International Journal of Sciences: Basic and Applied Research (IJSBAR)

ISSN 2307-4531

(Print & Online)

http://gssrr.org/index.php?journal=JournalOfBasicAndApplied

Multiple Paternity Detection of Olive Ridley Turtle, *Lepidochelys olivacea* Populations by Microsatellite Marker as a Genetic Conservation Strategy at Taman Buru Bena, Timor Island

Alfred O. M. Dima[a*], Dedy D. Solihin[b], Wasmen Manalu[c], Arief Boediono[d]

[a] *Bogor Agricultural University Doctoral Student, Department of Biology, Kampus IPB Dramaga Bogor, West Java, Indonesia*

[a] *Department of Biology, FST Nusa Cendana University (UNDANA), Kupang*

[b] *Department of Biology, Faculty of Mathematics and Science, Bogor Agricultural University, Kampus IPB Dramaga Bogor, West Java, Indonesia*

[c,d] *Department of Anatomy, Physiology and Pharmacology, Faculty of Veterinary Medicine, Bogor Agricultural University, Kampus IPB Dramaga Bogor, West Java, Indonesia*

[a]*Email : onny_dima@yahoo.co.id*

Abstract

Polyandry related to multiple paternities is one of the female reproduction behaviors of sea turtles. The multiple paternities are still difficult to be determined. We determined paternity in the offspring of olive ridley sea turtles (*Lepidochelys olivacea*) nesting at Taman Buru Bena, Timor Island. Five loci microsatellite markers that used in this research, namely OR-1, OR-4, OR-7, OR-11 and OR-14. DNA was extracted and isolated from the muscle tissues and amniotic fluid of the hatchlings. The amplicon were genotyped by using a GeneMapper genotyping software version 4.0. The result showed that the distribution of the paternal alleles were 3 (OR-11), 2 (OR-4 and OR-1), and 1 (OR-14). Four microsatellite loci OR-1, OR-14, OR-4, and OR-11 could show the multiple paternities of the turtles, this study observed that multiple paternities might contribute to the increase in genetic biodiversity in the sea turtle.

---

* Corresponding author.

E-mail address: onny_dima@yahoo.co.id

***Keywords**: Lepidochelys olivacea*; microsatellite; multiple paternities.

1. Introduction

Olive ridleys, one of the widely distributed marine turtle species has undergone population declines due to multiple anthropogenic factors. This turtle is currently listed as ‘‘Globally Endangered’’ due to direct observation of population reduction of at least 50% over the last 25 years (IUCN Red List of threatened Species). One of its characteristics is that after hatching, it may be drifting in the open ocean for several years and as adults migrate up to 2,700 miles on a regular basis between foraging and breeding grounds [1]. This condition can lead to overlapping niches with other turtle populations, considering the fact that the nature of turtle is loyalty to its nesting place (fidelity). Mating between populations can occur as consequences of this migration pattern. Genetic analyses have revealed that gene flow among nesting beaches is mediated by male turtles, and opportunities for gene flow probably occur when adult populations overlap in feeding areas and migratory corridors [2].

Male turtles show have limited opportunities because, different from females, they rarely come ashore; thus, most researches are carried out by simultaneously capturing and tagging males around both the breeding area and feeding area. The presence of this male turtle behavior makes little information of bioreproduction that can be obtained. On the other hand, females showed mating behavior by being faithful on their special courtship area near nesting beach chosen by females and they will return to their feeding area after mating [3]. In controlling their mating system, female turtles take an unnoticeable advantage by multiple mating.

Males turtles showed a wide range of ejaculated sperm volume between 1 to 2 mL up to 100 mL. Total sperm counts ranging between 88 and 645 per breeding season were observed with percent motility as high as 90%. Sperm motility and sperm volume were variable depend upon time and frequency of ejaculation and the individual male [4]. On the other hand, females lay eggs in several nests (4-7 times) within two weeks intervals during one breeding season and can produce 90 to 200 eggs per nest. Therefore, the idea that one females mates with only one male is not enough for fertilization of all eggs ovulated by the females during the breeding season [5, 6]. Moreover, the existence of a fixed area for feeding and breeding activity, and also highly migration ability of turtle allows high frequency and level of polyandry (one female mates with multiple males) or polygamy (a male mates with multiple females) in specific populations [5, 7, 8,9].

Mating patterns with one or multiple partners might be important as it significantly contribute to individual fitness [10]. Mating with multiple males may give direct beneficial for females such as providing fitness and positive selection, sperm selectivity by females, and also provide indirect benefits such as improving the genetic variation in offspring’s leading to increase in hatching success or offspring quality, providing access to resources and offspring’s parenting [11, 12]. Reproductive behavior of females for mating with multiple males and storing the sperm in the female’s reproductive tructs may affect male and female reproductive strategies. Male reproductive success is often limited by the ability to obtain female. Therefore, males should have a strategy to mate with many females. In contrast, the number of offspring produced by females is generally not limited by the quantity of mating pairs. Therefore, the selection of the multiple mating is expected to weaken the males.

Molecular studies on the origin of species provide basic data to investigate a number of biological subjects to be important, such as sex selection, ecology, behavior, and conservation genetics [13]. Microsatellite or simple sequence repeats (SSR) markers has been widely used to investigate paternity and mating detection of males and females in the sea turtle population [14, 15, 16, 17, 18, 19,20]. Several properties of microsatellite as molecular marker that makes it ideal for genetic diversity analysis are by having a high mutation rate, high polymorphism, codominant, and more importantly, the nature of its biparental inheritance that could display gene flow derived from both male and female [21].

Female turtles can store viable sperm in their reproductive tracts for as long as several years, but the extent to which this capacity is utilized in nature has remained unknown. Here, we employ microsatellite markers to assess genetic paternity in successive clutches of olive ridley sea turtles (*Lepidochelys olivacea*) in marine waters, Taman Buru Bena, Timor Island. The olive ridley sea turtles are among one of the most widely distributed and abundant sea turtles, however, a large population of this species in the marine waters of Timor is concentrated at the marine waters of Timor Bena.

The purpose of this study is to detect multiple paternity that occurs in natural populations of olive ridley sea turtles on Timor Bena beach by using five microsatellite markers, namely OR-1, OR-4, OR-7, OR-11, and OR-14. The results of this study are expected to improve the accuracy of prediction and control of the genetic contribution of male turtles in this population as genetic information for olive ridley turtle conservation.

2. Materials and Methods

2.1. Sampling

This study was conducted from May 2013 to May 2014 in Taman Buru Bena, Timor Island (124°13'E, 10°10'N; Figure. 1). Sample tissue of female turtle was taken from carapace and limb biopsy of adult female that came ashore after nesting. Sample of hatchlings was taken from amniotic fluid of the eggs obtained from the previous study phase. The amniotic fluid was then incubated in two incubation temperatures, masculine temperature: 26-27°C and feminine temperature: 30-33°C. The tissue of turtles was brought to the Molecular Biology Laboratory PSSP-LPPM, Bogor Agricultural University (IPB) for multiple paternity analyses.

2.2. DNA Extraction, Isolation, and Amplification

DNA extraction was performed by using DNeasy mini kit (Qiagen) according the company instruction. Five microsatellite loci were used in the analysis of multiple paternity [21], the OR-1 (NA: AY325422), OR-4 (NA: AY325425), OR-7 (NA: AY325427), OR-11 (NA: EU162580), and OR-14 (NA: EU162582). In general, 3 µL of DNA extraction used in a 25 µL mixture of PCR containing 1 µL forward primer labeled with Fam probe (OR-1; OR-7), Hex probe (OR-4; OR-11), Tet probe (OR -7), and 1 µL of reverse primer for each marker, 12.5 µL Gotaq master mix (Promega), and 8 µL of dH_2O. DNA Amplification was conducted in 96-well PCR thermal cycler (Verity, Applied Bio System) following the condition: 94°C for 5 min. as pre-PCR, then 45 cycles for amplification at 94°C 30 sec. for denaturation, 55°C 30 sec. for primer annealing, and 72°C 30 sec. for extention, lastly 72°C 7 min. for final extention.

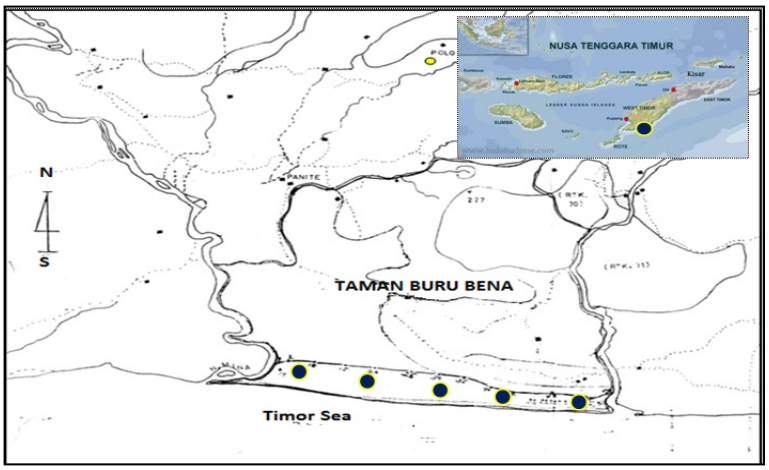

Figure 1: Map of the Taman Buru Bena, Timor Island (= Location of main olive ridley nesting beaches in the Taman Buru Bena).

2.3. Visualization of DNA bands

PCR products were electrophoresed by using 1.8% agarose gel containing ethidium bromide in 1x TAE buffer at 100 voltages for 45 minutes. Visualization was carried out in UV gel doc machine (Biored). For Further genotyping analysis, PCR products were sent to Ist BASE Malaysia.

2.4. Microsatellite and Genotyping Analysis

Maternal genotypes were determined directly from the sampled females, and observed in offspring genotypes. Paternal alleles were determined from genotype of the offspring when alleles of hatchling and maternal calculated after the determination of genotype (GeneMapper genotyping software version 4.0). The estimation of microsatellite variation is based on the five loci used in the study, namely OR-1, OR-4, OR-7, OR-11, and OR-14. Furthermore, the determination of paternal allele is done by eliminating maternal alleles of the offspring genotypes [8]. Multiple paternity in the nest is counted if there are more than two paternal alleles in one locus [23, 24].

3. Results and Discussions

Five microsatellite loci used in this study showed that the four loci OR-1, OR-14, OR-4, and OR-11 had more genetic variations than the locus OR-7 (Table 1). The allele sizes were obtained after genotyping with fragment length for locus OR-1: 175-187 bp, locus OR-4: 126-151 bp, locus OR-7: 188-200 bp, locus OR-11: 215-238 bp, and locus OR-14: 161-164 bp.

Information obtained from five microsatellite markers helped to detect multiple paternity of hatchlings that hatched in natural population. Based on the number of alleles at five loci, except for locus OR-7, four other loci showed different alleles of the female alleles. The analysis showed that loci OR-4 and OR-1 had two paternal alleles, whereas locus OR-14 had one paternal allele and locus OR-11 produced 3 paternal alleles. The result

where more than two paternal alleles were found in the locus OR-11(Figure.2), indicated that female turtles in the marine waters of the Taman Buru Bena, Timor Island showed a phenomenon of multiple paternity during the breeding season. From all the analyzed hatchling samples, the minimum numbers of males that contribute to donating a number of alleles for natural populations were two individuals. These alleles were detected in the locus OR-11, namely 215, 236 and 238 bp.

Table 1: Alleles Information Summary at Five Microsatellite Loci of Olive Ridley Sea Turtles

Microsatellite loci	Maternal Allele (bp)	Hatchling Allele (bp)	Frequency	Paternal Allele (bp)	Paternal Allele Number
OR-1	183/183	175/183	7	175	2
		183/187	17	187	
OR-4	140/146	126/140	4		
		126/126	2		
		151/151	2	126	
		140/146	9	151	
		146/151	6		2
OR-7	188/200	188/188	12	188	2
		188/200	12	200	
OR-11	224/232	224/232	1		
		232/238	7		
		215/232	7		
		215/224	6	215	
		224/238	3	236	
		232/236	1	238	3
OR-14	161/161	161/161	10		
		161/164	13	163	
		161/163	1	164	2

Based on data from the five microsatellite loci that were used to detect the phenomenon of multiple paternities, locus OR-7 contributed two alleles with the same size between females and hatchlings. In contrast, the four other loci, OR-1, OR-14, OR-4, and OR-11 could detect alleles from hatchlings which are different from the alleles possesed by the females. Empirically, locus OR-11 detected alleles in most hatchlings (5 alleles), followed by locus OR-4 (4 alleles), locus OR-14 (3 alleles), locus OR-1 (3 alleles), and OR-7 (2 alleles).

Analysis of the paternal allele pair combinations performed use definitive maternal genotype and hatchlings allele, showed that the mean maximum number of males were detected in olive ridley sea turtle populations using five microsatellite markers as much as 8 males with details on each locus sequentially, consist of OR-11

locus (14 males), OR-4 locus (9 males), OR-1 and OR-14 loci (6 males), and the OR-7 locus (3 males). In addition, maternal genotype homozygous, namely locus OR-1 and OR-14 with paternal allele number by 2 alleles, has the maximum number of males as much as 6 males (Table 2). Meanwhile, maternal heterozygous genotype, namely OR-4 locus and OR-11 locus (except OR-7 locus) with the amount of paternal alleles in a row as much as 2 and 3 alleles, produce the maximum amount that most males. Based on the analysis of the paternal allele possible combinations, it appears that the four loci can be used as microsatellite markers for population genetic studies on other sea turtle species in the waters of eastern Indonesia.

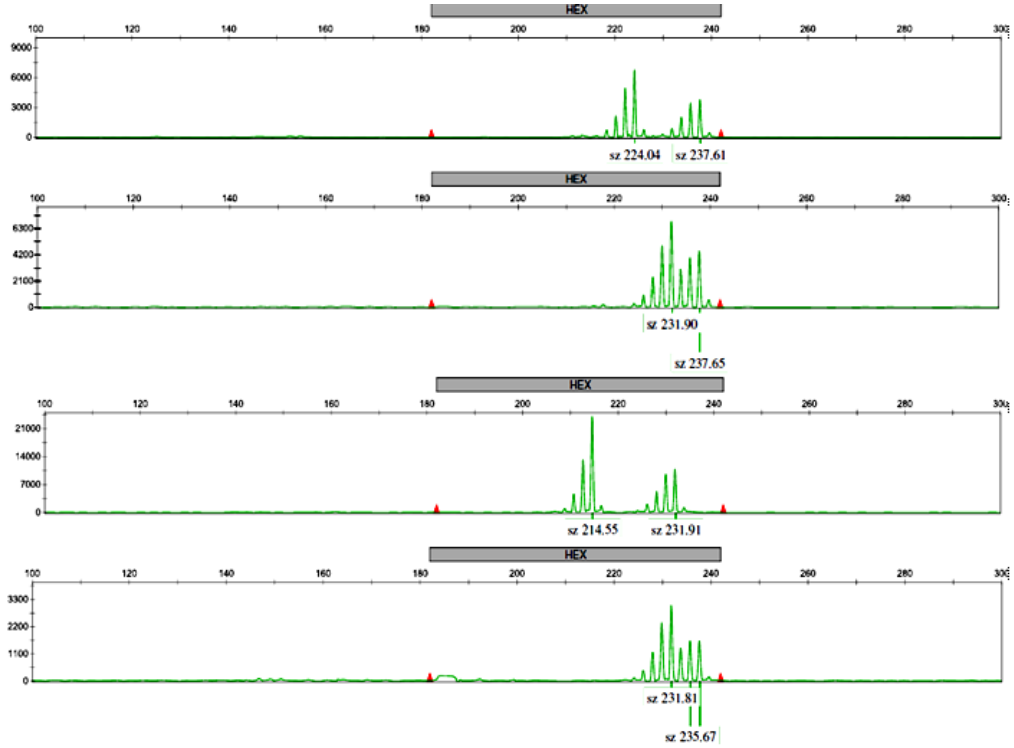

Figure 2: Chromatogram of allele's size and distribution that was detected in locus OR-11

In the phenomenon of multiple paternities, the presence of paternal genotypes showed that males could mate more than one female. [15] stated that nests having three to four paternal alleles indicated that the produced hatchlings came from the mating of one female and two males. If more than four paternal alleles occured, then the nest had a minimum of three males. The research data showed that OR-11 can detect the minimum number of two males (Table 2). It means that multiple paternity phenomenon can be detected in a population of olive ridley sea turtles in the marine waters of Taman Buru Bena, Timor island.

These five pairs of primers used in this study were similar to those used by [21, 22] at the olive ridley turtle with a very high polymorphism. In this study there were four polymorphic loci, namely OR-1, OR-14, OR-4, and OR-11 with a fewer number of alleles due to the limited number of detected nests. The number of alleles derived from the locus was less than the previous research on green turtles [7] and the *Dermochelys coriacea* species [7]. In addition, this is the first study that applied the use of microsatellite markers to reveal the poliandry of olive

ridley sea turtles population in the marine waters of Taman Buru Bena, Timor Island. Therefore, these four loci are expected to be useful microsatellite markers for genetic studies of other species of sea turtles in the marine waters of eastern Indonesia.

Table 2: The maximum number of males based on the number of paternal alleles of five microsatellite markers

Loci	Maternal genotype definitive (bp)	Hatchling Genotype (bp) (n= 24)	Combination of paternal alleles possibility that expected (bp)	Maximum number of males
OR-1	183/183	175/183,183/187	175/175, 175/183,175/187, 183/183, 183/187,187/187	6
OR-4	140/146	126/126,126/140, 140/146,146/151	126/126, 126/140,126/146, 126/151,140/146, 140/151, 146/146, 146/151,151/151	9
OR-7	188/200	188/188,188/200	188/188,188/200, 200/200	3
OR-11	224/232	215/224,215/232, 224/232,224/238, 232/232,232/236, 232/238	215/215, 215/224. 215/232, 215/236, 215/238, 224/224, 224/236, 224/238, 232/232, 232/236, 232/238, 236/236, 236/238, 238/238	14
OR-14	161/161	161/161,161/163, 161/164	161/161,161/163, 161/164, 163/163, 163/164,164/164	6
		Mean of males number		7.6 ∞ 8

Previous studies have also revealed multiple paternities phenomenon in natural populations of sea turtles, such as *Lepidochelys olivacea* [11,21, 22], *Lepidochelys kempy* [16], *Dermochelys coriacea* [7], *Caretta caretta* [12, 15, 19], and *Chelonia mydas* [5, 9, 23]. The females are expected to mate in the beginning of nesting season and store the sperms in a long period. The stored sperms have the ability to fertilize the egg during the next mating season. This condition allows the phenomenon of multiple paternities to prevail that resulted from the mixing and competition of sperms [5,25, 26].

Based on the explanation above, it can be concluded that females get direct benefit from polyandry mating patterns, which is to ensure the availability of sufficient number of sperm that enables fertilization of eggs. In addition, females may be able to avoid unsuccessful reproduction threat through a mechanism that allows the eggs to be fertilized by sperm of multiple males that are genetically similar [27].

Multiple paternities are believed to be able to decrease the rate of genetic drift in population compared to the population of the one female with one male mating pattern. The influences of multiple paternity on hatchling characteristics and population are the increasing of good genes, increasing of the genetic diversity among hatchlings, ensuring the availability of sufficient number of sperm that enables fertilization or allowing sperm competition, and thus improving hatchability and quality of hatchlings [12, 17].

4. Conclusion

The results of this study showed that the hatchlings of eggs laid by *Lepidochelys olivacea* were fertilized by multiple males. Microsatellite analyses showed that multiple paternities can be detected by using four microsatellite loci based the combination of paternal alleles possibility that expected, namely OR-11 locus (14 males), OR-4 locus (9 males), OR-1 and OR-14 loci (6 males). It can be occurred in one breeding season because females mate with multiple males, thus providing a new paternal allele among hatchlings. The phenomenon of multiple paternity of olive ridley sea turtle population in Taman Buru Bena significantly contributes in the improving of genetic diversity. To improve the precision of this study and determine the genetic contribution of males in this population, increasing the number of nests and samples are recommended.

Acknowledgments

We would like to thank all the staffs at the Balai Besar Konservasi Sumber Daya Alam NTT I, who have helped us to collect samples and provide facilities during this study. We would also like to thank the head and all the staffs of the Molecular Biology Laboratory, Primate Research Center, Bogor Agricultural University (IPB) in making this study possible.

References

[1] C.J. Limpus, J.D. Miller, C.J. Parmenter, D. Reimer, N. Mclachland, and R. Webb. (1992,Jan.)."Migration of green (*Chelonia mydas*) and loggerhead (*Caretta caretta*) turtles to and from eastern Australian rookeries." *Wildlife Research*. [On-line]. 19:pp 347-358. Available: www.researchgate.net/publication[Mar.1,2012].

[2] B.W. Bowen, and S.A. Karl. (2007,Oct.)."Population genetics and phylogeography of sea turtles." *Molecular Ecology*. [On-line].16:pp.4886-4907. Available: www.researchgate.net/publication [Jan.4,2012].

[3] C.J. Limpus. (1993,Jan.)."The green turtle, *Chelonia mydas*, in Queens land breeding males in the Southern Great Barrier Reef." Wildlife *Research*. [On-line]. 19:pp 347-358. Available: www.publish.csiro.au[Mar.1,2012].

[4] F. Wood, P. Carroll, C. Kim, and W. Jim.(1982,August.). "Semen collection by electroejaculation of the green turtle, *Chelonia mydas*."*British Journal of Herpetology*.[On-line]. 6:pp. 200-202. Available : www.turtle.ley/scientific/semencollection.html[Mar.20,2011].

[5] N.N. Fitzsimmons.(1998,May)."Single paternity of clutches and sperm storage in the promiscuous green turtle (*Chelonia mydas*)." *Molecular Ecology*. [On-line]. 7:pp. 575-584. Available :

www.researchgate.net[July.5,2012].

[6] D.E. Pearse, and J.C. Avise. (2001,June).”Turtle mating systems: behaviour, sperm storage and genetic paternity”.*Journal of Heredity*.[On-line]. 92:pp.206-211. Available : www.ncbi.nlm.nih.gov/pubmed[Oct.20,2011].

[7] J.L. Crim, L.D. Spotila, J.R. Spotila, M. O’Connor, R. Reina, C.J. Williams, and F.V. Paladino.(2002,Oct.).”The leatherback turtle, *Dermochylys coriacea*, exhibits both polyandry and polygyny.” *Molecular Ecology*. [On-line].11:pp.2097-2106. Available : www.opus.18fw.edu.cgi.viewcontent[June,3,2011].

[8] J.S. Ireland, A.C. Broderick, F. Glen, B.J. Godley, G.C. Hays, P.L.M. Lee,and D.O.F. Skibinski. (2011,July).”Multiple paternity assessed using microsatellite markers, in green turtles *Chelonia mydas* (Linnaeus, 1758) of Ascension Island, South Atlantic.” *Journal of Experimental Marine Biology and Ecology*. [On-line].291: pp.149-160. Available : www.researchgate.net[July.20,2012].

[9] P.L. Lee, and G.C. Hays.(2004,March).”Polyandry in a marine turtle: females make the best of a bad job.”*Proceedings of the National Academy of Sciences*. [On-line].101(17): pp.6530-6535. Available : www.pnas.org/cgi/doi/110.1073/pnas.0307982101[May.2,2011].

[10] T. Birkhead.(2000,Nov.).”Promiscuity: An Evolutionary History of Sperm Competition and Sexual Conflict.” *ISBE Newsletter*. [On-line].12 (2): pp.21-23. Available : www.danny.reviews.com [August.2,2010].

[11] D.J. Hosken, and P. Stockley.(2003,June).”Benefits of polyandry: a life history perspective.” *Evol. Biol*. [On-line].33:pp.173–194. Available : www.link.spinger.com[August.23,2010].

[12] J.L. Bollmer, M.E. Irwin, J.P. Rieder, and P.P. Parker.(1999,May).” Multiple paternity in loggerhead turtle clutches.” *Copeia*. [On-line]. 2: pp.475–478. Avalaible : www.jstor.org/stable/1447494 [Des.5,2010].

[13] J.C. Avise, A.G. Jones, D. Walker, andn J.A. DeWoody. (2002,June).”Genetic mating systems and reproductive natural histories of fishes: Lessons for ecology and evolution.” *Annual Review of Genetic*. [On-line]. 36:pp.19-45 36. Available : www.ncbi.nlm.nih.gov/pubmed[Nop.10,2010].

[14] N.N. Fitzsimmons, C. Moritz, L. Pope, and C.J. Limpus. (1997,Des.). “Geographic structure of mitochondrial and nuclear gene polymorphism in Australian green turtle populations and male-biased gene flow.“*Genetics*. [On-line]. 147:pp.1843-1854. Available : www.ncbi.nlm.nih.gov/pubmed [March,10,2013].

[15] M.K. Moore, and R.M. Ball.(2002,Feb.).”Multiple paternity in loggerhead turtle (*Caretta caretta*) nests on Melbourne Beach, Florida: a microsatellite analysis.” *Molecular Ecology*. [On-line].1:pp.281-288.Available: http://dx.doi.org/10.1046/j.1365-294X.2002.01426.x[Feb,12,2012].

[16] K.Kichler, M.T. Holder, S.K. Davis, R. Marquez-M, D.W. Owens.(1999,May).”Detection of multiple paternity in the Kemp’s ridley sea turtle with limited sampling.” *Molecular Ecology*. [On-line].8:pp.819-830.

Available : www.library.wiley.com[March,20,2013].

[17] T.Uller, and M. Olsson. (2008,April).”Multiple paternity in reptiles: patterns and processes.” *Molecular Ecology*. [On-line].17: pp.2566–2580. Available : http://doi: 10.1111/j.1365-294X.2008.03772.x[April,16,2013].

[18] W.E.J. Hoekert, H. Neuféglise, A.D. Schouten, and S.B.J. Menken.(2002,April).”Multiple paternity and female-biased mutation at a microsatellite locus in the olive ridley sea turtle (*Lepidochelys olivacea*).” *Heredity*. [On-line]. 89: pp.107-113. Available : www.nature.com.[April,12,2012].

[19] J.A. Zbinden, C.R. Largiadèr, F. Leippert, D. Margaritoulis, and R. Arlettaz.(2007,Sept.).”High frequency of multiple paternity in the largest rookery of Mediterranean loggerhead sea turtles.” *Molecular Ecology*. [On-line]. 16:pp.3703-3711.Available:www.dx.doi.org/10.1111/j.1365-294X.2007.03426.xiu[June,2,2013].

[20] C.M. Davy, T. Edwards, A. Lathrop, M. Bratton, M. Hagan, B. Henen, K.A. Nagy, J. Stone, L.S. Hillard, and R.W. Murphy.(2011,May).”Polyandry and multiple paternity in the threatened gassiz’s desert tortoise, Gopherus agassizii.” *Conserv.Genet*. [On-line].12:pp.1313-1322. Available: www.doi:10.1007/s10592-011-0232-y[June,2,2013].

[21] R.K. Aggarwal, A. Lalremruata, T.P. Velavan, A.P. Sowjanya, and L. Singh.(2008, August.).”Development and characterization of ten novel microsatellite markers from olive ridley sea turtle (*Lepidochelys olivacea*).” *Conserv. Genet*. [On-line]. 9:pp.981–984.Available:www.doi 10.1007/s10592-007-9421-0[Oct,6,2013].

[22] R.K. Aggarwal, T.P. Velavan, D. Udaykumar, P.S. Hendre, K. Shanker, B.C. Choudhury, and L. Singh.(2004,Nop.).”Development and characterization of novel Microsatellite markers from the olive ridley sea turtle (*Lepidochelys olivacea*).” *Molecular Ecology Notes*. [On-line].4:pp.77–79.Available :www. doi: 10.1046/j.1471-8286.2003.00574.x[July,6,2013].

[23] N. Mudsuk, S. Wansuk, M. Somchai, and K. Narongdet.”Detection of multiple paternity in green turtle clutches during a reproductive season at Khram Island, Thailand,”*inProc.of the International Symposium on SEASTAR 2000 and Bio-logging Science*, 2004, pp.1-6.

[24] S. Roques C. Díaz-Paniagua, and A.C. Andreu.(2004, May).”Microsatellite markers reveal multiple paternity and sperm storage in the Mediterranean spur-thighed tortoise, Testudo graeca.” Can. *J. Zool*. [On-line].82: pp.153–159. Available: www. nrcresearchpress.com. doi: 10.1139/z03-228[July,6,2013].

[25] J.D. Miller.”Reproduction in sea turtles,”in *The biology of sea turtles*. 1st ed. Vol. 1. CRC Press, Boca Raton, FL. 1997,pp. 51–81.

[26] M. Olsson, M. Healey, E. Wapstra, T. Shwartz, N. LeBas, and T. Uller.(2007,Des.).”Mating system variation and morph fluctuations in a polymorphic lizard.” *Molecular Ecology*. [On-line].16:pp.5307-5315.Available : www.library.wiley.com[March,25,2012].

[27] T. Tregenza, and N. Wedell. (2002,Jan.)."Polyandrous females, avoid costs of inbreeding."*Nature*. [Online].415:pp.71-73. Available : www.nature.com doi:10.1038/415071a[March,20,2013].