Life cycle and characterization of Neoglyphe locellus (Kossack, 1910)
(Digenea: Plagiorchiidae) from Bulgaria

V. RADEV, D. HRUSANOV, T. MUTAFOVA, V. DIMITROV

Institute of Experimental Pathology and Parasitology, Bulgarian Academy of Sciences, Bl. 25, Sofia 1113,
Bulgaria, E-mail: vradev@bas.bg

Summary

After experimental reproduction of Neoglyphe locellus (Kossack, 1910) life cycle was found that Planorbarius corneus (L., 1758) snail can be a second intermediate host for this species. All stages of parasite’s development were described, including argentophytic and chromosome structures. A comparative analysis of published data concerning N. locellus was performed. The degree of variation of some taxonomic important features in populations from different geographic areas was followed up.

Key words: Neoglyphe locellus (Kossack, 1910); Life cycle; chaetotaxy; karyotype

Introduction

Opisthiothylax locellus (Kossack, 1910) was described originally from the small intestines of Sorex foidicus. Schalaby (1953) divided the genus Opisthiothyle into two subgenera - Opisthiothylax, which includes amphibian parasites, and Neoglyphe, which includes mammalian parasites. Yamaguti (1958) declared Neoglyphe Schalaby, 1953, an independent genus and placed it in the subfamily Omphalometrinae Looss, 1899, of the family Plagiorchiidae (Lühe, 1901).

The life cycle of O. locellus was first studied by Macey and Moore (1958) in America. Bock (1982) described the life cycle of the species based on material from Europe (Germany) however Planorbarius corneus (L., 1758) was not recognized as a second intermediate host. In Bulgaria adult N. locellus was found in intestine of Crocidura leucodon, C. suaveolens, Neomys anomalus, Sorex araneus and S. minutus by Genov and Dimitrova (1966), Genov (1979, 1984), Prokopč and Genov (1974), but its life cycle has not been studied experimentally.

The cercarial chaetotaxy of N. locellus have been studied by Bock (1983). No data about karyological investigations of N. locellus.

The objective of this study was to provide full characterization of N. locellus from Bulgaria, using biological, morphological and cytogenetical methods.

Material and Methods

A total of 726 snails of the species P. corneus for natural invasion were studied. They were collected in autumn (October) along the banks of the Danube. Thirty-two of them (4.40 %) were infected with Xiphidiocercaria armata, belonging to the Plagiorchis-group, according to types of Grabda-Kazubska (1971). After sectioning, it was found that 325 snails (44.77 %) contained metacercariae of N. locellus localized in hepatopancreas, pericardial area and muscles. Five hamsters (Mesocricetus auratus) were fed individually with tissues containing metacercariae of N. locellus. Seven days post infection, 390 mature specimens were obtained and 70 were morphologically analyzed after fixation and staining used by Radev et al. (1999). Cultures from 250 eggs spontaneously laid by the mature parasite specimens were incubated for 80 days at 15 – 18°C and used to study embryogenesis. Thirty daughter sporocysts, 70 cercariae and 90 metacercariae of different localization in the tissues of the naturally infected P. corneus were morphologically studied.

Twenty-five cercariae impregnated according to Combes et al. (1976) were used to study the chaetotaxy. The topography of the surface tegumentary structures is described according to nomenclature of Buysse-Dufour (1979), with some modifications, which concern the head area, suggested by Grabda-Kazubska and Kiselienè (1989). Daughter sporocysts from 5 snails were used in the karyotype study. Twenty metaphase cells were karyometric analyzed statistically. Slides were prepared according to Mutafova et al. (2001).
The optical microscope "Zeiss Opton" with camera lucida, videocassette and automatic photo-camera was used for microscopic examinations, measurements, photographs and drawings. All metric data are given in micrometres.

Results

I. Morphology and life cycle

Eggs and miracidia. Spontaneously laid by adult tremato- des eggs (Fig. 1) are elliptical to oval, operculate, yellow to light brown, unembryonated 45.5 – 52.78 (47.86 ± 3.816) in length and 26.4 – 35.2 (27.35 ± 3.245) in width. The operculum base measured 13.2 – 21.12 (16.72 ± 4.033) and 3.16 – 3.69 (3.45 ± 0.271) in height.

Daughter sporocysts isolated from the digestive gland of snail are sausage-like (Fig 4), usually U-shape and measured 360.93 – 720.27 (565.480 ± 125.422) in length and 120.4 – 250.32 (192.90 ± 39.833) in width. They contain germinal balls and 3 to 5 cercariae.

Cercariae. Living spontaneously emerged Xiphidiocercaria armata had a spinose body 228.35 – 371.03 (295.63 ± 35.64) long and 111.23 – 130.32 (124.70 ± 5.83) width (Fig. 5). The tail is without finfolds and spines 62.91 – 265.34 (143.03 ± 54.46) long and 19.35 – 47.13 (30.74 ± 8.17) wide. The stylet is 28.76 – 32.75 (29.98 ± 0.98) in length and 7.5 – 8.2 (8.07 ± 0.3) in width (Figs. 6, 7). Oral sucker is subterminal, elliptical to oval 42.44 – 70.70

On the 12th day after egg lying, the miracidium has completed its development and showed active movements (Fig. 2). It is located near to the operculum and its dimensions are 42.84 in length, 22.70 in width. The miracidium does not leave the egg. Its mobility decreases from the 62nd to the 72nd day and after the 79th day it is immobile (Fig. 3).

Figs 1 – 9. Neoglyple jocellus (Kossack, 1910) - developmental stages: 1 – Egg at the moment of lay. Scale bar 10 μm; 2 – Egg after 12 days of incubation. Scale bar 10 μm; 3 – Egg containing unmovable embryo. Scale bar 10 μm; 4 – Sporocyst. Scale bar 50 μm; 5 – Cercaria. Scale bar 50 μm; 6 – Stylet. Scale bar 10 μm; 7 – Stylet. Scale bar 10 μm; 8 – Metacercaria. Scale bar 50 μm; 9 – Adult. Scale bar 50 μm


(52.28 ± 7.05) in length and 47.79 – 70.70 (53.37 ± 6.24) in width. Ventral sucker is oval just after or on the middle of the body 34.95 – 53.50 (41.60 ± 5.57) in length, 35.45 – 48.97 (41.64 ± 3.87) in width. Oral to ventral sucker ratio is 1.4. Prepharynx is distinct, 5.24 long. Pharynx is oval well developed 16.8 – 21.17 (17.35 ± 3.891) in diameter.
The flame-cell formula is $2 \cdot [(3+3+3) + (3+3+3)]$. Main excretory ducts lead into the lateral branches of the Y-shaped excretory bladder situated at posterior end of the body. Excretory pore is situated subterminal at the posterior body end. The penetration gland cells are located in two groups, symmetrically arranged beside and just anterior to the ventral sucker. Each group contains five to six gland cells and gland duct with openings at the base of the stylet. Cystogenous gland cells are spread all over the body. Cercariae survive for up to 20 - 40 h at 18°C. They have negative geotaxis and actively seek the second intermediate host. The infected snail host shedding cercariae during the twenty-four-hour period but most active process was observed in the morning between 06:00 and 08:00 am.

Metacercariae. Fully developed metacercariae isolated from naturally infected *P. corneus* are elliptical to oval 136.5 – 163.3 (152.43 ± 10.117) long and 95.2 – 118.4 (116.8 ± 8.7) wide (Fig. 8). Metacercariae are localized in the hepatopancreas, periappendicular and muscle tissues of the snail hosts. No morphological differences were found in metacercariae isolated from different sites.

Adults. Seven days old experimentally obtained specimens are with heartshaped to elliptical, dorsoventrally flattened, spinose body, 456.00 – 648.00 (565.00 ± 49.562) long and 240.00 – 300.00 (276.40 ± 16.320) wide (Fig. 9). Body spines are 2.00 – 3.00 long distinguished regularly on the body surface. Oral sucker is well developed, with elliptical to oval shape, subterminal 59.35 – 79.24 (68.15 ± 7.32) in length and 87.84 – 109.8 (97.40 ± 9.00) in width. Pristhynynch present. Pharynx is well developed, elliptical, just post oral sucker 23.96 – 47.134 (34.52 ± 9.17) long. The ratio of oral sucker to pharynx lengths is 1.97. Oesophagus is short, intestinal bifurcation is on the level between the first and second fourth of the body. Intestine ends are blindly near to the posterior body end. Ventral sucker is oval situated before middle of the body with diameter 49.41 – 65.88 (61.12 ± 4.26). Oral to ventral sucker length ratio is 1.11. Ovary is irregular in shape and located outside of the mediobody line, post ventral sucker. Vitellocytes are irregular in shape and different in size. Viteline fields are symmetrical, extraintestinal, beginning just post oral sucker, reaching to the posterior body end, curving around the end of intestinal branch and ending just post posterior testis. Cirrus is well developed, long 117.12 – 153.72 (140.60 ± 10.68), post ventral sucker before ovary, outside of the mediobody line, U-shaped around the ventral sucker margin. Body to cirrus pouch length ratio is 4.01. Male genital atrium is situated post intestinal bifurcation, before ventral sucker, outside of mediobody line. Testes are in posterior half of the body, tandem with rounded surface. Anterior testis is 42.09 – 64.05 (53.34 ± 5.792) long and 109.8 – 164.7 (140.501 ± 18.26) wide. Posterior testis is 42.07 – 76.35 (57.27 ± 13.39) long and 81.04 – 160.04 (150.70 ± 13.64) wide. Uterus is short, between anterior testis and ventral sucker, containing about 10 unembryonated yellowbrown eggs. Metraterm is around ventral sucker opposite of the cirrus pouch ending with female genital atrium.

II. Argentophilic structures of *N. locellus* cercariae.

The tegumentary papillae are situated as follow:

1. Cephalic region: (Fig. 10, 11)
   - $C_1 = C_1 V_1$, $S C_1 L_1$, $1 C_1 D_1$, $1 C_1 D_2$
   - $C_1 V_2 = 1 C_1 V_1$, $1 C_1 V_2$, $1 C_1 L_1$, $2 C_1 D_2$
   - $C_1 L_1 = 1 C_1 V_1$, $15-18 C_1 V_2 + C_1 L_1$, $14-22 C_1 D_1$. 

![Fig. 15. Neoglyphea locellus (Kosak, 1910) karyotype. Scale bar 10 µm.](image1)

![Fig. 16. Diagram of chromosomes: a – Neoglyphea locellus (constructed on the data in Table 1); b – Opisthobranchia rumens; c – Plagiocotyle mucinosa (by Mitrova, 1994).](image2)
2. Body papillae (Figs. 12, 13)
a) ventral papillae
   1AIV, 2AIV, 1AIIV
   2MIV
   1PIV
   b) dorsal papillae
   2AIID, 1AIID
   2MID
   1PID
   c) acetabular papillae
   9S1, 0-3S1
   d) lateral papillae
   about 20 papillae on each lateral body field.

3. Tail papillae (Fig. 14)
The papillae 2UD are in longitudinal line on the middle of
tail. Most of the papillae have constant number and location.
The papillae 1CIV are invaginated. Variations were obtained
mainly in PnD and Sn papillae.

Acetabular papillae on the circle Sn in most of the investigated
cercariae were not obtained. The papillae 1PIID in
some of investigated cercariae absent. The openings of the
penetration gland cells were impregnated together with
CnD1 papillae. They were irregular in shape and larger
from the papillae.

Table 1. Measurements (mean ± SD) and classification of the chromosones of Neoglyptobius locellus.

<table>
<thead>
<tr>
<th>Nr</th>
<th>Absolute length Lμ</th>
<th>Relative length L2</th>
<th>Centromere index E classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.17</td>
<td>18.26 ± 1.039</td>
<td>48.35 ± 2.333 (m)</td>
</tr>
<tr>
<td>2</td>
<td>2.82</td>
<td>12.50 ± 0.205</td>
<td>45.00 ± 7.070 (m)</td>
</tr>
<tr>
<td>3</td>
<td>2.72</td>
<td>12.04 ± 0.456</td>
<td>18.50 ± 2.120 (m)</td>
</tr>
<tr>
<td>4</td>
<td>2.33</td>
<td>10.22 ± 0.126</td>
<td>38.50 ± 2.120 (m-sm)</td>
</tr>
<tr>
<td>5</td>
<td>2.15</td>
<td>9.59 ± 1.019</td>
<td>41.40 ± 1.814 (m)</td>
</tr>
<tr>
<td>6</td>
<td>1.61</td>
<td>6.89 ± 0.990</td>
<td>36.66 ± 3.590 (sm-m)</td>
</tr>
<tr>
<td>7</td>
<td>1.61</td>
<td>6.89 ± 0.990</td>
<td>36.66 ± 3.590 (sm-m)</td>
</tr>
<tr>
<td>8</td>
<td>1.48</td>
<td>6.42 ± 0.326</td>
<td>35.00 ± 7.070 (sm-m)</td>
</tr>
<tr>
<td>9</td>
<td>1.43</td>
<td>6.26 ± 0.100</td>
<td>39.00 ± 1.400 (m-sm)</td>
</tr>
<tr>
<td>10</td>
<td>1.25</td>
<td>5.62 ± 0.759</td>
<td>39.00 ± 1.400 (m-sm)</td>
</tr>
<tr>
<td>11</td>
<td>1.16</td>
<td>5.31 ± 1.238</td>
<td>39.00 ± 1.400 (m-sm)</td>
</tr>
</tbody>
</table>

III. Karyological investigations
The diploid cells of N. locellus contain 22 chromosomes
with absolute length from 4.17 to 1.16. Mean genome
length is 22.43 (Table 1, Fig. 15). The first pair takes 18.26
% from the relative haploid length and differs from the
other. The differences in length between 2m to 11m chromosones are not statistically significant. According to the
centromere localization, the 1s, 2s and 5s chromosome pairs are typically metacentric, the 3s is subtelocentric,
and 7 pairs (4s, 6s to 11s) have median - submedian
classification.

Discussion

Our experimental studies showed that P. corneus is the
first and can be a second intermediate host for N. locellus.
According to Bock (1982) second intermediate host for N.
locellus are insect larve of the genus Aesha or freshwater
snail Lymanea stagnalis. Comparative analysis of published
data shows no essential differences between the morphometrical values of N. locellus (or O. locellus) larval stages
and adult from Bulgarian population, examined by us,
and the data for the same parasite species from Central and
South East Europe reported by Kossak, 1910; Zarnowski,
1966; Genov and Dimitrova, 1966; Genov, 1979, 1984;
Prokopiev and Genov, 1974; Bock, 1982.
Considerable differences between obtained morphological
characteristics and those by Macey and Moore (1958) for
N. locellus in America have been found. This supported the
opinion of Seese (1970), Bocks (1982) and Našinčová et
al. (1989) that the studied by Macey and Moore (1958)
trematodes probably belongs to another species of the
genus Neoglyptobius.
The basic chaetotaxy of N. locellus cercariae confirm a
plagiochete type of Bayssade-Dufour (1979). Regardless of
the use of different methods of impregnation (Combes et
al., 1976) and of papillae description (Richard, 1971;
Bayssade-Dufour, 1979) with some modifications suggested
by Grabda-Kazubská and Kisellinec (1989), models of
apipillae disposition used by Bock (1983) well correspon
liable criteria for the differentiation of *O. locellus* from *Opisthiglyphe rastellus* (Olsson, 1876) (according to Richard, 1971), *Opisthiglyphe megastomus* Baer, 1943 (according to Vaucher, 1972), and *Opisthiglyphe ranae* (Frölich, 1791), according to Dimitrov et al. (1989) were found.

Most of the studied genera included into the family Plagiocorchiidae have 22 diploid chromosomes (Barienê, 1993; Mutafowa, 1994) and this number can be the modal characteristic for the group. The karyotypes consist mainly of two-armed chromosomes with variation in I values in distinct taxons.

The comparative karyological analysis of *N. locellus* with *O. ranae* and *Plagiocorchi tus maculosus* studied by Mutafowa (1994) shows a significant difference in the localization of the centromere in some of the corresponding chromosome pairs (Fig. 16). Thus *N. locellus* has only one typical subtelocentric chromosome (No 3), *O. ranae* has 4, (No 3, 4, 5, 6) whereas karyotype of *P. maculosus* consists of two-arm chromosomes with variation of the centromere location in 4 of the chromosome pairs (No 2, 4, 6 and 7). These data show that *N. locellus* and *P. maculosus* have more similar karyotype characteristics than *O. ranae* and it could be suggested that they are phylogenetically closer genera.

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**References**


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