Sperm ultrastructure in *Culmenella rezvoji* (Lindholm, 1929) (Gastropoda: Hygrophila)

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ABSTRACT. The taxonomic position of the genus *Culmenella* within Hygrophila remains uncertain. The spermatozoa of *Culmenella rezvoji*, a species from the Far East of Russia, were examined using a combination of light, scanning and transmission electron microscopy with the objective to assess the utility of sperm characters for clarifying the phylogenetic relationships of the genus. The spermatozoa of *C. rezvoji* are divided into four regions: head, midpiece, glycogen piece and endpiece. The head contains a slender, cone-shaped acrosome and a conical nucleus with a sinistrally coiled keel. The acrosome consists of an apical vesicle and a thick-walled pedestal with an electron lucent canal partially filled with a patchy electron-dense material. The midpiece contains the mitochondrial derivative that encloses apically three parallel glycogen-filled tracts (helices) positioned in such a way that in the sperm cross section two helices lie opposite each other and equidistant from the third helix. The surface of the sperm above one of the helices forms a high, narrow ridge; the ridges above the remaining two helices have a much lower profile. The boundary between the midpiece and glycogen piece is demarcated by a constriction (annulus) consisting of an anterior electron-dense ring and a posterior conical cylinder connected to the ring with thin filaments. The structure of spermatozoa in *Culmenella* is consistent with the general pattern of sperm morphology common to all studied species of Hygrophila, but the spermatozoa of *Culmenella* also have distinctive characters (three glycogen helices and high-profile surface ridge in the apical portion of the midpiece) that should be potentially useful in resolving the taxonomic position of this genus.

Introduction

The taxonomic system of freshwater pulmonates (Hygrophila) has been reviewed and revised several times in recent years [Starobogatov *et al.*, 2004; Bouchet *et al.*, 2005, 2017; Vinarski, Kantor, 2016; Kiyashko *et al.*, 2016], but despite these attempts to refine the classification of the group a number of taxonomic problems remain unsolved. One of these problems is the taxonomic status, composition and phylogenetic relationships of the genus *Culmenella* Clench, 1927. Members of this genus live in Asia and have been studied by only a few authors [Clench, 1927, 1931; Lindholm, 1929; Zhadin, 1952; Hubendick, 1955a, b, 1967; Starobogatov, 1957, 1967; Starobogatov, Prozorova, 1990]. There have been different opinions on the taxonomic position of this genus: it was assigned either to Bulinidae [Starobogatov *et al.*, 2004] or to Planorbidae [Bouchet *et al.*, 2005; Vinarski, Kantor, 2016], even though the taxonomic boundaries of both families are still to be firmly established. Recently, *Culmenella* and closely related *Camptoceras* Benson, 1843 were shown by molecular data to be a sister group of *Ferrissia* (Tryon, 1863) (fam. Ancylidae) [Saito *et al.*, 2018], but these results should be regarded as preliminary due to limited taxon sampling. Overall, the taxonomic position of *Culmenella* remains uncertain warranting the search for new criteria that may shed light on morphology, taxonomy and phylogeny of this genus.

Sperm ultrastructure has been a consistently reliable source of phylogenetic markers in several groups of gastropods [Thompson, 1973; Kohnert, Storch, 1984; Healy, Willan, 1991; Hodgson *et al.*, 1991; Healy, 1993, 2001; Healy, Taylor, 1996; Hodgson, Healy, 1998; Röpstorf *et al.*, 2002] and was successfully employed in phylogenetic studies [Ponder, Lindberg, 1997; Dayrat, Tiller, 2002]. Among about 500 species of Hygrophila [Strong *et al.*, 2008], sperm ultrastructure is currently known only for 16 species [Bogitsh, 1974; Maxwell, 1975, 1977; Kitajima, Paraense, 1976; Rigby, 1982; Healy, 1983; Brackenbury, Appleton, 1991a, b; Appleton, Brackenbury, 1997; Hodgson, Healy, 1998; Soldatenko, Shatrov, 2016; Soldatenko *et al.*, 2016]. These studies have shown that the spermatozoa of Hygrophila have a complex morphology with a number

of traits common to all studied species. The spermatozoa are divided into four successive regions: head, midpiece, glycogen piece and endpiece. The head is occupied by a nucleus, usually in the shape of a slender cone, which is tipped by an acrosome consisting of an acrosomal pedestal capped with an apical vesicle. The midpiece, which is by far the longest region of the spermatozoon, is filled with a mitochondrial derivative surrounding the axoneme. The mitochondrial derivative encloses long strands of glycogen (glycogen helices) coiling in a helix-like fashion along the length of the midpiece. The surface of the nucleus and the midpiece is covered with spiralling ridges serving to add rotation to the forward movement of the sperm. The boundary between the midpiece and the glycogen piece is abrupt and is marked by a constriction known as annulus. The glycogen piece lacks the mitochondrial derivative and is filled instead with an amorphous mass of glycogen surrounding the axoneme. The posterior-most region of the sperm, the endpiece, contains only the tip of the axoneme enclosed in the plasma membrane.

The morphology of the acrosome, nucleus, helices, and ridges shows significant variation between species of Hygrophila suggesting that they can be used as a source of phylogenetic information. Since no information on sperm morphology in *Culmenella* is currently available, we have examined the spermatozoa of *Culmenella rezvoji* (Lindholm, 1929), a species from the Far East of Russia, with the objective to compare their morphology with that of other species of Hygrophila. The study used a combination of light, scanning and transmission electron microscopy to make an accurate assessment of the size and shape of the sperm structures. The information on sperm morphology presented in this study may provide the foundation for establishing new taxonomically informative characters and contribute to creating a more comprehensive dataset for the phylogenetic analysis of Hygrophila.

Material and Methods

The snails (sexually mature individuals) of *C. rezvoji* were collected by T.Ya. Sitnikova in July 2014, in marshes connected with an inlet of Lake Khanka (Primorsky Krai; 44°42'N, 132°04'E). All individuals were identified by E.V. Soldatenko and the voucher specimens were deposited at the Zoological Institute, Russian Academy of Sciences (St. Petersburg, Russia).

Since the spermatozoa of Hygrophila have a significant length (250 μm to 900 μm) and are packed in the seminal vesicles in a regular and highly compressed fashion [Soldatenko *et al.*, 2018], the detailed examination of sperm morphology and measurement of morphometric parameters were made possible by smearing the contents of the seminal vesicles on microscope slides or coverslips, which produced a great number of stretched, isolated spermatozoa.

For light microscopy (LM), the preparations were made as dry mounts [Soldatenko *et al.*, 2016]. The seminal vesicles were extracted from live molluscs and placed on a slide in a drop of distilled water, then teared apart and smeared on the slide with entomological pins. The smears were air-dried, rinsed in distilled water and then air-dried once again. Only fully mature spermatozoa or those at the final stages of maturation were used for measurements and the description of morphology. The preparations of spermatozoa were photographed on a Leica DM LS-2 light microscope equipped with a Leica EC-3 digital camera.

For scanning electron microscopy (SEM), the method of dry-mount preparations on coverslips was used. The seminal vesicles were spread out on a coverslip until spermatozoa were well separated from one another. Preparations were then washed several times in distilled water, air-dried, covered with a platinum layer in an Eiko IB-5 apparatus, and examined with SEM Quanta-250 (FEI) at 10-15 kV and magnification of up to x50000.

For transmission electron microscopy (TEM), the seminal vesicles were fixed with 2.5% glutaraldehyde in 0.1M phosphate buffer (pH 7.2-7.4) for no less than 4 h at 4-6°C. The specimens were then washed in several changes of 0.2M phosphate buffer, post-fixed with 2% osmium tetroxide in 0.1M phosphate buffer for 2-4 h at the same temperature, dehydrated in an ethanol and acetone series and embedded in an araldite mixture. Ultra-thin sections were made on a Leica UC-6 ultramicrotome and, after staining with uranyl acetate and lead citrate, were examined and photographed with a Morgagni-268D (FEI) transmission electron microscope.

The measurements of sperm cells were made from light microscopy images and SEM (Table 1) and TEM (Tables 2, 3) micrographs. The morphometric characteristics of spermatozoa and the abbreviations used in tables are shown schematically in Fig. 1.

Results

Light-microscopic observations. The total sperm length ranges from 372 to 439 μm (Fig. 2). The spermatozoa are divided into four regions: head, midpiece, glycogen piece and endpiece. The head (Fig. 2), comprising the acrosome and the nucleus, has the shape of a slender cone about 5 μm in length (Table 1). Most of the remaining sperm length (on average about 84%) is occupied by the midpiece (Table 1). Under oil immersion the midpiece shows
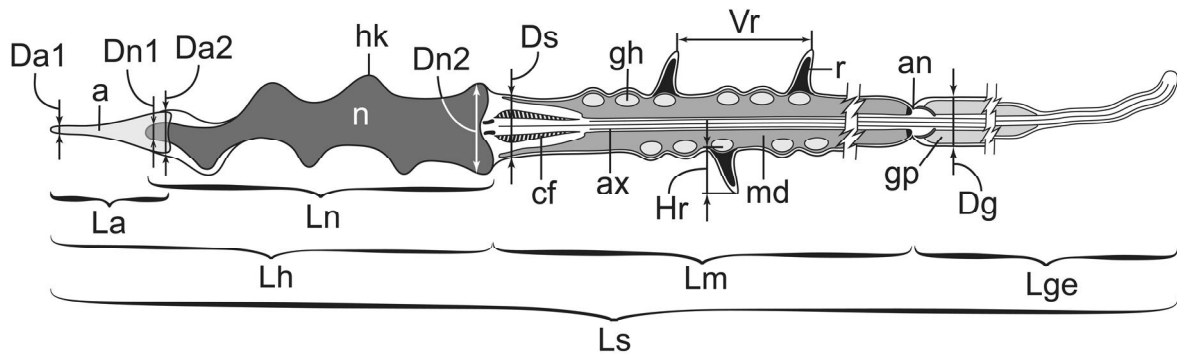
FIG. 1. Schematic representation of a spermatozoon of *Culmenella rezvoji* illustrating various sperm structures and morphometric parameters used in Tables 1-4. The midpiece and glycogen piece are shown shortened for clarity. Abbreviations: a, acrosome; an, annulus; ax, axoneme; cf, coarse fibres connected with axonemal doublets; Da1, apical acrosomal diameter; Da2, basal acrosomal diameter; Dg, diameter of glycogen region; Dn1, nuclear diameter near acrosome; Dn2, basal nuclear diameter; Ds, midpiece diameter at neck region; gh, glycogen helices; gp, glycogen particles; hk, helical nuclear keel; Hr, height of high surface ridge; La, acrosomal length; Lge, combined length of glycogen piece and endpiece; Lh, head length; Lm, midpiece length; Ln, nuclear length; md, mitochondrial derivative; n, nucleus; r, high surface ridge; Vr, pitch length of spiral followed by high surface ridge.

РИС. 1. Схематическое изображение сперматозоида *Culmenella rezvoji,* показывающее различные его структурные элементы и морфометрические параметры, используемые в таблицах 1-4. Для наглядности средний и гликогеновый отделы показаны значительно укороченными. Сокращения: a, акросома; an, аннулюс; ax, аксонема; cf, грубые волокна, связанные с дуплетами микротрубочек аксонемы; Da1, апикальный диаметр акросомы; Da2, базальный диаметр акросомы; Dg, диаметр гликогенового отдела; Dn1, диаметр ядра вблизи акросомы; Dn2, диаметр ядра у его основания; Ds, диаметр среднего отдела в области шейки; gh, гликогеновые спирали; gp, частицы гликогена; hk, спиральный ядерный киль; Hr, высота высокого гребня; La, длина акросомы; Lge, общая длина гликогенового и хвостового отделов; Lh, длина головки; Lm, длина среднего отдела; Ln, длина ядра; md, митохондриальная производная; n, ядро; r, высокий гребень; Vr, длина одного оборота спирали высокого гребня.

a single high ridge coiled along the length of the spermatozoon.

Electron-microscopic observations. The acrosome is composed of a long acrosomal pedestal and a small apical vesicle located at the tip of the pedestal (Fig. 3A–B) and has the shape of a slender cylindrical cone with a blunted apex (Fig. 4). The apical diameter of the acrosome is about 34% its basal diameter (Table 4). The pedestal caps the apical end of the nucleus and is slightly curved at the base so that the pedestal appears tilted relative to the apical portion of the nucleus (Fig. 3A–B). The pedestal has thick, electron dense walls surrounding an electron lucent canal (Fig. 3B), which is partially filled with a patchy network or irregular streaks of electron-dense material (Fig. 3B). The

Table 1. Morphometric characteristics of spermatozoa of *C. rezvoji.* Light microscopy (LM) and scanning electron microscopy (SEM). Data are expressed as mean ± standard deviation (minimum–maximum).

Табл. 1. Морфометрические характеристики сперматозоидов *C. rezvoji.* Световая (СМ) и сканирующая электронная микроскопия (СЭМ). Данные представлены в виде среднего арифметического ± стандартное отклонение (минимум–максимум).

n	Morphometric indices of sperm structures (μm)				
	Ls	Lh	Lm*	Lge*	Nh
45	419.29±15.34 (372.00-439.00)	5.23±0.37 (4.30-5.70)	352.40±18.16 (292.50-380.50)	61.66±9.92 (38.00-76.00)	3

Lge – combined length of glycogen piece and endpiece (LM); **Lh** – head length (SEM); **Lm** – midpiece length (obtained by subtracting Lh and Lge from Ls); **Ls** – total sperm length (LM). **Nh** – number of glycogen helices (SEM). *Indices **Lm** and **Lge** may have measuring errors because the boundary between the midpiece and the glycogen regions is almost indistinct by LM.

Lge – общая длина гликогенового и хвостового отделов (СМ); **Lh** – длина головки (СЭМ); **Lm** – длина среднего отдела (получена вычитанием Lh и Lge из Ls); **Ls** – общая длина сперматозоида (СМ). **Nh** – число гликогеновых спиралей (СЭМ).*Индексы **Lm** и **Lge** могут содержать погрешности измерений, так как граница между средним и гликогеновым отделами плохо различима на светомикроскопическом уровне.

Table 2. Morphometric ultrastructural characteristics of the sperm head of *C. rezvoji* (TEM). Data are expressed as mean ± standard deviation (minimum–maximum).

Табл. 2. Морфометрические ультраструктурные характеристики головок сперматозиодов *C. rezvoji* (просвечивающая электронная микроскопия). Данные представлены в виде среднего арифметического ± стандартное отклонение (минимум–максимум).

n	Morphometric indices of sperm structures (µm)

n	Ln	La	Lh	Dn1	Dn2	Da1	Da2
45	3.47±0.62 (2.54-4.44)	1.20±0.08 (1.00-1.39)	4.52±0.64 (3.60-5.67)	0.13±0.01 (0.09-0.14)	0.78±0.09 (0.60-0.93)	0.12±0.03 (0.07-0.18)	0.35±0.04 (0.26-0.43)

Da1 – apical acrosomal diameter; **Da2** – basal acrosomal diameter; **Dn1** – nuclear diameter near acrosome; **Dn2** – basal nuclear diameter; **La** – acrosomal length; **Lh** – head length; **Ln** – nuclear length.

Da1 – апикальный диаметр акросомы; **Da2** – базальный диаметр акросомы; **Dn1** – диаметр ядра вблизи акросомы; **Dn2** – диаметр ядра у его основания; **La** – длина акросомы; **Lh** – длина головки; **Ln** – длина ядра.

Table 3. Morphometric ultrastructural characteristics of the midpiece and glycogen piece of spermatozoa of *C. rezvoji* (TEM). Data are expressed as mean ± SD (minimum–maximum).

Табл. 3. Морфометрические ультраструктурные характеристики среднего и гликогенового отделов сперматозоидов *C. rezvoji* (просвечивающая электронная микроскопия). Данные представлены в виде среднего арифметического ± стандартное отклонение (минимум–максимум).

n	Morphometric indices of sperm structures (µm)

n	Ds	Dg	Hr	Vr
45	0.61±0.10 (0.43-0.77)	0.44±0.06 (0.32-0.54)	0.37±0.05 (0.26-0.54)	1.76±0.16 (1.41-1.99)

Dg – diameter of glycogen region; **Ds** – midpiece diameter at neck region; **Hr** – height of high surface ridge; **Vr** – pitch length of spiral followed by high surface ridge.

Dg – диаметр гликогенового отдела; **Ds** – диаметр среднего отдела в области шейки; **Hr** – высота высокого гребня; **Vr** – длина одного оборота спирали высокого гребня.

Table 4. Morphometric proportions of spermatozoa of *C. rezvoji* (n=45). Data are expressed as mean ± standard deviation (minimum–maximum).

Табл. 4. Отношения морфометрических характеристик сперматозоидов *C. rezvoji* (n=45). Данные представлены в виде среднего арифметического ± стандартное отклонение (минимум–максимум).

Sperm proportions

Lh/Ls	Lm/Ls	Lge/Ls	La/Ln	Ln/Dn2	Da1/Da2	Da2/Dn2	Dg/Dn2
0.013±0.001 (0.010-0.015)	0.84±0.03 (0.79-0.90)	0.15±0.03 (0.09-0.20)	0.35±0.06 (0.27-0.46)	4.52±1.13 (2.75-6.69)	0.34±0.08 (0.26-0.56)	0.45±0.06 (0.35-0.60)	0.57±0.11 (0.34-0.84)

Da1 – apical acrosomal diameter; **Da2** – basal acrosomal diameter; **Dg** – diameter of glycogen region; **Dn2** – basal nuclear diameter; **La** – acrosomal length; **Lge** – combined length of glycogen piece and endpiece; **Lh** – head length; **Lm** – midpiece length; **Ln** – nuclear length; **Ls** – total sperm length.

Da1 – апикальный диаметр акросомы; **Da2** – базальный диаметр акросомы; **Dg** – диаметр гликогенового отдела; **Dn2** – базальный диаметр ядра; **La** – длина акросомы; **Lge** – общая длина гликогенового и хвостового отделов; **Lh** – длина головки; **Lm** – длина среднего отдела; **Ln** – длина ядра; **Ls** – общая длина сперматозоида.

apical vesicle appears to be ovoid in some electron micrographs, but its exact shape and size cannot be properly resolved.

The nucleus has the shape of a slender cone and is about three times as long as the acrosome (Tables 2, 4). The basal portion of the nucleus is much wider than its apical portion (Table 2). The surface of the nucleus bears a wide sinistral helical keel that makes 2.5 turns along the length of the nucleus ending at about one-fifth the distance from the nuclear base (Figs 3A, 4). A narrow electron-lucent axial channel passes through the entire length of the nucleus. Basally, the nucleus has a shallow V-shaped fossa that encloses the proximal ends of the axoneme and coarse fibres (Fig. 3A, D).

The neck region of the midpiece is marked by an electron-dense ring closely apposed to the base of the nucleus and encircling the axial complex of the
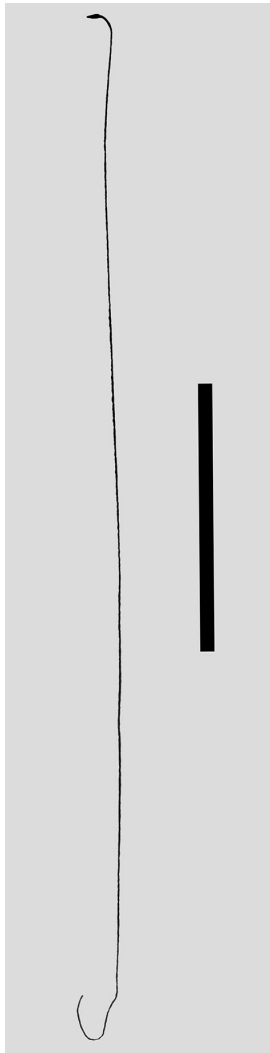
FIG. 2. General view of a spermatozoon of *Culmenella rezvoji*. Light microscopy. Scale bar = 100 μm.

РИС. 2. Световая микрофотография общего вида сперматозоида *Culmenella rezvoji*. Масштабная линейка = 100 мкм.

midpiece (Fig. 3A, D). The axial complex originates from the V-shaped fossa at the base of the nucleus and consists of an axoneme with a typical 9+2 microtubule pattern surrounded in the apical portion of the midpiece by nine thick coarse fibres. In the neck region, the mitochondrial derivative is filled with a single mass of glycogen granules (Fig. 3A, D), but in the apical portion of the midpiece, this glycogen mass becomes separated into three glycogen-filled tracts (helices) (Fig. 3D, E) that extend in a spiral fashion along the length of the midpiece. The helices run in parallel one after another and are arranged in such a way that the third (distal-most) helix is followed by a gap about the width of another helix (Fig. 3D, F). In the sperm cross section, the first and the third helices lie opposite each other and equidistant from the middle helix, with the gap located opposite the middle helix (Fig.

3E). The surface of the sperm above the third helix forms a high, narrow ridge filled with electron dense material (Fig. 3D–F); this is the only ridge that is clearly visible in a light microscope. The ridges above the remaining two helices have a much lower, triangular profile (Fig. 3F). The surface ridges gradually decrease in height posteriorly and finally disappear in the posterior portion of the midpiece (Fig. 3G–I). The glycogen helices terminate consecutively along the length of the midpiece leaving first two helices (Fig. 3G), then one (Fig. 3H) and finally no helices near the posterior end of the midpiece (Fig. 3I). The diameter of the midpiece only slightly decreases towards its posterior end (Table 3).

The boundary between the midpiece and the glycogen piece is marked by a constriction (annulus). The anterior part of the annulus consists of ring-shaped electron-dense material at the posterior end of the mitochondrial derivative (black arrows, Fig. 3J–L). The posterior part is a hollow, truncated electron-dense cone 0.1±0.02 μm (n=4) in length (white arrows, Fig. 3K–L) capping the glycogen mass of the glycogen piece. The cone narrows down posteriorly and flares out at the anterior end attaching with its flaring rim to the plasma membrane of the sperm (Fig. 3K–L). The anterior ring-shaped structure and the posterior cone are connected by thin filaments.

The axoneme in the glycogen piece is tightly surrounded by an amorphous mass of glycogen granules (Fig. 3J, K, M). The endpiece is short and has initially the classical 9+2 microtubule pattern (Fig. 3N), but further down the tail the microtubules terminate one after another until none are left at the distal tip of the sperm (Fig. 3O).

Discussion

The spermatozoa of *C. rezvoji* are typical in morphology for Hygrophila consisting of four distinct regions: head, midpiece, glycogen piece and endpiece [Anderson, Personne, 1967, 1970; Maxwell, 1975, 1977; Kitajima, Paraense, 1976; Dan, Takaichi, 1979; Rigby, 1982; Healy, 1983, 1988; Brackenbury, Appleton, 1991a, b; Schileyko, 1991; Healy, 2001; Appleton, Brackenbury, 1997; Hodgson, Healy, 1998]. Like all other Hygrophila, *C. rezvoji* has the spermatozoa with the heads containing an acrosome (apical vesicle and pedestal) and a nucleus with a sinistrally coiled keel. The acrosome of *C. rezvoji* is similar in its narrow, conical shape to those of *Ancylus fluviatilis* O.F. Müller, 1774 [Hodgson, Healy, 1998; Soldatenko, Shatrov, 2016], *Armiger crista* (Linnaeus, 1758) [Soldatenko *et al.*, 2016], *Biomphalaria glabrata* (Say, 1818) [Kitajima, Paraense, 1976], three species of *Bulinus* O. F. Müller, 1781 [Appleton, Brackenbury, 1997; Brack-
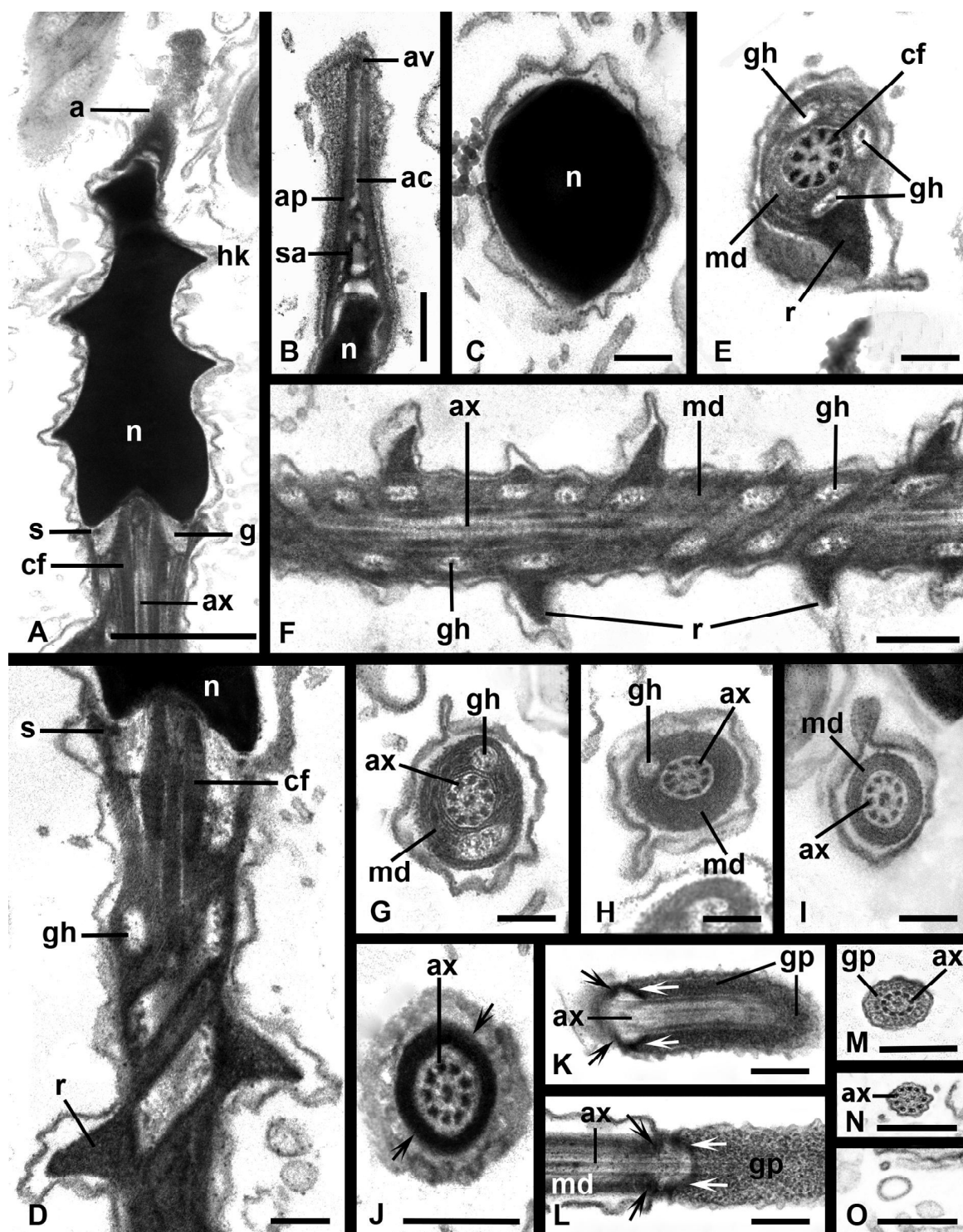
FIG. 3. Longitudinal (LS) and transverse (TS) TEM sections of spermatozoa of *Culmenella rezvoji*. **A.** LS, head of a spermatozoon at the final stage of maturation. **B.** LS, internal structure of the acrosome. **C.** TS, middle portion of the nucleus. **D.** LS, basal portion of the nucleus, the neck region and the anterior portion of the midpiece. **E.** TS, neck region showing coarse fibres, three glycogen helices enclosed within the mitochondrial derivative and a high surface ridge. **F.** LS, middle portion of the midpiece showing three glycogen helices, a high surface ridge, the mitochondrial derivative and the axoneme. **G.** TS, middle portion of the midpiece with two glycogen helices and the axoneme. **H.** TS, posterior portion of the midpiece with a single glycogen helix. **I.** TS, posterior portion of the midpiece without glycogen helices. **J.** TS, annulus region showing a ring at the tip of the mitochondrial derivative (black arrows). **K–L.** LS, annulus region showing a ring at the tip of the mitochondrial derivative (black arrows) and a cylinder at the anterior end of the glycogen piece (white arrows). **M.** TS, glycogen region. **N.** TS, axoneme in the tail region with a complete 9+2 pattern of microtubules. **O.** TS, tail region without an axoneme. Abbreviations: a, acrosome; ac, axial acrosomal canal; ap, acrosomal pedestal; av, apical vesicle; ax, axoneme; cf, coarse fibres connected with axonemal doublets; g, glycogen granules; gh, glycogen helices; gp, glycogen particles; hk, helical nuclear keel; md, mitochondrial derivative; n, nucleus; r, high surface ridge; s, sub-nuclear ring; sa, streaks of electron dense material in axial acrosomal canal. Scale bars: **A** = 1.0 µm; **B–L** = 0.25 µm; **M–O** = 0.5 µm.

enbury, Appleton, 1991a]*, Gyraulus albus* (O. F. Müller, 1774) [Soldatenko *et al.,* 2016], *Physa acuta* Draparnaud, 1805 [Brañkenbury, Appleton, 1991b]*, Planorbis planorbis* (Linnaeus, 1758) [Soldatenko, Shatrov, 2016], and *Planorbarius corneus* (Linnaeus, 1758) [Thompson, 1973; Soldatenko, Shatrov, 2016]. This contrasts with the spermatozoa of *Burnupia stenochorias* (Melvill et Ponsonby, 1903) [Hodgson, Healy, 1998], three species of *Lymnaea* Lamarck, 1799 [Thompson, 1973; Maxwell, 1975, 1977; Rigby, 1982; Healy, 1983], and *Segmentina nitida* (O.F. Müller, 1774) [Soldatenko, Shatrov, 2016]*,* which have short, widely conical or cap-shaped acrosomes. The length of the acrosome in Hygrophila varies from 0.7 µm in *Burnupia stenochorias* [Hodgson, Healy, 1998] to 2.2 µm in *Bulinus tropicus* (Krauss, 1848) [Brackenbury, Appleton, 1991a] and that of *C. rezvoji* (ca. 1.2 µm) is approximately in the middle of this range. In general, the size and shape of the acrosome does not provide any new evidence on the phylogenetic position of *Culmenella*.

Although the structure of the acrosomal pedestals is not always well resolved by TEM, it is possible to make some preliminary conclusions regarding the morphological differences of pedestals within Hygrophila. On the basis of their internal structure, the acrosomal pedestals in Hygrophila can be divided into two types. The first type (*Planorbis planorbis*, *Gyraulus albus*, and *Armiger crista*) is characterized by the presence of an electron dense cone-shaped plug that occupies the internal space in the pedestal near its base [Soldatenko, Shatrov, 2016; Soldatenko *et al.*, 2016]. The second type (*Ancylus fluviatilis, Planorbarius corneus, Segmentina nitida* and three species of *Lymnaea*) lacks an internal plug; the inner space in these pedestals is electron lucent or filled with a network of flocculent electron-dense material [Rigby, 1982; Healy, 1983; Soldatenko, Shatrov, 2016; Soldatenko *et al.*, 2016]. The pedestal with an internal plug has so far been described only in the species that belong
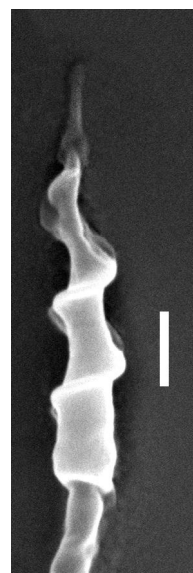

FIG. 4. Scanning electron micrograph of the sperm head of *Culmenella rezvoji*. Scale bar = 1 µm.

РИС. 4. Микрофотография (сканирующая электронная микроскопия) головки сперматозоида *Culmenella rezvoji*. Масштабная линейка = 1 мкм.

to the tribe Planorbini (fam. Planorbidae), which was shown by molecular evidence to be nested deep within Hygrophila [Albrecht *et al.,* 2006; Soldatenko, Petrov, 2019], and therefore this type of pedestal might constitute a synapomorphy of this tribe. In contrast, the pedestal without a plug is present across different hygrophilan groups and is likely to be the basal condition for Hygrophila. The pedestal of *C. rezvoji* lacks an internal plug and if this type is indeed a plesiomorphic condition, it cannot provide any information on the phylogenetic relationships of this species.

The sperm nucleus of *C. rezvoji* is narrowly conical in shape, which is the most common condition in Hygrophila. The exception is the nuclei of *Segmentina nitida* that have a characteristic hour-

РИС. 3 (Предыдущая страница). Микрофотографии (просвечивающая электронная микроскопия) продольных (LS) и поперечных (TS) срезов сперматозоидов *Culmenella rezvoji*. **A.** LS, головка сперматозоида на последней стадии созревания. **B.** LS, внутренняя структура акросомы. **C.** TS, средняя часть ядра. **D.** LS, базальная часть ядра, область шейки и передняя часть среднего отдела. **E.** TS, область шейки с грубыми волокнами, тремя гликогеновыми спиралями внутри митохондриальной производной и высоким гребнем. **F.** LS, средняя часть среднего отдела с тремя гликогеновыми спиралями, высоким гребнем, митохондриальной производной и аксонемой. **G.** TS, средняя часть среднего отдела с двумя гликогеновыми спиралями. **H.** TS, задняя часть среднего отдела с одной гликогеновой спиралью. **I.** TS, задняя часть среднего отдела без гликогеновых спиралей. **J.** TS, область аннулюса с кольцом на кончике митохондриальной производной (черные стрелки). **K–L.** LS, область аннулюса; черные стрелки указывают на кольцо на кончике митохондриальной производной, белые стрелки указывают на цилиндр на переднем конце гликогенового отдела. **M.** TS, гликогеновый отдел. **N.** TS, аксонема в хвостовом отделе с полным набором микротрубочек (9+2). **O.** TS, хвостовой отдел без аксонемы. Сокращения: a, акросома; ac, осевой канал акросомы; ap, акросомный пьедестал; av, апикальный пузырек; ax, аксонема; cf, грубые волокна, связанные с дуплетами микротрубочек аксонемы; g, гликогеновые гранулы; gh, гликогеновые спирали; gp, частицы гликогена; hk, спиральный ядерный киль; md, митохондриальная производная; n, ядро; r, высокий гребень; s, подъядерное кольцо; sa, волокна электронно-плотного материала в осевом канале акросомы. Масштабные линейки: **A** = 1.0 мкм; **B–L** = 0.25 мкм; **M-O** = 0.5 мкм.

glass shape, and widely conical nuclei of Lymnaeidae. In all Hygrophila, the nucleus bears one or several keels wound in a sinistral spiral along the length of the nucleus. *Culmenella rezvoji* has the sperm nucleus with a single keel, a condition, which is also found in *Burnupia stenochorias* [Hodgson, Healy, 1998]*, Armiger crista* [Soldatenko *et al.*, 2016]*, Gyraulus albus* [Soldatenko *et al.*, 2016] and species of *Bulinus* [Brackenbury, Appleton, 1991a; Appleton, Brañkenbury, 1997]. Two keels are present in *Ancylus fluviatilis* [Hodgson, Healy, 1998], *Biomphalaria glabrata* [Kitajima, Paraense, 1976]*, Physa acuta* [Brackenbury, Appleton, 1991b]*, and *Planorbarius corneus* [Soldatenko, Shatrov, 2016] and species of *Lymnaea* have seven parallel keels [Maxwell, 1975, 1977; Rigby, 1982]. Since *Culmenella* was suggested by molecular evidence to be related to *Ferrissia* (i.e. the family Ancylidae), the differences in the number of nuclear keels between *C. rezvoji* (one keel) and *Ancylus fluviatilis*, another representative of Ancylidae (two keels), is an indication that this character is likely to be variable within groups of familial rank and might be determined not only by phylogenetic constraints, but also by the hydrodynamic conditions within the reproductive tracts of each particular species.

In the midpiece, the spermatozoa of *C. rezvoji* have two characters that distinguish them from the spermatozoa of all other Hygrophila studied to date. One of these characters is the number and arrangement of glycogen helices. The maximum number of helices in *C. rezvoji* is three, while in most Hygrophila the spermatozoa have apically four helices that run equidistant from one another. In *C. rezvoji,* the glycogen helices run parallel side-by-side, with the third helix followed by a gap in place of a missing fourth helix. Three helices in the apical part of the midpiece have previously been described in *Planorbarius corneus* [Soldatenko, Shatrov, 2016] and *Physa acuta* [Brackenbury, Appleton, 1991], but unlike those in *C. rezvoji* the helices in these species appear to run at roughly equal distances from one another, with no additional gap between them.

The second unique feature of the sperm midpiece in *C. rezvoji* is a high and narrow ridge above the third helix; the surface ridges of other Hygrophila have a significantly lower profile. The two other surface ridges in *C. rezvoji* are much lower in height; this difference in height and shape between the ridges is also uncommon in Hygrophila and has so far been described only in *Planorbarius corneus* [Soldatenko, Shatrov, 2016].

The annulus region in different species of Hygrophila was described by different authors either as a simple constriction [Kitajima, Paraense, 1976], a single ring [Brackenbury, Appleton, 1991a, b; Appleton, Brackenbury, 1997] or a more complex structure composed of two or three elements [Soldatenko, Shatrov, 2016; Soldatenko *et al.*, 2016]. The differences in descriptions, however, may be due to accumulation of electron-dense material in the annulus regions, which makes these structures difficult to resolve by TEM. It is possible that most or all annuli in Hygrophila may in fact be composed of two elements: the anterior ring and the posterior hollow cylinder or cone, and the most obvious difference between the annuli in different species is in the length of the posterior structure, which can be either long or short. The annulus of *C. rezvoji* conforms to this bipartite morphology and belongs to the type with a long cylinder.

The comparative analysis of sperm morphology of *C. rezvoji* does not reveal any sperm characters that may be regarded as well-supported synapomorphies shared with any other species of Hygrophila, for which the sperm morphology is currently known. Several of the traits that characterize the spermatozoa of *C. rezvoji* are either very common in Hygrophila (narrow conical acrosome, conical nucleus with a single keel) or appear to be plesiomorphic for the whole group (acrosome without an electron dense plug). The spermatozoa of *C. rezvoji* are similar to those of *Planorbarius corneus* in having apically three glycogen helices and ridges of different shape and height, but this similarity is probably a result of convergence. Two characters that have so far been found only in *C. rezvoji* (three glycogen helices apically with a gap after the third helix and an exceptionally high surface ridge above the third helix) may prove to be particularly useful in clarifying the phylogenetic relationships of this species and genus. If found in the genera thought to be related to *Culmenella* (e.g. *Camptoceras* and *Ferrissia*), these characters may significantly strengthen the case for uniting these genera into a single monophyletic group.

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Ультраструктура сперматозоидов *Culmenella rezvoji* (Lindholm, 1929) (Gastropoda: Hygrophila)

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РЕЗЮМЕ. Вопрос о таксономическом положении рода *Culmenella* в пределах Hygrophila остается дискуссионным. Для выяснения возможности использования признаков морфологии спермиев в установлении филогенетических связей *Culmenella* было проведено комплексное исследование сперматозоидов дальневосточного представителя этого рода, *Culmenella rezvoji* (Lindholm, 1929), с использованием световой, сканирующей электронной и просвечивающей электронной микроскопии. Сперматозоиды *C. rezvoji* делятся на четыре отдела: головку, средний отдел, гликогеновый отдел и хвост. Головка спермия содержит узкую, конусовидную акросому и коническое ядро с левозакрученным килем. Акросома состоит из апикального пузырька и толстостенного пьедестала с проходящим сквозь него электронно-прозрачным каналом, частично заполненным хлопьевидным электронно-плотным материалом. Средний отдел содержит митохондриальную производную, которая в своей апикальной части включает три параллельные гликогеновые спирали, расположенные таким образом, что на поперечном срезе через спермий две спирали лежат противоположно друг другу, а третья находится на равном расстоянии от них. Поверхность спермия над одной из спиралей несет высокий, узкий гребень; гребни над двумя другими спиралями значительно ниже. Средний отдел отделен от гликогенового отдела перетяжкой (аннулюсом), содержащим в своей передней части электронно-плотное кольцо, а в задней – конический цилиндр, соединенный с кольцом тонкими филаментами. Спермии *Culmenella* соответствуют общей схеме организации, характерной для спермиев всех исследованных Hygrophila, но имеют также и отличительные особенности (три гликогеновые спирали и высокий гребень в апикальной части среднего отдела), которые следует учитывать при уточнении таксономического положения этого рода.