



UDC 595.12:597.8(477)

REDESCRIPTION AND VARIABILITY OF *POLYSTOMA MAZURMOVICI* AND *P. SKURATOVITCHI* (MONOGENEA, POLYSTOMATIDAE), WITH A KEY TO *POLYSTOMA* FROM ANURANS OF UKRAINE

N. Yu. Rubtsova

Institute of Parasitic Diseases (IPD), 11455 East Via Linda, #2-419,
Scottsdale, Arizona, USA 85259
E-mail: nyrubtsova@gmail.com

Redescription and Variability of *Polystoma mazurmovici* and *P. skuratovitchi* (Monogenea, Polystomatidae), with a Key to *Polystoma* from Anurans of Ukraine. Rubtsova, N. Yu. — New distributional records for *Polystoma mazurmovici* Buchvarov, 1980 from *Rana dalmatina* Fitzinger in Bonaparte, 1839 and *P. scuratovitchi* Buchvarov, 1984 from *Rana arvalis* Nicolsson, 1842 reported for the Ukrainian territory of Europe. *P. mazurmovici* and *P. skuratovitchi* confirmed as valid species, though their original descriptions were very brief and based on a mixed sub-adult and adult individuals. Metrical data of these species together with new morphological information are provided and incorporated in a dichotomous key to adult *Polystoma* from anurans of Ukraine. Redescription of an adult form of *P. skuratovitchi* and descriptions of sub-adult forms of *P. mazurmovici* and *P. skuratovitchi* are provided. Study confirms strict host specificity of polystomes and increases the number of already known species of *Polystoma* of Ukrainian fauna to four.

Key words: *Polystoma mazurmovici*, *P. skuratovitchi*, anurans, morphology, Ukraine.

Introduction

Pisanets (2007) reported the following species of anuran amphibians from the Ukrainian territory: European fire bellied toad, *Bombina bombina* (Linnaeus, 1761), yellow-bellied toad, *B. variegata* (Linnaeus, 1758), European common spadefoot, *Pelobates fuscus* (Laurenti, 1768), European tree frog, *Hyla arborea* (Linnaeus, 1758), common toad, *Bufo bufo* (Linnaeus, 1758), European green toad, *Bufo viridis* (Laurenti, 1768), natterjack toad, *Bufo calamita* Laurenti, 1768, common frog, *Rana temporaria* Linnaeus, 1758, moor frog, *R. arvalis* Nilsson, 1842, agile frog, *R. dalmatina* Fitzinger in Bonaparte, 1843, marsh frog, *Pelophylax ridibundus* Pallas, 1771, pool frog *P. lessonae* Camerano, 1882 «1881» and edible frog, *P. esculentus* Linnaeus, 1758.

Ryzhykov et al. (1980) reported only one species, *Polystoma integerrimum* (Fröhlich, 1798) from six species of anurans on the former Soviet Union territory, though they questioned the correctness of the findings of *P. integerrimum* in *P. fuscus* and *B. bufo*. Only two species of *Polystoma* have been known from the Ukrainian territory since 1981: *P. integerrimum* (Fröhlich, 1798) [= *P. integerrimum integerrimum* (sensu Euzet & Combes (1966))] from *R. temporaria* and *P. viridis* Euzet, Combes, Batchvarov, 1974 from *B. viridis* (Parokonyj et al., 1981). In 2008, a review of monogeneans from Ukraine confirmed the presence of *P. integerrimum* in *B. bufo* and

P. viridis from *B. viridis* and questioned the records of *P. integerrimum* in *P. fuscus*, *B. viridis*, *H. arborea*, *R. arvalis*, *R. temporaria* and *P. ridibundus*, considering them as misidentifications (Lisitsyna & Miroshnichenko, 2008). *Polystoma mazurmovici* Buchvarov, 1980 and *Polystoma skuratovitchi* Buchvarov, 1984 originally described from the Bulgarian and Polish territories of Europe (Buchvarov, 1980; Buchvarov, 1984 b), were never reported from the Ukrainian part of Europe. Both descriptions included a wide range of measurements of body structures, which suggests that they were based on specimens of various developmental stages (Rubtsova, Heckmann, 2017). This study was prompted by the need to clarify the species composition of polystomatids from *R. dalmatina* and *R. arvalis* and to distinguish adult and sub-adult forms of polystomatids from these hosts.

Materials and methods

We used museum material, collected by Dr. Viktor Sharpilo (I. I. Shmalhausen Institute of Zoology, National Academy of Sciences, Ukraine) in summer 1978 from water ponds of Lviv Region, Ukraine. Two specimens of *R. arvalis* were hosts for five polystomes [three sub-adults in one host and one adult in the other (Rubtsova, Heckmann, 2017)], and one specimen of *R. dalmatina* was a host of one sub-adult specimen of *Polystoma*. The whole mounts fixed in Canada balm were studied using light microscopy (Leica DM LB2 microscope), measured and photographed with Canon S50 Power Shot camera; drawings were made with Camera Lucida.

Results

Our review of museum collections from *R. arvalis* and *R. dalmatina*, that remained unidentified since 1978, showed that they belonged to *P. mazurmovici* Buchvarov, 1980 (fig. 1, A) and *P. skuratovitchi* Buchvarov, 1984 (fig. 1, B), respectively, see Buchvarov (1980; 1984 b). These monogeneans can be clearly identified by the morphology of anchors (hamuli) (fig. 2, A, B) and the ratio of their main parts. Measurements of five specimens of *P. skuratovitchi* collected from *R. arvalis* and one specimen of *P. mazurmovici* from *R. dalmatina*, and measurements from the original descriptions of *P. mazurmovici* and *P. skuratovitchi* are presented in the table 1.

Information on the male copulatory organ crown of spines, missing in the original descriptions of *P. mazurmovici* and *P. skuratovitchi* (Buchvarov, 1980, 1984 b), provided in the present study. Drawings of the crowns of spines of the copulatory organ of these two species are provided for the first time (fig. 2, A, B for *P. mazurmovici*; fig. 2, C for



Fig. 1. Fig. 1. Light microscopy photographs of *Polystoma mazurmovici* (from *Rana dalmatina*) and *Polystoma skuratovitchi* (from *Rana arvalis*): A — *P. mazurmovici*, dorsal view; B — *P. skuratovitchi*, ventral view. Scale bar 1 mm.

Table 1. Metric characteristics of *Polystoma mazurmovici* and *P. skuratovitchi* from original descriptions and present study

Parameter	<i>P. mazurmovici</i> (Buchvarov, 1980), mm	<i>P. mazurmovici</i> (present study, sub- adult, n = 1), mm	<i>P. skuratovitchi</i> (Buchvarov, 1984 b), mm	<i>P. skuratovitchi</i> (present study, total sample, n = 5), mm	<i>P. skuratovitchi</i> (present study, sub-adults, n = 4), mm	<i>P. skuratovitchi</i> (present study, adult, n = 1), mm
Body length	8.4 (5.4–11.9)	3.8	2.4	2.9 (2.3–4.3)	2.7 (2.3–3.25)	4.3
Maximum body width	3.5 (2.3–4.6)	1.55	1.1	0.99 (0.6–1.7)	0.81 (0.6–1.08)	1.7
Haptor length	1.8 (1.3–2.3)	1.15	0.887 (0.294–1.560)	0.87 (0.55–1.5)	0.72 (0.55–0.9)	1.2
Haptor width	3.4 (2.7–4.4)	1.8	1.261 (0.480–1.700)	1.18 (1.0–1.35)	1.09 (1.83–1.35)	1.8
Oral sucker length	0.412 (0.236–0.555)	0.25	0.197 (0.090–0.402)	0.17 (0.1–0.27)	0.15 (0.1–0.2)	0.27
Oral sucker width	0.647 (0.338–0.911)	0.32	0.294 (0.114–0.462)	0.28 (0.2–0.45)	0.24 (0.2–0.3)	0.45
Pharynx length	0.496 (0.422–0.562)	1.9	0.189 (0.030–0.306)	0.36 (0.23–0.72)	0.28 (0.23–0.36)	0.32
Pharynx width	0.312 (0.343–0.488)	1.5	0.170 (0.066–0.234)	0.35 (0.22–0.6)	0.27 (0.22–0.36)	0.25
a*	0.465	0.43	0.287	0.286 (0.26–0.32)	0.28 (0.26–0.32)	0.45
b	0.384	0.29	0.183	0.18 (0.16–0.23)	0.20 (0.19–0.23)	0.3
c	0.305	0.22	0.130	0.124 (0.11–0.14)	0.11 (0.09–0.14)	0.32
d	0.235	0.18	0.134	0.139 (0.12–0.16)	0.1 (0.06–0.14)	0.15
Sucker diameter	–	0.5	0.280	0.32 (0.2–0.6)	0.25 (0.2–0.32)	0.6
Crown of spines base diameter	–	0.7/0037	–	0.8 (0.7–0.9)	0.8 (0.7–0.9)	0.9
Spines length	–	0.04	–	0.04 (0.035–0.053)	0.04 (0.037–0.05)	0.039
Number of spines	–	11	–	9	9	9
Ovary length	1.1 (0.59–1.5)	0.5	0.476 (0.060–1.380)	0.39 (0.2–0.7)	0.31 (0.2–0.4)	0.7
Ovary width	0.56 (0.33–0.88)	0.18	0.203 (0.30–0.560)	0.17 (0.1–0.3)	0.13 (0.1–0.2)	0.25

* Hamuli parameters according to Euzet et al. (1974).



Fig. 2. Line drawings of anchors of *Polystoma mazurmovici* (from *Rana dalmatina*) and *Polystoma skuratovitchi* (from *Rana arvalis*): A — anchor of *P. mazurmovici*; B — anchor of *P. skuratovitchi*. Scale bar 0.1 mm.

P. skuratovitchi). Few morphological features that we consider significant for species diagnosis in *Polystoma* are discussed herein. Among them are the shape and diameter of the genital bulb crown of spines, the position of a constriction in the crown of spines and the shape of the intestine wall facing genital complex. A detailed description of the sub-adult form of *Polystoma mazurmovici*, with the additional previously missing information and correcting errors in the original description is provided below.

Description of a sub-adult form of *Polystoma mazurmovici* Buchvarov 1980 (fig. 1, A).

For measurements, see table 1. Body pyriform, about one-third as wide as long, tapering anteriorly. Haptor hexagonal, with three pairs of cup-like suckers. Hooklets not seen. Hamuli well developed (fig. 2, A), located between first pair of suckers. Blade of each hamulus ending with a sharp spike that raised above the body surface. Oral cavity subterminal. Pharynx muscular, pyriform. Esophagus short. Intestine bifurcate. Left caecum with 32 small external diverticula, right caecum with 31 small external diverticula. Caeca fusing anterior to haptor; posterior caecal union occupying central part of haptor, with nine external diverticula. Anastomoses four with 2–3 large internal diverticula extending beyond mid-line, often branched. Large diverticula interspersed by smaller, unbranched diverticula. Intestinal wall facing genital complex with anastomoses, 3 shorter ones in its anterior part on left and 5 larger ones in posterior part on the left (fig 1, A). Copulatory organ with palml-capital shaped crown of spines attached by bases forming shallow slots (fig. 2, A, B). Constriction part in the crown of the spines (fig. 3, A) at 1/5 length of spines, close to their dorsal ends. Testis not seen. Ovary sickle-shaped with narrowed and shorter anterior part, at right of mid-line in anterior third of body. Eggs not observed.

Type host: *Rana dalmatina* Fitzinger in Bonaparte, 1839.

Site of infection: urinary bladder.

Type locality: near towns of Plovdiv (42°08'31.8"N 24°41'33.1"E), Razlog (41°53'14.8"N 23°27'47.1"E), Jachoruda (42°01'07.7"N 23°39'55.8"E) and the village of Guljna bania (41°53'22.2" N 23°31'23.8" E), Bulgaria (Buchvarov, 1980).

Other localities: Çınarcık County, Yalova Province, Turkey (40°38'34.1" N 29°07'19.7" E) (Yildirimhan et al., 2016); Lviv Region, Ukraine (49°58'48.6" N 23°47'20.4" E) (present study).

Remarks. The description of a sub-adult form of *P. mazurmovici* provides new morphometric data that were missing in the original description. This description includes information on the morphology of crown of spines of copulatory organ, its base diameter, number and length of spines, depth of slots in the base of crown of spines and position of a constriction part in the crown of spines of the copulatory organ. Information on position of inner organs, such as intestine, number of its anastomoses, shape of the intestinal wall facing genital complex and shape of ovary is also provided. Ratio between major parameters of hamuli corresponds to that in the original description, which is considered a reliable diagnostic feature in *Polystoma* (Euzet et al, 1974; Buchvarov, 1980) (table 2). Parameters of hamuli in the original description are given as average, *a* and *b* parameters of the hamulus have close meanings, while *c* and *d* in specimen in our research are smaller. Dimensions of the body, pharynx and ovary in our specimen are smaller. That indicates that our specimen is a sub-adult form of *P. mazurmovici* as it is noted in the Discussion.

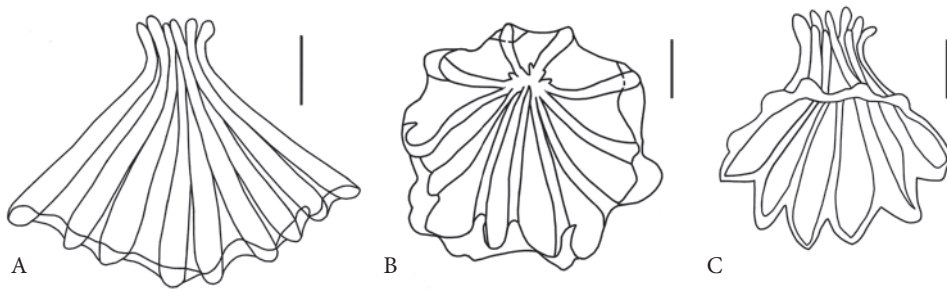


Fig. 3. Line drawings of crowns of genital spines of *Polystoma mazurmovici* (from *Rana dalmatina*) and *Polystoma skuratovitchi* (from *Rana arvalis*). A and B — lateral and dorsal views of genital crown of spines of *P. mazurmovici*; C — a dorso-lateral view of genital crown of spines of *P. skuratovitchi*. Scale bar 0.01 mm.

Table 2. Ratios of the main parameters of hamuli of *P. integerrimum*, *P. scuratovitchi* and *P. mazurmovici* used in the present study

Ratio	Adults	Sub-adults	Unspecified
		<i>P. integerrimum</i>	
	Rubtsova & Heckmann, 2017	Rubtsova & Heckmann, 2017	Euzet & Combes, 1966
a/b	2	1.68	1.8
b/c	0.64	0.98	0.8
b/d	0.78	1.19	0.9
		<i>P. scuratovitchi</i>	
	Present study	Present study	Buchvarov, 1984 b
a/b	1.5	1.39	1.56*
b/c	0.9	1.83	1.4
b/d	2	2.01	1.36
		<i>P. mazurmovichi</i>	
	Present study	Present study	Buchvarov, 1980
a/b	–	1.24	1.2
b/c	–	1.31	1.3
b/d	–	1.55	1.6

* Ratios for *P. skuratovitchi* are calculated by us, using data in Buchvarov (1984 b).

Below we provide a detailed redescription of an adult form and a description of a sub-adult form of *P. mazurmovici*, adding missing information and correcting errors in the original description.

Redescription of an adult form of *Polystoma scuratovitchi* Buchvarov 1984 with a description of sub-adult form (based on one adult and four sub-adults).

For measurements and metric differences between adult and sub-adult forms, see table 1. All redescription information of an adult form, unless specified separately, is true for the description of the sub-adult form.

Body pyriform, about one-third as wide as long, tapering anteriorly. Haptor hexagonal, with three pairs of cup-like suckers. Hooklets not seen. Hamuli well developed (fig. 2, B), located between first pair of suckers. Blade of each hamulus ending with a sharp spike raised above body surface. Oral cavity subterminal. Pharynx muscular, pyriform. Esophagus short. Intestine bifurcate. Left caecum with 27 external diverticula in adult and 26 (24–28) external diverticula in sub-adult. Right caecum with 25 small external diverticula extending in adult and 24 (22–26) in sub-adult. Large medial diverticula interspersed by 3–6 smaller, unbranched diverticula. Caeca fusing anterior to haptor; posterior caecal union occupying central part of haptor, with 10 external diverticula in adult and 10 to 12 external diverticula in sub-adults. Anastomoses five in adults and 4–5 in sub-adults, in both cases with 2–3 large internal diverticula extending beyond mid-line, often branched. Large diverticula interspersed by smaller, unbranched diverticula. In sub-adults, anastomoses sometimes not fused. The intestinal wall facing genital complex wavy, with 2–3 minor anastomoses in posterior part (fig 1, B). Copulatory organ with palm-capital shaped crown of spines attached by their bases forming deep slots (fig. 3, B). Constriction part in crown of spines (fig. 2, B) at 1/3 of length of spines, close to their dorsal ends. Testis not seen. Ovary sickle-shaped, with narrowed and shorter anterior part, at right of mid-line in anterior third of body. Eggs not observed.

Type host: *Rana arvalis* Nicolsson, 1842.

Site of infection: urinary bladder.

Type locality: Western Poland (the exact place is unknown) (Buchvarov, 1984 b).

Other localities: Lviv Region, Ukraine (49°58'48.6"N 23°47'20.4"E) (present study).

Remarks. Present redescription of an adult form provides new morphometric data on crown of spines of copulatory organ, its base diameter, number and length of spines, depth of slots in the base of crown of spines and position of a constriction part in the crown of spines of copulatory organ. Information on position of inner organs, such as intestine, number of its anastomoses, shape of the intestinal wall facing genital complex and ovary shape is also provided for the first time. Shape of hamuli is similar to that in the original description. All measurements from a total sample of five specimens of *P. scuratovitchi* are within the limits indicated in the original description. At the same time in our sample, we identified one adult specimen (based on body parameters and maturity of the reproductive system) and four sub-adults, and provided their measurements separately (tables 1, 2). The delineation of the two developmental stages in the original description was not made and the presence of a mixture of two age groups in the description of *P. scuratovitchi* is discussed below.

While the original descriptions of *P. mazurmovici* and *P. skuratovitchi* were very brief and obviously based on a mixture of monogeneans of different age groups, sub-adults and adults (see Discussion), we have no doubt on the validity of both species and include them in the Key of *Polystoma* from Ukraine. The following Key includes other two species of the genus known from the territory of the country, *P. integerrimum* and *P. viridis*. We

assume that in Ukraine at least two more *Polystoma* species, *P. skrjabini* Buchvarov, 1984 from *H. arborea* (Buchvarov, 1984 a) and *P. fuscus* Biserkov & Hadjinikolova, 1993 from *P. fuscus* (Biserkov, Hadjinikolova, 1993) may possibly be present because of the presence of their hosts. That is why we also include these species in the Key. We provide the Key only for adults of *Polystoma*. Ratios of main parameters of hamuli for adults of *P. integerrimum* and *P. scuratovitchi* used in the Key are discussed in Rubtsova & Heckmann (2017) and present study and given in table 2. Ratio information for adults of *P. mazurmovici* is not available, and not used in the Key. All other measurement are used as in their original descriptions.

Key to species of *Polystoma* from Ukraine

- 1. In Pelobatidae.2
- In other hosts.3
- 2. Hamuli underdeveloped. Hamuli present as primordia with length of 15 µm, situated between first pair of suckers and slightly anteriorly to marginal hooklets. Body length 6.574 (5.405–8.378) mm, body width 2.285 (1.703–2.756) mm. Near Lake Durankulak (Bulgaria). Parasite of common spadefoot, *Pelobates fuscus**P. fuscus* Biserkov & Hadjinikolova, 1993
- Hamuli developed.3
- 3. In Hylidae. Body length 3.4 (2.964–4.275) mm, body width 1.4 (1.235–1.651) mm. Hamuli (average): a 217, b 199, c 64, d 60 µm; ratio a/b 1.1, b/c 3.1, b/d 3.3. Parasite of common tree frog, *Hyla arborea*, Petrich (Bulgaria). *P. skrjabini* Buchvarov, 1984
- In other hosts.4
- 4. In Bufonidae. Body length 9.8 (8.9-10.8) mm. Hamuli (average): a 418, b 338, c 93, d 51µm; ratio a/b 1.1, b/c4.2, b/d 7.9. In Corsica (France), Plovdiv (Bulgaria), Kyiv, Kherson Region, Crimea (Ukraine); parasite of green toad, *Bufo viridis**P. viridis* Euzet, Combes, Batchvarov, 1974
- In Ranidae.....5
- 5. Ratio of hamuli measurements a/b about 2.0. Ratios b/c 0.7, b/d 0.8. Slots between spines on basal end of the crown deep. Average body length 10.1, body width 3.4 mm. Hamuli (average): a 480, b 233, c 359, d 298 µm. Parasite of common frog, *Rana temporaria*, wide spread throughout the distribution area of the host..... *P. integerrimum* (Fröhlich, 1798)
- Ratio of hamuli measurements a/b much smaller than 2.0.6
- 6. Slots between spines on basal end of the crown deep. A constriction part in the crown of the spines of copulatory organ is situated at 1/3 of the length of the spines, close to their dorsal ends. Ratio of hamuli measurements: a/b 1.5, b/c 0.9, b/d 2. In Western Poland and Lviv Region (Ukraine). Parasite of moor frog, *Rana arvalis*.....*P. scuratovitchi* Buchvarov 1984
- Slots between spines on basal end of the crown spines of copulatory organ shallow. A constriction part in the crown of the spines of copulatory organ situated at 1/5 of the length of the spines, close to their dorsal ends. In Plovdiv, Razlog, Jahoruda and Guljna bania (Bulgaria); Yalova Province, Çınarcik County (Turkey); Lviv Region (Ukraine). Parasite of agile frog, *Rana dalmatina*
..... *P. mazurmovici* Buchvarov 1980

Discussion

The spine crown of the genital bulb morphology

The shape of a crown of spines of the copulatory organ was noted as a significant feature in distinguishing of monogeneans (Bykhovsky, 1957). Gusev (1985) considered the shape of sclerotized parts of the copulatory organ as one of the substantial morphological features, together with attachment structures, in Monogenea. Recent descriptions of polystomes usually provide graphic, descriptive and metric information on spines of genital bulb (Biserkov & Hadjinikolova, 1993; Biserkov et al., 2001; Du Preez et al., 2007). That is why we deemed it necessary to add this information in the present study. Information on the position of a constriction of the crown of spines of the male copulatory organ was provided in the present study. Despite this interspecific difference was discovered in a limited number of specimens, it clearly distinguishes *P. mazurmovici* and *P. skuratovitchi* from each other

as well as from other species. For example, drawing provided in the original description of *P. macronemis* Biserkov et al., 2001 shows that the constriction part of the spine complex is at the dorsal tip of its crown (fig. 2, B in Biserkov et al. (2001)). Drawing in the description of *Polystoma fuscus* Biserkov & Hadjinikolova, 1993 shows that the constriction part of spine complex is at 1/5 of the length from the dorsal end of the crown of spines (fig. 1, C in Biserkov & Hadjinikolova (1993)). We consider this characteristic of the crown of spines to be a useful taxonomic feature and used it when creating the Key.

The presence of various developmental stages in the original description of *P. mazurmovici*

Morphometric analysis of one specimen of *P. mazurmovici* in the present study, its body size and proportionally smaller measurements of hamuli indicate that it is a sub-adult form, while the original description was apparently based on adults. Nevertheless, we found some inaccuracies in the original description. Sic, the original description of *P. mazurmovici* Buchvarov (1980) gives the range for the body length measurements as 5.4–11.9 mm, average 8.4 mm. While looking on the drawing of *P. mazurmovici* in Buchvarov (1980), that has a scale bar 1 mm, one could measure that body size of the worm is only 4.3 mm. That is very close to the size of the sub-adult specimen in our study. The size and the ratio between the length of the main part and the root of the hamuli in the description of *P. mazurmovici* (Buchvarov, 1980) show that the drawings in his research (and, obviously, measurements) represent different age groups of the same species. Four anchors from the left on plate “д” (fig. 8) have outer roots that are noticeably longer and inner roots are much wider, in comparison with the other six other anchors to the right. This graphic information of hamuli, together with the fact that Buchvarov (1980) provided only average values of hamuli measurements, gives us a reason to assume that there was a big range of variation in this one of the most reliable metric parameters in Monogenea. Range of variations in body parameters, dimensions of haptor, oral sucker and ovary also noticeably differ two or three fold in the description of *P. mazurmovici* (Buchvarov, 1980).

Sizes of hard parts of monogeneans, such as sclerotized structures of haptor and copulatory system may change with the age. For example, inner roots of anchors from mature dactylogyrid monogenean *Dactylogyrus auriculatus* (Nordmann, 1832) have more developed inner roots, than immature ones. Moreover, the most mature worms of the same species have “inner root very massive, with swelling” (Gusev & Kulemina, 1971). The shape of sclerotized structures of haptor and copulatory system, according to Gusev and Kulemina (1971), remains a more stable characteristic than metric parameters in monogeneans of the same species of different age groups. The same authors also showed that age differences are much bigger for sclerotized structures of haptor, than for sclerotized structures of copulatory system. Buchvarov (1980) drew attention to the fact that *c* and *d* parameters of hamuli show the best differences between the species of *Polystoma*. These parameters reflect the lengths of inner and outer roots of hamuli. Roots of hamuli grow during the worm's life (Gusev, Kulemina, 1971) and become bigger as the worm grows larger. Same observation have been documented in our study on a big sample of ancyrocephalid monogenean *Ligophorus vanbenedenii* (Euzet & Suriano, 1977) (Rubtsova et al., 2005). Accordingly, it is important to use only adult monogeneans in taxonomic studies (Rubtsova, Heckmann, 2017).

The presence of various developmental stages in the original description of *P. scuratovitchi*

There are few indicators that the original description of *P. scuratovitchi* was based on a mixture of worms of different age groups — sub-adults and adults (see Rubtsova, Heckmann, 2017). Body length and width in the original description of *P. scuratovitchi* was given as an average value. Most of the metric parameters in Butchvarov (1984 b) suggest a wide range of variation. Note variation of haptor length from 0.29 to 1.56 mm, oral sucker

length from 0.09 to 0.402 mm, pharynx length from 0.030 to 0.306 mm, ovary 0.060 to 1.380 mm and so on (table 1). In the description of *P. scuratovitchi* Buchvarov (1984 b) did not specify the minimum and maximum values for the size of the hamuli, but gave only the average; however, the difference could be obviously seen from the fig 3.1 (Buchvarov, 1982).

For another example of descriptions based on a mixture of two age groups, see the original description of *P. combesi* Buchvarov, 1982 from *R. graeca* Boulanger, 1897, that consists variation range of hamuli of two different size groups, one almost twice as large as the other (Buchvarov, 1982). Present study also raises the following question: why had the species *P. combesi* been mentioned in the 1980 paper (Buchvarov, 1980) while the description of the same species was published two years later, in 1982 (Buchvarov, 1982)?

Polystomes of Ukraine

Polystoma mazurmovici was initially described from *R. dalmatina* collected near Plovdiv, Bulgaria (Buchvarov, 1980) and recently reported from the same host from Turkey (Yildirimhan et al., 2016). *Polystoma scuratovitchi* was described from Western Poland from *R. arvalis* (Buchvarov, 1984 b), but was missing in the Keys for identification of Monogeneans of Poland (Dzika, 2008), in which *P. integerrimum* was reported from five host species (*R. temporaria*, *R. arvalis*, *R. esculenta*, *B. viridis* and *H. arborea*). The biology of polystomes is closely connected to that of the host, and each species of anuran harbors its specific species of *Polystoma* (Combes, 1966; Euzet et al., 1974). That's why we consider that the list of polystomes from Polish territory needs a revision. Results of our study increase the number of known species of *Polystoma* for the Ukrainian fauna and expands the boundaries of the range of distribution of these parasites. The question about the occurrence of specific polystomes on other anurans from the territory of Ukraine, *B. bufo*, *B. calamita*, *P. ridibundus* and *P. esculentus*, can only be solved at the later date.

We extend our appreciation to Dr. Vadym Korniyushin, Institute of Zoology of National Academy of Science, Kyiv (Ukraine) for providing a collection of museum material of Polystomatidae and valuable advice on the differences in crown of spines morphology. Our gratitude also goes to anonymous reviewers for their valuable advices and comments on the manuscript.

References

- Biserkov, V. Y., Hadjinikolova, R. I. 1993. *Polystoma fuscus* n. sp. (Polystomatidae) from the common spadefoot *Pelobates fuscus* (Pelobatidae) in Bulgaria. *Systematic Parasitology*, **25**, 145–151.
- Biserkov, V. Y., Yildirimhan, H. S., Buchvarov, G., Ugurtas, I. H. 2001. *Polystoma macrocnemis* n. sp. (Monogenea: Polystomatidae) from the Iranian longlegged wood frog *Rana macrocnemis* (Ranidae) in Turkey. *Systematic Parasitology*, **48** (1), 61–66.
- Buchvarov, G. 1980. *Polystoma mazurmovici* n. sp. (Polystomatidae Gambl, 1896), a parasite of *Rana dalmatina* Bonaparte, 1839 in Bulgaria. *Scientific Studies of the University of Plovdiv, Biology*, **18** (4), 183–190 [In Bulgarian].
- Buchvarov, G. K. 1982. *Polystoma combesi* n. sp. (Polystomatidae Gamble, 1896) — a parasite on *Rana graeca* Boulenger, 1897, in Bulgaria. *Comptes Rendus de l'Academie Bulgare des Sciences*, **35**, 249–251.
- Buchvarov, G. K. 1984 a. *Polystoma skrjabini* n. sp. (Polystomatidae Gambl, 1896) — a parasite of *Hyla arborea* (Linne, 1758), in Bulgaria. *Comptesrendus de l'Académie bulgare des Sciences*, **37** (5), 697–699.
- Buchvarov, G. K. 1984 b. *Polystoma skuratovitchi* n. sp. (Polystomatidae Gambl, 1896) — a parasite of *Rana arvalis* Nicolsson, 1842 from the West Poland. *Scientific Studies of the University of Plovdiv, Biology* **22** (2), 135–142 [In Bulgarian].
- Bykhovskiy, B. E. 1957. *Monogenetic flukes. Their system and phylogeny*. Academy of Sciences of USSR, Moscow, Leningrad, 1957, 1–509 [In Russian].
- Combes, C. 1966. Recherches expérimentales sur la spécificité parasitaire des polystomes de *Rana temporaria* et de *Pelobates cultripes* (Cuv.). *Bulletin de la Société Zoologique de France*, **91** (3), 439–444 [In French].
- Du Preez, L. H., Verneau, O., Gross, T. S. 2007. *Polystoma floridana* n. sp. (Monogenea: Polystomatidae) a parasite in the green tree frog, *Hyla cinerea* (Schneider), of North America. *Zootaxa*, **1663**, 33–45.
- Dzika, E. 2008. *Fish parasites of Poland (keys for identification)*. *Monogenetic flatworms — Monogenea*. Polish parasitological society, Warsaw, 1–189 [In Polish].

- Euzet, L., Combes, C. 1966. *Polystoma integerrimum pelobatis* n. subsp. (Monogenea), parasite of *Pelobates cultripes* (Cuvier, 1829). *Annales de Parasitologie Humaine et Comparee*, **41** (2), 109–18 [In French].
- Euzet, L., Combes, C., Batchvarov G. 1974. Sur un nouveau Polystomatidae Européen, parasite de l'amphibien *Bufo viridis* Laur. *Vie Milieu*, **24** (1), 129–139 [In French].
- Gusev, A. V. 1985. Class Monogenea. Nauka, Leningrad, 10–387. In: Bauer, O. N., ed. *Keys to parasites of freshwater fish of fauna of USSR. Vol. 2. Parasitic multicellular (The first part)* [In Russian].
- Gusev, A. V., Kulemina, I. V. 1971. Taxonomic features of some monogeneans from hosts of different ages. *Parasitologia*, **5** (2), 162–169 [In Russian].
- Lisitsyna, O. I., Miroshnychenko, A. I. 2008. *A catalogue of helminthes of vertebrates of Ukraine. Acanthocephalans. Monogeneans*. I. I. Schmalhausen Institute of Zoology National Academy of Science of Ukraine, Ukrainian Scientific Society of Parasitologists, Kiev, 1–138 [In Russian].
- Parokonnyj, A. S., Sharpilo, V. P., Lisitsyna O. I. 1981. *Polysloma viridis* Euzet, Combes et Batchvarov, 1974 (Monogenea, Polystomatidae) — a new species of the USSR Fauna. *Vestnik Zoologii*, **3**, 87–89 [In Russian].
- Pisanets, E. M. 2007. *Amphibians of Ukraine (Handbook and Key of amphibians of Ukraine and adjacent territories)*. Zoological museum NNPM NAS of Ukraine, Kiev, 1–312 [In Russian].
- Rubtsova, N. Yu., Heckmann, R. A. 2017. Morphological and structural differences of normal adult and sub-adult bladder forms of *Polystoma integerrimum* (Fröhlich, 1798) (Monogenea: Polystomatidae) from the common frog, *Rana temporaria*. *Scientia Parasitologica*, **18** (1–2), 38–53.
- Rubtsova, N. Yu., Sarabeev, V. L., Balbuena, J. A., Domnich, I. F. 2005. Influence of host on morphometric variability of *Ligophorus vanbenedenii* (Ancyrocephalidae, Monogenea). *Vestnik Zoologii*, Suppl. 19, part 2, 270–271 [In Ukrainian].
- Ryzhykov, K. M., Sharpilo V. P., Shevchenko, N. N. 1980. *Helminths of amphibians of fauna of the USSR*. Nauka, Moscow, 1–279 [In Russian].
- Yildirimhan, H. S., Sümer, N., Bursey, C. R. 2016. Helminth parasites of the agile frog, *Rana dalmatina* Fitzinger, 1839 (Anura: Ranidae), collected from two localities in Turkey. *Acta Zoologica Bulgarica*, **68** (3), 2016, 425–432.

Received 14 August 2017

Accepted 9 February 2018