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Population genetics implications for the conservation of the Philippine Crocodile *Crocodylus mindorensis* Schmidt, 1935 (Crocodylia: Crocodylidae)

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Abstract: Limited information is available on the Philippine Crocodile, *Crocodylus mindorensis*, concerning levels of genetic diversity either relative to other crocodilian species or among populations of the species itself. With only two known extant populations of *C. mindorensis* remaining, potentially low levels of genetic diversity are a conservation concern. Here, we evaluated 619 putative Philippine Crocodiles using a suite of 11 microsatellite markers, and compared them to four other crocodilian species sample sets. The two remaining populations from the island of Luzon and the island of Mindanao, representing the extremes of the former species' distribution, appear to be differentiated as a result of genetic drift rather than selection. Both extant populations demonstrate lower genetic diversity and effective population sizes relative to other studied crocodilian species. The 57 *C. mindorensis* and *C. porosus*, Saltwater Crocodile, hybrids identified earlier from the Palawan Wildlife Rescue and Conservation Center were revalidated with a suite of 20 microsatellite loci; however, the timing of the event and the prevalence of hybridization in the species had yet to be fully determined. We defined the hybrids as one first cross from a *C. porosus* female and a *C. mindorensis* male and 56 *C. mindorensis* backcross individuals. This hybridization event appears to be confined to the PWRCC collection.

Keywords: *Crocodylus*, hybrid detection, microsatellites, Philippine crocodile, population genetics.

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INTRODUCTION

The application of genetics in conservation efforts has increased dramatically over the past decades. Molecular genetic methodology has been used to address taxonomic issues, assess genetic variability and inbreeding, track gene flow and detect hybridization, all in an effort to conserve genetically healthy populations and aid in the identification of ecologically significant units (Fleischer 1998). The use of nuclear DNA (nucDNA) and mitochondrial DNA (mtDNA) sequence data in crocodilian research has increased our understanding of genetic variability (Flint et al. 2000; Ray et al. 2004; Russello et al. 2007), hybridization (FitzSimmons et al. 2002; Ray et al. 2004; Cedeño-Vásquez et al. 2008), differences between individuals (Farias et al. 2004), populations (Vasconcelos et al. 2006, 2008) and species (Li et al. 2007; Gatesy & Amato 2008; Meganathan & Dubey 2009; Meganathan et al. 2010). Microsatellites have been used to investigate population structure and gene flow in wild populations of Morelet's Crocodile *Crocodylus moreletii* Duméril & Bibron, 1851 (Dever & Densmore 2001; Dever et al. 2002), American Alligator *Alligator mississippiensis* Daudin, 1802 (Glenn et al. 1998; Davis et al. 2002) and Black Caiman *Melanosuchus niger* Spix, 1825 (de Thoisy et al. 2006). Microsatellites have also been useful in parentage analysis in Saltwater Crocodiles *C. porosus* Schneider, 1801 (Isberg et al. 2004), in determining and maintaining genetic variability in crocodiles bred for the leather trade (Flint et al. 2000; FitzSimmons et al. 2002) and to build the scaffolding for a genetic linkage map (Miles et al. 2009a).

Limited information exists concerning the Philippine Crocodile, *C. mindorensis,* and its comparative status with other crocodilian species. The Philippine Crocodile is a species of special concern and has already been the focus of a breeding program for many years (Banks 2005). A combination of hunting for commercial exploitation, extirpation because of fear, overfishing of prey, habitat loss and habitat fragmentation have severely diminished the range of this species and reduced the remaining populations to critical levels (van Weerd & van der Ploeg 2003). Fifteen years ago, the wild populations were estimated to contain less than 100 mature individuals (Ross 1998). The most recent Crocodile Specialist Group (CSG) status update assesses the populations of *C. mindorensis* in the wild to consist of less than 250 adults (van Weerd 2010). As a result, the Philippine Crocodile is currently listed as Critically Endangered A1c, C2a in the IUCN Red List (Crocodile Specialist Group 1996).

Silliman University in Dumaguete City, Philippines, in 1980, initiated the first captive breeding of the Philippine Crocodile for conservation purposes. In 1987, the Department of Environment and Natural Resources (DENR), in a collaboration substantially funded by the Japanese International Cooperation Agency, established the Crocodile Farming Institute (CFI). The CFI is now known as the Palawan Wildlife Rescue and Conservation Center (PWRCC) in Puerto Princessa City, Philippines, and operates under the Protected Areas and Wildlife Bureau (PAWB). The purpose of the facility was to conserve the two species of crocodiles found in the Philippines, the Saltwater Crocodile and the Philippine Crocodile (Sumiller 2000; Banks 2005). Both Silliman University and PWRCC succeeded in breeding *C. mindorensis*, and many of the resulting captive-bred stock have been sent to zoos in the Philippines and other countries via breeding loan agreements (Banks 2005). However, PWRCC temporarily discontinued captive breeding in 2001 due to financial constraints, limited space and ambiguities in the captive stock pedigrees (Rebong & Sumiller 2003; Banks 2005).

Philippine Crocodile reintroductions into suitable habitats have been planned by the Philippine Crocodile National Recovery Team (PCNRT; Banks 2005). A successful in situ Philippine Crocodile conservation program is in progress in the San Mariano municipality in Isabela Province (van Weerd & van der Ploeg 2003; van der Ploeg et al. 2011a,b,c). The Mabuwaya Foundation began a headstart program in 2005 where wild-born Philippine Crocodiles were captured, captive raised (i.e., headstarted) then released after two years

Abbreviations: ABI - Applied Biosystems, Inc.; bp - base pairs; CFI - Crocodile Farming Institute; CI - confidence interval; CSG - IUCN/SSC Crocodile Specialist Group; DENR - Department of Environment and Natural Resources; DNA - deoxyribonucleic acid; F_{IS} - within population fixation index; F_{ST} - between population fixation index; *He* - expected heterozygosity; *Ho* - observed heterozygosity; *I* - Shannon Information index; IUCN - International Union for the Conservation of Nature; LD - linkage disequilibrium; MSA - Microsatellite Analyzer; mtDNA - mitochondrial DNA; N - census size; *N* - average number of individuals genotyped per locus; *Na* - mean number of alleles; *Ne* - effective population size; *Nea* - effective number of alleles; *Neb* - number of effective breeders; nucDNA - nuclear DNA; PAWB - Protected Areas and Wildlife Bureau; PCA - Principal Coordinates Analysis; PCNRT - Philippine Crocodile National Recovery Team; PCR - polymerase chain reaction; PWRCC - Palawan Wildlife Rescue and Conservation Center; SSC - Species Survival Commission; t*I* - transformed Shannon entropy index; t*He* - transformed expected heterozygosity index; t*Ho* - transformed observed heterozygosity index; tU*He* - transformed unbiased expected heterozygosity index; U*He* - unbiased expected heterozygosity; WGA - whole genome amplification

thus increasing juvenile survival rates (van de Ven et al. 2009). In 2010, 50 PWRCC captive-bred Philippine Crocodiles were released into a lake in the Divilacan municipality, geographically separated from the wild Isabela crocodile population. This release served as a pilot project to assess the adaptability of captive-bred Philippine Crocodiles under wild conditions (van Weerd & General 2003; van Weerd et al. 2010).

Recent systematics studies identified hybrids between *C. mindorensis* and *C. porosus* at PWRCC from the analyses of both mtDNA (D-loop and ND4) and nucDNA (C-*mos*) gene sequences (Louis & Brenneman 2008; Tabora et al. 2012). These studies validated previous concerns regarding reintroduction candidate purity, thus warranting forensic diagnoses prior to release. Using data generated from microsatellite loci derived from crocodilian genomes by Miles et al. (2009b,c) and this study, we address three questions regarding the Philippine Crocodile: (1) how does the genetic diversity in *C. mindorensis* compare to other crocodilian species, (2) what are the population genetic inferences of the two populations in the current range distribution, and (3) to what extent has hybridization occurred between *C. mindorensis* and *C. porosus*.

MATERIALS AND METHODS

Sample collection

Tissue samples were collected from a total of 619 Philippine Crocodiles from 1999–2009. Once crocodiles were safely restrained, scute samples were obtained by cleaning the area with 70% isopropyl alcohol and cutting with a scalpel/razor blade. The samples were stored in 1.8ml NUNC® tubes containing a room temperature tissue preservative (Seutin et al. 1991). The majority of the Philippine Crocodile samples were collected from the captive population maintained at the PWRCC; the rest from Davao City Crocodile Park on Mindanao, Calauit Game Refuge and Wildlife Sanctuary on Palawan, Valera Square Mini Zoo in the Abra Province, Silliman University in Dumaguete City and individuals exported to the Gladys Porter Zoo in Brownsville, TX. Tissue samples from wild *C. mindorensis* were collected from the two extant populations in the Philippines: the San Mariano region in Isabela Province on Luzon and from the Liguasan (Ligawasan, Liguwasan) Marsh on Mindanao. These are two regions of the Philippine archipelago where indigenous cultural traditions offered some degree of protection to the Philippine Crocodile (van der Ploeg & van Weerd 2004; Mangansakan 2008; Pimentel et al. 2008). A single wild sample was collected on Dalupiri Island in the province of Cagayan north of Luzon. A list of the study areas, site descriptions and number of crocodiles sampled from each location are described in Tabora et al. (2012). Samples from *C. niloticus* Laurenti, 1768 (n = 12), *C. acutus* Cuvier, 1807 (n = 11), *C. siamensis* Schneider, 1801 (n = 12) and *C. porosus* (n = 37) were obtained from the Yale Peabody Museum of Natural History collection and from the St. Augustine Crocodile Farm for comparison to *C. mindorensis*.

DNA extraction

Genomic DNA from the great majority of the tissue samples was extracted and amplified using a whole genome amplification kit (WGA; Illustra TempliPhi®, GE Healthcare, Piscataway, NJ). The WGA yielded an average of 500ng of DNA per µL and all products were diluted to 50ng/µL. DNA from the remaining *C. mindorensis* tissue samples were extracted using a standard phenol/chloroform/isoamyl alcohol extraction method as described in Sambrook et al. (1989).

Microsatellite amplification

A subset of the sampled species was screened with an initial 31 microsatellite loci (Miles et al. 2009b,c) discovered in the *C. porosus* genome. A locus was eliminated from the comparative study if it failed to amplify in any one species or was monomorphic in at

Table 1. Primer sequences (5' to 3') with dye label, optimized annealing temperatures and microsatellite locus information including observed number of alleles detected (k), and size range in 527 *C. mindorensis*.

Locus	Primer Sequence	Repeat motif	Annealing Temp (ºC)	k	Size range	Gen Bank accession No.
4HDZ27	F: ^{HEX}GCACACATTCTCTGAGTAAAAAACC R: GGCACTGGTAGGCTTTGAAAT	$(CA)_{17}$	64	6	147–163	GU812903
4HDZ35	F: ^{FAM}GACAGTGTGGIGGGTGC R:TGCTGGCTGCTTGGGAC	$(CA)_8CG(CA)_{14}$	62	3	193–199	GU812904
4HDZ391	F: ^{FAM}ATGAGTCAGGTGGCAGGTTC R: CATAAATACACTTTTGAGCAGCAG	$(GT)_{12}$	60	4	133–143	GU812905

least two species. Microsatellite loci 4HDZ27, 4HDZ35 and 4HDZ391 were discovered in the *C. mindorensis* genome following the general protocol of Moraga-Amador et al. (2001) at Omaha's Henry Doorly Zoo and Aquarium's Center for Conservation and Research (Table 1).

PCR amplifications were performed in MBA Satellite 0.2G thermal cyclers (Thermo Fisher Scientific, Inc., Waltham, MA) in final reaction volumes of 25µL and containing 20–50 ng of DNA template. Final amplification conditions consisted of 12.5 pmol unlabeled reverse primer, 12.5 pmol fluorescently labeled forward primer, 1.5 mM $MgCl_2$, 200 µM each dNTP, and 0.5 units of *Taq* DNA polymerase (Promega; Madison, WI). One of two PCR thermal cycling methods were used depending on the microsatellite locus amplified. Stratified touchdown programs were used for three loci: TD55 for CpP4116 and TD65 for CpP302 and CpP2516 as described in Miles et al. (2009b). Standard PCR profile parameters for all other markers used in this study were: 34 cycles of 95^{0}C for 30s, a primer-specific annealing temperature for 45s, and 72^{0}C for 45s, and a final extension step of 72^{0}C for 10 min. Optimum annealing temperatures were determined as follows: 58°C for CpP305, CpP801 and CpP4004; 60^{0}C for CpP1708, CpP3008 and 4HDZ391; 62^{0}C for 4HDZ35; and 64^{0}C for 4HDZ27. PCR products were visualized to verify amplification on 2% agarose gels stained with ethidium bromide. For the comparison between *C. mindorensis* and *C. porosus* and hybridization analysis CpP305, CpP1708, CpP2516, CpP3008, CpP4004 and CpP4116 were amplified with the above standard conditions. An additional 12 loci were found to be informative for these analyses. The stratified touchdown programs TD55 for CpP3313 and CpP4301 and TD65 for CpP4311 were used as described in Miles et al. (2009b). The following loci were amplified with standard PCR as described above at the following annealing temperatures: 56^{0}C for CpP208 and CpP1610; 58^{0}C for CpP80 and CpP3601; 60^{0}C for CpP405, CpP1002 and CpP3220; and 62^{0}C for CpP203 and CpP610. Allele sizes were determined by separation of the PCR products via POP 4 capillary buffer electrophoresed on ABI 3100/ ABI 3130*xl* Genetic Analyzers (Applied Biosystems, Inc., Foster City, CA). Fragment length genotypes were assigned by GeneScan using GeneScan™ 500XL ROX™ size standard in the GeneMapper software version 4.0.

Data analysis

MICRO-CHECKER (Van Oosterhaut et al. 2004) and Microsatellite Analyzer (MSA; Dieringer & Schlötterer 2003) were used to analyze the data set for genotyping and typographical errors. Null allele frequencies were estimated using CERVUS 2.0 (Marshall et al. 1998; Slate et al. 2000). Excessive frequencies of null alleles can bias the data interpretation by either overestimating homozygosity or underestimating heterozygosity (Callen et al. 1993; Hoffman & Amos 2005). Loci with high null allele frequency estimates (*nf*>0.2) were removed from further analysis (Chapuis & Estoup 2007). The population genetic parameters: observed (*Ho*), expected (*He*), and unbiased expected heterozygosity (U*He*), mean number of alleles (*Na*), effective number of alleles (*Ne*), Shannon Information index (*I*; Shannon 1948), and the within population fixation index (F_{IS}) were estimated using GenAlEx 6.41 (Peakall & Smouse 2006). The Shannon entropy index was transformed by Diversity of Order 1 = exponential of *I* (Jost 2009). Heterozygosity estimates were transformed by Diversity of Order 2 = 1/ (1-*He*) (Jost 2008). The between population fixation index (F_{ST}) with significance was estimated with FSTAT 4.3 (Goudet 1995, 2001). For intraspecific diversity study, we neglected the captive populations because (1) the collections do not represent true populations; (2) the sample sizes for most were too small; and (3) hybrids had been previously discovered in PWRCC and thus we expect that *C. porosus* alleles would be present in the population inflating estimates reflecting intraspecific genetic diversity.

Effective population sizes were estimated with the linkage disequilibrium (LD) method using LDNe 1.31 (Waples & Do 2008) that corrects for small sample sizes bias (Waples 2006), an advantage over NeEstimator (Peel et al. 2004). The LD method is grounded on the principal that the loss of genetic variation is intensified by an increase in linkage disequilibrium. Testing allelic associations among multiple loci allows inbreeding estimation in the effective population size. Waples & Do (2008) determined that estimates of effective population size may become slightly less accurate but more precise as alleles with lower allele frequencies are included in the estimation. LDNe estimates effective population sizes excluding allele frequencies below the critical values of 0.05, 0.02, and 0.01 to assess the effects of rare alleles in the data. The ratio of the effective population size to the census size (*Ne*/N) can be used to predict inbreeding and genetic variation loss in wildlife populations (Frankham 1995).

Since it is possible that the two extant *C. mindorensis* populations, being from the northern and southern extremes of the distribution, might exhibit detectable selection, we tested for selection using both Lositran (Beaumont & Nichols 1996; Antao et al. 2008)

and BayeScan 2.0 (Foll & Gaggiotti 2008). Lositran implements an F_{ST} outlier method to identify loci likely under selection whereas BayeScan employs a maximum likelihood posterior probability. Relevance of the BayeScan posterior probabilities were interpreted with Jeffreys' scale of evidence (Jeffreys 1961). Considering that the extant populations are small, all within-population dyads were tested for relatedness (Queller & Goodnight 1989) using SPAGeDi (Hardy & Vekemans 2002) and compared to a simulation of 10,000 individuals of known pedigree relationships (Queller & Goodnight 1989).

Crocodylus porosus x *C. mindorensis* hybridization was identified in Tabora et al. (2012) where 57 captive crocodiles expected to be *C. mindorensis* by breeding records had inherited mtDNA haplotypes and nucDNA C-*mos* diagnostic sites found in *C. porosus*. We examined the microsatellite loci screened for the species diversity comparison to identify markers that would be informative in comparing the two species of crocodiles found in the Philippines. Eight additional loci found to be monomorphic in *C. mindorensis* and polymorphic in *C. porosus* for diagnostic alleles not present in the genotype data of *C. mindorensis* populations and collections exclusive of PWRCC (CpP2516, CpP208, CpP405, CpP610, CpP1002, CpP3601, CpP4301, and CpP4311) were included to test for evidence of hybridization. We generated multilocus data on 619 *C. mindorensis* from both wild populations and the captive collections comprising a great majority of the freshwater crocodiles in the Philippines and 37 *C. porosus* from samples collected in Republic of Palau (RP) by Russello et al. (2007).

Population structure was inferred using STRUCTURE v2.1 (Pritchard et al. 2000; Falush et al. 2003) to determine the differentiation between the northern and southern *C. mindorensis* populations and to test for potential hybridization in the populations with *C. porosus*. The program uses a Bayesian clustering based method to determine whether the two extant populations could be identified by genetic clustering and to determine if populations harboring allelic structure demonstrated genetic admixture of the parental species clusters. STRUCTURE attempts to identify population subsets that maximize Hardy Weinberg expectations and minimize LD from multilocus genotypes (Pritchard et al. 2000). We chose the ancestry model, correlated allele frequencies, different F_{ST} values assumed for each subpopulation, a uniform prior for alpha (max: 10, SD for updating: 0.025), constant lambda value of 1, prior F_{ST} mean (0.01) and standard deviation (0.05). We set the range to consider 1−11 genetic clusters as Evanno et al. (2005) suggests estimating over a range of at least three clusters more than sampling locations. The burnin period was set at 10^5 repetitions followed by 10^6 MCMC repetitions for 20 iterations of the Gibbs sampler for each K value. Occasionally STRUCTURE overestimates the optimal *K* value; hence, Evanno et al. (2005) developed an *ad hoc* test statistic ΔK to evaluate the output files in addition to approximating the asymptote of the posterior probability curve. At *K*-max, we applied a conservative threshold of $q \geq 0.05$ to the membership coefficient (*q*-value) of the cluster attributed to the introgressing species to identify hybrids (Hapke et al. 2011).

In addition, we used the Principal Coordinates Analysis (PCoA) in GenAlEx v6.41 to detect shifts in multilocus genotype groupings that might indicate individual affinity drifting away from expected parental groups. We charted the first two axes of inertia using genetic distance as the criteria with the covariance standardized method of calculation.

RESULTS

Eleven informative microsatellite loci amplified and were used to generate the data set from the two wild-sampled *C. mindorensis* populations and the samples of *C. acutus*, *C. niloticus*, *C. porosus* and *C. siamensis*. The average number of alleles ranged from 3.7 in the *C. mindorensis* samples from the population of Liguasan Marsh to six in *C. niloticus*. The number of effective alleles ranged from 2.159 in the *C. mindorensis* of Isabela to 3.847 in *C. niloticus*. The observed heterozygosity ranged from 0.408 in samples from the Isabela population to 0.630 in *C. porosus* and expected heterozygosity ranged from 0.423 in the Isabela population to 0.663 in *C. niloticus* (Table 2). Regardless of the estimate or index, the two extant *C. mindorensis* populations ranked lowest in genetic diversity compared to the sample collections of *C. acutus*, *C. niloticus*, *C. porosus* and *C. siamensis*. *F*-statistics measuring within population fixation or inbreeding (F_{IS}) ranged from -0.149 to 0.160 but were not significant. Population fixation indices (F_{ST}) and their significances are presented in Table 3.

Twenty loci were found to be informative for intraspecific evaluation and to compare *C. mindorensis* with *C. porosus*. Analysis of the estimated effective population sizes of the Isabela and Liguasan Marsh populations showed that those populations have much lower effective population sizes than the population of

Table 2. Average number of individuals genotyped per locus (*N*), average number of alleles per locus (*Na*), number of effective alleles (*Nea*), Shannon entropy index (*I*), observed heterozygosity (*Ho*), expected heterozygosity (*He*), unbiased expected heterozygosities (*UHe*), within population fixation index (F_{IS}), the transformed Shannon entropy index, observed heterozygosity, expected and unbiased expected heterozygosities into an index of genetic diversity (t*I*, t*Ho*, t*He* and t*UHe*, respectively) for the two extant populations of *C. mindorensis* (Isabela and Liguasan), *C. niloticus*, *C. siamensis*, *C. acutus*, and *C. porosus* derived from genotype data generated from a suite of 11 microsatellite loci.

Population		*N*	*Na*	*Nea*	*I*	*Ho*	*He*	*UHe*	F_{IS}	t*I*	t*Ho*	t*He*	t*UHe*
Isabela	Mean	84.000	3.900	2.159	0.751	0.408	0.423	0.425	0.055	2.119	1.689	1.739	1.739
	SE	0.558	0.900	0.384	0.170	0.101	0.082	0.083	0.129				
Liguasan	Mean	14.000	3.700	2.317	0.841	0.457	0.446	0.462	-0.004	2.319	1.842	1.805	1.859
	SE	0.000	0.920	0.499	0.181	0.085	0.070	0.073	0.088				
C. niloticus	Mean	12.000	6.000	3.847	1.407	0.583	0.663	0.691	0.198	4.084	2.398	2.967	3.236
	SE	0.000	0.856	0.616	0.170	0.104	0.064	0.066	0.108				
C. siamensis	Mean	11.000	4.700	2.982	1.101	0.609	0.539	0.565	-0.149	3.007	2.558	2.169	2.299
	SE	0.000	0.803	0.529	0.202	0.095	0.086	0.090	0.048				
C. acutus	Mean	10.800	4.600	3.428	1.104	0.473	0.543	0.569	0.160	3.020	1.808	2.188	2.320
	SE	0.133	1.147	0.955	0.231	0.109	0.097	0.101	0.094				
C. porosus	Mean	36.700	5.300	3.388	1.229	0.630	0.635	0.644	0.000	3.418	2.703	2.740	2.809
	SE	0.213	1.342	0.721	0.155	0.055	0.044	0.044	0.069				

Table 3 Fixation indices between populations (F_{ST}) below the diagonal (blue cells) with significance (after Bonferroni correction) above (orange cells).

	Isabela	Liguasan	*C. niloticus*	*C. siamensis*	*C. acutus*	*C. porosus*
Isabela		0.001	0.001	0.001	0.001	0.001
Liguasan	0.408		0.001	0.001	0.001	0.001
C. niloticus	0.449	0.363		0.001	0.001	0.001
C. siamensis	0.512	0.451	0.339		0.001	0.001
C. acutus	0.482	0.447	0.279	0.402		0.001
C. porosus	0.425	0.382	0.297	0.362	0.351	

Table 4 Effective population sizes estimated with LDNe (Waples & Do 2008) considering three thresholds for lowest allele frequency used in estimation and the corresponding harmonic mean of the sample size, the number of effective breeders (*Neb*) in the population and 95% confidence intervals (CI) for those estimations.

Lowest Allele Frequency Used	0.05	0.02	0.01
Isabela (*C. mindorensis*)			
Harmonic Mean Sample Size	100.5	100.3	100.3
Estimated *Neb*^	2.2	2.7	4.8
95% CIs for *Neb*^	1.7–2.8	2.1–3.3	3.5–7.3
Liguasan Marsh (*C. mindorensis*)			
Harmonic Mean Sample Size	14	14	14
Estimated *Neb*^	21.3	7.9	7.9
95% CIs for *Neb*^	6.5–Infinite	3–20.2	3–20.2
RP (*C. porosus*)			
Harmonic Mean Sample Size	36.7	36.7	36.7
Estimated *Neb*^	13.2	16.1	22.6
95% CIs for *Neb*^	10.8–16.2	13.4–19.4	18.8–27.6

C. porosus from Republic of Palau using the more precise 0.01 rare allele threshold (Table 4). The SPAGeDi dyad analysis revealed overall relatedness within the Isabela Philippine Crocodile population to be slightly more than what might be expected from matings of unrelated individuals (Fig. 1A). This trend was not detected, though, in the Liguasan Marsh population (Fig. 1B). The population of Saltwater Crocodiles showed little relatedness differing from the simulation of unrelated individuals (Fig. 2).

Both Lositran and BayeScan identified two outlier loci as potentially linked to genes that might be under some degree of selection. However, the two approaches agreed on only one locus (CpP801). Lositran found CpP801 to be a significant F_{ST} outlier whereas BayeScan found it "barely worth mentioning" using the Jeffreys' scale of evidence (data not shown). The sequences flanking the CpP801 repeat motif were submitted to the

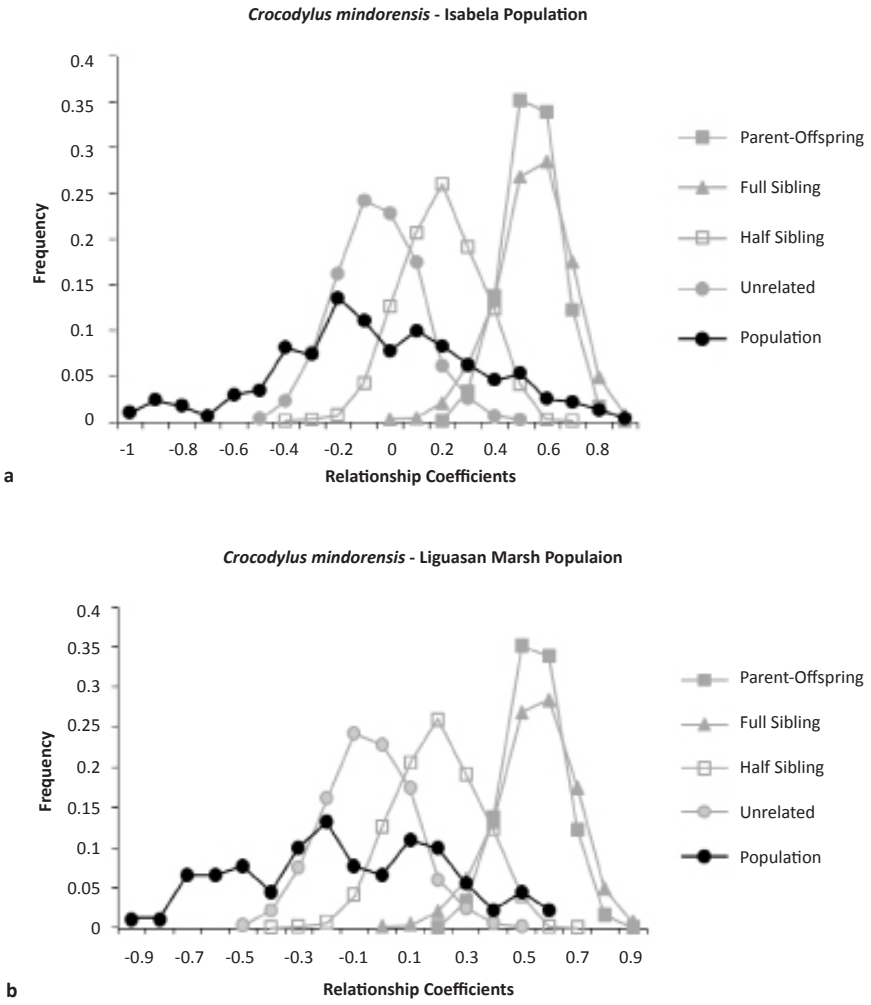

Figure 1. Relationship coefficient distirubtions of the two extant *Crocodylus mindorensis* populations from a - Isabela and b - Liguasan Marsh overlayed on a simulation of 10,000 individuals of known relationships by pedigree verification (Queller & Goodnight 1989).

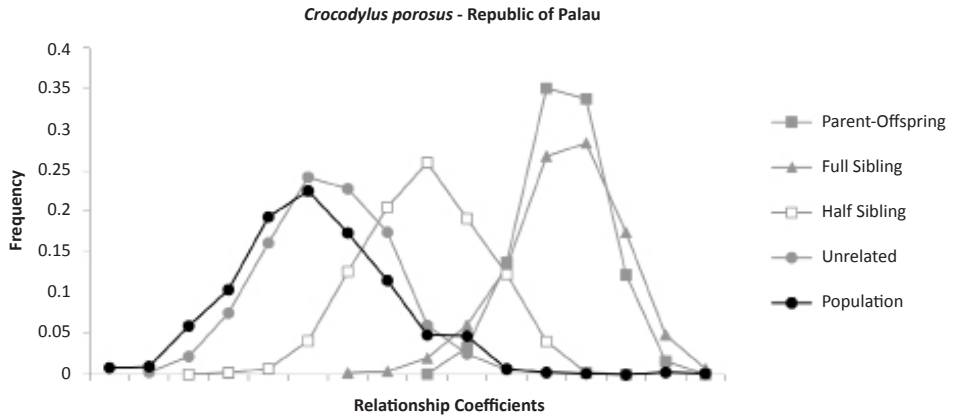

Figure 2. Relationship coefficient distirubtions of the *Crocodylus porosus* population from the Republic of Palau overlayed on a simulation of 10,000 individuals of known relationships by pedigree verification (Queller & Goodnight 1989).

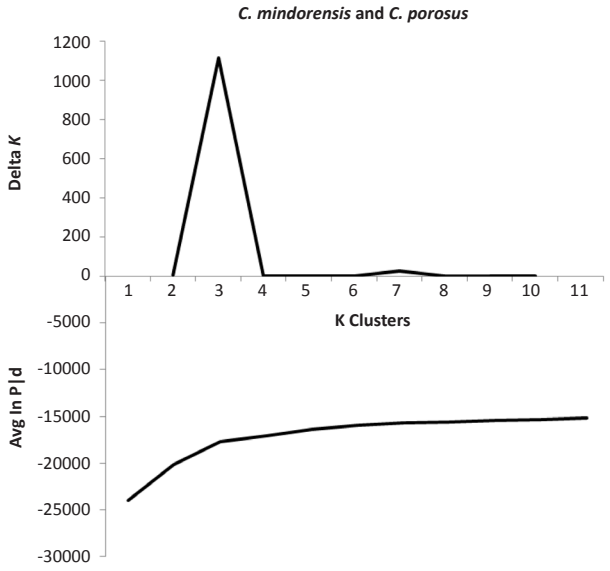

Figure 3. Evanno et al.'s (2005) Δ*K* and chart of the average logarithm of the probablity of the data for *K*-max, *K* = 3, for seven populations of *C. mindorensis* and one population of *C. porosus*.

BLASTn algorithm (http://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastn&BLAST_SPEC=WGS&BLAST_PROGRAMS=megaBlast&PAGE_TYPE=BlastSearch) to search for potential candidate genes that might be under selection. Minimal sequence fragments ranging 25−50 bp in length were found in other species but no long sequence homologies and none of the queries returned candidates common to both flanking regions. Two short sequences were found in multiple species although corresponding to different genes. They were also found on multiple chromosomes in a single species indicating that these two sequences were both conserved and duplicated in the genome.

From the STRUCTURE analysis, *K*=3 was found to be the optimal number of clusters represented in the data by Evanno et al.'s (2005) Δ*K* (Fig. 3). These clusters represent the Isabela *C. mindorensis* population, the Liguasan Marsh *C. mindorensis* population and the Republic of Palau *C. porosus* population. At *K*-max, a total of 59 putative *C. mindorensis* individuals had *q*-values above the noise threshold of 0.05 in the cluster represented by *C. porosus* (Fig. 4, see also Appendix 1). The PCoA suggested the same *C. mindorensis* individuals as previously identified with affinity to the *C. porosus* sample set (Fig. 5). The PCoA also identified individuals in the Isabela population that appear to group with the southern populations; a phenomenon which cannot be verified with records or observations. The PWRCC bred crocodiles reintroduced in Isabela were not included as Isabela members in this study.

DISCUSSION AND CONCLUSIONS

Previous studies have estimated genetic diversity in crocodilian species but making direct comparisons was difficult since the same marker systems were not applied across each study. Here, we used the same microsatellite loci to compare the genetic diversity of *C. mindorensis* to *C. acutus*, *C. niloticus*, *C. porosus* and *C. siamensis*. The heterozygosity estimates from our data for *C. acutus*, *C. niloticus*, *C. porosus* and *C. siamensis* fall within the ranges of estimates previously reported for captive purebred *C. siamensis*, *Ho* = 0.42±0.17 (FitzSimmons et al. 2002), farmed *C. porosus*, *Ho* = 0.59 (Isberg et al. 2004) and in wild populations of *C. niloticus*, *He* = 0.27–0.61 (Hekkala et al. 2010) and *Ho* = 0.51 (Bishop et al. 2009), *C. moreletti*, *Ho* = 0.49 (Dever et al. 2002) and *Melanosuchus niger*, *Ho* = 0.47–0.70 (de Thoisy et al. 2006). We found that genetic diversity measures for *C. mindorensis* were lower compared to *C. acutus*, *C. niloticus*, *C. porosus* and *C. siamensis*, whether using traditional F_{ST} and heterozygosity measures or by transforming such measures into diversity indices.

The LDNe analysis of the effective population sizes allows the interpretation at three levels dictated by thresholds for rare alleles in the data. Considering the lowest accepted frequency for rare alleles to be 0.01, the estimates of effective breeders were 4.8 (95% CI: 3.5−7.3) in Isabela, 7.9 (95% CI: 3.0−20.2) in Liguasan Marsh and 22.6 (95% CI: 18.8−27.6) in the collection of *C. porosus*

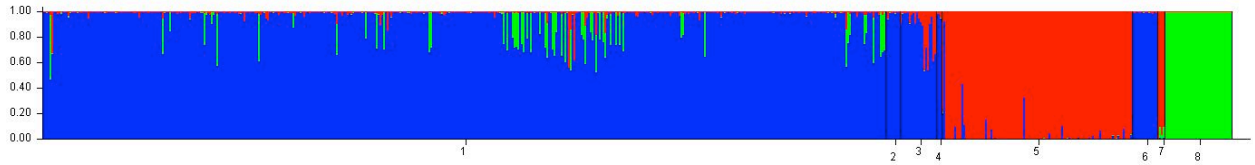

Figure 4. STRUCTURE bar graph of seven *C. mindorensis* populations and one *C. porosus* population at *K*-max, *K* = 3 clusters.
1 PWRCC, 2 Davao City Crocodile Park, 3 Silliman University, 4 Calauit Game Preserve and Wildlife Sanctuary, 5 Isabela Province, 6 Liguasan Marsh, 7 Valera Square Mini Zoo in Abra Province, 8 Republic of Palau (*C. porosus*).

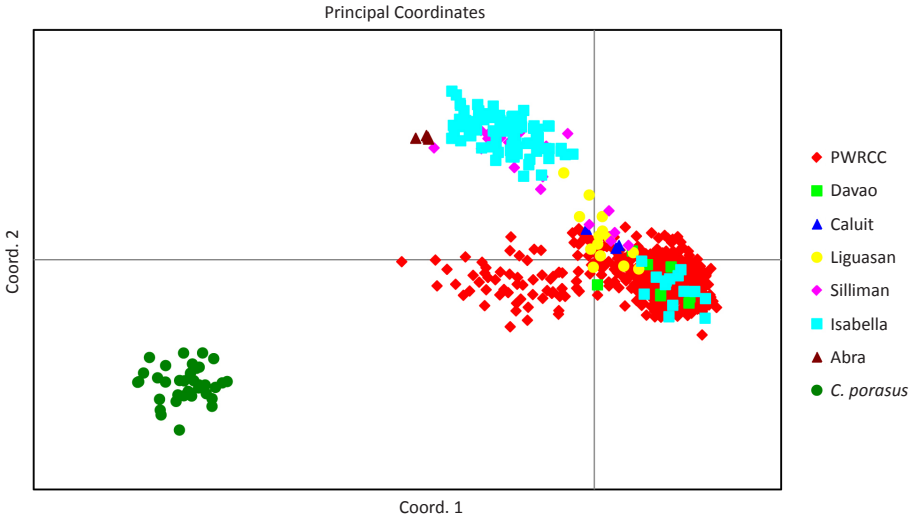

Figure 5. Principal Coordinate Analysis (PCoA) of the *Crocodylus mindorensis* populations sampled in the Philippines and the *C. porosus* population in Republic of Palau indicating the southern populations, the group of PWRCC *C. mindorensis* individuals with *C. porosus* introgression (red diamond cluster towards the *C. porosus* cluster) and the northern populations including individuals sampled in Isabela that were introduced from PWRCC (blue squares over the red diamond background).

from RP. In 2008, the minimum census of the Isabela population was 86 individual crocodiles comprised of 10 adults, 41 sub-adults/juveniles and 35 hatchlings with six nests in four distinct localities (van Weerd 2010 and van Weerd unpublished data). The Philippine Crocodile population in Liguasan Marsh remains poorly known but was estimated in 2008 to include at least 258 individuals in all age classes (Pomares et al. 2008). This estimate is based on interviews with the local inhabitants of the marsh, which in all likelihood contain multiple sightings of individual animals. The ratios of effective breeders to the estimated population sizes were determined to be 0.06 in Isabela and 0.03 in Liguasan Marsh. These estimates hover about the 0.05 ratio threshold which Frankham (1995) considers quite low, and is, when compared to recent studies in Steelhead Trout (*Oncorhynchus mykiss*, Araki et al. 2007) and the European Common Frog (*Rana temporaria*, Schmeller & Merila 2007), 0.10–0.40 and 0.23–1.67, respectively. We did find evidence for increasing relatedness in the small isolated Isabela population. This estimate would be expected as hatchlings were sampled from the nests. We did not find excessive F_{IS} values, but could expect those to rise in future generations if mating among related individuals becomes commonplace due to the small effective population sizes.

With only two extant populations of *C. mindorensis* known to remain today, it is imperative to evaluate the similarity or differences between the two. Biogeographic differences might exist since the Isabela population exists in the northern extreme of the distribution whereas the Liguasan Marsh population is found in the southern extreme. One might expect that if the populations were highly differentiated, molecular testing could detect a genetic selection signature associated with some of the neutral markers. We did find positive results using two testing methods, but for only one of the 11 loci. We searched the repeat motif flanking sequences against sequences stored in the BLASTn database, but we did not identify a potential candidate gene. In fact, in both flanking regions, small fragments (25–50 bp) were highly conserved among species and duplicated within genomes. With one method identifying this locus as a significant F_{ST} outlier and the other as marginal, we suggest that this locus is not under selection but a false positive in both tests. False positives can be the result of hierarchical structure perhaps created from the pooling of samples from four distinct breeding areas in the San Mariano area of the Isabela region (Excoffier et al. 2009). Likewise, the data set or the number of remaining Philippine Crocodiles in the wild may simply be too small to detect selection (Hohenlohe et al. 2010). Regardless, we cannot suggest that evidence was found to support selection that might be differentiating the populations. If the two populations differed greatly, then the populations might require separate management. However, the populations differ only slightly, which we assume may simply be caused by genetic drift thus mixing may reestablish or maximize genetic diversity supporting positive genetic health of the species.

Tabora et al. (2012) identified a total of 57 putative hybrids in that study. From the STRUCTURE analysis of the same set of samples, we identified 59 individuals with genotypic proportions exceeding a background noise level (q>0.05) in the cluster generated by the *C. porosus* samples (Appendix 1). The PCoA analysis also identified the same individuals to be closer to the *C. porosus* grouping than *C. mindorensis* below the nominal q-value threshold. Only two individuals approached the q = 0.50 genotypic proportions expected of an F1 individual (PWc005, q = 0.512; PWb097, q = 0.409). The former, PWc005, possesses both a *C. porosus* D-loop haplotype and the *C. porosus* C-*mos* diagnostic characters. We consider this individual to be an F1 from a *C. mindorensis* male and a *C. porosus* female. The latter, PWb097, possesses the *C. porosus* D-loop haplotype yet is homozygous for the *C. mindorensis* C-*mos* diagnostic sites. We consider this individual to be a *C. mindorensis* backcross falling in the upper tail of the backcross q-distribution. Two individuals from Abra (K7895 and K7897) exceeded the conservative 0.05 q-threshold for background noise though did not possess *C. porosus* D-loop or C-*mos* markers. We accept these to be *C. mindorensis* with slightly higher background noise than the conservative threshold we imposed in our criteria. The remaining 55 fell in a q-distribution around 0.25 (avg q = 0.253±0.067) which approximates the proportion of introgressed genes expected to be retained in the first backcross generation. Thus, we suggest one first generation hybrid cross and 56 backcross individuals only in the PWRCC-sampled group.

The morphological identification of hybrids, and particularly among the hybrids in this study, proves to be problematic. Hybrid detection through morphological characteristics is not always effective because hybrids can express mosaics of phenotypes (Campton 1987) due to incomplete penetrance or partial dominance of the diagnostic character. Hybrids in the PWRCC population were undetected since all express the post occipital scutes indicative of *C. mindorensis* (Image 1A). This suggests a single gene effect where the allele conferring the diagnostic scutes expressed in *C. mindorensis* is dominant over the allele fixed in *C. porosus* that suppresses the expression of that phenotype (Image 1B). Had F1 *inter se* mating occurred, one would expect that one fourth of the offspring should have inherited both *C. porosus* C-*mos* alleles and one fourth should express the absence of post occipital scutes. Neither scenario was detected in the data. Considering the multilocus allele frequency distributions, there is no indication that F1 *inter se* mating has occurred since the average of

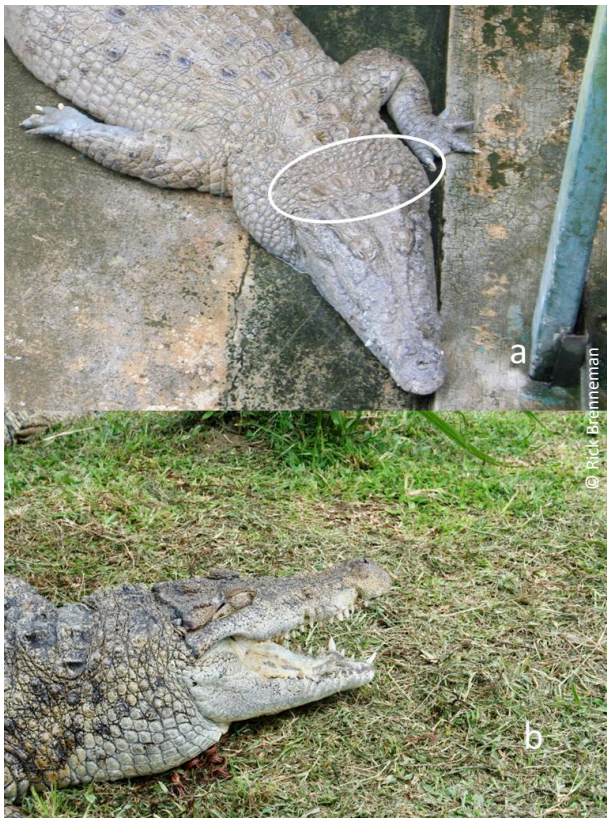

Image 1. a - *Crocodylus mindorensis* head showing post occipital scutes (encircled); b - *C. porosus* head showing lack of post occipital scutes.

the q-distribution of an F2 generation would be higher (closer to 0.50). Backcrossing to *C. mindorensis* would ensure at least one *C. mindorensis* allele at all loci which is exactly what the data shows. This comprehensive genetic testing identifies hybrids in the collection that can be separated out of the gene pool before a hybrid swarm is created that could have a detrimental effect on the conservation management of the species (Allendorf et al. 2001). The removal of suspected hybrids could protect the genetic integrity of the species, especially if used as reintroduction candidates or to augment the genetic diversity of the wild populations (Rhymer & Simberloff 1996).

The two distantly isolated extant populations of *C. mindorensis*, Isabela and Liguasan Marsh, present several concerns for long-term conservation management. Both show less genetic diversity than what has been detected in other crocodilian species in this and previous studies. Both populations have low effective population sizes and low effective population size to census ratios. The recent systematics study (Tabora et al. 2012) did not indicate branch lengths that would suggest more than population level differentiation. There is no

Appendix 1. Inferred ancestry of individuals: *K* 1 corresponds to northern *C. mindorensis* population ancestry, *K* 2 corresponds to *C. porosus*, *K* 3 corresponds to southern *C. mindorensis* population ancestry. Bold font indicates individuals exceeding the background noise threshold (0.05) in column *K* 2 inferring hybridization. Merging with information from Appendix 1 (Tabora et al. 2012), *italicized font* indicates individuals with *C. porosus* D-loop haplotypes and those with asterisks* were heterozygous for *C. porosus* diagnostic sites in the C-*mos* gene. Populations: 1) PWRCC, 2) Davao City Crocodile Park, 3) Silliman University, 4) Calauit Game Preserve and Wildlife Sanctuary, 5) Isabela Province, 6) Liguasan Marsh, 7) Valera Square Mini Zoo in Abra Province, 8) Republic of Palau *C. porosus*.

Sample No.	ID	Population	*K* 1	*K* 2	*K* 3
1	PwW001	1	0.006	0.001	0.992
2	PWc002	1	0.004	0.001	0.995
3	PWc003	1	0.007	0.001	0.992
4	PWc004	1	0.004	0.001	0.995
5	***PWc005****	**1**	**0.022**	**0.512**	**0.466**
6	PWc006	1	0.328	0.002	0.669
7	PWc007	1	0.004	0.001	0.995
8	PWc008	1	0.003	0.001	0.996
9	PWc009	1	0.018	0.001	0.981
10	PWc010	1	0.011	0.001	0.988
11	PWc011	1	0.003	0.013	0.983
12	PWc012	1	0.003	0.001	0.996
13	PWc013	1	0.004	0.001	0.995
14	PWx014	1	0.004	0.001	0.995
15	PWc015	1	0.030	0.016	0.954
16	PWc016	1	0.003	0.008	0.988
17	PWc017	1	0.003	0.001	0.996
18	PWc018	1	0.003	0.001	0.996
19	PWc019	1	0.003	0.001	0.996
20	PWb028	1	0.003	0.009	0.988
21	PWc021	1	0.003	0.001	0.996
22	PWc022	1	0.011	0.001	0.988
23	PWc023	1	0.003	0.001	0.996
24	PWc024	1	0.004	0.001	0.995
25	PWc025	1	0.005	0.001	0.994
26	PWc026	1	0.054	0.002	0.944
27	PWb027	1	0.004	0.001	0.995
28	PWc020	1	0.003	0.001	0.996
29	PWb029	1	0.004	0.001	0.995
30	PWb030	1	0.004	0.001	0.995
31	PWb031	1	0.003	0.001	0.996
32	PWb032	1	0.004	0.001	0.995
33	PWb033	1	0.004	0.001	0.995
34	PWb034	1	0.004	0.001	0.995
35	PWb035	1	0.005	0.007	0.988
36	PWb036	1	0.009	0.001	0.99
37	PWb037	1	0.005	0.001	0.994
38	PWb038	1	0.003	0.001	0.996
39	PWb039	1	0.004	0.001	0.995
40	PWb040	1	0.003	0.001	0.996

Sample No.	ID	Population	*K* 1	*K* 2	*K* 3
41	PWb041	1	0.007	0.001	0.992
42	PWb042	1	0.008	0.001	0.991
43	PWb043	1	0.004	0.001	0.995
44	PWb044	1	0.005	0.001	0.994
45	PWb045	1	0.004	0.001	0.995
46	PWb046	1	0.006	0.001	0.993
47	PWb047	1	0.005	0.001	0.994
48	PWb048	1	0.003	0.001	0.996
49	PWb049	1	0.006	0.001	0.993
50	PWb050	1	0.003	0.001	0.996
51	PWb051	1	0.008	0.001	0.991
52	PWb052	1	0.009	0.001	0.99
53	PWb053	1	0.003	0.001	0.996
54	PWb054	1	0.048	0.002	0.951
55	PWb055	1	0.003	0.001	0.996
56	PWb056	1	0.005	0.001	0.994
57	PWb057	1	0.003	0.001	0.996
58	PWb058	1	0.004	0.001	0.995
59	PWb059	1	0.010	0.001	0.989
60	PWb060	1	0.004	0.001	0.995
61	PWb061	1	0.004	0.001	0.995
62	PWb062	1	0.003	0.001	0.996
63	PWb063	1	0.004	0.001	0.995
64	PWb064	1	0.006	0.001	0.993
65	PWb065	1	0.003	0.001	0.996
66	PWb066	1	0.006	0.001	0.993
67	***PWb067****	**1**	**0.053**	**0.277**	**0.669**
68	PWb068	1	0.007	0.001	0.992
69	PWb069	1	0.003	0.001	0.996
70	PWb070	1	0.005	0.001	0.993
71	***PWb071****	**1**	**0.005**	**0.147**	**0.848**
72	PWb072	1	0.004	0.001	0.995
73	PWb073	1	0.004	0.001	0.995
74	PWb074	1	0.003	0.001	0.995
75	PWb075	1	0.019	0.001	0.979
76	PWb076	1	0.005	0.001	0.994
77	PWb077	1	0.008	0.001	0.991
78	PWb078	1	0.005	0.001	0.994
79	PWb079	1	0.004	0.002	0.995
80	PWb080	1	0.005	0.001	0.994

Sample No.	ID	Population	*K* 1	*K* 2	*K* 3
81	PWb081	1	0.004	0.001	0.995
82	PWb082	1	0.056	0.002	0.942
83	PWb083	1	0.009	0.001	0.990
84	PWb084	1	0.009	0.001	0.990
85	PWb085	1	0.008	0.001	0.991
86	PWb086	1	0.004	0.001	0.995
87	PWb087	1	0.004	0.001	0.995
88	PWb088	1	0.006	0.002	0.993
89	PWb089	1	0.006	0.002	0.993
90	***PWb090***	**1**	**0.011**	**0.248**	**0.741**
91	PWb091	1	0.006	0.017	0.977
92	PWb092	1	0.013	0.001	0.986
93	PWb093	1	0.023	0.001	0.976
94	***PWb094***	**1**	**0.003**	**0.096**	**0.901**
95	PWb095	1	0.007	0.018	0.976
96	PWb096	1	0.006	0.001	0.993
97	***PWb097***	**1**	**0.018**	**0.409**	**0.572**
98	PWb098	1	0.004	0.001	0.995
99	PWb099	1	0.005	0.001	0.994
100	PWb100	1	0.005	0.001	0.994
101	PWb101	1	0.012	0.001	0.987
102	PWb102	1	0.003	0.001	0.996
103	PWb103	1	0.006	0.001	0.993
104	PWb104	1	0.011	0.001	0.988
105	PWb105	1	0.003	0.001	0.995
106	PWb106	1	0.006	0.001	0.993
107	PWb107	1	0.008	0.001	0.991
108	PWb108	1	0.003	0.001	0.996
109	PWb109	1	0.018	0.001	0.981
110	PWb110	1	0.014	0.003	0.983
111	PWb111	1	0.004	0.001	0.995
112	PWb112	1	0.072	0.002	0.926
113	PWb113	1	0.003	0.001	0.996
114	PWb114	1	0.003	0.001	0.996
115	PWb115	1	0.004	0.001	0.995
116	PWb116	1	0.003	0.001	0.996
117	PWb117	1	0.004	0.001	0.995
118	PWb118	1	0.008	0.002	0.990
119	PWb119	1	0.008	0.001	0.991
120	***PWb120****	**1**	**0.016**	**0.372**	**0.612**
121	PWb121	1	0.023	0.001	0.976
122	PWb122	1	0.005	0.002	0.993
123	PWb123	1	0.007	0.001	0.992
124	PWb124	1	0.054	0.002	0.944
125	PWb125	1	0.069	0.002	0.930

Sample No.	ID	Population	*K* 1	*K* 2	*K* 3
126	PWb126	1	0.005	0.001	0.994
127	PWb127	1	0.003	0.001	0.996
128	PWb128	1	0.006	0.002	0.992
129	PWb129	1	0.006	0.001	0.993
130	PWb130	1	0.003	0.002	0.995
131	PWb131	1	0.024	0.003	0.973
132	PWb132	1	0.004	0.001	0.995
133	PWb133	1	0.004	0.001	0.995
134	PWb134	1	0.009	0.001	0.990
135	PWb135	1	0.011	0.001	0.988
136	PWb136	1	0.007	0.002	0.992
137	PWb137	1	0.011	0.001	0.988
138	PWb138	1	0.009	0.001	0.990
139	**PWb139**	**1**	**0.012**	**0.114**	**0.874**
140	PWb140	1	0.033	0.002	0.965
141	PWb141	1	0.003	0.001	0.995
142	PWb142	1	0.004	0.001	0.995
143	PWb143	1	0.007	0.001	0.992
144	PWb144	1	0.004	0.001	0.995
145	PWb145	1	0.037	0.001	0.962
146	PWb146	1	0.003	0.001	0.996
147	PWb147	1	0.003	0.001	0.996
148	PWb148	1	0.008	0.001	0.991
149	PWb149	1	0.005	0.001	0.994
150	PWb150	1	0.004	0.001	0.995
151	PWb151	1	0.004	0.001	0.995
152	PWb152	1	0.003	0.001	0.996
153	PWb153	1	0.003	0.001	0.996
154	PWb154	1	0.005	0.001	0.994
155	PWb155	1	0.004	0.001	0.995
156	PWb156	1	0.007	0.001	0.992
157	PWb157	1	0.006	0.001	0.992
158	PWb158	1	0.005	0.001	0.994
159	PWb159	1	0.004	0.001	0.995
160	PWb160	1	0.007	0.001	0.992
161	PWb161	1	0.009	0.001	0.990
162	PWb162	1	0.015	0.001	0.984
163	***PWb163***	**1**	**0.100**	**0.239**	**0.660**
164	PWb164	1	0.004	0.001	0.995
165	PWb165	1	0.003	0.001	0.996
166	PWb166	1	0.031	0.002	0.967
167	PWb167	1	0.003	0.001	0.996
168	PWb168	1	0.004	0.001	0.995
169	PWb169	1	0.026	0.002	0.973
170	PWb170	1	0.009	0.001	0.990

Sample No.	ID	Population	*K* 1	*K* 2	*K* 3
171	PWb171	1	0.020	0.003	0.977
172	PWb172	1	0.006	0.002	0.992
173	PWb173	1	0.004	0.001	0.995
174	PWb174	1	0.004	0.001	0.995
175	PWb175	1	0.004	0.001	0.995
176	PWb176	1	0.003	0.001	0.996
177	PWb177	1	0.007	0.001	0.992
178	PWb178	1	0.005	0.001	0.994
179	***PWb179***	**1**	**0.004**	**0.207**	**0.789**
180	PWb180	1	0.006	0.001	0.992
181	PWb181	1	0.004	0.001	0.994
182	PWb182	1	0.006	0.001	0.993
183	PWb183	1	0.003	0.002	0.995
184	PWb184	1	0.014	0.001	0.984
185	***PWb185****	**1**	**0.011**	**0.277**	**0.712**
186	PWb186	1	0.021	0.001	0.978
187	PWb187	1	0.102	0.002	0.897
188	PWb188	1	0.025	0.001	0.974
189	***PWb189***	**1**	**0.003**	**0.290**	**0.707**
190	PWb190	1	0.005	0.001	0.993
191	PWb191	1	0.143	0.002	0.855
192	PWb192	1	0.003	0.001	0.996
193	PWb193	1	0.042	0.002	0.956
194	PWb194	1	0.006	0.001	0.993
195	PWb195	1	0.006	0.001	0.993
196	PWb196	1	0.004	0.001	0.995
197	PWb197	1	0.004	0.001	0.995
198	PWb198	1	0.007	0.001	0.992
199	PWb199	1	0.003	0.001	0.996
200	PWb200	1	0.004	0.001	0.995
201	PWb201	1	0.006	0.001	0.993
202	PWb202	1	0.007	0.001	0.992
203	PWb203	1	0.007	0.001	0.992
204	PWb204	1	0.015	0.001	0.984
205	PWb205	1	0.004	0.001	0.995
206	PWb206	1	0.003	0.001	0.996
207	PWb207	1	0.009	0.001	0.990
208	PWb208	1	0.003	0.001	0.996
209	PWb209	1	0.003	0.001	0.996
210	PWb210	1	0.004	0.001	0.995
211	PWb211	1	0.014	0.001	0.985
212	PWb212	1	0.003	0.001	0.996
213	PWb213	1	0.003	0.001	0.996
214	***PWb214****	**1**	**0.005**	**0.310**	**0.686**
215	***PWb215****	**1**	**0.004**	**0.279**	**0.717**

Sample No.	ID	Population	*K* 1	*K* 2	*K* 3
216	PWb216	1	0.004	0.001	0.995
217	PWb217	1	0.003	0.001	0.996
218	PWb218	1	0.004	0.001	0.995
219	PWb219	1	0.006	0.001	0.993
220	PWb220	1	0.015	0.001	0.984
221	PWb221	1	0.005	0.010	0.985
222	PWb222	1	0.022	0.002	0.976
223	PWb223	1	0.016	0.001	0.983
224	PWb224	1	0.004	0.001	0.995
225	PWb225	1	0.007	0.001	0.992
226	PWb226	1	0.004	0.001	0.995
227	PWb227	1	0.003	0.001	0.996
228	PWb228	1	0.003	0.001	0.996
229	PWb229	1	0.004	0.001	0.995
230	PWb230	1	0.003	0.001	0.995
231	PWb231	1	0.004	0.001	0.995
232	PWb232	1	0.003	0.001	0.996
233	PWb233	1	0.004	0.001	0.995
234	PWb234	1	0.004	0.001	0.995
235	PWb235	1	0.011	0.001	0.988
236	PWb236	1	0.011	0.001	0.988
237	PWb237	1	0.007	0.001	0.992
238	PWb238	1	0.003	0.001	0.996
239	PWb239	1	0.003	0.001	0.996
240	PWb240	1	0.004	0.001	0.995
241	PWb241	1	0.005	0.001	0.994
242	PWb242	1	0.003	0.001	0.996
243	PWb243	1	0.004	0.001	0.995
244	PWb244	1	0.006	0.001	0.993
245	PWb245	1	0.003	0.001	0.996
246	PWb246	1	0.005	0.001	0.994
247	PWc247	1	0.009	0.001	0.99
248	PWc248	1	0.003	0.001	0.996
249	PWc249	1	0.004	0.001	0.995
250	PWc250	1	0.044	0.002	0.954
251	PWc251	1	0.003	0.001	0.996
252	PWc252	1	0.009	0.001	0.99
253	PWc253	1	0.003	0.006	0.991
254	PWx254	1	0.003	0.017	0.98
255	***PW255***	**1**	**0.005**	**0.246**	**0.75**
256	PWc256	1	0.003	0.001	0.996
257	***PWb257****	**1**	**0.008**	**0.296**	**0.697**
258	PWc258	1	0.003	0.001	0.996
259	PWb259	1	0.005	0.001	0.994
260	***PWb260***	**1**	**0.005**	**0.196**	**0.798**

Sample No.	ID	Population	*K* 1	*K* 2	*K* 3
261	***PWb261****	**1**	**0.008**	**0.275**	**0.717**
262	***PWb262****	**1**	**0.007**	**0.271**	**0.723**
263	***PWb263***	**1**	**0.008**	**0.285**	**0.707**
264	PWx264	1	0.004	0.001	0.995
265	***PWb265****	**1**	**0.003**	**0.254**	**0.743**
266	***PWb266***	**1**	**0.006**	**0.315**	**0.679**
267	PWc267	1	0.003	0.001	0.996
268	***PWb268****	**1**	**0.010**	**0.199**	**0.791**
269	PWx269	1	0.006	0.001	0.993
270	***PWb270***	**1**	**0.012**	**0.304**	**0.683**
271	PWb271	1	0.003	0.001	0.996
272	PWc272	1	0.007	0.001	0.991
273	PWb273	1	0.004	0.001	0.995
274	PWb274	1	0.002	0.001	0.997
275	***PWb275****	**1**	**0.006**	**0.166**	**0.828**
276	PWb276	1	0.003	0.001	0.996
277	PWc277	1	0.004	0.001	0.995
278	***PWb278****	**1**	**0.008**	**0.274**	**0.718**
279	***PWb279***	**1**	**0.088**	**0.272**	**0.640**
280	PWb280	1	0.004	0.001	0.995
281	PWb281	1	0.007	0.002	0.991
282	***PWb282****	**1**	**0.003**	**0.292**	**0.705**
283	***PWb283****	**1**	**0.005**	**0.344**	**0.651**
284	***PWb284***	**1**	**0.005**	**0.155**	**0.839**
285	PWc285	1	0.004	0.001	0.995
286	PWc286	1	0.003	0.001	0.996
287	***PWb287****	**1**	**0.020**	**0.328**	**0.652**
288	PWb288	1	0.003	0.001	0.996
289	***PWb289****	**1**	**0.010**	**0.386**	**0.604**
290	PWb290	1	0.007	0.001	0.992
291	PWb291	1	0.441	0.001	0.558
292	***PWb292***	**1**	**0.142**	**0.321**	**0.537**
293	PWc293	1	0.020	0.002	0.979
294	PWb294	1	0.382	0.002	0.616
295	PWb295	1	0.012	0.002	0.987
296	PWb296	1	0.003	0.001	0.996
297	PWb297	1	0.003	0.002	0.995
298	***PWb298****	**1**	**0.003**	**0.177**	**0.819**
299	***PWb299***	**1**	**0.005**	**0.211**	**0.783**
300	***PWb300***	**1**	**0.113**	**0.298**	**0.589**
301	PWc301	1	0.044	0.001	0.955
302	PWx302	1	0.012	0.001	0.987
303	***PWb303****	**1**	**0.004**	**0.160**	**0.836**
304	***PWb304****	**1**	**0.004**	**0.224**	**0.773**
305	PWb305	1	0.010	0.001	0.989

Sample No.	ID	Population	*K* 1	*K* 2	*K* 3
306	***PWb306***	**1**	**0.182**	**0.291**	**0.526**
307	PWb308	1	0.006	0.002	0.992
308	***PWb309****	**1**	**0.006**	**0.211**	**0.783**
309	PWb310	1	0.003	0.001	0.996
310	***PWb311***	**1**	**0.004**	**0.237**	**0.759**
311	***PWb312***	**1**	**0.165**	**0.195**	**0.640**
312	PWb313	1	0.022	0.033	0.945
313	PWb314	1	0.003	0.002	0.995
314	***PWb315****	**1**	**0.016**	**0.248**	**0.735**
315	PWb316	1	0.006	0.001	0.993
316	PWc317	1	0.003	0.001	0.996
317	P***Wb318***	**1**	**0.004**	**0.256**	**0.740**
318	PWc319	1	0.029	0.001	0.970
319	***PWb320****	**1**	**0.007**	**0.256**	**0.737**
320	PWc321	1	0.004	0.001	0.995
321	***PWb322****	**1**	**0.010**	**0.301**	**0.689**
322	PWb323	1	0.010	0.001	0.989
323	PWb324	1	0.003	0.001	0.996
324	PWb325	1	0.003	0.001	0.996
325	PWb326	1	0.003	0.001	0.996
326	PWc327	1	0.003	0.001	0.996
327	PWb328	1	0.003	0.001	0.996
328	PWb329	1	0.029	0.001	0.970
329	PWb330	1	0.038	0.002	0.961
330	PWb331	1	0.003	0.001	0.996
331	PWb332	1	0.006	0.001	0.993
332	PWb333	1	0.004	0.001	0.995
333	PWb334	1	0.003	0.001	0.996
334	PWb335	1	0.003	0.001	0.996
335	PWb336	1	0.005	0.001	0.994
336	PWb337	1	0.007	0.001	0.992
337	PWb338	1	0.026	0.001	0.972
338	PWb339	1	0.006	0.001	0.993
339	PWb340	1	0.004	0.001	0.995
340	PWb341	1	0.011	0.001	0.988
341	PWb342	1	0.003	0.001	0.996
342	PWb343	1	0.005	0.001	0.994
343	PWb344	1	0.005	0.001	0.993
344	PWb345	1	0.003	0.001	0.996
345	PWb346	1	0.004	0.001	0.995
346	PWb347	1	0.004	0.001	0.995
347	PWb348	1	0.030	0.002	0.969
348	PWb349	1	0.010	0.001	0.989
349	PWb350	1	0.006	0.001	0.993
350	PWb351	1	0.004	0.001	0.995

Sample No.	ID	Population	*K* 1	*K* 2	*K* 3
351	PWb352	1	0.003	0.001	0.996
352	PWb353	1	0.034	0.001	0.965
353	***PWb354****	**1**	**0.004**	**0.202**	**0.793**
354	***PWb355****	**1**	**0.014**	**0.167**	**0.819**
355	PWb356	1	0.017	0.001	0.982
356	PWb357	1	0.005	0.001	0.994
357	PWb358	1	0.015	0.001	0.984
358	PWb359	1	0.002	0.002	0.996
359	PWb360	1	0.003	0.001	0.996
360	PWb361	1	0.009	0.001	0.990
361	PWb362	1	0.003	0.001	0.996
362	PWb363	1	0.003	0.009	0.988
363	PWb364	1	0.065	0.001	0.934
364	PWb365	1	0.006	0.001	0.993
365	PWb366	1	0.004	0.001	0.995
366	***PWb367***	**1**	**0.007**	**0.350**	**0.642**
367	PWb368	1	0.005	0.001	0.994
368	PWb369	1	0.004	0.001	0.995
369	PWb370	1	0.004	0.001	0.995
370	PWb371	1	0.005	0.001	0.994
371	PWb372	1	0.003	0.001	0.996
372	PWb373	1	0.005	0.001	0.994
373	PWb374	1	0.012	0.001	0.987
374	PWb375	1	0.004	0.001	0.995
375	PWb376	1	0.041	0.002	0.957
376	PWb377	1	0.004	0.001	0.995
377	PWb378	1	0.007	0.001	0.992
378	PWb379	1	0.005	0.001	0.994
379	PWb380	1	0.003	0.001	0.996
380	PWb381	1	0.004	0.001	0.995
381	PWb382	1	0.008	0.001	0.991
382	PWc383	1	0.003	0.001	0.996
383	PWc384	1	0.020	0.001	0.979
384	PWc385	1	0.003	0.009	0.989
385	PWc386	1	0.002	0.001	0.997
386	PWc387	1	0.003	0.001	0.996
387	PWc388	1	0.003	0.001	0.996
388	PWc389	1	0.016	0.001	0.983
389	PWc390	1	0.003	0.001	0.996
390	PWc391	1	0.003	0.001	0.996
391	PWc392	1	0.004	0.001	0.995
392	PWc393	1	0.004	0.001	0.995
393	PWc394	1	0.003	0.001	0.996
394	PWc395	1	0.003	0.001	0.996
395	PWc396	1	0.003	0.001	0.996

Sample No.	ID	Population	*K* 1	*K* 2	*K* 3
396	PWc397	1	0.003	0.001	0.996
397	PWc398	1	0.003	0.001	0.996
398	PWc399	1	0.003	0.001	0.996
399	PWc400	1	0.003	0.001	0.996
400	PWc401	1	0.004	0.001	0.995
401	PWc402	1	0.003	0.001	0.996
402	PWc403	1	0.003	0.001	0.996
403	PWc404	1	0.008	0.001	0.99
404	PWc405	1	0.004	0.010	0.985
405	PWc406	1	0.004	0.001	0.994
406	PWw407	1	0.005	0.001	0.994
407	PWx408	1	0.004	0.001	0.995
408	PWc409	1	0.015	0.001	0.984
409	PWc410	1	0.005	0.001	0.994
410	PWc411	1	0.014	0.001	0.984
411	PWc412	1	0.004	0.001	0.995
412	PWc413	1	0.004	0.001	0.995
413	PWc414	1	0.013	0.002	0.985
414	PWc415	1	0.003	0.001	0.996
415	PWc416	1	0.008	0.001	0.991
416	PWc417	1	0.005	0.002	0.994
417	PWc418	1	0.003	0.001	0.996
418	PWc419	1	0.010	0.001	0.989
419	PWc420	1	0.004	0.001	0.995
420	PWc421	1	0.005	0.002	0.993
421	PWc422	1	0.003	0.001	0.996
422	PWc423	1	0.033	0.003	0.964
423	PWw424	1	0.003	0.001	0.996
424	PWc425	1	0.003	0.001	0.995
425	PWc426	1	0.003	0.001	0.996
426	PWc427	1	0.011	0.001	0.988
427	PWc428	1	0.009	0.001	0.990
428	PWc429	1	0.003	0.001	0.996
429	PWc430	1	0.003	0.001	0.996
430	PWc431	1	0.003	0.001	0.996
431	PWc432	1	0.003	0.001	0.996
432	PWc433	1	0.004	0.001	0.995
433	PWc434	1	0.011	0.002	0.987
434	PWc435	1	0.007	0.002	0.991
435	PWc436	1	0.003	0.001	0.995
436	PWc437	1	0.007	0.002	0.992
437	PWc438	1	0.009	0.002	0.990
438	PWc439	1	0.018	0.025	0.957
439	PWc440	1	0.003	0.001	0.995
440	PWc441	1	0.011	0.001	0.987

Sample No.	ID	Population	*K* 1	*K* 2	*K* 3
441	PWb442	1	0.007	0.001	0.992
442	PWb443	1	0.013	0.001	0.986
443	PWb444	1	0.003	0.001	0.996
444	***PWb445***	**1**	**0.140**	**0.294**	**0.566**
445	***PWb446***	**1**	**0.006**	**0.237**	**0.757**
446	***PWb447***	**1**	**0.020**	**0.165**	**0.816**
447	PWb448	1	0.003	0.001	0.996
448	PWb449	1	0.003	0.001	0.996
449	PWb450	1	0.003	0.001	0.996
450	PWb451	1	0.038	0.002	0.960
451	PWb452	1	0.005	0.001	0.994
452	PWb453	1	0.003	0.001	0.996
453	PWb454	1	0.003	0.001	0.995
454	***PWb456***	**1**	**0.022**	**0.231**	**0.747**
455	***PWb455***	**1**	**0.023**	**0.142**	**0.836**
456	PWb457	1	0.004	0.001	0.995
457	PWb458	1	0.011	0.001	0.988
458	PWb459	1	0.004	0.001	0.995
459	***PWb460****	**1**	**0.047**	**0.359**	**0.594**
460	PWb461	1	0.005	0.001	0.994
461	K7898	1	0.004	0.001	0.995
462	K7899	1	0.026	0.001	0.973
463	***K7900****	**1**	**0.007**	**0.343**	**0.649**
464	***K7901****	**1**	**0.023**	**0.294**	**0.683**
465	***K7902****	**1**	**0.006**	**0.297**	**0.697**
466	DCc001	2	0.004	0.001	0.995
467	DCc002	2	0.054	0.004	0.942
468	DCc003	2	0.007	0.007	0.986
469	DCc004	2	0.013	0.001	0.986
470	DCc005	2	0.003	0.001	0.996
471	DCc006	2	0.012	0.005	0.983
472	DCc007	2	0.003	0.001	0.996
473	DCc008	2	0.003	0.001	0.996
474	SU001	3	0.086	0.001	0.912
475	SU002	3	0.014	0.001	0.985
476	SU003	3	0.013	0.001	0.985
477	SU004	3	0.013	0.001	0.986
478	SU005	3	0.006	0.001	0.993
479	SU006	3	0.092	0.001	0.907
480	SU007	3	0.026	0.001	0.973
481	SU008	3	0.008	0.001	0.991
482	SU009	3	0.087	0.001	0.911
483	SU012	3	0.005	0.001	0.994
484	SU013	3	0.052	0.001	0.947
485	SU014	3	0.081	0.001	0.918

Sample No.	ID	Population	*K* 1	*K* 2	*K* 3
486	SU015	3	0.108	0.001	0.891
487	SU016	3	0.464	0.001	0.534
488	K7903	3	0.283	0.002	0.715
489	K7904	3	0.462	0.001	0.537
490	K7905	3	0.096	0.001	0.903
491	K7906	3	0.037	0.002	0.961
492	K7907	3	0.388	0.002	0.610
493	K7908	3	0.330	0.002	0.668
494	K7909	4	0.017	0.001	0.982
495	K7910	4	0.017	0.001	0.982
496	K7911	4	0.050	0.001	0.949
497	K7912	5	0.786	0.011	0.203
498	IS001	5	0.074	0.002	0.924
499	IS1232	5	0.996	0.001	0.003
500	IS1234	5	0.995	0.001	0.004
501	IS1235	5	0.995	0.001	0.004
502	IS1236	5	0.995	0.001	0.003
503	IS1237	5	0.995	0.001	0.004
504	IS1238	5	0.906	0.001	0.092
505	IS1239	5	0.996	0.001	0.003
506	IS1240	5	0.996	0.001	0.003
507	IS1241	5	0.995	0.001	0.004
508	IS1242	5	0.569	0.002	0.429
509	IS1244	5	0.890	0.001	0.109
510	IS1245	5	0.993	0.001	0.006
511	IS1246	5	0.995	0.001	0.004
512	IS1247	5	0.993	0.001	0.006
513	IS1248	5	0.995	0.001	0.004
514	IS1249	5	0.995	0.001	0.004
515	IS1250	5	0.995	0.001	0.003
516	IS1251	5	0.995	0.001	0.004
517	IS1252	5	0.995	0.001	0.004
518	IS1253	5	0.996	0.001	0.003
519	IS1254	5	0.995	0.001	0.004
520	IS1255	5	0.994	0.001	0.005
521	IS1256	5	0.848	0.001	0.151
522	IS1257	5	0.996	0.001	0.003
523	IS1258	5	0.996	0.001	0.003
524	IS1259	5	0.924	0.001	0.075
525	IS1260	5	0.996	0.001	0.003
526	IS1272	5	0.991	0.001	0.007
527	IS1273	5	0.995	0.001	0.004
528	IS1274	5	0.995	0.001	0.004
529	IS1275	5	0.996	0.001	0.003
530	IS1276	5	0.993	0.001	0.006

Sample No.	ID	Population	*K* 1	*K* 2	*K* 3
531	IS1277	5	0.993	0.001	0.006
532	IS1278	5	0.996	0.001	0.003
533	IS1279	5	0.995	0.001	0.004
534	IS1280	5	0.996	0.001	0.003
535	IS1281	5	0.995	0.001	0.004
536	IS1282	5	0.995	0.001	0.004
537	IS1283	5	0.995	0.001	0.004
538	IS1284	5	0.996	0.001	0.003
539	IS1285	5	0.996	0.001	0.003
540	IS1286	5	0.996	0.001	0.003
541	IS1287	5	0.995	0.001	0.004
542	IS1288	5	0.672	0.001	0.327
543	IS1289	5	0.995	0.001	0.004
544	IS1290	5	0.995	0.001	0.004
545	IS1291	5	0.995	0.001	0.004
546	IS1292	5	0.995	0.001	0.004
547	IS1293	5	0.995	0.001	0.004
548	IS1294	5	0.995	0.001	0.004
549	IS1295	5	0.996	0.001	0.003
550	IS1296	5	0.995	0.001	0.004
551	IS1297	5	0.994	0.001	0.005
552	IS1298	5	0.996	0.001	0.003
553	IS1299	5	0.992	0.001	0.007
554	IS1300	5	0.995	0.001	0.004
555	IS1301	5	0.996	0.001	0.003
556	IS1302	5	0.953	0.002	0.045
557	IS1303	5	0.996	0.001	0.003
558	IS1304	5	0.995	0.001	0.004
559	IS1305	5	0.994	0.001	0.005
560	IS1306	5	0.995	0.001	0.004
561	IS1307	5	0.996	0.001	0.003
562	IS1308	5	0.994	0.001	0.005
563	IS1309	5	0.893	0.001	0.106
564	IS1311	5	0.995	0.001	0.004
565	IS1312	5	0.996	0.001	0.003
566	IS1314	5	0.986	0.010	0.003
567	IS1315	5	0.989	0.001	0.009
568	IS1316	5	0.994	0.001	0.005
569	IS1317	5	0.995	0.001	0.004
570	IS1318	5	0.995	0.001	0.004
571	IS1319	5	0.994	0.001	0.005
572	IS1320	5	0.991	0.001	0.008
573	IS1321	5	0.995	0.001	0.004
574	IS1322	5	0.995	0.001	0.003
575	IS1323	5	0.995	0.001	0.004
576	IS1324	5	0.995	0.001	0.003
577	IS1326	5	0.994	0.001	0.005
578	IS1327	5	0.988	0.001	0.011
579	IS1328	5	0.994	0.001	0.005
580	IS1329	5	0.972	0.001	0.027
581	IS1330	5	0.995	0.001	0.004
582	IS1331	5	0.996	0.001	0.003
583	IS1332	5	0.995	0.001	0.004
584	IS1337	5	0.929	0.001	0.069
585	K7876	5	0.995	0.001	0.004
586	K7878	5	0.994	0.001	0.005
587	K7879	5	0.995	0.001	0.004
588	K7880	5	0.995	0.001	0.004
589	K7881	5	0.995	0.001	0.004
590	K7882	5	0.995	0.001	0.004
591	K7883	5	0.971	0.003	0.026
592	K7884	5	0.996	0.001	0.003
593	K7885	5	0.992	0.001	0.007
594	K7886	5	0.971	0.003	0.026
595	K7887	5	0.994	0.001	0.005
596	K7888	5	0.995	0.001	0.004
597	K7889	5	0.920	0.001	0.079
598	K7890	5	0.996	0.001	0.003
599	K7891	5	0.996	0.001	0.003
600	K7892	5	0.995	0.001	0.004
601	K7893	5	0.962	0.009	0.029
602	BU001	6	0.006	0.001	0.993
603	LM001	6	0.008	0.002	0.990
604	LM002	6	0.003	0.001	0.996
605	LM003	6	0.003	0.001	0.996
606	LM004	6	0.003	0.001	0.996
607	LM005	6	0.003	0.001	0.996
608	LM006	6	0.007	0.001	0.992
609	LM007	6	0.003	0.001	0.996
610	LM008	6	0.003	0.001	0.996
611	LM009	6	0.007	0.001	0.992
612	LM010	6	0.010	0.002	0.988
613	LM011	6	0.015	0.002	0.983
614	LM012	6	0.004	0.001	0.995
615	LM013	6	0.011	0.002	0.988
616	K7894	7	0.993	0.003	0.004
617	**K7895**	**7**	**0.905**	**0.086**	**0.010**
618	K7896	7	0.968	0.028	0.004
619	**K7897**	**7**	**0.904**	**0.082**	**0.014**
620	YPM14723	8	0.002	0.997	0.001

Sample No.	ID	Population	*K* 1	*K* 2	*K* 3
621	YPM14724	8	0.001	0.998	0.001
622	YPM14725	8	0.001	0.997	0.001
623	YPM14726	8	0.002	0.996	0.002
624	YPM14727	8	0.002	0.995	0.002
625	YPM14728	8	0.001	0.998	0.001
626	YPM14729	8	0.001	0.998	0.001
627	YPM14730	8	0.001	0.998	0.001
628	YPM14731	8	0.004	0.994	0.002
629	YPM14732	8	0.001	0.998	0.001
630	YPM14733	8	0.004	0.993	0.004
631	YPM14734	8	0.001	0.997	0.001
632	YPM14736	8	0.001	0.994	0.005
633	YPM14737	8	0.001	0.998	0.001
634	YPM14738	8	0.001	0.997	0.001
635	YPM14739	8	0.001	0.998	0.001
636	YPM14740	8	0.002	0.997	0.001
637	YPM14742	8	0.001	0.998	0.001
638	YPM14743	8	0.002	0.997	0.001
639	YPM14744	8	0.002	0.997	0.001
640	YPM14745	8	0.001	0.998	0.001
641	YPM14746	8	0.002	0.997	0.001
642	YPM14747	8	0.001	0.997	0.001
643	YPM14748	8	0.001	0.998	0.001
644	YPM14749	8	0.001	0.998	0.001
645	YPM14750	8	0.002	0.997	0.001
646	YPM14751	8	0.002	0.997	0.001
647	YPM14752	8	0.001	0.997	0.001
648	YPM14753	8	0.001	0.998	0.001
649	YPM14754	8	0.002	0.997	0.001
650	YPM14755	8	0.001	0.998	0.001
651	YPM14756	8	0.002	0.997	0.001
652	YPM14719	8	0.001	0.997	0.001
653	YPM14720	8	0.001	0.997	0.001
654	YPM14721	8	0.001	0.997	0.001
655	YPM14722	8	0.001	0.998	0.001
656	YPM14757	8	0.002	0.996	0.002

indication of selection being a differentiating factor but the distance and isolation would be expected to drive genetic drift. Slightly elevated relatedness estimates suggest that future generations within both populations could face unavoidable mating of related individuals and the potential consequences of inbreeding. Genetic augmentation should be considered to offset these potential problems, whether by reintroduction from captive populations or by translocation between the populations. The most difficult constraint for successful conservation is securing the necessary funding to engage and monitor the programs. Whether genetic mixing between the two extant populations, augmentation from captive collections, or reintroduction of headstarted or captive born candidates is decided upon, funding will be crucial to monitor the success of the effort and protect remaining habitats for the future of the species.

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Filipino Abstract: Limitado lamang ang kaalaman na mayroon ukol sa Philippine Crocodile (*Crocodylus mindorensis*), lalo na sa antas o lebel ng genetic diversity na mayroon ito kumpara sa iba pang uri ng buwaya o kahit mismo sa iba't-ibang populasyon ng Philippine crocodile sa bansa. Sa kasalukuyan, dalawang likas na populasyon na lamang ng Philippine crocodile ang matatagpuan sa ilang, at ang potensyal ng mababang antas ng genetic diversity na maaring matagpuan sa mga natitirang populasyon nito ay nagdudulot ng pangamba sa kanilang pangmatagalang kabutihan. Sa artikulong ito, aming sinuri ang 619 na Philippine Crocodile gamit ang labing-isang microsatellite markers at inihambing ang mga ito sa apat na pangkat na impormasyon mula sa ibang uri o species ng buwaya. Ang pagkakaibang genetiko ng dalawang natitirang populasyon mula sa isla ng Luzon at Mindanao na kumakatawan sa sukdulang distribusyon ng buwayang ito sa Pilipinas, ay waring dulot ng genetic drift at hindi seleksyon. Aming natuklasan na ang dalawang natitirang populasyon sa ilang ng Philippine Crocodile ay may mas mababang genetic diversity at effective population sizes kumpara sa ibang uri ng buwaya. Ang 57 hybrid na buwaya na natagpuan sa isang naunang pag-aaral ay muling napatotohanan na hybrid nga sa pag-aaral na ito gamit ang dalawampung microsatellite loci. Ganoon pa man, ang panahon na nangyari ang hybridization at kung gaano ito kalawak sa populasyon ng Philippine crocodile ay kailangan pa ng pagsisiyasat. Sa artikulong ito, aming minumungkahi na ang 57 hybrids na natagpuan ay binubuo ng isang unang henerasyon na supling ng lalaking C. mindorensis at babaeng C. porosus, at ang natitirang 56 na hybrid ay mga backcross na buwaya. Ang hybridization na natagpuan ay waring limitado lamang sa koleksyon ng Palawan Wildlife Rescue & Conservation Centre (PWRCC).

Author Contribution: Ma. Rheyda Hinlo was involved with the data generation and sample collection in the Philippines and was involved in every step. John A. G. Tabora was also involved with data generation especially the sequence data. Carolyn A. Bailey was involved with the sample acquisition from the outgroup crocodile samples, and generated the data on these samples. Steve Trewick, Glenn Rebong, Merlijn van Weerd, and Cayetano Pomares, provided overall project expertise of the Philippines and direction and academic rigour to the overall project for the participating student authors, and participated significantly in the final drafts of the manuscript. Shannon Engberg provided supervision and direction to the overall data generation and of the study. Dr. Brenneman was responsible for developing the collaborations with the Republic of the Philippines Department of Natural Resources' Protected Areas and Wildlife Bureau, the Palawan Wildlife Rescue and Conservation Center, corporate and private owners of the Crocodylus mindorensis individuals and archived samples, and for the collection of the *C. porosus* samples on Mindanao. He selected and performed the genetic analyses of the microsatellite data, the interpretations of the results, and wrote the majority of the manuscript. Edward Louis organized the collection of the majority of the outgroup crocodile samples, and was primary supervisor in the project overall design and the overall organization of the manuscript, including the revisions.

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