УДК 576.895.122

DESCRIPTION OF VIRGULATE *CERCARIA ETGESII* LARVA NOV. (XIPHIDIOCERCARIAE): A NEW TYPE OF VIRGULA ORGAN

© S. V. Shchenkov

Department of Invertebrate Zoology, Saint-Petersburg State University,
Universitetskaia nab., 7/9, Saint Petersburg, 199034
E-mail: sergei.shchenkov@gmail.ru
Submitted 07.12.2016

A new species of virgulate cercaria is described. The morphology and chaetotaxy of cercariae are studied. The new type of virgula organ is described. Every description is illustrated by drawings.
Key words: *Bithynia tentaculata*, *Cercaria etgesii*, Lecithodendroidea, sporocyst, morphology, virgula, biodiversity.

ОПИСАНИЕ ВИРГУЛИДНОЙ *CERCARIA ETGESII* LARVA NOV. (XIPHIDIOCERCARIAE): НОВЫЙ ТИП ВИРГУЛЫ

С. В. Щенков

Санкт-Петербургский государственный университет
Университетская наб., 7/9, С.-Петербург, 199034
E-mail: sergei.shchenkov@gmail.ru
Поступила 07.12.2016

В статье приведено описание новой виргулидной церкарии. Изучена ее морфология и хетотаксия. Описан новый тип виргулы. Описания сопровождены рисунками.
Ключевые слова: *Bithynia tentaculata*, *Cercaria etgesii*, Lecithodendrioidea, морфология, виргула, биоразнообразие.

Max Lühe (1909) allocated a very small subgroup Cercariae virgulae as a part of a large group of stylet cercariae (Xiphidiocercariae). Five small larvae were included in this group. Four of those cercariae possessed a pear-shaped structure in the anterior organ — «the virgula organ». Due to its shape this formation was called «the pear-shaped organ». The larvae of the fifth type had no typical pear-shaped organs, they only had a small bulge, bordering the opening of the anterior organ.

The first interpretation of virgula organ nature and processes of its formation has been proposed in series of articles of Kruidenier (1947, 1951). It was shown, that the virgula is a reservoir which stores the secret of special cells. These cells

function only in the cercarial embryogenesis. The main component of the secret (mucin) has also been revealed by Kruidenier. These cells were called «the mucoid glands». After the end of cercarial morphogenesis mucoid glands become functionally inactive. Kruidenier argued that the virgula is a fold of covers, which accumulates mucin, i. e. this is a kind of reservoir for the temporary deposition of the secret of mucoid glands.

Later, it was shown that mucin first enters the outer layer of the tegument of the cercarial body. Then it is transported to the tegument of the buccal cavity (Galaktionov, Dobrovolskij, 2003). Thus, the virgula is not a cavity for mucin accumulation but the specialized area of covers, which contains the secret of mucoid glands. According to the traditional view, folds of virgula form due to invagination of the tegument of buccal cavity into the anterior organ.

The biodiversity of virgulate cercariae is much higher than we thought but nowadays its morphology is studied incompletely (Manafov, 2010). Parasitologists used the limited number of publications for identification of virgulate and microcotylous cercariae (e. g., Cort, 1914; Sewell, 1922; Manafov, 2010).

In the course of studies of trematode fauna of gastropods *Bithynia tentaculata* L., 1758 in the area of the middle flow of the Volga River, a new form of virgulate cercaria were found and named *Cercaria etgesii* larva nov. Its morphological description is given below.

MATERIAL AND METHODS

Gastropods *B. tentaculata* were collected in the Lake Kruglen'koe (53°10′45.1′′ N; 49°25′49.9′′ E) near Mordovo village in September 2012. Molluscs were sampled from the bottom and from the surface of underwater substrates by hand. On the whole, 457 individuals of *B. tentaculata* were collected.

Molluscs were placed into small glass containers (15 ml) individually for 12—24 h or more for identification of infected specimens. Only 49 individuals of *B. tentaculata* were infected by *C. etgesii.*

Infected molluscs were kept in glass and plastic containers. Calcium carbonate was added into water. To prevent the fouling by symbionts, molluscs were totally dried every 3 days. Infected molluscs were deposited again individually into a small glass vessel for 24 h. Just emitted cercariae were placed on a glass slide in a small drop of water, and studied *in vivo* under Leica DM1000 and LOMO MBR—1 microscopes using 40x and 100x objectives. We used vital dyes — neutral red and Nile blau sulfate.

To identify cercarial chaetotaxy we used the traditional method of impregnation with silver nitrate (Ginetsinskaja, Dobrovolskij, 1963). Measurements were conducted in larvae, fixed in a 3 % solution of $AgNO_3$. All dimensions are given in millimeters.

All drawings were made with the DA-7 drawing tube.

RESULTS

Cercaria etgesii larva nov. (figs. 1—3; see table)

Cercariae are small, with elongate and contractile body. Tail length is less than a half of the body length when enlarged. Tegument of the body is covered by small spines, while tail has no spines. The diameter of the anterior organ is twice the diameter of the acetabulum, the latter is located in the posterior part of the body (fig. 1). Aperture of the acetabulum is elongate in longitudinal axis. The stylet has large bulb. Narrow mouth is situated subterminally on the ventral surface of the anterior organ.

About one third of the anterior organ is filled with virgula. It is a massive thickening of the tegument of the buccal cavity (fig. 2). Its ventral and lateral walls are thickened. Thickening of the dorsal wall is diminished in the region of the median line of the body (fig. 2, *d*). When buccal cavity is expanded it can be clearly seen, that the virgula of *C. etgesii* is ring-shaped. The proximal end of the buccal cavity is its broadest part. When the buccal cavity is contracted, both dorsomedial edges of the virgula move closer to its ventral wall, almost touching it. In this case, the lumen of the buccal cavity becomes slit and the pear-shaped organ looks like a structure which consists of 2 separated outgrowths; the latter are invaginated into the anterior organ (fig. 2, *a*).

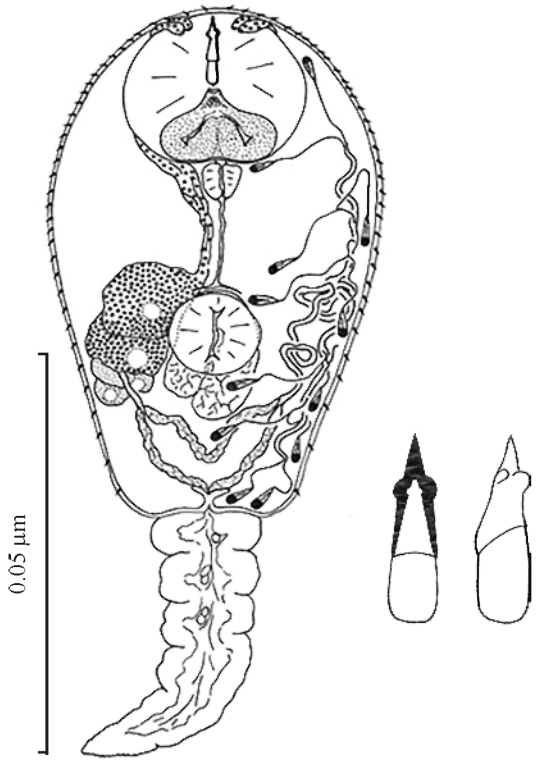

Fig. 1. General morphology of *Cercaria etgesii* larva nov.

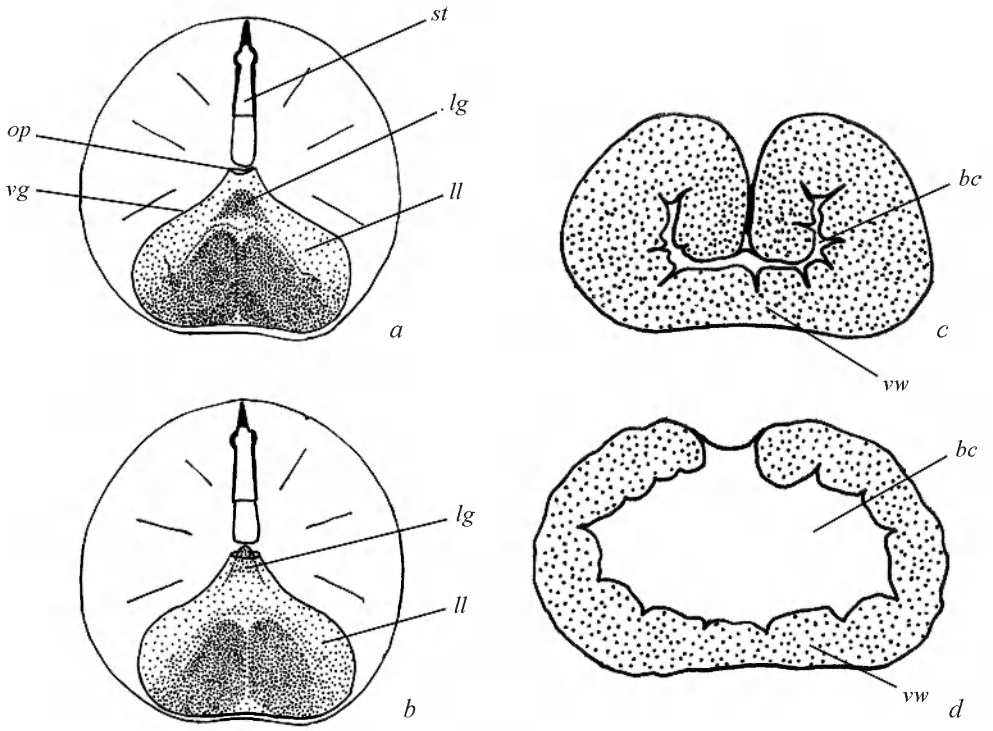

Fig. 2. Virgula organ of *Cercaria etgesii* larva nov.

a — ventral view. The ligula is inside the buccal cavity; *b* — ventral view. The ligula is protruding from the opening of the anterior organ; *c* — front view on virgula organ. Buccal cavity is contracted; *d* — front view on virgula organ. Buccal cavity is dilated. *bc* — buccal cavity, *lg* — ligula, *ll* — lateral lobes of virgula organ, *op* — opening of anterior organ, *st* — stylet, *vg* — virgula, *vw* — ventral wall of virgula organ.

A small ligula is situated on the ventral surface of the buccal cavity (fig. 2, *a*, *b*). The ligula (a thin fold of the tegument) can pull out of the mouth as far as 1/4—1/3 of its length. Its function is unknown. Short prepharynx goes into a small triangular pharynx. The pharynx lies often tight to the oral sucker so it looks like there is no prepharynx. The esophagus is long and narrow. The point of gut branches bifurcation is well seen. Lumen of the digestive system is visible through the esophagus and the anterior part of gut branches.

Cercariae have four pairs of penetration glands (fig. 1). They are located laterally to the acetabulum as two elongate cell groups. Cytoplasm of both anterior cell pairs is rough. Cytoplasm of both posterior cells possesses thin granules. Ducts of penetration glands are situated close to the esophagus. The round anterior organ lies dorsolaterally and opens near the stylet edge on the ventral surface of larvae (fig. 1).

Excretory formula: 2 [(2 + 2 + 2) + (2 + 2 + 2)] = 24. The distal end of anterior longitudinal collecting channels passes close to the ducts of penetration glands (fig. 1). Collecting channels round penetration glands from outer side. Proximal parts of the anterior longitudinal collecting channels run along the outer edge of penetration glands ducts. Then it's turn round penetration glands and merge with posterior longitudinal collecting channels at the level of posterior border of the third pair of penetration glands. The main collecting channels

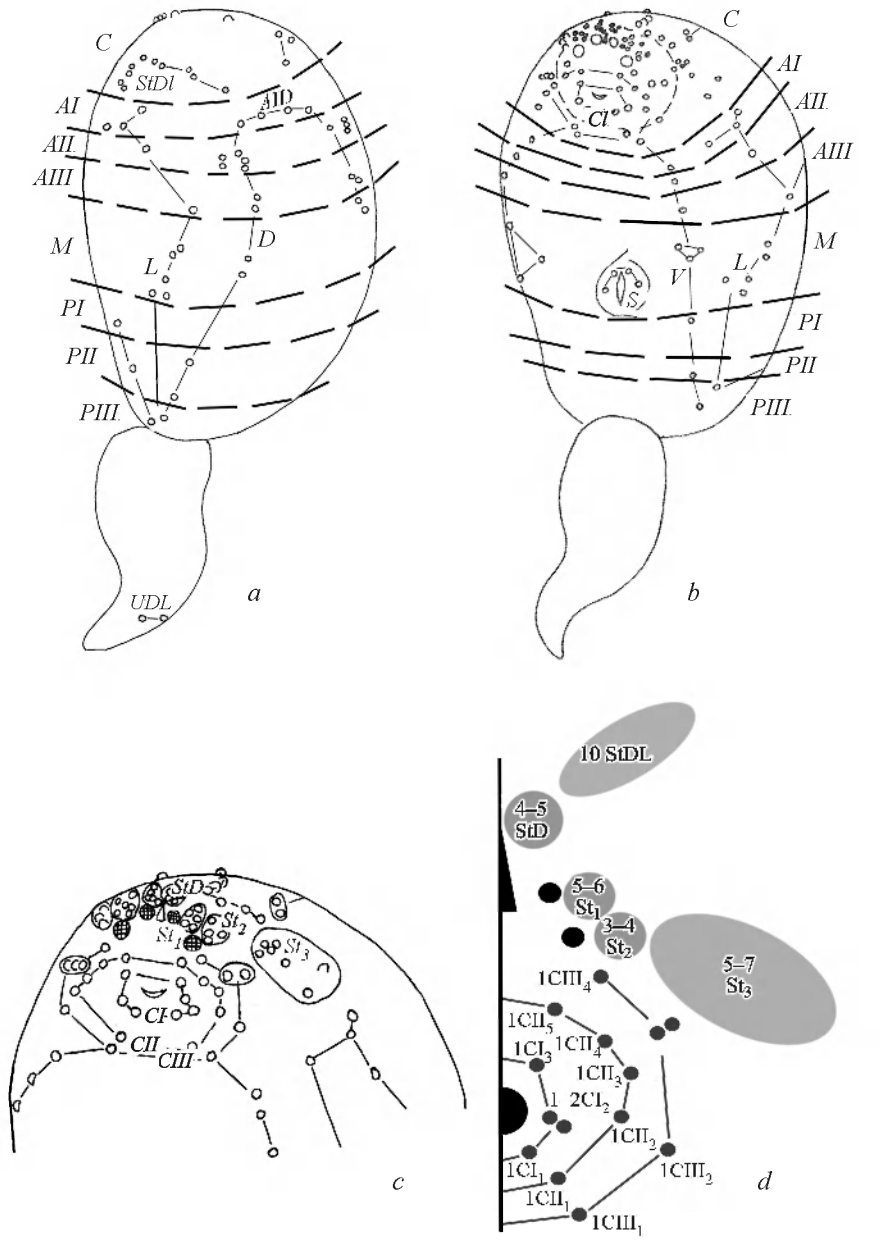

Fig. 3. Chaetotaxy of *Cercaria etgesii* larva nov.

a — dorso-lateral view, *b* — ventro-lateral view, *c* — sensilla on the anterior part of cercarial body, *d* — schematic drawing of groups of sensilla of the anterior part of cercarial body.

form several loops and fuse into the excretory bladder. The excretory bladder is V-shaped (fig. 1). Cytoplasm of its walls is granular. Genital primordium is triangular. It consists of two lobes. «Parenchyma» lacks visible fat droplets.

Chaetotaxy (fig. 3). Cycles CI, CII and CIII consist of small number of sensilla: 6—7 CI, 10 CII, 10—11 CIII. Groups of stylet region: 10 StDL, 4—5 StD,

Measurements of *Cercaria etgesii*, mm

Measurements	Size (min—max)	Mean size (M)	Mean-square deviation (S_x)	Coefficient of variation (C_v), %
Body length	0.997—1.219	1.075	0.073	0.068
Body width	0.042—0.058	0.053	0.005	0.091
Length of tail	0.029—0.043	0.037	0.004	0.118
Diameter of oral sucker	0.027—0.037	0.030	0.003	0.098
Diameter of acetabulum	0.013—0.019	0.016	0.002	0.119
Length of stylet	0.017—0.019	0.018	0.001	0.046

5—6 St_1, 3—4 St_2, 5—7 St_3. Ventral sensilla of body region are 1 AIV, 1 AIIV, 1 AIIIV, 3 MV, 1 PIV, 1 PIIV, 1 PIIIV. Lateral sensilla of body region are 3 AIL, 1AIIL, 1 AIIIL, 5 ML, 1 PIIIL. AID raw consists of 4 sensilla (4 AID). Dorsal sensilla of body region are 4—5 AIID, 2 AIID, 2 MD, 2 PIID, 1 PIIID. 4 sensilla are located on the anterior half of acetabulum (4S) and 2 UDL is present near the tip of tail.

Thus, argentophilic structures are absent in the majority of the area of the dorsal surface, being concentrated in the anterioventral part of the body.

DISCUSSION

Etges (1960) worked with similar cercariae in 1950's. He revealed that cercaria he had described belongs to *Prosthodendrium (Acanthatrium) anaplocami*. In the «Keys to the trematoda» (Bray et al., 2008), the genus *Prosthodendrium* Dollfus, 1931 is treated as the synonym of the genus *Paralecithodendrium* Odhner, 1911. Nevertheless we keep the traditional point of view and consider *Prosthodendrium* as a valid genus.

Etges (1960) found only three pairs of penetration glands in *P. anaplocami*. *C. etgesii* has 4 pairs of penetration glands, but the last (caudal) pair of glands is too small and poorly marked if the additional staining is absent.

The excretory formulae of *P. anaplocami* and *C. etgesii* are different. Etges (1960) reported that cercariae of *P. anaplocami* had 20 flame cells: 2[(2 + 2 + + 2) + (2 + 2)] = 20. There is one possible type of excretory formula among lecithodendrioid cercariae — 6 primary groups of flame cells (Galaktonov, Dobrovolskii, 2003). So it is more likely that the description of the excretory system of *P. anaplocami* cercaria is inaccurate.

Sensilla are very numerous and lie very close to each other on the ventral surface of the anterior part of the body. The chaetotaxy of *C. etgesii* is similar to that of *Pleurogenoides medians* (Richard, 1971). The most significant differences are: the presence of 6 CI in *C. etgesii* and 4 CI in *P. medians*, the lack of separated CII and CIII in *C. etgesii*, the presence of 6 AID in case of *P. medians* and 6—7 AID in *C. etgesii*, the presence of 10 StDL sensilla in *C. etgesii* and numerous unidentified StDL group in *P. medians*, the presence of 4 sensilla in S of *C. etgesii* and 6 to 8 sensilla in *P. medians*.

The only apparent similarity between cercaria of *P. anaplocami* and that of *C. etgesii* is the organization of the pear-shaped organ. The illustration of *P. anaplocami* cercaria is the synthesis of images of cercariae body parts from

different perspectives. The stylet is drawn in the horizontal plane, but the virgula organ is pictured as it lookes from the front end of cercaria (the characteristic condition of pear-shaped organ at the moment of maximal extension of buccal cavity is shown).

According to morphological features, *C. etgesii* is a larva of trematodes of the family Pleurogenidae.

References

Cort W. W. 1914. Larval trematodes from North American Freshwater snails. The Journal of Parasitology. 1 (1): 65—84.

Etges F. J. 1960. On the life history of *Prosthodendrium* (*Acanthatrium*) *anaplocami* n. sp. (Trematoda: Lecithodendriidae). The Journal of Parasitology. 46 (2): 235—240.

Galaktionov K. V., Dobrovolskij A. A. 2003. The biology and evolution of trematodes: an essay on the biology, morphology, life cycles, transmissions, and evolution of digenetic trematodes. London. Kluwer Academic Publishing. 594 p.

Ginetsinskaya T. A., Dobrovolskij A. A. 1963. A new method of the detection of sensilla of trematode larvae and its meaning for systematics. DAN SSSR. 151 (2): 460—463. [In Russian].

Bray R. A., Gibson D. I., Jones A. 2008. Keys to the Trematoda. Volume 3. London, CABI Publishing and the Natural History Museum. 805 p.

Kruidenier F. J. 1947. What is a virgula cercariae. The Journal of Parasitology. 33 (2): 12—13.

Kruidenier F. J. 1951. The formation and function of mucoids in Virgulate Cercariae including a study of virgula organ. The American Midland Naturalist Journal. 46 (3): 660—683.

Lühe M. 1909. Parasitische Plattwürmer. I. Trematodes. Süsswasserfauna Deutschlands. Jena. 17 (2): 1—217.

Manafov A. A. 2010. Parthenites and cercariae of trematodes of the mollusc *Melanopsis praemorsa* (L., 1758) in the Middle Kura river in Azerbaijan. Baku, NURLAR. 260 p. [In Russian]

Richard J. 1971. La chétotaxie des cercaires: valeur systématique et phylétique. Mémoires du Museum national d'histoire naturelle. 67: 1—179.

Sewell R. B. S. Cercariae Indica. Indian Journal of Medical Research. 10 (1): 1—370.