Ultrastructural Observations on Spermatozoa of the Soricidae, with Special Attention to a Subfamily Revision of the Japanese Water Shrew Chimarrogale himalayica

Takayuki MÖRI, Shusei ARAI*, Satoshi SHIRAISHI and Teru Aki UCHIDA

Zoological Laboratory, Faculty of Agriculture, Kyushu University 46-06, Fukuoka 812, Japan
*General Laboratory Center, Kyushu Dental College, Kitakyushu 803, Japan
(Accepted 22 February 1991)

Abstract. The sperm morphology of four shrew species, Sorex shinto and Chimarrogale himalayica (Soricinae), and Crocidura dsinezumi and Suncus murinus (Crocidurinae) belonging to the family Soricidae, was studied with the electron microscope. The Japanese water shrew Chimarrogale himalayica has been regarded as belonging to the subfamily Crocidurinae based on the teeth with white-colored tips. Spermatozoa of the Soricidae were characterized by the presence of a conspicuously long middle piece compared with those of other eutherians as far as we know, its outer dense fibers arranged in a horseshoe fashion and well-developed satellite fibers found in association with the inner aspect of fiber Nos. 5 and 6. However, the C. himalayica spermatozoon differed from other crocidurine spermatozoa in size and shape of the head; namely, the former head took a small spatulate shape, but the latter a large shield-like shape. In addition, the C. himalayica spermatozoon possessed an unusually slender, wavy and electron-dense apical body at the tip of the subacrosomal space surrounded by the smooth inner acrosomal membrane and the proximal centriole had a solid lumen filled with electron-dense materials, both of which are characteristic of the Soricidae. However, other crocidurine spermatozoa were devoid of such a peculiar apical body in the subacrosomal space surrounded by the serrated inner acrosomal membrane and the proximal centriole had a fistulous lumen. The evidence presented here strongly indicates that C. himalayica should be moved from the subfamily Crocidurinae to the subfamily Soricinae.

Key words: Spermatozoa; Ultrastructure; Taxonomic revision; Soricidae; Chimarrogale himalayica.

It has been well known that a spermatozoon exhibits the characteristic of a family, subfamily, genus and/or species (Friend, 1936; Hughes, 1965; Fawcett, 1970; Fawcett & Phillips, 1970; Uchida & Mori, 1972; Bedford, 1974). A comparative study of spermatozoon ultrastructure in the Soricidae of the most primitive order Insectivora within the eutherian mammals acquires much interest, because there exists some confusion as to the affinities of the genus Chimarrogale regarded as belonging to the subfamily Crocidurinae (Repenning,
Spermatozoon ultrastructure of shrews has been described only in the Asiatic musk shrew *Suncus murinus* (Green & Dryden, 1976; Cooper & Bedford, 1976; Koehler, 1977; Mori & Uchida, 1985) and the European common shrew *Sorex araneus* (Plöen et al., 1979). The aim of the present study was to examine in detail with the electron microscope the comparative morphology of epididymal spermatozoa from four shrew species belonging to two subfamilies, the Soricinae and Crocidurinae, and to discuss the taxonomic revision of the Japanese water shrew *C. himalayica* in accordance with our data.

### Materials and Methods

One male *Sorex shinto* captured in Nagano Prefecture in April, one male *Chimarrogale himalayica* in Kumamoto Prefecture in May, two male *Crocidura dsinezumi* in Fukuoka Prefecture in September and three male *Suncus murinus* in Okinawa Prefecture in October were killed by decapitation to use in this study. The epididymides were removed, and the tissues were promptly placed in cold 3% glutaraldehyde in 0.2 M-phosphate buffer (pH 7.4) for 4 hrs. After being thoroughly rinsed with the same buffer, the tissues were post-fixed with 1.3% osmium tetroxide in the same buffer for 3 hrs, dehydrated with acetone and embedded in epoxy resin. Thin sections (~60 nm) were doubly stained with uranyl and lead acetate before examination in a Hitachi HS-9 electron microscope (75 kV).

### Results

The sperm morphology of the shrews is described separately for the following four species, and the dimensions of the spermatozoa are summarized in Table 1.

<table>
<thead>
<tr>
<th>Species</th>
<th>Head (μm)</th>
<th>Tail (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total length</td>
<td>Width</td>
</tr>
<tr>
<td><em>Chimarrogale himalayica</em></td>
<td>6.1</td>
<td>2.9</td>
</tr>
<tr>
<td><em>Sorex shinto</em></td>
<td>5.7</td>
<td>4.0</td>
</tr>
<tr>
<td><em>Crocidura dsinezumi</em></td>
<td>14.0</td>
<td>11.5</td>
</tr>
<tr>
<td><em>Suncus murinus</em></td>
<td>20.0</td>
<td>17.1</td>
</tr>
</tbody>
</table>
Ultrastructure of Spermatozoa in Soricids

1. Chimarrogale himalayica

Sperm head

The spermatozoon had a dorsoventrally flattened, spatulate head (Figs. 1a and 2a, b). The head was 6.1 μm in length, whose posterior 3.6 μm was occupied by a nucleus, being about 2.9 μm in width (Table 1). The acrosome protruded for a length of 2.5 μm from the tip of the nucleus. The equatorial segment of the acrosome was situated on the middle portion of the nucleus. The apical body outlined by the smooth inner acrosomal membrane extended to the tip of the very large subacrosomal space as an electron-dense, wavy, fingerlike projection (0.8 μm) (Fig. 2c) unlike a common apical body in most other mammals.

Sperm tail

The total length of the sperm tail was long (77.0 μm) with a long middle piece (41.0 μm) (Table 1). The articular surface of the connecting piece was anomalous in lacking the roof of the capitulum consisting of the cross-banded dense matrix which emerges from an interspace between two triplets on the juxtanuclear side of the proximal centriole; thus, the juxtanuclear wall of the proximal centriole was directly attached to the basal plate lining the implantation.

Fig. 1. Planar views of spermatozoa from the caudal epididymis in four shrew species. a) Chimarrogale himalayica; b) Sorex shinto; c) Crocidura dsinezumi; d) Suncus murinus. A, acrosome; Mp, middle piece; N, nucleus; Pp, principal piece; →, terminal end of the middle piece.
Fig. 2. Electron micrographs showing a sperm head of *Chimarrogale himalayica*. a) and b) Horizontal and sagittal sections of the sperm head with a spatulate shape, respectively. c) The unusually long, slender and wavy apical body (Ab) in Fig. 2b at higher magnification. A, acrosome; Es, equatorial segment; Iam, inner acrosomal membrane; N, nucleus; Oam, outer acrosomal membrane; Pm, plasma membrane; Ss, subacrosomal space.

Furthermore, electron-dense granular materials which appear to be derived from the basal plate hung down into the lumen of the proximal centriole through the wide interspace (Fig. 3a, b).

In the cross section of the middle piece, the outer dense fibers were arranged in a horseshoe fashion: the profile was anomalously asymmetrical, with a major compartment containing four dense fibers (Nos. 4, 5, 6 and 7) on one side and a minor compartment including three (Nos. 1, 2 and 9) on the other side of the dorsoventral axis (X-Y) joining the central fibrils; in addition, fiber No. 1 was bilobed and Nos. 5 and 6 were larger than the others, and a pile of the satellite fibers were found only in association with the inner aspect of fiber Nos. 5 and 6 (Fig. 4).
Fig. 3. Electron micrographs showing the connecting piece of the *Chimarrogale himalayica* spermatozoon devoid of the capitulum roof, but bearing the proximal centriole (Pc) whose lumen is filled with the dense granular materials (Dg). a) and b) Cross and longitudinal sections of the proximal centriole, respectively. Bp, basal plate; N, nucleus; Ne, nuclear envelope; Od, outer dense fiber; Pr, posterior ring; Sc, segmented column.

2. *Sorex shinto*

*Sperm head*

The spermatozoon had a relatively small, flattened, shield-shaped head (Fig. 1b). A sperm nucleus occupied 3.4 μm of the head with a total length of 5.7 μm; the width of the head was about 4.0 μm (Table 1). The acrosome extended anteriorly beyond the leading edge of the nucleus for a length of 2.3 μm. The equatorial segment existed in the posterior portion of the nucleus. The electron-dense apical body was wavy and fingerlike, and the wide sub-acrosomal space was occupied by fine granules (Fig. 5) as in the *C. himalayica* spermatozoon (cf. Fig. 2c).
Fig. 4. Cross section through the middle piece of the *Chimarrugale himalayica* spermatozoon, showing the asymmetry of the outer dense fibers (Od) arranged in a horseshoe fashion, with four fibers on one side and three on the other side of the dorsoventral axis (X–Y). Notice also well-developed satellite fibers (Sf) associated with the inner aspect of fibers 5 and 6. Af, axial filament complex; Mt, mitochondrion; Pm, plasma membrane.

*Sperm tail*

The sperm tail was 62.0 μm in length, whose middle piece was 32.0 μm long (Table 1). The structure of the sperm neck and the proximal centriole (Fig. 6a, b), together with the unique arrangement of the outer dense fibers and a lot of satellite fibers, bore a striking resemblance to the form seen in the *C. himalayica* spermatozoon (cf. Fig. 3a, b).

3. *Crocidura dsinezumi*

*Sperm head*

The spermatozoon had a large, flattened, shield-shaped head measuring 14.0 μm in length and 11.5 μm in width, containing a nucleus with 3.4 μm in length (Figs. 1c and 7a; Table 1). The acrosome reached a length of 10.6 μm from the tip of the nucleus. The equatorial segment was present around the back portion of the nucleus. The spermatozoon had the common apical body in the very large subacrosomal space surrounded by the serrated inner
Fig. 5. Electron micrographs showing the unusually long, slender and wavy apical body (Ab) of the *Sorex shinto* spermatozoon. A, acrosome; Iam, inner acrosomal membrane; N, nucleus; Oam, outer acrosomal membrane; Pm, plasma membrane; Ss, subacrosomal space.

Fig. 6. Electron micrographs showing the connecting piece of the *Sorex shinto* spermatozoon. a) and b) Cross and longitudinal sections of the proximal centriole (Pc), respectively. Bp, basal plate; Dg, dense granular material; Mt, mitochondrion; N, nucleus; Ne, nuclear envelope; Pr, posterior ring; Sc, segmented column.
Fig. 7. Electron micrographs showing the head of the *Crocidura dsinezumi* spermatozoon. a) Horizontal section of the large, flat, shield shaped head with the nucleus (N), large acrosome (A) and the subacrosomal space (Ss) surrounded by the serrated inner acrosomal membrane (Iam) between them. b) The subacrosomal space (Ss) in Fig. 7a at higher magnification. c) Sagittal section through the common apical body (Ab) near the tip of the nucleus (N). Note the absence of a unique apical body. Es, equatorial segment; Ne, nuclear envelope; Oam, outer acrosomal membrane; Pm, plasma membrane.

Fig. 8. Electron micrographs showing the connecting piece of the *Crocidura dsinezumi* spermatozoon bearing the proximal centriole (Pc) with a fistulous lumen (Fl). a) and b) Cross and longitudinal sections of the proximal centriole, respectively. Bp, basal plate; N, nucleus; Ne, nuclear envelope; Sc, segmented column.
acrosomal membrane, but was lacking in such a peculiar apical body as seen in the spermatozoa of the above two species (Fig. 7a–c).

**Sperm tail**

The sperm tail was unusually long with a total length of 90.0 μm; the middle piece (55.0 μm) was longer than that of the *C. himalayica* and *S. shinto* spermatozoa (Fig. 1a–c; Table 1). The proximal centriole had the juxtanuclear wall coming in contact with the basal plate as in the spermatozoa of the above two species, but possessed a fistulous lumen with little electron dense granular materials (Fig. 8a, b; cf. Figs. 3a, b and 6a, b). The cross section of the middle piece showed a peculiar arrangement of the outer dense fibers and a number of well-developed satellite fibers (Fig. 9) as in that of the above two species (cf. Fig. 4).

4. **Suncus murinus**

**Sperm head and tail**

The spermatozoon had a giant, flattened, shield-shaped head (20.0 μm in length, 17.1 μm in width) with a nucleus (3.6 μm) (Table 1). The acrosome protruded for a length of 16.4 μm from the tip of the nucleus. The sperm tail was 102.0 μm in length, of which 63.0 μm were occupied by the unusually long middle piece (Fig. 1d). The other characters of the spermatozoon of this species were exactly similar to those in the *C. dsinezumi* spermatozoon.

![Figure 9](image_url)

**Fig. 9.** Cross section through the middle piece of the *Crocidura dsinezumi* spermatozoon, showing a strong similarity in structure to that of *Chimarrogale himalayica*. Af, axial filament complex; Mt, mitochondrion; Od, outer dense fiber; Pm, plasma membrane; Sf, satellite fiber. X–Y, dorsoventral axis.
Discussion

There has been no study from the viewpoint of the comparative spermatology within the Soricidae including the genera Chimarrogale, Sorex, Crocidura and Suncus, although fragmentary knowledge has been obtained from spermatozoa of two shrew species (Plöen et al., 1979 for Sorex araneus; Green & Dryden, 1976, Cooper & Bedford, 1976, Koehler, 1977, and Möri & Uchida, 1985 for Suncus murinus). Taxonomic revision of subfamily to which Chimarrogale in question belongs is discussed light and electron microscopically as follows.

The present study revealed that there is considerable variation in size and shape of the sperm head among the above four genera. In the subfamily Soricinae, which is one of the basic insectivores, the S. shinto spermatozoon seems to display a more conservative mold characterized by the standard shield-shaped head with the small acrosome, as previously described in Sorex araneus (Plöen et al., 1979). On the other hand, in the subfamily Crocidurinae, the C. dsinezumi and S. murinus sperm heads differed from each other in size, possessing the large and the giant acrosome, respectively. Such a great variation of acrosome size within the family Soricidae seems to provide a key with which to assess morphological specialization of the spermatozoa in relation to the possible phylogenetic relationships within this family. Furthermore, the Chimarrogale himalayica spermatozoon as well as the Sorex shinto spermatozoon was different from Crocidura dsinezumi and S. murinus spermatozoa in having a much shorter tail and middle piece at the subfamily level.

The ultrastructure of the sperm tail fell within a common peculiar pattern for spermatozoa of all the examined species belonging to the Soricidae, i.e. the outer dense fibers arranged in a horseshoe fashion and a lot of satellite fibers associated with the inner aspect of fibers 5 and 6. However, there were distinct differences according to subfamilies as follows: in S. shinto and C. himalayica, belonging to the Soricinae, the spermatozoa possessed the unusually electron-dense, slender and wavy apical body in the subacrosomal space outlined by the smooth inner acrosomal membrane; by contrast, in C. dsinezumi and S. murinus, belonging to the Crocidurinae, the spermatozoa are devoid of such a peculiar apical body in the subacrosomal space surrounded by the serrated inner acrosomal membrane. Fine filaments traversing the narrow, electron-lucent space between the capitulum and the basal plate are regarded as the structures mainly responsible for attachment of the head to the tail (Fawcett, 1975). In spermatozoa of the Soricinae, electron-dense granular materials instead of fine filaments were derived from the basal plate, and the lumen of the proximal centriole was filled with electron-dense materials, although such a morphological character has not been described for S. araneus (Plöen et al., 1979). On the other hand, spermatozoa of the Crocidurinae had the proximal centriole with a fistulous lumen. Thus, striking differ-
ences existed between the Soricinae and the Crocidurinae within the family Soricidae.

Repenning (1967) was the first to bring out a question on the taxonomic position of C. himalayica; he has considered C. himalayica to be closely related to the subfamily Soricinae, based on the detection of red-colored tips of the teeth under ultraviolet rays, and by difference in occlusal patterns of P₄ and in posterior patterns of mandibular condyles, all of which have been reconfirmed by Abe (1988). Taking also highly-decreased grade of the G-band homology between the C. himalayica karyotype and the C. dsinezumi karyotype (Tada & Obara, 1986), and close resemblance between C. himalayica and the European water shrew Neomys fodiens (Fredga & Levan, 1969) in their chromosome constitution (Obara & Tada, 1985) into account, we reach the conclusion on the above spermatological evidence that C. himalayica should be moved from the subfamily Crocidurinae to the Soricinae.

Acknowledgment

We thank Professor E. W. Jameson, Jr., University of California, for comments on the manuscript.

References


