

Phylogeny Study of 20 Selected Species of Zingiberaceae from *Ex situ* Collections in Peninsular Malaysia

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ABSTRACT

Zingiberaceae is widely distributed in Malaysia, with 750 species and 31 genera. This family comprises a different number of subfamilies and genera according to different taxonomic classification methods — classical taxonomy: one subfamily and four tribes vs molecular taxonomy: four subfamilies and six tribes. However, the taxonomic classification of Zingiberaceae is still debated, especially the classical taxonomy. It is due to some Zingiberaceae species showing cryptic morphologies that make it difficult to classify them through classical taxonomy, which refers to the unique morphological characteristics of a tribe/species. Therefore, accurate taxonomic classification is required by using a molecular approach. In this study, 20 selected species of Zingiberaceae collected from the Agricultural Conservatory Park, Institute of Bioscience (IBS), Universiti Putra Malaysia (UPM) were taxonomically classified using a molecular method with the help of three random amplified polymorphic DNA (RAPD) and three inter simple sequence repeat (ISSR) markers until

the tribe level. The combined RAPD and ISSR unweighted pair group method with arithmetic mean (UPGMA) phylogenetic tree was comparable to Zingiberaceae's current molecular and classical taxonomy. The 20 selected species were grouped into three tribes (Alpinieae, Zingiberaceae, and Globbeae). This finding has contributed additional biological information to better manage the 20 Zingiberaceae species in the Agricultural Conservatory Park, IBS,

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UPM. Further studies are needed to explore the genetic diversities and properties of Zingiberaceae species.

Keywords: ISSR, phylogenetic tree, RAPD, taxonomy, Zingiberaceae

INTRODUCTION

Zingiberaceae is the largest family in the order Zingiberales and is widely distributed all over the world, mainly in the tropical and subtropical countries: America, Indonesia, Indochina, Malaysia, and Thailand (Burt & Smith, 1972; Holttum, 1950; Kress et al., 2002; Larsen, 2007; Nagappan et al., 2019; Newman et al., 2004; Ridley, 1899; Schumann, 1904; Zahara, 2020). Zingiberaceae contains 55 genera with approximately 1,300 species (Royal Botanic Garden Edinburgh [RBGE], n.d.). In Malaysia, 750 species belonging to 31 genera can be found (Govaerts et al., 2022). At the same time, around 200 species belonging to 19 genera have been reported in Peninsular Malaysia (Nagappan et al., 2019).

The characteristics of the ovary, such as the number of locules and placentation, development of staminodia, and modifications of the fertile anther, as well as the rhizome and shoot-leaf orientation, have been used for classical taxonomy classification (Larsen et al., 1998). Accordingly, four tribes have been identified: Alpinieae, Hedychieae, Globbeae, and Zingiberaceae (Burt & Smith, 1972; Holttum, 1950; Larsen et al., 1998; Schumann, 1904). On the other hand,

Kress et al. (2002) classified Zingiberaceae by combining classical (used floral and vegetative characteristics) and molecular (used internal transcribed spacer [ITS] and *matK* markers) taxonomy classifications into four subfamilies and six tribes. The four subfamilies and six tribes are (i) subfamily Siphonochiloideae (tribe Siphonochileae), (ii) subfamily Tamijioideae (tribe Tamijieae), (iii) subfamily Alpinioideae (tribe Alpinieae and tribe Reidelieae), and (iv) subfamily Zingiberoideae (tribe Zingiberaceae and tribe Globbeae). However, discrepancies among the number of genera and species have remained for the family Zingiberaceae. Apart from ITS and *matK* markers, RAPD and ISSR have been used to assess the genetic diversity and genetic relationships/molecular taxonomy of Zingiberaceae species (Bidyaleima et al., 2019; Siriluck et al., 2014; Theanphong et al., 2016, 2018). RAPD is a dominant marker that allows random sampling of a marker over whole genomic DNA (Morell et al., 1995; Welsh & McClelland, 1990). ISSR relies on repeat motifs of DNA sequences and is also a dominant marker (Mohanta et al., 2015).

The 20 selected Zingiberaceae species used in this study were a part of the Agricultural Conservatory Park, Institute of Bioscience (IBS), Universiti Putra Malaysia (UPM) *ex situ* collection. The Agricultural Conservatory Park, IBS, UPM is an *ex situ* conservation centre for Malaysia's many important, endangered, and unique plant species. The park has about 100 species of Zingiberaceae, and almost half of the Zingiberaceae species can be found.

The 20 selected Zingiberaceae species included in this study are known for their traditional and medicinal value (Baruaha et al., 2019; Behera, 2006; Chan et al., 2011; Chattopadhyay et al., 2004; Habsah et al., 2005; Hussain et al., 1992; Larsen et al., 1999; Malek et al., 2011; Srivastava et al., 2006). There are threatened and endangered species: *Alpinia rafflesiana*, *Scaphochlamys kunstleri*, and *Zingiber puberulum* (International Union for Conservation of Nature [IUCN], n.d.). However, only a few species have been scientifically studied in relation to their biological value and characteristics (Awang et al., 2011; Md-Mustafa et al., 2014; Wijekoon et al., 2011). Meanwhile, the taxonomic information for the 20 selected species in this study also needs to be updated. It has been classified taxonomically (Larsen et al., 1998). Nevertheless, some have confusing cryptic morphological characteristics, such as *Etilingera* and *Hornstedtia*. It has caused difficulty classifying them according to their morphological characteristics, especially for those not experts in taxonomy. Hence, this study aims to construct an accurate phylogeny tree of the 20 selected Zingiberaceae species using the molecular

taxonomy method, which does not require the identification of tribes based on their morphological characteristics.

MATERIALS AND METHODS

Sampling Collection

Twenty species from three tribes and ten genera of Zingiberaceae (Table 1; 2–9 leaves for each plant — n = 1–3 per species) collected from the Agricultural Conservatory Park, IBS, UPM, Serdang, Selangor (3°00'01"N, 101°43'27"E). The samples were collected based on their morphological characteristics with the assistance of Dr Shamsul Khamis (a botanist) and Mr Rishzuan Talib (the park coordinator). Several references were used to identify them (Burt & Smith, 1976; Larsen et al., 1998, 1999; Lim, 2000; Nurainas & Arbain, 2017). All leaves were washed with tap water and rinsed with distilled water to get rid of any debris. Then leaves were left overnight to dry at room temperature (26–30°C). Each sample was photographed using a Redmi Note 7 (China) with a megapixel dual camera of 1,080 × 2,340 pixels. All samples were stored in a -20°C freezer with labelled plastic bags before DNA extraction.

Table 1

List of 20 collected Zingiberaceae species from the Agricultural Conservatory Park, IBS, UPM

Plant	Individuals	Label	Voucher number
<i>Alpinia mutica</i> Roxb.	1	AM/1	ACP110/2005
<i>Alpinia conchigera</i> Griff.	1	AC/1	ACP51/2003
<i>Alpinia rafflesiana</i> Wall. ex. Baker	1	AR/1	ACP43/2003
<i>Boesenbergia plicata</i> (Ridl.) Holttum	1	BP/1	ACP226/2017
<i>Boesenbergia rotunda</i> (L.) Mansf.	1	BR/1	ACP38/2002
<i>Curcuma longa</i> L.	1	CL/1	ACP27/2002

Table 1 (continue)

Plant	Individuals	Label	Voucher number
<i>Elettariopsis smithiae</i> Y. K. Kam	1	ES/1	ACP112/2010
<i>Elettariopsis smithiae</i> var. <i>rogusa</i> (Y. K. Kam) C. K. Lam	1	ES v. R/1	ACP119/2010
<i>Elettariopsis curtisii</i> Baker	1	EC/1	ACP164/2013
<i>Etilingera elatior</i> (Jack) R. M. Sm.	1, 2, 3	EE/1, EE/2, EE/3	ACP42/2002
<i>Etilingera terengganuensis</i> C. K. Lim	1, 2, 3	ET/1, ET/2, ET/3	ACP125/2015
<i>Globba nawawii</i> Ibrahim & K. Larsen	1	GN/1	ACP107/2014
<i>Hornstedtia leonurus</i> (J. Keonig) Retz	1, 2, 3	HL/1, HL/2, HL/3	ACP201/2016
<i>Kaempferia galanga</i> L.	1	KG/1	ACP39/2002
<i>Kaempferia pulchra</i> Ridl.	1	KP/1	ACP118/2007
<i>Scaphochlamys biloba</i> (Ridl.) Holttum	1	SB/1	ACP181/2017
<i>Scaphochlamys mat-kilau</i> C. K. Lim	1	SM/1	ACP190/2017
<i>Scaphochlamys kunstleri</i> (Baker) Holttum	1	SK/1	ACP219/2017
<i>Zingiber spectabile</i> Griff.	1, 2, 3	ZS/1, ZS/2, ZS/3	ACP178/2015
<i>Zingiber puberulum</i> Ridl.	1, 2, 3	ZP/1, ZP/2, ZP/3	ACP184/2015

Data Collection and Analysis

Genomic DNAs were extracted from fresh or frozen leaf samples (0.1 g) using the Qiagen DNeasy plant mini kit (Germany) following the manufacturer's protocol with some modifications. Modifications were made at the beginning of the DNA extraction protocol. The modifications included adding up 400 µl Buffer AP1 and 4 µl RNase into the tube containing the well-grinded sample before vortex vigorously and incubating the mixture at 65°C overnight to allow complete lysis of the cells. Samples were ground to a fine powder with the help of liquid nitrogen using sterilised pestles and mortars to begin the genomic DNA extractions. Successfully extracted genomic DNA was verified by using 0.8% agarose gel electrophoresis. Gel electrophoresis was run at 80 V, 250 current for 80 min. Clear and sharp visualisation of bands showed a successful DNA extraction. The bands were observed under UV light

and photographed using ENDURO™ GDS Gel Documentation System (USA). High polymorphic RAPD (n = 11) and ISSR (n = 6) markers that were used by Singh et al. (2012) to assess genetic diversity among and within the agro-climatic zones of several Zingiberaceae species were used for PCR amplification. RAPD and ISSR markers were selected due to their extensive use for assessing genetic variations at different DNA levels and their cost-effectiveness and sensitivity. Moreover, they provide concise genetic information for taxonomy classification, conservation, breeding, and genetic improvement strategies (Ismail et al., 2016).

Furthermore, both markers have been used to identify the species of Zingiberaceae, including *Alpinia* spp. (Siriluck et al., 2014), *Boesenbergia* spp. (Vanijajiva et al., 2005), *Curcuma* spp. (Basak et al., 2017; Das et al., 2011; Kitamura et al., 2007; Prashanth

et al., 2015; Sahoo et al., 2017; Senan et al., 2013; Siriluck et al., 2014; Theanphong et al., 2016; Zou et al., 2011), and *Kaempferia* spp. (Devi et al., 2015; Pojanagaroon et al., 2004; Theanphong et al., 2018; Vanijajiva et al., 2005). PCR amplification for RAPD was performed as follows: initial denature at 94°C for 5 min, followed by 34 cycles of denaturing at 92°C for 1 min, annealing at 50–30°C for 1 min and extension at 72°C for 1½ min with a final extension at 72°C for 7 min. Whilst for ISSR, PCR amplifications were performed as follows: initial denature at 94°C for 1 min, followed by 34 cycles of denaturing at 94°C for 5 min, annealing at 70–40°C for 1½ min and extension at 72°C for 2 min with a final extension at 72°C for 7 min. PCR amplifications contained ≥ 50 ng template DNA, 50 µmol primer, 25 mM magnesium chloride (MgCl₂), 1.5 mM Promega GoTaq® DNA Polymerase (USA), 7.5 mM buffer, 10 mM dNTPs mixed and topped up with ddH₂O to a final volume of 15 µL. PCR amplifications were performed using a BIO-RAD T100™ Thermal cycler (USA).

Amplified PCR regions were genotyped manually as “0” and “1,” which showed the absence and presence

of the bands for both RAPD and ISSR molecular markers. Only sharp bands were considered for the analysis, and then they were computerized/rechecked using PyElph 3.1 software as binary data (Table 2). The binary data were later used for computing similarity matrixes which were later used for building a dendrogram. A dendrogram was constructed using the SAHN module with a UPGMA in NTSYS-PC 2.10e software (Rohlf, 1998) to check the genetic relationship among 20 selected Zingiberaceae species. UPGMA is selected due to its common and valuable use for clustering trees using distance matrices (Nei & Kumar, 2000). UPGMA usually constructs a node at each stage and then makes a new node on a tree. This process continues from bottom to top and develops a new node at each step (Durbin et al., 1998; Rizzo & Eric, 2007). UPGMA combines a pair of operational taxonomic units (OTU) with high similarity into a new OTU, and OTU is composed of DNA or protein sequences (Nei & Kumar, 2000; Yujian & Xu, 2010). Furthermore, UPGMA constructs a tree which assumes a constant rate of evolution (Nei, 1987; Nei & Kumar, 2000).

Table 2

Binary data of combined data using three RAPD markers (*OPD16*, *OPD08*, and *OPD20*) and three ISSR (*SPS04*, *SPS07*, and *SPS08*) markers for 20 selected Zingiberaceae from Agricultural Conservatory Park, IBS, UPM

Primers	OPD16	OPD08	OPD20	SPS04	SPS07	SPS08
Expected band size (bp)	450-2,200	650-2,000	400-2,050	250-1,350	250-1,500	200-1,950
Species	Binary data using observed band size (bp)					
<i>Alpinia mutica</i>	1	1	1	1	1	1
<i>Alpinia conchigera</i>	1	1	1	1	1	1
<i>Alpinia rafflesiana</i>	1	1	1	1	1	1

Table 2 (continue)

Primers	OPD16	OPD08	OPD20	SPS04	SPS07	SPS08
Expected band size (bp)	450-2,200	650-2,000	400-2,050	250-1,350	250-1,500	200-1,950
Species	Binary data using observed band size (bp)					
<i>Boesenbergia plicata</i>	1	1	1	1	1	1
<i>Boesenbergia rotunda</i>	1	1	1	1	0	1
<i>Curcuma longa</i>	1	1	0	1	0	1
<i>Elettariopsis smithiae</i>	1	1	0	1	1	1
<i>Elettariopsis smithiae</i> var. <i>rugosa</i>	1	1	1	0	1	1
<i>Elettariopsis curtisii</i>	1	1	0	0	1	1
<i>Etilingera elatior</i>	1	1	1	1	1	1
<i>Etilingera terengganuensis</i>	1	1	0	1	1	1
<i>Globba nawawii</i>	1	1	0	1	0	0
<i>Hornstedtia leonurus</i>	1	1	1	1	1	1
<i>Kaempferia galanga</i>	1	1	1	1	1	0
<i>Kaempferia pulchra</i>	1	1	1	1	1	1
<i>Scaphochlamys biloba</i>	1	1	1	0	1	1
<i>Scaphochlamys mat-kilau</i>	1	1	1	1	1	1
<i>Scaphochlamys kunstleri</i>	1	1	1	1	1	1
<i>Zingiber spectabile</i>	1	1	1	1	1	1
<i>Zingiber puberulum</i>	1	1	1	1	1	1

There are three UPGMA phylogenetic trees (RAPD only, ISSR only, and combined RAPD and ISSR). However, RAPD-only and ISSR-only trees do not provide clear taxonomic classification until the tribe level. Some of the Alpinieae species were grouped into the Zingiberaceae tribe. Hence, RAPD-only and ISSR-only trees were rejected. However, the combined RAPD and ISSR tree provided clear taxonomic classification until the tribe level.

RESULTS

Among the three UPGMA phylogenetic trees, the combined tree of RAPD (OPD16, OPD08, and OPD20 markers) and ISSR (SPS04, SPS07, and SPS08 markers)

(Figure 1) was the best and comparable to current molecular and classical taxonomy of Zingiberaceae (Kress et al., 2002, 2005, 2007; Larsen et al., 1998; Pederson, 2004; Rangsiruji et al., 2000; Xia et al., 2004). When compared to the taxonomy studies of Zingiberaceae reported by Kress et al. (2005), Larsen et al. (1998), Rangsiruji et al. (2000), Pederson (2004), and Xia et al. (2004), the 20 studied species were grouped according to their tribes (Alpinieae, Zingiberaceae, and Globbeae) similarly in the studies mentioned above. In this study, three clades were formed. Clade i comprised the Alpinieae tribe, the clade comprised the Zingiberaceae tribe and clade iii comprised the Globbeae tribe.

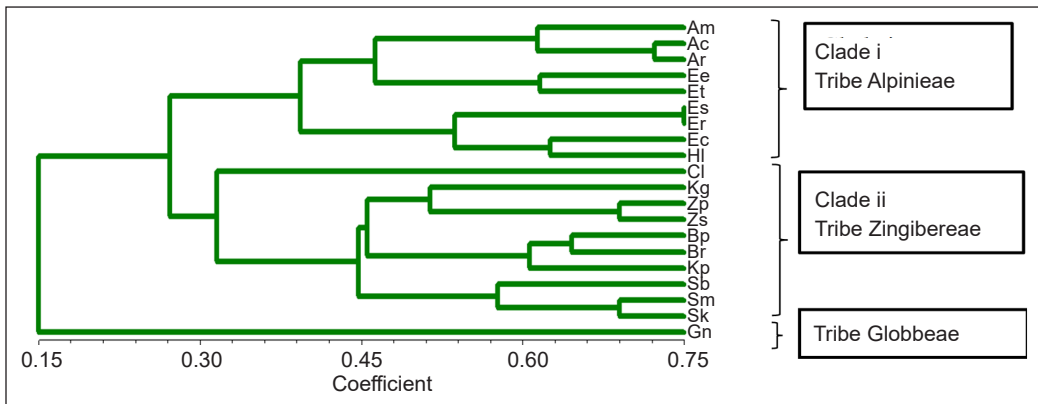


Figure 1. A UPGMA phylogenetic tree using combined data of three RAPD (OPD16, OPD08, and OPD20) and three ISSR (SPS04, SPS07, and SPS08) markers

Note. *Alpinia mutica* (Am), *Alpinia conchigera* (Ac), *Alpinia rafflesiana* (Ar), *Etilingera elatior* (Ee), *Etilingera terengganuensis* (Et), *Elettariopsis smithiae* (Es), *Elettariopsis smithiae* var. *rogusa* (Er), *Elettariopsis curtisii* (Ec), *Hornstedtia leonurus* (HI), *Curcuma longa* (Cl), *Kaempferia galanga* (Kg), *Zingiber puberulum* (Zp), *Zingiber spectabile* (Zs), *Boesenbergia plicata* (Bp), *Boesenbergia rotunda* (Br), *Kaempferia pulchra* (Kp), *Scaphochlamys biloba* (Sb), *Scaphochlamys mat-kilau* (Sm), *Scaphochlamys kunstleri* (Sk), and *Globba nawawii* (Gn)

Clade i comprised all nine species from four genera (*Alpinia*, *Elettariopsis*, *Etilingera*, and *Hornstedtia*) of the tribe Alpineae. All *Alpinia* species were grouped in one cluster. *Alpinia conchigera* (Ac) and *Alpinia rafflesiana* (Ar) were grouped in one cluster and shared the same root with *Alpinia mutica* (Am). The two *Etilingera* species, *Etilingera elatior* (Ee), and *Etilingera terengganuensis* (Et) were grouped in one cluster and connected to the genus *Alpinia*. Meanwhile, three *Elettariopsis* species and one *Hornstedtia* species were grouped in one cluster and connected to the genera *Alpinia* and *Etilingera*. *Elettariopsis smithiae* (Es) and *Elettariopsis smithiae* var. *rogusa* (Er) were grouped in one cluster and connected to *Elettariopsis curtisii* (Ec), which was grouped with *Hornstedtia leonurus* (HI).

Clade ii comprised all ten species from five genera (*Boesenbergia*, *Curcuma*,

Kaempferia, *Scaphochlamys*, and *Zingiber*) of the tribe Zingiberaceae. All *Scaphochlamys* species were grouped in one cluster. *Scaphochlamys mat-kilau* (Sm) and *Scaphochlamys kunstleri* (Sk) were grouped in one cluster and shared the same root with *Scaphochlamys biloba* (Sb). The two *Boesenbergia* species, *Boesenbergia plicata* (Bp) and *Boesenbergia rotunda* (Br) were grouped in one cluster and connected to *Kaempferia pulchra* (Kp). The two *Zingiber* species, *Zingiber puberulum* (Zp) and *Zingiber spectabile* (Zs) were grouped in one cluster and connected to *Kaempferia galanga* (Kg). The *Kaempferia* species, *Kaempferia pulchra* (Kp) and *Kaempferia galanga* (Kg) mixed with *Boesenbergia* and *Zingiber* species. Meanwhile, *Curcuma longa* (Cl) branched alone and connected to the four genera (*Boesenbergia*, *Kaempferia*, *Scaphochlamys*, and *Zingiber*) of the tribe

Zingiberaceae. Clade iii is comprised of one species of Globbeae tribe, which is *Globba nawawii*.

DISCUSSION

The RAPD and ISSR markers were used for studying the molecular taxonomy of 20 selected Zingiberaceae species from the Agricultural Conservatory Park, IBS, UPM. These 20 selected Zingiberaceae species are important to the endangered and unique *ex situ* collections of Zingiberaceae species from Peninsular Malaysia. A UPGMA phylogenetic tree built from three RAPD and three ISSR markers showed that the 20 selected Zingiberaceae species can be successfully grouped until the tribe level. Three clades were formed: (1) Clade i of tribe Alpinieae, (2) Clade ii of tribe Zingiberaceae, and (3) Clade iii of tribe Globbeae.

The combined RAPD and ISSR UPGMA phylogenetic tree were comparable to Zingiberaceae's recent molecular and classical taxonomy (Kress et al., 2002, 2005, 2007; Pederson, 2004; Rangsiruji et al., 2000; Xia et al., 2004). This UPGMA phylogenetic tree agrees with Kress et al. (2002, 2005, 2007) and Pederson (2004), whereby both have grouped the tribe Alpinieae and tribe Zingiberaceae as two major clades. Within the tribe Alpinieae, the genus *Alpinia* was separated from other genera (*Elettariopsis*, *Etlingera*, and *Hornstedtia*). Meanwhile, within the tribe Zingiberaceae, the genus *Scaphochlamys* was separated from other genera (*Boesenbergia*, *Kaempferia*, and *Zingiber*), and the genus *Curcuma* branched

out from other genera (*Scaphochlamys*, *Boesenbergia*, *Kaempferia*, and *Zingiber*). It agrees with other studies (Kress et al., 2002, 2005, 2007; Pederson, 2004; Xia et al., 2004). The present study grouped *Elettariopsis* and *Hornstedtia* in one cluster but separated it from *Etlingera*. Hence, they do not support *Etlingera* and *Hornstedtia* as two complex genera with confusing taxonomic classifications and cryptic morphologies (Kress et al., 2002; Pederson, 2004). *Etlingera* and *Hornstedtia* are sister groups because both have sterile bracts and a fertile bract that subtends a single flower (Kress et al., 2002), except for *Hornstedtia leonurus* (Pederson, 2004). *Hornstedtia leonurus* is unique in the genus, having more than one flower per bract and a tubular bracteole that is occasionally open to the base in Lambir plants. Flower of this species is not easily recognised in dark forests, while during the day, they can be easily identified due to dense brown hairs on leaf margins, truncate, subcordate leaf bases, and 1-2 cm prominent petiole (Sakai & Nagamasu, 2003). Genus *Kaempferia* was confounded with genera *Boesenbergia* and *Zingiber*. It is different from Kress et al. (2002). In addition, this study showed tribe Globbeae connected with the tribe Alpinieae and the tribe Zingiberaceae. However, this contradicts Kress et al. (2002, 2005) — the tribe Globbeae is not distinguished from the tribe Zingiberaceae.

The selected RAPD and ISSR markers used in this study have shown high success amplification in 55 accessions of *Curcuma longa* and five cultivars from different

agroclimatic zones of Zingiberaceae (Singh et al., 2012). One of the species studied by Singh et al. (2012), *Curcuma longa*, is used in this study. RAPD and ISSR markers are simple, dominant, highly reproducible, polymorphic, and easily handle molecular markers (RAPD: Zietkiewicz et al., 1994; Bornet & Branchard, 2001, 2004; ISSR: Welsh & McClelland, 1990; Williams et al., 1990). RAPD and ISSR have been used for genetic diversity and genetic relationship/taxonomy studies within Zingiberaceae species (RAPD: Das et al., 2011; Jatoi et al., 2008; Saha et al., 2016; Techaprasan et al., 2008; ISSR: Das et al., 2015; Siriluck et al., 2014; Taheri et al., 2012). Applications to molecular markers to identify and characterise species according to their genetic information differ according to their efficiency, including the level of polymorphism, locus specificity, genomic abundance, technical requirements, and reproducibility (Spooner et al., 2005). In the present study, RAPD is more reproducible than ISSR because all 11 RAPD markers amplified and produced clear and sharp bands.

Taxonomy classification of the family Zingiberaceae is still under debate because new species are being discovered in the 21st century and are taxonomically classified by referring to their morphological characteristics. Hence, there is a need to revise the taxonomy classification of the family Zingiberaceae to correctly place all newly identified species under their correct tribes and subfamilies. For instance, two new species of Zingiberaceae: *Amomum*

bungoensis and *Sundamomum corrugatum*, have been reported in Malaysia (Mohamad et al., 2020; Syazana et al., 2018). Another new Zingiberaceae species: *Etlingera terengganuensis* is an endemic species to Terengganu and was discovered in the year 2000 (Khaw, 2001; Lim, 2000) and 11 new species of Zingiberaceae from the genus *Scaphochlamys*: *Scaphochlamys durga*, *Scaphochlamys hasta*, *Scaphochlamys nigra*, *Scaphochlamys uniflora*, *Scaphochlamys multifolia*, *Scaphochlamys lucens*, *Scaphochlamys lanjakensis*, *Scaphochlamys penyama*, *Scaphochlamys graveolens*, *Scaphochlamys scintillans*, and *Scaphochlamys peuedoreticosa* have been investigated in Borneo (Im Hin et al., 2017). Five new species of Zingiberaceae including *Boesenbergia bella*, *Boesenbergia phenklaii*, *Boesenbergia putianai*, *Kaempferia phuphanensis*, and *Globba sirirugsae* have been discovered in Thailand (Mood et al., 2019). Two new Zingiberaceae species have been reported in Indonesia: *Zingiber alba* (Nurainas & Arbain, 2017) and *Etlingera megalocheilos* (Trimanto & Haspari, 2018).

All 20 selected species of Zingiberaceae have valuable uses for medicinal and economic ornamental purposes. Rhizome of *Alpinia conchigera* and *Alpinia mutica* is used to treat gastrointestinal disorders, and *Alpinia conchigera* is used in traditional Thai dishes (Awang et al., 2011; Malek et al., 2011). The fruit and rhizome of *Alpinia rafflesiana* have been used for their antimicrobial and anti-inflammatory properties (Ahmad et al., 2006; Jusoh et al., 2013). Rhizome of *Boesenbergia rotunda* is used

to treat dyspepsia, gastrointestinal disorders, mouth ulcers, dermatitis, diarrhoea, and dry cough (Md-Mustafa et al., 2014; Tewtrakul et al., 2003) and dried leaves are used in treating food poisoning and controlling allergies (Eng-Chong et al., 2012). Leaves of *Boesenbergia plicata* are used in treating human immunodeficiency viruses (HIV), protease inhibitory activity, and as an anti-inflammatory (Tewtrakul et al., 2003; Tuchinda et al., 2002). Rhizome of *Curcuma longa* is used in wound healing, common cold, stomachache, treatment of piles, antidiabetic, anti-chlorotic, anti-rheumatic, hypocholesterolemic, anti-fibrotic, antiviral, antihepatotoxic, antivenomous, anti-microbial, and for its anti-inflammatory and anti-cancerous properties (Akinyemi et al., 2015; Behera, 2006; Chattopadhyay et al., 2004; Hussain et al., 1992; Srivastava et al., 2006). Leaves of *Curcuma longa* are used in Peninsular Malaysia as a flavouring agent in spicy and savoury dishes (Larsen et al., 1999). Leaves, rhizomes, flower buds, and fruit of *E. elatior* are used to cure earaches and heal wounds and are used for their anticancer, antibacterial, antioxidant, antiproliferative, and cytotoxic activity. The flower bud is used in dishes, such as nasi kerabu, nasi ulam, and asam laksa in Peninsular Malaysia (Chan, Han, et al., 2007; Chan, Lim, et al., 2007; Jackie et al., 2011; Khaw, 2001; Lachumy et al., 2010; Larsen et al., 1999; Wijekoon et al., 2011). Furthermore, *E. elatior* is grown for ornamental and commercial purposes (Chan et al., 2007b; Khaw et al., 2001; Larsen et al., 1999). *Etilingera curtisii* whole plant

is used for carminative, antioxidant and antibacterial properties, and leaves are eaten as vegetables, added in chilly spices to enhance the flavour, as decoration and bath, and to stimulate gastric secretions (Chairgulprasert et al., 2008). *Keampferia galanga* leaves and rhizomes are used in cosmetic powder, flavouring agents, and traditional medicine to treat swelling, toothache, headache, stomach-ache, and rheumatism (Ibrahim, 1999; Larsen et al., 1999; Mitra et al., 2007). In addition, leaves, rhizome, and stem of *Zingiber spectabile* are eaten as salad and used as a flavouring agent in food, as an antiproliferative, antioxidant, and anticancer purpose (Lim, 2020; Rahman, Rasedee, Abdul, et al., 2014; Rahman, Rasedee, Yeap, et al., 2014).

CONCLUSION

This study provided additional biological information to the 20 selected Zingiberaceae species to help better conserve the species in the Agricultural Conservatory Park, IBS, UPM. The combined RAPD and ISSR UPGMA phylogenetic tree classed them according to their three tribes (Alpinieae, Zingiberaceae, and Globbeae). However, the 20 selected Zingiberaceae species cannot be classed until their genera. Hence, additional markers are required. In addition, further studies are needed to explore the genetic diversities and properties of Zingiberaceae species.

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