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

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우수리땃쥐 *Crocidura lasiura*의 치아 마모에 의한 연령결정과 번식의 계절적 변이의 조직학적 분석

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ABSTRACT

Captured wild specimens of the big white-toothed shrew, *Crocidura lasiura* were classified into three age classes by tooth wear and height of molars, and seasonal variations of breeding and reproductive organs were examined. Juveniles had not tooth wear in molars and height of the third molars were lower than the first and second molars, and had only non-breeding condition. Young adults had little tooth wear and the third molars reached to the first and second molars, and old adults had heavy tooth wear in molars, young adults and old adults had breeding or non-breeding condition according to the season. On the basis of histological examination, seasonal variations of breeding were confirmed that breeding condition of young and old adult males were continued from early February to early October although the breeding activity was the highest in April, that of females were continued from the end of March to October, males reached sexual maturity earlier than females. Whereas the breeding condition seems to cease for non-breeding season because of the deficiency of food resources, soil invertebrates. Young and old adult males of the breeding season had large testes with enlarged seminiferous tubules that were filled with numerous germ cells, and expanded caudal epididymides with a vast number of spermatozoa, and were more than 10.0 g in the body weight and 0.03 g in the testis and epididymis weight. The females of the breeding season were pregnant condition with 4～6 litters or had the Graafian follicles and the corpus lutea in the ovary, and were more than 9.6 g in the body weight.

Keywords : Age determination, Big white-toothed shrew, Breeding, *Crocidura lasiura*, Tooth wear

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INTRODUCTION

Shrews are small, short-legged, mouse-like animals with long, pointed snouts and short, dense fur, usually dark brown in color, and they are predatory, feeding mainly on small invertebrates (Churchfield, 1990). Shrews are placed in the order Insectivora and Insectivora including Soricidae is known as the most primitive mammal and the ancestor stock of the present mammals (Tsuchiya, 1985; Churchfield, 1990). Soricidae is generally considered to comprise two subfamilies, Soricinae and Crocidurinae. They were classified by morphometric characteristics, which is 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; Corbet, 1978) and distinctive reproductive characteristics (Churchfield, 1990). Crocidurinae living in Korea is composed of three species, *C. suaveolens*, *C. lasiura* and *C. dsinezumi* (Jones & Johnson, 1960; Won, 1967). Crocidurinae is not only a useful environmental indicator having the sensitivity about pollutions (Diamond & Sherburne, 1969; Braham & Neal, 1974; Robert et al., 1978; Andrew et al., 1984), but also is an important mediator controlling the population of the invertebrates and small vertebrates in ecosystem (Holling, 1959; Buckner, 1969; Churchfield, 1990). However, all of them inhabiting Korea is very rare in the wild and their populations also have been decreased, its breeding ecology has not been studied, with exception of the brief comments and taxonomical reviews (Jones & Johnson, 1960; Won, 1967), histological studies of sperm (Jeong & Yoon, 2001; Jeong et al., 2006) and salivary gland (Jeong et al., 2005a, b, c).

The aim of the present study was to determine the age class by tooth wear, to identify seasonal variations of breeding and reproductive organs and to discuss factors influencing the breeding of the big white-toothed shrew, *Crocidura lasiura*.

MATERIALS AND METHODS

1. Trapping procedures and investigated areas

Specimens of the big white-toothed shrew, *Crocidura lasiura* (Fig. 1) were captured with Sherman live traps at Daejeodong (latitude 35°12′30″, longitude 128°57′30″) of Busan from August 1994 to October 2000 with regularity and Mt. Jiri (latitude 35°17′ 30″, longitude 127°45′00″) of Gyeongsangnamdo in May 1885, November 1996, April and October 1998 at random and from October 1999 to September 2000 with regularity. 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 m 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 (abdominal or scrotal), weight and length of testis were recorded. For females, condition of nipples and uterus in relation to lactation, and number, size and weight of embryos were recorded.

2. Procedures for age determination

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

3. Histological procedures

Reproductive organs of the right side were fixed in the 4% formaldehyde (phosphate buffer, pH 7.2) for 12 hr. Specimens were dehydrated with series of graded ethyl alcohol and embedded in Paraplast. Blocks were cut to a thickness of 6 ~ 7 μm. 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. Specimens 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). Old adults had heavy tooth wear (Fig. 2e, 2f).

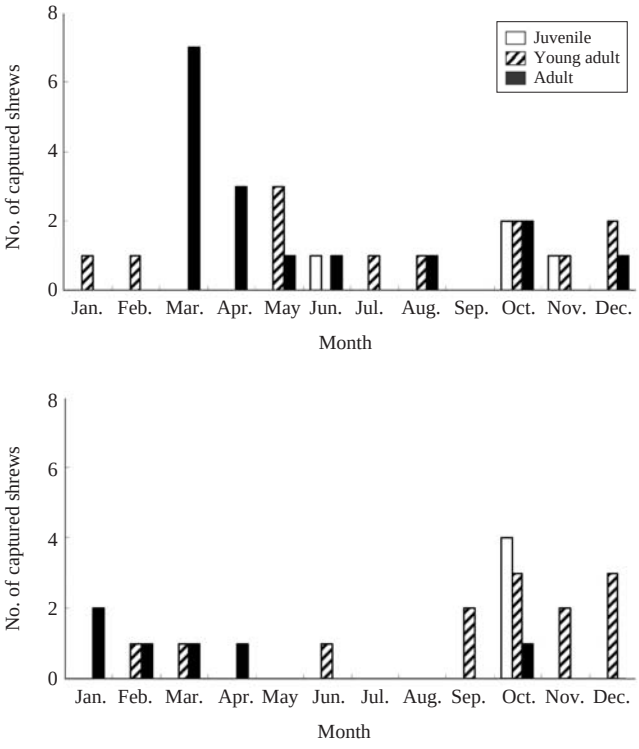

Fig. 3. Monthly variations in number of juvenile, young and old adults *C. lasiura* were captured in Busan (top) and Mt. Jiri (bottom).

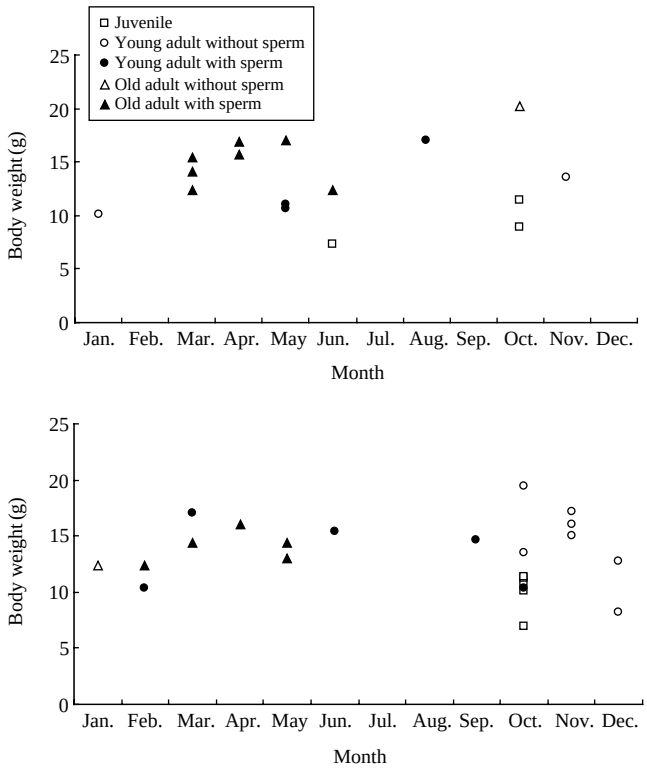

Fig. 5. Monthly variations of the body weight in males *C. lasiura* were captured in Busan (top) and Mt. Jiri (bottom).

2. Trapping records

Total of 721 small mammal specimens were captured using 4008 Sherman live traps, most of them were rodents (646 specimens; capture ratio 16.12%) and the specimen were mostly *Apodemus agrarius* (590 specimens; capture ratio 14.72%). Total of 55 *Crocidura lasiura* were captured, 32 specimens (16♂♂, 16♀♀) were captured at Busan and 30 specimens (22♂♂, 8♀♀) were captured at Mt. Jiri, capture ratios were 0.98% and 3.30% respectively. The capture ratios of each age class varied with the season. Juveniles were captured in June, October and November at Busan, in October at Mt. Jiri, young adults were captured in January, February, May, July, August and from October to December at Busan, in February, March, May, June and from September to December at Mt. Jiri, and old adults were captured in from February to June, August, October and December at Busan, from January to April, October and November at Mt. Jiri (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 March to August at Busan and from early February to early October 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 23 to January at Busan and from October 17 to January at Mt. Jiri had no spermatozoa in small testes and epididymides, that is, only had spermatogonia in the extremely slender seminiferous tubules of testes (Fig. 4b, 4e, 4f).

Seasonal variations in the body weight and the testis and epididymis weight of males as well as presence or absence of spermatozoa were shown in Figs. 5 and 6. The body weight of young adults and old adults males in both regions were similar (Busan, 14.3±2.5 g, n=13; Mt. Jiri, 14.0±2.8 g, n=18) and monthly variations in the body weight differed little. All specimens of young adults and old adults males of the breeding season had spermatozoa (Busan, 14.3±2.5 g, n=10; Mt. Jiri, 13.9±2.3 g, n=10), the lowest value of the body weight which had spermatozoa in the caudal epididymides was 10.7 g at Busan and 10.3 g at Mt. Jiri, but all specimens of the non-breeding season (Busan, 15.8±1.6 g, n=3; Mt. Jiri, 14.3±3.4 g, n=8) had no spermatozoa. The testis and epididymis weight

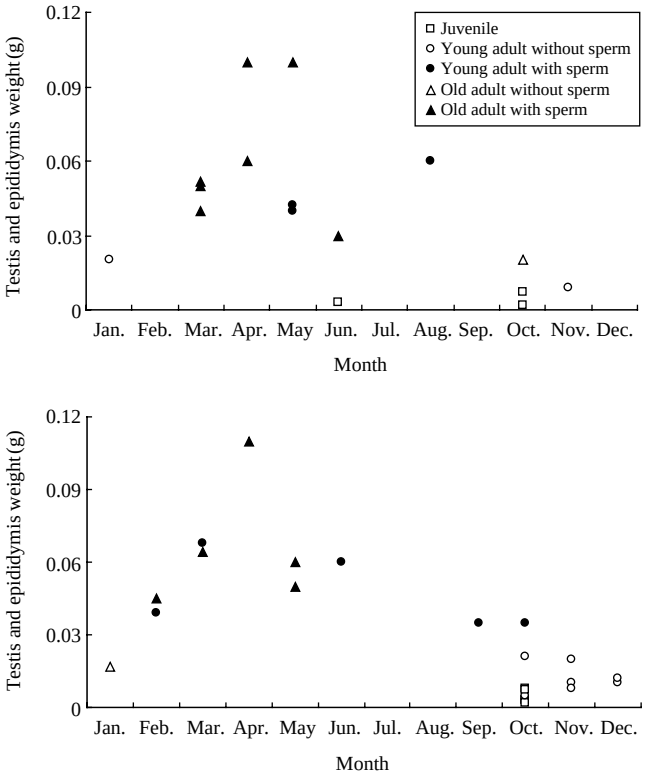

Fig. 6. Monthly variations of the testis and epididymis weight in males *C. lasiura* were captured in Busan (top) and Mt. Jiri (bottom).

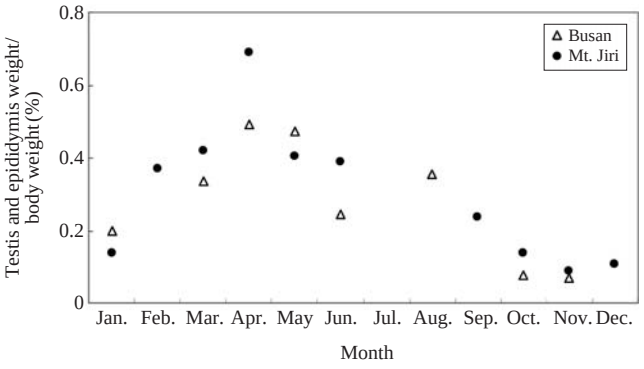

Fig. 7. Changes in the ratio of the testis and epididymis weight to the body weight in *C. lasiura* were captured in Busan and Mt. Jiri.

of young adults and old adults males in both regions were similar (Busan, 0.048±0.028 g, n=13; Mt. Jiri, 0.037±0.028 g, n=18), but there was monthly variations, namely, young adults and old adults of the breeding season which had spermatozoa in the caudal epididymides were more than 0.030 g in the testis and epididymis weight (Busan, 0.057±0.025 g, n=10; Mt. Jiri, 0.057±0.022 g, n=10), the largest value was seen in Apri (0.10 g) and May (0.10 g) at Busan and April (0.11 g) at Mt. Jiri, the testis and epididymis weight of young adults and old adults males of the non-breeding season (Busan, 0.016±0.006 g, n=3; Mt. Jiri, 0.013±0.006 g, n=8) which had no spermatozoa were less than those of the breeding season ($p < 0.05$). Monthly changes in the ratio of the testis and epididymis weight to the body weight in young adults and old adults were seen Fig. 7. The ratio was the highest in April (Busan, 0.49%; Mt. Jiri, 0.69%) but was the lowest in November (Busan, 0.07%; Mt. Jiri, 0.08%), namely, the breeding activity was the highest in April, form this month down to the non-breeding season, It has gradually decreased. Therefore, the breeding condition of adult males of *C. laiura* was continued from early February to early October although the breeding activity was the highest in April, the body weight seemed to be criterion of the breeding condition in adult males, from the fact that adult males weighing more than 10 g in body weight had breeding condition during the breeding season, whereas juveniles had not yet breeding condition even in the breeding season.

In order to make clear the breeding season of female, the ovary of young adults and old adults were histologically examined. In case of Busan, three of among the four captured specimens in March and all captured specimens in April, May, July, August and October, and in case of Mr. Jiri, all captured specimens in April, September and October were pregnant condition (Fig. 4g) or had the Graafian follicles and the corpus lutea in ovary (Fig. 4h). But, in case of Busan, all captured specimens in February and December and one of among the four captured specimens in March and in case of Mt. Jiri, all captured specimens in January, November and December had only a few primary follicles (Fig. 4i). namely, the breeding season of females *C. laiura* was form the end of March to October and the non-breeding season was from early November to the Middle of March. Seasonal variations in the body weight of females as well as the presence or absence of the Graafian follicle in the ovary or litter size were shown in Fig. 8. The body weight of non-pregnant young adults and old adults females captured in the breeding season were 13.0±2.1 g (n=4) at Busan and 15.3±6.0 g (n=4) at Mt. Jiri, but all specimens in the non-breeding season were 11.4±1.1 g (n=4) at Busan and 15.0±8.2 g (n=3) at Mt. Jiri. The body weight of non-pregnant young adult and old adult females in both regions were differed little but those of Mt. Jiri was relatively heavy than Busan. The lowest value of the body weight which was pregnant condition or had the Graafian follicles was 11.2 g at Busan and 9.6 g at Mt. Jiri. Pregnant females of Busan had 4~6 Litter sizes (4.8±1.0, n=6) and pregnant females of Mt. Jiri had 4 and 6 Litter sizes. Therefore, the breeding condition of females was contin-

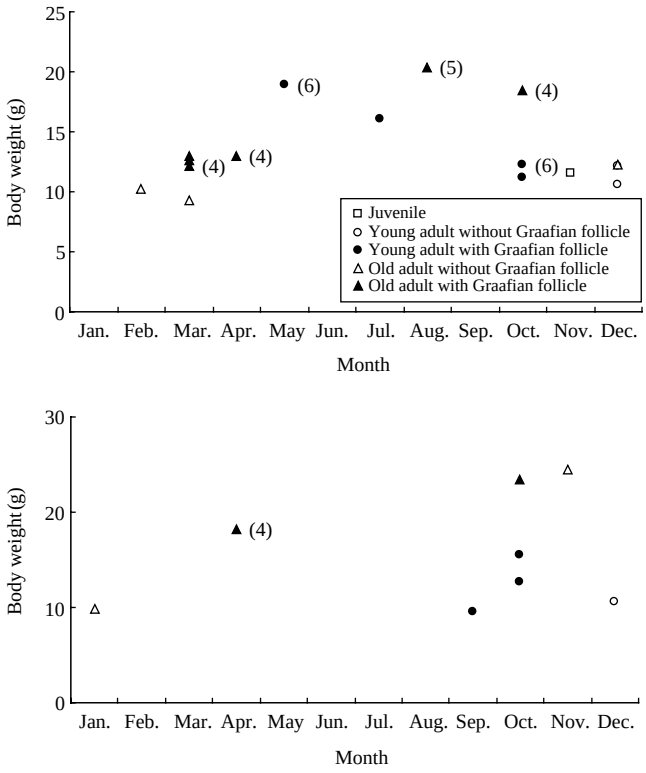

Fig. 8. Monthly variations of the body weight in females in *C. lasiura* were captured in Busan (top) and Mt. Jiri (bottom). ( ), Litter size.

ued from the end of March to October and females weighing more than 9.6 g in body weight had breeding condition during the breeding season.

DISCUSSION

1. Seasonal variation of capture ratio

Shrews are important constituents of small mammal communities in forests, grasslands and scrublands throughout much of the world, and the diversity of species is high, with as many as six or more occurring in single habitat, moreover many species of shrews coexist in the same habitate (Churchfield, 1980, 1990). Despite the diversity of shrew species in many local area, they 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 habitate, season and even year (Churchfield, 1981, 1990). Although research on the ecology of small wild mammals indicated that the capture ratio can be used an indicator representing density and fluctuation of wild populations (Pernetta, 1977; Churchfield, 1980, 1990; Baird et al., 1983; Yoshino & Abe, 1984), it was impossible to analyse population fluctuation in this study because the numeral of captured specimens was 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 (Churchfield, 1981, 1990). They are less diverse in drier forest and prairie or steppe ecosystems, and are least diverse and abundant in deserts (Churchfield, 1981, 1990). Moisture, then must be a principal factor in determining the regional and local diversity of shrews. This is consistent 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. 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. Mt. Jiri is not only close by forest to maintain moderate humidity but also has an abundant and diverse invertebrates in the soil, however Busan is open glassland where is difficult to maintain humidity and has only an abundant invertebrates in the soil.

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 (Pucek, 1960, 1965; Pernetta, 1977; Churchfield, 1980, 1981, 1990; Mock, 1982; Baird et al., 1983; Kress, 1984; Yoshino & Abe, 1984; Inoue T, 1988a, b, 1991; Genoud & Vogel, 1990). 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. Breeding of captive colony were apparently similar to that of 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, it was confirmed that the breeding condition of adult males of *C. laiura* inhabiting Korea was continued from early February to early October, but breeding reached a peak in April, whereas the breeding condition of females was continued from the end of March to October. Namely, breeding in *C. lasiura* showed seasonal variation. Individuals in which were born the late breeding season seems to be obtained the sexual maturity in spring of the following breeding season, on the grounds that young adults without breeding activity were captured From October to January and young adults with breeding activity were captured from February (Fig. 5), and the breeding of *C. lasiura* seems to cease for non-breeding season because of the deficiency of the food resources, soil invertebrates.

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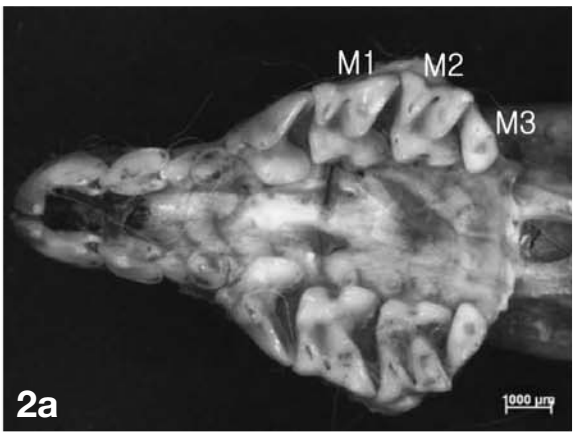
＜국문초록＞

포획된 야생의 우수리땃쥐 *Crocidura lasiura*는 구치의 마모와 높이를 이용하여 3개의 연령군으로 분류하였고 번식과 생식기관의 계절적 변이를 조사하였다. 유수들은 구치에서 마모를 가지지 않았고 세 번째 구치 높이가 첫 번째와 두 번째 구치보다 낮았고 비번식상태만을 가졌다. 아성수들은 약간의 마모를 가졌고 세 번째 구치가 첫 번째와 두 번째 구치 높이에 도달했고 성수들은 구치에 깊은 마모를 가졌으며 아성수와 성수는 계절에 따라 번식 혹은 비번식상태를 가졌다. 조직학적 관찰을 근거로 아성수와 성수 수컷의 번식상태는 2월 초에서 10월 초까지 유지되었고 4월에 가장 높은 번식활동을 보였으며 암컷의 번식 상태는 3월 말에서 10월까지 유지되어, 수컷이 암컷보다 빨리 성적 성숙에 이르는 것으로 밝혀졌다. 반면 번식상태는 비번식기 동안 중단되며 이것은 식량자원인 토양 무척추동물의 부족때문이라 생각된다. 번식기의 아성수와 성수들은 많은 생식세포들로 채워진 확장된 세정관을 가지는 큰 정소와 많은 정자들을 가진 팽창된 부정소 미부를 가졌고 10.0 g 이상의 몸무게와 0.03 g 이상의 정소와 부정소 무게를 가졌다. 번식기의 암컷들은 4～6개의 새끼를 가진 임신상태이거나 난소에 성숙난포들과 황체를 가졌고 9.6 g 이상의 몸무게를 가졌다.

FIGURE LEGENDS

Fig. 1. Photograph of the captured big white-toothed shrew, *Crocidura lasiura*. 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 reproductive organs of males and females of *C. lasiura* in the breeding and non-breeding seasons. (4a) Enlarged testes (T) and epididymides (E) in the breeding season. (4b) Small testes and epididymides in the non-breeding season. (4c) Light micrograph of the expanded seminiferous tubules 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. (4i) Light micrograph of the ovary with a few primary follicles in the non-breeding season. Scale bars=10 μm (4c～4f) and 25 μm (4h, 4i).

1



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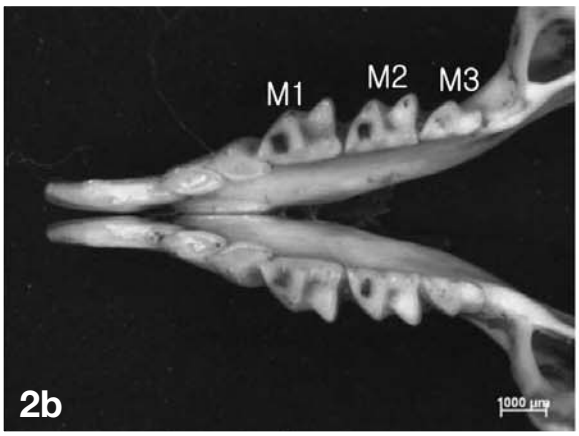

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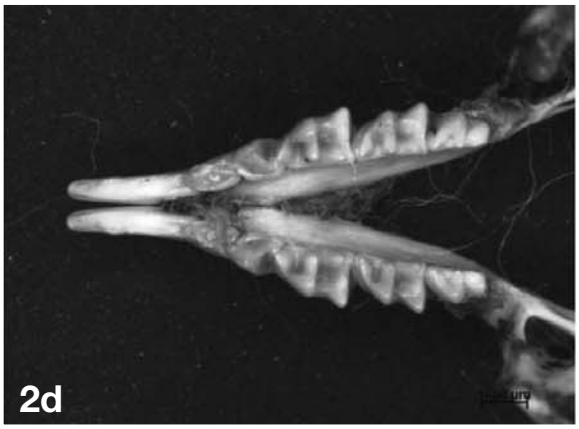

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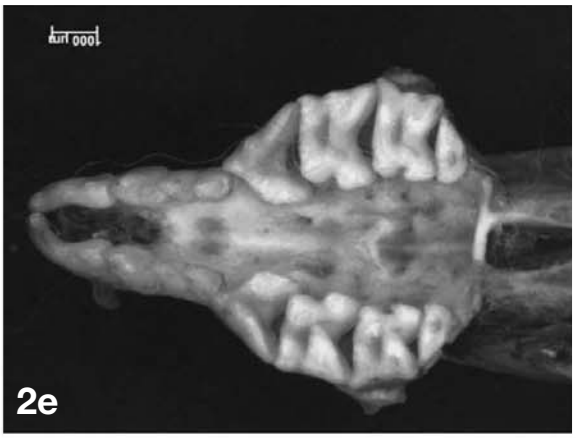

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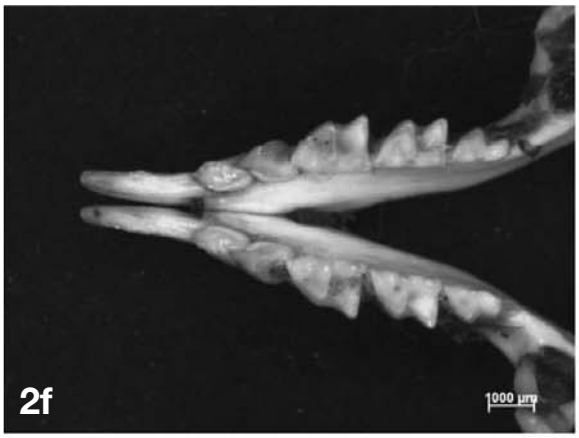

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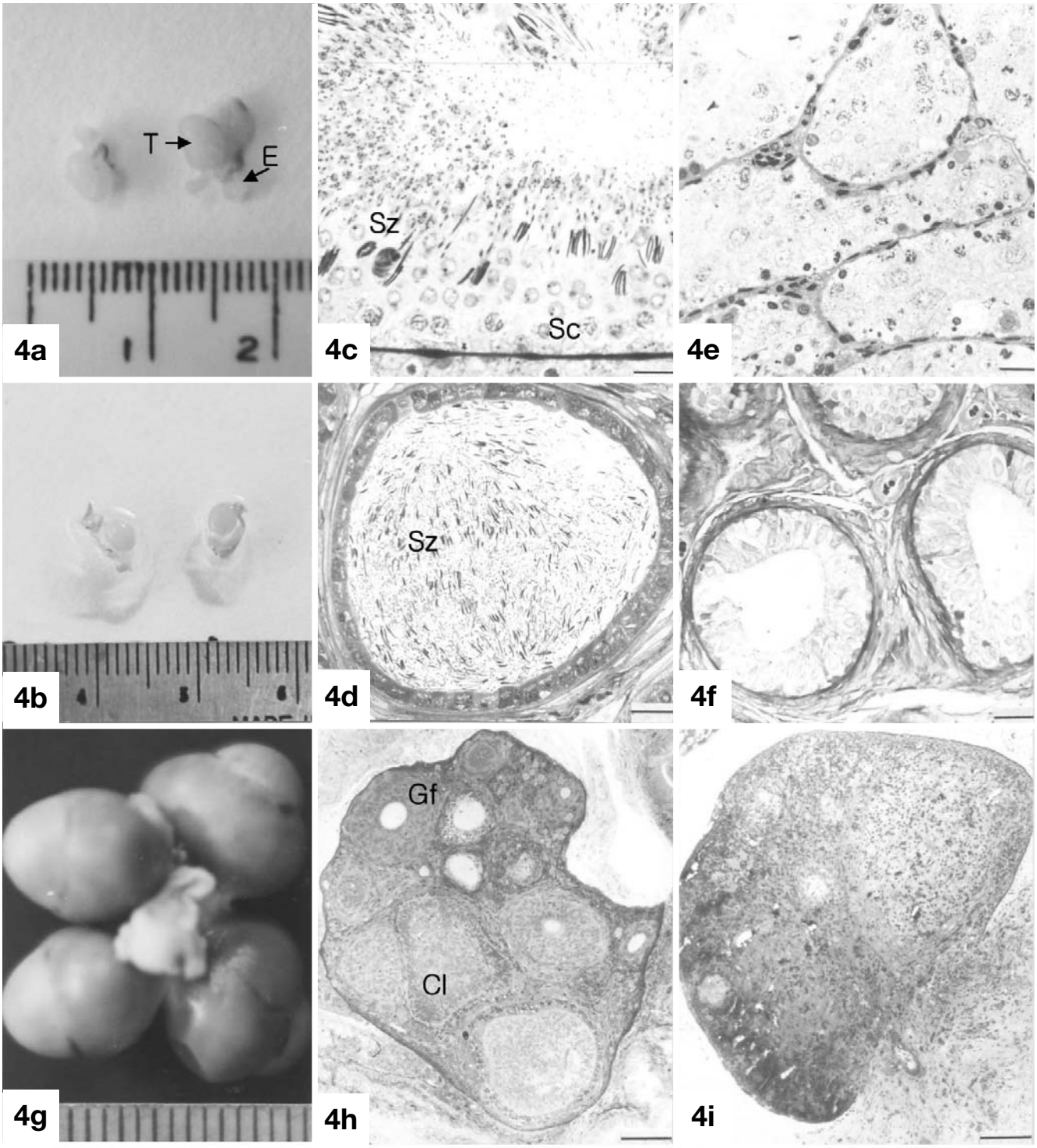
T
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