Reproduction of *Rhinolophus clivosus* (Microchiroptera) in Natal, South Africa

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Abstract

Investigated the reproductive cycle of *Rhinolophus clivosus*. The reproductive cycle of adults was characterized by copulation in May, sperm storage (in the oviducts and uterine horns) throughout winter hibernation, ovulation and fertilization in August, and parturition in December. Immature females came into oestrus in late September and parturition occurred in late January, after a three-and-a-half to four month gestation. Spermatogenesis and follicular development were similar to those previously described for members of this genus.

Introduction

Members of the genus *Rhinolophus* occur in tropical and temperate regions of the Old World, east to Australia, New Guinea, the Philippine Islands and Japan (Walker 1975).

Aspects of their reproduction have been described for species from northern Europe (Rollinat and Trouessart 1897; Matthews 1937; Gaisler 1966) and Australia (Dwyer 1966) where the reproductive cycles are characterized by a period of sperm storage during winter hibernation; from India (Ramakrishna 1950; Gopalakrishna and Ramakrishna 1977; Ramakrishna and Rao 1977) where the reproductive cycle is characterized by a period of delayed implantation; and from tropical Africa (Anciaux de Faveaux 1978) where reproduction is typically mammalian.

At least nine species of *Rhinolophus* occur in South Africa (Hayman and Hill 1971) yet precise details of the reproductive cycles are not available for any of them. Data for South Africa is limited to a report of lactating female *R. clivosus* in Natal in January (Harrison and Clancey 1952) and a brief description of the reproductive cycle of *R. clivosus* noting that sperm storage occurs (Laycock 1976).

The aim of this paper is to describe the reproductive cycle of *R. clivosus* and the work forms part of a larger study examining the reproduction of members of the genus *Rhinolophus* in South Africa.

Materials and methods

Specimens of *R. clivosus* were collected from several roosts (caves and disused mines) in the Natal Midlands area (c. 29° S) on an approximately two-weekly basis during 1981 (see Table). Specimens were killed by asphyxiation with carbon dioxide and the female reproductive tract or testes and epididymides removed. Tissues were fixed in Bouin's fluid for at least seven days and thereafter stored in 70 per cent alcohol. (All weighings were done from 70 per cent alcohol on a Sauter AR 100 analytical balance.) Following routine embedding and sectioning at 5 μm, sections were stained with Ehrlich's haematoxylin and eosin.

Changes in seminiferous tubule diameter were quantified by measuring two diameters at right angles in cross sections of ten seminiferous tubules per testis. Ovarian activity was quantified by plotting mean monthly diameters for secondary and Graafian follicles. Mean diameters were calcu-

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Table

Numbers of *R. clivosus* collected during 1981

	J	F	M	A	M	J	J	A	S	O	N	D
Males	5	4	4	6	6	5	5	6	4	4	6	5
Females	6	6	6	5	6	4	6	6	6	6	6	6

lated from two measurements at right angles for all secondary and Graafian follicles from both ovaries. All diameters were measured with an ocular micrometer. Throughout this report, where sample size was more than ten, the mean value has been given plus or minus two standard deviations. Where sample size was less than ten, the mean value alone has been given.

For the purpose of this study two age groups have been recognised; immatures (less than one year old) and adults (more than one year old). Three factors were used to separate immature from adult specimens. Firstly, colouration: immature specimens have a grey pelage while adults have a brown colouration. Secondly, ossification of the epiphyses: epiphyses appeared to be fully ossified after nine months. And thirdly, degree of wear of the canines. Using banded individuals this method was found to give an accurate distinction between immatures and adults in 22 out of 28 cases (79 %).

Results

In Natal, *R. clivosus* hibernated from May to August inclusive.

Male reproduction

Male *R. clivosus* became reproductively active in their second year and as a result this description covers adult reproduction only. The annual changes in testis weight, cauda epididymis length and seminiferous tuble diameter are shown in Figure 1.

Mean testis mass increased ten times between December and April, a period which corresponded with spermatogenesis. Between July and December the seminiferous tubules comprised a single layer of sertoli cells and scattered spermatogonia (Fig. 2). Mitotic divisions of the spermatogonia were apparent in January and February and during February and March, the spermatocytes underwent maturation and division (Fig. 3). Spermatids were produced in April and May (Fig. 4) and spermatozoa were released from the testes in late April, May and June.

The time of onset of spermatogenesis varied considerably from individual to individual and from one seminiferous tubule to another so that between January and July the condition of the seminiferous tubules varied and examples of most stages of spermatogenesis could be found.

The increase in length of the cauda epididymis in April and May is a result of the release of spermatozoa from the testes and enlargement of the epididymides. All males collected between May and September had large amounts of spermatozoa in their epididymides and spermatozoa that remained after September were destroyed by large phagocytic cells (Fig. 5).

Female reproduction

Female *R. clivosus* became reproductively active after about six months and as a result, reproduction of the immatures differed from that of the adults. *R. clivosus* was seasonally monoestrous and monotocous.

Ovarian cycle

There was no significant difference in mean monthly follicular diameter from the two ovaries, although only follicles from the right ovary ovulated, and in Figure 6 measure-

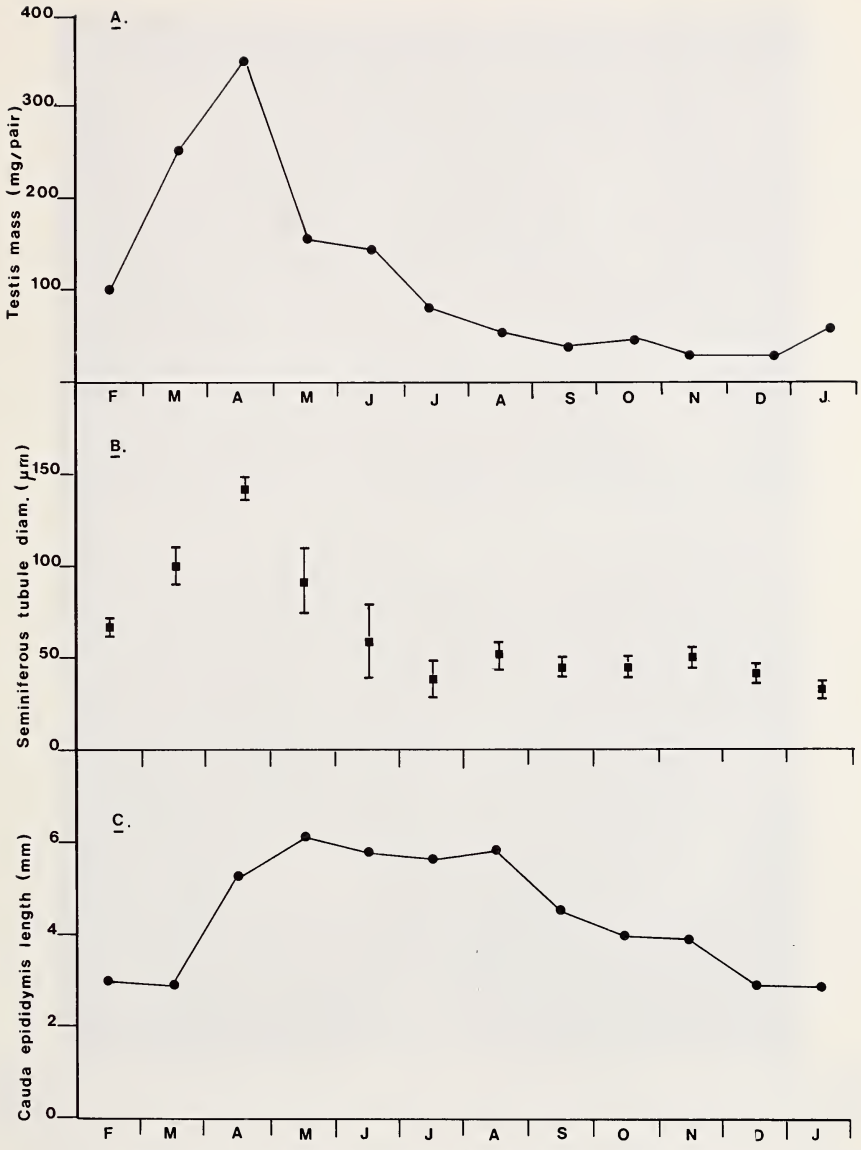

Fig. 1. Annual changes in, A: mean testis mass; B: mean seminiferous tubule diameter; and C: mean cauda epididymis length. (Vertical lines indicate mean plus or minus 2SD)

ments from the two ovaries have been pooled. In immatures, between January and June the ovaries were inactive, comprising a wide cortex filled with primordial and primary follicles, and a thin central medulla. The first cycle of follicular development began in July with the appearance of secondary follicles and the first Graafian follicles were present in August. Ovulation occurred in late September.

In adults, between October and February the ovaries were in a resting condition with very little development of secondary follicles and no Graafian follicles. During March and April there was a period of follicular development and in late March the first Graafian

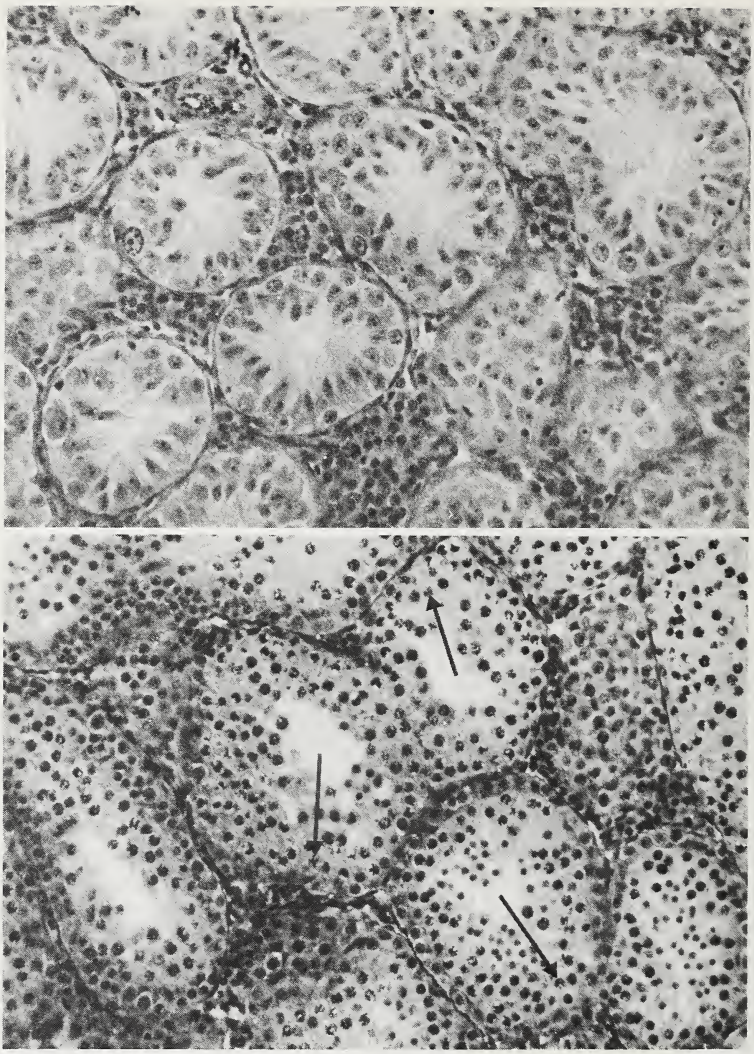

Fig. 2 (above). T. S. testis from a specimen collected in November, showing inactive seminiferous tubules lined by sertoli cells and spermatogonia (× 350). – *Fig. 3 (below).* T. S. testis from a specimen collected in March, showing dividing spermatocytes (arrows) (× 200)

follicles were present. Maximum Graafian follicle diameter was reached in June and ovulation occurred in August. (The Graafian follicles present during winter hibernation were not characterized by hypertrophied cumulus oophorus cells.) Between August and October there was a period of follicular atresia.

Female reproductive cycle

In immature specimens there was no reproductive activity during the first winter (Fig. 6). Oestrus, ovulation, copulation and fertilization occurred in late September and early October, and parturition occurred in late January after a three-and-a-half to four months gestation. Only the right uterine horn was functional.

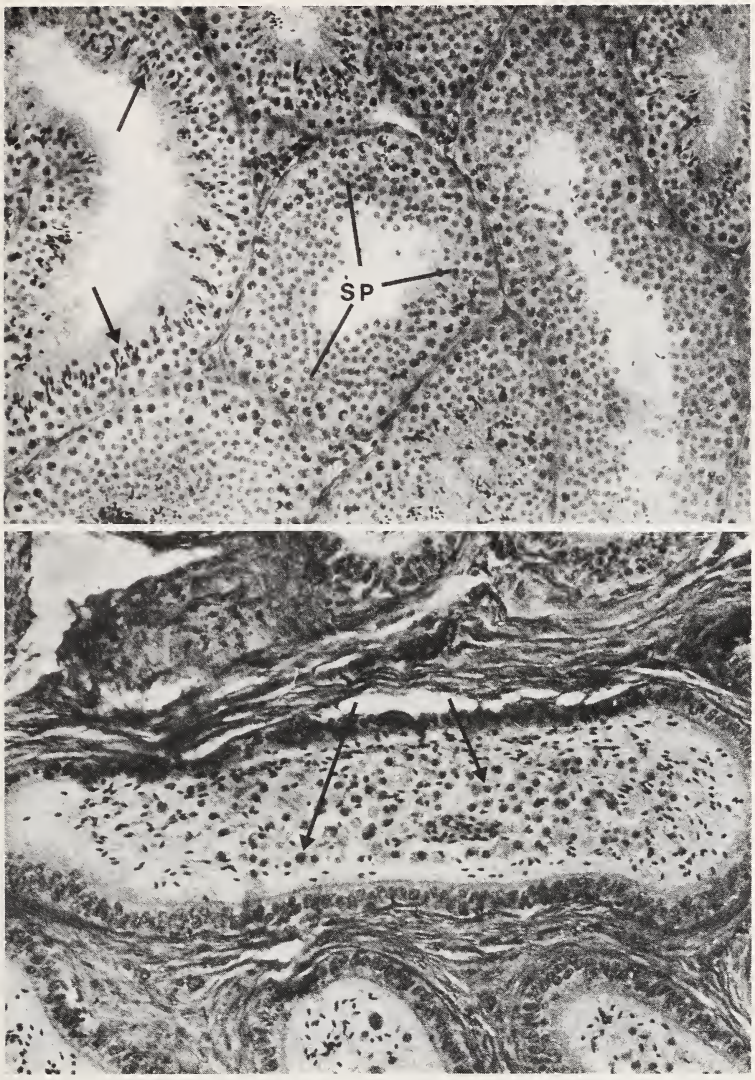

Fig. 4 (above). T. S. testis from a specimen collected in April, showing maturing spermatocytes (SP) and production of spermatids (arrows) (× 180). – *Fig. 5 (below).* T. S. cauda epididymis from a specimen collected in October showing phagocytic cells in the lumina (arrows) (× 250)

In adults, oestrus occurred in late April and early May and copulation, as indicated by the presence of spermatozoa in the female tract and the presence of a vaginal plug, first occurred in May. Spermatozoa were stored in the oviducts, uterotubal junction and uterine horns (Fig. 7, 8) of the females until ovulation and fertilization in August. The gestation was three-and-a-half to four months and parturition occurred throughout December. Only the right unterine horn was functional.

In adults and immatures lactation lasted for about two months and by April all females were postlactant.

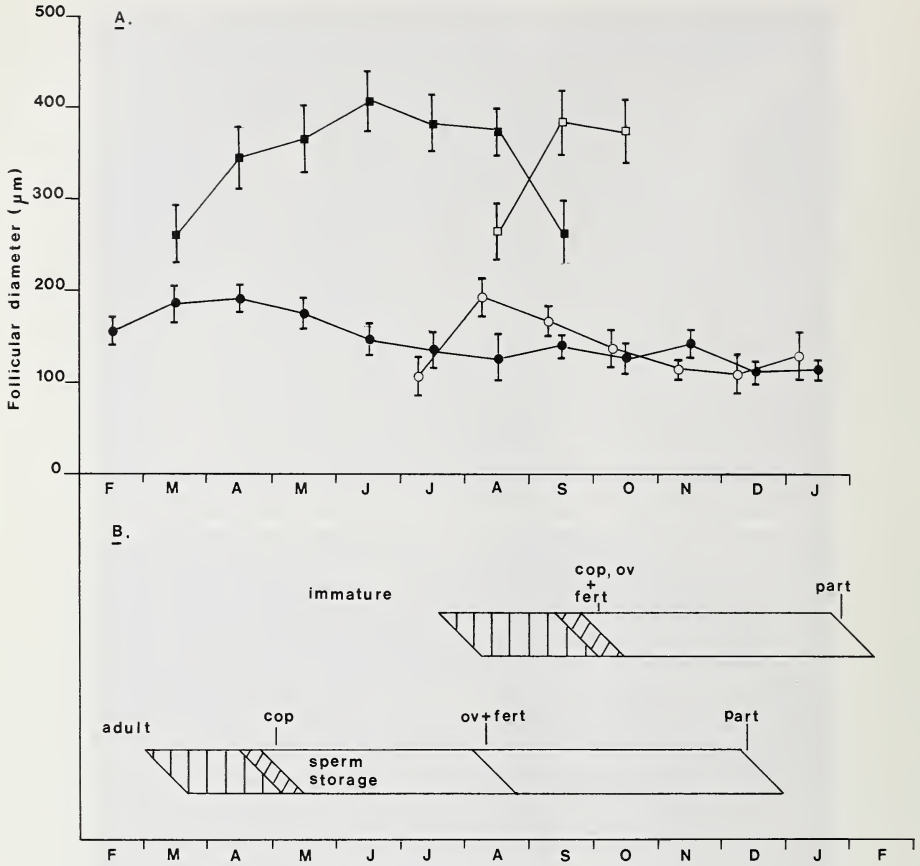

Fig. 6. A: Annual changes in secondary and Graafian follicle diameter (circular symbols indicate mean secondary follicle diameter and square symbols, mean Graafian follicle diameter. Open symbols are for immature and closed symbols for adult specimens); B: Female reproductive cycle for immature and adult specimens. Angled lines at beginning, middle and end of each "box" indicate imperfect synchrony in the various reproductive events. cop = copulation; ov = ovulation; fert = fertilliza-tion; part = parturition. = proestrus = oestrus

Discussion

The dextral structural and functional asymmetry described in *R. clivosus* falls into the "molossid pattern" described by Wimsatt (1979) and is the most widely encountered type of asymmetry in the Chiroptera. Dextral reproductive asymmetry appears to be a characteristic of the genus *Rhinolophus* and has been described for *R. ferrumequinum* and *R. hipposideros* (Matthews 1937) and *R. rouxi* (Ramakrishna 1950; Gopalakrishna and Ramakrishna 1977).

It is generally accepted that sexual maturity in male members of the genus *Rhinolophus* is attained during the second year. However, Rollinat and Trouessart (1897) and Matthews (1937) for *R. ferrumequinum* and *R. hipposideros* and Ramakrishna (1950) and Gopalakrishna and Ramakrishna (1977) for *R. rouxi* have reported that the females also, become reproductively active in their second year. The findings from the present

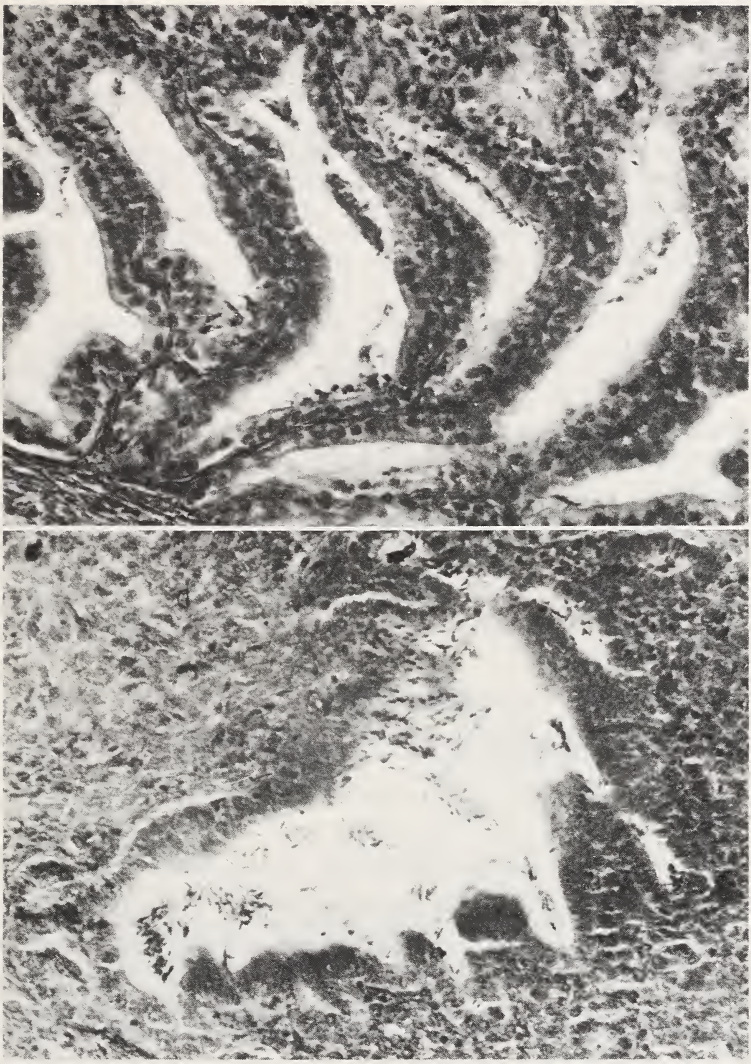

Fig. 7 (above). T. S. oviduct from a specimen collected in June, showing spermatozoa within the lumina (× 310). – *Fig. 8 (below).* T. S. right uterine horn of a specimen collected in July, showing spermatozoa in the lumen and in the necks of the uterine glands (× 420)

study, that female *R. clivosus* reach sexual maturity during their first year, are in accordance with those of Gaisler (1966) who reported that 15 per cent of female *R. hipposideros* were reproductively active during their first year. It is possible that this difference in age of sexual maturity in the species of *Rhinolophus* exists; however, such data should be treated with caution since they are reliant on accurate ageing and in the present study, the accuracy of ageing was estimated at 79 per cent.

The cycles of spermatogenesis and follicular development are similar to those described for north temperate members of this genus (Rollinat and Trouessart 1897; Matthews 1937; Dwyer 1966; Gaisler 1966; Gustafson 1979; Oxberry 1979).

Delayed ovulation and sperm storage (or delayed fertilization) as described for *R.*

clivosus in this study is a characteristic of most hibernating members of this genus (Rollinat and Trouessart 1897; Matthews 1937; Racey 1975 for *R. ferrumequinum* and *R. hipposideros*; Dwyer 1966 for *R. megaphylus*; Laycock 1976 for *R. clivosus*). There is however, one exception, being *R. rouxi* which has a 40 to 45 day period of delayed implantation after copulation (Ramakrishna and Rao 1977).

Reproduction of nonhibernating tropical members of this genus is typically mammalian and *R. clivosus, R. hilderbrandti* and *R. landeri* are monoestrous and monotocous with a gestation of about three months (Anciaux de Faveaux 1978). Menzies (1973) has suggested that there may be a two month period of delayed implantation in *R. landeri* from northern Nigeria. However, his data are inconclusive mainly due to a very small sample size.

The reported duration of sperm storage in the genus *Rhinolophus* varies from about three-and-a-half months in *R. clivosus* (from c. 29° S; present study) to seven to eight months in *R. hipposideros* (from c. 50° N; Gaisler 1966). This variation is probably a latitude related effect with species at higher latitudes experiencing longer winters and therefore requiring longer periods of sperm storage.

It was originally thought that the vaginal plug was the site of sperm storage in the Rhinolophidae (Rollinat and Trouessart 1897; Matthews; 1937). However, it is now generally accepted that the oviducts (Racey 1975) and uterus (Gaisler 1966) are the storage sites. (The findings of the present study are in accordance with presently held views.) The function of the vaginal plug is now thought to be to promote a monogamous mating system (Racey 1979). The importance of such a system is easy to understand when one considers that males store spermatozoa, and are therefore capable of fertile copulations, throughout winter.

Acknowledgements

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Zusammenfassung

Der Fortpflanzungszyklus von Rhinolophus clivosus (Microchiroptera) in Natal, Südafrika

An Fledermäusen der Art *Rhinolophus clivosus,* die in etwa 14tägigem Abstand im Jahre 1981 gesammelt wurden, wurden Hoden, Ovarien und weiblicher Genitaltrakt histologisch untersucht. Danach kopulieren die erwachsenen Tiere im Mai. Während des anschließenden Winterschlafs wird das Sperma in den Uteri und Oviduкten gespeichert. Ovulation und Befruchtung erfolgen im August, und die Jungen werden im Dezember geboren. Junge Weibchen haben ihren ersten Östrus im Alter von etwa acht Monaten gegen Ende September und bringen ihre Jungen nach einer Tragzeit von 3½ bis 4 Monaten im Januar zur Welt. Die Spermatogenese und die Follikelentwicklung von *Rh. clivosus* entsprechen denen anderer *Rhinolophus*-Arten.

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Taxonomy of the genus *Gerbillus* (Rodentia: Gerbillinae) with comments on the applications of generic and subgeneric names and an annotated list of species

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Abstract

The various usages of *Gerbillus, Dipodillus* and *Hendecapleura* as genera or subgenera are reviewed. Data are presented regarding variation in plantar morphology, tooth morphology, the ratio of tail length to head and body length, morphology of the auditory bulla and comparative karyology. These data fail to support any of the several concepts of genera and subgenera presently used in the taxonomy of this rodent group. These data reveal the need for a comprehensive revision and until such is available, it is suggested that rodent species of this group should be placed in a single genus, *Gerbillus,* with no subgenera.

An annotated list with provisional recognition of 62 species is provided. The criteria utilized to determine valid species are given. An appendix lists 113 named forms with citations and type localities.

Introduction

The genus *Gerbillus* includes an assemblage of species that occur in arid and semiarid environments of north and east Africa, Sinai, the Arabian Peninsula, Iran, Afghanistan, Pakistan and India. These rodents constitute a diverse and important component of the mammalian fauna of this large area.

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