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Age Determination by Tooth Wear and Histological Analysis of Seasonal Variation of Breeding in the Lesser White-Toothed Shrew, *Crocidura suaveolens*

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작은땃쥐 *Crocidura suaveolens*의 치아 마모에 의한 연령결정과 번식의 계절적 변이의 조직학적 분석

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ABSTRACT

Captured specimens of the lesser white-toothed shrew, *Crocidura suaveolens* were classified into three age classes by tooth wear and seasonal variations of reproductive organs were investigated. Molars of juveniles had not tooth wear and the height of the third molars were lower than the first and second molars, young adults had smooth tooth wear and the third molars reached to the first and second molars, and old adults had heavy tooth wear and the third molars also reached to the first and second molars. On the basis of histological examination, seasonal variation of breeding was confirmed that breeding season of adult males was from early February to early October, having a peak of the breeding in April and July, and non-breeding season was from in the middle of October to late January. Young and old adult males of the breeding season had large testes with enlarged seminiferous tubules filling with numerous germ cells and expanded caudal epididymides with a vast number of spermatozoa, Young and old adult males of the non-breeding season had the small testes with the extremely slender seminiferous tubules filling with only spermatogonia and the reduced caudal epididymides without spermatozoa. Males weighing more than 3.9 g in the body weight and 0.013 g in the testis and epididymis weight reached sexual maturation in breeding season, and the females weighing more than 3.8 g in body weight of the breeding season were pregnant condition having 5～6 litters or had the Graafian follicles and the corpus lutea in the ovary.

Keywords : Age, Breeding, *Crocidura suaveolens*, Lesser white-toothed shrew, Tooth wear

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INTRODUCTION

Insectivora including Soricidae is a primitive mammalian group, thought to be ancestors to many groups of mammals, exhibiting characteristics of specific interest for the study of mammal evolution (Eisenberg, 1981; Churchfield, 1990; Carson & Rose, 1993). Soricidae is generally considered to comprise two subfamilies, Soricinae and Crocidurinae by morphometric characteristics, which are determined by a color of the tooth tip, a length of the head and body, a length of the tail, a thickness of the tail base and a composition of the tail fur (Jones & Johnson, 1960; Won, 1967) and distinctive reproductive characteristics (Churchfield, 1990). Crocidurinae living in Korea is composed of three species, *C. suaveolens, C. lasiura.* and *C. dsinezumi* (Korean Society of Systematic Zoology, 1997). Crocidurinae is not only a useful environmental indicator having the sensitivity about pollutions (Braham & Neal, 1974; Roberts et al., 1978; Andrew et al., 1984), but also is an important mediator controlling the population of the invertebrates and small vertebrates in ecosystem (Buckner, 1969; Churchfield, 1990). However, all of them inhabiting Korea is very rare in the wild and their populations also have been decreased, little is known about breeding ecology in this shrew inhabiting Korea. Several studies has been made on brief comments and taxonomical reviews (Jones & Johnson, 1960; Korean Society of Systematic Zoology, 1977; Won, 1967), histological studies of sperm (Jeong et al., 2001, 2006) and salivary gland (Jeong et al., 2005a, b).

The aim of the present study was to classify into the age classes using tooth wear, to identify the seasonal variations of reproductive organs and to discuss the factors influencing the breeding of the lesser white-toothed shrew, *Crocidura suaveolens*.

MATERIALS AND METHODS

1. Trapping procedures and investigated areas

The lesser white-toothed shrew, *Crocidura suaveolens* (Fig. 1) were captured with the Sherman live traps at Daejeodong (latitude 35° 12′30″, longitude 128° 57′30″) in Busan from August 1994 to October 2000 and Mt. Jiri (latitude 35° 17′30″, longitude 127° 45′00″) in Gyeongsangnamdo from October 1999 to September 2000. The traps were set along the waterway of a rice field nearby stocks of *Phalaris arundinacea*, *Erigeron canadensis* and *Artermisia asiatica* at Daejeodong and were set around stream nearby a terraced farmland where is located in about 450～500 meters at Mt. Jiri. All captured animals were measured for body weight and lengths of head and body, tail, hind foot and ear. Each specimen was autopsied and breeding conditions of the specimen were noted down. For males, the position, weight and length of testis were recorded. For females, condition of nipples and uterus in relation to lactation, or number, size and weight of embryos were recorded.

2. Procedures for decision of age

Each skull was removed from the specimen and the carcass was kept in 70% alcohol. Skulls of the specimens were classified into three age classes, juvenile, young adult and old adult on the basis of the eruption and tooth wear of molars with reference to Koh (1983).

3. Histological procedures

The reproductive organs of the right side were fixed in the 4% formaldehyde (0.1 M phosphate buffer, pH 7.2) for 12 hr. Specimens were dehydrated with series of graded ethyl alcohol and embedded in Paraplast. The blocks were cut to a thickness of 6～7 μm. The sections were stained with Hematoxylin-Eosin for light microscopic observation. The reproductve organs of the other side were fixed in 2.5% glutaraldehyde and 2% paraformaldehyde in Millonig's phosphate buffer (pH 7.4) for 1 hr. Sepcimens were post-fixed with 1.3% osmium tetroxide in the same buffer for 2 hr, dehydrated with series of the graded ethyl alcohol and acetone and embedded in epoxy resin. Thick sections (0.5～1 μm) were stained with 5% toluidine blue.

RESULTS

1. Age determination

Molars of juveniles had not tooth wear and the height of the third molars were lower than the first and second molars (Fig. 2a, 2b). Young adults had little or smooth tooth wear and the third molars reached to the first and second molars (Fig. 2c, 2d), it was canyon stage or stream stage. Old adults had heavy tooth wear (Fig. 2e, 2f) and the third molars also reached to the first and second molars, it was lake stage or dish stage.

2. Trapping records

A total of 721 small mammal species were captured using

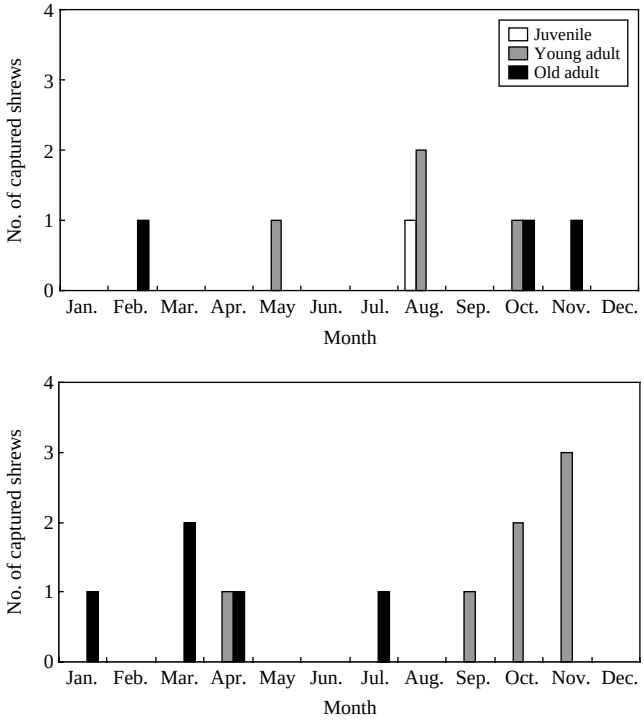

Fig. 3. Monthly variations in number of juvenile, young and old adults *C. suaveolens* captured in Busan (top) and Mt. Jiri (bottom).

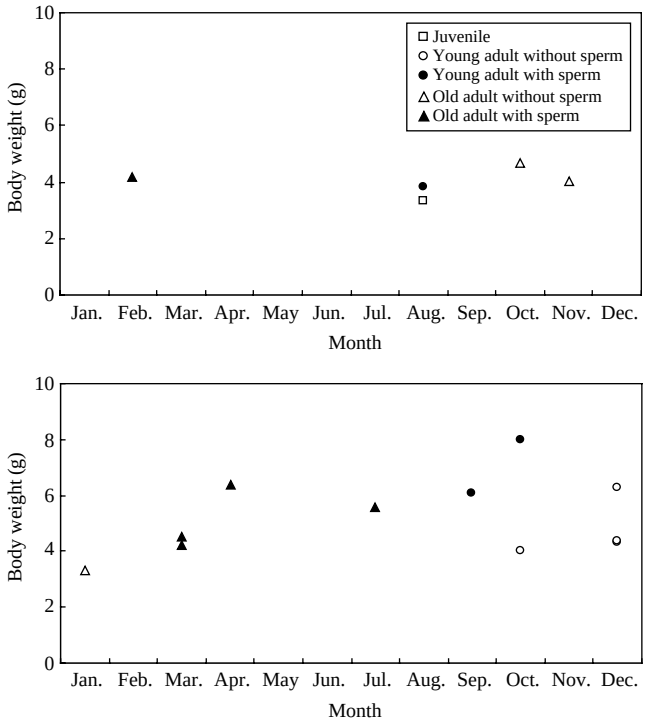

Fig. 5. Monthly variations of the body weight in males of *C. suaveolens* captured in Busan (top) and Mt. Jiri (bottom).

4008 Shermal live traps and the most of captured specimens were Rodentia (646 sepcimens, capture ratio 16.12%). Only 20 *Crocidura suaveolens* were captured and capture ratio was extremely low (Busan, 0.25%; Mt. Jiri, 1.60%). 8 specimens (5♂♂, 3♀♀) were captured at Busan and 12 specimens (11♂♂, 1♀) were captured at Mt. Jiri. Juveniles were captured in August at Busan, young adults were captured in May, August and October at Busan, in April and from September to November at Mt. Jiri, and old adults were captured in August at Busan (Fig. 3).

3. Seasonal changes of reproductive organs and breeding

According to the histological examinations of the testes and the caudal epididymides of males, captured specimens from February 14 and August 17 at Busan and from March to October 9 at Mt. Jiri had numerous germ cells such as intact spermatozoa and developing spermatocytes in enlarged seminiferous tubules of large testes and a vast number of spermatozoa in epididymides (Fig. 4a, 4c, 4d), but captured specimens from October 13 to January at Busan and from October 17 to January at Mt. Jiri had no spermatozoa in either small testes and the epididymides, that is, only spermatogonia in the extremely slender seminiferous tubules of testes (Fig. 4b, 4e, 4f).

Seasonal variations in the body weight of males and the testis and epididymis weight as well as the presence or absence of spermatozoa were shown in Figs. 5 and 6. The body weight of young adults and old adults males was significantly heavy at Mt. Jiri (5.2±1.4 g, n=11) than Busan (4.2±0.4 g, n=4) ($p<0.05$), but monthly variations in body weight differed little in each region. All specimens of young adults and old adults males of the breeding season had spermatozoa (Busan, 3.9 g, 4.2 g; Mt. Jiri, 5.8±1.4 g, n=6), the lowest value of the body weight which had spermatozoa in the caudal epididymides was 3.9 g at Busan and 4.2 g at Mt. Jiri, but all specimens of the non-breeding season (Busan, 4.05 g, 4.67 g; Mt. Jiri, 4.4±1.1 g, n=5) had no spermatozoa. The testis and epididymis weight of young adults and old adults males were 0.015±0.013 g (n=11) at Mt. Jiri and 0.010±0.007 g (n=4) at Busan. The testis and epididymis weight of young adults and old adults of the breeding season was more than 0.013 g (Busan, 0.013 g, 0.018 g; Mt. Jiri, 0.026±0.006 g, n=6), but that of the non-breeding season (Busan, 0.003 g, 0.006 g; Mt. Jiri, 0.003±0.001, n=5) was less than 0.006 g in both regions, the testis and epididymis weight of breeding season was heavy than those of non-breeding season ($p<0.05$). Monthly changes in

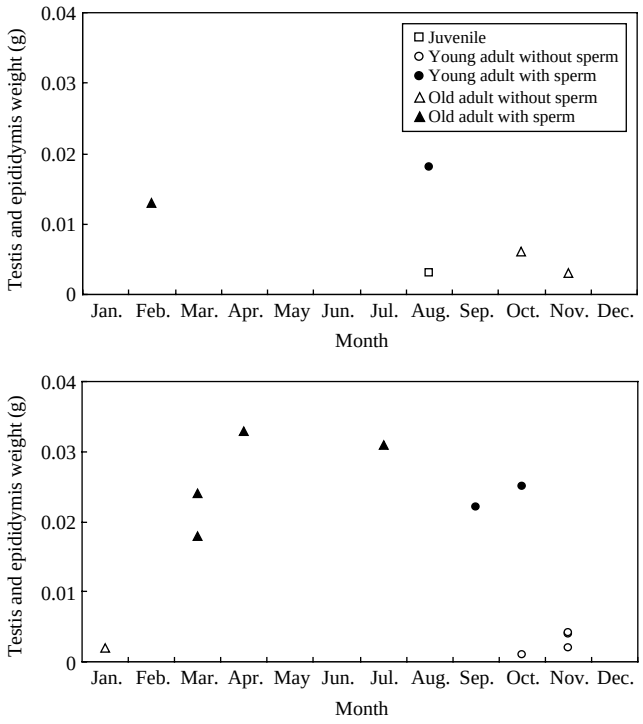

Fig. 6. Monthly variations of the testis and epididymis weight in *C. suaveolens* captured in Busan (top) and Mt. Jiri (bottom).

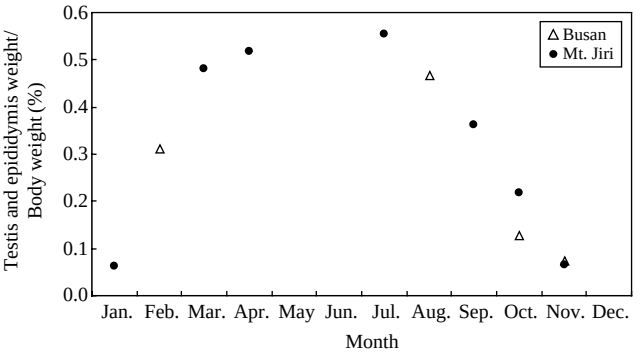

Fig. 7. Changes in the ratio of the testis and epididymis weight to the body weight in *C. suaveolens* captured in Busan and Mt. Jiri.

the ratio of the testis and epididymis weight to the body weight in young adults and old adults were seen Fig. 7. The ratios at Busan were relatively high in February (0.31%) and August (0.47%), form October (0.13%) down to the November (0.07%), it has gradually decreased. The ratios at Mt. Jiri were relatively high in March (0.48%), April (0.52%) and July (0.56%), the ratios began to decrease in September (0.36%), those of October (0.22%) and November (0.07%) were relatively low, the ratios of the testis and epididymis weight to the body weigh at Mt. Jiri were similar to those of Busan. The breeding activity was the highest in April and July, the peak of breeding seems to be Spring or Summer. Therefore, the breeding condition of adult males of *C. suaveolens* was continued from early February to early October, although the breeding activity was the highest in April and July, and non-breeding condition was continued from in the middle of October to late January. Adult males weighing more than 3.9 g in body weight and 0.013 g in the testis and epididymis weight had breeding condition during the breeding season.

Although captured females were four during the period for this investigation, all specimens was pregnant condition (Fig. 4g) or had the Graafian follicles and the corpus lutea in the ovary (Fig. 4h). Three females were captured at Busan in May, August and October and One female was captured at Mt. Jiri in April. The body weight of females were 4.3 ± 0.8 g ($n=3$) at Busan and 7.2 g at Mt. Jiri, the pregnant female that captured at Busan in May had 5 Litters and the pregnant female that captured at Mt. Jiri in April had 6 Litters. The number of captured females was so small, it was impossible to know the breeding season and the peak of breeding.

DISCUSSION

1. Seasonal variation of capture ratio

Shrews frequently comprise only a small proportion of the small mammal community in most geographical regions, and are usually outnumbered by the many species of rodents as mice and voles (Churchfield, 1980, 1990). The abundance of shrews in relation to other mammals may be highly variable, depending upon habitats, season and even year (Churchfield, 1981, 1990). Although the capture ratio in research on the ecology can be used an indicator representing density and fluctuation of wild populations (Pernetta, 1977; Churchfield, 1980, 1990; Barid et al., 1983; Yoshino & Abe, 1984), it was impossible to analyse fluctuation of population in this study because the numeral of captured specimens at Busan and Mt. Jiri was extremely poor. On the other hand, the capture ratio were significantly different depending on location, that is, the capture ratios in Mt. Jiri was higher than those of Busan. Shrews do tend to be most abundant and diverse in regions characterized by cool, moist, temperate forests and they are less diverse in drier forest and prairie or steppe ecosystems, and are least diverse and abundant in deserts (Churchfield, 1981, 1990). Moisture must be a principal factor in determining the regional and local diversity of shrews (Churchfield, 1990). This is con-

sistent with the fact that shrews have relatively high water requirements: they have a high evaporative respiratory loss compared with small rodents and seem unable to regulate evaporation at low humidities (Churchfield, 1990). This may be due to their high metabolic rates and their constant high level of activity (Churchfield, 1981, 1990). Mt. Jiri seems to be a moderate habitat for shrews than Busan, because Mt. Jiri is close by forest having moisture and aboundance and diversity of their invertebrate prey, however Busan is difficult to maintain moisture in the soil and has only an abundant invertebrates, and because the body weight of captured specimens at Mt. Jiri is heavier than those of Busan.

2. Breeding

Shrews in temperate regions have well-defined breeding season in the wild, generally speaking, they attain sexual maturity in the spring of their second calendar year, and breeding occurs in the late spring and summer, finishing by autumn at the latest (Pernetta, 1977; Mock, 1982; Barid et al., 1983; Kress, 1984; Yoshino & Abe, 1984; Inoue T, 1988a, b, 1991; Churchfield, 1990; Genoud & Vogel, 1990). Namely, most shrews pass the winter in an immature state, and the reproductive organs of both males and females are small and non-functional, both sexes are about equal in size through February and the beginning of March, although males may be slightly larger, then spring comes, both sexes undergo very rapid change to sexual maturity, with males reaching maturity earlier than females, often by some three weeks (Churchfield, 1980,1990). Winter breeding of young females in their first calendar year when they less than five months old, has rarely if ever been recorded in wild Sorex (Churchfield, 1990). Godfrey (1978) carried out a detailed study of breeding in captive colonies of *C. suaveolens* on the Channel Island of Jersey. She found that the first litter born in mid April and the last in late October, but breeding reached a peak in May. She concluded that the main breeding season extended for 8～9 months from February to October but that it could be extended under certain conditions (such as increased food supply). This apparently mirrors wild population of this species: in Scilly Isles, *C. suaveolens* has a similar breeding season and winter breeding has been recorded in unrelated to climatic factors (Rood, 1965). The breeding season of *C. russula* both in the Channel Islands and in France was also February-October (Bishop & Delany, 1963; Godfrey, 1978). On the basis of the histological examinations, breeding season of wild adult males of *C. suaveolens* living in Korea was from early February to early October, and breeding reached a peak in April and July, whereas the non-breeding season of adult males was from in the middle of October to late January, these results show that the breeding of *C. suaveolens* living in Korea had seasonal variation. Also males in which were born the late breeding season seems to obtain the sexual maturity in spring of the following breeding season, on the grounds that young adults without breeding activity were captured From late October and early November and Young adults with breeding activity were captured from March.

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＜국문초록＞

작은땃쥐 *Crocidura suaveolens*는 치아 마모도를 이용하여 3개의 연령군으로 분류하였고 생식기관의 계절적 변이를 조사하였다. 유수들의 구치는 치아 마모를 보이지 않았고 세 번째 구치의 높이가 첫 번째와 두 번째 구치보다 낮았으며, 아성수는 약간의 치아 마모를 보였고 세 번째 구치의 높이가 첫 번째와 두 번째 구치의 높이에 이르렀고, 성수들은 깊은 치아 마모를 보였으며 세 번째 구치 높이 역시 첫 번째와 두 번째의 구치에 도달하였다. 조직학적 조사를 근거로, 번식의 계절적 변이가 명확해졌으며, 수컷 성수의 번식 상태는 2월 초에서 10월 초까지 유지되었고 4월과 7월에 가장 높은 번식 활동을 보였고 비번식기는 10월 중순에서 1월 말까지였다. 번식기에 포획된 아성수와 성수 수컷은 다양한 생식세포들로 채워진 확장된 세정관을 가진 큰 정소와 많은 정자들을 가진 팽창된 부정소 미부를 가졌고 비번식기에 포획된 아성수와 성수 수컷들은 정원세포만으로 채워진 가늘어진 세정관을 가진 작은 정소와 정자를 가지지 않는 감소된 부정소를 가졌다. 몸무게가 3.9g 이상이고 정소와 부정소 무게가 0.013 g 이상인 수컷은 번식기에 성적 성숙에 도달하였고, 몸무게가 3.8 g 이상이고 번식기에 포획된 암컷들은 5～6마리의 새끼를 가진 임신상태이거나 난소에 성숙난포들과 황체를 가지고 있었다.

FIGURE LEGENDS

Fig. 1. Photographs of the captured lesser white-toothed shrew, *Crocidura suaveolens*. Scale bar=1 cm.

Fig. 2. Stereo micrographs showing teeth of the upper and lower jaws in the juvenile, young and old adults. (2a) The upper first molar (M1), second molar (M2) and third molar (M3) of juvenile without tooth wear. (2b) The lower first, second and third molar of juvenile without tooth wear. (2c) The upper molars of young adult with little and smooth tooth wear (Canyon stage or Stream stage). (2d) The lower molars of young adult with little and smooth tooth wear. (2e) The upper molars of old adult with heavy tooth wear (Lake stage or Dish stage). (2f) The lower molars of old adult with heavy tooth wear.

Fig. 4. Photographs and light micrographs showing the reproductive organs of males and females of *C. suaveolens* in the breeding and non-breeding seasons. (4a) Ventral view of the enlarged testes (T) in the breeding season. (4b) Small testes and epididymides in the non-breeding season. (4c) Light micrograph of the expanded seminiferous tubules filled with numerous germ cells, developing spermatocytes (Sc) and spermatozoa (Sz) in the breeding season. (4d) Light micrograph of the expanded caudal epididymis with heavy burden of spermatozoa in the breeding season. (4e) Light micrograph of the reduced seminiferous tubules with only spermatogonia in the non-breeding season. (4f) Light micrograph of the reduced caudal epididymis without spermatozoon in the non-breeding season. (4g) Litter in the uterus. (4h) Light micrograph of the ovary with the mature Graafian follicles (Gf) and corpus lutea (Cl) in the breeding season. Scale bars=10 μm (4c～4f) and 25 μm (4h).


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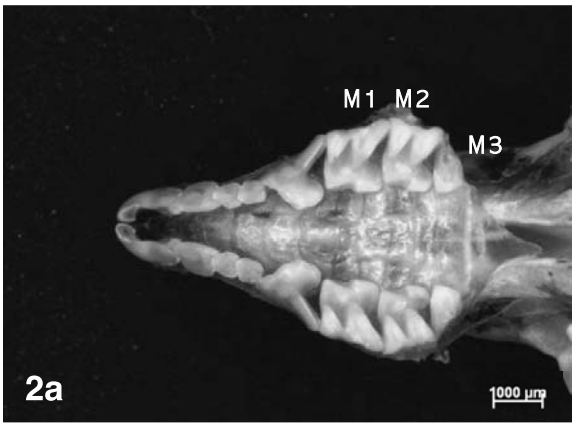
M1 M2
M3
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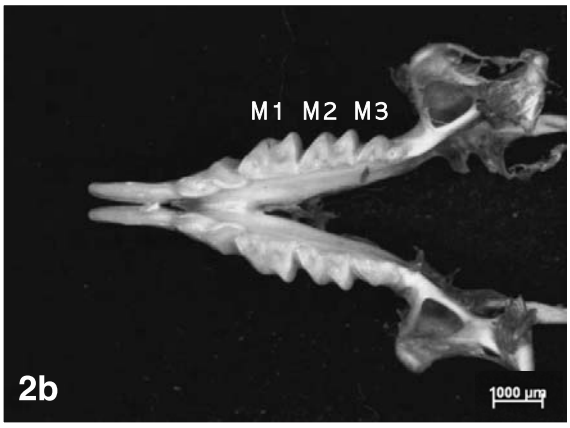
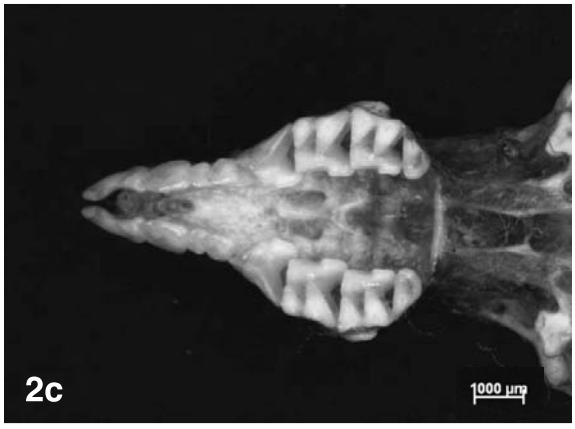
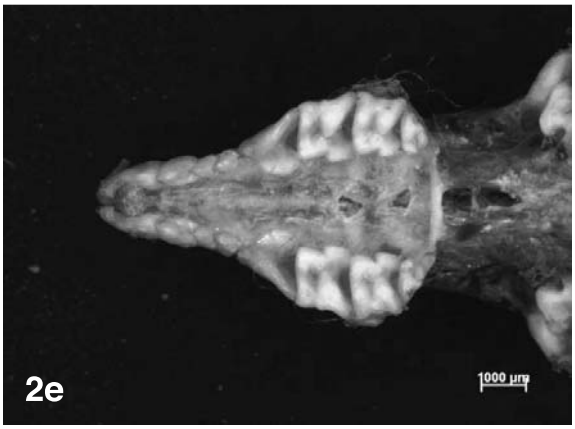
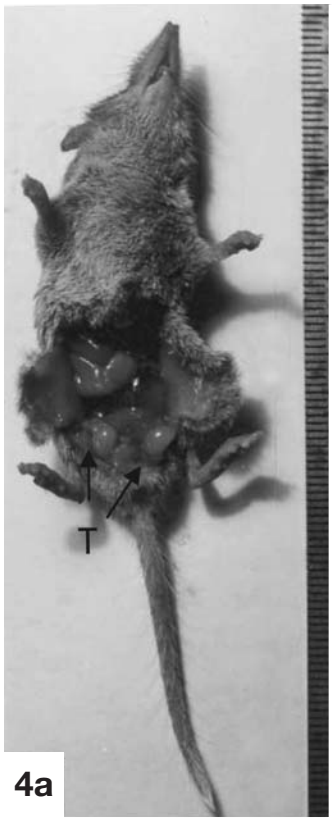

M1 M2 M3
2b
1000 μm


2c
1000 μm

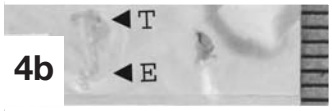

2d
1000 μm


2e
1000 μm

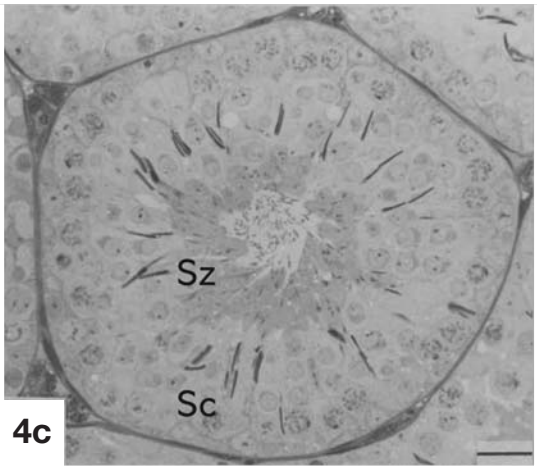

2f
1000 μm



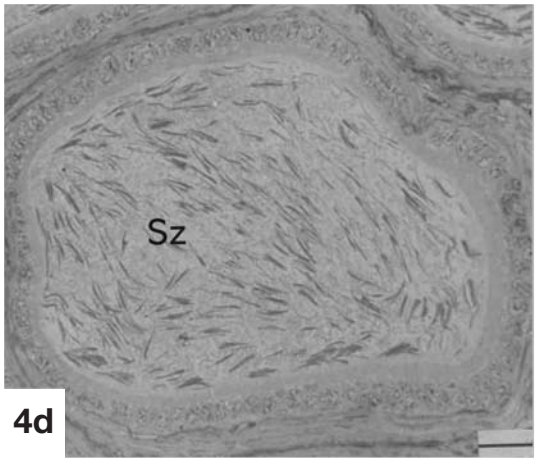
4a



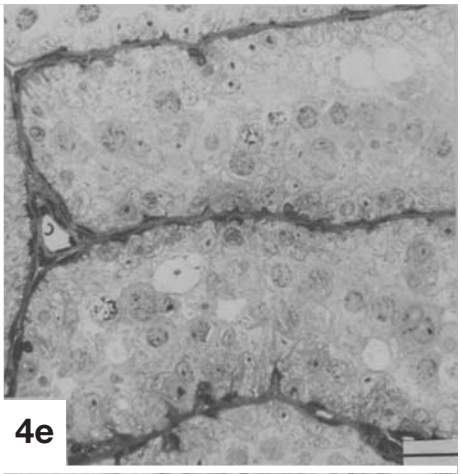
4b



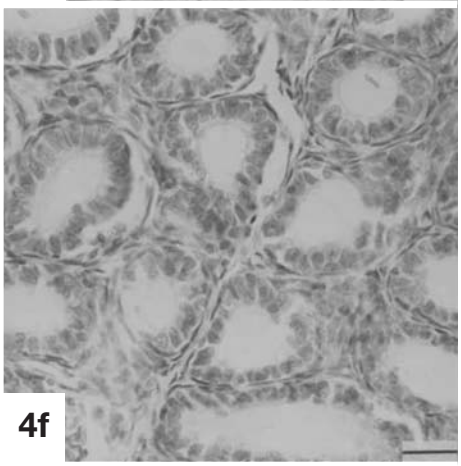
4c



4d

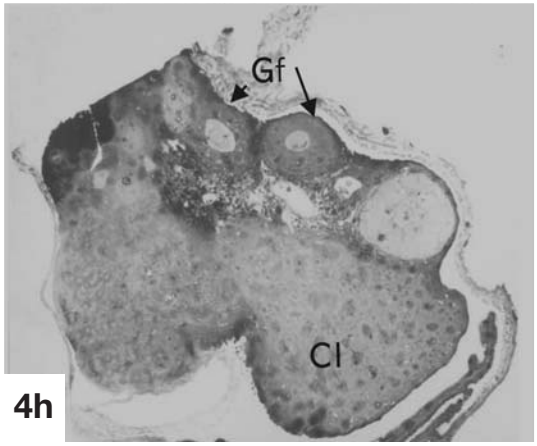


4e



4f

4g



4h