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NEW VIRGULID CERCARIA (TREMATODA, LECITHODENDROIDEA) FROM THE MOLLUSK *MELANOPSIS PRAEMORSA* (MELANOPSIDAE) FROM AZERBAIJAN WATER BODIES. MORPHOLOGY AND CHAETOTAXY OF CERCARIA *AGSTAPHENSIS 11*

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New Virgulid Cercaria (Trematoda, Lecithodendroidea) from the Mollusk *Melanopsis praemorsa* (Melanopsidae) from Azerbaijan Water Bodies. Morphology and Chaetotaxy of *Cercaria agstaphensis 11*. Manafov A. A. — An illustrated morphological description with chaetotaxy and differential diagnosis of the new form of virgulid cercaria — *Cercaria agstaphensis 11* from freshwater prosobranchial mollusks *Melanopsis praemorsa* (Linnaeus, 1758) are given. Special attention is paid to the tegument armament, the structure of glandular apparatus, excretory system, digestive system, the structure of the sensory apparatus and other larval morphological characters.

Key words: *Melanopsis praemorsa, Cercaria agstaphensis,* virgule, chaetotaxy.

Новые виргулидные церкарии (Trematoda, Lecithodendroidea) моллюска *Melanopsis praemorsa* (Melanopsidae) из водоемов Азербайджана. Морфология и хетотаксия *Cercaria agstaphensis 11*. Манафов А. А. — Приведены рисунки, описание морфологии, в том числе и хетотаксии, а также дифференциальный диагноз новой виргулидной церкарии — *Cercaria agstaphensis 11* из пресноводного переднежаберного моллюска *Melanopsis praemorsa* (Linnaeus, 1758). Особое внимание уделено вооружению тегумента, строению железистого аппарата, экскреторной системы, пищеварительной системы, строению сенсорного аппарата и других морфологических особенностей строения личинок.

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

Introduction

Host-parasite systems formed by mollusks and trematode parthenits are increasingly becoming now the basis for different population and environmental studies, one of the main directions in development of modern parasitology. Data on parthenits (sporocysts and rediae) and cercariae are of the same importance. It is impossible to create the phylogenetic system of Trematoda itself and Neodermata in general without such information. The faunistic works on morphological description of parthenits and cercariae with detailed analysis of chaetotaxy made by present-day requirements always were and remain the basis for studies of this kind. Despite the long traditions of researches on fauna of trematodes' larvae and parthenits, today their overall results are difficult to be considered as satisfactory. Fragmentary nature of our knowledge on mollusk parasites is mainly due to the fact that not all groups of mollusks were studied completely enough. The first objects of study were inhabitants of the temperate zone — pulmonary mollusks and limited number of prosobranchia mollusks (*Bithynia, Valvata, Viviparus*, etc.). At the same time trematode fauna of many groups of prosobranchia mollusks, especially the richest tropical and subtropical fauna, is studied scanty or even not investigated at all. From this point of view, the representatives of the ancient and archaic family Melanopsidae are of particular interest. *Melanopsis praemorsa* (fam. Melanopsidae) widespread in India, Central Asia and the Mediterranean — (Starobogatov, 1970; Izzatullaev, Starobogatov, 1984) remained practically unexplored.

Works done in the 1970th in small area in the Rhioni valley in the Western Georgia established surprising richness and diversity of *M. praemorsa* parasitic fauna, distinctive in its composition, and including a number of human and animal pathogens (Olenev, Dobrovolsky, 1972; Olenev, Dobrovolsky, 1975; Olenev, 1979). According to personal communication, A. A. Dobrovolsky found 18 species of cercariae in *Melanopsis praemorsa* belonging to, at least, 10 trematode families. However, only 7 species were identified: *Philophthalmus rhionica*, which life cycle was studied in detail by I. A. Tikhomirov (1980); *Cercaria rhionica VII*-larval stage of a species from the genus *Echinochasmus*; two species of cyathocotylid cercariae; heterophyid larvae identified as *Metagonimus yokogawai* Takahashi 1929, larva referred to the family Sanguinicolidae and furcocercaria relating to the family Strigeidae, and, by complex of morphological characters, close to cercariae from the genus *Cotylurus*. Later other authors (Galaktionov, 1980; Galaktionov et al., 1980) made detailed analysis of cyathocotylid cercariae and showed that, along with *C. rhionica XI*, there are other, morphologically similar, forms not differentiated previously. Deciphering of life cycle showed that it was *Mesostephanus appendiculatus*. M. G. Javelidze and E. A. Chiaberashvili (1973) worked in the same region and described two species of stiletto cercariae. However, more or less accurate description is only for *Cercaria ginetsinskaja* relating to "virgulae" group.

For the territory of Azerbaijan, mollusks *Melanopsis praemorsa* are common. However, before our studies, they have never been the objects of parasitological researches. Serious theoretical and practical significance of such investigations determined the main plot of our work — comprehensive study of trematode parthenites and cercariae which develop in freshwater mollusks *M. praemorsa* (Melanopsidae, Mesogastropoda — Prosobranchia) on the territory of Azerbaijan. The study showed that fauna of trematodes from Melanopsidae was surprisingly rich and diverse, had unique composition and was not comparable with the fauna of trematods parasitic in lung mollusks and common prosobranchia of temperate zone — *Bithynia, Valvata, Viviparus*, etc. This group includes a number of species, potentially and actually pathogenic to humans and animals.

In mollusk *M. praemorsa* from Azerbaijan water bodies, cercariae of 41 trematode species were found, of which 33 were studied and described for the first time, cercariae of 3 species were redescribed (Manafov, 2010). The vast majority of species found (23) refers to Xiphidiocercariae group (order Plagiorchiida). Of these, 21 species belong to Virgulee morphological group (superfam. Lecithodendroidea), and 2 — without virgule — to Microcotyle group. Order Heterophyida is represented by 7 species, order Schistosomatida — by 2 species (family Sanguinicolidae — 1; family Schistosomatidae — 1), order Strigeidida — by 5 species (suborder Cyathocotylata — 4, and suborder Strigeata — 1 species). Families Echinostomatidae, Notocotylidae and Philophthalmidae are represented by 1 or 2 species each. In this article morphological description and differential diagnosis of *Cercaria agstaphensis 11* are given.

Material and methods

Mollusks were collected from 1982 to 2008 in different water bodies of Azerbaijan: rivers Kura, Agstafachay, Dzhogaz, Kyurekchay; reservoirs Agstafa, Mingechavir, 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 examined 96, 718 mollusks and found cercariae of 41 trematode species belonging to at least 11 families.

To identify infected animals, collected mollusk were placed separately 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 parthenits, cercariae and metacercariae was studied on alive material. For this purpose, dissection microscopes MBI—3, MBI—15 with phase contrast were used. All drawings were made with aid of the drawing apparatus RA—4. To reveal sensillae in cercariae, traditional method of silver nitrate impregnation (Ginetsinskaya, Dobrovolsky, 1963) and its modifications (Alekperov, Manafov, 1995) were used. To analyze the chaetotaxy Richard (1971) nomenclature was used with additions of Bayssade-Dufour (1979).

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

The measurements were processed statistically (Plokhinsky, 1978). The error of infection prevalence (m_p) was calculated for each water body (Petrushevski, Petrushevskaya, 1960).

First described species were assigned with code name *Cercaria agstaphensis* with corresponding serial numbers by name of Akstafachay river.

Description of *Cercaria agstaphensis 11*

Cercaria has leaf-shaped body. Toward the posterior end it gradually narrows. The tail is strongly contractile. After contraction it becomes unusually short and wide (table 1, fig. 1). In larvae fixed in formalin its length is 1/4 of the body length, in larvae fixed in silver nitrate — 1/3. Oral sucker is large. Its size is 1.5 times more the size of abdominal sucker. Ventral sucker noticeably shifted to the posterior end of the larval body. Oval opening of the ventral sucker is elongated transversely.

Table 1. Measurements of *Cercaria agstaphensis 11*
Таблица 1. Размеры *Cercaria agstaphensis 11*

Parameters	Size, mm (min—max)	Median size, mm (M)	Mean square deviation (G)	Coefficient of variation (CV)
Body length A	0.095—0.112 (0.091—0.095)	0.104 (0.092)	0.003 (0.002)	2.89 (2.17)
Body width B	0.065—0.073 (0.065—0.068)	0.070 (0.065)	0.002 (0.001)	2.86 (1.54)
Tail length	0.022—0.033 (0.035—0.039)	0.028 (0.036)	0.003 (0.001)	10.71 (2.78)
Diameter of buccal sucker	0.033—0.036 (0.033—0.037)	0.035 (0.035)	0.001 (0.001)	2.86 (2.86)
Diameter of ventral sucker	0.020—0.021 (0.020—0.021)	0.020 (0.020)	0.001 (0.001)	5.0 (5.0)
Stiletto (length, width)	0.016—0.018 (0.017—0.018)	0.017 (0.018)	0.001 (0.0003)	5.88 (1.67)

Note. Measurements of larvae fixed in 4% formalin and measurements of larvae fixed 3% silver nitrate in parentheses.

Примечание. В таблице без скобок приводятся результаты измерения личинок, фиксированных в 4%-ном формалине, а в скобках — в 3%-ном нитрате серебра.

Tegument of the larval body is armed with small spines of the uniform size across the surface. Spines covering the tail are somewhat longer.

Oral sucker is armed with powerful thin-walled stiletto with length of almost 1/2 of sucker diameter. Stiletto shoulders are well distinct. Stiletto stalk significantly expands and ends with large bulb.

Mouth opening is subterminal. It leads to the gradually tapered cavity with walls forming massive virgule with two deep constrictions. Buccal cavity leads into the short, well-defined prepharinx followed by weakly muscular pharynx. Oesophagus is narrow and long. Intestine is bifurcated at the level of anterior border of genital bud. Branches of intestine reach the level of the middle of ventral sucker only. Oesophagus and intestinal branches look like thin strands without lumen.

Three pairs of penetration gland cells form a wide arc rounding the ventral sucker in anteriorly (fig. 1, *b*).

Cells of the first pair of glands are shifted to the midline of the body. This becomes especially apparent when the anterior end of the larval body is elongated and the ventral sucker, not involved in this movement, ceases to divide glands.

Cells of the second and the third pairs of glands lie on either side of the sucker, one after another. Their position relative to the sucker varies depending on the extent and nature of the constriction of the larval body. The first and the third pairs of cells contain coarsely granulated secret, highly light refracting. The secreta of the second pair of cells is also coarsely granulated, but with different index of light refraction, therefore these glands appear to be lighter. Ducts of penetration glands go separately to the level of the middle of buccal sucker. Each duct along the whole its length forms several unusually large “tanks” looking like single cells filled with secretion (fig. 1, *b*). Not far from the front end of the body they form two bundles rounding oral sucker. Ducts of the first and the third pairs of glands open near the tip of the stiletto, and the second is closer to the middle.

Excretory formula: 2 [(2+2+2) + (2+2+2)] = 24.

Anterior and posterior longitudinal collective channels merge and give rise to the main collective channel at the middle of ventral sucker, laterally. The main collective channels form several loops and flow into the side branches of the bladder. Bladder is U-shaped. Cells forming its walls contain granular cytoplasm. Excretory pore opens at the base of the tail.

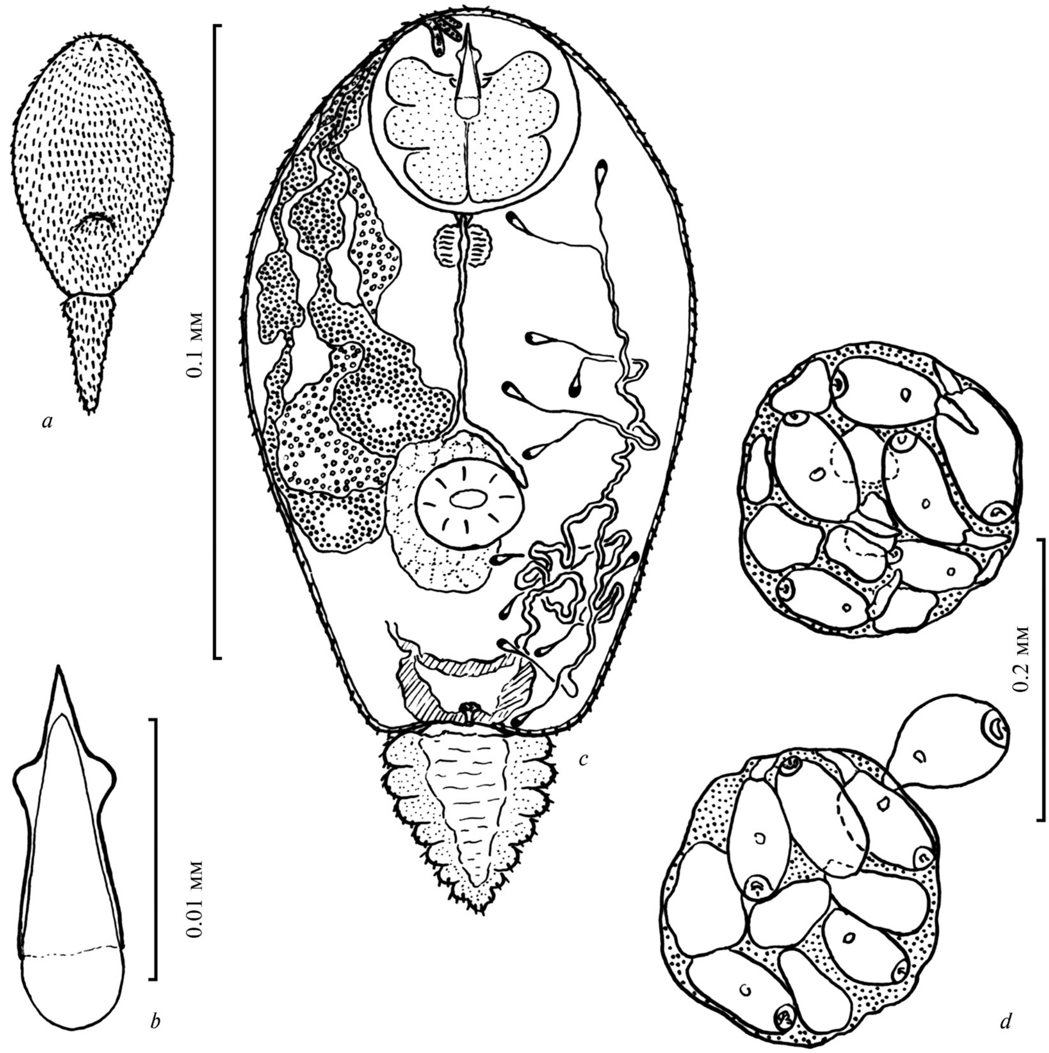

Fig. 1. *Cercaria agstaphensis 11*: *a* — general view and armament of tegument; *b* — scheme of cercaria structure; *c* — stiletto; *d* — sporocysts.

Рис. 1 *Cercaria agstaphensis 11*: *a* — общий вид и вооружение тегумента; *b* — схема строения церкарии; *c* — стилет; *d* — спороцисты.

Genital bud is undifferentiated and is composed of dense clumps of C-shaped cells. Regarding the ventral sucker it is located dorso-laterally.

Larval parenchyma is transparent, with no fat droplets.

Chaetotaxy (topography of the sensory apparatus) of the larva described (fig. 2, table 2) has a number of specific features. On the front end of the body, the fusion of StV and St_2 groups and formation of a single compact group StV + St_2 is observed. StDL group is represented by one zigzagging row. In AI area, L group is striking represented by three sensillae, and increased number of sensillae in the dorsal part (AID) where each additional group is represented by three sensillae (3+4+3). The rest of the dorsal surface bears very little sensillae. In AIIID area, only one pair is constant, while the second (fig. 2, *d*, marked by additional circles) are absent in most larvae. In AIIID area the only pair of sensillae is present. Beginning from M area, all other parts of the dorsal surface have no sensillae. Lateral rows are developed better. Beginning in AIL area, they lengthen to PIL area. On the ventral surface in preacetabular area sensillae

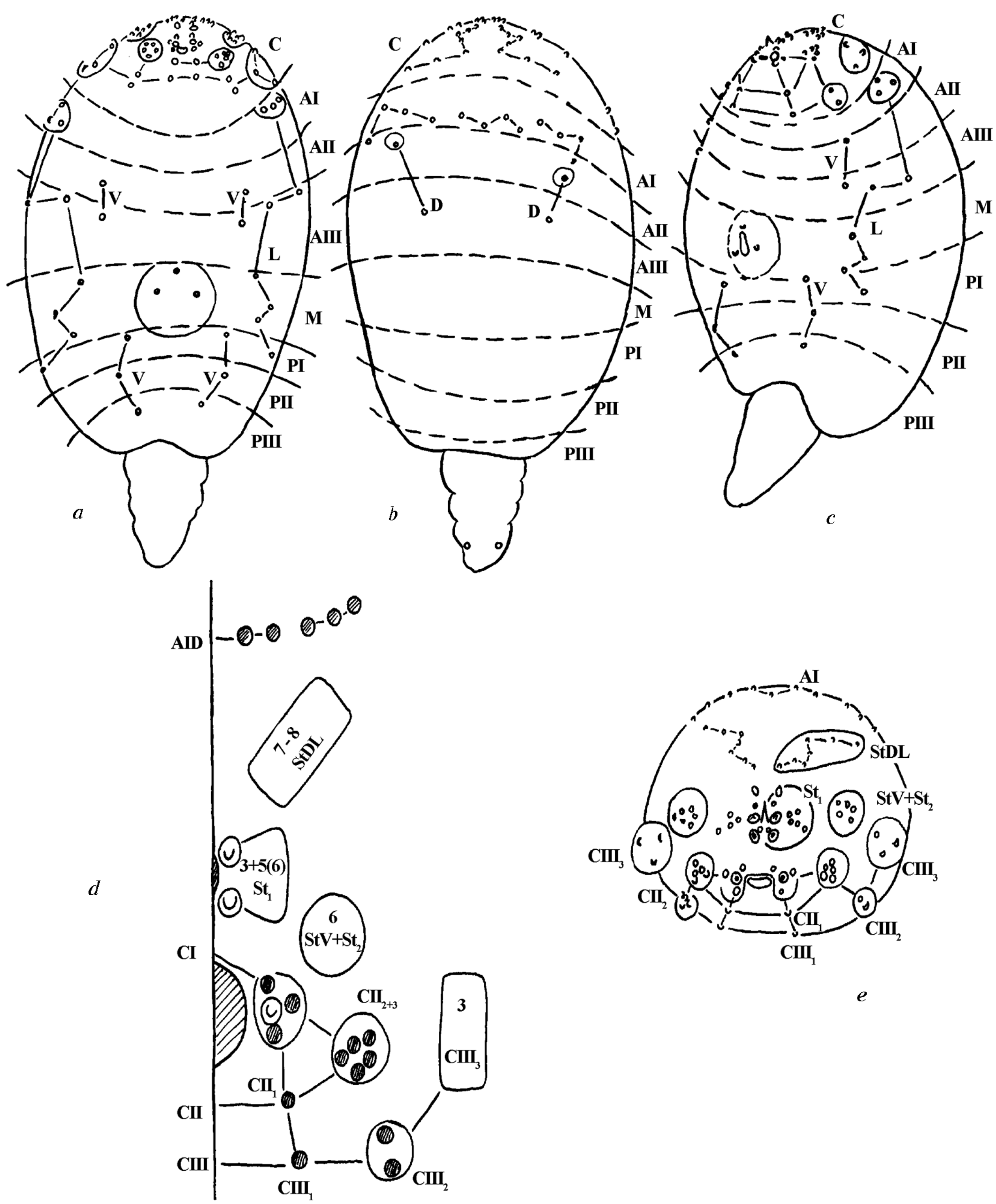

Fig. 2. Sensillae of *Cercaria agstaphensis 11*: *a* — ventral view; *b* — dorsal view; *c* — lateral view; *d–e* — scheme of sensilla structure in the anterior part of larvae.

Рис. 2. Сенсиллы *Cercaria agstaphensis 11*: *a* — вид с брюшной стороны; *b* — вид со спинной стороны; *c* — вид сбоку; *d–e* — схема строения сенсилл в головной части тела личинки.

are located in AIIV and AIIIV areas. In preacetabular area, only three pairs of sensillae are present.

The cercariae development is realized in thin walled sporocysts, which are oval or rounded (fig. 1, *d*). Sporocysts' size varies greatly. Sporocyst length is 0.099–0.253 mm, width — 0.066–0.132 mm. Number of sporocysts in the digestive gland of the mollusk can reach 2.000. Each sporocyst contains 4–7 formed cercariae and the same number of embryos at different stages of development.

Table 2. Description of sensillae arrangement in *Cercaria agstaphensis 11*
Таблица 2. Описание расположения сенсилл *Cercaria agstaphensis 11*

Head (fig. 2, *a, b, c*)
Area of the mouth opening:
3 CI DL (1 CI D + 2 CI L)
1 CII_1 ; 5 C II_{2+3}
1 $CIII_1$; 2 $CIII_2$; 3 $CIII_3$
Stiletto area:
6 StV + St_2 ; 3+5 (6) St_1
7 (8) StDL
Body (fig. 2, *a, b, c*)
- AI V ; 3 AI L ; 3+4+3 AI D
1 AII V ; - AII L ; 1 (2) AII D
1 AIII V ; 2 AIII L ; 1 AIII D
- MV ; 3 ML ; - MD
1 PI V ; 1 PI L ; - PI D
1 PII V ; - PII L ; - PII D
1 PIII V ; - PIII L ; - PIII D
3 (3/0) Act
Tail: 2 T

Discussion

Of all larvae found, *Cercaria agstaphensis 31* is the most similar to *Cercaria agstaphensis 11* having the same type of chaetotaxy, full armament of body and tail, and “light” cells of the second pair of penetration glands. Nevertheless, these larvae clearly differ in some characters. *C. agstaphensis 31* is significantly smaller, its first and third pairs of penetration glands are clearly distinguished by the type of secret granulation, and the cells of the second pair contain transparent hyaline secretion and are optically transparent. Significant differences are in excretory formula. These two larvae differ by the structure of stiletto and the presence of small fat droplets in parenchyma of *C. agstaphensis 31.*

In the literature some other larvae characterized by presence of “light” large cell of penetration glands are also described. Primarily, this is *C. indica XXXVII* Sewell, 1922 that differs from *C. agstaphensis 11* by larger size, slightly different ratio of the size of penetration glands. Moreover, the larvae described by Sewell (1922) are characterized by completely different ratio of stiletto length and oral sucker diameter (1 : 3) compared to that in *C. agstaphensis 11* (1 : 2).

Cercaria notura, described by Hall (1960) from *Goniobasis livescens* (family Pachychilidae) differs from *C. agstaphensis 11* by larger size and excretory formula. The same also applies to *Cercaria pinguisoma* (Hall, 1960). However, the virgule in *C. agstaphensis 11* is much larger than that in *C. pinguisoma*. *Cercaria pixiceps*, similar to *C. agstaphensis 11* by size and excretory formula, however, it differs markedly from the latter by the structure of the stiletto and in the nature of cell granulation of the first and the third pairs of penetration glands. Great number of characters allows us to distinguish *C. agstaphensis 11* from *C. apatema* described by Hall and Groves (1963).

Cercaria celatoglandis (Hall, 1960) differs from *C. agstaphensis 11* by excretory formula, relatively small virgule and very small cells of the second pair of penetration glands.

Consequently, we could not assign the larva described from Azerbaijan water bodies to any of previously described species, therefore we consider *C. agstaphensis 11* an independent species.

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