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Genetic variation and phylogenetic position of *Taxus sumatrana* (Miq.) de Laub. (Taxaceae) in Sumatra, Indonesia based on *trn*L-*trn*F region

IRSYAD KAMAL[1], NAWWALL ARROFAHA[2], SUSILA[3], KUSUMADEWI SRI YULITA[4], HENTI HENDALASTUTI RACHMAT[4], ARIDA SUSILOWATI[5,♥]

[1]Department of Biology, Faculty of Science and Technology, Universitas Islam Negeri Walisongo Semarang. Jl. Prof. Dr. Hamka (Kampus II), Ngaliyan, Semarang 50185, Central Java, Indonesia
[2]Department of Biology, Faculty of Science and Technology, Universitas Islam Negeri Syarif Hidayatullah Jakarta. Ir. H. Juanda Street No. 95, Ciputat, South Tangerang 15412, Banten, Indonesia
[3]Research Centre for Biosystematics and Evolution, National Agency for Research and Innovation. Jl. Raya Bogor km 46, Cibinong 16911, West Java, Indonesia
[4]Research Centre for Ecology and Ethnobiology, National Agency for Research and Innovation. Jl. Raya Bogor km 46, Cibinong 16911, West Java, Indonesia
[5]Faculty of Forestry, Universitas Sumatera Utara. Jl. Padang Bulan, Medan 20155, North Sumatra, Indonesia. Tel.: +62-61-8220605, ♥email: arida.susilowati@usu.ac.id

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Abstract. *Kamal I, Arrofaha N, Susila, Yulita KS, Rachmat HH, Susilowati A. 2023. Genetic variation and phylogenetic position of* Taxus sumatrana *(Miq.) de Laub. (Taxaceae) in Sumatra, Indonesia based on* trn*L*-trn*F region. Biodiversitas 24: 5155-5166. Taxus sumatrana* (Miq.) de Laub. is a synonym of *T. wallichiana* Zucc. by some authors based on morphological characters and its distribution. This species is a medicinal plant and contains taxol as an anti-cancer with slow natural regeneration rate and increasing threats to their existence. This study aimed to determine the genetic diversity and the phylogenetic analysis using *trn*L-*trn*F for *T. sumatrana* growing in Sumatra, Indonesia. The total of 37 individuals from three natural populations of *T. sumatrana* were studied, consisting of the current new collection from Toba - North Sumatra and previously studied population of Dempo - South Sumatra and Sungai Penuh, Kerinci Seblat National Park - Jambi. The Toba population did not show variation at individual level within the population as similar results were obtained from the previous study. However, one indel (- and A) was found at 217 bp from all three *T. sumatrana* populations from Sumatra, Indonesia. When the sequences were aligned with the reference sequence from GenBank, single base substitutions were found at 344, 478,722, and 757 bp consisting of one indel and three single nucleotide polymorphisms (SNPs). Phylogeny tree showed that *T. sumatrana* from Sumatra population clustered together with *T. mairei* and *T. wallichiana* var. *mairei* and later combined with other *T. sumatrana* available in NCBI.

Keywords: Genetic variation, phylogenetics, Sumatra, *Taxus sumatrana*, *trn*L-*trn*F

INTRODUCTION

Taxus sumatrana (Miq.) de Laub. as known as Sumatra yew or *kayu taji* is a medicinal plant. The species is distributed in Indonesia (Sumatra and Celebes), Philippines, Malaysia, Vietnam, Myanmar, China, India, and Afghanistan (de Laubenfels and Syracuse 1988; De Padua et al. 2003). This species is one of the sources of taxol drugs for cancer. Taxol is obtained by extracting parts of the *Taxus* plant from bark, leaves, twigs, and roots (Hidayat et al. 2014). The high level of exploitation for taxol extraction causes the population of *Taxus* in the world to decrease drastically. In addition to population decline in nature, the species within the genus also show slow regeneration rate. Hence, the species is included in Appendix 2 CITES (CITES 2005), and is considered Endangered according to the IUCN Red List (Thomas and Farjon 2011). The sustainable use of the species cannot be guaranteed when threats continue to happen.

There are 24 species of *Taxus* distributed in the world, *T. sumatrana* is considered a synonym of *T. wallichiana* (https://powo.science.kew.org/), but as accepted following an alternative taxonomy by Farjon (2001). The specimens from the Philippines, Sumatra, and Sulawesi were identified as *T. wallichiana* (Farjon et al. 2014), while several reported *T. celebica* and *T. sumatrana* experienced expansion from Malesia into Indochina and China, or even to Nepal, and the distribution area was also the spread of *T. wallichiana* (Thomas and Farjon 2011). Although *T. celebica* and *T. sumatrana* in Malesia are considered distinct in the latest treatments (Farjon 2001; Spjut 2007a), their characters did not differ consistently from *T. wallichiana.* However, Spjut (2007b) discriminated *T. sumatrana* with *T. Wallichiana*. Liu et al. (2011) determined *Taxus* species using *rbc*L, *mat*K, *trn*H-*psb*A, *trn*L-*trn*F, and internal transcribed spacer (ITS) including *T. sumatrana* that discriminated with other *Taxus* species including *T. wallichian*a. In other *Taxus* species, *T. baccata* show high genetic diversity based on simple sequence repeat (SSR) markers (Hematzadeh et al. 2023).

The *trn*L-*trn*F region is one of the recommended regions for DNA Barcode (Zanjanchi and Mehrvarz 2015; Sevindik et al. 2019; Sanna et al. 2019; Sevindik and Okan 2020; Yi et al. 2021; Hocaoglu-Ozyigit et al. 2022). The

region has a conservative secondary structure and contains homologous elements throughout land plants, the sequence is catalytic, and this region can be used for evolutionary studies at higher taxonomic levels (Chebet et al. 2022). The *trn*L-*trn*F gene is widely used since the 1990s (D'yachenko et al. 2015; Hartvig et al. 2015; Koohdar and Sheidai 2022; Kipkiror et al. 2023), and has shown successful application in plant molecular systematics in flowering plants (Chen et al. 2013), such as *Mangifera* spp. (Fitmawati et al. 2017), *Laurus nobilis* (Sevindik et al. 2020), *Taxus* (Liu et al. 2011, 2018), *Diospyros* (Wanda et al. 2021), and *Ziziphora* (Dündar and Tümen 2023). It also can be used to distinguish plant species, such as Pteridaceae (Chen et al. 2013), *Syzygium* sp. (Roslim 2019), Dipsacales (Zhang et al. 2003).

Rachmat et al. (2016) collected *T. sumatrana* from Sungai Penuh, Jambi and Mt. Dempo, South Sumatra, followed by amplification using *trn*L-*trn*F, *psb*C-*trn*S, and *rbc*L genes, but showed no genetic variation in both individuals and populations. However, the study was still lacking one more population growing in Sumatra and thus failed to figure out the comprehensive samples representing *Taxus* growing in Indonesia. This study serves as a complementary investigation aimed at bridging the gap in the *T. sumatrana* population that has previously eluded sampling efforts. By encompassing previously unsampled *Taxus* populations, the study endeavors to yield a more comprehensive understanding. This holistic sampling approach is expected to provide a comprehensive representation of the genetic variations and phylogenetic positioning of *Taxus sumatrana* in Sumatra, Indonesia. Therefore, this study aimed to analyze the genetic diversity of *T. sumatrana* from Toba, North Sumatra and compare with previous studies from Mt. Dempo, Jambi and Sungai Penuh, South Sumatra populations, and determine the phylogenetic position of *T. sumatrana* in the genus *Taxus* using *trn*L-*trn*F region.

MATERIALS AND METHODS

Study site

The leaf samples of genetic materials were collected from all natural populations of *T. sumatrana* growing in Sumatra, Indonesia (Figure 1, Table 1).

Plant materials

DNA isolation of *T. sumatrana* was conducted in the Plant Molecular Systematics Laboratory, National Research and Innovation Agency (BRIN), Cibinong, Indonesia. The ten dried leaf samples come from the only remaining natural population of *T. sumatrana* in Toba, North Sumatra, Indonesia. Reference individuals were acquired from GenBank (https://www.ncbi.nlm.nih.gov/nuccore) to facilitate phylogenetic analysis. Additionally, 27 sequence *trn*L-*trn*F from previously collected natural populations of *T. sumatrana* from Dempo in South Sumatra and Sungai Penuh in Jambi (Rachmat et al. 2016) were included in our analysis. All samples and sequences used as provided in Table 1.

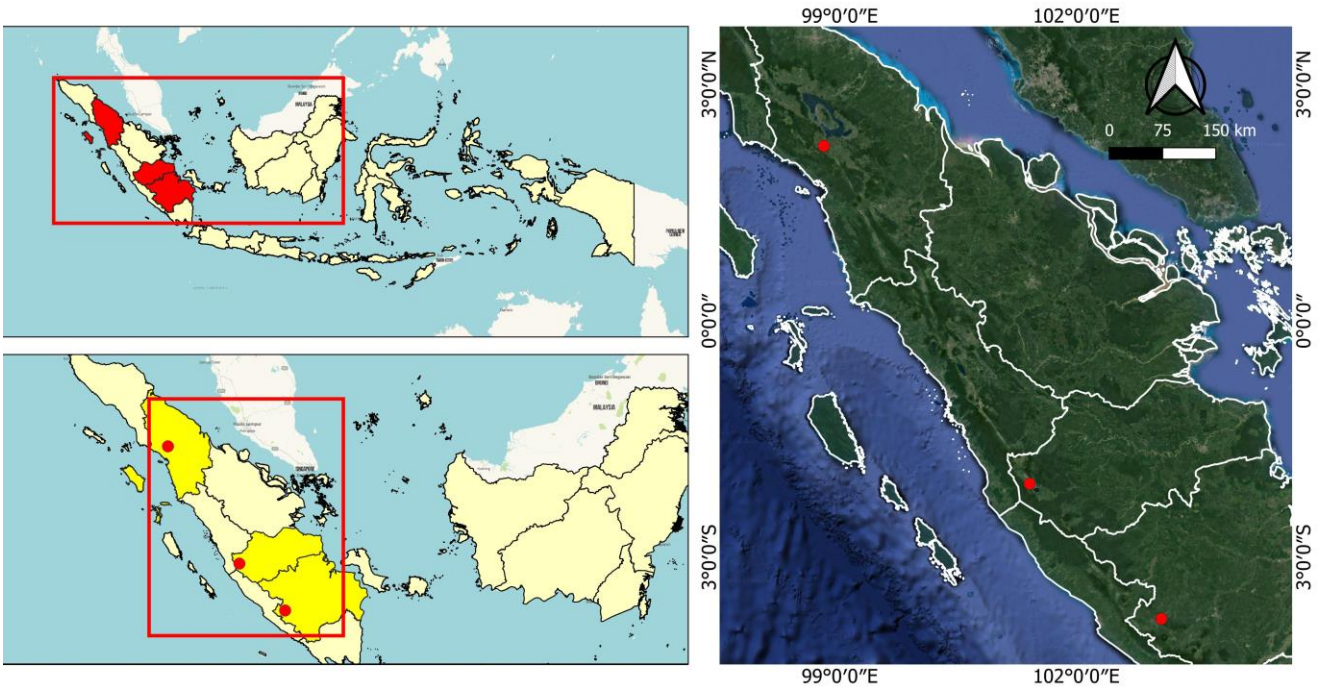

Figure 1. A map of study site of *Taxus sumatrana* in Sumatra, Indonesia

Table 1. Source of *Taxus sumatrana* from Sumatra, Indonesia and reference taxa were used for the phylogenetic analysis

Species	Locality	Sample Code / GenBank accession	Reference
Taxus sumatrana (Miq.) de Laub.	Toba, North Sumatra	Toba 1.1/ OR573938	*This study*
T. sumatrana (Miq.) de Laub.	Toba, North Sumatra	Toba 1.2/ OR573939	*This study*
T. sumatrana (Miq.) de Laub.	Toba, North Sumatra	Toba 1.3/ OR573940	*This study*
T. sumatrana (Miq.) de Laub.	Toba, North Sumatra	Toba 1.4/ OR573941	*This study*
T. sumatrana (Miq.) de Laub.	Toba, North Sumatra	Toba 1.5/ OR573942	*This study*
T. sumatrana (Miq.) de Laub.	Toba, North Sumatra	Toba 2.1/ OR573943	*This study*
T. sumatrana (Miq.) de Laub.	Toba, North Sumatra	Toba 2.2 OR573944	*This study*
T. sumatrana (Miq.) de Laub.	Toba, North Sumatra	Toba 2.3/ OR573945	*This study*
T. sumatrana (Miq.) de Laub.	Toba, North Sumatra	Toba 2.4/ OR573946	*This study*
T. sumatrana (Miq.) de Laub.	Toba, North Sumatra	Toba 2.5/ OR573947	*This study*
T. sumatrana (Miq.) de Laub.	Sungai Penuh, Kerinci, Jambi	Sungai Penuh 1/ OR487759	Rachmat et al. (2016)
T. sumatrana (Miq.) de Laub.	Sungai Penuh, Kerinci, Jambi	Sungai Penuh 2/ OR487760	Rachmat et al. (2016)
T. sumatrana (Miq.) de Laub.	Sungai Penuh, Kerinci, Jambi	Sungai Penuh 3/ OR487761	Rachmat et al. (2016)
T. sumatrana (Miq.) de Laub.	Sungai Penuh, Kerinci, Jambi	Sungai Penuh 4/ OR487762	Rachmat et al. (2016)
T. sumatrana (Miq.) de Laub.	Sungai Penuh, Kerinci, Jambi	Sungai Penuh 5/ OR487763	Rachmat et al. (2016)
T. sumatrana (Miq.) de Laub.	Sungai Penuh, Kerinci, Jambi	Sungai Penuh 6/ OR487764	Rachmat et al. (2016)
T. sumatrana (Miq.) de Laub.	Sungai Penuh, Kerinci, Jambi	Sungai Penuh 7/ OR487765	Rachmat et al. (2016)
T. sumatrana (Miq.) de Laub.	Sungai Penuh, Kerinci, Jambi	Sungai Penuh 8/ OR487766	Rachmat et al. (2016)
T. sumatrana (Miq.) de Laub.	Sungai Penuh, Kerinci, Jambi	Sungai Penuh 9/ OR487767	Rachmat et al. (2016)
T. sumatrana (Miq.) de Laub.	Sungai Penuh, Kerinci, Jambi	Sungai Penuh 10/ OR487768	Rachmat et al. (2016)
T. sumatrana (Miq.) de Laub.	Sungai Penuh, Kerinci, Jambi	Sungai Penuh 11/ OR487769	Rachmat et al. (2016)
T. sumatrana (Miq.) de Laub.	Sungai Penuh, Kerinci, Jambi	Sungai Penuh 12/ OR487770	Rachmat et al. (2016)
T. sumatrana (Miq.) de Laub.	Sungai Penuh, Kerinci, Jambi	Sungai Penuh 13/ OR487771	Rachmat et al. (2016)
T. sumatrana (Miq.) de Laub.	Sungai Penuh, Kerinci, Jambi	Sungai Penuh 14/ OR487772	Rachmat et al. (2016)
T. sumatrana (Miq.) de Laub.	Dempo, South Sumatra	Dempo 1/ OR487746	Rachmat et al. (2016)
T. sumatrana (Miq.) de Laub.	Dempo, South Sumatra	Dempo 2/ OR487747	Rachmat et al. (2016)
T. sumatrana (Miq.) de Laub.	Dempo, South Sumatra	Dempo 3/ OR487748	Rachmat et al. (2016)
T. sumatrana (Miq.) de Laub.	Dempo, South Sumatra	Dempo 4/ OR487749	Rachmat et al. (2016)
T. sumatrana (Miq.) de Laub.	Dempo, South Sumatra	Dempo 5/OR487750	Rachmat et al. (2016)
T. sumatrana (Miq.) de Laub.	Dempo, South Sumatra	Dempo 6/ OR487751	Rachmat et al. (2016)
T. sumatrana (Miq.) de Laub.	Dempo, South Sumatra	Dempo 7/ OR487752	Rachmat et al. (2016)
T. sumatrana (Miq.) de Laub.	Dempo, South Sumatra	Dempo 8/ OR487753	Rachmat et al. (2016)
T. sumatrana (Miq.) de Laub.	Dempo, South Sumatra	Dempo 9/ OR487754	Rachmat et al. (2016)
T. sumatrana (Miq.) de Laub.	Dempo, South Sumatra	Dempo 10/ OR487755	Rachmat et al. (2016)
T. sumatrana (Miq.) de Laub.	Dempo, South Sumatra	Dempo 11/ OR487756	Rachmat et al. (2016)
T. sumatrana (Miq.) de Laub.	Dempo, South Sumatra	Dempo 12/ OR487757	Rachmat et al. (2016)
T. sumatrana (Miq.) de Laub.	Dempo, South Sumatra	Dempo 13/ OR487758	Rachmat et al. (2016)
T. sumatrana (Miq.) de Laub.	Nueva Vizcaya, Philippines	HM591144.1	Liu et al. (2011)
T. sumatrana (Miq.) de Laub.	Nueva Vizcaya, Philippines	HM591145.1	Liu et al. (2011)
T. baccata L.	China	MH390453.1	Fu et al. (2019)
T. baccata L.	unknown	KR476375.1	Wu et al. (2015) (unpubl.)
T. baccata L.	China	MH390454.1	Fu et al. (2019)
T. baccata L.	China	MH390464.1	Fu et al. (2019)
T. baccata L.	Campagnano, Varese, Italy	EF680264.1	Shah et al. (2008)
T. baccata L.	Gorgan, Iran	EF680269.1	Shah et al. (2008)
T. baccata L.	Aberlady, Scotland	EF680267.1	Shah et al. (2008)
T. baccata L.	unknown	MK731952.1	Coughlan (2019) (unpubl.)
T. baccata L.	Oxford, UK	EF660635.1	Hao et al. (2009)
T. calcicola L.M.Gao & Mich.Möller	China	MH390451.1	Fu et al. (2019)
T. calcicola L.M.Gao & Mich.Möller	China	MH390489.1	Fu et al. (2019)
T. calcicola L.M.Gao & Mich.Möller	China	NC_041501.1	Fu et al. (2019)
T. calcicola L.M.Gao & Mich.Möller	China	MH390461.1	Fu et al. (2019)
T. canadensis Marshall	Montreal, Canada	EF660636.1	Hao et al. (2009)
T. canadensis Marshall	China	MH390483.1	Fu et al. (2019)
T. canadensis Marshall	China	MH390448.1	Fu et al. (2019)
T. canadensis Marshall	China	MH390466.1	Fu et al. (2019)
T. canadensis Marshall	China	NC_041499.1	Fu et al. (2019)
T. chinensis (Pilg.) Rehder	China	MH390442.1	Fu et al. (2019)
T. chinensis (Pilg.) Rehder	China	MH390476.1	Fu et al. (2019)
T. chinensis (Pilg.) Rehder	China	MH390478.1	Fu et al. (2019)
T. contorta Griff.	China	MH390443.1	Fu et al. (2019)
T. contorta Griff.	China	MH390449.1	Fu et al. (2019)
T. contorta Griff.	China	MH390455.1	Fu et al. (2019)

T. contorta Griff.	China	NC_041497.1	Fu et al. (2019)
T. contorta Griff.	Pakistan	KF765509.1	Poudel et al. (2014)
T. contorta Griff.	Pakistan	KF765507.1	Poudel et al. (2014)
T. contorta Griff.	Pakistan	KF765508.1	Poudel et al. (2014)
T. contorta Griff.	Pakistan	KJ768333.1	Poudel et al. (2014)
T. contorta Griff.	Pakistan	KJ768334.1	Poudel et al. (2014)
T. cuspidata Siebold & Zucc.	China	MH390447.1	Fu et al. (2019)
T. cuspidata Siebold & Zucc.	China	MH390465.1	Fu et al. (2019)
T. cuspidata Siebold & Zucc.	China	MH390477.1	Fu et al. (2019)
T. cuspidata Siebold & Zucc.	China	NC_041498.1	Fu et al. (2019)
T. cuspidata Siebold & Zucc.	unknown	MH463443.1	Wu et al. (2018) (unpubl.)
T. cuspidata Siebold & Zucc.	unknown	MF095888.1	Zhang et al. (2017) (unpubl.)
T. cuspidata Siebold & Zucc.	Russia	LT601776.1	Kozyrenko et al. (2017)
T. cuspidata Siebold & Zucc.	JiLin, China	EF660637.1	Hao et al. (2009)
T. cuspidata Siebold & Zucc.	Russia	LT601661.1	Kozyrenko et al. (2017)
T. cuspidata Siebold & Zucc.	Russia	LT601777.1	Kozyrenko et al. (2017)
T. cuspidata Siebold & Zucc.	Japan	EF660616.1	Hao et al. (2009)
T. florinii Spjut	China	MH390463.1	Fu et al. (2019)
T. florinii Spjut	China	MH390487.1	Fu et al. (2019)
T. florinii Spjut	Chins	MH390473.1	Fu et al. (2019)
T. florinii Spjut	China	NC_041504.1	Fu et al. (2019)
T. fuana Nan Li & R.R.Mill	India	EF680256.1	Shah et al. (2008)
T. fuana Nan Li & R.R.Mill	unknown	MF278259.1	Zhang et al. (2018) (unpubl.)
T. fuana Nan Li & R.R.Mill	unknown	NC_038099.1	Zhang et al. (2017) (unpubl.)
T. fuana Nan Li & R.R.Mill	Tibet, China	EF660638.1	Hao et al. (2009)
T. mairei (Lemée & H.Lév.) S.Y.Hu	unknown	AP014575.1	Hsu et al. (2014) (unpubl.)
T. mairei (Lemée & H.Lév.) S.Y.Hu	China	MH390479.1	Fu et al. (2019)
T. mairei (Lemée & H.Lév.) S.Y.Hu	unknown	JN867591.1	Li et al. (2011) (unpubl.)
T. mairei (Lemée & H.Lév.) S.Y.Hu	China	MH390482.1	Fu et al. (2019)
T. mairei (Lemée & H.Lév.) S.Y.Hu	China	MH390458.1	Fu et al. (2019)
T. mairei (Lemée & H.Lév.) S.Y.Hu	unknown	JN867586.1	Li et al. (2011) (unpubl.)
T. mairei (Lemée & H.Lév.) S.Y.Hu	unknown	KJ123824.1	Zhang et al. (2014) (unpubl.)
T. mairei (Lemée & H.Lév.) S.Y.Hu	unknown	JN867590.1	Li et al. (2011) (unpubl.)
T. mairei (Lemée & H.Lév.) S.Y.Hu	China	MH267558.1	Liu et al. (2018)
T. phytonii Spjut	China	MH390445.1	Fu et al. (2019)
T. phytonii Spjut	China	NC_041495.1	Fu et al. (2019)
T. phytonii Spjut	China	MH390441.1	Fu et al. (2019)
T. phytonii Spjut	China	MH390470.1	Fu et al. (2019)
T. sumatrana (Miq.) de Laub	unknown	EF660609.1	Hao et al. (2009)
T. wallichiana Zucc.	unknown	KR605490.1	Dinh et al. (2015) (unpubl.)
T. wallichiana Zucc.	unknown	KR605491.1	Dinh et al. (2015) (unpubl.)
T. wallichiana Zucc.	Tibet, China	EF660620.1	Hao et al. (2009)
T. wallichiana Zucc.	unknown	KR605492.1	Dinh et al. (2015) (unpubl.)
T. wallichiana Zucc.	unknown	KJ768336.1	Poudel et al. (2014)
T. wallichiana Zucc.	Solukhumbu District, Nepal	EF680275.1	Shah et al. (2008)
T. mairei (Lemée & H.Lév.) S.Y.Hu	China	EU052229.1	Gao et al. (2007)
T. mairei (Lemée & H.Lév.) S.Y.Hu	China	EU052227.1	Gao et al. (2007)
T. mairei (Lemée & H.Lév.) S.Y.Hu	China	EU052225.1	Gao et al. (2007)
T. mairei (Lemée & H.Lév.) S.Y.Hu	Taiwan	EU052230.1	Gao et al. (2007)
T. mairei (Lemée & H.Lév.) S.Y.Hu	China	EU052226.1	Gao et al. (2007)
T. mairei (Lemée & H.Lév.) S.Y.Hu	Taiwan	EU052231.1	Gao et al. (2007)
T. chinensis (Pilg.) Rehder	China	EU052213.1	Gao et al. (2007)
T. chinensis (Pilg.) Rehder	Hubei, China	EF660633.1	Hao et al. (2009)
T. chinensis (Pilg.) Rehder	China	EU052224.1	Gao et al. (2007)
T. chinensis (Pilg.) Rehder	China	EU052223.1	Gao et al. (2007)
T. chinensis (Pilg.) Rehder	Taiwan	EU052215.1	Gao et al. (2007)
T. chinensis (Pilg.) Rehder	China	KX431996.1	Jia and Liu (2017)
T. wallichiana Zucc.	Yunan, China	EU052221.1	Gao et al. (2007)
T. x hunnewelliana	Waterloo, Canada	EF017314.1	Hao et al. (2009)
T. x media	Delft, Netherland	EF660639.1	Hao et al. (2009)
T. sp. Emei type	China	MH390472.1	Fu et al. (2019)
T. sp. Emei type	China	MH390475.1	Fu et al. (2019)
T. sp. Emei type	China	MH390456.1	Fu et al. (2019)
T. sp. Huangshan type	China	MH390469.1	Fu et al. (2019)
T. sp. Huangshan type	China	MH390486.1	Fu et al. (2019)
T. sp. Qinling type	China	MH390444.1	Fu et al. (2019)
T. sp. Qinling type	China	MH390471.1	Fu et al. (2019)
T. sp. Qinling type	China	MH390481.1	Fu et al. (2019)
Cephalotaxus latifolia W.C.Cheng & L.K.Fu ex L.K.Fu & R.R.Mill	Hubei, China	EF017315.1	Hao et al. (2009)
Cephalotaxus oliveri Mast.	Guangxi, China	EF660619.1	Hao et al. (2009)

DNA isolation, amplification, and sequencing

The total DNA was isolated using Genomic DNA Plant Mini Kit (GeneAid) following the manufacturer's protocol. Amplification of the *trn*L-*trn*F region by the PCR technique was conducted using a universal pair primer of forward primer 'c' (CGAAATCGGTAGACGCTACG) and reverse primer 'f' (ATTTGAACTGGTGACACGAG) (Taberlet et al. 1991). This region is well-known as a universal primer and easy to amplify so it has a wide taxonomic range. The PCR mixture of a total volume of 12.5 µL consisted of 1.25 µL Green master mix (Promega) buffer, 1.25 µL MgCl, 0.25 µL dNTPs, 0.25 µL each primer forward and reverse, 0.07 µL Taq polymerase DNA (Promega), 1 µL DNA template, and 8.18 µL of nuclease-free water. The reaction was performed in a Sedi G thermal cycler (Wealtec) with the optimum condition as follows: a pre-denaturation at 94°C for 5 min, 35 cycle denaturation at 94°C for 1 min, annealing at 55°C for 1 min, extension at 72°C for 1 min, and final extension at 72°C for 8 min; and 4°C as holding temperature.

The amplified band was visualized on 1.0% agarose stained with GelRed (Biotium). Meanwhile, electrophoresis was executed with 100 V for 30 min in 1x TBE buffer, and the target bands were visualized under the UV light using a gel documentation system (Atto Bioinstrument). The PCR products were purified using Rapid Alkaline PhosphataseTM (Roche, Germany) and exonuclease I (New England Biolabs, Massachusetts, USA) following manufacturer instructions prior to sequencing. The purified PCR products were sent for Sanger Sequencing at the 1st Base Company, Singapore.

Data analysis

The *trn*L-*trn*F sequence data were assembled by the contig editor on MEGA X: Molecular Evolutionary Genetics Analysis version 11 (www.megasoftware.net) (Kumar et al. 2018). The forward and reverse sequences were observed to ensure there was no mismatch in the consensus produced, and then the *trn*L-*trn*F gene evaluated the nucleotide composition. Furthermore, the homology and identity of samples were examined using BLAST nucleotide on GenBank (https://blast.ncbi.nlm.nih.gov/Blast.cgi), and the data from GeneBank were downloaded and aligned using MEGA X software.

The evolutionary history was inferred by using the Maximum Likelihood method based on the Kimura 2-parameter model (K2P) (Kimura 1980; Kumar et al. 2018). Furthermore, the bootstrap consensus tree inferred from 1000 replicates represented the evolutionary history of the taxa analyzed (Felsenstein 1985). The branches corresponding to partitions reproduced in less than 50% of bootstrap replicates were collapsed. The percentage of the replicated trees in which the associated taxa clustered together (1000 replicates) was shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances. They were estimated using the Maximum Composite Likelihood (MCL) approach, and their topology was selected using a superior log-likelihood value. Additionally, we employed a discrete Gamma distribution with 5 categories (+G, with a parameter value of 6838.931) to characterize variations in evolutionary rates among sites. Bootstrap support (BS) values of ≥85% as strongly supported, 75-84% as moderately supported, 50-74% as weakly supported, and values <50% were not indicated (Devecchi et al. 2018).

Bayesian inference was performed using MrBayes 3.2.7a (Ronquist et al. 2012) as plugins in Geneious version 2023.0.1 (Geneious 2023), and the substitution model of HKY85 with rate variation of distributed gamma was applied for this analysis. Two independent MCMC runs of four chains for 1,100,000 generations and sampling tree topologies are for every 200 generations. Burn-in periods were set to 100,000 generations according to the standard deviation of split frequency values (<0.01). Meanwhile, a consensus topology and nodal support estimated as posterior probability values were calculated from the remaining trees. Posterior Probability (PP) values ≥0.95 as strongly supported and <0.95 as weakly supported (Devecchi et al. 2018).

RESULTS AND DISCUSSION

The sequence homology and identity of *T. sumatrana*

The amplicon size of the *trn*L-*trn*F chloroplast gene from the 10 samples of *T. sumatrana* from Toba population was 871 bp consisting of *trn*L intron and genes with the intergenic spacer. The *T. sumatrana* sequences were homolog to many sequences of the GenBank, 100 of which had similarities of more than 98% (data not shown). Furthermore, 94 reference species that will be used as ingroup taxa and 2 species as outgroups were incorporated into this study to build a phylogenetic tree based on Maximum Likelihood and Bayesian analyses.

Nucleotide composition and variations

The aligned DNA sequence of *trn*L-*trn*F is 802 bp. The *trn*L-*trn*F is an A/T rich region, composed of 32.6% thymine (T), 14.2% cytosine (C), 36.1% adenine (A), and 17.1% guanine (G) (Table 2). In this study, individuals of *T. sumatrana* samples from Toba show no variation, and similar pattern of no variation within population was determined for Dempo and Sungai Penuh populations (Rachmat et al. 2016). Nevertheless, we observed variations among populations. Specifically, we identified a single indel (- and A) at the 217th base pair in all three *T. sumatrana* populations from Sumatra, Indonesia. However, when the sequences were aligned with the reference sequence from GenBank, single base substitutions were found at 344, 478, 722, and 757 bp consisting of one indel and three single nucleotide polymorphisms (SNPs).

Phylogenetic analysis

The results of the phylogenetic tree reconstruction with *Cephalotaxus latifolia* (EF017315.1) and *C. oliveri* (EF660619.1) as outgroups using Maximum likelihood show that Toba populations formed a group together with Dempo and Sungai Penuh populations with bootstrap support 18 (Figure 2).

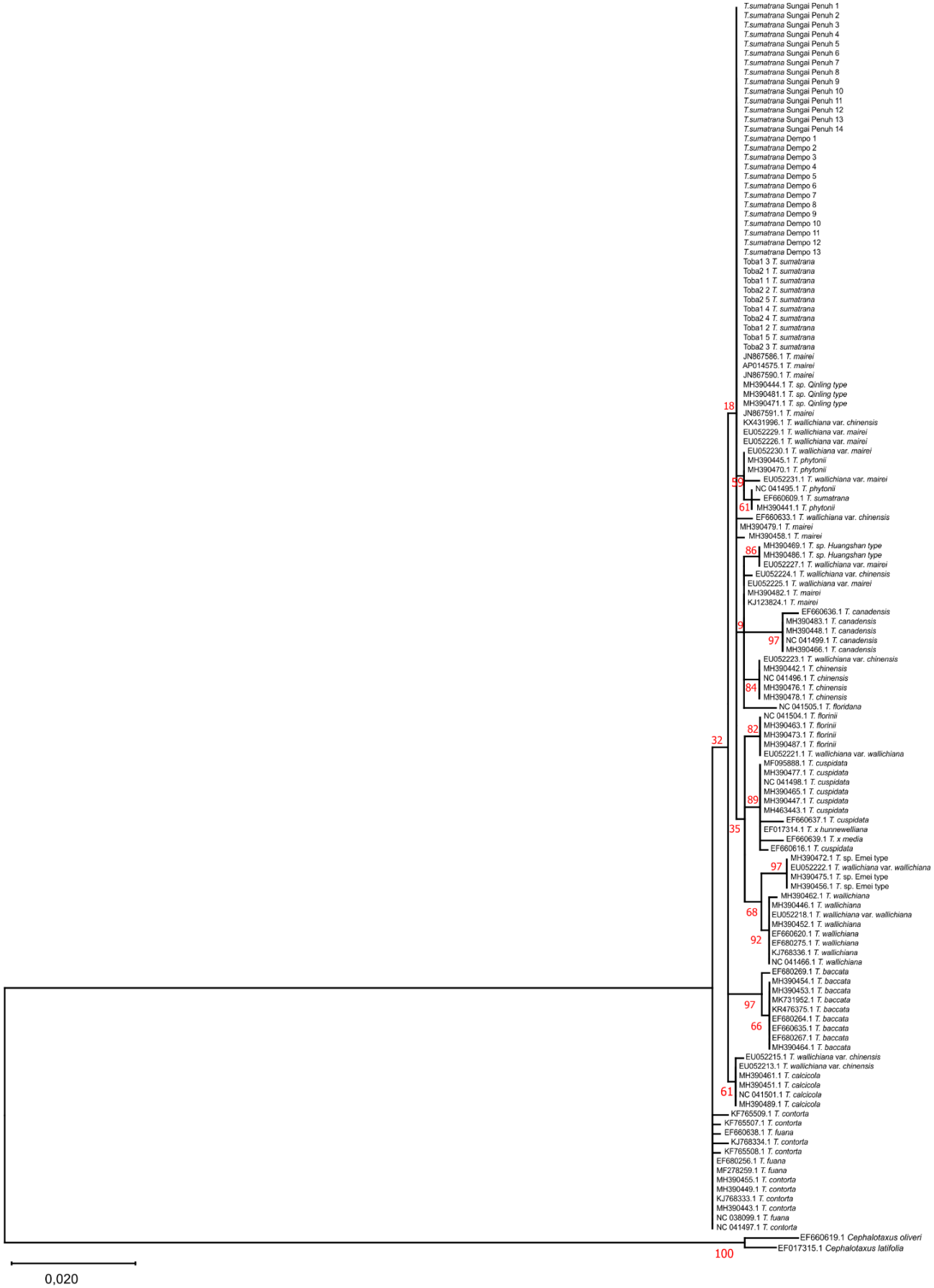

Figure 2. A phylogenetic tree generated by Maximum Likelihood method using *trn*L-*trn*F sequences. The tree with the highest log likelihood (-2250. 85) was shown, and branch supports were inferred using 1000 bootstrap replicates. The values are shown in the branches, and the tree is drawn to scale, with lengths measured in the number of substitutions per site

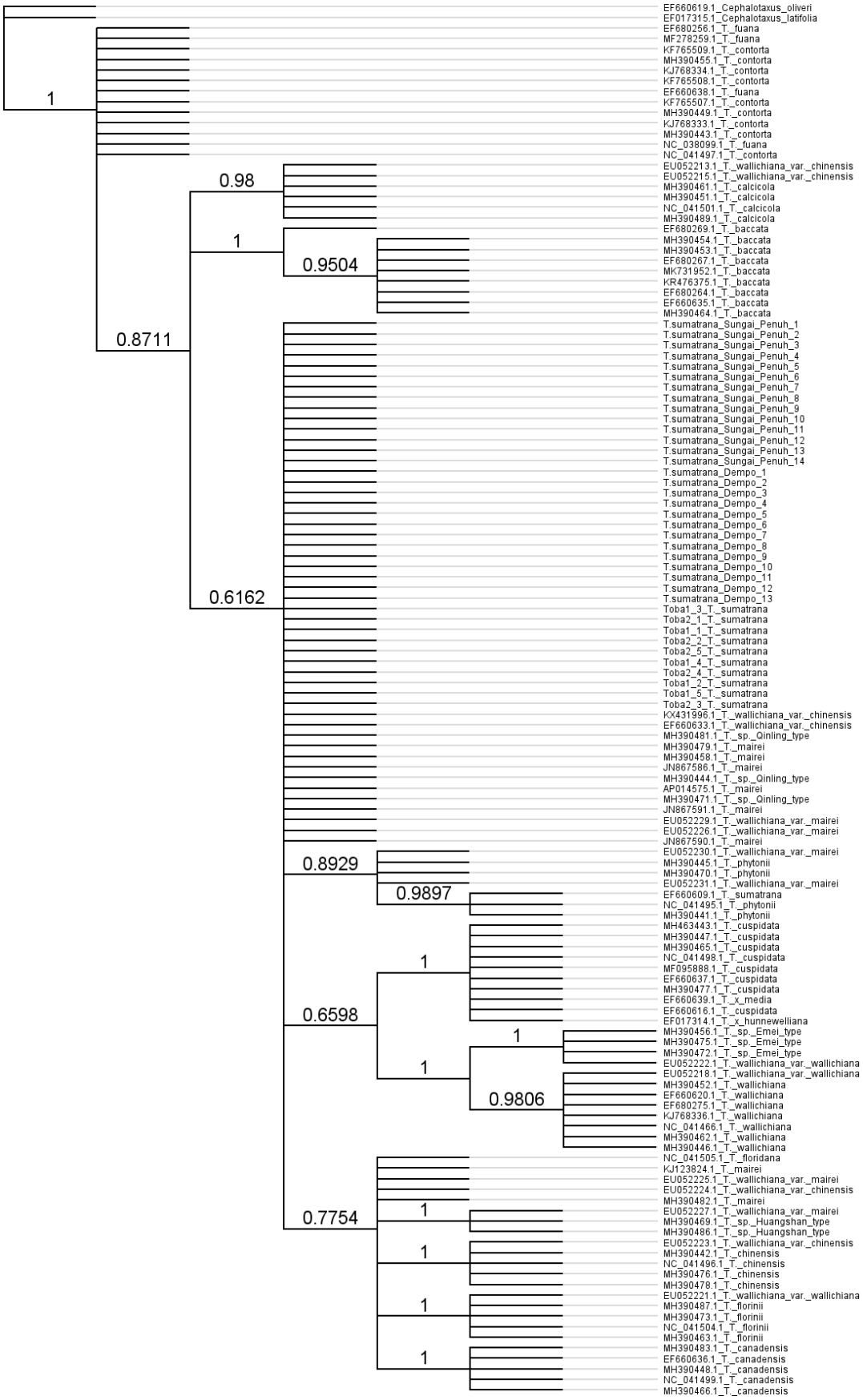

Figure 3. A phylogenetic tree generated by *trn*L - *trn*F sequences within genus *Taxus* based on Bayesian inference (Figure created from Geneious version 2021.0 created by Biomatters. Available from: https://www.geneious.com). The number above branches are Posterior Probability (PP) values

Table 2. Nucleotide composition and variation

Species	Nucleotide composition (%)				Position of point mutation				
	A	C	G	T	217	344	478	722	757
Reference nucleotide					-	T	G	-	C
Toba1 1 *T. sumatrana*	36,1	14,2	17,1	32,6	A	.	.	-	.
Toba1 2 *T. sumatrana*	36,1	14,2	17,1	32,6	A	.	.	-	.
Toba1 3 *T. sumatrana*	36,1	14,2	17,1	32,6	A	.	.	-	.
Toba1 4 *T. sumatrana*	36,1	14,2	17,1	32,6	A	.	.	-	.
Toba1 5 *T. sumatrana*	36,1	14,2	17,1	32,6	A	.	.	-	.
Toba2 1 *T. sumatrana*	36,1	14,2	17,1	32,6	A	.	.	-	.
Toba2 2 *T. sumatrana*	36,1	14,2	17,1	32,6	A	.	.	-	.
Toba2 3 *T. sumatrana*	36,1	14,2	17,1	32,6	A	.	.	-	.
Toba2 4 *T. sumatrana*	36,1	14,2	17,1	32,6	A	.	.	-	.
Toba2 5 *T. sumatrana*	36,1	14,2	17,1	32,6	A	.	.	-	.
T. sumatrana Dempo 1	36,0	14,3	17,1	32,6	-	.	.	-	.
T. sumatrana Dempo 2	36,0	14,3	17,1	32,6	-	.	.	-	.
T. sumatrana Dempo 3	36,0	14,3	17,1	32,6	-	.	.	-	.
T. sumatrana Dempo 4	36,0	14,3	17,1	32,6	-	.	.	-	.
T. sumatrana Dempo 5	36,0	14,3	17,1	32,6	-	.	.	-	.
T. sumatrana Dempo 6	36,0	14,3	17,1	32,6	-	.	.	-	.
T. sumatrana Dempo 7	36,0	14,3	17,1	32,6	-	.	.	-	.
T. sumatrana Dempo 8	36,0	14,3	17,1	32,6	-	.	.	-	.
T. sumatrana Dempo 9	36,0	14,3	17,1	32,6	-	.	.	-	.
T. sumatrana Dempo 10	36,0	14,3	17,1	32,6	-	.	.	-	.
T. sumatrana Dempo 11	36,0	14,3	17,1	32,6	-	.	.	-	.
T. sumatrana Dempo 12	36,0	14,3	17,1	32,6	-	.	.	-	.
T. sumatrana Dempo 13	36,0	14,3	17,1	32,6	-	.	.	-	.
T. sumatrana Sungai Penuh 1	36,0	14,3	17,1	32,6	-	.	.	-	.
T. sumatrana Sungai Penuh 2	36,0	14,3	17,1	32,6	-	.	.	-	.
T. sumatrana Sungai Penuh 3	36,0	14,3	17,1	32,6	-	.	.	-	.
T. sumatrana Sungai Penuh 4	36,0	14,3	17,1	32,6	-	.	.	-	.
T. sumatrana Sungai Penuh 5	36,0	14,3	17,1	32,6	-	.	.	-	.
T. sumatrana Sungai Penuh 6	36,0	14,3	17,1	32,6	-	.	.	-	.
T. sumatrana Sungai Penuh 7	36,0	14,3	17,1	32,6	-	.	.	-	.
T. sumatrana Sungai Penuh 8	36,0	14,3	17,1	32,6	-	.	.	-	.
T. sumatrana Sungai Penuh 9	36,0	14,3	17,1	32,6	-	.	.	-	.
T. sumatrana Sungai Penuh 10	36,0	14,3	17,1	32,6	-	.	.	-	.
T. sumatrana Sungai Penuh 11	36,0	14,3	17,1	32,6	-	.	.	-	.
T. sumatrana Sungai Penuh 12	36,0	14,3	17,1	32,6	-	.	.	-	.
T. sumatrana Sungai Penuh 13	36,0	14,3	17,1	32,6	-	.	.	-	.
T. sumatrana Sungai Penuh 14	36,0	14,3	17,1	32,6	-	.	.	-	.
EF660609.1 *T. sumatrana*	36,1	14,2	17,0	32,7	A	C	T	A	T
HM591144.1 *T. sumatrana*	36,1	14,1	17,0	32,8	A	.	T	A	T
HM591145.1 *T. sumatrana*	36,1	14,1	17,0	32,8	A	.	T	A	T
Average	36,03	14,24	17,11	32,62					

This Sumatra population was also clustered with *T. mairei*, *T. wallichiana* var. *mairei*, *T. wallichiana* var. *chinensis* and *Taxus* sp. *Qinling* type. Meanwhile, *T. sumatrana* (EF660609.1) from GeneBank originated from Nueva Vizcaya, Luzon, Philippines, but did not cluster with Sumatra population (Indonesia), but grouped with *T. wallichiana* var. *mairei* (EU052230.1 and EU052231.1), and *T. phytonii* (MH390470.1, MH390441.1, NC 041495.1, and MH390445.1). A similar topology was obtained by Bayesian analysis where Toba population was in a group with Dempo and Sungai Penuh populations, and group with *T. mairei*, *T. wallichiana* var. *mairei*, *T. wallichiana* var. *chinensis*, and *Taxus* sp. *Qinling* type with Bayesian support of 0.6162 (Figure 3).

Discussion

Our results show the *trn*L-*trn*F sequence of *T. sumatrana* was homolog to many sequences with 871 bp in NCBI GenBank. The sequence was homolog with *T. sumatrana*, *T. mairei*, and other *Taxus* species which had similarities of more than 98.00%. The high sequence similarity confirms that the sample sequences from three populations on the Sumatra belong to the *Taxus* species, establishing its southernmost natural distribution. The nucleotide composition of *T. sumatrana* from Toba population is different from Dempo and Sungai Penuh populations at A and T composition (0.1%), while G and C have the same composition. However, in general nucleotide composition *T. sumatrana* from Sumatra has a similarity with the sequence from GenBank. The GC content is higher than AT content, causing a more stable bond for this

region (Borisova et al. 1993; Chebet et al. 2022) explain that the *trn*L-*trn*F region is a conservative region, where this region that has remained essentially unchanged throughout evolution.

Taxus sumatrana is a threatened species by extinction and also suffers from limited natural existence and restricted natural breeding opportunities. Alongside in situ and ex-situ conservation approaches, genetic preservation also plays a pivotal role in safeguarding *T. sumatrana*. A genetic analysis targeted at the *trn*L-*trn*F non-coding region of the chloroplast DNA was performed on *T. sumatrana* samples collected from Toba, Sumatra, Indonesia. Although this study revealed no genetic variation within the Toba population, a comparison with a previous study encompassing different populations (Rachmat et al. 2016) identified a single insertion-deletion (A/-) at 217 bp. Based on its geographical location, the Toba population is located in the northern part, meanwhile the Dempo and Sungai Penuh populations are in the southern part of Sumatra, the distance among these populations is ± 882 km (Google Maps 2023). All three populations of *T. sumatrana* included in the Bukit Barisan mountain range, and there are natural barriers, including Bukit Kaba, Musi River, Bukit Daun, Bukit Belirang-Beriti, Mount Kunyit, Mount Sumbing, Mount Hutanpanjang, Batanghari River, Mount Talang, Mount Tandikat, Mount Sago, Mount Marapi, Mount Sarik Gajah, Mount Talamau, Mount Sorik Marapi, Mount Lubukraya, and Mount Sibualbuali (Setiawan et al. 2019; Hutchings and Mooney 2021; WorldAtlas 2023). The mountain range in the Bukit Barisan between the Toba population and the Dempo and Sungai Penuh populations plays an effective role as a physical barrier, contributing to the genetic differences among these populations.

During the last glacial period, which occurred approximately 20,000 years ago, certain areas in Sumatra including the western and northern parts, along with the northern and northeastern parts of Kalimantan, as well as the Mentawai Islands, acted as rainforest refugia. This has been supported by studies conducted by Ohtani et al. (2013), Roberts and Petraglia (2015), and Utomo et al. (2018). Similar investigations carried out in Sumatra have demonstrated a positive correlation between geographic and genetic distances. This phenomenon is evident in the population structuring of species like *Styrax sumatrana* (Rachmat et al. 2017), *Eurycoma apiculata* (Zulfahmi et al. 2021), *Hopea hainanensis* (Wang et al. 2020), dan *Dryobalanops aromatica* (Ritonga et al. 2018). Furthermore, several studies on *Taxus* also support the significant influence of physical barriers on the genetic structure of the species, as seen in *Taxus baccata* (Litkowiec et al. 2018; Maroso et al. 2021; Komárková et al. 2022; Hematzadeh et al. 2023), and *Taxus wallichiana* var. *mairei* in China (Luo et al. 2021). Therefore, these studies demonstrate that high mountains and large rivers can be considered effective barriers to gene flow (de Morais et al. 2015; Li et al. 2019).

The diversity among populations is influenced by the distance of gene flow and geographic isolation (Wu et al. 2015; Pérez-Alquicira et al. 2023). Populations that are distant from each other and separated by geographical features will differentiate due to adaptation to their respective environments and reproductive isolation, leading to greater genetic divergence. On the other hand, populations that are close to each other will have gene flow, resulting in lower diversity or higher levels of relatedness due to allele mixing (Choudhuri 2014; Sork 2016; Mihalik et al. 2020).

The examination of the *trn*L-*trn*F region in *Taxus* spp. conducted by Coughlan et al. (2020) yielded limited molecular variance for distinguishing between *Taxus* species. This outcome is consistent with our findings from *T. sumatrana* samples, which similarly exhibited no variations. Nevertheless, *trn*L-*trn*F remains the optimal barcode for *Taxus* as it is capable of effectively discerning all *Taxus* species, enabling the classification of distinct groups for each species (Liu et al. 2018).

The phylogenetic analyses with *trn*L-*trn*F reported here provide Bayesian support (0.6162) and bootstrap support (18) for groupings and indicate that *T. sumatrana* group with *T. wallichiana, T. wallichiana* var. *mairei, T. mairei*, and *T.* sp *Qinling* type. *Taxus sumatrana* from three populations in Sumatra didn't have one branch, but still in one group with different branches with *T. sumatrana* from GenBank originating from Nueva Vizcaya, Luzon, Philippines. This condition caused the differences in nucleotide compositions due to regional differences and differences in geographic conditions. Besides that *T. fuana* groups with *T. contorta. T. baccata* clustered into a group, but it was close to the group of *T. wallichiana* var. *chinensis (T. chinensis),* and *T. calcicola.* However, *T. wallichiana* group with *T. wallichiana* var. *wallichiana* and *T. emei type,* and *T. cuspidata* groups with *T.* × *media* and *T.* ×*hunnewelliana*. *Taxus canadensis* groups with *T. florini*, *T. chinensis*, *T. wallichiana* var. *mairei*, *T wallichiana* var. *wallichiana*, and *T. huangsan* type. In maximum likelihood tree *T. florinii* group with *T. cuspidata* group, then *T. canadensis* and *T chinensis* became their groups. That *T. sumatrana* from Genbank group with *T. phytonii* and *T. wallichiana* var. *mairei* was resolved as monophyletic but its varieties *T. wallichiana* var. *marie*, var. *chinensis* and var. *wallichiana* are not monophyletic, although individuals within variety do generally group. *Taxus baccata, T. canadensis*, and *T. cuspidata* are closely related but are not well resolved. There is little evidence for their monophyly. The networks support the clear separation of *T. wallichiana* from an unresolved group of *T. baccata, T. canadensis*, and *T. cuspidata* on the other side. Morphological identification that has been carried out on the *T. sumatrana* samples from the three populations of Sumatra showed that the leaf, bark, tree habitus, and fruit samples have similar characters, the more comprehensive study on molecular identification is needed to shear more light on the species identification.

Farjon (2017) and Spujt (2007b) employed morphological characteristics for the characterization of *Taxus* spp. Nevertheless, the utilization of morphological characters for such characterization poses challenges, leading to arguments suggesting the integration of these characters into a unified species. *Taxus sumatrana*, and *T.*

celebica are synonymous species *of T. wallichiana*, and *T. mairei* and *T. chinensis* are considered varieties of *T. wallichiana*, and through phylogenetic tree analysis, *T. sumatrana* is closely related to *T. mairei* (Liu et al. 2011). Twenty-four species and 55 varieties were categorized into three groups based on distinctions in leaf epidermal and stomatal characteristics as follows: (1) the Wallichiana group comprising the subgroups wallichiana and chinensis; (2) the Baccata group including the subgroups baccata and cuspidata; and (3) the undivided Sumatrana group. Elpe et al. (2018) developed a new identification key, based on leaf anatomical characters, using fluorescence microscopy. They found the presence of papillae on the abaxial midrib and on the adaxial leaf surface of *T. brevifolia*, to be a useful tool for separating *T. brevifolia*, *T. floridana*, *T. globose*, and *T. wallichiana* from other species. However, no differences were found between species, which had a papillose midrib, nor species which lacked this character.

In conclusion, our results showed the genetic diversity of the *trn*L-*trn*F region in *T. sumatrana* has five single substitutions consisting of three SNPs and two indels, single base substitutions were found at 217, 344, 478,722, and 757 bp. The population of Sungai Penuh, Jambi and Dempo, South Sumatra have a close relationship with no nucleotide variation and the absence of nucleotide variations from the two populations and these populations are different from the Toba by one indel (- and A) and was found at 217 bp from all three *T. sumatrana* populations from Sumatra, Indonesia. The nucleotide variation grouped the population of *T. sumatrana* into two groups, namely the first group was the population of Sungai Penuh and Dempo and the second group was Toba, North Sumatra population. Phylogeny tree from maximum Likelihood and Bayesian showed that *T. sumatrana* from Sumatra populations clustered together with *T. mairei* and *T. wallichiana* var. *mairei* and later combined with other *T. sumatrana* available in NCBI.

Based on the current study, several actions are needed to conserve the valuable *T. sumatrana* species. This includes conducting a deeper genetic assessment in Toba, Sungai Penuh, and Dempo to understand genetic diversity and connectivity among populations. Preserving suitable habitats is crucial to maintain gene flow and genetic identity. Establishing a seed collection program and researching effective germination and propagation methods are essential for long-term genetic diversity preservation. Lastly, creating long-term monitoring programs will help assess population dynamics and threats to ensure the species' survival.

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