

Genetic Population Structure of Two Threatened South American River Turtle Species, *Podocnemis expansa* and *Podocnemis unifilis*

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ABSTRACT. – An electrophoretic analysis of *Podocnemis expansa* from Peru and Brazil revealed significant allele frequency differences among populations at four of five polymorphic loci. One locus also demonstrated significant allele frequency differences among individuals between Brazilian nesting beaches separated by only 80 km. Electrophoretic analysis of *P. unifilis* from Peru and Brazil, as well as from three nesting sites along the Caquetá River in Colombia exhibited higher levels of genetic variability than *P. expansa*, differing significantly in allele frequencies at eight of nine polymorphic loci. The three Colombian sub-populations differed as much genetically from each other as they did from Peruvian and Brazilian populations. The results argue that current conservation management practices, such as protecting only a small number of nesting beaches within a reserve, may be inadequate, and that transplanting juveniles from their natal beaches may be inappropriate.

KEY WORDS. – Reptilia; Testudines; Pelomedusidae; Podocneminae; *Podocnemis expansa*; *Podocnemis unifilis*; turtle; genetics; population structure; geographic variation; conservation; management; Brazil; Colombia; Peru

River turtles of the genus *Podocnemis* (family Pelomedusidae) have been a traditional food source for the inhabitants of much of lowland South America for centuries (von Humboldt, 1859; Bates, 1863; Coutinho, 1868). For most species, ample habitat remains, but the three largest species in the genus (*P. expansa*, *P. unifilis*, and *P. lewyana*) are currently threatened by over-exploitation (Mittermeier, 1978; Smith, 1979; Johns, 1987; IUCN, 1989). *Podocnemis expansa* is particularly vulnerable to human hunting pressures due to its aggregated nesting behavior (Mosquera, 1945; Ramirez, 1956; Roze, 1964; Vanzolini, 1967; Ojasti, 1971; Alho and Padua, 1982a, b; von Hildebrand et al., 1988). The second largest species, *P. unifilis*, is sympatric with *P. expansa* throughout most of the Amazon and Orinoco drainages (Iverson, 1992), and becomes preferentially exploited when *P. expansa* is extirpated.

Information on the population structure of these two species is limited. Anecdotal evidence and some mark-recapture data suggest that female *P. expansa* in some populations may migrate hundreds of km each year between feeding ranges and nesting beaches (Roze, 1964; Ojasti, 1967; von Hildebrand et al., 1997). *Podocnemis unifilis* has less stringent nesting requirements than *P. expansa* (Foote, 1978; Fachin, 1992; Thorbjarnarson et al., 1993; Soini and Soini, 1995; Thorbjarnarson and Da Silveira, 1996) and radiotelemetry data from one population of *P. unifilis* (Bock et al., 1998) suggest that nesting females are less vagile.

In recent years, numerous management projects have been established for these two species in different areas (Brazil: Alho et al., 1979; Cantarelli and Herde, 1989; Cantarelli, 1997; Venezuela: Licata and Elgueza, 1997; Thorbjarnarson et al., 1997; Peru: Soini and Coppola, 1995;

Soini, 1996; Bolivia: Guayao, 1997; Colombia: Martinez and Muñoz, 1997; Páez and Bock, 1997). Most projects focus on guarding “key” nesting beaches and/or transferring nests to protect eggs from flooding and predation. In some projects, hatchling turtles are released immediately at the site of collection of the eggs, while other projects hold hatchlings for weeks or months before release. Several projects routinely transfer hatchlings considerable distances from their natal beaches for release in putatively suitable juvenile habitat.

The advisability of these management procedures depends to a great extent on the demographic and genetic structure of the populations being managed. What may be appropriate for one species or population may not work for another. Sites et al. (1999) recently compared three *P. expansa* populations in Brazil using microsatellite and mtDNA markers. In this study, we used protein electrophoresis to characterize the genetic population structure of *P. expansa* and *P. unifilis* populations from Brazil, Colombia, and Peru, thereby providing additional information on the population genetics of these highly exploited species.

METHODS

Recently hatched turtles were collected from three regions of South America (Fig. 1). Sample sizes and locations were the result of compromises between biological and statistical design considerations on the one hand and logistic feasibility and permit limitations on the other.

In Peru, 21 hatchling *P. expansa* were obtained randomly from a large pool of individuals from artificially incubated nests at the Cahuana Biological Station in the

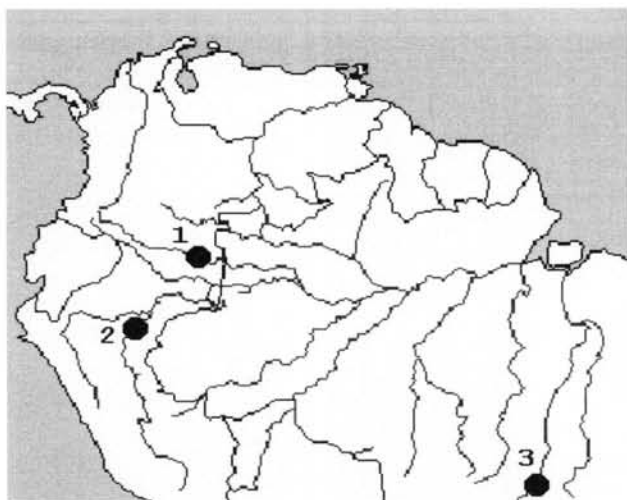


Figure 1. Location of the three geographic regions in South America from which juvenile *Podocnemis expansa* and *P. unifilis* individuals were collected. 1 = Río Caquetá, Colombia; 2 = Pacaya-Samiria reserve, Peru; 3 = Río Araguaia, Brazil.

Pacaya-Samiria Nature Reserve. Similarly, 24 hatchling *P. unifilis* were obtained randomly from nests incubated artificially by the Terecay Management Project of the Fundación Pronatura in the community of Manco Capac, located at the border of the reserve. All *P. expansa* nests had been collected from beaches on the Pacaya River, and all *P. unifilis* nests had been collected from beaches on the Yanayacú River (both tributaries of the Amazon drainage within the reserve).

In Brazil, turtles were obtained from a captive rearing facility maintained by the Centro Nacional dos Quelônios da Amazônia (CENAQUA). We obtained 28 *P. expansa* and 22 *P. unifilis* juveniles collected at a nesting beach on the Araguaia River ("Araguaia North"). This beach probably corresponds to the "Praia Rebojinho" beach of the "Ara-Praia" site studied by Sites et al. (1999). An additional 30 juvenile *P. expansa* were also collected from another nesting beach ("Araguaia South," or the "Ara-Faz" site of Sites et al., 1999) approximately 80 km upstream. As in Peru, the individuals were collected randomly from a large pool of individuals which had been obtained from numerous nests. However, the possibility exists that some individuals in each of these samples may have been siblings, which might introduce a bias into the estimates of the levels of genetic variability in these populations.

In Colombia, one hatchling *P. unifilis* was obtained from each of 27 nests from nesting beaches that were being monitored by the Proyecto Terecaya of the Fundación Natura at beaches on the Caquetá River in the Cahuinari National Park. Thirteen of these nests were from a beach at the confluence of the Caquetá and Bernardo rivers (Bernardo beach); the remaining 14 were from the Cahuinari beach, approximately 60 km upstream at the confluence of the Caquetá and Cahuinari rivers. In addition, park personnel provided one hatchling *P. unifilis* each from 5 different nests from Tres Islas beach located 100 km upriver from the Cahuinari beach.

Hatchlings were sacrificed to obtain muscle, liver, and blood samples. Most blood samples were separated into plasma and red blood cell fractions, and all samples were frozen in liquid nitrogen, transported on dry ice, and stored at -80°C at Ohio University. Samples were analyzed using standard horizontal starch gel electrophoresis (Murphy et al., 1996). The products of 23 presumptive structural loci were resolved, of which the following proved polymorphic in one or both species: Aconitate hydratase (ACOH, E.C. 4.2.1.3), Aspartate aminotransferase (AAT, E.C. 2.6.1.1), Esterase (EST), Fructose biphosphatase (FBP, E.C. 3.1.3.11), Fumarate hydratase (FUMH, E.C. 4.2.1.2), Glucose-6-phosphate isomerase (GPI, E.C. 5.3.1.9), Peptidase-B (PEP-B, E.C. 3.4.11.13), Phosphogluconate dehydrogenase (PGDH, E.C. 1.1.1.44), and Purine-nucleoside phosphorylase (PNP, E.C. 2.4.2.1). Allozyme data were analyzed using BIOSYS I (Swofford and Selander, 1981). Tests for deviations from Hardy-Weinberg genotype proportion expectations were conducted so as to achieve an experiment-wide significance level of $p < 0.05$ by adjusting the acceptance criterion level of tests at each locus using the sequential Bonferroni technique (Holm, 1979). Geographic and genetic distances between the sites were compared with a Mantel test (Mantel, 1967).

RESULTS

Five loci were polymorphic in *P. expansa* (0.95 common allele criterion). None of the 12 comparisons exhibited significant departures from Hardy-Weinberg expectations. Significant allele frequency heterogeneity was observed among the three populations at four loci (Table 1). Significant allele frequency differences were observed between the two Brazil sites at one locus (FBP, $\chi^2 = 9.15$, d.f. = 1, $p <$

Table 1. Allele frequencies at the three *Podocnemis expansa* populations sampled in Peru and Brazil, with values of P (percentage loci polymorphic) and H (mean heterozygosity) for each.

Locus	Peru			Brazil	
	Pacaya River	Araguaia North	Araguaia South	χ^2	p
ACOH					
A	0.361	0.659	0.583	7.42	<0.05
B	0.639	0.341	0.417		
EST					
A	0.789	1.000	1.000	22.35	<0.001
B	0.211	0.000	0.000		
FBP					
A	1.000	0.464	0.741	32.89	<0.001
B	0.000	0.536	0.259		
PEP-B					
A	0.952	0.881	0.817	4.18	n.s.
B	0.048	0.119	0.183		
PGDH					
A	0.150	0.433	0.536	12.24	<0.005
B	0.850	0.567	0.464		
mean n	20.7	20.6	28.6		
P	0.17	0.17	0.17		
H	0.043	0.072	0.078		
total χ^2				79.08	<0.001

Table 2. Unbiased Nei (1978) genetic distances between the three *Podocnemis expansa* populations sampled in Peru and Brazil (above the diagonal asterisks) and direct line geographic distances (km) between the sites (below the diagonal).

	Peru: Pacaya River	Brazil: Araguaia North	Brazil: Araguaia South
Peru: Pacaya River	****	0.022	0.013
Brazil: Araguaia North	2600	****	0.003
Brazil: Araguaia South	2600	80	****

0.005). There was no significant association between the magnitudes of the geographic and genetic distances among sites (Mantel, $r = 0.88$, $p > 0.10$; Table 2).

For *P. unifilis*, only one of the 21 comparisons for the nine polymorphic loci exhibited a significant departure from Hardy-Weinberg expectations (a deficiency of heterozygote individuals at the PNP locus in Brazil). Significant inter-population heterogeneity was detected among populations at eight loci (Table 3). A UPGMA cluster analysis of Nei (1978) genetic distances revealed a complex picture of differentiation among populations for *P. unifilis* (Fig. 2). Genetic variation was not statistically significantly related to geographic distances among the sites (Mantel, $r = -0.003$, $p > 0.10$; Table 4); as one Colombian population grouped with the Brazilian population and the remaining two Colombian populations grouped with the Peruvian population. However, the statistical power of both this and the *P. expansa* analyses were low. Sampling additional sites might well permit detection of significant geographic patterns in genetic structure for one or both species.

DISCUSSION

Despite evidence suggesting that *P. expansa* is a long-distance disperser (Roze, 1964; Ojasti, 1967; von Hildebrand et al., 1997), our results demonstrated significant regional genetic differences, as did the study by Sites et al. (1999). The suggestion of genetic differences among hatchlings obtained from nesting beaches some 80 km apart also implies either that adults in some populations may not migrate great distances, or that adults may wander extensively but return to breed at their natal beaches, as observed in sea turtles (Meylan et al., 1990; Bowen et al., 1992, 1993; Broderick et al., 1994; Allard et al., 1994; Bass et al., 1996; Encalada et al., 1996). Sites et al. (1999) also encountered significant genetic differences among three sites in the Araguaia River, Brazil, at one of the six microsatellite loci they examined.

In *P. unifilis*, inter-population differentiation was even more pronounced. Demes in Colombia separated by as little as 60 km exhibited genetic differences as great as those seen between populations separated by hundreds of km. In contrast to *P. expansa*, *P. unifilis* is less selective both in terms of its diet (Almeida et al., 1986; Fachin et al., 1995) and nesting microhabitat (Foote, 1978; Fachin, 1992; Thorbjarnarson et al., 1993; Soini and Soini, 1995; Thorbjarnarson and Da Silveira, 1996). This species may be

Table 3. Allele frequencies at the five *Podocnemis unifilis* populations sampled in Peru, Brazil, and Colombia.

Locus	Peru: Yanayacu River	Brazil: Araguaia North	Colombia:			χ^2	p
			Bernardo beach	Cahuinari beach	TresIsas beach*		
ACOH							
A	1.000	0.842	0.700	1.000	0.400		
B	0.000	0.158	0.300	0.000	0.600	17.74	<0.001
EST							
A	0.333	1.000	0.269	1.000	0.500		
B	0.667	0.000	0.731	0.000	0.500	71.85	<0.001
FBP							
A	1.000	0.909	1.000	1.000	1.000		
B	0.000	0.091	0.000	0.000	0.000	9.54	<0.050
PEP-B							
A	0.458	1.000	0.654	0.393	1.000		
B	0.542	0.000	0.346	0.607	0.000	35.15	<0.001
PGDH							
A	0.979	0.895	0.654	0.786	0.800		
B	0.000	0.105	0.346	0.214	0.200		
C	0.021	0.000	0.000	0.000	0.000	20.77	<0.005
AAT							
A	0.476	0.816	1.000	0.571	0.800		
B	0.524	0.184	0.000	0.429	0.200	25.49	<0.001
FUMH							
A	0.438	0.676	0.808	0.643	0.800		
B	0.563	0.324	0.192	0.357	0.200	11.04	<0.050
GPI							
A	1.000	0.977	1.000	1.000	1.000		
B	0.000	0.023	0.000	0.000	0.000	2.33	n.s
PNP							
A	1.000	0.786	1.000	1.000	1.000		
B	0.000	0.214	0.000	0.000	0.000	23.31	<0.001
mean							
n	23.9	21.1	12.7	14.0	5		
P	0.22	0.30	0.17	0.22			
H	0.071	0.059	0.065	0.069			
total							
χ^2						217.21	<0.001

* The Tres Islas beach site was excluded from the P, H, and χ^2 calculations due to the small sample size.

able to satisfy both needs within a much more restricted area. A preliminary radiotelemetry study of adult *P. unifilis* demonstrated that females dispersed little after nesting, until they moved into the *varzea* forests with the annual floods. One female relocated the following year when the river returned to its banks was first encountered almost exactly where she had disappeared into the forest nine months before (Bock et al., 1998). This evidence seems to suggest that, despite its wide geographic range, most *P. unifilis* populations may be comprised of multiple reproductively isolated demes, even in the absence of obvious dispersal barriers.

The levels of allozyme variability maintained by these populations of *Podocnemis* (proportion of polymorphic loci [P] varying from 0.17 to 0.30 and mean proportion of loci heterozygous per individual [H] varying from 0.043 to 0.075) were lower than those documented for populations of other freshwater turtles. Although this result could have been an artifact of possibly including siblings in the population samples, the concordance of observed genotype frequencies to those expected under Hardy-Weinberg equilibrium in both species, and for *P. unifilis* the similarity of the

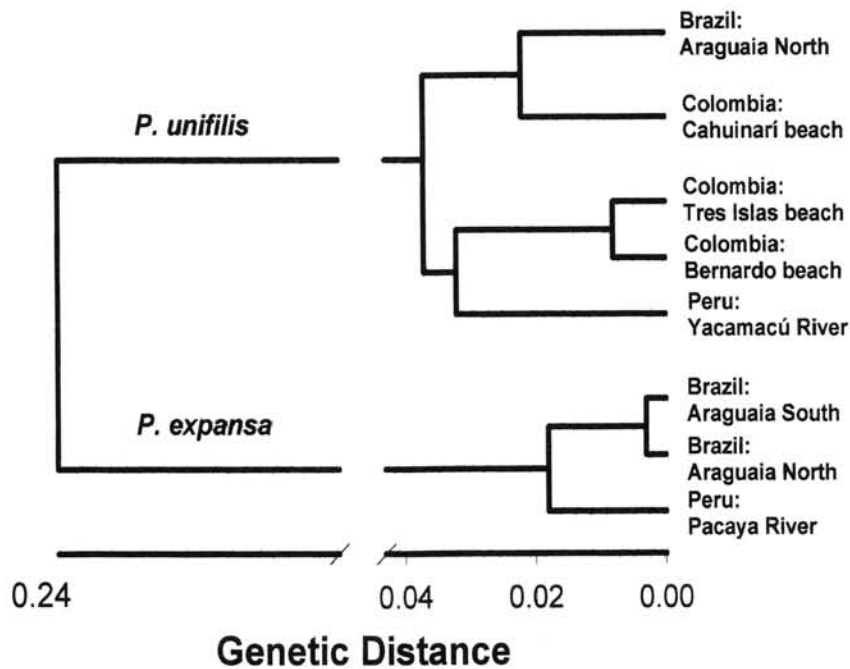


Figure 2. UPGMA cluster analysis of genetic distances between the three *Podocnemis expansa* populations sampled in Peru and Brazil and the five *Podocnemis unifilis* populations sampled in Peru, Brazil, and Colombia.

estimates of levels of genetic variability for the Brazilian, Peruvian, and Colombian populations (with the latter population sample comprised entirely on non-siblings), argues that these estimates are valid.

The low levels of genetic variability these populations express may be a reflection of their localized population structure, the exploitation they have faced in recent years, or both. The Peruvian population of *P. expansa* exhibited the lowest level of mean heterozygosity ($H = 0.043$) yet documented for a freshwater turtle population. This population has been estimated to contain only approximately 600 reproductive females (Soini, 1996) and has been maintained in recent years exclusively through artificial incubation of nests rescued from human depredation (Soini, 1995). However, more data on levels of genetic variation in other populations of this and other freshwater turtle species will be needed before the significance of these low levels of genetic variability may be confidently assessed.

Surprisingly few studies on allozyme variation in freshwater turtle populations have been conducted during the past three decades, in marked contrast to the situation for other vertebrate groups. This is especially true considering the

attention turtle biologists have given to intraspecific variation in life history characteristics. However, evidence for marked population structuring similar to our findings has been demonstrated with allozyme data for *Chrysemys picta* (Scribner et al., 1993) and *Trachemys scripta* (Scribner et al., 1984, 1986; Smith and Scribner, 1990). More recently, analyses of mtDNA restriction site and sequence data for freshwater turtles in the southeastern USA (Walker et al., 1995, 1997; Walker and Avise, 1998) also revealed extensive intraspecific geographic variation with strong local population structure. Thus, pronounced genetic differentiation among populations may be more typical of freshwater turtles than is generally assumed.

Until more information is available on the genetic population structure of freshwater turtle species, it would seem prudent for *Podocnemis* turtle management projects to refrain from releasing hatchlings long distances from their natal beaches. In addition, projects attempting to manage *P. expansa* should be aware that by holding hatchling turtles in captivity in attempts to prevent early juvenile mortality, they may also be disrupting key behavioral processes necessary for the development of typical migratory behavior at the time of first reproduction. Finally, *Podocnemis* management projects that focus on only one or a few nesting beaches in a region may not be providing sufficient protection to the overall local genetic diversity present in the regional population.

Table 4. Unbiased Nei (1978) genetic distances between the five *Podocnemis unifilis* populations sampled in Peru, Brazil, and Colombia (above the diagonal asterisks) and direct line geographic distances (km) between the sites (below the diagonal).

	Peru:	Brazil:	Colombia:		
	Yanayacú River	Araguaia North	Bernardo beach	Cahuinari beach	Tres Islas beach
Peru: Yanayacú River	****	0.046	0.028	0.023	0.040
Brazil: Araguaia North	2600	****	0.037	0.022	0.019
Colombia: Bernardo beach	600	2600	****	0.040	0.008
Colombia: Cahuinari beach	600	2600	60	****	0.045
Colombia: Tres Islas beach	600	2600	160	100	****

RESUMEN

Análisis electroforéticos de *P. expansa* de Brasil y Perú revelaron diferencias significativas en las frecuencias alélicas entre las poblaciones, para cuatro de cinco loci polimórficos. También demostramos diferencias significativas en las frecuencias alélicas de un locus entre individuos obtenidos

en diferentes playas de anidación en Brazil separadas por tan solo 80 km. Análisis electroforéticos de *P. unifilis* de Brazil, Perú, y de tres sitios de anidación a lo largo del río Caquetá en Colombia presentaron mayores niveles de variabilidad genética que *P. expansa*, difiriendo significativamente en las frecuencias alélicas de ocho de los nueve alelos polimórficos. Las tres subpoblaciones colombianas se diferencian en la misma magnitud unas de otras genéticamente, como lo hacen con respecto a las poblaciones de Perú y Brazil. Estos resultados indican que las prácticas de manejo conservacionista actuales, en las que se protegen únicamente algunas pocas playas de anidación dentro de las reservas, pueden estar siendo inadecuadas, y el transplante de juveniles desde sus playas natales a otros sitios podría ser inapropiado.

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LITERATURE CITED

- ALHO, C.J.R. AND PADUA, L.F.M. 1982a. Reproductive parameters and nesting behavior of the Amazon turtle *Podocnemis expansa* (Testudinata: Pelomedusidae) in Brazil. *Can. J. Zool.* 60:97-103.
- ALHO, C.J.R. AND PADUA, L.F.M. 1982b. Sincronia entre regime de vazante do rio e o comportamento de nidificação da tartaruga da Amazônia *Podocnemis expansa* (Testudinata: Pelomedusidae). *Acta Amazon.* 12:323-326.
- ALHO, C.J.R., CARVALHO, A.G., AND PADUA, L.F.M. 1979. Ecologia da tartaruga da Amazônia e avaliação de seu manejo na Reserva Biológica do Trombetas. *Brasil Forestal* 38:29-47.
- ALLARD, M.W., MIYAMOTO, M.M., BJORNDALE, K.A., BOLTON, A.B., AND BOWEN, B.W. 1994. Support for natal homing in green turtles from mitochondrial DNA sequences. *Copeia* 1994:34-41.
- ALMEIDA, S.S. DE, SÁ, P.G.S., AND GARCIA, A. 1986. Vegetais utilizados como alimento por *Podocnemis* (Chelonia) na região do baixo rio Xingu (Brazil-Para). *Bol. Mus. Para. Emilio Goeldi Ser. Bot.* 2:199-211.
- BASS, A.L., GOOD, D.A., BJORNDALE, K.A., RICHARDSON, J.I., HILLIS, A.M., HORROCKS, J.A., AND BOWEN, B.A. 1996. Testing models of female reproductive migratory behaviour and population structure in the Caribbean hawksbill turtle, *Eretmochelys imbricata*, with mtDNA sequences. *Molecular Ecol.* 5:321-328.
- BATES, H.W. 1863. *The Naturalist on the River Amazons*. John Murray, London, England, 383 pp.
- BOCK, B.C., PÁEZ, V.P., AND PÉREZ, N.F. 1998. Estudio preliminar con radiotelemetría sobre los desplazamientos de hembras de la tortuga *Podocnemis unifilis* en el río Caquetá, Amazonas, Colombia. *Actual. Biol.* 20:29-36.
- BOWEN, B.W., MEYLAN, A.B., ROSS, J.P., LIMPUS, C.J., BALAZS, G.H., AND AVISE, J.C. 1992. Global population structure and natural history of the green turtle (*Chelonia mydas*) in terms of matriarchal phylogeny. *Evolution* 46:865-881.
- BOWEN, B.W., AVISE, J.C., RICHARDSON, J.I., MEYLAN, A.B., MARGARITOU, D., AND HOPKINS-MURPHY, S. 1993. Population structure of the loggerhead turtle (*Caretta caretta*) in the northwest Atlantic Ocean and Mediterranean Sea. *Conserv. Biol.* 7:834-844.
- BRODERICK, D., MORITZ, C., MILLER, J.D., GUINEA, M., PRINCE, R.J., AND LIMPUS, C.J. 1994. Genetic studies of the hawksbill turtle: evidence for multiple stocks and mixed feeding grounds in Australian waters. *Pac. Conserv. Biol.* 1:121-131.
- CANTARELLI, V.H. 1997. The Amazon turtles: conservation and management in Brazil. In: Van Abbema, J. (Ed.). *Proceedings: Conservation, Restoration, and Management of Tortoises and Turtles - An International Conference*, New York: New York Turtle and Tortoise Society, pp. 407-410.
- CANTARELLI, V.H. AND HERDE, L.C. 1989. Projeto Quelônios da Amazônia. 10 Anos. Ministerio do Interior, Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis - IBAMA, Brasília, Brazil., 119 pp.
- COUTINHO, J.M. 1868. Sur les tortues de l'Amazone. *Bull. Soc. Imperiale Zoologique d'Acclimation* (2)5:147-166.
- ENCALADA, S.E., LAHANAS, P.N., BJORNDALE, K.A., BOLTON, A.B., MIYAMOTO, M.M., AND BOWEN, B.W. 1996. Phylogeography and population structure of the Atlantic and Mediterranean green turtle *Chelonia mydas*: A mitochondrial DNA control region sequence assessment. *Molecular Evol.* 5:473-483.
- FACHIN-T., A. 1992. Desove y uso de playas para nidificación de taricaya (*Podocnemis unifilis*) en el río Samiria, Loreto-Perú. *Boletín de Lima* 79:65-75.
- FACHIN-T., A., VOGT, R.C., AND DE FATIMA SOARES-G., M. 1995. Food habits of an assemblage of five species of turtles in the Rio Guapore, Rondonia, Brazil. *J. Herpetol.* 29:536-547.
- FOOTE, R.W. 1978. Nesting of *Podocnemis unifilis* (Testudines: Pelomedusidae) in the Colombian Amazon. *Herpetologica* 34:333-339.
- GUAYAO, E. 1997. Recuperación de la población de tortugas de río en la EBB en el río Maniquí. *Memorias - Primer Congreso Internacional. Investigación y Manejo en la Reserva de la Biosfera de la Estación Biológica del Beni: 10 Años de Aportes a la Gestión Ambiental Nacional*. La Paz, Bolivia: Artes Gráficas Latina, pp. 137-138.
- HOLM, S. 1979. A simple sequentially rejective multiple test procedure. *Scand. J. Statist.* 6:65-70.
- IUCN. 1989. *Tortoises and Freshwater Turtles: An Action Plan for their Conservation*. Gland, Switzerland: Tortoise and Freshwater Turtle Specialist Group, 47 pp.

- IVERSON, J.B. 1992. A revised checklist with distribution maps of the turtles of the world. Richmond, Indiana: Privately printed, 363 pp.
- JOHNS, A.D. 1987. Continuing problems for Amazon river turtles. *Oryx* 21:25-28.
- LICATA, L. AND ELGUEZABAL, X. 1997. Management plan for the giant Amazonian turtle, *Podocnemis expansa*, in De La Tortuga Arrua Wildlife Refuge, Orinoco River, Venezuela. In: Van Abbema, J. (Ed.). Proceedings: Conservation, Restoration, and Management of Tortoises and Turtles - An International Conference, New York: New York Turtle and Tortoise Society, pp. 171-173.
- MANTEL, N. 1967. The detection of disease clustering and a generalized regression approach. *Cancer Research* 27:209-220.
- MARTINEZ, E. AND MUÑOZ, D. 1997. El intercambio de saberes en una experiencia de manejo: la tortuga *Podocnemis expansa*, en el medio e bajo río Caquetá. Resúmenes, III Congreso Internacional sobre Manejo de Fauna Silvestre de la Amazonía. Santa Cruz, Bolivia: Museo Noel Kempff Mercado, pp. 124.
- MEYLAN, A.B., BOWEN, B.W., AND AVISE, J.C. 1990. A genetic test of the natal homing versus social facilitation models for green turtle migration. *Science* 248:724-727.
- MITTERMEIER, R.A. 1978. South America's river turtles: saving them by use. *Oryx* 14:222-230.
- MOSQUIERA-M, J.M. 1945. Las tortugas del Orinoco. Ensayos biológicos de la arrau (*Podocnemis expansa*). 3d Interam. Conf. Agr. Caracas 29:5-43.
- MURPHEY, R.W., SITES, J.W., JR., BUTH, D.G., AND HAUFLE, C.H. 1996. Proteins: Isozyme electrophoresis. In: Hillis, D.M., Moritz, C., and Mable, B.K. (Eds.). *Molecular Systematics*. Sunderland, Massachusetts: Sinauer Associates, pp. 51-120.
- NEI, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* 89:583-590.
- OJASTI, J. 1967. Consideraciones sobre la ecología y conservación de la tortuga *Podocnemis expansa* (Chelonia, Pelomedusidae). Atas do Simposio sobre a Biota Amazônica 7:201-206.
- OJASTI, J. 1971. La tortuga arrua del Orinoco. *Defensa de la Nat.* 1:3-9.
- PÁEZ, V.P. AND BOCK, B.C. 1997. Nesting ecology of the yellow-spotted river turtle in the Colombian Amazon. In: Van Abbema, J. (Ed.). Proceedings: Conservation, Restoration, and Management of Tortoises and Turtles - An International Conference, New York: New York Turtle and Tortoise Society, pp. 219-224.
- RAMIREZ-E., M.V. 1956. Estudio biológico de la tortuga "arrau" del Orinoco, Venezuela. *Agricultor Venezolano* 190:44-63.
- ROZE, J.A. 1964. Pilgrim of the river. *Nat. Hist.* 73:34-41.
- SCRIBNER, K.T., SMITH, M.H., AND GIBBONS, J.W. 1984. Genetic differentiation among local populations of the yellow-bellied slider turtle (*Pseudemys scripta*). *Herpetologica* 40:382-387.
- SCRIBNER, K.T., EVANS, J.E., MORREALE, S.J., SMITH, M.H., AND GIBBONS, J.W. 1986. Genetic divergence among populations of the yellow-bellied slider turtle (*Pseudemys scripta*) separated by aquatic and terrestrial habitats. *Copeia* 1986:691-700.
- SCRIBNER, K.T., CONGDON, J.D., CHESSER, R.K., AND SMITH, M.H. 1993. Annual differences in female reproductive success affect spatial and cohort-specific genotypic heterogeneity in painted turtles. *Evolution* 47:1360-1373.
- SITES, J.W., JR., FITZSIMMONS, N.N., DA SILVA, N.J., JR., AND CANTARELLI, V.H. 1999. Conservation genetics of the giant Amazon river turtle (*Podocnemis expansa*; Pelomedusidae) – inferences from two classes of molecular markers. *Chelonian Conservation and Biology* 3:454-463.
- SMITH, M.H. AND SCRIBNER, K.T. 1990. Population genetics of the slider turtle. In: Gibbons, J.W. (Ed.). *Life History and Ecology of the Slider Turtle*. Washington, DC: Smithsonian Institution Press, pp. 74-81.
- SMITH, N.J.H. 1979. Aquatic turtles of Amazonia: an endangered resource. *Biol. Conserv.* 16:165-176.
- SOINI, P. 1995. Investigación y manejo de la charapa (*Podocnemis expansa*) en 1993. In: Soini-N., P., Tovar-N., A., and Valdez-O., U. (Eds.). *Reporte Pacaya-Samiria: Investigaciones en la Estación Biológica Cahuana, 1979-1994*. Univ. Nac. Agraria La Molina, pp. 423-427.
- SOINI, P. 1996. Experiencias en el manejo de quelonios acuáticos en la Reserva Nacional Pacaya-Samiria, Perú. *Flora Fauna Areas Silvest.* 23:36-38.
- SOINI, P. AND CÓPULA, M. 1995. Estudio, reproducción, y manejo de los quelonios del género *Podocnemis* (charapa, cupiso, y taricaya) en la cuenca del río Pacaya, Loreto-Perú. In: Soini-N., P., Tovar-N., A., and Valdez-O., U. (Eds.). *Reporte Pacaya-Samiria: Investigaciones en la Estación Biológica Cahuana, 1979-1994*. Univ. Nac. Agraria La Molina, pp. 3-30.
- SOINI, P. AND DE SOINI, M. 1995. Un resumen comparativo de la ecología reproductiva de los quelonios acuáticos. In: Soini-N., P., Tovar-N., A., and Valdez-O., U. (Eds.). *Reporte Pacaya-Samiria: Investigaciones en la Estación Biológica Cahuana, 1979-1994*. Univ. Nac. Agraria La Molina, pp. 215-226.
- SWOFFORD, D.L. AND SELANDER, R.B. 1981. *Biosys I. A Computer Program for the Analysis of Allelic Variation in Population Genetic and Biochemical Systematics*. Champaign, Illinois: Illinois Natural History Survey, 43 pp.
- THORBJARNARSON, J.B. AND DA SILVEIRA, R. 1996. *Podocnemis unifilis* (yellow side-necked turtle). *Nesting. Herpetol. Review* 27:77-78.
- THORBJARNARSON, J.B., PEREZ, N., AND ESCALONA, T. 1993. Nesting of *Podocnemis unifilis* in the Capanaparo River, Venezuela. *J. Herpetol.* 27:344-347.
- THORBJARNARSON, J.B., PEREZ, N., AND ESCALONA, T. 1997. Biology and conservation of aquatic turtles in the Cinaruco-Capanaparo National Park, Venezuela. In: Van Abbema, J. (Ed.). Proceedings: Conservation, Restoration, and Management of Tortoises and Turtles - An International Conference, New York: New York Turtle and Tortoise Society, pp. 109-112.
- VANZOLINI, P.E. 1967. Notes on the nesting behavior of *Podocnemis expansa* in the Amazon valley (Testudines, Pelomedusidae). *Pap. Avuls. Zool. Sao Paulo* 20:191-215.
- VON HILDEBRAND, P., SAENZ, C., PAÑUELA, M.C., AND CARO, C. 1988. Biología reproductiva y manejo de la tortuga Charapa (*Podocnemis expansa*) en el bajo río Caquetá. *Colombia Amazónica* 3:89-112.
- VON HILDEBRAND, P., BERMUDEZ, N., AND PEÑUELA-M., M.C. 1997. La Tortuga Charapa (*Podocnemis expansa*) en el Río Caquetá, Amazonas, Colombia. Aspectos de la Biología Reproductiva y Técnicas para su Manejo. Bogotá, Colombia: Disloque, 159 pp.
- VON HUMBOLDT, A. 1859. *Reise in die Aequinoctial-Gegenden des neuen Continents*. Vol. 3. Stuttgart, Germany: Hermann Hauff, J.G. Cotta'scher Verlag.
- WALKER, D. AND AVISE, J.C. 1998. Principles of phylogeography as illustrated by freshwater and terrestrial turtles in the southeastern United States. *Ann. Rev. Ecol. Syst.* 29:23-58.
- WALKER, D., BURKE, V.J., BARÁK, I., AND AVISE, J.C. 1995. A comparison of mtDNA restriction sites vs. control region sequences in phylogeographic assessment of the musk turtle (*Sternotherus minor*). *Molecular Ecol.* 4:365-373.
- WALKER, D., NELSON, W.S., BUHLMANN, K.A., AND AVISE, J.C. 1997. Mitochondrial DNA phylogeography and subspecies issues in the monotypic freshwater turtle *Sternotherus odoratus*. *Copeia* 1997:16-21.

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