A publication of College of Natural and Applied Sciences, Fountain University, Osogbo, Nigeria.
Journal homepage: www.fountainjournals.com
ISSN: 2354-337X(Online),2350-1863(Print)

Phylogenetic Position of Nigerian Species of *Curcuma longa* (Zingiberaceae) in the Current Infrageneric Classification

[1]*Tiamiyu, B. B., [1]Lateef, A. A., [1]Sagaya, A., [1]AbdulKareem, K. A., [1]Olayinka, B. U., [2]Ayinla, A., [1]Adeyemi, S. B., [3]Amenu, S. G., [1]AbdulRahaman, A. A., and [1]Mustapha, O. T.

[1]Department of Plant Biology, Faculty of Life Sciences, University of Ilorin, Ilorin
[2]Department of Biological Sciences, Al-Hikmah University, Ilorin, Nigeria
[3]Key Laboratory of Plant Germplasm Enhancement and Specialty Agriculture, Wuhan Botanical Garden, Chinese Academy of Sciences, Wuhan 430074, China.

Abstract

Curcuma longa L. (commonly known as Tumeric) is the only species of the genus *Curcuma* found in Nigeria. It is of great economic importance to Nigeria, Africa, Asia, and other parts of the world, where it is widely used for ornamental and medicinal purposes, and as spices in food and beverages. However, the phylogenetic placement of the turmeric plant (*C. longa*) in Nigeria is far from being fully resolved, hence the need for this study. The rhizomes of turmeric were collected at the Forestry Research Institute of Nigeria, Ibadan, Oyo state. Genomic DNA was extracted, followed by the amplification of the ITS and *psb*A-*trn*H regions. Phylogenetic analysis was conducted using the Maximum likelihood method. The result resolved the phylogenetic position of Nigerian species and supported existing subgenera classification into three clades, all with high bootstrap support for the three clades. The result of this study supports the subgenera classification of the genus and further reveals the phylogenetic position of *C. longa*.

Keywords: *Curcuma, ITS, psb*A-*trnH*, Sanger *Sequencing, Zingiberaceae*

Introduction

Over the past decades, nuclear and plastid genes have been useful in assessing the phylogenetic relationship and taxonomic significance in various taxonomic groups (Oyebanji *et al*., 2019). The selection of a locus (DNA) for constructing phylogenies is based on characteristics such as copy number, ability to resolve relationships, character congruence (low homoplasy), suitable number of parsimony-informative characters, and rate of evolution in relation to the taxonomic group regardless of gene function (Mort *et al*., 2007).

The nuclear ribosomal DNA (nrDNA) internal transcribed spacer (nrDNA-ITS), and *psb*A-*trn*H spacer regions are among the most important regions in angiosperms that are commonly used as genetic informative regions for plant molecular systematic investigations (Miller *et al*., 2003; Kress and Kenneth, 2005), and they have been proven to be very useful in the understanding of plant phylogenetic placements and taxonomic studies (Yaradua *et al*., 2019; Lateef *et al*., 2019).

The family Zingiberaceae is monocotyledonous and highly natural with ca. 53 genera, which are mostly distributed in India to

* Corresponding Author: +2348060847600
Email address: tiamiyu.bb@unilorin.edu.ng

New Guinea. These genera are grouped into four tribes, mainly according to the nature of the lateral staminodes (Kress *et al*., 2002).

The genus *Curcuma* L. comprises about 160 species worldwide (Leong-Škorničková *et al*., 2007). It is a genus that has experienced hybridization and introgression in its closely related species (Mallet, 2005). On account of the flower parts, shape and colour of bracts, rhizome colour, and position of inflorescences in *Curcuma* are neither unique nor universal across all species, thus; the traditional diagnostic characters for the identification of *Curcuma* is poor and unreliable (Kress *et al*., 2002).

In Nigeria, *Curcuma longa* L. (Synonyms *Amomum Curcuma* Jacq., *Curcuma domestica* Valeton, *Curcuma purpurescens* Blume) is the only representative of the genus *Curcuma.* It is commonly known as Ajo, Laali pupa, and Ata ile pupa in Yoruba. The species has been reported to contain some essential oils, which makes the extract of its roots very useful in phytomedicine for the treatment of cancer, eczema, skin rashes, infections, etc., and as a spice in foods in Nigeria (Uchegbu *et al.,* 2014). It is also used in beauty care, where its juice is applied as a raw paste to soften and smoothen the skin give a glow to it and produce a fairer complexion. It is believed to have an antiseptic effect and promotes healing (Uchegbu *et al.,* 2014).

Before the advent of molecular data, the classification of the Zingiberaceae family was primarily based on floral and vegetative characteristics as well as karyological data (Larsen *et al*., 1998). Molecular techniques have evolved, leading to the emergence of more DNA regions, combining ITS and *psb*A-*trn*H for phylogenetic analysis. Despite these advances in plant systematics, the phylogenetic position of *Curcuma longa* in Nigeria is still far from being completely resolved. Hence, this study aimed to provide a clear phylogenetic position of the common turmeric species (*Curcuma longa*) in Nigeria.

Materials and Methods

Taxon sampling

The plant materials (leaves and rhizomes) were collected at the Forestry Research Institute of Nigeria (FRIN) Arboretum, Ibadan, Oyo State. Sample identification was carried out at the herbarium section in the Department of Plant Biology, University of Ilorin, and a voucher specimen (UILH/003/1403) was deposited at the same herbarium. To ensure that quality DNA from the samples were obtained, the rhizomes were planted in plastic buckets filled with sand, kept in the laboratory, and fresh leaves were obtained for molecular studies after a week of planting.

DNA Extraction, PCR and Sequencing

The total genomic DNA was extracted using a commercial Kit (Zymo Research) following the manufacturer's instructions. The selected plant parts were carefully cut from the plant using sterilized razor blades into a 2 ml Eppendorf tube, stored in an icebox, and transported to the laboratory. The extracted DNA was used for polymerase chain reaction (PCR) amplification using the *psb*A/ *trn*Hf_05 and ITS5/ITS4 primer pairs as forward/reverse primers for the ITS and chloroplast (*trnH—psbA*) regions respectively (Table 1).

A PCR mixture of 25 µl total volume was used for the amplification. The mixture was prepared by adding 12.5 µl of PCR Master Mix (containing 5X Mg free-PCR buffer, 2 µl of $MgCl_2$, 0.5 µl of 10mM dNTPs), 1.5 µl of each primer, 0.15 µl of Fermentas Taq DNA polymerase, 9.85 µl of double-sterilized distilled water (ddH_2O)), 1.0 µl of the extracted DNA and ddH_2O for the control reaction. The thermal cycling programme used was: 2 min at 94 ˚C, followed by 45 cycles at 94˚C for 1 min, annealing at varying temperatures for the different primers (55˚C for 1 min, 56 ˚C for 30 secs, and 64 ˚C for 30 secs), extension 72 ˚C for 1 min, 40 secs and 45 secs respectively; and a final extension at 72˚C for 10 mins, 7 mins, and 10 mins. Successful PCR products were sent to Inqaba Biotech sequencing company (South Africa) for forward and reverse primer Sanger sequencing.

Table 1: Primers used in this study and their respective sequences and melting points.

S/NO	NAME	SEQUENCE	MELTING TEMPERATURE (^{O}C)	Reference
1.	*psb*A_3 (forward)	GTT ATG CAT GAA CGT AAT GCT C	58.95	Kress et. al., 2005; CBOL plant working group, 2009
2.	*trn*Hf_05 (reverse)	CGC GCA TGG TGG ATT CAC AAT CC	66.33	Kress et. al., 2005; CBOL plant working group, 2009
3.	ITS 5(forward)	GGAAGTAAAAGTCGTAA CAAGG	58.95	White *et al*., 1990
4.	ITS 4 (reverse)	TCCTCCGCTTATTGATATGC	58.35	White *et al*., 1990

Phylogenetic Analysis

The forward and reverse sequences were assembled using BioEdit and consensus was obtained. *ITS* and *psbA-trnH* regions of 70 other *Curcuma* species were downloaded from Genbank and included in the analysis (see appendix).

PhyloSuite (Zhang *et al*., 2020) was used to conduct, manage, and streamline the analyses with the help of several plug-in programs. Batch alignment of sequences for each region was performed with MAFFT (Katoh and Standley, 2013) using the '--auto' strategy and normal alignment mode. Ambiguously aligned fragments were removed in batches using Gblocks (Talavera and Castresana, 2007) with the following settings: minimum number of sequences for a conserved/flank position (38/38), maximum number of contiguous non-conserved positions (8), minimum length of a block (10), allowed gap positions (with half). The best-fit model was selected in ModelFinder (Kalyaanamoorthy *et al*., 2017) using the BIC criterion. Maximum likelihood phylogenies were inferred using IQ-TREE (Nguyen *et al*., 2015) under the model automatically selected by IQ-TREE ('Auto' option in IQ-TREE) for 20000 ultrafast (Minh *et al*., 2013) bootstraps, as well as the Shimodaira–Hasegawa–like approximate likelihood-ratio test (Guindon *et al*., 2010). The trees were then visualized using iTOL (Letunic and Bork, 2021).

Results

The sequenced ITS region of our *Curcuma longa* species was 698 bp, while that of the chloroplast *psb*A-*trnH* was 787 bp. However, sequence homology was 99% for both regions.

The phylogenetic relationship of our *C. longa* with other *Curcuma* species was determined using Maximum Likelihood (ML) and Bayesian Inference (BI) approach. The total dataset included 74 species (3 outgroups inclusive), with 71 species representing 50% of the total species in the genus. The phylogenetic tree generated by ML based on ITS data revealed largely consistent tree topology but differed at deep phylogenetic levels with the concatenated data tree (Figure 1).

The results indicated that the *Curcuma* species consists of a monophyletic group (Figure 1), with the genus clustering into three monophyletic clades (subgenera): *Curcuma* clade, *Ecomatae* clade, and *Hitcheniopsis* clade.

From the maximum likelihood tree in Fig. 1, three clades are represented, all with high bootstrap supports (98%, 95 and 73%) based on ITS region. The first clade contained species in the subgenus

Table 2: Database search match for similarity

Barcode	Taxonomic Level	Sequence Length	BLAST Similarity	Query Cover	E-Value (BLAST)
ITS	Species	667 bp	*Curcuma longa* isolate 45.9	93%	0.0
***psb*A-*trn*H**	Species	760 bp	*Curcuma longa* voucher DQY2	88%	0.0
***psb*A-*trn*H**	Species	772bp	*Curcuma amada* voucher ZSD01	95%	0.0

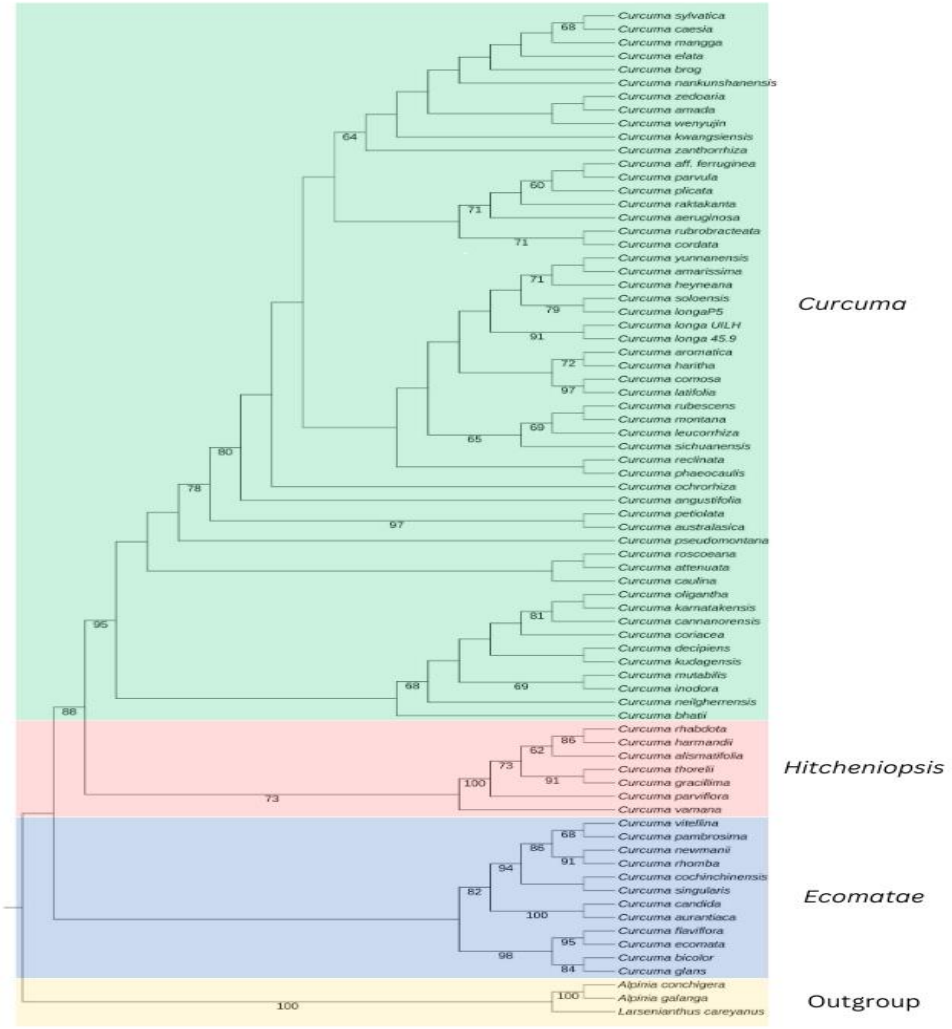

Figure 1. Phylogenetic tree of *Curcuma* based on Maximum likelihood analysis of the ITS region. The numbers in the nodes represent BS values (>50)

Curcuma species with 52 species, subgenus *Hitcheniopsis* with 7 species, and subgenus *Ecomatae* with 12 species with all the clades, showing monophyly. From the *Curcuma* clade, the position of the studied species (*C. longa*) across both ML (Fig. 1) has a support of 79% BS (Fig. 1), while the tree generated from the ML of the *psbA-trnH* (not shown), and concatenated data are not enough to resolve the relationship and clustering based on the infrageneric classification. The results indicated that single-gene (*psb*A-*trn*H) and combined gene data (ITS + *psb*A-*trn*H) revealed a general lack of clade support and topology across the three subgenera. Therefore, single-gene analysis for *psb*A and concatenated genes analysis are not shown here. However, the node of interest (*Curcuma longa*) in the current study is well supported.

Discussion

Previous studies have attempted the infrageneric classification of the genus *Curcuma*, using various characters for many years and were found problematic due to reliance on herbarium specimens when some of the member species need to be observed for a prolonged period especially the inflorescence and not all the species in the genus were available for critical studies.

Zaveska *et al*. (2012), in their studies, noted that the earlier proposed infrageneric classifications based on inflorescence positions as well as rhizome architecture are not tenable; hence, they proposed a classification based on a comprehensive study of the morphological diversity, geographical range, and phylogeny in the genus. They retained the existing subgenera *Curcuma* and *Hitcheniopsis* and added a new subgenus *Ecomatae,* but not all the species were accommodated. Leong-Skornickova *et al*. (2015), however, in their studies, continued with the outstanding transfers in the genus and resolved more than 80% of the total species population into different subgenera.

This study sampled more than 50% of the species population, and the results presented here showed continuous support for the current infrageneric classification as subgenera, *Curcuma, Ecomatae,* and *Hitcheniopsis.*

The position of our studied sample *C. longa* shows that it clustered with other species in the *Curcuma* subclade. This equally gained backing based on some morphological similarities such as Leaf-blade oblong, glabrous, and base attenuate. Inflorescences terminal on pseudostems, spike cylindric; fertile bracts green; coma bracts ovate; calyx white and labellum yellow in *C. yunnanensis, C. amarissima, C. sichuanensis* while *C. soloensis* exhibited similar rhizome colour with yellowish labellum; bracts pale yellow-green with brownish-purple apices.

Conclusion

In conclusion, the tree analysis showed that it is a good candidate gene for resolving the infrageneric classification in the genus and further supporting existing studies on phylogenetic placements of *C. longa* due to its PCR and sequencing success rates.

Although a number of classifications for *Curcuma* have been proposed, there is a need for further research on the global species diversity and re-classification to support the grouping of this genus.

Abbreviations

Not applicable

References

Guindon, S., Dufayard, J.F., Lefort, V., Anisimova, M., Hordijk, W., & Gascuel, O. (2010). New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. Syst. Biol. 59, 307-321.

Kalyaanamoorthy, S., Minh, B.Q., Wong, T.K.F., von Haeseler, A., & Jermiin, L.S. (2017). ModelFinder: fast model selection for accurate phylogenetic estimates. Nat. Methods 14, 587-589.

Katoh, K., & Standley, D.M. (2013). MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol. Biol. Evol. 30, 772-780.

Kress, W. J. & Kenneth, J. (2005). Proceedings of National Academy of Sciences, 8369: 837 [PMID: 15928076].

Kress, W.J., Prince, L.M., & Williams, K.K. (2002). The Phylogeny and a New Classification of the Gingers (Zingiberaceae): Evidence from Molecular Data. *American Journal of Botany*, 89 (11):1682-1696.

Larsen, K., Lock, J.M., Maas, H., & Maas, P. J. M. (1998). Zingiberaceae. In: Kubitzki K., editor. *The families and genera of vascular plants*. vol. 4. Berlin, Springer-Verlag: pp. 474–495.

Ivica Letunic, Peer Bork, Interactive Tree of Life (iTOL) v5: an online tool for phylogenetic tree display and annotation, Nucleic Acids Research, (2021); gkab301, https://doi.org/10.1093/nar/gkab301

Lateef, A. A., Garuba, T., Sa'ad, G., Olesin, M., Eperetun, G. G., & Tiamiyu, B. B. (2019). Isolation and molecular identification of dominant fungal endophytes from green leaves of physic nut (*Jatropha curcas*) from unilorin plantation, Ilorin, Nigeria.

Leong- Škorničková, J., Šída, O., Jarolímová, V., Sabu, M., Fér, T., Trávníček, P., *et al*. (2007). Chromosome numbers and genome size variation in Indian species of Curcuma (Zingiberaceae). *Ann. Bot.* 100, 505–526. doi: 10.1093/aob/

Leong-Škorničková, J., Šída, O., Záveská, E., & Marhold, K. (2015). History of infrageneric classification, typification of supraspecific names, and outstanding transfers in *Curcuma* (Zingiberaceae). *Taxon,64*(2), 362-373. Retrieved June 23, 2020, from www.jstor.org/stable/24639312t

Mallet, J. (2005). Hybridization as an invasion of Zthe genome. Trends Ecol. Evol 20, 229-237. DOI: 10.1016/j.tree2005.02.010.

Miller, J.T., Grimes, J.W., Murphy, D.J., Bayer, R.J., Ladiges, P.Y. (2003) A phylogenetic analysis of the Acacieae and Ingeae (Mimosoideae: Fabaceae) based on *trn*K, *mat*K, *psb*A-*trn*H, and *trn*L/*trn*F sequence data. Systematic Botany 28: 558-566.

Minh, B.Q., Nguyen, M.A., & von Haeseler, A., (2013). Ultrafast approximation for phylogenetic bootstrap. Mol. Biol. Evol. 30, 1188-1195.

Mort, M.E., Archibald, J.K., & Randle, C.P. (2007). Inferring phylogeny at low taxonomic levels: utility of rapidly evolving cpDNA and nuclear ITS loci. American Journal of Botany. 2007; 94:173–183.

Nguyen, L.T., Schmidt, H.A., von Haeseler, A., & Minh, B.Q. (2015). IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Mol. Biol. Evol. 32, 268-274.

Oyebanji, O. O., Chukwuma, E. C., Bolarinwa, K. A., Adejobi, O. I., Adeyemi, S. B., & Ayoola, A. O. (2020). Re-evaluation of the phylogenetic relationships and species delimitation of two closely related families (Lamiaceae and Verbenaceae) using two DNA barcode markers. *Journal of Biosciences*, *45*, 1-15.

Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D.L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M.A., & Huelsenbeck, J.P. (2012). MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Syst. Biol. 61, 539-542.

Talavera, G., & Castresana, J., 2007. Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. Syst. Biol. 56, 564-577.

Uchegbu, R.I., Ngozi–Oleh, L.C & Ogbuneke, R. U. (2014) Essential oils composition of ***Curcuma long***a rhizome from Nigeria. *Am Chem Appl* 1(1):1–5.

White, T. J., Bruns, T., Lee, S. & Taylor, J. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenies. In M. A. Innis, D. H. Gelfand, J. J. Sninsky, and T. J. White. PCR protocols: a guide to methods and applications, 315– 322. Academic Press, San Diego, California, USA.

Yaradua, S. S., Alzahrani, D. A., & Bello, A. (2019). Phylogenetic position of west African species of the genus *Crotalaria* l. (Crotalarieae, Fabaceae) based on the current

infrageneric classification. *Pak. J. Bot*, *51*(4), 1453-1458.

Zaveska, E., Fer, T., Sida, O., Krak, K., Marhold, K. & Leong Skornickova, J. (2012). Phylogeny of *Curcuma* (Zingiberaceae) based on plastid and nuclear sequences: Proposal of the new subgenus *Ecomata*. Taxon 61: 747-7.

Zhang, D., F. Gao, I. Jakovlić, H. Zou, J. Zhang, W.X. Li, G.T. & Wang (2020). PhyloSuite: An integrated and scalable desktop platform for streamlined molecular sequence data management and evolutionary phylogenetics studies. *Molecular Ecology Resources*, 20(1): p. 348–355. DOI: 10.1111/1755-0998.13096.

Appendix

Sequences obtained from NCBI with accession numbers.

	Family	**Genus**	**Species**	**ITS**	***psb*A-*trn*H**
1	Zingiberaceae	*Curcuma*	*aeruginosa*	JQ409983.1	JQ409757.1
2	Zingiberaceae	*Curcuma*	*alismatifolia*	KJ663945.1	JQ409721.1
3	Zingiberaceae	*Curcuma*	*amada*	MF076964.1	KP271441.1
4	Zingiberaceae	*Curcuma*	*amarissima*	KF694827.1	KC441318.1
5	Zingiberaceae	*Curcuma*	*angustifolia*	JQ409973.1	KC441322.1
6	Zingiberaceae	*Curcuma*	*aromatica*	KY129779.1	KY847872.1
7	Zingiberaceae	*Curcuma*	*attenuata*	KJ803130.1	KC441328.1
8	Zingiberaceae	*Curcuma*	*australasica*	KJ803132.1	KC441329.1
9	Zingiberaceae	*Curcuma*	*bhatii*	JQ409897.1	KY963942.1
10	Zingiberaceae	*Curcuma*	*bicolor*	JQ409883.1	JQ409781.1
11	Zingiberaceae	*Curcuma*	*brog*	JQ409919.1	KC441330.1
12	Zingiberaceae	*Curcuma*	*caesia*	MF076953.1	KR996156.1
13	Zingiberaceae	*Curcuma*	*candida*	JQ409988.1	JQ409773.1
14	Zingiberaceae	*Curcuma*	*cannanorensis*	JQ409892.1	JQ409742.1
15	Zingiberaceae	*Curcuma*	*caulina*	JQ409900.1	JQ409748.1
16	Zingiberaceae	*Curcuma*	*cochinchinensis*	DQ395334.1	GQ248280.1
17	Zingiberaceae	*Curcuma*	*comosa*	KY129781.1	HM748994.1
18	Zingiberaceae	*Curcuma*	*cordata*	KJ803135.1	MF348806.1
19	Zingiberaceae	*Curcuma*	*coriacea*	JQ409907.1	KY978409.1
20	Zingiberaceae	*Curcuma*	*decipiens*	MF595872.1	KY978412.1
21	Zingiberaceae	*Curcuma*	*ecalcarata*	MF595873.1	KY978414.1
22	Zingiberaceae	*Curcuma*	*ecomata*	JQ409881.1	JQ409727.1
23	Zingiberaceae	*Curcuma*	*elata*	HM236123.1	KC441341.1
24	Zingiberaceae	*Curcuma*	*ferruginea*	KJ803114.1	KC441307.1
25	Zingiberaceae	*Curcuma*	*flaviflora*	DQ395335.1	KC441343.1
26	Zingiberaceae	*Curcuma*	*glans*	JQ409885.1	KC441345.1
27	Zingiberaceae	*Curcuma*	*gracillima*	KF709128.1	JQ409720.1
28	Zingiberaceae	*Curcuma*	*haritha*	KY129782.1	KY963938.1
29	Zingiberaceae	*Curcuma*	*harmandii*	KJ663946.1	HM748996.1
30	Zingiberaceae	*Curcuma*	*heyneana*	KJ803142.1	KC441348.1
31	Zingiberaceae	*Curcuma*	*inodora*	KX148601.1	KY851764.1
32	Zingiberaceae	*Curcuma*	*karnatakensis*	MF595875.1	KY963941.1
33	Zingiberaceae	*Curcuma*	*kudagensis*	MF595876.1	KY963939.1
34	Zingiberaceae	*Curcuma*	*kwangsiensis*	KJ461762.1	MK227342.1

35	Zingiberaceae	*Curcuma*	*latifolia*	KY129783.1	HM748995.1
36	Zingiberaceae	*Curcuma*	*leucorrhiza*	KY129784.1	KR996161.1
37	Zingiberaceae	*Curcuma*	*longa*	MN998577.1	MK227316.1
38	Zingiberaceae	*Curcuma*	*mangga*	KY129786.1	KM521589.1
39	Zingiberaceae	*Curcuma*	*montana*	KM983495.1	KY851763.1
40	Zingiberaceae	*Curcuma*	*mutabilis*	JQ409890.1	KY978413.1
41	Zingiberaceae	*Curcuma*	*nankunshanensis*	KJ803150.1	KC441358.1
42	Zingiberaceae	*Curcuma*	*neilgherrensis*	JQ409893.1	KY963937.1
43	Zingiberaceae	*Curcuma*	*newmanii*	JQ409877.1	JQ409732.1
44	Zingiberaceae	*Curcuma*	*ochrorhiza*	JQ409966.1	JQ409761.1
45	Zingiberaceae	*Curcuma*	*oligantha var. oligantha*	MF611628.1	KX455853.1
46	Zingiberaceae	*Curcuma*	*pambrosima*	JQ409874.1	JQ409733.1
47	Zingiberaceae	*Curcuma*	*parviflora*	KF709138.1	HM748998.1
48	Zingiberaceae	*Curcuma*	*parvula*	KJ803152.1	KC441360.1
49	Zingiberaceae	*Curcuma*	*petiolata*	JQ409985.1	JQ409749.1
50	Zingiberaceae	*Curcuma*	*phaeocaulis*	KJ859225.1	MK227331.1
51	Zingiberaceae	*Curcuma*	*plicata*	JQ409979.1	JQ409760.1
52	Zingiberaceae	*Curcuma*	*pseudomontana*	JQ409895.1	KY851761.1
53	Zingiberaceae	*Curcuma*	*raktakanta*	KY129788.1	KY978411.1
54	Zingiberaceae	*Curcuma*	*reclinata*	JQ409969.1	KC441364.1
55	Zingiberaceae	*Curcuma*	*rhabdota*	JQ409854.1	HM748997.1
56	Zingiberaceae	*Curcuma*	*rhomba*	JQ409880.1	JQ409735.1
57	Zingiberaceae	*Curcuma*	*roscoeana*	JQ409887.1	KC441365.1
58	Zingiberaceae	*Curcuma*	*rubescens*	JQ409954.1	JQ409766.1
59	Zingiberaceae	*Curcuma*	*rubrobracteata*	JQ409986.1	HM748992.1
60	Zingiberaceae	*Curcuma*	*sichuanensis*	HM236126.1	KC441373.1
61	Zingiberaceae	*Curcuma*	*singularis*	JQ409872.1	HM749000.1
62	Zingiberaceae	*Curcuma*	*soloensis*	DQ445158.1	KC441376.1
63	Zingiberaceae	*Curcuma*	*sylvatica*	KY129789.1	KR996154.1
64	Zingiberaceae	*Curcuma*	*thorelii*	JQ409852.1	JQ409719.1
65	Zingiberaceae	*Curcuma*	*vamana*	JQ409867.1	KY851762.1
66	Zingiberaceae	*Curcuma*	*vitellina*	JQ409873.1	JQ409734.1
67	Zingiberaceae	*Curcuma*	*wenyujin*	HM236127.1	MK227352.1
68	Zingiberaceae	*Curcuma*	*yunnanensis*	HM236128.1	KC441406.1
69	Zingiberaceae	*Curcuma*	*xanthorrhiza*	KY129790.1	KY978408.1
70	Zingiberaceae	*Curcuma*	*zedoaria*	KY129791.1	MH069906.1
	Outgroups				
1	Zingiberaceae	*Alpinia*	*conchigera*	JQ409990.1	JN043828.1
2	Zingiberaceae	*Alpinia*	*galanga*	AF478715.1	EU552528.1
3	Zingiberaceae	*Larsenianthus*	*careyanus*	HM771393.1	HM771397.1