A Scanning Electron Microscope Study of Humboldt Penguin (*Spheniscus humboldti*) Spermatozoa

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走査電子顕微鏡によるフンボルトペンギン精子の観察

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ABSTRACT. The morphology and size of Humboldt penguin spermatozoa have not been reported. This study the spermatozoa of Humboldt penguin were examined by scanning electron microscope and their dimensions were determined. The spermatozoa of Humboldt penguin were long, narrow, with gently curved head. As the result of measurement, the total length was $73.8 \pm 3.6 \mu\text{m}$, head was $11.4 \pm 0.9 \mu\text{m}$, acrosome was $1.1 \pm 0.2 \mu\text{m}$, nucleus was $10.3 \pm 0.8 \mu\text{m}$, midpiece was $2.8 \pm 0.2 \mu\text{m}$ and tail was $62.6 \pm 3.8 \mu\text{m}$. respectively. The Humboldt penguin spermatozoa seemed to belong to the type of non-passerine birds. The spermatozoa size was slightly smaller than that of other species.

Key Words : Humboldt penguin, Scanning Electron Microscope, Spermatozoa

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The Humboldt penguin is a wild animal being on the verge of extinction and is the only species in the penguin order placed in Appendix of the CITES (Convention on International Trade in Endangered Species of Fauna and Flora) [1]. For conservation of such rare wild animals, conservation of the habitat and successive propagation outside the habitat are necessary. The propagation outside the habitat, i.e., in zoos or aquarium, is now in practice by natural mating. But in those circumstances, the ped-

igrees from a small number of individuals are apt to be preserved and problems of assortative mating and genetic diseases might occur in the future. To prevent such problems and to maintain the species permanently, artificial insemination [2,3] and preservation of spermatozoas as genetic resources [4-6] are needed. For that purpose, it is indispensable to understand the normal morphology of spermatozoas of this species, but no information is available so far. In the present study, we observed the spermat-

ozoas of Humboldt penguin under a scanning electron microscope (SEM).

Semen was collected by abdominal massage during the breeding season from 5 adult male Humboldt penguin (*Spheniscus humboldti*) aged 8 years, which were in an active breeding at Niigata City Aquarium, Japan. Samples were collected directly from the cloaca using glass tubes.

The semen collected was fixed by a mixture of 1.5% paraformaldehyde and 0.5% glutaraldehyde solution in PBS (pH 7.2) at 5°C. The solution containing the fixed spermatozoa were centrifuged at 700 rpm for 10 min at room temperature.

The precipitated spermatozoa were washed twice with PBS and finally resuspended in PBS. A drop of the spermatozoa solutions were mounted to poly-L

-lysine coated glass slides and subsequently dehydrated through a graded ethanol series (50%, 70%, 80%, 90%, 95%, 95% and 99% $\times$ 2-10 min/step) and lyophilized by t-butylalcohol freeze-drying method [7]. After platinum-palladium coating and samples were examined with SEM (HITACHI S 800 operated at 15 K ∇) and the each sizes of the respective segments were determined.

SEM was also employed for the morphometric analysis of the Humboldt penguin spermatozoa. The sizes of the head, acrosome, nucleus, midpiece, tail other than midpiece, tail and total length were determined on measurement of 10 spermatozoa chosen at random from each of the Humboldt penguin (a total of 50 spermatozoa). The measurement values were given an arithmetic averages and standard

Fig. 1 Scanning electron micrograph of the Humboldt penguin spermatozoa showing the head (H) and tail (T). Bar=10 μ m

Fig. 2 Scanning electron micrograph of the head and midpiece.

The midpiece showing the outline of mitochondria. The head is gently curved. acrosome (A), nucleus (N) and midpiece (M). Bar=2 μ m

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Fig. 3 Scanning electron micrograph of the acrosome and nucleus.

The acrosome forms a short cap-like structure. Note the distinct junction between the acrosome with the nucleus (arrow). Bar = $0.5\ \mu\text{m}$

Fig. 4 Scanning electron micrograph of nucleus (N) and midpiece (M).

A damaged spermatozoon with a denuded midpiece demonstrates helical arrangement of mitochondria (m). Bar = $0.5\ \mu\text{m}$

deviations (SD).

Humboldt penguin spermatozoa were long, narrow, and roughly divided into the head and the tail. The head was gently curved and acrosome and nucleus were distinguishable while the midpiece was observed in the part of the tail (Figs. 1 and 2). The acrosome had a short cap-like in shape (Fig. 3). The acrosome, nucleus and midpiece were clearly demarcated in most spermatozoa (Figs. 2 and 3). The midpiece usually appeared smooth, but in some spermatozoa the outlines of the mitochondria could be vaguely seen in beneath the plasmalemma (Fig. 2). The mitochondria were arranged in a helical pattern around the axoneme, which were most by observed in damaged spermatozoa lacking plasmalemma (Fig. 4).

The morphology of spermatozoa of birds is usually categorized 2 types: One is the non-passerine bird type, include species such as the chicken, turkey, crane, hawk, parrot and ostrich is, which are known as sauropsid form and similar to the spermatozoa of reptiles, and the other is the type seen in passerine bird, the order of perching or song-birds, which is similar to that of amphibians [8,9].

This study demonstrates that Humboldt penguin spermatozoa with a head and a long tail belongs to the type of non-passerine bird. The general morphology of spermatozoa was similar to that described for domesticated birds [10], white-head crane [11], budgerigar [12], ostrich [13] and tinamou [15].

The results of the morphometric measurement are

Table 1. Measurement of spermatozoa from 5 Humboldt penguins

No.	Head(μm)			Tail(μm)			Total Length(μm)
	Acrosome	Nucleus	Total	Tail other than Midpiece	Midpiece	Total	
1	1.1 $\pm$ 0.1	10.5 $\pm$ 0.6	11.7 $\pm$ 0.7	59.1 $\pm$ 3.0	3.0 $\pm$ 0.1	63.3 $\pm$ 3.5	74.9 $\pm$ 3.7
2	1.0 $\pm$ 0.2	10.8 $\pm$ 0.4	12.0 $\pm$ 0.8	61.4 $\pm$ 4.7	2.9 $\pm$ 0.3	64.3 $\pm$ 4.5	76.3 $\pm$ 3.7
3	1.2 $\pm$ 0.2	10.1 $\pm$ 1.1	10.8 $\pm$ 0.8	60.3 $\pm$ 3.7	2.7 $\pm$ 0.2	61.8 $\pm$ 2.8	72.5 $\pm$ 3.2
4	1.2 $\pm$ 0.1	10.1 $\pm$ 0.8	11.4 $\pm$ 0.8	59.3 $\pm$ 3.8	2.9 $\pm$ 0.3	61.6 $\pm$ 3.5	72.7 $\pm$ 3.9
5	0.9 $\pm$ 0.1	10.2 $\pm$ 1.0	11.1 $\pm$ 1.0	58.7 $\pm$ 3.6	2.8 $\pm$ 0.3	62.0 $\pm$ 3.7	72.8 $\pm$ 3.6
Average $\pm$ SD	1.1 $\pm$ 0.2	10.3 $\pm$ 0.8	11.4 $\pm$ 0.9	59.7 $\pm$ 3.8	2.8 $\pm$ 0.2	62.6 $\pm$ 3.8	73.8 $\pm$ 3.8

Table 2. Comparative dimensions of some non-passerine birds spermatozoa

Species	Acrosome (μm)	Nucleus (μm)	Head (μm)	Tail (μm)	Midpiece (μm)	Total length (μm)
Chicken [10]	≥ 2.0	10~14	14	≥ 70	—	> 90
Turkey [10]	1.6~1.8	7~9	9.7	60~65	—	—
[15]	1.0~2.6	7.2~11	11	—	4.0~6.0	76.7
Guinea fow [10]	1.6~1.8	10~14	13.7	60~65	—	76.2
[16]	1.8	12.8	14.6	—	3.3~4.5	—
Budgerigar [12]	1.42 $\pm$ 0.16	8.0 $\pm$ 1.97	9.4	54.3 $\pm$ 5.97	—	63.7
Ostrich [13]	1.91 $\pm$ 0.02	10.95 $\pm$ 0.08	12.86 $\pm$ 0.08	56.73 $\pm$ 0.26	3.16 $\pm$ 0.02	69.59 $\pm$ 0.31
Tinamou [14]	—	—	—	—	3	—
Duck [17,18]	2.2	10~11	12.7	—	3~4	—
Hawaiian gander [9]	—	—	—	—	—	100
Mallard duck [9]	—	—	—	—	—	100
Pigeon [9]	—	—	—	—	—	165
Humboldt penguin	1.1 $\pm$ 0.2	10.3 $\pm$ 0.8	11.4 $\pm$ 0.9	62.6 $\pm$ 3.8	2.8 $\pm$ 0.2	73.8 $\pm$ 3.6

shown in Table 1. The length of each part was 11.4 $\pm$ 0.9 μm in the head, 1.1 $\pm$ 0.2 μm in the acrosome, 10.3 $\pm$ 0.8 μm in the nucleus, 2.8 $\pm$ 0.2 μm in the midpiece, 62.6 $\pm$ 3.8 μm in the tail and 73.8 $\pm$ 3.8 μm in the total length, respectively. When the measurement of values various parts were compared with those of other non-passerine birds. It was found that the acrosome of Humboldt penguin spermatozoa was the smallest among the known species (Table 2).

The length of the nucleus in Humboldt penguin spermatozoa was longer than that observed in the turkey [10,15] and budgerigar [12], but was similar in length to that of other birds [10, 13, 16-18].

The tail of Humboldt penguin spermatozoa was

longer than that of the budgerigar [12], ostrich [13] and is similar in length to that of the turkey [10] and guinea fowl [10]. The midpiece was the smallest among the known species.

The total length of Humboldt penguin spermatozoa was longer than that of the budgerigar [12] and ostrich [13], similar to that of the turkey [15] and shorter than that of the Hawaiian gander [9], mallard duck [9], pigeon [9] and chicken [10].

As the results the present study showed that the spermatozoa of Humboldt penguin display the morphological characteristics typical of non-passerine birds the acrosome and midpiece were short, the total length was slightly smaller than that of other birds examined.

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This study defined the normal morphology of Humboldt penguin spermatozoa and the finding will give information for the future studies on semen collection, artificial insemination and preservation of spermatozoa as genetic resource.

要 約

フンボルトペンギンの精子形態と大きさに関する報告はない。今回、フンボルトペンギンの精子形態を走査電子顕微鏡(SEM)で観察し、また各部位の長さを測定した。精子は、紐状で頭部は細長くやや湾曲していた。全長ならびに各部位の長さを測定した結果、全長は $73.8 \pm 3.6 \mu\text{m}$ であった。頭部は $11.4 \pm 0.9 \mu\text{m}$ で、先体は $1.1 \pm 0.2 \mu\text{m}$ 、核は $10.3 \pm 0.8 \mu\text{m}$ であった。尾部は $62.6 \pm 3.8 \mu\text{m}$ で、中片部は $2.8 \pm 0.2 \mu\text{m}$ であった。フンボルトペンギンの精子形態は典型的な non-passerine birds のグループに属し、また全長は他種よりもやや小型であった。

キーワード: フンボルトペンギン, 走査電子顕微鏡, 精子

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