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Morphology and ultrastructure of *Brachymystax lenok tsinlingensis* spermatozoa by scanning and transmission electron microscopy

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ABSTRACT

This study was conducted to investigate *Brachymystax lenok tsinlingensis* spermatozoa cell morphology and ultrastructure through scanning and transmission electron microscopy. Findings revealed that the spermatozoa can be differentiated into three major parts: a spherical head without an acrosome, a short mid-piece, and a long, cylindrical flagellum. The mean length of the spermatozoa was 36.11 ± 2.84 μm, with a spherical head length of 2.78 ± 0.31 μm. The mean anterior and posterior head widths were 2.20 ± 0.42 μm and 2.55 ± 0.53 μm, respectively. The nuclear fossa was positioned at the base of the nucleus that contained the anterior portion of flagellum and a centriolar complex (proximal and distal centrioles). The short mid-piece was located laterally to the nucleus and possessed just one spherical mitochondrion with a mean diameter of 0.65 ± 0.14 μm. The spermatozoa flagellum was long and cylindrical, and could be separated into two parts: a long main-piece and a short end-piece. The main piece of the flagellum had short irregular side-fins. The axoneme composed the typical '9 + 2' microtubular doublet structure and was enclosed by the cell membran e. This study confirmed that *B. lenok tsinlingensis* spermatozoa can be categorized as teleostean "Type I" spermatozoa; 'primitive' or 'ect-aquasperm type' spermatozoa. To the best of the authers knowledge, this was the first study conducted on the morphology and ultrastructure of *B. lenok tsinlingensis* spermatozoa.

1. Introduction

Spermatozoa transport the male haploid chromosome set into the oocyte. For this purpose, spermatozoa compose four different compartments: the acrosome, the head, the mid-piece, and the tail or flagellum (Callard and Callard, 1999; Knobil and Neill, 1999). Spermatozoa structure in teleost fish is influenced by both the mode of reproduction and systematic position of the species (Knobil and Neill, 1999). In the teleost species studied to date, the spermatozoa structure has revealed a high diversity, predominately between systematic families. This diversity is reflected in the head shape, in the number, shape and location of mitochondra, and in the number, length and structure of the flagellum. The spermatozoa structure among *Osteichthyes* is diverse. Spermatozoa of *Actinistia*, *Dipnoi*, and *Chondrostei* spp. contain an acrosome, have an elongated head, and a nucleus pervaded by a channel along its longitudinal axis. The mid-piece is small in species with external fertilization (*Dipnoi* and *Chondrostei* spp.), but is well developed and of comparable size to those of elasmobranch fish in species with internal fertilization (*Actinistia* spp.) (Jamieson, 1991; Mattei, 1991). The head and mid-piece of the spermatozoa in Neopterygii spp. is very small (≤10 μm), the latter contains mitochondrion and is pervaded by a cytoplasmic channel. The structure of the flagellum typically exhibits a '9 + 2' microtubule pattern and may include lateral side-fins that can be considered as paddles that enhance the swimming ability of the flagellum (Lahnsteiner and Patzner, 2008).

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Table 1
Data of the broodfish and the spermatozoa.

Samples	Total length (cm)	Body length (cm)	Weight (g)	Density of the spermatozoa (10^{10}/ml)
Male 1	22.9	19.4	116.7	2.65 ± 0.072
Male 2	23.9	19.8	135.8	3.07 ± 0.043
Male 3	21.9	18.7	93.4	2.55 ± 0.025
Male 4	28.7	25.1	247.2	3.15 ± 0.110

Teleost spermatozoa exhibit a diverse range of structural features that makes it difficult to depict a common sperm type (Mattei, 1991). The structure of fish spermatozoa varies between families: from aflagellate to biflagellae, while shape, size, and structure can vary significantly according to whether a species adopts internal or external fertilization (Jones and Butler, 1988). Spermiogenesis is broadly categorized into two types (I and II); however, in teleosts it shows a wide variety of patterns (Mattei, 1991). In Type I, rotation of the nucleus occurs, the diplosome enters the nuclear fossa, and the flagellum is symmetrically located, while for in Type II, there is no nuclear rotation, the diplosome remains outside the fossa, and the flagellum is asymmetrically located (Mattei, 1970).

B. lenok tsinlingensis (Qinling lenok) is only found in the cold-water mountain streams and rivers of Qinling Mountains, Shaanxi, China. The fish is listed as a second class state-protected wild animal in the China Red Data Book of Endangered Animal (1988). The fine structures of the testis in *B. lenok tsinlingensis* have been studied by transmission and scanning electron microscopy (TEM and SEM, respectively), testicular structure is a lobular type, containing numerous cysts surrounded by a layer of Sertoli cells (Zhang and Wang, 1992). Despite the importance of this species, very little is known about its reproductive biology. Reports on the breeding of this fish in captivity are lacking (Liu et al., 2013) and hatchery production of this species is yet to be developed for large-scale farming. Considering the importance of protecting this fish, it is essential to understand its reproductive biology. Consequently, the present study aimed to investigate the ultrastructure of *B. lenok tsinlingensis* spermatozoa using SEM and TEM.

2. Materials and methods

2.1. Broodfish sperm collection

Fish were collected from the wild environment in Taibai River, located in the Baoji of Shaanxi province, China. They were placed in broodfish tanks in a hatchery for 10 days prior to spawning induction in May 2014. As an endangered salmon in China (Zhao and Zhang, 2009), it was difficulty to seize in the wild environment. Data of the broodfish and the spermatozoa used in the experiment can be seen in Table 1.Water tank temperature was maintained at 8–10 °C. Spermiation was stimulated by a single thoracic injection of carp pituitary powder dissolved in 0.9% NaCl at 4 mg per kg body weight. Spermatozoa were collected from 38 to 48 h after injection. The genital pore of the fish was carefully cleaned to remove water, urine, and feces. After wiping the genital area, the spermatozoa were expelled by applying gentle pressure on the abdomen and collected into a 2.0 ml Elmendorf tube (Eppendorf® Safe-Lock micro-centrifuge tubes volume 2.0 ml, green) at 4 °C. As a result, four samples of spermatozoa were got (one sample from each fish). Then each sample of spermatozoa were then fixed with 1 ml 2.5% glutaraldehyde and cacodylate buffer for SEM and TEM analysis.

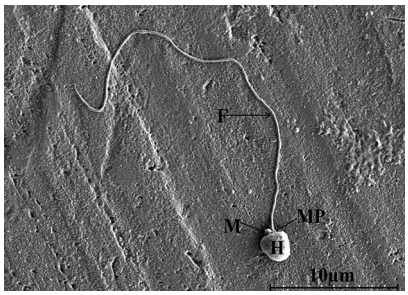

Fig. 1. Ultrastructure of *Brachymystax lenok tsinlingensis* spermatozoa: head (H); mid-piece (MP); mitochondrion (M); flagellum (F). Scale bar: 10 μm.

2.2. Ultrastructural study

Electron microscopy studies were carried out at the Laboratory of Electron Microscopy of the Institute of Hydrobiology, Chinese Academy of Sciences.

The samples were fixed in 0.1 M cacodylate buffer (pH 7.5) containing 2.5% glutaraldehyde (Karnovsky, 1965) for 2 h at 4.0 °C. The samples were then washed in cacodylate buffer for 1 h, and post-fixed in 1% cacodylate-buffered osmium tetroxide for 1 h at 4.0 °C. Following double fixation, the spermatozoa pellets were processed for SEM and TEM. For SEM, the spermatozoa samples were dehydrated through an ascending series of ethanol (30%, 50%, 70%, 80%, 100%, 10mins for every concentration and doubled for 100%), and the dehydrated samples were critical-point dried, mounted on specimen holders, and sputter-coated with 20 nm gold palladium. Preparations were examined using a SEM (Hitachi S-4800, Tokyo, Japan) at 20 kV (Greven and Schmahl, 2006). For TEM, the samples were dehydrated as above, and embedded in epoxide resin. Ultra-thin sections, 60–100 nm thick, were collected using glass knives. The sections were then placed on copper grids, stained with uranyl and lead citrate, and screened under a TEM (Hitachi HT-700) (Ravaglia and Maggese, 2003).

All SEM and TEM measurements were evaluated using MicroImage software (version 4.0.1 for Windows, Olympus Optical Co., Hamburg, Germany). Spermatozoa morphological characteristics were assessed and expressed as mean ± standard deviation, number, and range. The experiment of SEM and TEM has been repeated four times.

3. Results

Morphologically, the spermatozoa of *B. lenok tsinlingensis* comprised three major parts: a head without an acrosome, a mid-piece, and a single flagellum (Fig. 1). Morphological measurements of the spermatozoa are shown in Table 2. The mean total length of the spermatozoa was 36.11 ± 2.84 μm (n = 29). The head of the spermatozoa was small, spherical, and 2.78 ± 0.31 μm (n = 42) in length, and width of the anterior and posterior heads was 2.20 ± 0.42 μm (n = 42) and 2.55 ± 0.53 μm (n = 42), respectively.

The nucleus occupied most of the head and was composed of electron-dense, granular materials (chromatin) surrounded by a nuclear envelope. At the base of the nucleus, a depression termed the nuclear fossa was formed by the invaginated nuclear envelope (Figs. 2–5). It contained the anterior portion of flagellum and a centriolar complex (proximal and distal centrioles) (Figs. 2–5). The nuclear fossa promotes connection of the posterior part of the spermatozoa nucleus with the mid-piece and the axial components of the flagellum. The centriolar complex comprised a proximal (anterior region) and distal centriole (posterior region) inside the nuclear fossa. At the base of the head, the centrioles were vertically aligned with each other with at 90°. The distal centriole functions as a

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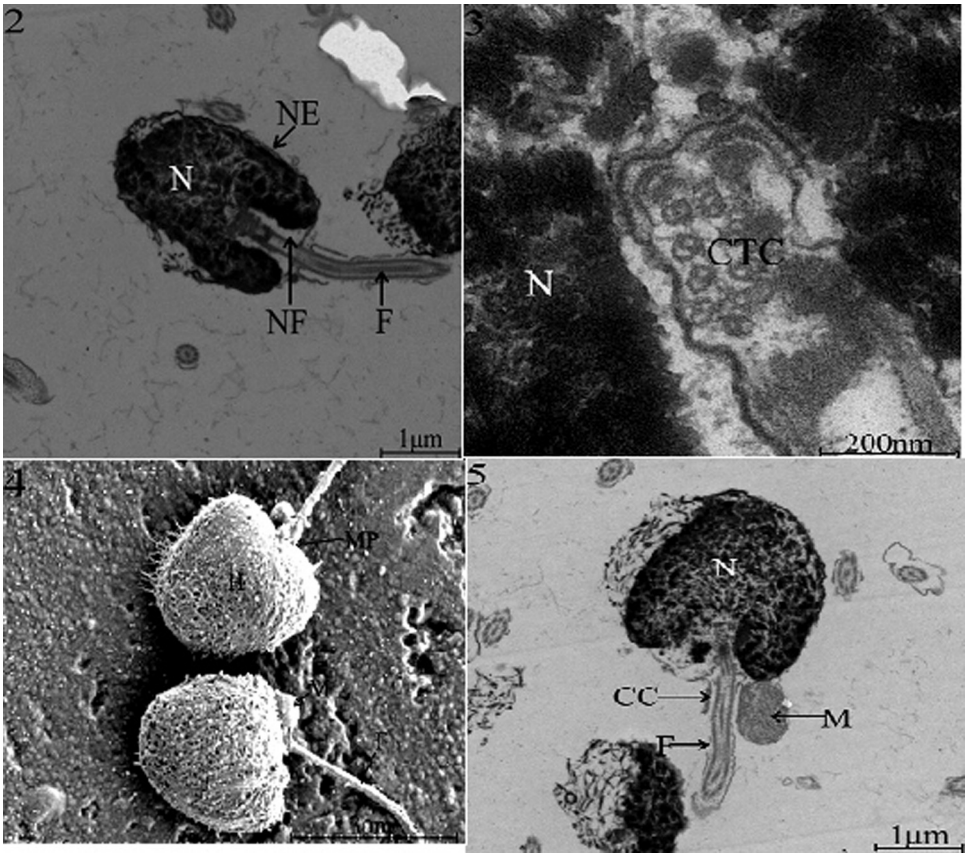

Figs. 2–5. Ultrastructure of Brachymystax lenok tsinlingensis spermatozoa (I).
2, a longitudinal section of the spermatozoa; 3, a longitudinal section of the mid-piece; 4, ultrastructure of the head and mid-piece; 5, a longitudinal section of the spermatozoa. nucleus (N); nuclear fossa (NF); nuclear envelope (NE); centriolar complex (CTC);head (H); mid-piece (MP); flagellum (F); mitochondrion (M); cytoplasmic canal (CC). Scale bar: 2, 1 μm; 3, 200 nm; 4, 3 μm; 5, 1 μm.

Table 2
Ultrastructural variables of *Brachymystax lenok tsinlingensis* spermatozoa.

Spermatozoa	Variables				
Sperm size (μm)	Total length 36.11 ± 2.84[29] (30.7–45.74)				
Head (μm)	Length 2.78 ± 0.31[42] (2.37–3.79)	AHW 2.20 ± 0.42[42] (1.31–3.43)	PHW 2.55 ± 0.53[42] (1.30–3.46)		
Mid-piece (μm)	Length 0.50 ± 0.1 [16] (0.35–0.70)	Width 0.77 ± 0.19 [17] (0.37–1.02)	MCD 0.65 ± 0.14[21] (0.44–0.94)	MN 1	
Flagellum	Length (μm) 32.9 ± 3.31[31] (22.4–41.7)	Width (μm) 0.29 ± 0.06[44] (0.16–0.43)	AW (μm) 0.17 ± 0.008[20] (0.15–0.18)	CCW (nm) 90.6 ± 12.6[13] (80.47–104.68)	LCPB (nm) 23.17 ± 4.81[20] (15.55–35.56)
Axoneme (nm)	PDM 64.3 ± 3.22[29] (45.10–70.43)	CDM 84.79 ± 6.8[19] (70.87–90.89)	LRS 41.81 ± 2.31[32] (29.45–47.12)	MTD 20.37 ± 1.08[31] (15.58–21.92)	Axoneme pattern 9 + 2

Measurements are means ± standard deviation for n (square brackets). The range of measurements is given in parentheses. anterior head width (**AHW**); posterior head width (**PHW**); mitochondrion diameter (**MCD**); mitochondrion number (**MN**); axoneme width (**AW**); cytoplasmic canal width (**CCW**); peripheral doublets of microtubules width (**PDM**); central doublets of microtubules width (**CDM**); length of radial spoke (**LRS**); microtubule diameter (**MTD**); length of central pair bridge (**LCPB**).

primary structure of the flagellum. An electron-dense zone surrounded the anterior end of the distal centriole and was connected to the pair of centrioles.

The mid-piece of the spermatozoa was short and had an irregular shape (Figs. 2–5). It had a mean length and width of 0.50 ± 0.1 μm (n = 16) and 0.77 ± 0.19 μm (n = 17), respectively (Table 2). Unequally sized spherical mitochondrion (Figs. 2–5) was found in the posterior region of the nucleus with a mean diameter of 0.65 ± 0.14 μm (n = 21) (Table 2). In the mid-piece, a cytoplasmic canal (Fig. 5) was located between the plasma and the flagellum, with a mean width of 90.6 ± 12.6 nm (n = 13) (Table 2). It also separated the plasma membrane from the axoneme, located posterior to the distal centriole.

The flagellum of the spermatozoa was long and cylindrical with a mean length of 32.9 ± 3.31 μm (n = 31) (Table 2). The mean width of the flagellum was 0.29 ± 0.06 μm (n = 44) (Table 2). The flagel-

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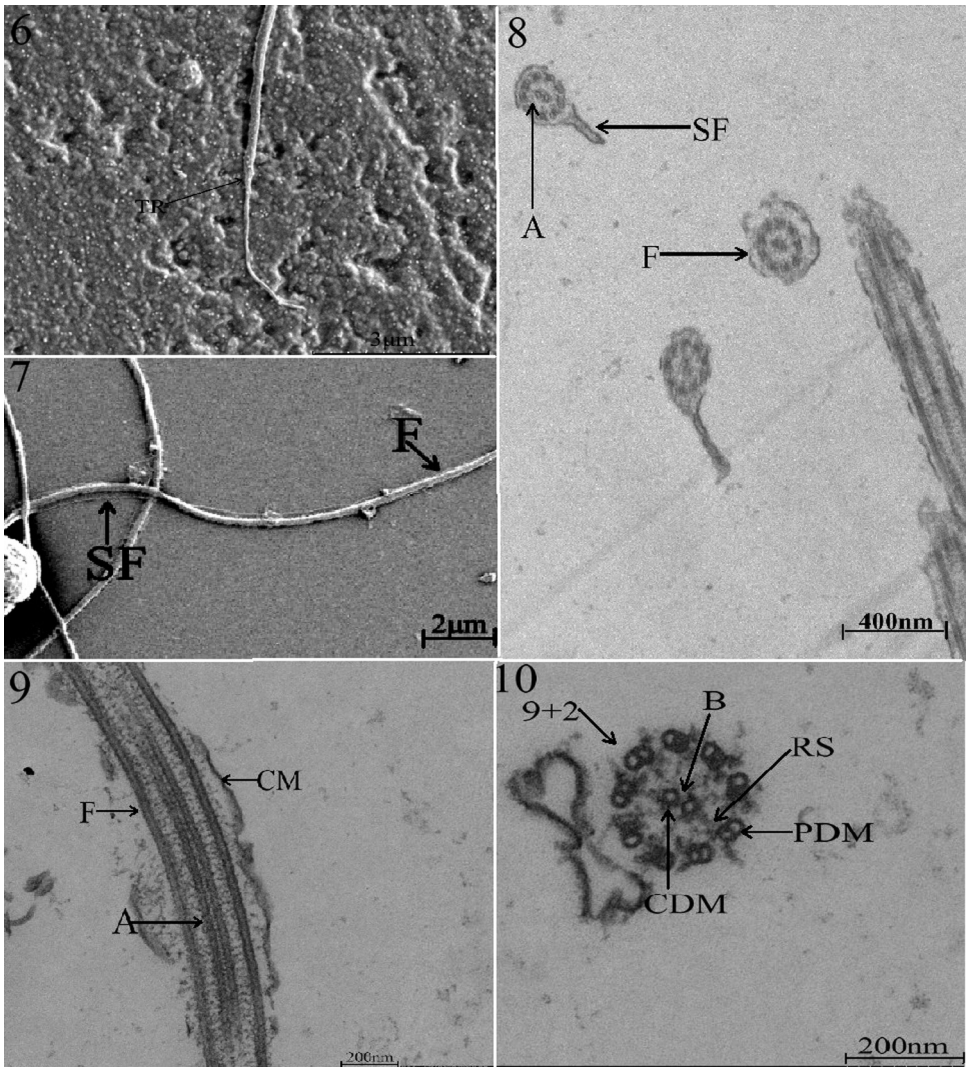

Figs. 6–10. Ultrastructure of Brachymystax lenok tsinlingensis spermatozoa (II).
6, ultrastructure of the flagellum; 7, ultrastructure of a flagellum; 8, ultrastructure of a cross section of flagellum; 9, ultrastructure of a longitudinal section of flagellum; 10, ultrastructure of a cross section of flagellum. transition region (TR); flagellum (F); side-fin (SF); axoneme (A); bules (PDM); radial spoke (RS); bridge (B); axoneme pattern (9+2). Scale bar: 6, 3 μm; 7, 2 μm; 8, 400 nm;9, 200 nm;10, 200 nm.

lum could be separated in to two main parts: a long main-piece and a short end-piece. A short transition region was found between these two regions (Figs. 6–10). The flagellum was surrounded by a cell membrane, that projected to form two side-fins located on the both sides of the flagellum, unequal in size (Figs. 6–10). To the best of the authors' knowledge, this is the first time unequal sized side-fins have been reported. The distal centriole stretched from inside the basal nuclear fossa to the anterior end of the cytoplasmic canal, acting as a primary structure of the flagellum axoneme (Figs. 6–10). The mean width of the axoneme was 0.17 ± 0.008 μm (n = 20) (Table 2). The flagellum axoneme comprised microtubule configurations in a '9 + 2' microtubule pattern (Figs. 6–10), encompassed by a cell membrane (Fig. 9). The cross-section of the flagellum axoneme was comparable to that of a vehicle wheel, with the radial spokes extending from the central doublets of microtubules to the peripheral doublets of microtubules (Fig. 10). Two of the microtubules of the central doublet were connected by a bridge (Figs. 6–10). Radial spokes were situated between peripheral and central pairs (Figs. 6–10). The accessory microtubules were connected to each other by radial spokes and electron-dense microfilaments. The mean width of the central doublet and the outer microtubule pair was 84.79 ± 6.8 nm (n = 19) and 64.3 ± 3.22 nm (n = 29), respectively. The mean diameter of the microtubule is 20.37 ± 1.08 nm (n = 31) and the mean length of radial spokes was 41.81 ± 2.31 nm (n = 32). The mean length of the central pair bridges was 23.17 ± 4.81 nm (n = 20) (Table 2).

Based on these SEM and TEM observations, a schematic reconstruction of *B. lenok tsinlingensis* spermatozoa ultrastructure is presented in Fig. 11.

4. Discussion

This study confirmed that *B. lenok tsinlingensis* spermatozoa have three major parts: the head, mid-piece, and flagellum. The spermatozoa possess an acrosome-less aquasperm, and exhibit structural variations that can be valuable in taxonomy (Baccetti et al., 1984; Jamieson, 1991; Mattei, 1991). Since the spermatozoa cells of *B. lenok tsinlingensis* have a head without an acrosome, they can be categorized as a primitive type of cell. Anacrosomal aquasperm can also participate in external fertilization once released into the water environment (Fuerboeck et al., 2010). Spermatozoa without an acrosome can also be found in many other teleost species such as Turbot (Suquet et al., 1993) and Tilapia (Don and Avtalion, 1993). Spermatozoa without an acrosome in the head

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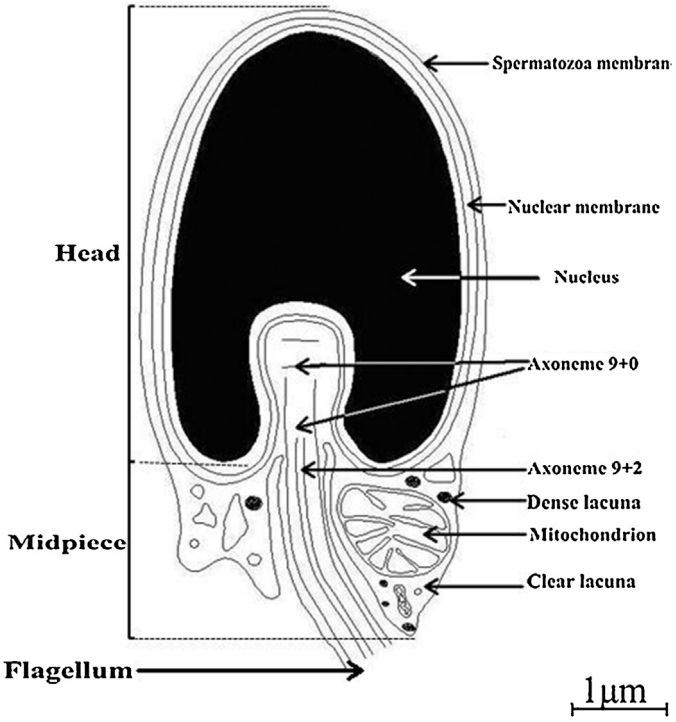

Fig. 11. Schematic reconstruction of *Brachymystax lenok tsinlingensis* spermatozoa based on the findings of the present study.

is likely to be the consequence of concerted evolution of micropyle possession: shedding in the zona pellucida allowing spermatozoa to enter and fertilize the egg (Medina et al., 2000). The dimensions of an acrosome-less head are closely related to the egg micropyle diameter of the species (Kim et al., 2011). The present study confirmed that the spermatozoa of this fish possess an uniflagellate, anacrosomal aquasperm, typical of spermatozoon of the neopterygian fish with external fertilization (Jamieson, 1991). Based on the ultrastructure description above, the spermatozoa of *B. lenok tsinlingensis* can be classified as 'Type I' or 'primitive type'.

It has been reported that head shape is highly variable among teleost spermatozoa. The head of *B. lenok tsinlingensis* spermatozoa is spherical in shape and contains an ovoid nucleus, comprising heterogeneously granular, strongly electron-dense chromatin and an irregular-shaped small nuclear fossa. Most of the salmon spermatozoa that have been studied have been shown to possess an ovoid-shaped head (Gwo et al., 1996; Lahnsteiner et al., 1991a, 1992). The presence of the spherical head in spermatozoa has also been reported in many other teleost species such as Gilthead Sea Bream (Maricchiolo et al., 2007), Pikeperch (Kristan et al., 2014), and *Scatophagus argus* (Madhavi et al., 2015). However, according to Maricchiolo et al. (2010), the shape of the nucleus in fish spermatozoa is species specific and can vary considerably between species (Maricchiolo et al., 2010). Generally, the hydrodynamic shape of the head in fish spermatozoa can reduce swimming ability and speed (Malo et al., 2006), these spermatozoa are considered primitive or ect-aquasperm. The small ovoid-shaped head found in the present study is the consequence of a plain spermiogenesis process, the elongated head found in Siberian Sturgeon indicates a more complex spermiogenic process, and is regarded as an advanced morphological spermatozoa characteristic (Psenicka et al., 2007). In *B. lenok*, *tsinlingensis*, the nucleus almost fully occupies the entire head portion and the nuclear fossa is located at the base of the head; the depress aspect of which is deep, with a length of approximately one-third that of the nucleus. The relative position of the centriolar complex is species specific and it can range from parallel to perpendicular (Morisawa, 1999). In *B. lenok tsinlingensis*, the centriolar complex lies inside the nuclear fossa with vertically aligned centrioles at 90°.

The present study demonstrates that *B. lenok tsinlingensis* has an irregular and short mid-piece. A cross-section of the mid-piece in *B. lenok tsinlingensis* shows that there is only one irregular-sized spherical mitochondrion comprising a ring in the posterior of region of the nucleus. There is only one mitochondrion in all species of the salmon family such as Formosan Landlocked Salmon (Gwo et al., 1996), Brook Trout (*Salvelinus fotinalis*), and grayling (*Thymallus thymallus*) (Lahnsteiner et al., 1991a,b). However, the present investigation confirmed that interspecific differences in the organization of the mid-piece exist among the Salmonidae family. The shape of the mitochondrion is species-specific, for example, the mitochondrion of the Grayling spermatozoa is helical (Lahnsteiner et al., 1991a), while in most investigated Salmoninae spp., spermatozoa the mitochondrion are cylindrical (Gwo et al., 1996). In other species of fish, the number of mitochondrion present in the mid-piece of the spermatozoa ranges from one to nine. For example, there are six to nine mitochondria in the Longtooth Grouper (*Epinephelus bruneus*) (Kim et al., 2013), five to six in the Leopard Coralgrouper (*Plectropomus leopardus*) (Gwo et al., 1994), four to six in the Common Barbell (*Barbus barbus*) (Alavi et al., 2008), and six in the Chinese Sturgeon (*Acipenser sinensis*) (Wei et al., 2007). The function of mitochondrion in the mid-piece of the spermatozoa is comparable to an energy factory; it produces adenosine triphosphate (ATP) for spermatozoa motility (Billard et al., 2000). According to Maricchiolo et al. (2004), ATP is used by the dynein arms that can assist the self-oscillatory bending behavior of the flagellar axoneme (Maricchiolo et al., 2004). Therefore, as a source of energy for the spermatozoa, the number of the mitochondra is a key factor in spermatozoa motility and plays an important role in reproductive activity of the fish (Lahnsteiner and Patzner, 1995). With the esception of energy, the swimming speed and endurance of spermatozoa motility are also affected by the size and shape of the mid-piece (Baccetti et al., 1984). As previously reported, spermatozoa in species with internal fertilization have a complicated mid-piece, while those with an external fertilization have a short and small mid-piece (Lahnsteiner et al., 1997). In *B. lenok tsinlingensis*, the spermatozoa have a short, irregular mid-piece and a long flagellum; this can help the spermatozoa move quickly through the water towards the floating egg waiting to be fertilized.

The spermatozoa of *B. lenok tsinlingensis* have a long and cylindrical flagellum, comprising two pats: a long main-piece and a short end-piece. The single flagellum, found in *B. lenok tsinlingensis* spermatozoa, has also been observed in other salmon species such as Grayling (Lahnsteiner et al., 1991a), Formosan Landlocked Salmon (*Oncorhynchus masou formosanus*) (Gwo et al., 1996), Rainbow Trout (*Oncorhynchus mykiss*), and Brown Trout (*Salmo trutta F. fario*) (Drokin et al., 1998). The spermatozoa with a single flagellum perpendicular to the nucleus can be classified into Type I spermatozoa. Spermatozoa in the present study exhibited a pattern comprising flagellum placed perpendicularly to the nucleus, closely resembling that of Type I spermatozoa (Mattei, 1991). According to Mattei (1991), the spermatozoa of Type I aquasperm contain an axoneme with a '9 + 2' configuration pattern (Mattei, 1991). As a result, the spermatozoa of *B. lenok tsinlingensis* can be categorized as Type I aquasperm. The axoneme forms the cytoskeletal structure with the flagellum, providing support and structure to the flagellum primarily during swimming (Gwo, 1995). The length of the flagellum is considerably long, nearly 12-fold that of the head. It has been reported that a long spermatozoa flagellum and mid-piece may be selected to optimize energetic demands under conditions of increased spermatozoa competition intensity (Vladic et al., 2002). The two side-fins found outside the main-piece of the flagellum is not order- nor family-specific, as reported by Gwo et al. (1996) in

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Table 3
Variations in the fine structure of spermatozoa between *Brachymystax lenok tsinlingensis*, Salmoninae spp., Coregoninae spp., and Thymallinae spp. (family Salmonidae).

Parameter	B. lenok tsinlingensis	Salmoninae	Coregoninae	Thymallinae
		Oncorthynuhus mykiss[1,3], O. masou formosanus[2], O. masou masou[3], *Salvelinus fontinalis*[4], *S. alpinus*[5], *Hucho hucho*[6]	*Coregonus lavaretus*[7]	*Thymallus thymallus*[8]
Shape of the head	ovoid	ovoid	ovoid	ovoid
Dimensions of head (μm)	2.78 ± 0.31[a];2.53 ± 0.46[b]	2.01 ± 0.04[a];1.71 ± 0.03[b]	2.62 ± 0.09[a];1.69 ± 0.16[b]	2.02 ± 0.05[a];1.66 ± 0.15[b]
Dimensions of mid-piece (μm)	0.50 ± 0.1[a];0.77 ± 0.19[b]	circa0.25[a];circa0.37[b]	1.09 ± 0.12[a];0.47 ± 0.08[b]	1.54 ± 0.02[a];0.35 ± 0.05[b]
Nuclear fossa	bell shaped	bell shaped	cylindrical	cylindrical
Number of mitochondrion	1	1	absent	1
Shape of mitochondrion	unequal sized spherical	cylindrical or helical surrounding cytoplasmic channel in 1.5windings	cylindrical	helical, surrounding cytoplasmic channel in 2–3windings
Flagellar structure	Typical 9 + 2 axoneme	Typical 9 + 2 axoneme	Typical 9 + 2 axoneme	Typical 9 + 2 axoneme
Flagellar length (μm)	32.9 ± 3.31	absent	absent	absent
Peculiarities	a long main piece and a short end piece of the flagellar	osmiophilic body laterally to each centriole	fibrous ring at the caudal of midpiece	absent
References	present study	Lahnsteiner and Patzner (2008)	Lahnsteiner and Patzner (2008)	Lahnsteiner and Patzner (2008)

[a] Diameter in longitudinal axis.
[b] Diameter in lateral axis.
[1] Data from Billard (1983).
[2] Data from Gwo et al. (1996).
[3] Data from Hara and Okiyara (1998).
[4] Data from Fribourgh (1978).
[5] Data from Lahnsteiner and Patzner (1991).
[6] Data from Radziun and Tomasik (1985).
[7] Data from Lahnsteiner et al. (1991a,b).
[8] Data from Lahnsteiner et al. (1992).

Formosan Landlocked Salmon (Gwo et al., 1996); Wei et al. (2007) in Chinese Sturgeon (Wei et al., 2007); and Kristan et al. (2014) in Percidae spp. (Kristan et al., 2014). Although the function of the two side-fins is unclear, they may help accelerate flagellar movement and increase friction with the surrounding environment, increasing the potential of egg fertilization (Maricchiolo et al., 2004; Psenicka et al., 2007; Zhang et al., 1993).

Variations in the fine structure of spermatozoa between *B. lenok tsinlingensis*, Salmoninae, Coregoninae, and Thymallinae spp. are presented in Table 3 (Billard, 1983; Fribourgh, 1978; Gwo et al., 1996; Hara and Okiyama, 1998; Radziun and Tomasik, 1985; Lahnsteiner and Patzner, 1991, 2008; Lahnsteiner et al., 1991a, 1992). Salmonidae spp. has simply constructed aquasperm. These types of spermatozoa have many fine structural features in common. Salmonidae spp. spermatozoa have an ovoid head and the flagellar structure of all species is the typical '9 + 2' pattern. There is only one mitochondrion in Salmonidae spp.; however, the shape varies species.

5. Conclusion

The findings of this study increase our understanding of *B. lenok tsinlingensis* spermatozoa. The spermatozoa possess the configuration of a uniflagellate acrosome-less aquasperm and can therefore be categorized as Type I, with a pattern of external fertilization. Detailed spermatozoa characteristics have been revealed in this study including an ovoid-shaped head, a short and irregular mid-piece, and a long and cylindrical flagellum comptising two parts: a long main-piece and a short end-piece. This information on the ultrastructure of *B. lenok tsinlingensis* spermatozoa improves our understanding of its reproductive biology, with the potential to contribute to the protection of this endangered species. In addition, this knowledge can assist in the development of artificial reproduction for enhanced production of this species.

Author contributions

Wei Guo and **Jian Shao** contributed equally to this work.
Qiwei Wei is the corresponding author.
Ping Li and **Jinming Wu** contributed to collecting samples and modifying the paper.

Conflict of interest

The authors declare no conflict of interest to any of the internal or external funding sources.

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