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A study on reproductive specialization of many lined Sun Skink *(Mabuya multifasciata)*

Uma Dutta[1] and Nabanita Medhi[2]
[1]Department of Zoology, Cotton College, Guwahati, India
[2] Department of Zoology, Gauhati University, Guwahati, India

Abstract

The scincid lizard *Mabuya multifasciata* contributes its part to the biodiversity of North-Eastern region of India. However, its distribution, diversity and reproductive behaviour is least reported in this part of country. Studies on reproductive pattern is fundamental point for the conservation of the species, population studies and also for the balance of entire ecosystem. Therefore, the present study has been designed with an objective to evaluate the specificity of female reproductive organs in Mabuya multifasciata, if any, by assessment of histological architecture of reproductive organs and embryonic nutritional pattern during breeding period. The study was carried out during March-May of a year. Following routine technique the histological structure of the ovary and the oviduct had been attempted. Ovarian histology reflected various stages of follicular development. Ovary of gravid female showed vitellogenic follicle. Villi like infoldings were found to be projected into oviduct lumen, which may probably helpful in propelling the egg through oviduct. Histology of uterine oviduct in gravid female showed placental structure, which may lead to placentotrophy. The presence of yolk sac with direct connection to the developing embryos has established the occurrence of lecithotrophy. Therefore, *M. multifasciata* can be stated as a lecitho cum placentotrophic viviparous species, or an intermediate species, which may represents a link between both oviparous and viviparous form.

Keywords : Mabuya multifasciata, Ovary, Lecithotrophy, Placentotrophy,

1. Introduction

Reproductive mode has been remarkably labile among squamate reptiles, since an evolutionary transitional range from oviparity to viviparity is expressed within this group. Squamate reproduction typically involves these two distinct generalized pattern i.e., oviparity where all or most of the development of the embryos in the egg takes place after the egg has been laid; and viviparity where females gestate developing eggs inside their reproductive tract and gives birth to young ones (Blackburn,2000). The evolutionary transition of viviparity from oviparity commonly has been accompanied by a shift in the pattern of embryonic nutrition (Stewart et al, 2004). Viviparity has been relatively evolved quickly through few structural and functional modifications. Lizards of the family Scincidae (skink) is a useful model for studying morphological and physiological adaptation to viviparity (Thomson et al, 2002). Among Skinks 70% species are viviparous and inhabit a wide range of placental complexities (Stewart and Thomson, 2002). The viviparous skink Eluamprus tympanum is totally lecithotrophic, with only water and some inorganic ions supplied by simple placenta (Thomson et al, 2001). On the other hand, the genus Mabuya have highly specialized placenta depicting a degree of placentotrophy similar to that of eutherian mammals.

The scincid lizard *Mabuya multifasciata* (synonymous to Eutropis multifasciata) commonly known as Many Lined Sun Skink occupies an important position in the nature because of its evolutionary species diversity. However, detailed study of distribution, diversity and reproductive behaviour of this species has not yet been reported in North East region of India. It is also evident that the

Corresponding author : umadutta@yahoo.com

study of reproductive pattern of a particular species is most essential for species diversity, survival, conservation and balance of entire ecosystem (Molinia et al, 2010). Therefore, in the present study, an attempt was made to investigate the specificity of female reproductive organ through histological architectural changes of oviduct and ovary during the breeding period along with embryonic nutritional pattern.

2. Materials and Methods

2.1 Animal collection : Twelve (12 no.) of individuals of the specimen Mabuya multifasciata were collected during the month of March to May (2011) from the Gauhati University campus Guwahati of Kamrup district and from the Mangaldai area of Darrang district with the help of collecting net.

2.2 Materials : Ethanol (95%) and Rectified spirit purchased from Bengal chemicals, Kolkata, India. Paraffin, xylene, Eosin, Phloxin and Haematoxyline purchased from Hi-Media, Mumbai and Loba Med, India.

2.3 Methods : After collection of the specimen, body measurements of all individual specimens were recorded which includes the length of Snout to Vent (SVL), Total Body Length (TBL), Tail Length (TL) as well as external morphological feature and body colour.

Six specimens were dissected ventrally to open up the abdominal cavity, dividing into 2 groups – three(3) in the earlier period (month of March) and three(3) in the later period (month of May). External morphology and gross anatomical features were studied and photographs were taken using Canon digital camera. The ovary and oviduct from non-gravid female as well as extra embryonic membrane together with ovary and oviduct from gravid female were carefully excised for histological preparation.

The tissues of respective reproductive organs were trimmed into small pieces (0.5 cm), fixed in freshly prepared Carnoy's fixative for 24 hours for histological studies. The tissues were rehydrated in descending grades of alcohol and then washed thoroughly in running tap water. After thorough washing, the tissues were dehydrated in ascending grades of alcohol, cleared in xylene and embedded in paraffin and blocks were made. Sections were cut at 4-5 micron thickness and stained by routine technique following Haematoxyline and Eosin (H & E) method (Luna, 1968). Micro photographs were taken with the help of Bright-field microscope.

3. Results

3.1 General body pattern and morphology

The body of the collected specimens were bronzy brownish or olive green in colour with 5-7 dark stripes on its dorsal surface (Plate1.A). Presence of bright and beautiful spots in the lateral side of the body indicated reproductive activity in females. Well developed limbs with 5 digits and smooth hexagonal scales present in the dorsal body surface. Teeths were relatively small, pleurodont in dentition. The individual measurement of each animal with their total body length (TBL), snout to vent length (SVL) and tail length were given in Table-1. The average length of total body (TBL), length of snout to vent (SVL) as well as tail length were 29.68 cm, 10.72 cm and 18.96 cm respectively (Fig-1).

Table -1: Measurement of the relative proportion of length of total body, snout to vent and tail

Specimen no.	Total Body Length (TBL) (cm)	Snout to Vent Length (SVL) (cm)	Tail Length (cm)
1	30.0	11.5	18.5
2	33.0	14.0	19.0
3	29.5	12.0	17.5
4	31.0	11.5	19.5
5	28.0	9.4	18.6
6	26.4	8.0	18.4
7	28.6	8.5	20.1
8	30.0	10.8	19.2
9	32.8	12.0	20.8
10	27.3	7.5	19.8
11	29.0	12.4	16.6
12	30.5	11.0	19.5
	Average length= 29.68 cm	Average length=10.72cm	Average length=18.95cm

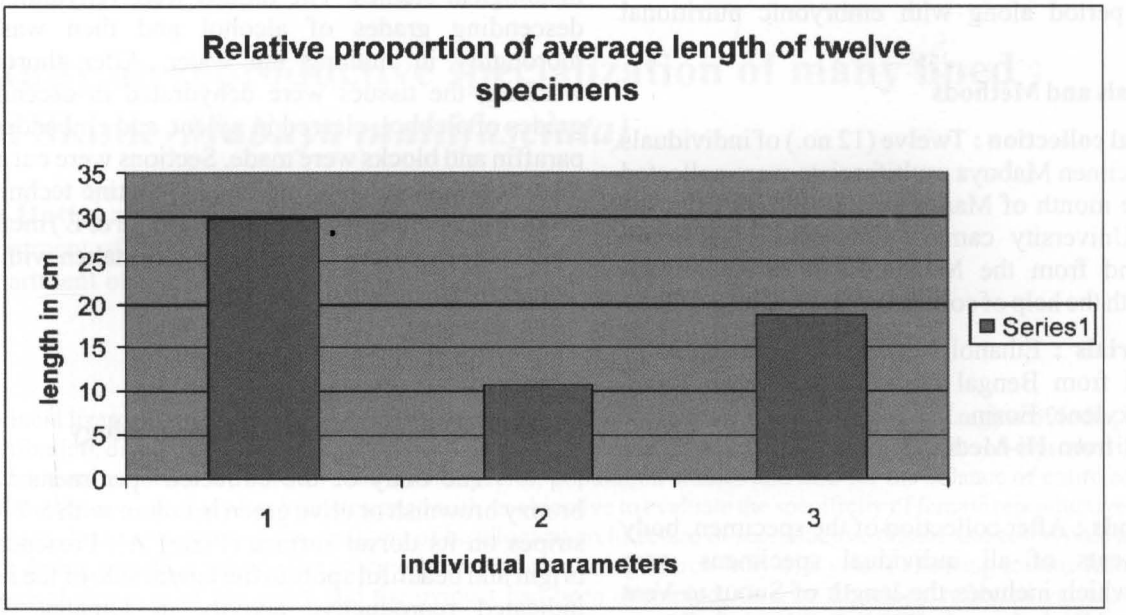
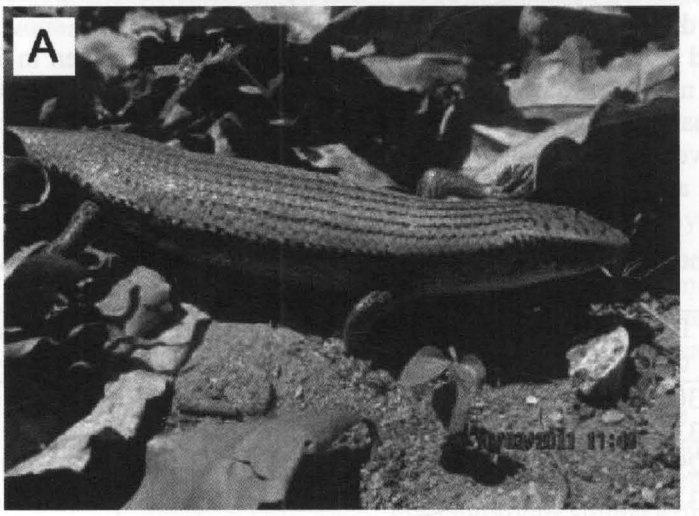


Fig-1: Relative proportion of average body length, Snout-vent length and tail length of twelve(12) specimens



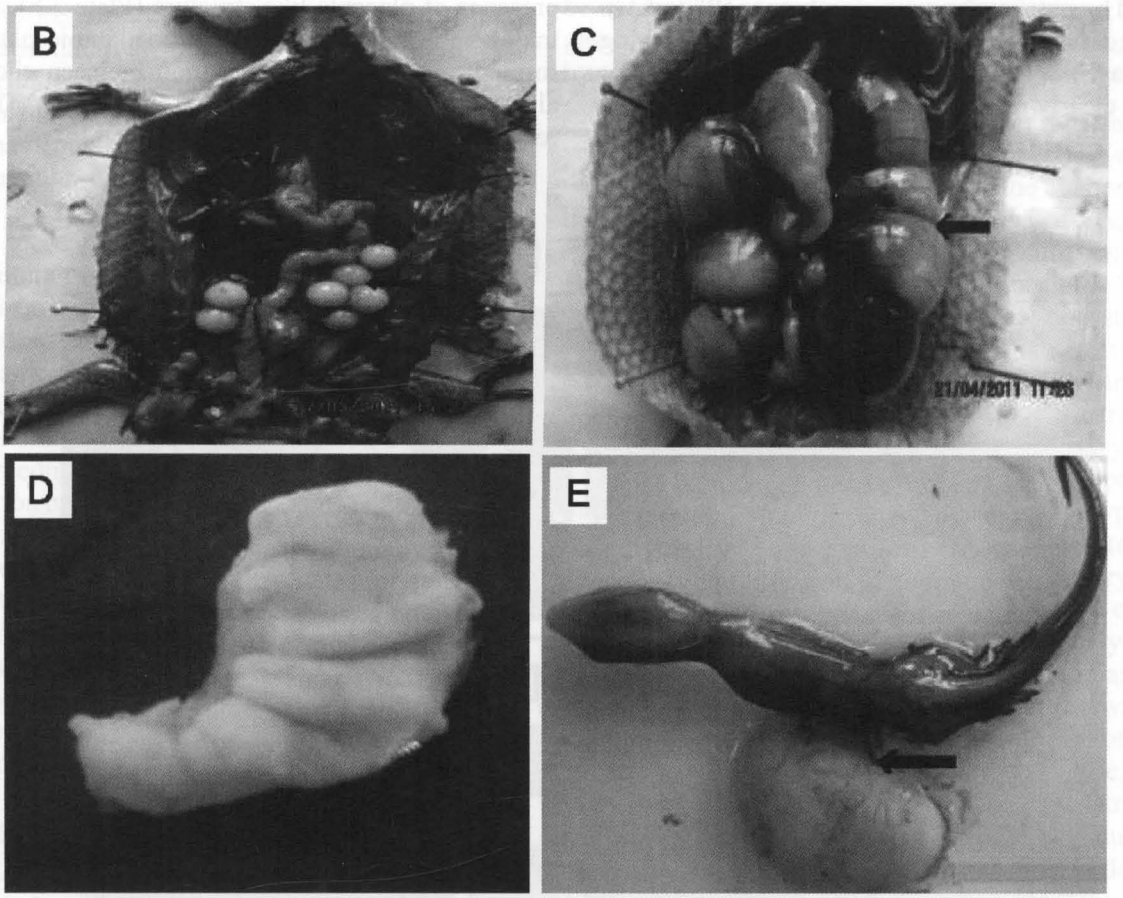


Plate-1(A-E): A. Shows external morphology of the species Mabuya multifasciata collected in month of March, 2011 ; B. Gross anatomy of non-gravid female showing eggs in the abdominal cavity (arrow), C. Gravid female showing retention of embryo in the oviduct (arrow); D. Highly folded structure of oviduct in non-gravid female; and E. Direct vascular connection of developing embryo with the yolk sac (arrow).

3.2 Histological architecture of ovary and oviduct

3.2.1 Ovary

The non-gravid animals collected in the month of March were found to be polyautochronic due to ovulation of number of ova ranges from 3-10 from both ovaries simultaneously (Plate 1.B). Even after completion of ovulation, eggs were retained in the abdominal cavity for sometime that indicative for ready to the passage through oviduct. The sizes of ova were 5 mm in diameter in a clustered form, the ovary resembles a cluster of spheres and reflecting various stages of follicular development (Plate 2.A).

The gravid female collected in the month of May showed the the presence of vitellogenic follicles in the ovary. These follicles were larger in size due to accumulation of yolk, and some of them showed eccentric germinal vesicles in the ooplasm (Plate 2.D). Large vitellogenic follicle showed clearly distinct zona pellucida and thecal layer separated by a layer of intermediate cells (Plate 2.E).

3.2.2 Oviduct

Gross morphology of oviduct from non gravid females revealed highly folded structure (Plate 1.D). Histologically, presence of numerous villi like infoldings from the wall of oviduct that were projected into lumen (Plate 2.B). It was lined by a single layer of epithelial cells followed by a thin layer of vascularized connective tissue.

In the gravid females, the oviducts were found

to retain embryos inside ranges from 7-9 numbers and protected by extra embryonic membranes (Plate 1.C). Presence of large amount of yolk in the yolk sac having direct vascular connection (Plate 1.E) with the embryos clearly revealed lecithotrophic nature of the animal. Histological observation revealed the presence of placenta like structure (Plate 2.F) which may be the typical type-iv chorioallantoic placenta formed as a result of association between chorion, allantois, yolk sac and oviducal epithelium. This is restricted to Mabuya group only.

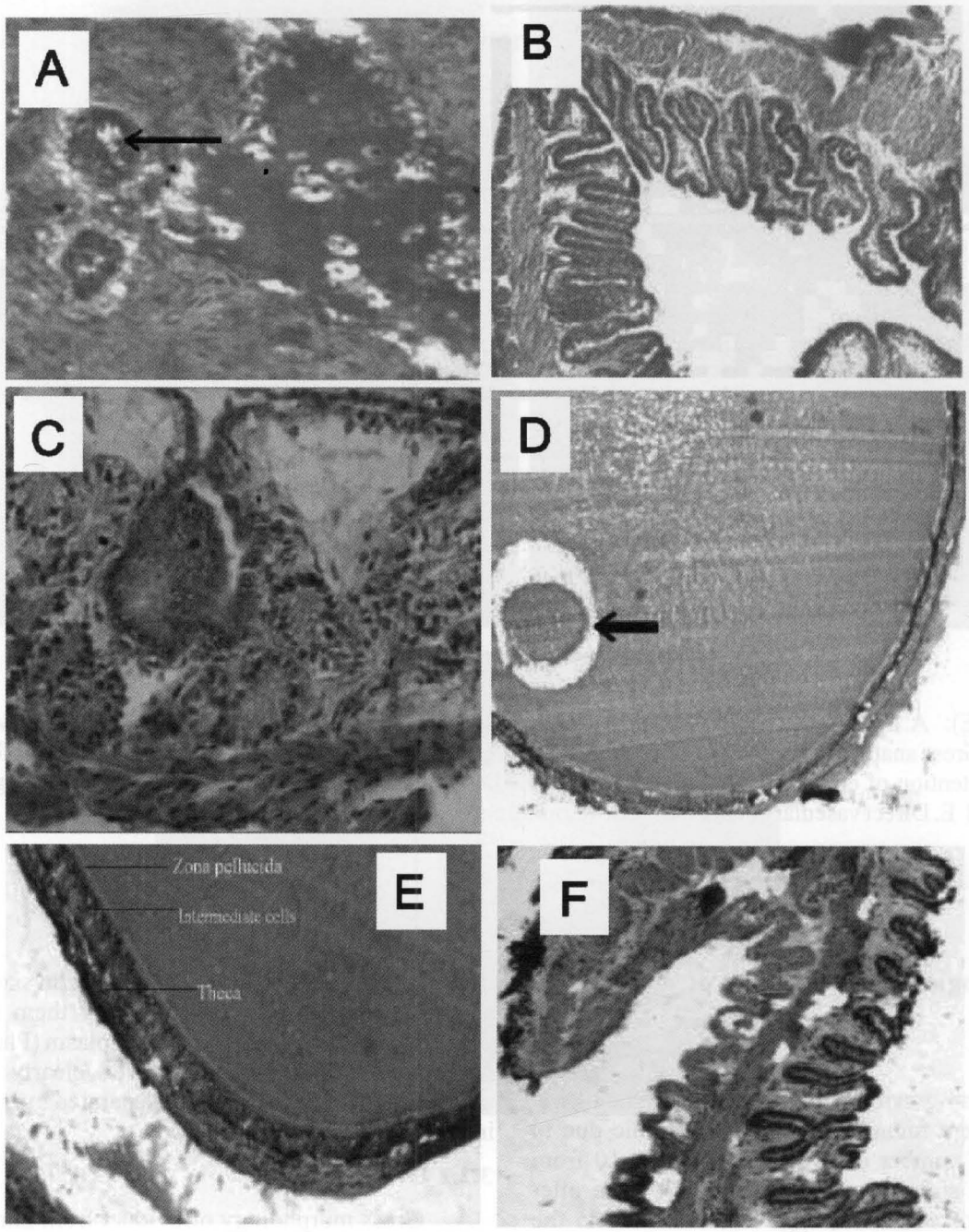


Plate 2(A-F): A. Growing follicles in the ovary of non-gravid female Mabuya multifasciata (arrow mark) (X 400, H and E). B. Villi like infoldings in the oviduct of non gravid female (X 150, H & E) . C. Oviduct shows rounded appearance of villi in gravid female (X 400, H & E). D. Arrow indicates vitellogenic follicle with eccentric germinal vesicle in ovary of gravid female (X 400, H & E). E. Show a large vitellogenic follicle with zona pellucida and theca separated by intermediate cells(X 400) and F. Placental structure shows villous folds of uterine endometrium protruding into chorioallantois.(X 150, H & E).

4. Discussion

The present findings suggest an overall feature about reproductive pattern of species *Mabuya multifasciata.* The present histological study showed development of follicular structure in ovarian cortex, which may move towards the central cavity as the development proceeds. Similar kind of study was reported in *Mabuya brevicollis* (Dokhi, 1998). The studied specimen *Mabuya multifasciata* showed infundibulum of the oviduct as funnel like structure with ciliated margin. Such kind of ciliary structure may probably helpful during the transfer of ovum from abdominal cavity into the oviduct. This was also supported by Fox, (1997) and Guillette, (1985). The gross anatomical features of the dissected specimen during breeding period showed some sign of special organization by retention of developing embryo inside the oviduct with maternal-fetal vascular connection. This feature clearly indicated the viviparous mode of reproduction. Earlier studies by Murphy (2000, 2004) and John *at al.,* (1982) stated that the microvilli of uterine epithelium undergo changes by plasma membrane transformation prior to implantation to allow the newly hatched blastocyst to implant in the wall of the uterine lining, giving rise to a somewhat rounded appearance of microvilli. Those findings extend support to the present histological finding.

The viviparity in the skink species Mabuya multifasciata revealed the presence of placenta as specialized structure. Previous workers Stewart and Thomson (2000) demonstrated placental complexities in the species Eulamprus tympanum. Earlier report showed that squamate placentation can be divided into 3 generalized patterns – (i) Chorioplacentation, (ii) Yolk sac placentation and (iii) Allanto placentation. Blackburn (1993) added a type-iv placenta found in most skinks of the genus Mabuya, which bears superficial similarities to type-iii category but is highly distinctive by presence of chorionic areolae. This type-iv placenta contains villous folds of the uterine lining, the endometrium that protrude deeply into invegination of chorioallantoic membrane. The present study also revealed somewhat placenta like structure bearing such special features, and hence may be assumed as placenta that actively takes part in the gaseous exchange and some amount of nutrient transfer.

Another important finding is the presence of yolk in the yolk sac with direct connection to the developing fetus. This indicates the pattern of lecithotrophy. In addition, the presence of placental structure associated with embryonic nutrition may also suggest to the pattern of placentotrophy. Thus, this species *Mabuya multifasciata* is not strictly lecithotrophic but exhibit some capacity of placentotrophy. Therefore, it may be an intermediate group. Again in contrast to oviparous lecithotrophic species, where all nutrients for embryonic development must be present in egg, the viviparous animal carry on the opportunity for both type of nutrition- lecithotrophy as well as placentotrophy, thereby occupying an intermediate type of embryonic nutrition. The molecules required from outside of the yolk are oxygen and water and sometimes may be a small amount of Na or other inorganic ions as per previous data obtained from earlier reports (Stewart et al, 2009). Egg nutrients may contain lipid, proteins, some inorganic ions, Ca, Na, P, Mg, Chloride, and Vitamins etc. Any deficiency in the nutrients contained within the egg in viviparous form, must be compensated by uptake across the placenta during development.

5. Conclusion

The present investigation attempted on reproductive specialization through histological techniques clearly revealed that Mabuya multifasciata is a lecitho cum placentotrophic viviparous species which may represent a connecting link between both oviparous and viviparous form. This species bears enormous significance as it contributes an important part to the biodiversity of North east India. Study on reproductive strategy may helpful for the conservation of the species and balance of the ecosystem. Therefore, thorough investigation is required in this line for the study of its diversity.

References

Andrews, R. M., Mathies, T.. Natural history of reptilian development : Constraints on the Evolution of viviparity Bioscience Vol. 50, No.3.227.

Al-Dokhi, O. A. 1998. Ovarian structures and follicle development in the lizard Mabuya brevicollis. Saudi J. Bio. Sci., Vol.5, No.2.

Blackburn, D. G. 1999. Placenta and placental analogs in Reptiles and Amphibians. Encyclopedia of Reproduction, Vol 3.

Blackburn, D. G., Vitt, L.J. and Beuchat, C.A.. Eutherian like reproductive specialization in a viviparous reptile. Proc. Acad. Sci. USA, vol.81, pp. 4860-4863.

Blackburn, D. G. 1994 ; Standard criteria for recognition of embryonic nutritional patterns in Squamate Reptile. Copeia (4), pp 925-935.

Blackburn, D.G.; Flemming, A.F. 2010; Reproductive specialization in a viviparous African Skink and its implication for evolution and conservation ., Herpetol. Conserv. And Biol. 5(2) : 263-270.

Ferner, K., Mess, A. 2011. Evolution and development of fetal membranes and placentation in amniote vertebrates. Resp. Physiol. And Neurobiol .

Murphy, C. R., Shaw, T.J. 1994. Plasmamembrane transformation: a common response of uterine epithelial cells during the pre implantation period. Cell. Biol, int 18, 1115-1128.

Masson, G. R., Guillete, L.J. 1987, Changes in oviductal vascularity during the reproductive cycle of 3 oviparous lizards (Eumeces obsoletus, Sceloporus undulates and Crotaphytus collaris). J. Reprod. Fert. 80, 361-371.

Molinia, F. C.; Bell, T; Norbury, G.; Cree. A.2010 assisted breeding of Skinks or how to teach alizard old tricks. Herpatol cons. And Biol.5(2): 311-319

Packard, G.C.; Tracy, C.R.; Roth, J.J 1977. The physiology, ecology of reptilian eggs and embryos, and the evolution of viviparity within the class reptilian. Biol. Rev. 52, 71-105.

Radder, R., Shine, R. 2006, germinal bed condition in a Polyautochronic single clutched lizard, Bassiana duperreyi (Scincidae). Amphibia- Reptilia 28: 159-162.

Speake, B.K; Herbert, J.F.; Thomson, M.B 2004. Evidence for placental transfer of lipids during gestation in the viviparous lizard, Pseudomoia entrecasteauxii. Comp. Physiol. Biol. Part A 139 : 213-220

Thomson, M.B.; Stewart, J.R.; Speake, B.K.; Hoise, M.J.; Murphy, C. R., (2002). Evolution of viviparity : what can Australian Lizard tell us ? Comp. Biochem. Physiol., B 131 , 631-643.

Thompson, M. B.; Speake, B.K.; Russel, K.J.; McCartney, R. J 2001 a. Nutrient Uptake by Embryos of the Australian viviparous lizard, Eulamprus tympanum . Physiol. Biochem. Zool. 74, 560-567.

Whiting, A.S., SitesJr, J.W, Pellegrino, K. C.M., Rodrigues. M. T.2005. Comparing alignment method for inferring the history of new world genus Mabuya (Squamata: Scincidae). Mol. Phylo. And Evol. 38. 719-730.