Relationship between multiple paternity and reproductive parameters for *Podocnemis sextuberculata* (Testudines: Podocnemididae) in the Trombetas River, Brazil

F.P. Freda[1], V.C.D. Bernardes[1], C.C. Eisemberg[3], C. Fantin[2] and R.C. Vogt[1]

[1]Instituto Nacional de Pesquisas da Amazônia, Manaus, AM, Brasil
[2]Universidade do Estado do Amazonas, Manaus, AM, Brasil
[3]Institute for the Environment and Livelihoods, Charles Darwin University,
Darwin-Northern Territory, Australia

Corresponding author: F.P. Freda
E-mail: ferfreda@gmail.com

ABSTRACT. Genetic studies of multiple paternity are a valuable tool to gain information on the reproductive biology of turtles. We analyzed paternity type in *Podocnemis sextuberculata* and related number of fathers per nest to nesting period (beginning, middle, or end of nesting season); clutch size (number of eggs); female size; and hatchling success. Females were captured and maximum linear carapace lengths measured during the 60 days that encompass the nesting season at Rio Trombetas Biological Reserve (Pará, Brazil). Nests were marked and blood samples collected from hatchlings. Six heterologous loci were used: five from *Podocnemis unifilis* and one from *Podocnemis expansa*. Hatchlings were analyzed from 23 nests, and the rate of multiple paternity was 100%. The mean number of fathers per nest was six (± 0.9), and no significant difference between number of fathers in a nest and nesting period. Similarly there was no significant relationship between number of fathers in a nest and female

size or hatchling success rate. Number of fathers was, however, positively correlated with clutch size (Spearman correlation rho = 0.47; P > 0.05). To our knowledge, this is the first study to test the relationship between multiple paternity and ecological aspects of the reproductive ecology of turtles in the genus *Podocnemis*.

Key words: Amazon; Multiple paternity; *Podocnemis sextuberculata*; Reproductive parameters; Trombetas River; Turtles

INTRODUCTION

Advanced genetic analysis has revealed that multiple paternity is common in marine (Galbraith et al., 1993; Bollmer et al., 1999) and freshwater (Valenzuela, 2000; Pearse et al., 2006; Fantin et al., 2008) turtles. The study of multiple paternity is particularly interesting in chelonians, since it provides indirect information on mating of turtles, which can be challenging to observe in the field, under the water (Carpenter and Ferguson, 1977; Ireland et al., 2003).

Reproductive behavior studies of marine turtles in their natural habitat are relatively common (Booth and Peters, 1972; Broderick and Godley, 1997) compared to those conducted on Amazon freshwater turtles (Nascimento, 2002; Vogt, 2008). In the Amazon, *Podocnemis* spp turtles migrate from lakes to rivers in the beginning of the nesting season, once water levels decrease. Courtship and copulation occur in lakes before migration, or near nesting beaches along main river channels (Vogt, 2008).

Paternity studies have shown polygamy (one male copulating with more than one female) and polyandry (one female copulating with more than one male) among chelonians (Gist et al., 2002; Fisher et al., 2006). Bowen et al. (1992) described a polyandrous reproduction system in male chelonians, where males mate with the maximum number of females possible, with no territory delimitation. According to Fisher et al. (2006), polyandry guarantees benefits to the offspring and field studies have shown that polyandrous female progeny usually present a lower juvenile mortality rate (Madsen et al., 1992; Jennions and Petrie, 2000). The genetic benefits obtained through polyandry are in line with the "good-sperm hypothesis", which states that paternity is granted to males that produce the most viable descendants (Fisher et al., 2006).

In species with a polyandrous mating system, females have the capacity to stock sperm in the oviduct (Gist and Jones, 1989). This mechanism is fairly common among birds and reptiles. Sperm storage promotes a post-copula selection that can potentially benefit offspring through the inheritance of "good genes", increasing population reproductive viability and genetic heterozygosity (Jennions and Petrie, 2000). This reproductive strategy allows genetic contribution of more than one male during ovule fertilization.

Polyandry was first observed in the genus *Podocnemis* in studies using microsatellite DNA markers to reveal multiple paternity in *Podocnemis expansa*, *P. unifilis*, and *P. erythrocephala* (Valenzuela, 2000; Pearse et al., 2006; Fantin et al., 2008; Fantin et al., 2010). These microsatellites are highly polymorphic (Faleiro, 2007; Fantin et al., 2008) and are commonly used in paternity studies. So far, multiple paternity studies in Amazon freshwater turtles have focused on analysing the number of males that contribute genetically to female progeny (Valenzuela, 2000; Pearse et al., 2006; Fantin et al., 2008; Fantin et al., 2010). Nevertheless, studies on the influence multiple paternity in the ecological aspects of the population of turtles need to be more studied. It is possible to use these genetic tools to answer ecological questions with regard to the reproductive biology of Amazon turtles.

The six-tubercled Amazon river turtle, *Podocnemis sextuberculata*, has a limited distribution in the Amazon Basin from Colombia in the southeast to Peru in the northeast. It is restricted to white and clear water rivers and tributaries. It is the second smallest species in the genus *Podocnemis*, with a maximum carapace length of 34 cm and maximum weight of 3.5 kg (Vogt, 2008). The nesting season for this species varies geographically and can occur between June and July in the Purus River; July to November in the lower Solimões; or August and September in the Trombetas River (Vogt, 2008). Nesting takes place during the dry season on river sandbanks and clutches ranges from 6 to 25 eggs. Hatchling sex and incubation period are determined by incubation temperature; hatchlings emerge from the nest between 45 and 60 days after nesting (Haller and Rodrigues, 2006; Vogt, 2008).

In this study we verified cases of multiple paternity in nests of *P. sextuberculata* in the Rio Trombetas Biological Reserve (Pará, Brazil). We tested whether if 1) the number of males in a given clutch was related to the nesting period, and 2) the number of males is correlated to female size, clutch size, and hatchling success rate.

MATERIAL AND METHODS

Study area and data collection

Data were recorded from October to November 2013, for 60 nights (7:00 pm to 7:00 am), at the Farias beach located at the Rio Trombetas Biological Reserve (1°15'S, 56°50'W, WGS84) (city of Oriximiná, Pará, Brazil). This period encompassed the nesting season, when we were able to capture females and mark nests following egg laying. For all captured females, the maximum linear carapace length was measured. And blood (100-500 µL) collected from the femoral vein. Because of the difficulty of capturing females after nesting, female size was also inferred by measurement of tracks to and from freshly laid nests using a metric tape (± 0.1 mm). This is commonly used procedure, has proven effective in many turtle nesting biology studies (Valenzuela, 2001; Bonach et al., 2006). We also recorded the number of eggs and nesting date for each female.

After 40 days incubation, nests were fenced to prevent exit of hatchlings. For each nest, blood samples (50-100 µL) were collected via the femoral vein from all hatchlings. Blood samples from females and hatchlings were stored in microtubules containing ethanol and solution EDTA preservative. Material collection and transport were carried out under authorization of the ICMBio/SISBIO (approval No. 40084-3) and the Ethics and Research Committee for Animal Use (INPA No. 037/2013).

DNA extraction and genotyping

Only nests with more than ten hatchlings were included for final analysis; 23 nests met this criterion. Total DNA was isolated from blood samples using a detergent solution CTAB protocol (Doyle and Doyle, 1987). Specific regions of loci were amplified by polymerase chain reaction (PCR), following the protocol described by Schuelke (2000), where each forward primer had an M13 sequence tail added to its 5' end to allow for dynamic fluorescent labelling (TET-labelled M13 primer). PCRs were performed in 12.5 µL reaction volumes containing 4.6 µL H_2O; 1.5 µL buffer 10X with $(NH_4)_2SO_4$; 1.5 µL 25 nM $MgCl_2$; 1.5 µL 0.2 µM reverse primer; 0.75 µL 0.2 µM forward primer; 0.75 µL 0.2 µM FAM-6 labeled M13 label primer; 1.5 µL 0.2 µM each dNTP mix; 0.4 µL 1 U/µL Taq DNA Polymerase; and 1 µL 10 ng DNA. The initial denaturation temperature was 94°C

per minute, which was followed by 25 cycles for 30 s at 94°C, 30 s at 55°C, and 30 s at 68°C, and final extension for 15 min at 72°C. Amplified DNA fragments were visualized by electrophoresis on a 1% agarose (w/v) gel. We used six loci for PCRs, five were developed for *P. unifilis* (Fantin et al., 2008) and one for *P. expansa* (Valenzuela, 2000) as shown in Table 1.

Table 1. Loci used in multiple paternity analysis of *Podocnemis sextuberculata*.

Loco	Repeat motif	References
Puni_2E7	$(GA)_5gc(GA)_8$	Fantin et al. (2008)
Puni_1B11	$(GA)_7gg(GA)_9$	Fantin et al. (2008)
Puni_2A9	$(GA)_{12}$	Fantin et al. (2008)
Puni_1H9	$(GA)_{12}$	Fantin et al. (2008)
Puni_1D12	$(GA)_{10}$	Fantin et al. (2008)
PE_344	$(AG)_{13}$	Valenzuela (2000)

Products amplified by PCR were diluted in water 1:100, and the size marker ROX pUC-19, modified from Dewoody et al. (2000), was added to determine sizes of observed alleles. Genotyping of amplified DNA used an ABI 3130xl automatic sequencer (Applied Biosystems, Foster City, CA, USA).

Genetic analysis

Allele length and peak patterns were analyzed using the GeneMarker v.2.4 software (SoftGenetic, 2012). The probability of paternity exclusion (Q) was determined using the method described by Weir (1996). The probability of genetic identity (I), which indicates the probability of two individuals not related, having the same genotype, was calculated according to Paetkau et al. (1995). Observed (H_O) and expected (H_E) heterozygosity values were calculated using the Arlequin v.3.5 software (Excoffier and Lischer, 2010). The presence of null alleles and large allele drop out was evaluated using the program MicroChecker (Oosterhout et al., 2004), which estimates the values of the null alleles through the Brookfield (1996) method.

Three methods were used to analyse the type of paternity to confirm our observation. Initially, we used the method of simple allelic counting, which assumes a normal pattern of Mendelian inheritance. The inference of multiple paternity was based on the presence of five alleles per locus sampled among the hatchlings of each nest. Secondly, we used the program KINALYZER (Ashley et al., 2009), which uses a minimum 2-allele set cover approach based on Mendelian properties to find the lowest possible number of siblings groups in a given nest (Ashley et al., 2009). We also used the GERUD 2.0 program to reconstruct parental alleles, infer the relative contribution of individual males to each nest (Jones, 2005), and confirm minimum number of fathers per nest results obtained using the previous analysis techniques.

Statistical analysis

We used the number of fathers estimated using the program KINALYZER to perform subsequent ecological analysis. A Kruskal-Wallis test was used to evaluate the relationship between the number of fathers at different points in the nesting season (beginning, middle, or end) (Ayres and Ayres Júnior, 2007). Spearman correlations were used to analyze relationships between number of fathers and female size, clutch size (number of eggs per nest) and hatching success rate (Vanzolini, 1992).

RESULTS

For all six loci the I value was 1.84 x 10^{-13} and Q was 99.27% (Table 2). For each locus, H_O and null alleles are presented in Table 2. Null alleles were observed in five of the six loci, only the locus Puni_1D12 did not present null alleles. However, observed values were similar to the indices calculated by Brookfield (1996). The population was found to be in Hardy-Weinberg equilibrium.

Table 2. Genetic characteristics of the heterologous loci used in multiple paternity analysis of *Podocnemis sextuberculata*.

Loco	H_O	H_E	I	Q	Null alleles	
					Observed	Brookfield (1996)
Puni_2E7	0.788	0.949	0.005	0.896	0.064	0.062
Puni_1B11	0.235	0.916	0.013	0.833	0.374	0.363
Puni_2A9	0.546	0.916	0.013	0.776	0.155	0.150
Puni_1H9	0.043	0.868	0.030	0.748	0.463	0.446
Puni_1D12	0.950	0.941	0.006	0.856	-0.019	-0.018
PE_344	0.817	0.969	0.001	0.732	0.060	0.059
Total			1.84 x 10^{-13}	0.999		

H_O = observed heterozygosity; H_E = expected heterozygosity; I = probability of identity; Q = paternity exclusion.

The 2013 nesting season started on September 30 and ended on November 27 (59 days). This period was divided into beginning, middle, and end, with approximately 20 days for each period. Eight nests each were sampled from the beginning and middle, and seven from the end of the nesting season.

In total, 402 hatchlings from 23 nests were analyzed. Blood samples were obtained from 10 females that laid nests with less than 10 eggs; these were not used in subsequent analysis. There was evidence of multiple paternity in all nests, using the three methods described to assess paternity type (Table 3). Analyzed nests presented a high number of alleles, with minimum 9 and maximum 16 alleles per nest. The mean of fathers per nest were 6, with minimum 4 and maximum 8 fathers per nest (Table 3). There was a minimum of two to three fathers, according to the program GERUD 2.0.

Table 3. Number of alleles ratio and number of fathers in *Podocnemis sextuberculata* nests, using three methods (minimum count alleles, Kinalyzer, and Gerud) for analysis.

Nest	No. hatchlings	No. alleles	MMCA* No. fathers	Kinalyzer No. brothers groups	Gerud No. fathers minimum
N1	14	15	7	6	3
N2	12	10	4	6	2
N3	12	14	6	6	3
N4	12	11	5	5	3
N5	14	13	6	7	2
N6	12	11	5	6	2
N7	10	10	4	5	2
N8	13	14	6	7	2
N9	13	11	5	7	2
N10	11	12	5	5	3
N11	16	12	5	6	2
N12	12	13	6	6	2
N13	14	15	7	7	2
N14	12	10	4	5	2
N15	10	9	4	4	2
N16	14	11	5	7	3
N17	11	9	4	5	2
N18	17	11	5	8	3
N19	16	12	5	7	2
N20	13	13	6	7	3
N21	12	12	5	5	3
N22	18	11	5	8	2
N23	15	16	7	7	2
Total	302				
Mean	13	12	5	6	2.3

*MMCA = Method Minimum Count Alleles.

Data are reported as means ± SD. The number of fathers per nest was not significantly different during the three periods of the nesting season (Kruskal-Wallis P = 0.33, d.f. = 2, H = 2.9), beginning = 6 ± 0.76, middle 6 ± 1.20, end = 6.7 ± 1.25, and total nesting period = 6.14 ± 1.11. There was no significant relationship between female size [track width (mm) = 125.17 ± 25.24] and the number of fathers per nest (Spearman correlation: rho = -0.10; P = 0.76). There was however, a significant positive relationship between clutch size (number of eggs per nest = 16.04 ± 2.77) and the number of fathers per nest (Spearman correlation: rho = 0.47; P ≤ 0.05; Figure 1). There was no significant relationship between the hatching success (hatchlings/ eggs = 0.69 ± 0.12) and the number of fathers per nest (Spearman correlation: rho = 0.04; P = 0.85).
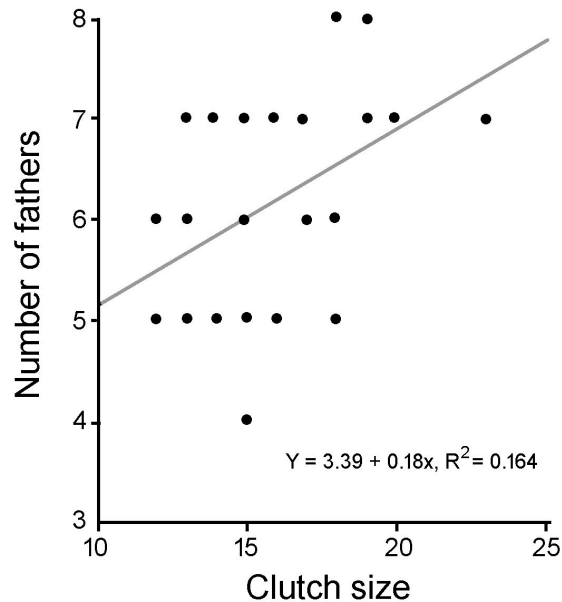
Figure 1. Relationship between clutch size of *Podocnemis sextuberculata* and the numbers of fathers participating in fertilization of the clutch.

DISCUSSION

To our knowledge, this is the first study to test the relationship between multiple paternity and ecological aspects of the reproductive ecology of turtles in the genus *Podocnemis*. We found that using heterologous loci was satisfactory for multiple paternity analysis and the probabilities of I and Q values were within the acceptable range for genetic analysis by using these loci. Because of the presence of null alleles, H_O was below H_E for some loci. We conclude that using heterologous loci is a viable option for the analysis of multiple paternity in *P. sextuberculata,* but because of the non-specificity of loci, there may be a high occurrence of null alleles, suggesting that the loci are less polymorphic. We found Puni_1B11 and Puni_1H9 to be the less polymorphic loci, as H_O was lower than H_E in both cases. Fantin et al. (2008) found higher heterozygosity using the same loci in paternity analysis in *P. unifilis*, a finding that may be specific to the species studied.

In our study, multiple paternity was observed in 100% of nests and the number of alleles has high variability. High occurrence of alleles and multiple paternity are probably because this population of *P. sextuberculata* is located in a biological reserve, where the chelonian population nesting areas have been protected for more than 30 years. In a previous study, the *P. sextuberculata* population in Barreirinha (Amazonas State, Brazil) presented a less variation from three to 11 alleles and there was multiple paternity in all nests analyzed with a minimum of two fathers per nest (Fantin, 2008). Barreirinha is located in a relatively densely populated region, with low or no official protective measures to prevent the harvest of turtle eggs and meat, both of which are considered local delicacies. Chelonian populations in areas that are fully protected, there are probably higher numbers of males contributing genetically in comparison to populations in areas with a lower degree of environmental protection. These findings indicate the importance of conservation and management actions to the maintenance of the gene pool for *P. sextuberculata* and other chelonians in the Amazon. According to Lasala et al. (2013), high rates of multiple paternity are good indicators of population stability and/or growth, since they suggest a high number of adult males. While female adult populations are easy to count through the number of nests on a beach, genetic studies of paternity are good to estimate the male population of a given chelonian species (Jensen et al., 2006). In a similar study of *Lepidochelys olivacea* (the olive ridley sea turtle), a strong correlation between paternity rate and population size was observed, indicative of a larger number of individuals available to participate in the mating system (Jensen et al., 2006). This difference was attributed to the larger number of individuals available to participate in the mating system, with a strong correlation between paternity rate and population size (Jensen et al., 2006).

In previous studies that the correlation between female size and paternity rates was observed, the authors declared that larger females have more capacity to store sperm and consequently, present a higher occurrence of different fathers in their offspring (van Buskirk and Crowder, 1994; Lasala et al., 2013). In the current study, we did not find a correlation between female size and number of fathers per nest. However, a higher number of fathers was observed for nests with a higher number of eggs.

A higher degree of multiple paternity in a given nest is probably related to an increase in the genetic variability of the offspring, which might enhance hatchling survivorship rates. According to Madsen et al. (1992), genetic variability reduces juvenile mortality but does not increase the hatching success rate. We did not observe a correlation between hatching success rate and the number of fathers per nest in the current study. This finding reflects that the Lasala et al. (2013), who expected a positive relationship between these parameters for a population of *Caretta caretta* (loggerhead sea turtle) in Wassaw Island, USA, but found no correlation. This is probably because hatching success rates are more strongly influenced by environmental factors (Ferreira, 2009) than by genetic heterozygosity.

CONCLUSIONS

Heterologous loci to *P. sextuberculata* can be used in studies of multiple paternity. However, the development of specific primers is highly recommended to improve the efficacy of the tests. We found multiple paternity in 100% of analyzed nests, a high allele variation and similar numbers of fathers per nest across different periods of the nesting season. Female size had no relationship to the number of fathers per nest. However, there was a significant positive relationship between the number of fathers per nest and clutch size, indicating larger clutches represent more males

contributing genetically to the offspring. Hatchling success rate was not significantly correlated with the number of fathers per nest. This study confirms the polyandrous mating system between the genus *Podocnemis* turtles and showed some effects of multiple paternity in reproductive ecology *P. sextuberculata* species. However, it is necessary for most field studies are conducted to assess the effect of multiple paternity in other aspects of the species behavior.

ACKNOWLEDGMENTS

REFERENCES

Ashley MV, Caballero IC, Chaovalitwongse W, Dasgupta B, et al. (2009). KINALYZER, a computer program for constructing sibling groups. *Mol. Ecol. Resour.* 9: 1127-1131. http://dx.doi.org/10.1111/j.1755-0998.2009.02562.x

Ayres M and Ayres Júnior M (2007). Bio-Estat - Aplicações Estatísticas nas Áreas das Ciências Bio-Médicas. Sociedade Civil Mamirauá, Pará.

Bollmer JL, Irwin ME, Rieder JP and Parker PG (1999). Multiple paternity in loggerhead turtles clutches. *Copeia* 1999: 475-478. http://dx.doi.org/10.2307/1447494

Bonach K, Piña CI and Verdade LM (2006). Allometry of reproduction of *Podocnemis expansa* in Southern Amazon basin. *Amphibia-Reptilia* 27: 55-61. http://dx.doi.org/10.1163/156853806776052191

Booth J and Peters J (1972). Behavioral studies on the green turtle (*Chelonia mydas*) in the sea. *Anim. Behav.* 20: 808-812. http://dx.doi.org/10.1016/S0003-3472(72)80155-6

Bowen B, Meylan A, Ross J, Limpus C, et al. (1992). Global population structure and natural history of the green turtle (*Chelonia mydas*) in terms of maternal phylogeny. *Evolution* 46: 865-881. http://dx.doi.org/10.2307/2409742

Broderick A and Godley B (1997). Observations of reproductive behavior of male green turtles (*Chelonia mydas*) at a nesting beach in Cypurs. *Chelonian Conserv. Biol.* 2: 615-616.

Brookfield JF (1996). A simple new method for estimating null allele frequency from heterozygote deficiency. *Mol. Ecol.* 5: 453-455. http://dx.doi.org/10.1111/j.1365-294X.1996.tb00336.x

Carpenter CC and Ferguson GW (1977). Variation and evolution of stereotyped behavior in reptiles. In: Biology of the Reptilian (Pough FH, ed.). Academic Press, USA, 335-554.

Dewoody JA, Dewoody YD, Fiumera AC and Avise JC (2000). On the number of reproductives contributing to a half-sib progeny array. *Genet. Res.* 75: 95-105. http://dx.doi.org/10.1017/S0016672399004000

Doyle JL and Doyle JJ (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochem. Bull.* 19: 11-15.

Excoffier L and Lischer H (2010). Arlequin 3.5.1.2: An Integrated Software Package for Population Genetics Data Analysis. Available at [http://cmpg.unibe.ch/software/arlequin3512/man/Arlequin35.pdf]. Accessed November 15, 2014.

Faleiro FG (2007). Marcadores genético-moleculares aplicados a programas de conservação e uso de recursos genéticos. Embrapa Cerrado, Brasil.

Fantin C (2008). Análise do sistema reprodutivo de três espécies de quelônios da Amazônia (*Podocnemis* spp.) através de marcadores moleculares de microssatélites. Doctor's thesis, Universidade Federal do Amazonas - UFAM, Manaus.

Fantin C, Farias IP, Monjeló LAS and Viana LS (2008). Polyandry in *Podocnemis unifilis* (Pleurodira:Podocnemididae), the vulnerable yellow-spotted Amazon river turtle. *Amphibia-Reptilia* 29: 479-486. http://dx.doi.org/10.1163/156853808786230361

Fantin C, Farias IP, Monjeló LAS and Hrbek T (2010). Polyandry in the red-headed river turtle *Podocnemis erythrocephala* (Testudines, Podocnemididae) in the Brazilian Amazon. *Genet. Mol. Res.* 9: 435-440. http://dx.doi.org/10.4238/vol9-1gmr749

Ferreira Junior PD (2009). Efeitos de Fatores Ambientais na Reprodução de Tartarugas. *Acta Amaz.* 39: 319-334.

Fisher DO, Double MC, Blomberg SP, Jennions MD, et al. (2006). Post-mating sexual selection increases lifetime fitness of polyandrous females in the wild. *Nature* 444: 89-92. http://dx.doi.org/10.1038/nature05206

Galbraith DA, White BN, Brooks RJ and Boag PT (1993). Multiple paternity in clutches of snapping turtles (*Chelydra serpentina*) detected using DNA fingerprints. *Can. J. Zool.* 71: 318-324. http://dx.doi.org/10.1139/z93-044

Gist DH and Jones JM (1989). Sperm storage within the oviduct of turtles. *J. Morphol.* 199: 379-384. http://dx.doi.org/10.1002/jmor.1051990311

Gist DH, Dawes SM, Turner TW, Sheldon S et al. (2002). Sperm Storage in Turtles: A Male Perspective. *J. Exp. Zool.* 292: 180-186. http://dx.doi.org/10.1002/jez.1153

Haller ECP and Rodrigues MT (2006). Reproductive Biology of the six-tubercled Amazon river turtle, *Podocnemis sextuberculata* (Testudines:Podocnemididae), in the Biological Reserve of Rio Trombetas, Pará, Brazil. *Chelonian Conserv. Biol.* 5: 280-284. http://dx.doi.org/10.2744/1071-8443(2006)5[280:RBOTSA]2.0.CO;2

Ireland JS, Broderick AC, Glen F, Godley BJ, et al. (2003). Multiple paternity assessed using microsatellite markers, in green turtles *Chelonia mydas* (Linnaeus, 1758) of Ascension Island, South Atlantic. *J. Exp. Mar. Biol. Ecol.* 291: 149-160. http://dx.doi.org/10.1016/S0022-0981(03)00118-7

Jennions MD and Petrie M (2000). Why do females mate multiply? A review of the genetic benefits. *Biol. Rev. Camb. Philos. Soc.* 75: 21-64. http://dx.doi.org/10.1017/S0006323199005423

Jensen MP, Abreu-Grobois F, Frydenberg J and Loeschcke V (2006). Microsatellite provide insight into contrasting mating patterns in arribada vs. non-arribada olive Ridley sea turtle rookeries. *Mol. Ecol.* 15: 2567-2575. http://dx.doi.org/10.1111/j.1365-294X.2006.02951.x

Jones AG (2005). Gerud 2.0: a computer program for the reconstruction of parental genotypes from half-sib progeny arrays with known or unknown parents. *Mol. Ecol. Notes* 5: 708-711. http://dx.doi.org/10.1111/j.1471-8286.2005.01029.x

Lasala JA, Harrison JS, Williams KL and Rostal DC (2013). Strong male-biased operational sex ratio in a breeding population of loggerhead turtles (*Caretta caretta*) inferred by paternal genotype reconstruction analysis. *Ecol. Evol.* 3: 4736-4747. http://dx.doi.org/10.1002/ece3.761

Madsen T, Shine R, Loman J and Hakansson T (1992). Why do female adders copulate so frequently? *Nature* 355: 440-441. http://dx.doi.org/10.1038/355440a0

Nascimento SP (2002). Observações sobre o comportamento de nidificação de três espécies de *Podocnemis* Wagler (Testudinata, Pelomedusidae) no Baixo Rio Branco, Roraima, Brasil. *Rev. Bras. Zool.* 19: 201-204. http://dx.doi.org/10.1590/S0101-81752002000100018

Paetkau D, Calvert W, Stirling I and Strobeck C (1995). Microsatellite analysis of population structure in Canadian polar bears. *Mol. Ecol.* 4: 347-354. http://dx.doi.org/10.1111/j.1365-294X.1995.tb00227.x

Pearse DE, Dastrup RB, Hernandez O and Sites Junior JW (2006). Paternity in an Orinoco population of endangered Arrau river turtles, *Podocnemis expansa* (Pleurodira; Podocnemididae) from Venezuela. *Chelonian Conserv. Biol.* 5: 232-238. http://dx.doi.org/10.2744/1071-8443(2006)5[232:PIAOPO]2.0.CO;2

Schuelke M (2000). An economic method for the fluorescent labeling of PCR fragments. *Nat. Biotechnol.* 18: 233-234. http://dx.doi.org/10.1038/72708

SoftGenetic LLC (2012). GeneMarker: The Biologist Friendly Software. Available at [http://www.softgenetics.com/PDF/GeneMarker_2-4_UserManual_July2012.pdf]. Accessed July 4, 2014.

van Buskirk JV and Crowder LB (1994). Life-history Variation in Marine Turtles. *Copeia* 1994: 66-81. http://dx.doi.org/10.2307/1446672

van Oosterhout CV, Hutchinson WF, Wills DPM and Shipley P (2004). Micro-Checker: software for identifying and correcting genotyping errors in microsatellite data. *Mol. Ecol. Notes* 4: 535-538. http://dx.doi.org/10.1111/j.1471-8286.2004.00684.x

Valenzuela N (2000). Multiple paternity in side-neck turtles *Podocnemis expansa*: evidence from microsatellite DNA data. *Mol. Ecol.* 9: 99-105. http://dx.doi.org/10.1046/j.1365-294x.2000.00806.x

Valenzuela N (2001). Maternal effects on life-history traits in the Amazonian giant river turtle *Podocnemis expansa*. *J. Herpetol.* 35: 368-378. http://dx.doi.org/10.2307/1565954

Vanzolini PE (1992). Métodos estatísticos elementares em sistemática zoológica. Hucitec, Brasil.

Vogt RC (2008). Tartarugas da Amazônia. Gráfica Biblos, Peru.

Weir BS (1996). Genetic data analysis II: methods for discrete population genetic data. Sinauer Associates, Sunderland.