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Two new records of *Alpinia* Roxb. (Zingiberaceae) in Sumatra, Indonesia and phylogenetic relationship to their allied species

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Abstract
Alpinia is one genus of Zingiberaceae that is distributed throughout the tropical regions. Nine species of *Alpinia* have been recorded in Sumatra. In this article, we report 2 new record species for Sumatra, which are *Alpinia submutica* Roxb. and *A. denticulata* (Ridl.) Holttum. We also registered 3 sequences of them as new data in GenBank under accession number: MH087456, MH087457, and MH218561. Phylogenetic analysis using a molecular approach with their allied species in Sumatra is also presented.

Key words
Ginger, molecular analysis, new occurrences, Sumatra, taxonomy.

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Introduction

Alpinia Roxb. is a genus with the highest diversity in Zingiberaceae family that is widespread from Sri Lanka to northern New South Wales (Smith 1990, Larsen et al. 1998). Some species of this genus, like *A. galanga* (L.) Willd., *A. malaccensis* (Burm.f.) Roscoe**,** and *A. nutans* (L.) Roscoe have been used as medicine, rhizome of *A. galanga* (L.) Willd. is also used as a spice, and the beautiful *A. purpurata* (Vieill.) K.Schum. flowers are known as ornamental flowers (Heyne 1987, Larsen et al. 1999, van Valkenburg and Bunyapraphatsara 2001, Nurainas 2007, Namsa et al. 2009).

Alpinia is a native genus of Southeast Asia and related information about the species has been reported in some publications. Twenty-five *Alpinia* species are noted from the Malay Peninsula region. Seven species are distributed throughout Java. Borneo region has 9 species, out of which, 6 endemic, Sarawak, which is the closest area to Sumatra, has 8 species. The latest report for Sumatra has five species: *A. aquatica* (Retz.) Roscoe, *A. capitellata* Jack, *A. galanga* (L.) Willd., *A. mutica* Roxb., *A. sumatrana* (Miq.) K.Schum. (Newman et al. 2004, Poulsen 2006, Lamb et al. 2013).

Exploration of the Sumatran Zingiberaceae including *Alpinia* has been carried out quite throughly since 2006. In that period, several new discoveries have been reported, such as the new species, namely, *Zingiber engganoensis* Ardiyani, *Zingiber alba* Nurainas, and *Scaphoclamys perakensis* Holtt. which is a new record for Sumatra (Ardiyani 2016, Nurainas and Arbain 2017). Information related to the distribution or morphological description of *Alpinia* in Sumatra has not been recorded properly, and there is a big chance for new findings.

Based on a survey conducted in Sijunjung and Harau, West Sumatra, several populations of *Alpinia* have been found. They have morphological similarities with *A. submutica* which was last found on Java and *A. denticulata* which was reported previously in Perak, Terengganu, Peninsular Malaysia. The objective of this study is to report the distribution and morphological description as well as the phylogenetic relationship of 2 new records of *Alpinia* in Sumatra with their allied species.

Methods

Taxonomy work. The surveys and exploration were carried out in the field from December 2016 until September 2017 followed by samples collection at various sites in Sumatra, which referred to Herbarium ANDA specimen information. Sample collection and preservation techniques referred to Smith (1981) and character note referred to Smith (1990). The description follows de Vogel (1987), where the description starts from general to specific characters. Collected specimens were deposited as voucher specimens at ANDA Herbarium of Andalas University.

Identification process, information related to taxonomy and geographic distribution to confirm species identities were obtained from the related literature (Schumann 1904b, Holttum 1950, Newman et al. 2004). Additional specimen information based on Herbarium Bogoriense (BO) Cibinong, virtual online database Plantlist (http://www.theplantlist.org/), Global Biodiversity Information Facility (https://www.gbif.org/) and Kew's Herbarium (http://www.kew.org/herbcat).

Molecular work. The genomic DNAs were extracted from fresh or silica dried tissue using some modification of Doyle and Doyle (1987) CTAB (hexadecyltrimethylammonium bromide) method. The amplification of Internal Transcribed Spacer (ITS) was accomplished using primer pair namely ITS4–ITS5 and the *trnH-psbA* intergenic spacer was amplified with trnHf_05–psbA3_f (White et al. 1990, Kress et al. 2005b). We used MyTaq™ Red Mix for amplification according to the manufacturer's directions and following the same conditions as described in (Kress et al. 2005a, Kress and Erickson 2007), with annealing temperatures of 54–58 °C. Consensus sequences were aligned using Multiple Sequence Alignment tool Clustal X v. 2.0 (Thompson et al.1997) and concatenation was done using Geneious Prime 2019.0.4 (http://www.geneious.com, Kearse et al. 2012).

Maximum Parsimony (MP) and Maximum Likelihood (ML) analyses of the ITS and *trnH-psbA* sequence data were carried out using MEGA 7 with Tamura 3-Parameter model (Kumar et al.2016), with equally weighted characters and 1000 random-sequence-additional-replicates. A total of 13 ITS and *trnH-psbA* sequences from GenBank were used as ingroups and 3 sequences of *Curcuma longa* L., *Hedychium coccineum* Buch.-Ham. ex Sm., *Zingiber mioga* (Thunb.) Roscoe were chosen as an outgroup. Bootstrap support was categorized according to criteria by Kress et al. (2002), i.e., strong (> 85%), moderate (70–85%), weak (50–70%), or poor (< 50%) support.

Results

***Alpinia submutica* K.Schum.** *Alpinia submutica* K. Schum., Bot. Jahrb. Syst. 27 (1899) 280. — Type: J.E. Teijsmann 2040 (holo BO!).

Figure 1

New record. Indonesia. Sumatra: West Sumatra, Payakumbuh, Lima Puluh Kota regency, Harau village, Batang Harau riverside near Aka Barayun waterfall,

Table 1. Sources, and voucher specimens of ingroup and outgroup were obtained from GenBank.

No.	Species	GenBank accession number		Voucher	
		ITS	trnH-psbA	ITS	trnH-psbA
Ingroup					
1	*Alpinia aquatica*	KJ507884	JN043815	N9	—
2	*Alpinia conchigera*	AF478712	JN043826	Kress #00-6706 US	ZL305-3
3	*Alpinia pusilla*	KT280465	KT280463	WI 97	WI 97
4	*Alpinia macrocrista*	KT280464	KT280462	WI 96	WI 96
5	*Alpinia officinarum*	AF478718	GU180428	Kress #00-6614 US	PS0519MT04
6	*Alpinia zerumbet*	KX018020	JN043874	—	ZL355-1
7	*Alpinia japonica*	AF254474	KF694867	LM	OUT2
8	*Alpinia mutica*	KJ507924	—	N84	—
9	*Alpinia blepharocalyx*	AF478709	JN043817	Kress #98-6136 US	ZL302-3
10	*Alpinia foxworthyi*	AF478714	JN043836	Kress #98-6293 US	Kress #94-5539
11	*Alpinia oxyphylla*	AY742372	JN043863	—	—
12	*Alpinia polyantha*	AY745692	JN043873	Kress #94-3744 US	—
13	*Alpinia calcarata*	KJ871912	JN043820	86558	—
Outgroup					
1	*Hedychium coccineum*	AY424758	KC597934	—	ZMN06
2	*Curcuma longa*	JQ409956	FJ687416	JLS 73222	—
3	*Zingiber mioga*	KJ025068	GQ435047	TKM-1-000049	PS0528MT01

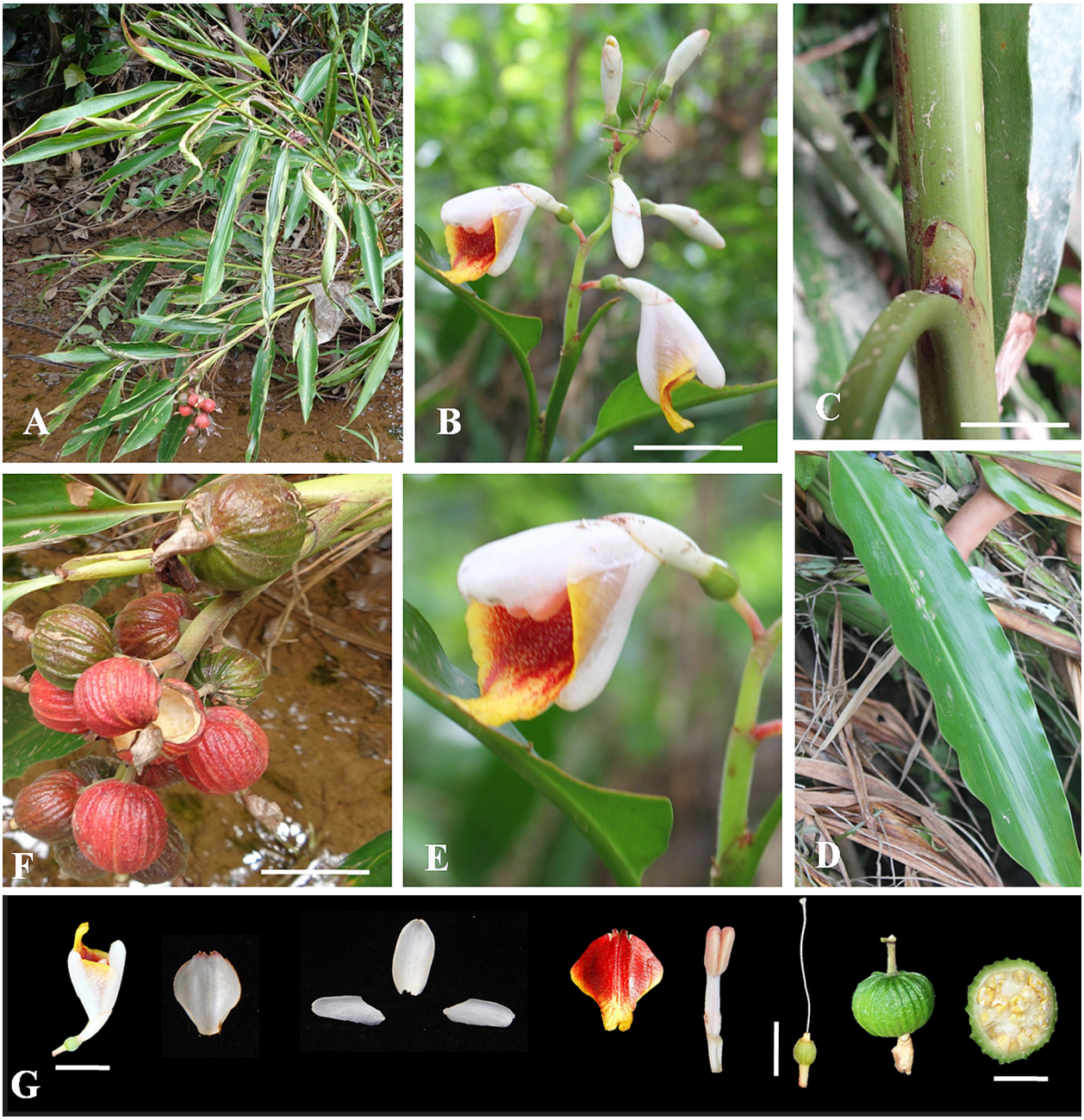

Figure 1. *Alpinia submutica* K.Schum. (S.E. Fitri et al. 04-Sc AL HR, ANDA). **A.** Habit. **B.** Inflorescence. **C.** Flower. **D.** Ligule. **E.** Leaf. **F.** Fruits. **G.** Dissected perianth (from left): flower, calyx, corolla lobes, labellum, stamen, pistilum, fruit. Scale bars: B = 2 cm; C= 2 cm; F = 2 cm; G = 1 cm. Photographed by S.E. Fitri.

(00°06′21.82″ S, 099°88′56.4″ E), 500 m a.s.l., 23-IV-2016, fl., S.E. Fitri et al. 04-Sc AL (ANDA); Sijunjung regency, Muaro Sijunjung village, Kulampi customary forest, (00°39′ S, 100°58′ E), 150 m a.s.l., 2-IV-2016, fl.&fr., S.E. Fitri et al. 07 Al SC SJJ (ANDA).

Description. Herbs 1–2.5 m tall, rhizome subsurface, ca 2.1 cm in diameter, cream inside, fleshy, aromatic. Pseudostem 2.0–3.0 cm in diameter, erect, thick. Leaves 16–21 in 1 plant, petioles 0.5–4 cm, glabrous, ligule 0.5–01.0 × 0.5–1.0 mm long, green with red pattern; laminae 14.0–34 × 4–6.0 cm long, lanceolate, green, with hairy lower side and midrib, upper side glabrous, apex caudate, base unequal, margins entire. Inflorescences terminal, erect or drooping, rachis 10.5–16.0 cm, 6–25 cincinni, primary bract absent. Flower ca 5.5 cm long, 2–4 flowers open at a time; bracteole absent; calyx white, obovate; corolla tube shorter than calyx; dorsal lobe white, ovate, apex rounded, 0.1 × 2.5 cm, glabrous; lateral lobe white, like ship, 0.5 × 3.0 cm; labellum obovate, 2.5 × 3.5 cm, basally concave with red spots and lines, apex bilobed, yellow, with wrinkled margin throughout; stamen ca 3.5 cm long; anther 0.5 × 1.0 mm, cream, not crested; style glabrous, white, 3–4.0 cm long; ovary green, ca 1 cm long, hairy; epyginous glands ca 4 mm long. Fruit 2.0–3.0 cm in diameter, tricoccous, round, red when ripe, wavy surface, calyx persistent, 7–21 fruit in one stem; 35–36 seeds in 1 fruit, seed black with aril.

Distribution. Java and Sumatra

Ecology. *Alpinia submutica* is found in riverside areas of secondary forest.

Additional Information. Previously, *Alpinia submutica* was identified as *A. mutica* Roxb. but differs in its fruit surface. *Alpinia submutica* has a wavy surface, while *A. mutica*has a flat surface. This result was confirmed based on specimen of Herbarium Bogoriense *Alpinia submutica* K.Schum. Bot. Jahrb. Syst. 27 (1899) 280 [Type: *J.E. Teijsmann 2040* (holo BO!)] and Archip. Ind. Sumatra Sidjoendjoeng. There was no sequence data for this species in GenBank, and we registered it for the first time on 20 March 2018 under accession number MH087457.

***Alpinia denticulata* (Ridl.) Holttum**, *Alpinia denticulata* (Ridl.) Holttum, Gard. Bull. Singapore 13 (1950) 143. — Basionym: *Hedychium denticulatum* Ridl., J. Straits Branch Roy. Asiat. Soc. 32 (1899) 102. — *Odontychium denticulatum* (Ridl.) K. Schum., Pflanzenr. IV, 46 (1904) 60. — Lectotype: *H.N. Ridley 9455* (SING!,designated by Turner, 2000), Perak.

Figure 2

New record. Indonesia. Sumatra: West Sumatra, Payakumbuh, Lima Puluh Kota regency, Harau village, (00°06′19.55″ E, 100°40′23.88″ S), ca 720 m a.s.l., 23-IV-2016, fl.&fr., S.E. Fitri et al. 01-Sc Al HR (ANDA).

Description. Herbs 1–2 m tall, rhizome subsurface, 1–1.5 cm in diameter, cream inside, fleshy, aromatic. Pseudostem 1–3 cm in diameter, erect, thick. Leaves 11–13 in 1 plant, petioles 0.5–1.5 cm, green, ligule 0.5–1.0 × 0.1–0.5 cm long, green, hairy; laminae 7.5–32 × 1–5.5 cm long, lanceolate, apex caudate, base unequal, entire margins, green, hairy. Inflorescences terminal, erect, rachis 13.0–14.0 cm, 26–35 cincinni (1–3 flowers per cincinni), 2 primary bracts, 3.5–6 cm and 8–12 cm long. Flower ca 5.0 cm long, 1–6 flowers open at a time; bracteole obovate, brown; calyx tubular, tube 1.0 ×**1.**5 cm long, apex acute, brown, stiff; corolla: dorsal lobe acute, ca 1.5 cm long, red, apex acute, thick, glabrous; lateral lobe 1.0 ×**1.**5 cm, brownish-cream, apex rounded, thick, glabrous; labellum ca 2.6 cm long, apex trilobed, from base to middle red, thick, glabrous, middle to top light green; stamen ca 4 cm; staminodes ca 9 mm long, like long horn; anther 8 mm long, reddish-cream, not crested; style ca 2.6 cm long, white; ovary ca 10 mm long, light green, glabrous; epyginous glands ca 2 mm long. Fruits round, tricoccous, green when unripe, hairy, ca 6 in 1 stem.

Distribution. Perak, Terengganu and Harau Valley, West Sumatra.

Ecology. Found in limestone hills at ca 720 m a.s.l. in the Harau Valley. Vernacular name “Langkueh Rimbo”.

Additional Information. *Alpinia denticulata* was collected in Lumut, Perak in the period 1896–1898 by Ridley and collected in Bauk Hill, Terengganu by Lewis in 1977 (Holttum 1950, Royal 2018). There was no sequence data for this species in GenBank, and we registered it for the first time on 20 March 2018 under the accession numbers MH087456 and MH218561.

Twenty-three sequences of ITS and *trnH-psbA* were obtained from GenBank and taken as ingroup and outgroup in this study. *Alpinia submutica* from *trnH-psbA* region is not included in this analysis because of the poor sequencing result. The phylogenetic tree building methods Maximum Parsimony (MP) and Maximum Likelihood (ML) produced similar ITS and *trnH-psbA* tree topologies (Figs 3, 4). Based on the phylogenetic analysis, it was seen that the Internal Transcribed Spacer had a total aligned length of 572 bp with the mean GC content of 55.67%, meanwhile, *trnH-psbA* had a total aligned length of 509 bp with 29.87% mean GC content. Combined alignments of ITS and *trnH-psbA* produce a total aligned length of 1081 bp with mean GC content of 44.13%.

Discussion

Seven species of *Alpinia* species that have already been reported are spread across several regions in Sumatra. These results increase the number of species reported from Sumatra to 11 species, with 2 new records, namely *A. submutica* (Fig. 1) and *A. denticulata* (Fig. 2). They were not initially recorded in Sumatra region, where *A. submutica* was reported to be distributed in Java (Newman et al. 2004), while *A. denticulata* was last found in Perak and Terengganu-Malaysia (Holttum 1950).

In 2009, *A. submutica* (Fig. 1) was collected in Harau, Lima Puluh Kota District, West Sumatra, and the specimens were stored in ANDA Herbarium, and identified as *A. mutica* (Nelvita 2009). The difference in fruit surface of these 2 species caused doubts about the results of the identification. After tracing and recollecting this species in the same area, it was re-identified and the similarities were found with that of the specimen of Herbarium Bogoriense *Alpinia submutica* K.Schum.Bot. Syst. 27 (1899) 280.– Type: J.E. Teijsmann 2040 (holo! BO!) And Archip Ind Sumatra Sidjoendjoeng, with the same wavy fruit surface and this species can be confirmed as *A. submutica* (Fig. 1). In this study, *A. submutica* was also found in Kulampi Customary Forest, Muaro Sijunjung, Sijunjung District, West Sumatra.

Alpinia denticulata was collected in Lumut, Perak in the period 1896–1898 by Ridley and collected in Bauk Hill, Terengganu by Lewis in 1977 (Holttum1950, Royal Botanic Garden Edinburgh 2018). Initially, this species was identified as *Hedycium* because of its narrowed ligule and long staminodes. These characters did not have similarities with *Alpinia* in general. Schumann (1904b) was doubtful of that result because of the similarity of characters of inflorescences and fruit with that of Dieramalpinia section of *Alpinia* prepared by him.

Figure 2. *Alpinia denticulata* (Ridl.) Holttum (S.E. Fitri et al. 01–SC Al HR, ANDA). **A.** Habit. **B.** Inflorescence. **C.** Ligule. **D.** Young leaf. **E.** Flower. **F.** Fruits. **G.** Dissection of flower (from left): flower, calyx, bract, corolla lobes, labellum, stamen, pistilum. Scale bars: B = 2 cm; C = 2 mm; F = 2 cm; G = 1 cm. Photographed by S.E. Fitri.

Staminodes of this species are not much different from some species of *Alpinia*, only the curved shape of ligule makes a striking distinguishing character compared to other *Alpinia*. These results were referred to Kew Herbarium specimen (http://specimens.kew.org/herbarium/K000292387) and illustration of *A. denticulata* (Schumann, 1904a). The existence of these 2 species in new locations that have not been previously reported is possible, this can be attributed to the distribution area in the Malay Peninsula which is geographically very close to Sumatran Island.

Maximum Likelihood (ML) analysis, which is commonly used, provides the most robust result and Maximum Parsimony (MP) that uses character-based method, is chosen to construct the phylogenetic tree. The phylogenetic analysis carried out shows the position of the 2 new records of *Alpinia* along with other existing species (Figs 3–6). The ITS+*trnH-psbA* combination gave a better result than single marker analysis of ITS and *trnH-psbA* (Figs 5, 6). This result can be seen from the percentage of bootstrap value.

Based on 7 species that were sequenced, the results of sequence divergence range are 0.2–12.3%. *Alpinia denticulata* and *A. submutica* were added for the first time

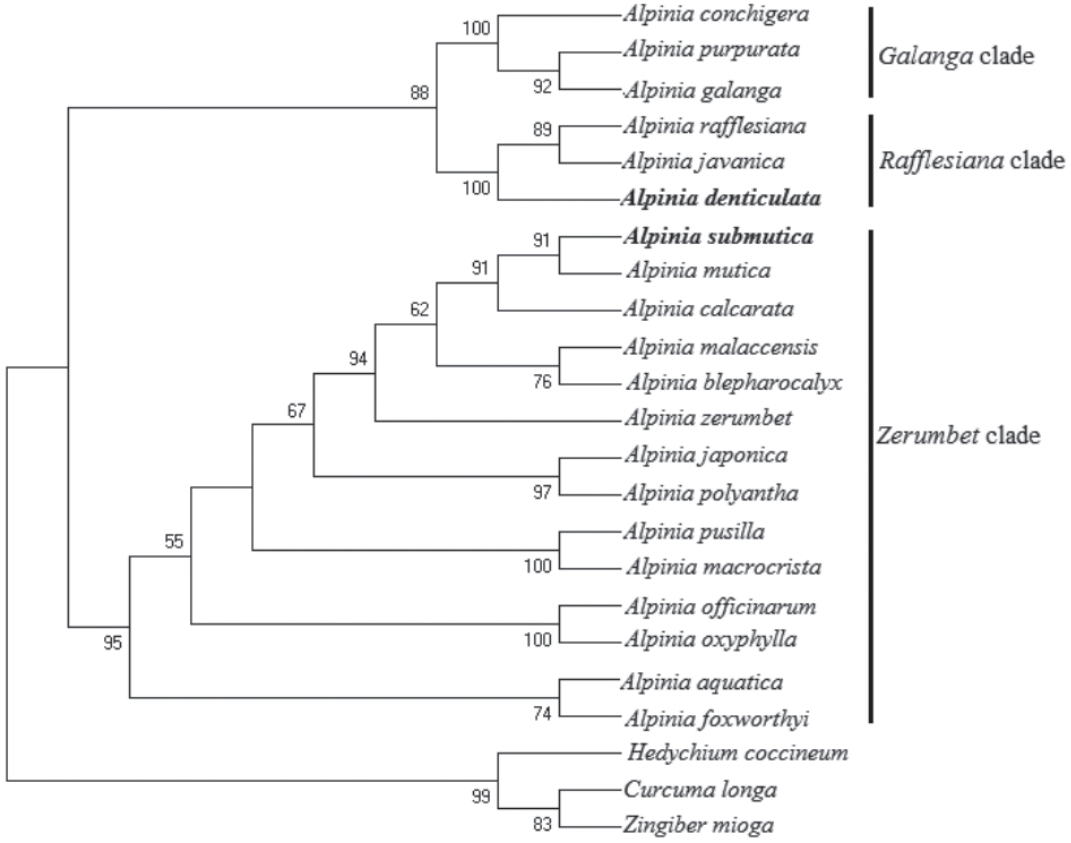

Figure 3. *Maximum Parsimony* phylogenetic tree (length = 245) based on complete sequences of Internal Transcribed Spacer (ITS) region of *Alpinia* from this study and GenBank data sequences with *boostrap* values (≥ 50%). Cluster name based on Kress et al. (2005a).

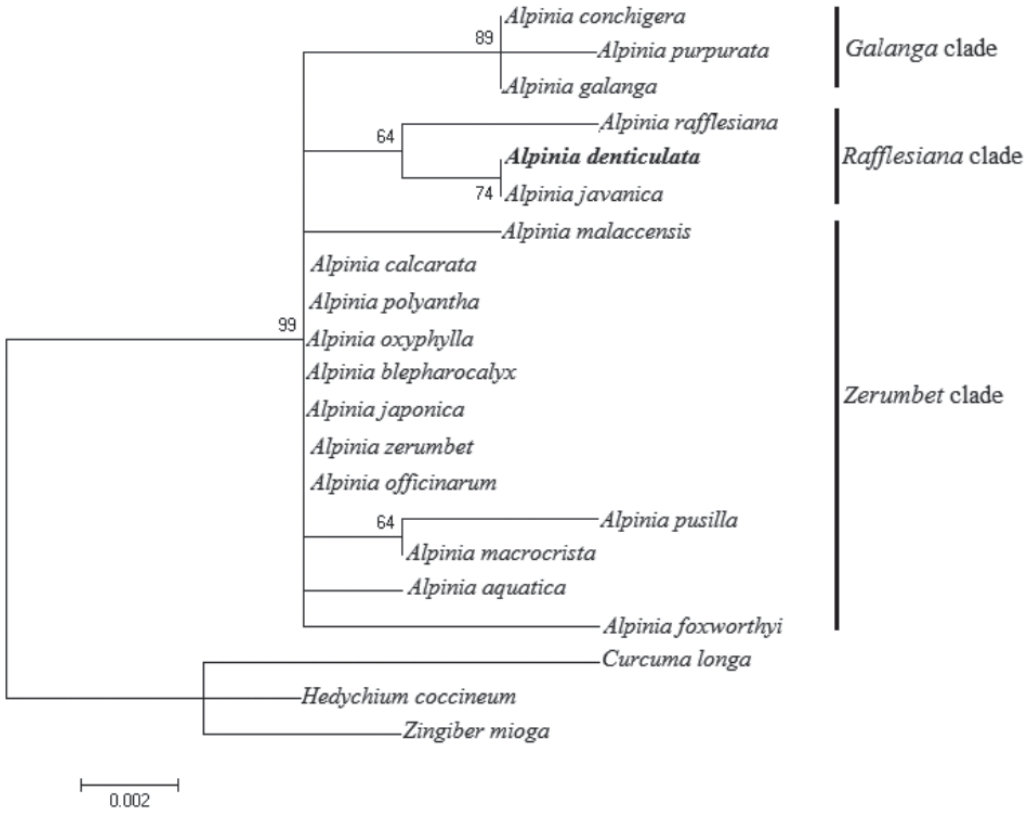

Figure 4. Maximum Likelihood phylogenetic tree (–log likelihood = –848.30) based on complete sequences of *trnH-psbA* Intergenic Spacer region of *Alpinia* from this study and GenBank data sequences with bootstrap values (≥ 50%). Cluster name based on Kress et al. (2005a).

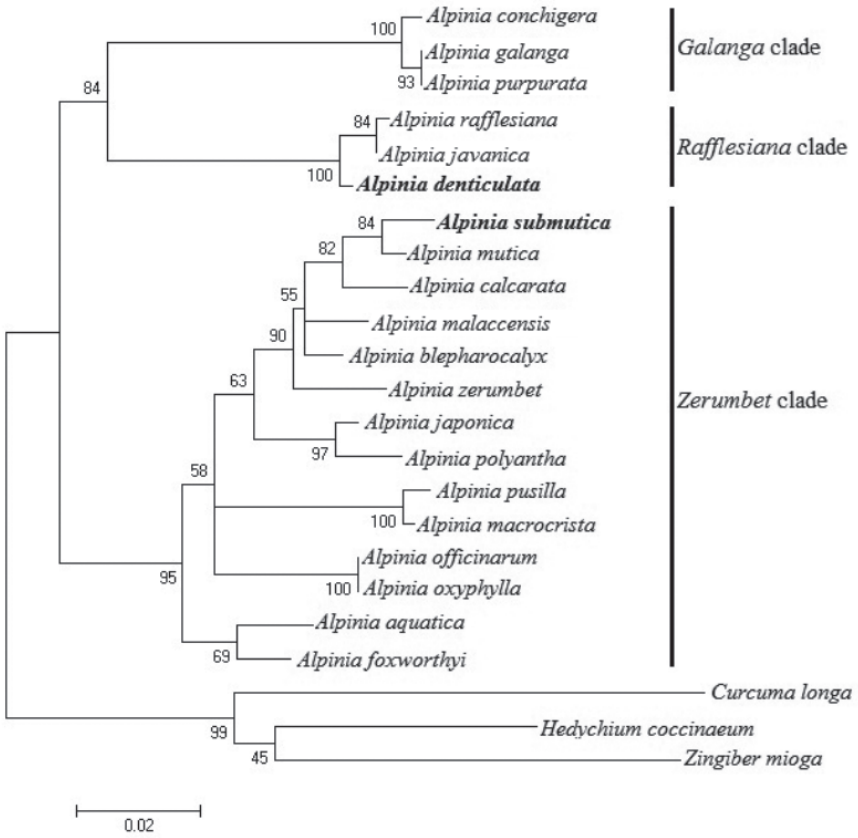

Figure 5. Maximum Likelihood phylogenetic tree (–log likelihood = –1988.44) based on combined ITS and *trnH-psbA* Intergenic Spacer region from this study and GenBank sequences data with bootstrap values (≥ 50%). Cluster name based on Kress et al. (2005a).

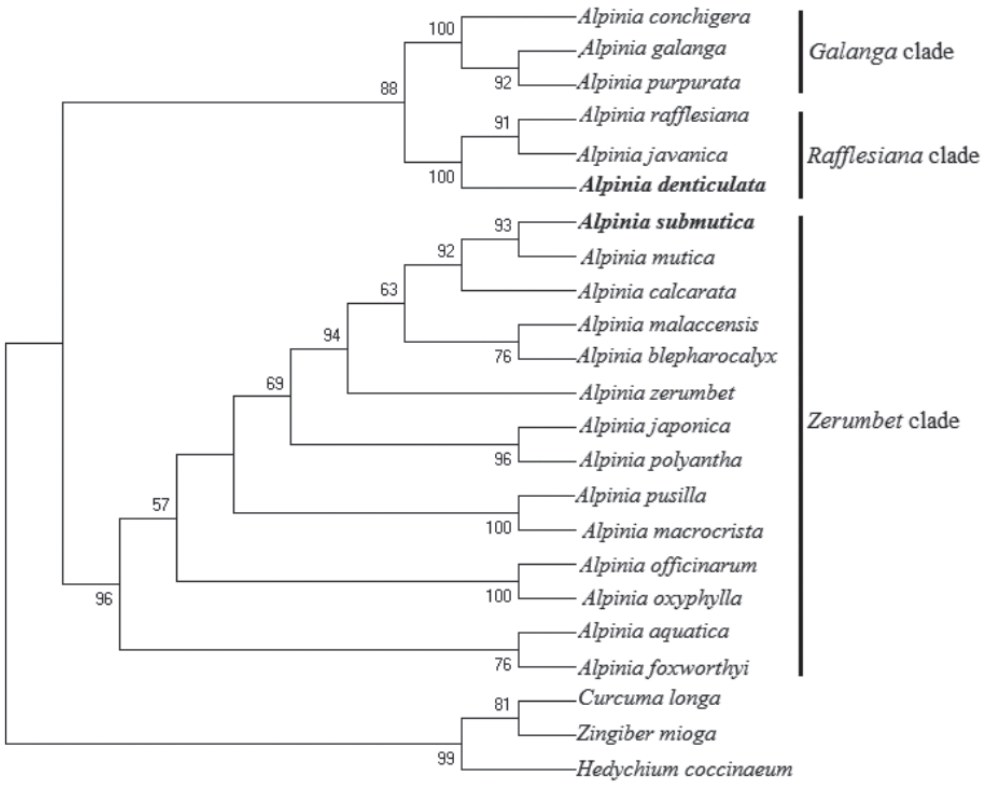

Figure 6. Maximum Parsimony phylogenetic tree (length = 245) based on combined ITS and *trnH-psbA* Intergenic Spacer region from this study and GenBank sequences data with bootstrap values (≥ 50%). Cluster name based on Kress et al. (2005a).

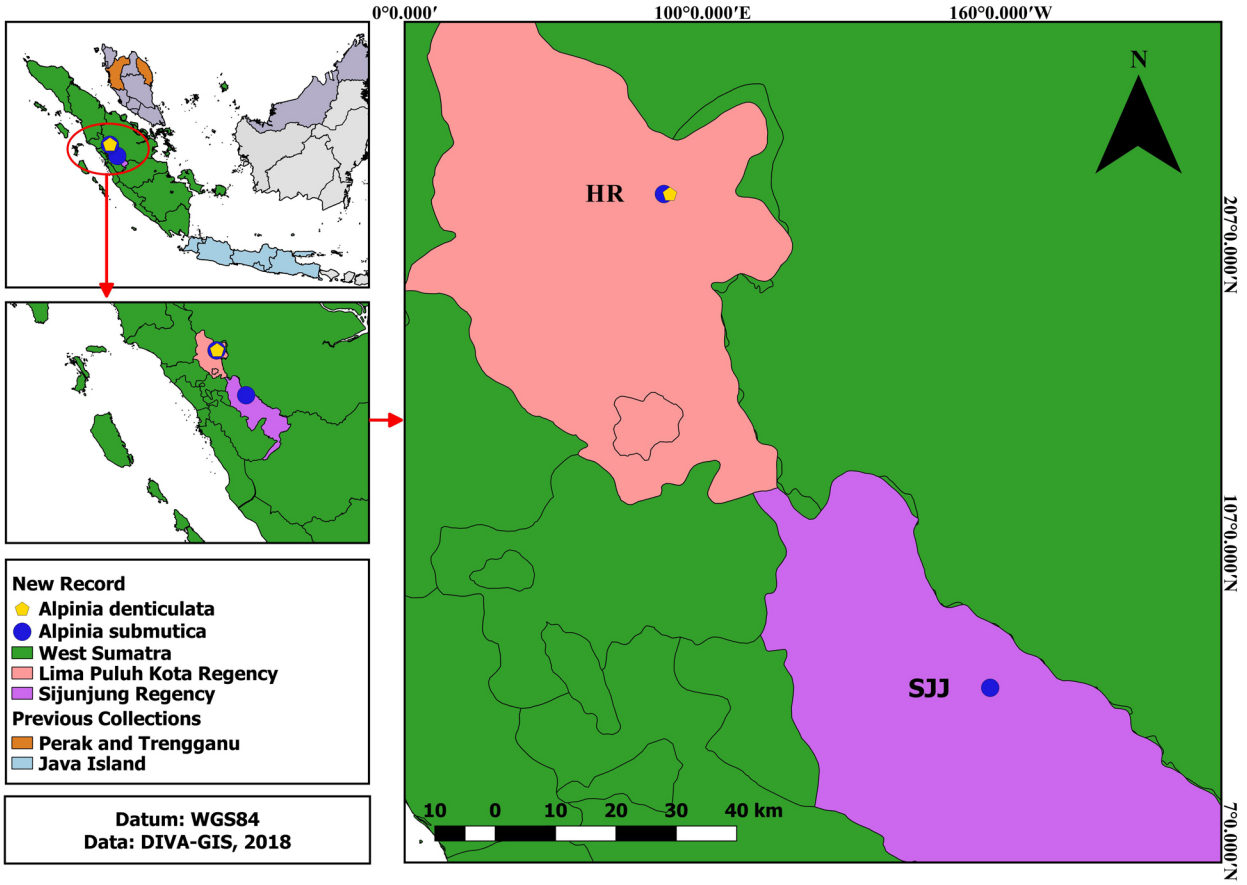

Figure 7. Collection side of 2 new records in Sumatra and previous collections of them based on Royal (2018) database and Schumann (1904b).

in the grouping of the the *Alpinia supertree* by Kress et al. (2005a). Based on its position, *A. submutica* is a sister taxon of *A. mutica* with a sequence divergence value of 1.2% and percentage bootstrap value more than 84% for ITS and combined ITS+*trnH-psbA*. They have similar morphological characteristics, but in previous studies were considered as distinct species because of the differences in fruit morphology. From this morphological and molecular character, it can be ascertained that both are different species.

Meanwhile, *A. denticulata* is in the same cluster with *A. rafflesiana* and *A. javanica* in Rafflesian Clade (Figs 3, 4). The sequence divergence value range of this cluster is 0.8–1% with strong bootstrap support of 100% for ITS and combined ITS+*trnH-psbA*, but weak bootstrap support (64%) for *trnH-psbA*. Rafflesian Clade group is not based on similarity of morphological characters, because *A. denticulata* has a small flowers and cylindrical ovary, while *A. rafflesiana* and *A. javanica* have large flowers with the square ovary. This cluster is clearly formed more on the similarity of distribution area in the Malay Peninsula (Kress et al. 2005a).

Conclusion. *Alpinia denticulata* and *A. submutica* are newly recorded species of *Alpinia* in Sumatra, supported by their morphological descriptions and a phylogenetic analysis.

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