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## LIFE CYCLE OF THE TREMATODE *NEODIPLOSTOMUM ORIOLINUM* (DIPLOSTOMATIDAE) UNDER NATURAL CONDITIONS IN PRIMORYE REGION (RUSSIA)

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**Жизненный цикл трематод *Neodiplostomum oriolinum* (Diplostomatidae) в условиях Приморского края (Россия). Беспрозванных В. В.** — Приведено описание жизненного цикла и стадий развития трематод *Neodiplostomum oriolinum* Oschmarin, 1963. Экспериментально установлено, что в условиях Приморского края роль первого промежуточного хозяина трематод выполняют моллюски *Polypylis semiglobosa* и *Helicorbis suffunensis*, а второго — головастики лягушки *Rana dybowskii*. Половозрелые черви выращены у цыпленка и крысы. На основании сравнительного анализа морфологических характеристик выращенных в ходе эксперимента половозрелых червей, а также описаний *N. oriolinum* и *N. paraoriolinum* Oschmarin, 1963 высказано мнение о том, что *N. oriolinum* и *N. paraoriolinum* являются синонимами.

**Ключевые слова:** *Neodiplostomum oriolinum*, церкария, метацеркария, марита, жизненный цикл.

**Life Cycle of the Trematode *Neodiplostomum oriolinum* (Diplostomatidae) under Natural Conditions in Primorye Region (Russia). Besprozvannykh V. V.** — The life cycle and developmental stages of the trematode *Neodiplostomum oriolinum* Oschmarin, 1963 are described. It was experimentally established that under natural conditions of Primorye Region, the first intermediate hosts of this trematode are snails *Polypylis semiglobosa*, *Helicorbis suffunensis*, and the second are tadpoles of *Rana dybowskii*. Adult worms were developed in chicken and rat. Based on comparative morphological analysis of adult worms developed under experimental conditions and data given in P. G. Oshmarin (1963) paper it is assumed that *N. oriolinum* and *N. paraoriolinum* Oschmarin, 1963 are synonyms.

**Key words:** *Neodiplostomum oriolinum*, cercaria, metacercaria, adult worms, life cycle.

### Introduction

In 2002, in mollusks *Polypylis semiglobosa* Dvorjadkin, 1980 and *Helicorbis suffunensis* Starobogatov, 1957 collected in water body from Vladivostok city, we found trematode parthenits and cercariae from family Diplostomatidae Poirier, 1886. It was experimentally established that these trematodes belong to the species *Neodiplostomum oriolinum* Oschmarin, 1963.

### Materials and methods

Spontaneously infested freshwater lung mollusks *Polypylis semiglobosa* (100 specimens examined) and *Helicorbis suffunensis* (120 specimens examined) served as working materials. Life cycle of these worms was studied in laboratory at water temperature 18–22°C. Cercariae behaviour was observed on individuals placed in Petri dishes with high boards. To establish a range of the second intermediate hosts, mollusks *Lymnaea* sp., *Anisus centrifugops* Prosorova et Starobogatov, 1997, *Boreoelona ussuriensis* (Ehrmann, in: Buettner et Ehrmann, 1927), fishes *Perccottus glehni* Dybowski, 1877 (10 spec. each) and tadpoles of *Rana dybowskii* Guenther, 1876 (15 spec.) were placed to mollusk *H. suffunensis* releasing furcocercariae. All animals were grown up in laboratory or caught in a water bodies without any source of invasion (to control, 50% animals used in experiment were autopsied). Adult trematodes were developed in incubatory chicken and albino rat. Marites were processed as whole mounts and stained with aluminous carmine.

Parthenits, cercariae and metacercariae were measured being alive, and cercariae were preliminary immobilized. Sensillas on cercaria body were revealed by T. A. Ginetsinskay and A. A. Dobrovolskiy (1963) technique.

*Neodiplostomum oriolinum* Oschmarin, 1963

First intermediate hosts: Planorbidae, *Helicorbis sujfunensis* and *Polipylys semiglobosa*. Prevalence of infection is 14% and 7.5%, respectively.

Locality: water body in Vladivostok city.

Parthenita (5 spec. each). Threadlike sporocysts sized 0.72–1.24 : 0.056–0.112 mm (fig. 1, a). Maternity pore is terminal. Sporocysts contain cercariae on different stages of development. In large sporocysts, metacercariae of trematode *Cotylurus hebraicus* Dubois, 1934 were found in addition to cercariae (metacercariae were attributed to species by adult worms developed under experimental conditions). Invasion intensity was 1–3 metacercariae.

Cercaria (10 spec. each). Body 0.11–0.13 : 0.042–0.060 mm (fig. 1, b). Anterior part, up to the pharynx, is covered with spines. There are numerous grainy cells inside the body, from posterior end up to the oesophagus. Oral organ is 0.025–0.027 mm in diameter. There are short prepharynx, pharynx 0.011 : 0.013 mm and oesophagus. Intestine is bifurcated before ventral sucker. Intestinal branches are short, reach the

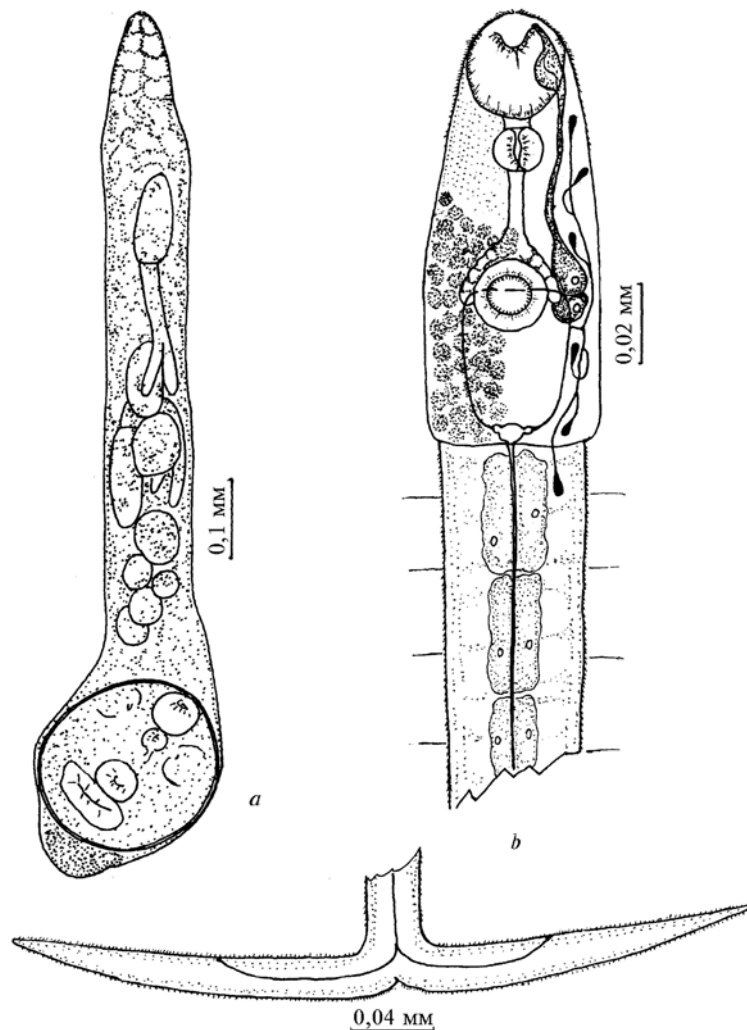


Fig. 1. *Neodiplostomum oriolinum*: a — sporocyst, b — cercaria.

Рис. 1. *Neodiplostomum oriolinum*: a — спороциста, b — церкария.

middle of ventral sucker. Ventral sucker is 0.017–0.020 mm in diameter, in 0.064–0.076 mm from anterior end of body. There are spines on internal side of the sucker, along its opening. Penetrative glands are two pairs of cells located on the level of ventral sucker. Their ducts open near oral opening. Sensory apparatus (fig. 2): CI = 1V<sub>0</sub>, 10V; CII = 5V, 4D; CIII = 7V, 2D, 3L; AI = 1V, 2D, 2L; AII = 1V, 2D, 3L; AIII = 1V, 2L; M = 1–2V, 1D, 3L; S = 6; on tail stem, 6 sensilles in median row, and 17–18 sensilles in each lateral row; on furcae, 6 sensilles in median row, and 8–9 sensilles in each lateral row. Excretory system includes small excretory bladder, the first-order ducts connected by transversal commissure near abdominal sucker, the second-order ducts and caudal duct. The latter penetrates the tail stem and, before furcae, splits in two ducts reaching the half of furcas length where opened in pores. Excretory formula is  $2[(1+1+1)+(1+1+[1])] = 12$ . Tail stem is 0.22–0.26 : 0.035–0.045 mm, contains 7 pairs of caudal cells, has sensible hairs and, together with furcae measures 0.20 : 0.02–0.03 mm, is covered with small spines.

The second intermediate host: *Rana dybowskii*. Prevalence of invasion under natural conditions is 100% (n = 30), intensity is 25–50 metacercariae; in experiment (n = 15) it is 100% and 10–25 metacercariae, respectively.

Localization: body cavity.

Metacercaria (10 spec. each). Body is grey due to pigmentation, consists of two segments: large anterior measures to 0.39–0.41 : 0.32–0.41 mm, and small posterior (fig. 3, c). Anterior segment is leaf-shaped, with ventral hollow. Oral sucker measures 0.056–0.067 : 0.045–0.067 mm, prepharynx is absent, pharynx is 0.028–0.034 : 0.022–0.039 mm, oesophagus is 0.07 mm long. Intestine is bifurcated in the middle between pharynx and ventral sucker. Thin intestinal branches reach the posterior margin of Brandes' organ. Ventral sucker is 0.039–0.050 mm in diameter, situated at

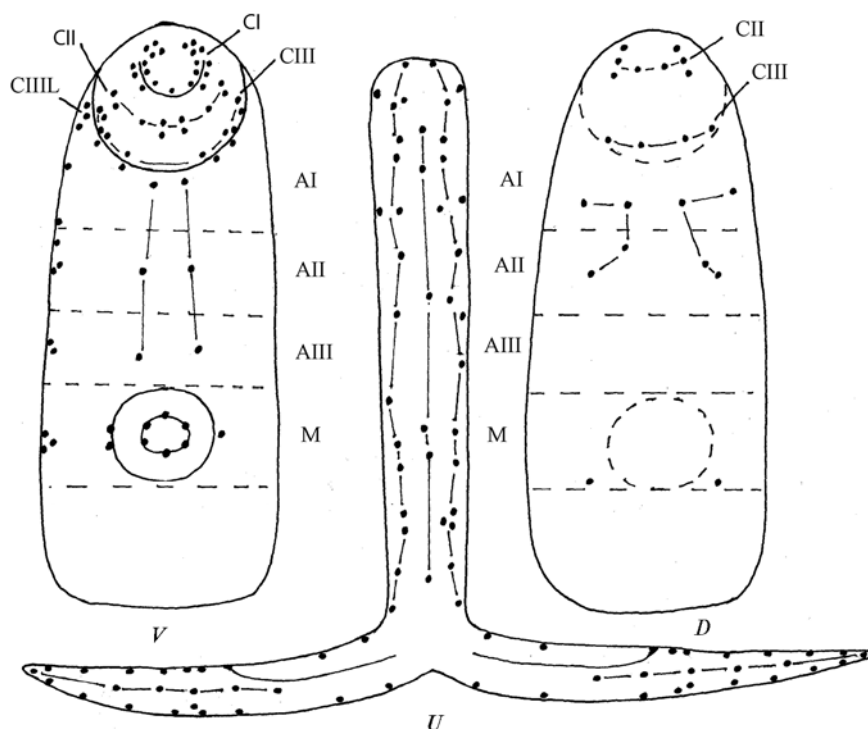


Fig. 2. Sensilles of cercaria *Neodiplostomum oriolinum*: V — ventral; D — dorsal; U — tail.

Рис. 2. Сенсорный аппарат церкарии *Neodiplostomum oriolinum*: V — вентрально, D — дорсально, U — хвост.

0.19–0.25 mm from anterior end of the body. Brandes' organ is 0.056–0.078 : 0.045–0.084 mm. Bud reproductive system consists of two parts, measuring 0.036–0.045 : 0.056–0.067 mm and 0.028–0.031 : 0.017–0.031 mm, lies between Brandes' organ and excretory bladder. From excretory bladder, 4 ducts go aside, two of them are blind and reach Brandes' organ, and two others are ramified forming duct network in metacercaria body.

The final host (experimental): *Gallus gallus* dom. (intensity of invasion 25 trematode), *Rattus norvegicus* (2 trematodes).

Localization: small intestine.

Adult worm (marita). Body (measurements are in the table 1) consists of two segments (fig. 3, *d*). Anterior segment is leaf-shaped with ventrally declinated lateral and back margins (fig. 3, *d, e*). The surface of anterior segment is covered with small spines. Posterior segment is oval, rounded in section. Its posterior end is declinated dorsally. Oral sucker is rounded or funnel like, prepharynx is 0.011–0.017 mm in length, in some individuals it is present, in others it is not visible, pharynx is almost rounded, oesophagus is 0.061–0.067 mm in length. Intestinal branches reach posterior end of body. Ventral sucker is situated 0.42–0.49 mm from anterior end of the body. It is either adjacent to Brandes' organ, or lies 0.04–0.05 mm before it, or is partly covered with its front margin (fig. 3, *d, e*). Ovary is transversal-oval, located on segments' border. Mehlis' gland lies behind the ovary, near the middle of anterior testis. Seminal receptacle is on the left of ovary. Testes are close to each other, transversally elongated, with hollows on anterior, posterior and ventral sides. Vitelline glands consist of small follicles. They begin either at the level of intestinal ramification, or in the middle between ventral sucker and intestinal bifurcation, or near anterior margin of ventral sucker (fig. 3, *d, e*). In anterior segment, vitelline follicles are aggregated in 8 longitudinal ribbon fields (4 on each side of median line), and after Brandes' organ they merge into the single field. In posterior segment, vitelline glands form two median ribbons and follicle aggregations on each body side, before anterior testis and near posterior testis. Volume seminal vesicle is behind posterior testis, adjoining its dorsal side. Genital cloaca is 0.11 mm in diameter, located dorsally, near posterior end of body. Eggs are yellow, thin-walled, with a lid.

Life cycle. It was experimentally established that the second intermediate host of trematodes *N. oriolinum* are tadpoles of *Rana dybowskii*. Other animals used in experiments were not infected. As observations showed, free cercariae life consists of alternating periods of active swimming and rest. At that, they display of positive rheotaxis. After contact with tadpoles, cercariae fixed themselves on the body or tail of amphibians with ventral sucker and, using the oral organ, penetrated tissues directly in places of fixation. Having penetrated the host, cercariae migrated into its body cavity where metacercariae were formed. Metacercariae developed without encystation. During the first seven days (fig. 3, *a*), body and suckers increased in size, intestinal branches and first-order excretory ducts became longer. The latter reached pharynx. Spines disappeared from body surface, but remained on internal side of ventral sucker. Immediately behind ventral sucker, cells aggregation forming Brandes' organ were seen. Metacercariae measurements: body 0.28–0.32 : 0.19–0.20 mm, oral sucker 0.030–0.045 : 0.040–0.045 mm, pharynx 0.020–0.025 : 0.025–0.028 mm, ventral sucker 0.028–0.31 : 0.030–0.036 mm, Brandes' organ 0.045–0.048 : 0.050 mm. On the 10th day of development, no significant changes in size of metacercaria body and organs were seen, except for Brandes' organ (fig. 3, *b*). In this period, Brandes' organ increased almost two times (to 0.076 : 0.081 mm) and got its inherent shape. The duct network of the second excretory system was formed. In the area of Brandes' organ, ventral cavity was visible. On the 21st day from the beginning of experiment, infected tadpoles were fed to a chicken and a rat. On 6th day, adult worms were found in small

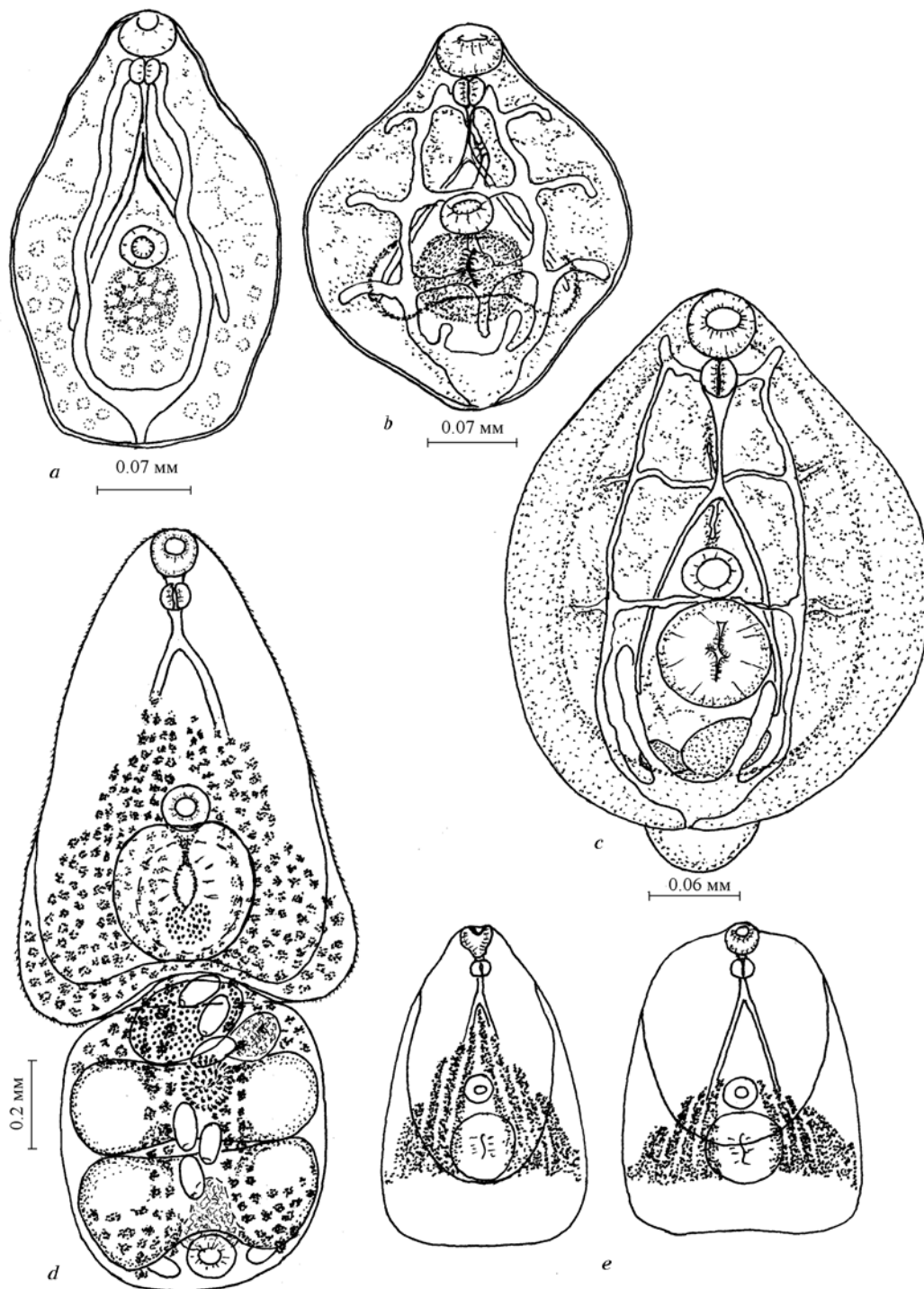


Fig. 3 *Neodiplostomum oriolinum*: a — metacercaria on the 7th day of development; b — metacercaria on the 10th day of development; c — infective metacercaria; d — adult worm; e — variations in location of vitelline follicles in anterior segment of adult worms and position of ventral sucker relatively to Brandes' organ.

Рис. 3 *Neodiplostomum oriolinum*: a — метацеркария на 7-е сутки развития; b — метацеркария на 10-е сутки развития; c — инвазионная метацеркария; d — марита; e — варианты расположения желточников в переднем сегменте марит и брюшной присоски относительно органа Брандеса.

**Table 1. Measurements (mm) of adult worms of *Neodiplostomum oriolinum* and *N. paraoriolinum***  
**Таблица 1. Размеры (мм) половозрелых червей видов *Neodiplostomum oriolinum* и *N. paraoriolinum***

Parameters	<i>N. oriolinum</i>		<i>N. paraoriolinum</i> (by Oshmarin, 1963)
	our data (7 spec.)	by Oshmarin, 1963	
Body length	1.08–1.68	2.0* (1.980–2.610)**	2.286 (1.98–2.610)
Anterior segment	0.67–1.03 : 0.64–0.82	1.2 : 0.9 (1.134–1.26 : 0.9)	1.440 : 0.865 (1.3 : 0.81–1.62 : 0.92)
Posterior segment	0.63–0.75 : 0.56–0.68	0.8 : 0.640 (0.846–0.9 : 0.650–0.684)	0.846 : 0.670 (0.68 : 0.594–0.99 : 0.57)
Oral sucker	0.08–0.10 in diameter	0.150 in diameter (0.140–0.155 : 0.155–0.160)	0.056 : 0.065 (0.090 : 0.085–0.094 : 0.092)
Pharynx	0.061–0.067 : 0.067–0.072	0.086 : 0.075 (0.095–0.102 : 0.070–0.095)	0.070 : 0.055 (0.085 : 0.065–0.090 : 0.065)
Ventral sucker	0.09–0.10 in diameter	0.155 : 0.165 (0.140–0.164 : 0.170–0.178)	0.108 : 0.135 (0.105 : 0.126–0.108 : 0.130)
Brandes' organ	0.28–0.31 : 0.24–0.31	0.310 : 0.270 (0.400–0.415 : 0.280–0.360)	0.380 : 0.420 (0.285 : 0.305 0.360 : 0.365)
Anterior testis	0.23–0.25 : 0.47–0.50	0.230 : 0.520 (0.225–0.235 : 0.450–0.590)	0.180 : 0.450 (0.215 : 0.360–0.225 : 0.396)
Posterior testis	0.22–0.29 : 0.38–0.56	0.230 : 0.520 (0.225–0.235 : 0.450–0.590)	0.175 : 0.540
Ovary	0.11–0.16 : 0.20–0.26	0.140 : 0.150 (0.160–0.170 : 0.250–0.270)	0.145 : 0.215 (0.115 : 0.360–0.225 : 0.395)
Eggs	0.10–0.11 : 0.056–0.061	0.070–0.094 : 0.050–0.056	0.098 : 0.057–0.104 : 0.062

\* Measurements of holotype.

\*\* Variability limits.

intestine of both animals. Consequently, it was established that at the water temperature 18–22°C, metacercariae (fig. 3, *c*) became invasive on 21st day from the moment of cercariae penetration into the second intermediate host.

## Discussion

In 1963, P. G. Oshmarin described two trematodes *Neodiplostomum oriolinum* and *N. paraoriolinum* from one black-headed oriole *Oriolus chinensis* Linnaeus, 1766 bagged in Primorye region. Totally, there were 20 trematodes — 10 specimens of each species. As Oshmarin pointed out, both these species were close both by their structure and size (tabl. 1). However, they were recognized as independent species based on different shape and size of the oral sucker (spherical in *N. oriolinum* and funnel like, smaller in size, in *N. paraoriolinum*), different position of ventral sucker in relation to Brandes' organ (in *N. oriolinum* the ventral sucker overlap with anterior margin of Brandes' organ, in *N. paraoriolinum* it is at a short distance from it) and different extension of vitelline glands (in *N. oriolinum* they reach posterior end of oesophagus, in *N. paraoriolinum* do not reach intestinal bifurcation).

In our material, developed in experiment adult worms had features characteristic for the both aforementioned trematode species, except for some metrical differences (tabl. 1). Among adult worms, we found specimens with rounded and funnel like oral sucker (its shape depends on position in mount), and with location of ventral sucker in relation to Brandes' organ characteristic both for *N. oriolinum* and *N. paraoriolinum* (fig. 3, *d*, *e*). Size of the oral sucker is rather variable. In *N. paraoriolinum* holotype, the oral sucker is almost two times less than that in other specimen of this species (tabl. 1). Extension of vitelline fields in anterior body segment is also variable (see description of adult worm and fig. 3, *d*, *e*). Moreover, based on Oshmarin's (1963) fi-

gure of *N. oriolinum*, only one of eight vitelline fields begins near the pharynx posterior margin, but not at its level.

Significant difference in developed adult worms of *N. oriolinum* and *N. paraoriolinum* described by P. Oshmarin is spines on anterior segment. Spines are rather small and hardly visible, and in some specimen they are seen on few areas of segment surface only. During collection, fixation and mounting, the superficial layer of worm tegument is probably destroyed and spines are lost.

Basing on the aforementioned, we can not exclude that *N. oriolinum* and *N. paraoriolinum* described by P. G. Oshmarin (1963) may be synonyms.

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*Ginetsinskaya T. A., Dobrovolskiy A. A.* [New method of finding sensillas in trematode larvae and significance of these formations for taxonomy] // Dokl. AN SSSR. — 1963. — **151** (2). — P. 460–463. — Russian : *Гинецинская Т. А., Добровольский А. А.* Новый метод обнаружения сенсилл личинок трематод и значение этих образований для систематики // Докл. АН СССР. — 1963. — 151 (2). — С. 460–463.

*Oshmarin P. G.* [Parasitic worms of mammals and birds in Primorye region]. — M. : AN SSSR, 1963. — 323 p. — Russian : *Ошмарин П. Г.* Паразитические черви млекопитающих и птиц Приморского края. — М. : Изд-во АН СССР, 1963. — 323 с.