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Ultrastructural observations on the sperm of two *Apodemus* species, *Apodemus agrarius coreae* and *Apodemus speciosus peninsulae*, in Korea

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Sperm morphology of *Apodemus agrarius coreae*, *Apodemus speciosus peninsulae*, laboratory mouse and rat were studied. The total length of the sperm of *A. a. coreae*, *A. s. peninsulae*, the mouse and rat was about 133 μ m, 124 μ m, 131 μ m and 192 μ m, respectively. Two *Apodemus* sperm heads had a fish hook shape, while the mouse sperm head had a hook shape; thus, the sperm heads of genus *Apodemus* were somewhat like those of the mouse. The rat sperm head was unlike the other species in that it had a falciform shape. In addition, two *Apodemus* spermatozoa had two well-developed ventral spurs (Vs), and the mouse sperm head also had two similar small Vs, but rat sperm head had low ridge line-like Vs. All species had a separated head-cap segment in the ventral side of the anterior acrosomal portion of sperm. In the neck region, the vault formerly occupied by the proximal centriole was retained in a niche in the dense substance of the connecting piece in the spermatozoa of two *Apodemus* species, the mouse and the rat. The neck region of two *Apodemus* spermatozoa was surrounded by the scroll of a redundant nuclear envelope and a few large mitochondria. In contrast, in the mouse and rat spermatozoa, the neck region was devoid of mitochondria, except a fold of the redundant nuclear envelope. Since a very similar sperm head morphology occurs in the two *Apodemus* species and the mouse, it suggests that these genera are sister groups, to the exclusion of the rat.

Key Words: acrosome, *Apodemus*, sperm morphology, separated head-cap segment, spermatozoa, ventral spur.

INTRODUCTION

The subfamily Murinae contains more than 400 species of rats and mice, mainly in tropical Africa, Asia and Australia. Only two genera, *Apodemus* and *Micromys*, are predominantly Palaearctic, but the representatives of two others, *Mus* and *Rattus*, have become global through their association with people (Corbet and Harris, 1991). Except for an excellent and extensive report describing the sperm morphology of the mice and rats using the light microscope by Friend (1936) and sperm morphological feature of *Apodemus sylvaticus* by Moore *et al.* (2002), ultrastructural observations of the spermatozoa of two species – *Apodemus a. coreae* and *A. s. peninsulae* – are not known.

The aim of the present study was to investigate in detail the particular ultrastructure of the sperm of two *Apodemus* species, and to make a comparison with those of the laboratory mouse and the laboratory rat belonging muroid rodent.

MATERIALS AND METHODS

Adult specimens of the striped field mouse *A. agrarius coreae*, Korean field mouse *A. speciosus peninsulae*, the mouse (ddY strain) and rat (Wistar strain) were used in the present study. Specimens were

killed by asphyxiation and the epididymides removed. For light microscopy, spermatozoa of two *Apodemus* species, mouse and rat were flushed from the cauda epididymis, were smeared on slide glasses at room temperature for 5 min., and washed in 0.1 M Milloing's buffer (pH 7.4). They were then fixed in 3% glutaraldehyde (0.1 M Milloing's buffer, pH 7.4) and dehydrated in a graded series of ethanol for 10 min, respectively. These samples were observed without staining under the light microscopy.

In scanning electron microscopy, the samples smeared on slide glasses were fixed and dehydrated as light microscopic methods, and coated with gold, and observed under Akashi SX-40 scanning electron microscope at 15 kV. For transmission electron microscopy, after following fixation in 3% glutaraldehyde in 0.1 M Milloing's buffer (pH 7.4) for 4 hr, the tissues were washed in the same buffer, postfixed in 1.3% osmium tetroxide in the same buffer for 2 hr, and dehydrated in a graded series of ethanol and embedded in Epoxy resin. Thin sections (60–90 nm) were stained with uranyl acetate and lead citrate, and examined with an H-600 (Hitachi) transmission electron microscope at 75 kV.

RESULTS

The sperm morphology of *Apodemus agrarius coreae*, *Apodemus speciosus peninsulae*, mouse and rat were described (Figs 1–17, Text-fig. 1), and the dimensions of the spermatozoa were summarized in Table 1.

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Table 1. Comparison of the sperm morphology of *Apodemus agrarius coreae*, *Apodemus speciosus peninsulae*, mouse and rat

| Species | Head (μm) | | | | Tail (μm) | | | | Total length of the sperm |
|---------------------------------|-----------|-----|------|----|-----------|---------|------|-------|---------------------------|
| | L | W | Nul | S | Tl | Nl | Mp | Pp+Ep | |
| <i>Apodemus agrarius coreae</i> | 8.0 | 4.0 | 7.6 | FH | 125.5 | 0.8–1.0 | 29.5 | 95.0 | 133.3 |
| <i>A. speciosus peninsulae</i> | 7.8 | 3.6 | 7.6 | FH | 116.3 | 0.8–1.0 | 22.5 | 93.0 | 124.1 |
| Mouse (ddY strain) | 7.3 | 3.4 | 6.6 | H | 124.2 | 0.8–1.0 | 23.5 | 99.5 | 131.5 |
| Rat (Wistar strain) | 12.0 | 1.6 | 10.6 | F | 180.0 | 0.8–1.0 | 63.0 | 116.2 | 192.0 |

F, falciform; FH, fish hook; H, hook; L, length; Mp, middle piece; Nl, neck length; Nul, nuclear length; Pp + Ep, principal piece plus end piece; S, sperm head shape; Tl, tail length; W, width.

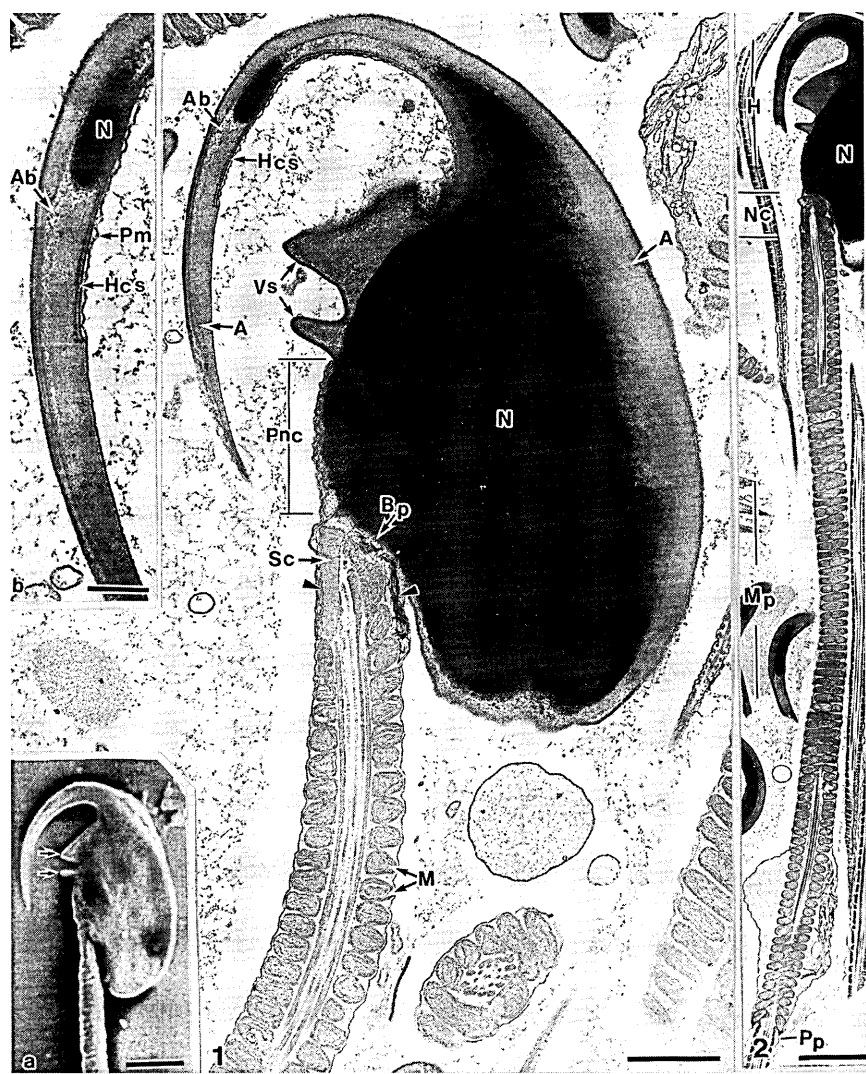


Fig. 1. Transmission and scanning electron micrographs showing the caudal epididymal spermatozoa of *Apodemus agrarius coreae*. Sperm head had a sharply curved fish hook shape, and acrosome (A) covering the apical body (Ab) is seen. The sperm head has two ventral spurs (Vs) on the ventral region of sperm head with an opener-like form (Fig. 1, arrows in Fig. 1a), and the separated head-cap segment (Hcs) is clearly visible (Fig. 1, 1b). The post-nuclear cap (Pnc) occupied about a third of nucleus. The basal plate (Bp) was adherent to the nuclear envelope, defining the implantation fossa. The segmented columns (Sc) were surrounded by redundant membranous scroll (arrowheads in Fig. 1), and adjacent outer membrane of the first mitochondria of the middle piece. The proximal centriole had degenerated. M, mitochondria; Pm, plasma membrane. N, nucleus. Scale bars=1 μm (Fig. 1), 2 μm (inset a), 0.2 μm= (inset b).

Fig. 2. Longitudinal section of the middle piece of in *A. agrarius coreae*. Mitochondrial gyres were 188 approximately. H, head; Mp, middle piece; N, nucleus; Nc, neck; Pp, principal piece. Scale bar =2 μm.

Apodemus agrarius coreae**Sperm head**

The shape of the sperm head of *A. a. coreae* had a sharply curved fish hook (Fig. 1, inset a in Fig. 1). The sperm head was $8.0\mu\text{m}$ in length, whose posterior $7.6\mu\text{m}$ was occupied by a nucleus, being about $4.0\mu\text{m}$ in width (Table 1). The spermatozoon had two well-developed ventral spurs (Vs) with an opener-like form on the ventral region of the sperm head (Fig. 1, inset a in Fig. 1, Fig. 2). The acrosome covered about two-thirds of the anterior portion of the sperm head. The apical body outlined by the inner acrosomal membrane extended to the tip of the very long subacrosomal space as an finger like projection (Fig. 1, inset b in Fig. 1). The sperm head of this species was characterized by the presence of a separated head-cap segment (Hcs) on the ventral side of anterior acosomal portion of sperm nucleus (Fig. 1, inset b in Fig. 1). The post-nuclear cap (Pnc) occupied about a third of nucleus (Fig. 1, Text-figs 1, 2). The equatorial segment of acrosome was situated on the middle portion of the nucleus (Text-figs 1, 2).

Sperm tail

The total length of the sperm tail of *A. a. coreae* was $125.3\mu\text{m}$ with a long middle piece. The sperm tail consisted of four segments: the neck ($0.8\text{--}1.0\mu\text{m}$), middle piece ($29.5\mu\text{m}$), and principal piece plus end piece ($95.0\mu\text{m}$) (Fig. 2).

The neck region consisted of the basal plate, the capitulum, the persistence of the proximal centriole, traces of the distal centriole and a few mitochondria (Figs 1, 6).

The basal plate adhering to the nuclear envelope, defining the implantation fossa, was a band of electron dense materials. The segmented columns were surrounded by redundant membranous scroll of nuclear envelope that extended into the neck region (Fig. 6), were adjacent to one or two longitudinal oriented mitochondria that project forward from the mitochondrial sheath of the middle piece (Fig. 6).

Major segmented columns were seen in the neck (Fig. 7a). Nos. 1, 5, 6 and 9 of the outer dense fibers were larger than the others (Fig. 7b). The total number of mitochondrial gyres of *A. a. coreae* was 188. A fibrous sheath (Fs) and longitudinal column (Lc) of the principal piece were in evidence (Fig. 7c, d), but the fibrous sheath was not seen at the end piece (Fig. 7e).

The end of the middle piece was marked by the annulus, a thin dense ring to which the flagellar membrane is firmly adhered.

Apodemus speciosus peninsulae**Sperm head**

The sperm head of the *A. s. peninsulae* had a sharply curved fish hook shape, and was slimmer than that of *A. a. coreae* (Fig. 8, cf. Fig. 1, Text-figs 1, 2). The sperm head of *A. s. peninsulae* was $7.8\mu\text{m}$ in length, whose posterior $7.6\mu\text{m}$ was occupied by a nucleus, being about $3.6\mu\text{m}$ in width (Table 1). *A. s.*

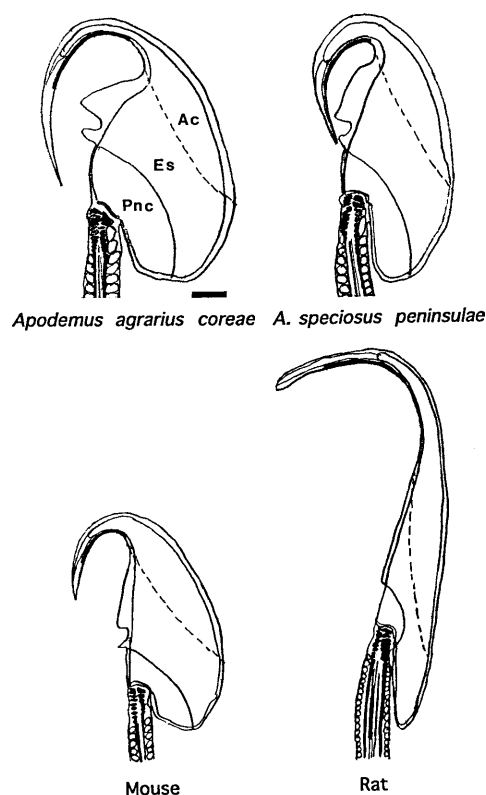
peninsulae spermatozoon were two well-developed ventral spurs with an opener-like form on the ventral region of sperm head (Fig. 8, inset a in Fig. 8), as *A. a. coreae* (Fig. 1, inset a in Fig. 1). A separated head-cap segment existed on the ventral side of anterior acrosomal portion of sperm nucleus such as *A. a. coreae*. The post-nuclear cap occupied about a third of nucleus.

Sperm tail

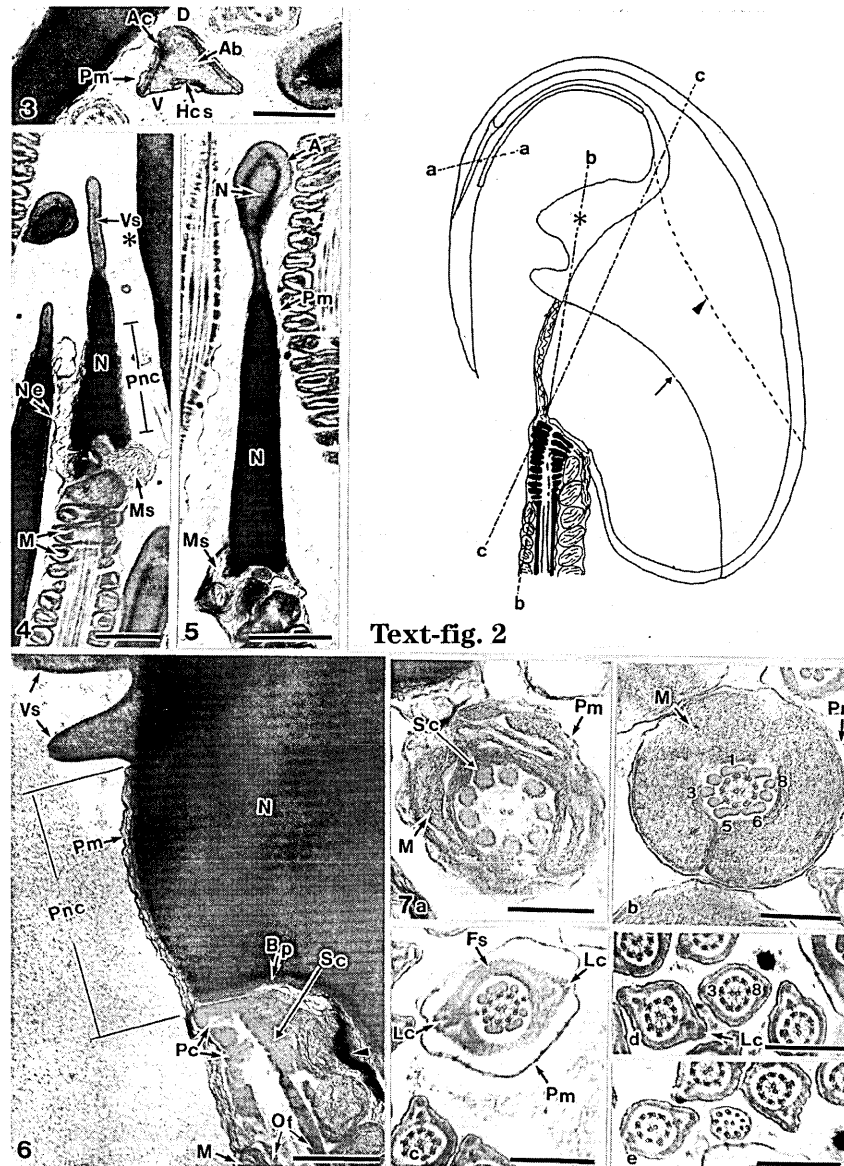
The length of the sperm tail of *A. s. peninsulae* was $116.3\mu\text{m}$, and the sperm tail consisted of four major segments: the neck ($0.8\text{--}1.0\mu\text{m}$), middle piece ($22.5\mu\text{m}$), and principal piece plus end piece ($93.0\mu\text{m}$). The total number of mitochondrial gyres of *A. s. peninsulae* was 184.

The basal plate was adhered to the nuclear envelope, defining the implantation fossa, and consisted of a band of electron dense materials (Figs 8, 10). The segmented columns were surrounded by redundant membranous scroll of nuclear envelope, adjacent to one or two mitochondria that project forward from the mitochondrial sheath of the middle piece (Figs 8, 10, and 11a). The persistence of the proximal centriole and traces of the distal centriole were observed (Fig. 10).

The shape and number of the outer dense fibers were same as in *A. a. coreae* (Fig. 11b, c). The end of



Text-fig. 1. Comparison of two *Apodemus*, mouse and rat sperm head. Two *Apodemus* species and mouse are very similar in the shape of the sperm head, but rat was different to the others. a, *Apodemus agrarius coreae*; b, *Apodemus speciosus peninsulae*; c, mouse; d, rat. Ac, acrosomal cap; Es, equatorial segment; Pnc, post-nuclear cap. Scale bar = $1\mu\text{m}$.



Text-fig. 2. Diagrammatic representation, based on scanning and transmission electron microscopy, of horizontal sections (such as Fig. 1) of the epididymal spermatozoon in *A. agrarius coreae*. **a-a**, Section passing through the separated head-cap segment (Hcs). **b-b**, Section passing through ventral spur (Vs) on the ventral region of sperm head. **c-c**, Section passing through the nucleus to ventral spur. A part of equatorial segment (Es) is located between the post-nuclear cap segment (arrow) and acrosomal cap (arrowhead) on the nuclear surface.

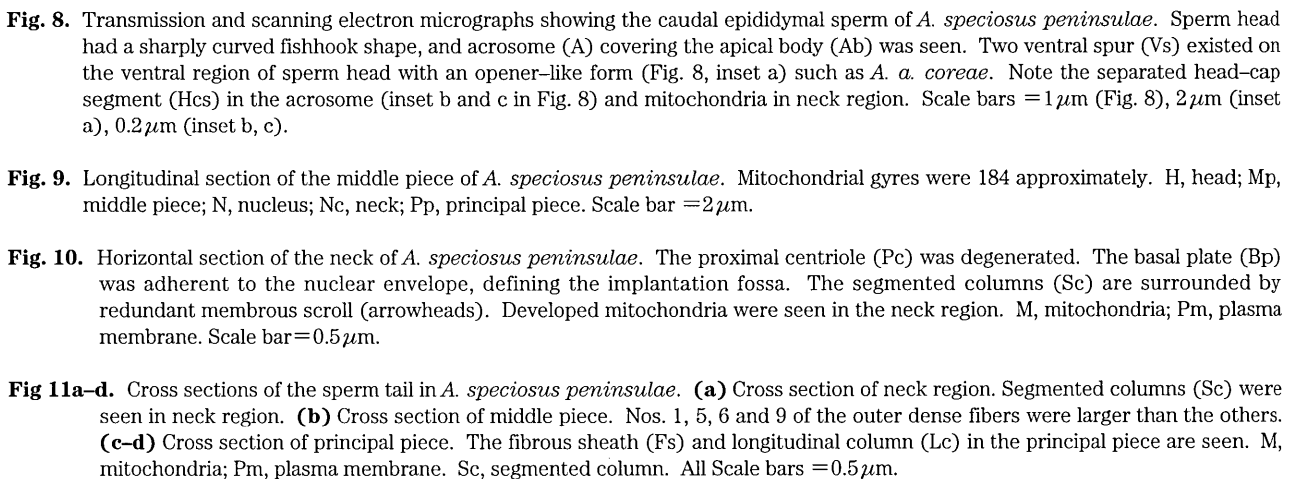
Fig. 3. Cross section through the anterior region of sperm head (cf. Text-fig. 2, a-a). The separated head-cap segment (Hcs) was seen in ventral region of sperm head. The apical body (Ab) was covered by acrosomal cap (Ac). D, dorsal part; V, ventral part; P, plasma membrane. Scale bar = 0.5 μ m.

Fig. 4. Sagittal section through the ventral spur (Vs) of sperm head in *A. agrarius coreae* (cf. Text-fig. 2, b-b). Note the ventral spur is a part of post-nuclear cap (Pnc). An acrosome (A) covers the anterior portion of the sperm head. M, mitochondria; Ms, membranous scroll; N, nucleus; Ne, nuclear envelope; Pm, plasma membrane. Scale bar = 1 μ m.

Fig. 5. Parasagittal section of sperm head in *A. agrarius coreae* (cf. Text-fig. 2, c-c). The nucleus (N) was surrounded by the acrosome (A). A part of ventral spur (Vs) are seen. Ms, membranous scroll; N, nucleus; Pm, plasma membrane. Scale bar = 1 μ m.

Fig. 6. Horizontal section of neck of *A. agrarius coreae*. The proximal centriole (Pc) was degenerated. Two ventral spur (Vs) and post-nuclear cap (Pnc) are clearly visible. The mitochondria were presented between segmented column and redundant membruous scoll (Ms). Bp, basal plate; M, mitochondria; Pm, plasma membrane; Se, segmented column. \blacktriangle , membranous scroll. Scale bar = 0.5 μ m.

Fig. 7a-e. Cross sections of the sperm tail in *A. agrarius coreae*. **(a)** Cross section of neck region. Segmented columns (Sc) and mitochondria (M) were seen. **(b)** Cross section of middle piece. Nos. 1, 5, 6 and 9 of the outer dense fibers were larger than the others. **(c-e)** Cross section of principal piece and end piece. The fibrous sheath (Fs) and longitudinal column (Lc) in the principal piece were seen, but fibrous sheath (Fs) was not seen at the end piece (fig. 7e). M, mitochondria; Pm, plasma membrane; Sc, segmented column. All scale bars = 0.5 μ m.



the middle piece was marked by an annulus (Fig. 9), a thin dense ring to which the flagellar membrane firmly adhered (Fig. 11c, d).

Mouse

Sperm head

The shape of the sperm head of the mouse had a hook shape (Fig. 12). The sperm head was $7.3\mu\text{m}$ in length, with a posterior $6.6\mu\text{m}$ occupied by a nucleus, being about $3.4\mu\text{m}$ in width (Table 1). The mouse sperm head had two ventral spurs with an opener-like form on the ventral region of the sperm head, as in the two *Apodemus* sperm (Text-fig. 1). Mouse sperm had the separated head-cap segment on the ventral side of the anterior acrosomal portion of the sperm nucleus (inset in Fig. 12), as in the two *Apodemus* sperm (cf. Fig. 3, Fig. 8b, c). In the post-nuclear cap, the mouse sperm occupied about a quarter of the nucleus.

Sperm tail

The length of the sperm tail of the mouse was $124.2\mu\text{m}$, and the sperm tail consisted of four major seg-

ments: the neck ($0.8\text{--}1.0\mu\text{m}$), middle piece ($23.5\mu\text{m}$), and principal piece plus end piece ($99.5\mu\text{m}$).

The segmented columns were surrounded by redundant, membranous scroll of nuclear envelope (Fig. 13). The proximal centriole had degenerated (Fig. 13), as in the two *Apodemus* species.

Segmented columns were seen in the neck (Figs 12, 13). The cross section of the middle piece showed a singular arrangement of the outer dense fibers, with similar numbers and central tubules. Nos. 1, 5, and 6 of the outer dense fibers were distinctly larger than the others (Fig. 14b). In the principal piece, a fibrous sheath and longitudinal column were observed in evidence (Fig. 14c).

Rat

Sperm head

The shape of the sperm head of the rat had a slender falciform (Fig. 15). The sperm head was $12.0\mu\text{m}$ in length, with a posterior $10.6\mu\text{m}$ occupied by a nucleus of about $1.6\mu\text{m}$ width (Table 1). Rat spermatozoa had one ventral spur on the ventral region of the

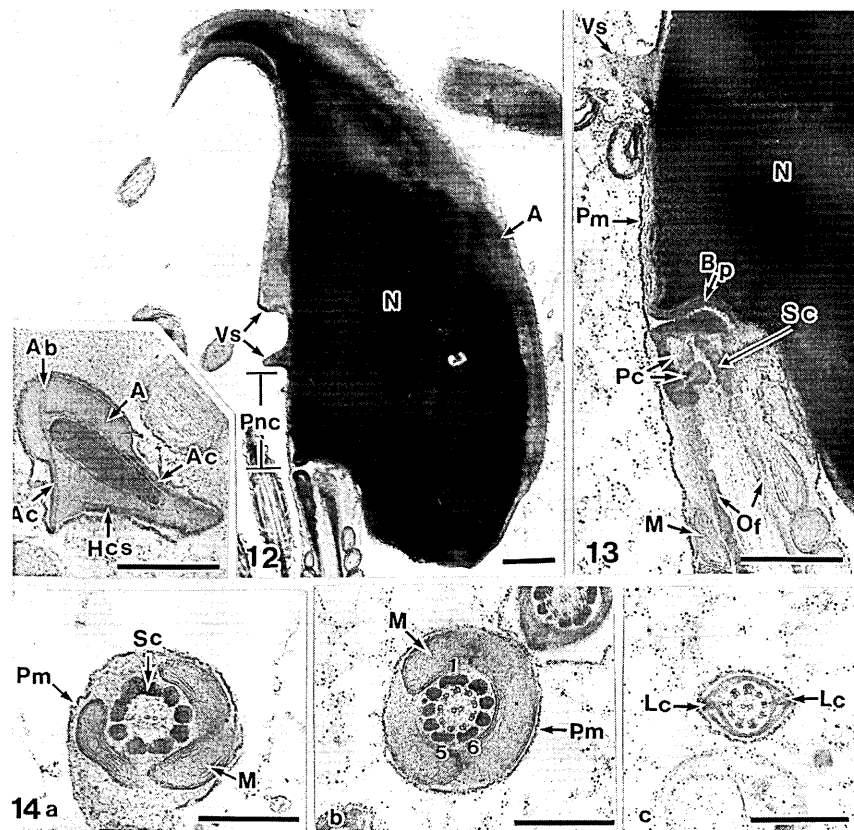


Fig. 12. Transmission electron micrographs showing the caudal epididymal spermatozoa of mouse. Sperm head had a curved hook shape, and acrosome (A) covering the apical body (Ab) is seen. Note two ventral spurs (Vs) on the ventral region of the sperm head. The separated head-cap segment (Hcs) was clearly visible (inset in Fig. 12). All Scale bars = $0.5\mu\text{m}$.

Fig. 13. Horizontal section of neck region in the mouse. Note the degenerated proximal centriole. Bp, basal plate; M, mitochondria; N, nucleus; Of, outer dense fiber; pm, plasma membrane; Pnc, post-nuclear cap; Sc, segmented column. Scale bar = $0.5\mu\text{m}$.

Fig. 14a-c. Cross sections of the sperm tail in mouse. (a) Cross section of lower part of neck region. Major segmented columns (Sc) were seen. (b) Cross section of middle piece. Nos. 1, 5 and 6 of the outer dense fibers were larger than the others. (c) Cross section of principal piece. The fibrous sheath (Fs) and longitudinal column (Lc) are seen (Fig. 14c). M, mitochondria; Pm, plasma membrane. All scale bars = $0.5\mu\text{m}$.

sperm head (**Fig. 15**). The head had a separated head-cap segment on the ventral side of the acrosome of the anterior acrosomal portion of sperm nucleus, as in the two *Apodemus* species and mouse sperm (inset b in **Fig. 1**, inset b and inset c in **Fig. 8**, inset in **Fig. 12**). The post-nuclear cap of the rat occupied about a seventh of nucleus (**Fig. 15**, **Text-fig. 1**).

Sperm tail

The length of the sperm tail of the rat was $180.0\mu\text{m}$, and the sperm tail consisted of four major segments: the neck ($0.8\text{--}1.0\mu\text{m}$), middle piece ($63\mu\text{m}$), and principal piece plus end piece ($116.2\mu\text{m}$).

The segmented columns were surrounded by mem-

branous scroll of nuclear envelope, adjacent to one or two mitochondria that project forward from the mitochondria sheath of the middle piece (**Fig. 16**). The persistence of the proximal centriole and traces of the distal centriole were observed.

Several major segmented columns are fused (**Fig. 17a**). Nos. 1, 5 and 6 fibers of the outer dense fibers were larger than the others. The mitochondria were smaller in size than those of the two *Apodemus* and mouse sperm (cf. **Figs 7a**, **11b** and **14b**). There were numerous satellite fibers around the adaxial surfaces of the outer dense fibers in the middle piece (**Fig. 17b**). A fibrous sheath and longitudinal column of the principal piece were evident (**Fig. 17c**).

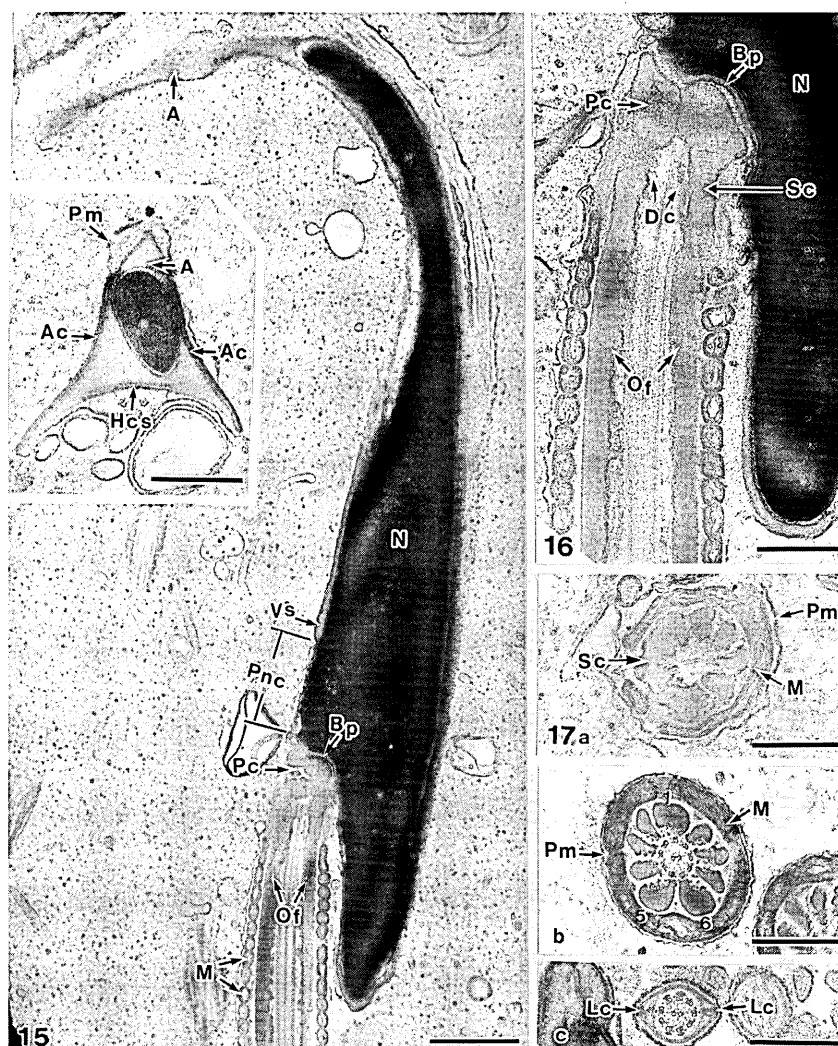


Fig. 15. Transmission electron micrographs showing the rat caudal epididymal spermatozoa. Sperm head had a falciform shape. Spermatozoon has only one small a loof-like ventral spur (Vs) on the ventral region of sperm head. The separated head-cap segment (Hcs) existed in the ventral side of anterior portion of sperm nucleus. A, acrosome; Ac, acrosomal cap; Bp, basal lamina; M, mitochondria; N, nucleus; Of, outer denser fiber; Pm, plasma membrane. Scale bars = $1\mu\text{m}$. Fig. 15 inset, $0.5\mu\text{m}$.

Fig. 16. Horizontal section of neck region of the rat. Note the degenerated proximal centriole, the two major segmented columns (Sc) and anterior extension of the central pair of microtubules (Mt) through the distal centriole. B, basal plate; M, mitochondria; N, nucleus; Of, outer dense fiber. Scale bar = $0.5\mu\text{m}$.

Fig. 17a–c. Cross sections of the sperm tail in rat. (a) Cross section of neck region. Several segmented columns (Sc) were fused. (b) Cross section of middle piece. Nos. 1, 5 and 6 of the outer dense fibers were larger than the others, and the dimension of mitochondria was slander than two *Apodemus* species and mouse. Note the electron dense granules in the dense fiber cortex. (c) Cross section of principal piece. The fibrous sheath and longitudinal column (Lc) in the principal piece were seen. M, mitochondria; Pm, plasma membrane. All scale bars = $0.5\mu\text{m}$.

DISCUSSION

In most mammalian species, the head shape is ovoid or spatulate, but in murid rodents there is usually an apical hook consisting of nuclear, subacrosomal and acrosomal material (Friend, 1936; Fawcett, 1970; Lalli and Clermont, 1981; Breed, 1983; Flaherty and Breed, 1983; Breed, 1984). In the sperm morphology and the ultrastructure of the striped field mouse *Apodemus agrarius coreae* and the Korean field mouse *Apodemus speciosus peninsulae* belonging to the subfamily Murinae, the sperm heads had a sharply curved fish hook shape, having two, well-developed ventral spurs (Vs) on the ventral region of sperm head. In Murinae, the two major subfamilies (Muridae and Cricetidae) have species with the same type spermatozoa, and the two families evolve separately, and within each family at least two separate evolutionary trends can be detected in Muridae, that is, the *Rattus* lineage and Hydromyinae lineage (Roldan, *et al.*, 1991).

The sperm morphology of the Australian hydromyine rodents is characterized by the presence of three curved hooks on the apical margin of the sperm head (Breed and Sarafis, 1979; Breed, 1980). In the plains mouse, *Pseudomys australis*, during Step13, spermatogenic step, the two ventral hooks elongate to full length (Flaherty and Breed, 1987), and the dorsal hook contains nuclear, acrosomal, and subacrosomal material, whereas the two ventral hooks appear to consist largely of an extension of the material in the subacrosomal space (Breed, 1983, 1984, Flaherty and Breed, 1983, Breed *et al.*, 2000). In *Apodemus spermatozoa*, ultrastructural studies have revealed that the two ventral spurs contain subacrosomal material, in a fine structure similar to the two large ventral processes (hooks) of the plains mouse, *Pseudomys australis* (Flaherty, 1987).

The ventral spurs have been shown in the present study to consist of a continuation of the post-nuclear cap region. Although the postacrosomal region has been implicated as the initial site of sperm-egg fusion (Yanagimachi and Noda, 1970), recent studies suggest that this is primarily a function of the equatorial segment (Bedford *et al.*, 1979; and Moore and Bedford, 1983). On the basis of their shape, inherent rigidity, and position, it has been postulated that the ventral spurs may function in assisting the mechanical penetration of the zona pellucida (Flaherty *et al.*, 1983; Breed, 1984; Flaherty, 1987). The functional significance of the well-developed ventral spurs of *Apodemus* sperm remains unknown.

In the neck region of two *Apodemus* sperm, a degenerated proximal centriole occupied a niche in the dense substance of the connecting piece, as it does in the mouse and the rat. The neck region of two *Apodemus* spermatozoa is surrounded by a mass of redundant nuclear envelope and large mitochondria that project forward from the mitochondrial sheath of the middle piece, but in the mouse and rat spermatozoa, the neck region is slender and devoid of mitochondria

except for a fold of redundant nuclear envelope.

The sperms of the genus *Apodemus* are similar to those of laboratory mouse, in having a sperm head with a curved hook and thus being mouse-like, although the detailed ultrastructure of their neck region on features of the redundant nuclear envelope and mitochondria varies *Apodemus* and mouse. The rat is unlike the mouse and *Apodemus spermatozoa* in having a falci-form shaped head as well as a very long tail with different ultrastructure.

From the viewpoint of taxonomic implications, since a very similar morphology occurs in the two *Apodemus* species and the mouse, it suggests that these genera are sister groups, to the exclusion of the rat.

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