Consorcio para la Conservación de Magnolias del Neotrópico

Genetic and ecological divergence of species of the *Magnolia pacifica* A.Vázquez complex in western Mexico

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Mexico

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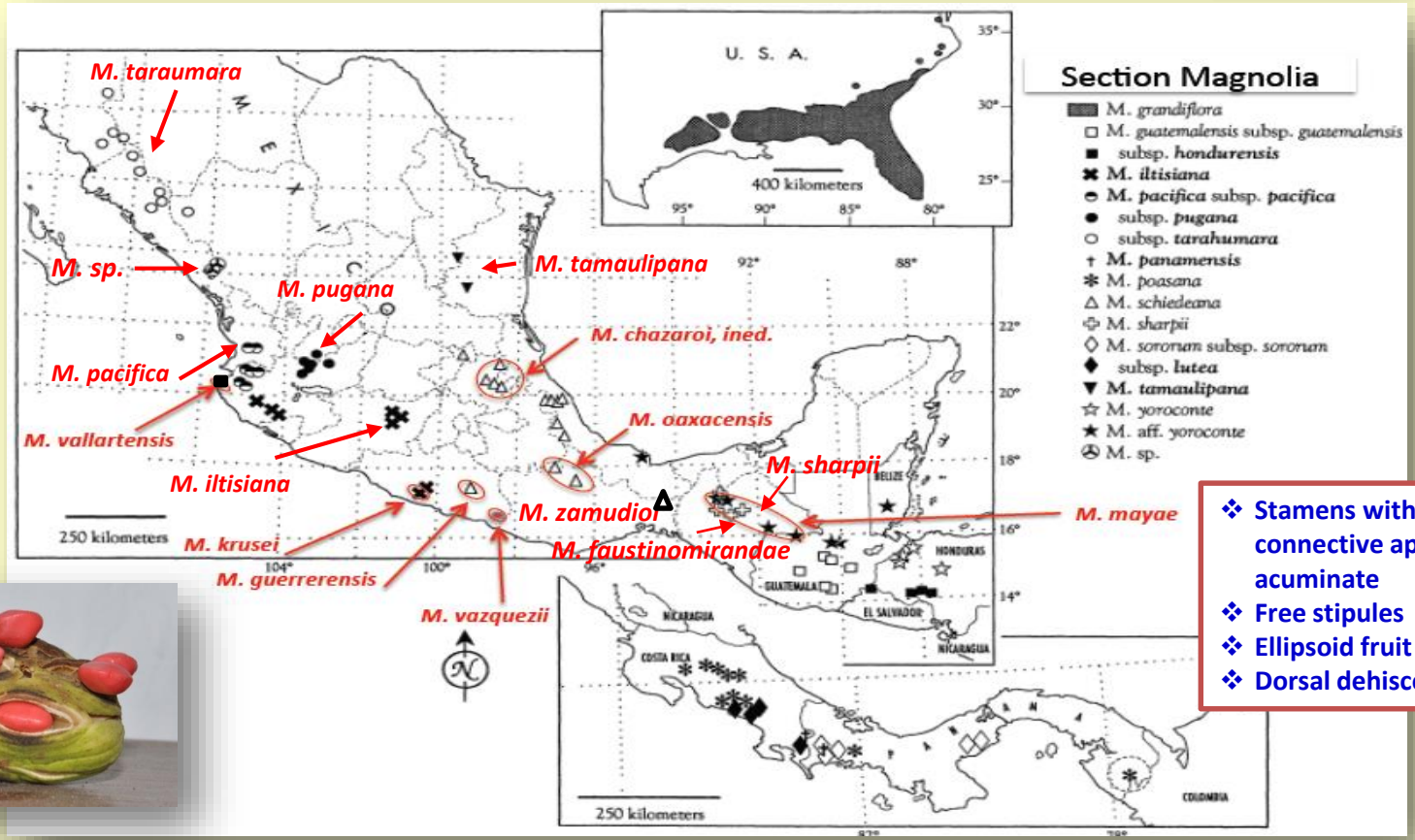
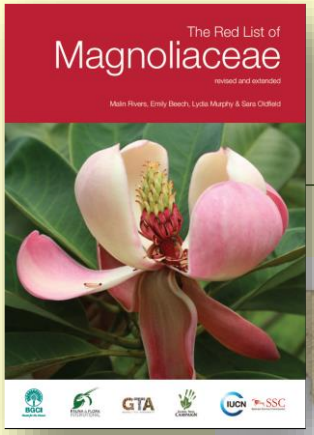
Genus *Magnolia* L., Section *Magnolia* in México y Central America

In Mexico, there are *ca*. **40 species** of the Magnoliaceae family
19 species belong to Section *Magnolia*



- Stamens with short connective apex acute or acuminate
- Free stipules
- Ellipsoid fruit
- Dorsal dehiscence

(Cruz-Durán et al., 2008; Jiménez-Ramírez y Cruz-Durán, 2005; Jiménez-Ramírez et al., 2007; Vázquez-García, 1990, 1994; Vázquez-García et al., 2002, 2012a, 2012b, 2012c, 2013a, 2013b, 2013c, 2013d, 2014, 2015)



Magnolia pacifica A.Vázquez *species* complex in SW Mexico

Composed by four species

Vulnerable

Magnolia tarahumara

Endangered

Magnolia vallartensis

Critically Endangered

Magnolia pugana

Magnolia pacifica s.s.

Endangered

Because species are the fundamental units of evolutionary biology their delimitation is an essential task of current systematics

Rivers et al. 2016; Vázquez-García *et al.,* 2012; Vázquez-García *et al.,* 2002; Vázquez-García, 1994

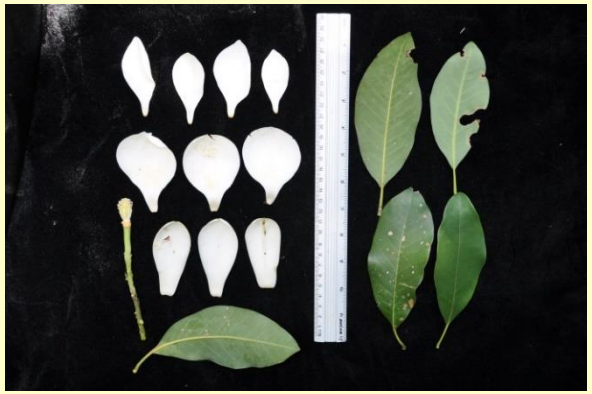
Magnolia vallartensis

Carpels 14-19

Magnolia pacifica s.s.

Carpels 18-25

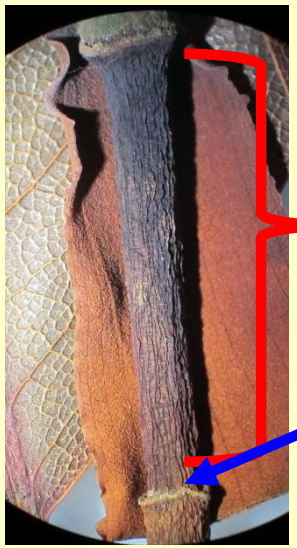
Magnolia pugana



Carpels 25-35

Peduncle indumentum

M. vallartensis



Glabrous

Pubescent

Glabrous

M. Muñiz-Castro 1312

M. pacifica s.s.

J.Curiel 69

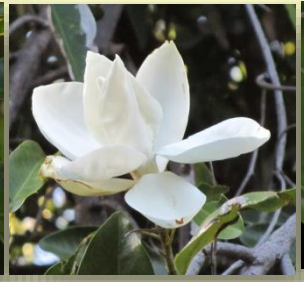
M. pugana

Essentially Glabrous

Iltis 29722 (Isotype)

Taxonomy complexes


***Cycas segmentifida* complex**


***Magnolia pacifica* complex**

Species identification can be troublesome when taxa are closely related

Population genetics approach

Defining boundaries between species complexes

For reliable identification, it is necessary to validate both morphological and molecular markers.

Rieseberg et al., 2006; Nie et al., 2008, Sánchez-Velásquez et al., 2006; Duminil y Di Michele 2009.

Molecular markers

The development of **neutral single locus molecular markers** has the advantage of **infer recent events of speciation** helping to understand delimitation of species

Neutral molecular markers

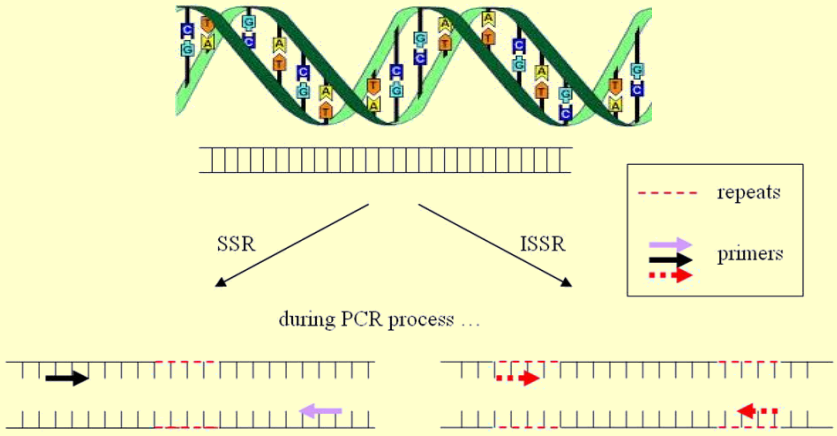
NO

Environment

Natural selection

ISSR ***(Inter Simple Sequence Repeats)***

The ISSR allows observing the variation in inter-microsatellite regions of the genome.



Advantages

- High genetic variability (polimorphism)
- Small DNA concentrations
- Fast and easy to get
- Eficients
- Low cost

Disadvantages

- Fragment homology
- Dominant marker. Assumes HW equilibrium

The last ones sorted out by statistical corrections

Azfeifa, *2*006; Tanksley, 1983; Aguirre *et al.,* 2002; Karp *et al.*,1997

Objectives

1. Analyze species boundaries and genetic relationships within the *M. pacifica* complex under a population genetics approach.

2. Evaluate ecological divergence of the three species by using ecological niche models

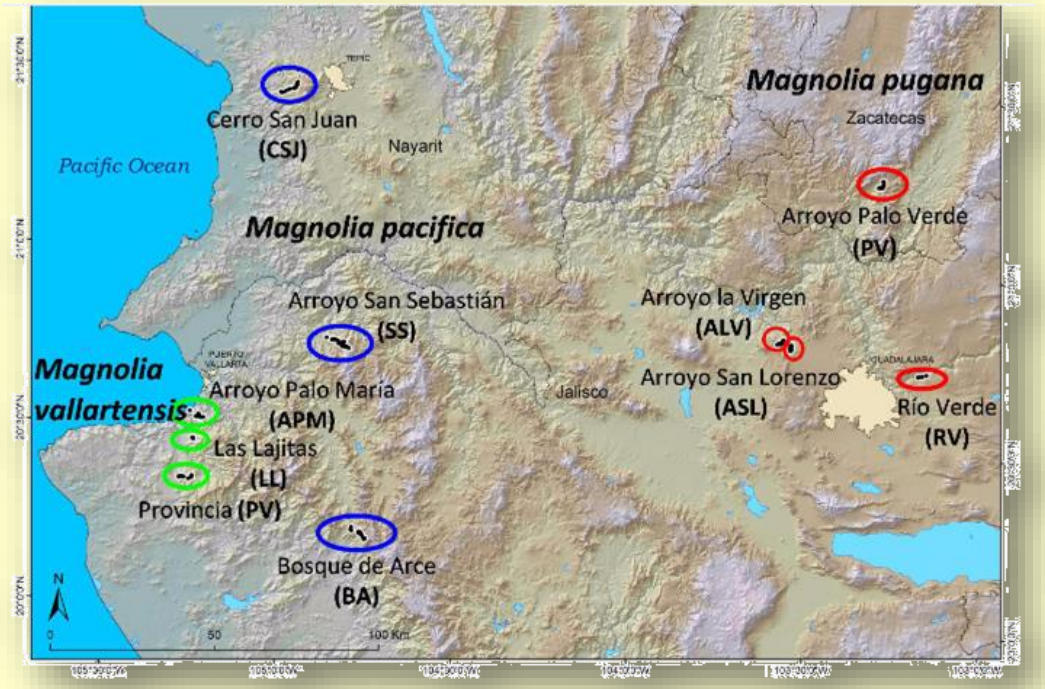
M. vallartensis

M. pacifica s.s.

M. pugana

Materials and methods

1 Sample collection



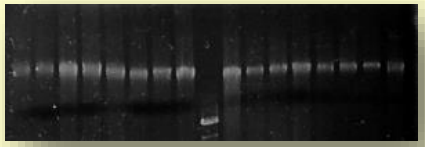
M. vallartensis

M. pacifica s.s.

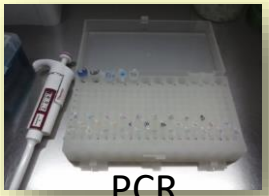
M. pugana

2 Sample Processing

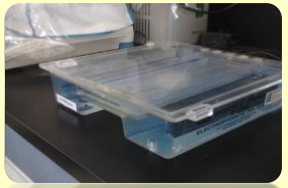
DNA extraction (Cota-Sánchez CTAB method)



Amplification of ISSR markers

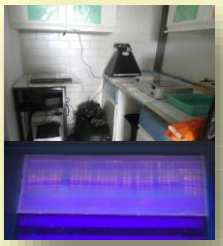


PCR



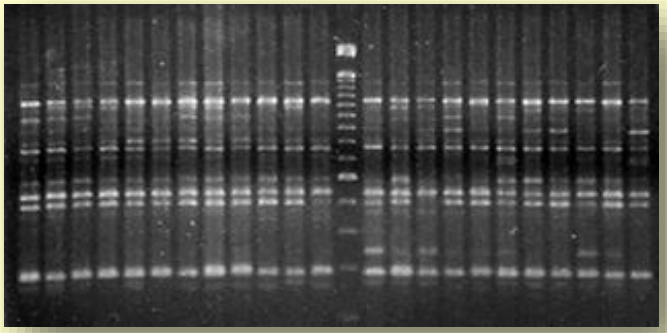
Electrophoresis

810-814-834-836-855-857

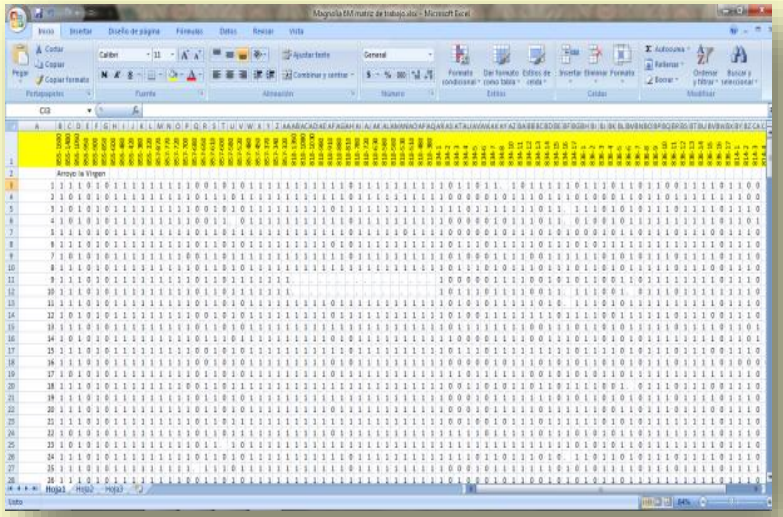


Gels Photo-documentation

3 Data Analysis



Binary matrix



Genetic structure

- Bayesian clustering to detect number of clusters **STRUCTURE 2.3.4**
- UPGMA Nei´s genetic distances **TFPGA 1.3**
- Genetic similarity between localities **Exact Test for Population Differentiation TFPGA**
- Distribution of the genetic variance for groups **AMOVA GenAlEx 6.5**

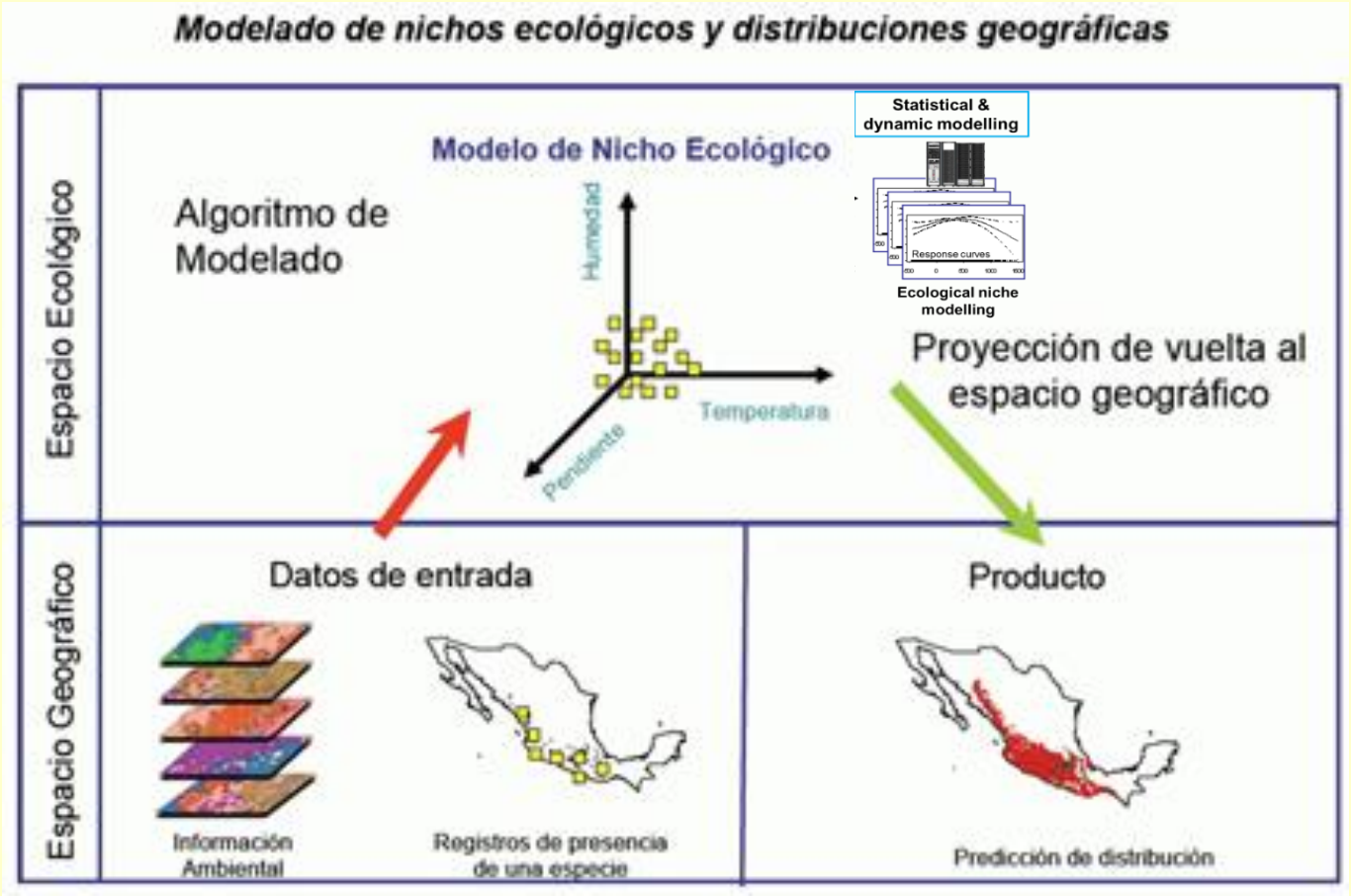
Genetic diversity

- PB(%), I, H_E, HS, HT, **POPGENE 1.31**

Genetic differentiation

- Jost's D and G''ST corrected standardized fixation index **GenoDive β 2.0**

Ecological niche modelling



MAXENT

CROSS-VALIDATION

10 REPLICATES

500 ITERATIONS

1000 BACKGROUND POINTS

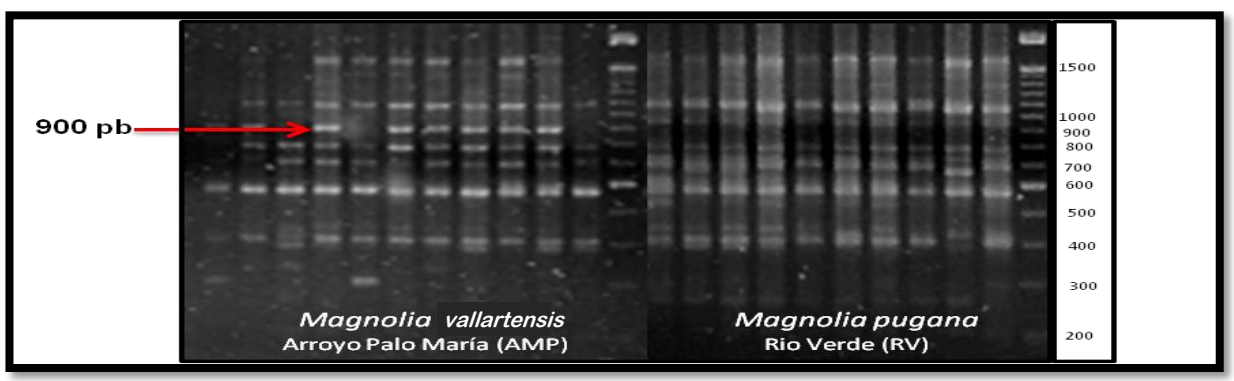
---19 bioclimatic layers WorldClim V. 2.0 (*Fick & Hijmans, 2017)*

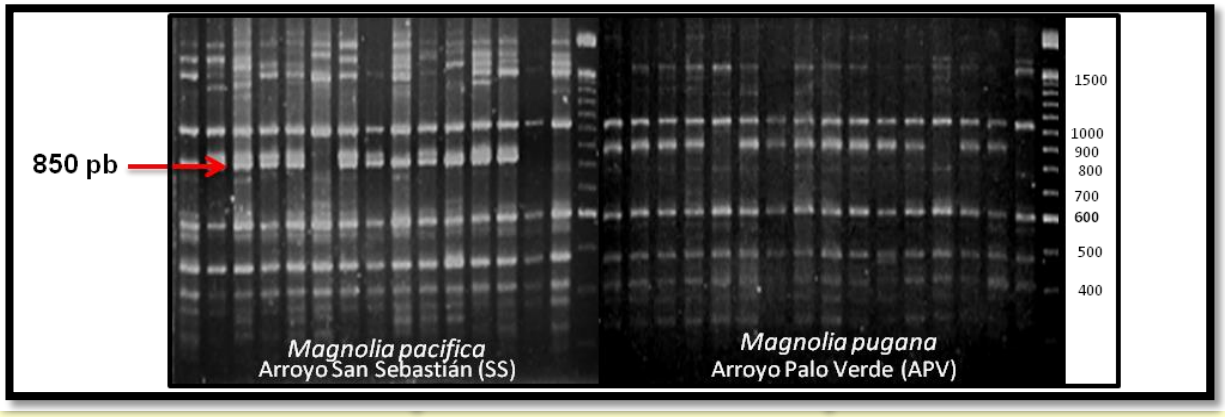
---*M. Pugana* 39 records

***M. Pacifica* 49 & *M. vallartensis* 22**

Potential Geographic Distribution

Results





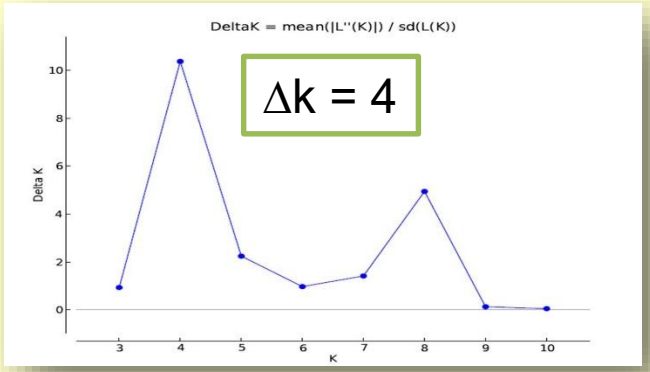
- 76 fragments
- 41 *loci* polimorphic
- Size 290 to 1650 pb
- Two exclusive fragments for *M. pacifica* s.s. and *M. vallartensis*

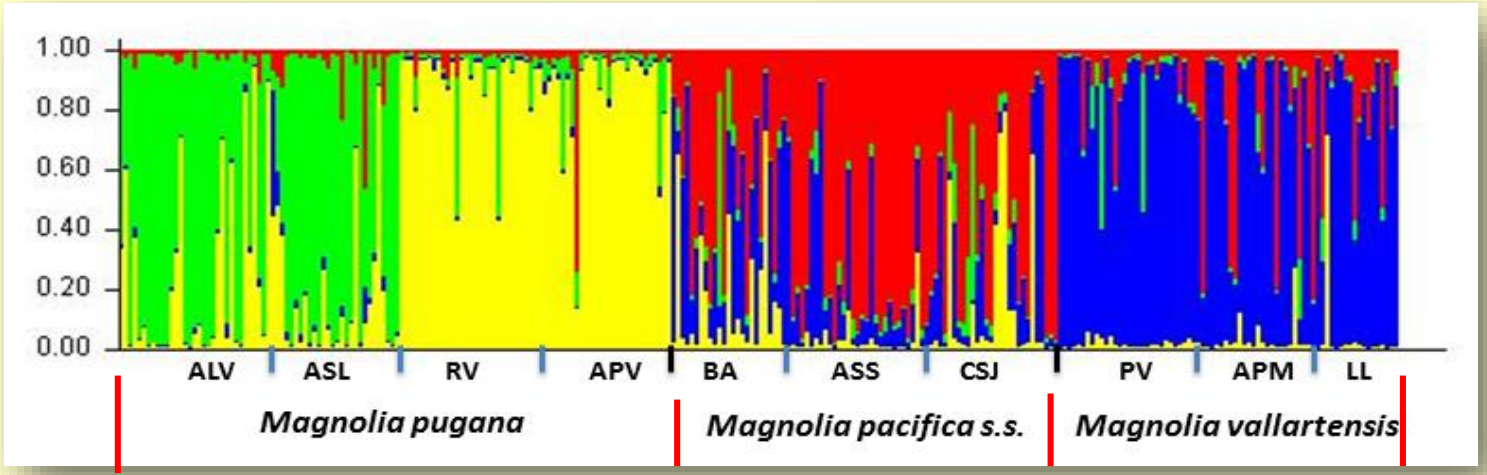
ISSR usually **produce a high number of private bands** (loci)
(Maltagliati et al. 2006, Casu et al. 2009)

M. pugana, the most divergent species of ***M. pacifica*** **complex**

Genetic Structure

Estimated genetic clustering STRUCTURE 2.3.4





Genetic component

1
2
3
4

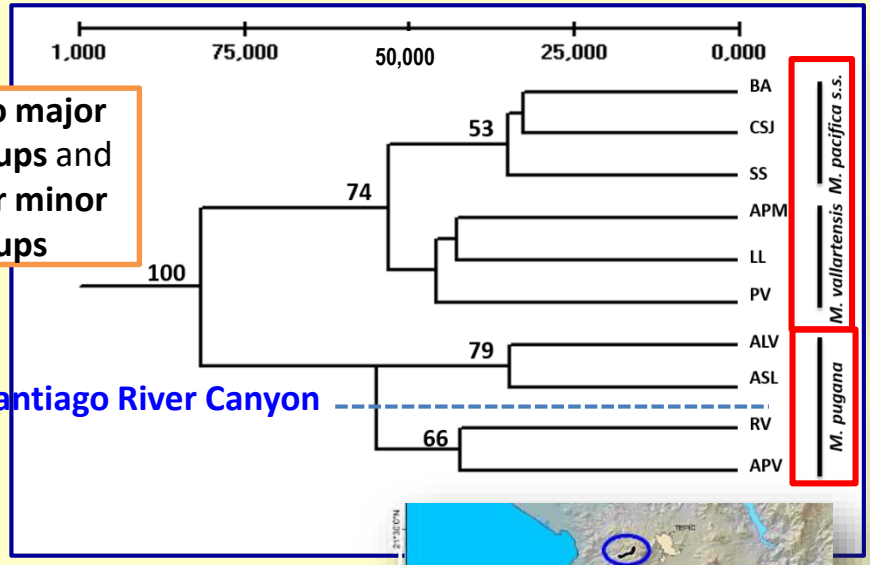
Four genetic goups

Two panmictic subpopulations of one species: ***M. pugana***

Two taxa: ***M. pacifica* s.s** and ***M. vallartensis***

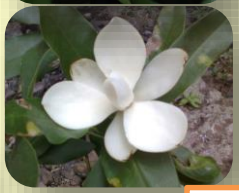
Genetic relationships

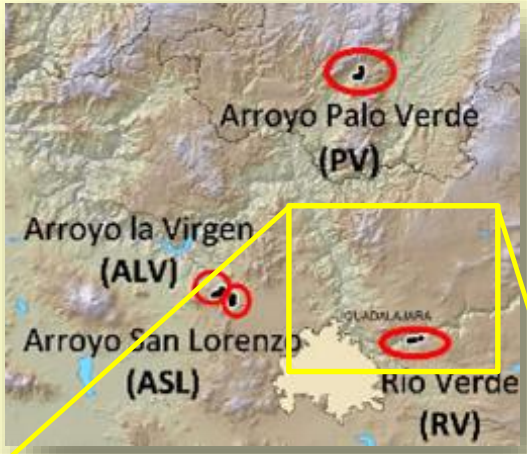
UPGMA dendrogram based on Nei's genetic distances
Bootstrap (%) at each node (1000 replicates)

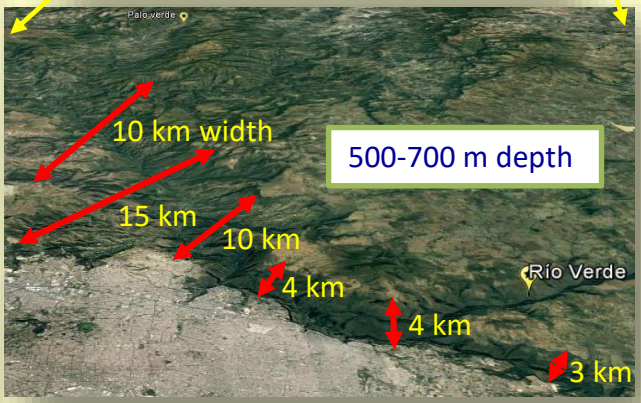


Two major groups and **four minor groups**

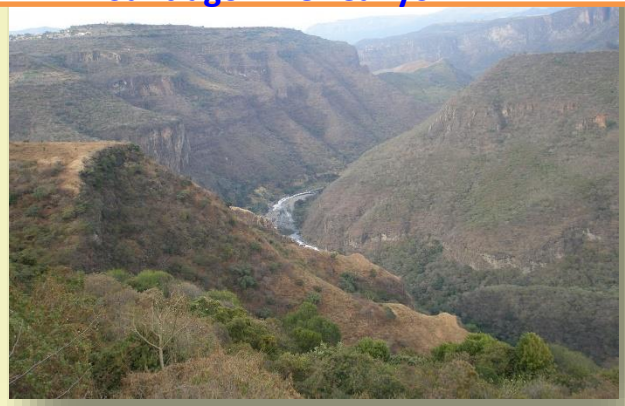
Santiago River Canyon





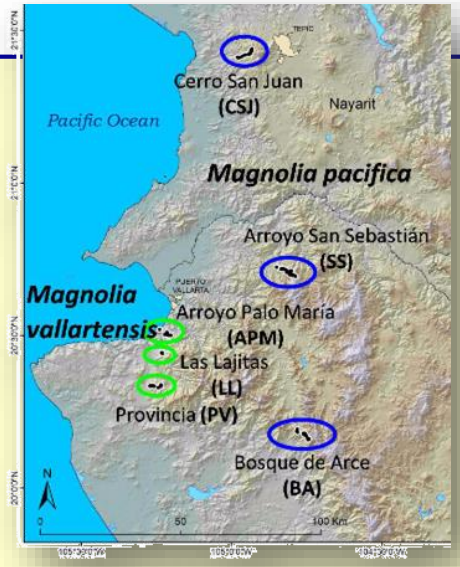


Two allopatric subpopulations of ***M. pugana*** separated by a physiographical barrier:
Santiago River Canyon



M. vallartensis **in the same major group of *M. pacifica s.s.***

Current process of **parapatric divergence from *M. pacifica s.s.***



Pairwise genetic differentiation among *M. pacifica* complex localities

Nei's unbiased genetic distance

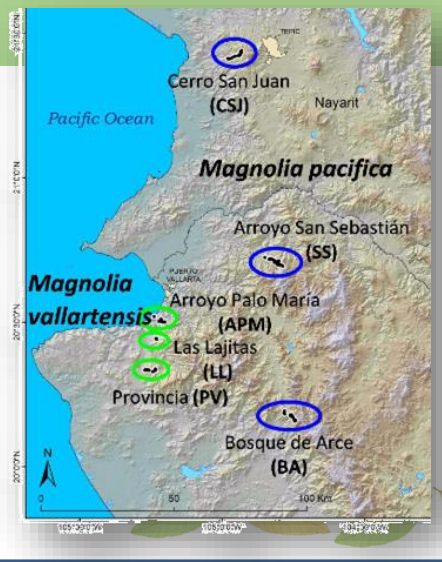
Exact test of differentiation probability

Species	*Magnolia pugana*				*Magnolia pacifica s.s.*			*Magnolia vallartensis*		
Locality	ALV	ASL	RV	APV	BA	ASS	ASJ	**PV**	APM	LL
ALV	-	**0.032**	0.052	0.05	0.069	0.07	0.066	0.099	0.08	0.097
ASL	0.144	-	0.054	0.051	0.055	0.06	0.05	0.077	0.072	0.079
RV	0	0.001	-	**0.039**	0.07	0.074	0.073	0.11	0.088	0.112
APV	0.013	0.006	0.079	-	0.057	0.069	0.06	0.098	0.088	0.097
BA	0	0	0	0.002	-	**0.031**	**0.03**	0.058	**0.049**	**0.042**
ASS	0	0.002	0	0	0.52	-	**0.032**	0.065	**0.045**	**0.042**
ASJ	0	0	0	0	0.703	0.312	-	0.056	**0.04**	**0.042**
PV	0	0	0	0	**0.052**	**0.06**	**0.05**	-	**0.043**	**0.039**
APM	0	0	0	0	0.21	0.062	0.162	0.55	-	**0.038**
LL	0	0	0	0	0.475	0.06	0.119	0.976	0.36	-

Nei's unbiased (1978) distance
Close genetic relationships among localities are presented in red bold

There are not differentiation of most of *M. pacifica* localities with *M. vallartensis* ones

Among the ***M. vallartensis*** localities, **Provincia (PV) the most differentiated from the *M. pacifica* s.s.** group

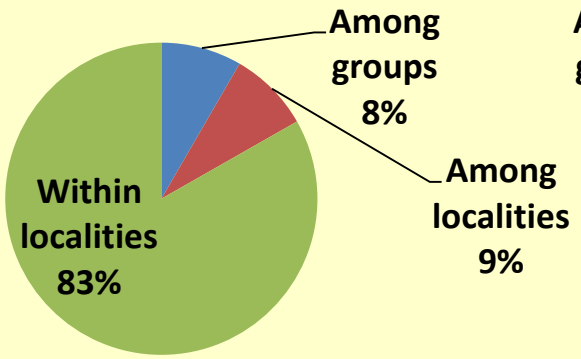


Genetic Structure

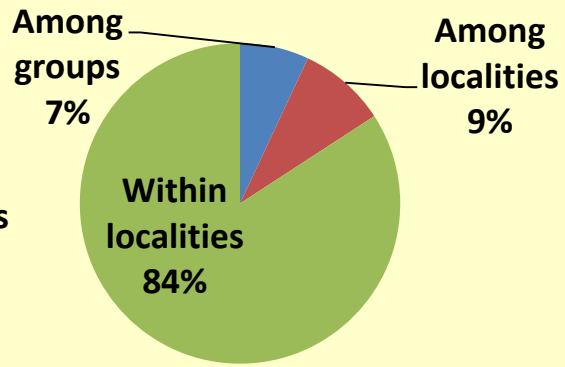
Molecular variance analysis (AMOVA) for taxonomical/Bayesian analysis groups and species of the *Magnolia pacifica* complex

	Percentage of variation %					
	Groups		Species			
Variation source	Taxonomic (3)	Bayesian analysis (4)	*M. pugana*	*M. pacifica s.s.*	*M. vallartensis*	*M. coriacea* (Zhao et al. 2012)
Among groups	8	7				
Among localities/groups	9	9	12	7	7	23
Within localities/all localities	83	84	88	93	93	77
ΦST	0.168	0.158	0.118	0.070	0.070	
p(rand>= data)	0.001	0.001	0.001	0.001	0.001	

0.168 > 0.158 > 0.118 > 0.070 = 0.070



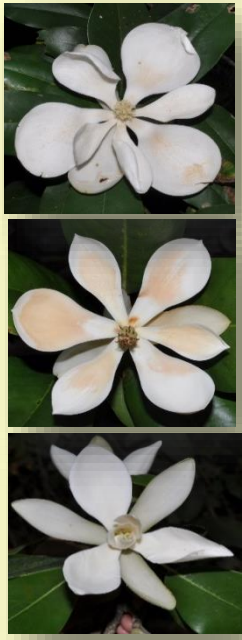
3 taxonomic groups



4 Bayesian groups

- The population genetic approach assumes a **higher genetic structure between species than within species** (Drummond &Hamilton 2007).It supports the current **taxonomical status of three species.**
- ***M. pacifica* complex spp. has moderate to low genetic structure**
- **Lower ΦST** than perennial (0.25), cross-polinated (0.27) and widely distributed plant species (0.34) (Nybom 2004)

Genetic diversity and differentiation parameters in species of the *Magnolia pacifica* complex

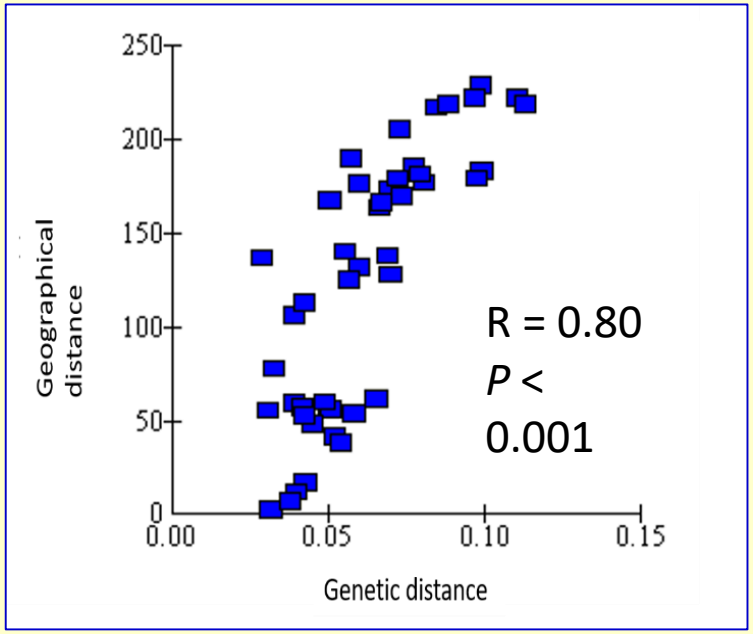


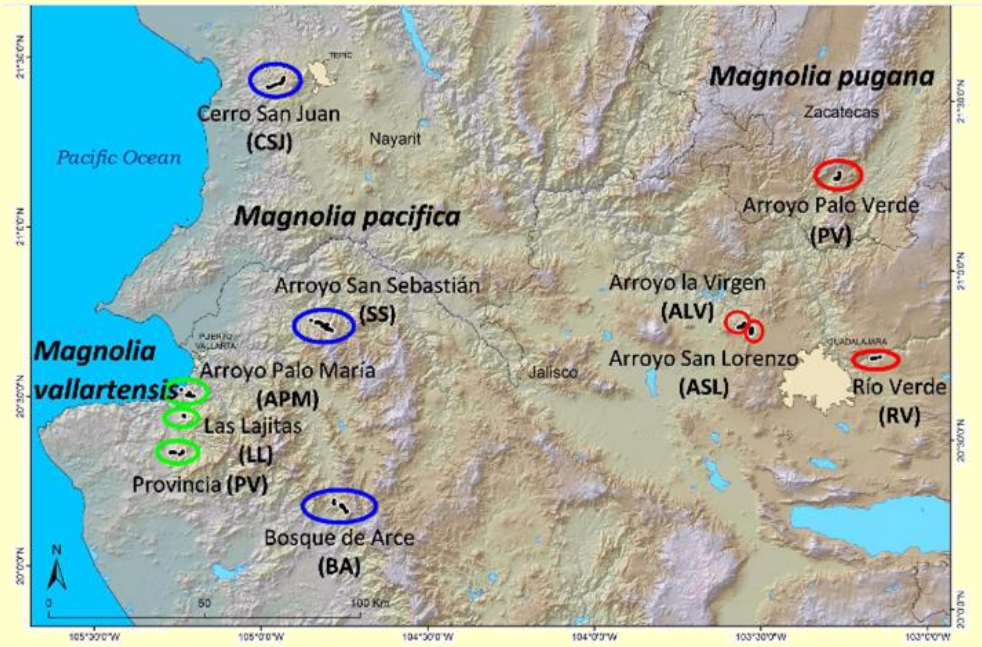
Species	Locality	P(%)	I	H_E	H_T	H_S	G''_{ST}	D (Jost)
	ALV	38	0.208	0.138				
	ASL	39	0.23	0.14				
	RV	38	0.218	0.121				
	APV	38	0.227	0.136				
M. pugana		51	0.268(0.288)		0.158(0.023)	0.134 (0.020)	0.178(0.030)	0.028(0.007)
	BA	47	0.28	0.178				
	ASS	38	0.209	0.14				
	ASJ	41	0.233	0.159				
M. pacifica s.s.		51	0.272(0.286)		0.175(0.025)	0.159(0.023)	0.105(0.026)	0.018(0.005)
	PV	39	0.244	0.159				
	APM	39	0.241	0.147				
	LL	39	0.236	0.152				
M. vallartensis		46	0.275(0.310)		0.171(0.024)	0.153(0.022)	0.124(0.027)	0.021(0.006)
Total		**64**	**0.309(0.285)**		**0.178(0.023)**	**0.147(0.20)**	**0.222(0.039)**	**0.040(0.009)**

P polymorphism, I Shannon index, H_E expected heterozygosity, H_T total heterozygosity, H_S intrapopulation heterozygosity, G''_{ST} corrected and standardized fixation index, D Jost's differentiation index, standard deviation in parentheses

- **G''_{ST} produce higher values than G_{ST} (0.12, 0.06, 0.07),** these latter are lower than *M. coriacea* (G_{ST} =0.187) (Zhao et al. 2012).
- ***M. pacifica* complex Shannon Diversity (I=0.31) was lower** than *M. sharpii* (0.56) and *M. schiedeana* (0.50) (Newton et al. 2008).
- ***M. pugana* is the more endangered species, because their low genetic diversity (I=0.27), higher population differentiation and isolation, so they need more protection priority**.

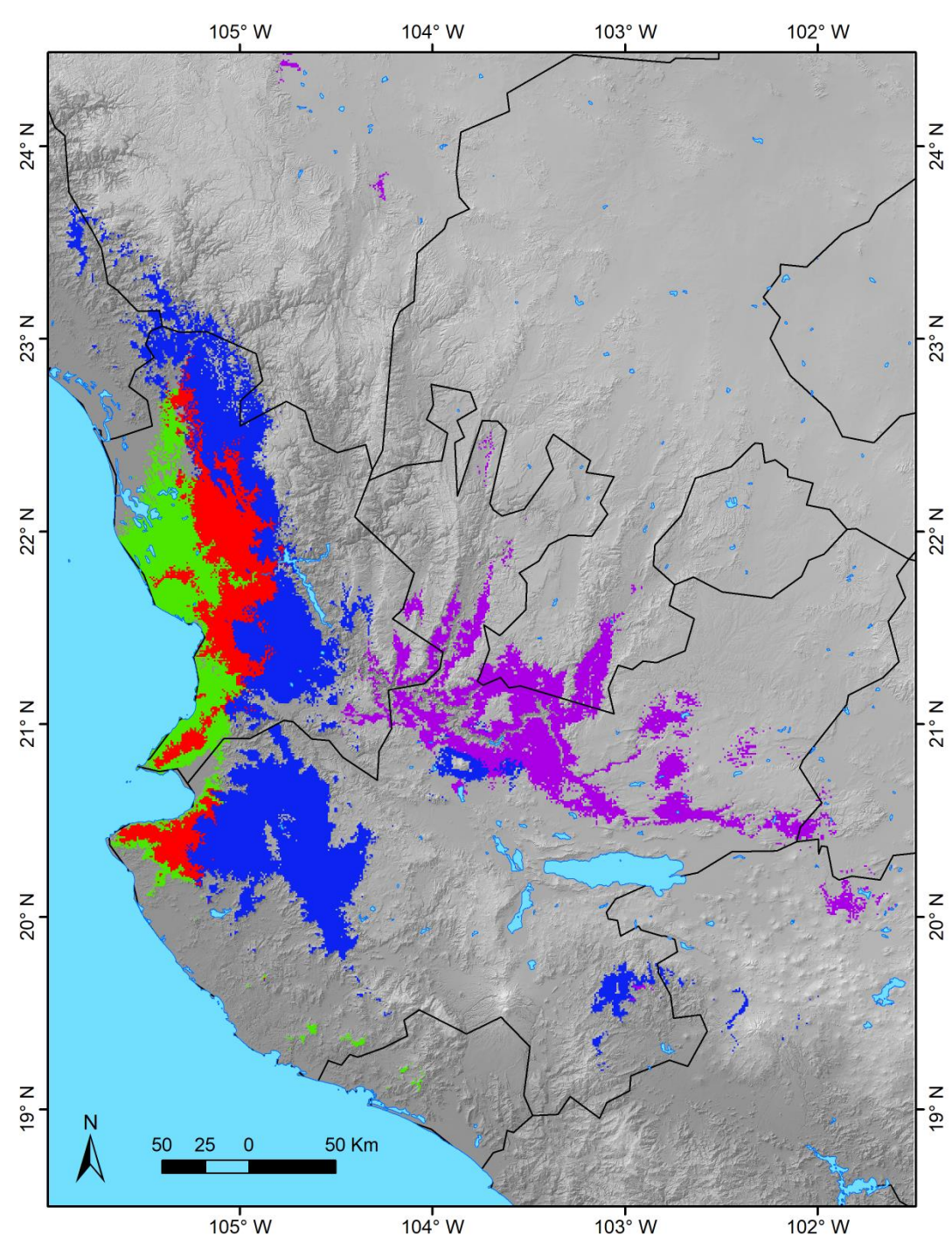
Mantel test





Significant positive correlation between genetic and geographical distance:

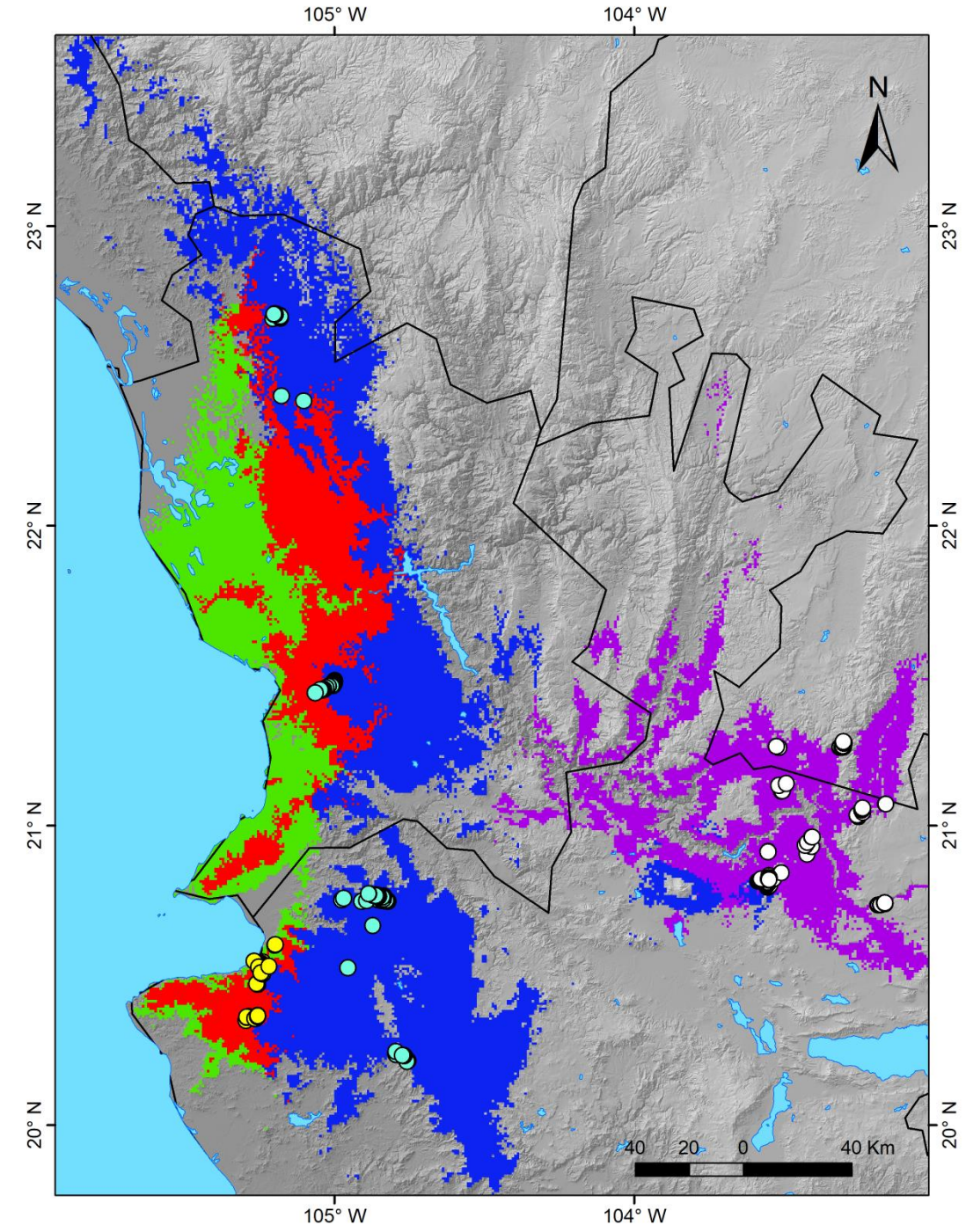
A process of **isolation by distance (IBD)**



RESULTS
SPECIES POTENTIAL GEOGRAPHIC DISTRIBUTION MODELS

POTENTIAL DISTRIBUTION AREAS

- ***M. pugana***
- ***M. pacifica***
- ***M. vallartensis***
- **Overlap area for *M. pacifica* and *M. vallartensis***



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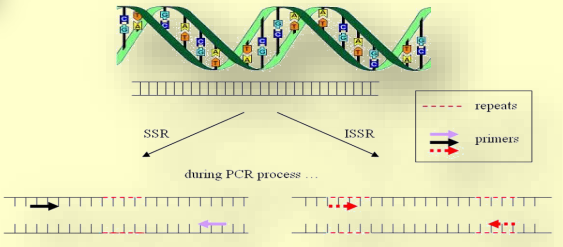
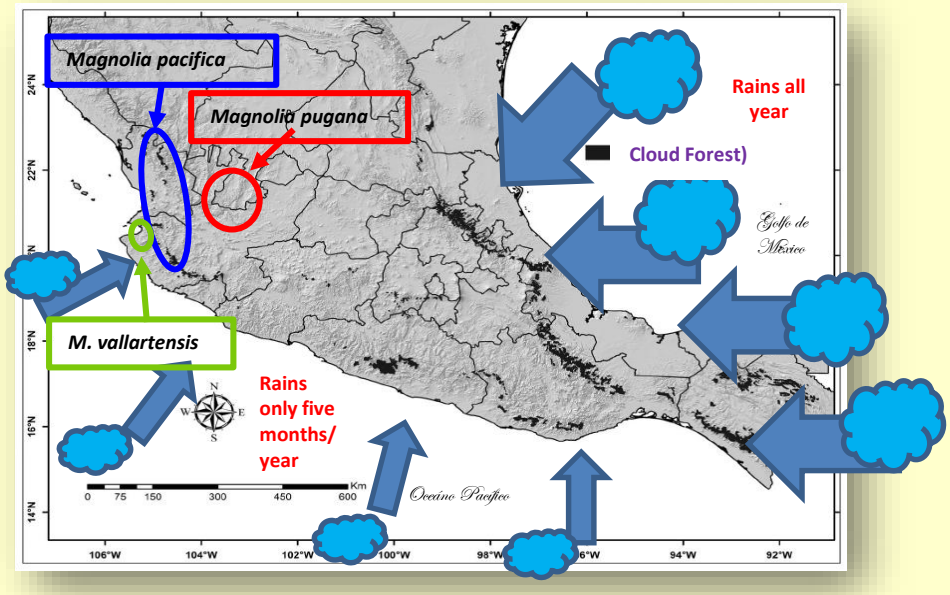
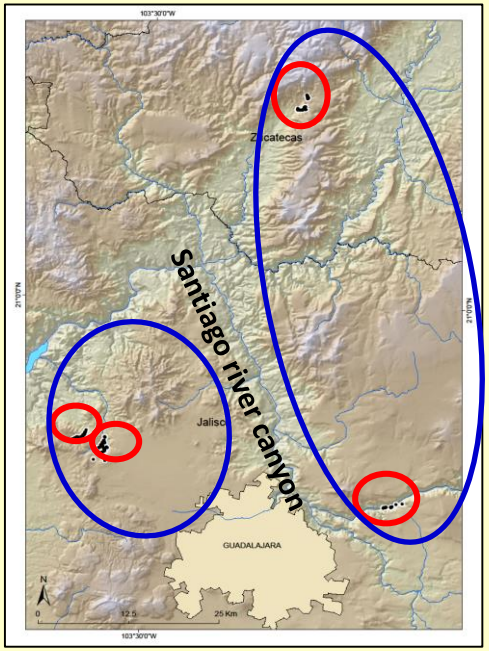
Conclusions

1. **ISSR markers are a useful and complementary tool helping to species delimitation in taxonomy complexes of closely related species.**



2. **Two major genetic groups** in the ***Magnolia pacifica*** **complex in SW Mexico:**
   1. ***M. vallartensis*** **and** ***M. pacifica*** **s.s**. form a group and are in current process of **parapatric and ecological divergence**.
   2. A second group: **two allopatric panmictic subpopulations of** ***M. pugana*** separated by **Santiago Canyon partial barrier.**





Conclusions

3. The current taxonomical status (three species) is supported by the higher genetic structure between these species than within species,
4. but the ISSR genetic differentiation between *M. vallartensis* and *M. pacifica* s.s. subpop. is not significant.

5. The four genetic groups deserves especific conservation efforts, but *M. pugana* ones are imore endangered, because their lower genetic diversity and higher isolation, so they need more protection priority.

***M. pacifica* s.s. and *M.vallartensis* habitat**
Cloud forest

***M. pugana* habitat**
Riparian gallery forest surrounded by seasonally dry forest

CONCLUSIONS

6. The results for the differentiation of ecological niches and areas of potential geographical distribution **support the results of genetic divergence.**

7. The ecological niche and the geographical distribution of ***M. pugana*** **are completely separated from the other two species**.

8. **The potential geographical distribution of *M. pacifica* and *M. vallartensis*,** although different in their greater proportion**, present a partial overlap**. The foregoing indicates an ecological divergence not very far between these two species.

Thanks !!!

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