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SEASONAL VARIATION OF ATTACHMENT APPARATUS AND COPULATORY ORGAN MORPHOMETRIC VARIABLES OF *DACTYLOGYRUS CRUCIFER* WAGENER, 1857 (MONOGENEA: DACTYLOGYRIDAE) ON THE GILLS OF ROACH (*RUTILUS RUTILUS* L.) IN LATVIAN WATER BODIES

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There are many abiotic factors, such as oxygen, salinity, pH etc. that inpact to phenotypic plasticity of parasitic organisms. In the present studies, we are focused on seasonal variation in morphometry of monogenea from *Dactylogyrus* genus. Seasonal variation in measurments of attachment apparatus and copulatory organ of *Dactylogyrus crucifer* Wagener, 1857 were studied on museum colection material from 1956 and the period from 1984 to 1998. The main results indicate that difference between winter and spring conditions basically cause significant ($p<0.05$) changes in morphometrical variables of measurements of anchors, dorsal connective bar and copulatory organ.

Key words: Monogenea, *Dactylogyrus crucifer*, attachment apparatus, copulatory organ, seasonality, morphometric variables.

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INTRODUCTION

Dactylogyrus crucifer belongs to the Dactylogyridae family where the genus *Dactylogyrus* is the most diverse genus of the family with more than 900 species (Gibson et al. 1996, Gusev 1985). At least 48 *Dactylogyrus* species parasitize on 19 Latvian fish species (Kirjušina & Vismanis 2007). *D. crucifer* is highly specific species, that localize only on roach`s gills (Gusev 1985). For the first time *D. crucifer* was detected in Latvian water bodies - in lakes Rāznas and Rušons, Ķegums Water Reservoir and Daugava River by S. S. Shulman (Шульман 1949). The further investigation of the roach from 18 lakes shows that prevalence of *D. crucifer* grows up to 100% in some lakes. Parasite is less frequent in the rivers with prevalence from 40% to 86.6%. The intensity range of infection in different types of water bodies also varied (Кирюшина & Висманис 2004).

According Gusev (1985) *D. crucifer* is spread in almost all water bodies where roach habitat.

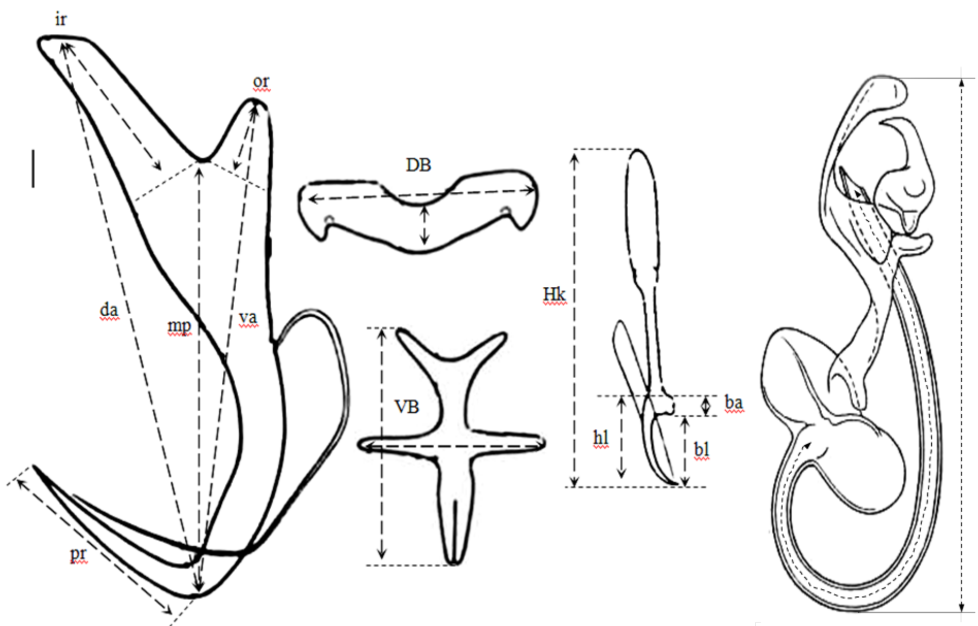

Fig. 1. Metric parameters of the *Dactylogyrus* attachment apparatus and copulatory organ according to Gussev 1983 with the additions.

Öztürk & Altunel (2006) recorded seasonal variation of occurrence of *D. crucifer* from Lake Manyas in Turkey. The prevalence of infection rapidly increase in autumn season and gradually decrease in winter and spring till reach the lowest point in summer season. The mean intensity trend of infecting follows the prevalence except spring season when mean intensity increase while prevalence decrease. The prevalence of *D. sphyrna, D. cornu, D. difformis* rapidly increase in summer period and the lowest value reach in spring and autumn or winter period that does not coincide with *Dactylogyrus crucifer* occurrence. Meanwhile, Soylu et al. (2010) confirmed seasonal variation of occurrence of *D. crucifer* on the roach from Lake Sapanca in Turkey. The highest prevalence of infection was recorded in period from March to October and the lowest in January and February with the highest mean intensity in March and the lowest in November. There are several morphological and anatomical characteristics of monogenea that are used for species determination. The main morphological parameters are morphometric characteristics of attachment apparatus and copulatory organ. Similar species, like *D. crucifer*, *D. caballeroi* and *D. erhardovae* are difficult for species definition using only the shape and measurement of attachment apparatus and copulatory organ, because some measurements often overlap, while shape is variable. Because the mistaken description of an already existing species as newly discovered is also occurs, it is important to know which factors affect morphometrical variation to avoid incorrect determination of species (Gusev 1985).

The aim of this study is to investigate the differences of measurements of attachment apparatus and copulatory organs between *D. crucifer* individuals in four seasons: winter, spring, summer and autumn. It is expected to detect metrical variation in morphology of *D. crucifer* considering seasonal changes in water temperature.

MATERIAL AND METHODS

The research material consists of museum collection from 1956 and the period from 1984 to 1998. The all museum collection samples have following information: the name of fish species, parasite localization on the fish, the date of investigation and name of waterbody. Data about water temperature are not available.

There were used 14 morphometric parameters of the attachment apparatus and two of the copulatory organ: ir – inner root length; or – outer root length; da – dorsoapical total length; va – ventroapical total length; mp – length of main part; pr – length of point recurved; DB – length and width of dorsal connective bar; VB – length and width of ventral connective bar; bl – blade length; ba – base length; hl – hooklet length; Hk – length of total hook; copulatory organ total length and copulatory organ tube length. The attachment apparatus and copulatory organs were measured according to Gusev (Гусев 1983) (Fig. 1).

The number of *D. crucifer* specimens for each season was measured as follow: winter 21, spring 113, summer 23 and autumn 10. A total 167 specimens of *D. crucifer* were measured and photographed with Nicon 90i microscope by using the NIS-elements basic research software. It was not always possible to measure all 16 characters, because some prepared specimens were destroyed during the preparation or because with time had deteriorated (of time-dependent crystallization of glycerol - gelatin which was usually used for slide preparation). Some attachment apparatus had inconvenient position or were unsuccessfully compressed between the coverslip and the slide therefore, have not been recorded, all envisaged measurements. Unequal number of measurements and low number of specimens in autumn period was taken into account when statistical analysis was (Table 1).

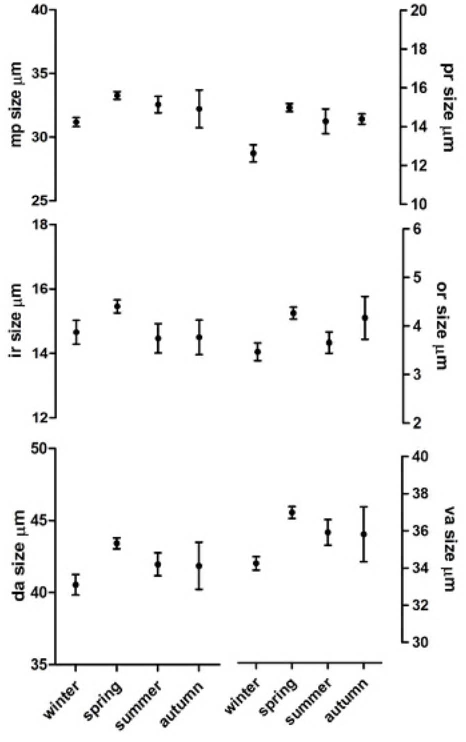

Fig. 2. Seasonal variation of anchor variables – mean with SEM.

Statistical analyses were performed by SPSS (version 20.0) software using descriptive statistic tools, parametric and non-parametric tests, but figures were made by GraphPad Prism 5 software. All the measurements values were initially tested for normal distribution using Kolmogorov – Smirnov test and for homogeneity using Levene test. Depending on mentioned tests results, the following tests were selected: one – way ANOVA or Kruskal – Wallis H test. We used the Brown – Fosythe test to compare means in situation when the data is normally distributed and non-homogeneous. After ANOVA test the post hoc multiple comparison with Bonferroni correction were assessed, when it was determined that there is a difference among the means. If it was significant difference between the groups using Kruskal – Wallis test we performed Mann – Whitney test. There was designed a new critical level of significance which was 0.0085 (by the formula P=1–0.951/n, where n – number of pairwise comparisons). The significance level of 5% was selected for all tests except Mann - Whitney.

RESULTS

Obtained results suggest that seasonal changes have contrastive impact on different structures of attachment apparatus and copulatory organ.

Table 1. Seasonal variation of attachment apparatus and copulatory organ morphometric variables of *Dactylogyrus crucifer*

	Winter (n=21)				Spring (n=113)				Summer (n=23)				Autumn (n=10)			
	N	Mean±SEM	SD	Range	N	Mean±SEM	SD	Range	N	Mean±SEM	SD	Range	N	Mean±SEM	SD	Range
da	18	40.54±0.72	3.04	33-45	86	43.42±0.37	3.47	36-51	17	41.96±0.80	3.28	35-47	6	41.86±1.63	3.99	37-46
ir	18	14.66±0.37	1.56	12-17	86	15.46±0.20	1.89	11-21	17	14.47±0.45	1.87	10-19	6	14.50±0.54	1.32	13-16
va	18	34.25±0.36	1.52	31-37	86	36.99±0.31	2.91	31-45	17	35.91±0.69	2.86	30-39	6	35.82±1.48	3.62	31-39
or	18	3.46±0.18	0.77	2-5	86	4.26±0.12	1.12	2-7	17	3.66±0.22	0.90	2-5	6	4.17±0.44	1.08	3-5
mp	18	31.18±0.35	1.50	28-33	86	33.27±0.30	2.74	27-41	17	32.56±0.65	2.67	28-36	6	32.22±1.48	3.63	28-36
pr	17	12.63±0.44	1.80	8-15	86	14.99±0.21	1.92	11-20	17	14.29±0.64	2.63	8-18	6	14.40±0.26	0.64	14-16
bl	17	5.32±0.09	0.36	4.5-6	84	5.24±0.05	0.49	4-7	16	5.34±0.09	0.35	4.5-6	6	5.40±0.08	0.20	5-6
ba	18	1.78±0.06	0.27	1-2	84	1.83±0.03	0.27	1-2.5	15	1.94±0.09	0.35	1-3	6	1.80±0.06	0.15	1.5-2
hl	18	7.04±0.11	0.45	6-8	84	7.09±0.06	0.53	6-9	15	7.29±0.14	0.54	6-8	6	7.21±0.11	0.27	7-8
Hk	18	29.30±0.72	3.05	25-36	85	31.75±0.45	4.15	24-43	16	30.03±0.88	3.53	22-34	6	29.89±1.62	3.96	25-34
DB length	18	24.94±0.57	2.42	20-28	82	28.21±0.31	2.78	23-39	16	27.25±0.76	3.05	21-34	6	28.32±1.09	2.67	26-32
DB width	17	4.08±0.15	0.60	3-6	80	4.71±0.11	0.96	2-8	16	4.42±0.16	0.64	4-6	6	4.43±0.24	0.59	4-5
VB length	6	24.76±0.51	1.24	23-27	43	24.72±0.40	2.61	21-33	8	27.30±1.19	3.37	22-31	4	23.41±1.98	3.96	20-28
VB width	6	22.04±0.33	0.80	21-23	41	22.93±0.45	2.85	17-30	8	23.47±0.78	2.21	21-27	4	20.52±2.32	4.65	16-25
Copulatory total length	20	52.50±0.72	3.20	47-57	106	55.52±0.43	4.41	44-71	22	52.56±0.76	3.58	46-57	10	55.36±1.12	3.55	50-59
Copulatory tube length	20	68.80±1.37	6.14	57-78	105	71.87±0.68	6.93	59-92	20	65.96±1.52	6.78	55-80	10	69.62±1.89	5.99	58-76

N – number of measured variable; n – number of investigate specimens; SEM – standard error of mean; SD – standard deviation

Generally, sequential seasonal changes (from winter to autumn) don't cause the same sequential changes for *D. crucifer* attachment apparatus and copulatory organ morphometrical parameters.

All 14 morphometric parameters of the attachment apparatus and two parameters of the copulatory organ were normally distributed except width of dorsal connective bar and length of copulatory tube in winter season, but the length of main part, length and width of ventral connective bar were non-homogeneous.

According to ANOVA test there was no effect of season on all hook variables (bl, ba, hl and Hk), and length and width of ventral connective bar (VB) P>0.05. Almost all anchor morphometric variables differ between seasons except inner root length (Fig. 2): dorsoapical total length (da) $F_{3,\ 123}$=4.057 P=0.009; ventroapical total length (va) $F_{3,\ 123}$=5.115 P=0.002; outer root length (or) $F_{3,\ 123}$=3.852 P=0.011; length of main part (mp) $F_{3,\ 123}$=3.299 P=0.023; length of point recurved (pr) $F_{3,\ 122}$=6.815 P<0.000 and inner root length (ir) $F_{3,\ 123}$=2.316 P=0.079. The most significant difference in anchor variable measurements is between winter and spring seasons, but following sequential seasonal changes do not affect anchor size. There is 2.88±0.89 µm (mean ± std. error) mean difference for dorsoapical total length P=0.009, but for ventroapical total length mean difference is 2.74±0.72 µm P=0.001. Outer root length mean difference is 0.8±0.27 µm P=0.024, length of main part 2.09±0.68 µm P=0.017 and length of point recurved 2.36±0.53 µm P<0.000. The length ($F_{3,\ 118}$=7.123 P<0.000 ANOVA) and width (x^2=9.69 df=3 P=0.021 Kruskal – Wallis) of dorsal connective bar differs between seasons and like anchor variables the size of dorsal connective bar is significantly larger only in spring season comparing with winter season (Fig. 3). There is 3.27±0.72 µm mean difference P<0.000 for length of dorsal connective bar, bur for width of dorsal connective bar mean difference is 0.85±0.5 µm (U=382.5 Z=-2.87 P=0.004).

The size of length ($F_{3,\ 154}$=5.37 P=0.002 ANOVA) of copulatory organ and tube (x^2=10.70 df=3 P=0.013 Kruskal – Wallis) significantly differs between seasons (Fig. 4). The length of copulatory organ is smaller only in winter season comparing with spring to 3.03±1.00 µm P=0.018, but length of tube differs between spring and summer seasons U=606.00 Z=-3.07 P=0.002. The mean difference is 4.56±2.57 µm.

All measurements of the attachment apparatus and copulatory organ of investigated *Dactylogyrus* specimens was within the size range described in Key of Monogenea by Gusev (1985)

DISCUSSION

Gyrodactylus and *Dactylogyrus* genus species are ectoparasites that habitat on the fish in similar conditions of abiotic impact. Although the both genus are related, the scrutiny level of abiotic factors result of the influence on metrical parameters of attachment apparatus and copulatory organ differ. Several species of *Gyrodactylus* were investigated for variation

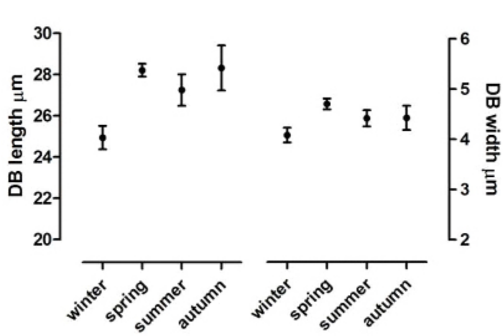

Fig. 3. Seasonal variation of dorsal connective bar – mean with SEM.

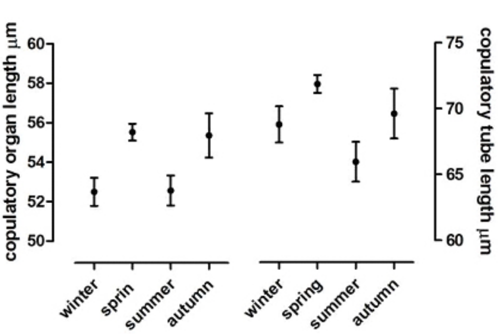

Fig. 4. Seasonal variation of copulatory organ length and tube – mean with SEM.

in the size and shape of attachment apparatus. Investigations concerning rapport between seasons, temperature, host-dependent, host body size and metrical variables of monogenea attachment apparatus were carried out (Malmberg 1970, Ergens 1976, 1991, Ergens & Gelnar 1985, Mo 1991a, 1991b, 1991c, 1991d, 1993;, Geets et al. 1999, Jackson & Tinsley 1995, Dmitrieva & Dimitrov 2002, Karaivanova et al. 2003, Dávidová et al. 2005).

It was established a negative correlation between water temperature and metrical variables of attachment apparatus of the several gyrodactylid (Ergens & Gelnar 1985, Mo 1991b, Geets et al. 1999, Dávidová et al. 2005). By increasing water temperature the size of attachment apparatus gradually decrease. This relationship was verified in the field (Mo, 1991b, 1993, Dávidová et al. 2005) and in the laboratory conditions too (Mo, 1991b,). In both cases some variables of attachment apparatus showed significant regression to the different water temperature. It means that in high water temperature the *Gyrodactylus* species develop smaller attachment apparatus than in cold temperature.

The *D. crucifer* is highly host specific parasite that excludes metrical variation considering different hosts, in spite of this species is spread in almost all water bodies where the roach habitat. In the present studies seasonal effect on metrical variation in measurements of attachment apparatus and copulatory organ of *D. crucifer* was investigated.

The results of investigation confirmed the existence of the changes in size of anchor, dorsal connective bar and copulatory organ of morphometrical parameters of *D. crucifer* attachment apparatus and copulatory organ considering seasonal changes. Changes in winter and spring conditions cause significant difference in measure of anchor parts, dorsal connective bar and copulatory organ length, but copulatory tube length change between spring and summer seasons. Almost the same results recorded by Dávidová et al. (2005) investigating water temperature impact to total length of marginal hooks, the sickle length of marginal hooks and the width of ventral bar of *Gyrodactylus rhodei*. Similar results reported by Ergens&Gelnar (1985) and Mo (1991) making experiments with *Gyrodactylus katharineri* and *G. salaris* respectively. However interrelation between seasons and total length of marginal hooks, the sickle length of marginal hooks and the width of ventral bar was not noticed for *D. crucifer* in present study, but seasonal variation of anchor measure coincide for mentioned *Gyrodactylus* species.

Because Öztürk & Altunel (2006) and Soylu et al. (2010) recorded seasonal variation of occurrence of *D. crucifer* we suggest that metrical variations in attachment apparatus can be connected with optimal temperature for *D. crucifer* new generation development. In summers and winter when temperature amplitudes are usually larger than in spring and autumn period *D. crucifer* reproduces less intensely and individuals develop smaller attachment apparatus. Meanwhile, Mo (1991) recorded significant regression of attachment apparatus to water temperature for *Gyrodactylus* species. Because of absence data about water temperature the correlation between temperature and attachment apparatus and copulatory organ of *D. crucifer* metrical parameters are not processed.

Research results suggest that the further investigation of *Dactylogyrus* species require investigating correlation between water temperature and attachment apparatus and copulatory organ morphometrical parameters using new speciments of monogenea.

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REFERENCES

Dávidová M., Jarkovský J., Matějusová I., Gelnar M., 2005. Seasonal occurrence and metrical variability of *Gyrodactylus rhodei* Žitňan 1964 (Monogenea, Gyrodactylidae). *Parasitology.* 95. p. 398 – 405.

Dmitrieva E. and Dimitrov G. 2002. Variability in the taxonomic characters of Black Sea gyrodactylids (Monogenea). *Syst. Parasitol.* 51. p. 199–206.

Gibson D. I., Timofeeva T. A., Gerasev P. I. 1996. A catalogue of the nominal species of the monogenean genus *Dactylogyrus* Diesing, 1850 and their host genera. *Syst. Parasitol*. Vol. 35. p. 3 – 48.

Gusev A.V., 1985. Monogenea. In: Bauer O. N. (ed) Identification key to parasties of fresh – water fishes (in Russian). *Nauka* Vol. 2., Leningrad p. 425.

Soylu E., Rüzgar B., Soylu M., 2010. Seasonal Dynamics and spatial distribution os *Dactylogyrus crucifer* Wagener, 1857 on the gills of roach (*Rutilus rutilus*) from Lake Sapanca. *Turkey. Turk J Zool.* 34. p. 393 – 398.

Öztürk M. O., Altunel F. N., 2006. Occurrence of *Dactylogyrus* infection linked to seasonal changes and host fish size on four cyprinid fishes in lake Manyas, Turkey. *Acta Zoologica Academiae Scientiarum Hungaricae* 52 (4), p. 407–415.

Kirjušina M., Vismanis K., 2007. Checklist of the parasties of fishes of Latvia. *FAO Fisheries Technical Paper.* No. 369/3. p. 106.

Malmberg G. 1970. The excretory systems and the marginal hooks as a basis for the systematics of *Gyrodactylus* (Trematoda, Monogenea). *Ark Zool* 23: 1–235.

Ergens R. 1976. Variability of hard parts of opisthaptor of two species of *Gyrodactylus* Nordmann, 1832 (Monogenoidea) from *Phoxinus phoxinus* (L). *Folia Parasitol* 23:111–126.

Ergens R., 1991. Variability of the hard parts of opisthaptor of *Gyrodactylus leucisci* Žitnan, 964 (Monogenea: Gyrodactylidae). *Folia Parasitol* 38:23–28.

Ergens R., Gelnar M. 1985. Experimental verification of the effect of temperature on the size of hard parts of opisthaptor of *Gyrodactylus katharineri* Malmberg, 1964 (Monogenea). *Folia Parasitol* 32:377–380.

Geets A., Appleby C., Ollevier F. 1999. Host-dependent and seasonal variation in opisthaptoral hard parts of *Gyrodactylus cf. arcuatus* from three *Pomatoschistus* spp. and *C. arcuatus* from *Gasterosteus aculeatus*: a multivariate approach. *Parasitology* 119: 27–40.

Jackson J. A. and Tinsley R. C. 1995. Sclerite growth and morphometric variation in *Gyrdicotylus gallieni* Vercammen Grandjean, 1960 (Monogenea: Gyrodactylidae) from *Xenopus laevis laevis* (Anura). *Systematic Parasitology* 31: 1 – 9.

Karaivanova E., Nedeva I., Atanassov G. 2003. Analyses of the influence of the host body size on the morphometrical characters of *Dactylogyrus extensus* (Mueller et Van Cleave, 1932). *Proceedings of the 2nd Congress of Ecologists of Macedonia,* 6: 286 – 291.

Mo T. A., 1991a. Variations of opisthaptoral hard parts of *Gyrodactylus salaris* Malmberg, 195 (Monogenea: Gyrodactylidae) on rainbow trout *Oncorhynchus mykiss* (Walbaum, 1792) in fish farm, with comments on the spreading of the parasite in southeastern Norway. *Syst. Parasitol.* 20: 1–9.

Mo T. A. 1991b. Seasonal variations of opisthaptoral hard parts of *Gyrodactylus salaris* Malmberg, 1957 (Monogenea:

Gyrodactylidae) on parr of Atlantic salmon *Salmo salar* (L) in the River Batnfjordselva, Norway. *Syst. Parasitol.* 19: 231–240.

Mo T. A. 1991c. Variations of opisthaptoral hard parts of *Gyrodactylus salaris* Malmberg, 1957 (Monogenea: Gyrodactylidae) on parr of Atlantic salmon *Salmo salar* (L) in laboratory experiments. *Syst. Parasitol.* 20: 11–19.

Mo T. A. 1991d. Seasonal variations of opisthaptoral hard parts of *Gyrodactylus salads* Malmberg, 1957 (Monogenea: Gyrodactylidae) on parr of Atlantic salmon *Salmo salar* L. in the River Batnfjordselva, Norway. *Systematic Parasitology* 19: 231 – 240.

Mo T. A. 1993. Seasonal variations of opisthaptoral hard parts of *Gyrodactylus derjavini* Mikailov, 1975 (Monogenea: Gyrodactylidae) on brown trout *Salmo trutta* L. parr and Atlantic salmon *S salar* L. parr in the River Sandvikselva, Norway. *Systematic Parasitology* 26. p. 225 – 231.

Шульман С. С., 1949. Паразиты рыб Латвийской СССР (канд. дисс.). Л.: 337 с.

Кирюшина М., Висманис К., 2004. Паразиты пресноводных и морских рыб Латвии. Систематический каталог. *Научные тетради.* Выпуск № 8. 100 с.

Гусев А. В., 1983. Методика сбора и обработки материала по моногенеям, паразитирующим у рыб. Л.: *Наука*. 47с.

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