Nagam.) A.D.Poulsen & M.F.Newman, **comb. nov.** ≡ *Amomum durum* S.Sakai & Nagam. in Edinburgh J. Bot. 55(1): 55. 1998.

Sundamomum flavoalbum (R.M.Sm.) A.D.Poulsen & M.F.Newman, **comb. nov.** ≡ *Amomum f lavoalbum* R.M.Sm. in Notes Roy. Bot. Gard. Edinburgh 42(2): 310. 1985.

****Sundamomum hastilabium*** (Ridl.) A.D.Poulsen & M.F. Newman, **comb. nov.** ≡ *Amomum hastilabium* Ridl. In J. Straits Branch Roy. Asiat. Soc. 32: 137. 1899.

= *Amomum holttumii* Ridl., Fl. Malay Penins. 4: 264. 1924.

= *Amomum xanthoglossum* Ridl. in J. Fed. Malay States Mus. 10: 153. 1920.

Sundamomum laxesquamosum (K.Schum.) A.D.Poulsen & M.F.Newman, **comb. nov.** ≡ *Amomum laxesquamosum* K.Schum. in Bot. Jahrb. Syst. 27(3): 315. 1899.

Sundamomum longipedunculatum (R.M.Sm.) A.D.Poulsen & M.F.Newman, **comb. nov.** ≡ *Amomum longipedunculatum* R.M.Sm. in Notes Roy. Bot. Gard. Edinburgh 42(2): 309. 1985.

Sundamomum luteum (R.M.Sm.) A.D.Poulsen & M.F.Newman, **comb. nov.** ≡ *Amomum luteum* R.M.Sm. in Notes Roy. Bot. Gard. Edinburgh 42(2): 310. 1985.

Sundamomum macroglossa (K.Schum.) A.D.Poulsen & M.F. Newman, **comb. nov.** ≡ *Amomum macroglossa* K.Schum. in Bot. Jahrb. Syst. 27(3): 314. 1899.

****Sundamomum oligophyllum*** (A.J.Droop) A.D.Poulsen & M.F.Newman, **comb. nov.** ≡ *Amomum oligophyllum* A.J. Droop in Edinburgh J. Bot. 71(2): 238. 2014.

Sundamomum paucifolium (R.M.Sm.) A.D.Poulsen & M.F. Newman, **comb. nov.** ≡ *Amomum paucifolium* R.M.Sm. in Notes Roy. Bot. Gard. Edinburgh 42(2): 307. 1985.

****Sundamomum pseudofoetens*** (Valeton) A.D.Poulsen & M.F. Newman, **comb. nov.** ≡ *Amomum pseudofoetens* Valeton in Bull. Inst. Bot. Buitenzorg 20: 23. 1904.

Sundamomum somniculosum (S.Sakai & Nagam.) A.D. Poulsen & M.F.Newman, **comb. nov.** ≡ *Amomum somniculosum* S.Sakai & Nagam. in Edinburgh J. Bot. 55(1): 53. 1998. **Fig. 8.** *Sundamomum*. **A,** *Sundamomum dictyocoleum* (flower); **B,** *Sundamomum oligophyllum* (flower); **C,** *Sundamomum hastilabium* (flower); **D,** *Sundamomum dictyocoleum* (grooved fruit); **E,** *Sundamomum oligophyllum* (ribbed fruit); **F,** *Sundamomum hastilabium* (ribbed fruit). Photos: A & D, Axel D. Poulsen; B & E, A. Jane Droop; C & F, Jana Leong-Škorničková.

Wurfbainia Giseke, Prael. Ord. Nat. Pl.: 199. 1792 – Type: *W. uliginosa* (J.Koenig) Giseke (≡ *Amomum uliginosum* J.Koenig) ≡ *Cardamomum* Rumph. ex Kuntze, Revis. Gen. Pl. 2: 685. 1891 – **by type designation here** of *C. uliginosum* (J.Koenig) Kuntze.

= *Paludana* Giseke, Prael. Ord. Nat. Pl.: 199. 1792 – Type: *Amomum globba* J.F.Gmel., Syst. Nat., ed. 1791: 6. 1791, nom. rej. vs. *Amomum* Roxb. 1820, nom. cons. Fig. 1, clade VII; Fig. 9.

Description. – Mostly medium-sized to large herbs, often with creeping rhizomes and forming large colonies, occasionally loosely clump-forming. Leafy shoots distichous, with sessile or almost sessile (petiole to 1 cm long) leaf blades. Peduncles usually short, creeping or ascending, holding flowering heads at ground level, sometimes more or less erect (e.g., *A. staminidivium*, *A. tenellum*, *A. testaceum*). Flowering heads fewto many-flowered, lax or compact. Fertile bracts

always subtending a single flower; bracteoles tubular. Labellum white with yellow patch and red marking, rarely cream white to pale yellow with purple, dark pink or red markings (*A. staminidivum*, *A. tenellum*). Flowers always of exposed type with labellum more or less flat and reflexed margins, or spoon-shaped, never of gullet type. Staminodes mostly absent, sometimes small, linear or scale-like. Anther crest composed of three small lobes, the side lobes usually pointing upwards and the mid lobe positioned behind stigma (crown-like appearance). Fruit prominently echinate in the majority of species, spines smaller in species such as *A. glabrifolium* and *A. schmidtii*, and fruits smooth with a few round lobes and appressed hairs in the subclade containing *A. compactum*, *A. testaceum* and *A. verum*.

Distribution. – Wurfbainia with 27 species and 2 varieties is most diverse in the Indochinese floristic region with only a few and often cultivated species extending into Sundaland, Philippines and one or two species across Wallace's Line in Sulawesi.

Etymology. – Johann Siegmund Wurffbain or Wurfbain (1613–ca. 1661), Bavarian (German) soldier and then merchant for the Netherlands' Vereenigde Oost-Indische Compagnie, stationed at various places in the Dutch East Indies, ca. 1632–1646 (see Linnaeus 1792: 226).

Wurfbainia aromatica (Roxb.) Škorničk. & A.D.Poulsen, **comb. nov.** ≡ *Amomum aromaticum* Roxb., Fl. Ind. 1: 44. 1820.
Wurfbainia bicorniculata (K.Schum.) Škorničk. & A.D. Poulsen, **comb. nov.** ≡ *Amomum bicorniculatum* K.Schum. in Engler, Pflanzenr. IV. 46 (Heft 20): 229. 1904.
Wurfbainia biflora (Jack) Škorničk. & A.D.Poulsen, **comb. nov.** ≡ *Amomum biflorum* Jack in Malayan Misc. 1(1): 2. 1820.
= *Amomum elettarioides* Baker in Hooker, Fl. Brit. India 6: 240. 1892 ("*elatterioides*")
≡ *Elettariopsis pubescens* Ridl. in J. Straits Branch Roy. Asiat. Soc. 32: 155. 1899, nom. illeg.
≡ *Cyphostigma pubescens* (Ridl.) K.Schum. in Engler, Pflanzenr. IV. 46 (Heft 20): 274. 1904, nom. illeg.
Wurfbainia blumeana (Valeton) Škorničk. & A.D.Poulsen, **comb. nov.** ≡ *Amomum blumeanum* Valeton in Bull. Jard. Bot. Buitenzorg, sér. 3, 2: 354. 1920.
****Wurfbainia compacta*** (Sol. ex Maton) Škorničk. & A.D. Poulsen, **comb. nov.** ≡ *Amomum compactum* Sol. ex Maton in Trans. Linn. Soc. London 10: 251. 1811 ≡ *Zingiber compactum* (Sol. ex Maton) Stokes, Bot. Mat. Med. 1: 68. 1812.
= *Amomum kepulaga* Sprague & Burkill in Gard. Bull. Straits Settlem. 6: 10. 1929.
Wurfbainia elegans (Ridl.) Škorničk. & A.D.Poulsen, **comb. nov.** ≡ *Amomum elegans* Ridl. in Publ. Bur. Sci. Gov. Lab. 35: 84. 1906.
****Wurfbainia glabrifolia*** (Lamxay & M.F.Newman) Škorničk. & A.D.Poulsen, **comb. nov.**
≡ *Amomum glabrifolium* Lamxay & M.F.Newman in Edinburgh J. Bot. 69(1): 135. 2012.
Wurfbainia gracilis (Blume) Škorničk. & A.D.Poulsen, **comb. nov.** ≡ *Amomum gracile* Blume, Enum. Pl. Javae: 49. 1827. ***Wurfbainia graminea*** (Wall. ex Baker) Škorničk. & A.D. Poulsen, **comb. nov.** ≡ *Amomum gramineum* Wall. ex Baker in Hooker, Fl. Brit. India 6: 233. 1892.
Wurfbainia hedyosma (I.M.Turner) Škorničk. & A.D.Poulsen, **comb. nov.** ≡ *Amomum trilobum* Ridl. in Publ. Bur. Sci. Gov. Lab. 35: 85. 1905, nom. illeg. ≡ *A. hedyosmum* I.M.Turner in Asian J. Trop. Biol. 4: 18. 2000.
Wurfbainia jainii (S.Tripathi & V.Prakash) Škorničk. & A.D.Poulsen, **comb. nov.** ≡ *Amomum*

jainii S.Tripathi & V.Prakash in Nordic J. Bot. 19(5): 609. 1999.

Wurfbainia longiligularis (T.L.Wu) Škorničk. & A.D.Poulsen, **comb. nov.** ≡ *Amomum longiligulare* T.L.Wu in Chun, Fl. Hainan. 4: 533. 1977.

Wurfbainia micrantha (Ridl.) Škorničk. & A.D.Poulsen, **comb. nov.** ≡ *Amomum micranthum* Ridl. in J. Straits Branch Roy. Asiat. Soc. 32: 138. 1899.

Wurfbainia microcarpa (C.F.Liang & D.Fang) Škorničk. & A.D.Poulsen, **comb. nov.** ≡ *Amomum microcarpum* C.F.Liang & D.Fang in Acta Phytotax. Sin. 16(3): 49. 1978.

Wurfbainia mindanaensis (Elmer) Škorničk. & A.D.Poulsen, **comb. nov.** ≡ *Amomum mindanaense* Elmer in Leaf l. Philipp. Bot. 8: 2894. 1915.

Wurfbainia mollis (Ridl.) Škorničk. & A.D.Poulsen, **comb. nov.** ≡ *Amomum molle* Ridl. in J. Fed. Malay States Mus. 10: 120. 1920.

= *Amomum rivale* Ridl., Fl. Malay Penins. 5: 338. 1925.

Wurfbainia neoaurantiaca (T.L.Wu, K.Larsen & Turland) Škorničk. & A.D.Poulsen, **comb. nov.** ≡ *Amomum aurantiacum* H.T.Tsai & S.W.Zhao in Acta Phytotax. Sin. 17(4): 91. 1979, nom. illeg. ≡ *Amomum neoaurantiacum* T.L.Wu, K.Larsen & Turland in Novon 10(1): 90. 2000.

Wurfbainia palawanensis (Elmer) Škorničk. & A.D.Poulsen, **comb. nov.** ≡ *Amomum palawanense* Elmer in Leaf l. Philipp. Bot. 8: 2897. 1915.

Wurfbainia quadratolaminaris (S.Q.Tong) Škorničk. & A.D. Poulsen, **comb. nov.** ≡ *Amomum quadratolaminare* S.Q. Tong in Acta Phytotax. Sin. 27(4): 279. 1989.

Wurfbainia schmidtii (K.Schum.) Škorničk. & A.D.Poulsen, **comb. nov.** ≡ *Elettariopsis schmidtii* K.Schum. in Bot. Tidsskr. 24: 269. 1902 ≡ *Amomum schmidtii* (K.Schum.) Gagnep. in Lecomte, Fl. Indo-Chine 6: 111. 1904.

Wurfbainia staminidiva (Gobilik, A.L.Lamb & A.D.Poulsen) Škorničk. & A.D.Poulsen, **comb. nov.** ≡ *Amomum staminidivum* Gobilik, A.L.Lamb & A.D.Poulsen in Sandakania 14: 51. 2004.

Wurfbainia tenella (Lamxay & M.F.Newman) Škorničk. & A.D.Poulsen, **comb. nov.** ≡ *Amomum tenellum* Lamxay & M.F.Newman in Edinburgh J. Bot. 69(1): 179–182, fig. 30, 31. 2012.

Wurfbainia testacea (Ridl.) Škorničk. & A.D.Poulsen, **comb. nov.** ≡ *Amomum testaceum* Ridl. in J. Straits Branch Roy. Asiat. Soc. 32: 135. 1899.

Wurfbainia uliginosa (J.Koenig) Škorničk. & A.D.Poulsen, **comb. nov.** ≡ *Amomum uliginosum* J.Koenig in Retzius, Observ. Bot. 3: 56. 1783 ≡ *Cardamomum uliginosum* (J.Koenig) Kuntze, Revis. Gen. Pl. 2: 687. 1891.

= *Amomum ovoideum* Pierre ex Gagnep. in Bull. Soc. Bot. France 53: 140. 1906.

= *Amomum robustum* K.Schum. in Engler, Pflanzenr. IV. 46 (Heft 20): 253. 1904.

Wurfbainia vera (Blackw.) Škorničk. & A.D.Poulsen, **comb. nov.** ≡ *Amomum verum* Blackw., Herb. Blackwell.: t. 371. 1757.

= *Amomum krervanh* Pierre ex Gagnep. in Bull. Soc. Bot. France 53: 138. 1906, nom. Illeg.

Wurfbainia villosa (Lour.) Škorničk. & A.D.Poulsen, **comb. nov.** ≡ *Amomum villosum* Lour., Fl. Cochinch.: 4. 1790, nom. cons. ≡ *Zingiber villosum* (Lour.) Stokes, Bot. Mat. Med. 1: 63. 1812 ≡ *Cardamomum villosum* (Lour.) Kuntze, Revis. Gen. Pl. 2: 687. 1891.

= *Amomum echinosphaera* K.Schum. in Bot. Jahrb. Syst. 27(3): 322. 1899.

Wurfbainia villosa var. ***nana*** (H.T.Tsai & S.W.Zhao) Škorničk. & A.D.Poulsen, **comb. nov.**

≡ *Amomum villosum* var. *nanum* H.T.Tsai & S.W.Zhao in Acta Phytotax. Sin. 17(4): 92. 1979.

Wurfbainia villosa var. ***xanthioides*** (Wall. ex Kuntze) Škorničk. & A.D.Poulsen, **comb. nov.**

≡ *Cardamomum xanthioides* Wall. ex Kuntze in Revis. Gen. Pl. 2: 687. 1891 ≡ *Amomum xanthioides* Wall. ex Baker in Hooker, Fl. Brit. India 6: 239. 1892 ≡ *A. villosum* var. *xanthioides* (Wall. ex Baker) T.L.Wu & S.J.Chen in Acta Phytotax. Sin. 16(3): 38. 1978.

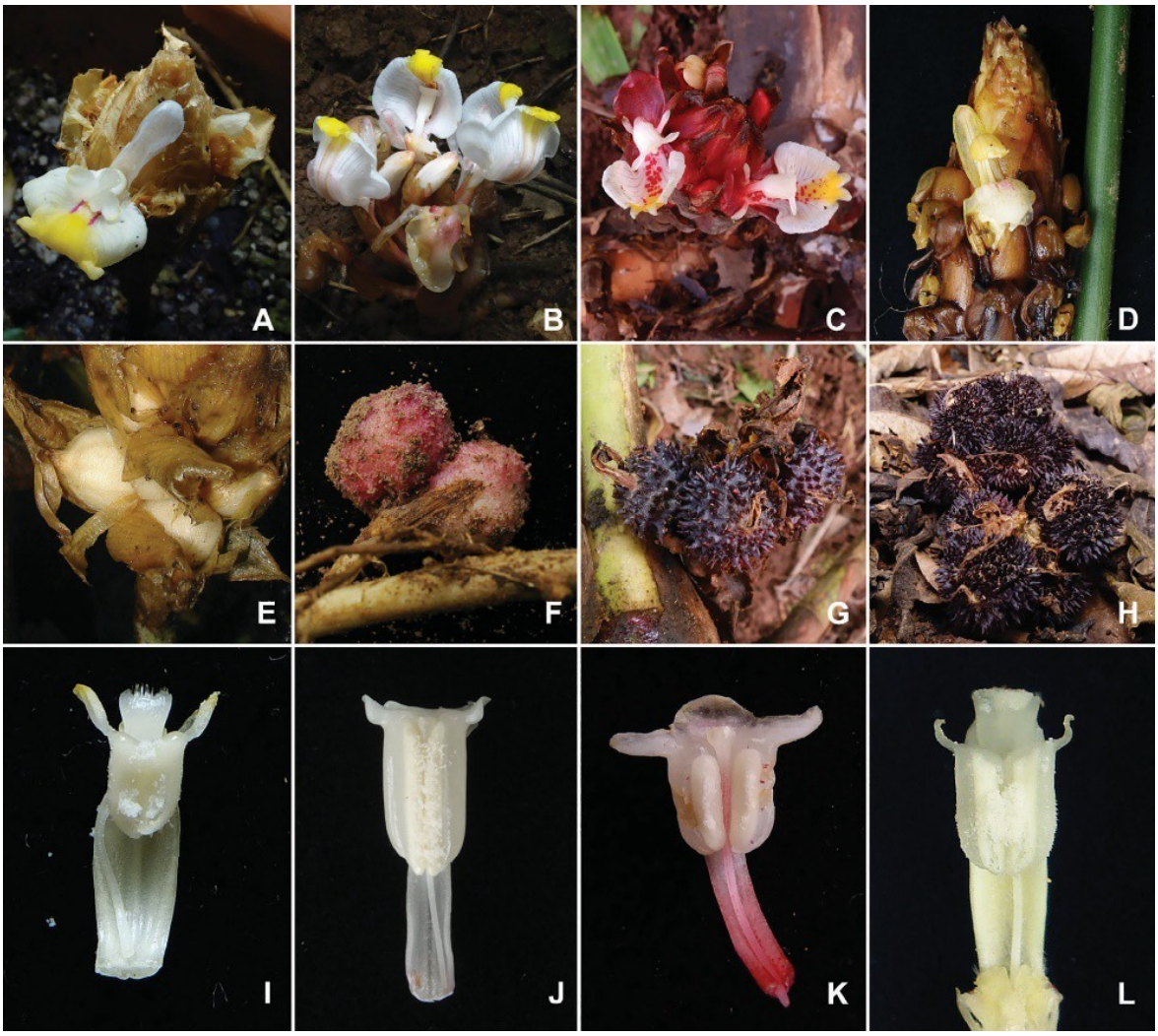


Fig. 9. *Wurfbainia*. **A,** *Wurfbainia testacea* (flower); **B,** *Wurfbainia longiligularis* (flower); **C,** *Wurfbainia microcarpa* (flower); **D,** *Wurfbainia tenella* (flower); **E,** *Wurfbainia testacea* (ribbed fruit); **F,** *Wurfbainia longiligularis* (echinate fruit); **G,** *Wurfbainia microcarpa* (echinate fruit); **H,** *Wurfbainia villosa* (echinate fruit); **I.** *Wurfbainia testacea* (stamen with eared anther crest); **J,** *Wurfbainia longiligularis* (stamen with eared anther crest); **K,** *Wurfbainia microcarpa* (stamen with eared anther crest); **L,** *Wurfbainia schmidtii* (stamen with eared anther crest). Photos: A–L, Jana Leong-Škorničková.

Incertae sedis

The following names in *Amomum* listed as incertae sedis include those for which the protologue and original material do not provide sufficient information to allow a new generic placement. Some of these names may even belong in other genera of Alpinioideae.

Amomum alborubellum K.Schum. & Lauterb., Fl. Schutzgeb. Südsee: 230. 1900.
Amomum apiculatum K.Schum. in Bot. Jahrb. Syst. 27(3): 315. 1899.
Amomum bilabiatum S.Sakai & Nagam. in Edinburgh J. Bot. 55(1): 57. 1998.

Amomum centrocephalum A.D.Poulsen in Blumea 48(3): 524. 2003.
Amomum cephalotes Ridl. in J. Fed. Malay States Mus. 10: 154. 1920.
Amomum deuteramomum K.Schum. in Bot. Jahrb. Syst. 27(3): 313. 1899.
Amomum flavorubellum K.Schum. & Lauterb., Fl. Schutzgeb. Südsee: 229. 1900.
Amomum kingii Baker inHooker, Fl. Brit. India 6: 241. 1892.
Amomum kingii var. ***oblongum*** V.P.Thomas & M.Sabu in Phytotaxa 220(1): 89–94. 2015.
Amomum longipes Valeton in Bull. Inst. Bot. Buitenzorg 20: 73. 1904.
Amomum luzonense Elmer in Leaf l. Philipp. Bot. 8: 2976. 1919.
Amomum macrodons Scort. in Nuovo Giorn. Bot. Ital. 18: 309. 1886.
Amomum nemorale (Thwaites) Trimen, Syst. Cat. Fl. Pl. Ceylon: 92. 1885.
Amomum procurrens Gagnep. in Bull. Soc. Bot. France 49: 254. 1903.
Amomum sabuanum V.P.Thomas, Nissar & U.Gupta in Phytotaxa 159(2): 122. 2014.
Amomum stenocarpum Valeton in Bull. Jard. Bot. Buitenzorg, sér. 3, 2: 354. 1920.
Amomum tephrodelphys K.Schum. in Engler, Pflanzenr. IV. 46 (Heft 20): 248. 1904.
Amomum warburgianum K.Schum. & Lauterb., Fl. Schutzgeb. Südsee: 230. 1900.
Amomum warburghii (K.Schum.) K.Schum. in Engler, Pflanzenr. IV. 46 (Heft 20): 257. 1904 ≡ *Costus warburghii* K. Schum. in Bot. Jahrb. Syst. 27(3): 346. 1899.

Geocharis (K.Schum.) Ridl. in J. Straits Branch Roy. Asiat. Soc. 50: 143. 1908 – **Type (designated here):** *G. macrostemon* (K.Schum.) Holttum (≡ *Alpinia macrostemon* K.Schum.). Fig. 1, clade *Geocharis*; Fig. 10.

Geocharis aurantiaca Ridl. in J. Straits Branch Roy. Asiat. Soc. 50: 144. 1908.
Geocharis fusiformis (Ridl.) R.M.Sm. in Notes Roy. Bot. Gard. Edinburgh 43(3): 458. 1986 ≡ *Amomum fusiforme* Ridl. in Philipp. J. Sci., C 4: 171. 1909 ≡ *Elettariopsis fusiformis* (Ridl.) Loes. in Engler & Prantl, Nat. Pflanzenfam., ed. 2, 15a: 603. 1930.
Geocharis fusiformis var. ***borneensis*** R.M.Sm. in Notes Roy. Bot. Gard. Edinburgh 43(3): 58. 1986.
Geocharis macrostemon (K.Schum.) Holttum in Gard. Bull. Singapore 13: 221. 1950 ≡ *Alpinia macrostemon* K.Schum. in Bot. Jahrb. Syst. 27(3): 297. 1899 ≡ *Riedelia macrostemon* (K.Schum.) Loes. in Engler & Prantl, Nat. Pflanzenfam., ed. 2, 15a: 627. 1930.
Geocharis radicalis (Valeton) B.L.Burtt & R.M.Sm. in Notes Roy. Bot. Gard. Edinburgh 31(2): 315. 1972 ≡ *Rhynchanthus radicalis* Valeton in Bull. Jard. Bot. Buitenzorg, sér. 3, 3: 141. 1921.
Geocharis rubra Ridl. in J. Straits Branch Roy. Asiat. Soc. 50: 146. 1908.
Geocharis secundiflora (Ridl.) Holttum in Gard. Bull. Singapore 13: 223. 1950 ≡ *Alpinia secundiflora* Ridl. in J. Straits Branch Roy. Asiat. Soc. 32: 165. 1899.

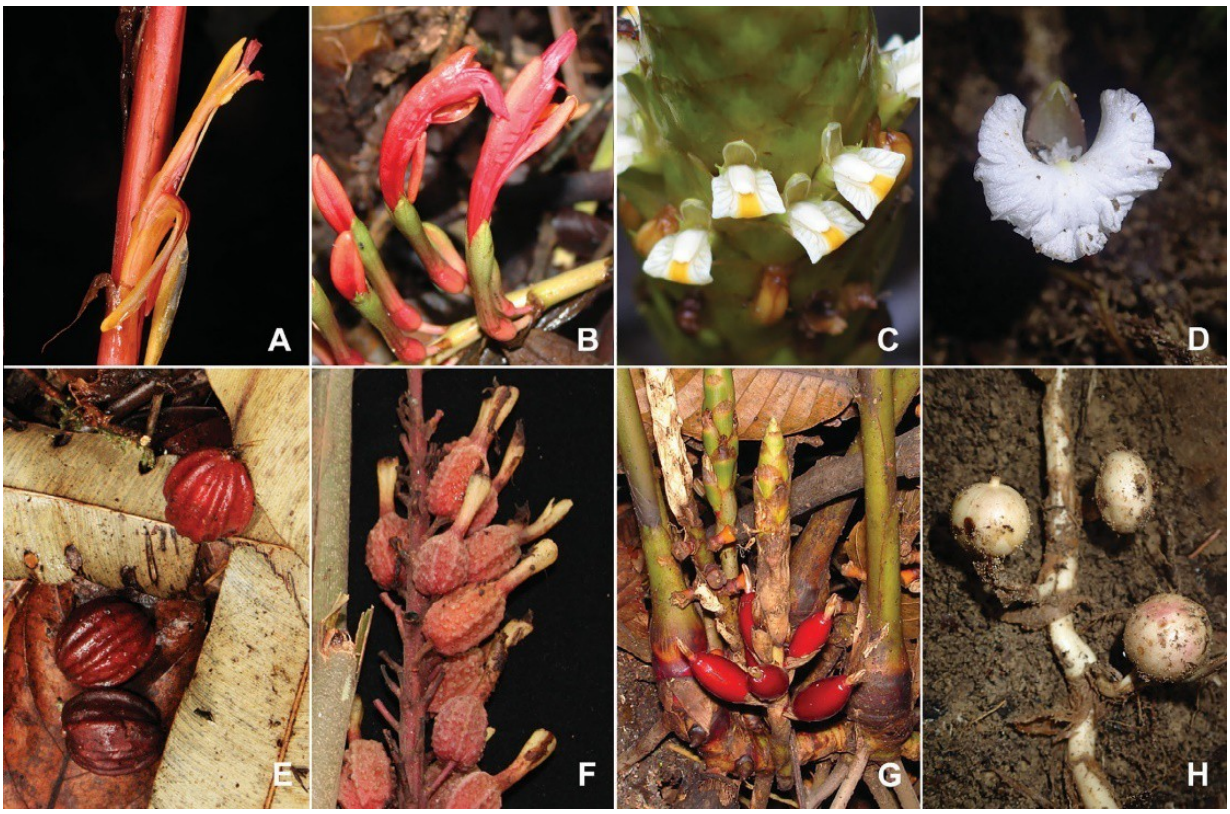


Fig. 10. *Geocharis* and *Amomum* clade V. **A,** *Geocharis fusiformis* (flower); **B,** *Geocharis macrostemon* (flower); **C,** *Amomum dimorphum* (flower); **D,** *Elettaria longituba* (flower); **E,** *Geocharis rubra* (grooved rugose fruit); **F,** *Geocharis macrostemmon* (grooved rugose fruit); **G,** *Amomum anomalum* (smooth fruit); **H,** *Elettaria longituba* (smooth fruit). — Photos: A, Pieter Pelser (see Pelser *et al.*, 2011); B, E & F, A. Jane Droop; C, Januarius Gobilik; D & H, Axel D. Poulsen; G, Jana Leong-Škorničková.

Geostachys (Baker) Ridl. in J. Straits Branch Roy. 32: 157. 1898 ≡ *Alpinia* subg. *Geostachys* Baker in Hooker, Fl. Brit. India 6: 257. 1892 – **Type (designated here):** *G. secunda* (Baker) Ridl. (≡ *Alpinia secunda* Baker). Fig. 1, clade *Geostachys*; Fig. 6. = *Carenophila* Ridl. in J. Fed. Malay States Mus. 4: 78. 1909 – Type: *C. montana* Ridl.

Geostachys angustifolia K.Larsen in Nordic J. Bot. 6: 31(1). 1986.
Geostachys annamensis Ridl. in J. Nat. Hist. Soc. Siam 4: 112. 1921.
Geostachys belumensis C.K.Lim & K.H.Lau in Fol. Malaysiana 6(3–4): 84–85, fig. 1, 3–9. 2005.
Geostachys chayanii Mayoe in Taiwania 55(1): 8–11, fig. 1, 2. 2010.
Geostachys decurvata (Baker) Ridl. in J. Straits Branch Roy. Asiat. Soc. 32: 158. 1898 ≡ *Alpinia decurvata* Baker in Hooker, Fl. Brit. India 6: 257. 1892.
Geostachys densiflora Ridl. in J. Straits Branch Roy. Asiat. Soc. 82: 201. 1920.
Geostachys elegans Ridl. in J. Straits Branch Roy. Asiat. Soc. 32: 160. 1898.
Geostachys erectifrons K.H.Lau, C.K.Lim & Mat-Salleh in Fol. Malaysiana 6(3–4): 85–86, fig. 2, 10–14. 2005.
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Geostachys kerrii K.Larsen in Notes Roy. Bot. Gard. Edinburgh 31: 241. 1972 ≡ *G. densiflora*

K.Larsen in Bot. Tidsskr. 58: 45. 1962, nom. illeg.
Geostachys leucantha B.C.Stone in Malaysian J. Sci. 6A: 77. 1980.
Geostachys maliauensis C.K.Lim & K.H.Lau in Fol. Malaysiana 7(1–2): 34. 2006.
Geostachys megaphylla Holttum in Gard. Bull. Singapore 13: 228. 1950.
Geostachys montana (Ridl.) Holttum in Gard. Bull. Singapore 13: 229. 1950 ≡ *Carenophila montana* Ridl. in J. Fed. Malay States Mus. 4: 78. 1909.
Geostachys penangensis Ridl. in J. Straits Branch Roy. Asiat. Soc. 32: 159. 1898.
Geostachys pierreana Gagnep. in Bull. Soc. Bot. France 53: 147. 1906.
Geostachys primulina Ridl. in J. Straits Branch Roy. Asiat. Soc. 82: 201. 1920.
Geostachys rupestris Ridl. in J. Straits Branch Roy. Asiat. Soc. 32: 159. 1898.
Geostachys secunda (Baker) Ridl. in J. Straits Branch Roy. Asiat. Soc. 32: 158. 1898 ≡ *Alpinia secunda* Baker in Hooker, Fl. Brit. India 6: 257. 1892.
Geostachys sericea (Ridl.) Holttum in Gard. Bull. Singapore 13: 229. 1950 ≡ *Conamomum sericeum* Ridl. in J. Fed. Malay States Mus. 6: 185. 1915.
Geostachys smitinandii K.Larsen in Thai Forest Bull., Bot. 29: 17. 2001.
Geostachys sumatrana Valeton in Bull. Jard. Bot. Buitenzorg, sér. 3, 3: 146. 1921.
Geostachys tahanensis Holttum in Gard. Bull. Singapore 13: 232. 1950.
Geostachys taipingensis Holttum in Gard. Bull. Singapore 13: 230. 1950.
Geostachys tratensis Picheans. & Mayoe in J. Jap. Bot. 86(3): 133–138, fig. 1, 2. 2011.

CONCLUSIONS

Amomum as previously perceived was diverse in morphology, showing differences in habit, inflorescence and capsule, and phylogenetic studies have shown the genus to be paraphyletic (Xia & al., 2004; Kress & al., 2007). Morphological studies have also highlighted the limited differences between some genera in Alpinieae, such as *Elettariopsis* and *Amomum* (Kam, 1982; Lamxay & Newman, 2012). Previous molecular and morphological studies have been unable to resolve relationships and taxonomy in the Alpinieae, either due to lack of accessions of the type of *Amomum*, or in the face of the great morphological variability. The targeted sampling in this study combined with the molecular data, phylogenetic analyses and examination of morphological characters allows recircumscription of the 10 clades of the paraphyletic genus *Amomum* as separate genera. This will provide a framework to facilitate detailed taxonomic revisions and creates nomenclatural stability. Three genera, *Conamomum*, *Meistera* and *Wurfbainia*, are resurrected, and three new genera *Epiamomum*, *Lanxangia* and *Sundamomum* are described. Further studies and specific sampling will be needed to resolve relationships within and among *Alpinia*, *Elettaria*, *Etlingera* and *Hornstedtia*.

AUTHOR CONTRIBUTIONS

HdB and MN contributed equally to this work. HdB and MN initiated the study. ADP, JL-Š, MN, VL and AJP collected and identified material. AJD, KH, LTTH and KS carried out the laboratory work. HdB, TF and LTTH did the phylogenetic analysis. MN, ADP, JL-Š, AJD, KH and VL did the taxonomic research. HdB, MN, ADP, JER and JL-Š drafted the manuscript and figures. All

authors have read, commented and approved the final manuscript. — HdB, https://orcid.org/0000-0003-1985-7859; JL-Š, https://orcid.org/0000-0003-4490-3490

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Appendix 1. List of taxa sampled with voucher information and GenBank accession numbers (nrITS, *matK*). Sequences denoted with an asterisk (*) were generated for this study. Numbers in parentheses refer to the branch labels in the phylogeny (Fig. 1).

Aframomum angustifolium (Sonn.) K.Schum., Madagascar, *W.J. Kress 92-3403* (US), AF478704, AF478804; ***Aframomum sceptrum*** (Oliv. & D.Hanb.) K.Schum., Gabon, *W.J. Kress 98-6268* (US), AF478706, AF478806; ***Alpinia abundif lora*** B.L.Burtt & R.M.Sm., Sri Lanka, *A. Weerasooriya s.n.* (K, PDA), AY742334, AY742393; ***Alpinia aenea*** B.L.Burtt & R.M.Sm., Indonesia, South Sulawesi, *G. Argent 0016* (E), AY742351, AY742394; ***Alpinia calcarata*** (Haw.) Roscoe, China, *W.J. Kress 94-3657* (US), AF478710, AF478810; ***Alpinia conchigera*** Griff., China, *W.J. Kress 00-6706* (US), AF478712, AF478812; ***Alpinia elegans*** (C.Presl) K.Schum., Philippines, *W.J. Kress 99-6412* (US), AF478713, AF478813; ***Alpinia fax*** B.L.Burtt & R.M.Sm., Sri Lanka, *A. Weerasooriya s.n.* (K, PDA), AY742348, AY742405; ***Alpinia galanga*** (L.) Willd., Cult., *Lyon Arbor. 83.505* (HLA), AF478715, AF478815; ***Alpinia javanica*** Blume, Indonesia, *A. Rangsiruji 53* (E), AY742358, AY742413; ***Alpinia luteocarpa*** Elmer, Philippines, *W.J. Kress 99-6403* (US), AF478717, AF478817; ***Alpinia malaccensis*** (Burm.f.) Roscoe, India, Kerala, Kakkattode, *M.R. Vinitha 86426* (CALI), KY438058*, KY510020*; ***Alpinia*** aff. ***melichroa*** K.Schum., Indonesia, Sulawesi Tengah, *A.D. Poulsen & Sharp 2834* (E) **(1)**, KY438060*, KY620226*; ***Alpinia*** aff. ***melichroa*** K.Schum., Indonesia, Sulawesi, *M.F. Newman & J. Leong- Škorničková 1478* (E) **(2)**, KY438045*, KY620248*; ***Alpinia monopleura*** K.Schum., Indonesia, Sulawesi, *S.M. Scott 02-101* (E), KY438054*, KY620264*; ***Alpinia murdochii*** Ridl., Malaysia, Pahang, *O. Šída, T. Fér & E. Záveská M-11-1* (PR), KY438007*, KY620260*; ***Alpinia nieuwenhuizii*** Valeton, Borneo [ex cult. SBG], *J. Leong-Škorničková GRC-192* (SING), KY438031*, KY620256*; ***Alpinia nigra*** (Gaertn.) Burtt, India, Arunachal Pradesh, *M.R. Vinitha 92522* (CALI) **(1)**, –, KY510017*; ***Alpinia nigra*** (Gaertn.) Burtt, Thailand, Chiang Mai, *O. Šída, T. Fér & P. Suksathan T-11-105* (PR) **(2)**, KY438091*, KY620220*; ***Alpinia officinarum*** Hance, China, *W.J. Kress 00-6614* (US), AF478718, AF478818; ***Alpinia pumila*** Hook.f., China, *W.J. Kress 97-6119* (US), AF478719, AF478819; ***Alpinia purpurata*** (Vieill.) K.Schum., Solomon Islands, Guadalcanal, *A.D. Poulsen 2467* (AAU, BSIP, E), KY438102*, KY620252*; ***Alpinia raff lesiana*** Wall. ex Baker, Malaysia, *Ibrahim & Jong s.n. (Kress 97-6119)* (E), AY742376, AY742430; ***Alpinia cylindrocephala*** K.Schum., Indonesia, Gorontalo, *M.F. Newman & J. Škorničková 1467* (E), AY742345, AY742403; ***Alpinia tonkinensis*** Gagnep., China, *Liao 020709* (IBSC), AY742386, AY742439; ***Amomum aculeatum*** Roxb., Indonesia, West Sumatra, *A.J. Droop 96* (ANDA, BO, E) **(1)**, KY438036*, KY620254*; ***Amomum aculeatum*** Roxb., Thailand, Krabi, *M.F. Newman & J. Škorničková 1458* (E) **(2)**, KY438062*, –; ***Amomum angustipetalum*** S.Sakai & Nagam., Malaysia, Sarawak, Lambir Hills, *S. Sakai 389* (KYO), AB097245, JF715466; ***Amomum anomalum*** R.M.Sm., Malaysia, Sarawak, Hose Mts., *A.D. Poulsen et al. 2033* (AAU, SAR), KY438106*, –; ***Amomum apiculatum*** K.Schum., Indonesia, West Sumatra, *A.D. Poulsen & Hatta 2275* (ANDA, AAU, BO), KY438083*, KY620224*; ***Amomum calcaratum*** Lamxay & M.F.Newman, Laos, Khammouan, *V. Lamxay 2065* (NLS), KY438053*, KY510004*; ***Amomum calcicolum*** Lamxay & M.F.Newman, Laos, Khammouan, *V. Lamxay 2066* (NLS), –, KY510000*; ***Amomum calyptratum*** S.Sakai & Nagam., Malaysia, Sarawak, Lambir Hills, *S. Sakai 363* (KYO), AB097239, JF715467; ***Amomum celsum*** Lamxay & M.F.Newman, Laos, Attapeu, *V. Lamxay 1189* (E, NLS, P, UPS), KY438033*, KY510011*; ***Amomum centrocephalum*** A.D.Poulsen, Indonesia, North Sumatra, *A.J. Droop 29* (BO, E), KY438010*, KY620247*; ***Amomum cerasinum*** Ridl., Indonesia, West Sumatra, *A.J. Droop 160* (ANDA, BO, E) **(1)**, KY438109*, KY620251*; ***Amomum cerasinum*** Ridl., Malaysia, Sarawak, Kubah National Park, *A.D. Poulsen et al. 2945* (E, SAR) **(2)**, KY438080*, –; ***Amomum chinense*** W.Y.Chun, Laos, Bolikhamxai, *V. Lamxay 2071* (NLS), KY438044*, KY510019*; ***Amomum chryseum*** Lamxay & M.F.Newman, Laos, Bolikhamxai, *V. Lamxay 1171* (E, NLS), –, KY510002*; ***Amomum compactum*** Sol. ex Maton, Malaysia, Sabah, *J. Mood 753* (UH), KY438038*, –; ***Amomum coriaceum*** R.M.Sm., Malaysia, Sarawak, Lambir Hills, *S. Sakai 357* (KYO), AB097240, JF715468; ***Amomum coriandriodorum*** S.Q.Tong & Y.M.Xia, China, *Y.M. Xia 721* (HITBC), AY351987, AY352017; ***Amomum dealbatum*** Roxb., Laos, Vientiane Capital, *V. Lamxay 1119* (E, NLS), KY438014*, –; ***Amomum dictyocoleum*** K.Schum., Malaysia. Sarawak, Semenggoh Forest Reserve, *A.D. Poulsen et al. 2936* (E, SAR), KY438039*, –; ***Amomum dimorphum*** M.F.Newman, Malaysia, Sarawak, Lambir Hills, *S. Sakai 372* (KYO), JF715469, AB097244; ***Amomum durum*** S.Sakai & Nagam., Malaysia, Sarawak, Lambir Hills, *S. Sakai 362* (KYO), JF715470, AB097241; ***Amomum echinocarpum*** Alston, Laos, Houaphan, *V. Lamxay 1315* (E, NLS), KY438068*, –; ***Amomum glabrifolium*** Lamxay & M.F.Newman, Laos, Khammouan, *V. Lamxay 2068* (NLS), KY438049*, KY510005*; ***Amomum glabrum*** S.Q.Tong, Laos, Louangnamtha, *V. Lamxay 1157* (E, NLS) **(1)**, KY438070*, –; ***Amomum glabrum*** S.Q.Tong, China, *Y.M. Xia 722* (HITBC) **(2)**, AY351989, AY352019; ***Amomum gyrolophos*** R.M.Sm., Malaysia, Sarawak, Lambir Hills, *S. Sakai 352* (KYO), JF715471, AB097242; ***Amomum*** aff. ***hasti- labium*** Ridl., Indonesia, West Sumatra, *A.J. Droop 76* (ANDA, E) **(1)**, KY438067*, KY620266*; ***Amomum hastilabium*** Ridl., Indonesia, West Sumatra, *A.D. Poulsen et al. 2262* (ANDA, BO, E) **(2)**, KY438022*, KY620246*; ***Amomum hastilabium*** Ridl., Singapore, *J. Leong-Škorničková et al. SNG-160* (SING) **(3)**, KY438085*, KY620261*; ***Amomum kinabaluense*** R.M.Sm., Malaysia, Sabah, *J. Mood 418* (UH), KY438096*, –; ***Amomum koenigii*** J.F.Gmel., Laos, Champasak, *V. Lamxay 2078* (NLS) **(1)**, KY438112*, KY510012*; ***Amomum koenigii*** J.F.Gmel., Laos, Oudomxai, *J. Leong-Škorničková et al. JLS-1731* (SING) **(2)**, KY438048*, –; ***Amomum lappaceum*** Ridl., Malaysia, Pahang, *J. Leong-Škorničková et al. JLS-3173* (SING), KY438063*, KY620230*; ***Amomum*** aff. ***laxesquamosum*** K.Schum., Indonesia, Banten, *A.D. Poulsen et al. 2346* (BO, E), KY438043*, KY620268*; ***Amomum longiligulare*** T.L.Wu, Laos, Champasak, *V.*

Lamxay 2081 (NLS) **(1)**, KY438101*, –; ***Amomum longiligulare*** T.L.Wu, Vietnam, Kontum, *J. Leong-Škorničková et al. JLS-1602* (E, P, PR, SING, VNMN) **(2)**, KY438041*, KY510021*; ***Amomum longiligulare*** T.L.Wu, Laos, Champasak, *V. Lamxay 2083* (NLS) **(3)**, KY438108*, KY620234*; ***Amomum longipetiolatum*** Merr., China, *W.J. Kress 99-6353* (US), AF478722, AF478822; ***Amomum maximum*** Roxb., China, *Y.M. Xia 725* (HITBC), AY351995, AY352025; ***Amomum menglaense*** S.Q.Tong, China, *Y.M. Xia 726* (HITBC), AY351996, AY352026; ***Amomum mentawaiense*** A.J.Droop, Indonesia, West Sumatra, *A.D. Poulsen et al. 2249* (ANDA, BO, E), KY438075*, KY620223*; ***Amomum micranthum*** Ridl., Malaysia, Penang, *J. Leong-Škorničková*
et al. JLS-2010 (SING), KY438042*, KY620231*; ***Amomum microcarpum*** C.F.Liang & D.Fang, Laos, Louangnamtha, *V. Lamxay 2055* (NLS) **(1)**, KY438066*, KY510006*; ***Amomum microcarpum*** C.F.Liang & D.Fang, Laos, Bolikhamxai, *V. Lamxay 2091* (NLS) **(2)**, KY438025*, KY510010*; ***Amomum ochreum*** Ridl., Indonesia, North Sumatra, *A.D. Poulsen 2365* (BO, E), KY438050*, –; ***Amomum odontocarpum*** D.Fang, Laos, Xiangkhoang, *V. Lamxay 1300* (E, NLS) **(1)**, KY438046*, –; ***Amomum odontocarpum*** D.Fang, Laos, Phongsali, *V. Lamxay 1322* (E, NLS) **(2)**, KY438006*, –; ***Amomum oliganthum*** K.Schum., Malaysia, Sarawak, Lambir Hills, *S. Sakai 370* (KYO), AB097243, JF715472; ***Amomum oligophyllum*** A.J.Droop, Indonesia, West Sumatra, *A.J. Droop 155* (ANDA, BO, E) **(1)**, KY438020*, –; ***Amomum oligophyllum*** A.J.Droop, Indonesia, West Sumatra, *A.J. Droop 92* (ANDA, BO, E) **(2)**, KY438089*, KY620219*; ***Amomum paratsaoko*** S.Q.Tong & Y.M.Xia, China, *W.J. Kress 98-6197* (US), AY351997, AY352027; ***Amomum petaloideum*** (S.Q.Tong) T.L.Wu, China, *Li Qing jun ZL365-4* (KUN) **(1)**, –, JF953210; ***Amomum petaloideum*** (S.Q.Tong) T.L.Wu, China, Yunnan, *W.J. Kress, T. Wood, Li 95-5508* (E) **(2)**, KY438055*, KY620228*; ***Amomum pierreanum*** Gagnep., Cambodia, Kaoh Kong, *J. Kanstrup 222* (E) **(1)**, KY438009*, KY510023*; ***Amomum pierreanum*** Gagnep., Thailand, Chantaburi, *M.F. Newman 929* (E) **(2)**, KY438094*, KY620232*; ***Amomum plicatum*** Lamxay & M.F.Newman, Laos, Attapeu, *V. Lamxay 1191* (E, NLS, P, UPS), KY438064*, –; ***Amomum prionocarpum*** Lamxay & M.F.Newman, Laos, Houaphan, *V. Lamxay 1303* (E, NLS, P), KY438037*, –; ***Amomum propinquum*** Ridl., Philippines, *Lyon Arbor. 93.0558* (HLA), AY351999, AY352029; ***Amomum pseudofoetens*** Valeton, Indonesia, West Java, *A.D. Poulsen 2284* (AAU, BO, E), KY438008*, KY620229*; ***Amomum pterocarpum*** Thwaites, India, Kerala, Calicut Univ. campus, *M.R. Vinitha 75254* (CALI) **(1)**, KY438065*, KY509999*; ***Amomum pterocarpum*** Thwaites, India, Kerala, Nilakkal, *M.R. Vinitha 95679* (CALI) **(2)**, KY438081*, KY510009*; ***Amomum purpureorubrum*** S.Q.Tong & Y.M.Xia, China, *Y.M. Xia 727* (HITBC), AY352000, AY352030; ***Amomum putrescens*** D.Fang, China, *Y.M. Xia 728* (HITBC) **(1)**, AY352002, AY352032; ***Amomum putrescens*** D.Fang, Vietnam, *J. Leong-Škorničková et al. JLS-2146* (SING) **(2)**, KY438017*, –; ***Amomum quadratol- aminare*** S.Q.Tong, India, Nagaland, *J. Mood 3208* (ASSAM) **(1)**, KY438028*, –; ***Amomum quadratolaminare*** S.Q.Tong, China, *Y.M. Xia 729* (HITBC) **(2)**, AY352003, AY352033; ***Amomum queenslandicum*** R.M.Sm., Australia, *Lyon Arbor. Kmn 1428* (HLA), AY352004, AY352034; ***Amomum*** aff. ***repoeense*** Pierre ex Gagnep., Vietnam, Thua Thien-Hue, *J. Leong-Škorničková et al. JLS-1619* (E, PR, SING, VNMN) **(1)**, KY438040*, KY620222*; ***Amomum*** aff. ***repoeense*** Pierre ex Gagnep., Vietnam, Thua Thien-Hue, *J. Leong-Škorničková et al. JLS-1637* (E, PR, SING, VNMN) **(2)**, KY438019*, KY620243*; ***Amomum repoeense*** Pierre ex Gagnep., Vietnam, Lam Dong, *H.Đ. Trần et al. 67* (E) **(3)**, KY438059*, KY620240*; ***Amomum repoeense*** Pierre ex Gagnep., Cambodia, Kaoh Kong, *J. Kanstrup 223* (E) **(4)**, KY438056*, KY620237*; ***Amomum roseisquamosum*** Nagam. & S.Sakai, Malaysia, Sarawak, *S. Sakai 188* (KYO), AB097246, JF715473; ***Amomum sceletescens*** R.M.Sm., Malaysia, Sabah, *J. Mood 1154* (UH), KY438111*, –; ***Amomum schmidtii*** (K.Schum.) Gagnep., Laos, Bolikhamxai, *V. Lamxay 2069* (NLS) **(1)**, KY438069*, KY510016*; ***Amomum schmidtii*** (K.Schum.) Gagnep., Vietnam, Tay Ninh, *H.Đ. Trần et al. 28* (E) **(2)**, KY438103*, KY509998*; ***Amomum sericeum*** Roxb., Thailand, *J. Mood 2019* (BKF) **(1)**, KY438097*, –; ***Amomum sericeum*** Roxb., Laos, Louangnamtha, *V. Lamxay 1155* (E, NLS) **(2)**, KY438034*, –; ***Amomum sericeum*** Roxb., Laos, Louangphabang, *V. Lamxay 2108* (NLS) **(3)**, KY438052*, KY510018*; ***Amomum somniculosum*** S.Sakai & Nagam., Malaysia, Sarawak, Lambir Hills, *S. Sakai 354* (KYO), AB097247, JF715474; ***Amomum staminidivum*** Gobilik, A.L.Lamb & A.D.Poulsen, Indonesia, East Kalimantan, *A.D. Poulsen et al. 2113* (AAU, BO, L, WAN), KY438023*, KY620225*; ***Amomum stephanocoleum*** Lamxay & M.F.Newman, Laos, Bolikhamxai, *V. Lamxay 1250* (E, NLS), KY438082*, –; ***Amomum subcapitatum*** Y.M.Xia, Laos, Oudomxai, *V. Lamxay* 2060 (NLS) **(1)**, –, KY510022*; ***Amomum subcapitatum*** Y.M.Xia, Laos, Louangnamtha, *V. Lamxay* 2058 (NLS) **(2)**, KY438076*, KY510007*; ***Amomum subulatum*** Roxb., India, Nagaland, *J. Mood 3218* (ASSAM) **(1)**, KY438086*, –; ***Amomum subulatum*** Roxb., India, Sikkim, Rongli, *M.R. Vinitha 92765* (CALI) **(2)**, KY438013*, KY510015*; ***Amomum subulatum*** Roxb., India, Nagaland, Kohima, *M.R. Vinitha 103627* (CALI) **(3)**, KY438107*, KY620253*; ***Amomum su- bulatum*** Roxb., India, West Bengal, Darjeeling, *J. Škorničková 71468* (CALI, SING) **(4)**, KY438057*, KY510013*; ***Amomum subulatum*** Roxb., India, Sikkim, Gangtok, *M.R. Vinitha 92709* (CALI) **(5)**, KY438095*, KY510003*; ***Amomum tenellum*** Lamxay & M.F.Newman, Laos, Attapeu, *V. Lamxay 1192* (E, NLS, P), KY438072*, –; ***Amomum testaceum*** Ridl., Malaysia, Pahang [ex cult. SBG], *J. Leong-Škorničková GRC-213* (SING), KY438110*, KY620250*; ***Amomum tomrey*** Gagnep. var. ***tomrey***, Laos, Savannakhet, *V. Lamxay 1196* (NLS), KY438004*, KY510008*; ***Amomum trianthemum*** K.Schum., Indonesia, Gorontalo, *M.F. Newman et al. 118* (E), KY438090*, –; ***Amomum tsaoko*** Crevost & Lemarié, China, *Y.M. Xia 734* (HITBC), AY352007, AY352037; ***Amomum uliginosum*** J.Koenig, Laos, Khammouan Province, *V. Lamxay 1021* (E, NLS) **(1)**, KY438071*, KY620227*; ***Amomum uliginosum*** J.Koenig, Laos, Khammouan, *V. Lamxay 2067* (NLS) **(2)**, KY438073*, KY510001*; ***Amomum uliginosum*** J.Koenig, Vietnam, Lam Dong, *H.Đ. Trần et al. 68* (E) **(3)**, KY438098*, KY509997*; ***Amomum*** aff. ***utriculosum*** Ridl., Malaysia, Penang, *J. Leong-Škorničková et al. JLS-3170* (SING), –, KY629731*; ***Amomum verum*** Blackw., Indonesia,

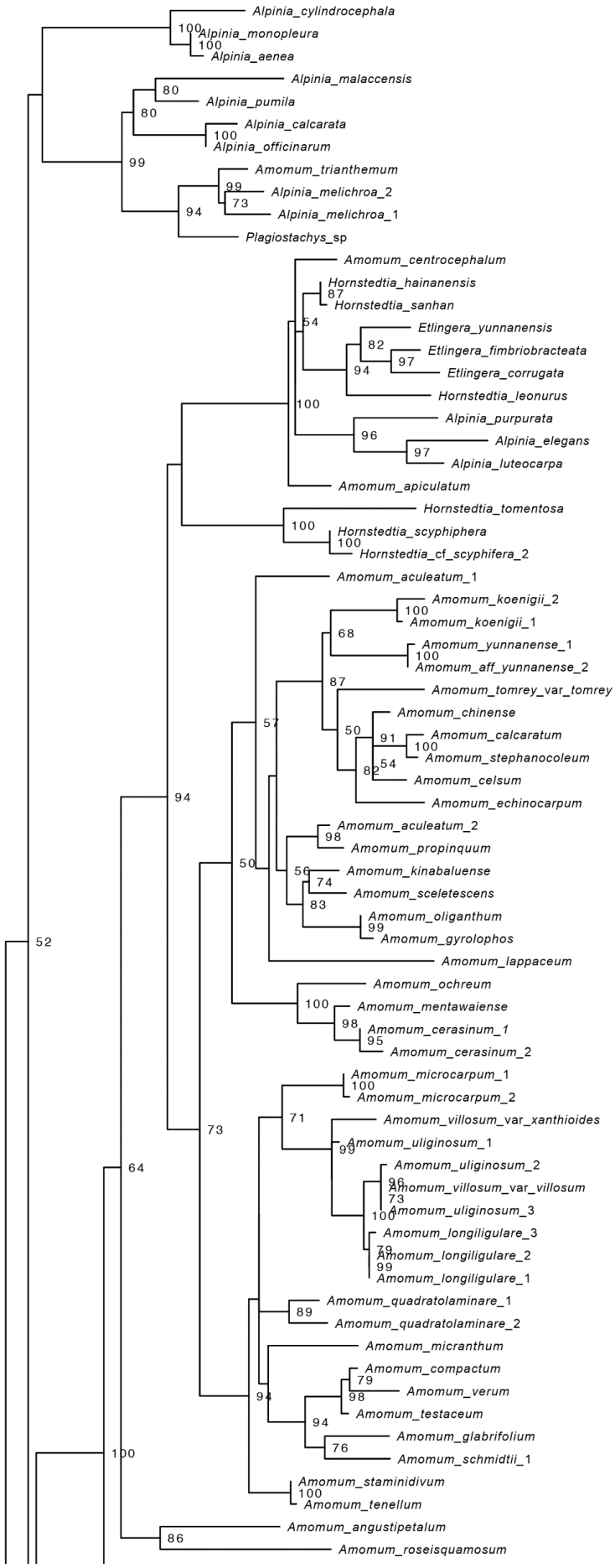
West Sumatra, *A.J. Droop 10* (BO, E), KY438099*, KY620241*; ***Amomum villosum*** Lour. var. ***villosum***, Laos, Champasak, *V. Lamxay 2079* (NLS), KY438016*, KY510014*; ***Amomum villosum*** var. ***xanthioides*** (Wall. ex Baker) T.L.Wu & S.J.Chen, Vietnam, Ha Noi, *Nguyen Quoc Binh VMN-B0000487* (VNMN), KY438092*, –; ***Amomum*** cf. ***xanthophlebium*** Baker, Malaysia, Penang, *J. Leong-Škorničková et al. JLS-1663* (SING) **(1)**, KY438051*, KY510024*; ***Amomum xanthophlebium*** Baker, Indonesia, West Sumatra, *A.J. Droop 81* (BO, E) **(2)**, KY438018*, –; ***Amomum xanthophlebium*** Baker, Singapore, *J. Leong- Škorničková et al. SNG-139* (SING) **(3)**, KY438026*, KY620242*; ***Amomum*** aff. ***yunnanense*** S.Q.Tong, India, Nagaland, *J. Mood 3226* (ASSAM) **(1)**, AY352012, AY352042; ***Amomum yunnanense*** S.Q.Tong, China, *Y.M. Xia 737* (HITBC) **(2)**, KY438027*, –; ***Burbidgea schizocheila*** Hackett, Malaysia, Sarawak, *W.J. Kress 01-6867* (US), AF478729, AF478829; ***Burbidgea stenantha*** Ridl., Borneo [ex cult. SBG], *J. Leong-Škorničková & H.Đ. Trần GRC-88* (SING), KY438061*, KY620236*; ***Elettaria cardamomum*** (L.) Maton, Malaysia, Sarawak, *J. Leong-Škorničková JLS-432* (SING), KY438100*, –; ***Elettaria longipilosa*** S.Sakai & Nagam., Malaysia, Sarawak, Lambir Hills, *S. Sakai 380* (KYO), AB097229, JF715480; ***Elettaria longituba*** (Ridl.) Holttum, Malaysia, Sarawak, Lambir Hills, *S. Sakai 201* (KYO), AB097228, JF715481; ***Elettariopsis curtisii*** Baker, Thailand, Trang, *M.F. Newman s.n.* (E), KY438105*, –; ***Elettariopsis elan*** C.K.Lim, Malaysia [ex cult. SBG], *J. Leong-Škorničková GRC-79* (SING), KY438087*, KY620262*; ***Elettariopsis kerbyi*** R.M.Sm., Malaysia, Sarawak, *W.J. Kress 96-5746* (US), AF478746, AF478845; ***Elettariopsis rugosa*** (Y.K.Kam) C.K.Lim, Malaysia [ex cult. SBG], *J. Leong-Škorničková GRC-362* (SING), KY438032*, KY620267*; ***Elettariopsis stenosiphon*** (K.Schum.) B.L.Burtt & R.M.Sm., Malaysia, Sarawak, *W.J. Kress 01-6847* (US), AF478748, AF478847; ***Elettariopsis triloba*** (Gagnep.) Loes., Vietnam, Dong Nai, *M.F. Newman & J. Škorničková 1455* (E), KY438077*, –; ***Elettariopsis unifolia*** (Gagnep.) M.F.Newman, Vietnam, Dong Nai, *M.F. Newman & J. Škorničková 2002* (E), KY438015*, KY620257*; ***Etlingera corrugata*** A.D.Poulsen & Mood, Malaysia, Sabah, *J. Leong-Škorničková JLS-220* (SING), KY438084*, KY620239*; ***Etlingera fimbriobracteata*** (K.Schum.) R.M.Sm., Borneo [ex cult. SBG], *J. Leong-Škorničková GRC-362* (SING), KY438005*, KY620255*; ***Etlingera yunnanensis*** (T.L.Wu & S.J.Chen) R.M.Sm., China, *Y.M. Xia 738 (W.J. Kress 95-5511)* (HITBC), AY352014, AY352044; ***Geocharis fusiformis*** var. ***borneensis*** R.M.Sm., Malaysia, Sabah (cult.), *L.B. Pedersen 1141* (C), AF414487, –; ***Geocharis rubra*** Ridl., Indonesia, West Sumatra, *A.J. Droop 106* (E), KY438079*, KY620258*; ***Geocharis macrostemon*** (K.Schum.) Holttum, Indonesia, North Sumatra, *A.J. Droop 19* (E), KY438104*, KY620249*; ***Geostachys densif lora*** Ridl., Malaysia, Pahang, *O. Šída, T. Fér & E. Záveská M-11-2* (PR), KY438011*, KY620238*; ***Geostachys megaphylla*** Holttum, Malaysia, Pahang, *O. Šída, T. Fér & E. Záveská M-11-10* (PR), KY438078*, KY620244*; ***Hornstedtia hainanensis*** T.L.Wu & S.J.Chen, China, Hainan, *W.J. Kress 97-5769* (US), AF478766, AF478865; ***Hornstedtia leonurus*** (J.Koenig) Retz., Singapore, *J. Leong-Škorničková et al. SNG 72* (SING), –, KY620269*; ***Hornstedtia sanhan*** M.F.Newman, Vietnam, *M.F. Newman 202* (E), AY769844, –; ***Hornstedtia*** cf. ***scyphifera*** (J.Koenig) Steud., Indonesia, West Sumatra, *A.J. Droop 4* (ANDA, BO, E) **(1)**, –, KY620235*; ***Hornstedtia scyphifera*** (J.Koenig) Steud., Singapore, *J. Leong-Škorničková et al. SNG-21* (SING) **(2)**, KY438021*, –; ***Hornstedtia tomentosa*** (Blume) Bakh.f., Borneo [ex cult. SBG], *J. Leong- Škorničková GRC-169* (SING), KY438074*, KY620265*; ***Plagiostachys*** **sp.**, Borneo [ex cult. SBG], *J. Leong-Škorničková JLS-1882* (SING), KY438024*, KY620259*; ***Pleuranthodium f loribundum*** (K.Schum.) R.M.Sm., Papua New Guinea, *Waimea W75S1701 (Kress 94-5337)* (US), AF478774, AF478875; ***Pleuranthodium schlechteri*** (K.Schum.) R.M.Sm., Papua New Guinea, *W.J. Kress 00-6725* (US), AF478775, AF478876; ***Renealmia alpinia*** (Rottb.) Maas, Tropical America, *W.J. Kress 99-6407* (US), AF478778, AF478879; ***Renealmia battenbergiana*** Cummins ex Baker, Ghana, *W.J. Kress 94-5277* (US), AF478779, AF478880; ***Renealmia polypus*** Gagnep., Central African Republic, *D.J. Harris 7298* (E), KY438047*, KY620263*; ***Renealmia thyrsoidea*** (Ruiz & Pav.) Poepp. & Endl., Tropical America, *Lyon 80.0721 (Kress 99-6406)* (US), AF478783, AF478884; ***Riedelia*** **sp.**, Papua New Guinea, *GH #96-281* (US), AF478785, AF478886; ***Siamanthus siliquosus*** K.Larsen & J.Mood, Thailand, *W.J. Kress 99-6349* (US), AF478790, AF478891; ***Siliquamomum oreodoxa*** N.S.Lý & Škorničk., Vietnam, Lam Dong, *S. Hul & N.S. Lý 3583* (E, P, SING, VNM), KY438093*, KY620221*; ***Siliquamomum tonkinense*** Baill., Vietnam, Ha Noi, *J. Leong-Škorničková et al. JLS-846* (SING), KY438088*, KY620233*; ***Siphonochilus kirkii*** (Hook.f.) B.L.Burtt, Tanzania, *W.J. Kress 94-3692* (US), AF478794, AF478895.

Table S1. Historical classifications of *Amomum* from Schumann (1899) onwards, including indication of placement of the species as supported by the present study.

Clades in present study	Schumann 1899 - based on species from Malaysia & Papuasia				Schumann 1904 - based on entire geographical distribution				Loesner 1930 - based on entire geographical distribution		Smith 1985 & 1989, Bornean species only Sakai & Nagamasu (1998) - species added in blue font					
	Subgenus III. Autamomum - Inflorescence short, peduncle no more than 15 cm long, sterile bracts absent Species included: III, VII, IX, UNPLACED, *Alpinia* IV, aff. *Alpinia* IV, *Etlingera*, *Hornstedtia*				Sect. Geanthus - Anther appendage absent Species included: III, VII, UNPLACED, *Alpinia* I, *Etlingera*		Sect. Euamomum (*Amomum*) - Anther appendage present Species included: I, III, IV, VII, VIII, IX, UNPLACED,*Alpinia* IV, *Etlingera*, *Hornstedtia*									
	Series 1. Densiflorae - Inflorescence flat on top, on a laxly imbricate peduncle. Species included: III & UNPLACED	Series 2. Multiflorae - Inflorescence rounded on top, sessile or on a short, densely imbricate peduncle. Species included: III, VII, IX, UNPLACED, *Etlingera*, *Hornstedtia*	Series 3. Laxiflorae - Flowers numerous, lax, basically not hidden by the bracts. Species included: *Etlingera*	Series 4. Pauciflorae - Flowers fewer, not completely covered. Species included: I, III, *Etlingera*, *Alpinia*, aff. *Alpinia*	Series 1. Oliganthae - Flowers few, 3–4 in spike Species included: *Etlingera*	Series 2. Polyanthae - Flowers many in spike Species included: III, VII, UNPLACED, *Etlingera*, *Alpinia* I	Series 3 Lobulatae - Anther appendage bi- or trilobed Species included: III, VII, VIII, UNPLACED, *Elettaria*, *Etlingera*, IX + *Hornstedtia* III	Series 4 Integrae (*Amomum*) - Anther appendage entire Species included: I, III, IV, VII, VIII, UNPLACED, *Hornstedtia*, *Etlingera*, *Alpinia* IV	Series Integrae (*Amomum*) - Anther appendage entire Species included: I, III, IV, VII, VIII, UNPLACED, *Hornstedtia*, *Etlingera*, *Alpinia* IV	Series Lobulatae - Anther appendage bi- or trilobed Species included: III, IV, VII, VIII, UNPLACED, *Elettaria*	Group I - Flowers borne in cincinni; bracteoles always tubular; corolla tube equal to the calyx; lateral petals free from the labellum; lateral staminodes absent; anther-thecae dehiscing in the upper half only; connective crested or not Species included: V	Group II. Flowers borne singly; bracteoles open to the base; corolla tube long exserted from the calyx; lateral petals centrally connate to each other and to the labellum in the lower part; lateral staminodes present or not; anther-thecae dehiscing throughout their length; connective crested Species included: VI	Group III - Flowers borne singly; bracteoles open to the base; corolla tube long exserted from the calyx; lateral petals free; lateral staminodes absent; anther-thecae dehiscing throughout their length but with basal spurs which are adnate to the broad filament; connective crested Species included: VI, but see remark	Group IV: Flowers borne singly; bracteoles open to the base or tubular; corolla tube ± equal to or shorter than the calyx; lateral petals free; lateral staminodes almost always present; anther-thecae dehiscing throughout their length; connective usually crested Species included: III, IV, VII, VIII	Group V (added by R.M.Smith 1989) Flowers borne singly; bracteoles open to the base; corolla tube long exserted from the calyx; lateral petals free; lateral staminodes absent; anther-thecae dehiscing throughout their length; connective crested; capsule flattened laterally Species included:	Incertae sedis (added by Sakai & Nagamasu 1998) Species included: VI, UNPLACED
clade I				*A. stenosiphon* (I)				*A. dealbatum* (I) *A. hypoleucum* (I) *A. maximum* (I) *A. microstephanum* (I) *A. pauciflorum* (I) *A. pterocarpum* (I) *A. sericeum* (I) *A. stenosiphon* (I) *A. subulatum* (I)	*A. dealbatum* (I) *A. maximum* (I) *A. pterocarpum* (I) *A. robertsonii* (I) *A. siamense* (I) *A. stenosiphon* (I) *A. subulatum* (I)							
Clade II																	
Clade III	*A. dictyocalenum* (III)	*A. laxesquamosum* (III) *A. macroglossa* (III)		*A. oliganthum* (III)		*A. dictyocalenum* (III) *A. macroglossa* (III)	*A. laxesquamosum* (III)	*A. gracilipes* (III) *A. hastilabium* (III) *A. oliganthum* (III)	*A. hastilabium* (III)	*A. laxesquamosum* (III)				*A. calyptratum* (III) *A. dictyocalenum* (III) *A. durum* (III) *A. flavoalbum* (III) *A. laxesquamosum* (III) *A. longipedunculatum* (III) *A. luteum* (III) *A. macroglossum* (III) *A. oliganthum* (III) *A. paucifolium* (III) *A. rostellatum* (III)			
Clade IV								*A. cylindraceum* (IV) *A. stenoglossum* (IV)	*A. cylindraceum* (IV) *A. stenoglossum* (IV)	*A. cylindrostachys* (IV)				*A. coriaceum* (IV) *A. floridulum* (IV) *A. xanthophlebium* (IV)			
Clade V											*A. anomalum* (V) *A. burttii* (V) *A. dimorphum* (V; listed as *A. polycarpum*) *A. lambirense* (V) *A. ligulatum* (V)						
Clade VI												*A. angustipetalum* (VI) *A. hansenii* (VI) *A. pungens* (VI)	*A. sarawacense* sensu R.M. Sm. non Schuman (VI) Note: *Amomum sarawacense* sensu Schuman is possibly *Hornstedtia* , the specimen Anderson S.30713 at E, thought to be by R.M.Smith *A. sarawacense* is most likely species from clade VI.		*A. barneense* (VI) *A. epiphyticum* (VI)	*A. roseisquamosum* (VI)	
Clade VII		*A. compactum* (VII)				*A. bicorniculatum* (VII) *A. koppaeum* (VII)	*A. aromaticum* (VII) *A. compactum* (VII) *A. gracile* (VII) *A. uliginosum* (VII) *A. villosum* (VII)	*A. echinosphaera* (VII) *A. micranthum* (VII) *A. robustum* (VII) *A. testaceum* (VII) *A. xanthioides* (VII)	*A. echinosphaera* (VII) *A. micranthum* (VII) *A. mindanaense* (VII) *A. xanthioides* (VII)	*A. aromaticum* (VII) *A. gracile* (VII) *A. flavum* (VII) *A. elegans* (VII) *A. krervanh* (VII) *A. palawanense* (VII) *A. trilobum* (sensu Ridely, VII) *A. villosum* (VII)				*A. bicorniculatum* (VII) *A. testaceum* (VII)		
Clade VIII							*A. aculeatum* (VIII) *A. benthamianum* (VIII) *A. citatum* (VIII) *A. dallachyi* (VIII) *A. echinatum* Willd. (VIII) *A. flavum* (VIII) ?*A. kingii* (VIII)	*A. acuminatum* (VIII) *A. cannicarpum* (VIII) *A. corynostachyum* (VIII) *A. graminifolium* (VIII) *A. holmeii* (VIII) *A. loheri* (VIII) *A. masticatorium* (VIII) *A. echreum* (VIII) *A. parakense* (VIII) *A. thyrsoideum* (VIII) *A. vespertilio* (VIII)	*A. corynostachyum* (VIII) *A. loheri* (VIII) *A. tonroy* (VIII) *A. acuminatum* (VIII) *A. masticatorium* (VIII) *A. muricatum* (VIII)	*A. aculeatum* (VIII) *A. ciliatum* (VIII) *A. dallachyi* (VIII) *A. elephantorum* (VIII) *A. echinatum* (VIII) *A. propinquum* (VIII)			*A. borealiborneense* (VIII; listed as *A. ridleyi*) *A. cerasinum* (VIII) *A. gyrolophus* (VIII)		
	Unplaced or in other clades/genera: *A. alborubellum* (unplaced) *A. flavorubellum* (unplaced) *A. heteranthum* (unplaced) *A. pausodipsus* (*Etlingera*) *A. polycarpum* (*Etlingera*)	Unplaced or in other clades/genera: *A. apiculatum* (III) *A. deuteramomum* (unplaced) *A. trichanthera* (*Etlingera*) *A. calophrys* (*Etlingera*) *A. sarasinorum* (*Etlingera*) *A. validum* (*Etlingera*) *A. rubrum* (*Hornstedtia*)	Unplaced or in other clades/genera: *A. stenophyllum* (*Etlingera*) *A. nasutum* (*Etlingera*)	Unplaced or in other clades/genera: *A. foetens* (*Etlingera*) *A. labellosum* (*Etlingera*) *A. gymnopodum* (aff *Alpinia* IV) *A. trianthemum* (*Alpinia* IV)	Unplaced or in other clades/genera: *A. labellosum* (*Etlingera*) *A. nasutum* (*Etlingera*) *A. stenophyllum* (*Etlingera*) *A. xanthoparyphe* (*Etlingera*)	Unplaced or in other clades/genera: *Amomum nemorale* (unplaced) *A. floribundum* (*Alpinia abundiflora*) *A. involucratum* (*Alpinia fax*) *A. brachypadanthum* (*Etlingera*) *A.calophrys* (*Etlingera*) *A. cevuga* (*Etlingera*) *A. helicandifolium* (*Etlingera*) *A. littorale* (*Etlingera*) *A. longifolium* (*Etlingera*) *A. pausodipsus* (*Etlingera*) *A. polycarpum* (*Etlingera*) *A. roseum* (*Etlingera*) *A. sarasinorum* (*Etlingera*) *A. trachycarpum* (*Etlingera*) *A. trichanthera* (*Etlingera*) *A. truncatum* (*Etlingera*) *A. validum* (*Etlingera*)	Unplaced or in other clades/genera: *A. apiculatum* (III) *A. alborubellum* (unplaced) *A. deuteramomum* (unplaced) *A. flavorubellum* (unplaced) *A. procurrens* (unplaced) *A. cardomon* (*Elettaria cardamomum*) *A. chaunocephalum* (*Etlingera*)	Unplaced or in other clades/genera: *A. teiphrodelphys* (unplaced) *A. warburgii* (unplaced) *A. trianthemum* (*Alpinia* IV) *A. gymnopodum* (*?Alpinia* IV) *A. fimbriobractea* (*Etlingera*) *A. helicalyx* (*Etlingera*) *A. vestitum* (*Etlingera*) *A. harmsii* (*Hornstedtia rubra*) *A. longibracteo* (*Hornstedtia rubra*) *A. rubrum* (*Hornstedtia rubra*) *A. padangense* (cf. *Hornstedtia*)	Unplaced or in other clades/genera: *A. macrodus* (unplaced) *A. trianthemum* (*Botryamomum* clade) *A. gymnopodum* (*?Botryamomum* clade) *A. chrysogynium* (*Etlingera alba*) *A. harmsii* (*Hornstedtia rubra*) *A. longibracteo* (*Hornstedtia rubra*) *A. padangense* (cf. *Hornstedtia*)	Unplaced or in other clades/genera: *A. alborubellum* (unplaced) *A. flavorubellum* (unplaced) *A. longipes* (unplaced) *A. cardamom* (*Elettaria cardamomum*)					Unplaced or in other clades/genera: *A. bilobiatum (unplaced)*
					Note: species previously included by Schuman 1899 in Laxiflorae and Pauciflorae												
	Subgenus I. *Hornstedtia* **Inflorescence fusiform, very thick; labellum at first narrow, held outwards** All species included *Hornstedtia* and *Etlingera* : *A. sarawacense* (*Hornstedtia*) *A. elongatum* (*Hornstedtia*) *A. reticulatum* (*Hornstedtia*) *A. havilandii* (*Hornstedtia*) *A. phaeochaenum* (*Hornstedtia*) *A. minus* (*Hornstedtia*) *A. lycostomum* (*Hornstedtia*) *A. paludosum* (*Hornstedtia*) *A. penicillatum* (*Etlingera*) *A. coccineum* (*Etlingera*)				**GENUS *Hornstedtia*** All species from subgen. *Hornstedtia* transferred to genus *Hornstedtia* .												
	Subgenus II. *Nicolaia* **Inflorescence on a very long peduncle with bright coloured sterile bracts, broadly cup or bowl-shaped** All Species included now in *Etlingera*: *A. chrysocalyx* (*E. solaris*) *A. magnificum* (*E. elatior*) *A. grandibigulatum* (*E. grandibigulata*) *A. hemisphaericum* (*E. hemisphaerica*) *A. pyramidosphaera* (*E. pyramidosphaera*) *A. solare* (*E. solaris*)				**GENUS *Phaeomeria*** All species from subgen. *Nicolaia* transferred to genus *Phaeomeria* .												
	Subgenus IV. *Botryamomum* **Inflorescence cluster-like** Species included: *A. villosum* (VII) Unplaced or in other clades/genera: *A. chrysogynium* (*Etlingera alba*) *A. mellichraum* (*Alpinia mellichrea*)				**GENUS *Alpinia*** *Amomum villosum* Schumann transferred under *Amomum* , series 3 *Lobulatae* ; the other two species transferred into *Alpinia*												
	Subgenus V. *Mastigamomum* **Flowers subtended in small numbers in the axils of large, distant bracts on sideshoots** Species included: *Amomum gracile* (VII) *A. kondariense* (V; *Elettariopsis kondariense*) *A. stolonifarum* , (V; *Elettaria*), *A. surculosum* (V; *Elettaria*)				**GENUS *Cyphostigma*** *Amomum gracile* Schumann transferred under *Amomum* , series 3 *Lobulatae* , all other *Mastigamomum* species under genus *Cyphostigma* in Schuman 1904												

Fig. S1. RAxML maximum likelihood gene tree for nrITS sequences. Bootstrap values are given at nodes.

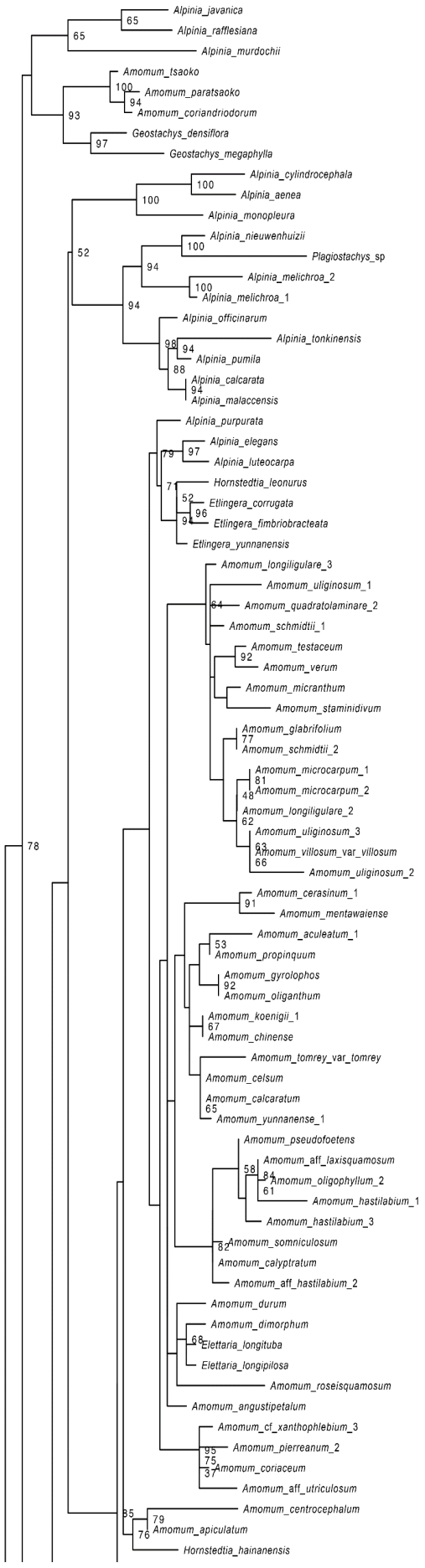
(Figure continues on next page)

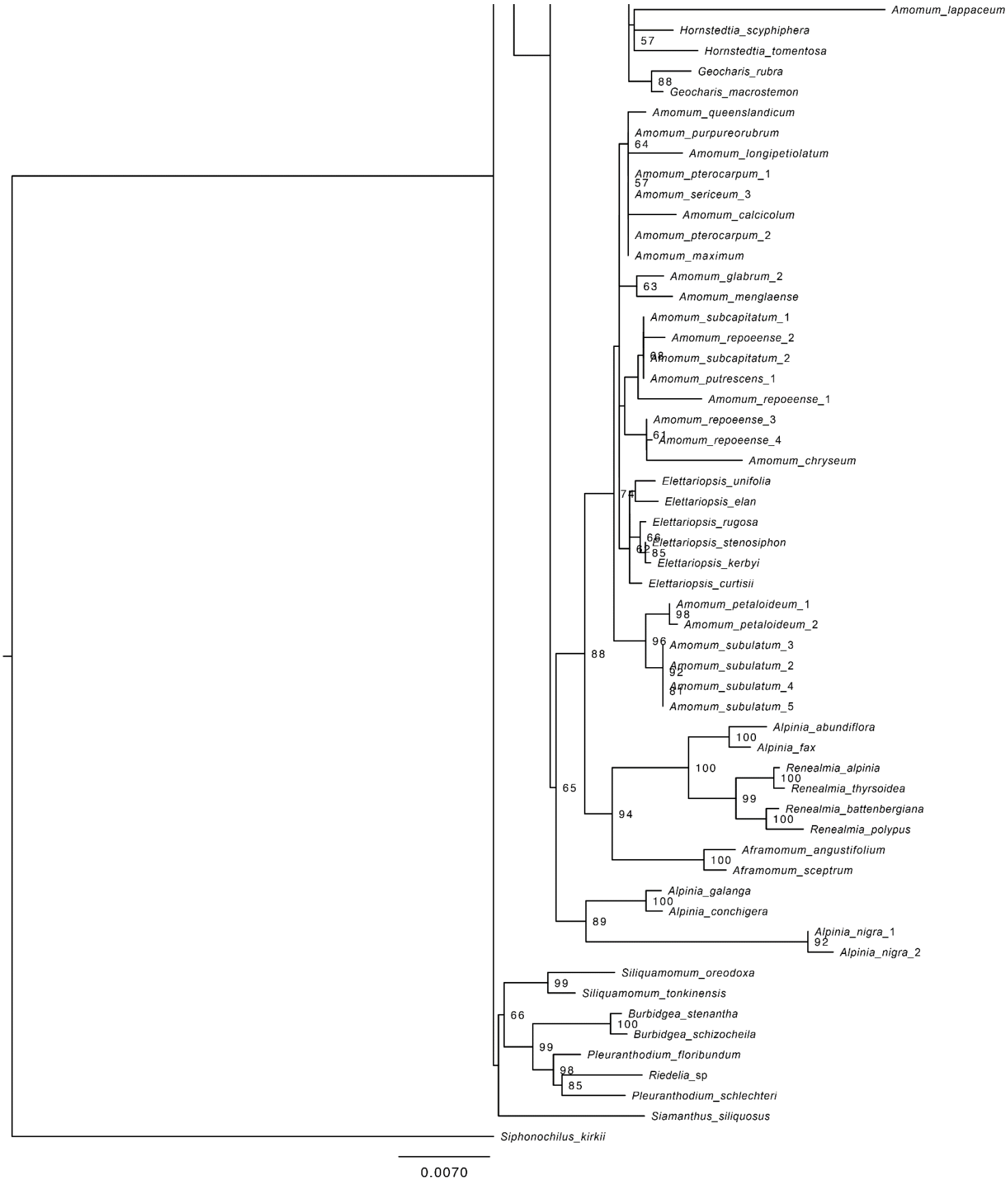



Geocharis_fusiformis_var_borneensis
Geocharis_rubra
Geocharis_macrostemon
Amomum_anomalum
Elettaria_longituba
Elettaria_longipilosa
Amomum_dimorphum
Amomum_cf_xanthophlebium_3
Amomum_xanthophlebium_2
Amomum_xanthophlebium_1
Amomum_coriaceum
Amomum_pierreanum_2
Amomum_dictyocoleum
Amomum_durum
Amomum_oligophyllum_1
Amomum_aff_hastilabium_2
Amomum_hastilabium_1
Amomum_aff_laxisquamosum
Amomum_pseudofoetens
Amomum_calyptratum
Amomum_hastilabium_3
Amomum_somniculosum
Amomum_coriandriodorum
Amomum_paratsaoko
Amomum_tsaoko
Geostachys_megaphylla
Geostachys_densiflora
Amomum_pterocarpum_2
Amomum_pterocarpum_1
Amomum_queenslandicum
Amomum_maximum
Amomum_purpureorubrum
Amomum_odontocarpum_1
Amomum_odontocarpum_2
Amomum_longipetiolatum
Amomum_glabrum_1
Amomum_glabrum_2
Amomum_prionocarpum
Amomum_menglaense
Amomum_sericeum_3
Amomum_sericeum_1
Amomum_sericeum_2
Amomum_repoeense_4
Amomum_repoeense_3
Amomum_repoeense_2
Amomum_putrescens_1
Amomum_subcapitatum_2
Amomum_repoeense_1
Amomum_plicatum
Amomum_putrescens_2
Amomum_petaloideum_2
Amomum_petaloideum_1
Amomum_subulatum_3
Amomum_subulatum_1
Amomum_subulatum_2
Amomum_subulatum_4
Amomum_subulatum_5
Elettariopsis_unifolia
Elettariopsis_triloba
Elettariopsis_elan
Elettariopsis_curtisii
Elettariopsis_rugosa
Elettariopsis_kerbyi
Elettariopsis_stenosiphon
Aframomum_sceptrum
Aframomum_angustifolium
Alpinia_abundiflora
Alpinia_fax
Elettaria_cardamomum
Renealmia_battenbergiana
Renealmia_polypus
Renealmia_thyrsoidea
Renealmia_alpinia
Alpinia_nigra_2
Alpinia_galanga
Alpinia_conchigera
Alpinia_javanica
Alpinia_rafflesiana
Alpinia_murdochii
Siliquamomum_oreodoxa
Siliquamomum_tonkinensis
Siamanthus_siliquosus
Burbidgea_stenantha
Burbidgea_schizocheila
Pleuranthodium_schlechteri
Pleuranthodium_floribundum
Riedelia_sp
Alpinia_nieuwenhuizii
Siphonochilus_kirkii
0.03

Fig. S2. RAxML maximum likelihood gene tree for *matK* sequences. Bootstrap values are given at nodes.

Figure continues on next page)

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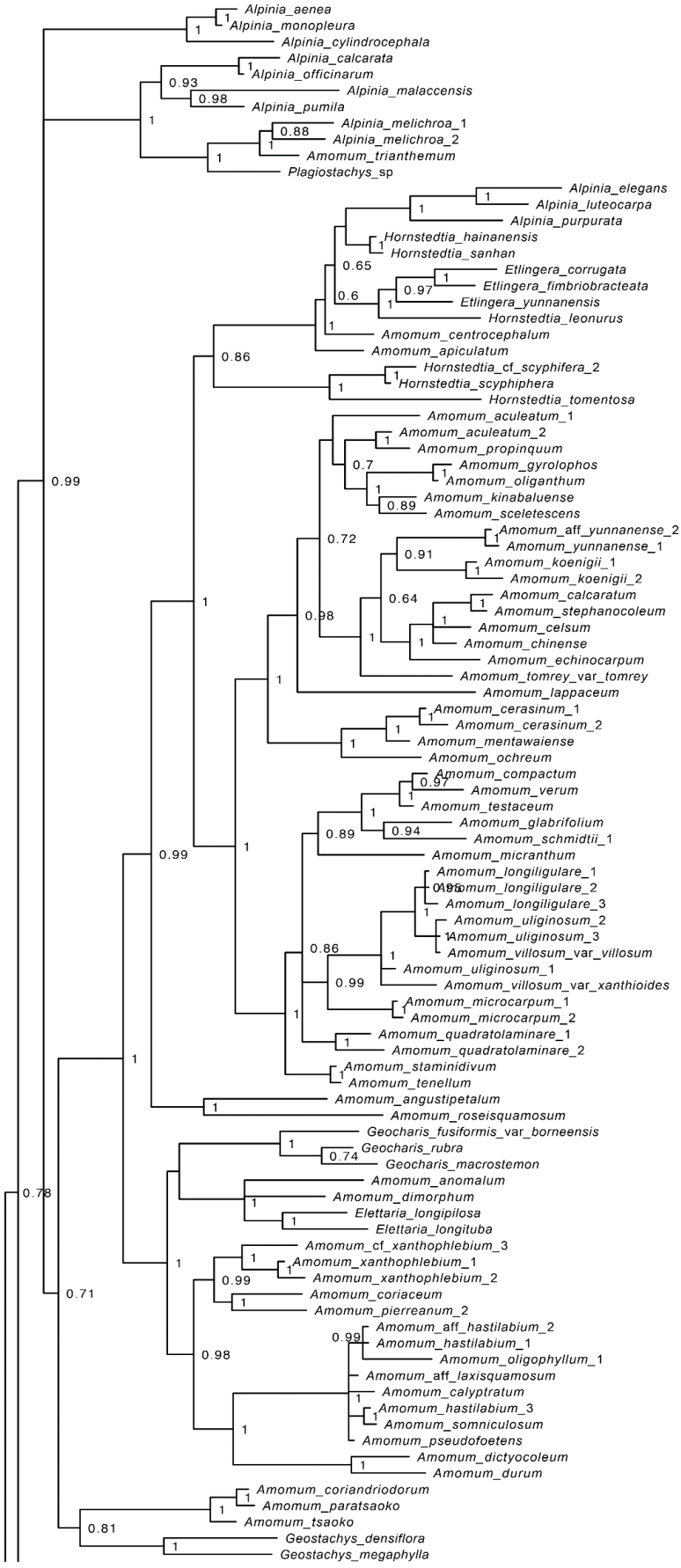
Hornstedtia_scyphiphera
57
Hornstedtia_tomentosa
Geocharis_rubra
88
Geocharis_macrostemon
Amomum_queenslandicum
Amomum_purpureorubrum
64
Amomum_longipetiolatum
Amomum_pterocarpum_1
57
Amomum_sericeum_3
Amomum_calcicolum
Amomum_pterocarpum_2
Amomum_maximum
Amomum_glabrum_2
63
Amomum_menglaense
Amomum_subcapitatum_1
Amomum_repoeense_2
Amomum_subcapitatum_2
Amomum_putrescens_1
Amomum_repoeense_1
Amomum_repoeense_3
61
Amomum_repoeense_4
Amomum_chryseum
Elettariopsis_unifolia
74
Elettariopsis_elan
Elettariopsis_rugosa
Elettariopsis_stenosiphon
85
Elettariopsis_kerbyi
Elettariopsis_curtisii
Amomum_petaloideum_1
98
Amomum_petaloideum_2
96
Amomum_subulatum_3
88
Amomum_subulatum_2
92
Amomum_subulatum_4
Amomum_subulatum_5
Alpinia_abundiflora
100
Alpinia_fax
100
Renealmia_alpinia
100
Renealmia_thyrsoidea
99
Renealmia_battenbergiana
100
Renealmia_polypus
65
94
Aframomum_angustifolium
100
Aframomum_sceptrum
Alpinia_galanga
100
Alpinia_conchigera
89
Alpinia_nigra_1
92
Alpinia_nigra_2
Siliquamomum_oreodoxa
99
Siliquamomum_tonkinensis
66
Burbidgea_stenantha
100
Burbidgea_schizocheila
99
Pleuranthodium_floribundum
98
Riedelia_sp
85
Pleuranthodium_schlechteri
Siamanthus_siliquosus
Siphonochilus_kirkii
0.0070

Fig. S3. MrBayes Bayesian gene tree for nrITS sequences. Posterior probabilities are given at nodes.

(Figure continues on next page)



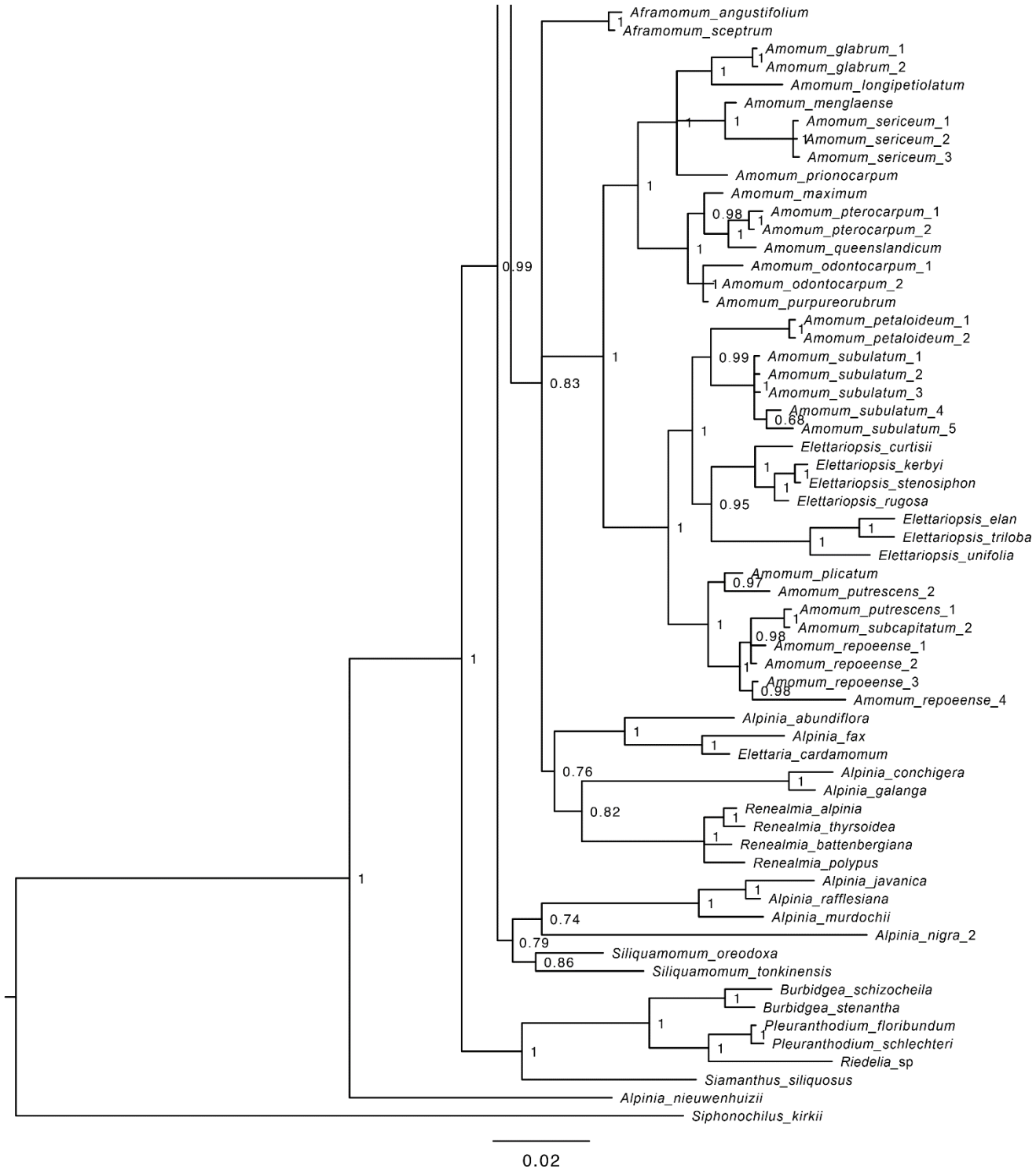
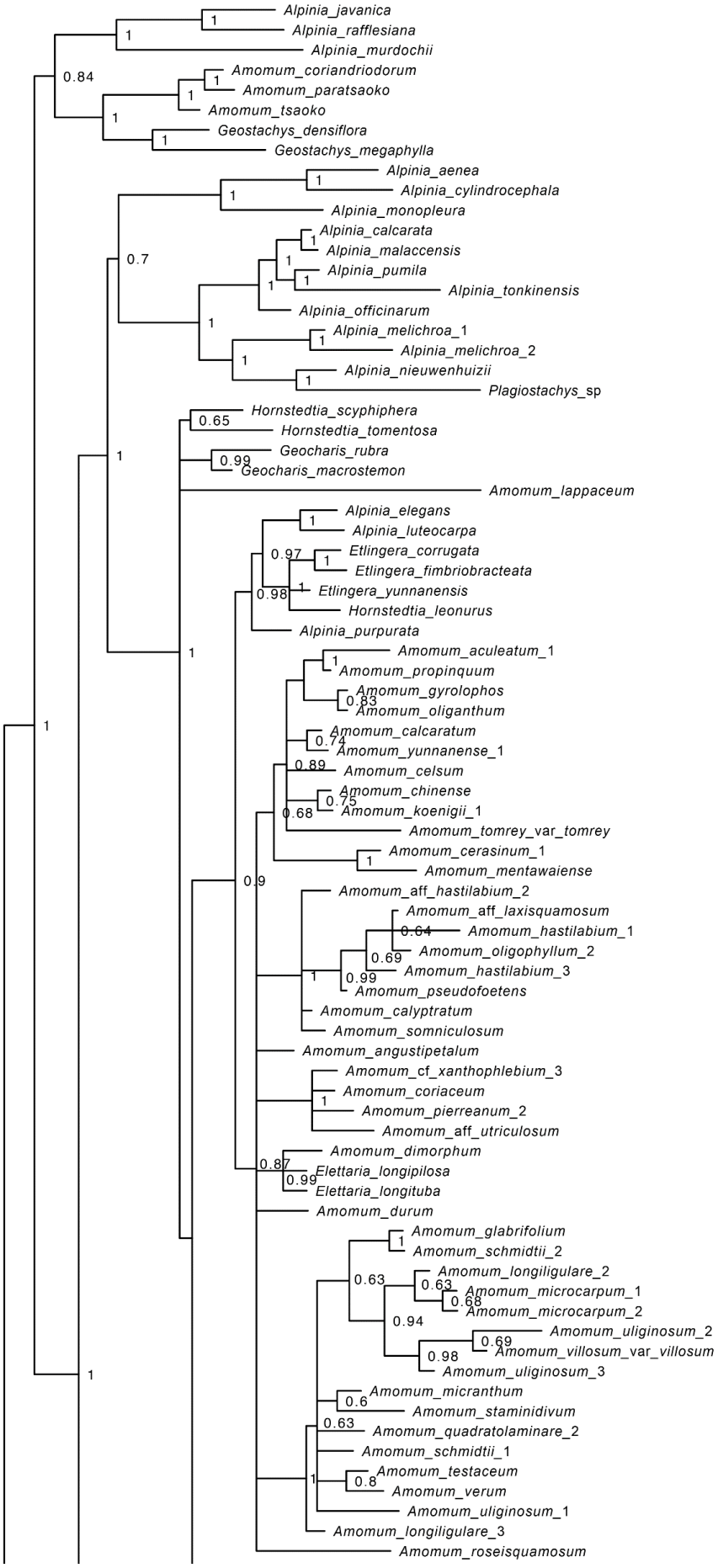
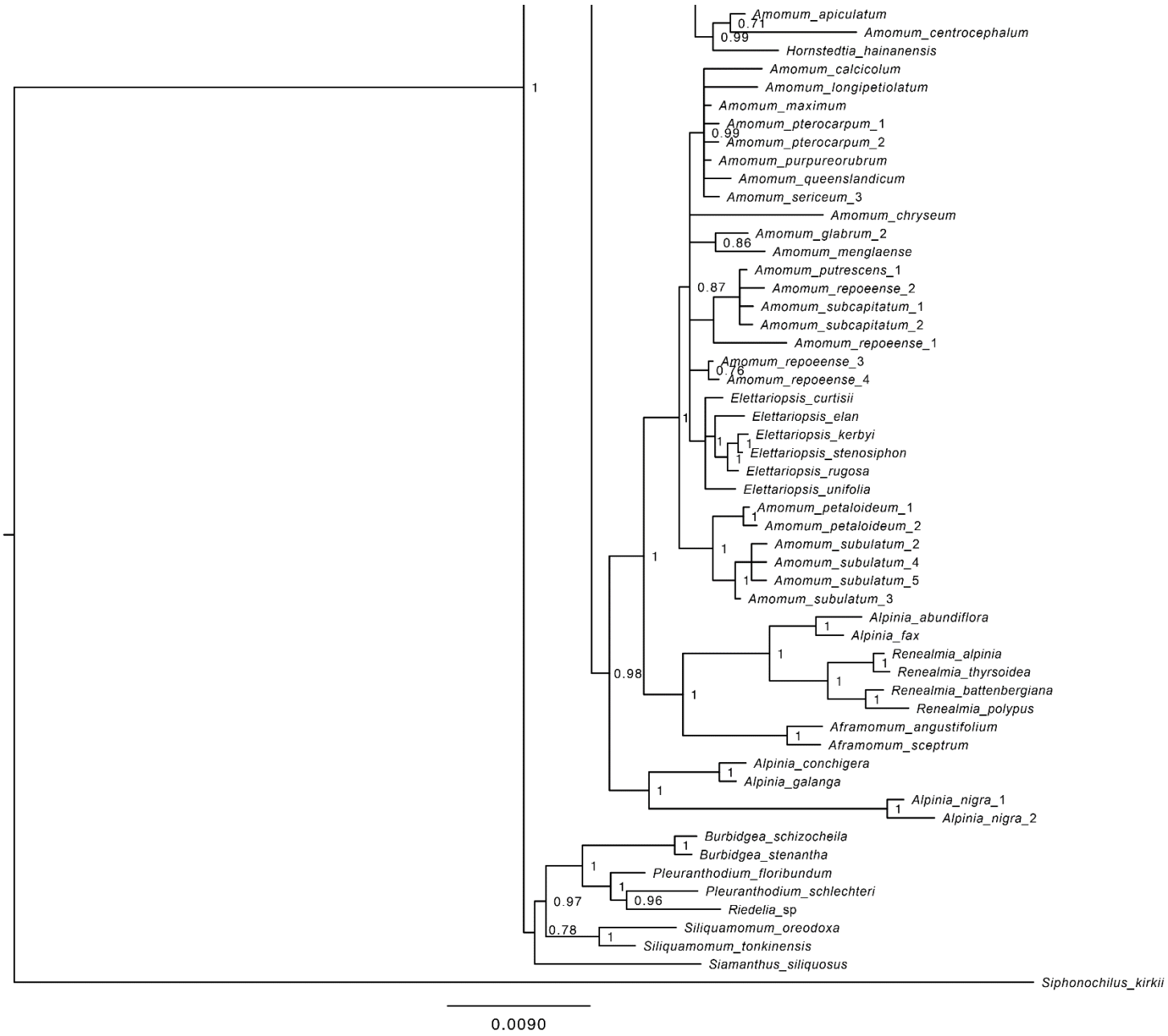

Aframomum_angustifolium
Aframomum_sceptrum
Amomum_glabrum_1
Amomum_glabrum_2
Amomum_longipetiolatum
Amomum_menglaense
Amomum_sericeum_1
Amomum_sericeum_2
Amomum_sericeum_3
Amomum_prionocarpum
Amomum_maximum
Amomum_pterocarpum_1
Amomum_pterocarpum_2
Amomum_queenslandicum
Amomum_odontocarpum_1
Amomum_odontocarpum_2
Amomum_purpureorubrum
Amomum_petaloideum_1
Amomum_petaloideum_2
Amomum_subulatum_1
Amomum_subulatum_2
Amomum_subulatum_3
Amomum_subulatum_4
Amomum_subulatum_5
Elettariopsis_curtisii
Elettariopsis_kerbyi
Elettariopsis_stenosiphon
Elettariopsis_rugosa
Elettariopsis_elan
Elettariopsis_triloba
Elettariopsis_unifolia
Amomum_plicatum
Amomum_putrescens_2
Amomum_putrescens_1
Amomum_subcapitatum_2
Amomum_repoeense_1
Amomum_repoeense_2
Amomum_repoeense_3
Amomum_repoeense_4
Alpinia_abundiflora
Alpinia_fax
Elettaria_cardamomum
Alpinia_conchigera
Alpinia_galanga
Renealmia_alpinia
Renealmia_thyrsoidea
Renealmia_battenbergiana
Renealmia_polypus
Alpinia_javanica
Alpinia_rafflesiana
Alpinia_murdochii
Alpinia_nigra_2
Siliquamomum_oreodoxa
Siliquamomum_tonkinensis
Burbidgea_schizocheila
Burbidgea_stenantha
Pleuranthodium_floribundum
Pleuranthodium_schlechteri
Riedelia_sp
Siamanthus_siliquosus
Alpinia_nieuwenhuizii
Siphonochilus_kirkii
0.02

Fig. S4. MrBayes Bayesian gene tree for *matK* sequences. Posterior probabilities are given at nodes.

(Figure continues on next page)

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Hornstedtia_hainanensis
Amomum_calcicolum
Amomum_longipetiolatum
Amomum_maximum
Amomum_pterocarpum_1
Amomum_pterocarpum_2
Amomum_purpureorubrum
Amomum_queenslandicum
Amomum_sericeum_3
Amomum_chryseum
Amomum_glabrum_2
Amomum_menglaense
Amomum_putrescens_1
Amomum_repoeense_2
Amomum_subcapitatum_1
Amomum_subcapitatum_2
Amomum_repoeense_1
Amomum_repoeense_3
Amomum_repoeense_4
Elettariopsis_curtisii
Elettariopsis_elan
Elettariopsis_kerbyi
Elettariopsis_stenosiphon
Elettariopsis_rugosa
Elettariopsis_unifolia
Amomum_petaloideum_1
Amomum_petaloideum_2
Amomum_subulatum_2
Amomum_subulatum_4
Amomum_subulatum_5
Amomum_subulatum_3
Alpinia_abundiflora
Alpinia_fax
Renealmia_alpinia
Renealmia_thyrsoidea
Renealmia_battenbergiana
Renealmia_polypus
Aframomum_angustifolium
Aframomum_sceptrum
Alpinia_conchigera
Alpinia_galanga
Alpinia_nigra_1
Alpinia_nigra_2
Burbidgea_schizocheila
Burbidgea_stenantha
Pleuranthodium_floribundum
Pleuranthodium_schlechteri
Riedelia_sp
Siliquamomum_oreodoxa
Siliquamomum_tonkinensis
Siamanthus_siliquosus
Siphonochilus_kirkii
0.0090

Paper II

The identity of *Amomum trilobum* and *Amomum unifolium* (Zingiberaceae: Alpinioideae), and description of four new related species from Vietnam

Jana Leong-Škorničková, Trần Hữu Đăng, Nguyễn Quốc Bình, Kristýna Hlavatá, Lưu Hồng Trường, Nguyễn Quốc Đạt, Nguyễn Thành Trung & Mark Newman

Phytotaxa 401(3), April 2019: 149–165

Paper II

The identity of *Amomum trilobum* and *Amomum unifolium* (Zingiberaceae: Alpinioideae), and description of four new related species from Vietnam

Jana Leong-Škorničková[1], Trần Hữu Đăng[2], Nguyễn Quốc Bình[3], Kristýna Hlavatá[4], Lưu Hồng Trường[2,5], Nguyễn Quốc Đạt[2], Nguyễn Thành Trung[6] & Mark Newman[7]

[1]The Herbarium, Singapore Botanic Gardens, 1 Cluny Road, 259569 Singapore.

[2]Southern Institute of Ecology, Vietnam Academy of Science and Technology, 1 Mạc Đĩnh Chi Street, District 1, Hồ Chí Minh City, Vietnam.

[3]Vietnam National Museum of Nature, Vietnam Academy of Science and Technology, 18 Hoàng Quốc Việt Street, Cầu Giấy, Hanoi, Vietnam.

[4]Department of Botany, Faculty of Science, Charles University, Benátská 2, 128 00 Prague, Czech Republic.

[5]Graduate University of Science and Technology, Vietnam Academy of Science and Technology, 18 Hoàng Quốc Việt, Cầu Giấy, Hà Nội, Vietnam.

[6]Núi Chúa National Park, Thái An Commune, Vĩnh Hải Ward, Ninh Hải District, Ninh Thuận Province, Vietnam.

[7]Royal Botanic Garden Edinburgh, 20A Inverleith Row, Edinburgh EH3 5LR, Scotland, U.K.

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Abstract

The identities of *Amomum trilobum* and *Amomum unifolium*, described from material originating from uncertain location in Indochina, are discussed. A lectotype of *Amomum unifolium* is designated here and epitypes with complete flowers are designated to ensure unambiguous application of both names. In addition, four new related species are presented in this paper, *Amomum cinnamomeum, A. corrugatum, A. lutescens* and *A. miriflorum*. Detailed descriptions, colour plates including flower dissections, notes on habitat and preliminary IUCN assessments are given for all six species.

Keywords: Alpinieae, *Amomum cinnamomeum, A. corrugatum, A. lutescens*, *A. miriflorum, Elettariopsis,* Hòn Bà Nature Reserve, Núi Chúa National Park

Introduction

As we continue the extensive field exploration of Vietnamese Zingiberales which we began in 2008, we also revise existing herbarium material and literature to improve our understanding of the historical names in various genera. As a result, numerous taxa have already been described by us (e.g. Lamxay & Newman 2012; Leong-Škorničková *et al.*, 2010, 2011, 2013, 2015, 2016;

Leong-Škorničková & Luu, 2013; Leong-Škorničková & Lý, 2010; Leong-Škorničková & Trần, 2013; Luu *et al.*, 2015; Lý & Leong-Škorničková, 2018; Lý *et al.*, 2010; Nguyen & Leong-Škorničková, 2012; Trần *et al.* 2018).

An introduction to the phytogeography of the Indochinese region was given by Averyanov *et al.* (2003) and an introduction to the family Zingiberaceae can be found in Leong-Škorničková & Newman (2015), as well as in some of the papers cited above.

The delimitation of *Elettariopsis* Baker (1892: 251) from *Amomum* s.l. (Roxburgh 1820: 75) has always been problematic as no single character distinguishes the two genera with absolute fidelity. The earliest studies to include phylogenetic analyses (e.g. Kress *et al.* 2002, 2007; Xia *et al.* 2004) indicated that *Amomum* s.l. as previously delimited was highly polyphyletic, and that *Elettariopsis* was probably nested in one of the *Amomum* clades. This was confirmed in the most recent study of De Boer *et al.* (2018) which included the type species of *Amomum* and *Elettariopsis* and which re-circumscribed the genera, basing them on clades involving most of the species previously classified under *Amomum* s.l. and *Elettariopsis*. According to this study, *Amomum* s.s. is a group of species which mainly possesses winged or angled fruits and fan-shaped or extended anther crests. *Elettariopsis* formed a monophyletic group which was well nested in the clade of *Amomum* containing the type of the genus *Amomum subulatum* Roxb. (Roxburgh, 1820: 43) and was therefore placed in synonymy under *Amomum* s.s. This generic concept is followed here.

In this paper we provide an overview of the six *Amomum* species in Vietnam which would previously have been classified in *Elettariopsis*. Two of these, *Amomum trilobum* Gagnep. (Gagnepain, 1904: 453) and *A. unifolium* Gagnep. (Gagnepain, 1907: 403), were described long ago but their identity remained unclear because the exact collecting locality of their type specimens was unknown. This meant that new collections could not be made in the type localities so it was not possible to distinguish these species from several similar ones known from the region. In order to stabilise the use of these two names, we propose to epitypify them on recent collections from southern Vietnam which match the original descriptions and the type material perfectly. In addition, we formally describe and illustrate four new species, *A. cinnamomeum* Škorničk., Luu & H.Đ.Trần, *A. corrugatum* Škorničk., H.Đ.Trần & Luu, *A. lutescens* Škorničk. & Luu and *A. miriflorum* Škorničk. & Q.B.Nguyen, from southern and central Vietnam. Two of these were informally mentioned but not validly published as *Elettariopsis 'lutescens'* and *E. 'mirantha'* in *Gingers of Cambodia, Laos and Vietnam* (Leong-Škorničková & Newman, 2015). Photographs of these species can be seen there (Leong-Škorničková & Newman, 2015, p. 138).

The species descriptions are based on living material and follow the style and terminology of our papers cited above and Beentje (2016). The preliminary conservation assessments follow the guidelines of IUCN (2017). Herbarium materials (or high resolution images of specimens) from E, HN, HNL, K, P, SING, VNM, VNMN were consulted to compare the new material to the most similar species within the respective genera and to see if they had been previously collected. The herbarium codes follow Thiers (continuously updated).

Identity and typification of *Amomum trilobum* & *A. unifolium*

These two species, until recently classified in the now synonymous *Elettariopsis*, were originally described in *Amomum* by F. Gagnepain from material cultivated in the glasshouses of the Muséum national d'histoire naturelle, Paris. The living plants of both species originated in French Indochina.

Amomum trilobum flowered in Paris on 5 May 1901 and 26 June 1904. Its origin was recorded simply as French Indochina. Only one specimen, collected from the plant which flowered in 1904, has been found at P, and is therefore treated here as the holotype.

Amomum unifolium flowered in Paris in May 1907. Gagnepain noted in the protologue that it was unclear whether the plant had been sent to Paris by Pierre from Cochinchina (southern Vietnam) or by Bon from Tonkin (northern Vietnam) (Gagnepain, 1907; Newman 1997).

During our fieldwork since 2008 in Vietnam, we have made numerous collections in various parts of Vietnam with unifoliate leafy shoots, but these were growing in habitats from lowlands to high altitude, and somewhat differed in overall habit. We have very few flowering accessions, so it is hard to conclude whether all these collections represent one fairly variable taxon, or a group of cryptic species. A similar situation, although to a lesser extent, applies also to *Amomum trilobum*.

To address the ambiguity in application of these names, we designate epitypes from recent collections which perfectly match the type material and attached sketches, and the original descriptions. The collections were made by M. Newman in southern Vietnam in December 1989 and January 1990, and were brought into cultivation at the Royal Botanic Garden Edinburgh where they still regularly flower, and have been documented with specimens including flowers preserved in spirit, and photographs of flower dissections (Fig. 1 & 2). These accessions were also sampled in the most recent phylogenetic study dealing with the generic delimitation of *Amomum* and *Elettariopsis* (de Boer *et al.*, 2018; Hlavatá *et al.*, in prep). The collection of *Amomum unifolium* selected here was also the basis for the generic transfer of this species to *Elettariopsis* by Newman in 1997.

Amomum trilobum **Gagnep.**, Bull. Soc. Bot. France 51:453 (1904) ≡ *Elettariopsis triloba* (Gagnep.) Loes. in H.G.A.Engler, Nat. Pflanzenfam. Ed. 2, 15a: 603 (1930)

Holotype:—French Indochina, unspecified location, cultivated in the glasshouses of the Museum [Muséum national d'Histoire naturelle Paris], flowered 26 June 1904, sine coll., sine dat. [P032744].

Epitype (designated here):—cultivated at Royal Botanic Garden Edinburgh under Acc. Number 19901444A, 27 April 2006, *Newman & Škorničková 1455,* E [E00228079], including flowers preserved in alcohol [E00830414]; isoepitype SING. Original collection data: Vietnam, Đồng Nai Province, Cát Tiên National Park, 31 December 1989, *Newman, M.F. L2.* **Fig. 1 & 7.**

Rhizomatous spreading herb up to 65 cm tall. ***Rhizome*** 2.5–5 mm in diam. (when fresh), sheathed by scales, scales ovate with broadly acute to obtuse apex, 20–30 mm long, c. 12 mm broad, white, tinged with pink-red when young, soon turning papery brown and decaying with age, glabrous. ***Leafy shoots*** with 3–4 leaves per shoot, 10–25 cm apart; *leafless sheaths* c. 3, reddish green when young, turning papery with age; *leaf sheaths* green, almost smooth; *ligule* 2–2.5 mm, slightly bilobed, hyaline, cream to greenish, glabrous; *petiole* to 9.5 cm long, green, shallowly canaliculate, glabrous; *lamina* elliptic 21–24 × 4–5.5 cm, green and glabrous on both sides,

slightly coriaceous, margin glabrous, apex caudate (cauda 3–4 cm long) ciliate distally, base attenuate. ***Inflorescence*** radical, arising from base of leafy shoot; *peduncle* to 6–15 mm long, peduncular bracts cream to red, 4–9 mm long, overlapping, covering peduncle; *spike* 2–5.5 × 0.9–1.5 cm (excl. exserted flowers); composed of 5–8 fertile bracts; *bract* c. 19–42 × 5–10 mm, narrowly ovate, apex mucronate (mucro 1–3 mm), outermost bracts dark red, shorter and broader, innermost bract pale red, longer and narrower, glabrous, subtending a single flower; *bracteole* 19–32 × 7–8 mm, narrowly triangular, cream with reddish tinge, glabrous. ***Flower*** (6–)7–9 cm long; *calyx* tubular 34–51 mm long, cream with slight red tinge, unilaterally incised 8–22 mm,

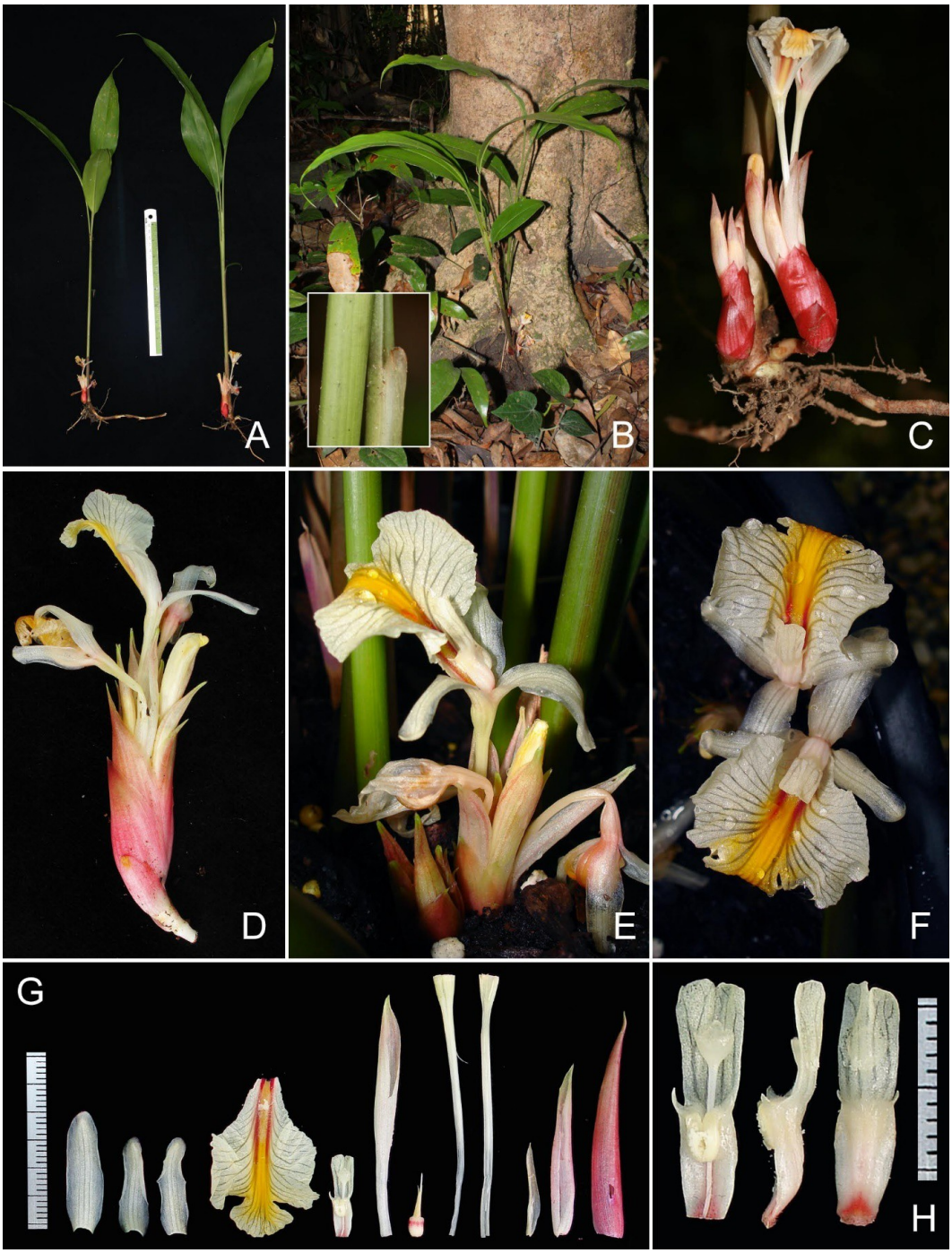

FIGURE 1. *Amomum trilobum* Gagnep. A. Habit. B. Habit *in situ* (detail of ligule in inset). C. Basal part of leafy shoot and rhizome with inflorescences. D. Inflorescence. E. Flower (top and semi-side view). F.

Flowers (top view). G. Dissection (from left): dorsal corolla lobe, lateral corolla lobes, labellum, stamen, calyx, ovary with epigynous glands, floral tube, bracteole, fertile bract and outer sterile bract (scale bar in mm). F. Detail of stamen (front, side and back view; scale bar in mm). *Photo:* A–C *Trần Hữu Đăng*, D–H *Jana Leong-Škorničková.*

3-toothed, teeth c. 0.5 mm long, ciliate, pale green; *floral tube* 4.5–5 mm long, whitish cream at base, cream to pale yellow towards apex; *dorsal corolla lobe* 17–22 × 4.5–7 mm, semi-translucent white, with translucent stripes, apex slightly hooded, margin hyaline, glabrous; *lateral corolla lobes* 15–18 × 4.5–6 mm, semi-translucent white, with translucent stripes, apex slightly hooded, margin hyaline, glabrous; *labellum* 28–30 mm long, 19–23 mm wide at broadest point, broadly obovate, pale yellow at sides, yellow in centre, basal half with two prominent red lines, densely hairy between these lines, apex more or less prominently 3-lobed, undulate, reflexed; *lateral staminodes* absent. ***Stamen*** 12–15 mm long; *filament* 3.5–5 × 3–3.3 mm, cream with red base, abaxially with a few hairs at base, adaxially densely hairy; *anther* c. 10–12 mm long (including crest), connective tissue white, glabrous; anther crest 3- lobed, mid-lobe 7–8 × 4–5 mm, rectangular (slightly broader at tip), side lobes minute, needle-shaped, c. 2 mm long; *anther thecae* 3–3.5 mm long, dehiscing longitudinally almost for entire length. ***Epigynous glands*** two, c. 4–6 mm long, narrowly conical. ***Ovary*** 2.5–3.5 × 2.5–3.5 mm, cylindrical, pale green with red tinge towards apex, puberulous. ***Style*** white, glabrous; *stigma* 2 × 2 mm, funnel- to cup-shaped, with a lobule on dorsal side, slightly dorso-ventrally compressed, ostiole densely ciliate. ***Fruit*** not seen.

Etymology:—The specific epithet refers to the three prominent lobes of the labellum.

Distribution & IUCN preliminary assessment:—*Amomum trilobum* has a narrow distribution with populations known from Cát Tiên National Park and Tân Phú Protection Forest in Đồng Nai Province and a single small population in Di Linh, Lâm Đồng Province. The estimated EOO is 1266 km2 and AOO is 12 km2. Although the populations in Cát Tiên National Park and Tân Phú Protection Forest are well protected, the population in Di Linh is not protected and faces imminent threat from burning forests to clear land for coffee plantations. Moreover, the area of occupancy of this species is only 12 km2 so we propose to treat this species as Endangered B1ab(i,ii,iv)+B2ab(iv).

Ecology and phenology:—Growing in the understorey of semi-deciduous lowland broad-leaved forest. Flowering occurs shortly after the first monsoonal showers in March and lasts into April. Fruiting has not been observed.

Other specimens examined:—VIETNAM. Lâm Đồng Province, Di Linh District, 1046 m asl, 11°30’4.26’’N, 108°4’13.96’’E, 9 May 2016, *Nguyễn Quốc Đạt et al. LBA-016 (SGN);* Bảo Lâm District, Lộc Bắc Municipality, 14 km WNW from Lộc Thắng Town, 11°44’25’’N, 107°42’20’’E, 900 m asl, 12 April 2013, *Nuraliev M.S. 814* (MW [MW0751093]); Đồng Nai Province, Cát Tiên National Park, 138 m asl, 11°25’51.91’’N, 108°4’13.96’’E, 14 March 2009, *Trần Hữu Đăng et al. TRAN-146* (E, SING)

Other field records:—Lâm Đồng Province, Bảo Lâm District, Lộc Bắc Municipality, 7 km NW from Lộc Bảo Town; 11°50’10’’N,107°38’30’’E, 600 m, *Nuraliev M.S. 588a* (photo, MW-DigiPic0000010); Đồng Nai Province, Định Quán District, Tân Phú Protection forest, 123 m a.s.l., 11°6’33.27’’N, 107°23’25.45’’E, 17 June 2008, *Trần Hữu Đăng s.n.* (pers. observation).

Note:—In addition to the epitype, two other specimens at E originate from the same living accession (*Rangsiruji & Newman 19;* E00211494-specimen, E00211256-liquid collection, collected on 9 April 1997) and (*Newman M.F. L2*; E00211494-liquid collection, collected on 15 May 2007).

According to our observation of this species at various locations, the shape of the labellum may vary from very prominently trilobed (as seen in Fig. 1) to obscurely trilobed as seen on some material from Lâm Đồng Province.

Amomum unifolium **Gagnep.**, Bull. Soc. Bot. France 54:403 (1907) ≡ *Elettariopsis unifolia* (Gagnep.) M.F.Newman, Edinburgh J. Bot. 54: 111 (1997)

Lectotype (designated here):—Vietnam, unspecified location, cultivated in the glasshouses of the Museum [Muséum national d'Histoire naturelle, Paris], flowered May 1907, sine coll., sine dat. [P00143807].

Epitype (designated here):—cultivated at Royal Botanic Garden Edinburgh under Acc. Number 19901449A, 30 April 2007, *Newman & Škorničková 2002,* E [E00269270], including flowers preserved in alcohol (E00211575); isoepitype SING. Original collection data: VIETNAM, Đồng Nai Province, Cát Tiên National Park, 4 km S of Đắk Lua village, 5 January 1990, *Newman, M.F. L7.* **Fig. 2 & 7.**

Rhizomatous spreading herb 30–70(–80) cm tall. ***Rhizome*** 3–6 mm in diam. (when fresh), sheathed by scales, scales ovate, 0.7–1 cm long, white with pink-red tinge when young, soon turning papery brown and decaying with age, glabrous. ***Leafy shoots*** with only 1(–2) leaves per shoot, 7–17.5 cm apart; *leafless sheaths* 3, 2–14 cm long, pale straw to pale green when young, weakly striate, glabrous, turning papery with age (striae more prominent); leaf sheaths pale yellow-green, smooth, glabrous; *ligule* small (c. 1–2 mm) hardly visible, hidden below sheathing bracts; *petiole* 9–22 cm long, green, canaliculate, glabrous; *lamina* elliptic with sides unequal in width, 22–32 × 7–10.5 cm, mid to dark green and shiny above, slightly lighter and shiny below, weakly plicate, glabrous on both sides, apex acute, base obtuse to attenuate, margin glabrous throughout including apex. ***Inflorescence*** radical, arising from base of leafy shoot; *peduncle* 1.5–4.5 cm long, horizontally creeping, covered by cream pinkish sheathing bracts (turning papery with age); *spike* 2–3.2 × 1–1.3 cm (excl. exserted flowers); composed of 3–5 fertile bracts; *bract* c. 7–20 mm long, ovate to broadly ovate, tinged pink-red in young inflorescences (soon becoming brown), both sides glabrous, subtending a single flower; *bracteole* 11 mm long. ***Flower*** 7.5–12 cm long; *calyx* 2.1–4.3 cm long, white at base, turning pinkish towards apex, mostly glabrous, unilaterally incised 10–13 mm, 3-toothed, teeth c. 2 mm long with hairy margins; *floral tube* 4–8 cm long, cream white, externally glabrous, internally puberulous towards apex; *dorsal corolla lobe* 16–22 × c. 8 mm, cream white to pale yellow, bluntly hooded (not mucronate), occasionally with slight pink tinge at apex, glabrous both sides; *lateral corolla lobes* 17–21 × 5.5–7.5 mm, cream white to pale yellow, occasionally with slight pink tinge at apex, slightly bluntly hooded, glabrous both sides; *labellum* 20–26 mm long, 20–25 mm wide at broadest point, almost orbicular with short claw, sides cream white, dark yellow to yellow-orange patch at centre, bordered by thick dark red lines at base, which radiate gradually towards margins, labellum glabrous distally but densely hairy at basal half of midline; *lateral staminodes* absent. ***Stamen*** (12–)16–17 mm

long; *filament* 3–5 mm long, 3–4 mm wide, white with pink tinge especially at centre and towards base, glabrous; *anther* 8.5–11.5 mm long (including crest), connective tissue cream white, glabrous; *anther thecae* c. 4.5 mm long, dehiscing longitudinally along entire length; anther crest 5–8 × 4–6 mm, bluntly rectangular with irregular apex, cream white with yellowish apex. ***Epigynous glands*** two, 6–8 mm long, narrowly conical. ***Ovary*** 4–5 × 3–4 mm, ovate, cream with pink-red tinge, shortly puberulent. ***Style*** white, glabrous; *stigma* c. 3 mm long, c. 2.6 mm broad, funnel-shaped, dorso-laterally compressed, dorsal rim with a lobe, ostiole ciliate, upward facing; stigma exserted above anther by c. 2.5 mm. ***Fruit*** not seen.

Etymology:—Refers to the mostly unifoliate habit of this species.

Distribution & IUCN preliminary assessment:—This species is fairly widespread and locally common. EOO = 4,251km2, AOO = 20 km2. Populations in Cát Tiên National Park, Bidoup—Núi Bà National Park and Phước Bình National Park are well protected. The population at Yang Bay waterfall, Khánh Hoà Province, is not in a protected area but the population is robust and there are currently no threats to it. Although the EOO and AOO meet the criteria for Endangered, the known populations are either well protected or experience no threats so we treat this species as of Least Concern.

Other specimens examined:—VIETNAM. Gia Lai Province, Mang Yang District, A Yun Municipality, Kon Ka Kinh National Park, 31 km WNW of K‘Bang Town, 14°12‘35‘‘N, 108°19‘00‘‘E, 900 m asl., 13 May 2016, *Nuraliev M.S. 1481* (MW [MW0754352]); Đắk Lắk Province, Chu Yang Sin National Park, 6 km S from Krong Kmar village, 12°27’10’’N, 108°20’15’’E, 700 m, 14 May 2014, *M.S. Nuraliev 906* (MW [MW0751094]); Lâm Đồng Province, Bidoup—Núi Bà National Park, 698 m asl, 12°15’39.52’’N, 108°26’42.84’’E, 16 August 2018, *Nguyễn Quốc Đạt et al. BDJC-396* (SGN); Khánh Hoà Province, 81 m asl, 12°12’11.52’’N, 108°54’38.16’’E, 5 May 2018, *Trần Hữu Đăng & Nguyễn Hiếu Cường KH2-335* (SGN), Khánh Vĩnh District, Khánh Trung Commune, 12°21’13.64”N, 108°51’37.34”E, 570 m asl., 20 April 2013, *Lưu Hồng Trường & Trần Giỏi KH-622* (SGN); Ninh Thuận Province, Phước Bình National Park, 331 m asl, 11°59’16.88’’N, 108°44’47.64’’E, 8 May 2018, *Trần Hữu Đăng & Nguyễn Hiếu Cường PL-520* (SGN).

Note: We have located only a single sheet at P, clearly annotated *Amomum unifolium* Gagnep. *sp. nov.* in Gagnepain’s handwriting. The specimen consists of a leafy shoot, an inflorescence with flower, pressed labellum and, as usual on Gagnepain’s original material, a small pencil drawing with flower details. Newman (1997) noted that the type specimen consisted of two unnumbered and undated sheets in Paris, one of them being a drawing of the habit of the living plant, which we were unable to find. We designate the present specimen as the lectotype.

Another specimen at E is made from the same living accession as the epitype (*Newman 747*; E00211296, E00211297-specimens, E00211255-liquid collection).

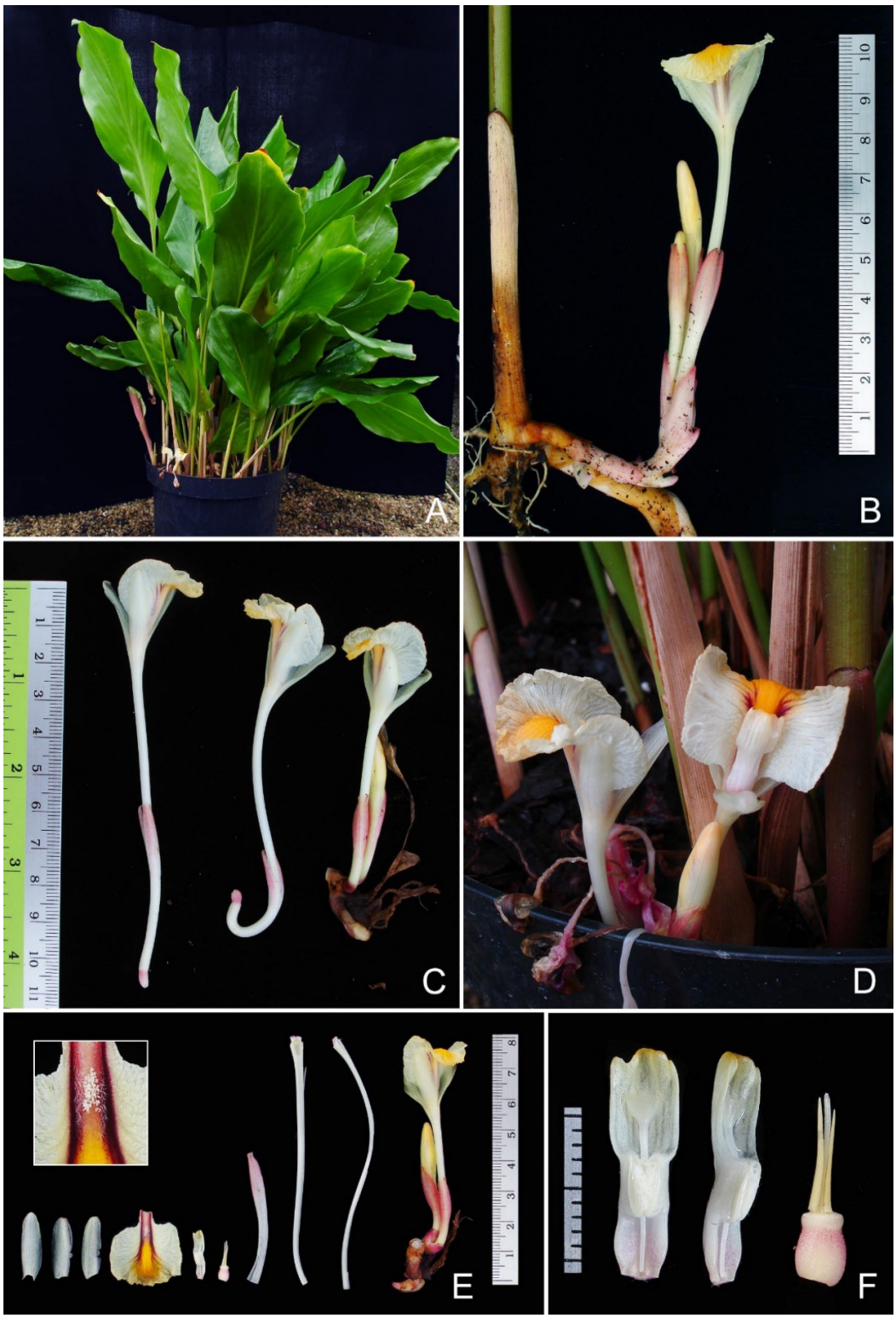

FIGURE 2. *Amomum unifolium* Gagnep. A. Habit. B. Detail of inflorescence (scale bar in cm). C. Flowers in side view (scale bar in cm). D. Flower (top and semi-side view). E. Dissection (from left): calyx, ovary with epigynous glands, corolla lobes and labellum, floral tube with stamen (scale bar in cm). F. Detail of stamen (front and side view) and ovary with epigynous glands (scale bar in mm). *Photo: Jana Leong-Škorničková.*

Descriptions of new taxa

Amomum cinnamomeum* Škorničk., Luu & H.Đ.Trần, *sp. nov.

Similar to *Amomum trilobum* Gagnep. in general habit of leafy shoots bearing up to four leaves with elliptic laminae, but distinguished by dark green laminae, labellum broadly oblong c. 28 × 30 mm, anther connective extending into elongated anther crest without two teeth above anther thecae, and stigma without dorsal appendage (compared to lamina bright mid green, labellum oblong c. 29 × 21 mm with prominently trilobed apex, anther connective extending into elongated anther crest with two teeth above anther thecae and stigma with dorsal appendage in *A. trilobum*). It is also unique in *Amomum* by its strong smell and taste of cinnamon, a character which is very striking but only of use in fresh material.

Type:—VIETNAM, Ninh Thuận Province, Bình Tiên District, Công Hải Commune, Núi Chúa National Park, 300 m a.s.l., 11°46'17''N, 109°10'5.0016''E, 20 May 2012, *Lưu Hồng Trường et al. NC-1006* (holotype SING, isotype SGN, VNMN). **Fig. 3 & 7.**

Rhizomatous spreading herb up to 50 cm tall, all parts of plant strongly aromatic when crushed, releasing smell and taste of cinnamon. ***Rhizome*** up to 1 cm in diam. (when fresh), sheathed by scales, scales ovate with broadly acute to obtuse apex, to 3 cm long, c. 1.2 cm broad, white with pink-red when young, soon turning papery brown and decaying with age, glabrous. ***Leafy shoots*** with 2–4 leaves per shoot, 10–12 cm apart; *leafless sheaths* 1–2, dull green when young, turning papery with age, shortly puberulent (scaberulous); *leaf sheaths* green, shortly puberulent, (rough to touch), weakly ribbed to weakly reticulate; *ligule* 1.5–2.5 mm, bilobed, dull green, glabrous with ciliate margin; *petiole* to 9.5 cm long, green, shallowly canaliculate, glabrous; *lamina* elliptic (12–)15–25 × 3–5.5(–7) cm, dark green above, slightly lighter below, smooth (not plicate), glabrous on both sides, apex long caudate (4–6.5 cm long), base obtuse, margin glabrous, hyaline, smooth througout but with a few sharp small teeth spaced well apart at apex (rough to touch). ***Inflorescence*** radical, arising from base of leafy shoot; *peduncle* to 2.5 cm long, covered by sheathing bracts; *spike* 3 × 1.5 cm (excl. exserted flowers); composed of c. 5 fertile bracts; *bract* c. 20–35 × 15–27 mm, ovate with acute, mostly mucronate apex, almost white at base, pale green, variously tinged pink (outermost bracts usually richer in tinge, innermost greener), glabrous, subtending a single flower; *bracteole* 3–5 × 2–3 mm, triangular, translucent white, glabrous with ciliate margin. ***Flower*** c. 8.5 cm long; *calyx* c. 4.3 cm long, translucent cream white, puberulent at basal half (rough to touch), nearly glabrous in apical half, unilaterally incised 12–15 mm, 3-toothed, teeth c. 1.2 mm long with densely pubescent margins; *floral tube* 5.9–6.5 cm long, externally white and glabrous at base, cream-white and puberulous towards apex; *dorsal corolla lobe* c. 22 × 8 mm, semi-translucent white at base, pale yellow at apex, prominently hooded, glabrous both sides; *lateral corolla lobes* 20–21 × 5–5.5 mm, semi-translucent white at base, pale yellow at apex, slightly hooded, glabrous both sides; *labellum* c. 28 mm long, c. 30 mm wide at broadest point, broadly obovate, white throughout with yellow midline bordered by red lines, densely hairy at centre in lower half; *lateral staminodes* absent. ***Stamen*** 17–18 mm long; *filament* broad, c. 5 mm long, 3.5 mm broad at base, glabrous but with puberulous pinkish patch running along midline of inner surface, 5 mm broad at apex; *anther* c. 13 mm long (including crest), connective tissue white, glabrous; *anther thecae* 3.5–4 mm long, dehiscing longitudinally almost for entire length; anther crest 7–8 × 6.5–7 mm, bluntly rectangular.

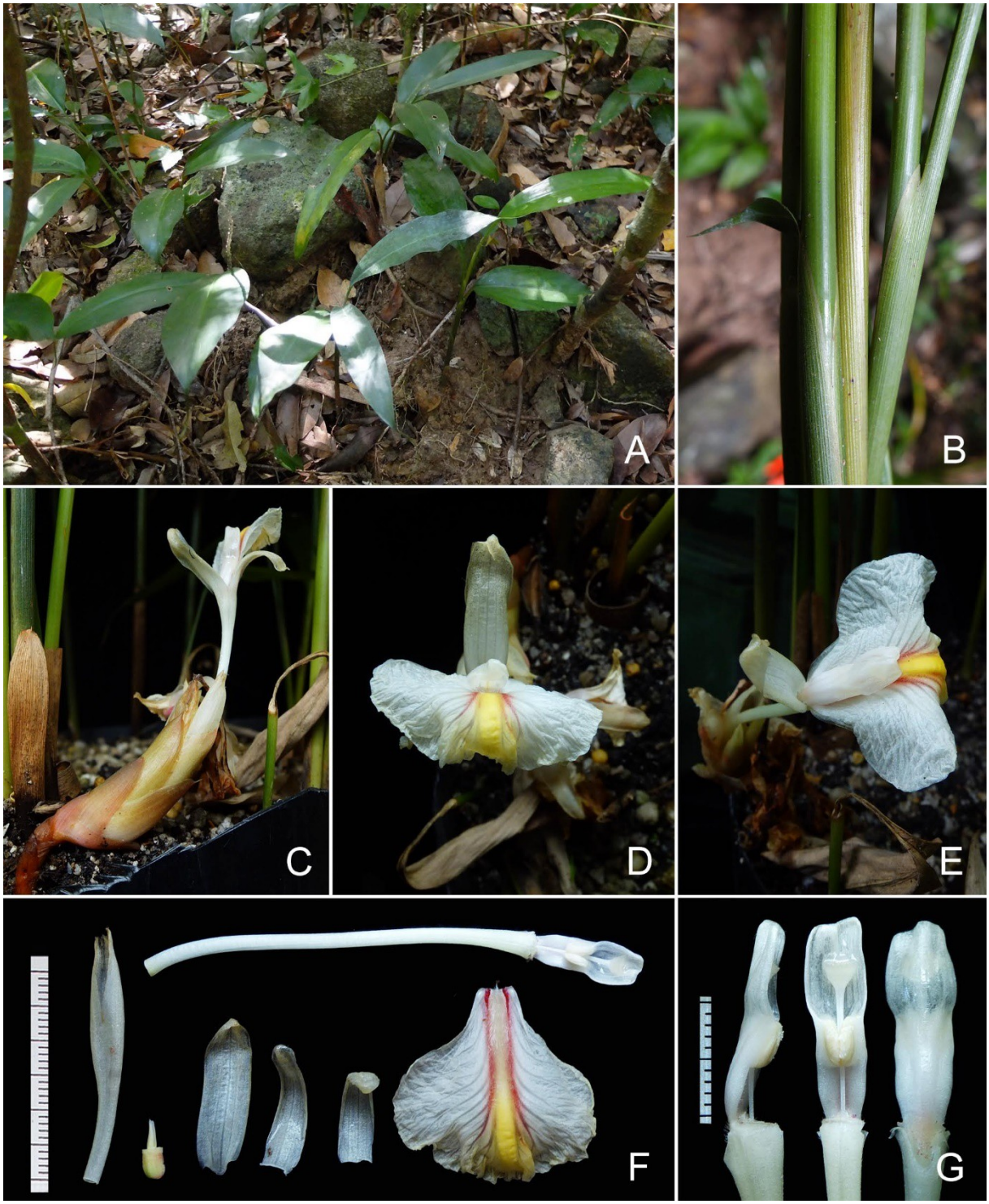

FIGURE 3. *Amomum cinnamomeum* Škorničk., Luu & H.Đ.Trần. A. Habit. B. Detail of ligules. C. Inflorescence (side view). D. Flower (front view). E. Flower (top view). F. Dissection (from left): calyx, ovary with epigynous glands, corolla lobes and labellum, floral tube with stamen (scale bar in cm). G. Detail of stamen (side, front and back view; scale bar in mm). *Photo: Jana Leong-Škorničková.*

Epigynous glands two, c. 3 mm long, narrowly conical. *Ovary* 4–4.5 × 3 mm, cylindrical, pale green with pink tinge, puberulous. ***Style*** white, glabrous; *stigma* 3 mm long, 3 mm broad, funnel-shaped but dorso-laterally compressed (triangular), back half consisting of three obscure lobes, ostiole densely ciliate. ***Fruit*** not seen.

Etymology: All parts of this plant, when crushed, emit a strong smell and taste of cinnamon.

Distribution & IUCN preliminary assessment:—*Amomum cinnamomeum* is a common species locally. It occurs at five locations in Lâm Đồng (Trần Hữu Đăng, pers. obs.), two in Núi Chúa National Park [Ninh Thuận Province], one at Suối Cát (Khánh Hoà Province) and one at Cam Lập (Khánh Hoà Province, Lưu Hồng Trường, pers. obs.) but only the two locations in Núi Chúa National Park are under legal protection. The others are all threatened by burning to clear the land for farming. The EOO is estimated at 4,391 km2, AOO is estimated at 24 km2 and there is only legal protection at two locations so we assess this species as EN B1ab(i,ii,iv)+B2ab(i,ii,iv).

Ecology and phenology:—Understory of deciduous dry lowland forest, from 90–350 m a.s.l. Flowering has been observed from June to August.

Other specimens examined (paratypes):—VIETNAM, Ninh Thuận Province, Bình Tiên District, Công Hải Commune, Núi Chúa National Park, 11°46’44.2”N, 109°10’42.9”E, 106 m a.s.l., 31 Oct 2013, *Leong-Škorničková et al. JLS-2582.* Khánh Hoà Province, Cam Lâm District, Suối Cát, 311 m a.s.l., 12°11’04.2”N 109°05’09.5”E, 20 April 2018, *Trần Hữu Đăng et al. KH-299.*

Amomum corrugatum **Škorničk., H.Đ.Trần & Luu,** ***sp. nov.***

Similar to *Amomum exsertum* in its upright and mostly single-leaved habit with distinctly corrugate lamina, but differs by its height to 100 cm, lamina to 50 × 15 cm, compact inflorescence, longer calyx 4–-4.5 cm and longer floral tube 10–12 cm, tooth-like staminodes present (compared to larger habit 120–180 cm, lamina c. 90 × 15 cm, lax inflorescence, shorter calyx c. 2.5 cm, shorter floral tube c. 7.5 cm, staminodes absent).

Type:—VIETNAM, Lâm Đồng Province, Đạ Hoai District, Đèo Chuối, 11º24’11.9”N 107º34’15.2”E, 216 m a.s.l., 20 June 2008, *Trần Hữu Đăng, Jana Škorničková, Loïc Cecilio, Mark Newman, Thi Thuận Phước, Đặng Quốc Quân, Lý Ngọc Sâm & Vichith Lamxay 53* (holo SING including material in spirit, iso E (incl. spirit), P, VNM). **Fig. 4 & 7.**

Rhizomatous spreading herb up to 100 cm tall. ***Rhizome*** up to 1 cm in diam. (when fresh), sheathed by scales, whitish at first, soon turning brown and decaying. ***Leafy shoots*** with 1–3 leaves per shoot, 10–20 cm apart; *leafless sheaths* 2–3, dull green, sometimes with red tinge, weakly reticulate, puberulent externally; *leaf sheaths* green, very sparsely puberulent, almost glabrous; *ligule* to c. 5 mm long, bilobed, semi-transparent green, soon becoming brown and brittle, pubescent externally; *petiole* 6–20 cm long, green, canaliculate, very sparsely puberulent, almost glabrous; *lamina* elliptic, to 50 × 15.5 cm, mid to dark green, semi-matte in appearance, slightly lighter below, prominently plicate, glabrous above, almost glabrous beneath with shortly puberulent lines along main veins, and pubescent midrib (particularly on sides), base attenuate,

apex acuminate, margin glabrous above, sparsely shortly strigose throughout including apex below. ***Inflorescence*** radical, arising from base of leafy shoot; *peduncle* 1–2 cm long, covered by greenish sheathing bracts; *spike* c. 1.5 cm long (excl. flowers), composed of 3–5 fertile bracts; *bract* c. 14–16 × c. 8 mm, ovate to triangular, cream white to pale greenish, sometimes with faint red tinge in very young inflorescences (soon becoming brown), both sides glabrous, subtending a single flower; *bracteole* c. 13 × 4 mm, triangular with incurved margins, white with red tinge at apex, both sides glabrous. ***Flower*** 12–14 cm long; *calyx* 4–5 cm long, white at base, pale yellow and sometimes with red tinge at apex, glabrous, unilaterally incised c. 10 mm, 3-toothed, teeth c. 1 mm long; *floral tube* 10–12cm long, externally white; *dorsal corolla lobe* c. 20 × 7 mm, semi-translucent greenish yellow, hooded, glabrous both sides; *lateral corolla lobes* 18–20 × c. 5 mm, semi-translucent greenish yellow, rounded, with weakly concave apex, glabrous both sides; *labellum* c. 28 mm long, 24–25 mm wide at broadest point, broadly bluntly triangular with basal claw, white with yellow-orange centre extending to apex and bright crimson red lines at sides of midline, glabrous throughout; *lateral staminodes* sharply narrowly triangular, c. 3–4 mm long, 0.5–1 mm wide at base, white with red tinge basally. ***Stamen*** c. 13 mm long; *filament* 3–4 mm long, 2–3 mm broad at base, c. 4.5 mm distally, white with red tinge towards base, glabrous with sparse glandular hairs at base; *anther* 9–10 mm long (including crest), connective tissue white, glabrous; *anther thecae* c. 5.5 mm long, dehiscing longitudinally along entire length; anther crest round and concave, 3.5–4 mm long, 5 mm wide at base, white; ***Epigynous glands*** two, c. 4–5 mm long, narrowly conical, cream to pale yellow. ***Ovary*** 4–6 × 3–4 mm, cylindrical, pure white, glabrous. ***Style*** white, glabrous; *stigma* 2–2.5 mm long, c. 2.5 mm broad, broadly diamond-shaped, dorso-laterally compressed, ostiole front-facing, densely ciliate. ***Fruit*** a hesperidium developing underground, globose with prominent blunt shoulders, c. 2.5 cm in diam., cream white, glabrous.

Etymology:—The epithet refers to the prominently corrugate lamina.

Distribution & IUCN preliminary assessment:—This species is only known from three collections. The population in Bù Gia Mập National Park is protected, while the two populations in Lâm Đồng Province have no degree of protection and face threats. One of the collections is from forested areas in a tourist resort, which is being constantly developed. The second population is threatened by land clearance for agriculture. The EOO is estimated at 2,453 km2, and AOO is 16 km2 so we treat this species as Endangered B1ab(ii, iii)+B2ab(ii, iii).

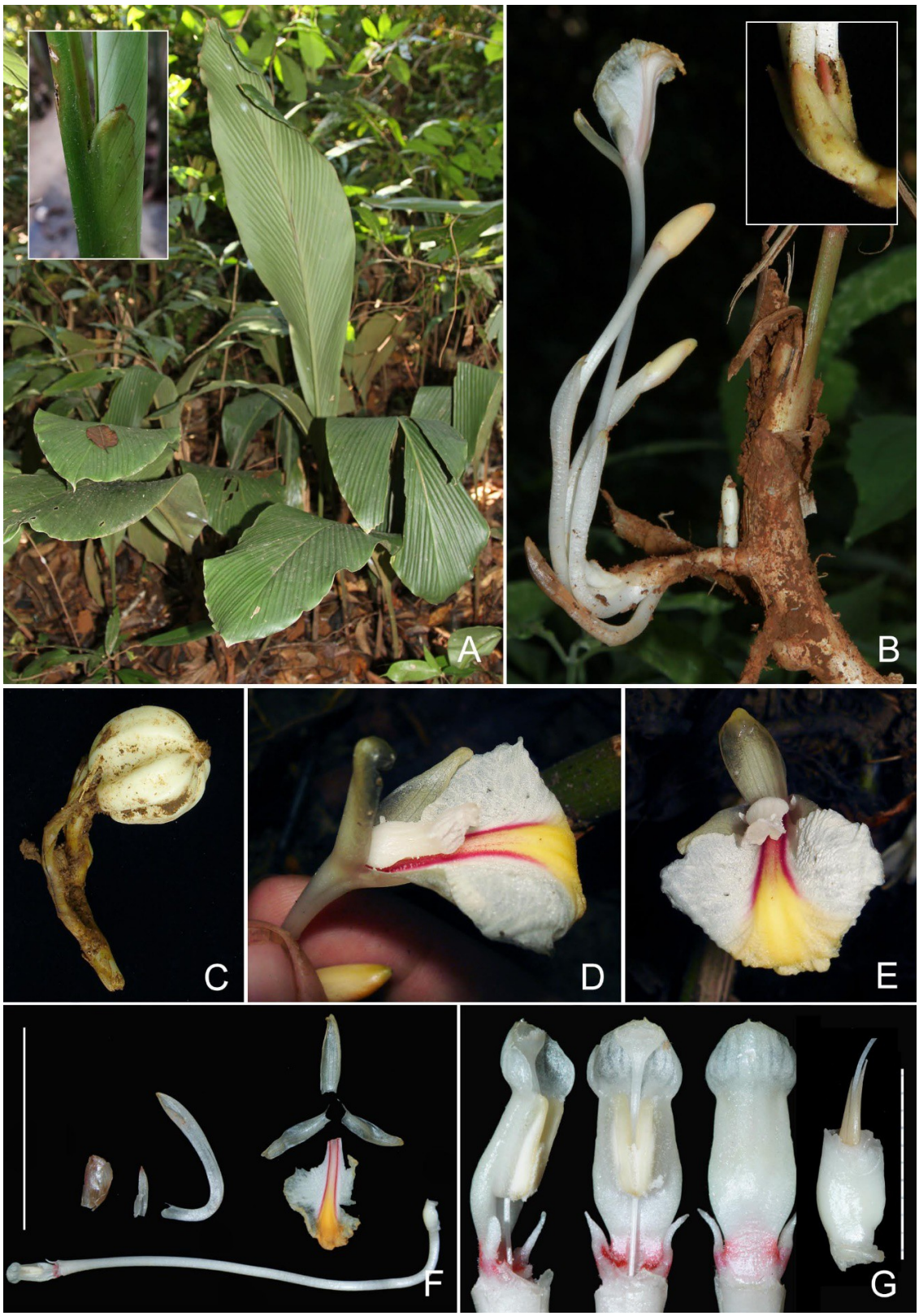

FIGURE 4. *Amomum corrugatum* Škorničk., H.Đ.Trần & Luu. A. Habit, and ligule in inset. B. Inflorescence, details of bracts in inset. C. Fruit. D. Flower (semi-side view). E. Flower (front view). F. Dissection (from left): bract, bracteole, calyx, labellum and corolla lobes, ovary, floral tube with ovary and stamen attached (positioned horizontally) (scale bar 5 cm). G. Detail of stamen (side, front and back view) and ovary with epigynous glands (scale bar in mm). *Photo: A (main image), B (main image), F & G Lưu Hồng Trường; A (inset), B (inset), C, D & E Jana Leong-Škorničková.*

Ecology and phenology:—Growing in partially to fully shaded understorey of lowland broadleaved evergreen forest, in clay substrate, often near streams.

Other specimens examined (paratypes):—VIETNAM, Lâm Đồng Province, Đạ Hoai District, Madaguoi resort, 11º25'15.2"N, 107º35'08.9"E, 172 m, 20 June 2008, *Trần Hữu Đăng et al. 52* (E, FOF, P, RUPP, SING VNM,), Bình Phước Province, Bù Gia Mập National Park, 370 m asl, 20 May 2013, 12°13'01.7"N, 107°08'33.2"E, *Lưu Hồng Trường et al. LUU-1046* (SGN).

Amomum lutescens* Luu & Škorničk., *sp. nov.

This species is unique among yellow-flowered *Amomum* species by its small habit, leafy shoots with 3–5 leaves, the presence of small flat obovate staminodes held upright, and the anther with very small semi-circular anther crest.

Type:—VIETNAM. Khánh Hòa Province, Cam Lâm District, Khánh Phú, Hòn Bà Nature Reserve, 12°07'54"N 108°56'33"E E, 1310 m a.s.l., 3 August 2012, *Lưu Hồng Trường & Trần Giỏi, KH-188* (holotype SGN, isotypes E, SGN, SING). **Fig. 5 & 7.**

Rhizomatous spreading herb up to 50 cm tall. ***Rhizome*** up to 1 cm in diam. (when fresh), sheathed by scales, scales ovate, tubular in basal 1–2 mm, with obtuse apex, c. 1.5 cm long, 5–6 mm broad (flattened at apical incised part), cream-coloured at first, very soon turning rusty brown and striate, decaying with age, glabrous. ***Leafy shoots*** with 3–5 leaves per shoot, 3–15 cm apart; *leafless sheaths* 1–2, dull yellow-green, strigose externally; *leaf sheaths* green, glabrous; *ligule* c. 3 mm long, obscurely bilobed, dull yellow-green, strigose externally; *petiole* 4–20 cm long, green, canaliculate, glabrous; *lamina* elliptic, (10–)20–26 × (3–)5–7 cm, mid to dark green and shiny above, slightly lighter below, prominently plicate, glabrous on both sides, base obtuse, apex narrowly acuminate to caudate (1–3 cm), margin almost glabrous above, below sparsely strigose throughout including apex. ***Inflorescence*** radical, arising from rhizome, at 3–8 cm from leafy shoot; *peduncle* (1.5–)3–5 cm long, often branching, covered by pink sheathing bracts; *spike* 1.5–2 cm long (excl. flowers), composed of 2–5 fertile bracts; *bract* c. 15–20 × 6–7 mm, ovate to triangular, pale pink in very young inflorescences (soon becoming brown), both sides glabrous, subtending a single flower; *bracteole* 8–16 × 1–3 mm, narrowly triangular with incurved margins, cream white, both sides glabrous. ***Flower*** 7.5–9 cm long; *calyx* 4–4.3 cm long, semi-translucent pale pink, almost glabrous in basal half, sparsely puberulent in apical half, unilaterally incised c. 5 mm, 3-toothed, teeth c. 1.5 mm long with a few hairs at apices; *floral tube* 6.5–7 cm long, externally white to pale yellow, almost glabrous at base, puberulent towards apex; *dorsal corolla lobe* 18–20 × c. 6 mm, semitranslucent pale yellow at base, darker yellow at apex, hooded, glabrous both sides; *lateral corolla lobes* 16–18 × 3–4 mm, semi-translucent pale yellow, darker towards rounded, concave apex, glabrous both sides; *labellum* 20–22 mm long, 11–12 mm wide at broadest point, broadly oblong, yellow throughout, darker towards apex, central part of base with red tinge radiating towards margins, hairy in centre at base; *lateral staminodes* oblong to narrowly obovate, 2–4 × 1.5 mm, yellow, erect. ***Stamen*** 8–9 mm long; *filament* much narrower than anther, 1.5–2 mm long, c. 1.5 mm broad, white, sparsely hairy; *anther* c. 7 mm long (including crest), connective tissue cream white to pale yellow, glabrous; *anther thecae* 4–4.5 mm long, dehiscing longitudinally along entire length; anther crest small, semicircular, 1.5 mm long, 3 mm wide at base, pale yellow.

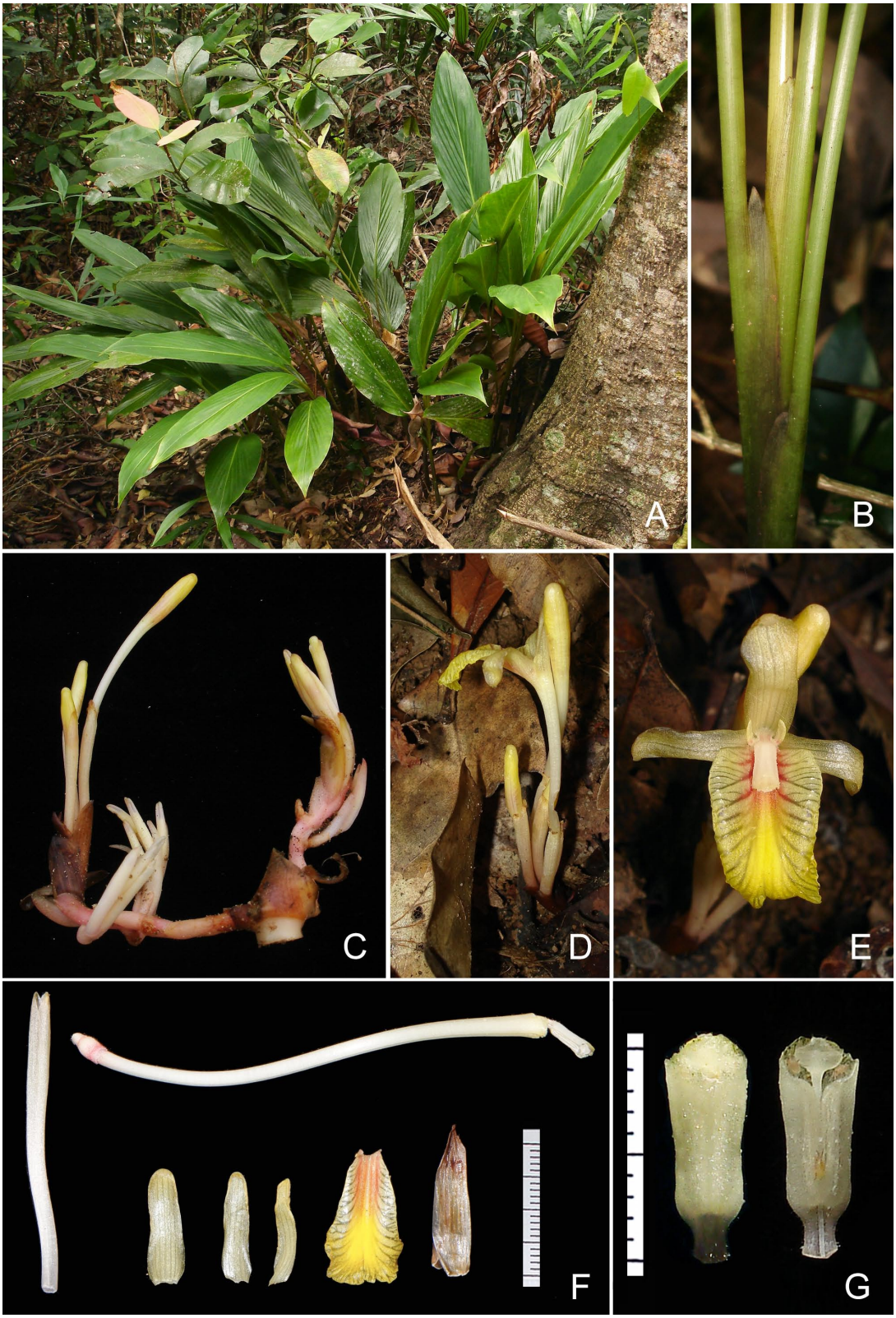

FIGURE 5. *Amomum lutescens* Luu & Škorničk. A. Habit. B. Detail of ligules. C. Inflorescences arising from rhizome. D. Flower (side view). E. Flower (front view). F. Dissection (from left): calyx, corolla lobes and labellum, bract, floral tube with ovary and stamen (scale bar in cm). G. Detail of stamen (side and front view; scale bar in mm). *Photo: A-E, G Jana Leong-Škorničková; F Lưu Hồng Trường.*

Epigynous glands two, c. 6 mm long, narrowly conical, pale yellow. ***Ovary*** 3.5 × 3 mm, globose, cream with pink tinge, puberulous. ***Style*** white, glabrous; *stigma* 1.5 mm long, 2 mm broad, broadly diamond-shaped, dorso-laterally compressed, ostiole front-facing, densely ciliate. ***Fruit*** not seen. **Etymology:—**The epithet reflects the yellow colour throughout the labellum.

Distribution & IUCN preliminary assessment:—This species is only known from two collections at differing locations in Hòn Bà Nature Reserve and one more location at Khánh Phú (Khánh Hòa Province) where there is no legal protection. The EOO and AOO are estimated at 13.88 km2 and 12 km2 respectively. This species may yet be found in Bidoup—Núi Bà National Park, which seems to have many Zingiberaceae species that we have previously seen in Hòn Bà Nature Reserve but the evidence available to us now indicates that this species is EN B1ab(i,ii) +B2ab(i,ii).

Ecology and phenology:—Broad-leaved evergreen montane forest, at elevations between 800–1310 m a.s.l. Flowering has been observed from June to August.

Other specimens examined (paratypes):—VIETNAM. Khánh Hòa Province, Cam Lâm District, Khánh Phú, Hòn Bà Nature Reserve, 4 July 2011, *Leong-Škorničková et al*. *JLS-1088* (SING); ibid., 12°0’6.43’’N, 109°0’17.44’’E, 953 m asl, 18 August 2017, *Trần Hữu Đăng et al. KH2-101* (SGN).

Amomum miriflorum **Škorničk. & Q.B.Nguyen, *sp. nov.***

Similar to *Amomum rugosum* (Y.K.Kam) Škorničk. & Hlavatá from Peninsular Malaysia, but differs in longer petioles (10–30 cm vs. up to 6 cm in *A. rugosum*), flowers larger overall, i.e. corolla lobes 25–27 × 11–13mm, larger and rhomboid labellum 45 × 42 mm, stamen 30 mm long, anther crest 17 × 11 mm (vs. corolla lobes up to 15 × 7 mm, labellum obovate 30 × 18 mm, stamen 14 mm long, anther crest 6 × 5 mm in *A. rugosum*) and colour of bracts, bracteoles and ovary which are with rich red tinge compared to pale green in *A. rugosum.*

Type:—VIETNAM. Kontum Province, Kon Plong District, Xã Hiếu, 1231 m, 14°40’48.7”N 108°24’05.5”E, 26 April 2012, *Jana Leong- Škorničková, Nguyễn Quốc Bình, Trần Hữu Đăng, Eliška Záveská JLS-1589* (holotype SING!, isotypes E!, K!, P!, PR!, VNMN!).

Fig. 6 & 7.

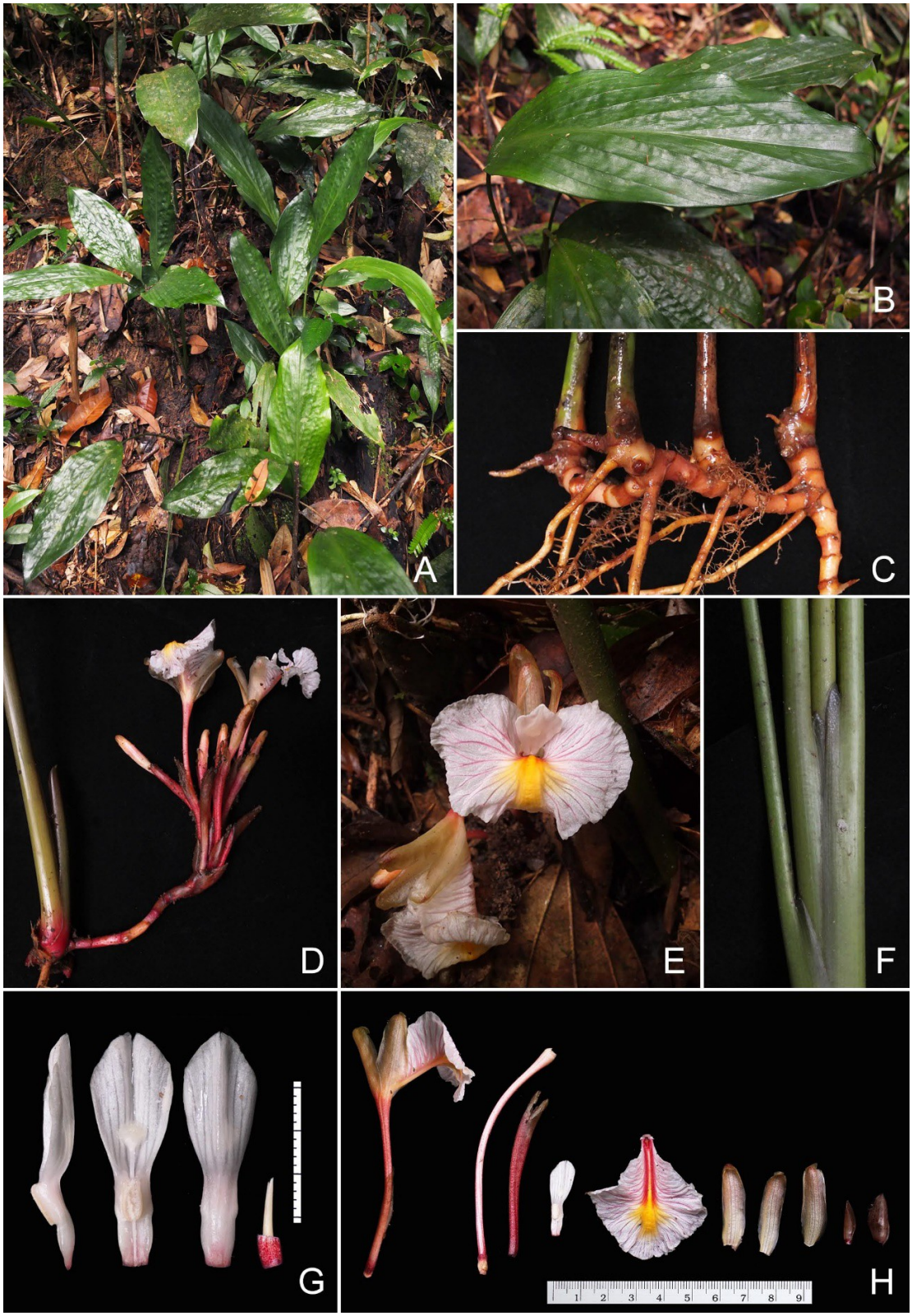

FIGURE 6. *Amomum miriflorum* Škorničk. & Q.B.Nguyen. A. Habit. B. Detail of lamina. C. Rhizome. D. Inflorescence arising from rhizome. E. Flower (front view). F. Detail of ligules. F. Detail of stamen (side, front and back view) and ovary with epigynous glands (scale bar in mm). G. Flower in side view and dissection (from left): ovary with floral tube, calyx, stamen, labellum, corolla lobes and bracts (scale bar in cm). *Photo: Jana Leong-Škorničková.*

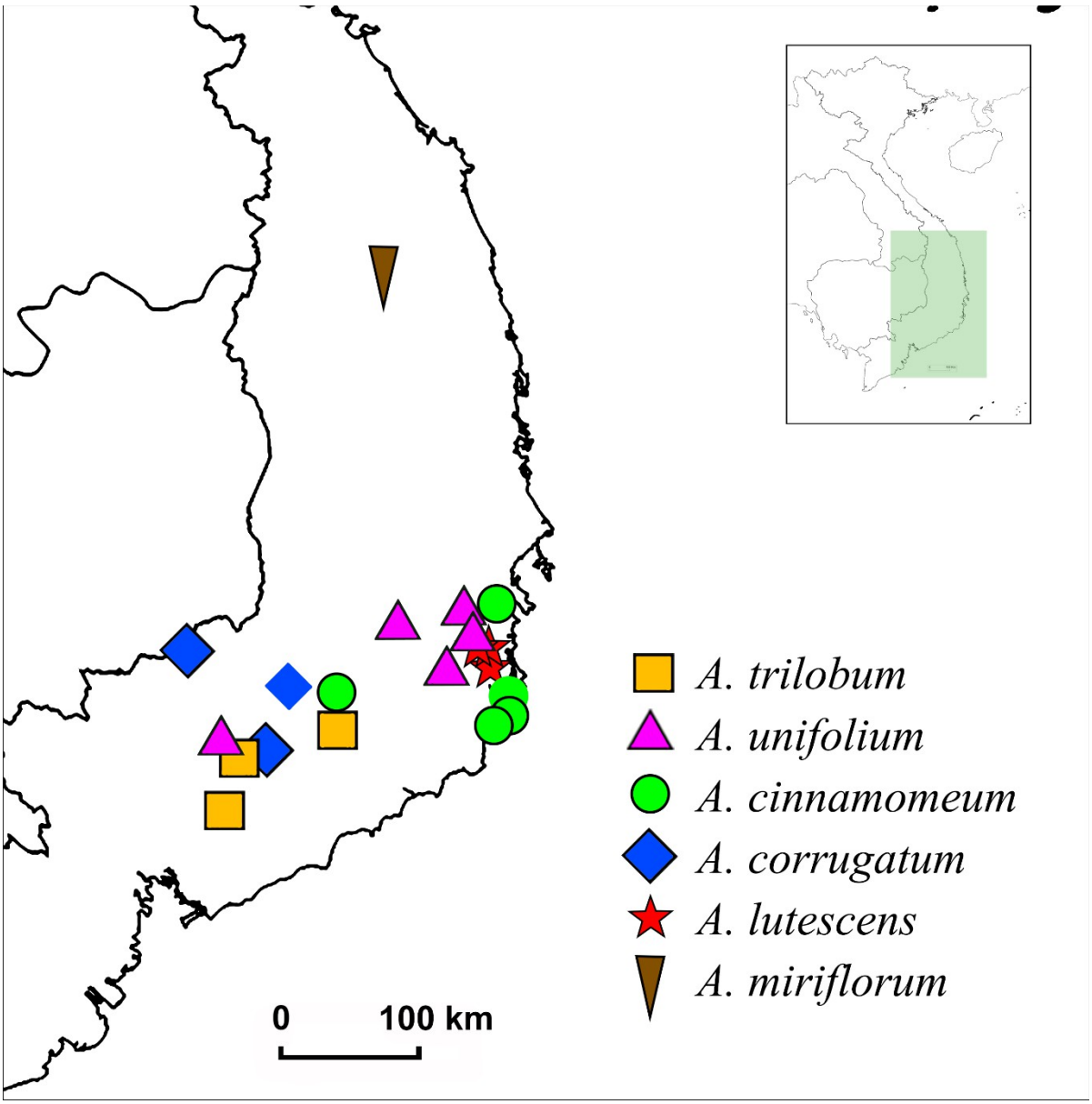

FIGURE 7. Distribution map of the six species treated in this paper. Symbols of records supported by specimens have black borders. Symbols of sightings supported by photographic records only have no borders.

Rhizomatous spreading herb up to 70 cm tall. ***Rhizome*** up to 1 cm in diam. (when fresh), sheathed by scales, scales ovate with obtuse apex, c. 1.5 cm long, 1.2 cm broad, bright pink-red when young, turning brown and decaying with age, glabrous. ***Leafy shoots*** with 2–4(–5) leaves per shoot, 3–5 cm apart; *leafless sheaths* 1–2, green (often reddish at base), glabrous; *leaf sheaths* green, glabrous; *ligule* 2–3 mm long, obscurely bilobed, grey–green, glabrous including margin; *petiole* 10–30 cm long, green, canaliculate, glabrous; *lamina* elliptic to weakly obovate, 25–43 × 6–11 cm, mid to dark green and shiny above, slightly lighter and matte below, weakly bullate, glabrous on both sides, base obtuse, apex caudate, margin glabrous throughout including the apex. ***Inflorescence*** radical, arising from base of leafy shoot; *peduncle* 3–5 cm long, rarely branching, covered by sheathing bracts; *spike* c. 2.5 cm long (excl. flowers), composed of 5–8 fertile bracts; *bract* c. 15 × 12 mm, ovate to triangular with acute apex, red in very young inflorescences (soon

becoming brown), both sides glabrous, subtending a single flower; *bracteole* c. 14 × 6 mm, triangular, folded (with prominent keel), pale red, both sides glabrous. ***Flower*** 8–12 cm long; *calyx* 3–5.5 cm long, bright red (but decaying rapidly from apex after anthesis), glabrous but sparsely puberulous towards base, unilaterally incised c. 15 mm, apex with 3 small teeth, puberulent at very tips; *floral tube* 4.5–8.5 cm long, externally red at base, gradually lighter towards pale pink apex, glabrous; *dorsal corolla lobe* c. 27 × 13 mm, pale yellow at base, with slight red tinge towards apex, bluntly hooded (not mucronate), glabrous both sides; *lateral corolla lobes* c. 25 × 11 mm, pale yellow at base, with slight red tinge towards apex, slightly bluntly hooded, glabrous both sides; *labellum* c. 45 mm long, c. 42 mm wide at broadest point, broadly trullate with entire round apex, white throughout with dark yellow patch in centre and bright red thick lines at base, which radiate as pink veins towards sides of labellum, glabrous throughout; *lateral staminodes* <1 mm, sharply triangular, white with bright red tinge at base. ***Stamen*** c. 3 cm long; *filament* 5–6 mm long, 4 mm wide at apex (2.5 mm wide at base), white with pink tinge at base, glabrous; *anther* 22 mm long (including crest), connective tissue white or pale pink, glabrous; *anther thecae* c. 5 mm long, dehiscing longitudinally for their entire length; anther crest 17 × 11 mm, obovate, pure white. ***Epigynous glands*** two, 5–6 mm long, narrowly conical with sharp apex. ***Ovary*** 5 × 3.5 mm, cylindrical, bright red, pubescent. ***Style*** white, glabrous; *stigma* 3 mm long, 3 mm broad, ostiole ciliate, top-front facing; stigma exserted above anther by c. 3 mm. ***Fruit*** not seen.

Etymology:—The Latin-derived specific epithet ‘miriflorum’ denotes its beautiful large flowers.

Distribution & IUCN preliminary assessment:—Only known from the type locality in Kon Tum Province which is an active logging area, so we assess this species as CR B1ab(i) +B2ab(i,ii,iii,iv).

Ecology and phenology:—Growing in montane evergreen broad-leaved forest on slopes near streams. Flowering in April to May, fruiting not observed.

Notes:—This species can be easily recognized by its large showy flowers and leafy shoots with 2–4 fairly glossy bullate leaves so far not seen in any other Vietnamese species. The length of the corolla tube and calyx is highly variable in this and a few other species we have observed, and seems to depend on the position of the flower in the inflorescence (outer flowers are longer), and on how deep the inflorescence is buried in the leaf litter.

We have not seen any additional herbarium material, which we could place with great confidence to this taxon. Photographs of flowers provided by Prof. Leonid Averyanov (collection *HAL 7889* from A Lưới, Thừa Thiên - Huế Province) may belong to this species as may photographs provided by Maxim Nuraliev (MW-DigiPic0000012 from Thạch Nham Protection Forest in Kon Tum Province). In both cases, the anther crest is smaller and has a different shape. New collections at the localities of these sightings are needed.

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Paper III

Phylogenomics and genome size evolution in *Amomum* s. s. (Zingiberaceae): Comparison of traditional and modern sequencing methods

Kristýna Hlavatá, Jana Leong-Škorničková, Eliška Záveská, Otakar Šída, Mark Newman, Terezie Mandáková, Martin A. Lysak, Karol Marhold, Tomáš Fér

Molecular Phylogenetics and Evolution 178, 2023: 107666

Paper III

Phylogenomics and genome size evolution in *Amomum* s. s. (Zingiberaceae): Comparison of traditional and modern sequencing methods

Kristýna Hlavatá[1], Jana Leong-Škorničková[2], Eliška Záveská[3], Otakar Šída[4], Mark Newman[5], Terezie Mandáková[6], Martin A. Lysak[6], Karol Marhold[7], Tomáš Fér[1]

[1]Department of Botany, Faculty of Science, Charles University, Benátská 2, CZ-128 01 Prague, Czech Republic

[2]The Herbarium, Singapore Botanic Gardens, 1 Cluny Road, 259569 Singapore.

[3]Institute of Botany, Czech Academy of Science, Zámek 1, CZ-252 43, Průhonice, Czech Republic

[4]Department of Botany, National Museum in Prague, Cirkusová 1840, CZ-193 00 Prague, Czech Republic

[5]Herbarium, Royal Botanic Garden Edinburgh, 20A Inverleith Row, Edinburgh EH3 5LR, UK

[6]Central European Institute of Technology (CEITEC), and Faculty of Science, Masaryk University, Kamenice 5, CZ-625 00 Brno, Czech Republic

[7]Department of Vascular Plant Taxonomy, Institute of Botany, Plant Science and Biodiversity Centre, Slovak Academy of Sciences, SK-845 23 Bratislava, Slovak Republic

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Abstract

Background and Aims: A targeted enrichment NGS approach was used to construct the phylogeny of *Amomum* Roxb. (Zingiberaceae). Phylogenies based on hundreds of nuclear genes, the whole plastome and the rDNA cistron were compared with an ITS-based phylogeny. Trends in genome size (GS) evolution were examined, chromosomes were counted and the geographical distribution of phylogenetic lineages was evaluated.

Methods: In total, 92 accessions of 54 species were analysed. ITS was obtained for 79 accessions, 37 accessions were processed with Hyb-Seq and sequences from 449 nuclear genes, the whole cpDNA, and the rDNA cistron were analysed using concatenation, coalescence and supertree approaches. The evolution of absolute GS was analysed in a phylogenetic and geographical context. The chromosome numbers of 12 accessions were counted.

Key Results: Four groups were recognised in all datasets though their mutual relationships differ among datasets. While group A (*A. subulatum* and *A. petaloideum*) is basal to the remaining groups in the nuclear gene phylogeny, in the cpDNA topology it is sister to group B (*A. repoeense* and related species) and, in the ITS topology, it is sister to group D (the *Elettariopsis* lineage).

The former *Elettariopsis* makes a monophyletic group. There is an increasing trend in GS during evolution. The largest GS values were found in group D in two tetraploid taxa, *A. cinnamomeum* and *A.* aff. *biphyllum* (both 2n = 96 chromosomes). The rest varied in GS (2C = 3.54–8.78 pg) with a constant chromosome number 2n = 48. There is a weak connection between phylogeny, GS and geography in Amomum.

Conclusions: *Amomum* consists of four groups, and the former *Elettariopsis* is monophyletic. Species in this group have the largest GS. Two polyploids were found and GS greatly varied in the rest of *Amomum*.

Keywords: Chromosome counts, cpDNA, *Elettariopsis*, Hyb-Seq, ITS, polyploidy

INTRODUCTION

The Zingiberaceae (ginger family; 58 genera, >1,800 species) is the largest family in the order Zingiberales with its centre of diversity in South and South-East Asia with only four genera extending to Africa and South America (Kress *et al.*, 2002). The most recent phylogenetic study of the entire family (Kress *et al.*, 2002) proposed a new classification into four subfamilies: Zingiberoideae, Alpinioideae, Siphonochileae and Tamijioideae and made clear that generic delimitation within Alpinioideae was very problematic and that several genera, including the two largest, *Alpinia* Roxb. and *Amomum* Roxb., were polyphyletic. This has been confirmed by several subsequent studies (Droop and Newman, 2014; Kress *et al.*, 2007, 2005; Xia *et al.*, 2004).

The most recent study by de Boer *et al.* (2018) re-circumscribed *Amomum* s.s. as monophyletic and recognised six additional monophyletic genera (*Conamomum* Ridl., *Epiamomum* A.D.Poulsen & Škorni

k., *Lanxangia* M.F.Newman & Škorni

k., *Meistera* Giseke, *Sundamomum* A.D.Poulsen & M.F.Newman, and *Wurfbainia* Giseke) based on an analysis of ITS and *mat*K sequences. *Amomum* s.s. is distributed from China and India through South-East Asia to Australia (*A. queenslandicum*). It consists of approximately 64 species, of which almost 30 were previously placed in *Elettariopsis* Baker. The type species of *Amomum* is *A. subulatum* Roxb., known commercially as black cardamom. *Amomum* s.s. is sister to *Renealmia* L.f., *Elettaria* Maton and clade I of *Alpinia* according to de Boer *et al.* (2018), and it is divided into two main lineages, but these are only weakly supported, probably because of insufficient molecular characters to allow resolution of diverging lineages. The first lineage comprises species of the *A. maximum* clade by Droop (2012). The second lineage contains, among others, the type species, *A. subulatum* and all accessions of the previously recognised genus *Elettariopsis*. Since the morphological delimitation of *Elettariopsis* from *Amomum* remained problematic even after the recircumscription, and since retaining *Elettariopsis* would lead to a paraphyletic *Amomum* s.s., de Boer *et al.* (2018) united both genera under *Amomum* s.s.

Previously published molecular studies included only a few samples of *Elettariopsis* (de Boer *et al.*, 2018; Droop, 2012; Kress *et al.*, 2005, 2002; Xia *et al.*, 2004) and none of these studies encompassed the whole range of morphological and geographical distribution of this previously

recognised genus. The highest number of *Elettariopsis* samples (13) was included in the PhD thesis of Droop (Droop, 2012). Since then, several new species apparently belonging to this group have been discovered and described in *Elettariopsis* (e.g. Picheansoonthon and Yupparach, 2012; Lim, 2013; Saensouk and Saensouk, 2014).

Phylogenetic analyses in Zingiberaceae using ITS and one or a few chloroplast markers only (de Boer *et al.*, 2018; Kress, 1990; Kress *et al.*, 2005, 2002; Vinitha *et al.*, 2014; Xia *et al.*, 2004) have failed to reconstruct robust phylogenies at the infrageneric level (Záveská *et al.*, 2011, 2016; Ngamriabsakul, *et al.*, 2003; Williams *et al.*, 2004). With the development of next generation sequencing (NGS) methods, it has become possible to construct phylogenies based on hundreds of genes, e. g. the Hyb-Seq method (Schmickl *et al.*, 2016; Weitemier *et al.*, 2014) which allows the acquisition of several hundred single-copy genes, as well as the nearly-complete chloroplast genome. This facilitates the construction of a strongly supported phylogeny and permits detailed examination of selected genes or parts of organelles. Furthermore, a large quantity of repetitive sequences is obtained which has also been found to aid in phylogenetic analyses (Dodsworth *et al.*, 2015; Macas *et al.*, 2015; Novák *et al.*, 2014).

The estimation of genome size (GS for short; nuclear DNA content) in Zingiberaceae has proven itself as a useful marker for the interpretation of phylogeny and decision making leading to new taxonomic treatment (Leong-Škorni

ková *et al.*, 2007; Záveská *et al.*, 2011; Schönswetter *et al.*, 2007). Generally, GS in plants often correlates with the phenotype and ecology (Herben *et al.*, 2012; Kang *et al.*, 2014; Knight *et al.*, 2005; Levin and Funderburg, 1979; Rayburn, 1990; Šímová and Herben, 2012). Absolute genome sizes (2C; the amount of DNA in the unreplicated nucleus) vary greatly in angiosperms, ranging from 0.13 to 304.46 pg (Leitch *et al.*, 2013), and the distribution across orders and families is uneven. In *Zingiberaceae* the 2C value is so far known to vary from 1.66 to 48.70 pg (Leitch *et al.*, 2019). Differences in GS are caused by changes in chromosome number and structure (polyploidisation, partial or whole chromosome loss or gain) and by other factors such as epigenetic effects on transcription (Leitch *et al.*, 2013) but also by accumulation of repetitive sequences in the genome (e.g. Gill *et al.*, 2010; Piednoël *et al.*, 2012; Piegu *et al.*, 2006; Sanmiguel and Bennetzen, 1998; Zedek *et al.*, 2010). GS in the Alpinioideae has not yet been intensively studied, and our study is one of the first to address this topic.

In Zingiberaceae, the range of chromosome numbers is very wide in the subfamily Zingiberoideae (from 2n = 20 in *Globba* sect. *Mantisia* to 2n = 105 in *Curcuma zedoaria* (Christm.) Roscoe; Leong-Škorni

ková *et al.*, 2007; Sharma *et al.*, 2012), whereas in Alpinioideae it is usually 2n = 48 (Beltran and Kam, 1984; Chen, 1989; Eksomtramage *et al.*, 2002; Mahanty, 1970) with only a few exceptions, such as *Renealmia* 2n = 44 (Beltran and Kam, 1984) and some doubtful counts unsupported by vouchers. Reports of polyploidy in the Alpinioideae are scarce and no polyploids have been reported in *Amomum* s.s. (sensu de Boer *et al.*, 2018). The only polyploid reported so far is *Alpinia austrosinensis* (D. Fang) P.Zou & Y.S.Ye (2n = 96, Chen *et al.*, 1988).

The aims of this study, focusing on *Amomum* s.s. including the majority of known species previously classified in *Elettariopsis*, are: (i) to reconstruct a phylogeny based on several hundred genes from targeted enrichment and compare it with phylogeny based on ITS as well as

with phylogeny based on the whole rDNA cistron and whole chloroplast, (ii) to examine the evolutionary trends in GS of *Amomum* s.s., (iii) to investigate chromosome numbers and their correlation with GS and phylogeny, and (iv) to interpret all this evidence in a geographical context.

MATERIALS AND METHODS

Plant material

Living material of 92 accessions belonging to 54 species (incl. 26 species formerly classified in *Elettariopsis*) were analysed in this study. Samples were determined to species level using characters from proto- logues and additional taxonomic literature, combining morphological and molecular characters. Some samples could not be confidently determined and are treated here as informal units (*Amomum* sp. 1–9). The accessions of A. trilobum (Z92) and A. unifolium (Z123) originate from the epitypes of the respective species names (Leong-Škorničková *et al.*, 2019).

Raw reads of Z575 (Renealmia polypus) were obtained from SRA (SAMN08971237; Carlsen *et al.*, 2018). The other accessions were obtained from plants cultivated in botanic gardens (Royal Botanic Garden Edinburgh, Singapore Botanic Gardens and Prague Botanical Garden), and from collections made across South-East Asia (Table 1).

Genome size estimation

Absolute GS was measured using flow cytometry (Doležel *et al.*, 2007) in 63 accessions in the flow cytometry laboratory of the Department of Botany of the National Museum in Prague. Remaining accessions were not measured due to lack of living material (including all accessions from the GenBank). Bellis perennis was used as the internal standard; its GS was calibrated by repeated measurements on different days (N = 10) against *Pisum sativum* (2C = 8.84 pg; Greilhuber and Ebert, 1994) and estimated to be 2C = 3.45 pg. Samples were stained using propidium iodide. Measurements were carried out and analysed using a Partec CyFlow® ML cytometer equipped with a green stable Cobolt laser (532 nm) and Partec FloMax® software. Each sample was measured on three different days. The GS was calculated as the arithmetic mean of three measurements made on different days. Only measurements with CV below 4.0 were accepted and values differing by more than 2 % were discarded and repeated on another day.

Chromosome preparations and counting

Root tips of 12 accessions for chromosome counts were collected mostly from plants grown in a controlled environment greenhouse at the Central European Technological Institute, Masaryk University, Brno, Czech Republic and in the glasshouses of the Royal Botanic Garden, Edinburgh.

Actively growing, young roots were harvested from cultivated plants, pre-treated with ice-cold water for 12 h, fixed in ethanol/acetic acid (3:1, v/v) for 24 h at 4 ◦C and stored in this mix at - 20

◦C until further use. Selected root tips were rinsed in distilled water (twice for 5 min) and citrate buffer (10 mM sodium citrate, pH 4.8; twice for 5 min), and digested in 0.3 % (w/v) cellulase, cytohelicase and pectolyase (all Sigma-Aldrich, St Louis, MO, USA) in citrate buffer at 37 ◦C for 90 min.

After digestion, individual root tips were dissected on a microscope slide in approximately 10 µl acetic acid and covered with a cover slip. The cell material was then spread evenly using tapping, thumb pressure and gentle heat from a flame. Finally, the slide was quickly frozen in liquid nitrogen and the cover slip flicked off with a razor blade. Slides were fixed in ethanol/acetic acid (3:1) and dried in air. The chromosomes were counterstained with 2 µg/ml DAPI in Vectashield (Vector Laboratories, Peterborough, UK). The preparations were analysed and photographed using an Olympus BX-61 epifluorescence microscope and CoolCube CCD camera (Metasystems, Altlussheim, Germany). At least 20 mitotic chromosome spreads were counted from each accession analysed.

DNA sequencing

ITS sequencing

An Invisorb® Spin Plant Mini Kit (Invitek Inc., Hayward, CA, USA) was used to extract genomic DNA from dry leaf tissue. ITS4 and ITS5 primers (White *et al.*, 1990) were used to amplify the ITS region in a PCR reaction containing 10.35 µl Milli-Q water, 3.0 µl 5 × MyTaq™ Reaction Buffer Red (Bioline GmbH, Germany), 10 pmol each of the ITS4 and ITS5 primers, 0.75U MyTaq™ polymerase (Bioline) and 4 ng DNA. PCR reactions were carried out in an Eppendorf Mastercycler or Eppendorf Mastercycler ep S with an initial denaturation of 1 min at 95 ◦C followed by 35 cycles of denaturation for 20 s at 95 ◦C, annealing for 50 s at 55 ◦C and an extension of 1 min at 72 ◦C, with a final extension of 15 min at 72 ◦C. PCR fragments were purified using a Gel/PCR DNA Fragments Extraction kit (Geneaid) and sequenced with the same primers used for PCR in the DNA sequencing laboratory of the Faculty of Science, Charles University, Prague on a 3130xl Genetic Analyzer sequencer (Applied Biosystems). Sixty-three ITS sequences were newly sequenced and uploaded to NCBI GenBank (Table 1).

Table 1

Amomum and outgroup accessions used in this study. Lineages are marked according to their labels in the phylogeny. Genome size is in pg as 2C value and chromosome count as 2n. Standard GenBank accession numbers and Short Read Archive identifiers are given.

Taxon name	Sample code	Collection no. (Herbarium)	Country	Lineage	2C (pg)	2*n*	ITS accession nr.	NGS (SRR code)
Amomum sp.	AY351985	Y.M. Xia 719 (HITBC)	China	C	–	–	AY351985	–
Amomum biphyllum (Saensouk & P. Saensouk) Škorničk. & Hlavatá	Z902 (S311)	ex cult. Mahasarakham University	Thailand	D	9.61	–	MW905342	SRR12824540
Amomum biphyllum aff. (Saensouk & P. Saensouk) Škorničk. & Hlavatá	Z644 (S312)	V. Lamxay VL2222 (NLS)	Laos	D	12.79	96	MW905315	SRR12824530
Amomum calcicola Lamxay & M.F. Newman	Z949 (S227)	M.F. Newman et al. LAO 1302 (E)	Laos	C	–	–	–	SRR12824535
Amomum cinnamomeum Škorničk., Luu & H.Đ.Trần	Z303 (S13)	J. Leong-Škorničková GRC-393 (SING)	Vietnam	D	15.66	96	MW905294	SRR12824532
Amomum cinnamomeum Škorničk., Luu & H.Đ.Trần	Z724	ex cult. SI W.J. Kress & Q.J. Li 05–7779	Vietnam	D	15.36	–	MW905325	–
Amomum chryseum Lamxay & M.F. Newman	HB_016	V. Lamxay VL1171 (E, NLS)	Laos	B	–	–	MW928599	–
Amomum corrugatum Škorničk., H.Đ. Trần & Luu	Z299 (S310)	H.Đ.Trần et al. 53 (E, SING, VNM)	Vietnam	D	5.40	–	MW905293	SRR12824531
Amomum corrugatum Škorničk., H.Đ. Trần & Luu	Z645	H.Đ.Trần et al. 52 (E, P, RUPP, SING, VNM, NLS)	Vietnam	D	5.44	48	MW905316	–
Amomum curtisii (Baker) Škorničk. & Hlavatá	Z847 (S296)	ex cult. Singapore Botanic Gardens	Pen. Malaysia	D	6.08	–	MW905338	SRR12824539
Amomum curtisii aff. (Baker) Škorničk. & Hlavatá	Z490 (S399)	M.F. Newman s.n. RBGE20001425	Thailand	D	5.69	48	MW905304	SRR12824538
Amomum curtisii aff. (Baker) Škorničk. & Hlavatá	Z721	W.J. Kress 99–6322	Thailand	D	5.67	–	MW905323	–
Amomum curtisii aff. (Baker) Škorničk. & Hlavatá	Z739	J. Mood 13P14 (PRC)	Malaysia	D	5.94	–	MW905335	–
Amomum dealbatum Roxb.	Z964 (S273)	ex cult. SI W.J. Kress 06–8414	China	C	6.36	–	–	SRR12824524
Amomum elan aff. (C.K. Lim) Škorničk. & Hlavatá	Z24 (S368)	J. Leong-Škorničková GRC-079 (SING)	Malaysia	D	7.90	48	MW905291	SRR12824528
Amomum elan aff. (C.K. Lim) Škorničk. & Hlavatá	Z732	J. Mood 1286 (PRC)	Malaysia, Sabah	D	7.33	–	MW905332	–
Amomum elan aff. (C.K. Lim) Škorničk. & Hlavatá	AF478747	W.J. Kress 00–6720 (US)	Malaysia?	D	–	–	AF478747	–
Amomum glabrum S.Q. Tong	Z861 (S294)	V. Lamxay VL1157 (E, NLS)	Laos	C	6.36	–	–	SRR12824516
Amomum glabrum S.Q. Tong	AF478721	W.J. Kress 00–6715 (US)	China	C	–	–	AF478721	–
Amomum glabrum aff. S.Q. Tong	AY351990	Y.M. Xia 73 (HITBC)	China	C	–	–	AY351990	–
Amomum glabrum aff. S.Q. Tong	Z442 (S166)	J. Leong-Škorničková et al. JLS-1598 (E, PR, SING, VNMN)	Vietnam	C	6.37	–	MW905300	SRR12824517
Amomum kerbyi (R.M. Sm.) Škorničk. & Hlavatá	AF478746	W.J. Kress 96–5746 (US)	Indonesia	D	–	–	AF478746	–
Amomum kerbyi aff. (R.M. Sm.) Škorničk. & Hlavatá	Z851 (S293)	M. Dančák 2015/3004 (OL)	Brunei	D	6.35	–	MW905340	SRR12824537
Amomum latiflorum (Ridl.) Škorničk. & Hlavatá	Z121	H.Đ. Trần et al. SNG-46 (SING)	Singapore	D	6.45	–	MW905287	–
Amomum latiflorum (Ridl.) Škorničk. & Hlavatá	Z642	H. Ibrahim & S. Teo SNG-113 (SING)	Singapore	D	6.31	–	MW905344	–
Amomum latiflorum (Ridl.) Škorničk. & Hlavatá	Z90 (S79)	J. Leong-Škorničková et al. SNG-13 (SING)	Singapore	D	6.42	–	MW905313	SRR12824536
Amomum limianum aff. (Picheans. & Yupparach) Škorničk. & Hlavatá	Z635	ex cult. SBG20123383	Laos	D	6.88	–	MW905307	–
Amomum limianum aff. (Picheans. & Yupparach) Škorničk. & Hlavatá	Z722	W.J. Kress 99–6313 (US)	Thailand	D	7.09	–	MW905324	–
Amomum limianum aff. (Picheans. & Yupparach) Škorničk. & Hlavatá	Z726	J. Mood 3247 (PRC)	Thailand	D	7.14	–	MW905327	–
Amomum longipetiolatum Merr.	AF478722	W.J. Kress 99–6353 (US)	China	C	–	–	AF478722	–
Amomum lutescens Škorničk. & Luu	Z925	J. Leong-Škorničková et al. JLS-1088 (SING)	Vietnam	D	6.72	–	MW905345	–
Amomum maximum Roxb.	AY351995	Y.M. Xia 725 (HITBC)	China	C	–	–	AY351995	–
Amomum maximum Roxb.	Z950 (S256)	A.D. Poulsen 2892 (E)	Papua New Guinea	C	5.62	–	–	SRR12824515
Amomum maximum Roxb.	Z686	Leong-Škorničková et al. JLS-1726 (SING, PR, P, E)	Laos	C	5.62	–	MW905321	–
Amomum menglaense S.Q. Tong	AY351996	Y.M. Xia 726 (HITBC)	China	C	–	–	AY351996	–
Amomum miriflorum Škorničk. & Q.B. Nguyen	Z439 (S172)	J. Leong-Škorničková et al. JLS-1589 (E, K, P, PR, SING, VNMN)	Vietnam	D	8.78	48	MW905299	SRR12824534
Amomum odontocarpum D. Fang	Z862 (S295)	V. Lamxay VL1300 (E, NLS)	Laos	C	–	–	–	SRR12824514
Amomum petaloideum (S.Q.Tong) T.L. Wu	Z96 (S139)	W.J. Kress et al. 95–5508 (US)	China	A	6.29	–	MW905347	SRR12824513
Amomum pterocarpum Thwaites	KF304443	M.R. Vinitha 75,254 (CALI)	India	C	–	–	KF304443	–
Amomum pterocarpum Thwaites	KF304442	M.R. Vinitha 94,731 (CALI)	India	C	–	–	KF304442	–

(*continued on next page*)

Table 1 (*continued*)

Taxon name	Sample code	Collection no. (Herbarium)	Country	Lineage	2C (pg)	2*n*	ITS accession nr.	NGS (SRR code)
Amomum purpureorubrum S.Q. Tong & Y.M. Xia	AY352000	Y.M. Xia 727 (HITBC)	China	C	–	–	AY352000	–
Amomum putrescens D. Fang	AY352002	Y.M. Xia 728 (HITBC)	China	B	–	–	AY352002	–
Amomum putrescens D. Fang	Z614 (S396)	J. Leong-Škorničková et al. JLS-2146 (VNM, SING)	Vietnam	B	5.30	–	–	SRR12824545
Amomum queenslandicum R.M. Sm.	AY352004	Kmn 1428 (HAW (HLA))	Australia	C	–	–	AY352004	–
Amomum ranongense (Picheans. & Yupparach) Škornič. & Hlavatá	Z731	J. Mood 3377 (PRC)	Thailand	D	6.17	–	MW905331	–
Amomum ranongense aff. (Picheans. & Yupparach) Škornič. & Hlavatá	Z727	J. Mood 08P288 (PRC)	Thailand	D	5.96		MW905328	–
Amomum repoeense aff. Pierre ex Gagnep.	Z450	J. Leong-Škorničková JLS-1619 (SING, E, P, VNMN)	Vietnam	B	5.65	–	MW905301	–
Amomum repoeense aff. Pierre ex Gagnep.	Z456	J. Leong-Škorničková JLS-1637 (SING, E, P, VNMN)	Vietnam	B	5.27	–	MW905302	–
Amomum repoeense aff. Pierre ex Gagnep.	Z659	V. Lamxay VL2223 RBGE20111045	Laos	B	4.79	–	MW905317	–
Amomum repoeense aff. Pierre ex Gagnep.	Z665 (S67)	H.Đ. Trần et al. 67 (E, SING, VNM)	Vietnam	B	5.04	–	MW905320	SRR12824544
Amomum rugosum (Y.K.Kam) Škornič. & Hlavatá	Z7 (S80)	J. Leong-Škorničková GRC-363 (SING)	Malaysia	D	5.95	–	MW905336	SRR12824533
Amomum sericeum Roxb.	Z559	J. Leong-Škorničková et al. JLS-1675 (E, SING)	Laos	C	7.46	–	MW905306	–
Amomum sericeum Roxb.	Z662 (S68)	M.F. Newman 2397 (E)	Cambodia	C	6.49	–	MW905319	SRR12824543
Amomum smithiae (Y.K.Kam) Škornič. & Hlavatá	Z720	ex cult. SBG20001092	Malaysia	D	6.50	–	MW905322	–
Amomum stenosiphon K.Schum.	AF478748	W.J. Kress 01–6847 (US)	Malaysia	D	–	–	AF478748	–
Amomum stenosiphon aff. K.Schum.	Z872 (S173)	Conlon et al. 41 (E)	Indonesia	D	6.21	–	MW905326	SRR12824526
Amomum stenosiphon aff. K.Schum.	Z725	J. Mood 89P43 (PRC)	Malaysia, Sabah	D	6.21	–	MW905341	–
Amomum subcapitatum Y.M. Xia	HB_079	V. Lamxay VL2058 (NLS)	Laos	B	–	–	MW928600	–
Amomum subulatum Roxb.	KF304452	M.R. Vinitha 103,627 (CALI)	India	A	–	–	KF304452	–
Amomum subulatum Roxb.	KF304453	M.R. Vinitha 92,765 (CALI)	India	A	–	–	KF304453	–
Amomum subulatum Roxb.	Z81 (S51)	J. Škorničková CU71468 (CALI, SING)	India	A	3.54	48	MW905337	SRR12824541
Amomum trilobum Gagnep.	Z92 (S12)	M.F. Newman & J. Škorničková 1455 (E, SING)	Vietnam	D	7.67	48	MW905346	SRR12824525
Amomum trilobum aff. Gagnep.	Z308 (S366)	ex cult. SBG20110993	Vietnam	D	7.69		MW905296	SRR12824529
Amomum unifolium Gagnep.	Z123 (S11)	M.F. Newman & J. Škorničková 2002 (E, SING)	Vietnam	D	5.99	48	MW905288	SRR12824523
Amomum unifolium aff. Gagnep.	Z304	ex cult. SBG20110994	Vietnam	D	6.05	–	MW905295	–
Amomum unifolium aff. Gagnep.	Z636	ex cult. SBG20123278	Vietnam	D	5.93	–	MW905308	–
Amomum unifolium aff. Gagnep.	Z638	H.Đ. Trần 257 (VNM)	Vietnam	D	5.91	–	MW905309	–
Amomum unifolium aff. Gagnep.	Z661	H.Đ. Trần s.n. RBGE20081123	Vietnam	D	6.12	–	MW905318	–
Amomum unifolium aff. Gagnep.	Z850	ex cult. SBG20111709	Vietnam	D	6.38	–	MW905339	–
Amomum velutinum X.E.Ye, Škornič. & N.H.Xia	Z429 (S400)	J. Leong-Škorničková et al. JLS-1557 (E, PR, SING, VNMN)	Vietnam	B	5.23	–	MW905298	SRR12824512
Amomum wandokthong (Picheans. & Yupparach) Škornič. & Hlavatá	Z903 (S309)	K. Hlavatá 1 (PRC)	Thailand	D	7.98	–	MW905343	SRR12824522
Amomum wandokthong aff. (Picheans. & Yupparach) Škornič. & Hlavatá	Z730	J. Mood 3000 (PRC)	Thailand	D	7.85	–	MW905330	–
Amomum sp. 1	Z104	H.Đ.Trần et al. 383 (VNM)	Vietnam	D	8.08	–	MW905286	–
Amomum sp. 2	Z458	J. Leong-Škorničková et al. JLS-1640 (E, PR, SING, VNMN)	Vietnam	D	7.78	–	MW905303	–
Amomum sp. 3	Z641	ex cult. SBG20050385	Malaysia	D	6.48	48	MW905312	–
Amomum sp. 3	Z643	ex cult. SBG20060610	Malaysia	D	6.31	–	MW905314	–
Amomum sp. 3	Z729	J. Mood 08P289 (PRC)	Thailand	D	6.23	–	MW905329	–
Amomum sp. 4	Z738	J. Mood 3181 (PRC)	Malaysia	D	8.10	–	MW905334	–
Amomum sp. 5	Z639	J. Leong-Škorničková GRC-50 (E, SING)	Malaysia	D	7.93	–	MW905310	–
Amomum sp. 6	Z329 (S369)	ex cult. SBG20110992	Vietnam	D	6.40	48	MW905297	SRR12824527
Amomum sp. 7	Z734 (S308)	J. Mood 3387 (PRC)	Thailand	C	4.78	–	MW905333	SRR12824542
Amomum sp. 8	Z640	ex cult. SBG20122011	Laos	C	4.89	–	MW905311	–
Amomum sp. 9	Z491	H.Đ.Trần 366 (VNM)	Vietnam	B	4.78	–	MW905305	–
Aframomum alboviolaceum (Ridl.) K. Schum.	Z236 (S118)	D.J. Harris 5745 (E)	Central African Rep.	outgroup	–	–	MW905290	SRR12824547
Aframomum chrysanthum Lock	Z22	J. Leong-Škorničková GRC-173 (SING)	Ex cult. Singapore BG	outgroup	–	–	MW905289	–
Aframomum melegueta K. Schum.	Z743 (S70)	M. Antheuisse s.n. (E)	Côte d'Ivoire	outgroup	–		–	SRR12824546
Geostachys densiflora Ridl.	Z947 (S254)	J. Leong-Škorničková et al. JLS-1662 (SING)	Malaysia	outgroup	–		–	SRR12824521
Hedychium aureum C.B.Clarke & G. Mann ex Baker	Z495 (S129)	T. Fér & O. Šída 48 (PR)	Thailand	outgroup	–	–	–	SRR12824520
Renealmia battenbergiana Cummins ex Baker	Z253	A. Rangsiruji & M. Newman s. n. (E)	Ghana	outgroup	–	–	MW905292	–
Renealmia polypus Gagnep.	Z575 (S66)	M. Newman & J. Škorničková 2009 (E)	Central African Rep.	outgroup	–	–	–	SRR7058219
Riedelia arfakensis Valeton	Z3 (S49)	J. Leong-Škorničková GRC-394 (SING)	Papua New Guinea	outgroup	–	–	–	SRR12824519
Zingiber officinale Roscoe	Z942 (S242)	M.V. Mathew (E)	India	outgroup	–	–	–	SRR12824518

Hyb-Seq

A subset of 37 samples (including seven outgroup accessions; covering all lineages recognised with ITS-based phylogeny) was chosen for the Hyb-Seq method (Table 1). First, DNA was sonicated to obtain fragments 500–600 bp long using a M220 Focused-ultrasonicator™ (Covaris). The fragment length was verified by gel electrophoresis with O'GeneRuler™ 100 bp DNA Ladder Plus (Thermo Fisher Scientific) and Quick-Load® 1 kb DNA Ladder (New England BioLabs) used as standards. The Hyb-Seq procedure followed (Cronn *et al.*, 2012; Straub *et al.*, 2012; Weitemier *et al.*, 2014) with slight modifications described below. Libraries were prepared using a NEBNext Ultra DNA Library Prep Kit for Illumina (New England BioLabs), purified using QIAquick PCR Purification Kit (Qiagen), and the DNA was dissolved in 30 µl ddH2O and visualised by gel electrophoresis using O'GeneRuler™ DNA Ladder Mix (Thermo Fisher Scientific) as standard. Products of 500–600 bp in length were cut from the gel, purified using QIAquick Gel Extraction Kit (Qiagen), and the DNA was eluted into 20 µl ddH2O. The products were PCR amplified, indexed using Q5 Hot Start HiFi PCR Master Mix (New England BioLabs) and NEBNext Multiplex Oligos for Illumina index primers (Set 1, NEB #E7335), purified twice using an Agencourt AMPure XP kit (Beckman Coulter) in the ratio of 0.75:1 (kit:DNA), and checked on a gel. The samples were quantified using a Qubit® 2.0 fluorometer (Invitrogen), in equimolar proportions and concentrated to 6 µl using an IR Concentrator NB 503CIR (*N*-Biotek). The pooled libraries were enriched for 26 h with custom probes (targeting 1,180 loci from 4,618 exons; Carlsen *et al.*, 2018; https://github. com/tomas-fer/gingers) using a MYbaits® In-Solution Sequence Capture for Targeted High-Throughput Sequencing kit v.2 (MYcroarray, Michigan, USA), PCR amplified with 2 × KAPA HiFi HotStart ReadyMix (Roche) with P5 and P7 primers (Illumina), purified with QIAquick PCR Purification Kit (Qiagen) and quantified using a Qubit® 2.0 fluorometer (Invitrogen). The final enriched library was sequenced on an Illumina MiSeq using 300 cycle sequencing kit to obtain 150 bp paired-end reads in the Central European Institute of Technology (CEITEC), Brno, Czech Republic. Raw Hyb-Seq reads were uploaded to SRA (BioProject PRJNA668878).

Phylogeny inference

Two samples of *Renealmia* (*R. battenbergiana* Cummins ex Baker, *R. polypus* Gagnep.) and two samples of *Aframomum* K.Schum. (*A. alboviolaceum* (Ridl.) K.Schum., *A. chrysanthum* Lock) were used as the outgroup in the ITS analysis; for Hyb-Seq, seven outgroup species from the subfamily Alpinioideae (*Aframomum alboviolaceum, Aframomum melegueta* K.Schum., *Renealmia battenbergiana*, *Renealmia polypus, Riedelia arfakensis* Valeton and *Geostachys densiflora* Ridl.) and two outgroup species from the subfamily Zingiberoideae (*Hedychium aureum* C.B.Clarke & H.Mann ex Baker and *Zingiber officinale* Roscoe) were used.

ITS

Contigs were manually created using Geneious 7.0.6 (Kearse *et al.*, 2012), intra-individual variations were coded according to IUPAC standards (only ambiguities in two or more accessions were treated). Sixteen ITS sequences were obtained from GenBank (Kress *et al.*, 2002, Vinitha *et*

al., 2014, and de Boer *et al.* 2018; https://www.ncbi.nlm.nih. gov/genbank/); missing regions were replaced by Ns. In total, 80 sequences were aligned using MAFFT ver. 7 (Katoh and Standley, 2013) online server (http://mafft.cbrc.jp/alignment/server/index.html) with default settings. The resulting alignment was manually improved in BioEdit v. 7.0.5 (http://www.mbio.ncsu.edu/bioedit/bioedit.html). A Bayesian analysis was conducted in MrBayes 3.2.2 (Ronquist *et al.*, 2012) using 10 million generations with GTR model (best model suggested by MrModeltest2*;* Nylander, 2004). The Maximum likelihood tree was estimated in RAxML 8.2.4 (Stamatakis, 2014) under GTRGAMMA model, with 100 bootstrap replicates mapped on the best tree. Analyses were run on the Czech National Grid Infrastructure (Metacentrum; htt ps://metavo.metacentrum.cz/).

The phylogeny was supplemented with biogeographical information, i.e. the distribution of each species in four biogeographical regions: SubHimalayan, North Indochina, South Indochina and Sundaland.

Hyb-Seq data analysis

Nuclear genes

Analyses were performed using the HybPhyloMaker pipeline (Fér and Schmickl, 2018): Raw reads were adaptor- and quality- trimmed with Trimmomatic 0.32 (Bolger *et al.*, 2014), deduplicated using FastUniq v. 1.1 (Xu *et al.*, 2012) and mapped to a 'pseudoreference' (made from 4,680 target exons sequences separated by 400 'Ns') using BWA v. 0.7.15 (Li and Durbin, 2009). Exon sequences were aligned with MAFFT 7.402 (Katoh *et al.*, 2019; Kuraku *et al.*, 2013), exons belonging to the same gene were concatenated and filtered according to missing data (samples with more than 10 % of missing data were discarded from the respective gene and only genes with more than 90 % of samples left were kept). Gene trees were constructed using RAxML 8.2.4 (Stamatakis, 2014) with the GTRGAMMA model and 100 standard bootstrap replicates. Supermatrix (concatenation with maximum likelihood using ExaML 3.0.15 and per-exon partitioning; Kozlov *et al.*, 2015), coalescence-based (ASTRAL v. 4.11.1; Mirarab *et al.*, 2014) and supertree (maximum representation using maximum likelihood with BINGAMMA model in RAxML with 100 standard bootstrap replicates; MRL, Nguyen *et al.*, 2012) species trees were estimated. Biogeographical information was plotted next to the tree as for the ITS phylogeny.

cpDNA and rDNA

Off-target reads including chloroplast and ribosomal DNA reads (Maia *et al.*, 2014) were quality-filtered (see above), paired and mapped to the *Alpinia oxyphylla* plastome (NC_035895; 161,351 bp) and *Elettaria cardamomum* rDNA reference (T. Fér, Charles University, Prague, Czech Republic, unpubl. res.; 7,530 bp) in Geneious v. 7.0.6 using the settings described in the HybPhyloMaker manual (Fér and Schmickl, 2018). For cpDNA, the second inverted repeat was deleted from the reference before mapping. The rDNA analysed consisted of partial ETS (cA. 754 bp), 18S, ITS1, 5.8S, ITS2, 26S, and partial NTS (cA. 700 bp) regions. The consensus sequences were aligned in MAFFT v. 7.402 (Katoh *et al.*, 2019; Kuraku *et al.*, 2013) and phylogenetic trees were reconstructed using RAxML ver. 8.2.4 with the GTRGAMMA model and 500 standard bootstrap replicates.

Genome size evolution

Absolute holoploid GS (2C) was measured in 63 accessions, i.e., only for ingroup samples. Because the inner nodes of the phylogeny were better resolved using the NGS nuclear gene analysis, GS was mapped on the ASTRAL NGS phylogenetic tree (28 samples) to obtain the best estimate of GS evolution in the genus. The full set of GS measured was also reconstructed on an ITS Bayesian majority rule consensus tree (59 samples). The reconstruction was done in phytools 0.6–60 (Revell, 2012) for R 3.5.0 (R Core Team, 2018) using the *contMap* function. In order to test for phylogenetic association, mode, and tempo of GS evolution, *lambda*, *kappa*, and *delta* scaling parameters (Pagel, 1999, 1997), respectively, were estimated using the phylogenetic generalised least squares (PGLS) method in caper (Orme *et al.*, 2013) for R using functions *comparative.data* and *pgls*. For accessions with missing 2C values, a measurement of another accession of the same species, if available, was used. Branches with missing data were coloured in grey. A boxplot visualisation of 2C GS in individual groups (defined by phylogeny; see Results), using all available accessions, was made in R 3.5.0. The spatial distribution of the GS of all accessions together with their group membership was plotted on the map.

RESULTS

Sequencing

Nuclear genes

For 37 samples, 53,248,014 raw reads were obtained in total (mean 1,401,264 reads per sample), of which 89.78 % (47,807,628) of non-duplicate reads of the required quality were retained, i.e. 1,292,098 reads per sample on average. The number of filtered reads ranged from 380,301 in Z123 *Amomum unifolium* to 2,964,641 in Z743 *Aframomum melegueta* (Supplement Table S1). Of these, 37.8 % (494,338 reads) on average mapped onto the targeted nuclear genes (the lowest mapped number being 205,258 in sample Z123 *Amomum unifolium* and the highest 1,609,510 in sample Z743 *Aframomum melegueta*). At the minimum 4 × coverage and 90 % taxon-specific missing data cut-off, 449 genes were recovered. An alignment of 640,488 positions was constructed with 117,920 (18.4 %) variable and 58,767 (9.2 %) parsimony informative sites (Table 2), where the average coverage per sample ranged from 19 × in Z92 *Amomum trilobum* to 152 × in Z743 *Aframomum melegueta* with a mean of 44 ×.

Chloroplast DNA

For 37 samples, a mean of 18,154 reads (1.34 %) per sample was mapped onto the reference used, ranging from 2,097 (0.36 %) in Z123 *Amomum unifolium* to 50,504 (2.75 %) in Z575 *Renealmia polypus*. An alignment of 135,040 positions was constructed with 14,408 (10.4 %) variable and 5,594 (4.1 %) parsimony informative sites (Table 2). The completeness of the plastome was 98.7 % on average, ranging from 97.3 % in Z442 *Amomum* aff. *glabrum* to 99.6 % in Z862 *Amomum odontocarpum*.

Ribosomal DNA

For 37 samples, a mean of 7,617 reads (1.45 %) per sample was mapped onto the reference used,

ranging from 1,824 reads (0.59 %) in Z308 *Amomum* aff. *trilobum* to 76,292 reads (4.74 %) in Z743 *Aframomum meleguetA.* An alignment of 7,362 positions was constructed with 1,536 (20.9 %) variable and 963 (13.1 %) parsimony informative sites (Table 2; Supplement Table S1).

ITS

Sequences of 80 accessions were used to construct an alignment of 595 positions, an average of 3.63 % of missing data, and 173 (29.1 %) variable and 136 (22.8 %) parsimony informative positions.

Table 2 . Properties of DNA alignments used in the analyses of the genus *Amomum* s.s..

Alignment	No. of accessions	Alignment length	Missing data (%)	No. of variable positions	% of variable positions	No. of parsimony informative positions	% of parsimony informative positions
Nuclear	37	578,439	1.00	103,483	17.9	48,401	8.4
cpDNA	37	135,040	7.93	14,048	10.4	5,594	4.1
rDNA	37	7,362	0.86	1,536	20.9	963	13.1
ITS	81	595	3.63	173	29.1	136	22.9

Phylogenetic analyses

For nuclear genes, all phylogenetic approaches applied (coalescence-based ASTRAL, supertree MRL and concatenation using ExaML) yielded identical results (Fig. 1). Four basic groups (see below) were recovered in analyses of all four datasets (nuclear genes, ITS, cpDNA and rDNA; Fig. 2); however, some species were only included in the ITS analysis (mostly downloaded from GenBank).

The outgroup (O) consists of Alpinioideae and Zingiberoideae accessions. Group A contains *A. petaloideum* (S.Q.Tong) T.L.Wu and the type species of *Amomum*, *A. subulatum*. Group B contains *A. chryseum* Lamxay & M.F. Newman, *A. subcapitatum* Y.M. Xia, *A.* sp. 9, *A. repoeense* Pierre ex Gagnep., *A. plicatum* Lamxay & M.F. Newman and *A. putrescens* D. Fang. Group C corresponds to the "*A. maximum clade"* sensu Droop and Newman (2014) and is further divided into two subgroups: (1) *A. maximum* Roxb., *A. dealbatum* Roxb., *A. odontocarpum* D.Fang, *A. queenslandicum* R.M.Sm., *A. pterocarpum* Thwaites, *A, purpureorubrum* S.Q. Tong & Y.M. Xia, *A.* sp. 7 and *A.* sp. 8; (2) *A. sericeum* Roxb., *A. calcicola* Lamxay & M.F. Newman, *A. longipetiolatum* Merr., *A. menglaense* S.Q. Tong and *A. glabrum* S.Q. Tong. Group D comprises solely accessions of the former genus *Elettariopsis* and is further divided into three subgroups (D1, D2 and D3). In the nuclear gene phylogeny group D1 is sister to D2 and D3 whereas, in the rDNA and ITS phylogenies, D3 is sister to a group formed by D1 and D2. The relationships among groups differ between analyses (Fig. 2) but species composition of the groups does not change. In the nuclear gene phylogeny, group A is basal to the remaining *Amomum* s.s. accessions, whereas in the ITS topology it is sister to group D (former *Elettariopsis*). In the cpDNA and rDNA topologies it makes a group with B, however, with low support in rDNA analysis. Group C is strongly supported as basal, i.e. sister to all other groups in

both ITS and rDNA phylogenies whereas, in the nuclear gene and cpDNA analyses, it is sister to group D. Group D is supported as monophyletic in all analyses; in the nuclear gene and cpDNA topology, group C is in the sister position whereas, in the ITS and rDNA analysis, it is sister to groups A and B.

Genome size evolution

GS (measured as 2C) ranged from 3.54 pg in Z81 *A. subulatum* to 15.66 pg in Z303 *A. cinnamomeum*, showing 4.5-fold variation. The smallest genomes are found in group A (*A. subulatum*), and rather small genomes also occur in group B. Group D contains accessions with the highest absolute GS. The pattern of GS distribution supports the phylogenetic division of group D into three subgroups with smaller genomes in D1 and D2 and larger genomes in D3 (Fig. 3). Two subgroups of group C correlate with differences in GS; however, only 6 measurements exist of the 16 accessions in this group.

The reconstruction of GS evolution along the tree based on both nuclear genes and ITS showed generally an increasing trend in GS with decreases in some subgroups (Fig. 4; Supplement Fig. S1, S2). *Amomum subulatum*, which is in the basal position, has the smallest GS. The largest genomes are found in group D, especially in subgroup D3 which is in the crown position in the phylogeny. In subgroup D1, the GS of the *A.* aff. *curtisii*/*A. corrugatum* lineage decreases while the GS of the *A. curtisii*/ *A. latiflorum* lineage increases. Subgroup D2 shows a decreasing trend, as do lineages of D3 without polyploid taxa (with a prominent decrease in Z439 *A. miriflorum* and Z92 *A. trilobum*). A predominantly increasing trend can only be seen in group C, where the only taxon with a prominent decrease is Z734 *A.* sp. 7. The overall largest genomes were found in two polyploid species (*A. cinnamomeum* and *A.* aff. *biphyllum*; see below), however high GS are also found in some diploids (*A. wandokthong*, *A. biphyllum*, *A.* aff. *elan*) from subgroup D3. Lambda (λ) was estimated to be 1 for both reconstructions showing phylogenetic association of GS with phylogeny; however, it was insignificant for the analysis based on nuclear genes. Kappa (κ) was significantly higher than 1 for the analysis with the ITS tree but insignificant when the tree based on nuclear genes was used. Kappa (κ) > 1 generally indicates gradual rather than punctuated evolution. Delta (δ) estimates were 4.7 and 3.9 for nuclear genes and ITS, respectively; the value for the analysis with nuclear genes was not significantly higher than 1. Values higher than 1 indicate temporally later GS evolution, i.e. species-specific adaptation.

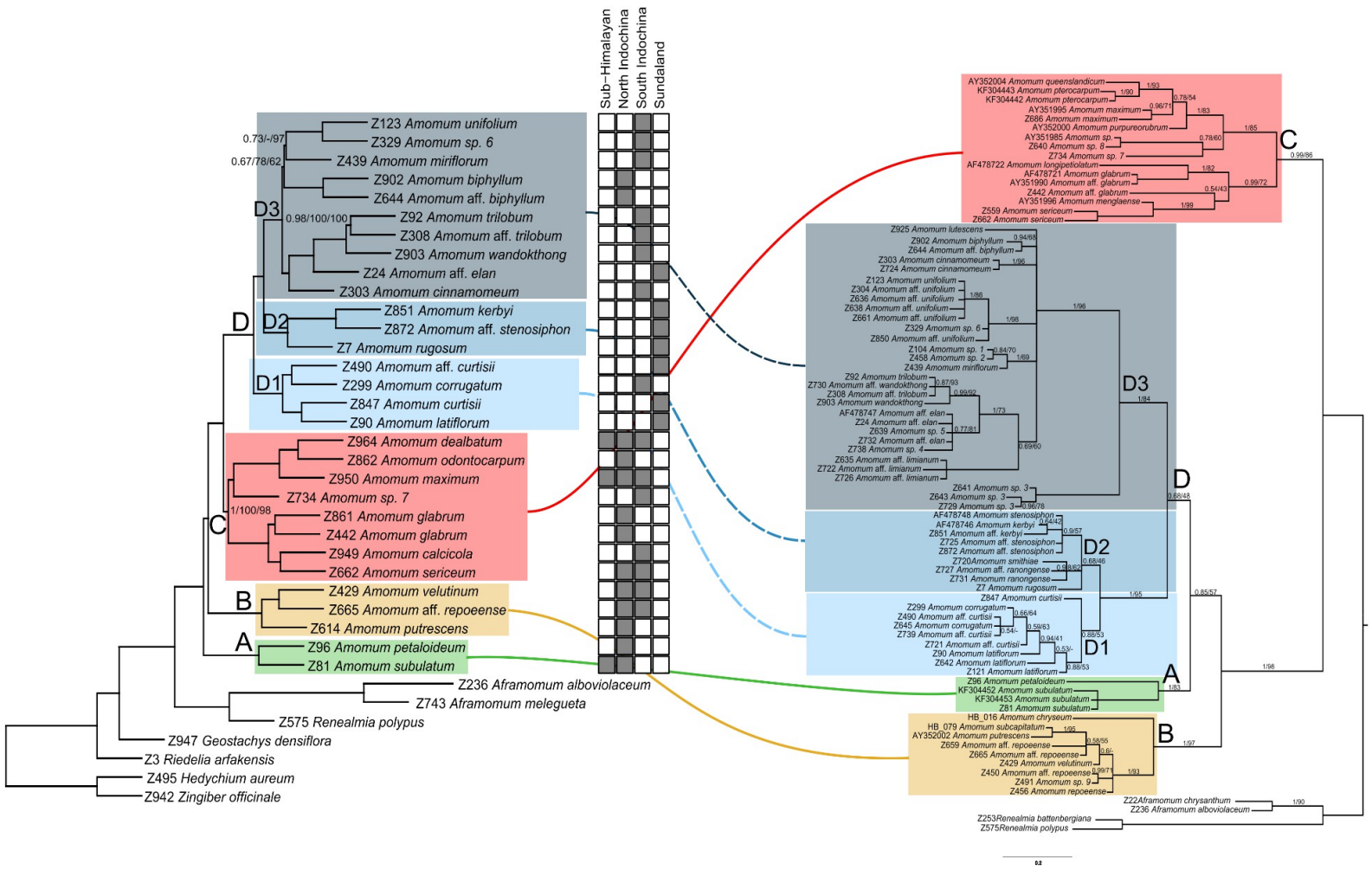

Fig. 1. Left side: An ASTRAL phylogeny of *Amomum* based on 449 genes obtained by Hyb-Seq. Bootstrap values from ASTRAL, ExaML and concatenation analyses, respectively, mapped on the branches. Only support values lower than full (1.00/100/100) are displayed. **Right side:** Bayesian inference (majority rule consensus) phylogeny based on ITS datA. Posterior probability and bootstrap values from RAxML mapped above branches. Only support values lower than 1.00/100 % displayed. Corresponding groups joined by lines.

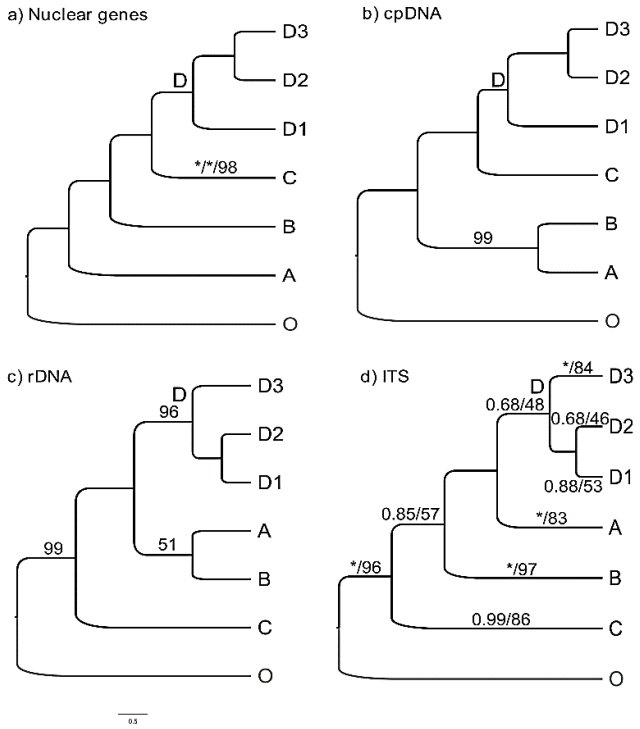

Fig. 2. A comparison of *Amomum* phylogenies based on different datasets. Support values mapped above the branches: a) bootstrap values from ASTRAL, ExaML and concatenation analysis, b) and c) bootstrap values from RAxML, d) posterior probabilities from BI and bootstrap values from RAxML analyses. Only branches with less than full support marked, branches with full support in individual analyses marked by asterisks.

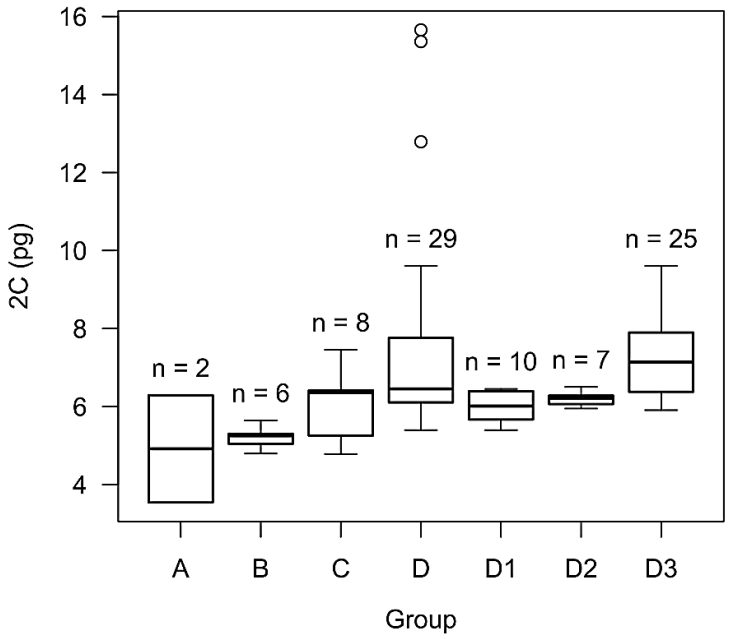

Fig. 3. Absolute genome size (2C) variation among groups of *Amomum* (A–D) showing increasing tendency during evolution.

Chromosome counts

Chromosome numbers were determined in 12 accessions (Fig. 5). All but two accessions had 2n = 48. Two tetraploids with 2n = 96 were identified (Z303 *A. cinnamomeum*, and Z644 *A.* aff. *biphyllum*), correlating with the largest GS in the dataset (2C = 15.66 pg in Z303 and 2C = 12.79 pg in Z644; Fig. 4).

DISCUSSION

This is the first comprehensive study of *Amomum* s.s. which implements state-of-the-art sequencing approaches (Hyb-Seq, target enrichment) and also the first study to compare the phylogeny with absolute GS measurements in this genus.

The infrageneric structure and relationships within Amomum

Our results recovered two main topologies differing in the placement of group C (Fig. 2). The first main topology was recovered from the plastid and nuclear gene datasets and shows group C as sister to group D. The nuclear gene dataset differs from the chloroplast dataset in the basal topology of groups A and B. Although these branches have absolute bootstrap support (common in NGS datasets; e.g. Anderson *et al.*, 2017; Degnan and Rosenberg, 2009; Gardner *et al.*, 2016), they are very short, probably reflecting rapid radiation in the past and indicating nearly concurrent diversification of these lineages. The second main topology recovered group C as the basal group in the ITS and rDNA analyses. The differences between these two trees are unsupported. Although a number of reports in the literature state that reticulate evolution and cytonuclear discordance are quite common in the evolution of plants (e.g. García *et al.*, 2017; Huang *et al.*, 2014; Vitales *et al.*, 2014), it seems that this phenomenon may not play a significant role in the early diversification of *Amomum* where we discovered nearly congruent nuclear gene and chloroplast phylogenies. Cytonuclear discordance is considered to arise from hybridisation (Renoult *et al.*, 2009), incomplete lineage sorting or chloroplast capture. Hybridisation in the Zingiberaceae is rather poorly documented, however, having been reported only in *Curcuma* (Záveská *et al.*, 2016), *Alpinia* (Liu *et al.*, 2009; Liu and Wang, 2009) and possibly *Renealmia* (Valderrama *et al.*, 2018) and *Roscoea* (Zhao *et al.*, 2017). No hybridisation has been reported in *Amomum* so additional investigation should be undertaken to determine whether it occurs in the genus.

Both the NGS-based analyses (Fig. 1; Supplement Figs. S1 and S2) and the analysis of ITS (Fig. 1) support the splitting of group D (former *Elettariopsis*) into three distinct subgroups, D1, D2 and D3. In the NGS phylogenies, group D1 contains three stenoendemic species, *A. curtisii*, *A. corrugatum* and *A. latiflorum* from Penang, southern Vietnam and Singapore respectively, group D2 includes predominantly Sundaland species, *A. kerbyi*, *A. stenosiphon* and *A. rugosum*, and group D3 encompasses mostly Indochinese species, *A. cinnamomeum*, *A. biphyllum*, *A.* aff. *elan*, *A. trilobum*, *A. unifolium*, and *A. wandokthong*. The basal

topology of these three groups within group D differs between the two main topologies recovered, where nuclear gene and cpDNA topologies group D2 and D3 together.

Our finding that nuclear gene and chloroplast results showed significantly different topologies than that of the rDNA- and ITS-based tree (but congruent with each other; Fig. 2) supports the hypothesis that the ITS region (and the whole rDNA region) may not be a suitable and reliable marker for reconstructing the evolution of deeper/basal nodes (Álvarez and Wendel, 2003). Using rDNA could result (as in our study) in a supported tree which may not accurately reflect the phylogeny of individual lineages as also previously suggested (Yang *et al.*, 2016). This may be due to the fact that rDNA is a multiple-copy marker subjected to a certain degree of concerted evolution and despite its frequent use, it is often criticised as problematic (Álvarez and Wendel, 2003), especially its ability to show the phylogenetic signal of the basal and deep internal nodes (Carbone and Kohn, 1993; Kay *et al.*, 2006). On the other hand, phylogeny based on low-copy nuclear genes provides significantly more robust signal as it is based on hundreds of loci, providing more parsimony-informative sites by two orders of magnitude as compared to the phylogeny based on rDNA. Similarly, for the phylogeny derived from whole plastome sequences, the number of parsimony-informative sites is nearly 6 × higher (Table 2). However, further investigations to elucidate what processes cause the strongly supported discrepancy between rDNA and nuclear genes are required.

Patterns in GS, its correspondence to phylogeny and evolutionary trends

Genome size in angiosperms is highly variable, with almost 2,400- fold diversity unevenly distributed among groups (Leitch *et al.*, 2013).

Complex relationships with evolution, ecology and other factors have been observed (Dos Santos *et al.*, 2019; Dušková *et al.*, 2010; Guignard *et al.*, 2016; Herben *et al.*, 2012; Kang *et al.*, 2014). The largest genomes have been reported in monocots, especially in species in derived groups. In basal groups, GS was found to be small and several increases and decreases have taken place through the evolution of many lineages, leading to great variability in the extant monocots (Leitch *et al.*, 2010). Compared to other monocot families, a small average GS is reported in *Zingiberaceae* (2C = 3.86 pg; Pellicer and Leitch, 2019), with polyploidy known in several generA. In *Zingiberaceae*, GS is known to correspond to the evolution of lineages in *Curcuma* (Záveská *et al.*, 2011) where it varies greatly. However, it is clearly connected with extensive occurrence of polyploidy there.

In *Amomum*, the evolution of GS is correlated with phylogeny ($\lambda = 1$); the non-significant value in the analysis with nuclear genes could be due to a lower number of samples. *Amomum* belongs among genera with higher absolute GS than other genera in the Alpinioideae (Leitch *et al.*, 2019). Within *Amomum*, GS seems to increase gradually from group A (mean 4.92 pg) through B (mean 5.21 pg) and C (mean 6.04 pg) to D (mean 7.51 pg) but this trend is not significant. A similar gradual increase has also been reported in, for example, *Filago* L. (Andrés-Sánchez *et al.*, 2013) and *Chenopodium* L. (Kolano *et al.*,

2015) and is probably connected to the accumulation of repetitive elements during evolution (as in e.g. Macas *et al.*, 2015; Zedek *et al.*, 2010; Zuccolo *et al.*, 2007). On the other hand, decreases in GS appearing in many subgroups of *Amomum* suggest that some lineages are undergoing genome downsizing (as demonstrated in other groups, e.g. Leitch and Bennett, 2004; Lysak *et al.*, 2008; Simonin and Roddy, 2018).

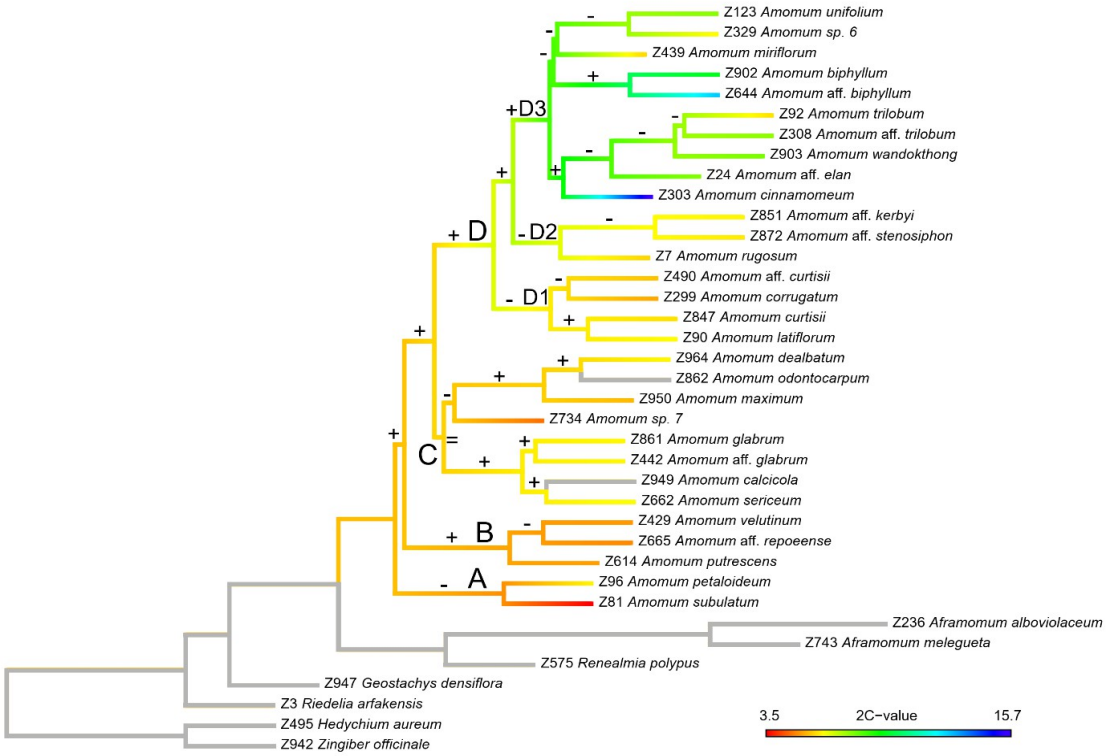

Fig. 4. Reconstruction of absolute genome size (2C) evolution in *Amomum* mapped onto the ASTRAL phylogeny (see Fig. 1). Branches with missing genome size data depicted in grey. Increases and decreases in genome size marked by '+' and '-' signs, respectively; no change marked by '='.

The "large genome constraint" hypothesis suggests that selection exists against large GS, as distribution of taxa with large genomes is usually ecologically limited, i.e. plants with larger genomes are supposedly less adaptable and less morphologically variable, and therefore need a more stable environment (Knight *et al.*, 2005). This has been confirmed by Simonin and Roddy (2018), who suggested that genome downsizing facilitated the evolutionary success of angiosperms.

The limits of our sampling did not allow us explicitly to test the correlation between biotic factors and genome size. Within the geographical range of *Amomum*, stable conditions over the whole year are found in the evergreen, moist and shady tropical rainforest as opposed to dry and light seasonal semideciduous dipterocarp forests on poor, shallow soils. In theory, species with larger genome sizes should occur in biotopes with stable conditions and *vice versA*. Our biogeographical data show that, while some samples with larger genome sizes

occurred in wet environments (e.g. Z92 *A. trilobum*, Dong Nai, Vietnam; Z439 *A. miriflorum*, Kon Tum, Vietnam; Z458 *A.* sp. 2, Thua Thien-Hue, Vietnam; Z732 *A.* aff. *elan*, Sabah, Malaysia; and Z104 *A.* sp. 1, Quang Nam, Vietnam), other accessions with larger genome sizes, including some with the largest genome sizes, were found in seasonally dry locations (both the polyploid Z303 *A. cinnamomeum* from Khanh Hoa, Vietnam and Z644 *A.* aff. *biphyllum* from Bolikhamsai, Laos, and diploid Z903 *A. wandokthong*, Prachinburi, Thailand). On the other hand, some species with smaller genomes were found in evergreen rainforest as well, such as Z847 *A. curtisii* (Penang, Malaysia) and Z450 *A.* aff. *repoeense* (Thua Thien-Hue, Vietnam). These examples show that, in *Amomum*, genome size is not clearly correlated with the amount of precipitation and light availability. In group D, however (which corresponds to the former genus *Elettariopsis*), there does seem to be some connection between geography and genome size. Groups D1 and D2, distributed predominantly south of the Isthmus of Kra, have lower GS than species from group D3 with larger GS and distribution mostly north of the Isthmus. The pattern in group D does not follow the usual distribution within *Zingiberaceae*, where species with larger genomes grow predominantly in evergreen forests, while those with smaller genomes also occur in locations with more variable conditions (Šída *et al.*, unpubl. data).

Chromosome counts in the group

High values of absolute GS in some samples may indicate polyploidy but polyploidy has only been confirmed by chromosome counting in two accessions (Z303 *A. cinnamomeum* and Z644 *A.* aff. *biphyllum*; 2n = 96). These accessions may be functional tetraploids (4x). Polyploidy is also expected in *A. biphyllum* (Z902) but its root tips unfortunately could not be obtained. This accession could be triploid as its GS is 25 % lower than that of Z644. These two accessions are the first polyploids to be found in *Amomum*. Furthermore, *A. cinnamomeum* may have the largest detected GS in the order Zingiberales (Šída *et al.*, unpubl. data).

The whole genus (like the whole subfamily) may be of paleopolyploid (paleotetraploid) origin, considering that its usual chromosome count 2n = 48 is tetraploid relative to Mahanty's determination of the basic chromosome number in the Zingiberaceae as x = 12 (Mahanty, 1970). At the same time, two cases of neopolyploidisation have been found, as mentioned above.

The polyploid accessions had the highest GS in *Amomum* but, despite their fairly broad GS range (from ca 6 to ca. 9 pg), all other accessions counted had 2n = 48 chromosomes. Such a broad GS range in species with constant chromosome number has also been found, for example, in *Aesculus* (Krahulcová *et al.*, 2017) and *Anacyclus* (Vitales *et al.*, 2020). Significant differences in GS are known to correlate to different proportions of retrotransposons in the genome (e.g. Piednoël *et al.*, 2012). A future genome scale analysis of repetitive DNA (e.g. da Costa *et al.*, 2019; Lee *et al.*, 2018; Yang *et al.*, 2019) might elucidate whether this is also the case in *Amomum*.

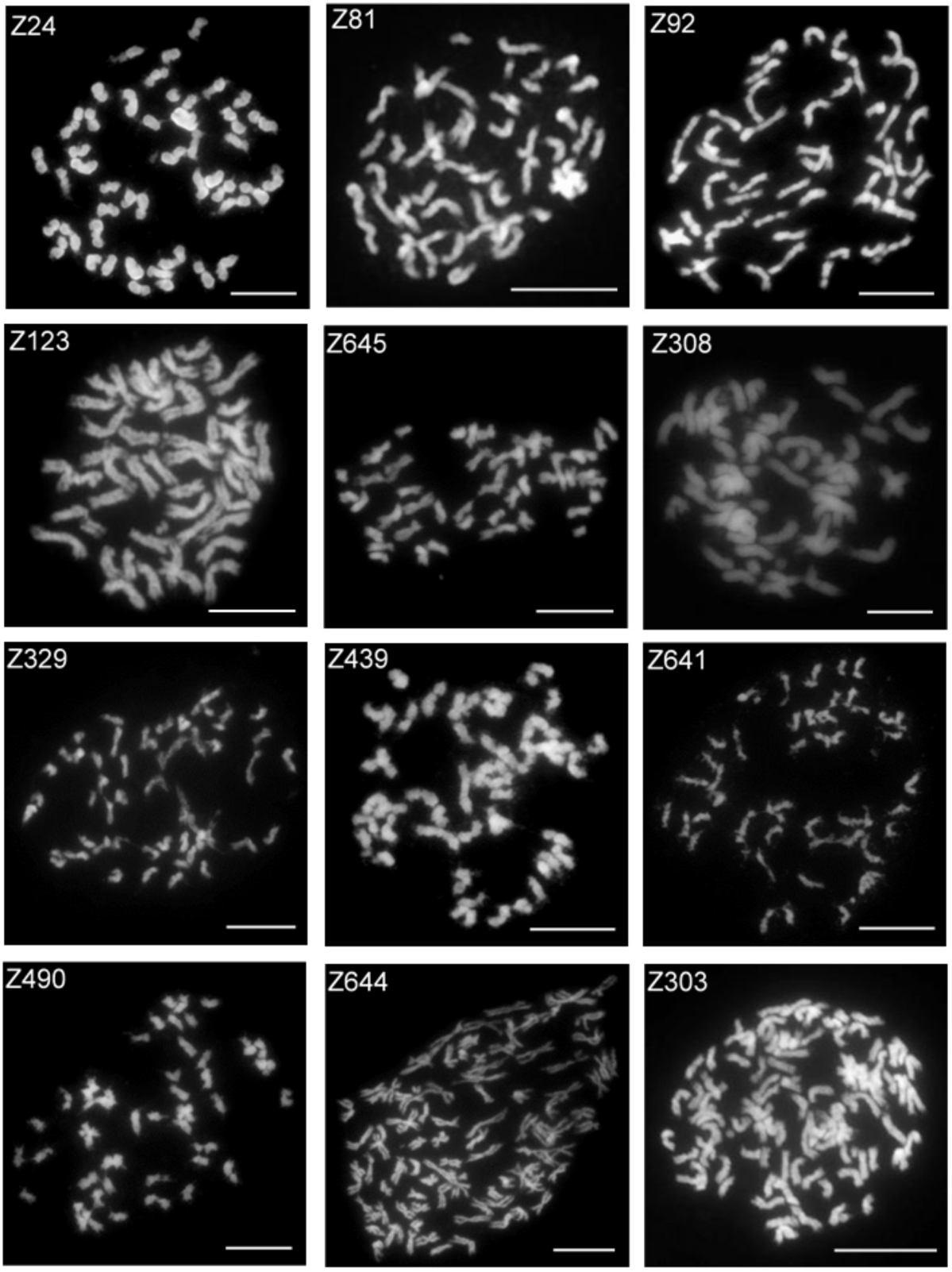

Fig. 5. Chromosomes counterstained by DAPI: Z24 – *A.* aff. *elan*, Z81 – *A. subulatum*, Z92 – *A. trilobum*, Z123 – *A. unifolium*, Z645 – *A. corrugatum*, Z308 – *A.* aff. *trilobum*, Z329 – *A.* sp. 6,

Z439 – *A. miriflorum*, Z641 – *A.* sp. 3, Z490 – *A.* aff. *curtisii* (all 2n = 48), and Z644 – *A.* aff. *biphyllum*, Z303 – *A. cinnamomeum* (both 2n = 96). Scale bars 10 µm.

Relationships between the phylogeny of Amomum s.s. and its geographical distribution

Like many other members of the Zingiberaceae, *Amomum* is only distributed west of Wallace's line (Fig. 6), with the exception of a few species in Papua New Guinea and wet tropical Australia which are most likely of anthropogenic origin (de Boer *et al.*, 2018). The geographical data (Figs. 1 and 4) show that groups A, B and C are mostly distributed in southern China and northern Indochina, with occasional expansion to the south (Indochina) or north-west (foothills of the Himalayas), while group D has nearly the range of the whole genus. Groups D1 and D2 contain predominantly species from Sundaland (with a few exceptions crossing the Isthmus of Kra back to southern Indochina); on the contrary, species in group D3 occur mostly in Indochina. It seems possible (based on the nuclear gene topology, which we consider the most reliable) that, during the evolution of group D, the ancestral lineage diversified south of the Isthmus of Kra. While subgroups D1 and D2 predominantly remained there, the ancestor of subgroup D3 migrated back to southern Indochina. There it diversified and some species migrated further to northern Indochina (*A. biphyllum*) while others returned to Sundaland (*A. elan*, *A.* sp. 4 and *A.* sp. 5).

While many species are documented crossing the Isthmus of Kra from north to south (Hughes *et al.*, 2003; Parnell, 2013; Parnell *et al.*, 2013)**,** only a few are known to disperse back northwards. Some species of *Musa* (*M. ornata, M. laterita* and *M. exotica*) returned from Malesia to Indo-Burma in the Pliocene, approximately 5–6 Mya (Janssens *et al.*, 2016).

Dispersal of *Amomum* back north may have been possible if the exposed Sundaland was indeed overgrown with rainforest in the Pleistocene (Cannon *et al.*, 2009; Morley, 1999). Little is known about seed dispersal in the Zingiberaceae (García-Robledo and Kuprewicz, 2009; Pfeiffer *et al.*, 2004), but the fleshy fruits (hesperidia) of *Amomum* with seeds enclosed in sour to sweet aril signify possible dispersal by mammals (Howe and Smallwood, 1982), which may have facilitated its migration. More recent studies (Bird *et al.*, 2005; Meijaard, 2003; Wurster *et al.*, 2019, 2010) suggest the existence of a savannah corridor which would obstruct east–west rainforest species migration and facilitated speciation in Borneo, Sumatra and JavA. However, according to their data, an area of forest still remained at the east coast of Sundaland, which rainforest species may have used to migrate north.

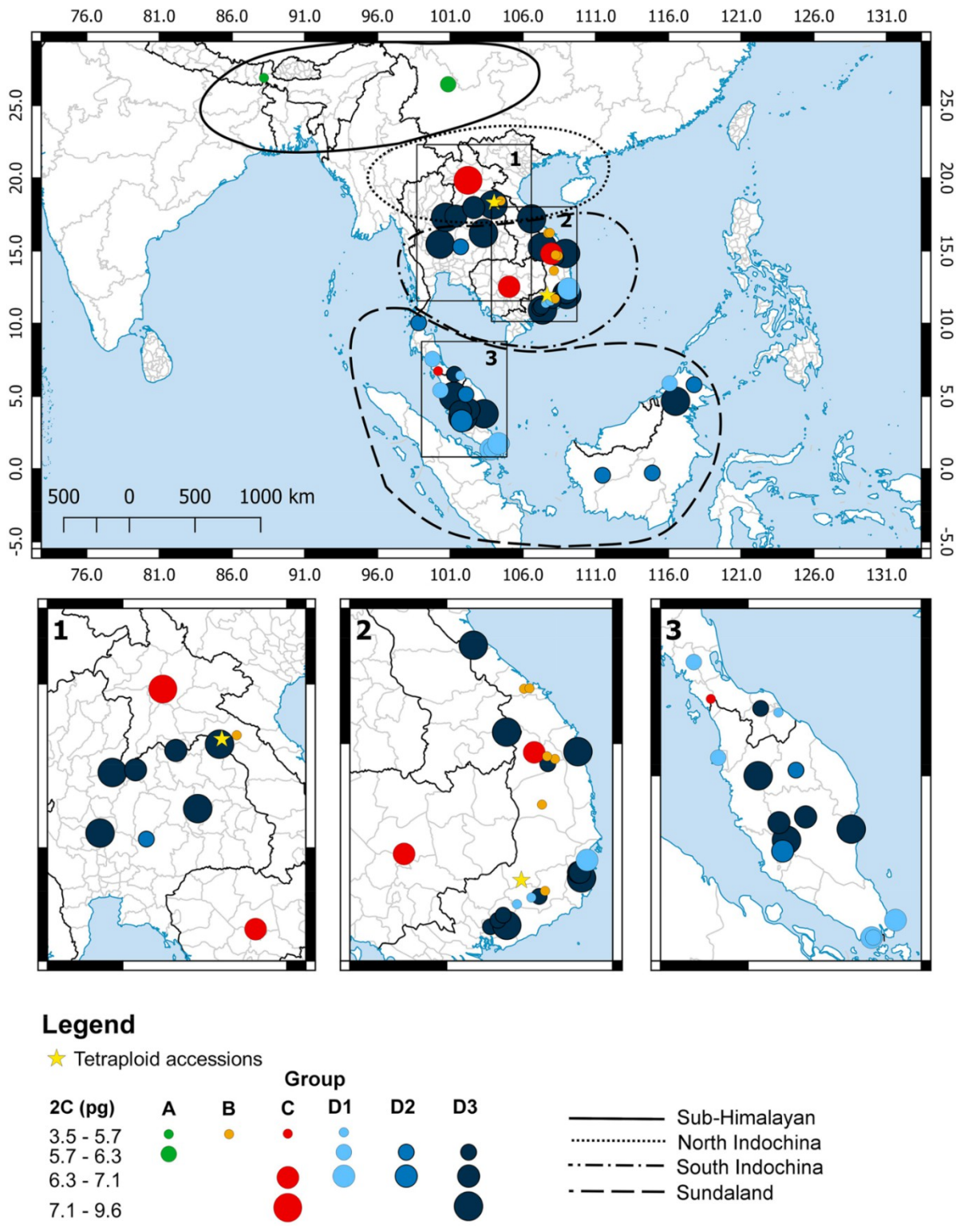

Fig. 6. The geographical distribution of *Amomum* accessions used in this study. Available 2C genome size data are expressed for respective samples by dot size (samples without GS data not mapped), phylogenetic groups by colour and biogeographical regions by lines, all according to the legend.

CONCLUSION

Analyses were performed on 92 accessions in total (45 species of *Amomum* Roxb. and 9 of the outgroup) using molecular (next generation sequencing/Hyb-Seq and ITS) and phylogenetic (coalescence, concatenation, Bayesian inference and maximum likelihood) methods. The absolute genome size (2C) was measured, and the analysis showed its evolution correlated with the phylogeny. No clear geographical pattern was found in the genome size of the whole genus. However, in group D (former *Elettariopsis*), the distribution of GS, itself correlated with phylogeny, seems to be divided at the Isthmus of Kra with larger GS being found mostly north of the Isthmus. *Amomum subulatum* Roxb., the type species of *Amomum*, is strongly supported together with *A. petaloideum* (S.Q. Tong) T.L. Wu as basal to the remaining taxa in the NGS nuclear gene and chloroplast phylogenies while, in the ribosomal DNA and ITS phylogenies, they are embedded in the group in accordance with previous results (de Boer *et al.*, 2018). This shows that analyses based on rDNA markers alone can be misleading due to their different evolutionary rates and unevenly distributed concerted evolution. Chromosomes were counted in 12 accessions and, while almost all accessions had 2n = 48 chromosomes despite their different absolute GS, two tetraploid accessions with 2n = 96 chromosomes were found. Large variation in GS should be studied in future in order to quantify the differences in composition of repetitive sequences that might be responsible for larger genomes in *Amomum*.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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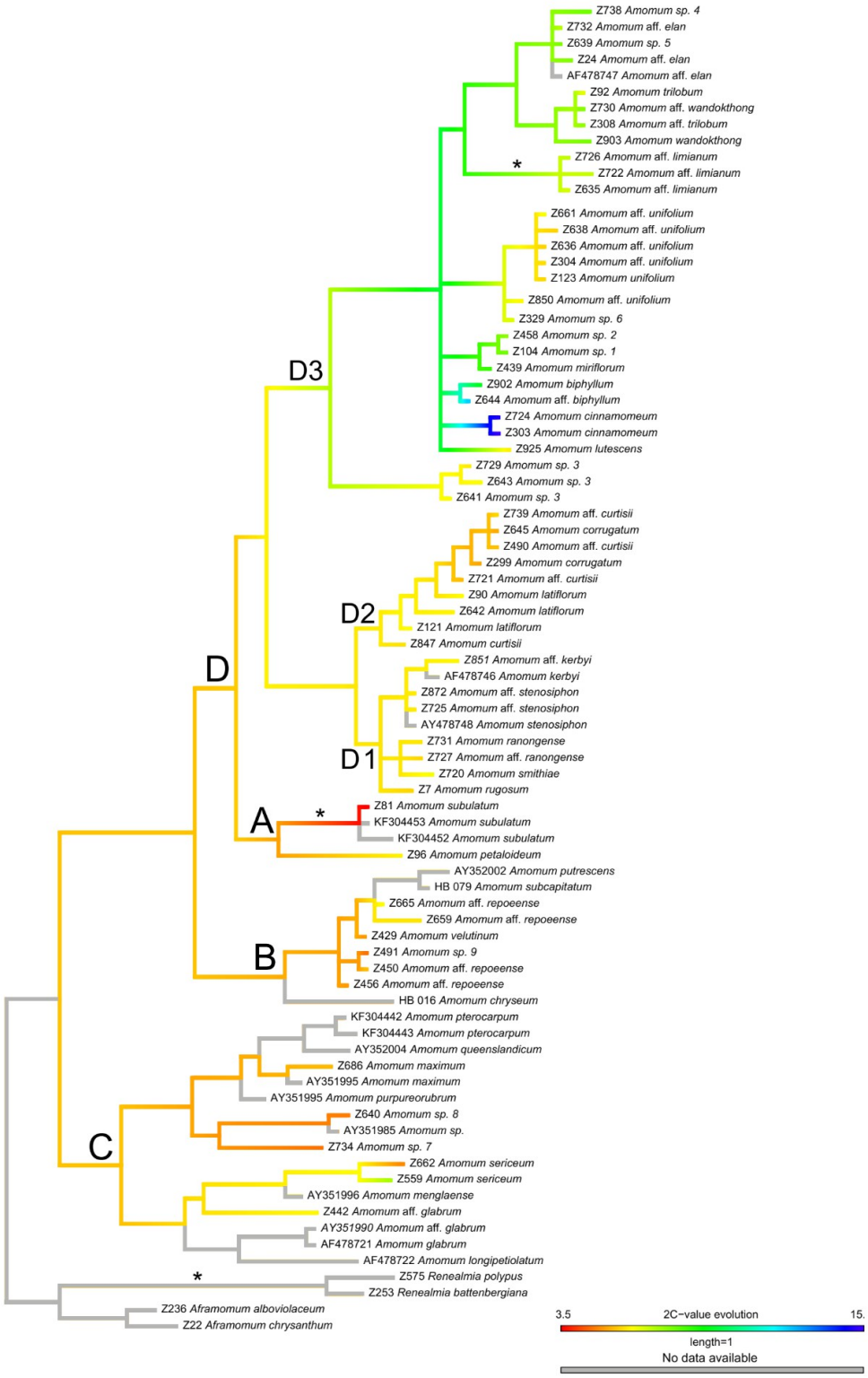
SUPPLEMENTARY MATERIAL

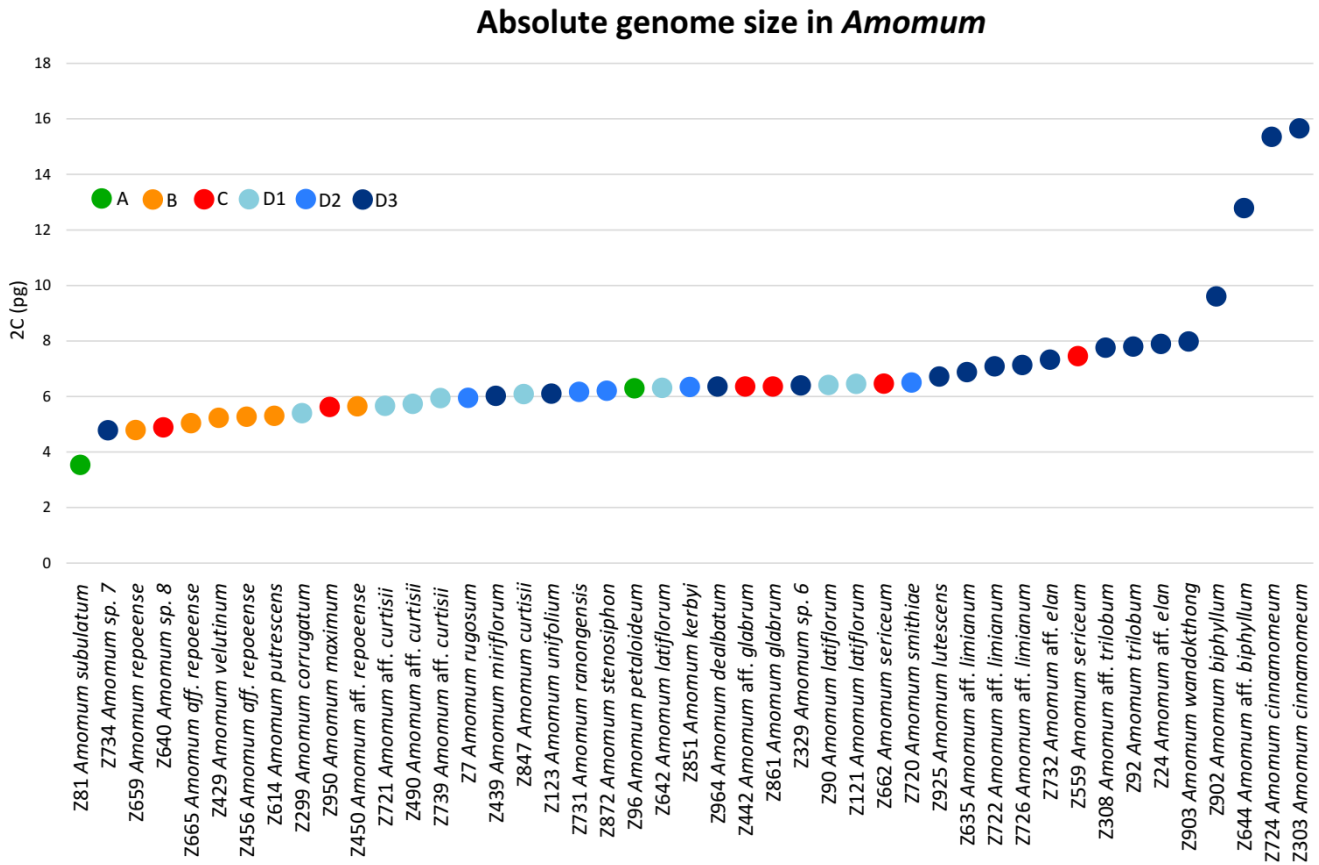
Supplementary Table S1. Sequencing read-related statistics for Amomum accessions used in Hyb-Seq (NGS) analyses (nuclear genes, cpDNA, rDNA) including accession codes in Sequence Read Archive (SRA).

					Number of reads		Nuclear genes					cpDNA			rDNA	
Sample no. (S)	Accession (Z)	Genus	Species	GB accession no.	Raw reads	Trimmed without duplicates	Number of mapped reads	% of mapped reads	No. of recovered genes	% of recovered genes	Average coverage	Number of mapped reads	% of mapped reads	completeness (%)	Number of mapped reads	% of mapped reads
S118	Z236	*Aframomum*	*alboviolaceum*	SRR12824547	2 572 132	2 230 914	1 280 664	56.83	1004	96.72	115	33 547	0.45	99.55	11 066	0.86
S70	Z743	*Aframomum*	*melegueta*	SRR12824546	3 354 268	2 964 641	1 609 510	53.78	992	95.57	152	12 032	0.45	99.55	76 292	4.74
S227	Z949	*Amomum*	*calcicola*	SRR12824535	892 870	810 453	299 133	36.69	762	73.41	28	9 902	1.39	98.61	2 576	0.86
S273	Z964	*Amomum*	*dealbatum*	SRR12824524	1 309 414	1 216 184	493 147	40.31	872	84.01	42	22 518	1.30	98.70	3 288	0.67
S166	Z442	*Amomum*	aff. *glabrum*	SRR12824517	924 288	848 052	296 154	34.71	721	69.46	28	9 768	2.75	97.25	2 634	0.89
S294	Z861	*Amomum*	*glabrum*	SRR12824516	1 735 008	1 584 468	516 430	32.51	640	61.66	45	9 179	1.21	98.79	5 514	1.07
S256	Z950	*Amomum*	*maximum*	SRR12824515	1 561 640	1 394 830	476 621	33.97	913	87.96	44	22 447	0.93	99.07	3 952	0.83
S295	Z862	*Amomum*	*odontocarpum*	SRR12824514	1 819 320	1 676 937	720 431	42.79	753	72.54	64	8 994	0.36	99.64	4 885	0.68
S139	Z96	*Amomum*	*petaloideum*	SRR12824513	1 701 316	1 585 989	470 399	29.50	838	80.73	43	22 475	0.84	99.16	6 315	1.34
S400	Z429	*Amomum*	*velutinum*	SRR12824512	976 496	910 073	249 683	27.31	789	76.01	23	15 358	2.39	97.61	2 411	0.97
S396	Z614	*Amomum*	*putrescens*	SRR12824545	1 591 590	1 486 941	392 238	26.26	907	87.38	36	22 478	1.30	98.70	8 868	2.26
S67	Z665	*Amomum*	aff. *repoeense*	SRR12824544	1 657 084	1 433 823	442 567	30.72	872	84.01	41	20 091	1.15	98.85	7 370	1.67
S68	Z662	*Amomum*	*sericeum*	SRR12824543	1 236 940	1 073 206	328 053	30.41	777	74.86	30	11 483	1.32	98.68	6 193	1.89
S308	Z734	*Amomum*	*sp. 7*	SRR12824542	822 424	747 692	217 708	28.98	662	63.78	20	17 733	1.06	98.94	6 794	3.12
S51	Z81	*Amomum*	*subulatum*	SRR12824541	839 618	715 005	248 564	34.58	588	56.65	23	10 882	2.51	97.49	5 954	2.40
S311	Z902	*Amomum*	*biphyllum*	SRR12824540	1 049 286	945 756	310 007	32.60	770	74.18	28	15 408	0.98	99.02	4 021	1.30
S296	Z847	*Amomum*	*curtisii*	SRR12824539	2 240 992	2 084 700	1 017 754	48.49	910	87.67	94	26 315	1.11	98.89	9 737	0.96
S399	Z490	*Amomum*	aff. *curtisii*	SRR12824538	1 282 094	1 184 956	341 580	28.69	841	81.02	29	19 334	1.86	98.14	10 669	3.12
S293	Z851	*Amomum*	*kerbyi*	SRR12824537	2 102 904	1 925 323	785 442	40.56	825	79.48	73	13 475	0.70	99.30	8 809	1.12
S79	Z90	*Amomum*	*latiflorum*	SRR12824536	1 347 292	1 247 621	401 280	31.98	867	83.53	35	11 337	1.44	98.56	10 490	2.61
S172	Z439	*Amomum*	*miriflorum*	SRR12824534	1 593 784	1 510 875	443 715	29.22	799	76.97	41	39 981	1.72	98.28	6 713	1.51
S80	Z7	*Amomum*	*rugosum*	SRR12824533	1 166 200	1 078 404	306 918	28.31	768	73.99	27	27 825	0.64	99.36	9 624	3.14
S13	Z303	*Amomum*	*cinnamomeum*	SRR12824532	1 107 036	797 893	419 002	52.01	814	78.42	40	2 815	0.53	99.47	2 100	0.50
S310	Z299	*Amomum*	*corrugatum*	SRR12824531	892 216	803 257	306 621	37.94	741	71.39	26	9 807	0.49	99.51	4 885	1.59

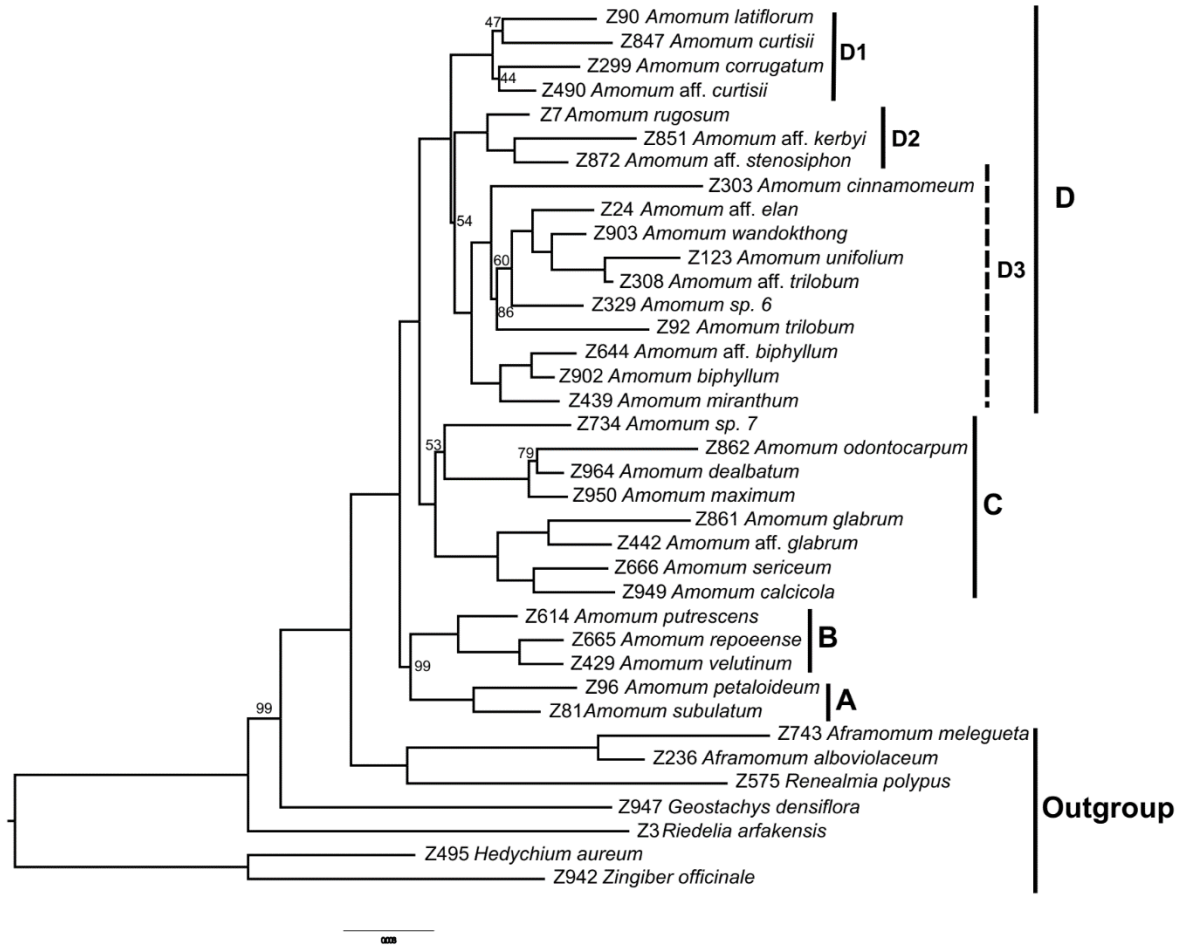
S312	Z644	*Amomum*	aff. *biphyllum*	SRR12824530	999 220	908 062	307 973	33.71	753	72.54	29	17 302	0.41	99.59	4 203	1.36
S366	Z308	*Amomum*	aff. *trilobum*	SRR12824529	900 276	819 660	308 495	37.34	496	47.78	28	17 733	2.16	97.84	1 824	0.59
S368	Z24	*Amomum*	aff. *elan*	SRR12824528	1 198 004	1 082 430	325 372	29.89	552	53.18	30	25 620	2.63	97.37	2 292	0.70
S369	Z329	*Amomum*	*sp. 6*	SRR12824527	1 094 038	956 462	352 287	36.66	608	58.57	33	22 765	1.10	98.90	2 274	0.65
S173	Z872	*Amomum*	aff. *stenosiphon*	SRR12824526	1 317 616	1 248 120	419 354	33.40	844	81.31	38	12 944	1.47	98.53	6 706	1.60
S12	Z123	*Amomum*	*unifolium*	SRR12824523	464 352	380 301	205 358	53.48	471	45.38	19	2 097	1.73	98.27	1 980	0.96
S11	Z92	*Amomum*	*trilobum*	SRR12824525	795 344	588 159	294 606	49.44	646	62.24	27	3 601	0.62	99.38	2 610	0.89
S309	Z903	*Amomum*	*wandokthong*	SRR12824522	1 126 696	1 020 878	319 726	31.08	695	66.96	29	29 606	1.97	98.03	3 639	1.14
S254	Z947	*Geostachys*	*densiflora*	SRR12824521	837 576	749 641	258 682	34.33	755	72.74	23	5 858	2.14	97.86	2 930	1.13
S129	Z495	*Hedychium*	*aureum*	SRR12824520	2 381 112	2 189 217	1 183 809	53.57	1038	100.00	103	27 355	2.08	97.92	7 816	0.66
S66	Z575	*Renealmia*	*polypus*	SRR7058219	1 836 472	1 598 492	699 928	43.45	929	89.50	67	50 504	1.41	98.59	10 100	1.44
S49	Z3	*Riedelia*	*arfakensis*	SRR12824519	1 652 048	1 441 921	621 889	42.83	922	88.82	57	22 960	1.51	98.49	6 678	1.07
S242	Z942	*Zingiber*	*officinale*	SRR12824518	1 464 022	1 308 893	557 733	42.33	982	94.61	48	27 295	1.57	98.43	4 437	0.80
				TOTAL	50 378 966	45 241 336	-	-	-	-	-	-	-	-	-	-
				AVERAGE	1 399 416	1 256 704	490 864	37.45	782	75.29	45	18 154	1.34	98.66	7 617	1.45
				MAX	3 354 268	2 964 641	1 609 510	57	1 038	100.00	152	50 504	2.75	99.64	76 292	4.74
				MIN	464 352	380 301	205 358	26.26	471	45.38	19	2 097	0.36	97.25	1 824	0.50



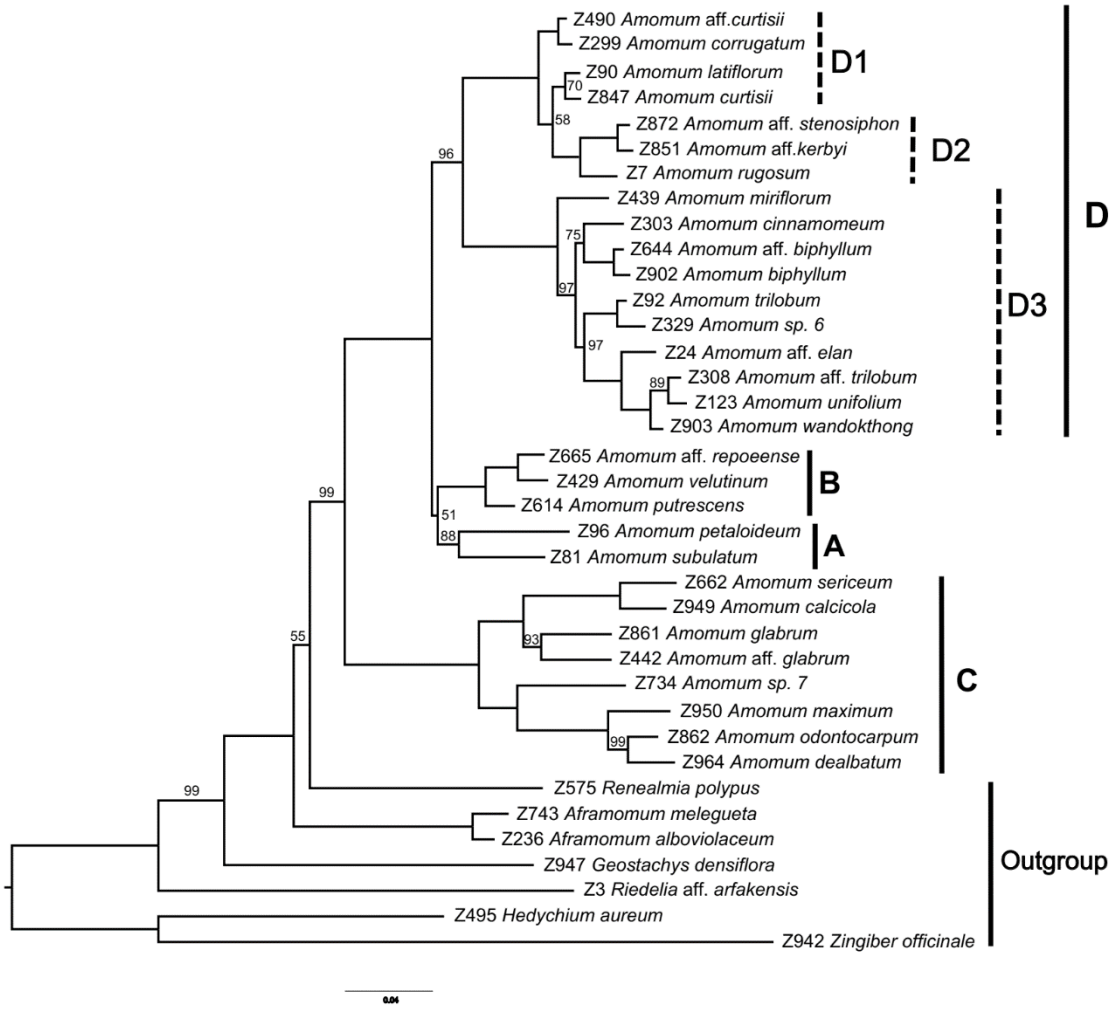
Supplementary Fig. S1. Absolute genome size mapped onto the BI phylogeny based on ITS data, made in phytools. Fully supported branches are marked by asterisks. Branches without genome size data are in grey.



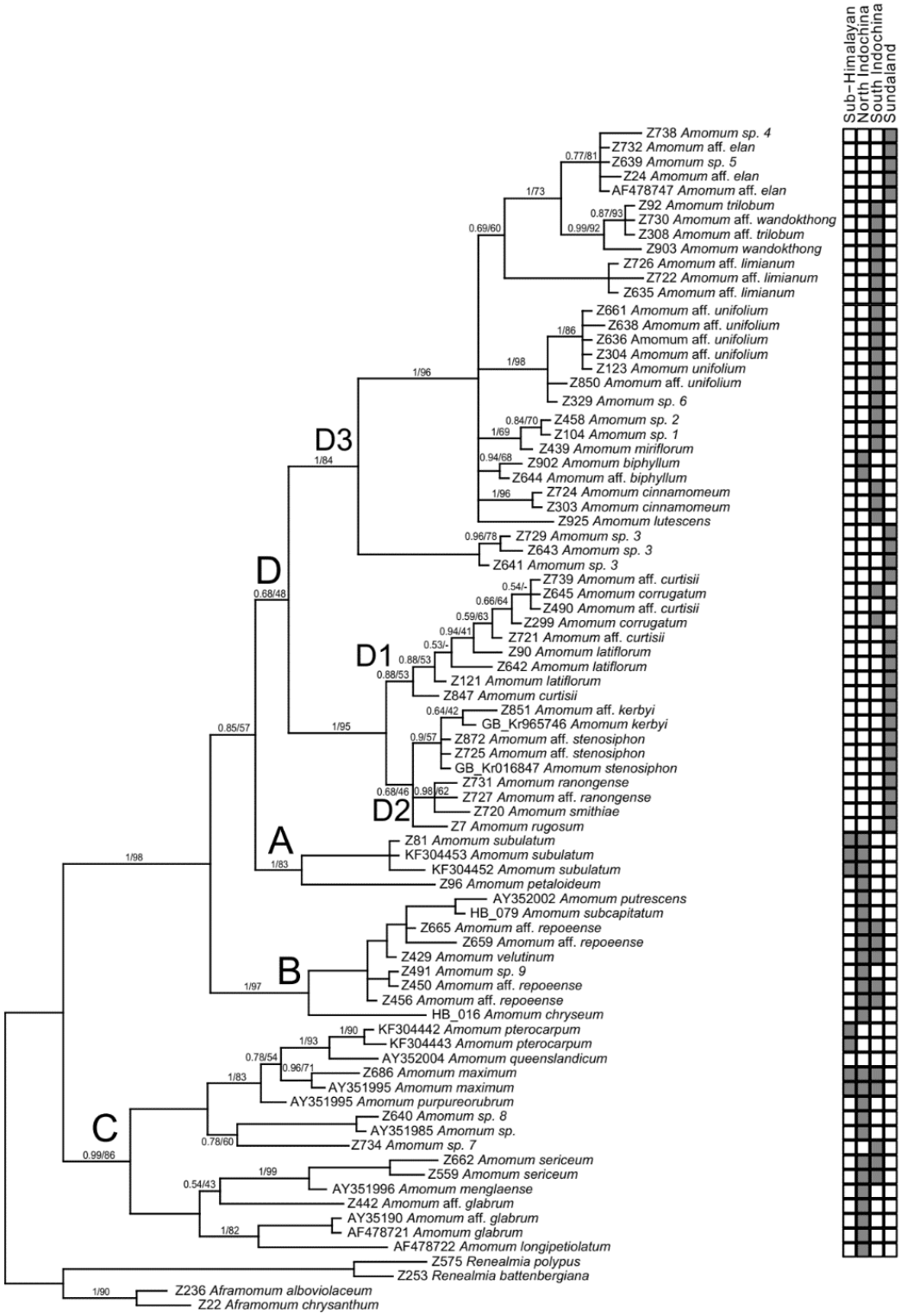
Supplementary Fig. S2. Absolute genome size in groups of Amomum.



Supplementary Fig. S3. A maximum likelihood (RAxML) phylogeny based on chloroplast DNA data. Bootstrap values (500 replicates) are mapped on the branches. Only supports lower than 100 % are displayed.



Supplementary Fig. S4. A maximum likelihood (RAxML) phylogeny based on ribosomal DNA data. Bootstrap values (500 replicates) are mapped on the branches. Only supports lower than 100% are displayed.



Supplementary Fig. S5. Geographical distribution of Amomum samples mapped onto the BI phylogeny based on ITS data, made in phytools. Posterior probability and bootstrap values from RAxML are mapped above branches. Only support values lower than 1.00/100% are displayed.

Paper IV

Ancient Hybridization and Repetitive Element Proliferation in the evolutionary history of the Monocot Plant Genus *Amomum* (Zingiberaceae)

Kristýna Hlavatá, Eliška Záveská, Jana Leong-Škorničková[3], Milan Pouch, Axel Dalberg Poulsen, Otakar Šída, Bijay Khadka, Terezie Mandáková and Tomáš Fér

Frontiers in Plant Science

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Kristýna Hlavatá[1†], Eliška Záveská[1,2†], Jana Leong-Škorničková[3,4], Milan Pouch[5,6], Axel Dalberg Poulsen[7], Otakar Šída[8], Bijay Khadka[1], Terezie Mandáková[5,8*] and Tomáš Fér

[1]Department of Botany, Faculty of Science, Charles University, Prague, Czech Republic
[2]Institute of Botany, Czech Academy of Science, Průhonice, Czech Republic
[3]Herbarium, Singapore Botanic Gardens, National Parks Board, Singapore
[4]Department of Biological Sciences, National University of Singapore, Singapore
[5]Central European Institute of Technology, Masaryk University, Brno, Czech Republic [6]National Centre for Biomolecular Research (NCBR), Masaryk University, Kamenice 5, Brno 625 00, Czech Republic
[7]Tropical Diversity Section, Royal Botanic Garden Edinburgh, Edinburgh, United Kingdom
[8]Department of Botany, National Museum in Prague, Prague, Czech Republic
[8]Department of Experimental Biology, Faculty of Science, Masaryk University, Brno, Czech Republic

[†]**Equal contribution and first authorship:** These authors contributed equally to this work and share first authorship

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Abstract

Genome size variation is a crucial facet of plant evolution, influenced by a complex interplay of factors. Repetitive elements, integral components of genomic architecture, often contribute to genome expansion through the selective amplification of specific repeat motifs. This study focuses on the genus *Amomum*, a member of the ginger family (Zingiberaceae), known for its remarkable 4.4-fold variation in genome sizes. Using a robust methodology involving PhyloNet reconstruction, RepeatExplorer clustering, and repeat similarity-based phylogenetic network construction, we systematically examine the repeatome composition, dissect repeat dynamics, and unveil potential hybridization events within the genus. Our analysis confirms the presence of four major infrageneric clades (A–D) within *Amomum*, with clades A–C exclusively comprising diploid (2n = 48) species and clade D encompassing both diploid and tetraploid species (2n = 48 and 96). Within the genus, levels of repeat content, ranging from 84% to 89%, increased compared to the outgroup species with 75% of the repeatome. The SIRE lineage of the *Ty1-Copia* repeat superfamily is prevalent in all analyzed ingroup genomes. We observe significant difference in repeatome structure between basal *Amomum* clades (A, B, C) and the most diverged clade D, where 20% and 13% of all repeat clusters significantly decreased and increased, respectively, along the phylogeny. Our investigation uncovers evidence of ancient interspecific hybridization events within clade D, coinciding with a significant proliferation of multiple repeat groups. This discovery supports the hypothesis that ancient hybridization is a driving force behind the genomic evolution mediated by repeats in *Amomum*. Furthermore, we contextualize our findings within the broader context of genome size variations and repeatome dynamics observed across major monocotyledonous plant lineages. *Amomum* stands out among these species due to its

exceptionally high repeatome proportions. This study enhances our understanding of evolutionary processes within monocots by highlighting the pivotal roles of repetitive elements in shaping genome size and suggesting the mechanisms that drive these changes.

Keywords: genome evolution, genome size, interspecific hybridization, repetitive DNA, repeatome, phylogeny, 5S rDNA, Zingiberaceae

INTRODUCTION

Genome size, also known as C-value or haploid nuclear DNA content (hereafter referred to as GS), is a fundamental parameter in the study of organismal evolution. In land plants, GS exhibits remarkable variation, spanning up to 2,400-fold (Pellicer *et al.*, 2018). This wide range in GS has profound implications for the evolution of various biological traits (Bhadra *et al.*, 2023). Both genome expansion and contraction have been recognized as major driving forces of diversification in land plants (Cheng *et al.*, 2014; Meudt *et al.*, 2015; Simonin and Roddy, 2018). Genome expansion, often linked to whole genome duplication events, has been a historical precursor to speciation and the emergence of novel morphological features in various plant lineages (Qiao *et al.*, 2022). Another mechanism that is profoundly shaping GS is amplification of repetitive sequences, in which transposable elements play a pivotal role (Pulido and Casacuberta, 2023).

Repetitive elements, often referred to as "tuning knobs of evolution" (King *et al.*, 1997; hereafter referred to as repeats), are integral components of plant genomes. They can constitute as little as 3% in *Utricularia gibba* or as much as 91% of the entire genome in *Allium sativum* (Sun *et al.*, 2020). They play the key roles in gene expression regulation (Garrido-Ramos, 2012; Bennetzen and Wang, 2014) and can evolve into new genes due to their rapid evolutionary rates (Mehrotra and Goyal, 2014). From the evolutionary perspective, the proliferation of repeats has been associated with diversification of new phylogenetic groups (Gaiero *et al.*, 2019; Hloušková *et al.*, 2019) and facilitates adaptation to changing environments (Jansz, 2019; Kumar and Mohapatra, 2021). For example, the proliferation of the *Ty1-Copia* superfamily has been linked to an increase in GS and correlated with the evolution of dioecy in the genus *Asparagus* (Harkess *et al.*, 2016). Similarly, in the family Brassicaceae, the amplification of LTR retrotransposons appears to be related to life cycle adaptations, with genome downsizing occurring as plants adapt to an ephemeral or annual life cycle (Hloušková *et al.*, 2019). In palms (Schley *et al.*, 2022), the SIRE repeat lineage was shown to be activated as a response to stress. While many studies have explored the evolutionary significance of repeat proliferation, there is still a scarcity of research specifically investigating the biotic or abiotic factors responsible for stimulating or constraining repeat amplifications or reductions. Given the linear relationship between repeat content and GS within specific ploidy level (Lee and Kim, 2014), it is plausible to hypothesize that factors influencing repeat amplification align with those governing changes in GS.

Interspecific hybridization, a widespread phenomenon throughout the angiosperms (Mallet, 2005), plays a pivotal role in GS changes through repeatome dynamics and allopolyploidization. Genomic shock following the subgenome merger can further result in genome reorganization, including repeat activation and proliferation (O'Neill *et al.*, 1998; Ungerer *et al.*, 2006; Wei *et al.*, 2021). However, hybridization events may also lead to repeat deactivation and genome downsizing (Renny-Byfield *et al.*, 2013; Heyduk *et al.*, 2021), through processes such as illegitimate recombination, unequal homologous recombination, and epigenetic regulation via DNA methylation (Devos *et al.*, 2002; Bennetzen *et al.*, 2005; Grover and Wendel, 2010; Staton *et al.*, 2012; Pachamuthu and Borges, 2023). Recent advancements in phylogenetics and phylogenomics now enable the robust identification of hybrid species and lineages through the

analysis of extensive genomic datasets. Large-scale genomic data, including target enrichment techniques (Cao *et al.*, 2019), have become instrumental in this regard.

The tropical genus *Amomum*, as circumscribed with black cardamom (*A. subulatum*) as its taxonomic type species (de Boer *et al.*, 2018), represents a distinctive and pivotal case for investigation into the processes linked with GS amplification. *Amomum* exhibits the most significant GS variation within the entire family Zingiberaceae, ranging from 1,731 to 7,656 Mb, representing a 4.4-fold difference (Záveská *et al.*, 2023). In the genus, only two tetraploid species are known (2n = 96), with GS values of 6,254 and 7,656 Mb (Hlavatá *et al.*, 2023). However, even the diploid species (2n = 48) display substantial GS variation, ranging from 1,731 to 4,699 Mb, representing a 2.7-fold difference. A well-supported Hyb-Seq-based phylogeny based on 449 nuclear genes revealed four main clades (A, B, C, and D Hlavatá *et al.*, 2023). Nevertheless, cyto-nuclear discordance, which might indicate interspecific hybridization, was indicated (Hlavatá *et al.*, 2023) and remains to be further explored. Indeed, hybridization and polyploidization processes are common within the Zingiberaceae (Leong-Škorničková *et al.*, 2007; Lim, 2008; Záveská *et al.*, 2016; Sangvirotjanapat *et al.*, 2022). In this context, we hypothesize that the enlargement of GS in diploid *Amomum* species is a result of an expansion of repeats triggered by interspecific hybridization. Particularly, we aim to answer the following questions: i) what is the repeatome composition in the genus *Amomum*?, ii) does interspecific hybridization play a role in the evolution of *Amomum* and its repeatome?; and iii) is the evolution of repeats correlated with phylogenetic relationships in *Amomum*? To answer these questions, we use a wide range of analyses starting with a revision of GS variation within the genus based on 52 *Amomum* accessions (33 species), continuing with a phylogenetic network reconstruction of 30 *Amomum* species, complemented with a qualitative and quantitative analysis of repeats in a subset of 11 *Amomum* species. The genus *Amomum* serves as an exemplary model system for scrutinizing the genomic mechanisms underpinning alterations in GS within tropical genera. Limited evidence has been available to date regarding hybridization, polyploidization, and repeatome compositions in these genera. Moreover, by situating our findings within a broader context encompassing major monocotyledonous (hereafter referred to as monocot) families, this study provides a valuable overview and comparison of GS and repeatome dynamics across this entire evolutionary lineage of plants.

METHODS

Plant material

A total of 52 accessions, corresponding to 30 distinct *Amomum* species and encompassing the documented morphological, phylogenetic, and cytological spectrum of the genus (Hlavatá *et al.*, 2023), were employed in the present study to analyze genome size (GS) data. Reticulate relationships were reconstructed for these 30 species, while a subset of 11 accessions (plus two outgroup species included for comparative purposes) was further designated for an in-depth examination of repeat content. The selection of these subsets was meticulously devised to ensure that they represented the following aspects i) the primary phylogenetic clades within the genus *Amomum*, ii) variability in GS within and among these clades and iii) variation in ploidy levels observed across the genus. A comprehensive listing of all samples and their characteristics is provided in Supplementary Table 1.

Genome size estimation and chromosome counts

Nuclear GS (referred to as nuclear DNA 1C values in Mb) data were sourced from our previous study (Hlavatá *et al.*, 2023) for a total of 52 *Amomum* accessions plus 2 outgroup species. Chromosome numbers, and ploidy levels were also sourced from Hlavatá *et al.* (2023) for 12 *Amomum* accessions representing 12 species (Supplementary Table 1).

Sequencing data from target enrichment (HybSeq) for species networks reconstruction

Raw data derived from Hyb-Seq encompassing 30 *Amomum* accessions were obtained from Hlavatá *et al.* (2023) and were processed similarly as in the previous study using HybPhyloMaker 1.6.4. (Fér and Schmickl, 2018) up to the reconstruction of gene trees based on a total of 448 loci employing RAxML 8.2.4 (Stamatakis, 2014) with 1000 standard bootstrap replicates and per exon partitioning. In cases where gene trees contained uncertain nodes with bootstrap support below 50, branches were collapsed. These gene trees were then employed in the reconstruction of species networks using a maximum pseudo-likelihood (MPL) framework function 'InferNetwork_MPL' (Yu and Nakhleh, 2015) and implemented in PhyloNet 3.6.1 (Than *et al.*, 2008). Since the comprehensive exploration of a dataset comprising 30 accessions with a larger number of reticulations (> 2) utilizing a MPL approach would be limited by prohibitive runtime costs (Than *et al.*, 2008; Skopalíková *et al.*, 2023) we adopted a sequential, stepwise approach for the analysis of our dataset. Initially, we constructed a species network for the entire dataset of 30 accessions, hereafter referred to as '*complete dataset*', allowing for a maximum of two reticulations. Subsequently, we conducted a separate analysis on a subset comprising 17 accessions, which represented 16 species belonging to clade D, hereafter termed the *'clade D dataset'*, again allowing for a maximum of two reticulations. Prior to the Phylonet analyses, the gene trees were rooted using Newick Utilities 1.6 (Junier and Zdobnov, 2010). For the *complete dataset*, *A. subulatum* and *A. petaloideum* served as rooting taxa, while *A.* aff. *curtisii*, *A. latiflorum* and *A. corrugatum* were employed for rooting the *clade D dataset.* Each analysis involved ten runs with default settings, resulting in the generation of five optimal networks per analysis. The selection of the best-fitting network was accomplished by applying the Akaike information criterion (AIC, AIC = 2*k - 2*L). Here, 'k' represented the number of parameters, which included the number of branches and the number of reticulations, while 'L' denoted the likelihood value (Keuler *et al.*, 2020). To present the findings effectively, we combined the best-fitting models from both datasets into a unified phylogenetic network.

Low-coverage sequencing

Genome skimming was conducted on a subset of 13 accessions, consisting of 11 *Amomum* species and two closely related outgroup species (*Aframomum melegueta* and *Renealmia polypus*, Supplementary Table 1). DNA was sonicated to yield fragments 500-600 bp long, using a M220 Focused-ultrasonicator™ (Covaris). Verification of fragment length was accomplished through gel electrophoresis, with O'GeneRuler™ 100bp DNA Ladder Plus (Thermo Fisher Scientific) and Quick-Load® 1 kb DNA Ladder (New England BioLabs) employed as reference standards. Subsequently, libraries were prepared utilizing the NEBNext Ultra II DNA Library Prep Kit for Illumina (New England BioLabs). After library preparation, purification was carried out using QIAquick PCR Purification Kit (Qiagen), with the resulting DNA dissolved in 30 µl ddH2O. The quality and integrity of the DNA were assessed through gel electrophoresis, with O'GeneRuler™ 100bp DNA Ladder Plus (Thermo Fisher Scientific) and Quick-Load® 1 kb DNA Ladder (New England BioLabs) as reference markers. DNA fragments falling within the 500-600 bp range were excised from the gel, subjected to purification using QIAquick Gel Extraction Kit (Qiagen), and eluted into 20 µl ddH2O. Subsequent to gel extraction, the DNA products underwent PCR amplification and indexing. This was achieved using Q5 Hot Start HiFi PCR Master Mix (New England BioLabs) and NEBNext Multiplex Oligos for Illumina index primers (96 Unique Dual Index Primer Pairs, E6440S). Following amplification and indexing, the samples were purified twice using an Agencourt SPRI kit (Beckman Coulter), maintaining a kit-to-DNA ratio of 0.75:1, and were subsequently verified by gel electrophoresis. To determine the concentration of the samples, a Qubit® 2.0 fluorometer (Invitrogen) was employed, ensuring equimolar proportions. The final library was then subjected to sequencing on an

Illumina NextSeq, utilizing a 300-cycle sequencing kit to generate 150 bp paired-end reads. The sequencing process was conducted at the Central European Institute of Technology (CEITEC), Masaryk University in Brno, Czech Republic. Raw reads resulting from this process were subsequently uploaded to the Sequence Read Archive (SRA) under the BioProject designation (ID PRJNA1029323).

Repeatome analysis

Read clustering and subsequent automated quantification of repetitive elements (repeats) were performed on the Galaxy platform (Afgan *et al.*, 2018; https://repeatexplorer-elixir.cerit-sc.cz/) following the established protocol as described by Novák *et al.* (2020). Following an initial quality check using FastQC (Andrews, 2010), the reads were trimmed to 150 bp. Subsequently, the paired-end reads from each species were subjective to separate analysis within the similarity-based clustering RepeatExplorer pipeline. Default settings were maintained, with read sampling disabled, and the processing queue was configurated to "extra long" to accommodate the maximum possible number of reads. Repeats were classified into distinct groups, such as Long Terminal Repeats (LTRs) and DNA transposons, superfamilies like *Ty1-Copia* and *Ty3-Gypsy*, and lineages including SIRE and Tekay. This classification was carried out in accordance with the automatic procedure of RepeatExplorer (REXdb; Neumann *et al.*, 2019) and was subject to manual verification. For repeat identification, BLAST (Altschul *et al.*, 1990) was employed to search against a comprehensive repeat library compiled from various publicly accessible sources, including msRep (Liao *et al.*, 2022; https://msrepdb.cbrc.kaust.edu.sa/), PlantRep (Luo *et al.*, 2022; http://www.plantrep.cn/), RepeatMasker (Smit *et al.*, 2013; https://www.repeatmasker.org/), and Musaceae-specific repeat database (Novák *et al.*, 2014; https://olomouc.ueb.cas.cz/en/content/dna-repeats/). The manually reviewed file of each accession was subsequently used for the quantification of repeats, taking into account the known GS of the respective accession. Barplots representing the main repeat groups were constructed using Microsoft Excel (Microsoft Excel 365, 2018).

An additional analysis of tandem repeats was conducted using Tandem Repeat Analyzer (TAREAN; Novák *et al.*, 2017) for all accessions. This analysis aimed to identify potential satellite sequences that may not have been detected by the RepeatExplorer analysis and to provide insights into the presence and organization of 5S rDNA clusters. In TAREAN, the cluster size threshold was established at 0.01, and the processing queue was configured for "extra long" run times to accommodate the analysis of the maximum feasible number of reads. In the context of 5S rDNA, diploid specimens typically exhibit as single-looped circular graphs, whereas accessions of hybrid and/or polyploid origin may present more complex multi-looped graphs, as detailed by Garcia *et al.* (2020).Therefore, the examination of 5S rDNA can serve as an indicator of hybridization (Garcia *et al.*, 2020).

Comparative analysis of repeats

A comparative analysis, involving the simultaneous clustering of reads from all accessions, was performed in RepeatExplorer. This analysis adhered to the established protocol of Novák *et al.* (2020) and employed default settings. A random subsample of 1,000,000 reads was selected from each accession for this analysis. From this analysis, the distribution of the 225 most prevalent comparative repeat clusters, hereafter referred to as "sub-lineages", was graphically represented, excluding clusters originating from plastid-derived sequences. To integrate these repeat sub-lineage abundances onto the phylogeny, the "*contMap*" function from the "*phytools*" package (Revell, 2012) was employed. Additionally, a measure of phylogenetic signal, namely Pagel's λ (Pagel, 1997, 1999), and its statistical significance were calculated using the *"phytools*" package within the R environment (R 4.2.1, R Core Team, 2022).

Phylogenetic signal and correlation of repeat proportions

In the representation of phylogenetic relationships among the 13 accessions studied for repeats, the ASTRAL species tree, as constructed based on HybSeq data and presented in Hlavatá *et al.* (2023), served as the foundation. This tree was appropriately tailored by pruning using the "*drop.tip*" function in the "*ape*" package within the R environment to exclusively encompass the specific subset of sampled accessions. To assess the phylogenetic signal, represented as Pagel's λ (Pagel, 1997, 1999), associated with the proportions of repeats (for lineages, superfamilies and groups), the "*phylosig*" functions within "*phytools*" package in R was employed. The degree of simple correlation (adjusted R-squared) between the quantity of repeats and 1C GS (both considering and not considering the phylogenetic context) was computed. This was achieved using the "*geiger*" (Pennell *et al.*, 2014) and "*caper*" (Orme *et al.*, 2018) packages in the R environment. The specific functions utilized for this purpose included "*comparative.data*", "*model.pgls*", and "*anova*". These correlations were calculated for both the overarching repeat groups and individual repeat lineages.

Similarity based consensus network

The matrices, originally indicating the observed/expected number of edges between species as derived from *RepeatExplorer* clustering analysis, were transformed into distance matrices as described by Vitales *et al.* (2020b). In this process, matrices that represented clusters without any edges connecting species were entirely excluded from the analysis. Additionally, both outgroup species were entirely omitted from consideration since they exhibited very few connections with the ingroup. For the construction of neighbour-joining trees, we employed the "*ape*" package (Paradis and Schliep, 2019) within the R environment. Furthemore, a consensus network was established using the SplitsTree (Huson and Bryant, 2006), based on the method by Holland and Moulton (2003). Only splits that garnered support in a minimum of 10% of the trees were taken into account for subsequent analysis.

Comparative analysis of the repeatome structure across monocots

To contextualize the repeatome structure of *Amomum* in a broader context, we conducted an extensive data collection exercise encompassing various genomic and repeatomic characteristics across diverse monocot genera. This endeavor leveraged previously published studies employing diverse methodologies. Our primary data source included plant genome information available up to September 2023 from https://www.plabipd.de. We employed this resource to gather a comprehensive array of genomic features, conduct a repeatome analysis, and facilitate comparison across 17 monocot families. In the pursuit of comprehensive data, we thoroughly examined documented repeats from over 150 monocot plant species with a particular focus on multiple publications available for individual species when accessible. Notably, several species featured multiple publications, such as *Musa acuminata* (see Supplementary Table 4). To establish comparison data, we exclusively considered articles that presented information on the various repeat superfamilies and transposable elements, particularly LTR/*Ty1-Copia* and LTR/*Ty3-Gypsy*, which were annotated, quantified, and expressed as percentages relative to the entire genome. Publications utilizing the RepeatExplorer pipeline were excluded, as this method diverges from the approaches employed in the majority of other selected papers. For studies lacking essential details necessary for comparison, we meticulously reviewed the findings, supplementary materials, and other available data. In some instances, we recalculated several repeat families based on the published data. For the purposes of comparison, we used the percentage of the entire repeat content of the particular genome, as well as the percentages of LTR/*Ty1-Copia* and LTR/*Ty3-Gypsy* elements, LTRs, LINEs and DNA transposons (Supplementary Table 4). Additionally, we extracted and log-transformed published GS for various

monocot plant species and families from https://cvalues.science.kew.org/ for further analysis (Supplementary Table 4). For Zingiberaceae and its subfamilies, we used our own GS measurements.

DNA probes for fluorescent *in situ* hybridization (FISH)

Genomic DNA was extracted from silica-dried or freshly collected leaves of selected accessions using the NucleoSpin Plant II kit (Macherey-Nagel). The highly variable GAG domains of retrotransposable elements (REs) SIRE and Tekay were sequentially chosen for FISH probe.

Firstly, multiple sequence DNA alignments of GAG domains were performed using MAFFT v7.490 (Katoh and Standley, 2013) implemented in Geneious Prime 2022.1.1 (https://www.geneious.com). Subsequently, distance tables showing pairwise % identities of sequences were generated. Maximum Likelihood (ML) phylogenetic trees for the two selected elements were inferred using IQ-TREE v2.2.0 (Nguyen *et al.*, 2015; Hoang *et al.*, 2018) with 1000 ultrafast bootstrap (UF bootstrap) replicates. The ML trees were manipulated and graphically modified in FigTree v1.4.4 (http://tree.bio.ed.ac.uk/software/figtree/). In both trees, main clades were defined as clusters of sequences with a sequence identity greater than 80% (Supplementary Figure 1, Supplementary Figure 2). The subsequent step involved designing probes specific to particular clades of the ML trees. The details of the probes utilized for targeting GAG domains of SIRE and Tekay elements are provided in Supplementary Table 5. Two types of probes were designed and tested. (I) Oligonucleotide probes spanning 60 nucleotides, with an optimal GC content ranging from 30% to 50%, were designed from DNA alignments via Geneious Prime. Sequences with minimized risk of self-annealing and hairpin structure formation were selected. (II) PCR primers were designed to GAG domains to obtain theoretical amplicons longer than 200 bp using Primer3 v2.3.7 implemented in Geneious Prime. The PCR amplification consisted of 1 cycle (95˚C for 5 min), 35 cycles (95˚C for 20s, 58˚C for 20s, and 72˚C for 20s), and 1 cycle (72˚C for 5 min). PCR products were purified using the NucleoSpin Gel and PCR Clean-up kit (Macherey-Nagel). The preparation and labeling of DNA probes followed the published protocol (Mandáková and Lysak, 2016).

RESULTS

Revealing hybridization events in the evolutionary history of *Amomum*

The PhyloNet analyses of the *'complete dataset*', comprising 30 *Amomum* species, consistently yielded the same topology with a single reticulation, irrespective of the number of predefined reticulation events. The optimal model, determined by the highest log-probability and the lowest AIC score over multiple runs with varying priors on the number of reticulations, was obtained from a run specifying two predefined reticulations (Table 1, Supplementary Figure 3). The network's topology closely aligns with the genus's phylogeny as previously reported by Hlavatá *et al.* (2023), distinguishing four primary clades, denoted as A, B, C, and D, along with three subclades within clade D (D1–D3). Additionally, it introduces a reticulation indicating introgression from *Amomum* sp. 7 (1-□=0.1, where □represent the inheritance probability, Yu and Nakhleh, 2015) of clade C (or its ancestor) into ancestor of clade D. For the '*clade D dataset',* the most suitable network also resulted from a run specifying two predefined reticulations (Table 1, Supplementary Figure 4). This network reveals i) introgression from the ancestor of D1 (1-□= 0.2) into a specific lineage within subclade D3, here referred to as 'D3 hybrid'; and ii) introgression from the ancestors of *Amomum* sp. 6 and *A. unifolium* within the D3 subclade (1-□= 0.3) into the tetraploid *A. cinnamomeum*. The group of species that were not affected by hybridization and form monophylum within the D3 subclade are further called the 'D3 parental' subclade. Figure 1A summarizes the outcomes of PhyloNet analyses on these two datasets, highlighting three significant hybridization events within the genus. As the hybridization events occurred prior to the diversification of specific groups (clade D and

clade ‘D3 hybrid’), they align with the definition of 'ancient hybridization' proposed by Stull *et al.* (2023). Subsequently, in the following text, we also employ this term in the same context.

Genomic variation and repeat composition in *Amomum* and outgroup species

The GS (1C value) of *Amomum* species exhibited considerable variation, ranging from 1,731 Mb in *A. subulatum* to 7,656 Mb in *A. cinnamomeum* (Figure 1B), whereas the outgroup species displayed lower GS, with 1,006 Mb in *Aframomum melegueta* and 1,224 Mb in *Renealmia polypus*. Diploid *Amomum* species exhibited total repeat percentages ranging from 84% in *A. miriflorum* to 88% in *A. calcicola* and *A. subulatum* (Supplementary Table 2). The proportions of repeat content in tetraploids are well within the diploid range, with 86% and 87% in *A.* aff. *biphyllum* and *A. cinnamomeum*, respectively.

Among the 13 species analyzed by RepeatExplorer (Figure 2), the repeat composition of the outgroup species exhibited significant divergence from that of *Amomum* (Figure 2B, D). Several sub-lineages, prevalent in *Amomum*, either underwent substantial amplification or emerged anew within the genus. In the majority of *Amomum* genomes, a significant portion was found to be dominated by LTR retrotransposons of the *Ty1-Copia* superfamily, with *Ty3-Gypsy* lineages representing the second most prevalent element. Unclassified LTRs constituted a substantial portion of the genome in certain species, particularly in the tetraploid *A. cinnamomeum* and diploid *A. miriflorum*. Tandem repeats were more abundant in some species (*A.* aff. *curtisii*, *A*. aff. *biphyllum*, *A. elan*) while their proportion remained notably low in others (*A. subulatum*, *A.* aff. *repoeense*, *A. unifolium*). *A. cinnamomeum* and *A. miriflorum* moreover exhibited a relatively high proportion of unclassified repeats). Single-copy genome content and “small clusters” (comprising less than 0.01 % of reads from the dataset) constituted substantial portions of the genome; nevertheless, their proportions displayed minimal variability among species. For detailed quantification data, see Supplementary Table 2.

Table 1. PhyloNet outcomes and AIC assessments for the determination of the optimal network in the '*complete dataset*' and '*clade D dataset*'. The optimal network is indicated in bold. In this context, 'lnL' denotes the likelihood value, while 'k' represents the cumulative count of reticulations and branch lengths, serving as the number of parameters involved in the AIC computation.

	# reticulations	lnL	ΔlnL	# branch lengths	k	AIC	ΔAIC
complete dataset	0	-1374253.32	-	32.2	32.2	2748571.05	1069
	1	-1373843.13	410	35.6	36.6	2747757.46	255
	2	**-1373715.25**	**128**	**35.8**	**37.8**	**2747502.11**	**0**
clade D	0	-261422.00	-	18.8	18.8	522881.61	433
	1	-261291.72	130	21	22	522625.44	177
	2	**-261202.79**	**89**	**21.4**	**23.4**	**522448.37**	0

Comparative analysis reveals genomic distinctions and repeat composition in *Amomum*

The comparative analysis (Figure 2D) unveiled stark disparities between the outgroup and ingroup, as well as variations among individual clades within the genus *Amomum*. Most repeat sub-lineages shared with the outgroup showed a reduction in *Amomum*, while some experienced amplification. Notably, certain lineages

such as Angela (Ty1-Copia) or Athila (Ty3-Gypsy) exhibited different sub-lineage compositions in the outgroup compared to Amomum. Amomum featured several sub-lineages of unclassified LTR repeats not present in the outgroup. Clades A, B and C within *Amomum* exhibited highly similar repeat compositions, with minor distinctions in less abundant repeats, such as Athila and Retand (Ty3-Gypsy). In contrast, clade D showcased the emergence of a new, abundant, unclassified LTR sub-lineage, along with reductions in several other sub-lineages within this clade. Notably, clade D exhibited a pronounced amplification of numerous sub-lineages, including SIRE (Ty1-Copia), Tekay (Ty3-Gypsy), 45S rDNA, and unidentified LTRs, while experiencing reductions in other sub-lineages, particularly within Angela, and to a lesser extent, some SIRE and unidentified LTR sub-lineages. The distinctions in repeatome between subclades D1 (represented solely by *A.* aff. *curtisii*) and D3 were relatively minor, except for the notable amplification of specific SIRE and Tekay sub-lineages in subclade D3. Within subclade D3, unclassified LTRs and unclassified repeats seemed to contribute to the observed increase in GS in select taxa, such as *A. miriflorum* and tetraploid *A. cinnamomeum*. The 45S rDNA displayed variable amplification within certain species in clade D, with larger genome sizes observed in *A. unifolium* and *A. trilobum*, but lesser amplification in others such as *A. miriflorum* and *A.* aff. *elan*. Surprisingly, *A.* aff. *curtisii*, despite having a smaller genome, exhibited notable 45S rDNA amplification (Figure 2B, D). Ribosomal DNA content demonstrated variation across the genus, with the smallest amount observed in clade A.

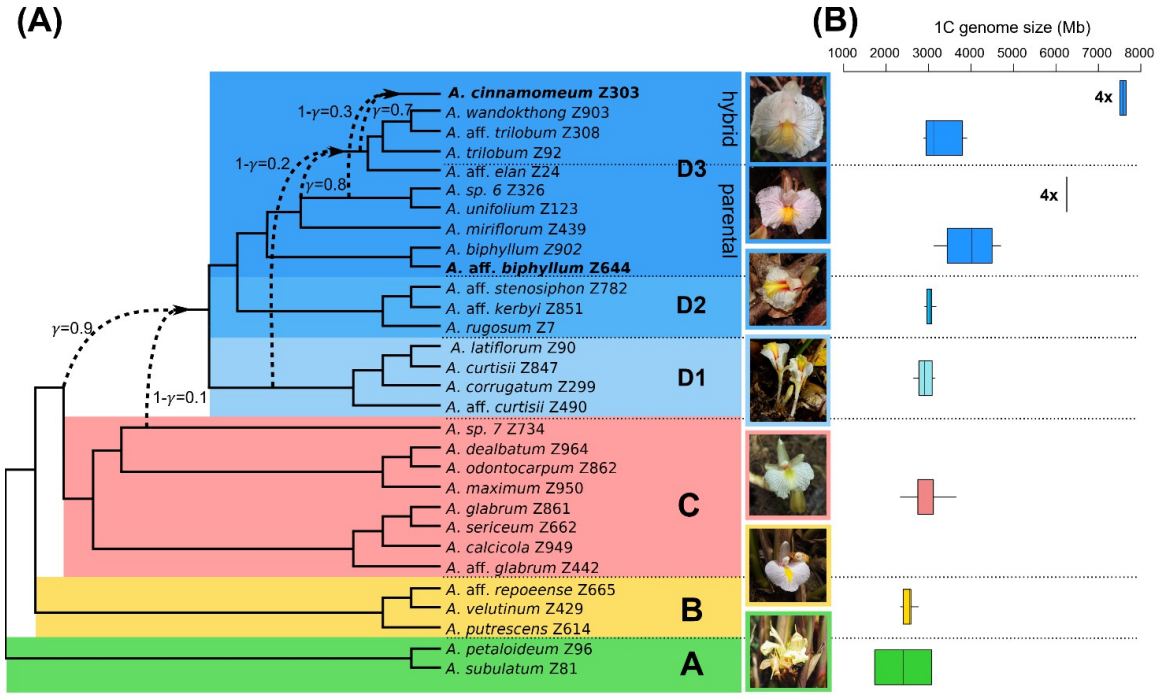

Figure 1. Phylogenetic network and genome size variation in the genus *Amomum*. A. The phylogenetic network illustrates the interrelationships among 30 species in the genus *Amomum*. It was constructed based on optimal networks derived from PhyloNet maximum pseudo-likelihood analysis of both the '*complete dataset*' and '*clade D dataset*' (see methods). All species are diploid (2n = 48), except for two tetraploids (2n = 96) highlighted in bold. The primary clades (A, B, C, D) and subclades (D1, D2, D3), as originally defined in Hlavatá *et al.* (2023), are visually distinguished through distinct color-coding. Dashed lines highlight instances of ancient hybridization predating diversification of clade D and within clade D, particularly in part of the D3 subclade ('D3 hybrid'), as well as the recent hybrid origin of tetraploid *A. cinnamomeum*. The γ value signifies the probability of inheritance from one potential ancestor, while 1-γ represents inheritance from the second ancestor. Photographs showcasing the flowers of clade representatives are presented (clade A: *A. subulatum*, B: *A.* aff. *repoeense*, C: *A.* aff. *glabrum*, D1: *A. curtisii*, D2: *A. rugosum*, and D3: *A. cinnamomeum*; photographed by J.L.-Š. and K.H.). **B.** The analysis of genome size variation, as indicated by 1C values in Mb, within the examined clades. The data is drawn from 52 *Amomum* accessions (33 species). Notably, the genome size variations of the two tetraploid species (*A. biphyllum* and *A. cinnamomeum*) are presented in separate box plots within the 'D3 parental' and 'D3 hybrid' clades and denoted by '4x' labels.

Regarding satellite regions, in the comparative analysis, a prominent satellite, cluster 48, was shared by most species in the D3 subclade, while a less abundant satellite, cluster 185, was exclusively found in *A. repoeense* of clade B (Supplementary Figure 5). However, in the comparative analysis, some species (e.g., *A.* aff. *curtisii*, *A. miriflorum*) showed no satellite presence. In a dedicated analysis employing TAREAN, numerous additional satellites marked as "high confidence" by RepeatExplorer were identified, although none appeared to be shared among different *Amomum* species. Instead, species-specific satellites were discovered in distinct clades and subclades of the genus, including *A. subulatum* (clade A), *A. petaloideum* (clade A), *A. repoeense* (clade B), and several others such as *A. trilobum*, *A.* aff. *biphyllum*, *A. miriflorum*, and *A. cinnamomeum*, all belonging to subclade D3 (Figure 2A). Notably, the clusters of 5S rDNA, analyzed in relation to phylogeny and hybridization estimates (Figure 2A, C), exhibited increased complexity. In clade A (represented by A. subulatum) and clade C (represented by *A. calcicola*), 5S rDNA clusters exhibited a one-looped configuration, whereas in subclade D1 (represented by *A.* aff. *curtisii*), subsequent to a presumed ancient hybridization event, the number of loops increased to two. Within subclade D3, two and more loops were observed. Among diploids, the maximum number of loops reached three in *A. miriflorum* and *A. unifolium*, while in the tetraploid *A. cinnamomeum*, the maximum number of loops extended to four (Figure 2).

Assessing phylogenetic signal in repeat content and its correlations with GS

We conducted a comprehensive examination of the phylogenetic signal at various levels, encompassing overall repeat content, superfamilies, and specific lineages. The overall repeat content demonstrated significant phylogenetic signal (Supplementary Table 3, $p < 0.05$) as well as the superfamily *Ty3-Gypsy* ($p < 0.05$). We further examined the lineages in detail and identified significant phylogenetic signals in the quantities of Ale ($p < 0.01$) and Ivana ($p < 0.05$), both belonging to the *Ty1-Copia* superfamily and Tekay ($p < 0.05$) from Ty3-Gypsy. Among the total 225 repeat clusters corresponding to sub-lineages in the comparative analysis, 75 (33.3%) displayed significant phylogenetic signals, as indicated by the presence of red and green bars in the barplot shown in Figure 2D. Of these, 28 sub-lineages (12.4%) displayed an increasing trend, while 47 (20.9%) exhibited a decreasing trend from clade A towards clade D, i.e. from early to late diverging group in *Amomum* as suggested by our rooted Hyb-Seq phylogeny. The remaining 172 clusters (76.4%) did not demonstrate any significant phylogenetic signals (Supplementary Table 3). Diverse trends were observed within specific lineages, exemplified by the SIRE lineage, where 10 sub-lineages displayed an increase, while 12 showed a decrease (Figure 2D; Supplementary Figure 6). This variability within lineages may account for the absence of phylogenetic signals at the lineage level. In certain lineages, all sub-lineages carrying phylogenetically significant signals displayed an increasing trend. For instance, the lineages Tekay (3 clusters) and LINEs (also 3) exhibited such a pattern, suggesting that these lineages expanded in abundance from clade A to subclade D3. Conversely, all 17 sub-lineages within Angela and both Athila sub-lineages, which conveyed phylogenetic signals, demonstrated a decreasing trend. This observation indicates a reduction in the presence of Angela and Athila from clade A to subclade D3.

Phylogenetically adjusted correlation tests were performed to assess the relationship between GS and the total amount of repeats, repeat superfamilies and lineages. Notably, a significant correlation was observed between GS and the overall quantity of repeats. Furthermore, significant positive correlations were found between GS and the repeat quantities at the superfamily level for, Ty1-Copia, Ty3-Gypsy and LINEs (Supplementary Table 3). Similarly, positive correlations were shown for multiple lineages within above mentioned superfamilies, for satellites, for the group of pararetrovirus and unclassified Class I repeats, and for a group of unclassified repeats.

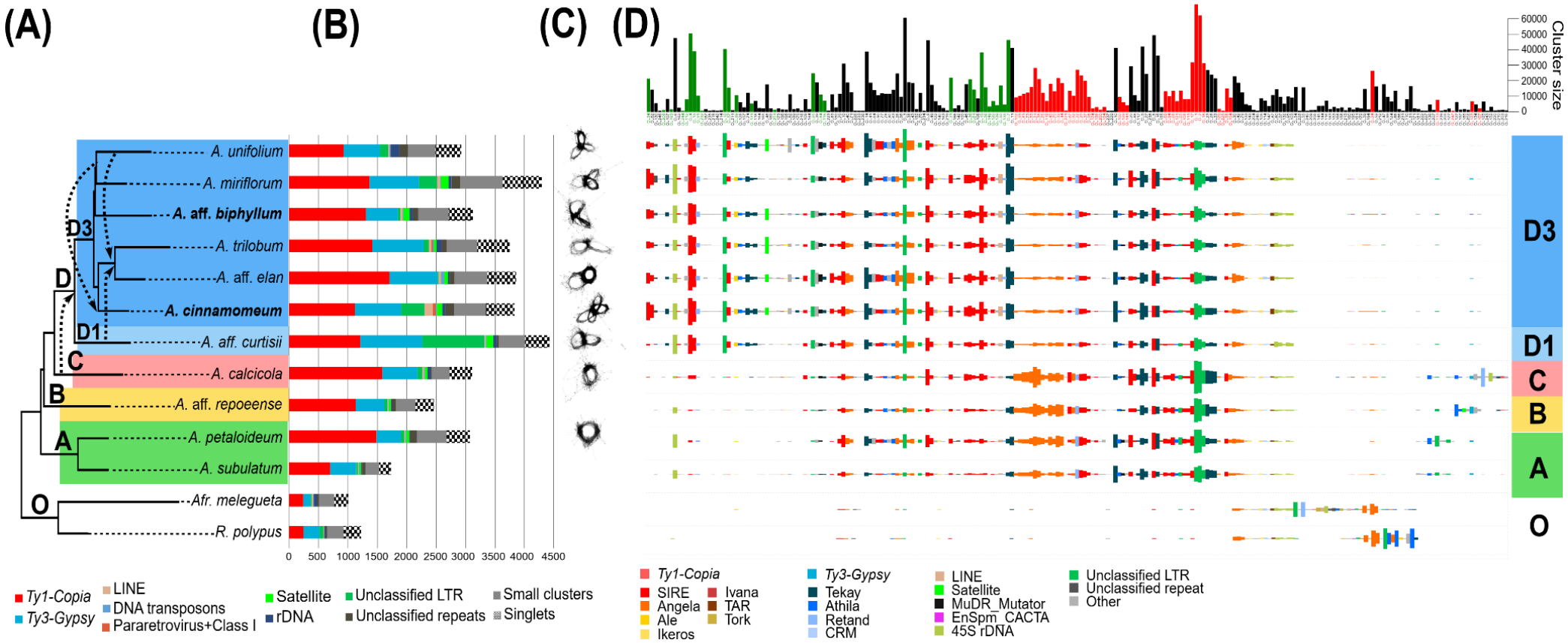

Figure 2. Comprehensive repeatome analysis in *Amomum* species. A. A species tree constructed using Hyb-Seq data, encompassing eleven *Amomum* species and two outgroup species, which were subjects of repeatome exploration. Hybridization events, as revealed by PhyloNet analysis with a broader sampling, are represented by dashed arrows. The major phylogenetic clades (A, B, C, D) and subclades (D1 and D3), as originally characterized by Hlavatá *et al.* (2023), are indicated with discrete color-coding; "O" indicates the outgroup species. All species are diploid (2n = 48), except for two tetraploids (2n = 96) highlighted in bold. *A.* = *Amomum*, *Afr.* = *Aframomum*, *R.* = *Renealmia*. **B.** Results from the RepeatExplorer clustering, quantified in Mb. The legend below the graph explains the repeat lineages. **C.** Visualization of 5S rDNA clusters in individual species, illustrating increasing complexity in clade D. The number of loops in 5S rDNA increases to two or more following ancient hybridization events. **D.** Comparative analysis of repeats in *Amomum* species, adjusted to GS, conducted using RepeatExplorer. The graph illustrates the abundances of 225 repeat clusters (sub-lineages) in individual species. Size barplots of clusters displaying significant phylogenetic signals (Pagel's $\lambda = 1$, $p < 0.05$) are color-coded as green (indicating amplification) and red (indicating reduction).

Consensus network analysis

In the creation of a consensus network, utilizing 179 matrices representing the observed/expected number of edges between species from the RepeatExplorer clustering analysis (Figure 3B), distinct patterns emerged. Specifically, two accessions from clade A exhibited close clustering within the network, while accessions representing clades B and C similarly formed a consolidated cluster. Accessions originating from subclade D3 constituted a distinctive cluster, adjacent to the accession representing subclade D1. Notably, the consensus network, constructed based on cluster similarity, demonstrated a remarkable alignment with the nuclear-gene based phylogeny, exhibiting congruence across all major clades (Figure 3A).

Genome size and repeatome structure across monocot families

Within monocots, the range of GS varies from 196 Mb (as observed in *Amorphophallus rivieri*; Zhang *et al.*, 2013) to 80,343 Mb (as evidenced in *Galanthus lagodechianus*; Zonneveld *et al.*, 2003). However, when considering only whole-genome sequencing data in our comparison, the GS range significantly narrows, falling within the 2 to 5 Mb range (Figure 4B). This phenomenon can be attributed to the technical challenges associated with whole-genome sequencing for species with larger genomes. Notably, among the subset of monocots examined in our comparison, GS ranges exhibit considerable diversity, with the most pronounced variations occurring within the Poaceae (Poales) and Asparagaceae (Asparagales) families. The highest absolute GS values are encountered in families belonging to Asparagales.

In our comparison of repeatomes in monocots (Figure 4B), we observe the lowest proportion of repeats among monocots (10.5%) in *Korthalsia laciniosa* (Arecaceae; Ghosh Dasgupta *et al.*, 2021), while the highest proportion (91.3%) is observed in *Allium sativum* (Amaryllidaceae; Sun *et al.*, 2020). While garlic displays the most elevated proportion of repeats within the realm of monocots, the average repeat percentage within the Amaryllidaceae family ranks third, trailing behind the Asphodelaceae and Alpinioideae (Zingiberaceae). Due to the limited selection and availability of studies, our comparison includes only two species from Asphodelaceae, which may render this result somewhat inconclusive.

The overall pattern of repeat proportion in monocot genomes is inherently reflected in the distribution of LTR proportions. While relatively less data is available for the proportion of LINEs, it is generally observed that their proportions are lower, with the maximum proportion reaching less than 20% of the genome. However, Orchidaceae genomes exhibit a remarkable exception to this trend by displaying the highest proportion of LINEs as well as the broadest range among monocot families. Notably, the pattern of LINE proportions closely aligns with that of *Ty3-Gypsy* proportions, with a conspicuous divergence observed in Poaceae genomes, where both the proportion and range of LINEs are notably lower than those of *Ty3-Gypsy* (Supplementary Figure 7).

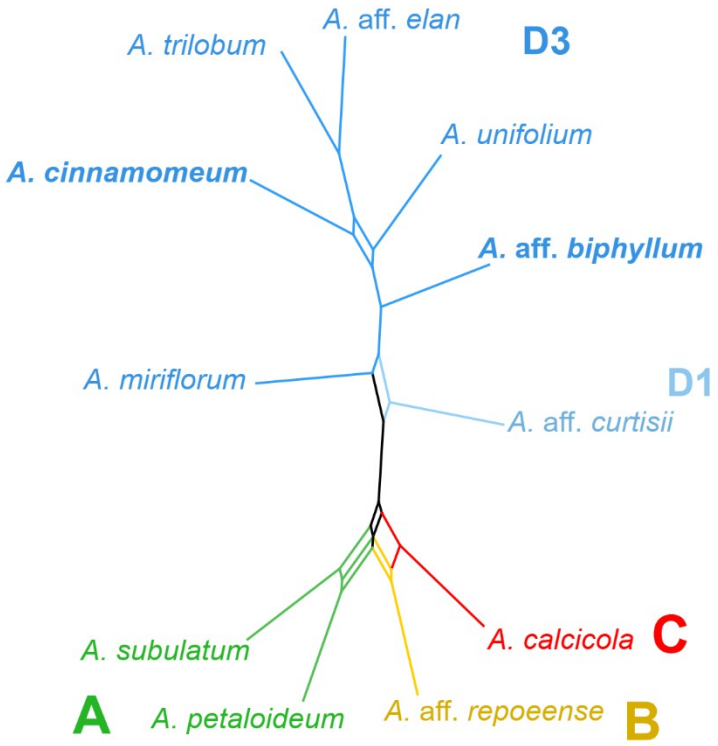

Figure 3. Repeat similarity network in *Amomum* species. A repeat similarity network was constructed based on 179 similarity matrices derived from repeats. The network represents the primary clades (A, B, C, D) and subclades (D1 and D3), as originally defined in Hlavatá *et al.* (2023), using distinct color-coding. All species are diploid (2n = 48), except for the two tetraploids (2n = 96), highlighted in bold. Clades are delineated by colors and letters. *A.* = *Amomum*.

Regarding DNA transposons, which have been more extensively documented, they are generally observed in relatively low proportions in most monocot families, typically within the range of up to 20% of the genome. Notably, the Cyperaceae genomes exhibit the highest proportion of DNA transposons, while the Bromeliaceae genomes showcase the most extensive range of proportions (Supplementary Figure 7).

The broadest ranges of repeatome proportions are observed in Poaceae genomes, spanning from 21.9% in *Eleusine indica* (Zhang *et al.*, 2019) to 90.3% in *Secale cereale* (Li *et al.*, 2021). Arecaceae genomes also exhibit significant variability, with *Korthalsia laciniosa* having the lowest percentage, and *Areca catechu* reaching 82.2% (Zhou *et al.*, 2022). The distribution of *Ty1-Copia* and *Ty3-Gypsy* superfamilies varies among different monocot groups, with Arecaceae, Juncaceae, Musaceae, and Zingiberaceae displaying higher proportions of *Ty1-Copia*, while other families exhibit higher proportions of *Ty3-Gypsy*. The most significant quantities of *Ty1-Copia* are found in the family Zingiberaceae, while genomes with the highest Ty3-Gypsy proportions are identified in Poaceae.

Arecaceae, Poaceae, and Musaceae appear to exhibit broader ranges of both *Ty1-Copia* and *Ty3-Gypsy* percentages in comparison to other monocot groups, although this observation may partly arise from the limited datasets available for some of these groups. Poaceae and Orchidaceae stand out with the widest ranges of *Ty3-Gypsy* proportions, varying from 12.8% in *Brachypodium*

distachyon (Tanaka *et al.*, 2016) to 54.9% in *Secale cereale* (Li *et al.*, 2021) within Poaceae, and from 11.8% in *Apostasia shenzhenica* (Zhang *et al.*, 2017) to 39.7% in *Phalaenopsis equestris* (Cai *et al.*, 2015) within Orchidaceae. It's worth noting that our comparison includes genomic data for only these two Orchidaceae species, and the actual range in *Ty3-Gypsy* proportions may be even more extensive, given the recognized diversity in repeat amounts within Orchidaceae (Chumová *et al.*, 2021).

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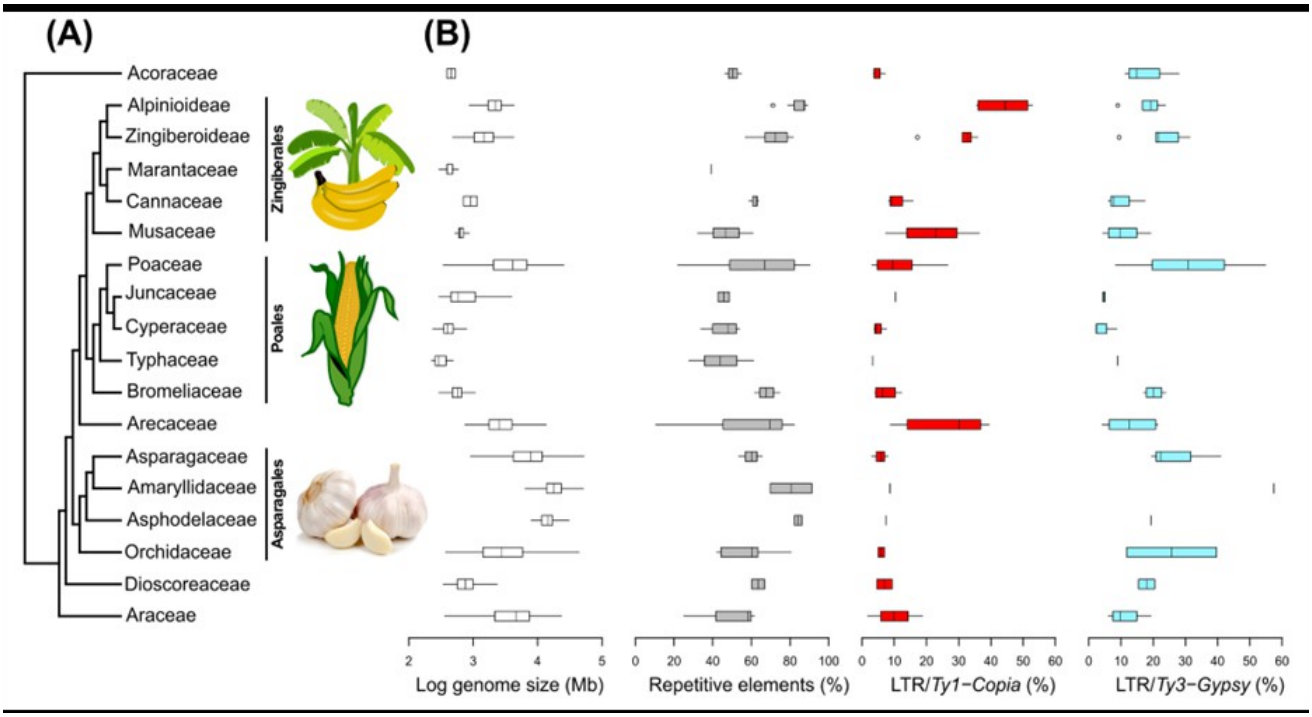

Figure 4. Phylogenetic relationships among 18 monocot plant families and selected genomic characteristics. A. Monocot phylogenetic relationships based on APG IV. Images sourced from Wikimedia Commons. **B.** Logarithm of GS, the percentages of overall repeat content, and the representation of *Ty1-Copia* and *Ty3-Gypsy* superfamilies in individual monocot families. These data were extracted from the Plant DNA C-values Database (https://cvalues.science.kew.org/) and selected genomic studies listed in Supplementary Table 4. Additional repeat lineages are presented in Supplementary Figure 5.

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DISCUSSION

Repeatome proportion in *Amomum* genome is among the largest within monocots

Our comprehensive analysis of repeat proportions across monocot families (Figure 4, Supplementary Figure 7, Supplementary Table 4) places our data on *Amomum*'s repeat content, serving as a representative of Zingiberaceae: Alpinioideae, within a broader context. Notably, *Amomum*'s repeat content stands out for its exceptional richness, rivaling representatives from Amaryllidaceae, such as *Allium sativum*, which exhibits one of the highest recorded repeat percentages at 91% (Sun *et al.*, 2020). It's worth emphasizing that *Allium*'s genome is notably larger than other Amaryllidaceae species, as well as those in *Amomum*, with a 1C value of 15,844 Mb.

Intriguingly, the repeat content in *Amomum* species consistently accounts for 84–88% of the genome size (GS) in diploids, with proportions of 86-87% in polyploids falling within the range. This striking characteristic sets *Amomum* apart from Amaryllidaceae and the broader Asparagales and Poales groups, where the *Ty3-Gypsy* superfamily overwhelmingly dominates the repeatome. Conversely, in Alpinioideae, including *Amomum*, the *Ty1-Copia* superfamily takes the lead in shaping the repeatome. This pattern of a robust correlation between the total repeat proportion and the proportion of the *Ty1-Copia* superfamily appears to persist within other closely related families in the Zingiberales order. These families typically feature lower absolute repeat quantities, in line with their smaller genome sizes, and generally exhibit lower repeat proportions. For instance, within Musaceae, diploid species such as *Musa troglodytarum* exhibit a maximum repeat proportion of approximately 61% (Li *et al.*, 2022). Conversely, various studies have reported proportion estimates as low as approximately 32% in species like *Ensete ventricosum* (Harrison *et al.*, 2014) and *Musa itinerans* (Wu *et al.*, 2016). Within Musaceae, similar to *Amomum*, the *Ty1-Copia* superfamily predominates, with the SIRE lineage significantly represented in *Musa* and Angela in *Ensete* (Novák *et al.*, 2014).

It is important to emphasize that comparing repeatomes among species across a wide phylogenetic spectrum poses inherent challenges. These challenges arise primarily due to significant variations in the depth and scope of data analysis in specific studies. Consequently, this important limitation constrains our ability to achieve a comprehensive understanding of the fundamental factors

contributing to variations in the predominant repeatome compositions among distinct species groups.

Repeat dynamics' influence on genome size in *Amomum*

Our investigation underscores the pivotal role of repeatome dynamics in shaping genome size (GS) changes within *Amomum*, revealing the complex interplay between repeats and GS. While some species exhibit minimal repeat activity affecting GS (e.g., *Anacyclus*; Vitales *et al.*, 2020a), our model group exemplifies a scenario where repeat amplification is a primary mechanism underlying GS variation.

In the context of *Amomum*, we observe a remarkably strong correlation between overall repeat abundance and GS (Adj. R2 = 0.40, $p < 0.01$), leading to a noteworthy 2.7-fold range of GS variation among diploid species. We find that specific repeat lineages, particularly Unclassified LTR, exhibit pronounced amplification within *Amomum* compared to outgroup species (Figure 2B, D). Furthermore, within basal clades A, B, and C, the Angela (*Ty1-Copia*) repeats show substantial amplification (Figure 2D) in contrast to clade D where Angela repeats decrease, while multiple other lineages increase, including SIRE (*Ty1-Copia*), Tekay (*Ty3-Gypsy*), and tandem repeats including 45S rDNA. These lineage-specific repeat changes within clade D strongly correlate with GS and represent the predominant contributors to GS increases (Supplementary Table 3). Notably, this GS increase due to *Ty1-Copia* elements, especially the SIRE and Angela lineages, is not unique to *Amomum*; similar observations have been made in the closely related Musaceae family (Novák *et al.*, 2014). More compellingly, in the grass subtribe Loliinae (family Poaceae), which is more distantly related to *Amomum*, GS experiences a 1.5-fold increase (Moreno-Aguilar *et al.*, 2022). This substantial change is attributed to the abundant presence of Retand (*Ty3-Gypsy*) and Angela (*Ty1-Copia*), with Angela being the primary driver of GS differences on average (Moreno-Aguilar *et al.*, 2022). However, it is important to highlight that the dynamics of specific repeat types within diploids differ from their polyploid counterparts, making direct comparisons with *Amomum*'s repeat dynamics challenging.

General trend of gradual GS increase along the phylogeny was further supported by discernible phylogenetic signal (Pagel's λ) in the total amount of repeats as well as in several repeat lineages. Interestingly, no phylogenetic signal was detected for the most abundant superfamily Ty1-Copia as well as for many other lineages. The absence of signal can be likely attributed to counteracting trends within specific repeat lineages. For instance, within the SIRE lineage, certain sub-lineages exhibit an increasing trend in repeat abundance ($\lambda = 1$, $p < 0.001$; Supplementary Figure 4), while others reveal a decreasing trend ($\lambda = 1$, $p < 0.001$; Supplementary Figure 4). Particularly noteworthy is the behavior of phylogenetically significant sub-lineages of the Angela lineage (*Ty1-Copia*), which remarkably demonstrate a decreasing trend. This is unconventional since Angela is typically associated with amplification events, often significantly impacting GS (e.g., in *Heloniopsis*, Pellicer *et al.*, 2021; or *Passiflora*, Sader *et al.*, 2021). In *Amomum*, repeat dynamics predominantly occur at the sub-lineage level, aligning with findings in *Setaria italica*, which described the evolution of various sub-lineages within SIRE, Angela, and other lineages (Suguiyama *et al.*, 2019). Our results support the notion that analyses at this detailed level can complement phylogenetic analyses when studying repeat evolution, shedding light on the intricate mechanisms driving GS changes.

Hybridization as a potential trigger of repeat amplification

Intriguing patterns emerge when scrutinizing the GS dynamics within the genus *Amomum*. As previously discussed, a gradual increase in GS becomes evident from outgroup species through early branching lineages A and C, reaching a noteworthy expansion within the latest branching lineage, D3 (Figure 1). This observed GS increase is not only due to the presence of tetraploid individuals, as the analysis of diploid individuals reveals a consistent pattern. Our data thus strongly suggests that the accumulation of repetitive elements (repeats) plays a pivotal role in instigating GS changes.

Our detailed examination of the repeatome composition within clade D revealed the most substantial increase in multiple repeat lineages (Figure 2D). Strikingly, clade D was also identified as having an ancient hybrid origin, as previously suggested by cyto-nuclear discordance observed in Hlavatá *et al.* (2023). While causality cannot be definitively proven, it is plausible that the significant increase in multiple repeat lineages within clade D is closely associated with its hybrid lineage origin. Another clue to this hypothesis comes from the pattern of 5S rDNA clustering. Species within early derived clades, such as A and C, displayed a single loop of 5S rDNA, consistent with non-hybridogenous species (Garcia *et al.*, 2020). In contrast, the analyzed species from clade D exhibited two or more loops, suggesting at least one hybridization event at the base of clade D, and potentially more within the diversification of subclade D3. Notably, tetraploid *A. cinnamomeum* displayed four loops of 5S rDNA, indicating recent (allopolyploid) as well as ancient hybridization events, contrasting with the pattern observed in recent allopolyploid Loliineae species where a maximum of two loops were detected (Moreno-Aguilar *et al.*, 2022). This finding supports the idea of additional past hybridization events in *Amomum*. As the complexity of 5S rDNA structure increased after hybridization event(s), we hypothesize that other sub-lineages of repeats may have evolved in a similar manner. This could explain the proliferation of repeats in the genome, particularly in abundant lineages like SIRE. In various studies, GS increase after hybridization events has been attributed to a burst of specific repeat lineages. Examples include a burst of the *Gorge3* element in *Gossypiu*m (Hawkins *et al.*, 2009), chromovirus-like retro elements in *Nicotiana* (Renny-Byfield *et al.*, 2013), two Gypsy-like retrotransposons in *Phalaenopsis* (Hsu *et al.*, 2020), or one satellite in *Spartina* (Giraud *et al.*, 2021). In the case of *Amomum*, our analysis revealed that 12.4% of sub-lineages from various lineages were significantly amplified in clade D compared to other clades (Figure 2D, Supplementary Table 6). This finding indicates the independent evolution of individual sub-lineages and suggests higher repeat lineage complexity in *Amomum*.

As an alternative to the hypothesis of a repeat burst resulting from ancient hybridization, the unique repeatome composition in clade D could also be attributed to its distinct and long-term independent evolutionary history. Notably, clade D was previously recognized as a separate genus, *Elettariopsis*, based on its distinct morphology (Hooker, 1894; de Boer *et al.*, 2018). Currently, it shares a biogeographic overlap with the basal clades of *Amomum* to the north of the Isthmus of Kra in seasonal, monsoonal regions of Southeast Asian tropics (Hlavatá *et al.*, 2023). Additionally, many species from clade D dispersed to the south of the Isthmus in evergreen areas of Southeast Asia. If the diversification of clade D occurred sympatrically with clades A, B, and C (in the north of the Isthmus of Kra), our theory of the ancient hybrid origin of clade D and the subsequent boost of repeats due to genomic shock gains substantial support. However, if the diversification took place allopatrically to the south of the Isthmus, a distinct repeat composition could have evolved due to long-term isolation. Once the biogeographic history of the genus is fully reconstructed, and further research corroborates allopatric vs. sympatric diversification within clade D, we will be able to secure more definitive evidence for either of these hypotheses.

Exploring the utility of repeats as molecular markers and phylogenetic tools in *Amomum*

Repeats have proven to be valuable resources in molecular biology and phylogenetics, with specific applications in discerning species-specific or group-specific markers. In the case of Musaceae, repeats have served as effective molecular markers, as the proliferation of certain groups often accompanies speciation (Novák *et al.*, 2014). This concept aligns with (Rebollo *et al.*, 2010)'s review in 2010, emphasizing the usefulness of repeats in speciation studies.

To address insufficient resolution when using other markers, Dodsworth *et al.* (2015) proposed the utilization of repeats as molecular markers, highlighting the versatility of repeats in molecular phylogeny. Vitales *et al.* (2020b) recently reconstructed phylogenetic relationships by employing matrices of similarity between repeat clusters, a part of the RepeatExplorer results introduced by Novák *et al.* (2020). We adopted this approach to construct a phylogenetic network, mirroring a nuclear gene-based phylogeny based on 449 genes. Remarkably, despite the smaller sample size, the repeat-based method provided congruent results, affirming that cluster similarities within *Amomum* can be effectively used to estimate phylogeny or complement other phylogenetic markers. Furthermore, our phylogenetic network analysis provided compelling support for the hybrid origin of the D3 'hybrid' subclade, given its most distinct position within the network. This corroborates previous evidence indicating the hybrid origin of clade D3, strengthening our understanding of *Amomum*'s evolutionary history. On the other hand, we noted that this repeat-based phylogenetic method exhibited higher proportions of uncertainties in the relationships between clades A, B, and C (**Figure 4**), which mirrors the topological incongruences observed in previous studies involving chloroplast DNA, ribosomal DNA, and nuclear DNA (Hlavatá *et al.*, 2023). These findings suggest that while the repeat-based phylogenetic approach is promising for resolving shallower evolutionary events, it may encounter limitations when addressing deeper phylogenetic relationships. This limitation aligns with the outcomes of a similar method applied to Loliinae, which yielded networks that, although largely congruent with other phylogenies, displayed reduced resolution at deeper phylogenetic levels (Moreno-Aguilar *et al.*, 2022). Our findings underscore the potential and limitations of using repeats as molecular markers and phylogenetic tools within *Amomum*, ultimately contributing to our understanding of the genus's complex evolutionary history.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author Contributions

T.F. and T.M. conceived the project. K.H., E.Z., T.F., B.K., M.P. and T.M. conducted the analyses and assessed the data. J.L.-Š., A.D.P and O.Š. contributed plant materials. T.M., K.H. and E.Z. wrote the manuscript. All authors edited and approved of the manuscript.

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Data Availability Statement

The datasets analyzed for this study can be found in the Sequence Read Archive (https://www.ncbi.nlm.nih.gov/sra) under the ID PRJNA1029323.

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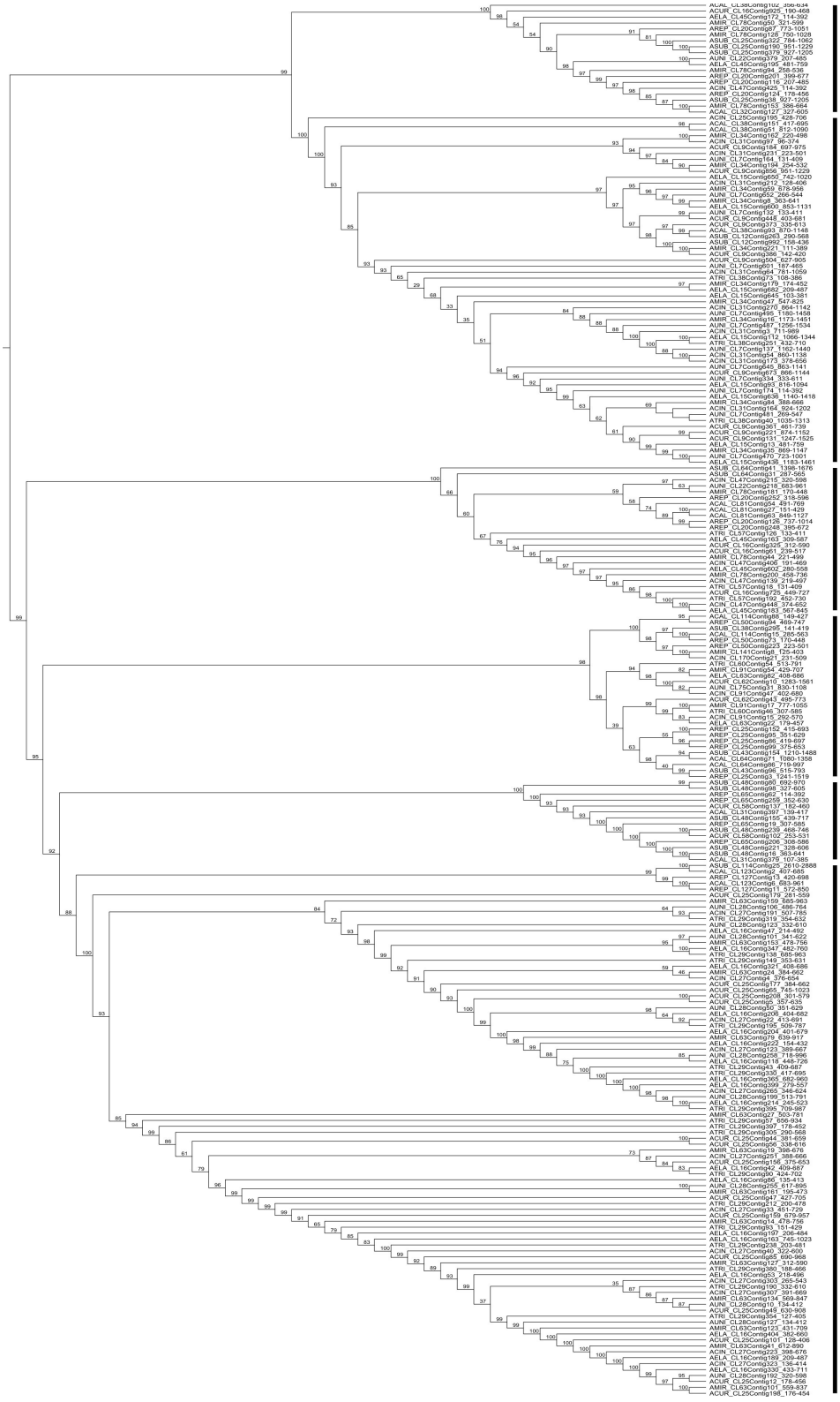
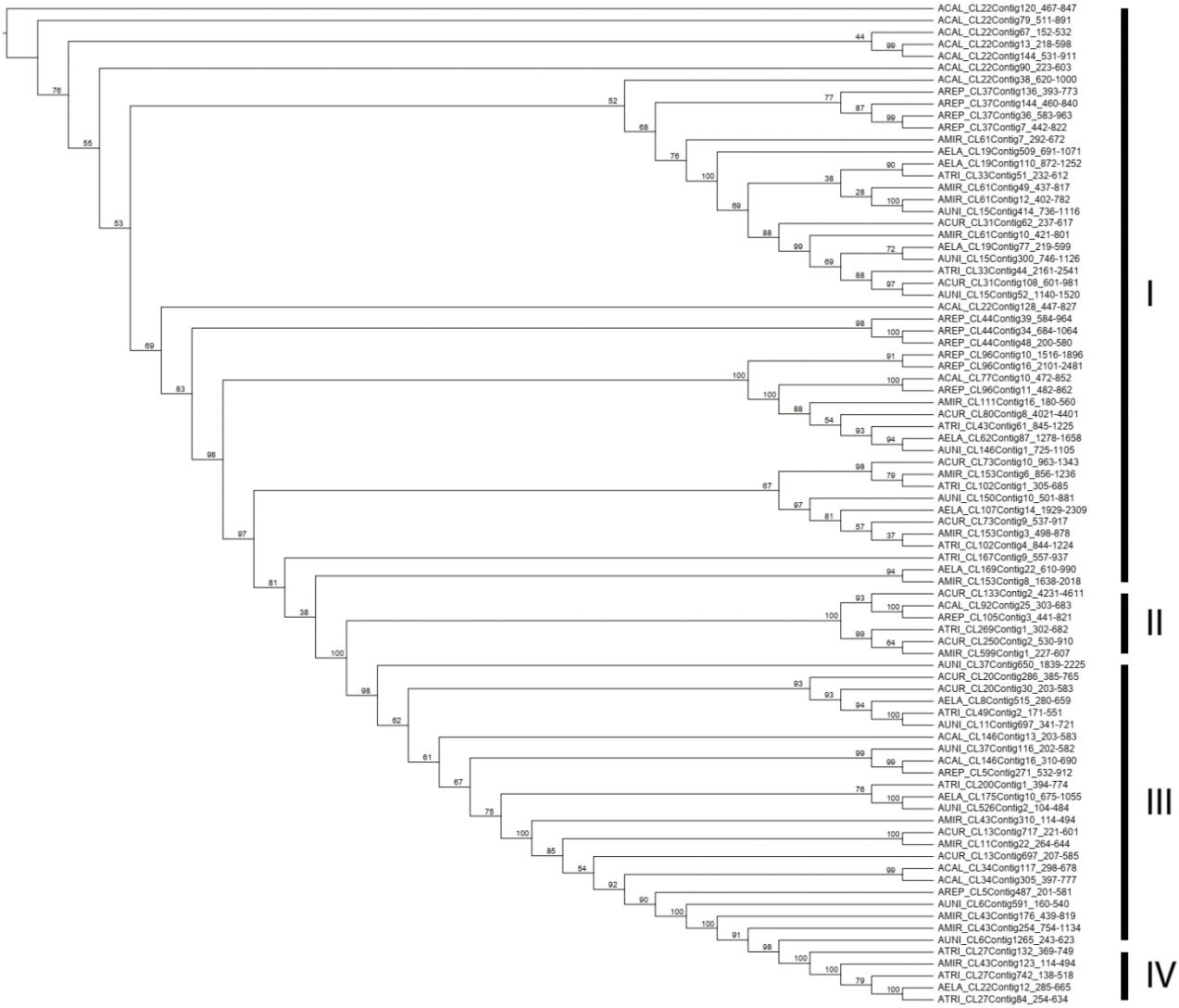
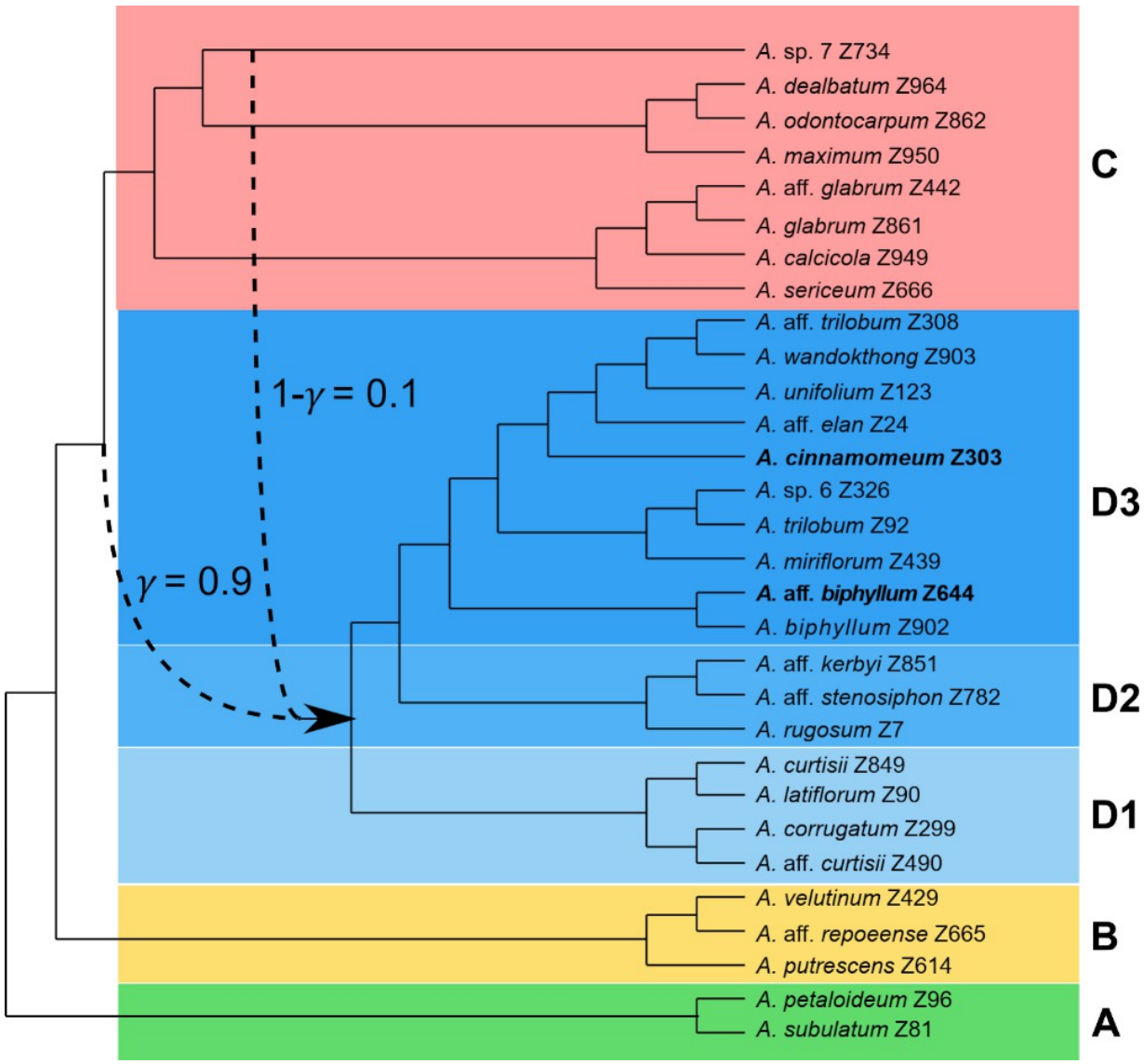
SUPPLEMENTARY FIGURES



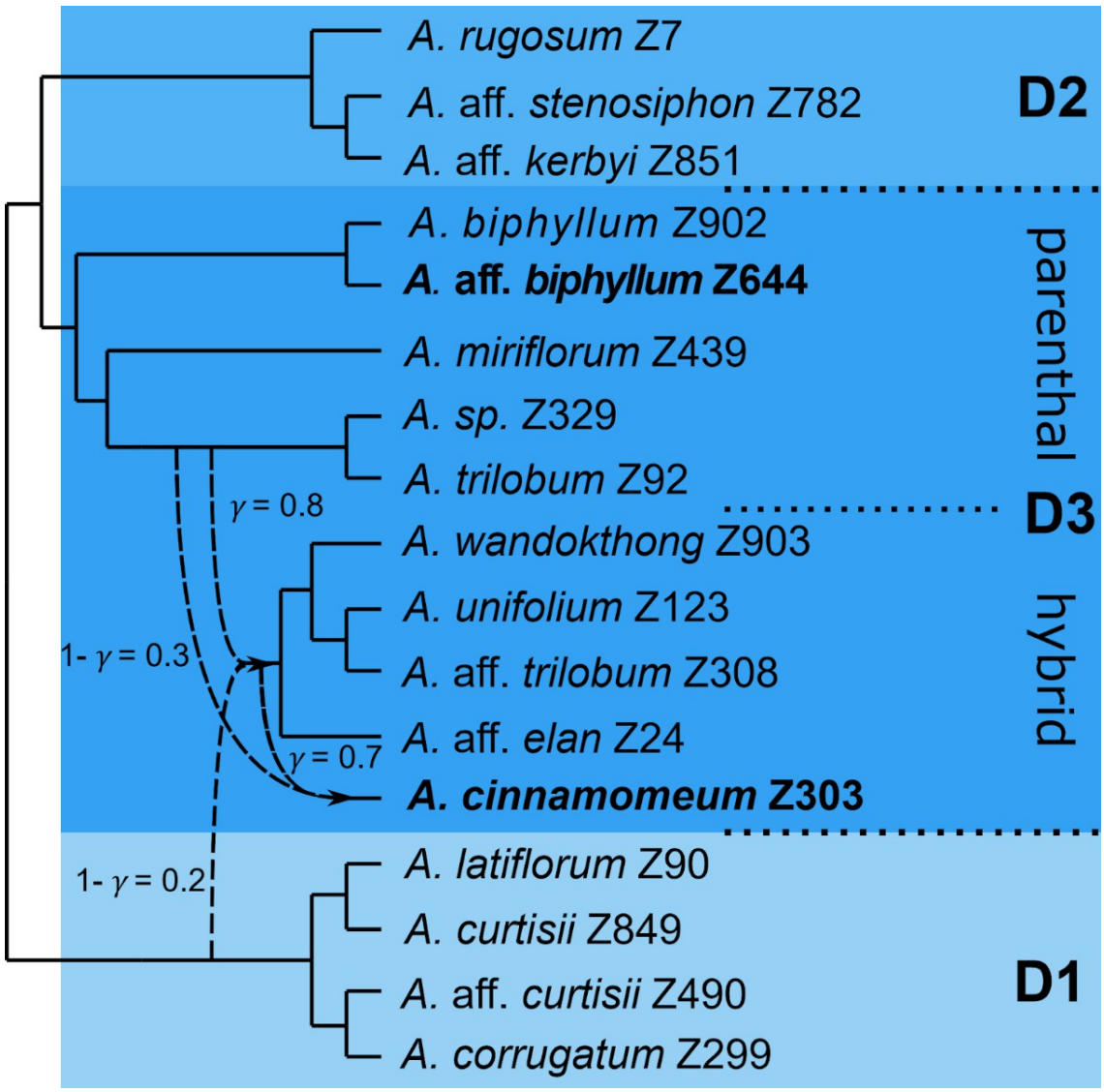
Supplementary Figure 1. Maximum likelihood phylogeny inferred from the GAG domain of SIRE element of selected Amomum species.



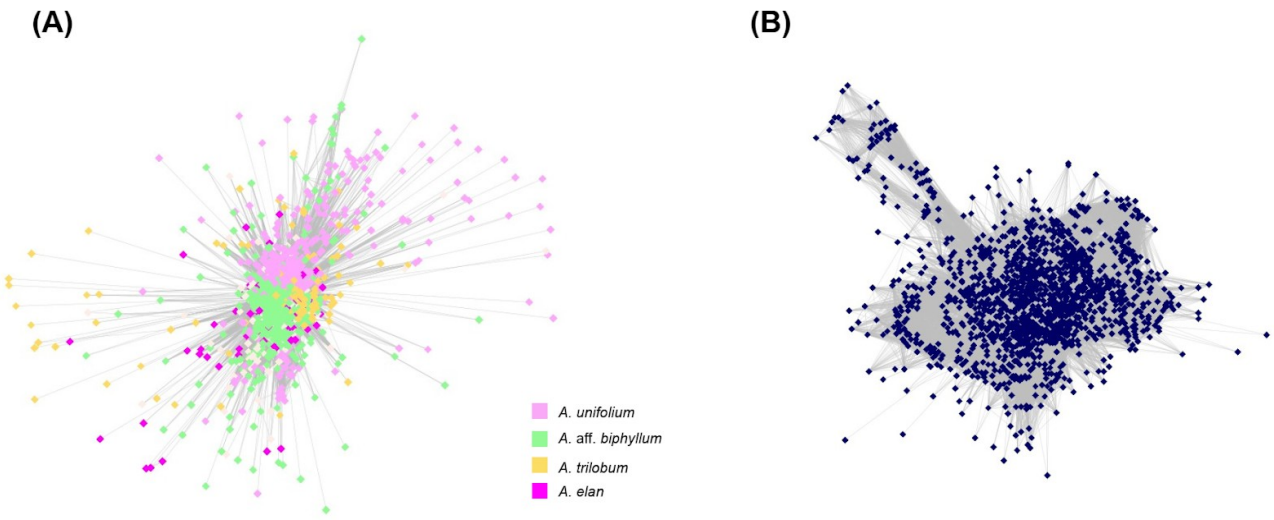
Supplementary Figure 2. Maximum likelihood phylogeny inferred from the GAG domain of Tekay element of selected Amomum species.



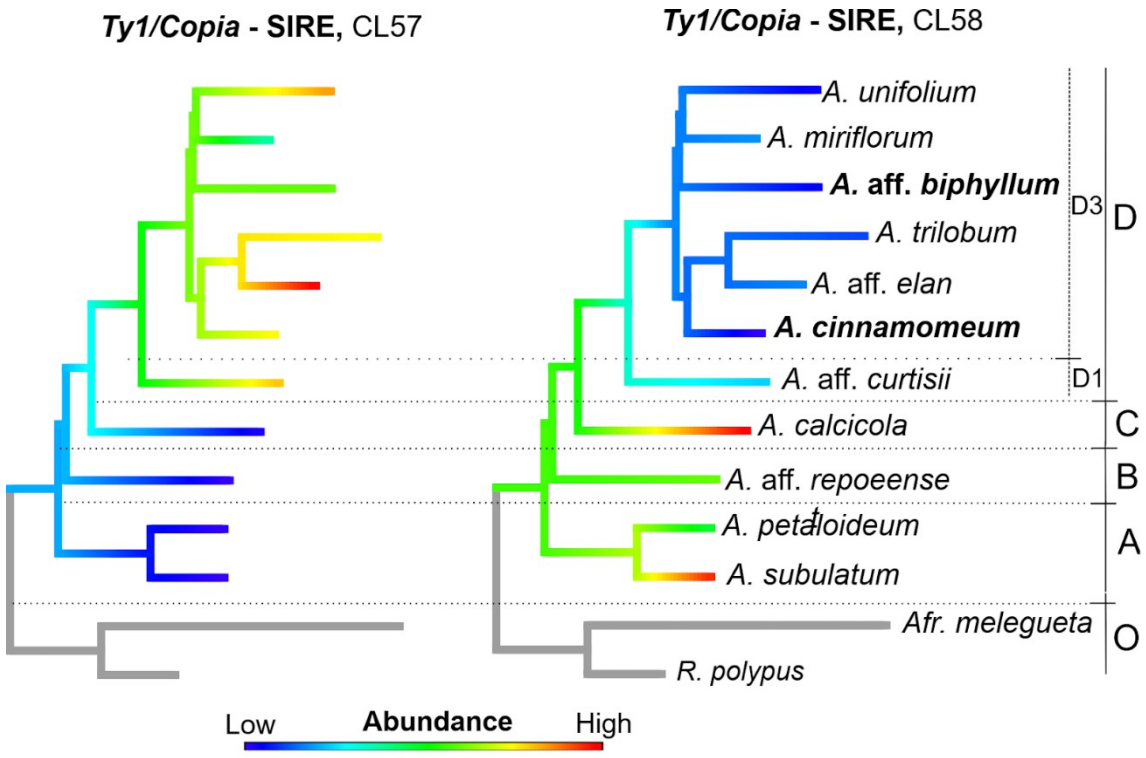
Supplementary Figure 3. PhyloNet network for 30 *Amomum* species. The best network resulting from the maximum pseudo-likelihood analysis of the '*complete dataset*' in Hyb-Seq (see Methods) represents the relationships among 30 species within the genus *Amomum*. Main clades (A, B, C, D) and subclades (D1, D2, D3) within the genus, as defined in Hlavatá et al. (2023), are distinguished by colors . Blue curves indicate the ancient hybrid origin of clade D. The □ and 1-□ values denote the inheritance probability from the first and second possible ancestors, respectively. Tetraploids are indicated in bold.



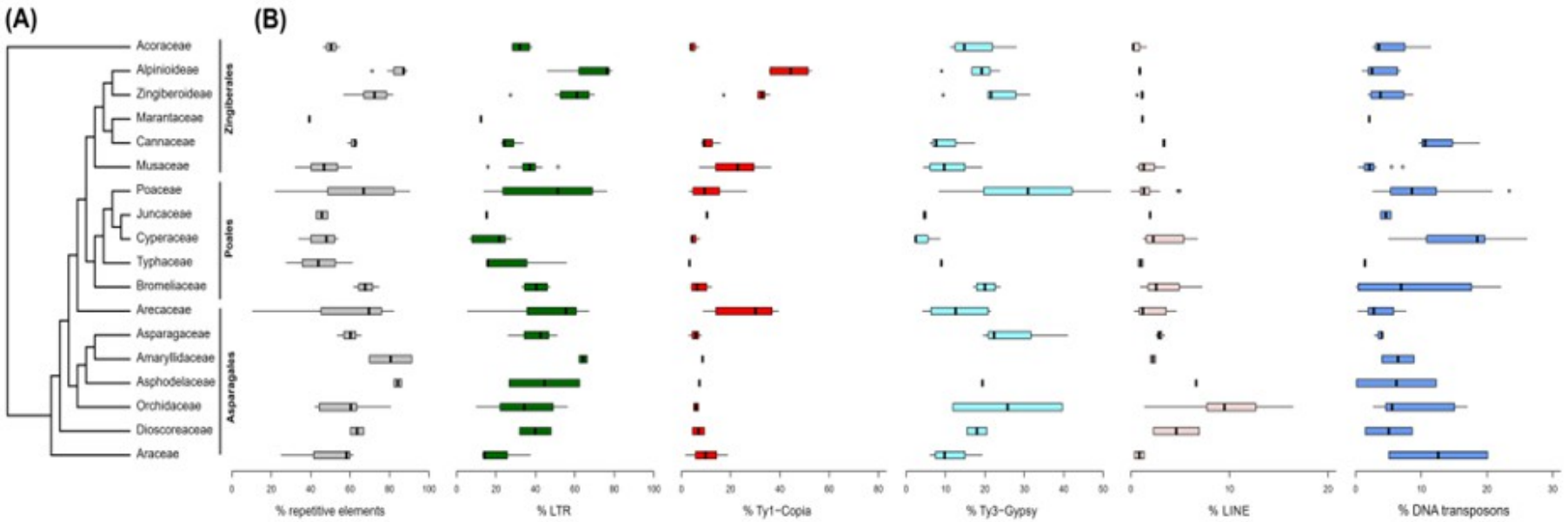
Supplementary Figure 4. PhyloNet network for *Amomum* clade D. The best network resulting from PhyloNet maximum pseudo-likelihood analysis of Hyb-Seq '*clade D dataset*' (see Methods), illustrating relationships within 17 *Amomum* species. Subclades within clade D (D1, D2, and D3) are distinguished by different shades of blue color. Tetraploids are highlighted in bold. *A.* = *Amomum*. Blue curves indicate the ancient hybrid origin of the 'D3 hybrid' subclade and the hybrid origin of tetraploid *A. cinnamomeum*. The □□and 1-□□values denote the inheritance probability from the first and second possible ancestors, respectively.



Supplementary Figure 5. Satellite cluster analysis in *Amomum* clades D3 and B. Satellite cluster CL48 is shared among multiple *Amomum* species within clade D3 (**A**), while satellite cluster CL185 is unique to*A. repoeense* within clade B (**B**). Adapted from RepeatExplorer. *A.* = *Amomum*.



Supplementary Figure 6. Repeat sub-lineage abundances mapped onto *Amomum* phylogeny. Repeat sub-lineage (corresponding to clusters in comparative RepeatExplorer analysis) abundances are mapped onto the Hyb-Seq-based *Amomum* phylogeny. Two SIRE sub-lineages exhibit opposing trends (CL 57 amplification, CL 58 reduction), with abundances displaying a significant phylogenetic signal (Pagel's $\lambda = 1$ and $p < 0.05$). Grey branches indicate missing data, and tetraploids are highlighted in bold. Phylogenetic clades are marked by lines and letters *Afr.* = *Aframomum*. *R.* = *Renealmia*. O = outgroup.



Supplementary Figure 7. Monocot plant family relationships and repeat abundances. (**A**) Phylogenetic relationships among 18 monocot plant families based on APG IV. (**B**) Percentages of overall repeat contents, along with the representation of LTRs, *Ty1-Copia*, *Ty3-Gypsy* superfamilies, LINEs, and DNA transposons in individual plant families, with data sources from selected genomic studies listed in Suppl. Table S4.

SUPPLEMENTARY TABLES

Table S1. List of investigated *Amomum* accessions and collection data. Accessions used in the study are listed, with herbarium specimen abbreviations in brackets where applicable. Herbarium abbreviations conform to the Index Herbariorum, except for NLS, which corresponds to the herbarium of Faculty of Sciences, National University of Laos as per Lamxay & Newman (2012). Collection details include: SBG - Singapore Botanic Gardens, SI - Smithsonian Institution, RBGE - Royal Botanic Gardens Edinburgh, PBG - Prague Botanic Garden. Abbreviations for different analyses are as follows: GS - 1C genome size visualisation in Figure 1, RE - RepeatExplorer analysis, PNc - PhyloNet *'complete dataset'*, PND = PhyloNet *'clade D dataset'*, SRR - number in Sequence Read Archive (SRA).

		collection data			genomic characteristics		presence in analyses					
accession	**clade**	**accession no.**	**herbarium specimen**	**country of origin**	**genome size (Mb/1C)**	**chromosome number (2n)**	**ploidy level**	**GS**	**RE**	**PNc**	**PND**	**HybSeq**
***Aframomum alboviolaceum* (Ridl.) K.Schum.**	outgroup	Z236	D.J. Harris 5745 (E)	Central African Rep.	**1213**		-	x	-	x	-	SRR7058219
***Aframomum melegueta* K. Schum.**	outgroup	Z743	ex cult. RBGE 19982065	Côte d'Ivoire	**1006**	-	-	x	x	x	-	SRR12824546
***Amomum biphyllum* (Saensouk & P.Saensouk) Škorničk. & Hlavatá**	D3	Z902	ex cult. Mahasarakham University	Thailand	**4699**	-	-	x	-	x	x	SRR12824540
***Amomum biphyllum* aff. (Saensouk & P.Saensouk) Škorničk. & Hlavatá**	D3	Z644	V. Lamxay VL2222 (NLS)	Laos	**6253**	96	4x	x	x	x	x	SRR12824530
***Amomum calcicola* Lamxay & M.F.Newman**	C	Z949	M. Newman et al. LAO 1302 (E)	Laos	**3115**	-	-	-	x	x	x	SRR12824535
***Amomum cinnamomeum* Škorničk., Luu & H.Đ.Trần**	D3	Z303	J. Leong-Škorničková GRC-393 (SING)	Vietnam	**7656**	96	4x	x	x	x	x	SRR12824532
***Amomum cinnamomeum* Škorničk., Luu & H.Đ.Trần**	D3	Z724	ex cult. SI W.J. Kress & Q.J. Li 05-7779	Vietnam	**7510**	-	4x	x	-	-	-	-
***Amomum corrugatum* Škorničk., H.Đ.Trần & Luu**	D1	Z299	H.Đ.Trấn et al. 53 (E, SING, VNM)	Vietnam	**2641**	48	2x	x	-	x	x	SRR12824531
***Amomum corrugatum* Škorničk., H.Đ.Trần & Luu**	D1	Z645	H.Đ.Trấn et al. 52 (E, SING, VNM)	Vietnam	**2659**	48	2x	x	-	-	-	-
Amomum curtisii (Baker) Škorničk. & Hlavatá	D1	Z847	ex cult. Singapore Botanic Gardens	Pen. Malaysia	2975	-	-	x	-	-	-	SRR12824539
***Amomum curtisii* aff. (Baker) Škorničk. & Hlavatá**	D1	Z490	ex cult. RBGE 20001425	Thailand	**2782**	48	2x	x	-	x	x	SRR12824538
***Amomum curtisii* aff. (Baker) Škorničk. & Hlavatá**	D1	Z721	W.J. Kress 99-6322	Thailand	**2771**	-	-	x	-	-	-	-
***Amomum curtisii* aff. (Baker) Škorničk. & Hlavatá**	D1	Z739	J. Mood 13P14 (PRC)	Malaysia	**2906**	-		x	-	-	-	-

***Amomum dealbatum* Roxb.**	C	Z964	ex cult. SI W.J. Kress 06-8414	China	**3110**	-	-	x	-	x	x	SRR12824524
***Amomum elan* aff. (C.K. Lim) Škorničk. & Hlavatá**	D3	Z024	J. Leong-Škorničková GRC-079 (SING)	Malaysia	**3863**	-	-	x	x	x	x	SRR12824528
***Amomum elan* aff. (C.K. Lim) Škorničk. & Hlavatá**	D3	Z732	J. Mood 1286 (PRC)	Malaysia, Sabah	**3585**	-	-	x	-	-	-	-
***Amomum glabrum* aff. S.Q. Tong**	C	Z442	J. Leong-Škorničková et al. JLS-1598 (E, PR, SING, VNMN)	Vietnam	**3115**	-	-	x	-	x	x	SRR12824517
***Amomum glabrum* S.Q. Tong**	C	Z861	V. Lamxay VL1157 (E, NLS)	Laos	**3110**	-	-	x	-	x	x	SRR12824516
***Amomum kerbyi* aff. (R.M. Sm.) Škorničk. & Hlavatá**	D2	Z851	ex cult. PBG M. Dančák 2015/3004	Brunei	**3105**	-	-	x	-	x	x	SRR12824537
***Amomum latiflorum* (Ridl.) Škorničk. & Hlavatá**	D1	Z090	J. Leong-Škorničková et al. SNG-13 (SING)	Singapore	**3139**	-	-	x	-	x	x	SRR12824536
***Amomum latiflorum* (Ridl.) Škorničk. & Hlavatá**	D1	Z121	W.J. Kress 99-6322	Thailand	**3156**	-	-	x	-	-	-	-
***Amomum latiflorum* (Ridl.) Škorničk. & Hlavatá**	D1	Z642	H. Ibrahim & S. Teo SNG-113 (SING)	Singapore	**3086**	-	-	x	-	-	-	-
***Amomum maximum* Roxb.**	C	Z950	A.D. Poulsen 2920 (E)	Papua New Guinea	**2748**	-	-	x	-	x	x	SRR12824515
***Amomum maximum* Roxb.**	C	Z686	Leong-Škorničková et al. JLS-1726 (SING, PR, P, E)	Laos	**2749**	-	-	x	-	-	-	-
***Amomum miriflorum* Škorničk. & Q.B.Nguyen**	D3	Z439	J. Leong-Škorničková et al. JLS-1589 (E, K, P, PR, SING, VNMN)	Vietnam	**4294**	48	2x	x	x	x	x	SRR12824534
***Amomum odontocarpum* D. Fang**	C	Z862	V. Lamxay VL1300 (E, NLS)	Laos	**-**	-	-	-	-	x	x	SRR12824514
***Amomum petaloideum* (S.Q.Tong) T.L.Wu**	A	Z096	W.J. Kress et al. 95-5508 (US)	China	**3078**	-	-	x	x	x	x	SRR12824513
***Amomum putrescens* D. Fang**	B	Z614	J. Leong-Škorničková et al. JLS-2146 (VNM, SING)	Vietnam	**2592**	-	-	x	-	x	x	SRR12824545
Amomum ranongense (Picheans. & Yupparach) Škorničk. & Hlavatá	D2	Z731	J. Mood 3377 (PRC)	Thailand	**3016**	-	-	x	-	-	-	-
Amomum ranongense* aff. *(Picheans. & Yupparach) Škorničk. & Hlavatá	D2	Z727	J. Mood 08P288 (PRC)	Thailand	**2914**	-	-	x	-	-	-	-
***Amomum repoeense* aff. Pierre ex Gagnep.**	B	Z665	H.Đ. Trần et al. 67 (E, SING, VNM)	Vietnam	**2465**	48	2x	x	x	x	x	SRR12824544
***Amomum repoeense* aff. Pierre ex Gagnep.**	B	Z450	J. Leong-Škorničková JLS-1619 (SING, E, P,	Vietnam	**2761**	-	-	x	-	-	-	-

			VNMN)									
***Amomum repoeense* aff. Pierre ex Gagnep.**	B	Z456	J. Leong-Škorničková JLS-1637 (SING, E, P, VNMN)	Vietnam	**2578**	-	--	x	-	-	-	-
***Amomum repoeense* aff. Pierre ex Gagnep.**	B	Z659	V. Lamxay VL2223 RBGE20111045	Laos	**2344**	-	-	x	-	-	-	-
***Amomum rugosum* (Y.K.Kam) Škorničk. & Hlavatá**	D2	Z007	J. Leong-Škorničková GRC-363 (SING)	Malaysia	**2910**	-	-	x	-	x	x	SRR12824533
***Amomum sericeum* Roxb.**	C	Z662	M.F. Newman 2397 (E)	Cambodia	**3174**	-	-	x	-	x	x	SRR12824543
***Amomum sericeum* Roxb.**	C	Z559	J. Leong-Škorničková et al. JLS-1675 (E, SING)	Laos	**3646**	-	-	x	-	-	-	-
***Amomum smithiae* (Y.K.Kam) Škorničk. & Hlavatá**	D2	Z720	ex cult. SBG20001092	Malaysia	**3179**	-	-	x	-	-	-	-
Amomum sp. 6	D3	Z329	ex cult. SBG20110992	Vietnam	**3130**	48	2x	x	-	x	x	SRR12824527
Amomum sp. 7	C	Z734	J. Mood 3387 (PRC)	Thailand	**2339**	-	-	x	-	x	-	SRR12824542
Amomum sp. 8	C	Z640	ex cult. SBG20122011	Laos	**2389**	-	-	x	-	-	-	-
Amomum sp. 9	B	Z491	H.Đ.Trấn 366 (VNM)	Vietnam	**2337**	-	-	x	-	-	-	-
***Amomum stenosiphon* aff. K.Schum.**	D2	Z872	Conlon et al. 41 (E)	Indonesia	**3037**	-	-	x	-	x	x	SRR12824526
***Amomum stenosiphon* aff. K.Schum.**	D2	Z725	J. Mood 89P43 (PRC)	Malaysia, Sabah	**3038**	-	-	x	-	-	-	-
***Amomum subulatum* Roxb.**	A	Z081	J. Škorničková CU71468 (CALI, SING)	India	**1731**	48	2x	x	x	x	x	SRR12824541
***Amomum trilobum* aff. Gagnep.**	D3	Z092	M.F. Newman & J. Škorničková 1455 (E, SING)	Vietnam	**3750**	48	2x	x	x	x	x	SRR12824525
***Amomum trilobum* aff. Gagnep.**	D3	Z308	ex cult. SBG 20110993	Vietnam	**3760**	48	2x	x	-	x	x	SRR12824529
***Amomum unifolium* aff. Gagnep.**	D3	Z304	ex cult. SBG20110994	Vietnam	**2958**	-	-	x	-	-	-	-
***Amomum unifolium* aff. Gagnep.**	D3	Z636	ex cult. SBG20123278	Vietnam	**2900**	-	-	x	-	-	-	-
***Amomum unifolium* aff. Gagnep.**	D3	Z368	H.Đ. Trấn 257 (VNM)	Vietnam	**2890**	-	-	x	-	-	-	-

***Amomum unifolium* aff. Gagnep.**	D3	Z661	H.Đ. Trấn s.n. RBGE20081123	Vietnam	**2993**	-	-	x	-	-	-	-
***Amomum unifolium* aff. Gagnep.**	D3	Z850	ex cult. SBG20111709	Vietnam	**3120**	-	-	x	-	-	-	-
***Amomum unifolium* Gagnep.**	D3	Z123	M.F. Newman & J. Škorničková 2002 (E, SING)	Vietnam	**2927**	48	2x	x	x	x	x	SRR12824523
***Amomum velutinum* X.E.Ye, Škorničk. & N.H.Xia**	B	Z429	J. Leong-Škorničková et al. JLS-1557 (E, PR, SING, VNMN)	Vietnam	**2557**	-	-	x	-	x	x	SRR12824512
***Amomum wandokthong* (Picheans. & Yupparach) Škorničk. & Hlavatá**	D3	Z903	K. Hlavata 1 (PRC)	Thailand	**3902**	-	-	x	-	x	x	SRR12824522
***Amomum wandokthong* (Picheans. & Yupparach) Škorničk. & Hlavatá**	D3	Z730	J. Mood 3000 (PRC)	Thailand	**7679**	-	-	x	-	-	-	-
***Renealmia polypus* Gagnep.**	outgroup	Z575	M. Newman & J. Škorničková 2009 (E)	Central African Rep.	**1224**	-	-	-	x	x	-	SRR7058219

Table S2. Repeat percentages of individual species calculated in the RepeatExplorer analysis. Subtotals that are part of the final repeatome percentage are highlighted in bold. Subtotals of *Ty1-Copia* and *Ty3-Gypsy* superfamilies are bolded and italicized. O = outgroup. *R. = Renealmia*. *Afr. = Aframomum*. *A. = Amomum*.

Species		***R. polypus***	***Afr. melegueta***	***A. subulatum***	***A. petaloideum***	***A.* aff. *repoeense***	***A. calcicola***	***A.* aff. *curtisii***	***A. cinnamomeum***	***A.* aff. *elan***	***A. trilobum***	***A. aff. biphyllum***	***A. mirifl orum***
Clade		**O**	**O**	**A**	**A**	**B**	**C**	**D1**	**D3**	**D3**	**D3**	**D3**	**D3**
Chromosome number (2n)		**NA**	**NA**	**48**	**NA**	**48**	**NA**	**48**	**96**	**48**	**48**	**96**	**48**
Repeat group	**Lineage**	**Proportion (%)**											
***Ty1-Copia* – total**		***19.83***	***23.71***	***40.34***	***48.26***	***45.94***	***50.80***	***27.28***	***29.15***	***44.10***	***37.72***	***41.74***	***31.82***
	Ty1-Copia unclassified	0.11	6.79	11.60	3.03	13.85	1.24	7.16	0.00	0.17	0.00	0.64	14.01
	Alesia	0.00	0.22	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Ale	0.67	0.00	0.05	0.07	0.00	0.11	0.63	0.77	0.58	0.65	0.80	0.42
	Angela	17.29	12.26	14.65	18.77	19.90	17.61	9.68	4.53	9.98	9.46	6.90	5.99
	Ikeros	0.69	0.82	0.59	0.43	0.42	0.35	0.40	0.54	0.43	0.40	0.43	0.32
	Ivana	0.47	0.39	0.02	0.02	0.00	0.00	0.03	0.04	0.06	0.07	0.06	0.02
	SIRE	0.08	0.04	13.16	25.67	11.57	31.24	25.19	22.66	32.42	26.79	32.44	10.77
	TAR	0.40	0.78	0.27	0.28	0.19	0.27	0.35	0.58	0.45	0.32	0.45	0.29
	Tork	0.13	2.42	0.00	0.00	0.00	0.00	0.01	0.03	0.02	0.03	0.02	0.00
***Ty3-Gypsy* – total**		***22.42***	***12.12***	***25.15***	***13.41***	***19.70***	***19.23***	***23.81***	***20.59***	***21.17***	***23.26***	***17.3***	***19.54***
	Ty3-Gypsy unclassified	1.58	0.81	1.28	1.15	1.26	0.26	1.64	1.67	0.36	0.78	0.85	2.28
	Chromovirus unclassified	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00
	CRM	1.19	1.22	0.12	0.18	0.15	0.27	0.39	0.21	0.48	0.54	0.27	0.12
	Galadriel	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Tekay	1.29	0.88	17.47	7.17	10.98	13.10	9.75	15.25	15.08	16.71	11.93	12.97
	OTA unclassified	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Athila	18.11	0.69	2.67	1.89	3.15	2.31	1.49	1.20	3.73	3.92	1.12	1.86
	Retand	0.26	8.52	3.61	3.02	4.16	3.29	1.81	2.25	1.53	1.31	3.14	2.31
LTR unclassifed		5.60	2.33	2.01	1.98	2.25	2.56	23.81	10.29	0.53	2.40	1.53	7.24
LTR – total		**47.47**	**38.16**	**67.50**	**63.66**	**67.89**	**72.60**	**60.03**	**60.04**	**65.80**	**63.38**	**60.58**	**58.59**
LINE		**0.43**	**2.48**	**0.81**	**0.59**	**0.65**	**1.01**	**0.42**	**3.73**	**0.92**	**0.88**	**1.10**	**1.23**
Pararetrovirus and Class I unclassified		**0.32**	**0.10**	**0.00**	**0.02**	**0.00**	**0.02**	**0.01**	**1.32**	**0.02**	**0.87**	**0.00**	**0.00**
DNA transposons – total		**0.45**	**0.45**	**0.64**	**0.51**	**0.27**	**0.38**	**0.56**	**0.59**	**1.43**	**0.47**	**0.44**	**0.27**
	DNA transp. unclassified	0.00	0.00	0.03	0.00	0.00	0.00	0.10	0.00	0.00	0.00	0.00	0.00
	EnSpm_CACTA	1.15	3.59	2.74	8.03	4.05	5.11	6.32	7.95	14.86	6.53	5.90	7.48
	hAT	4.45	0.94	0.00	0.00	0.00	0.00	1.09	3.86	2.27	1.87	0.00	0.00
	Mudr_Mutator	0.00	0.00	7.77	7.55	2.51	6.73	5.26	10.72	38.29	9.08	8.16	4.00
rDNA – total		**2.01**	**6.00**	**0.78**	**0.46**	**0.73**	**1.91**	**2.34**	**1.37**	**0.86**	**2.39**	**1.70**	**1.20**
	rDNA unclassified	0.80	0.00	0.07	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	45S_rDNA	0.00	5.88	0.71	0.45	0.73	1.89	2.30	1.35	0.84	2.37	1.68	1.18
	18S_rDNA	1.16	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00
	5S_rDNA	0.05	0.12	0.00	0.01	0.00	0.02	0.02	0.02	0.02	0.01	0.02	0.02
Satellite		**0.00**	**0.06**	**1.72**	**1.52**	**1.39**	**1.38**	**4.14**	**2.36**	**1.66**	**1.16**	**3.38**	**2.95**
Small clusters		**22.46**	**26.06**	**13.43**	**16.54**	**13.49**	**9.85**	**16.15**	**14.20**	**14.36**	**13.90**	**16.55**	**16.86**
Other unclassified		**1.88**	**2.64**	**3.40**	**3.69**	**2.68**	**0.52**	**0.98**	**3.85**	**2.06**	**2.33**	**2.95**	**3.55**
Single copy sequences		24.59	24.07	11.72	13.02	12.91	12.33	15.11	12.56	12.89	14.63	13.30	15.36
1C genome size (Mbp)		1224	1006	1731	3078	2465	3081	2783	7656	3863	3750	6254	4294
Total repeats (%)		**75.43**	**75.96**	**88.27**	**86.99**	**87.08**	**88.64**	**84.89**	**87.44**	**87.12**	**85.37**	**86.7**	**84.65**

Table S3. Repeat correlations. Correlation between individual repeat groups, superfamilies, and lineages with GS and their phylogenetic signal (Pagel's lambda). Significant values are denoted in bold.

Repeats				Correlation with GS		Phylogenetic signal
Group	**Superfamily**	Family	**Lineage**	**Adj. R2**	**λ**	
LTR-retrotransposons	*Ty1-Copia*		Total *Ty1-Copia*	**0.23**	**0.06**	0.70
			Ale	0.55	0.002	**1.00**
			Angela	-0.08	0.76	0.42
			Ikeros	**0.77**	**<0.001**	0.35
			Ivana	-0.05	0.50	**1.00**
			SIRE	**0.40**	**0.01**	0.08
			TAR	**0.76**	**<0.001**	0.77
			Tork	-0.06	0.62	0.49
	Ty3-Gypsy		Total *Ty3-Gypsy*	**0.23**	**1.00**	
		Chromovirus	CRM	-0.08	0.19	
		Chromovirus	Tekay	**0.48**	0.96	
		Non-chromovirus	Athila	-0.01	0.00	
		Non-chromovirus	Retand	0.10	0.00	
LINE				**0.66**	**<0.001**	0.00
Unclassified LTR				-0.08	0.76	0.00
Pararetrovirus and Class I unclassified				**0.33**	**0.02**	0.00
DNA transposons			Total DNA transposons	0.03	0.44	0.11
			EnSpm/CACTA	0.21	0.07	0.15
			hAT	-0.04	0.50	0.22
			MuDR-Mutator	-0.06	0.62	0.00
Other unclassified				**0.35**	**0.02**	0.09
Satellites				**0.42**	**0.01**	0.65
rDNA				-0.08	0.74	0.36
Total repeats				**0.40**	**0.01**	**1.00**

Table S4. Repeat content in monocot families. Data sourced for repeat abundances in monocot (sub)families from selected genomic studies up to September 2023, accessible at https://www.plabipd.de, with references and links provided.

(sub)family	species	repetitive sequences (%)	LTRs (%)	Ty1-Copia (%)	Ty3-Gypsy (%)	LINE (%)	DNA transposons (%)	reference	download link
Acoraceae	Acorus calamus	46.30	28.50	3.57	11.27	0.24	3.55	Ma, L., Liu, K.W., Li, Z., Hsiao, Y.Y., Qi, Y., Fu, T., Tang, G.D., Zhang, D., Sun, W.H., Liu, D.K. and Li, Y. 2023. Diploid and tetraploid genomes of Acorus and the evolution of monocots. Nature Communications, 14(1), p.3661.	https://www.nature.com/articles/s41467-023-38829-3
Acoraceae	Acorus calamus	54.82	28.20	3.77	13.64	NA	11.46	Shi, T., Huneau, C., Zhang, Y., Li, Y., Chen, J., Salse, J. and Wang, Q. 2022. The slow-evolving Acorus tatarinowii genome sheds light on ancestral monocot evolution. Nature Plants, 8(7), pp.764-777.	https://www.nature.com/articles/s41477-022-01187-x
Acoraceae	Acorus gramineus	50.07	38.40	7.20	28.00	1.59	NA	Guo, X., Wang, F., Fang, D., Lin, Q., Sahu, S.K., Luo, L., Li, J., Chen, Y., Dong, S., Chen, S. and Liu, Y. 2023. The genome of Acorus deciphers insights into early monocot evolution. Nature Communications, 14(1), p.3662.	https://www.nature.com/articles/s41467-023-38836-4#Sec2
Acoraceae	Acorus gramineus	50.56	35.61	3.91	15.93	0.17	2.57	Ma, L., Liu, K.W., Li, Z., Hsiao, Y.Y., Qi, Y., Fu, T., Tang, G.D., Zhang, D., Sun, W.H., Liu, D.K. and Li, Y. 2023. Diploid and tetraploid genomes of Acorus and the evolution of monocots. Nature Communications, 14(1), p.3661.	https://www.nature.com/articles/s41467-023-38829-3
Amaryllidaceae	Allium fistulosum	69.81	62.18	NA	NA	2.00	4.00	Liao, N., Hu, Z., Miao, J., Hu, X., Lyu, X., Fang, H., Zhou, Y.M., Mahmoud, A., Deng, G., Meng, Y.Q. and Zhang, K. 2022. Chromosome-level genome assembly of bunching onion illuminates genome evolution and flavor formation in Allium crops. Nature Communications, 13(1), p.6690.	http://dx.doi.org/10.1038/s41467-022-34491-3
Amaryllidaceae	Allium sativum	91.30	66.20	8.60	57.50	2.50	8.90	Sun, X., Zhu, S., Li, N., Cheng, Y., Zhao, J., Qiao, X., Lu, L., Liu, S., Wang, Y., Liu, C. and Li, B. 2020. A chromosome-level genome assembly of garlic (Allium sativum) provides insights into genome evolution and allicin biosynthesis. Molecular Plant, 13(9), pp.1328-1339.	http://dx.doi.org/10.1016/j.molp.2020.07.019

Araceae	Lemna minor	61.50	NA	18.79	10.59	1.35	5.08	Van Hoeck, A., Horemans, N., Monsieurs, P., Cao, H.X., Vandenhove, H. and Blust, R. 2015. The first draft genome of the aquatic model plant Lemna minor opens the route for future stress physiology research and biotechnological applications. Biotechnology for Biofuels, 8(1), pp.1-13.	http://dx.doi.org/10.1186/s13068-015-0381-1
Araceae	Lemna minuta	58.20	37.40	9.80	19.30	NA	20.07	Abramson, B.W., Novotny, M., Hartwick, N.T., Colt, K., Aevermann, B.D., Scheuermann, R.H. and Michael, T.P. 2022. The genome and preliminary single-nuclei transcriptome of Lemna minuta reveals mechanisms of invasiveness. Plant Physiology, 188(2), pp.879-897.	http://dx.doi.org/10.1093/plphys/kiab564
Araceae	Spirodela intermedia	25.00	14.10	NA	8.96	0.37	NA	Hoang, P.T., Fiebig, A., Novák, P., Macas, J., Cao, H.X., Stepanenko, A., Chen, G., Borisjuk, N., Scholz, U. and Schubert, I. 2020. Chromosome-scale genome assembly for the duckweed Spirodela intermedia, integrating cytogenetic maps, PacBio and Oxford Nanopore libraries. Scientific reports, 10(1), p.19230.	https://www.nature.com/articles/s41598-020-75728-9
Araceae	Spirodela polyrhiza	NA	13.00	1.72	6.06	NA	NA	Wang, W., Haberer, G., Gundlach, H., Gläßer, C., Nussbaumer, T.C.L.M., Luo, M.C., Lomsadze, A., Borodovsky, M., Kerstetter, R.A., Shanklin, J. and Byrant, D.W. 2014. The Spirodela polyrhiza genome reveals insights into its neotenous reduction fast growth and aquatic lifestyle. Nature communications, 5(1), p.3311.	https://www.nature.com/articles/ncomms4311
Arecaceae	Areca catechu	82.20	55.20	NA	NA	3.58	5.80	Yang, Y., Huang, L., Xu, C., Qi, L., Wu, Z., Li, J., Chen, H., Wu, Y., Fu, T., Zhu, H. and Saand, M.A. 2021. Chromosome-scale genome assembly of areca palm (Areca catechu). Molecular ecology resources, 21(7), pp.2504-2519.	https://onlinelibrary.wiley.com/doi/10.1111/1755-0998.13446
Arecaceae	Areca catechu	69.19	61.68	NA	NA	1.05	NA	Zhou, G., Yin, H., Chen, F., Wang, Y., Gao, Q., Yang, F., He, C., Zhang, L. and Wan, Y. 2022. The genome of Areca catechu provides insights into sex determination of monoecious plants. New Phytologist, 236(6), pp.2327-2343.	https://nph.onlinelibrary.wiley.com/doi/10.1111/nph.18471
Arecaceae	Cocos nucifera	74.48	67.10	NA	NA	0.87	2.64	Xiao, Y., Xu, P., Fan, H., Baudouin, L., Xia, W., Bocs, S., Xu, J., Li, Q., Guo, A., Zhou, L. and Li, J. 2017. The genome draft of coconut (Cocos nucifera). Gigascience, 6(11), p.gix095.	https://academic.oup.com/gigascience/article/6/11/gix095/4345653

Arecaceae	Cocos nucifera	78.33	60.26	27.27	20.77	0.34	7.67	Lantican, D.V., Strickler, S.R., Canama, A.O., Gardoce, R.R., Mueller, L.A. and Galvez, H.F. 2019. De novo genome sequence assembly of dwarf coconut (Cocos nucifera L.'Catigan Green Dwarf') provides insights into genomic variation between coconut types and related palm species. G3: Genes, Genomes, Genetics, 9(8), pp.2377-2393.	https://academic.oup.com/g3journal/article/9/8/2377/6026782
Arecaceae	Cocos nucifera	77.29	58.85	36.80	21.44	NA	NA	Muliyar, R.K., Chowdappa, P., Behera, S.K., Kasaragod, S., Gangaraj, K.P., Kotimoole, C.N., Nekrakalaya, B., Mohanty, V., Sampgod, R.B., Banerjee, G. and Das, A.J. 2020. Assembly and annotation of the nuclear and organellar genomes of a dwarf coconut (Chowghat Green Dwarf) possessing enhanced disease resistance. OMICS: A Journal of Integrative Biology, 24(12), pp.726-742.	https://www.liebertpub.com/doi/10.1089/omi.2020.0147
Arecaceae	Elaeis guineensis	NA	54.00	33.00	8.00	NA	2.00	Singh, R., Ong-Abdullah, M., Low, E.T.L., Manaf, M.A.A., Rosli, R., Nookiah, R., Ooi, L.C.L., Ooi, S.E., Chan, K.L., Halim, M.A. and Azizi, N. 2013. Oil palm genome sequence reveals divergence of interfertile species in Old and New worlds. Nature, 500(7462), pp.335-339.	https://www.nature.com/articles/nature12309
Arecaceae	Elaeis guineensis	73.70	55.79	39.46	17.19	NA	NA	Wang, L., Lee, M., Wan, Z.Y., Bai, B., Ye, B., Alfiko, Y., Rahmadsyah, R., Purwantomo, S., Song, Z., Suwanto, A. and Yue, G.H. 2022. Chromosome-level reference genome provides insights into divergence and stress adaptation of the African oil palm. Genomics, Proteomics & Bioinformatics.	https://www.sciencedirect.com/science/article/pii/S1672022922001437?via%3Dihub
Arecaceae	Phoenix dactylifera	38.41	21.99	14.03	4.17	0.46	0.32	Al-Mssallem, I.S., Hu, S., Zhang, X., Lin, Q., Liu, W., Tan, J., Yu, X., Liu, J., Pan, L., Zhang, T. and Yin, Y. 2013. Genome sequence of the date palm Phoenix dactylifera L. Nature communications, 4(1), p.2274.	https://www.nature.com/articles/ncomms3274
Arecaceae	Phoenix dactylifera	52.25	NA	8.78	6.35	2.58	1.93	Hazzouri, K.M., Gros-Balthazard, M., Flowers, J.M., Copetti, D., Lemansour, A., Lebrun, M., Masmoudi, K., Ferrand, S., Dhar, M.I., Fresquez, Z.A. and Rosas, U. 2019. Genome-wide association mapping of date palm fruit traits. Nature Communications, 10(1), p.4680.	https://www.nature.com/articles/s41467-019-12604-9
Arecaceae	Calamus simplicifolius	54.15	47.85	NA	NA	3.77	4.23	Zhao, H., Wang, S., Wang, J., Chen, C., Hao, S., Chen, L., Fei, B., Han, K., Li, R., Shi, C. and Sun, H. 2018. The chromosome-level genome assemblies of two rattans (Calamus simplicifolius and Daemonorops jenkinsiana). Gigascience, 7(9), p.giy097.	https://academic.oup.com/gigascience/article/7/9/giy097/5067873

Arecaceae	Daemonorops jenkinsiana	70.00	61.09	NA	NA	4.62	5.82	Zhao, H., Wang, S., Wang, J., Chen, C., Hao, S., Chen, L., Fei, B., Han, K., Li, R., Shi, C. and Sun, H. 2018. The chromosome-level genome assemblies of two rattans (Calamus simplicifolius and Daemonorops jenkinsiana). Gigascience, 7(9), p.giy097.	https://academic.oup.com/gigascience/article/7/9/giy097/5067873
Arecaceae	Korthalsia laciniosa	10.49	5.54	NA	NA	1.40	0.65	Dasgupta, M.G., Dev, S.A., Parveen, A.B.M., Sarath, P. and Sreekumar, V.B. 2021. Draft genome of Korthalsia laciniosa (Griff.) Mart., a climbing rattan elucidates its phylogenetic position. Genomics, 113(4), pp.2010-2022.	https://www.sciencedirect.com/science/article/pii/S0888754321001506?via%3Dihub
Arecaceae	Nypa fruticans	38.05	23.59	NA	NA	0.91	2.89	He, Z., Feng, X., Chen, Q., Li, L., Li, S., Han, K., Guo, Z., Wang, J., Liu, M., Shi, C. and Xu, S. 2022. Evolution of coastal forests based on a full set of mangrove genomes. Nature ecology & evolution, 6(6), pp.738-749.	https://www.nature.com/articles/s41559-022-01744-9
Asparagaceae	Asparagus setaceus	65.59	42.51	8.08	19.50	2.90	4.12	Li, S.F., Wang, J., Dong, R., Zhu, H.W., Lan, L.N., Zhang, Y.L., Li, N., Deng, C.L. and Gao, W.J. 2020. Chromosome-level genome assembly, annotation and evolutionary analysis of the ornamental plant Asparagus setaceus. Horticulture Research, 7.	http://dx.doi.org/10.1038/s41438-020-0271-y
Asparagaceae	Dracaena cambodiana	53.45	26.13	2.97	22.31	2.56	2.85	Ding, X., Mei, W., Huang, S., Wang, H., Zhu, J., Hu, W., Ding, Z., Tie, W., Peng, S. and Dai, H. 2018. Genome survey sequencing for the characterization of genetic background of Dracaena cambodiana and its defense response during dragon's blood formation. PLoS One, 13(12), p.e0209258.	http://dx.doi.org/10.1371/journal.pone.0209258
Asparagaceae	Dracaena cochinchinensis	60.15	51.06	5.90	41.00	3.43	4.05	Xu, Y., Zhang, K., Zhang, Z., Liu, Y., Lv, F., Sun, P., Gao, S., Wang, Q., Yu, C., Jiang, J. and Li, C. 2022. A chromosome-level genome assembly for Dracaena cochinchinensis reveals the molecular basis of its longevity and formation of dragon's blood. Plant Communications, 3(6).	http://dx.doi.org/10.1016/j.xplc.2022.100456
Asphodelaceae	Aloe vera	82.26	26.71	7.34	19.37	NA	0.13	Jaiswal, S.K., Mahajan, S., Chakraborty, A., Kumar, S. and Sharma, V.K. 2021. The genome sequence of Aloe vera reveals adaptive evolution of drought tolerance mechanisms. Iscience, 24(2).	http://dx.doi.org/10.1016/j.isci.2021.102079
Asphodelaceae	Hemerocallis citrina	86.20	62.40	NA	NA	6.63	12.27	Qing, Z., Liu, J., Yi, X., Liu, X., Hu, G., Lao, J., He, W., Yang, Z., Zou, X., Sun, M. and Huang, P. 2021. The chromosome-level Hemerocallis citrina Borani genome provides new insights into the rutin biosynthesis and the lack of colchicine. Horticulture Research, 8, p.89.	http://dx.doi.org/10.1038/s41438-021-00539-6

Bromeliaceae	Aechmea fasciata	61.72	33.00	3.97	23.92	0.96	0.70	Li, Z., Wang, J., Zhang, X., Zhu, G., Fu, Y., Jing, Y., Huang, B., Wang, X., Meng, C., Yang, Q. and Xu, L. 2022. The genome of Aechmea fasciata provides insights into the evolution of tank epiphytic habits and ethylene-induced flowering. Communications Biology, 5(1), p.920.	https://www.nature.com/articles/s42003-022-03918-4
Bromeliaceae	Ananas bracteatus	74.66	35.98	4.46	17.05	7.23	22.12	Chen, L.Y., VanBuren, R., Paris, M., Zhou, H., Zhang, X., Wai, C.M., Yan, H., Chen, S., Alonge, M., Ramakrishnan, S. and Liao, Z. 2019. The bracteatus pineapple genome and domestication of clonally propagated crops. Nature Genetics, 51(10), pp.1549-1558.	https://www.nature.com/articles/s41588-019-0506-8
Bromeliaceae	Ananas comosus	68.20	44.80	8.23	21.29	2.57	13.18	Feng, L., Wang, J., Mao, M., Yang, W., Adje, M.O., Xue, Y., Zhou, X., Zhang, H., Luo, J., Tang, R. and Tan, L. 2022. The highly continuous reference genome of a leaf-chimeric red pineapple (Ananas comosus var. bracteatus f. tricolor) provides insights into elaboration of leaf color. G3, 12(2), p.jkab452.	https://academic.oup.com/g3journal/article/12/2/jkab452/6501447
Bromeliaceae	Puya raimondii	67.02	47.30	12.32	18.73	NA	0.07	Liu, L., Tumi, L., Suni, M.L., Arakaki, M., Wang, Z.F. and Ge, X.J. 2021. Draft genome of Puya raimondii (Bromeliaceae), the Queen of the Andes. Genomics, 113(4), pp.2537-2546.	https://www.sciencedirect.com/science/article/pii/S0888754321002160?via%3Dihub
Cannaceae	Canna edulis	62.62	22.80	8.20	7.65	3.46	9.63	Fu, Y., Jiang, S., Zou, M., Xiao, J., Yang, L., Luo, C., Rao, P., Wang, W., Ou, Z., Liu, F. and Xia, Z. 2022. High-quality reference genome sequences of two Cannaceae species provide insights into the evolution of Cannaceae. Frontiers in Plant Science, 13.	https://www.frontiersin.org/articles/10.3389/fpls.2022.955904/full
Cannaceae	Canna indica	63.60	24.15	9.25	6.05	3.26	10.59	Fu, Y., Jiang, S., Zou, M., Xiao, J., Yang, L., Luo, C., Rao, P., Wang, W., Ou, Z., Liu, F. and Xia, Z. 2022. High-quality reference genome sequences of two Cannaceae species provide insights into the evolution of Cannaceae. Frontiers in Plant Science, 13.	https://www.frontiersin.org/articles/10.3389/fpls.2022.955904/full
Cannaceae	Canna indica	58.72	33.83	15.84	17.46	NA	18.87	Yang, P., Ling, X.Y., Zhou, X.F., Chen, Y.X., Wang, T.T., Lin, X.J., Zhao, Y.Y., Ye, Y.S., Huang, L.X., Sun, Y.W. and Qi, Y.X. 2023. Comparing genomes of Fructus Amomi-producing species reveals genetic basis of volatile terpenoid divergence. Plant Physiology, p.kiad400.	https://academic.oup.com/plphys/advance-article/doi/10.1093/plphys/kiad400/7222149
Cyperaceae	Carex cristatella	40.04	6.86	4.27	2.43	1.37	9.97	Planta, J., Liang, Y.Y., Xin, H., Chansler, M.T., Prather, L.A., Jiang, N., Jiang, J. and Childs, K.L. 2022. Chromosome-scale genome assemblies and annotations for Poales species Carex cristatella, Carex scoparia, Juncus effusus, and Juncus inflexus. G3, 12(10), p.jkac211.	https://academic.oup.com/g3journal/article/12/10/jkac211/6670624

Cyperaceae	Carex kokanica	52.47	23.84	NA	NA	6.77	26.10	Qu, G., Bao, Y., Liao, Y., Liu, C., Zi, H., Bai, M., Liu, Y., Tu, D., Wang, L., Chen, S. and Zhou, G. 2022. Draft genomes assembly and annotation of Carex parvula and Carex kokanica reveals stress-specific genes. Scientific reports, 12(1), p.4970.	https://www.nature.com/articles/s41598-022-08783-z
Cyperaceae	Carex littledalei	53.93	27.87	NA	NA	5.42	18.53	Can, M., Wei, W., Zi, H., Bai, M., Liu, Y., Gao, D., Tu, D., Bao, Y., Wang, L., Chen, S. and Zhao, X. 2020. Genome sequence of Kobresia littledalei, the first chromosome-level genome in the family Cyperaceae. Sci Data 7: 175.	https://www.nature.com/articles/s41597-020-0518-3
Cyperaceae	Carex myosuroides	51.89	21.68	7.55	8.66	2.27	20.74	Ning, Y., Li, Y., Dong, S.B., Yang, H.G., Li, C.Y., Xiong, B., Yang, J., Hu, Y.K., Mu, X.Y. and Xia, X.F. 2023. The chromosome-scale genome of Kobresia myosuroides sheds light on karyotype evolution and recent diversification of a dominant herb group on the Qinghai-Tibet Plateau. DNA Research, 30(1), p.dsac049.	https://academic.oup.com/dnaresearch/article/30/1/dsac049/6887608
Cyperaceae	Carex parvula	47.97	25.47	NA	NA	5.34	18.60	Qu, G., Bao, Y., Liao, Y., Liu, C., Zi, H., Bai, M., Liu, Y., Tu, D., Wang, L., Chen, S. and Zhou, G. 2022. Draft genomes assembly and annotation of Carex parvula and Carex kokanica reveals stress-specific genes. Scientific reports, 12(1), p.4970.	https://www.nature.com/articles/s41598-022-08783-z
Cyperaceae	Carex scoparia	39.93	6.32	3.68	2.28	1.24	11.68	Planta, J., Liang, Y.Y., Xin, H., Chansler, M.T., Prather, L.A., Jiang, N., Jiang, J. and Childs, K.L. 2022. Chromosome-scale genome assemblies and annotations for Poales species Carex cristatella, Carex scoparia, Juncus effusus, and Juncus inflexus. G3, 12(10), p.jkac211.	https://academic.oup.com/g3journal/article/12/10/jkac211/6670624
Cyperaceae	Cyperus esculentus	33.90	8.67	NA	NA	1.79	5.02	Zhao, X., Yi, L., Ren, Y., Li, J., Ren, W., Hou, Z., Su, S., Wang, J., Zhang, Y., Dong, Q. and Yang, X. 2023. Chromosome-scale genome assembly of the yellow nutsedge (Cyperus esculentus). Genome Biology and Evolution, 15(3), p.evad027.	https://academic.oup.com/gbe/article/15/3/evad027/7049323
Dioscoreaceae	Dioscorea alata	66.82	32.00	9.35	15.47	2.31	1.50	Bredeson, J.V., Lyons, J.B., Oniyinde, I.O., Okereke, N.R., Kolade, O., Nnabue, I., Nwadili, C.O., Hřibová, E., Parker, M., Nwogha, J. and Shu, S. 2022. Chromosome evolution and the genetic basis of agronomically important traits in greater yam. Nature communications, 13(1), p.2001.	http://dx.doi.org/10.1038/s41467-022-29114-w

Dioscoreaceae	Dioscorea zingiberensis	60.17	47.87	4.56	20.46	6.93	8.62	Li, Y., Tan, C., Li, Z., Guo, J., Li, S., Chen, X., Wang, C., Dai, X., Yang, H., Song, W. and Hou, L. 2022. The genome of Dioscorea zingiberensis sheds light on the biosynthesis, origin and evolution of the medicinally important diosgenin saponins. Horticulture Research, 9, p.uhac165.	http://dx.doi.org/10.1093/hr/uhac165
Juncaceae	Juncus effusus	48.64	14.95	10.39	4.39	1.98	3.82	Planta, J., Liang, Y.Y., Xin, H., Chansler, M.T., Prather, L.A., Jiang, N., Jiang, J. and Childs, K.L. 2022. Chromosome-scale genome assemblies and annotations for Poales species Carex cristatella, Carex scoparia, Juncus effusus, and Juncus inflexus. G3, 12(10), p.jkac211.	https://academic.oup.com/g3journal/article/12/10/jkac211/6670624
Juncaceae	Juncus inflexus	42.73	15.62	10.39	5.04	1.94	5.41	Planta, J., Liang, Y.Y., Xin, H., Chansler, M.T., Prather, L.A., Jiang, N., Jiang, J. and Childs, K.L. 2022. Chromosome-scale genome assemblies and annotations for Poales species Carex cristatella, Carex scoparia, Juncus effusus, and Juncus inflexus. G3, 12(10), p.jkac211.	https://academic.oup.com/g3journal/article/12/10/jkac211/6670624
Marantaceae	Thalia dealbata	39.34	12.36	NA	NA	1.17	2.09	Tang, M., Huang, J., Ma, X., Du, J., Bi, Y., Guo, P., Lu, H. and Wang, L. 2023. A near-complete genome assembly of Thalia dealbata Fraser (Marantaceae). Frontiers in Plant Science, 14, p.1183361.	https://www.frontiersin.org/articles/10.3389/fpls.2023.1183361/full
Musaceae	Ensete glaucum	55.02	37.20	17.64	19.25	0.70	7.18	Wang, Z., Rouard, M., Biswas, M.K., Droc, G., Cui, D., Roux, N., Baurens, F.C., Ge, X.J., Schwarzacher, T., Heslop-Harrison, P.J. and Liu, Q. 2022. A chromosome-level reference genome of Ensete glaucum gives insight into diversity and chromosomal and repetitive sequence evolution in the Musaceae. GigaScience, 11.	https://academic.oup.com/gigascience/article/doi/10.1093/gigascience/giac027/6576245
Musaceae	Ensete glaucum	45.17	33.01	13.92	11.79	NA	1.31	Wang, Z.F., Rouard, M., Droc, G., Heslop-Harrison, P. and Ge, X.J. 2023. Genome assembly of Musa beccarii shows extensive chromosomal rearrangements and genome expansion during evolution of Musaceae genomes. GigaScience, 12, p.giad005.	https://academic.oup.com/gigascience/article/doi/10.1093/gigascience/giad005/7049365
Musaceae	Ensete ventricosum	32.65	NA	NA	NA	NA	NA	Harrison, J., Moore, K.A., Paszkiewicz, K., Jones, T., Grant, M.R., Ambacheew, D., Muzemil, S. and Studholme, D.J. 2014. A draft genome sequence for Ensete ventricosum, the drought-tolerant "tree against hunger". Agronomy, 4(1), pp.13-33.	https://www.mdpi.com/2073-4395/4/1/13

Musaceae	Musa acuminata	53.55	40.03	28.86	10.98	2.03	1.93	Wang, Z., Rouard, M., Biswas, M.K., Droc, G., Cui, D., Roux, N., Baurens, F.C., Ge, X.J., Schwarzacher, T., Heslop-Harrison, P.J. and Liu, Q. 2022. A chromosome-level reference genome of Ensete glaucum gives insight into diversity and chromosomal and repetitive sequence evolution in the Musaceae. GigaScience, 11.	https://academic.oup.com/gigascience/article/doi/10.1093/gigascience/giac027/6576245
Musaceae	Musa acuminata	41.85	NA	20.36	14.92	0.81	2.03	Wang, Z., Miao, H., Liu, J., Xu, B., Yao, X., Xu, C., Zhao, S., Fang, X., Jia, C., Wang, J. and Zhang, J. 2019. Musa balbisiana genome reveals subgenome evolution and functional divergence. Nature plants, 5(8), pp.810-821.	https://www.nature.com/articles/s41477-019-0452-6
Musaceae	Musa acuminata	46.70	39.45	29.48	6.11	NA	1.06	Wang, Z.F., Rouard, M., Droc, G., Heslop-Harrison, P. and Ge, X.J. 2023. Genome assembly of Musa beccarii shows extensive chromosomal rearrangements and genome expansion during evolution of Musaceae genomes. GigaScience, 12, p.giad005.	https://academic.oup.com/gigascience/article/doi/10.1093/gigascience/giad005/7049367
Musaceae	Musa acuminata	35.43	32.72	15.62	18.34	0.95	2.13	Wu, W., Yang, Y.L., He, W.M., Rouard, M., Li, W.M., Xu, M., Roux, N. and Ge, X.J. 2016. Whole genome sequencing of a banana wild relative Musa itinerans provides insights into lineage-specific diversification of the Musa genus. Scientific reports, 6(1), pp.1-11.	https://www.nature.com/articles/srep31586
Musaceae	Musa acuminata V2	38.38	26.38	8.96	4.67	2.74	2.18	Belser, C., Baurens, F.C., Noel, B., Martin, G., Cruaud, C., Istace, B., Yahiaoui, N., Labadie, K., Hřibová, E., Doležel, J. and Lemainque, A. 2021. Telomere-to-telomere gapless chromosomes of banana using nanopore sequencing. Communications biology, 4(1), p.1047.	https://www.nature.com/articles/s42003-021-02559-3
Musaceae	Musa balbisiana	49.35	34.12	12.43	5.14	2.94	2.15	Belser, C., Baurens, F.C., Noel, B., Martin, G., Cruaud, C., Istace, B., Yahiaoui, N., Labadie, K., Hřibová, E., Doležel, J. and Lemainque, A. 2021. Telomere-to-telomere gapless chromosomes of banana using nanopore sequencing. Communications biology, 4(1), p.1047.	https://www.nature.com/articles/s42003-021-02559-3
Musaceae	Musa balbisiana	55.75	NA	28.04	12.88	1.30	2.12	Wang, Z., Miao, H., Liu, J., Xu, B., Yao, X., Xu, C., Zhao, S., Fang, X., Jia, C., Wang, J. and Zhang, J. 2019. Musa balbisiana genome reveals subgenome evolution and functional divergence. Nature plants, 5(8), pp.810-821.	https://www.nature.com/articles/s41477-019-0452-6

Musaceae	Musa balbisiana	41.53	35.59	25.90	6.32	NA	1.35	Wang, Z.F., Rouard, M., Droc, G., Heslop-Harrison, P. and Ge, X.J. 2023. Genome assembly of Musa beccarii shows extensive chromosomal rearrangements and genome expansion during evolution of Musaceae genomes. GigaScience, 12, p.giad005.	https://academic.oup.com/gigascience/article/doi/10.1093/gigascience/giad005/7049364
Musaceae	Musa beccarii	51.79	43.47	25.35	9.07	NA	2.79	Wang, Z.F., Rouard, M., Droc, G., Heslop-Harrison, P. and Ge, X.J. 2023. Genome assembly of Musa beccarii shows extensive chromosomal rearrangements and genome expansion during evolution of Musaceae genomes. GigaScience, 12, p.giad005.	https://academic.oup.com/gigascience/article/doi/10.1093/gigascience/giad005/7049363
Musaceae	Musa itinerans	32.12	15.89	7.30	4.37	NA	2.85	Wang, Z.F., Rouard, M., Droc, G., Heslop-Harrison, P. and Ge, X.J. 2023. Genome assembly of Musa beccarii shows extensive chromosomal rearrangements and genome expansion during evolution of Musaceae genomes. GigaScience, 12, p.giad005.	https://academic.oup.com/gigascience/article/doi/10.1093/gigascience/giad005/7049365
Musaceae	Musa itinerans	38.95	34.07	16.70	16.25	1.45	3.15	Wu, W., Yang, Y.L., He, W.M., Rouard, M., Li, W.M., Xu, M., Roux, N. and Ge, X.J. 2016. Whole genome sequencing of a banana wild relative Musa itinerans provides insights into lineage-specific diversification of the Musa genus. Scientific reports, 6(1), pp.1-11.	https://www.nature.com/articles/srep31586
Musaceae	Musa schizocarpa	56.34	39.38	13.77	5.85	3.48	1.74	Belser, C., Baurens, F.C., Noel, B., Martin, G., Cruaud, C., Istace, B., Yahiaoui, N., Labadie, K., Hřibová, E., Doležel, J. and Lemainque, A. 2021. Telomere-to-telomere gapless chromosomes of banana using nanopore sequencing. Communications biology, 4(1), p.1047.	https://www.nature.com/articles/s42003-021-02559-3
Musaceae	Musa schizocarpa	52.26	43.00	30.06	7.23	NA	0.83	Wang, Z.F., Rouard, M., Droc, G., Heslop-Harrison, P. and Ge, X.J. 2023. Genome assembly of Musa beccarii shows extensive chromosomal rearrangements and genome expansion during evolution of Musaceae genomes. GigaScience, 12, p.giad005.	https://academic.oup.com/gigascience/article/doi/10.1093/gigascience/giad005/7049366
Musaceae	Musa textilis Née var. Abuab	53.60	NA	34.46	10.03	0.92	2.13	Galvez, L.C., Koh, R.B.L., Barbosa, C.F.C., Asunto, J.C., Catalla, J.L., Atienza, R.G., Costales, K.T., Aquino, V.M. and Zhang, D. 2021. Sequencing and de novo assembly of abaca (Musa textilis Née) var. Abuab genome. Genes, 12(8), p.1202.	https://www.mdpi.com/2073-4425/12/8/1202

Musaceae	Musa troglodytarum	60.83	51.48	36.40	15.10	0.55	0.41	Li, Z., Wang, J., Fu, Y., Jing, Y., Huang, B., Chen, Y., Wang, Q., Wang, X.B., Meng, C., Yang, Q. and Xu, L. 2022. The Musa troglodytarum L. genome provides insights into the mechanism of non-climacteric behaviour and enrichment of carotenoids. BMC biology, 20(1), p.186.	https://bmcbiol.biomedcentral.com/articles/10.1186/s12915-022-01391-3
Musaceae	Musa acuminata	46.41	39.97	30.09	9.28	NA	5.48	Yang, P., Ling, X.Y., Zhou, X.F., Chen, Y.X., Wang, T.T., Lin, X.J., Zhao, Y.Y., Ye, Y.S., Huang, L.X., Sun, Y.W. and Qi, Y.X. 2023. Comparing genomes of Fructus Amomi-producing species reveals genetic basis of volatile terpenoid divergence. Plant Physiology, p.kiad400.	https://academic.oup.com/plphys/advance-article/doi/10.1093/plphys/kiad400/7222150
Orchidaceae	Apostasia shenzhenica	42.05	22.06	4.97	11.84	12.68	6.46	Zhang, G.Q., Liu, K.W., Li, Z., Lohaus, R., Hsiao, Y.Y., Niu, S.C., Wang, J.Y., Lin, Y.C., Xu, Q., Chen, L.J. and Yoshida, K. 2017. The Apostasia genome and the evolution of orchids. Nature, 549(7672), pp.379-383.	http://dx.doi.org/10.1038/nature23897
Orchidaceae	Cremastra appendiculata	59.15	56.25	NA	NA	1.38	2.70	Wang, J., Xie, J., Chen, H., Qiu, X., Cui, H., Liu, Y., Sahu, S.K., Fang, D., Li, T., Wang, M. and Chen, Y. 2022. A draft genome of the medicinal plant Cremastra appendiculata (D. Don) provides insights into the colchicine biosynthetic pathway. Communications Biology, 5(1), p.1294.	http://dx.doi.org/10.1038/s42003-022-04229-4
Orchidaceae	Cymbidium ensifolium	80.58	48.98	NA	NA	16.46	15.08	Ai, Y., Li, Z., Sun, W.H., Chen, J., Zhang, D., Ma, L., Zhang, Q.H., Chen, M.K., Zheng, Q.D., Liu, J.F. and Jiang, Y.T. 2021. The Cymbidium genome reveals the evolution of unique morphological traits. Horticulture research, 8.	http://dx.doi.org/10.1038/s41438-021-00683-z
Orchidaceae	Dendrobium officinale	63.33	22.23	NA	NA	8.17	4.59	Yan, L., Wang, X., Liu, H., Tian, Y., Lian, J., Yang, R., Hao, S., Wang, X., Yang, S., Li, Q. and Qi, S. 2015. The genome of Dendrobium officinale illuminates the biology of the important traditional Chinese orchid herb. Molecular plant, 8(6), pp.922-934.	http://dx.doi.org/10.1016/j.molp.2014.12.011
Orchidaceae	Phalaenopsis equestris	61.53	46.47	6.95	39.66	7.70	4.63	Cai, J., Liu, X., Vanneste, K., Proost, S., Tsai, W.C., Liu, K.W., Chen, L.J., He, Y., Xu, Q., Bian, C. and Zheng, Z. 2015. The genome sequence of the orchid Phalaenopsis equestris. Nature genetics, 47(1), pp.65-72.	http://dx.doi.org/10.1038/ng.3149
Orchidaceae	Vanilla planifolia	44.30	10.00	NA	NA	10.80	17.00	Hasing, T., Tang, H., Brym, M., Khazi, F., Huang, T. and Chambers, A.H. 2020. A phased Vanilla planifolia genome enables genetic improvement of flavour and production. Nature Food, 1(12), pp.811-819.	http://dx.doi.org/10.1038/s43016-020-00197-2

Poaceae	Streptochaeta angustifolia	66.82	42.90	8.90	28.16	NA	23.39	Seetharam, A.S., Yu, Y., Belanger, S., Clark, L.G., Meyers, B.C., Kellogg, E.A. and Hufford, M.B. 2021. The Streptochaeta genome and the evolution of the grasses. Frontiers in Plant Science, 12, p.710383.	http://dx.doi.org/10.3389/fpls.2021.710383
Poaceae	Phragmites australis	56.19	36.42	NA	NA	1.74	11.43	Oh, D.H., Kowalski, K.P., Quach, Q.N., Wijesinghege, C., Tanford, P., Dassanayake, M. and Clay, K. 2022. Novel genome characteristics contribute to the invasiveness of Phragmites australis (common reed). Molecular Ecology, 31(4), pp.1142-1159.	http://dx.doi.org/10.1111/mec.16293
Poaceae	Raddia guianensis	54.15	29.86	9.01	20.85	2.37	6.01	Guo, Z.H., Ma, P.F., Yang, G.Q., Hu, J.Y., Liu, Y.L., Xia, E.H., Zhong, M.C., Zhao, L., Sun, G.L., Xu, Y.X. and Zhao, Y.J. 2019. Genome sequences provide insights into the reticulate origin and unique traits of woody bamboos. Molecular plant, 12(10), pp.1353-1365.	http://dx.doi.org/10.1016/j.molp.2019.05.009
Poaceae	Cynodon dactylon	37.91	22.79	6.38	9.44	4.95	4.28	Zhang, B., Chen, S., Liu, J., Yan, Y.B., Chen, J., Li, D. and Liu, J.Y. 2022. A high-quality haplotype-resolved genome of common bermudagrass (Cynodon dactylon L.) provides insights into polyploid genome stability and prostrate growth. Frontiers in Plant Science, 13, p.890980.	http://dx.doi.org/10.3389/fpls2022.890980
Poaceae	Eleusine indica	21.90	13.80	NA	NA	1.40	2.90	Zhang, H., Hall, N., Goertzen, L.R., Bi, B., Chen, C.Y., Peatman, E., Lowe, E.K., Patel, J. and McElroy, J.S. 2019. Development of a goosegrass (Eleusine indica) draft genome and application to weed science research. Pest management science, 75(10), pp.2776-2784.	http://dx.doi.org/10.1002/ps.5389
Poaceae	Eragrostis curvula	28.70	16.97	3.14	13.62	NA	4.00	Carballo, J., Santos, B.A.C.M., Zappacosta, D., Garbus, I., Selva, J.P., Gallo, C.A., Díaz, A., Albertini, E., Caccamo, M. and Echenique, V. 2019. A high-quality genome of Eragrostis curvula grass provides insights into Poaceae evolution and supports new strategies to enhance forage quality. Scientific Reports, 9(1), p.10250.	http://dx.doi.org/10.1038/s41598-019-46610-0
Poaceae	Leersia perrieri	26.83	NA	3.91	8.32	2.04	10.07	Stein, J.C., Yu, Y., Copetti, D., Zwickl, D.J., Zhang, L., Zhang, C., Chougule, K., Gao, D., Iwata, A., Goicoechea, J.L. and Wei, S. 2018. Genomes of 13 domesticated and wild rice relatives highlight genetic conservation, turnover and innovation across the genus Oryza. Nature genetics, 50(2), pp.285-296.	https://www.nature.com/articles/s41588-018-0040-0#Tab1

Poaceae	Oryza sativa	30.78	19.85	3.08	16.39	1.11	5.82	Jia, J., Zhao, S., Kong, X., Li, Y., Zhao, G., He, W., Appels, R., Pfeifer, M., Tao, Y., Zhang, X. and Jing, R. 2013. Aegilops tauschii draft genome sequence reveals a gene repertoire for wheat adaptation. Nature, 496(7443), pp.91-95.	http://dx.doi.org/10.1038/nature12028
Poaceae	Oryza sativa	48.70	21.60	3.30	17.70	1.00	13.70	Tanaka, H., Hirakawa, H., Kosugi, S., Nakayama, S., Ono, A., Watanabe, A., Hashiguchi, M., Gondo, T., Ishigaki, G., Muguerza, M. and Shimizu, K. 2016. Sequencing and comparative analyses of the genomes of zoysiagrasses. DNA Research, 23(2), pp.171-180.	http://dx.doi.org/10.1093/dnares/dsw006
Poaceae	Oryza sativa	32.02	23.49	3.45	18.55	1.05	8.53	Jain, R., Jenkins, J., Shu, S., Chern, M., Martin, J.A., Copetti, D., Duong, P.Q., Pham, N.T., Kudrna, D.A., Talag, J. and Schackwitz, W.S. 2019. Genome sequence of the model rice variety KitaakeX. BMC genomics, 20, pp.1-9.	https://bmcgenomics.biomedcentral.com/articles/10.1186/s12864-019-6262-4
Poaceae	Oryza sativa	49.04	NA	4.08	21.89	1.51	20.80	Stein, J.C., Yu, Y., Copetti, D., Zwickl, D.J., Zhang, L., Zhang, C., Chougule, K., Gao, D., Iwata, A., Goicoechea, J.L. and Wei, S. 2018. Genomes of 13 domesticated and wild rice relatives highlight genetic conservation, turnover and innovation across the genus Oryza. Nature genetics, 50(2), pp.285-296.	https://www.nature.com/articles/s41588-018-0040-0#Tab1
Poaceae	Alloteropsis semialata	51.00	NA	4.63	31.05	NA	3.44	Dunning, L.T., Olofsson, J.K., Parisod, C., Choudhury, R.R., Moreno-Villena, J.J., Yang, Y., Dionora, J., Quick, W.P., Park, M., Bennetzen, J.L. and Besnard, G. 2019. Lateral transfers of large DNA fragments spread functional genes among grasses. Proceedings of the National Academy of Sciences, 116(10), pp.4416-4425.	http://dx.doi.org/10.1073/pnas.1810031116
Poaceae	Saccharum spontaneum	57.52	40.64	11.47	28.87	1.55	8.45	Zhang, J., Zhang, X., Tang, H., Zhang, Q., Hua, X., Ma, X., Zhu, F., Jones, T., Zhu, X., Bowers, J. and Wai, C.M. 2018. Allele-defined genome of the autopolyploid sugarcane Saccharum spontaneum L. Nature genetics, 50(11), pp.1565-1573.	http://dx.doi.org/10.1038/s41588-018-0237-2
Poaceae	Sorghum bicolor	65.83	49.70	6.81	42.85	0.98	7.17	Jia, J., Zhao, S., Kong, X., Li, Y., Zhao, G., He, W., Appels, R., Pfeifer, M., Tao, Y., Zhang, X. and Jing, R. 2013. Aegilops tauschii draft genome sequence reveals a gene repertoire for wheat adaptation. Nature, 496(7443), pp.91-95.	http://dx.doi.org/10.1038/nature12028
Poaceae	Sorghum bicolor	64.80	47.90	6.50	41.40	1.40	9.10	Tanaka, H., Hirakawa, H., Kosugi, S., Nakayama, S., Ono, A., Watanabe, A., Hashiguchi, M., Gondo, T., Ishigaki, G., Muguerza, M. and Shimizu, K. 2016. Sequencing and comparative analyses of the genomes of zoysiagrasses. DNA Research, 23(2), pp.171-180.	http://dx.doi.org/10.1093/dnares/dsw006

Poaceae	Zea mays	82.48	75.52	26.55	48.43	0.80	5.39	Jia, J., Zhao, S., Kong, X., Li, Y., Zhao, G., He, W., Appels, R., Pfeifer, M., Tao, Y., Zhang, X. and Jing, R. 2013. Aegilops tauschii draft genome sequence reveals a gene repertoire for wheat adaptation. Nature, 496(7443), pp.91-95.	http://dx.doi.org/10.1038/nature12028
Poaceae	Zea mays	85.00	74.60	23.70	46.40	1.00	8.60	Schnable, P.S., Ware, D., Fulton, R.S., Stein, J.C., Wei, F., Pasternak, S., Liang, C., Zhang, J., Fulton, L., Graves, T.A. and Minx, P. 2009. The B73 maize genome: complexity, diversity, and dynamics. science, 326(5956), pp.1112-1115.	http://dx.doi.org/10.1126/science.1178534
Poaceae	Zea mays	64.00	59.98	18.30	34.88	0.01	4.01	Jiao, Y., Peluso, P., Shi, J., Liang, T., Stitzer, M.C., Wang, B., Campbell, M.S., Stein, J.C., Wei, X., Chin, C.S. and Guill, K. 2017. Improved maize reference genome with single-molecule technologies. Nature, 546(7659), pp.524-527.	http://dx.doi.org/10.1038/nature22971
Poaceae	Pharus latifolius	78.86	64.30	7.60	46.70	1.32	13.12	Ma, P.F., Liu, Y.L., Jin, G.H., Liu, J.X., Wu, H., He, J., Guo, Z.H. and Li, D.Z. 2021. The Pharus latifolius genome bridges the gap of early grass evolution. The Plant Cell, 33(4), pp.846-864.	http://dx.doi.org/10.1093/plcell/koab015
Poaceae	Aegilops longissima	82.50	70.50	15.50	33.80	0.30	11.10	Avni, R., Lux, T., Minz-Dub, A., Millet, E., Sela, H., Distelfeld, A., Deek, J., Yu, G., Steuernagel, B., Pozniak, C. and Ens, J. 2022. Genome sequences of three Aegilops species of the section Sitopsis reveal phylogenetic relationships and provide resources for wheat improvement. The Plant Journal, 110(1), pp.179-192.	http://dx.doi.org/10.1111/tpj.15664
Poaceae	Aegilops sharonensis	82.30	70.40	15.50	33.70	0.30	11.10	Avni, R., Lux, T., Minz-Dub, A., Millet, E., Sela, H., Distelfeld, A., Deek, J., Yu, G., Steuernagel, B., Pozniak, C. and Ens, J. 2022. Genome sequences of three Aegilops species of the section Sitopsis reveal phylogenetic relationships and provide resources for wheat improvement. The Plant Journal, 110(1), pp.179-192.	http://dx.doi.org/10.1111/tpj.15664
Poaceae	Aegilops speltoides	78.70	69.40	18.30	30.80	0.50	8.30	Avni, R., Lux, T., Minz-Dub, A., Millet, E., Sela, H., Distelfeld, A., Deek, J., Yu, G., Steuernagel, B., Pozniak, C. and Ens, J. 2022. Genome sequences of three Aegilops species of the section Sitopsis reveal phylogenetic relationships and provide resources for wheat improvement. The Plant Journal, 110(1), pp.179-192.	http://dx.doi.org/10.1111/tpj.15664

Poaceae	Aegilops tauschii	80.50	63.80	15.80	26.90	0.40	15.90	Avni, R., Lux, T., Minz-Dub, A., Millet, E., Sela, H., Distelfeld, A., Deek, J., Yu, G., Steuernagel, B., Pozniak, C. and Ens, J. 2022. Genome sequences of three Aegilops species of the section Sitopsis reveal phylogenetic relationships and provide resources for wheat improvement. The Plant Journal, 110(1), pp.179-192.	http://dx.doi.org/10.1111/tpj.15664
Poaceae	Avena atlantica	82.97	66.69	17.26	47.89	0.92	6.00	Maughan, P.J., Lee, R., Walstead, R., Vickerstaff, R.J., Fogarty, M.C., Brouwer, C.R., Reid, R.R., Jay, J.J., Bekele, W.A., Jackson, E.W. and Tinker, N.A. 2019. Genomic insights from the first chromosome-scale assemblies of oat (Avena spp.) diploid species. Bmc Biology, 17(1), pp.1-19.	http://dx.doi.org/10.1186/s12915-019-0712-y
Poaceae	Avena eriantha	83.64	NA	13.84	48.44	1.20	7.40	Maughan, P.J., Lee, R., Walstead, R., Vickerstaff, R.J., Fogarty, M.C., Brouwer, C.R., Reid, R.R., Jay, J.J., Bekele, W.A., Jackson, E.W. and Tinker, N.A. 2019. Genomic insights from the first chromosome-scale assemblies of oat (Avena spp.) diploid species. Bmc Biology, 17(1), pp.1-19.	http://dx.doi.org/10.1186/s12915-019-0712-y
Poaceae	Avena sativa	86.95	70.63	5.74	33.03	4.78	8.96	Peng, Y., Yan, H., Guo, L., Deng, C., Wang, C., Wang, Y., Kang, L., Zhou, P., Yu, K., Dong, X. and Liu, X. 2022. Reference genome assemblies reveal the origin and evolution of allohexaploid oat. Nature Genetics, 54(8), pp.1248-1258.	http://dx.doi.org/10.1038/s41588-022-01127-7
Poaceae	Brachypodium distachyon	37.48	18.38	4.46	13.77	2.94	5.33	Jia, J., Zhao, S., Kong, X., Li, Y., Zhao, G., He, W., Appels, R., Pfeifer, M., Tao, Y., Zhang, X. and Jing, R. 2013. Aegilops tauschii draft genome sequence reveals a gene repertoire for wheat adaptation. Nature, 496(7443), pp.91-95.	http://dx.doi.org/10.1038/nature12028
Poaceae	Brachypodium distachyon	37.70	17.10	4.10	12.80	1.50	2.60	Tanaka, H., Hirakawa, H., Kosugi, S., Nakayama, S., Ono, A., Watanabe, A., Hashiguchi, M., Gondo, T., Ishigaki, G., Muguerza, M. and Shimizu, K. 2016. Sequencing and comparative analyses of the genomes of zoysiagrasses. DNA Research, 23(2), pp.171-180.	http://dx.doi.org/10.1093/dnares/dsw006

Poaceae	Brachypodium distachyon	28.01	21.39	4.86	16.05	1.94	4.77	DNA sequencing and assembly Barry Kerrie 5 Lucas Susan 5 Harmon-Smith Miranda 5 Lail Kathleen 5 Tice Hope 5 Schmutz (Leader) Jeremy 4 Grimwood Jane 4 McKenzie Neil 7 Bevan Michael W. michael. bevan@ bbsrc. ac. uk 7 k, Gene analysis and annotation Haberer Georg 16 Spannagl Manuel 16 Mayer (Leader) Klaus 16 Rattei Thomas 17 Mitros Therese 6 Rokhsar Dan 6 Lee Sang-Jik 18 Rose Jocelyn KC 18 Mueller Lukas A. 19 York Thomas L. 19 and Comparative genomics Salse (Leader) Jerome 27 Murat Florent 27 Abrouk Michael 27 Haberer Georg 16 Spannagl Manuel 16 Mayer Klaus 16 Bruggmann Remy 13 Messing Joachim 13 You Frank M. 8 Luo Ming-Cheng 8 Dvorak Jan 8, 2010. Genome sequencing and analysis of the model grass Brachypodium distachyon. Nature, 463(7282), pp.763-768.	http://dx.doi.org/10.1038/nature08747
Poaceae	Hordeum spontaneum	86.50	74.30	19.30	27.00	NA	12.30	Zhang, W., Tan, C., Hu, H., Pan, R., Xiao, Y., Ouyang, K., Zhou, G., Jia, Y., Zhang, X.Q., Hill, C.B. and Wang, P. 2023. Genome architecture and diverged selection shaping pattern of genomic differentiation in wild barley. Plant biotechnology journal, 21(1), pp.46-62.	http://dx.doi.org/10.1111/pbi.13917
Poaceae	Poa annua	74.50	39.23	12.35	25.88	2.12	3.33	Benson, C.W., Sheltra, M.R., Maughan, P.J., Jellen, E.N., Robbins, M.D., Bushman, B.S., Patterson, E.L., Hall, N.D. and Huff, D.R. 2023. Homoeologous evolution of the allotetraploid genome of Poa annua L. BMC Genomics, 24(1), p.350.	http://dx.doi.org/10.1186/s12864-023-09456-5
Poaceae	Poa infirma	81.20	53.00	14.53	29.03	1.87	2.98	Benson, C.W., Sheltra, M.R., Maughan, P.J., Jellen, E.N., Robbins, M.D., Bushman, B.S., Patterson, E.L., Hall, N.D. and Huff, D.R. 2023. Homoeologous evolution of the allotetraploid genome of Poa annua L. BMC Genomics, 24(1), p.350.	http://dx.doi.org/10.1186/s12864-023-09456-5
Poaceae	Secale cereale	90.31	76.20	15.30	54.90	1.16	11.80	Li, G., Wang, L., Yang, J., He, H., Jin, H., Li, X., Ren, T., Ren, Z., Li, F., Han, X. and Zhao, X. 2021. A high-quality genome assembly highlights rye genomic characteristics and agronomically important genes. Nature genetics, 53(4), pp.574-584.	http://dx.doi.org/10.1038/s41588-021-00808-z
Poaceae	Triticum aestivum	82.30	68.90	16.70	32.40	0.40	12.50	Avni, R., Lux, T., Minz-Dub, A., Millet, E., Sela, H., Distelfeld, A., Deek, J., Yu, G., Steuernagel, B., Pozniak, C. and Ens, J. 2022. Genome sequences of three Aegilops species of the section Sitopsis reveal phylogenetic relationships and provide resources for wheat improvement. The Plant Journal, 110(1), pp.179-192.	http://dx.doi.org/10.1111/tpj.15664

Poaceae	Triticum aestivum	81.70	66.60	10.20	44.10	NA	14.50	International Wheat Genome Sequencing Consortium (IWGSC), Mayer, K.F., Rogers, J., Doležel, J., Pozniak, C., Eversole, K., Feuillet, C., Gill, B., Friebe, B., Lukaszewski, A.J. and Sourdille, P. 2014. A chromosome-based draft sequence of the hexaploid bread wheat (Triticum aestivum) genome. Science, 345(6194), p.1251788.	http://dx.doi.org/10.1126/science.1251788
Poaceae	Triticum aestivum	81.20	63.00	10.30	45.50	NA	17.50	International Wheat Genome Sequencing Consortium (IWGSC), Mayer, K.F., Rogers, J., Doležel, J., Pozniak, C., Eversole, K., Feuillet, C., Gill, B., Friebe, B., Lukaszewski, A.J. and Sourdille, P. 2014. A chromosome-based draft sequence of the hexaploid bread wheat (Triticum aestivum) genome. Science, 345(6194), p.1251788.	http://dx.doi.org/10.1126/science.1251788
Poaceae	Triticum aestivum	79.90	59.90	8.20	39.80	NA	19.30	International Wheat Genome Sequencing Consortium (IWGSC), Mayer, K.F., Rogers, J., Doležel, J., Pozniak, C., Eversole, K., Feuillet, C., Gill, B., Friebe, B., Lukaszewski, A.J. and Sourdille, P. 2014. A chromosome-based draft sequence of the hexaploid bread wheat (Triticum aestivum) genome. Science, 345(6194), p.1251788.	http://dx.doi.org/10.1126/science.1251788
Poaceae	Triticum urartu	66.88	46.66	9.89	36.57	2.34	9.77	Ling, H.Q., Zhao, S., Liu, D., Wang, J., Sun, H., Zhang, C., Fan, H., Li, D., Dong, L., Tao, Y. and Gao, C. 2013. Draft genome of the wheat A-genome progenitor Triticum urartu. Nature, 496(7443), pp.87-90.	http://dx.doi.org/10.1038/nature11997
Typhaceae	Sparganium stoloniferum	61.19	55.71	NA	NA	0.77	1.55	Zou, Y., Wei, Z., Xiao, K., Wu, Z. and Xu, X. 2023. Genomic analysis of the emergent aquatic plant Sparganium stoloniferum provides insights into its clonality, local adaptation and demographic history. Molecular Ecology Resources.	https://onlinelibrary.wiley.com/doi/10.1111/1755-0998.13850
Typhaceae	Typha angustifolia	27.60	15.65	3.32	8.93	NA	NA	Liao, Y., Zhao, S., Zhang, W., Zhao, P., Lu, B., Moody, M.L., Tan, N. and Chen, L. 2023. Chromosome-level genome and high nitrogen stress response of the widespread and ecologically important wetland plant Typha angustifolia. Frontiers in Plant Science, 14, p.1138498.	https://www.frontiersin.org/articles/10.3389/fpls.2023.1138498/full
Typhaceae	Typha latifolia	43.84	15.35	NA	NA	1.22	1.30	Widanagama, S.D., Freeland, J.R., Xu, X. and Shafer, A.B. 2022. Genome assembly, annotation, and comparative analysis of the cattail Typha latifolia. G3, 12(2), p.jkab401.	https://academic.oup.com/g3journal/article/12/2/jkab401/6433155

Alpinioideae	Alpinia oxyphylla	88.06	61.70	NA	NA	1.04	2.44	Pan, K., Dai, S., Tian, J., Zhang, J., Liu, J., Li, M., Li, S., Zhang, S. and Gao, B. 2023. Chromosome-level genome and multi-omics analyses provide insights into the geo-herbalism properties of Alpinia oxyphylla. Frontiers in Plant Science, 14, p.1161257.	https://www.frontiersin.org/articles/10.3389/fpls.2023.1161257/full
Alpinioideae	Elettaria cardamomum	71.15	46.00	36.00	9.00	NA	0.97	Gaikwad, A.B., Kumari, R., Yadav, S., Rangan, P., Wankhede, D.P. and Bhat, K.V. 2023. Small cardamom genome: development and utilization of microsatellite markers from a draft genome sequence of Elettaria cardamomum Maton. Frontiers in Plant Science, 14, p.1161499.	https://www.frontiersin.org/articles/10.3389/fpls.2023.1161499/full
Alpinioideae	Lanxangia tsao-ko	78.90	62.50	44.44	16.69	NA	NA	Li, P., Bai, G., He, J., Liu, B., Long, J., Morcol, T., Peng, W., Quan, F., Luan, X., Wang, Z. and Zhao, Y. 2022. Chromosome-level genome assembly of Amomum tsao-ko provides insights into the biosynthesis of flavor compounds. Horticulture Research, 9.	https://academic.oup.com/hr/article/doi/10.1093/hr/uhac211/6705571
Alpinioideae	Lanxangia tsao-ko	89.15	76.28	35.55	19.16	0.91	1.93	Sun, F., Yan, C., Lv, Y., Pu, Z., Liao, Z., Guo, W. and Dai, M. 2022. Genome Sequencing of Amomum tsao-ko Provides Novel Insight Into Its Volatile Component Biosynthesis. Frontiers in Plant Science, 13.	https://www.frontiersin.org/articles/10.3389/fpls.2022.904178/full
Alpinioideae	Wurfbainia longiligularis	85.27	76.32	51.44	21.30	NA	6.85	Yang, P., Ling, X.Y., Zhou, X.F., Chen, Y.X., Wang, T.T., Lin, X.J., Zhao, Y.Y., Ye, Y.S., Huang, L.X., Sun, Y.W. and Qi, Y.X. 2023. Comparing genomes of Fructus Amomi-producing species reveals genetic basis of volatile terpenoid divergence. Plant Physiology, p.kiad400.	https://academic.oup.com/plphys/advance-article/doi/10.1093/plphys/kiad400/7222147
Alpinioideae	Wurfbainia villosa	87.14	78.94	52.92	23.80	NA	6.40	Yang, P., Ling, X.Y., Zhou, X.F., Chen, Y.X., Wang, T.T., Lin, X.J., Zhao, Y.Y., Ye, Y.S., Huang, L.X., Sun, Y.W. and Qi, Y.X. 2023. Comparing genomes of Fructus Amomi-producing species reveals genetic basis of volatile terpenoid divergence. Plant Physiology, p.kiad400.	https://academic.oup.com/plphys/advance-article/doi/10.1093/plphys/kiad400/7222151
Alpinioideae	Wurfbainia villosa	87.23	78.26	NA	NA	0.73	2.59	Yang, P., Zhao, H.Y., Wei, J.S., Zhao, Y.Y., Lin, X.J., Su, J., Li, F.P., Li, M., Ma, D.M., Tan, X.K. and Liang, H.L. 2022. Chromosome-level genome assembly and functional characterization of terpene synthases provide insights into the volatile terpenoid biosynthesis of Wurfbainia villosa. The Plant Journal, 112(3), pp.630-645.	https://onlinelibrary.wiley.com/doi/10.1111/tpj.15968

Zingiberoideae	Boesenbergia rotunda	72.51	67.16	NA	NA	NA	3.29	Taheri, S., Teo, C.H., Heslop-Harrison, J.S., Schwarzacher, T., Tan, Y.S., Wee, W.Y., Khalid, N., Biswas, M.K., Mutha, N.V., Mohd-Yusuf, Y. and Gan, H.M. 2022. Genome assembly and analysis of the flavonoid and phenylpropanoid biosynthetic pathways in Fingerroot ginger (Boesenbergia rotunda). International Journal of Molecular Sciences, 23(13), p.7269.	https://www.mdpi.com/1422-0067/23/13/7269
Zingiberoideae	Curcuma alismatifolia	72.31	62.92	33.86	27.82	NA	8.76	Yang, P., Ling, X.Y., Zhou, X.F., Chen, Y.X., Wang, T.T., Lin, X.J., Zhao, Y.Y., Ye, Y.S., Huang, L.X., Sun, Y.W. and Qi, Y.X. 2023. Comparing genomes of Fructus Amomi-producing species reveals genetic basis of volatile terpenoid divergence. Plant Physiology, p.kiad400.	https://academic.oup.com/plphys/advance-article/doi/10.1093/plphys/kiad400/7222148
Zingiberoideae	Curcuma alismatifolia	79.28	68.45	NA	NA	1.24	2.46	Dong, Q., Zou, Q.C., Mao, L.H., Tian, D.Q., Hu, W., Cao, X.R. and Ding, H.Q. 2022. The Chromosome-Scale Assembly of the Curcuma alismatifolia Genome Provides Insight Into Anthocyanin and Terpenoid Biosynthesis. Frontiers in Plant Science, 13, p.899588.	https://www.frontiersin.org/articles/10.3389/fpls.2022.899588/full
Zingiberoideae	Curcuma alismatifolia	NA	52.60	31.79	20.81	NA	NA	Liao, X., Ye, Y., Zhang, X., Peng, D., Hou, M., Fu, G., Tan, J., Zhao, J., Jiang, R., Xu, Y. and Liu, J. 2022. The genomic and bulked segregant analysis of Curcuma alismatifolia revealed its diverse bract pigmentation. Abiotech, 3(3), pp.178-196.	https://link.springer.com/article/10.1007/s42994-022-00081-6
Zingiberoideae	Curcuma longa	64.16	27.37	17.19	9.42	1.13	2.26	Chakraborty, A., Mahajan, S., Jaiswal, S.K. and Sharma, V.K. 2021. Genome sequencing of turmeric provides evolutionary insights into its medicinal properties. Communications Biology, 4(1), p.1193.	https://www.nature.com/articles/s42003-021-02720-y
Zingiberoideae	Curcuma longa	70.00	50.00	31.09	21.40	0.64	8.40	Yin, Y., Xie, X., Zhou, L., Yin, X., Guo, S., Zhou, X., Li, Q., Shi, X., Peng, C. and Gao, J. 2022. A chromosome-scale genome assembly of turmeric provides insights into curcumin biosynthesis and tuber formation mechanism. Frontiers in Plant Science, 13, p.3685.	https://www.frontiersin.org/articles/10.3389/fpls.2022.1003835/full
Zingiberoideae	Zingiber officinale	77.81	69.92	35.95	31.43	NA	6.31	Yang, P., Ling, X.Y., Zhou, X.F., Chen, Y.X., Wang, T.T., Lin, X.J., Zhao, Y.Y., Ye, Y.S., Huang, L.X., Sun, Y.W. and Qi, Y.X. 2023. Comparing genomes of Fructus Amomi-producing species reveals genetic basis of volatile terpenoid divergence. Plant Physiology, p.kiad400.	https://academic.oup.com/plphys/advance-article/doi/10.1093/plphys/kiad400/7222147

Zingiberoideae	Zingiber officinale	81.70	56.57	33.66	21.69	1.29	4.30	Cheng, S.P., Jia, K.H., Liu, H., Zhang, R.G., Li, Z.C., Zhou, S.S., Shi, T.L., Ma, A.C., Yu, C.W., Gao, C. and Cao, G.L. 2021. Haplotype-resolved genome assembly and allele-specific gene expression in cultivated ginger. Horticulture Research, 8.	https://academic.oup.com/hr/article/doi/10.1038/s41438-021-00599-8/6446763
Zingiberoideae	Zingiber officinale	56.70	61.06	NA	NA	1.05	1.85	Li, H.L., Wu, L., Dong, Z., Jiang, Y., Jiang, S., Xing, H., Li, Q., Liu, G., Tian, S., Wu, Z. and Wu, B. 2021. Haplotype-resolved genome of diploid ginger (Zingiber officinale) and its unique gingerol biosynthetic pathway. Horticulture Research, 8.	https://academic.oup.com/hr/article/doi/10.1038/s41438-021-00627-7/6446767

Table S5. The list of probes targeted to the GAG domains of Sire and Tekay elements in genus *Amomum*.

Marker	Type of probe	Element (domain)	ML tree inferred from GAG domain (clade specificity)	Sequence	Probe GC content (%)
sire_oligo_cl_I	Oligonucleotide	SIRE - GAG	I	AACTTCAAAGCTTCACCATGAAGAGCAATGAGTCCGTG AGTCAAATGCATGGACGSTTCA	45.0
sire_oligo_cl_II	Oligonucleotide	SIRE - GAG	II	MTTCAGCAGTGCTCAAGAWCTCTGGAGGAAACTGATG GAACTMCATGAGGGCACCCGAGA	51.7
sire_oligo_cl_III	Oligonucleotide	SIRE - GAG	III	AAAGGTATTATCTTTRCATTCTAGATTTAAAGAAATTATT AATGGWTTGTCAAGTGTAGG	25.4
sire_oligo_cl_IV	Oligonucleotide	SIRE - GAG	IV	GACCTCTGGGACAAGTTGGTTGAGCTACACGAGGGTA CTTCGGATACCAAGGTATGYAAA	49.2
sire_oligo_cl_V	Oligonucleotide	SIRE - GAG	V	ATGAAGGRACAAATGATTCTAAAATTGCAAAAAGAGA CATGTTACTTAATAAATTATTTA	22.0
sire_oligo_cl_VI	Oligonucleotide	SIRE - GAG	VI	ATAAAAATGCAGGAAGGWGAGTCRGCWAGTCAGCTCC ACGCCCGGATCAAGGAGATCCTC	52.5
tekay_oligo_cl_I	Oligonucleotide	Tekay - GAG	I	TTCTGCCTKACTGGAGATGCCAGAATGTGGTGGGAACG AGTAAAGGCAAAGAGAGTGGTY	50.0
tekay_oligo_cl_II	Oligonucleotide	Tekay - GAG	II	CTGACTTGGAGAGAGTTCAAGGAAGTGTTCTACCGGA AATACTTTACGGAGGATGTGCGT	46.7
tekay_oligo_cl_IV	Oligonucleotide	Tekay - GAG	IV	AGGCCACCACTTGGTGGGAGACTCAGCAGACAGTTTA TGGCGGGCAGGAAATTTCTTGGT	53.3
tekay_oligo_cl_III	Oligonucleotide	Tekay - GAG	III	GATCAGGCCGTCACYTGGTGGAAGACSCAGARGACKG TATTYGGHGAGCAGGAGRTYWCH	57.7

				Forward primer	Sequence	Reverse primer	Sequence
sire_pcr_cl_II	PCR	SIRE - GAG	II	sire_pcr_cl_II_F	TCCATTCAGCAGTGCTCAGG	sire_pcr_cl_II_R	AACATACCTGGCGAGATCCC
sire_pcr_cl_VI	PCR	SIRE - GAG	VI	sire_pcr_cl_VI_F	CGGTCTAACCAAGGAGGAGC	sire_pcr_cl_VI_R	GTGCAGCCCATTCAGGATCT
tekay_pcr_cl_I	PCR	Tekay - GAG	I	tekay_pcr_cl_I_F	ATGCTCAGGCCTGGTTCAAG	tekay_pcr_cl_I_R	GTTGCCTTGACGAAACTCCG
tekay_pcr_cl_III	PCR	Tekay - GAG	III	tekay_pcr_cl_III_F	ATCATTTCCGGGATCAGGCC	tekay_pcr_cl_III_R	CCCTGCTTTAGGCCCAGAAA

Table S5. Phylogenetically significant repeat clusters. Seventy-five phylogenetically significant clusters, along with their Pagel's lambda and p values, quantities in clades A+B+C versus clade D, and trends marked as increasing (+) or decreasing (-). These clusters are sourced from the RepeatExplorer comparative analysis.

superfamily/group	lineage	λ	p-value	amount in A+B+C (Mb)	amount in D (Mb)	trend	cluster no.
Ty1-Copia	Ale	1	0.003	155	1386	+	89
-	other	1	0.001	17	1086	+	112
-	other	1	0.003	164	479	+	139
-	other	1	0.006	518	243	-	141
-	other	0.967	0.036	428	158	-	154
-	other	1	0.009	406	43	-	167
-	other	1	0.008	270	102	-	174
-	other	1	0.038	15	178	+	193
-	other	1	0.001	169	59	-	200
-	other	1	0.022	4	116	+	208
-	other	1	0.016	81	40	-	221
-	other	1	0.008	81	28	-	225
-	other	1	0.006	5	66	+	232
-	other	1	0.021	10	51	+	244
-	other	1	0.004	95	1	-	247
Ty1-Copia	Angela	1	0.007	5121	1009	-	21
Ty1-Copia	Angela	1	0.027	185	77	-	24
Ty1-Copia	Angela	1	0.002	3796	865	-	32
Ty1-Copia	Angela	1	0.008	3929	698	-	35
Ty1-Copia	Angela	1	0.01	2875	924	-	44
Ty1-Copia	Angela	1	0.007	2963	815	-	45
Ty1-Copia	Angela	1	0.006	2854	557	-	53
Ty1-Copia	Angela	1	0.007	2112	627	-	70
Ty1-Copia	Angela	1	0.009	2232	437	-	73
Ty1-Copia	Angela	1	0.006	1970	565	-	75
Ty1-Copia	Angela	1	0.009	2209	317	-	81
Ty1-Copia	Angela	1	0.005	1842	367	-	92
Ty1-Copia	Angela	1	0.007	1827	368	-	93
Ty1-Copia	Angela	1	0.023	1610	363	-	102
Ty1-Copia	Angela	1	0.006	1402	446	-	104
Ty1-Copia	Angela	1	0.006	1182	273	-	119
Ty1-Copia	Angela	1	0.007	314	58	-	179
Ty3-Gypsy	Athila	1	0.016	1151	646	-	100
Ty3-Gypsy	Athila	1	0.003	1018	353	-	121
-	LINE	1	0.008	741	1918	+	50
-	LINE	1	0.017	168	357	+	149
-	LINE	0.86	0.046	38	133	+	201
-	unclassified LTR	1	0.008	9448	4329	-	1

-	unclassified LTR	1	0.003	9422	3340	-	2
-	unclassified LTR	1	0.014	50	5643	+	12
-	Unclassified LTR	1	0.004	4472	1838	-	18
-	Unclassified LTR	0.937	0.044	416	3219	+	25
-	Unclassified LTR	1	0.001	1943	757	-	65
-	Unclassified LTR	1	0.014	1662	113	-	113
DNA transposons	MuDR_Mutator	0.87	0.043	433	1282	+	84
DNA transposons	MuDR_Mutator	1	0.049	736	407	-	129
Ty3-Gypsy	Retand	1	0.033	3505	1783	-	22
Ty3-Gypsy	Retand	1	0.003	253	1284	+	91
Ty3-Gypsy	Retand	1	0.01	201	513	+	135
Ty1-Copia	SIRE	1	0.002	634	6718	+	4
Ty1-Copia	SIRE	1	0.003	333	5273	+	13
Ty1-Copia	SIRE	1	0.008	1348	4592	+	15
Ty1-Copia	SIRE	1	0.02	3896	2235	-	17
Ty1-Copia	SIRE	1	0.021	2959	1572	-	29
Ty1-Copia	SIRE	1	0.03	1272	2348	+	31
Ty1-Copia	SIRE	1	0.044	4	2969	+	34
Ty1-Copia	SIRE	1	0.028	1193	2245	+	36
Ty1-Copia	SIRE	1	0.031	2628	1292	-	37
Ty1-Copia	SIRE	0.963	0.029	871	1685	+	56
Ty1-Copia	SIRE	1	0.01	32	2145	+	57
Ty1-Copia	SIRE	1	0.014	2164	860	-	58
Ty1-Copia	SIRE	0.963	0.034	785	1538	+	61
Ty1-Copia	SIRE	1	0.024	1982	729	-	67
Ty1-Copia	SIRE	1	0.001	2400	485	-	68
Ty1-Copia	SIRE	1	0.006	1815	800	-	69
Ty1-Copia	SIRE	1	0.031	1292	640	-	94
Ty1-Copia	SIRE	1	0.031	1174	649	-	98
Ty1-Copia	SIRE	1	0.001	1237	410	-	108
Ty1-Copia	SIRE	1	0.002	1165	445	-	111
Ty1-Copia	SIRE	1	0.002	255	795	+	120
Ty1-Copia	SIRE	1	0.001	435	143	-	156
Ty3-Gypsy	Tekay	1	0.011	1539	5618	+	7
Ty3-Gypsy	Tekay	1	0.005	272	824	+	115
Ty3-Gypsy	Tekay	1	0.023	20	715	+	131
Ty3-Gypsy	All	1	0.016	1309	173	-	123