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Conservation genetics of harvested river turtles, *Podocnemis expansa* and *Podocnemis unifilis*, in the Peruvian Amazon: All roads lead to Iquitos

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Abstract
We present a mtDNA analysis of *Podocnemis expansa* ($n = 81$) and *Podocnemis unifilis* ($n = 228$) turtles traded in Peru to evaluate the potential origin of these animals. In particular, we were interested in the relationship between samples reported in the Iquitos markets (IMs) and a Pacaya Samiria Natural Reserve (PSNR) where illegal hunting is presumed. Our mtDNA data showed that, for both species, all haplotypes found within the PSNR were observed in the IM, and that these markets also displayed haplotypes not documented in the reserve. This suggests that the IMs are recipients of *Podocnemis* turtles from within and outside the PSNR. The fact that most of the haplotype diversity observed in the markets was not found within the PSNR strongly suggests that *Podocnemis* genetic diversity is exploited in areas where conservation actions are limited. Hence, we recommend expanding *Podocnemis* conservation efforts outside of protected areas.

Keywords: *Amazon, conservation genetics,* Podocnemis, *turtles, urban markets, wildlife harvesting*
Abbreviations: *CITES, Convention on International Trade in Endangered Species of Wild Fauna and Flora; IUCN, International Union for the Conservation of Nature; INRENA, National Resources Institute from Peru; PSNR, Pacaya Samiria National Reserve; PPUARS, Pacaya-Puinahua-Ucayalli-Amazon River System*

Introduction

The Giant South American Turtle (*Podocnemis expansa* Schweigger, 1812) and the Yellow-spotted Sideneck Turtle (*Podocnemis unifilis* Troschel, 1848) inhabit three of the main hydrological systems in South America: the Amazon, the Orinoco, and the Essequibo or Magdalena river basins (Pritchard et al. 1984; Ernst and Barbour 1989). *P. unifilis* is considered vulnerable by the International Union for the Conservation of Nature (IUCN 2009). *P. expansa* is currently classified as low-risk conservation dependent; however, its situation in the wild requires urgent re-evaluation (IUCN 2009). Both species are regulated under Appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES 2010) and listed as endangered under the US Endangered Species Act (USFWS 2010). Currently, the distribution of the natural populations of both species is limited mainly to protected areas (Hernandez and Espín 2003;

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Coway-Gomez 2007; Kemenes and Pezzuti 2007). These species have been severely impacted by habitat destruction and centuries of overexploitation due to their use in local communities as sources of food, raw materials for handcrafts, and to obtain profits from wildlife trade (Moll and Moll 2004).

Efforts for protecting *Podocnemis* species have been implemented since the 1970s (Ojasti 1967; Mittermeier 1978; Smith 1979). Conservation programs have focused mainly on the protection of nesting beaches, nest relocation, and headstarting programs (Soini 1999; Caputo et al. 2005; Hernandez and Espín 2006; Jaffe et al. 2008). Although important results have been achieved through the implementation of these programs, legal and illegal harvesting of adults, juveniles, and eggs is still prevalent throughout their entire current range (Hernandez and Espín 2003; Kemenes and Pezzuti 2007). Urban trade of these species has been documented but the impact on their natural populations is unknown (Coway-Gomez 2007). Restriction of conservation activities mainly to protected areas may be rendered meaningless by harvesting of the same populations in adjacent unprotected areas, especially for migratory species, such as these freshwater turtles. For these species, genetic studies are an important tool for assessing their population connectivity, for improving our understanding of the role of biogeographic processes on their genetic structure, and for evaluating the potential genetic and demographic impacts of harvesting (DeSalle and Amato 2004).

There are a few published population genetic studies of *Podocnemis* turtles. Two studies evaluated the population structure of *P. expansa* and *P. unifilis* throughout their range, in part overlapping with our study area (Bock et al. 2001; Pearse et al. 2006; Fantin et al. 2007; Escalona et al. 2009). These studies identified significant differences in haplotype frequencies among river basins in both species, suggesting the presence of semi-isolated populations in each major tributary. In contrast, turtles within each river basin typically lacked population structure and exhibited high gene flow. Despite the high connectivity within river basins, both studies showed evidence of genetic diversity loss and recent bottlenecks, and suggested there was potential for population fragmentation as a result.

Notwithstanding the knowledge about regional connectivity patterns of Podocnemis expansa, little is known about finer spatial scale movement patterns and resulting population structure for this and other Podocnemis species. In order to shed light on such within-basin scale of population structure for *P. expansa* and *P. unifilis* in the Peruvian Amazon, we monitored harvesting of both species along the Pacaya-Puinahua-Ucayalli-Amazon River System (PPUARS; Figure 1), from Iquitos (S3°45′; W73°15′), the largest city in the Peruvian Amazonia, to the Pacaya Samiria Natural Reserve (PSNR; S5°26′; W74°34′), the largest protected area in Peru. Although the PSNR has been the focus of most of the local conservation activities protecting *Podocnemis* turtles since the 1970s, continuous harvesting has taken place in the area and its surroundings since the nineteenth century (von Humboldt and Bonpland 1941; Smith 1979). Most of the natural resources exploited regionally are concentrated in Iquitos. This city is the center of an urban network of smaller urban areas and hundreds of rural settlements connected within the Amazon River hydrological system. Hence, we assumed that by sampling Iquitos markets (IMs), it would be possible to obtain a collection of *Podocnemis* turtle populations inhabiting the northeast region of the Peruvian Amazon, and that their populations and associated genetic diversity would be represented proportionally to the level of harvesting pressure in each locality. The objectives of our study were (a) to evaluate the population structure and genetic diversity of *Podocnemis* samples collected along the PPUARS, (b) to evaluate whether the IMs receive specimens from outside the PPUARS, and (c) to estimate a set of demographic parameters associated with population size fluctuations of *Podocnemis* turtles in the study area.

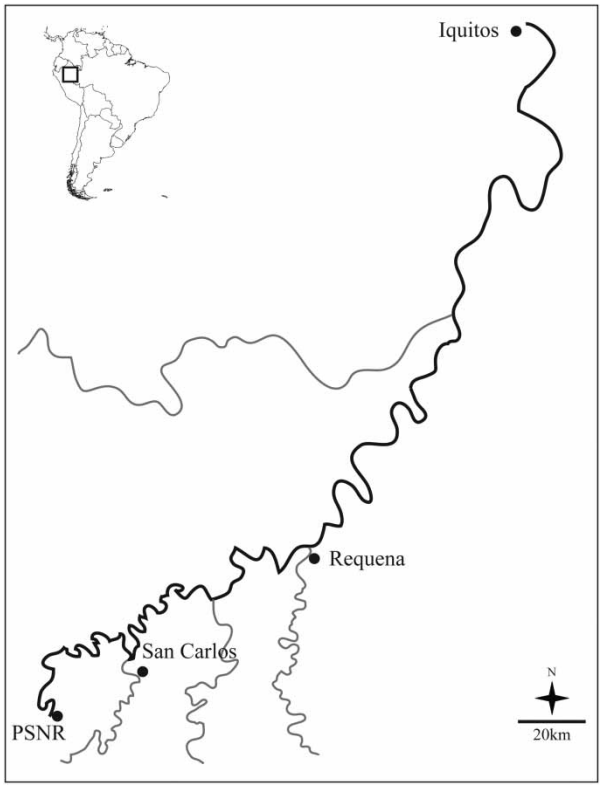

Figure 1. Map illustrating the PPUARS (dark line) and the localities (black dots) where the *Podocnemis* turtle samples were collected. Grey lines depict other important rivers associated to the system studied here.

Table I. Polymorphic sites and haplotype frequency of *P. expansa* mtDNA-CR.

	Nucleotide position												
Haplotype	3	4	78	79	153	154	196	415	569	763	807	829	Haplotype frequency
Pe-4	–	–	T	A	T	A	A	C	A	T	T	A	43*
Pe-2	T	A	–	–	·	·	·	·	·	·	·	·	13
Pe-5	–	–	–	–	·	·	·	·	·	·	·	·	12
Pe-1	T	A	·	·	·	·	·	·	·	·	·	·	4
Pe-6	·	·	T	A	·	·	·	T	·	·	·	·	1
Pe-7	·	·	·	·	·	·	T	·	G	·	G	G	1
Pe-8	?	?	T	A	·	·	·	·	·	C	·	·	1
Pe-3	?	?	?	?	·	·	·	·	·	·	·	·	6

Notes: Haplotype names correspond to designations used in Figure 2a. Haplotypes with missing data (i.e. Pe-3 and 8) were not included in the analysis of population structure and detection of population size expansion; *Three of these samples were collected in the PSNR.

Materials and methods

Sample collection and laboratory techniques

Sampling took place during the regional nesting period of both *P. expansa* and *P. unifilis*. Between June and September of 2007 and 2008, blood, skin, or muscle tissue samples of 81 *P. expansa* and 228 *P. unifilis* were collected from captive animals in the IMs and from the PSNR (Figure 1 and Table I). *P. unifilis* samples were also obtained from two additional localities between Iquitos and the PSNR, Requena and San Carlos, where turtles are locally harvested from the surrounding waters (Table II). Wild turtles were captured in oxbow lakes, marked with scale notches to avoid duplicate sampling, and released immediately after sampling. Blood samples were collected from the femoral sinus using sterilized 5 ml syringes and $1.5' \times 21$ gauge needles following standard procedures (Barrows et al. 2004; Campbell 2004). Blood samples were kept to a maximum 0.8% of the animal's weight (100 g of body weight is approximately equal to 0.8 ml of blood), but never exceeded 5 ml of total sample for each individual. All samples were preserved in 95% ethanol, stored at room temperature prior to arrival and processed at the laboratory and at −80°C thereafter. Collection and transportation of samples were conducted under appropriate National Resources Institute from Peru (INRENA), US Fish and Wildlife Services, and CITES import and export permits.

Total genomic DNA was extracted using the DNeasy Blood and Tissue Kit (QIAGEN, Germantown, MD,

Table II. Polymorphic sites and haplotype distribution of *P. unifilis* mtDNA-CR.

	Nucleotide position															Haplotype frequency				
Haplotype	4	37	39	135	182	215	220	302	303	367	381	461	564	605	618	Total	IMs	Requena	San Carlos	PSNR
Pu-1	T	A	–	T	A	A	T	G	T	A	T	G	G	–	A	152	129	6	3	14
Pu-9	C	·	–	·	·	·	·	·	·	·	·	·	·	A	–	14	7	2	1	4
Pu-20	·	·	A	·	·	·	·	·	·	·	·	·	·	–	·	12	9	1		2
Pu-3	·	·	–	·	·	·	·	·	·	·	·	·	·	A	–	8	7		1	
Pu-2	C	·	–	·	·	·	·	·	·	·	·	·	·	–	·	5	5			
Pu-8	·	·	–	·	·	·	·	·	·	·	·	·	C	–	·	5	5			
Pu-14	·	·	–	G	·	·	·	·	·	·	·	·	·	–	·	5	5			
Pu-17	·	·	–	·	T	·	·	·	·	·	·	·	·	–	·	2	2			
Pu-25	·	·	–	·	·	·	·	·	·	·	·	A	·	–	·	2	2			
Pu-26	·	C	–	·	·	·	·	·	·	·	·	·	·	–	·	2	2			
Pu-28	·	·	–	·	·	·	·	·	·	G	·	·	·	A	–	2	2			
Pu-5	·	·	–	·	·	·	·	·	C	·	·	·	·	–	·	1	1			
Pu-23	C	·	–	·	·	·	C	·	·	·	·	·	·	A	–	1	1			
Pu-15	·	·	–	·	·	·	·	T	·	·	·	·	·	A	·	1	1			
Pu-24	·	·	–	·	·	·	·	·	·	·	·	·	·	–	·	1	1			
Pu-37	·	·	–	·	·	·	·	·	·	G	C	·	·	A	–	1				1
Pu-6	?	·	–	·	·	·	·	·	·	·	·	·	·	–	·	6	4	1		1
Pu-21	?	·	–	·	·	·	·	·	·	·	·	·	·	A	–	1	1			
Pu-11	?	?	?	·	·	·	·	·	·	·	·	·	·	–	·	2	1			1
Pu-16	?	?	?	·	·	·	·	·	·	·	·	A	·	–	·	2	2			
Pu-34	?	?	?	·	·	·	·	·	·	·	·	·	·	A	–	1		1		
Pu-36	?	?	?	·	·	·	·	·	·	·	·	·	·	?	?	1				1
Pu-30	·	·	A	·	·	·	·	·	·	·	·	·	?	?	?	1	1			

Notes: Haplotype names correspond to designations used in Figure 2b. Haplotypes with missing data (i.e. Pu-11, 16, 30, 34, and 36) were not included in the analysis of population structure and detection of population size expansion.

USA) following manufacturer's instructions. A 1120-bp DNA fragment of the *P. expansa* mitochondrial control region (mtDNA-CR) was amplified using two sets of primers that generate overlapping sequences: Pro (5′-CCCATCACCCACTCCCAAAGC-3′) – DRL (5′-GGGATGCTGGTTTCTTGAG-3′) and PodF (5′-TAATCTATCGCATCTTCAG-3′) – CSB (5′-TTATAGTGCTCTTCCCCATATTATG-3′; Pearse et al. 2006). A fragment of 630 bp of the *P. unifilis* mtDNA-CR was amplified using Pro-DRL and a set of primers, CR2F (5′-AGCCTTCGTGG-TCCTAGCGGT-3′) – CR2R (5′-GGGGTCCGGG-GTGGGATCAT-3′), designed by our team using Primer3 (Rozen and Skaletsky 2000) as implemented in Geneious Pro v4.8.3 (Biomatters, Auckland, New Zealand; http://www.geneious.com).

All mtDNA-CR polymerase chain reaction (PCR) amplifications were done using 0.5 μM of each primer, 200 μM each dNTP, 0.5 U *Taq* DNA polymerase (Fisher BioReagents, Pittsburgh, PA, USA), 1.25 μl of 10 × buffer A (Fisher BioReagents), 1 μl (1–2 ng) template DNA, and ddH_2O to a total volume of 12.5 μl. The following PCR conditions were used: initial denaturation for 1 min at 94°C, followed by 25 cycles of 50 s at 94°C, 50 s at primer-specific annealing temperature (Pro-DRL: 56°C, CSB-PodF: 50°C, or CR2F-CR2R: 59°C), 1 min at 72°C, and final extension for 15 min at 72°C. PCR products were cycle-sequenced in forward and reverse directions with dye-labeled terminators using the BigDye chemistry kit v3.1 and conditions recommended by the manufacturer [Applied Biosystems, Inc. (ABI), Carlsbad, CA, USA]. The thermal profile was used as follows: initial denaturation for 5 min at 94°C followed by 30 cycles of 10 s at 96°C, 5 s at 50°C, and final extension for 4 min at 60°C. All samples were then cleaned up by filtration in a matrix of Sephadex/water or alternatively by ethanol precipitation, and analyzed using an ABI 3730xl DNA Analyzer. Sequences for each individual were assembled and edited in Geneious, and then aligned in Geneious using MUSCLE v3.6 (Edgar 2004; maximum number of iterations set at 10).

Genetic diversity estimates

For both study species, sequences were collapsed into haplotypes and estimations of haplotype diversity, H_d (Nei 1987); the mean number of pairwise differences among sequences, k (Kimura 1980; Tajima 1983); nucleotide diversity, π (Nei et al. 1975; Kimura 1980; Tajima 1983); and Watterson's estimate of nucleotide diversity, θ_W (Watterson 1975) were carried out in DnaSP v5.10.01 (Librado and Rozas 2009).

Analysis of population structure

We tested for population differentiation among the following sampling localities: PPUARS (i.e. PSNR + San Carlos), Requena, and the IMs. The diversity and geographic variation of CR haplotypes were quantified using F_{ST} and Φ_{ST} statistics (Weir and Cockerham 1984). Φ_{ST} was estimated using the Jukes–Cantor model accounting for multiple hits (Jukes and Cantor 1969).The significance of the observed Φ and F statistics was tested using 5000 random permutations of the data matrix. The extent of geographical heterogeneity in haplotype frequency distributions was further assessed through a χ^2-test conducted in DnaSP and an exact test of population differentiation (Raymond and Rousset 1995) implemented in Arlequin v3.5.1.2 (Excoffier et al. 2005). Relationships among the identified haplotypes were depicted using a median-joining network (Bandelt et al. 1999) implemented in Network v4.516 (http://www.fluxus-engineering.com).

Detection of population size expansion from DNA sequence data

We investigated recent demographic events in both *Podocnemis* species using Ramos-Onsins and Rozas' (2002) R_2, Tajima's (1989) D, and Fu's (1997) F_S test statistics as implemented in DnaSP. The R_2 and D statistics evaluate information concerning the mutation frequency spectrum based on the differences between the number of singleton mutations and the average number of nucleotide differences. When a population has grown recently, multiple singletons are present causing Tajima's D to acquire negative values and R_2 to be positive near zero. Fu's F_S statistic draws information from the haplotype distribution and acquires negative values with the excess of singleton mutations caused by a recent population expansion. Statistical significance was tested by means of 100,000 coalescent simulations. We evaluated these parameters for each of the sampling localities and in the IM. Assessing demographic stability/instability in this market would be analogous to pooling its collection sites together for the analysis. Because statistical tests of population size change are impacted by subpopulation structure (Tajima 1989), we employed such tests where genetic differentiation was not statistically evident.

Results

Genetic diversity

Eighty-one mtDNA-CR sequences were obtained for *P. expansa* (~976 bp length) and 228 sequences were obtained for *P. unifilis* (~630 bp length). *P. expansa* samples presented eight polymorphic/segregating sites and two dinucleotide insertion/deletions defining eight haplotypes (Table I), two of which had not been previously described (i.e. Pe-6 and 7). Haplotype Pe-4, the most common haplotype, was observed in

43 animals (40.6%), followed by Pe-2, found in 13 turtles (12.3%). All three samples from the PSNR belong to haplotype Pe-4 (Table I). Three haplotypes (i.e. Pe-6, 7, and 8) were found in only one turtle each.

From *P. unifilis*, six polymorphic sites and three single-nucleotide insertion/deletions defined 16 different haplotypes, nine of which have not been previously described (i.e. Pu-5, 15, 17, 23, 25, 24, 26, 28, and 37; Table II). The most common haplotype was Pu-1 (153 samples, 67.7%) found in all sampled populations at different frequencies. The second most abundant haplotype (Pu-9) was found only in 6.2% of the samples (14 turtles), observed also in all populations but in different frequencies. Several private haplotypes were found for *P. unifilis* (i.e. Pu-2, 5, 8, 14, 15, 17, 21, 23-26, and 28; Pu-37). Reference sequences for new haplotypes found in this study were deposited in GenBank (accessions: HQ641038-641070).

Haplotype diversity was similar in both species (Table III). *P. unifilis* sampled at Requena and San Carlos presented higher haplotype diversity than turtles sampled at other localities. In contrast, nucleotide diversity and nucleotide polymorphism were lower in *P. unifilis* than in *P. expansa* (Table III).

The median-joining network showed that most haplotypes for both species appeared to be related by a short distance to their nearest neighbor. The *P. expansa* network consisted of a simple cluster of haplotypes without reticulations (Figure 2a). In this network, the PSNR sequences could not be differentiated from those obtained in the IMs. Conversely, *P. unifilis* haplotypes grouped around a basal haplotype (Pu-1) with multiple short branches and a few distant haplotypes, such as Pu-11, 30, or 36 (Figure 2b). Geographically, all of the haplotypes observed in *P. expansa* and most for *P. unifilis* were found in the IMs (Figure 2). All haplotypes not previously reported in GenBank were collected from turtles sampled at these markets. In contrast, at least one *P. unifilis* haplotype was not observed at IMs.

Population structure

Limited differentiation was found among the samples collected along the PPUARS for *P. unifilis* (Table IV). F and Φ statistics were not significant among Requena, San Carlos, and the PSNR. However, all three localities showed significant F_{ST} values when compared to the IMs. Φ_{ST} was only significant for the PSNR–Iquitos comparison.

Population size changes estimated from sequence data

Significant negative values for Tajima's D were obtained for both *P. expansa* and *P. unifilis* showing an excess of rare polymorphisms (Table III); the latter species had significant values only when all samples were evaluated altogether. In contrast, only *P. unifilis* generated significant values for R_2 when evaluated as a single group and when the IMs and Requena were evaluated individually. No significant values were obtained for F_S.

Discussion

The results obtained in this study suggest that populations of *P. expansa* and *P. unifilis* are not genetically structured along the PPUARS, that the IMs receive *Podocnemis* turtles harvested in this river system as well as from other unknown areas likely surrounding Iquitos, and that populations of both *Podocnemis* species seem to have suffered a bottleneck in the study area and possibly throughout the Northeast Peruvian Amazonia.

The geographic pattern of genetic variation observed indicates that movement through the complex system of channels and lakes in this flooded forest region prevents the isolation of *Podocnemis* turtle populations and facilitates gene flow among groups of animals living in distant habitats (>50 km). Similar results have been obtained for other Amazonian freshwater vagile species such as *Arapaima gigas* (arapaima fish; Hrbek et al. 2005), *Trichechus inunguis*

Table III. Genetic diversity summary statistics and neutrality test statistics from mtDNA-CR sequence data in both *Podocnemis* species.

	Location	n	h	h_p	S	k	H_d	π	θ_W	F_S	D	R_2
P. unifilis	IMs	188	15	8	5	0.1	0.093	0.00023	0.00204	−8.672 ($P = 0.576$)	−1.721 ($P > 0.05$)	**0.026 ($P = 0.002$)**
	Requena	11	3	0	1	0.33	0.327	0.00056	0.00058	0.356 ($P = 0.456$)	−0.100 ($P > 0.10$)	**0.164 ($P = 0.043$)**
	San Carlos	5	3	0	1	0.4	0.4	0.00064	0.00077	0.090 ($P = 0.199$)	−0.817 ($P > 0.10$)	0.400 ($P = 0.746$)
	PSNR	24	4	1	2	0.17	0.083	0.00033	0.00105	−0.192 ($P = 0.427$)	−1.514 ($P > 0.10$)	0.199 ($P = 0.585$)
	Total	228	16	9	6	0.1	0.086	0.00023	0.00273	−11.001 ($P = 0.569$)	**−1.805 ($P < 0.05$)**	**0.022 ($P = 0.001$)**
P. expansa	IMs	78	8	5	6	0.154	0.076	0.00016	0.00125	−3.365 ($P = 0.519$)	**−2.046 ($P < 0.05$)**	0.079 ($P = 0.328$)
	PSNR	3	1	0	0	0	0	0	0	–	–	–
	Total	81	8	5	6	0.136	0.067	0.00014	0.00122	−3.600 ($P = 0.497$)	**−2.031 ($P < 0.05$)**	0.074 ($P = 0.332$)

Notes: Sequence length: *P. expansa* = 976 bp; *P. unifilis* = 630 bp. All values were calculated excluding sites with gaps. N, number of individuals; h, number of haplotypes; h_p, number of private haplotypes; S, number of segregating/polymorphic sites; k, average number of pairwise nucleotide differences; H_d, haplotype diversity; π, nucleotide diversity; and θ_W, Watterson's estimate of nucleotide diversity. Values for Fu's (1997) F_S, Tajima's (1989) D, and Ramos-Onsins and Rozas' (2002) R_2 statistics evaluating neutrality against population growth. Significant values are indicated in bold fonts ($P < 0.05$).

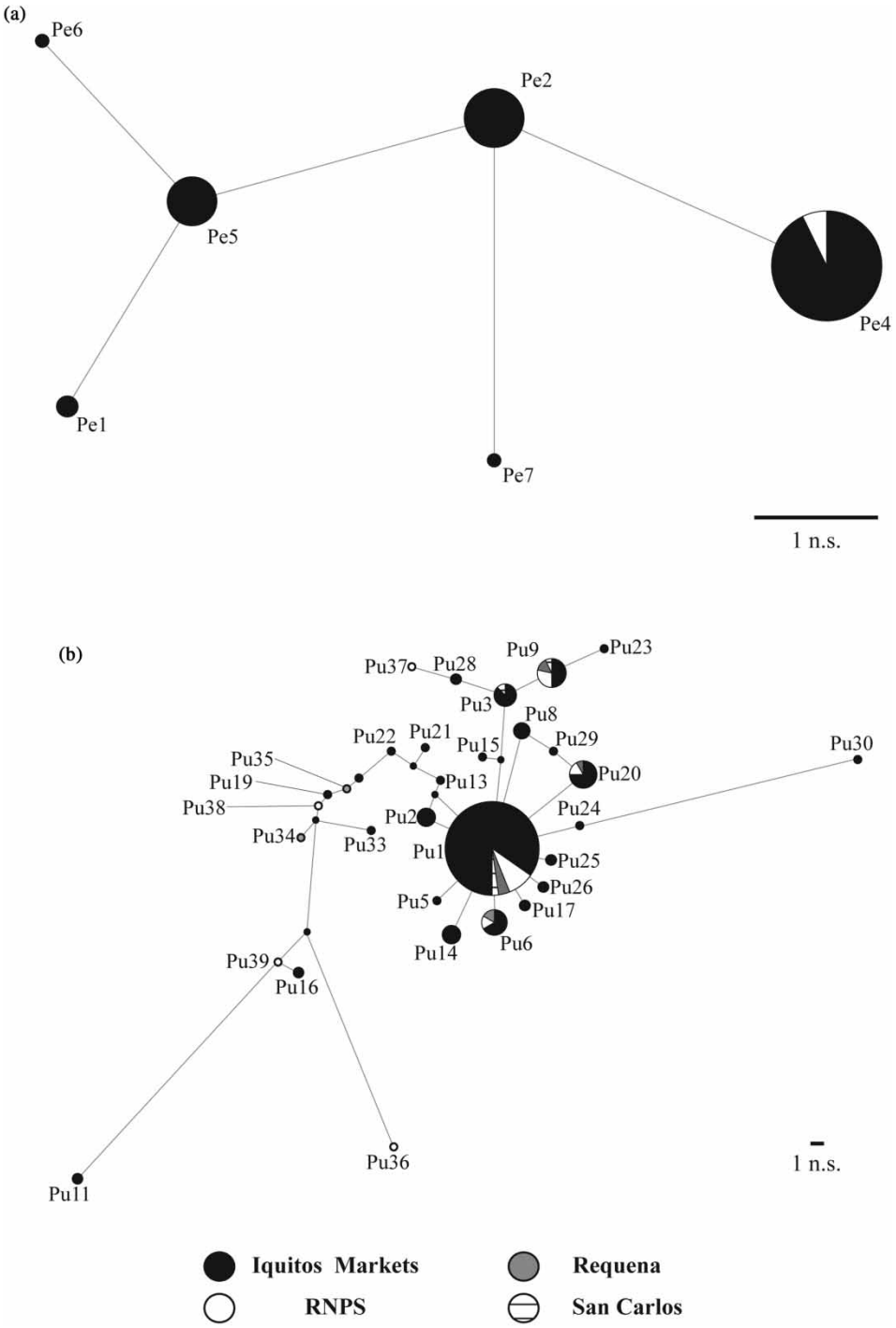

Figure 2. Median-joining network of mtDNA-CR haplotypes: (a) *P. expansa* and (b) *P. unifilis*. Haplotype names correspond to designations in Table I for *P. expansa* and Table II for *P. unifilis*. Branch lengths are proportional to number of nucleotide changes (scale bar = one nucleotide substitution, n.s.) and circle size corresponds to overall haplotype frequency.

(Amazonian manatee; Cantanhede et al. 2005), and *Inia geoffrensis* (Amazon River dolphin; Banguera-Hinestroza et al. 2002). Given that the Amazonian flood plain forms a largely continuous ecosystem during the wet season, one might expect little geographical structuring between localities in mobile species. However, the dispersal patterns of these species present can be influenced by directionality of water bodies, connectivity among lentic bodies by lotic components, and channel distances among water bodies (Willis et al. 2010). Population structure is thus expected to depend on the strength of physical geographic structuring in the river system.

Our estimates of haplotype diversity (h) and nucleotide diversity (π) are smaller than the diversity found by Engstrom (2003) for *P. unifilis* ($h = 0.086$ vs.

Table IV. Fixation indices between all sampling locations for *P. unifilis*.

	IMs	Requena	San Carlos
Requena	**0.405 (<0.001)**	–	
	0.091 (0.087)		
San Carlos	**0.414 (<0.001)**	0 (0.900)	–
	0 (0.303)	0 (0.590)	
PSNR	**0.421 (<0.001)**	0 (0.900)	0.900 (0.690)
	0.061 (0.023)	0 (0.970)	0 (0.770)

Notes: Top row shows F_{ST} and lower row shows Φ_{ST}. *P*-values are shown in parentheses. Significant values are indicated in bold fonts ($P < 0.05$).

0.405 and $\pi = 0.0002$ vs. 0.0014) and by Pearse et al. (2006) for *P. expansa* ($h = 0.067$ vs. 0.733 and $\pi = 0.0001$ vs. 0.0000). Our results constitute the smallest estimations of genetic diversity reported to date for *Podocnemis*' mitochondrial CR sequences. This is a striking result, especially when compared with data obtained by Engstrom in the same sampling areas in the PSNR. One explanation could be that our sample size is smaller than his ($n = 24$ vs. 468). Additionally, our samples were obtained only from the Pacaya River, one of the main river basins found in the protected area, whereas Engstrom obtained his samples from the three main river basins found in the PSNR: the Pacaya, Samiria, and Yanayacu rivers. To focus on the sampling effort in one river basin inside, PSNR could potentially reduce the genetic diversity observed in our study.

The median-joining network showed that most haplotypes for both species appeared to be related by a short genetic distance from their nearest neighbor haplotype. Similar networks have been observed previously in these species using mtDNA sequences (Engstrom 2003; Pearse et al. 2006). The IMs contained all of the haplotypes observed in *P. expansa* and most for *P. unifilis* (Figure 2 and Tables I and II). The genetic diversity observed in IMs for both *Podocnemis* species included all the haplotypes observed in the PPUARS, with the exception of Pu-37 observed in the PSNR, and several other haplotypes not observed in these river system. This observation suggests that the IMs are recipient of *Podocnemis* diversity harvested outside our study area, but the exact origin is unknown. These results are consistent with independent information of active *Podocnemis* harvesting in other river basins located in the PSNR, as well as the Napo, Marañon, and other regional river systems, which were not sampled in this study (personal observation, O.P.C.).

Neutrality test results (D, R_2, and F_S) suggest that genetic drift, the effect of which is exacerbated by population size reductions, is the primary force influencing genetic differentiation among populations of *P. unifilis* in the area of study. Tajima's D statistic was negative and significant ($P < 0.05$) for both species, indicating an excess of low- and high-frequency polymorphisms. Processes that could account for the observed patterns include recent selective sweeps or purifying selection with a recent population bottleneck eliminating low-frequency alleles. Such a recent bottleneck would not allow sufficient time to restore the mutation-drift equilibrium (Tajima 1989; Pearse and Crandall 2004; Hartl and Clark 2007). It is also important to mention that F_S and R_2 were not statistically significant for most of the localities, except for the IMs and Requena, where R_2 was significant. These two neutrality tests are the most powerful statistical evaluations of recent population growth events for small sample sizes and cases of small numbers of segregating sites (Ramos-Onsins and Rozas 2002), indicating no population growth following a bottleneck in our case. In our study, most sample sizes were small, as were the number of segregating sites (<25 individuals and 6 segregating sites, respectively), with the IM being the only exception to small samples. Such mutation-drift disequilibrium is expected considering independent demographic and census data indicating sustained population declines of *Podocnemis* over the last two centuries (Soini 1991, 1994; von Hildebrand et al. 1997).

Exploitation of *Podocnemis* turtles has been documented throughout Amazonia, and severe population declines have become evident since the middle of the twentieth century (Mittermeier 1978; Smith 1979). Despite regulation of hunting for trade, harvest is still observed throughout the year in urban markets, mainly during the local nesting season (Hernandez and Espín 2003; Coway-Gomez 2007). The importance of these species as a source of food and income for the local communities is considerable, but unregulated and unmonitored harvesting threatens their survival. Results from a survey conducted by our team (data not shown) showed depletion of *P. expansa* around Iquitos (in a radius of 90 km), whereas fishermen living around PSNR (>120 km away from Iquitos) still capture *P. expansa*. *P. unifilis* is still harvested near Iquitos, but fishermen have reported an increasing effort per turtle every year. If, as a consequence, stocks near major Amazonian cities became depleted, fishermen could have to move further away from traditional grounds to harvest *Podocnemis* turtles, mainly *P. expansa*. Our data from IM suggest that urban markets function as hubs receiving animals from a network of localities settled throughout the region. For instance, we see that every year *Podocnemis* turtles are harvested from areas with important genetic diversity. These animals represent the sustained harvesting effort of hundreds of fishermen scattered throughout the northeast region of the Peruvian Amazonia, an area covering approximately 350,000 km^2, almost one-quarter of the whole Peruvian territory.

In conclusion, conservation strategies have so far focused on protected areas. Given the linkages between such protected areas and urban hubs (evidenced by the lack of population structure found in *Podocnemis* turtles in the area of study), it is necessary to expand conservation efforts to nonprotected areas. In addition, our demographic data suggest that it is essential to promote protection of adults and to motivate the sustainable management and exploitation of these species, possibly through captive breeding.

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