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Ultrastructure and morphology of spermatozoa in Chinese sturgeon (*Acipenser sinensis* Gray 1835) using scanning and transmission electron microscopy

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Abstract

The Chinese sturgeon (*Acipenser sinensis* Gray 1835) is an endangered anadromous sturgeon inhabiting the Yangtze River in China. In this study, the ultrastructure and morphology of spermatozoa was studied using transmission and scanning electron microscopy with a cryo-holder. The spermatozoon consisted of an elongated head with a distinct acrosome and nucleus region, a midpiece and a flagellum. The mean length of the head and midpiece, the flagellum and total length of spermatozoon were 4.48, 33.3 and 37.8 μm, respectively. The nucleus was an elongated trapezoid shape with anterior (acrosome) end narrower than the posterior. Granular material and an actin filament were observed within the anterior acrosome. Three to five endonuclear canals were present. The midpiece was eudipleural along its longitudinal axis. Compared to other sturgeon species, the data from the present study suggest a more recent evolutionary linkage between Chinese sturgeon and white sturgeon (*Acipenser transmontanus* Richardson 1836).

Keywords: *Acipenser sinensis*; SEM; TEM; Spermatozoon; Acrosome; Fish biology

1. Introduction

Study of the ultrastructure and morphology of fish spermatozoa provides information for understanding their possible taxonomic and evolutionary relationships at family [1,2], subfamily and species [3] levels, as well as for optimizing artificial reproduction, prevention of polyspermy problems and development of cryopreservation techniques [4–6]. Several different types of spermatozoa have been distinguished in fish. Nevertheless, little research has been carried out to describe sturgeon diversity as revealed by spermatozoa structure.

The order *Acipenseriformes* consists of two families comprising 27 species [7], but ultrastructure and morphology of spermatozoa have been described only for Russian sturgeon (*Acipenser gueldenstaedti* Brandt & Ratzeburg 1833) [4], stellate sturgeon (*A. stellatus* Pallas

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1771) [8], white sturgeon (*A. transmontanus* Richardson 1836) [9], Atlantic sturgeon (*A. oxyrhynchus* Mitchill 1815) [10], shortnose sturgeon (*A. brevirostrum* Lesueur 1818) [11], lake sturgeon (*A. fulvescens* Rafinesque 1817) [12], pallid sturgeon (*Scaphirhynchus albus* Forbes & Richardson 1905) [13], Siberian sturgeon (*A. baerii* Brandt 1869) [14] and paddlefish (*Polyodon spathula* Walbaum 1792) [15].

Chinese sturgeon (*Acipenser sinensis* Gray 1835) is an endangered anadromous species [16] belonging to the family *Acipenseridae* [17] and found only in China at Yangtse River [18]. As with other sturgeon species, its stock has declined dramatically due to overfishing, loss of natural habitat for reproduction and interference by other human activities [16,18–20]. Since the 1990s, several attempts have been made to conserve the species by artificial culture and restocking with fingerlings [21,22].

The main objective of this study was to investigate the morphology and ultrastructure of Chinese sturgeon spermatozoa by scanning and transmission electron microscopy.

2. Materials and methods

Electron microscopy was carried out in China (Laboratory of Electron Microscopy, Institute of Hydrobiology, Chinese Academy of Sciences) and the Czech Republic (Laboratory of Electron Microscopy, Institute of Parasitology, Academy of Sciences of the Czech Republic in Ceske Budejovice).

2.1. Sperm sampling

Three mature male Chinese sturgeons were captured during their spawning migration (October–December) in the Yangtze River. To induce spermiation, the males were injected intramuscularly with 3.5 μg/kg luteinizing hormone releasing hormone analogue (LHRHa). Sperm was collected by catheter 6–7 h after hormone injection from the urogenital papilla, stored in sealed plastic bags with oxygen, placed on ice, and transported to the laboratory for controlling sperm motility and collection of samples. Non-active sperm samples were used to study the morphology and ultrastructure of spermatozoa. The percentage of motile spermatozoa and total period of motility were in range of 20–100% and 4–8 min.

2.2. Electron microscopy

For SEM study, sperm samples were fixed for 3 h in freshly prepared 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) at 0–4 °C, rinsed in the same buffer and immersed for 2 h in 1.0% osmium tetroxide in 0.1 M phosphate buffer (pH 7.4) at 20 °C. After rinsing in the buffer, sperm were dehydrated in a graded ethanol series and critical-point dried. Samples were examined and photographed using a scanning electron microscope (SEM) (Hitachi-S570, Tokyo, Japan and JSM 6300, JEOL Ltd., Akishima, Tokyo, Japan) for morphological parameters and a JSM 7401 S (JEOL Ltd., Akishima, Tokyo, Japan) in cryo-regime for fine details.

For TEM study, sperm was centrifuged at 200 × *g* for 30 min and the supernatant discarded. It was prepared with a two-step fixation following the method described above. After several rinses the sample was dehydrated in a graded ethanol series and embedded in Epon812. Hardened blocks were sectioned at 50–70 nm and sections mounted on copper grids. These were post-stained with 2% uranyl acetate in 50% ethanol, followed by staining with Reynolds' lead citrate. Finally, the grids were examined and photographed using transmission electron microscopy (TEM) (JEM-1230, Tokyo, Japan and JEOL 1010, JEOL Ltd., Tokyo, Japan).

2.3. Measurements and data analysis

Measurements of spermatozoa parameters were taken using CorelDRAW 12™ software with the reported scale from our electron microscopy. The measurement parameters, and methods used, are in Psenicka et al. [14], and include: acrosome length and width; anterior and posterior head width; midpiece length and width; head length (nucleus + acrosome length) and total length (total head length + midpiece length + flagellum). Other parameters, including diameter of the endonuclear canal and microtubules and length of flagellum, were also measured. Average values of the measured parameters and standard deviation (S.D.) were calculated from 12 measurements of 3 fish.

3. Results

3.1. General morphology

The Chinese sturgeon spermatozoon was an elongate, slightly conical cylindrical shape with radial symmetry (Fig. 1a and b). It consisted of a head, midpiece and flagellum. The head was elongated with an umbrella-like acrosome, a common structural feature of sturgeon spermatozoa, and an elongate nuclear region with the posterior end wider than the anterior. The Chinese sturgeon spermatozoon possessed a small midpiece, filler-like in shape, and a single flagellum

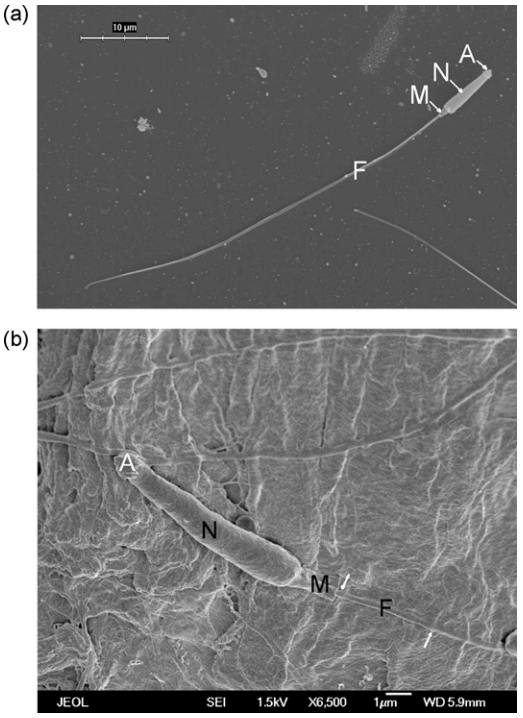

Fig. 1. (a) SEM [JSM 6300] showing sperm cell morphology (scale bar 10 μm); (b) SEM [JSM 7401 S] showing sperm cell morphology, arrows point to the origin of the fins along the flagellum (scale bar 1 μm). Acrosome (A), nucleus (N), midpiece (M), flagellum (F).

surrounded by a plasma membrane. Morphological parameter measurements are given in Table 1.

3.2. Head

The head contained an acrosome and a nucleus (Fig. 1a and b and Fig. 2a–f). In longitudinal section, the acrosome covered the anterior end of the spermatozoon. There was a granular material in the anterior end of the acrosome and probably an actin filament reaching out from its center (Fig. 2c). The acrosomal vesicle bounded by the acrosomal membrane was located in the outermost portion. Ten posterolateral projections (PLP) arise from the posterior edge of the acrosome (Fig. 2a, b and f). These PLPs were 0.37 μm in length (Table 1), extending along the anterior sides of the nucleus. Between the nucleus and the acrosomal vesicle was the subacrosome region (Fig. 2b and e). Fig. 2d shows the surface of the anterior nucleus, under the acrosome, after removing of the acrosome.

The nucleus, extending from the rear of the granular material to the midpiece, consisted of electron-dense homogeneous chromatin surrounded by a nuclear membrane. It was an elongate trapezoid in longitudinal section and circular in cross section. Three intertwining endonuclear canals, which measured 0.082 μm in diameter, traversed the nucleus from the acrosomal end to the basal nuclear fossa region (Fig. 2b and e–g). The implantation fossa, an organelle connecting the axial portion of nucleus with the midpiece, was located at the posterior end of the nucleus (Fig. 3a and e).

3.3. Midpiece

The midpiece was small, filler-like in longitudinal section and cuneiform in transverse section (Fig. 3a–d), and contained mitochondria and the centriolar apparatus. Around the flagellum there was a cytoplasmic channel, extracellular space between the midpiece and the flagellum, formed by an invagination at the plasmalemma (Fig. 3a). Three to eight ovoid mitochondria in one slice that deliver the ATP required for flagellar beatings were found in the midpiece. Ridges of the mitochondria were well developed (Fig. 3c). The centriolar apparatus consisted of proximal and distal centrioles. The ovoid proximal centriole (196.46 ± 22.22 nm × 198.76 ± 20.70 nm) was located immediately posterior to the implantation fossa. The proximal centriole was connected to the implantation fossa by a structure resembling a fibrous body. The distal centriole was observed near the flagellar base. The axoneme was located immediately posterior to the distal centriole. Both

Table 1
Measurements of Chinese sturgeon sperm components from SEM and TEM

Characteristic	Dimension (μm)	Characteristic	Dimension (μm)
Acrosome length	0.54 ± 0.15	Endonuclear canal diameter	0.08 ± 0.03
Acrosome width	0.68 ± 0.06	Posterolateral projections	0.37 ± 0.04
Anterior head width	0.59 ± 0.05	Flagellum diameter	0.32 ± 0.01
Posterior head width	1.84 ± 0.45	Lateral extensions of flagellum	0.34 ± 0.11
Total head length	3.27 ± 0.20	Microtubule diameter	0.01 ± 0.00
Midpiece length	2.17 ± 0.36	Flagellum length	33.26 ± 2.74
Midpiece width	1.57 ± 0.27	Total length	38.7 ± 0.37

Dimensions are the mean ± S.D.

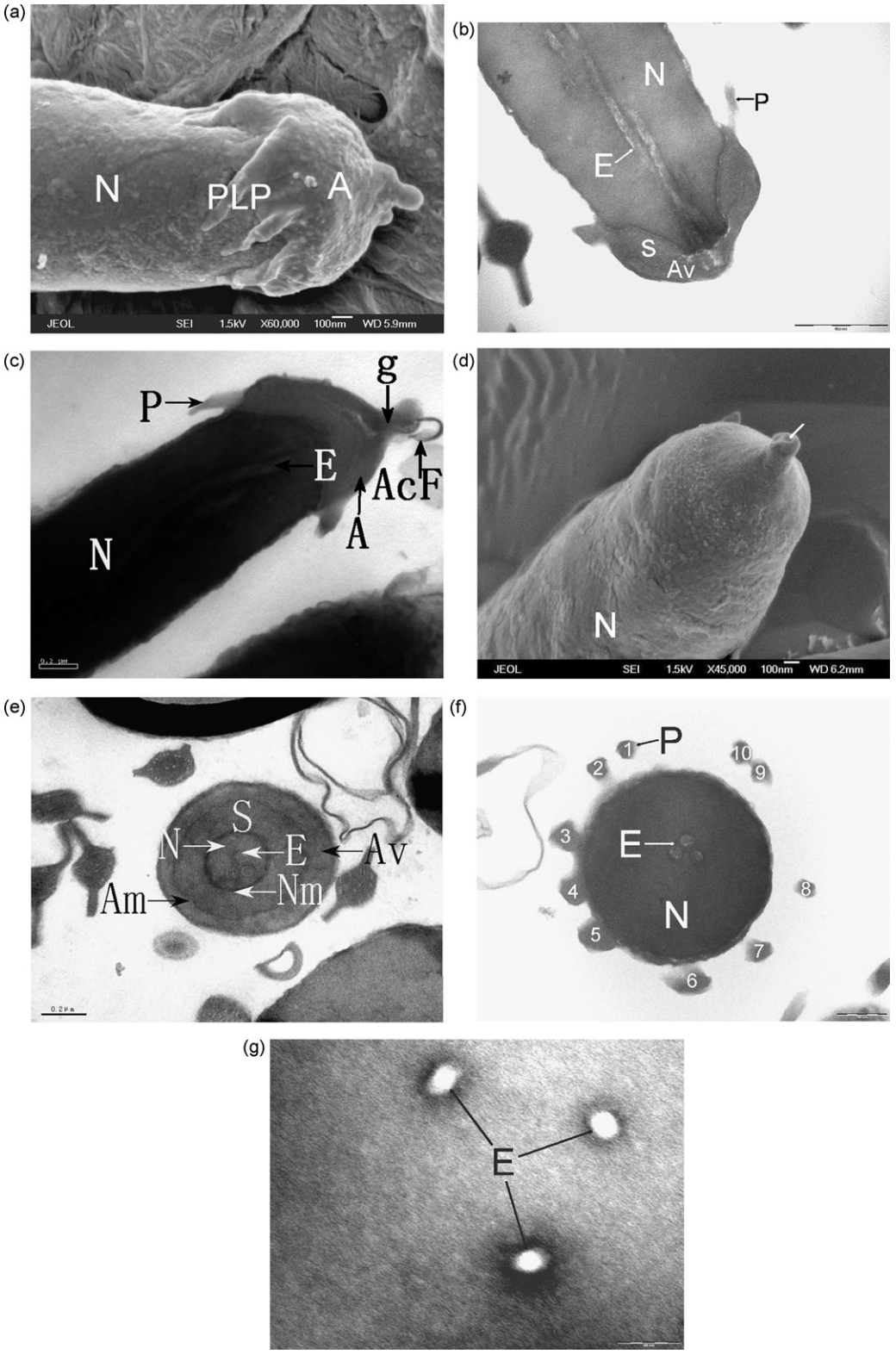

Fig. 2. (a) SEM [JSM 7401 S] of the acrosome showing posterolateral projections (PLP) (scale bar 100 nm); (b) TEM [JEOL 1010] sagittal section between the nucleus and acrosome, scale bar 500 nm; (c) TEM [JEM-1230] sagittal section between the nucleus and acrosome showing the

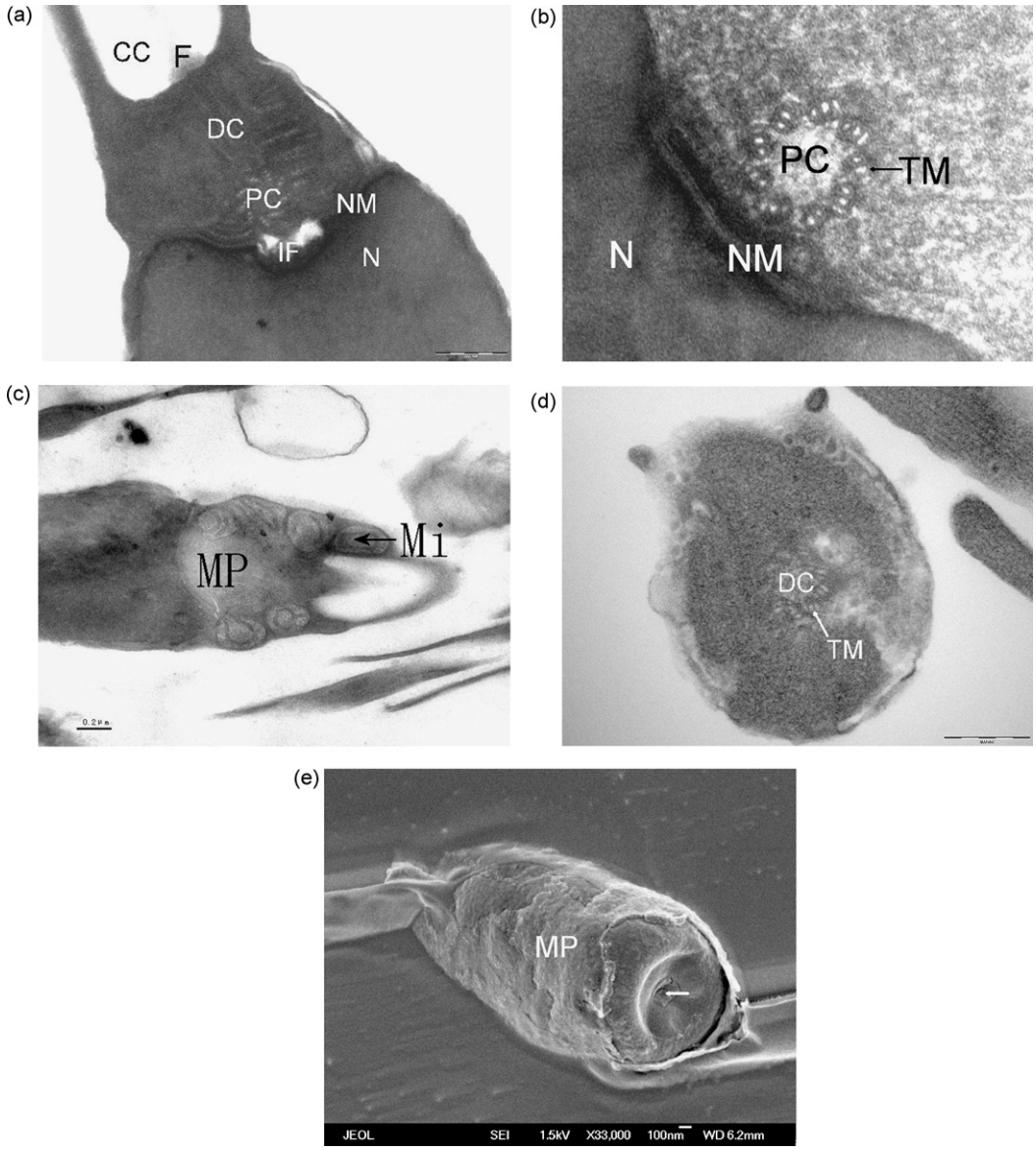

Fig. 3. (a) TEM [JEOL 1010] sagittal section of the midpiece showing the distal (DC) and the proximal centriole (PC), the implantation fossa (IF), the nucleus (N) surrounded by the nuclear membrane (NM) and the flagellum (F) separated by the cytoplasmic channel (CC) (scale bar 500 nm); (b) TEM [JEM-1230] showing the proximal centriole (PC) with triplets of microtubules (TM) (scale bar 100 nm); (c) TEM showing the midpiece (MP) with numerous mitochondria (Mi) irregularly dispersed in the cytoplasm (scale bar 200 nm); (d) TEM [JEOL 1010] transverse section at midpiece showing distal centriole (DC) with nine triplets of microtubules (TM) (scale bar 200 nm); (e) SEM [JSM 7401 S] of the midpiece after abruption of nucleus using. showing the proximal centriole (arrow) under the implantation fossa region (scale bar 100 nm).

acrosome vesicle (Av) and the subacrosome (S) with the posterolateral projections (PLP) and the endonuclear canals (ENC) traversed in the nucleus (N), actin filament (AcF), granular material (g) (scale bar 200 nm); (d) SEM [JSM 7401 S] of the anterior surface of nucleus (N) after abruption of the acrosome, scale bar 100 nm. The arrow shows an opening of an endonuclear canal; (e) TEM [JEM-1230] transverse section between the nucleus and the acrosome showing acrosome membrane (Am), acrosome vesicle (Av), subacrosome (S), nuclear membrane (Nm), nucleus (N), and endonuclear canals (E) (scale bar 200 nm); (f) TEM [JEOL 1010] transverse section between the nucleus and the acrosome showing posterolateral projections (PLP) 1–10 (scale bar 200 nm); (g) TEM [JEOL 1010] transverse section of the nucleus showing the endonuclear canals traversed in the nucleus (scale bar 100 nm).

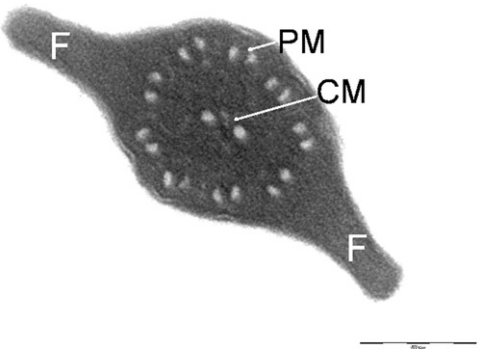

Fig. 4. TEM [JEOL 1010] transverse section of the flagellum showing the peripheral doublets of microtubules (PM) and central doublets of microtubules (CM). The fins (F) make the flagellum more effective (scale bar 50 nm).

orthogonally centrioles were formed of nine peripheral triplets of microtubules arranged in a cylindrical pattem (Fig. 3a–d). The distal centriole was connected with the anterior of the proximal centriole, and the axoneme was immediately posterior to the distal centrioles. The diameter of the cytoplasmic channel, an invagination of the plasma membrane in the midpiece separating the cytoplasm of the midpiece almost completely from the flagellum, measured 479.18 ± 38.16 nm and was located between the cytoplasmic sheath and the flagellum.

3.4. Flagellum

The flagellum contained a cytoplasmic membrane and an axoneme comprising 9 + 2 microtubules (Fig. 4). The internal diameter of the microtubules measured 9 nm. Dynein arms are not visible in the micrograph. The mean width of the peripheral and the central microtubules were 37.42 ± 8.07 and 50.26 ± 12.22 nm, respectively. The flagellum unthreaded in the cytoplasmic canal and gradually developed into two independent lateral extensions of cytoplasmic membrane (Figs. 1b and 4). These fins were observed parallel to the plane of the two central microtubules of the flagellum. The first and second fins originated approximately 0.65 and 4.01 μm post midpiece, respectively and extended along the flagellum terminating 5.58 and 7.97 μm from the end of flagellum, respectively. The average length and diameter of flagellum were 33.26 ± 2.74 and 0.33 ± 0.01 μm, respectively.

4. Discussion

The spermatozoa of sturgeons are primitive in organization, with cells having almost radial symmetry

Table 2
Comparison of sperm cell morphology of stellate [8], white [9], Atlantic [10], shortnose [11], lake [12], pallid [13], Siberian [14] and Chinese sturgeon (present study)

Species	AL	AW	NL	ANW	PNW	ML	MW	AHML	FL	TL	n
Stellate sturgeon	0.97	1.22	6.66	0.98	1.49	3.43	1.38	11.05	40–70	51.05–81.05	1
White sturgeon	1.31	1.34	9.21	1.25	1.44	2.13	1.08	11.82	30–40	41.82–51.82	1
Atlantic sturgeon	0.83 (0.11)	1.00 (0.07)	3.15 (0.36)	0.92 (0.06)	0.55 (0.08)	1.37 (0.16)	0.51 (0.07)	5.66 (0.37)	37.08	42.74	12
Shortnose sturgeon	0.78 (0.08)	0.91 (0.06)	6.99 (0.83)	0.75 (0.11)	1.21 (0.12)	1.91 (0.35)	0.81 (0.09)	9.71 (0.73)	36.7	46.41	15
Lake sturgeon	0.73 (0.14)	0.81 (0.07)	5.69 (0.43)	0.68 (0.07)	1.04 (0.08)	2.68 (0.43)	0.70 (0.08)	9.10 (0.53)	47.53	56.63	14
Pallid sturgeon	1.07 (0.10)	0.82 (0.06)	3.78 (0.33)	0.68 (0.04)	0.89 (0.06)	1.23 (0.16)	0.67 (0.08)	6.07 (0.48)	37.16	43.23	16
Siberian sturgeon	0.95 (0.17)	0.93 (0.12)	5.87	0.87 (0.13)	1.14 (0.18)	1.09 (0.43)	0.81(A), 0.57(P)	7.01 (0.83)	44.75 (4.93)	51.76	8
Chinese sturgeon	0.54 (0.15)	0.68 (0.06)		0.59 (0.05)	1.835 (0.45)	2.17 (0.36)	1.57 (0.27)	5.44	33.26 (2.74)	38.7	3

Data are the means and S.D. AL, acrosome length, AW, acrosome width; NL, nuclear length; ANW, anterior nucleus width; PNW, posterior nucleus width; ML, midpiece width; AHML, length of acrosome, head and midpiece; FL, flagellum length; TL, total length.

[23,14]. Sperm is discharged directly into the water and sperm motility is long in terms of sperm activation, 4–8 min [24]. The Chinese sturgeon spermatozoon is similar in shape to those of most other sturgeon species (Table 2). It is very close to shortnose and lake sturgeon in terms of head shape and to Atlantic sturgeon in terms of head size (Table 2). The present study further revealed differences among sturgeons in terms of spermatozoon morphometry. Morphological parameters of spermatozoon in previous studies of sturgeons are summarized in Table 2. The observed differences suggest species-specific characteristics among sturgeons similar to those reported for teleosts [3], which could be useful for taxonomic purposes [25,1,13]. For example, DiLauro et al. [12] suggested that white and lake sturgeons are similar phylogenetically and systematically based on observed similarities in structure of spermatozoon. However, in the case of the Chinese sturgeon spermatozoa, we could not conclude which species it is closer to evolutionarily (Table 2).

The longest flagellum and total length have been reported in stellate sturgeon [8], but the shortest flagellum and total length were observed in the Chinese sturgeon in the present study. In addition, the present study confirmed the presence of an acrosome in spermatozoa of sturgeon, a group of ancient animals called ''living fossils''. This is similar to elasmobranch and teleost fishes with internal fertilization, but in contrast to other fishes, such as other teleosts with external fertilization [26,1,10,3], indicating the evolutionary development of fish reproductive systems [3].

4.1. Acrosome

A common characteristic of higher vertebrate sperm-cell acrosomes is that they possess an acrosome vesicle [27], as, for example, in tree frogs [28], birds [29] and mammals [30]. These structures are not found in the spermatozoa of all sturgeons. Some species possess only an acrosome vesicle, a subacrosome and posterolateral projections. This is the case for white sturgeon [9,31], stellate sturgeon [8], Atlantic sturgeon [10] and Siberian sturgeon [14]. The acrosome, possibly functioning to aid in egg penetration during fertilization, is considered to be derived from the Golgi apparatus and is commonly cap-shaped [32]. The present study showed some variations in length and width of the acrosome among sturgeon species (Table 2). This may reflect an inter-specific diversity.

The physio-biochemical function of the acrosome in fertilization of sturgeon eggs is unclear. Granular material and probably actin filament, which describe Gary et al. [33] in white sturgeon, are noted within the anterior end of the acrosome of spermatozoon in Chinese sturgeon as well as in white sturgeon [9]. However, the acrosome of lake, shortnose and Atlantic sturgeon exhibits granular, but not filamentous, material in this region [12]. Fine granular material is evenly dispersed within the anterior end of the spermatozoon of lamprey acrosome, with distinct clumps present against the posterior wall [34]. It is known that such structure corresponds to acrosomal function, so the granular material and actin filament may adapt the spermatozoon to fertilization mode. Further studies are needed to understand its role during sperm penetration into the egg and egg activation, or egg activation alone.

The sturgeon spermatozoon head usually terminates in posterolateral projections (PLP) that are radially distributed lobes [10–14] and present study. As with the acrosome, there are no data to explain PLP function in sperm physiology. DiLauro et al. [12] speculated they might function as barbs to assist effective penetration into the egg. The literature shows variations in the number and size of PLPs. The present study showed the presence of 10 PLPs in Chinese sturgeon, which differs from the pallid, which have 8 PLPs [13]. The length of PLPs in lake, Atlantic, shortnose, pallid and Siberian sturgeon has been reported to be 0.324, 0.233, 0.246, 0.760 and 0.950 μm, respectively. The length of PLPs in the Chinese sturgeon (0.369 μm) is thus close to that of the lake sturgeon.

4.2. Nucleus

The nucleus morphology and the presence or absence of nuclear vesicles are key parameters in terms of teleost taxonomy [35,3]. Nuclear vesicles have been observed in the spermatozoon of teleost fish, such as the yellow catfish (*Pseudobagrus fulvidraco* Richardson 1846) [36] and tench (*Tinca tinca* Linnaeus 1758) [37]. It has been suggested that they may decrease by stress of mechanical injury, and thus the impact on the germ plasma was reduced when the spermatozoon swam incessantly during fertilization [30]. Interestingly, no nuclear vesicles are reported in sturgeons, which contain only electron-dense homogeneous chromatin, confirming that sturgeons are more primitive than teleosts.

One or more endonuclear canals have been reported in teleosts, with the exception of garfish [38,3]. In the case of sturgeons and paddlefish, three endonuclear canals are reported in white [39], stellate [8], shortnose [11], and Siberian sturgeon [14], and in paddlefish [15], but two canals in Atlantic sturgeon [10] spermatozoa. Spermatozoa of Chinese sturgeon have three to five

endonuclear canals that traverse the entire nucleus in a helical arrangement. Further studies are needed to determine whether the number of canals is an intra- or inter-specific characteristic and why the arrangement of canals is helical. Cherr and Clark [9] presumed the endonuclear canals assisted in transferring a centriole into the egg after sperm-egg fusion. In general, it seems that the endonuclear canals traversing the nucleus from the nuclear fossa to the acrosome act on the eggs through the granular material of the acrosome. The spermatozoa of Atlantic sturgeon is the smallest among the species studied and its chromatin and nucleic acids are the most densely packed, suggesting a more advanced evolutionary arrangement [11].

Similar to other sturgeon species [8–12,14,15], the implantation fossa is present in the Chinese sturgeon spermatozoon and is located at the posterior end of the nucleus. Mattei and Mattei [40] described this structure as an enlargement of the endonuclear canal and DiLauro et al. [12] suggested it connects the endonuclear canals with the centrioles of the midpiece. DiLauro et al. [10] observed a structure in Atlantic, lake and shortnose sturgeon that is similar to the fibrous body of the garfish spermatozoon [38] and proposed that the structure, which has nearly the same diameter as the endonuclear canals, might serve as a channel for the transport of genetic material between the centriole and the nuclear fossa.

4.3. Midpiece

In previous studies, the midpiece of sturgeon spermatozoa was considered to be an elongate and slightly conical cylinder with nearly radial symmetry [10]. The present study showed that the midpiece of Chinese sturgeon spermatozoa is eudipleural (bilaterally symmetrical) about the longitudinal axis (Figs. 3d and 4).

There is a great diversity in the shape and the structure of the spermatozoa midpiece between fish species. The symmetry of the midpiece, the arrangement of the centriole, the number of mitochondria, the distribution of vacuoles and the presence of vacuoles are all regarded as important systematic characteristics for teleosts [35,3]. Normally, there are a few vacuoles with membranes between mitochondria and nucleus in most teleost fish, such as *Sebastiscus marmoratus* Cuvier 1829, Nile tilapia (*Tilapia nilotica* Linnaeus 1758) and common carp (*Cyprinus carpio* Linnaeus 1758) [29]. No similar structures were observed in the midpiece of Chinese sturgeon in the present study, or in white [9], lake [12] or stellate [8] sturgeon. Vacuoles containing lipid droplets have been reported in Atlantic [10], shortnose [11] and Siberian [14] sturgeon.

In all sturgeon species, both proximal and distal centrioles have been observed in the midpiece, with variation in size but with similar function [8–15]. The proximal centriole is apparently attached to the nucleus by several thin strands in the center of the nuclear fossa, called the fibrous body [10,14], which connects the nuclear fossa with the proximal centriole. The distal centriole is located adjacent to the proximal centriole; posterior to the distal centriole is the axoneme. The arrangement of the centriolar complex in Chinese sturgeon spermatozoon was T-shaped as in other sturgeons.

The number of mitochondria may differ among sturgeon species [11] as in teleosts [39]. The number of sperm mitochondria in other sturgeon is reported to be more than one [11]. For example, three to six mitochondria have been observed in the Siberian sturgeon, whilst six mitochondria were present in the periphery of the midpiece of the Chinese sturgeon (Fig. 3a). However, to determine developmental changes of structure and behavior of spermatozoa in *Acipenseriformes*, there is a need to study and compare the mitochondrial number, their physiological function during spermatozoa motility and ATP generation, and the effects of storage procedures, both short and long term (cryopreservation) among species.

4.4. Flagellum

In the mid stage of spermatozoa ontogeny, the distal centriole outgrows the axoneme, extending to form the flagellum. In the Chinese sturgeon, the flagellum is separated from the midpiece by the cytoplasmic channel as in other fish spermatozoa [40,1,3] with the characteristic 9 + 2 structure of microtubules. The mean length of the Chinese sturgeon flagellum is 33.26 μm (Table 1) and is the shortest flagellum of those sturgeons examined, although close to that of white sturgeon (30–40 μm; [9]).

In both teleosts and sturgeons [1,3,14] the flagellum is separated from the midpiece by the cytoplasmic channel as in other fish spermatozoa. The axoneme originates from the posterior end of the distal centriole in the classical 9 + 2 arrangement of microtubules, giving rise to the flagellum in all described sturgeon spermatozoa. The present study and those of Ginsburg [8], Cherr and Clark [9], DiLauro et al. [10,11] and Psenicka et al. [14] revealed that the sturgeon spermatozoon has a flagellum with a paddle- or fin-like shape, which is adapted for locomotion during external fertilization. The same structure has been also observed in some teleosts [41,36,42]. In the Siberian

sturgeon, it has been shown that the fins were flat, indicating no rotation, so that the first and second fins started 0.7 and 5.3 μm post midpiece, respectively, and extended along the flagellum. The first and second fins terminated 3.4 and 5.1 μm, respectively, from the end of Siberian sturgeon spermatozoon flagellum [14]. Compared to the Siberian sturgeon, both first and second fins of Chinese sturgeon arose closer to the midpiece (0.65 and 4.01 μm post midpiece, respectively) and extended farther along the end of flagellum (terminating 5.58 and 7.97 μm from the end of flagellum, respectively). As with the Siberian sturgeon, the observed fins were flat, not in a spiral configuration.

5. Conclusion

(1) The present study shows the diversity of spermatozoa ultrastructure between sturgeon species, similar to that observed and reviewed in teleost fishes [1,3], due to adaptation to various modes of fertilization according to the physio-chemical, environmental and related parameters, which regulate fertilization mechanism in fishes.
(2) Comparison of spermatozoa between Chinese and other sturgeons in Table 2 suggests four characteristics as parameters that will be considered to study phylogenetic relationships among species: (1) the shape and size of the sperm head with its organelles, (2) the presence of granular material and the actin filament, (3) the number of endonuclear canals and (4) the absence of fibrous body and vacuole in the midpiece. Taken together, based on ultrastructural similarities, the Chinese sturgeon may be more closely related to white sturgeon than to other sturgeons.
(3) Compared to teleost fishes, data on physiological functions of sturgeon spermatozoa organelles are rare and need further study especially to understand the roles of the acrosome, PLP, and fin structure of the flagellum.

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