Spermatozoa of Several Frog Species from Japan and Adjacent Regions

MITSURU KURAMOTO

Abstract: Sperm shape and size of a total of 25 frog species belonging to five families are described. Spermatozoa of Bombina orientalis were peculiar in having a ventral undulating membrane. Its shape was similar to that of B. bombina, but differed from that of B. variegata. Three species of the genus Bufo had spermatozoa with an elongated head and a tail equipped with an undulating membrane. Spermatozoa of two species of the genus Hyla had a crescent-like head, and it was observed that the tail contains two flagellar units, presumably an axoneme and an axial rod. In all the above taxa, mitochondrial spheres were observed at the base of the tail, where they usually constituted a middle piece. Most species of the genus Rana had spermatozoa with a cylindrical head and a simple thin tail, but three species of the Rana narina complex and R. ishikawae had a very long sperm head. In the latter four species, mitochondrial spheres were evident at the neck region. Platymantis petrewensis had also slender spermatozoa. Spermatozoa of Microhyla ornata had a cone-shaped head and a distinct middle piece. These findings are compared with previous studies, and several taxonomic implications are discussed.

Key words: Spermatozoa; Sperm size; Discoglossidae; Bufonidae; Hylidae; Ranidae; Microhylidae

Sperm shape and size are extremely variable among animal taxa, and it is assumed that the differences in sperm shape reflect phylogenetic relationships between taxa (Jameison, 1991). I have described shape and size of spermatozoa of several salamanders and rhacophorid tree frogs (Kuramoto, 1995, 1996a, 1997; Kuramoto and Tanaka, 1997). In salamanders, hynobiid and cryptobranchid species have many characteristics in common, although sizes are variable between species or even between populations, whereas salamandrids have a distinctive structure, the acrosomal barb. In rhacophorids, a remarkable generic differentiation was observed, which does not correlate with the breeding mode of each genus.

Spermatological data for anurans are still fragmentary (see table in Lee and Jameison, 1992). For Japanese frogs, fine sperm structures of Bufo japonicus (Yoshizaki and Katagiri, 1982), Rana japonica (Yoshizaki, 1987), Rhacophorus arboreus and R. schlegeli (Mizuhira et al., 1986) have been described. Morita (1928) carried out light microscopic studies on spermatogenesis in Rana nigromaculata. Recently, Kwon and Lee (1992, 1995), Kwon et al. (1993), and Lee and Kwon (1992) clarified fine sperm structures of six Korean frog species belonging to the genera Bombina, Bufo, Hyla and Rana. Spermatozoa of Chinese Bufo gargarizans and Rana nigromaculata were investigated by Mo (1985), using TEM.

Most of the recent spermatological studies on anurans give much information about internal structures, but only a few supply external features including sperm sizes. The present study deals with external sperm morphology of a total of 25 frog species of the Discoglossidae, Bufonidae, Hylidae, Ranidae, and Microhylidae, obtained from Japan and adjacent regions.

MATERIALS AND METHODS

Species used were: Bombina orientalis from the suburbs of Seoul, Korea (Discoglossidae), Bufo japonicus from Mt. Hikosan, Fukuoka Pref., B. gargarizans from Mt. Mientien-Shan, Taipei, Taiwan, B. melanostictus from Nankang, Taipei (Bufonidae), Hyla japonica from Mt. Inunaki, Fukuoka Pref., H. chinensis from Mt. Inunaki, Fukuoka Pref., H. chinensis from Nankang (Hylidae), Rana japonica from Munakata, Fukuoka Pref., R. tsushimensis from Tsushima Island, Nagasaki Pref., R. okinavana from Yona, Okinawa Island, Okinawa Pref., R. tagoi from Mt. Hikosan, R. ornativentris from Oita, Oita Pref., R. dybowskii from Tsushima Island, R. nigromaculata from

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Mt. Inunaki and from the suburbs of Chengdu, Suchuan Prov., China, *R. latouchii* from Chiaoshii, Ilan Prov., Taiwan, *R. rugulosa* from Taichung, Taiwan, *R. rugosa* from Seihi, Nagasaki Pref., *R. limnocharis* from Seihi and from the suburbs of Chengdu, *R. catesbeiana* from Munakata, *R. amamiensis* from Sumiyo, Amami Island, Kagoshima Pref., *R. narina* from Yona, *R. supranarina* from Iriomote Island, Okinawa Pref., *R. ishikawae* from Sumiyo, *R. namiyi* from Yona, *Platymantis pelewensis* from Koror and Pelelieu, Palau Islands (Ranidae), and *Microhyla ornata* from Iriomote Island and from Nankang (Microhylidae). *Rana catesbeiana* is an introduced species. Specimens of *Bombina orientalis* were taken from a strain which has been kept in the Laboratory for Amphibian Biology, Hiroshima University, and all the other specimens were collected in the field during breeding seasons from 1992 to 1994. At least two males for each species or population were examined except *R. ishikawae* for which only one male specimen was available.

For each specimen, sperm suspension was prepared by squashing testis in a small quantity of water; the supernatant was put on a slide glass and a cover slip, and 2% glutaraldehyde was added. For light microscopy (LM), spermatozoa were stained with Giemsa or alum carmine, and for scanning electron microscopy (SEM) spermatozoa were coated with gold. Sizes of spermatozoa were measured on enlarged photomicrographs using a digitizer, and fine structures, such as mitochondrial spheres and width of tail fibers, were measured on enlarged electron photomicrographs. The scanning electron microscope used was a JSM-T200 (JEOL).

**RESULTS**

The oriental fire-bellied toad, *Bombina orientalis*, had peculiar spermatozoa. The main body (head) was bent slightly; one end formed a truncated short rod, apparently an acrosome, and the other end tapered gradually. A flagellum and an undulating membrane were always observed along the convex (ventral) side of the head (Fig. 1a, d). The flagellum originated at the anterior portion of the head (Fig. 1c). In many spermatozoa, the posterior part bifurcated

**Fig. 1.** Spermatozoa of *Bombina orientalis*. (a) Normal spermatozoon. (b) Spermatozoon with axial rod detached at the posterior portion of the head. (c) Deformed spermatozoon with axial rod and flagellum detached. (d) Ventral part of spermatozoon showing extensively waved flagellum. (e) Ventral part of spermatozoon with slightly detached axial rod and flagellum, showing many spherical mitochondria around axial rod. Scale bars in (a), (b) and (c) equal 10 μm and those in (d) and (e) 1 μm.
TABLE 1. Sperm size in six species belonging to the Bufonidae, Hylidae and Microhylidae (x̄ ± SD, in μm). Asterisk denotes a population from Taiwan.

<table>
<thead>
<tr>
<th>Species</th>
<th>N</th>
<th>Acrosome length</th>
<th>Head length</th>
<th>Middle piece length</th>
<th>Tail length</th>
<th>Total length</th>
<th>Head width</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Bufo japonicus</em></td>
<td>15</td>
<td>2.6 ± 0.4</td>
<td>26.8 ± 2.0</td>
<td>1.4 ± 0.3</td>
<td>58.4 ± 9.4</td>
<td>89.3 ± 10.5</td>
<td>1.0 ± 0.1</td>
</tr>
<tr>
<td><em>B. gargarizans</em></td>
<td>15</td>
<td>2.7 ± 0.6</td>
<td>23.8 ± 2.5</td>
<td>1.3 ± 0.4</td>
<td>44.0 ± 5.9</td>
<td>71.9 ± 5.5</td>
<td>1.1 ± 0.1</td>
</tr>
<tr>
<td><em>B. melanostictus</em></td>
<td>10</td>
<td>2.3 ± 0.4</td>
<td>18.6 ± 1.1</td>
<td>1.4 ± 0.4</td>
<td>41.5 ± 6.2</td>
<td>63.7 ± 6.3</td>
<td>1.1 ± 0.1</td>
</tr>
<tr>
<td><em>Hyla japonica</em></td>
<td>20</td>
<td>2.5 ± 0.4</td>
<td>16.7 ± 2.3</td>
<td>6.9 ± 2.4</td>
<td>41.6 ± 5.6</td>
<td>67.7 ± 5.1</td>
<td>1.5 ± 0.2</td>
</tr>
<tr>
<td><em>H. chinensis</em></td>
<td>20</td>
<td>1.7 ± 0.2</td>
<td>18.4 ± 1.4</td>
<td>6.0 ± 1.9</td>
<td>46.6 ± 4.9</td>
<td>72.7 ± 5.2</td>
<td>1.5 ± 0.3</td>
</tr>
<tr>
<td><em>Microhyla ornata</em></td>
<td>20</td>
<td>1.6 ± 0.2</td>
<td>11.2 ± 1.1</td>
<td>1.5 ± 0.1</td>
<td>39.5 ± 5.9</td>
<td>53.7 ± 6.4</td>
<td>1.7 ± 0.1</td>
</tr>
<tr>
<td><em>M. ornata</em></td>
<td>20</td>
<td>1.5 ± 0.3</td>
<td>10.7 ± 1.5</td>
<td>1.0 ± 0.1</td>
<td>41.1 ± 2.8</td>
<td>54.3 ± 2.7</td>
<td>2.0 ± 0.1</td>
</tr>
</tbody>
</table>

to various degrees, exposing an axial rod to which a flagellum and an undulating membrane were attached (Fig. 1b). This structure clearly corresponds to the sperm tail, which is normally put in a groove along the ventral side of the head. Many small spheres, about 0.3 μm in diameter, were observed on the axial rod (Fig. 1b, e). In unbifurcated spermatozoa, the axial rod usually extends beyond the end of the head (Fig. 1a). Size of the head was 28.9 ± 2.5 μm in length and 1.5 ± 0.1 μm in width (x̄ ± SD, N = 20). Width of the axial rod was about 0.5 μm and that of the flagellum 0.3 μm. In a few extremely deformed spermatozoa (Fig. 1c), the base of the axial rod was traced to near the attached site of the flagellum. In such a spermatozoon the flagellum (ca. 80 μm in length) was more than twice the length of the head.

Spermatozoa of the three species of the genus *Bufo* had an elongated head and a long tail with

FIG. 2. Spermatozoa of Bufonidae and Hylidae. (a) Spermatozoon of *Bufo japonicus*. (b) and (c) Spermatozoa of *Hyla japonica*. (d) Neck part of spermatozoon of *B. gargarizans* showing several mitochondrial spheres. (e) – (g) Neck parts of *H. chinensis* showing separation of tail fibers and mitochondrial spheres. Scale bars in (a) – (c) equal 10 μm and those in (d) – (g) 1 μm.
an undulating membrane (Fig. 2a). The acrosome could not be discriminated from the head proper in SEM, but was discernible as a heterochromatic part in LM, as exemplified by *Hyla japonica* sperm in Fig. 2b. In *B. japonicus*, more than half of the spermatozoa had a swollen posterior end of the head, presumably corresponding to the middle piece. Width of the middle piece was about 1.5 μm, much wider than the head. Probably the middle piece is readily detached, because the plain head end is more common for spermatozoa of the other two *Bufo* species. Several spherical bodies, presumably mitochondria, were observed near the plain posterior end of head (Fig. 2d). This implies that the middle piece consists of a loose clump of mitochondria. The tail was composed of an axial rod, a flagellum, and an undulating membrane. The axial rod was rather straight and slightly thicker (ca. 0.34 μm in diameter) than the flagellum (ca. 0.26 μm) at the base, and became thinner toward the posterior end (Fig. 2a). The flagellum was only slightly wavy and extended beyond the point of attachment to the axial rod. Width of the undulating membrane (distance between axial rod and flagellum) was about 1.5 μm.

The sperm head of *B. melanostictus* was shorter and thicker than those of *B. japonicus* and *B. gargarizans* (Table 1). The spermatozoon of *B. japonicus* was longer than the other two species, largely due to its longer tail. *Hyla japonica* and *H. chinensis* had spermatozoa which were very similar to each other in both shape and size (Fig. 2b, c; Table 1). The sperm head was crescent-like. Under LM, the acrosome was observed as a thin heterochromatic part at the tip of the sperm head (Fig. 2b). The acrosome was significantly longer in *H. japonica* than in *H. chinensis*. In most spermatozoa, a middle piece was clearly recognized (Fig. 2b, c), but its length was very variable. The middle piece is apparently composed of many spherical mitochondria and covered with a thin envelope. In some spermatozoa, the envelope of the middle piece was destroyed and the inner clump of mitochondria was clearly seen (Fig. 2f, g). The tail was composed of a single
fiber, but in *H. chinensis* and, to a lesser extent, in *H. japonica*, there were a few spermatozoa with two flagellar units (Fig. 2f, g). Because the diameter of the tail fiber was thicker in single-fibered spermatozoa (ca. 0.36 μm in diameter) than in bi-fibered ones (ca. 0.19 μm), and because there were a few cases in which a normal single fiber branched off into two thin units (Fig. 2e, f, g), it was concluded that the tail fiber of the two *Hyla* species contains intrinsically two flagellar units, which apparently correspond to an axoneme and an axial rod, described by Lee and Kwon (1992).

The 13 species of the genus *Rana*, excluding three species of the *Rana narina* complex and *R. ishikawae*, had an elongated cylindrical sperm head (Fig. 3g, h) and a simple tail (Fig. 3a-f). Head size and shape varied from one species to another, and head length/width ratio ranged from 16.3 in *R. ornativentris* to 6.8 in *R. catesbeiana*. In most species, the acrosome and the middle piece were not clearly discernible, so the measurements were made on the head as a whole. The tail was thin, and often detached from the head or cut off randomly. Mean tail diameter ranged from 0.15 μm in *R. rugulosa* to 0.24 μm in *R. tsushimensis* and *R. ornativentris*. In several species, such as *R. rugulosa*, *R. limnocharis*, and *R. namiyoi*, the tail contained several cytoplasmic droplets (Fig. 3d). Mean tail lengths were about 25–45 μm, and mean sperm length about 40–60 μm (Table 2).

Three species of the *Rana narina* complex, *R. amamiensis*, *R. narina*, and *R. supranarina*, had a thin, slender sperm head, with a mean length/width ratio of 22 to 31 (Fig. 4a; Table 2). Because of the longer sperm head, total sperm length was longer than those of the above-mentioned ranid species. *Rana ishikawae* had a very long, thread-like sperm head and a relatively longer tail (Fig. 4b), and thus total length was more than twice those of the other species of the genus *Rana*. The tail fiber of *R. ishikawae* was much thicker (ca. 0.34 μm in diameter) than that of *R. narina* (ca. 0.20 μm). Spermatozoa of the three species of the *Rana narina* complex and *R. ishikawae* had a middle piece composed of spherical mitochondria (Fig. 4g).
TABLE 2. Sperm size in 18 species belonging to the Ranidae (x±SD, in μm). Asterisks denote populations from Sichuan, China.

<table>
<thead>
<tr>
<th>Species</th>
<th>N</th>
<th>Head length</th>
<th>Tail length</th>
<th>Total length</th>
<th>Head width</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rana japonica</td>
<td>20</td>
<td>16.9±1.1</td>
<td>35.0±8.1</td>
<td>51.9±8.0</td>
<td>1.3±0.1</td>
</tr>
<tr>
<td>R. tsushimensis</td>
<td>20</td>
<td>15.1±1.8</td>
<td>25.3±4.3</td>
<td>40.4±5.0</td>
<td>1.3±0.1</td>
</tr>
<tr>
<td>R. okinavana</td>
<td>20</td>
<td>16.1±1.2</td>
<td>35.1±5.9</td>
<td>51.2±5.7</td>
<td>1.2±0.1</td>
</tr>
<tr>
<td>R. taigoi</td>
<td>20</td>
<td>18.2±2.1</td>
<td>30.7±5.9</td>
<td>48.9±6.9</td>
<td>1.2±0.1</td>
</tr>
<tr>
<td>R. ornativentris</td>
<td>20</td>
<td>19.1±2.0</td>
<td>39.6±7.1</td>
<td>58.7±7.4</td>
<td>1.2±0.1</td>
</tr>
<tr>
<td>R. dybowskii</td>
<td>20</td>
<td>14.3±1.0</td>
<td>36.1±8.4</td>
<td>50.4±8.4</td>
<td>1.3±0.1</td>
</tr>
<tr>
<td>R. nigromaculata</td>
<td>20</td>
<td>14.6±2.2</td>
<td>37.7±5.6</td>
<td>52.3±5.6</td>
<td>1.4±0.1</td>
</tr>
<tr>
<td>R. nigromaculata*</td>
<td>20</td>
<td>12.3±2.2</td>
<td>28.1±4.8</td>
<td>40.5±5.9</td>
<td>1.4±0.1</td>
</tr>
<tr>
<td>R. limnocharis</td>
<td>20</td>
<td>11.6±1.1</td>
<td>45.8±3.0</td>
<td>57.4±3.4</td>
<td>1.4±0.1</td>
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<tr>
<td>R. limnocharis*</td>
<td>20</td>
<td>11.6±0.9</td>
<td>39.4±4.8</td>
<td>51.0±5.0</td>
<td>1.5±0.1</td>
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<td>R. rugosa</td>
<td>20</td>
<td>14.4±1.7</td>
<td>40.7±6.2</td>
<td>55.1±5.6</td>
<td>1.8±0.1</td>
</tr>
<tr>
<td>R. catesbeiana</td>
<td>20</td>
<td>11.7±1.6</td>
<td>29.9±4.1</td>
<td>41.6±4.3</td>
<td>1.7±0.2</td>
</tr>
<tr>
<td>R. latouchii</td>
<td>20</td>
<td>15.0±1.7</td>
<td>39.7±5.8</td>
<td>54.7±5.8</td>
<td>1.7±0.1</td>
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<td>R. rugulosa</td>
<td>20</td>
<td>13.5±1.9</td>
<td>34.9±6.7</td>
<td>48.3±6.8</td>
<td>1.1±0.1</td>
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<tr>
<td>R. namiyei</td>
<td>20</td>
<td>15.5±1.2</td>
<td>39.8±4.4</td>
<td>55.3±4.9</td>
<td>1.2±0.1</td>
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<tr>
<td>R. amamiensis</td>
<td>20</td>
<td>32.3±3.5</td>
<td>38.8±5.6</td>
<td>71.1±6.2</td>
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<tr>
<td>R. narina</td>
<td>20</td>
<td>26.4±2.3</td>
<td>40.4±4.5</td>
<td>66.8±5.6</td>
<td>0.9±0.1</td>
</tr>
<tr>
<td>R. supranarina</td>
<td>20</td>
<td>24.1±0.7</td>
<td>41.3±3.5</td>
<td>65.3±3.6</td>
<td>1.1±0.1</td>
</tr>
<tr>
<td>R. ishikawae</td>
<td>20</td>
<td>83.5±6.8</td>
<td>55.8±17.8</td>
<td>139.4±17.4</td>
<td>0.5±0.1</td>
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<tr>
<td>Platymantis pelewensis</td>
<td>20</td>
<td>38.0±2.9</td>
<td>57.4±12.9</td>
<td>95.4±11.2</td>
<td>0.6±0.1</td>
</tr>
</tbody>
</table>

Platymantis pelewensis had a slender sperm head (Fig. 4c, f), and a long, thick (ca. 0.45 μm in diameter) tail fiber.

Microhyla ornata had a cone-shaped sperm head and a long, thin tail fiber (Fig. 4d, e, h). Under LM, a distinct acrosome and a middle piece were recognized (Fig. 4e). Populations from Iriomote and Taiwan did not differ in sperm shape or size (Table 1).

DISCUSSION

Undoubtedly, spermatozoa of Bombina orientalis swim with a ventral undulating membrane as several protozoans do. This mode of propulsion is unique among animal spermatozoa. The axial rod may be concealed within the head, as shown by Kwon and Lee (1992, 1995), or slightly exposed. Extensive deformation of spermatozoa seem to be caused by the fixative.

Sperm shape of B. orientalis is very similar to that of B. bombina (Broman, 1900; Retzius, 1906), but the size is smaller, since the head of B. bombina was reported to be 40 μm in length and 2 μm in width (Broman, 1900).

Furieri (1975) described detailed structures of the spermatozoa of B. variegata, and pointed out that the axial rod, undulating membrane, and flagellum are attached longitudinally along the side of the head and that the acrosome is cylindrical with a truncated apex. The elongated nucleus is not compact and the head contains a relatively large mass of cytoplasm. The axial rod is located just beneath the groove along one side of the head, from which an undulating membrane extends. The centriole generating a flagellum is situated at the anterior portion of the head. These characteristics are very similar to the sperm structure of B. orientalis and B. bombina, but there are several differences. First, the structures of movement (axial rod, undulating membrane, and flagellum) constitute a "tail" in B. orientalis, and thus are readily observable in fixed specimens. Mitochondria, usually forming the middle piece in many animal spermatozoa, are dispersed in the cytoplasm in B. variegata, but form a cluster around the axial rod in B. orientalis and B. bombina (Champy, 1913). Second, the sperm head of B. orientalis is curved as in B. bombina (Broman, 1900), and tapered toward the posterior end, whereas that of B. variegata is straight with a round posterior end. Folliot (1979) noted that spermatozoa with two sets of movement structures are not uncommon in B. variegata, but I could not observe such spermatozoa in B. orientalis.

Discoglossus pictus has typical spermatozoa with an elongated head and a long tail with an undulating membrane, the total length being...
2—3 mm (Ballowitz, 1903; Favard, 1955; Sandoz, 1974). Spermatozoa of Alytes obstetricians have an elongated head and a long tail with an undulating membrane like those of D. pictus (Retzius, 1906). Obviously, the basic sperm configurations of the genus Bombina differ from those of Discoglossus and Alytes. Although Furieri (1975) agreed with Lanza et al. (1955) in separating the Bombinidae including Bombina and Alytes from the Discoglossidae, available spermatological data support the separation of Bombina from Discoglossus and Alytes. This treatment agrees with Ford and Cannatella (1996a), as well as to that of the genus Bombina and Barbourula from the Discoglossidae (Discoglossus and Alytes).

Sperm structures of the three species of the genus Bufo, B. japonicus, B. gargarizans and B. melanostictus, are essentially identical with those of previous studies, in that all have an elongated head and a tail with an undulating membrane (Burgos and Fawcett, 1956; Champy and Coujard, 1939; Kwon et al., 1993; Mo, 1985; Retzius, 1906; Sharma and Dhindsa, 1955; Swan et al., 1980). Differing from the spermatozoa of salamanders and Bombina orientalis, the flagellum does not wave strongly. This means that the length of the axial rod and that of the flagellum, from the base to the point where it fuses with the axial rod, are nearly the same. Swan et al. (1980) showed that the mean sperm velocity of B. marinus was 22 μm/s, much slower than ram or oyster sperm cells which have no undulating membrane. Extent of the flagellum waving may be related with sperm velocity, but no data are available for sperm velocity of salamanders or frogs with membrane-bearing spermatozoa. Sperm size has not been given for those of previous studies, and examined toad species, and sperm morphology of other genera of the family Bufonidae has not been reported.

The curved sperm head in the two species of the genus Hyla is very similar to that of the rhacophorid genus Chirixalus (Kuramoto, 1996a), as well as to that of the genus Bombina. Delahoussaye (1966) described sperm morphology of 10 hydrid frogs (genera Acris, Hyla and Pseudacris) from Louisiana. Acrosome length and middle piece length of the two Hyla species here examined are comparable to those of six Hyla species he examined, but the sperm head (nucleus) is much longer in the former than in the latter (8.7—11.1 μm). He recognized three major shapes of head portion, slightly curved, almost “S” shaped, and sickle shaped. A more or less curved sperm head may be a common feature of the subfamily Hylinae. Although crescent- and spiral-shaped sperm heads (Kuramoto, 1996a) may be produced by some cytological mechanisms during spermiogenesis, it is completely unknown which factor constrains head shape.

In H. arborea, Retzius (1906) described two fibers in the sperm tail. Typically, the tail appeared as a single fiber, but two fibers were observed to a varying extent. Lee and Kwon (1992) showed that the sperm tail of H. japonica contains an axoneme and an axial rod (but without an undulating membrane). Thus, it is highly probable that the one of the two fibers observed in spermatozoa of H. arborea, H. japonica and H. chinensis is an axonemal fiber and the other an axial rod. Fouquette and Delahoussaye (1977) found many hylid species with bi-flagellated spermatozoa, and presented a revised taxonomic system based on sperm morphology. If a single tail fiber involves two flagellar components as indicated in the present study, it seems not essential to separate “two-flagellated” from “single-flagellated” species, until further studies reveal that each of the two “flagellas” contains an axoneme. Several rhacophorid genera have been proven to contain actually two flagellar apparatuses (axonemes) in a single tail fiber (Mainoya, 1981; Mizuhira et al., 1986). Although most hylid spermatozoa lack an undulating membrane, Rostogi et al. (1988) reported spermatozoa with an undulating membrane in Pachymedusa dacnicolor. It is totally unknown whether the other phyllomedusine tree frogs have this type of sperm in common.

Relatively few studies are available for comparison of spermatozoa in the genus Rana. Forier and Spink (1971) reported the size of the sperm head of R. clamitans to be 17—18 μm by 1.2—1.6 μm, and of R. pipiens 12—13 μm by 1.7—2.0 μm. These values are within the range of common ranid frogs in the present study. Rana sylvatica has a relatively long sperm head ranging from 21.7 to 24.7 μm (Witschi, 1953). Broman (1997) reported the sperm head length of R. temporaria to be about 45 μm and tail length 60 μm. He described sperm shape of R. esculenta, R. arvalis and R. catesbeiana. Although no numerical data were given, the head shapes of these three species are very similar to those of the common ranid species here presented. Champy and Coujard (1939) illustrated several ranid spermatozoa without numerical data, in which R. temporaria has an elongated head whereas other ranids have a cylindrical
head. Sperm shapes of *R. japonica* and *R. nigromaculata* previously reported by Morita (1929), Kwon and Lee (1995), and Yoshizaki (1987) are identical to those of the present study. No clear distinctions are found among various species groups of the genus *Rana*, except three species of the *Rana narina* complex and *R. ishikawai*. *Rana limnocharis* and *R. rugulosa* are currently allocated to the genera *Limnonectes* and *Hoplobatrachus*, respectively, in the subfamily Dicroglossinae (Dubois, 1992), but their spermatozoa are very similar to those of the other species of the genus *Rana*. The sperm shape of *R. tigerina* (Sharma and Sekhri, 1955) is essentially identical with that of *R. rugulosa*, a close relative. The Japanese and Chinese populations of *R. nigromaculata* and *R. limnocharis* did not differ in sperm shape, but showed significant differences in size. As revealed in *Hynobius nebulosus* (Kuramoto, 1997), geographic variations in sperm size are expected to occur in other frog species here examined, which remain for future studies.

The *Rana narina* complex has specialized spermatozoa with a slender sperm head. *Rana amamienensis* seems to be differentiated from the other two species in head size, and this is supported by karyological evidence (Kuramoto, 1996b). So far as the present spermatological data are concerned, *R. ishikawai* has unique spermatozoa, very similar to the thread-like spermatozoa of the rhacophorid genus *Polypedates* and *Buergeria buergeri* (Kuramoto, 1996a). Obviously, the *Rana narina* complex and *R. ishikawai* are distinct from the other members of the genus *Rana* and from each other. Spermatozoa of *P. pelewensis* have a long head and thick tail, which are shared with spermatozoa of *R. ishikawai*. All of these ranids with long spermatozoa lay large, yolky eggs, and their long, slender sperm may be favorable for fertilizing yolky eggs. Van der Horst et al. (1995) made a distinction between aquatic and terrestrial fertilizers in South African frogs: the sperm head of the former is short (16.0 ± 2.2 μm in average) as compared with that of the latter (29.6 ± 6.0 μm). This trend seems to be inapplicable to the present material, because only *P. pelewensis* is terrestrial in reproductive mode.

Retzius (1906) reported sperm morphology of an Asiatic microhylid *Kaloula pulchra*. Its head is long and cone-shaped, and a short middle piece is very distinct. These characteristics are very similar to those of *M. ornata* here examined, suggesting common features in the subfamily Microhylinae. No size data were given for *K. pulchra*. The Microhylidae is an extremely diverse family comprising 10 subfamilies. It may be advantageous to compare spermatozoa of different subfamilies for elucidating relationships in this diverse family.

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**Literature Cited**


KURAMOTO—SPERMATOZOA OF FROGS

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要旨 日本と周辺地域のカエル数種の精子

倉本 満

5科25種のカエルについて精子の形と大きさを記載した。スズガエルの精子は腹側に波動模をもつ点で特異であり、その形態はBombina bombinaに似ているが、B. variegataとは異なっていた。ヒキガエル属3種の精子は頭部が長く、尾に波動模をもつ。アマガエル属2種の精子は三日月形の頭部をもつ、尾には軸条と軸桿を含む。以上の種では尾の基部に球状のミトコンドリアが観察され、通常中片を形成する。アカガエル属の多くの種は精子頭部が円筒状で単純な細い尾をもつが、ハナサキガエル群の種とインカワガエルは精子頭部が非常に長く、頭部に球状のミトコンドリアがある。Platymantis pelewensisも細長い精子をもっていた。ヒメアマガエルの精子頭部は円錐状で、中片は明瞭である。これらの知見を以前の研究と比較し、分類との関連について論じた。

（811-4146 福岡県宗像市赤間729 福岡教育大学生物学教室；現住所：811-3403 福岡県宗像市ひかりヶ丘3-6-15）