DENVER BOTANIC GARDENS

Genetic diversity analysis of *Eligmocarpus cynometroides*, an endangered priority species for conservation in the littoral forests of Madagascar
Final Report
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1. Background & Introduction

The littoral forest of Madagascar is a distinctive type of humid evergreen forest restricted to unconsolidated sand located within a few kilometers from the Indian Ocean (Lowry et al. 2008). The littoral forests, which once occupied much of the coastal fringe of eastern Madagascar and were contiguous with the dense humid lowland evergreen forests, now, persist only in small fragments (de Gouvenain and Silander 2003). The original size of this habitat was less than 1% of Madagascar's total surface area, and today exists in only about 10% of its original range (Moat and Smith 2007) with only about 1.5% of the remaining fragments included within the existing protected areas network (Consiglio et al. 2005). Even though the habitat range is very small, the littoral forests harbor about 13% of Madagascar‟s total native flora, of which 25% are endemic to this habitat (Moat and Smith 2007). The littoral region of southeastern Madagascar in Tolagnaro (Fort Dauphin) is dominated by the Vohimena Mountains and a rolling coastal plain extending several kilometers to the Indian Ocean (Vincelette et al. 2007a).

One of the most threatened ecosystems in Madagascar with less than 2,835 ha remaining, the littoral forests of the Tolagnaro region are expected to lose numerous plant and animal species in the near future as a result of deforestation and consequent habitat changes (Bollen and Donati 2006). The remaining littoral forests of southeastern Madagascar are under severe pressure from various threats from the local human population such as tavy (shifting slash and burn agriculture), bushfires as a result of the practice of tavy, and harvest of timber and non-timber forest products (e.g. charcoal for cooking, wood for construction) for both subsistence and commercial activities (Bollen and Donati 2006). The three main remaining groups of littoral forest fragments are located in Mandena, Petriky, and Sainte Luce with fragment sizes ranging from 1 to 377 ha (Bollen and Donati 2006). The most imminent threat to these forests is the plan to extract ilmenite by QIT Madagascar Minerals (QMM) (Bollen and Donati 2006).

QIT Madagascar Minerals (QMM), a company jointly owned by Rio Tinto, UK, and the Malagasy State represented by the Office des Mines Nationales et des Industries Strategiques de Madagascar (OMNIS) started an extensive exploration program in 1986 for heavy mineral sands containing titanium dioxide in the form of ilmenite and rutile along the eastern coast of Madagascar (Vincelette et al. 2007b). Major sediments were located underneath the littoral forests in Mandena, Sainte Luce, and Petriky (Lowry et al. 2008). Over the following 20 years, before the start of mining activities in 2009, QMM launched an extensive biodiversity assessment project addressing the potential impact of mining on economic, technical, and

cultural issues with ramifications for environmental conservation (Vincelette et al. 2007b). Mining activities started in Mandena in 2009 and is scheduled to start in Petriky and Sainte Luce 20-45 years later, lasting up to 60 years (Bollen and Donati 2006). The impact of these activities would result in the loss of littoral forests in Mandena, Sainte Luce, and Petriky at 62.8 ha, 661.8 ha, and 705.8 ha, respectively (Bollen and Donati 2006). To mitigate this loss, the environmental impact assessment conducted by QMM has led to the establishment of tree nurseries and plantations, seed banks, and extensive research into reforestation (Bollen and Donati 2006).

Eligmocarpus cynometroides Capuron is a tree species endemic to the littoral forests of southeastern Madagascar, where it grows in the narrow, transitional area between the humid and sub-arid bioclimatic zones. It is classified as critically endangered (CR) due to habitat loss, selective harvesting, and mining activities. It is documented as occuring within an area of only 77 km^2 in only two subpopulations, neither of which are within protected areas (Randriatafika et al. 2007). Randriatafika et al. (2007) documented 27 known individual plants in the littoral forests of Petriky with a concern that they may become extinct if rescue strategies are not developed soon. Since documenting these 27 trees, they report that four trees were felled in 2003 for timber, leaving only 23 trees. These 23 trees occupy an area of only 0.01 km^2. Randriatafika et al. (2007) mention distribution of this species in a second subpopulation at Parcel 3 of the Parc National d'Andohahela, though Lowry et al. (2008) report this population as extinct. This species is a QMM priority species for conservation. The purpose of this project is to develop conservation strategies for long term conservation of this species by ensuring that the entire genetic diversity of the existing populations is preserved.

The specific research objectives of this project were to:

1. Understand the genetic diversity of the existing *in situ* and *ex situ* populations of *Eligmocarpus cynometroides*.
2. Develop propagation protocols to enhance nursery production.
3. Develop reintroduction strategies.

2. Materials and Methods

Plant material

Travel to Madagascar to collect leaf samples for genetics work was done Feb. 27 – March 9, 2013. Collecting permits from Malagasy Forestry department were acquired with assistance from Missouri Botanical Garden, Madagascar. In addition to collection of leaf and herbarium samples, permission to collect seeds was also provided from the Directorate General of Forest Malagasy (Permit Number: 048/13/MEF/SG/DGF/DCB.SAP/SCB). Collecting in the littoral forests of Petriky and Mandena in South Eastern Madagascar was performed in collaboration with Qit Madagascar Minerals (QMM). Figure 1 shows the littoral forest habitat in Petriky.

Figure 1: Littoral forest habitat in Petriky

Appendix 1 gives the list of collections made. Table 1 lists a summary of populations sampled for genetic diversity analysis. Location coordinates were recorded using WGS 84 map datum using a Magellan Meridian Color Handheld GPS. Several leaves of each individual plant were collected and placed in a plastic bag with silica gel. Leaf samples were collected from seedlings growing in the QMM nursery in Mandena (Figure 2). Leaves from forest trees were collected in Manambaro village in Pertriky. Forest trees were divided into two populations, those east of the village and west of the village respectively. Voucher specimens of selected samples were collected in replicates of three, one each for Denver Botanic Gardens (KHD), Parc Botanique et Zoologique de Tsimbazaza (TAN), and Missouri Botanical Garden (MO). Select trees had fallen fruit pods at the base of the trees (Figures 3 and 4). Pods were shaken at random to confirm the presence of seeds in the pods. A majority of the fallen pods did not make the sound of seeds within the pod when shaken and were discarded. Only those that made sound were collected. Additionally, seeds from the QMM seed bank were also brought back for germination testing (Figure 5).

Table 1: Summary of *Eligmocarpus cynometroides* populations sampled

Population Name	**Collection Numbers***	**# Samples**
Pop 1 - QMM seedlings	SK 723 – SK 729	7
Pop 2 – Trees east of village	SK 730; SK 732 – SK 739	9
Pop 3 – Trees west of village	SK 740 – SK 741	2

*Detailed collections list with location coordinates and herbarium and seed sample information is provided in Appendix 1.

Figure 2: Sampling of seedlings grown at QMM nursery in Mandena

Figure 3: Mature *Eligmocarpus cynometroides* tree with fruit pods in the littoral forest in Petriky

Figure 4: Fallen fruit pods at the base of an *Eligmocarpus cynometroides* tree

Figure 5: Fruit pods at the QMM Seed Bank collection, seeds of which were used in germination testing

Exportation permits to bring the samples from Madagascar was acquired through Missouri Botanical Garden (permit numbers: 088N-EV04/MG13 and 144N-EV07/MG13). USDA APHIS requires separate permits to import seeds and leaf/herbarium samples. The seed importation permit was not issued until late April, 2013. Since leaves/herbarium and seeds cannot be shipped together, the leaves/herbarium were shipped and received at Denver in May, 2013. The seeds were shipped and received in August, 2013.

DNA extraction and ISSR amplification

Genomic DNA was extracted using GenCatch™ Plant Genomic DNA purification kit (Epoch Biolabs) in the Conservation Genetics lab at Denver Botanic Gardens. Slight modifications were made to the extraction protocols.

Initially, 30 ISSR primers were selected based on general success we have had in our lab. After testing for amplifications, 20 were discarded. The remaining 10 primers with their optimal PCR reaction conditions are listed in Table 2.

PCR reactions were carried out in a total volume of 17 µl containing 1 µl of genomic DNA, 1.7 µl of 10x PCR reaction buffer, 1.36 µl of 10mM dNTPs, and $MgCl_2$, primer, BSA, and Taq DNA Polymerase concentrations as listed in Table 2. Amplification was performed in an Eppendorf Mastercycler proS (Hamberg, Germany) under the following conditions: initial denaturation at 94°C for 5 min followed by 35 cycles of denaturation at 94°C for 30 s; primer annealing for 90 s at varying annealing temperatures (48.1ºC – 58ºC) for each primer as

noted in Table 2; extension at 72°C for 90 s; and final extension at 72°C for 8 min. PCR products were analyzed by electrophoresis on 1.5% agarose gel for 100 minutes at 70mA containing 4 µl of EZ-Vision dye in 1X TAE buffer. The PCR products were visualized using the UV transilluminator (Syngene InGenius Bioimager) and documented using the GeneSnap software program. Figure 6 shows an example of a gel image.

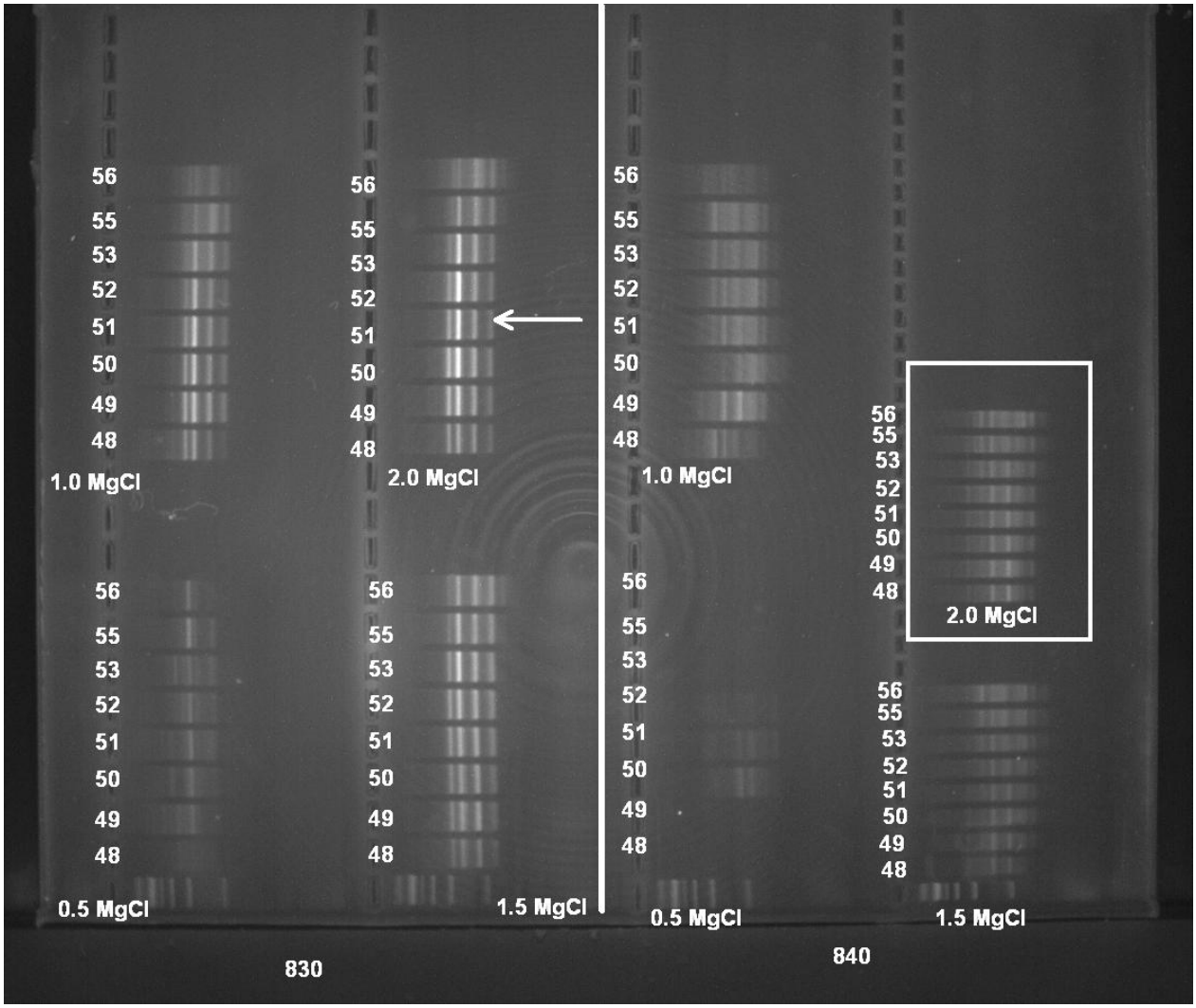
Table 2: Primers used in ISSR analysis with their sequence information, annealing temperatures, $MgCl_2$ concentrations and reagent combinations.

Primer	Primer Sequence (5' – 3')	Annealing Temp. (°C)	$MgCl_2$ Conc. (µl)	Reagent Combo*
UBC807	(AG)8T	58.0	2.0	Combo 1
UBC810	(GA)8T	52.0	1.5	Combo 1
UBC811	(GA)8C	54.7	1.5	Combo 3
UBC812	(GA)8A	55.7	1.5	Combo 4
UBC817	(CA)8A	56.0	2.0	Combo 1
UBC819	(GT)8A	53.0	1.0	Combo 1
UBC823	(TC)8C	50.0	2.0	Combo 1
UBC830	(TG)8G	51.0	2.0	Combo 1
UBC840	(GA)8YT	48.1	2.0	Combo 4
UBC841	(GA)8YC	50.1	2.0	Combo 2

***Reagent Combinations:**

	Combo 1 (µl)	**Combo 2 (µl)**	**Combo 3 (µl)**	**Combo 4 (µl)**
Primer	0.7	0.8	0.9	1
BSA	0.8	0.7	0.6	0.5
Taq	0.135	0.14	0.145	0.17

Figure 6: A gel representing optimization experiments of two primers (UBC830 and UBC840) to identify the best combination of annealing temperature and reagent mix combinations.



Data analysis

The ISSR products were scored for the presence (1) and absence (0) of homologous DNA bands using GelCompar II (Applied Maths). Gene diversity (H) and Shannon's information index (I) (Shannon and Weaver 1949) were computed using the software POPGENE 1.32 (Yeh et al. 1997). The Nei's estimate of gene diversity and Shannon's information index were computed assuming Hardy-Weinberg disequilibrium with fixation indices of 0.95. Hierarchical genetic structure was examined through an analysis of molecurlar variance (AMOVA) (Excoffier et al. 1992) with 999 permutations using GenAlEx V.6.501 (Peakall and Smouse 2006). AMOVA was applied to estimate the components of variance among and within populations based on PhiPT (which is analogous to F_{ST}) for each population. GenAlEx was also used to calculate Nei's genetic distance (Nei 1978) between the three populations to estimate genetic distance among them. Structure ver. 2.2 (Pritchard et al. 2000), a probabilistic based-clustering method for multi-locus genotype data, was used to investigate population structure. Parameters included admixture model with allele frequencies correlated among populations and each run with 50,000 MCMC repetitions after 50,000 burn-in. The determination of the best *K* value was considered as the modal value of ΔK, an ad hoc quantity as proposed by Evanno et al. (2005). Structure Harvester was used to determine *K*=2.

Seed germination testing

Three different treatments were performed to see which one gives best germination:

- Control - Scarification pretreatment done by soaking the seeds for 4 hours in water
- Treatment 1 – Scarification pretreatment done by nicking the seeds with clippers
- Treatment 2 - Scarification pretreatment done by nicking the seeds with clippers and soaking for 4 hours in water

There were a total of 18 seeds, with 6 seeds per treatment. The seeds were sown in a germination tray containing a germination mix. The germination mix consisted on one part peat + vermiculite + perlite and one part sand. Seeds were sown on November 10, 2013 and the tray was placed on bottom heat mats at a soil temperature of 74°F (23.33°C).

3. Results and Discussion

Of the 18 total samples, one of the samples (SK 739) gave poor amplification across all primers and so this sample was dropped from the study. The 10 primers produced a total of 194 loci, of which, loci with less than four occurrences across all samples were dropped, leaving 128 scorable bands. Of these, 119 bands (92.97%) were polymorphic across the 17 samples from three populations (Table 3). The total number of bands ranged from five for UBC819 to 17 for UBC807 and UBC810. The polymorphism index ranged from 53.85% for UBC811 to 100% for UBC807, UBC812, UBC823, UBC830, UBC840 and UBC841 (Table 3).

Table 3: ISSR polymorphism exhibited for *Eligmocarpus cynometroides*.

Primer	No. of bands	No. of polymorphic bands	Polymorphism index (%)
UBC807	17	17	100.00
UBC810	17	16	94.12
UBC811	13	7	53.85
UBC812	14	14	100.00
UBC817	13	12	92.31
UBC819	5	4	80.00
UBC823	9	9	100.00
UBC830	14	14	100.00
UBC840	16	16	100.00
UBC841	10	10	100.00
Total	**128**	**119**	**92.97**

Table 4 gives a summary of the measures of genetic diversity for each population as measured by the percentage of polymorphic loci, Shannon's information index (I) and Nei's gene diversity (H).

Table 4: Measures of genetic diversity in each population of *Eligmocarpus cynometroides*.

Population	No. of individuals	% polymorphic loci	Shannon's Index (I)	Nei's Gene Diversity (H)
Seedlings	7	79.69	0.48	0.33
East	8	82.03	0.45	0.31
West	2	43.75	0.27	0.18
Mean	**17**	**68.49**	**0.40**	**0.27**

The genetic diversity of the QMM seedlings was higher than both the *in-situ* trees growing East and West of the village with a higher Shannon's information index of 0.48 and Nei's gene diversity of 0.33. The East population had higher genetic diversity compared to the West population with Shannon's Index of 0.45 and Nei's Gene Diversity of 0.31. These same indices were 0.27 and 0.18 respectively for the West population. This lower genetic diversity of the West population could be due to the small sample size of only two individuals in this population. Overall the extant populations of *Eligmocarpus cynometroides* exhibit high genetic diversity with a mean Shannon's Index of 0.40 and Nei's gene diversity of 0.27.

To understand the genetic partitioning among the three populations, Analysis of Molecular Variation (AMOVA) was performed, which is presented in Table 5. Ninety one percent of the variation was within population and 9% among populations. AMOVA was performed again to compare the genetic differentiation between the *ex situ* seedling population and the *in situ* forest population, which is presented in Table 6. The among-population variation was higher at 11% between the *ex situ* and *in situ* populations. The variation within population was 89%.

Table 5: Summary of partitioning of genetic variation among three populations of *Eligmocarpus cynometroides* using AMOVA

Source of variation	***df***	**Sum of squares**	**Est. Variance**	**Percentage of variation**
Among populations	2	68.56	2.18	9
Within populations	14	325.32	23.38	91
Total	16	393.882	25.42	100

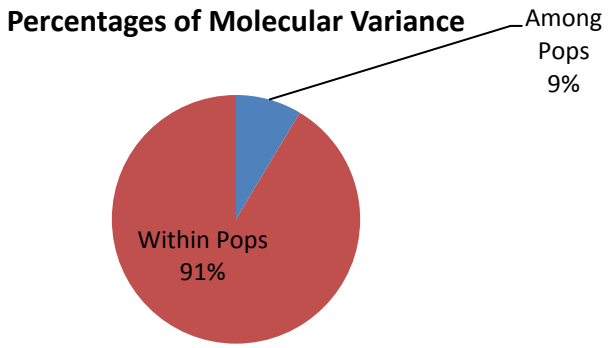


Table 6: Summary of partitioning of genetic variation among the *ex situ* and *in situ* populations of *Eligmocarpus cynometroides* using AMOVA

Source of variation	***df***	**Sum of squares**	**Est. Variance**	**Percentage of variation**
Among populations	1	46.11	2.78	11
Within populations	15	347.77	23.19	89
Total	16	393.88	25.97	100

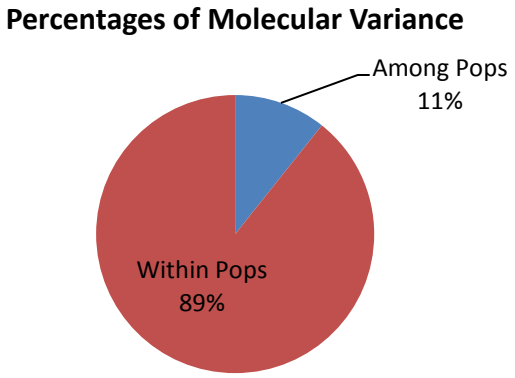


Table 7 gives the Nei's Genetic Distance, showing the pairwise matrix of genetic distance between the three populations. The seedling population (Pop 1) was more distant from the West population (Pop 3) with a Nei's genetic distance of 0.26 compared to 0.12 between the seedling and East populations (Pop 2). The Nei's genetic distance between the East and West populations was 0.17 suggesting some differentiation among these two *in situ* populations.

Table 7: Pairwise population matrix of Nei's Genetic Distance among populations

	Pop 1	**Pop 2**	**Pop 3**
Pop 1	0.0000		
Pop 2	0.1207	0.0000	
Pop 3	0.2627	0.1739	0.0000

Pop 1 – Seedlings
Pop 2 – East of village
Pop 3 – West of village

Figure 7 gives an analysis of the genotypes of the three populations using Structure. Bar plots representing the genotypes are given on the X-axis, distributed in predefined populations and colored according to their membership of the *K*-dependent clustering. For each set analyzed, the probability membership for each genotype in each *K*-cluster is given on the Y-axis.

Individuals with multiple colors have admixed genotypes. The mean likelihood for ancestral inference for these populations was $\Delta K = 46.01$. The seedlings are genotypically different from both the East and West populations corresponding with the results indicated by the Nei's genetic distance testing and AMOVA.

Figure 7: Analysis of *Eligmocarpus cynometroides* genotypes using Structure.

Seed germination testing resulted in one seedling (5.56%). The results of the germination test are listed in Table 8. Germination of this one seedling was recorded during the week of February 17, 2014 (14 weeks after sowing) and the seedling was transplanted to a larger pot the week of March 3, 2014 (Figure 8). Treatment 2 whereby the seeds are scarified by nicking the seeds with clippers, followed by soaking in water for four hours prior to sowing seems to provide the best germination results. Seeds that remain firm and viable (38.89%) will continue to be retained in the greenhouses and monitored frequently for germination.

Table 8: Germination test results.

Treatment	# Seeds Germinated	# Seeds Firm & Viable	# Seeds Rotted
Control	0	3	3
Treatment 1	0	0	6
Treatment 2	1	4	1

Figure 8: The germinated seedling that was treated to Treatment 2 (Scarification pretreatment done by nicking the seeds with clippers and soaking for 4 hours in water)

4. Conservation Implications

Based on the results, the following conclusions can be reached:

i. The remaining extant populations and seedlings of *Eligmocarpus cynometroides* retain high genetic diversity as indicated through 10 ISSR markers.

ii. The seedlings being grown at the QMM nursery exhibit higher genetic diversity compared to the East and West forest populations using the genetic diversity parameters of Shannon's Index and Nei's Gene Diversity.

iii. The East population has higher genetic diversity compared to the West population as indicated by the genetic diversity parameters of Shannon's Index and Nei's Gene Diversity. This could possibly be due to small sample size of the West population.

iv. Partitioning of genetic variation was 91% within populations and 9% among populations when all three populations were compared.

v. When the *ex situ* seedling population was compared with the *in situ* forest populations (East and West combined), the partitioning of genetic variation was higher among population at 11% and within population variation of 89%.

vi. The Nei's Genetic Distance was greater between the seedling population and the East population.
vii. There was a moderate amount of genetic differentiation between the East and West populations as indicated by a Nei's Genetic Distance of 0.17.
viii. Structure analysis shows the genotypic difference of the seedling population from the East and West populations.
ix. Seed germination was poor (5.6%). The only successful germination was achieved by scarifying the seeds by nicking them with clippers, followed by soaking in water for four hours prior to sowing.

Based on these results, it may be concluded that the current seedlings grown at the QMM nursery probably came from seeds from trees that are currently extinct in the wild or not sampled during this study. Every effort should be made to conserve these seedlings and use them in restoration and future propagation programs. The East and West *in situ* populations were well differentiated and so every effort should be made to conserve the existing extant trees in the East and West locations and developing propagation programs to increase the production of saplings for restoration programs. A genetic distance of 0.17 between the East and West populations indicate a need to conserve these two populations as separate entities. Studies on propagation by cuttings and micropropagation should be undertaken.

The 2007 survey of *Eligmocarpus cynometroides* (Randriatafika et al. 2007) documented 23 extant trees in the littoral forests of Petriky. During field work in 2013, we documented and collected from 11 trees in Petriky. One additional outlier tree was reported to be located southwest of our collection site, where we were unable to collect. Additionally a restoration site where saplings grown at the QMM nursery had been transplanted to was also not sampled. Analyzing the genetic diversity of these individuals will be important in documenting any additional genetic variation present within these individuals that is not present in those sampled.

Another subpopulation was reported in Parcel 3 of the Parc National d'Andohahela (Randriatafika et al. 2007), though specific population information is not available. Lowry et al. (2008) report this population as extinct. Attempts should be made to scout for presence of trees or seedlings at Parcel 3 of the Parc National d'Andohahela.

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Acknowledgements:
Thanks to Association of Zoological Horticulture for support of this project through their conservation grant. At QMM, key people that were instrumental in making this project happen were Johny Rabenantoandro, Faly Randriatafika, David Rabehevitra and Roger Ramison. Assistance of staff at Missouri Botanical Garden (MBG), Madagascar, was critical in acquiring the required permits. Thanks to Faranirina Lantoarisoa and Christian Camara of MBG. Thanks to Vololotahina Razafindrahaja for field work assistance. Finally thanks to Denver Botanic Gardens' Conservation Genetics team of Dr. Jenny Neale and Anna Schwabe for lab assistance and data review and to Michael Bone for greenhouse germination assistance.

Appendix 1: *Eligmocarpus cynometroides* Collections List

Eligmocarpus cynometroides

2013 Collections List

Collectors:	Sarada Krishnan (DBG)	**GPS Datum:**	WGS 84	**State:**	Toliara		
	David Rabehevitra (QMM)			**District:**	Tolagnaro		
	Roger Ramison (QMM)			**Area:**	Petriky		Mandena
	Vololotahina Razafindrahaja (PBZT)			**Locality:**	Manambaro		QMM Nursery

Coll. #	Plant ID	Population ID	Date of Collection	Lat / Long	Ht: (M)	DNA Sample	# Herb.	# Fruits	Notes
SK 723	*Eligmocarpus cynometroides*	QMM seedling	March 4, 2013	24°57'09S 47°00'11E	0.15	x			Seeds collected in Pertiky on Feb 1, 2011; planted on April 1, 2011. 16-20 leaves.
SK 724	*Eligmocarpus cynometroides*	QMM seedling	March 4, 2013	24°57'09S 47°00'11E	0.75	x			Seeds collected in Pertiky on Feb 1, 2011; planted on April 1, 2011. Largest seedling; numerous leaves; healthy.
SK 725	*Eligmocarpus cynometroides*	QMM seedling	March 4, 2013	24°57'09S 47°00'11E	0.30	x			Seeds collected in Pertiky on Feb 1, 2011; planted on April 1, 2011. Numerous leaves
SK 726	*Eligmocarpus cynometroides*	QMM seedling	March 4, 2013	24°57'09S 47°00'11E	0.30	x			Seeds collected in Pertiky on Feb 1, 2011; planted on April 1, 2011. Numerous leaves
SK 727	*Eligmocarpus cynometroides*	QMM seedling	March 4, 2013	24°57'09S 47°00'11E	0.15	x			Seeds collected in Pertiky on Feb 1, 2011; planted on April 1, 2011. Fewer leaves
SK 728	*Eligmocarpus cynometroides*	QMM seedling	March 4, 2013	24°57'09S 47°00'11E	0.15	x			Seeds collected in Pertiky on Feb 1, 2011; planted on April 1, 2011. Smallest seedling with very few leaves - only 7.
SK 729	*Eligmocarpus cynometroides*	QMM seedling	March 4, 2013	24°57'09S 47°00'11E	0.30	x			Seeds collected in Pertiky on Feb 1, 2011; planted on April 1, 2011. 12-15 leaves
SK 730	*Eligmocarpus cynometroides*	Pop 1 - E of village	March 5, 2013	24°03'10S 46°53'48E	19.0	x	3		Huge tree; next to path
SK 731	*Erythroxylum nitidulum*		March 5, 2013	24°03'10S 46°53'48E	1.80	x	3		Associated species; next to path; white flowers present; fruits present
SK 732	*Eligmocarpus cynometroides*	Pop 1 - E of village	March 5, 2013	24°03'13S 46°53'47E	3.00	x	3		Well branched. Healthy; next to *Coffea commersoniana*
SK 733	*Eligmocarpus cynometroides*	Pop 1 - E of village	March 5, 2013	24°03'13S 46°53'47E	4.00	x	3	3	A few feet from SK 732. Fruits present at the base of the tree; few fruits on tree. Collected only fruits that were rattling.
SK 734	*Eligmocarpus cynometroides*	Pop 1 - E of village	March 5, 2013	24°03'11S 46°53'50E	7.00	x			Well branched; fruits on tree and on the ground, though did not find any with seeds (rattling).
SK 735	*Eligmocarpus cynometroides*	Pop 1 - E of village	March 5, 2013	24°03'10S 46°53'44E	3.00	x			Next to the path and close to SK 730. Young leaves tinged red. Shooting from a cut tree; no fruits.
SK 736	*Eligmocarpus cynometroides*	Pop 1 - E of village	March 5, 2013	24°03'09S 46°53'44E	15.00	x			100 meters from the previous trees with fragmented forest in between.
SK 737	*Eligmocarpus cynometroides*	Pop 1 - E of village	March 5, 2013	24°03'14S 46°53'41E	2.50	x			Young tree
SK 738	*Eligmocarpus cynometroides*	Pop 1 - E of village	March 5, 2013	24°03'15S 46°53'42E	17.00	x			Huge tree; leaves present way up - collected with a long stick.
SK 739	*Eligmocarpus cynometroides*	Pop 1 - E of village	March 5, 2013	24°03'14S 46°53'41E	3.00	x			Sparsely leaved
SK 740	*Eligmocarpus cynometroides*	Pop 2 - W of village	March 5, 2013	25°03'25S 46°53'27E	8.00	x		1	Highly degraded habitat. Fruits at base of tree and on tree.
SK 741	*Eligmocarpus cynometroides*	Pop 2 - W of village	March 5, 2013	24°03'36S 46°53'19E	6.00	x	3	2	Highly degraded habitat. Fruits at base of tree and on tree.
QMM 1646	*Eligmocarpus cynometroides*	QMM seed collection	Sept 19, 2011					17	Fruits from QMM's seed collection. Collected from Petriky from various trees (mixed).

Habitat Description:

Littoral Forest. Sandy soil. Assosicated vegetation: *Rhopalocarpus coriaceus* (Sphaerosepalaceae), *Croton* sp. (Euphorbiaceae), *Strychnos* sp. (Loganiaceae), *Tricalysia cryptocalyx* (Rubiaceae), *Dracaena* sp. (Asparagaceae), *Garcinia* sp. (Clusiaceae), *Coffea commersoniana* (Rubiaceae), *Erythroxylum* sp. (Erythroxylaceae)