

## A MOLECULAR PHYLOGENY OF *ALPINIA* ROXB. (ZINGIBERACEAE): AN OVERVIEW FOR PHYLOGENY OF VIETNAMESE *ALPINIA*

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ARTICLE INFO	ABSTRACT
<p><b>Received:</b> 23/02/2021</p> <p><b>Revised:</b> 19/3/2021</p> <p><b>Published:</b> 06/4/2021</p>	<p>The genus <i>Alpinia</i> is the largest member of Zingiberaceae, but its phylogeny is still unclear. The studies in Vietnam have been focusing only on new records and new species of <i>Alpinia</i> for the Vietnamese flora. Thus, an overview of the phylogeny of Vietnamese <i>Alpinia</i> is necessary. This work based on molecular data from <i>matK</i> and ITS regions of 149 species confirms that <i>Alpinia</i> is supported to be a polyphyletic group with six different clades. 25/37 Vietnamese <i>Alpinia</i> species were included in the molecular analyses, results indicated that Vietnamese <i>Alpinia</i> formed non-monophyletic group and was placed in clades II, IV and V of <i>Alpinia</i>. Most Vietnamese <i>Alpinia</i> are placed in clade IV, which also included species from Southern China, and South East Asia. Based on the molecular analyses, Vietnamese <i>Alpinia</i> is genetically closely related even though they are distributed in the whole of Vietnam including subtropical and tropical regions and their morphological apomorphies are difficult to find.</p>
<p><b>KEYWORDS</b></p> <p>Molecular phylogeny</p> <p>Nonmonophyletic</p> <p><i>Alpinia</i></p> <p>Overview</p> <p>Vietnam</p>	

## PHÁT SINH LOÀI PHÂN TỬ CỦA CHI RIỀNG *ALPINIA* ROXB. (ZINGIBERACEAE): GÓC NHÌN TỔNG QUAN VỀ PHÁT SINH LOÀI CỦA CHI RIỀNG VIỆT NAM

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THÔNG TIN BÀI BÁO	TÓM TẮT
<p><b>Ngày nhận bài:</b> 23/02/2021</p> <p><b>Ngày hoàn thiện:</b> 19/3/2021</p> <p><b>Ngày đăng:</b> 06/4/2021</p>	<p>Chi Riêng <i>Alpinia</i>, thành viên lớn nhất của Zingiberaceae, có mối quan hệ phát sinh loài chưa rõ ràng. Các nghiên cứu hiện tại ở Việt Nam thường chỉ tập trung vào các ghi nhận loài bổ sung và loài mới của <i>Alpinia</i> cho hệ thực vật Việt Nam. Vì vậy, cần có một cái nhìn tổng quan về phát sinh loài của <i>Alpinia</i> ở Việt Nam. Công trình này dựa trên dữ liệu phân tử từ các vùng gen <i>matK</i> và ITS của 149 loài xác nhận rằng <i>Alpinia</i> được ủng hộ là nhóm phát sinh đa ngành với sáu nhánh khác nhau. 25/37 loài <i>Alpinia</i> Việt Nam được đưa vào phân tích phân tử, kết quả chỉ ra rằng <i>Alpinia</i> Việt Nam không phải nhóm đơn phát sinh và chúng nằm tại các nhánh II, IV và V của <i>Alpinia</i>. Hầu hết các loài <i>Alpinia</i> Việt Nam tập trung nằm tại nhánh IV nơi bao gồm các loài từ miền Nam Trung Quốc và Đông Nam Á. Dựa trên các phân tích phân tử, <i>Alpinia</i> Việt Nam có mối quan hệ di truyền chặt chẽ với nhau mặc dù chúng phân bố trên toàn lãnh thổ Việt Nam với các vùng khí hậu khác nhau như cận nhiệt đới và nhiệt đới và khó tìm thấy các đặc điểm hình thái đặc trưng chung của chúng.</p>
<p><b>TỪ KHÓA</b></p> <p>Phát sinh loài phân tử</p> <p>Không đơn ngành</p> <p><i>Alpinia</i></p> <p>Tổng quan</p> <p>Việt Nam</p>	

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## 1. Introduction

Molecular phylogenetics derived from the traditional method for classifying organisms according to their similarities and differences, was first practiced in a comprehensive fashion by Linnaeus in the 18th century. The naturalists of the 18th and early 19th centuries likened this hierarchy to a "tree of life", an analogy that was adopted by Darwin [1] in *The Origin of Species* as a means of describing the interconnected evolutionary histories of living organisms. The classificatory scheme therefore became reinterpreted as a phylogeny indicating not just the similarities between species but also their evolutionary relationships [2]. The molecular phylogenetic tree can provide many information of phylogenetic relationship and evolutionary history of species.

*Alpinia* Roxb. is the largest and widely spread member of Zingiberaceae, which includes ca. 250 species and distribute in tropical and subtropical Asia, Australia, and Pacific Islands [3], [4].

Several phylogenetic studies of *Alpinia* were conducted during the past two decades; however, the phylogenetic relationship between *Alpinia* is still unclear due to the missing data especially poor sampling. Rangsiruji et al. [5] performed a study of molecular phylogeny for *Alpinia* based on dataset from nuclear and chloroplast DNA of 47 species. The study recognized nine clades within *Alpinia*. Additionally, the authors demonstrated significantly statistical support for several monophyletic groups of species of *Alpinia*, but suggested that the genus may not be monophyletic.

Kress et al. [3], [6] addressed the relationships among genera in the Zingiberaceae. These studies used data from both ITS and *matK* DNA regions. Results of the studies showed a well-resolved phylogenetic relationship among the genera, and provided a new classification of the Zingiberaceae with four subfamilies and four tribes. Kress et al. [6] recognized four clades of *Alpinia*, and demonstrated that a number of the larger genera in the family such as *Amomum*, *Alpinia*, *Etlingeria*, *Boesenbergia*, and *Curcuma* could be nonmonophyletic, and suggested that more extensive sampling is necessary for these genera. Results of Kress et al. [3] recognized that *Alpinia* is polyphyletic; six clades within *Alpinia* were recognized based on the molecular data of 72 taxa of *Alpinia*. A new classification of the tribe Alpinieae was also provided by this study. However, the monophyly and phylogenetic position of a number of *Alpinia*'s members have not yet been established.

In the most recent phylogenetic research of *Alpinia*, Vu et al. [4] reconstructed the phylogeny of *Alpinia* to investigate the phylogenetic position of *A. coriandriodora* within the genus. Result of this study indicated that *Alpinia* is not a monophyletic group, and the species *A. coriandriodora* is closely related to the southern Chinese *Alpinia*.

In Vietnam, *Alpinia* was recognized with 37 species [4], [7]-[9]. In recent years, the studies have been focusing on new records and new species of *Alpinia* for Vietnamese flora. Thus, an overview of the phylogeny of *Alpinia* is necessary.

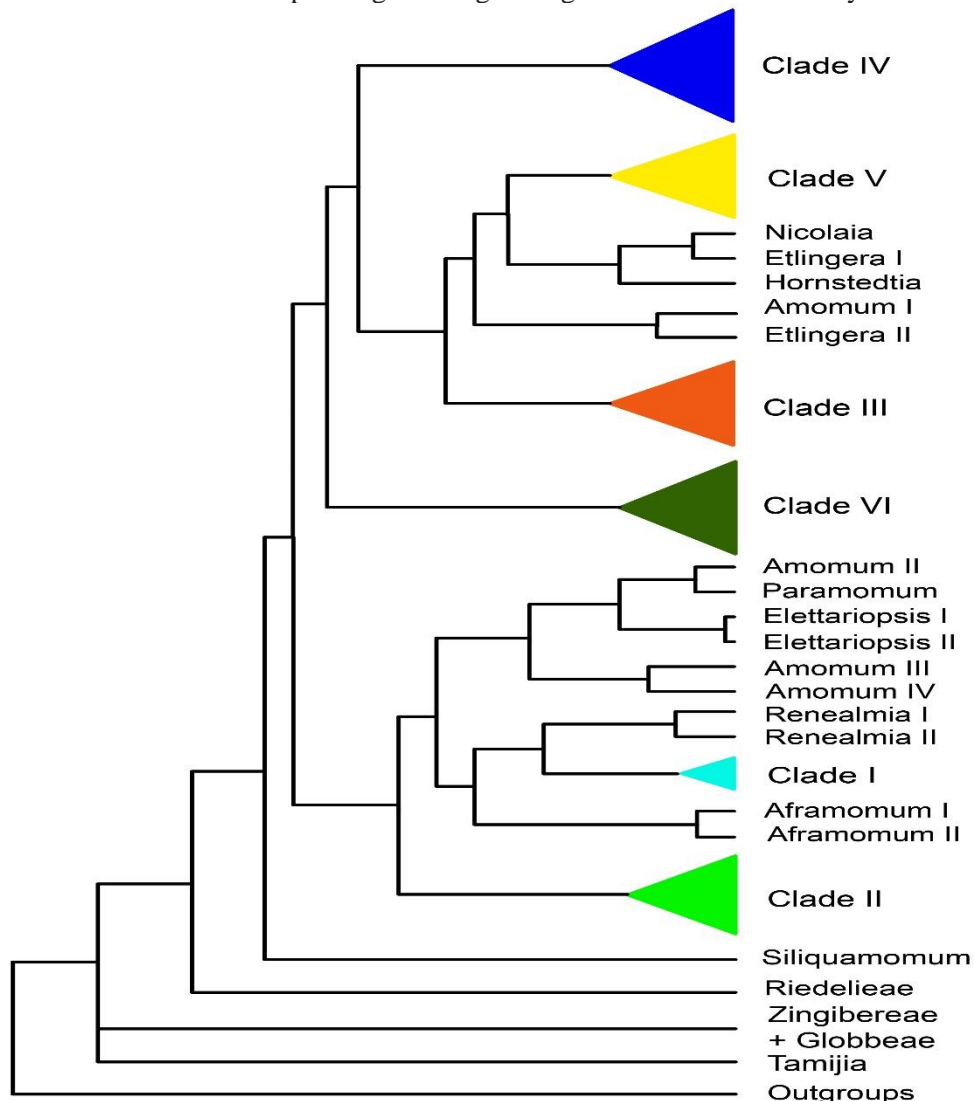
Here we focus on phylogenetic analyses using both nuclear and chloroplast DNA regions based on intensive taxon sampling of *Alpinia*. Our major aims are to reconstruct the phylogeny of *Alpinia* and discuss molecular phylogenetics of *Alpinia* in Vietnam.

## 2. Materials and methods

### 2.1. Taxon sampling

To fully understand the phylogeny of *Alpinia*, we sampled all sections present in *Alpinia* based on the previous phylogenetic results [3], [4], [6]. Approximately 74% of the genus *Alpinia* were sampled, and all relevant sequences were downloaded from GenBank (last downloaded on 6 August 2020). The combined dataset contained 110 species of *Alpinia*. In addition, 39 non-*Alpinia* species of 24 genera following Kress et al. [3], [6] were included for molecular analyses. Two markers *matK* and ITS were used for molecular analyses. All the sequences were carefully

checked using BLAST, and then aligned in Geneious v.8.0.5 [10]. The combined dataset was partitioned into two subsets corresponding to two gene regions for molecular analyses.



**Figure 1.** Summary topology of the molecular tree from maximum likelihood indicating the relationship of clades of *Alpinia*

## 2.2. Molecular phylogenetic analyses

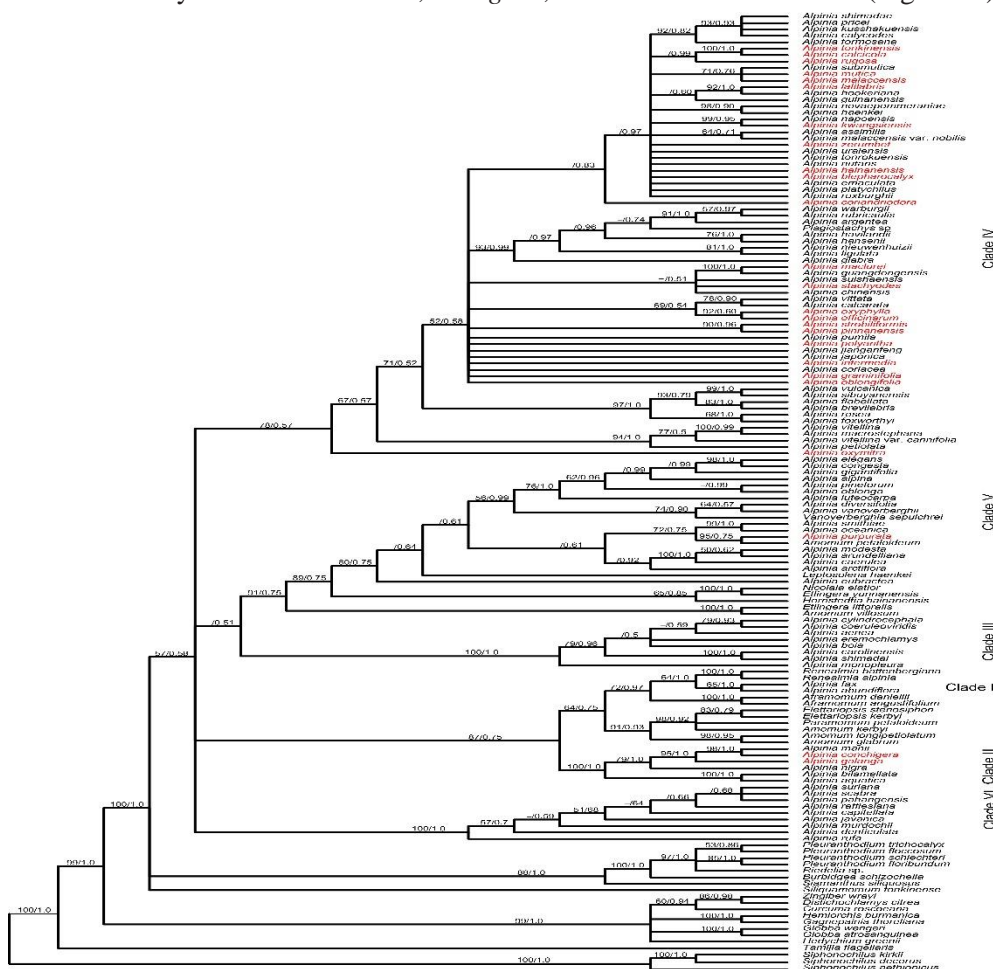
Phylogenetic analyses of *Alpinia* were carried out using maximum likelihood (ML) and Bayesian Inference (BI) methods. ML analyses were conducted in RAxML 8.2.12 [11], applying 1,000 bootstrap replicates with the substitution model selected in jModeltest 2.1.6 [12]. The Bayesian analysis was conducted in MrBayes 3.2.6 [13] as implemented on the CIPRES Science Gateway Portal [14] based on the same models as in the ML analysis. The Markov chain Monte Carlo (MCMC) algorithm was run for 10,000,000 generations with a total of four chains, starting from a random tree, and trees were sampled every 1000 generations. The program Tracer v.1.4 [15] was used to check that effective sample sizes (ESSs) were attained for all relevant parameters assessed (>200). With the first 15% of sampled generations discarded as burn-in, the 50% majority-rule consensus tree and Bayesian posterior probabilities (PP) were obtained using the remaining trees.

### 3. Results and discussion

The combined dataset of 149 taxa including 3752 pb was used in the analyses and discussion. Our molecular results from ML and BI analyses based on combined dataset of different taxon and character sampling strategies were similar, with differences only in clades that were weakly supported. Thus, we summarized the phylogeny of *Alpinia* under maximum likelihood tree in Figure 1. The molecular relationship in detail of *Alpinia* from the ML and BI analyses was presented under Bayesian tree with BS and PP values and shown in Figure 2.

Our molecular results were congruent with the previous studies [3], [6], *Alpinia* was supported to be polyphyletic group. Six clades were recognized within the phylogeny of *Alpinia* (Figure 1). However, the relationships among these clades are not well resolved and show a similar level of resolution to previous results [3], [4], [6].

*Alpinia* was placed into two main clades, the first clade includes clade I and clade II of *Alpinia*; while, the second main clade includes clades III, IV, V and VI of *Alpinia*. Clade I of *Alpinia* was strongly supported as close relative to *Renalmia* (Figures 1 and 2), and clade II of *Alpinia* was supported as sister to all the first main clade (Figures 1). The clade VI of *Alpinia* was supported as sister to the all second main clade (Figures 1), while, the remaining clades of *Alpinia* were closely related to *Nicolaia*, *Etingera*, *Amonum* and *Hornstedtia* (Figures 1).



**Figure 2.** Phylogenetic tree of *Alpinia* from Bayesian inference of the combined datasets of *matK* and *ITS*. ML bootstrap values and posterior probabilities (PP) of the BI analysis are presented above the branches. “–” indicates the support values less than 50%. Vietnamese *Alpinia* species are highlighted in red. The circumscription of clades follows Kress et al. [3].

37 *Alpinia* species were recognized in Vietnam, our molecular phylogenetic analyses indicated that Vietnamese *Alpinia* was a non-monophyletic group and they were placed in clades II, IV and V of *Alpinia* (Figure 2). Clade II includes *Alpinia conchigera* (Riềng rừng) and *A. galanga* (Riềng nếp), the two species were close relative to *A. manii*, *A. nigra*, *A. bilamellata* and *A. aquatica* (Figure 2). These species share morphological characteristics such as: bracteoles funnellform, persistent, enclosing flower buds, bracts small, corolla white or pale blue-green, abaxially pubescent; tube equaling calyx [16].

All the remaining Vietnamese *Alpinia* are placed in clade IV. This clade was weakly supported by molecular data, due to the poorly resolved position of *A. oxymitra*, which is sister to all remaining species in the clade. The clade IV consists of several polytomies due to the highly conservative of the DNA sequences, this result also meets in Kress et al. [3], [6]. This molecular result is congruent with morphology but very difficult to find morphological apomorphies of the clade IV [3]. Most *Alpinia* members of the clade IV were distributed in southern China (Guangdong, Guangxi, Hainan and Yunnan), SE Asia (Thailand, Laos, Cambodia, Myanmar, Vietnam, etc.), in which, several species are endemic to Vietnam such as: *Alpinia vietnamica*, *A. newmanii*, *A. phuthoensis*, *A. calcicola*, *A. brevilligulata*, *A. gagnepainii*, *A. hirsuta*, etc. [9], [17]-[20]. Based on the molecular analyses, members of Vietnamese *Alpinia* are genetic closely related eventhough they are distributed in the whole of Vietnam including subtropical and tropical regions. Furthermore, this result also indicated that *Alpinia* species of southern China and SE Asia are genetically closely related.

Clade V was strongly supported by molecular data (Figure 2). Members of this clade are distributed in the Philippines, Australia, the Bismarck Archipelago, and the tropical Pacific [3]. In clade V, *Alpinia purpurata* was closely related to *Amomum petaloideum* and some other *Alpinia* (Figure 2). This result is advanced than Kress et al. [3]. *Amomum* was supported as non-monophyletic group [3], [this study], thus the placement of *Amomum petaloideum* and the relationship between *Amomum petaloideum* and *Alpinia purpurata* are uncertain and that needs to clarify in a study with more samples of the two genera. Similar to clade IV, it is difficult to characterize this clade morphologically.

#### 4. Conclusion

The study based on molecular analyses confirms that *Alpinia* is supported to be a polyphyletic group with six different clades. 25/37 Vietnamese *Alpinia* species were included in molecular analyses, results indicated that Vietnamese *Alpinia* was non-monophyletic group and they were placed in clades II, IV and V of *Alpinia*. Most of Vietnamese *Alpinia* are placed in clade IV which included species from southern China, and SE Asia. Based on molecular analyses, Vietnamese *Alpinia* has close genetic relationship even they are distributed in the whole of Vietnam including subtropical and tropical regions and difficult to find morphological apomorphies of them.

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