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NEW VIRGULID CERCARIA (TREMATODA, LECITHODENDROIDEA) FROM FRESHWATER MOLLUSK MELANOPSIS PRAEMORSA (MELANOPSIDAE) FROM AZERBAIJAN WATER BODIES. MORPHOLOGY OF *CERCARIA AGSTAPHENSIS 27*

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New Virgulid Cercaria (Trematoda, Lecithodendroidea) from Freshwater Mollusk *Melanopsis praemorsa* (Melanopsidae) from Azerbaijan Water Bodies. Morphology of *Cercaria agstaphensis 27*. Manafov A. A. — Illustrated morphological description and differential diagnosis of a new virgulid cercaria, *Cercaria agstaphensis 27,* from the freshwater prosobranchial mollusk *Melanopsis praemorsa* (Linneus, 1758) are given. Special attention is paid to the arming of tegument, the structure of glandular apparatus, excretory system, digestive system and other individual morphological characters important for taxonomy.

Key words: virgulae, *Melanopsis praemorsa, Cercaria agstaphensis.*

Новые виргулидные церкарии (Trematoda, Lecithodendroidea) пресноводного моллюска *Melanopsis praemorsa* (Melanopsidae) из водоемов Азербайджана. Морфология *Cercaria agstaphensis 27*. Манафов А. А. — Приведены рисунки, описание морфологии и дифференциальный диагноз новой виргулидной церкарий, *Cercaria agstaphensis 27* (Trematoda: Lecithodendroidea) из пресноводного переднежаберного моллюска *Melanopsis praemorsa* (Linneus, 1758). Особое внимание уделено вооружению тегумента, строению железистого аппарата, экскреторной системе, пищеварительной системе и другим морфологическим особенностям индивидуального строения церкарий, имеющим важное таксономическое значение.

Ключевые слова: виргула, *Melanopsis praemorsa, Cercaria agstaphensis.*

Introduction

Mollusks *Melanopsis praemorsa* (Linneus, 1758) are rather common in Azerbaijan. However, until the time of our researches they have never been the object of parasitological studies. Serious theoretical and practical importance of such a study determined the overall goal of our work — the comprehensive study of trematode fauna, parthenits and cercariae of which develop in freshwater mollusks *Melanopsis praemorsa* (Melanopsidae, Mesogastropoda — «Prosobranchia») in this country. Since 1982, the study of cercariae and parthenits fauna of the freshwater mollusks *Melanopsis praemorsa* of Azerbaijan revealed that melanopsid trematodofauna is surprisingly rich and diverse, has unique composition and not comparable to the fauna of trematodes parasitizing the lung mollusks and common prosobranchias of temperate zone — Bithynia, Valvata, Viviparous mollusks. This fauna includes a number of species potentially and actually pathogenic for humans and animals.

In the first article, the summary on some results of our researches (1982–2008) was presented along with pictures and descriptions of *Cercaria agstaphensis 11* morphology and chaetotaxy. Particularly, there were some notes on parthenitae and cercariae fauna of trematodes from the freshwater mollusk *Melanopsis praemorsa*, short systematic review of the species found. There we stated that in mollusks *Melanopsis praemorsa* from reservoirs in Azerbaijan, 41 species of trematode cercariae were found, of which 33 were studied and described for the first time, and 2 cercaria species were redescribed.

The vast majority of species found (23) belong to group Xiphidiocercariae or stiletto cercariae (order Plagiorchiida). Of them 21 species belong to the morphological group Virgulae (superfam. Lecithodendroidea), and 2 — without virgule − to the group Microcotylae. Order Heterophyida is represented by 7 species, order Schistosomatida — 2 species (fam. Sanguinicolidae — 1; fam. Schistosomatidae — 1), order Strigeidida — 5 species (suborder Cyathocotylata — 4, and suborder Strigeata — 1 species). Families Echinostomatidae, Notocotylidae and Philophthalmidae are represented by 1−2 species each. Many resistant nidi of metagonimosis, heterophyasis, opisthorchiasis, haplorchiasis, notocotylosis were revealed, and also presents potential for the outbreaks of schistosomiasis (as dermatitis form), phyilophthalmosis, etc.

Cercaria agstaphensis 27, larva described in this communication, is one of the most widespread in Azerbaijan lecithodendroid cercaria.

Material and methods

Mollusks were collected from 1982 to 2008 in different water bodies of Azerbaijan (rivers Kura, Akstafachay, Dzhogaz, Kyurekchay; reservoirs Akstafa, Mingechaur, Varvara, Shemkir, Enikend; streams, springs, artesian waters, canals and other waterways of the South Slope of the Greater Caucasus and the north-eastern slope of the Lesser Caucasus). Totally, we have examined 96,718 mollusks. In the process we have found cercariae of 41 trematode species belonging to at least 11 families.

To identify infected animals, collected mollusks were placed one by one into 25 cm^3 glass vessels filled with water for 12−24 hours or more. Mollusks with cercariae were selected under dissection microscope MBI−1. Morphology of parthenitae, cercariae and metacercariae was studied on living material of fully mature specimens. For this purpose, dissection microscopes MBI−3, MBI−15 with phase contrast PC−4 were used. All drawings were made with the aid of drawing tubes RA−4 and RA−7. To reveal sensillae in cercariae, traditional method of silver nitrate impregnation was used (Ginetsinskaya, Dobrovolsky, 1963) as well as its various modifications (Alekperov, Manafov, 1995). To analyze the chaetotaxy, Richard nomenclature (1971) was used with additions of Bayssade-Dufour (1979).

Measurements of parthenitae and larvae were carried out on material fixed in 4% formalin, and 3% silver nitrate solution. In each case, the measurement was made on 15 larvae.

The measurements were processed statistically (Plokhinsky, 1978): arithmetical mean (M), standard deviation (G), and coefficient of variation (CV) were calculated (Plokhinskiy, 1978). The error of invasion prevalence (m_p) was calculated for every water body (Petrushevski, Petrushevskaya, 1960).

First described species were assigned with code name *Cercaria agstaphensis* with corresponding serial numbers by name of Akstafachay River. One species found in Kura River was named as *Cercaria kurensis.*

Description of *Cercaria agstaphensis 27*

Cercaria has oval body (fig. 1, *a, b*). The tail is broad and massive. When fixed, its length is somewhat more than 1/2 of larval body length (table).

Oral sucker is big. Ventral sucker is much less and shifted a little to the posterior end of the body. Its external opening is triangular and elongated in longitudinal direction.

Integument of the larval body is armed with small spines. Internal surface of the ventral sucker also bears small spines. Tail armament is unusual. The front two thirds

Table 1. Measurements of *Cercaria agstaphensis 27*, mm

Таблица 1. Размеры *Cercaria agstaphensis 27*, мм

Parameters	Size (min-max)	Median size (M)	Mean quadratic deviation (G)	Coefficient of variation (CV)
Body length	0.087−0.101 (0.091−0.096)	0.097 (0.093)	0.004 (0.002)	4.12 (2.15)
Body width	0.070−0.078 (0.065−0.072)	0.072 (0.069)	0.003 (0.002)	4.17 (2.90)
Tail length	0.049−0.096 (0.049−0.061)	0.059 (0.055)	0.013 (0.003)	22.03 (5.45)
Diameter of buccal sucker	0.031−0.034 (0.027−0.029)	0.033 (0.028)	0.002 (0.001)	6.06 (3.57)
Diameter of ventral sucker	0.018−0.022 (0.018−0.020)	0.021 (0.020)	0.001 (0)	4.76 (0)
Stiletto	0.009−0.010 (0.009−0.010)	0.010 (0.010)	0 (0)	0 (0)

Note. Measurements of larvae fixed in 4% formalin are given without brackets, and in parentheses are measurements for larvae fixed 3% silver nitrate.

Примечание. Результаты измерения личинок фиксированных в 4%-ном формалине даны без скобок, в скобках — результаты измерения личинок, фиксированных в 3%-ном нитрате-серебра.

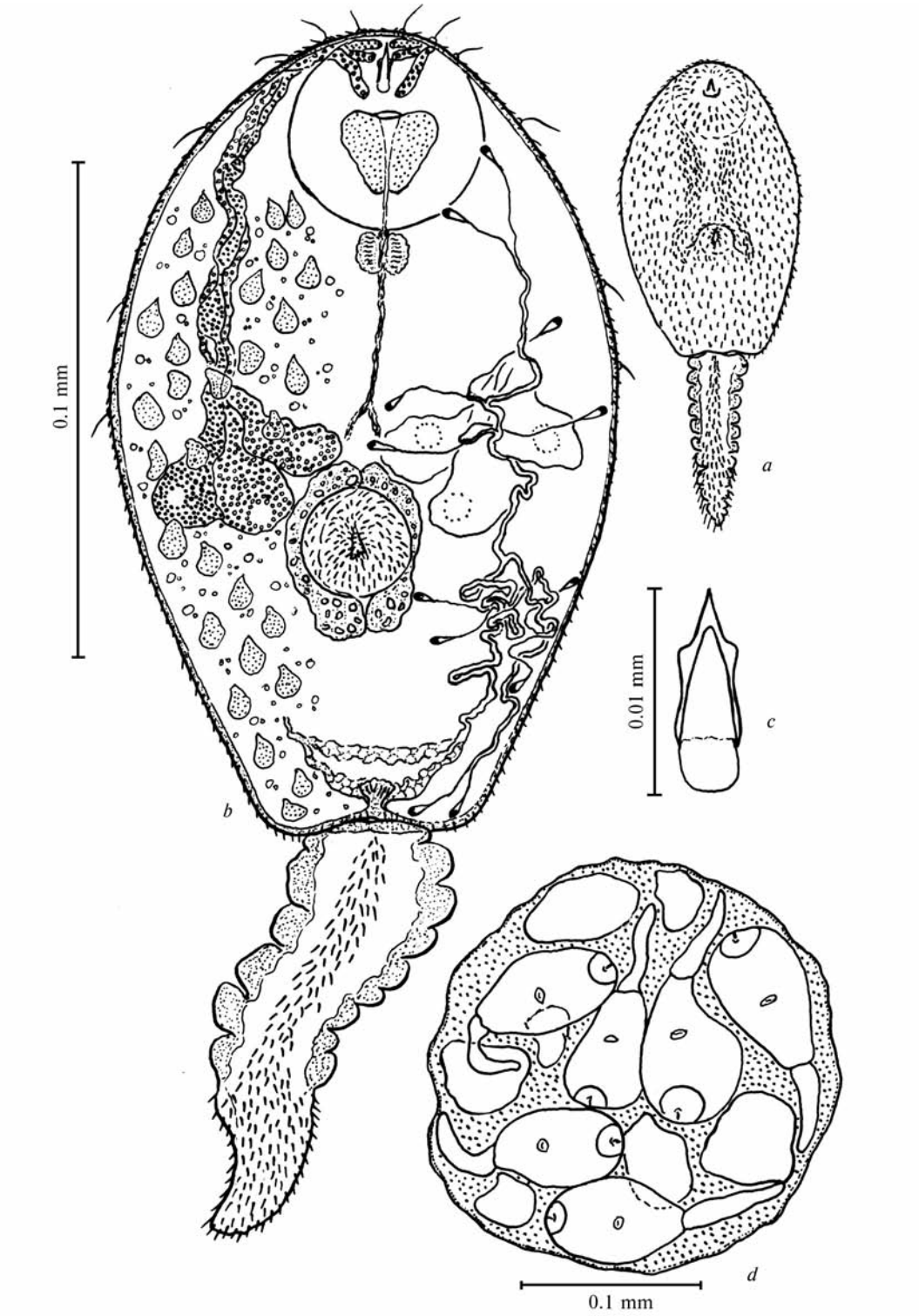

Fig. 1. *Cercaria agstaphensis 27*: *a* — larval armament; *b* — composition of cercaria; *c* — stiletto; *d* — sporocyst.

Рис. 1. *Cercaria agstaphensis 27*: *a* — вооружение личинки; *b* — строение церкарии; *c* — стилет; *d* — спороциста.

of the tail bear two relatively narrow bands of spines located dorsally and ventrally, respectively. The distal third is armed with spines through the whole surface. From the front part of the tail towards its end the length of spines is markedly increased.

Oral sucker is armed with powerful thin-walled stiletto with length of less than 1/3 diameter of sucker. Stiletto shoulders are well developed. Stiletto stalk gradually expands posteriorly ending with large bulb (fig. 1, *c*). Stiletto length is stable, but the shape of shoulders and stalk are varied a little.

Round mouth opening is subterminal. It leads to narrow, gradually tapered cavity with walls forming germinal virgule of simple construction. The virgule looks like thick-walled hyaline cone surrounding buccal cavity and has no lobes with space inside. Prefarinx is not visible.

Poorly developed pharynx is closely adjacent to the oral sucker. Thin-walled oesophagus is narrow and relatively long. Intestinal bifurcation is at the level of anterior border of the first pair of penetration glands. Intestines are not developed. Only the initial parts of its branches can be traced.

Penetration glands are three pairs of cells of the same size. The first pair is situated entirely over the ventral sucker. The second and third pairs are at the level of its front half. Position of penetration glands is fairly constant, and with a strong cercarian body constriction the lateral pair of glands is significantly shifted ahead and reaches the first pair of glands.

Two pairs of penetration glands, laying median, contain coarse secret with larger granules slightly refracting light. Thus these cells appear to be more transparent than the laterally located cells of the third pair (fig. 1, *b*). The secret of the latter strongly refracts light, although their size do not differ from secretion granules of the first two pairs of cells.

Ducts of penetration glands are directed to stiletto as two lateral beams and dorsolaterally bend the oral sucker round. Ducts of median glands open at stiletto shoulders, and lateral cells — at its base.

Ducts of the lateral pair form several nodules (reservoirs) on their length. Ducts of the median pair are much narrower.

The whole cercarian body is filled with large transparent cystogenic cells with small secret granules.

Excretory formula: 2 [(2+2+2)+(2+2+2)] = 24. Longitudinal collective channels merge and give rise to the main collective channel at the middle of ventral sucker. The main collective channels form several loops and flow into the side branches of the bladder. Bladder is widely U-shaped with short branches, thick-walled. Its outer surface is smooth while the inner surface has numerous irregular protrusions. Distal part of bladder is formed by continuation of cercarian surface tegument and looks like a funnel. Anterior margin is slightly scalloped. Excretory pore opens at the base of tail.

Sexual bud is big, poorly differentiated, and composed of two almost equal in size C-shaped areas, enveloping ventral sucker dorsally.

Larval parenchyma has many fat droplets.

Cercariae develop into round or oval sporocysts of very different size (fig. 1, *d*). Length of sporocysts is 0.132–0.374 mm, width — 0.110–0.198 mm. Each sporocyst contains 4–7 mature cercariae and the same number of embryos at different stages of development.

Discussion

Due to profound difficulties while establishing the taxonomic status of any virgulid cercaria and taking into account their alleged reasons, in this paper we cover all essential cercarian details facilitating the compilation of complete morphological characteristics of our findings.

From all known and, moreover, adequately described virgulid cercariae (that seems needed to be particularly emphasized) (Sewell, 1922; Hall, 1959, 1960; Hall, Groves, 1963; Seytner, 1945) with three pairs of penetration glands, *C. agstaphensis 27* differs by shape, size and virgule structure, as well as very unusual tail armament.

The shape of stiletto and the tail armament of *C. agstaphensis 27* are very close to those of *C. agstaphensis 32* and *C. agstaphensis 36* larvae in our description (Manafov, 1990, 2009). However, differences in size, especially in ratio of stiletto length to the body length are especially significant. Moreover, these larvae vary greatly by the degree of virgule development: in *C. agstaphensis 27* the ratio of larval length to stiletto length is about 1 to 9, and in *C. agstaphensis 32* and *C. agstaphensis 36* it is approximately 1 to 6. Besides, *C. agstaphensis 36* is almost 1.5 times larger and has only germinal, weakly expressed, virgule.

C. agstaphensis 27 and *C. agstaphensis 32* are very close in appearance and armament characteristics (especially unusual tail armament). Today, however, it is impossible to identify these two larval forms until decoding their life cycle. These differences are not great, but fairly stable. First of all, these cercariae have different structure and size of stilettos. Despite variable shape of shoulders, length of stilettos is constant: stiletto in *C. agstaphensis 27* is noticeably shorter than that in *C. agstaphensis 32.* Also, overall size of larvae is different. The differences in location of penetration glands are quite stable: in *C. agstaphensis 27* they never form longitudinal series normally seen in actively crawling larvae of *C. agstaphensis 32.* Cystogenic cells in *C. agstaphensis 27* are relatively small, normally oval or drop-shaped, with no visible nuclei. In *C. agstaphensis 32* they are large without definite shape, and nuclei are almost always clearly visible. Besides, *C. agstaphensis 27* has a thin and long oesophagus with bifurcation at the middle of larval body, directly at the anterior edge of penetration glands cells. *C. agstaphensis 32* has not developed digestive system, the beginning of a short oesophagus is only seen. By maturity of the digestive system, *C. agstaphensis 36* is very close to *C. agstaphensis 27.* However, the latter has entirely different nature of armament of body and tail, and has germinative virgule only. Parthenitae of the larvae described are also substantially different in shape, size, number of cercariae and embryos.

Today it is difficult to estimate the rank of the above-mentioned differences between these two trematode larvae: whether they are species, or there are two morphs of one species. Until we have no clear answer to this question, we would like to consider these two types of larvae as separate species.

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