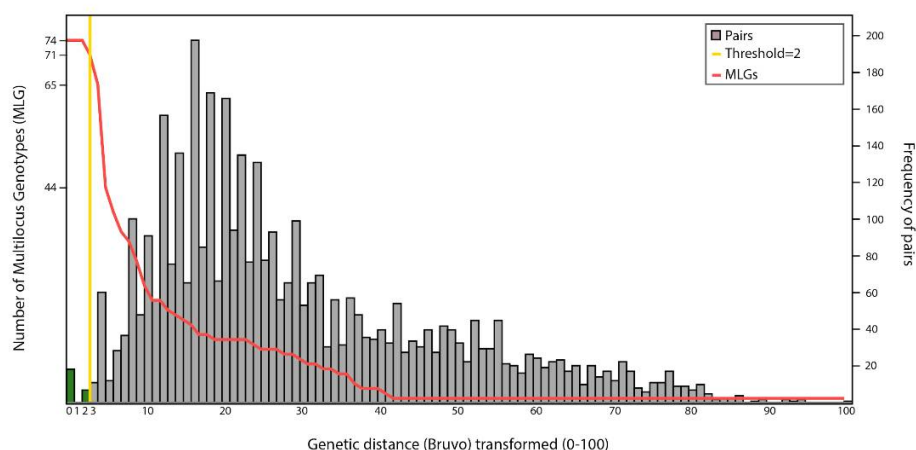
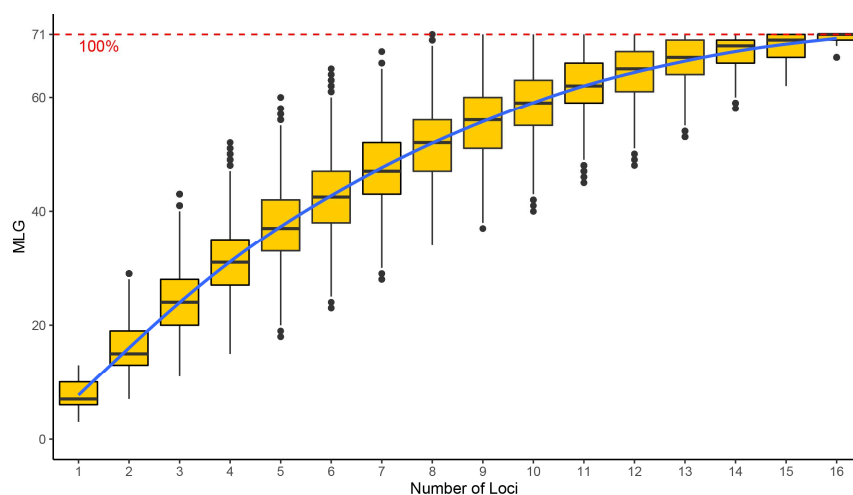


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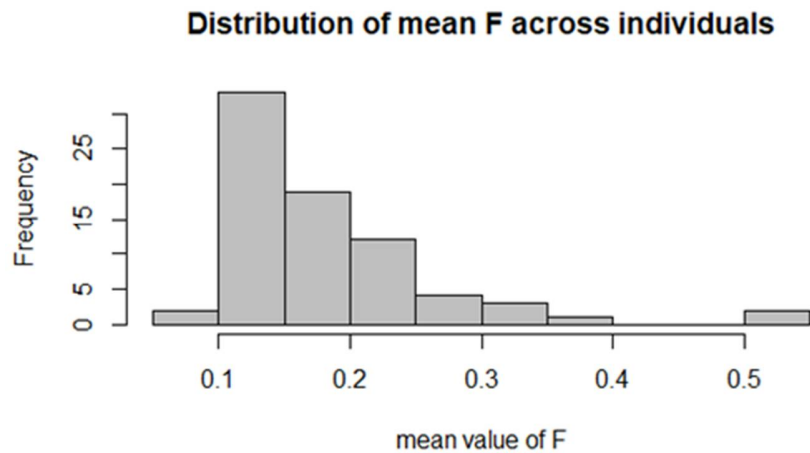
# Assessing the Genetic Diversity of *Ilex guayusa* Loes., a Medicinal Plant from the Ecuadorian Amazon



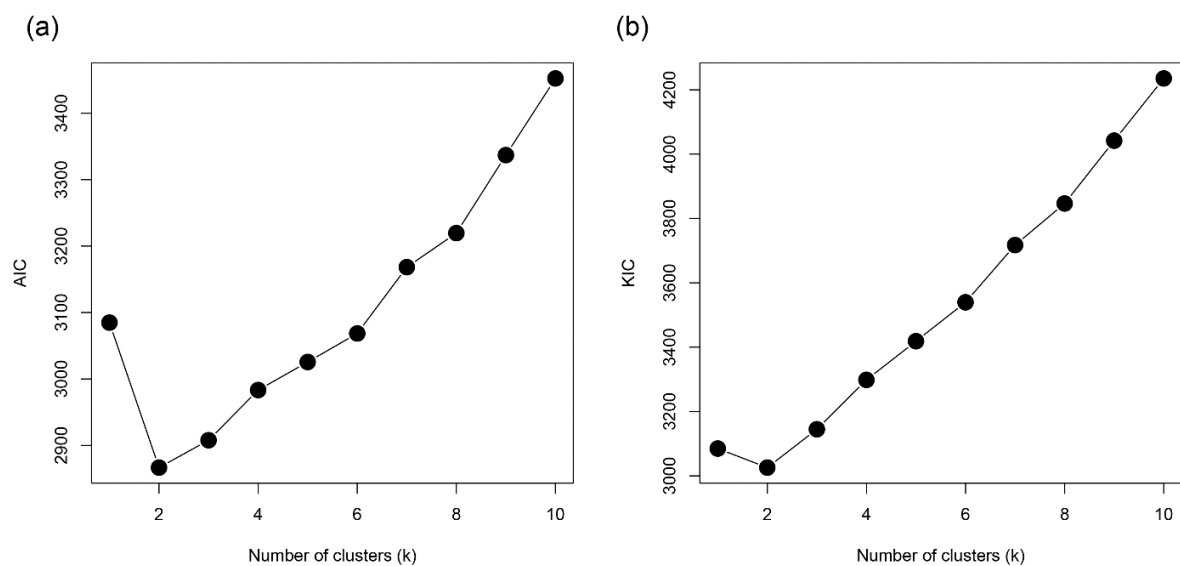
**Figure S1.** Frequency distribution of the pairwise Bruvo's genetic distances (transformed between 0 and 100) for 88 *Ilex guayusa* individuals genotyped with 17 SSR markers. Following the assumption that the histograms of the pairwise comparisons are often multimodal, the valley between the first and second peak was considered as a good candidate to use as a threshold. At a threshold=2, the inferred number of MLGs was 71.



**Figure S2.** Genotype accumulation curve describing the genotypic resolution of the 17 SSR markers used in this study. All the employed markers were sufficient to identify and discriminate between the 71 MLGs.



**Figure S3.** Histogram of the frequency of the F (inbreeding coefficient) values observed within the 76 *Ilex guayusa* individuals selected after clone correction. The data follows a right-skewed non-normal distribution.



**Figure S4.** Goodness-of-fit statistics obtained for the determination of the optimal number of genetic clusters (K) using a maximum-likelihood approach. (a) Akaike information criterion (AIC) values plotted against a determined number of K; (b) Kullback Information Criterion (KIC) values plotted against a determined number of K.

**Table S1.** Associated information to the *Ilex guayusa* samples collected for this study in the Ecuadorian Amazon

Sample	Region	Province	Locality	Longitude	Latitude	Altitude (m.a.s.l.)
SG1	Northern	Sucumbíos	El Eno	-76.88531	-0.00914	308
SG8	Northern	Sucumbíos	El Eno	-76.93972	-0.01122	304
SG9	Northern	Sucumbíos	El Eno	-76.93989	-0.01147	304
SG10	Northern	Sucumbíos	El Eno	-76.93975	-0.01161	302
SG4	Northern	Sucumbíos	El Eno	-76.91542	-0.01267	308
SG22	Northern	Sucumbíos	El Eno	-76.95981	-0.02719	319
SG23	Northern	Sucumbíos	El Eno	-76.96006	-0.02739	324
SG21	Northern	Sucumbíos	El Eno	-76.95383	-0.02831	322
SG24	Northern	Sucumbíos	El Eno	-76.95947	-0.02881	315
SG25	Northern	Sucumbíos	El Eno	-76.83806	-0.02900	298
SG16	Northern	Sucumbíos	El Eno	-76.91814	-0.03431	326
SG30	Northern	Sucumbíos	El Eno	-76.98981	-0.05822	301
SG39	Northern	Sucumbíos	El Eno	-77.03550	-0.05969	322
SG45	Northern	Sucumbíos	El Eno	-77.07114	-0.08383	340
OG9	Northern	Orellana	San Sebastián del Coca	-77.14461	-0.10325	377
OG8	Northern	Orellana	San Sebastián del Coca	-77.14447	-0.10336	373
OG6	Northern	Orellana	San Sebastián del Coca	-77.13383	-0.10469	373
OG10	Northern	Orellana	San Sebastián del Coca	-77.13106	-0.10661	359
OG3	Northern	Orellana	San Sebastián del Coca	-77.11800	-0.10792	358
OG5	Northern	Orellana	San Sebastián del Coca	-77.11814	-0.10842	364
OG4	Northern	Orellana	San Sebastián del Coca	-77.11806	-0.10856	363
OG13	Northern	Orellana	San Sebastián del Coca	-77.11014	-0.11064	355
OG14	Northern	Orellana	San Sebastián del Coca	-77.11044	-0.11064	360
OG15	Northern	Orellana	San Sebastián del Coca	-77.10939	-0.11114	360
OG12	Northern	Orellana	San Sebastián del Coca	-77.11036	-0.11392	356
OG17	Northern	Orellana	San Sebastián del Coca	-77.10636	-0.11442	357
OG16	Northern	Orellana	San Sebastián del Coca	-77.10717	-0.11575	349
OG20	Northern	Orellana	San Sebastián del Coca	-77.10775	-0.11625	347
OG19	Northern	Orellana	San Sebastián del Coca	-77.10622	-0.11658	346
SG26	Northern	Orellana	San Sebastián del Coca	-76.97747	-0.12317	302
GUA1	Northern	Napo	Hatun Sumaku	-77.60970	-0.71820	1197
MUS2	Northern	Napo	Cotundo	-77.64860	-0.79010	799
20MC3	Northern	Napo	Cotundo	-77.81040	-0.83190	819
RUK2	Northern	Napo	Archidona	-77.79470	-0.89650	604
INC4	Northern	Napo	Tena	-77.83800	-0.92200	630
PAR1	Northern	Napo	San Pablo de Ushpayacu	-77.72200	-0.92350	819
ALTC1	Northern	Napo	San Pablo de Ushpayacu	-77.69470	-0.94000	872
ATT4	Northern	Napo	Tena	-77.88120	-0.94010	677
SD3	Northern	Napo	San Pablo de Ushpayacu	-77.76050	-0.94840	499
LH1	Northern	Napo	Tena	-77.80500	-0.98990	506
SICP2	Northern	Napo	Ahuano	-77.46530	-1.01750	343

MACH1	Northern	Napo	Napo	-77.71910	-1.03150	503
ATA2	Northern	Napo	Napo	-77.75440	-1.04760	415
SVN1	Northern	Napo	Carlos Julio Arosemena Tola	-77.79750	-1.14080	496
PCT1	Central	Napo	Carlos Julio Arosemena Tola	-77.73110	-1.15600	479
PG3	Central	Pastaza	Santa Clara	-77.88275	-1.22783	543
PG5	Central	Pastaza	San José	-77.91328	-1.33675	993
PG6	Central	Pastaza	Teniente Hugo Ortiz	-77.98017	-1.38553	1047
PG7	Central	Pastaza	Fátima	-77.99447	-1.45275	966
PG21	Central	Pastaza	Puyo	-77.99717	-1.47619	959
PG37	Central	Pastaza	Shell	-78.07458	-1.49086	1075
PG16	Central	Pastaza	Veracruz	-77.97133	-1.52442	938
PG8	Central	Pastaza	Tarqui	-78.02144	-1.55303	946
PG9	Central	Pastaza	Tarqui	-78.01636	-1.55428	930
PG10	Central	Pastaza	Tarqui	-78.01181	-1.55594	944
PG32	Central	Pastaza	Canelos	-77.80975	-1.59461	622
PG29	Central	Pastaza	Canelos	-77.75544	-1.59592	506
PG42	Central	Pastaza	Madre Tierra	-78.03392	-1.59669	909
PG28	Central	Pastaza	Canelos	-77.75364	-1.59972	475
PG45	Central	Pastaza	Madre Tierra	-78.00611	-1.63706	860
MSG58	Central	Morona Santiago	Sinaí	-78.05094	-2.09647	1204
MSG81	Central	Morona Santiago	Sinaí	-78.03297	-2.12319	1202
MSG44	Central	Morona Santiago	Sinaí	-78.06606	-2.12692	1266
MSG67	Central	Morona Santiago	Sinaí	-78.10169	-2.14356	1207
MSG27	Central	Morona Santiago	Sinaí	-78.08225	-2.18450	1176
MSG5	Central	Morona Santiago	Sevilla don Bosco	-78.08808	-2.28436	1039
MSG100	Central	Morona Santiago	Sevilla don Bosco	-78.11764	-2.30461	1038
MSG25	Central	Morona Santiago	Sevilla don Bosco	-78.10183	-2.30817	1000
MSG96	Central	Morona Santiago	Sevilla don Bosco	-78.13669	-2.32456	968
MSG22	Central	Morona Santiago	Sevilla don Bosco	-78.07392	-2.34172	959
MSG13	Central	Morona Santiago	Sevilla don Bosco	-78.11017	-2.34439	950
MSG84	Central	Morona Santiago	Santa Marianita de Jesús	-78.15561	-2.40667	894
MSG86	Central	Morona Santiago	Santa Marianita de Jesús	-78.15444	-2.41625	892
MSG90	Central	Morona Santiago	Santa Marianita de Jesús	-78.15328	-2.42853	890
MSG92	Central	Morona Santiago	Santa Marianita de Jesús	-78.16344	-2.44003	865
ZG6	Southern	Zamora Chinchipe	Yanzatza	-78.76006	-3.83319	842
ZG5	Southern	Zamora Chinchipe	Yanzatza	-78.76031	-3.83497	817
ZG10	Southern	Zamora Chinchipe	Panguitza	-78.81500	-3.90231	842
ZG11	Southern	Zamora Chinchipe	Panguitza	-78.81464	-3.90375	840
ZG9	Southern	Zamora Chinchipe	Cumbaratza	-78.86514	-3.99356	879
ZG8	Southern	Zamora Chinchipe	Cumbaratza	-78.86558	-3.99450	882
ZG7	Southern	Zamora Chinchipe	Zamora	-78.98856	-4.04422	1015
ZG4	Southern	Zamora Chinchipe	Zamora	-78.96567	-4.05989	973
ZG3	Southern	Zamora Chinchipe	Zamora	-78.96631	-4.06039	989
ZG12	Southern	Zamora Chinchipe	Valladolid	-79.13203	-4.55019	1595

ZG13	Southern	Zamora Chinchipe	Valladolid	-79.13272	-4.55272	1594
ZG1	Southern	Zamora Chinchipe	Palanda	-79.13161	-4.64753	1137
ZG2	Southern	Zamora Chinchipe	Palanda	-79.13331	-4.64853	1146

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**Table S2.** Information of SSR specific primers designed for *Ilex guayusa*.

Primer		Sequences (5'-3')	Motifs	Expected size (bp)	Annealing Temperature (°C)	Overall PIC
GYS02	Fw	GCGTAGCGATCAATGAACCTTTAAGTTAGC	ACC(30)	172-289	64	0.48
	Rv	TTTGCCAAATCCCTCTCTCAACC				
GYS03	Fw	TGTAGTGGTGGTGGATTCAAAAACG	ATT(33)	177-270	62	0.39
	Rv	ACGACCATGGCACAAGGATGC				
GYS05	Fw	ACCACCACCAACATCGTTGCTACC	ACC(24)	224-299	64	0.13
	Rv	TAGTGGTGGGGCATTGGTAGGG				
GYS07	Fw	CAAGAATCCATGATACAGTAGG	TC(24)	292-306	64	0.27
	Rv	ATTTACATACACACAGGTACCC				
GYS08	Fw	GCTCTCTTCTTAGACAATACC	TC(30)	154-176	58	0.11
	Rv	GAATTAGGTACACTTCATCTCC				
GYS09	Fw	TATGTCATAAGAGGAGGTTAGG	TC(60)	299-333	62	0.096
	Rv	CTCCTAATATTTACCCTCACC				
GYS11	Fw	CCATAGAGGGTATTAGTTAAGG	AT(24)	224-262	64	0.53
	Rv	CTAGTTATCCCTTGGTTATCC				
GYS12	Fw	ATAAATGTGGAGTGGTAGACC	TC(38)	246-302	60	0.56
	Rv	GAGTCTAACTATGCAGAAACC				
GYS14	Fw	ATGGACTACTAGAGAAGACTCG	TC(18)	348-350	59	0.074
	Rv	CTGGTCTTAGATAGTTCATCC				
GYS17	Fw	GTTATCTGATCTCTCTCATCG	TC(36)	160-180	62	0.38
	Rv	ATGTACATAACTCAAGGAGTGC				
GYS20	Fw	CACTAACACCAAGATTAAGACC	TC(20)	256-302	64	0.36
	Rv	ACTTCAGTTGTCTACATGAGC				
GYS21	Fw	AGTCTCTTACTCACCTGAACG	TC(22)	175-209	62	0.51
	Rv	GTAGAGAATGAGGAATGAACC				
GYS22	Fw	CTTTGCAGTAGAGTTGTTACC	TC(22)	402-408	60	0.39
	Rv	AATAGGGATAAGAGTGAAGTACC				
GYS23	Fw	GTACCAACTATATTCACCTCTCG	TC(34)	273-289	62	0.51
	Rv	CAACTGTAATCATCCTCTTCC				
GYS25	Fw	CGTAGATGAAGGTAGATAAAGG	TC(40)	194-206	62	0.24
	Rv	CCAGATCTAGACTTTCACTCC				
GYS26	Fw	AGAGAGTGTGTGTAGAAATGG	TC(38)	239-261	60	0.48
	Rv	CGATAGTAGTTGCTAAATAGGG				
GYS28	Fw	TATGACAGTAGGCTAGTGTGG	TC(28)	176-190	58	0.42
	Rv	TTCAGTGTTAGCTGAGTTAGG				

**Table S3.** Inferred MLG for each sample as calculated by GenoDive, using a genetic distance threshold of 2.

Sample	Region	MLG	Sample	Region	MLG	Sample	Region	MLG
SG1	Northern	MLG1	PAR1	Northern	MLG28	ZG9	Southern	MLG66
SG25	Northern	MLG2	ALTC1	Northern	MLG29	ZG8	Southern	MLG67
SG8	Northern	MLG2	ATT4	Northern	MLG30	ZG7	Southern	MLG68
SG9	Northern	MLG2	MACH1	Northern	MLG30	ZG3	Southern	MLG69
SG10	Northern	MLG3	SD3	Northern	MLG31	ZG12	Southern	MLG70
SG4	Northern	MLG4	SICP2	Northern	MLG32	ZG13	Southern	MLG71
SG22	Northern	MLG5	ATA2	Northern	MLG33			
OG17	Northern	MLG6	PG37	Central	MLG34			
SG23	Northern	MLG6	SVN1	Northern	MLG34			
ZG11	Southern	MLG6	PG3	Central	MLG35			
SG21	Northern	MLG7	PG5	Central	MLG36			
SG24	Northern	MLG8	PG6	Central	MLG37			
ZG1	Southern	MLG8	PG7	Central	MLG38			
SG16	Northern	MLG9	PG9	Central	MLG38			
SG30	Northern	MLG10	PG21	Central	MLG39			
SG39	Northern	MLG11	PG16	Central	MLG40			
SG45	Northern	MLG12	PG8	Central	MLG41			
OG9	Northern	MLG13	PG10	Central	MLG42			
OG8	Northern	MLG13	PG32	Central	MLG43			
OG19	Northern	MLG13	PG29	Central	MLG44			
OG6	Northern	MLG14	PG42	Central	MLG45			
OG13	Northern	MLG14	PG28	Central	MLG46			
OG14	Northern	MLG14	PG45	Central	MLG47			
ZG4	Southern	MLG14	MSG58	Central	MLG48			
ZG2	Southern	MLG14	MSG81	Central	MLG49			
OG10	Northern	MLG15	MSG44	Central	MLG50			
OG3	Northern	MLG16	MSG67	Central	MLG51			
OG4	Northern	MLG16	MSG27	Central	MLG52			
OG5	Northern	MLG17	MSG5	Central	MLG53			
OG15	Northern	MLG18	MSG100	Central	MLG54			
OG12	Northern	MLG19	MSG25	Central	MLG55			
OG16	Northern	MLG20	MSG96	Central	MLG56			
OG20	Northern	MLG21	MSG22	Central	MLG57			
SG26	Northern	MLG22	MSG13	Central	MLG58			
GUA1	Northern	MLG23	MSG84	Central	MLG59			
MUS2	Northern	MLG24	MSG86	Central	MLG60			
LH1	Northern	MLG24	MSG90	Central	MLG61			
20MC3	Northern	MLG25	MSG92	Central	MLG62			
RUK2	Northern	MLG26	ZG6	Southern	MLG63			
PCT1	Central	MLG27	ZG5	Southern	MLG64			

INC4   Northern   MLG27   ZG10   Southern   MLG65

**Table S4.** Results of the Hardy-Weinberg Equilibrium (HWE) test for each locus, after a Bonferroni correction.

Loci	Northern	Central	Southern	Overall
GYS25	Monomorphic	***	Monomorphic	***
GYS22	***	*	*	***
GYS2	>0.05	>0.05	>0.05	***
GYS7	***	>0.05	Monomorphic	***
GYS9	>0.05	**	Monomorphic	***
GYS11	***	***	Monomorphic	***
GYS12	***	***	Monomorphic	***
GYS14	Monomorphic	*	Monomorphic	**
GYS17	***	*	*	***
GYS20	*	**	Monomorphic	***
GYS21	***	**	Monomorphic	***
GYS28	***	**	>0.05	***
GYS26	Monomorphic	**	Monomorphic	***
GYS3	>0.05	Monomorphic	Monomorphic	*
GYS5	*	*	Monomorphic	***
GYS8	Monomorphic	>0.05	Monomorphic	*
GYS23	***	>0.05	>0.05	***

Note: \*indicates a significance of <0.05 ; \*\*indicates a significance of <0.01; \*\*\*indicates a significance of <0.001

**Table S5.** Null allele frequencies estimation for each analyzed region and SSR locus.

Locus	Northern	Central	Southern	Mean
GYS02	0.03477	0.09883	0.00000	0.04942
GYS03	0.13480	0.06169	0.18258	0.12214
GYS05	0.11885	0.00005	0.00100	0.00053
GYS07	0.26045	0.09793	0.00001	0.04897
GYS08	0.00006	0.13570	0.00100	0.06835
GYS09	0.00006	0.17785	0.00100	0.08943
GYS11	0.00000	0.11876	0.00000	0.05938
GYS12	0.09055	0.00000	0.00000	0.00000
GYS14	0.00100	0.19944	0.00100	0.10022
GYS17	0.00000	0.00000	0.00000	0.00000
GYS20	0.15758	0.15000	0.00001	0.07501
GYS21	0.00000	0.00189	0.00000	0.00095
GYS22	0.00000	0.00000	0.00000	0.00000
GYS23	0.00000	0.18290	0.00000	0.09145
GYS25	0.00100	0.23536	0.00100	0.11818



GYS26	0.08741	0.17900	0.00001	0.08951
GYS28	0.00000	0.00000	0.00000	0.00000

**Table S6.** Pairwise  $F_{ST}$  values calculated for the three Ecuadorian Amazon regions.

Regions	Northern	Central	Southern
<b>Northern</b>	0.00000	0.02389	0.01749
<b>Central</b>	0.02389	0.00000	0.02866
<b>Southern</b>	0.01749	0.02866	0.00000