



Evaluating threats and providing effective tools to conserve Coquí Guajón (*Eleutherodactylus cooki*)

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Cooperative Agreement - Proyecto Coquí & USFWS

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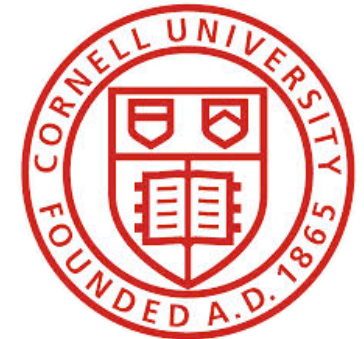
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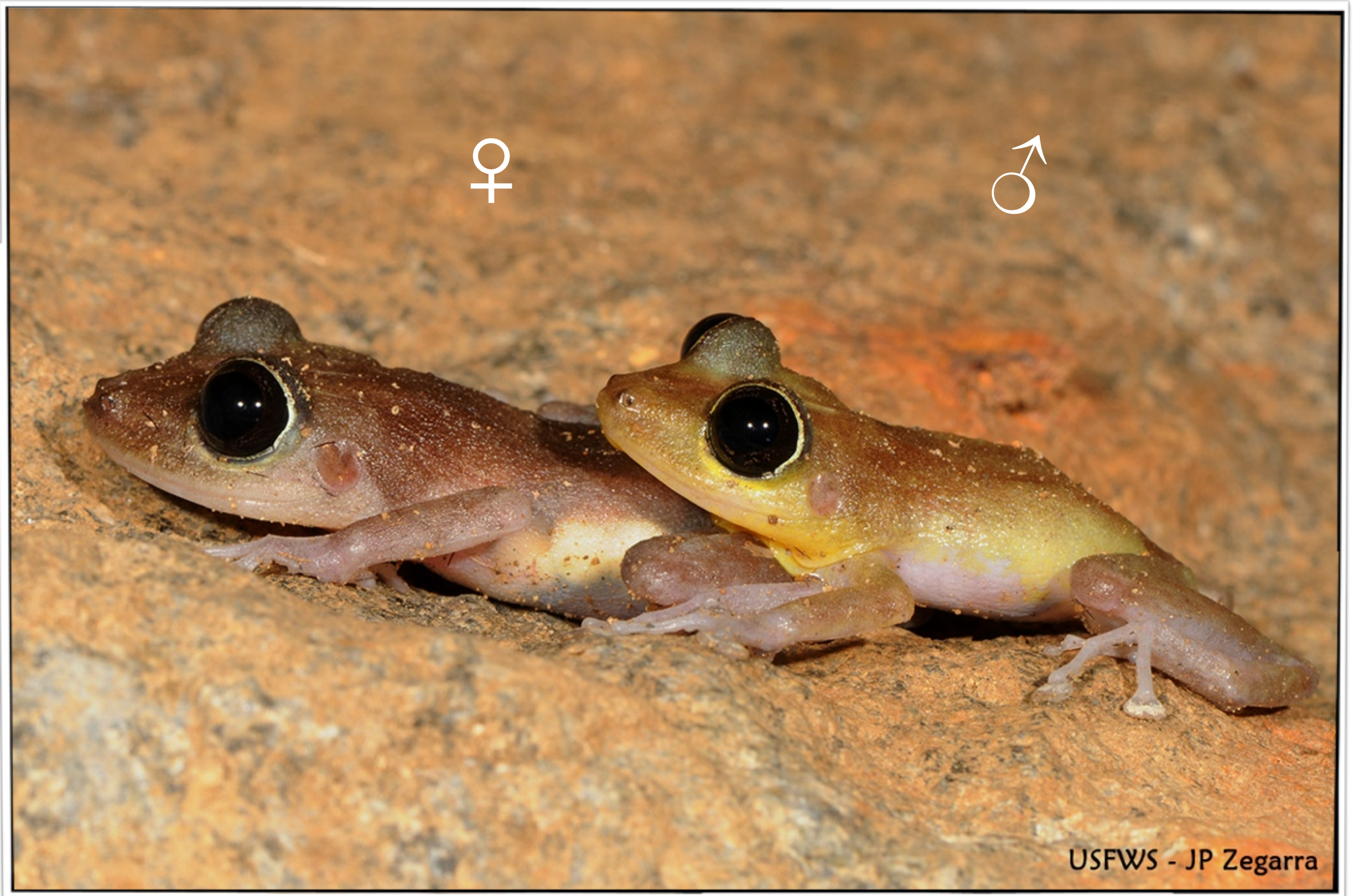
Special thanks to all the landowners who gave us permission to work in their lands!



Photo: Louis Santiago



Granodiorite is essential for Guajón's reproduction





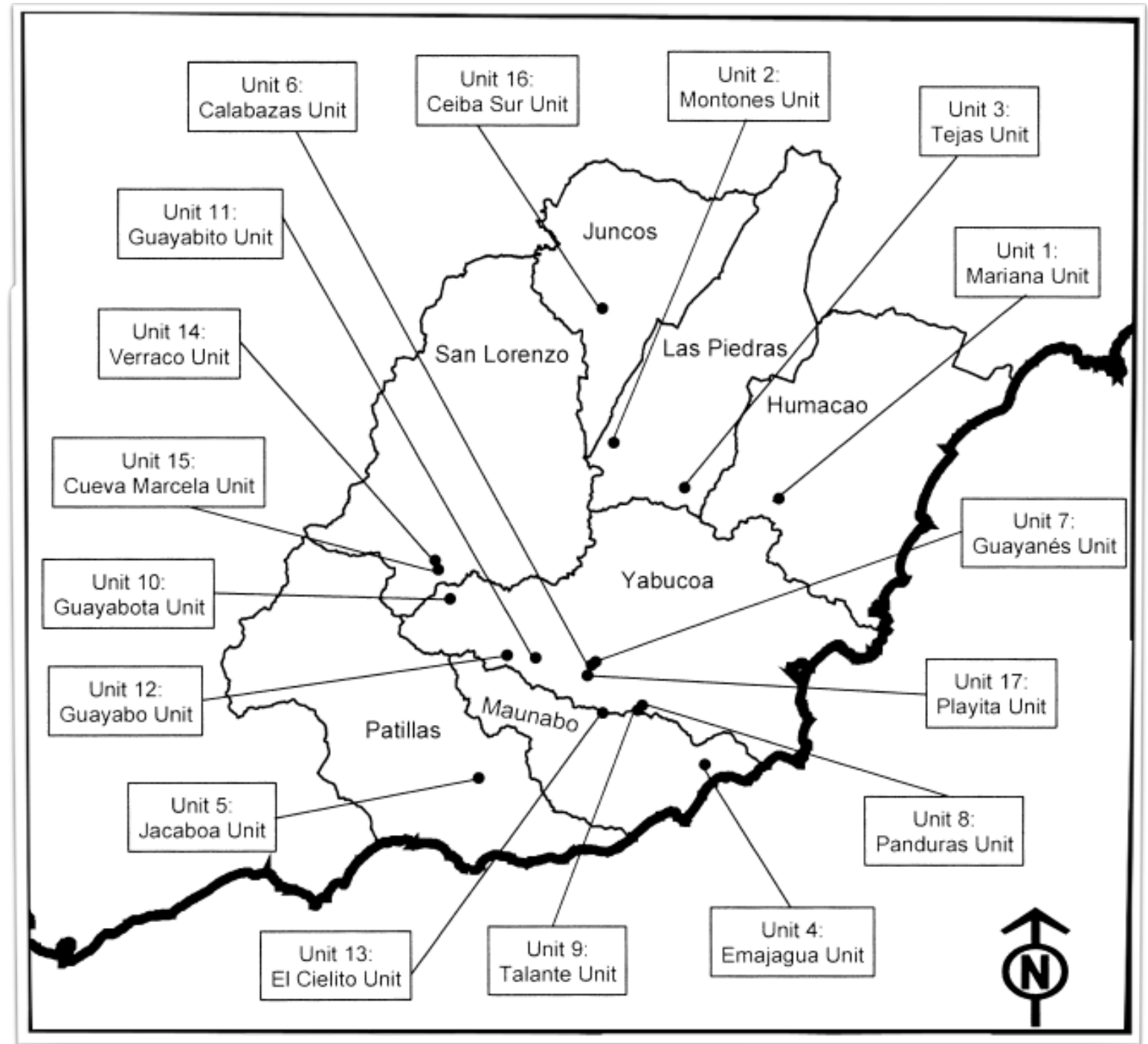
♂ guarding eggs

Alberto López ©



Coquí Guajón (*Eleutherodactylus cooki*)

- Listed as threatened in 1997 by USFWS.
 - ◆ Vulnerable: DNR
- Recovery plan released in 2004.
- 17 critical habitats declared in 2007.
 - ◆ Restricted to private lands



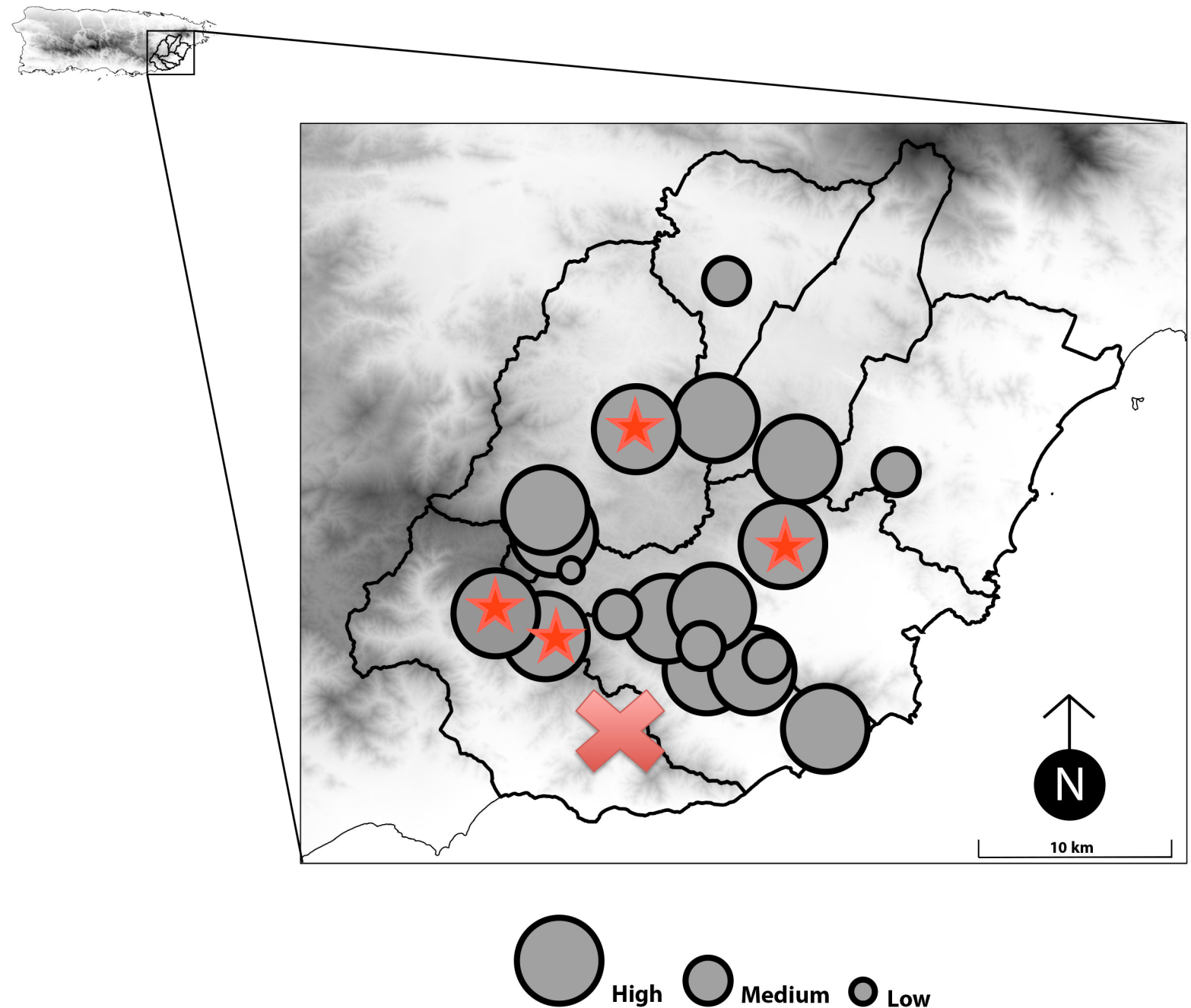
USFWS, 2007

Aims of the cooperative agreement

1. Monitor the population status by performing demographic studies.
2. Determine the impacts of the fungal pathogen *Batrachochytrium dendrobatidis* (Bd) and parasitic tick *Ornithodoros talaje* (OT).
3. Assess the genetic diversity and patterns of gene flow (connectivity) between localities.
4. Develop an outreach program to promote species awareness and to improve habitat quality.

Monitor the population status of Coquí Guajón.

- 16 habitats (+4 additional localities ★) visited twice: November 2012 & 2013 (wet); and March 2013 & 2014 (dry).
- Audio & visual-encounter surveys.
- Collected tissues: swabs, toe clips for genetic analyses, and ticks.
- No individuals found in Jacabo.



Monitor the population status of Coquí Guajón.

Locality	Trash	Invasive Predator	Parasites	Bd	Habitat Modification	Agriculture	Erosion	Abundance Score	Threat Level Score	Abundance Category	Threat Level Category
Claudio Hernández	0	1	0	1	0	2	3	1	8	High	Moderate
Casas de la Selva	0	1	0	1	0	0	0	1	3	High	Low
Car. 181 Patillas	2	0	0	2	0	0	3	1	8	High	Moderate
Fuentes	2	1	1	1	0	2	0	1	8	High	Moderate
Unit_01_Mariana	0	0	0	2	3	0	0	2	7	Medium	Moderate
Unit_02_Montones	0	1	1	2	0	2	0	1	7	High	Moderate
Unit_03_Tejas	2	0	0	2	3	2	3	1	13	High	High
Unit_04_Emajagua	2	1	1	2	3	0	0	1	10	High	Moderate
Unit_05_Jacabo*	2	1	0	2	3	2	3	3	16	Low	High
Unit_07_Guayanes	0	1	1	1	0	0	0	1	4	High	Low
Unit_08_Pandura	2	1	1	1	0	0	0	2	7	Medium	Moderate
Unit_09_Talante	2	0	1	1	0	0	3	1	8	High	Moderate
Unit_10_Guayabota	2	1	1	1	3	2	3	3	16	Low	High
Unit_11_Guayabito	2	0	1	1	0	0	0	1	5	High	Low
Unit_12_Guayabo	0	0	1	1	0	2	0	2	6	Medium	Moderate
Unit_13_Cielito	0	1	1	1	0	0	0	1	4	High	Low
Unit_14_Verraco	0	1	1	1	0	0	0	1	4	High	Low
Unit_15_Marcela	0	1	1	1	0	2	3	1	9	High	Moderate
Unit_16_Ceiba Sur	2	1	0	1	3	0	3	2	12	Medium	High
Unit_17_Playita	0	1	0	1	3	2	0	2	9	Medium	Moderate
Number of locations with threat	10	14	12	20	7	9	8	Higher numbers indicate higher threat level			

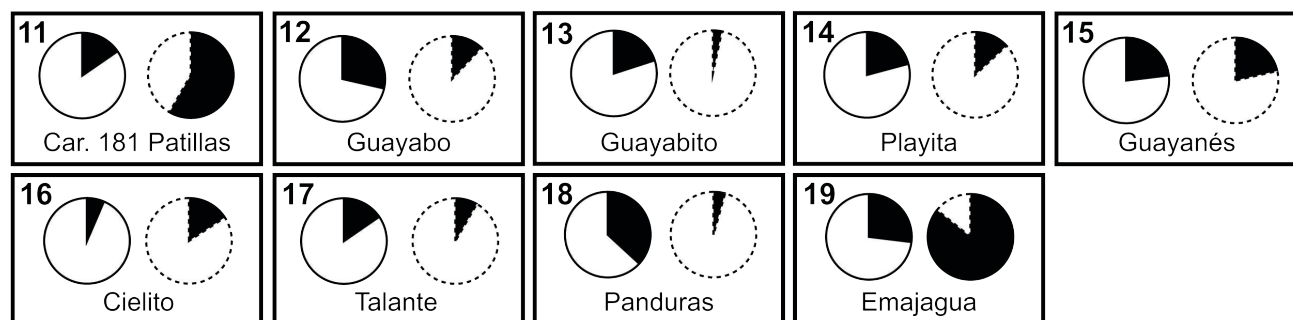
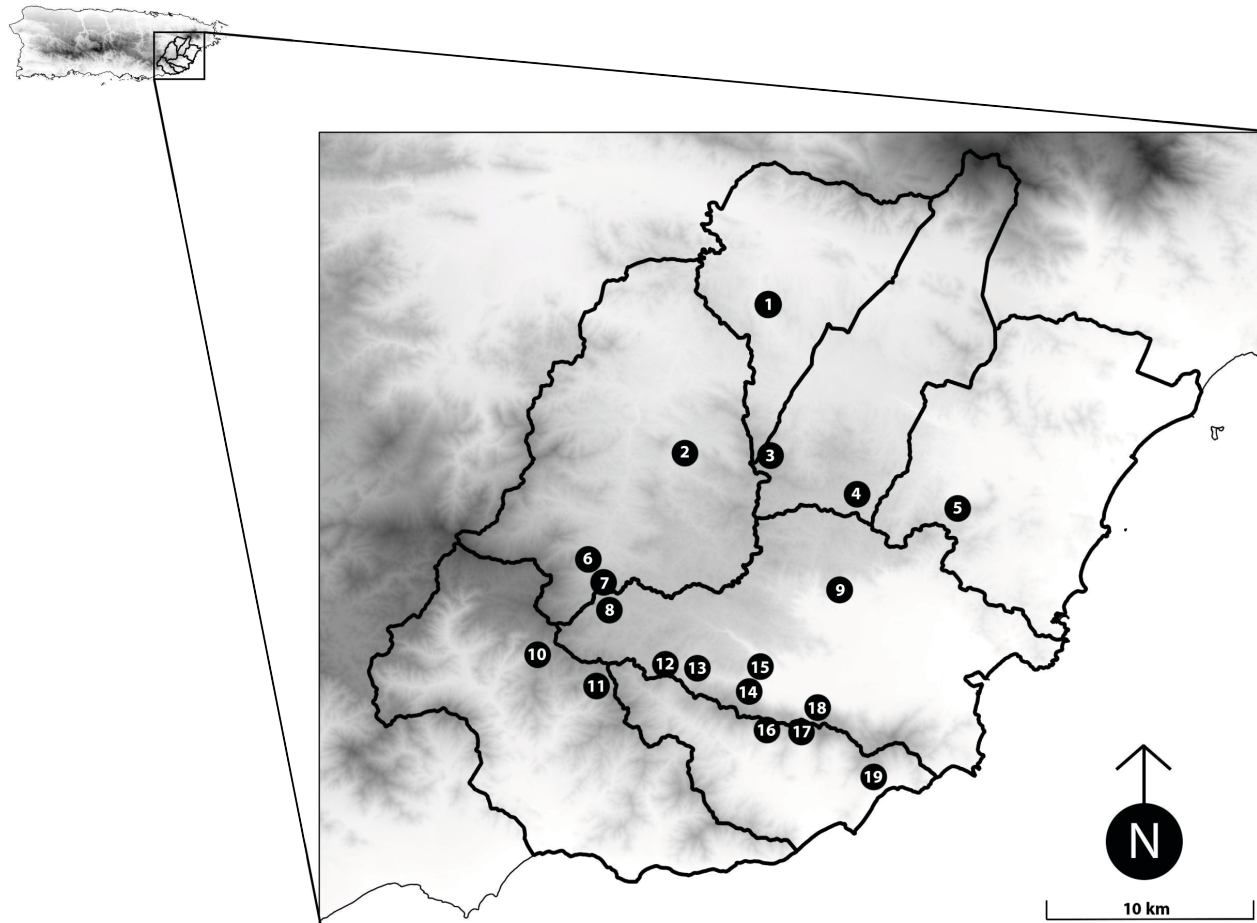
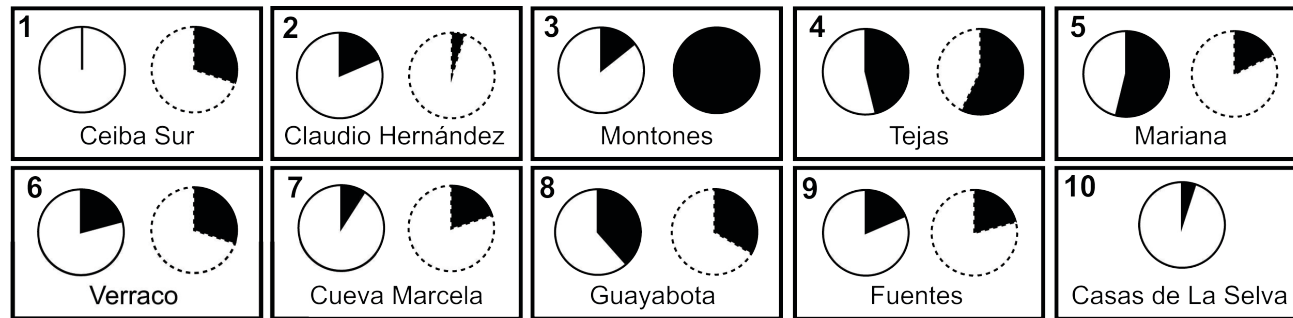
* No E cooki were found & Bd Prevalence on Common Coqui 77%

Threat Level	Score
High	11 -- 16
Moderate	6 -- 10
Low	1 -- 5

Type of Threat	Threats	Values*
Human Induced	Agriculture	2
	Erosion	3
	Habitat Modification	3
	Trash	2
Natural	Bd prevalence < 50%	1
	Bd prevalence > 50%	2
	Invasive Predators	1
	Parasites	1
	Low Abundance	3
	Medium Abundance	2
	High Abundance	1

*The higher the number the larger the threat

Pathogen prevalence and load significantly increased during the cool-dry season.



100% of populations are infected:

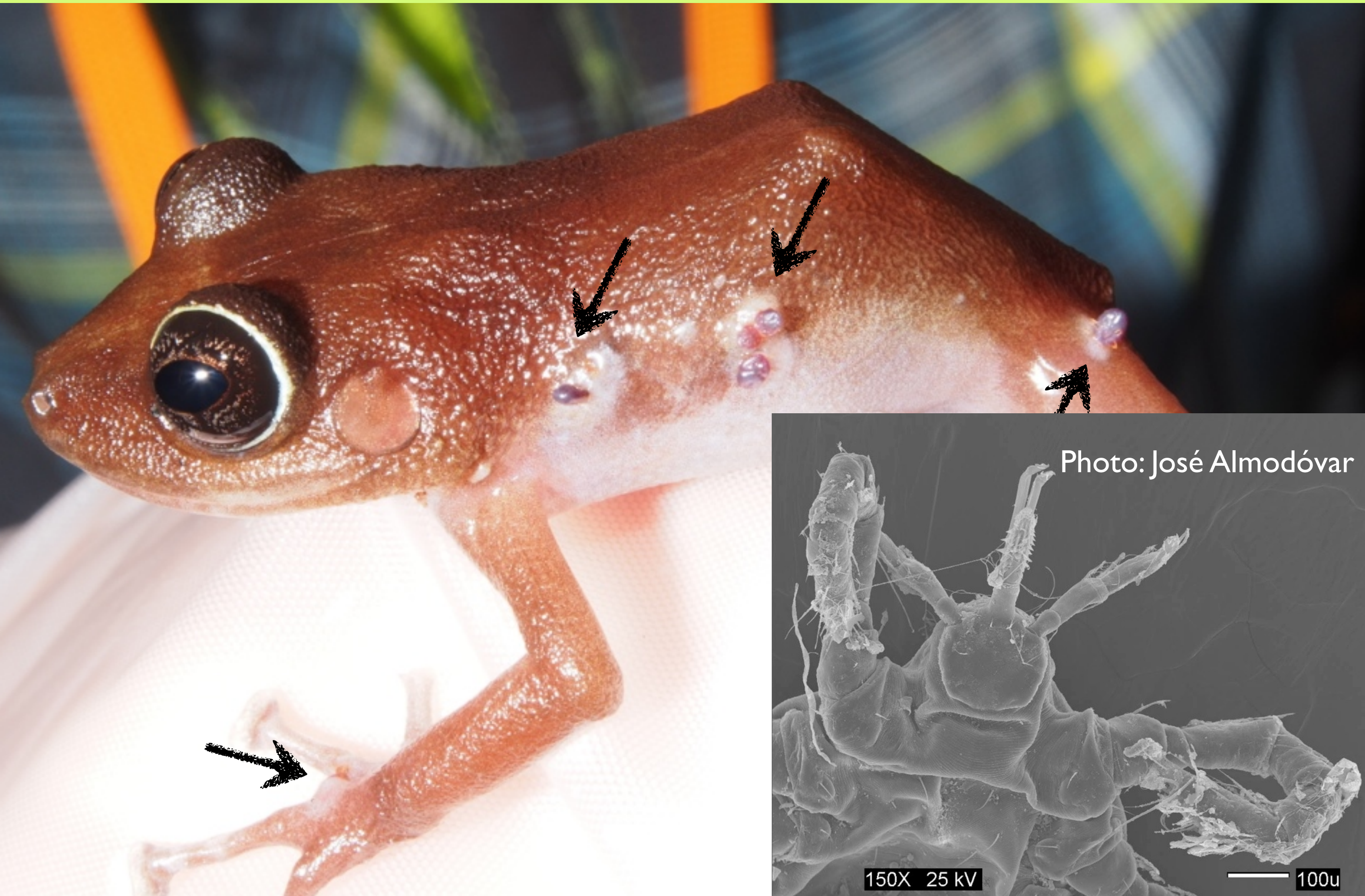
- Prevalence $> 50\%$ at 6 localities (30% of total)

Patterns are not associated to:

- Elevation
- Lat/Long
- Habitat type (caves vs streams)

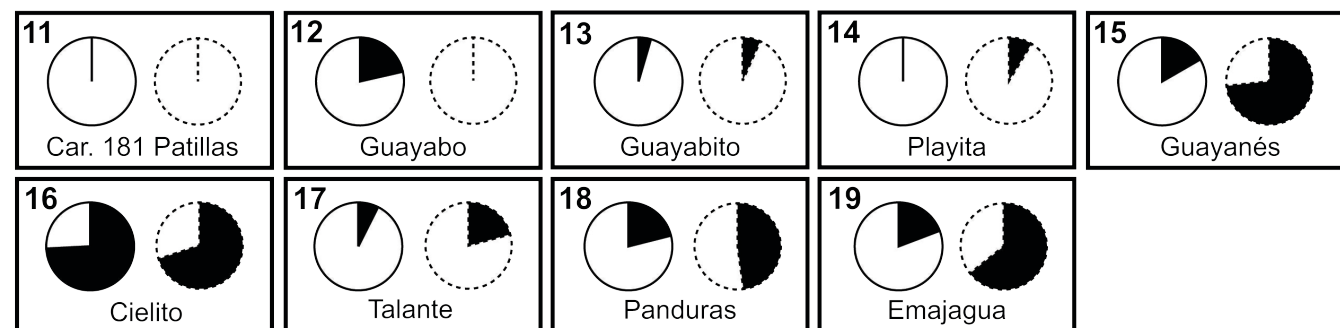
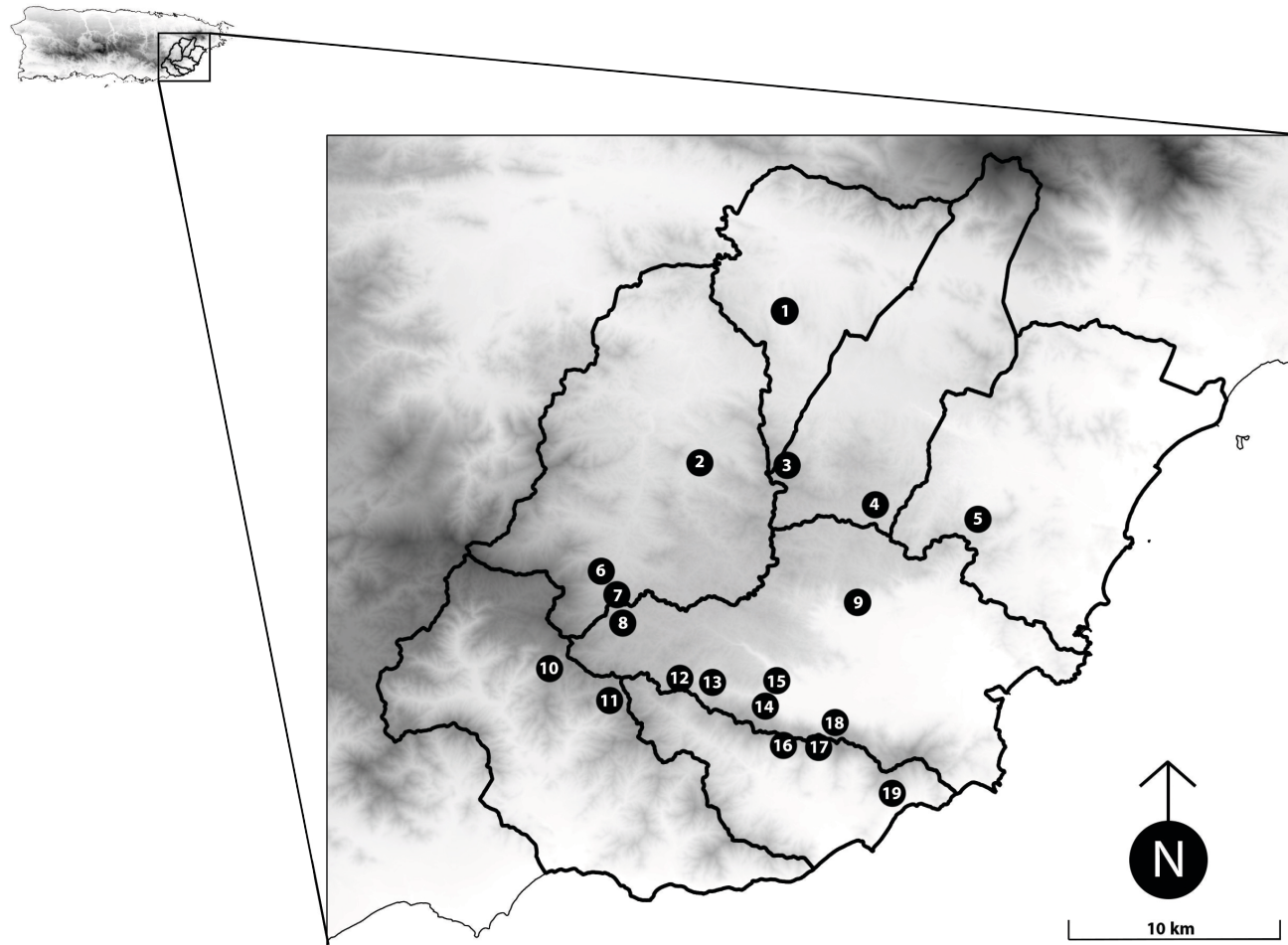
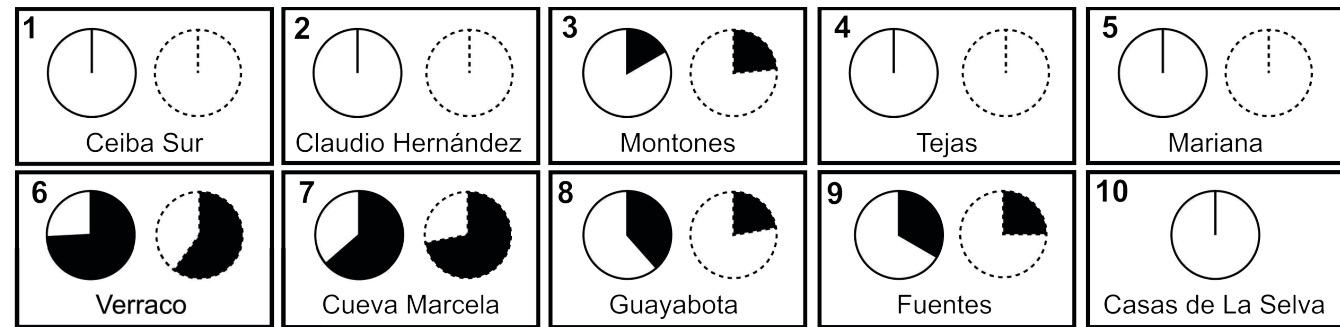


Ticks mostly infect adult frogs.





Parasite prevalence significantly increased during the cool-dry season.



Ticks also showed seasonal patterns:

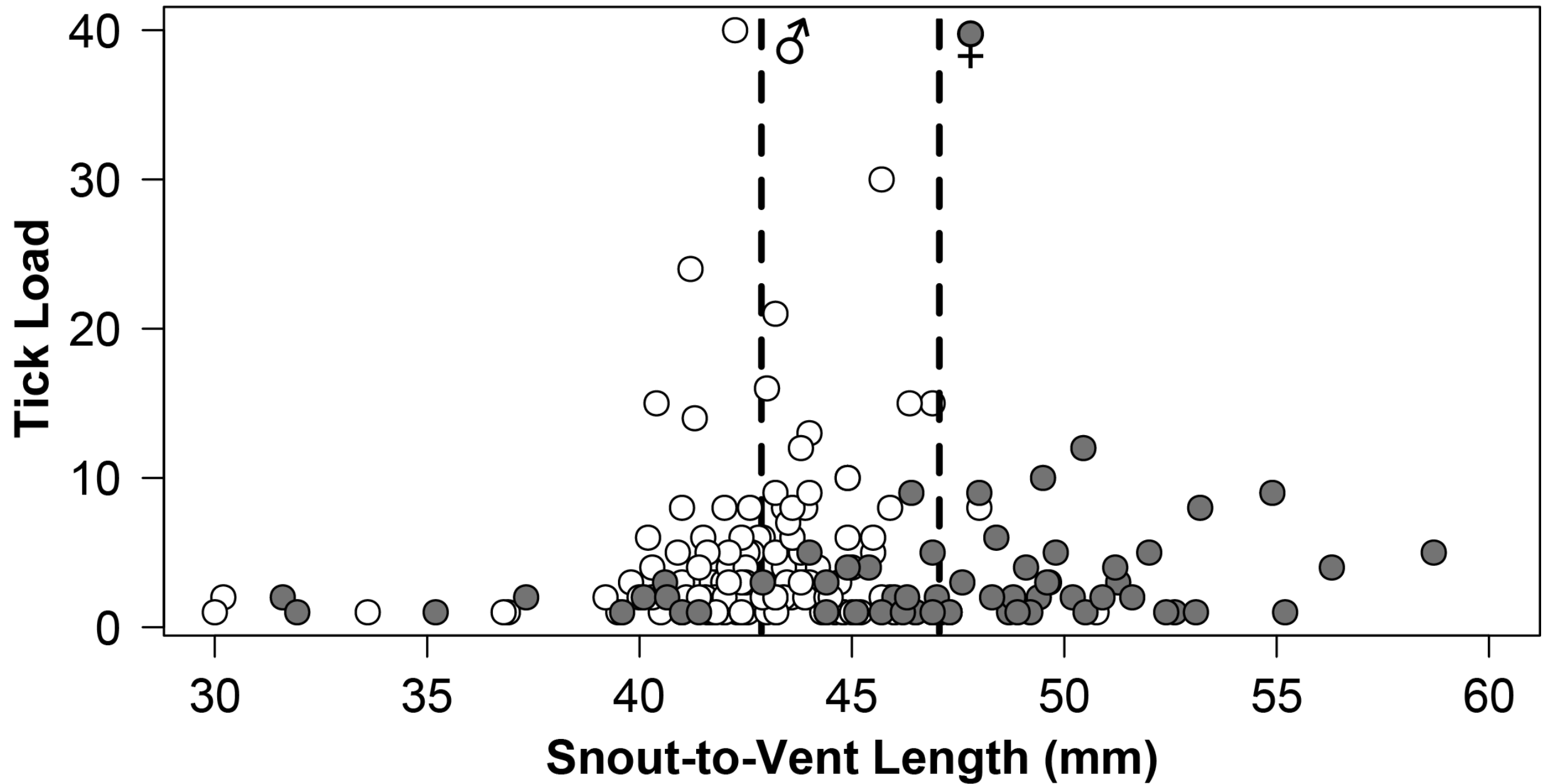
Avg. of 4 (1-30 ticks).

Some localities were “tick-free”

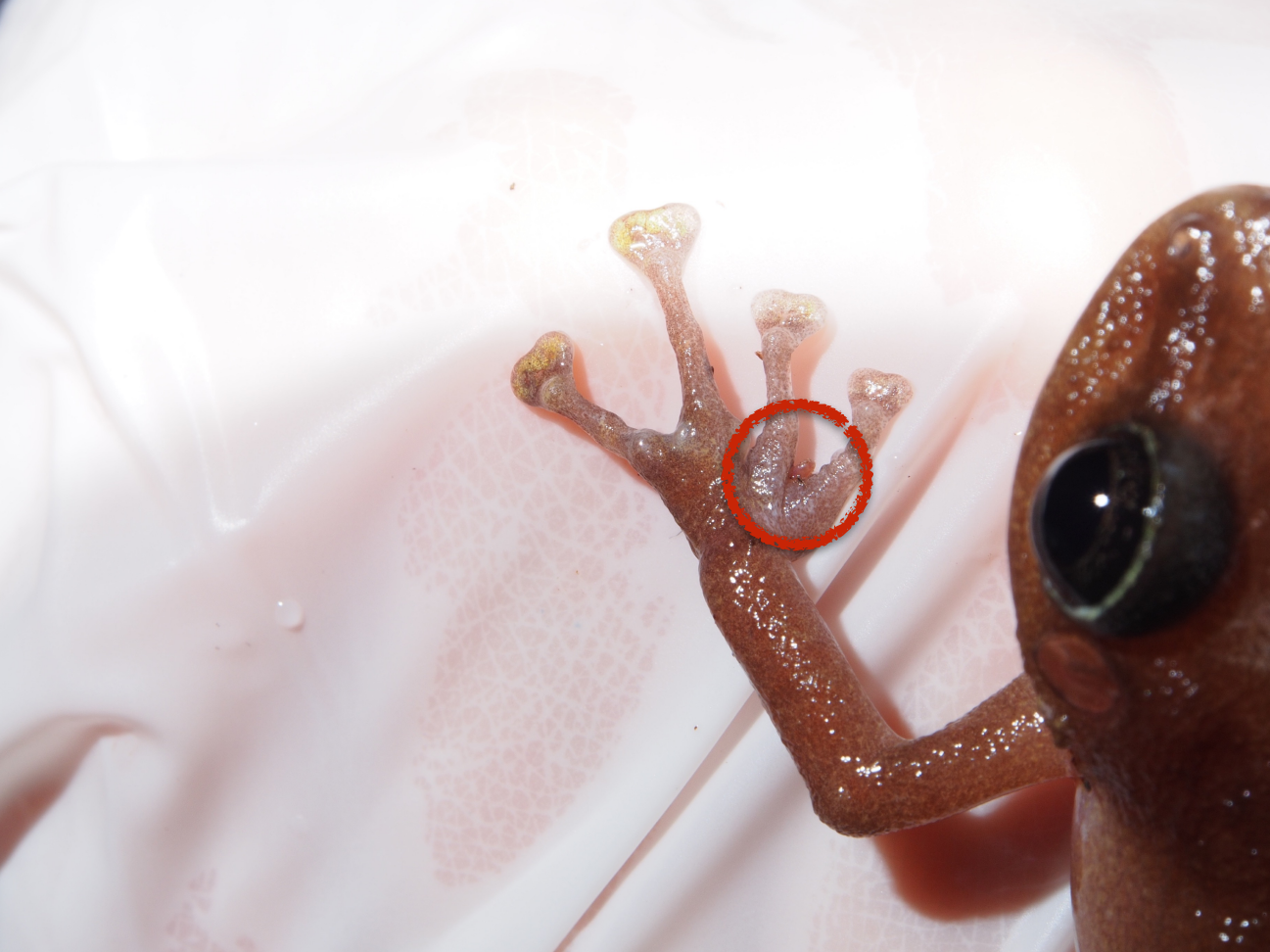
68% of populations were parasitised by ticks.

Skin discoloration marks

Ticks mostly infect male frogs.

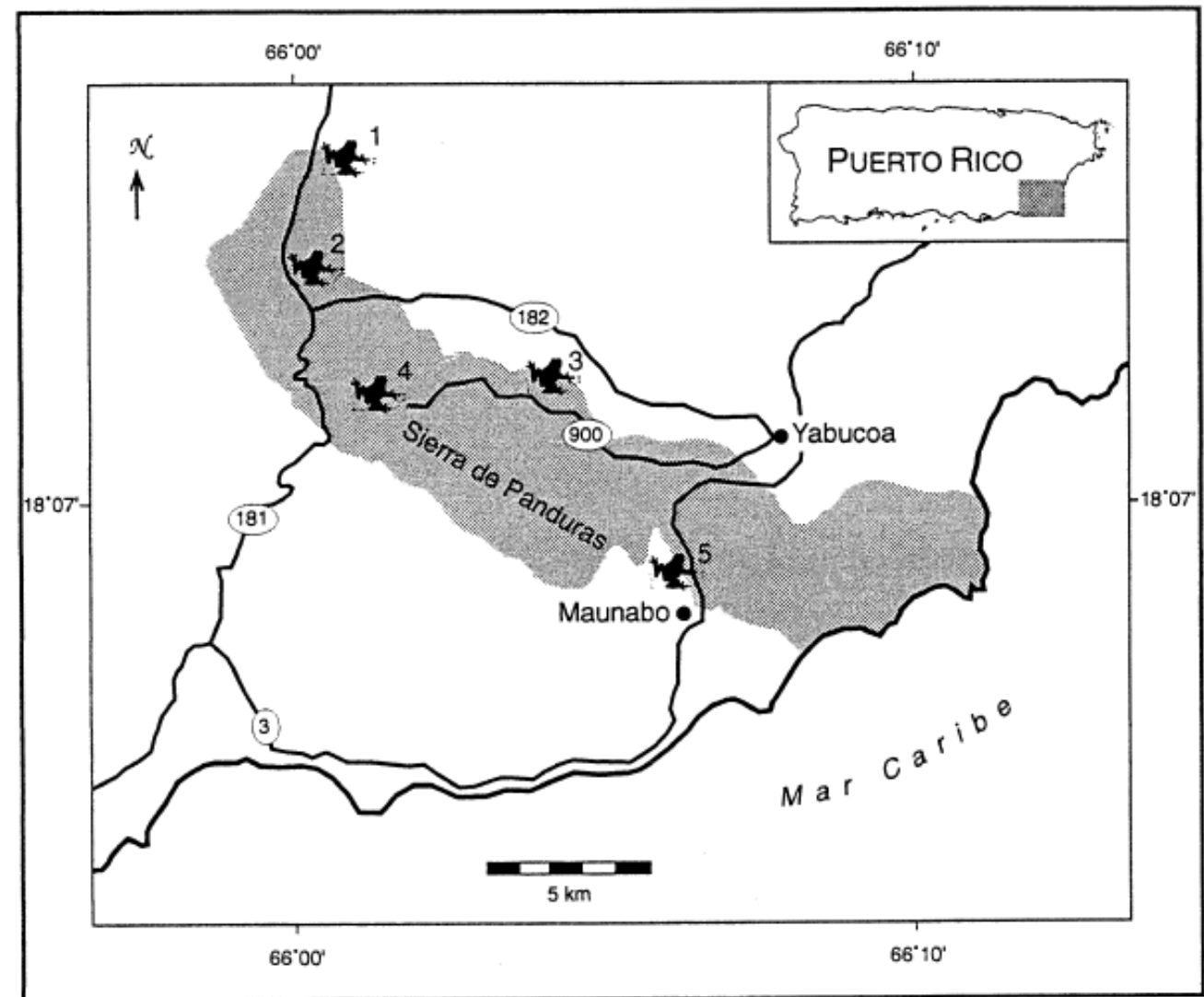




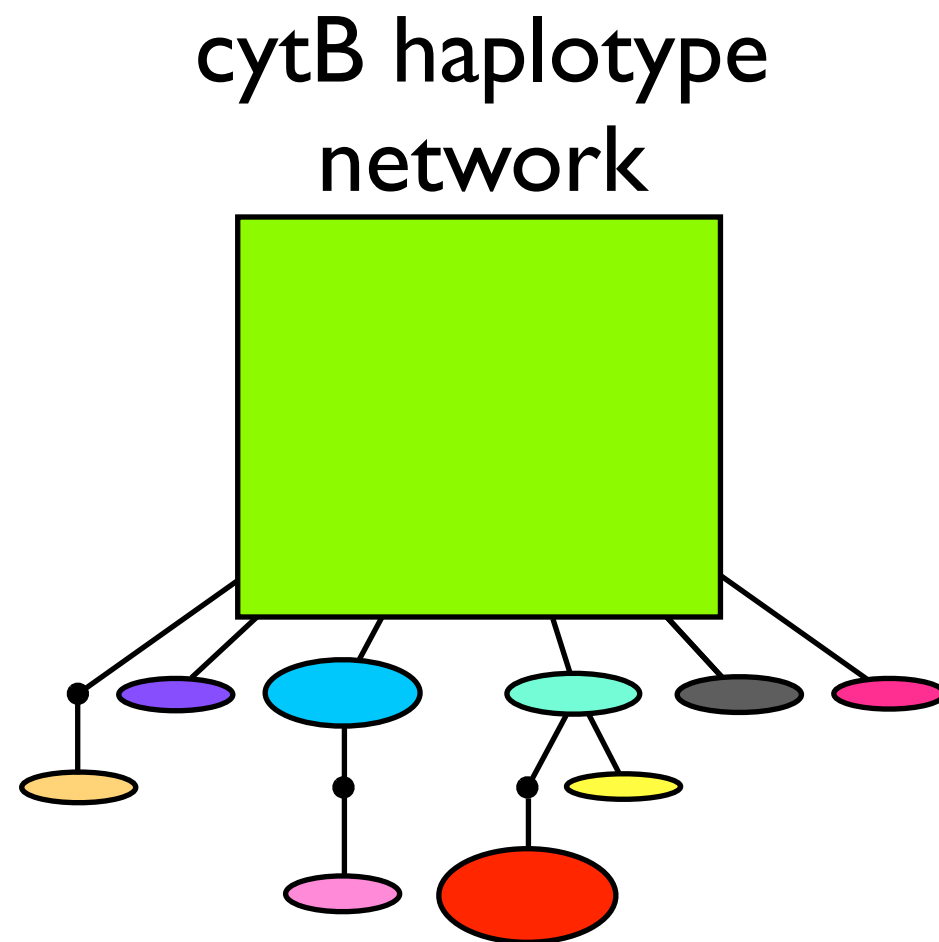


Cave-dwelling species should show genetic structure

- Burrowes & Joglar (1999) did not find genetic differentiation among 5 populations in Sierra Pandura.
- Guajones move approximately 30m to forage (Vega-Castillo, 2001).



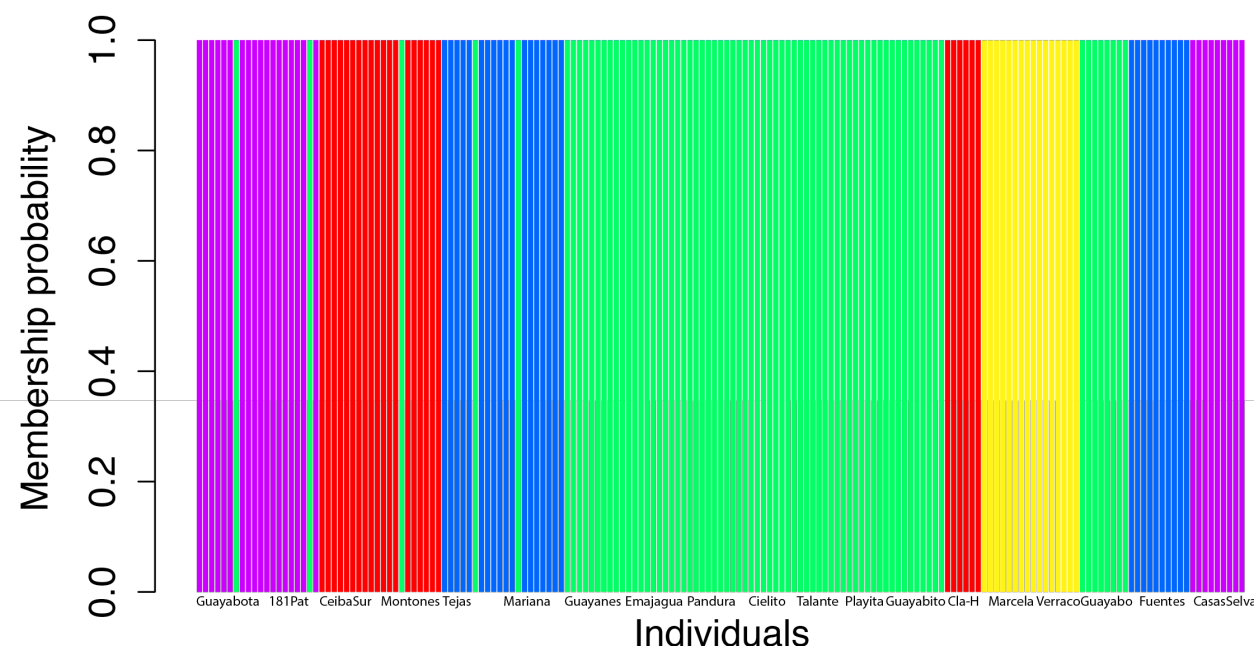
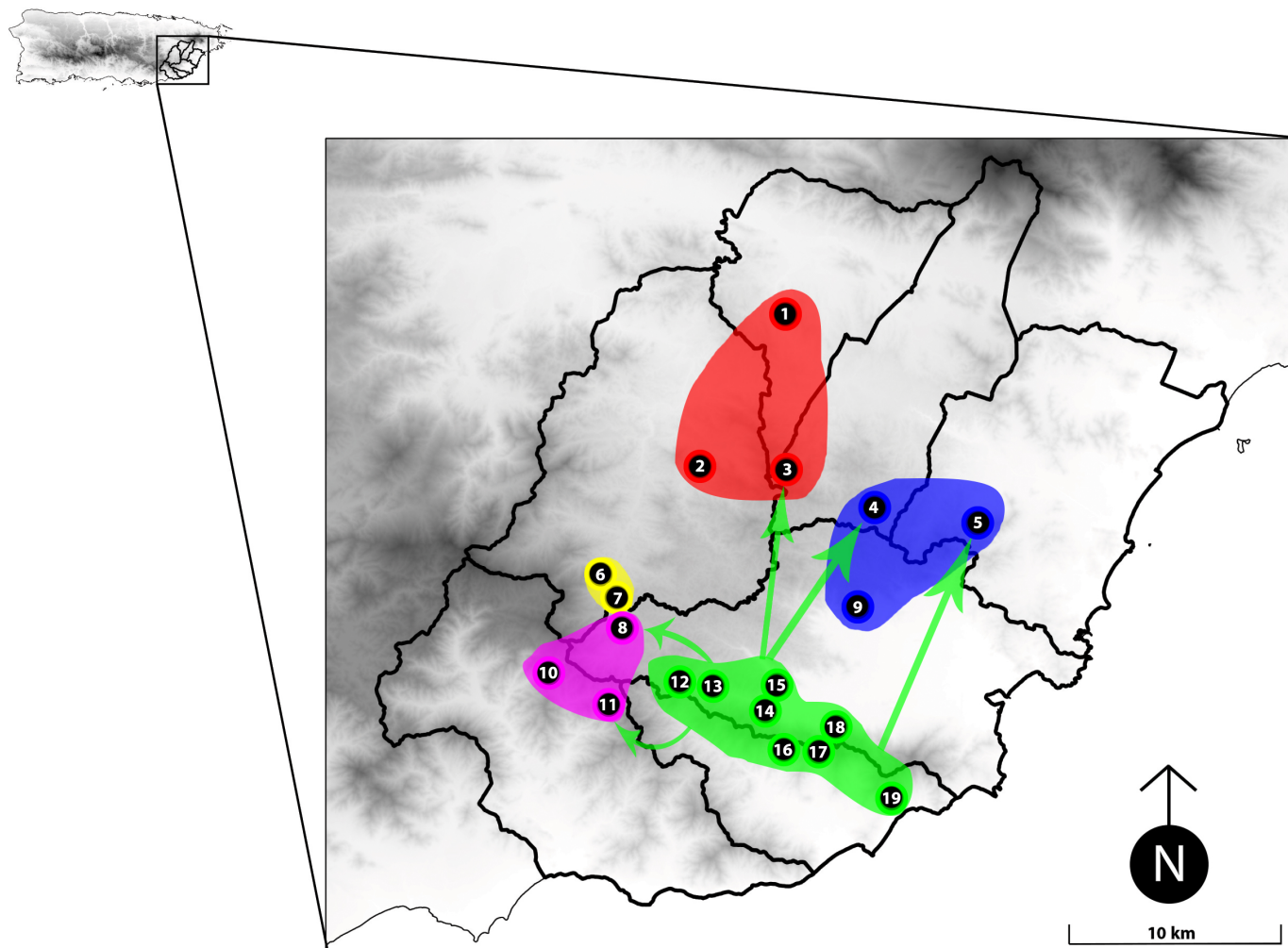
Mitochondrial DNA: only 10 haplotypes!



- 10 haplotypes in 96 individuals.
- ◆ 49 haplotypes in 77 individuals (*E. coqui*-Velo-Antón et al. 2007)
- 17% divergence from *E. coqui*.
- Typical pattern of population expansion followed by bottlenecks.

Very little genetic variation with no apparent population structure.

High population structure among critical habitats

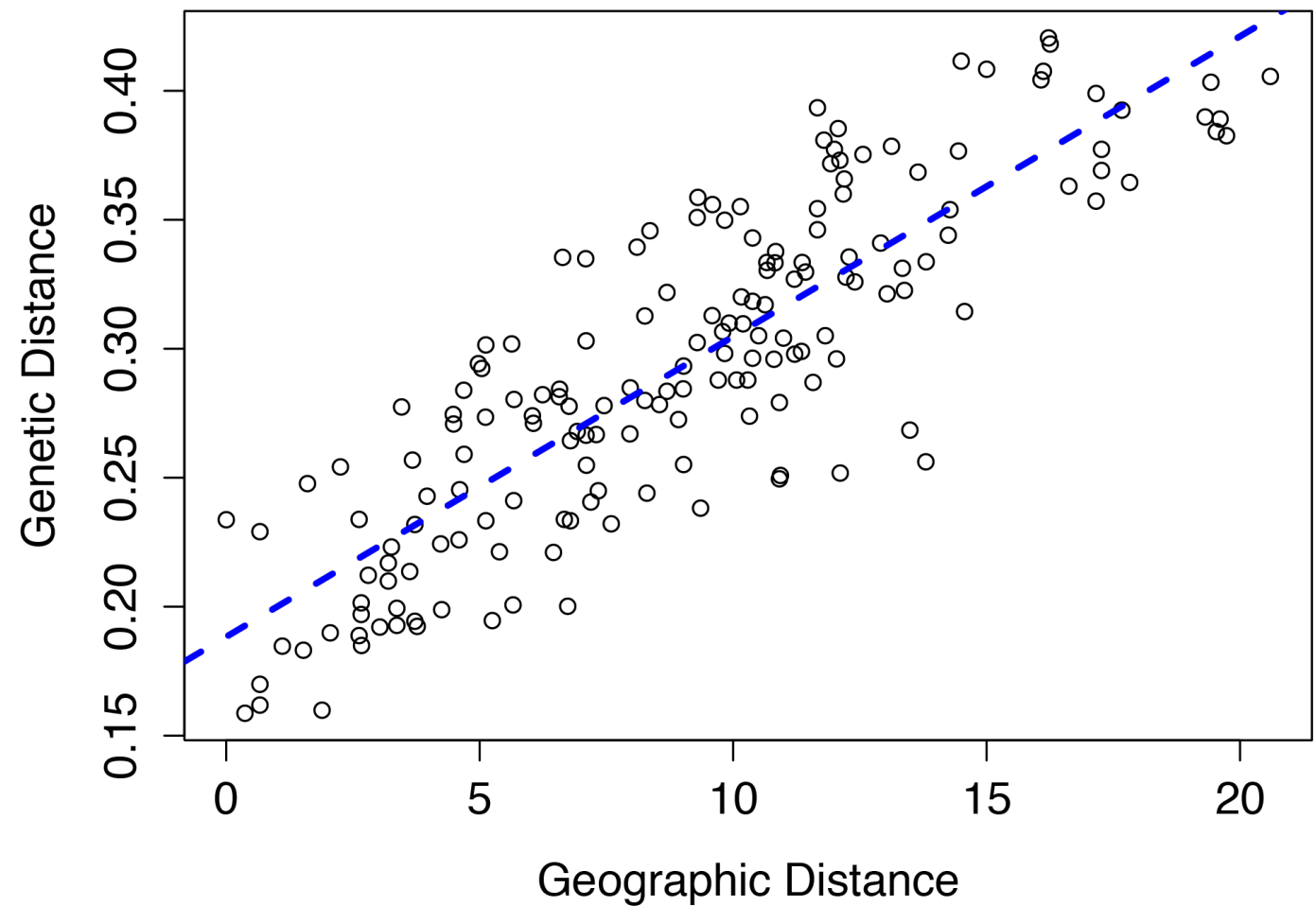


We used genotyping-by-sequencing (GBS) to genotype 175 *E. cooki* individuals at 3125 SNP loci throughout their entire genome. We then performed multivariate analyses to identify genetic clusters using the variation in allele frequencies across all 3125 SNP loci. Discriminant analyses revealed that the 19 populations of *E. cooki* are divided into five genetic clusters.

Isolation by distance only in North-eastern habitats

We found that gene flow was limited to very short distances, which led to increased genetic differentiation as the geographic distance became greater.

The largest geographic distance is between Mariana and Casas de la Selva (20.6 km), and it corresponds with individuals showing the highest genetic distances.



Increase awareness among landowners and students

¿Conoces al Coquí Guajón?

www.coquiguajon.org



Hábitat	Distribución	Importancia	Amenazas
 <p>¿Dónde vive? Vive exclusivamente en terrenos sombreados y húmedos, en zonas de bosque primario y secundario. En las zonas urbanizadas, se encuentra en áreas verdes y parques.</p> <p>¿Dónde se encuentra? El Coquí Guajón se encuentra en las zonas de bosque primario y secundario, en las zonas urbanizadas, en las zonas verdes y parques.</p>	 <p>¿Dónde se encuentra? El Coquí Guajón se encuentra en las zonas de bosque primario y secundario, en las zonas urbanizadas, en las zonas verdes y parques.</p>	 <p>¿Por qué es importante? El Coquí Guajón es una especie endémica de Puerto Rico, lo que significa que solo se encuentra en esta isla. Es una especie vulnerable y su conservación es importante para la biodiversidad de Puerto Rico.</p>	 <p>¿Cuáles son sus amenazas? Las principales amenazas del Coquí Guajón son la pérdida de su hábitat natural, la contaminación y la introducción de especies invasoras.</p>
 <p>Coquí Guajón (<i>Eleutherodactylus cooki</i>)</p>			
 <p>Protégelo</p> <p>¿Qué puedes hacer? 1. Evitar la destrucción de su hábitat. 2. Evitar la contaminación de su hábitat. 3. Evitar la introducción de especies invasoras.</p>	 <p>Conservación</p> <p>¿Cómo se está protegiendo? El Coquí Guajón está protegido por la Ley de Especies en Peligro de Extinción de Puerto Rico.</p>	 <p>Éducate</p> <p>¿Dónde puedes aprender más? En el sitio web www.coquiguajon.org.</p>	 <p>¡Ayudemos al Coquí Guajón!</p>



Increase awareness among landowners and students



Next steps

1. Genotype more individuals around cueva marcela and verraco.
2. Examine patterns of genetic differentiation from males vs. females.
3. Link phenotypes with genotypes to find resistant individuals.

Questions?

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