GEOGRAPHIC PATTERN OF GENOTYPIC VARIATION OF RAMIN IN SARAWAK: TOWARDS THE IDENTIFICATION OF THE ORIGIN OF TIMBER

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ITTO-CITES Activity: Use of DNA for Identification of *Gonystylus* species and Timber Geographical Origin in Sarawak

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List of Abbreviations

EUTR	European Union Timber Regulation
FLEGT	Forest Law Enforcement, Governance and Trade
RFID	Radio Frequency Identification
cpDNA	Chloroplast DNA
INDELs	Insertions and deletions
NJ	Neighbor-joining
PSF	Peat Swamp Forest

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Summary

International concern on the drastic reduction of Peat Swamp Forest and the sustainable production of Ramin has caused the listing of Ramin in CITES Appendix II in which all export and import of Ramin timber and products will require a CITES Certificate of Origin. Trading of Ramin and other CITES-listed species in The European Union countries is governed by the European Union Timber Regulation (EUTR) mandatory regulation. Meanwhile in Sarawak, the export of Ramin log is totally prohibited and in some states in Malaysia, log tracking system is implemented, where logs from legal and illegal sources can be distinguished.

Despite all the control system introduced, there are reports of illegal timber from Indonesia being re-exported through Malaysia with false documents. A tracking system to check and verify the origin of timber is very important to monitor and control the trading of Ramin timber.

During this study, the geographic pattern of genotypic variation for Ramin populations in Sarawak is assessed and examined to confirm whether the pattern is applicable for tracing Ramin in Sarawak. A total of eight rare haplotypes were found of which six haplotypes were only found in a single population. In addition, when assignment test was conducted to analyse the capability of the samples to be assigned back, the percentage of correct assignment rates were 98.91% and 99.23% when the Ramin populations in Sarawak were compiled into two large populations based on the geographical

distance. These findings indicated the possibility of the use of molecular DNA in tracing the origin of timber. Molecular DNA technology could be a powerful and important tool in preventing and combating international trafficking of timber and timber products from illegal sources.

1.0 Introduction

Pear Swamp Forests (PSF) is very important ecosystem as it is a true carbon sink where carbon is being sequestered and converted into peat. The volume of carbon pool in tropical peat land in South East Asia is estimated to be 68.5 Gigatonnes (11-14% of the global peat carbon pool). Of this, 57.4 Gigatonnes is in Indonesia and 9.1 Gigatonnes is in Malaysia (Page *et al.* 2011). PSF is also important as the habitat for threatened taxa especially *Gonystylus bancanus* (Ramin). The species is valuable for its timber which is used for the production of mouldings, furniture, picture frames and billiard cues. In 2001 a total of 39,285 m^3 of Ramin sawn timber was been exported to countries especially Hong Kong, Italy, China, Singapore and Japan as stated in the UNEP-WCMC CITES Trade database.

However, over the years the area of PSF has shown drastic reduction. At the end of the seventies, the PSF in Sarawak covered an area of 1,455,000 ha (Lee, 2004). Almost twenty years later, the satellite images of 1995/1996 showed that the area covered by PSF has reduced to 1,126,834 ha (Wong, 2003). Then in 2004 only slightly more than 320,000 ha of PSF were left in the Permanent Forest Estate (PFE) (Chai, 2004). More than 300, 000 ha of PSF in PFE has been converted to agricultural plantation and a further 300,000 ha were earmarked for conversion to oil palm plantation in 2000 (Sawal, 2004). To date, only a total of 78,481 ha of PSF are gazetted as national parks namely: Loagan Bunut National Park, Maludam National Park, Sedilu National Park and Ulu Sebuyau National Park (NP).

The degradation of PSF has shown an impact on the supply of Ramin from Sarawak. Ramin timber production in 1990 was 521,000 m^3 and in 2000 was only 67,999 m^3. To sustain Ramin production, the Sarawak Government banned the export of Ramin logs in 1980. In 2005, Ramin was included in CITES Appendix II. The international trade of Ramin timber and products thus far has to be regulated under CITES. All export and import of Ramin require a CITES Certificate of Origin. During the 15th session of United Nation (UN) Commission on Crime Prevention and Criminal Justice and at the Conference of State Parties to the Convention in Transnational Organised Crime held in Vienna in 2006, international cooperation was called to prevent and combat international trafficking in timber and timber products from illegal logging. Measures to control and prevent exportation of endangered tree species were established and the log tracking system that can trace logs from source to their final destination was implemented in some Malaysian states.

In 2012, the European Union Commission adopted the European Union Timber Regulation (EUTR) mandatory regulation. The EUTR prohibits the sale of illegally harvested timber and products to EU and only timber accompanied by the Forest Law Enforcement, Governance and Trade (FLEGT) and CITES permits will be accepted as legal.

Efforts to strengthen the control in the trading of CITES-listed species are continuously being discussed internationally. Timber tracking technologies including paint markings, plastic tagging, barcoding, Radio Frequency Identification (RFID), DNA technique and Isotopic sampling to monitor the illegal trade were introduced and research to enhance the tracking technologies is being conducted by many research institutions in many countries. Among them, the DNA technique is one of the most promising tools to prove the legality of timber.

2.0 Objective

The objective of this study was to assess the geographic pattern of genotypic variation in Ramin populations and to examine if the geographic pattern is applicable for tracing the origin of Ramin in Sarawak. We used a widely-distributed species, *G. bancanus,* collected from the nine localities as the reference species and examined the haplotype of cpDNA and genotype of the microsatellite.

3.0 Materials and Method

3.1 Data retrieval

Samples of template DNA of *Gonystylus bancanus* which were collected from Kayangeran Forest Reserve (F.R)., Loagan Bunut N.P., Naman F.R., Manggut F.R., Betong F.R., Serapau-Lingga F.R., Lingga water catchment area, Sedilu N.P. and Maludam N.P during the previous ITTO CITES project “Development of DNA database of *Gonystylus bancanus* in Sarawak” was used. The sequence data from 16 microsatellite regions (Gba028, Gba092, Gba108, Gba129, Gba147, Gba348, Gba430, Wgb06, Wgb17, Wgb22, Wgb24, Wgb29, Wgb32, Wgb37, Wgb38 and Wgb39) were analysed. In addition, DNA sequence data from five chloroplast DNA (cpDNA) regions [*trnE-T* (654 – 664bp); *trnK-rps16* (616 – 621bp); *psbM-trnD* (640- 641bp); *trnG-rps14* (456-462bp) and *ycf9-trnG* (631 – 638bp)] generated by Forest Research Institute Malaysia (FRIM) were also included in this analysis.

3.2 Data analysis

Haplotypes were first determined after the chloroplast DNA sequence data were retrieved. Then, the distribution of haplotypes in each location was plotted on a map. At the same time, the microsatellite sequence data were assigned for self-assignment test. The data was analyzed using STRUCTURE 2.3.4 (Falush *et al.*, 2003) to determine the best number of populations (K) using admixture model, a burn-in of 10000 iteration and ten simulations for each K. Then, using the generated result, a delta K graph was plotted by an online program named Structure Harvester (Earl and von Holdt, 2012). After determining the number of population genetic clusters to be formed, the resolution percentage was calculated for each population.

4.0 Results and discussion

4.1 Chloroplast DNA polymorphism

A total of nine haplotypes (H1-H9) were defined for the five cpDNA in Sarawak (Table 1). We found a total of 28 polymorphic characters (13 substitutions and 15 insertions and deletions (INDELs)) over the five cpDNA region among the 152 samples. The distribution of each haplotype was plotted on the map (Figure 1). H1 (with 77.6% occurrence) is the most common haplotype and is found in every population. This is followed by H2 (13.2%), which is found in three populations (Kayangeran, Loagan Bunut and Sedilu), and H3 (3.9%), which is found in Naman and Sedilu. The rarer haplotypes which are located in restricted areas, such as H4, H7 and H8 are only found in Naman, H6 and H9 are only found in Serapau-Lingga and H5 is only found in Lingga. These rare haplotypes are all found in the southwest of Sarawak. This suggested that *G. bancanus* might have been well distributed in this area for a long period of time in the past for new mutations to occur and have not yet dispersed to other areas (Duminil *et al.*, 2010). However, no clear division of regions in Sarawak was revealed.

Haplotype	PA4_trnE-T				PA1_trnK-rps16									
	197	198-202	203-206	261-266	81	86	201	375	406	484	485-488	490-494	497	505-509
H1	A	:	ATAT	GTGAT	C	A	A	T	C	:	:	ATAAG	A	:
H2	A	:	ATAT	:	C	A	A	T	C	:	:	ATAAG	A	AAATA
H3	A	:	ATAT	GTGAT	T	G	A	T	C	T	TATT	ATAAG	A	AAATA
H4	A	:	ATAT	GTGAT	C	A	A	T	C	:	:	ATAAG	A	:
H5	:	:	:	GTGAT	C	A	C	T	T	T	:	CTTAT	:	:
H6	A	GAATA	ATAT	GTGAT	T	G	A	T	C	:	:	ATAAG	A	AAATA
H7	A	:	ATAT	GTGAT	C	A	A	T	C	T	:	CTTAT	:	:
H8	A	:	ATAT	GTGAT	C	A	A	T	C	:	:	ATAAG	A	:
H9	A	:	ATAT	GTGAT	C	A	A	A	C	:	:	ATAAG	A	:

Table 1: Haplotypes of *Gonystylus bancanus* in Sarawak.

Haplotype	PV_psbM-trnD			PX_trnG-rps14		PA5_ycf9-trnG								
	19	49	115	96	302	62	83	119-123	125	126-127	131-135	137-138	183-186	200-203
H1	:	G	C	G	A	C	C	CTAAA	T	TA	TTTAG	AA	:	:
H2	:	G	T	G	A	A	C	CTAAA	T	TA	TTTAG	AA	:	:
H3	T	G	T	G	A	A	A	CTAAA	T	TA	:	:	:	:
H4	:	T	C	G	A	C	C	CTAAA	T	TA	TTTAG	AA	:	:
H5	:	G	T	T	G	A	C	:	A	:	TTTCT	AA	ATTT	ATTT
H6	:	G	T	G	A	A	C	CTAAA	T	TA	TTTAG	AA	:	:
H7	:	G	C	G	A	C	C	CTAAA	T	TA	TTTAG	AA	:	:
H8	T	G	C	G	A	C	C	CTAAA	T	TA	TTTAG	AA	:	:
H9	:	G	C	G	A	C	C	CTAAA	T	TA	TTTAG	AA	:	:

Table 1: Haplotypes of *Gonystylus bancanus* in Sarawak. (continued)

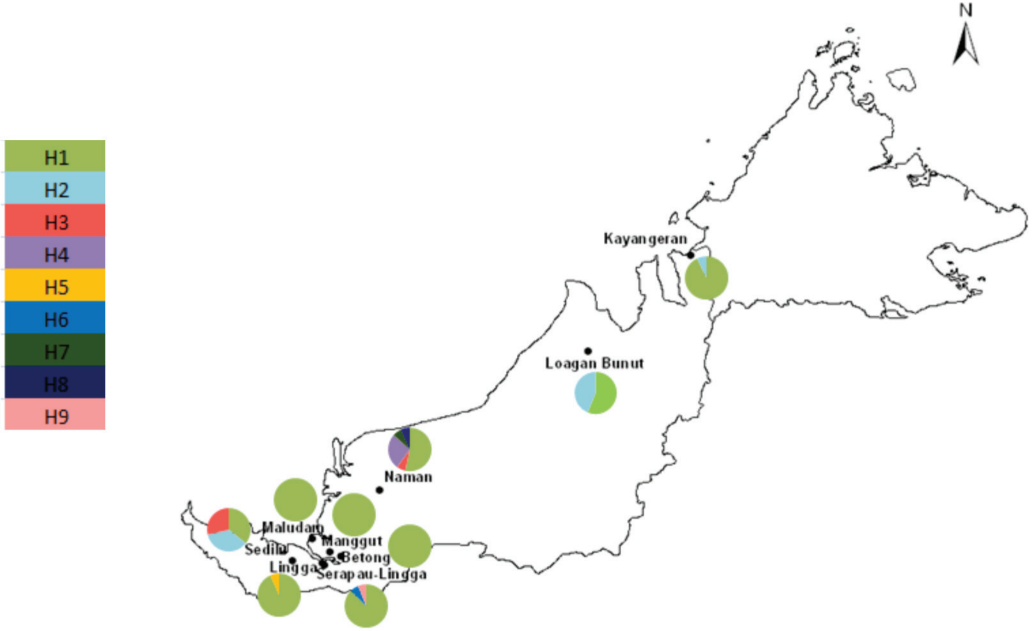


Figure 1: Distribution of haplotypes in Sarawak.

4.2 Self-assignment test

The assignment test was done to analyze the capability of the samples to be assigned back to the original populations. After the microsatellite data of *G. bancanus* in Sarawak was analyzed using STRUCTURE 2.3.4, a delta K graph (Figure 2) was plotted. The peak of the graph is at K value of two. Hence, the analyzed data are best to be integrated into two larger populations. A bar plot of data division into two populations was obtained, as well as the neighbor-joining (NJ) tree (Figure 3). The bar plot and NJ tree showed that the populations are generally divided into two groups where Loagan Bunut and Kayangeran are grouped together (red) and the remaining populations are in another group (green). However, the Naman column in the bar plot contained a mixture of red and green with a majority of green. The generated NJ tree also placed Naman between the two populations but closer to the population which is green in the bar plot. This indicated that Naman served as the intermediate population between the two divided populations. This result is in accordance with the location of the populations in the map as Kayangeran and Loagan Bunut are located a distance from the other populations in Sarawak, whereas Naman is located in the central region of Sarawak but closer to the populations in the southwest of Sarawak.

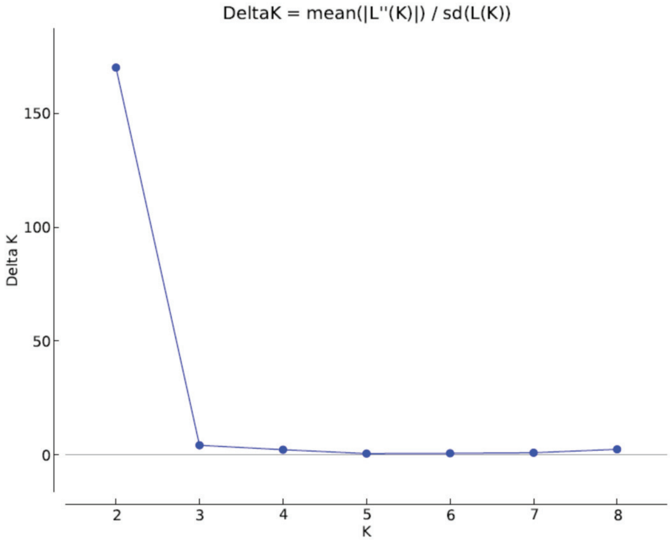


Figure 2: Delta K graph which determines the best K value (number of population).

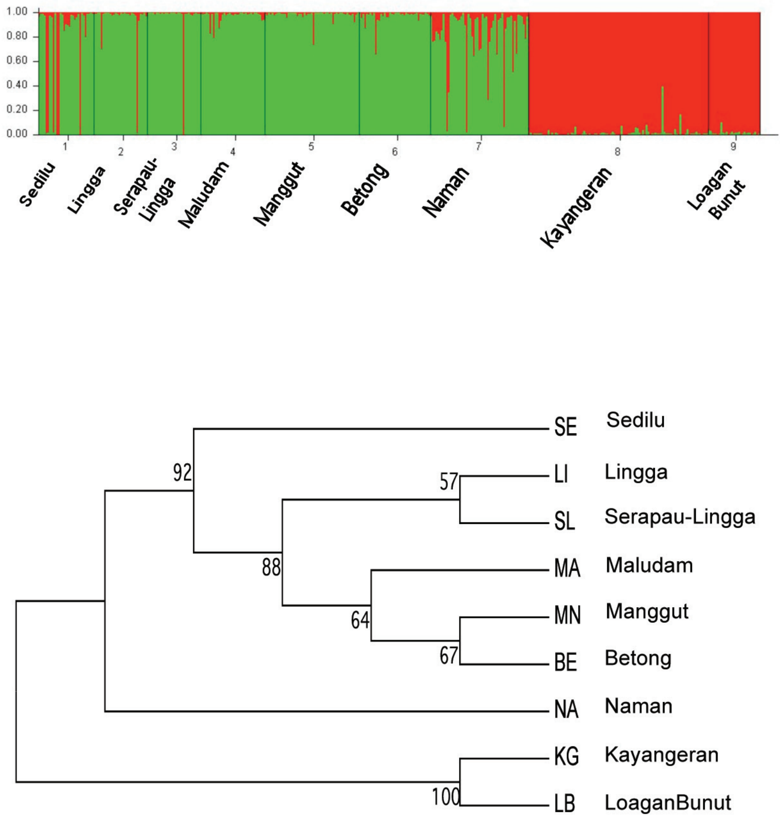


Figure 3: Bar plot and Neighbor-Joining tree of the *Gonystylus bancanus* populations in Sarawak.

However, in order to generate a more precise database or reference for geographical origin detection, several trials were conducted by altering the grouping of the populations, each giving different resolution (Table 2). All the populations were first analysed individually and the mean value of the correct assignment rate was 58.78% (Table 2a). Approximately half of the samples could be assigned back to the original populations where the populations from Naman, Loagan Bunut and Kayangeran have the correct assignment rates higher than 80%.

As Naman, Loagan Bunut and Kayangeran populations had high correct assignment rates, they remained as individual populations and only the populations along the Lupar River were further compiled into larger populations in different combinations. The populations located on the western side of the Lupar River (Sedilu, Lingga and Serapau-Lingga) were grouped as R1, populations on the eastern side of the River (Maludam, Manggut and Betong) as R2. The mean percentage of correct assignment rate was increased to 84.00% (Table 2b). The mean percentage was then further increased to 92.91% when all populations along the river were grouped together as R3 (Table 2c).

Moreover, when the populations were compiled into two larger populations (R4 and R5) as defined by the STRUCTURE software and NJ tree, the percentages were 98.91% and 99.23%, respectively (Table 2d). The results obtained correspond to the findings by Degen *et al.* (2012) where the percentage of samples assigned back to the original populations will be increased when the geographical scale covered is increased. This database can be used as a reference for tracking or tracing the possible origin of *G. bancanus* timber or timber products in Sarawak.

The findings from this study prove the ability of using molecular DNA technology to differentiate the origin of Ramin. A study conducted by FRIM also showed that Ramin in Peninsular Malaysia, Sarawak and Sabah are genetically different (Kevin Ng, pers. comm.). Thus, if assignment test is to be conducted, the samples tested could be assigned back to that from Peninsular Malaysia, Sarawak or Sabah respectively. Similarly if the assignment test is to be conducted to trace the timber origin between Malaysia and Indonesia, we believe that due to the geographical separation between the two countries, the test samples will show higher correct assignment rate, indicating the difference in the timber origin.

Population	By population
Sedilu	54.84
Lingga	26.67
Serapau-lingga	36.67
Maludam	44.44
Manggut	54.72
Betong	30.00
Naman	83.64
LoaganBunut	98.02
Kayangeran	100.00
Mean	58.78

(a)

	Population	By genetic cluster
R1	Sedilu	58.24
	Lingga	
	Serapau-lingga	
R2	Maludam	83.72
	Manggut	
	Betong	
	Naman	80.00
	LoaganBunut	98.02
	Kayangeran	100.00
	Mean	84.00

(b)

	Population	By genetic cluster
R3	Sedilu	95.45
	Lingga	
	Serapau-lingga	
	Maludam	
	Manggut	
	Betong	
	Naman	78.18
	LoaganBunut	98.02
	Kayangeran	100.00
	Mean	92.91

(c)

	Population	By genetic cluster
R4	Sedilu	98.91
	Lingga	
	Serapau-lingga	
	Maludam	
	Manggut	
	Betong	
	Naman	
R5	LoaganBunut	99.23
	Kayangeran	
	Mean	99.07

(d)

Table 2: Self-assignment tests of *Gonystylus bancanus* in Sarawak with different combination of the populations.

5.0 Conclusion

Today, with increased concern on the sustainable management of Ramin production and harvesting, EU has made it mandatory that the global trade of Ramin timber and products in the EU market has to be accompanied with FLEGT or CITES Certificate of Origin from the exporters. Timber species name and country of origin must be indicated precisely. Authorities concerned particularly CITES and EU, have continuously put efforts on the conservation of ramin and enforcement of regulations by controlling the illegally traded timber and timber products in the global market. It is very important to prove whether the information (species name and origin) stated in the documents is true or false. It is also vital that the figures for timber export and import volumes tally in both exporting and importing countries. For example, in 2002 Italy reported imports of 142,285 m^3 of Ramin from Malaysia while Malaysia only reported exports to Italy as 8,429 m^3 in 2001 and 11, 608 m^3 in 2002. In 2003, Italy reported similarly high levels of Ramin imports from Malaysia of 102, 505 m^3 (Lim *et. al.* 2004). Although export data in 2003 was not available, with the reduction of PSF throughout Malaysia, it is impossible that the production of Ramin from Malaysia in 2003 would be that high. Later, Chen (2004) and EIA & Telapak (2005) reported illegal timber from Indonesia was re-exported through Malaysia with false documents as they were marked as Malaysian timber. The ability to trace timber origin is thus very important to check the precision of the declared origin claimed by the timber exporters or importers.

The molecular DNA technology for timber forensic although is always considered as expensive, is the most powerful tool in the timber tracing system. The haplotypes found in the present chloroplast DNA data were widely distributed in Sarawak where no clear regions could be differentiated; however, microsatellite DNA data are able to divide the sample locations into regions. This database may not be able to assign the sample back to a specific location but it gives a clue on the origin of the sample. The analysis and database will be more useful if it is used together with the Ramin database from other geographical ranges (eg. Indonesia, Brunei), making it more informative for law enforcement to uncover deceptions in the Ramin trade. This study indicates that tracing the timber using DNA markers back to the source or exact origin may be difficult but is possible to trace to its geographical range (for example, Malaysia or Indonesia). The application of DNA technology together with the existing control system in place, can reduce and prevent the illegal timber laundering, and enhance control and enforcement measures of timber trading of endangered species.

References

Anon (2004), Species survival network, Washington.www.ssn.org.

Chai, E.O.K. (2004). Restoration of ramin in peat swamp forest of Sarawak: Sustainable management of peat swamp forests of Sarawak with species reference to ramin. Alterra, The Netherlands; Forest Department Sarawak and Sarawak Forestry Corporation.

Chen H.K. (2004). Report on forest law enforcement and governance in Malaysia in the context of sustainable forest management. Prepared by TRAFFIC for the Government of Malaysia. ITTC 37th Session ITTC Doc XXXVII/9. 11 November 2004.

Degen, B., Ward, S.E., Lemes, M.R., Navarro, C., Cavers, S. and Sebbenn, A.M. (2012). Verifying the geographic origin of mahogany (*Swietenia macrophylla* King) with DNA-fingerprints.*Forensic Science International: Genetics*, 7, 55-62. DOI: 10.1016/j.fsigen.2012.06.003.

Duminil, J., Heuertz, M., Douce, J.L., Bourland, N., Cruaud, C., Gavory, F., Doumenge, C., Navascués, M. and Hardy, O.J. (2010).CpDNA-based species identification and phylogeography: application to African tropical tree species. *Molecular Ecology*, 19(24), 5469-5483. DOI: 10.1111/j.1365-294X.2010.04917.x.

Earl, D.A. & von Holdt, B.M. (2012) STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources*, 4(2), 359-361. DOI: 10.1007/s12686-011-9548-7.

EIA & Telapak 2005. *Stemming the tide: halting the trade in stolen timber in Asia*. [accessed 14 May 2007]
http://www.eia-international.org/cgi/reports/reports.cgi?t=template&a=114

Falush, D., Stephens, M. & Pritchard, J.K. (2003). Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. *Genetics*, 164(4), 1567-1587.

Khali Aziz, H., Ismail, P., Ani, S., Mohd.Azahari, F., Harry, Y. and Ihsan, S.K. (2010). *Gonystylus bancanus*: jewel of the peat swamp forest. Forest Research Institute Malaysia (FRIM). ISBN: 978-967-5221-46-0.

Kremer, A., Ronce, O., Robledo-Arnuncio, J.J., Guillaume, F., Bohrer, G., Nathan, R., Bridle, J.R., Gomulkiewicz, R., Klein, E.K., Ritland, K., Kuparinen, A., Gerber, S. and Schueler, S. (2012). Long-distance gene flow and adaptation of forest trees to rapid climate change. *Ecology Letters*, 15, 378-392. DOI: 10.1111/j.1461-0248.2012.01746.x.

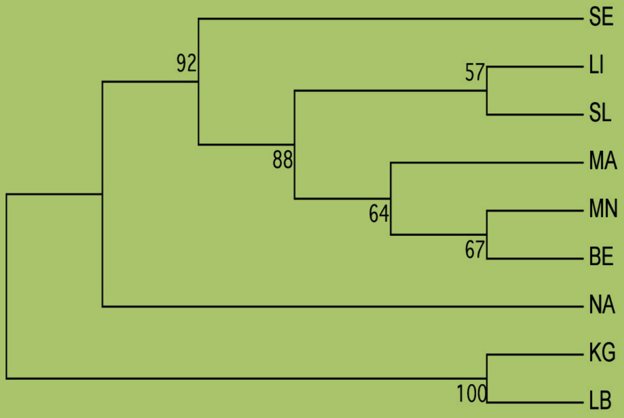
Lee H.S. (2004).Improving biodiversity and sustainability of peat swamp forests of Sarawak: The sustainable management of peat swamp forest in Sarawak with special reference to ramin. Alterra, The Netherlands; Forest Department Sarawak and Sarawak Forestry Corporation.

Lim T.W., Tonny S. and Chen H.K. (2004). Framing the picture: and assessment of ramin trade in Indonesia, Malaysia and Singapore. Traffic South East Asia.

Page S.E., Rieley J.O., and Banks C.J. (2011). Global and regional importance of the tropical peatland carbon pool. In *Global Change Biology.* Vol. 17, Issue 2.Pg: 798 – 818.

Sawal P. (2004). Threats of peat swamp forest of Sarawak: The sustainable management of peat swamp forest in Sarawak with special reference to ramin. Alterra, The Netherlands; Forest Department Sarawak and Sarawak Forestry Corporation.

Wong J.L.M. (2003) Status of peat swamp forest in Sarawak: The sustainable management of peat swamp forest in Sarawak with special reference to ramin. Alterra, The Netherland; Forest Department Sarawak and Sarawak Forestry Corporation.



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