

**MOLECULAR PHYLOGENETIC RELATIONSHIP
OF SOME *CURCUMA* SPECIES (ZINGIBERACEAE) IN VIETNAM****Le Chi Toan***Hanoi Pedagogical University 2*

ARTICLE INFO	ABSTRACT
Received: 04/12/2023 Revised: 05/3/2024 Published: 11/3/2024	The genus <i>Curcuma</i> L. is one of the largest members of Zingiberaceae with about 120 species. Species of <i>Curcuma</i> are of economic, medicinal, ornamental and cultural importance. Several studies to explore the species diversity and phylogenetic relationship of <i>Curcuma</i> have been conducted, but the phylogenetic relationship of <i>Curcuma</i> in Vietnam still remains unclear. This study, based on the comprehensive taxon sampling of <i>Curcuma</i> and molecular data from 4 DNA regions (ITS, <i>matK</i> , <i>psbA-trnH</i> , and <i>trnL-F</i>) supported the monophyly of <i>Curcuma</i> . Three major clades corresponding to three subgenera within the genus were recognized. The <i>Curcuma</i> species recorded in Vietnam are placed in all the three subgenera, but subgenus <i>Ecomata</i> has advantage of quantity. The phylogenetic relationship of <i>Curcuma</i> in Vietnam is well supported by molecular data.
KEYWORDS	
Molecular phylogeny <i>Curcuma</i> Monophyly Zingiberaceae Vietnam	

**NGHIÊN CỨU MỐI QUAN HỆ PHÁT SINH
CỦA MỘT SỐ LOÀI NGHỆ *CURCUMA* TẠI VIỆT NAM****Lê Chí Toàn***Trường Đại học Sư phạm Hà Nội 2*

THÔNG TIN BÀI BÁO	TÓM TẮT
Ngày nhận bài: 04/12/2023 Ngày hoàn thiện: 05/3/2024 Ngày đăng: 11/3/2024	Chi nghệ <i>Curcuma</i> L. là một trong những thành viên lớn nhất của họ Gừng Zingiberaceae với khoảng 120 loài. Các loài <i>Curcuma</i> có vai trò quan trọng về kinh tế, dược liệu, cảnh quan và văn hóa. Một số nghiên cứu nhằm tìm hiểu sự đa dạng thành phần loài và mối quan hệ phát sinh của <i>Curcuma</i> đã được tiến hành, tuy vậy mối quan hệ phát sinh loài của <i>Curcuma</i> ở Việt Nam vẫn chưa được làm rõ. Nghiên cứu này dựa trên việc lấy mẫu phân loại toàn diện của <i>Curcuma</i> và dữ liệu phân tử từ 4 vùng DNA (ITS, <i>matK</i> , <i>psbA-trnH</i> và <i>trnL-F</i>) đã ủng hộ nghệ là nhóm đơn phát sinh. Ba nhánh phát sinh chính tương ứng với ba phân chi của <i>Curcuma</i> đã được ghi nhận. Các loài <i>Curcuma</i> ghi nhận ở Việt Nam có mặt ở cả 3 phân chi, nhưng dường như tập trung chủ yếu tại phân chi <i>Ecomata</i> . Mối quan hệ phát sinh loài của <i>Curcuma</i> ở Việt Nam được hỗ trợ tốt và rõ ràng bởi dữ liệu phân tử.
TỪ KHÓA	
Phát sinh loài phân tử <i>Curcuma</i> Đơn phát sinh Zingiberaceae Việt Nam	

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1. Introduction

Zingiberaceae is the largest member of Zingiberales with 53 genera and over 1300 species [1]. The species of Zingiberaceae are mainly distributed in South and Southeast Asia and some species of the family are found in America and Africa [1], [2]. Within the 53 genera of Zingiberaceae, the genus *Curcuma* L. is one of the largest members with about 120 species [3]-[5]. The main diversity hotspot of the genus is South East Asia, but some *Curcuma* species are also distributed in Australia and the South Pacific Islands [6]. Species of *Curcuma* are of economic, medicinal, ornamental and cultural importance. Several *Curcuma* species are used as food and medicines to treat abdominal pain, stomach, and indigestion diseases [7].

Although this genus was considered to have economic potential, its phylogeny remains poorly understood, an evidence from the studies working on the genus especially for *Curcuma* in Vietnam. Kress et al. [1] conducted the phylogeny and classification of gingers family (Zingiberaceae) based on ITS and *matK* sequences. Results of the study indicated that the genus *Siphonochilus* from Africa and genus *Tamijia* from Borneo are basal clades. Four major clades were recognized within Zingiberaceae, and based on these phylogenetic investigations a new classification of the Zingiberaceae that recognized four subfamilies and four tribes: Siphonochiloideae (Siphonochileae), Tamijioideae (Tamijieae), Alpinioideae (Alpinieae, Riedelieae), and Zingiberoideae (Zingibereae, Globbeae) was proposed. However, the study sampled only three species of *Curcuma* and did not discuss the phylogeny of the genus.

Záveská et al. [5] performed the phylogeny of *Curcuma* based on plastid and nuclear sequences. Sixty-two species of *Curcuma* and four DNA regions (*trnL-trnF*, *psbA-trnH*, *matK* and ITS) were used for the molecular analyses. Result of the study supported a broad generic boundary for *Curcuma*. Four main groups in *Curcuma* s.l. corresponding to three subgenera were documented. Additionally, some species of *Laosanthus*, *Paracautleya*, *Stahlianthus*, *Smithatris*, *Kaempferia* and *Hitchenia* placed within *Curcuma* were treated as members of *Curcuma*. The study also mentioned the vegetative reproduction and hybridization to have likely affected polymorphism of ITS sequences. Further studies are therefore needed to uncover complex relationships in this subgenus.

Gui et al. [8] analyzed the chloroplast genome of some *Curcuma* species, and based on the results, analysis and comparison of gene structures, the study uncovered 7 variable genes that can be used as potential specific DNA barcodes for species identification. The 4 genes *psbA*, *psbD*, *PetA* and *rbcL* closely related to photosynthesis are significant and can contribute to understanding the genetic basis, phylogeny and adaptive evolution of *Curcuma*.

Recently, some new species and new record species of *Curcuma* from Vietnam were published (Figure 1) such as *Curcuma arida* and *C. sahuynhensis* [9], *C. cotuana* [10], *C. singularis* [11], *C. sixsensesensis* and *C. vinhlinhensis* [12], *C. tuanii* [6]. However, few studies have used molecular data to investigate the phylogenetic relationships among taxa. Van et al. [13] only described six sequences of *C. xanthella* and *C. cotuana*. But the study did not reconstruct the phylogeny of *Curcuma*. Thus, the phylogenetic relationship of Vietnamese *Curcuma* still remains unresolved.

The present work aims to (1) reconstruct the phylogeny of genus *Curcuma*, and (2) discuss the phylogenetic relationship of *Curcuma* species from Vietnam.

2. Materials and methods

2.1. Taxon sampling, DNA extraction, amplification, and sequencing

The study sampled 65 species of Zingiberaceae including 59 species of *Curcuma* (61 individuals) to represent all four major groups. Four species *Alpinia conchigera*, *A. galanga*, *Camptandra parvula*, and *Larsenianthus careyanus* were used as outgroups. The samples from Vietnam were collected during field surveys from 2019, and 2023 in Lao Cai province and

additional sequences assembled from NCBI. The 4 primers ITS, *matK*, *psbA-trnH*, and *trnL-F* used for PCR and sequencing for two *Curcuma* individuals in this study followed the protocols described by Závěská et al. [5]. The sequences used in this study are shown in Table 1. Sequences were aligned in Geneious v.8.0.5 using MUSCLE [14].



Figure 1. *Curcuma sahuynhensis* Škorničk. & N.S.Lý from Vietnam. A: Habit and flower, B: Inflorescence

Table 1. Voucher information and GenBank accession numbers for DNA sequences generated or used in this study. “–” indicates missing data, and the sequences generated in this study begin with “*”

Species	Source	ITS	<i>matK</i>	<i>psbA-trnH</i>	<i>trnL-F</i>
<i>Alpinia conchigera</i>	India	JQ409990	AF478812	JN043827	–
<i>Alpinia galanga</i>	–	AF478715	AF478815	EU552528	AY424775
<i>Camptandra parvula</i>	Thailand	AF478730	AF478830	–	AY424780
<i>Curcuma aeruginosa</i>	India	JQ409920	JQ409689	JQ409757	JQ409824
<i>Curcuma alismatifolia</i>	Thailand	JQ409860	JQ409654	JQ409721	JQ409788
<i>Curcuma amarissima</i>	–	KJ803123	KC441212	KC441318	KJ803044
<i>Curcuma angustifolia</i>	India	JQ409972	JQ409685	JQ409753	JQ409820
<i>Curcuma aromatica</i>	India	JQ409910	JQ409699	JQ409767	JQ409834
<i>Curcuma attenuata</i>	Thailand	JQ409978	JQ409691	JQ409759	JQ409826
<i>Curcuma aurantiaca</i>	India	JQ409906	JQ409672	JQ409739	JQ409806
<i>Curcuma bhatii</i>	India	JQ409897	JQ409679	JQ409747	JQ409814
<i>Curcuma bicolor</i>	Thailand	JQ409882	JQ409710	JQ409780	JQ409847
<i>Curcuma borg</i>	Indonesia	JQ409913	JQ409696	JQ409764	JQ409831
<i>Curcuma campanulatus</i>	China	JQ409864	JQ409651	JQ409718	JQ409785
<i>Curcuma candida</i>	Thailand	JQ409988	JQ409704	JQ409773	JQ409840
<i>Curcuma cannanorensis</i>	India	JQ409892	JQ409675	JQ409742	JQ409809

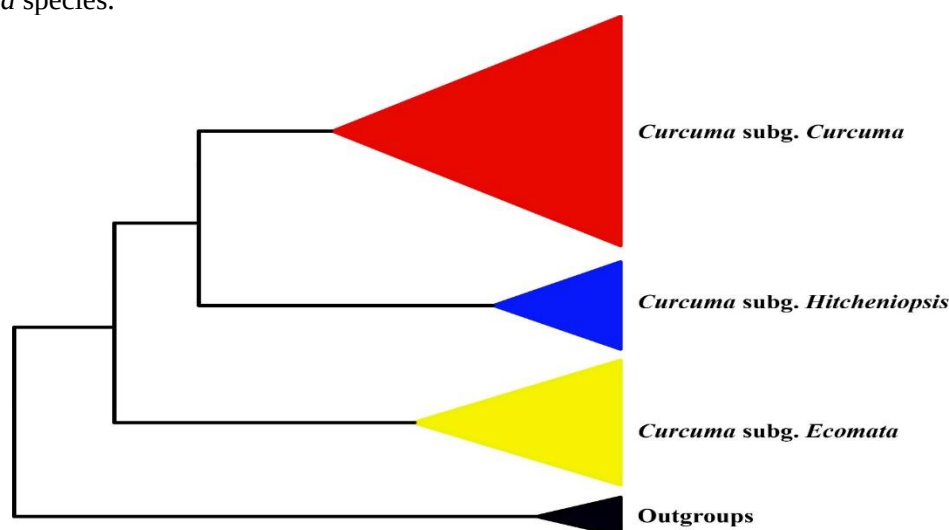
Species	Source	ITS	matK	psbA-trnH	trnL-F
<i>Curcuma caulina</i>	India	JQ409898	JQ409680	JQ409748	JQ409815
<i>Curcuma cochinchinensis</i>	Thailand	JQ409871	JQ409662	JQ409729	JQ409796
<i>Curcuma coriacea</i>	India	JQ409902	JQ409673	JQ409740	JQ409807
<i>Curcuma cotuana</i>	Vietnam	ON545846	ON600879	–	ON564474
<i>Curcuma ecomata</i>	Thailand	JQ409881	JQ409660	JQ409727	JQ409794
<i>Curcuma flaviflora</i>	Thailand	KJ803141	KC441234	KC441342	KJ803066
<i>Curcuma glans</i>	Thailand	JQ409884	JQ409712	JQ409782	JQ409849
<i>Curcuma gracillima</i>	Vietnam	JQ409851	JQ409653	JQ409720	JQ409787
<i>Curcuma graminifolius</i>	Laos	JQ409861	JQ409709	JQ409778	JQ409845
<i>Curcuma harmandii</i>	Thailand	JQ409853	JQ409657	JQ409724	JQ409791
<i>Curcuma inodora</i>	India	JQ409899	KX650809	KY851764	KY303750
<i>Curcuma involucratus</i>	India	JQ409862	JQ409650	JQ409717	JQ409784
<i>Curcuma leucorrhiza</i>	India	JQ409912	JQ409695	JQ409763	JQ409830
<i>Curcuma longa</i>	Sri Lanka	JQ409924	JQ409688	JQ409756	JQ409823
<i>Curcuma montana</i>	India	JQ409932	JQ409701	JQ409769	JQ409836
<i>Curcuma mutabilis</i>	India	JQ409890	JQ409714	JQ409745	JQ409812
<i>Curcuma myanmarensis</i>	Myanmar	JQ409908	JQ409708	JQ409777	JQ409844
<i>Curcuma neilgherrensis</i>	India	JQ409891	JQ409678	JQ409746	JQ409813
<i>Curcuma newmanii</i>	Vietnam	JQ409876	JQ409665	JQ409732	JQ409799
<i>Curcuma ochrorhiza</i>	Indonesia	JQ409916	JQ409693	JQ409761	JQ409828
<i>Curcuma oligantha</i>	Sri Lanka	JQ409889	JQ409715	JQ409772	JQ409839
<i>Curcuma pambrosima</i>	Vietnam	JQ409874	JQ409666	JQ409733	JQ409800
<i>Curcuma parviflora</i>	Thailand	JQ409855	JQ409655	JQ409722	JQ409789
<i>Curcuma petiolata</i>	Thailand	JQ409985	JQ409681	JQ409749	JQ409816
<i>Curcuma pierreana</i>	Vietnam	JQ409879	–	–	–
<i>Curcuma plicata</i>	Vietnam	JQ409979	JQ409692	JQ409760	JQ409827
<i>Curcuma prakasha</i>	India	JQ409923	JQ409702	JQ409770	JQ409837
<i>Curcuma pseudomontana</i>	India	JQ409894	KX650818	KY851761	KY303753
<i>Curcuma reclinata</i>	India	JQ409968	JQ409676	JQ409743	JQ409810
<i>Curcuma rhabdota</i>	Laos	JQ409854	JQ409658	JQ409725	JQ409792
<i>Curcuma rhomba</i>	Vietnam	JQ409878	JQ409668	JQ409735	JQ409802
<i>Curcuma roscoeana</i>	Thailand	JQ409887	JQ409670	JQ409737	JQ409804
<i>Curcuma rubescens</i>	India	JQ409911	JQ409698	JQ409766	JQ409833
<i>Curcuma rubrobracteata</i>	Thailand	JQ409984	JQ409682	JQ409750	JQ409817
<i>Curcuma sahuynhensis</i>	Vietnam	OR139856	–	OR148374	–
<i>Curcuma scaposa</i>	India	JQ409896	–	–	–
<i>Curcuma singularis</i>	Thailand	JQ409869	JQ409664	JQ409731	JQ409798
<i>Curcuma</i> sp.	Vietnam	OR783957*	–	–	–
<i>Curcuma</i> sp.	Vietnam	OR783958*	–	–	–
<i>Curcuma sparganiifolia</i>	Cambodia	JQ409858	–	–	–
<i>Curcuma supraneeana</i>	Thailand	JQ409870	JQ409663	JQ409730	JQ409797
<i>Curcuma thorelii</i>	Vietnam	JQ409852	JQ409652	JQ409719	JQ409786
<i>Curcuma tuanii</i>	Vietnam	OR139855	–	OR148373	OR148376
<i>Curcuma vamana</i>	India	JQ409867	JQ409659	JQ409726	JQ409793
<i>Curcuma vitellina</i>	Vietnam	JQ409873	JQ409667	JQ409734	JQ409801
<i>Curcuma xanthella</i>	Vietnam	OR139857	–	–	OR148377
<i>Curcuma zanthorrhiza</i>	India	JQ409914	JQ409697	JQ409765	JQ409832
<i>Curcuma zedoaria</i>	India	JQ409967	JQ409683	JQ409771	JQ409818
<i>Larsenianthus careyanus</i>	India	JQ409992	KJ872442	KC441413	KJ803106

2.2. Phylogenetic analyses

The study carried out the phylogeny of *Curcuma* by using both maximum likelihood (ML) and Bayesian inference (BI) methods. The phylogenetic trees of *Curcuma* were constructed on

Additionally, the study also evaluated the proportion of variable sites in single molecular data sets by the maximum parsimony method in PAUP* [22].

Manzanilla et al. [23] and Le [24] suggested that the numbers of variable sites and pairwise distances are proportional to the species divergence, though a previous study [25] suggested that the proportion of variable sites may not affect a marker's classification ability. Based on the phylogenetic results in this study (see below section), the low proportion of variable sites in the single and combined data sets of *Curcuma* in this study indicates the close relationship of *Curcuma* species.



from combined dataset had a higher resolution among species within *Curcuma* than single datasets. Thus, the summary and detail of the phylogeny of *Curcuma* is shown in the ML tree with BS and PP values in Figures 2, and 3, respectively. The molecular analysis results strongly support the monophyly of *Curcuma* (Figures 2 and 3). Three subgenera corresponding to three major clades within *Curcuma* were recognized. Subgenus *Ecomata* was placed at the basal clade, and *Curcuma* as sister to *Hitcheniopsis*. These results are in agreement with Závěská et al. [5].

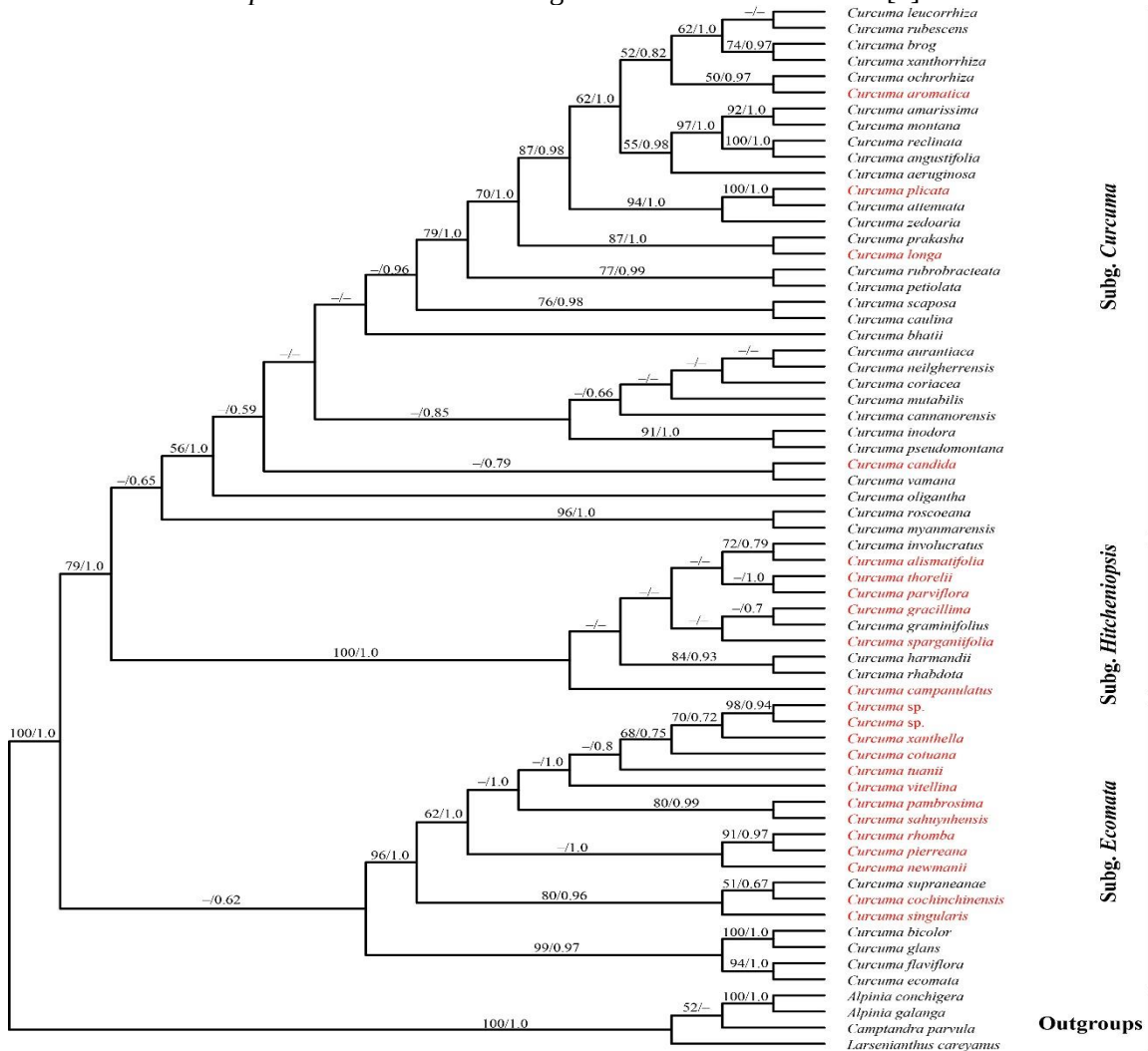


Figure 3. Maximum likelihood (ML) tree derived from analysis of combined dataset of four genes (*ITS*, *matK*, *psbA-trnH*, and *trnL-F*) representing *Curcuma* and outgroups. The *Curcuma* species recorded in Vietnam are in red. ML bootstrap values and posterior probabilities (PP) of the BI analysis are presented above the branches. Support values less than 50% are indicated with “-”.

The *Curcuma* subg. *Ecomata* was supported as sister to the two other subgenera (Figure 2 and 3) [5]. Závěská et al. [5] noted that the species in this subgenus are characterized by the lack of a coma, predominantly more-or-less open flowers, the presence of epigynous glands, and the presence of variably shaped anther spurs. Most members of this subgenus are distributed in SE Asia like Thailand, Laos, Cambodia, and Vietnam. Nguyen et al. [6] suggested that the center of species richness of subgenus *Ecomata* is Thailand with 29 species. Our results indicated that most *Curcuma* species in Vietnam belong to the subgenus *Ecomata* including recently described species such as *Curcuma sahyunhensis*, *C. cotuana*, *C. singularis* and *C. tuanii* (Figure 3). The species in Vietnam are placed at the crown of this clade and they are closely related (Figure 3). In

general, the species in Vietnam of this subgenus are genetically closely related with species from Thailand (Figure 3). Our results suggests that Vietnam and Thailand are the diversity hotspots for the subgenus. However, some nodes in this clade are weakly supported by molecular data, similar to the results in both Závěská et al. [5] and Nguyen et al. [6]. This is likely caused by natural hybridization as mentioned by Závěská et al. [5], and thus can be explored in the future study with comprehensive taxon sampling and data.

The *Curcuma* subg. *Hitcheniopsis* was strongly recognized as monophyletic group (Figure 3). The subgenus can be distinguished from the two other subgenera by the lack of epigynous glands and the lack of anther spurs. The species in this subgenus share the distribution areas with the subgenus *Ecomata* except for few species reaching further westwards to Myanmar, northeast India, and also southern China. Our molecular analysis supported *Curcuma campanulatus* as sister to remaining species in this subgenus. The Vietnamese species in this subgenus seem to have an unstable position in the phylogenetic trees (Figure 3), this result is likely from the hybridization in historical evolution between species in this subgenus with other genera in Zingiberaceae [5].

The *Curcuma* subg. *Curcuma* is the biggest clade in the phylogenetic trees, however, the phylogenetic relationship of species within this subgenus are very complex, it resulted from the natural polyploidization and hybridization during the speciation [3], [26]. The center of diversity of this group is India, Bangladesh and Myanmar, nevertheless some species of the subgenus are widely distributed and cultivated in South and Southeast Asia and elsewhere in the tropics. Our results indicated that only four species of this subgenus are recorded in Vietnam, but they are not closely related, and are placed far from each other (Figure 3). However, these species are popular species and commonly used in the lives of local people such as *Curcuma longa*. Similar to the two subgenera *Hitcheniopsis* and *Ecomata*, our results noted the polytomy and low support values in this clade. This issue seems corresponding to the variation of monoploid genome size between subclades in *Curcuma* subg. *Curcuma* [5].

4. Conclusions

The study supported the monophyly of *Curcuma* based on molecular data from nuclear and chloroplast sequences. Three major clades corresponding to three subgenera within the genus were recognized. The *Curcuma* species recorded in Vietnam are placed in all the three subgenera, but most of them likely place within subgenus *Ecomata*. The phylogenetic relationship of *Curcuma* in Vietnam is well supported by molecular data.

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