HERPETOLOGICAL JOURNAL, Vol. 15, pp. 9-13 (2005)

REPRODUCTIVE BIOLOGY OF THE "GLASS SNAKE" *OPHIODES FRAGILIS* (SQUAMATA: ANGUIDAE) IN SOUTH-EAST BRAZIL

L. PIZZATTO

Pós-graduação em Ecologia, Depto. de Zoologia, Universidade Estadual de Campinas, Campinas, SP, Brazil

The reproductive cycles of tropical lizards can be continuous or seasonal, depending on a wide variety of factors, some of which are historical. Most studies on reproductive biology of lizards have been conducted on species in the Iguanidae, Gekkonidae, Teiidae, Lacertidae, and Scincidae. The few studies on anguid lizards have been on various alligator lizards and one species of *Ophisaurus*. This study presents data on sexual maturity, adult body size, neonate body size and body mass, fecundity, and male and female reproductive cycles of a population of *Ophiodes fragilis* from São Paulo and Paraná states in south-east Brazil. Females are larger and attain sexual maturity at a larger SVL than males. The female reproductive cycle is highly seasonal with secondary vitellogenesis starting in the middle of the rainy season (February). Embryos in early stages of development occur in the dry season (June) and parturition occurs at the transition from the dry season to the rainy season (August-December). Clutch size averages 7.5 young and is not related to maternal female size as in many other lizard species. Neonates were 33-57 mm in SVL and 0.45-0.85 g in mass. The residual volume of testes does not vary throughout the year suggesting a continuous spermatogenic cycle. Diameter of the deferent duct is largest from January to March (onset of the rainy season), suggesting that even though males produce sperm continuously, they store sperm until mating season.

Key words: legless lizard, reproduction, clutch size, reproductive cycle, spermatogenic cycle

INTRODUCTION

In temperate areas lizard reproduction is seasonal with mating and egg-laying often occurring from spring to summer (Fitch 1970). Some species mate in autumn and females of some of these species can store sperm over winter (Fox, 1963; Conner & Crews, 1980; Kwait & Gist, 1987). However, tropical lizard species reproduce continuously in some areas (Inger & Greenberg, 1966; Fitch, 1982; James & Shine, 1985; Vitt, 1986; Patterson, 1990) and seasonally in some areas where rainfall is seasonal (Fitch, 1982; James & Shine, 1985; Patchell & Shine, 1986; Clerke & Alford, 1993; Vrcibradic & Rocha, 1998). Male and female reproductive cycles can differ with males producing sperm through the year whereas females may produce eggs only seasonally (Clerke & Alford, 1993; Wilhoft, 1993*a,b*; Van Sluys *et al*., 2002). In addition, intraspecific variation in the reproductive patterns may occur across different latitudes or climatic areas (Fitch, 1982; Clerke & Alford, 1993). More recent analyses of existing squamate reproductive data demonstrate that a considerable phylogenetic signal exists in lizard reproductive strategies. Species within clades tend to be more similar to each other in their reproductive strategies than they are to species in distant clades (Dunham and Miles, 1985). The ability to discern evolutionary strategies in lizard reproduction requires reasonable data on reproductive variables of species in all major clades. One shortcoming of the study by Dunham and Miles (1985) and earlier studies (Tinkle *et al*., 1970) has been a lack of data on several important lizard clades, such as the Anguidae.

This kind of gap is also seen in Neotropical areas. Although several studies about lizard reproduction are available in this region, they are limited to only some families: Polychrotidae (Vitt & Lacher Jr., 1981), Scincidae (Vitt & Blackburn, 1991; Vrcibradic & Rocha, 1998; Rocha & Vrcibradic, 1999), Tropiduridae (Rocha, 1992; Van Sluys, 1993; Vieira *et al*., 2001; Van Sluys *et al*., 2002; Wiederhecker *et al*., 2002) and Gekkonidae (How *et al*., 1986; Vitt, 1986). The few reproductive studies on anguid lizards have been conducted on various alligator lizards (Vitt, 1973; Goldberg, 1972, 1975) and one species of *Ophisaurus* (Fitch, 1989), all in the northern temperate zone.

The genus *Ophiodes* (Anguidae) is exclusively Neotropical and are distributed in the east Andes, central, east and south-east South America. The genus contains about seven species of limbless, elongate lizards (Martins, 1998). *Ophiodes fragilis* Raddi, 1820 (after revision of Martins, 1998) occurs in south and south-east Brazil, in coastal south Bahia state, Minas Gerais and Mato Grosso do Sul. In Argentina it is registered in north-east of Missiones (Martins, 1998). This species is common in anthropic areas and, similar to North American *Ophisaurus*, are commonly known as glass snakes. As in other anguids, their cryptozooic habits make it difficult to conduct ecological studies on *Ophiodes*.

In the present study, I present data on the reproductive biology of *O. fragilis* in south-east Brazil, including

Correspondence: L. Pizzatto, Depto. de Zoologia, Universidade Estadual de Campinas, CP. 6109, cep. 13083-970, Campinas, SP, Brazil.
E-mail: ligia_oceanica@yahoo.com

size at sexual maturity, body size of males and females, sexual dimorphism, reproductive cycles, fecundity and neonate size.

MATERIAL AND METHODS

A total of 115 (32 females, 42 males and 40 juveniles) specimens were examined from the collections of the Museu de História Natural da Universidade Estadual de Campinas (ZUEC) and Museu de História Natural do Capão da Imbuia (MHNCI). Areas where these specimens were collected are covered mainly by open vegetation, disturbed areas and plantations in São Paulo and Paraná states, south-east Brazil (between latitudes 19.9º and 26.6ºS). Climate is seasonal. The warm and rainy season extends from October to March and the cold and dry season from April to September (Nimer, 1989). During the rainy season mean temperature varies from 21 to 24ºC and mean rainfall varies from 123 to 240 mm. In the cold and dry season mean temperature varies from 17 to 22ºC and mean rainfall varies from 36.8 to 121 mm (Nimer, 1989).

The following data were taken for each specimen: (1) snout-vent length (SVL – to the nearest 1mm); (2) sex; (3) reproductive status: mature or immature. Females were considered mature when the diameter of their ovarian follicles was greater than 3 mm or if they had embryos; males were considered mature if the testes were large and turgid or if the deferent ducts were opaque and convoluted, indicating the presence of sperm (see Shine, 1977); (4) diameter of the largest ovarian follicles or embryos (plus yolk) in females (to the nearest 0.1 mm); (5) embryonic stage (from one to three according to the quantity of yolk, see below); (6) largest, medium and smallest diameters of testes (testicular volume –TV– was estimated using the ellipsoid volume formula $TV = 4/3ab^2$ where a = largest radius, b = smallest radius – *cf.* James and Shine, 1985); (7) deferent duct diameter near the cloaca (see Almeida-Santos et al., 2003). Development of embryos was categorized as: Stage 1 – just following ovulation (and probably fecundation) when only yolk was visible; Stage 2 – a small embryo could be observed but yolk was still very abundant, and Stage 3 – a large embryo was present and totally developed, without yolk.

Birth data were obtained from gravid females received at Instituto Butantan (IB), São Paulo, Brazil. Pregnancy was detected by palpation and these females were maintained in captivity (at room temperature 19.3-31.0°C) until birth. Food (cockroaches and lycosid spiders) and water were provided *ad libitum*. After birth, each neonate was measured in SVL (to the nearest 1 mm) and weight (to the nearest 0.1 g). Clutch size was estimated based on the number of embryos in preserved specimens. Because captivity may affect gestation and clutch size, data on number of neonate lizards from captive females were not used in fecundity estimate. Neonate SVL was recorded from individuals received at IB on birth time and individuals born in captive. An index of the degree of sexual size dimorphism (SSD) was "(mean adult SLV of the larger sex/mean adult SLV of the smaller sex) –1" (*cf.* Shine, 1994).

Analysis of volume of testes and diameter of deferent duct was made by combining data from each of four seasons: (1) onset of rainy season (October-December), (2) end of rainy season (January-March), (3) onset of dry season (April-June) and (4) end of dry season (July-September). Because only testes volumes were correlated with SVL by seasons, residuals from the regression of testes volume to SVL were used to indicate spermatogenic activity. Diameter of deferent duct was used to indicate sperm release (*cf.* Volsøe, 1944; Shine, 1977).

RESULTS

SEXUAL MATURITY AND BODY SIZE

The smallest mature female measured 156 mm SVL and the smallest mature male measured 126 mm SVL. Females (SVL = 192.5±28 mm, range 56-300 mm) are significantly larger than males (SVL = 158.9±22.8 mm, range 126-221 mm) (t=5.87, df=1, P<0.0001). The degree of sexual size dimorphism (SSD) was 0.211.

FEMALE REPRODUCTIVE CYCLE

Secondary vitellogenesis starts in February and ovulation occurs just after secondary vitellogenesis, from March until June. At this time much yolk is visible in ova (stage 1). Embryos in stage 2 are found in June and early August (Fig. 1). By the end of August, embryos in stage 3 begin to appear and continue until December (Fig. 1). In September, one female undergoing parturition was received at IB. Another gravid female gave birth in August (after five months and one week in captivity) and another one in October (after four months and two weeks; Fig. 1). Gestation lasts at least five months (Fig. 1) and all embryos within a given female were in the same developmental stage.

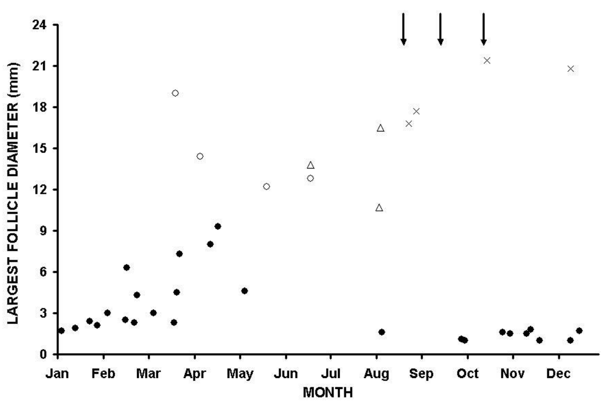

FIG 1. Seasonal variation in the diameter of the largest ovarian follicle in adult females of *Ophiodes fragilis* from south-east Brazil. Solid circles = ovarian follicle; open circles = embryo at stage one of development; triangles = embryo at stage two of development, crosses = embryo at stage three of development; arrows = birth.

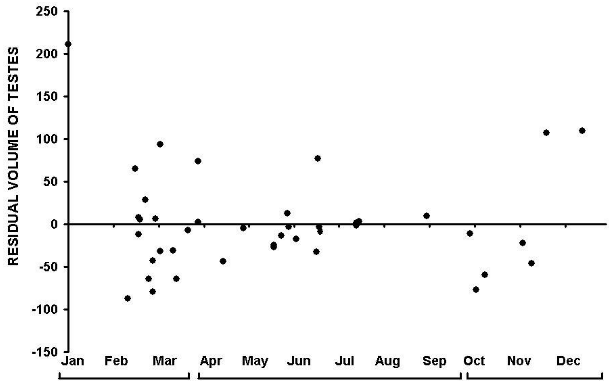

FIG 2. Seasonal variation in the residuals of volume of testes in adult male *Ophiodes fragilis* from south-east Brazil.

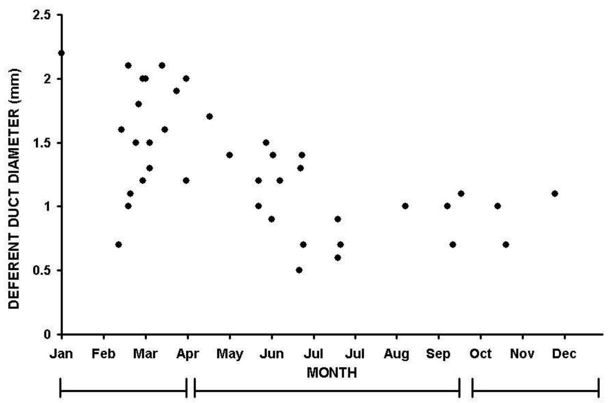

FIG 3. Seasonal variation in the diameter of deferent duct in adult male *Ophiodes fragilis* from south-east Brazil.

Fecundity and Neonate Size

Clutch size averaged 7.5±2.3 embryos (range 5-13, n=18) in preserved specimens. In captive specimens (females collected gravid), mean clutch size was 3.6±1.53 (range 2-5, n=3). Clutch size was not related to female SVL (r=0.23; P=0.366, n=18). Neonate SVL averaged 44.1±9.4 mm (range 33-57 mm, n=11 individuals from three clutches) and weighed 0.69±0.18 g (range 0.45-0.85 g, n=6 individuals from two clutches).

Male Cycle

Size-adjusted testes volume did not differ among seasons (Kruskal-Wallis test: H=1.34, df= 3, P=0.718, n=41, Fig. 2). However, diameter of deferent duct decreased significantly from July to December (Kruskal-Wallis test: H=18.98, df=3, P=0.0003, n=41) and reached maximum size from January to June (Fig. 3).

DISCUSSION

Females of *Ophiodes fragilis* are larger in SVL than males, as in some other lizard species (and also in snakes) (Patchell & Shine, 1986; Shine, 1994; Vrcibradic & Rocha, 1998). The SSD index to *O. fragilis* was higher than in viviparous skinks of the genus *Mabuya* (range 0.055-0.145; Rocha & Vrcibradic, 1999) and in many oviparous geckos (range 0.053-0.164; *cf.* How *et al*., 1986; Vitt, 1986). However, it is similar to that of some oviparous legless Australian pygopodids (*Lialis burtonis:* 0.242; *Aprasia pulchella:* 0.236; *cf.* Patchell & Shine, 1986). SSD can be related to both ecological traits and phylogenetic lineage. As in many other lizards, including the oviparous legless anguid *Ophisaurus attenuatus*, clutch size is not significantly related to maternal SVL (How *et al*., 1986; Patchell & Shine, 1986; Fitch, 1989; Clerke & Alford, 1993; Rocha & Vrcibradic, 1999). In many snakes the primary selective force causing larger SLV in females in relation to males is the potential increase in fecundity (clutch size) associated with increased body size (Shine, 1994). This explanation may apply to lizard species in which clutch size increases with maternal female size, but does not apply to species in which there is no clutch size-female size relationship. In these species, a tradeoff between present and future reproductive success may exist, particularly if females have a high probability of surviving to the next reproductive season Shine, 1988). As female body size increases, frequency of reproduction may increase thus driving the evolution of female-biased sexual dimorphism in the absence of a clutch size-female size relationship (*cf.* Shine, 1988).

Clutch size in *O. fragilis* is larger than that in legless oviparous pygopodid lizards but similar to the oviparous legless *Ophisaurus attenuatus* (Patchell & Shine, 1986; Fitch, 1989). Low clutch size in pygopodids probably reflects their origins within the Gekkota, a group of lizards typically having clutch sizes of one or two eggs (Fitch, 1970; Dunham and Miles, 1985; Vitt, 1986). Although relative clutch mass and clutch size may be influenced to some degree by body shape (e.g. Vitt, 1981), clutch size may be conservative in anguid lizards, even when the reproductive mode differs. In captivity, high mortality of *Ophiodes fragilis* embryos (at stage 1) is suggested by the lower clutch size in captive females than in wild ones. Circumstantial evidence suggests that early-stage ova can be reabsorbed. One gravid female (detected by palpation) collected and killed some months after being held captive, contained no vestiges of embryos, and she had regressed ova in the oviducts.

The female reproductive cycle in *Ophiodes fragilis* was highly seasonal, as is expected for viviparous reptiles. Oviparous lizards usually tend to lay eggs from late spring to early summer both in temperate (Mayhew, 1963; Fitch, 1989) and tropical areas (How *et al*., 1986; Patchell & Shine, 1986; Rocha, 1992; Clerke & Alford, 1993; Van Sluys, 1993, Vitt, 1973). Hatching may occur in late summer or early autumn. However, in *O. fragilis* secondary vitellogenesis and ovulation starts in late summer and early autumn, pregnancy lasts at least five months (March to August) and parturition starts in late winter (August). This pattern is similar to that of some tropical viviparous skinks of the genus *Mabuya* (Vitt & Blackburn, 1991; Vrcibradic & Rocha, 1998; Rocha & Vrcibradic, 1999) and differs significantly from that of the viviparous anguid *Gerrhonotus coeruleus principis* in temperate areas (Vitt, 1973).

Barbosa *et al*. (1991) recorded parturition of a captive female *O*. cf. *striatus* collected in Rio de Janeiro State in October. Although this record is consistent with the present study, Barbosa *et. al*. (1991) did not describe the specimen in his work. Because *O. striatus* is a large complex of species, in which *O. fragilis* was included (Martins, 1998) it is not possible to recover its actual identification. Marques & Sazima (2003) recorded parturition of two *O. fragilis* (O.A.V. Marques, pers. com) collected in the Atlantic forest in November. Additionally, I recorded parturition, in captivity, of an individual from Minas Gerais State. This female was collected on 22 July and gave birth to six lizards on 4 October indicating that its reproductive period is similar to that of other populations close to the studied area.

Although some tropical species have seasonal spermatogenesis (Rocha, 1992; Vrcibradic & Rocha, 1998) many others produce sperm continuously (Van Sluys, 1993; Van Sluys *et al.*, 2002), similar to *O. fragilis*. However, in this species, mating appears to be seasonal, occurring at the end of the rainy season (at least from February to March) as indicated by the increase in deferent duct diameter. Mating during this season is synchronous with fecundation and thus, sperm storage by females is not essential to reproductive cycle in this species.

ACKNOWLEDGMENTS

I thank to Otavio A. V. Marques for suggesting this study. Fernanda Stender de Oliveira, João C. Ferreira, and Donizetti for help in reception of specimens at IB. Julio C. Moura-Leite for permission to access the collection of Museu de História Natural do Capão da Imbuia (MHNCI). Paulo Roberto Manzani and Fátima B. Souza for permission to access the collection of Museu de História Natural da Universidade Estadual de Campinas (ZUEC). Marcio B. Martins for confirmation on species identity. Jivanildo P. Miranda, Carlos Frederico Duarte Rocha, Monique Van Sluys and Otavio A. V. Marques for reading and commenting on this manuscript. Laurie J. Vitt and Otavio A. V. Marques for reviewing this work. I thank to FAPESP (00/13654-9 and 00/12339-2) for financial support.

REFERENCES

Almeida-Santos, S. M., Pizzatto, L. & Marques, O. A. V. (2003). Intra-sex synchrony and inter-sex coordination in the reproductive timing of the coral snake *Micrurus corallinus* (Elapidae). *Herpetological Natural History,* in press.

Barbosa O. R., Machado, C. & Fernandes, L. (1991). Notas sobre a parturição da "cobra-de-vidro", *Ophiodes striatus* (Spix, 1825) (Sauria, Anguidae). *Revista Brasileira de Biologia* **51**, 285-287.

Clerke R. B. & Alford, R. A. (1993). Reproductive biology of four species of tropical Australian lizards and comments on the factors regulating lizard reproductive cycles. *Journal of Herpetology* **27**, 400-406.

Conner, J. & Crews, D. (1980). Sperm transfer and storage in the lizard, *Anolis carolinensis. Journal of Morphology* **163**, 331-348.

Dunham, A. E., & Miles D. B. (1985). Patterns of covariation in life history traits of squamate reptiles: The effects of size and phylogeny reconsidered. *American Naturalist* **126**, 231-257.

Fitch, H. S. (1970). Reproductive cycles in lizards and snakes. *Miscellaneous Publications of the Museum of Natural History University of Kansas* **52**, 1-247.

Fitch, H. S. (1982). Reproductive cycles in tropical reptiles. *Occasional Papers of the Museum of Natural History University of Kansas* **96**, 1-53.

Fitch, H. S. (1989). A field study of the slender glass lizard, *Ophisaurus attenuatus*, in northern Kansas. *Occasional Papers of the Museum of Natural History University of Kansas* **125**, 1-50.

Fox, W. (1963). Special tubules for sperm storage in female lizards. *Nature* **198**, 500-501.

Goldberg, S. R. (1972). Reproduction in the southern alligator lizard, *Gerrhonotus multicarinatus. Herpetologica* **28**, 267-273.

Goldberg, S. R. (1975). Reproduction in the Arizona alligator lizard, *Gerrhonotus kingi. Southwestern Naturalist* **20**, 409-420.

How, R. A., Dell, J. & Wellington B. D. (1986). Comparative biology of eight species of *Diplodactylus* gecko in western Australia. *Herpetologica* **42**, 471-482.

Inger R. F. & Greenberg, B. (1966). Annual reproductive patterns of lizards from a Bornean rainforest. *Ecology* **47**, 1007-1021.

James C. & Shine, R. (1985). The seasonal timing of reproduction: a tropical-temperate comparison in Australian lizards. *Oecologia* **67**, 464-474.

Kwait, G. A & Gist, D. H. (1987). Annual reproductive cycle of an introduced population of European wall lizard (*Podarcis muralis*) in Ohio. *Journal of Herpetology* **21**, 205-209.

Marques, O. A. M. & Sazima, I. (2003). História Natural dos Répteis da Estação Ecológica Juréia-Itatins. In Estação Ecológica Juréia-Itatins: Ambiente Físico, Flora e Fauna, 254-274. Marques, O. A. V. & Duleba W. (Eds.). Ribeirão Preto: Editora Holos.

Martins, M. B. (1998). Revisão taxonômica e sistemática filogenética do gênero *Ophiodes* Wagler, 1828 (Sauria: Anguidae: Diploglossinae). Umpubl. PhD. Thesis, PUCRS, Porto Alegre, Brasil.

Mayhew, W. W. (1963). Reproduction in the granite spiny lizard, *Sceloporus orcutii*. *Copeia* **1963**, 144-152.

Nimer, E. (1989). Climatologia do Brasil. 2nd ED. IBGE. Departamento de Recursos Naturais e Estudos Ambientais. Rio de Janeiro, RJ.

Patchell, F. C. & Shine, R. (1986). Food habits and reproductive biology of the Australian legless lizard (Pygopodidae). *Copeia* **1**, 30-39.

Patterson, J. K. (1990). Female reproductive cycles of the tropical lizard *Mabuya striata*. *Oecologia* **44**, 168-172.

Rocha, C. F. D. (1992). Reproductive and fat body cycles of the tropical sand lizard (*Liolaemus lutzae*) of southeastern Brazil. *Journal of Herpetology* **26**, 17-23.

Rocha, C. F. D. & Vrcibradic, D. (1999). Reproductive traits of two sympatric vivivparous skinks (*Mabuya macrorhyncha* and *Mabuya agilis*) in Brazilian restinga habitat. *Herpetological Journal* **9**, 43-53.

Shine, R. (1977). Reproduction in Australian elapid snakes I – Testicular cycles and matting seasons. *Australian Journal of Zoology* **25**, 647-53.

Shine, R. (1988). The evolution of large body size in females: a critique of Darwin's "Fecundity advantage" model. *American Naturalist* **131**, 124-131.

Shine, R. (1994). Sexual dimorphism in snakes revised. *Copeia* **1994**, 326-346.

Tinkle, D. W., Wilbur, H. M. & Tilley, S. G. (1970). Evolutionary strategies in lizard reproduction. *Evolution* **24**, 55-74.

Van Sluys, M. (1993). The reproductive cycle of *Tropidurus itambere* (Sauria: Tropiduridae) in southeastern Brazil. *Journal of Herpetology* **27**, 28-32.

Van Sluys, M, Mendes, H. M. A., Assis, V. B. & Kiefer, M. C. (2002). Reproduction of *Tropidurus montanus* Rodrigues, 1987 (Tropiduridae), a lizard from a seasonal habitat of south-eastern Brazil, and a comparison with other *Tropidurus* species. *Herpetological Journal* **12**, 89-97.

Vieira, G. H. C., Wiederhecker, H. C., Colli, G. R., Bao, S. N. (2001). Spermiogenesis and testicular cycle of the lizard *Tropidurus torquatus* (Squamata, Tropiduridae) in the Cerrado of central Brazil. *Amphibia-Reptilia* **22**, 217-233.

Vitt, L. J. (1973). Reproductive biology of the anguid lizard, *Gerrhonotus coeruleus principis*. *Herpetologica* **29**, 176-184.

Vitt, L. J. (1981). Lizard reproduction: habitat specificity and constraints on relative clutch mass. *American Naturalist* **117**, 506-514.

Vitt, L. J. (1986). Reproductive tactics of sympatric gekkonid lizards with a comment on the evolutionary and ecological consequences of invariant clutch size. *Copeia* **1986**, 773-786.

Vitt, L. J. & Blackburn, D. G. (1991). Ecology and life history of the viviparous lizard *Mabuya bistriata* (Scincidae) in Brazilian Amazon. *Copeia* **1991**, 916-927.

Vitt, L. J. & Lacher Jr., T. E. (1981). Behavior, habitat, diet and reproductive of the iguanid lizard *Polychrus acutirostris* in the caatinga of northeastern Brazil. *Herpetologica* **37**, 53-63.

Volsøe, H. (1944). Seasonal variation of the male reproductive organs of *Vipera berus* (L.). *Spolia Zoologica Musei Hauniensis* **5**, 1-157

Vrcibradic, D. & Rocha, C. F. D. (1998). Reproductive cycle and life-history traits of the viviparous skink *Mabuya frenata* in southeastern Brazil. *Copeia* **1998**, 612-619.

Wiederhecker, H. C., Pinto, A. C. S. & Colli, G. R. (2002). Reproductive ecology of *Tropidurus torquatus* (Squamata: Tropiduridae) in the highly seasonal Cerrado biome of central Brazil. *Journal of Herpetology* **36**, 82-91.

Wilhoft, D. C. (1993*a*). Reproduction in the tropical Australian skink, *Leiolopisma rhomboidalis. American Midland Naturalist* **70**, 442-461.

Wilhoft, D. C. (1993*b*). Gonadal histology and seasonal changes in the tropical Australian lizard, *Leiolopisma rhomboidalis. Journal of Morphology* **116**, 185-203.

Accepted: 22.8.03

APPENDIX 1

SPECIMENS EXAMINED

MHNCI: 198, 808, 849, 1438, 1697, 1699, 2075, 2076, 2106, 5166, 6756, 6984, 6985, 7119, 7227, 7272, 7394, 7442, 7660, 7761, 8156, 8228, 9026, 9164, -641. ZEUC: 2511 – 2548, 2550 – 2569, 2572, 2574, 2576, 2610 – 2614, 2616 – 2618, 2807 – 2829.