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HAPTORAL HARD PART VARIABILITY IN GYRODACTYLUS TEUCHIS LAUTRAITE, BLANC, THIERY, DANIEL & VIGNEULLE, 1999 (MONOPISTHOCOTYLA: GYRODACTYLIDAE) PARASITIZING CAGE-REARED RAINBOW TROUT IN KARELIA

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Variability of the marginal hooks of *Gyrodactylus teuchis* parasitizing rainbow trout *Oncorhynchus mykiss* (Walbaum, 1792) was studied in cage farms situated in water bodies of the Baltic and White Sea drainage basins. Differences between groups proved to be predicated on two characters – marginal hook total length (*MHTL*) and marginal hook sickle length (*MHSL*). The probability of correctly identifying a specimen as belonging to the White Sea or Baltic group relying on the discriminant function based on these predictors is 98.6%. Meanwhile, when the function was tested on the worms whose samples were not involved in discriminant analysis, its predictions were correct only in 73% cases. These results indicate substantial intraspecific variability in the above mentioned characters in *G. teuchis*.

Keywords: *Oncorhynchus mykiss*, aquaculture, ectoparasites, haptoral hooks

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In Europe, *Gyrodactylus teuchis* Lautraite, Blanc, Thiery, Daniel & Vigneulle, 1999 is a widespread parasite of wild and farmed salmonids (Harris et al., 2004; Rokicka et al., 2007). For a long time, the lack of adequate genetic characterization and morphological similarity led to confusion between *G. teuchis* and the widely known potentially epizootic parasite of salmonids *Gyrodactylus salaris* Malmberg, 1957. Some authors (Cunningham et al., 2001) described these species as cryptic to each other, so the species identification of *G. teuchis* still required a combination of the morphological and molecular approaches. Advancements in optical and digital techniques as well as latest multivariate statistical analysis packages have enabled convincing visualization of morphological differences between gyrodactylid species, namely *G. teuchis* vs. *G. salaris* (Hahn et al., 2011). As we know, morphological analysis in the traditional taxonomy of gyrodactylids largely builds upon the assessment of a stable species characters – the shape of the hamuli and marginal hooks of the opisthaptor (Ergens, 1985). On the other hand, morphometric analysis of individual structural elements demonstrated high variability of their values, which was sharper for hamuli and smoother for marginal hooks (Kulemina, 1977). The variability vector depends on external environmental impacts, most importantly water temperature (Ergens, 1976; Kulemina, 1977).

The aim of this study was to assess the distinctive morphometric features of marginal hooks in *G. teuchis* worms collected from rainbow trout reared in cages in lakes of Karelia (Baltic and White Sea basins) during the cold season.

MATERIALS AND METHODS

Fish were sampled on 11–30 May 2023 from cages located in "Tulguba" (Lake Onego), "Raiguba" (Lake Sundozero, Lake Onego catchment), "Rautalahti", "Lamberg" (Lake Ladoga), and "Tiksha" (Lake Kalmozero, catchment of the Kem River, draining to the White Sea) (Table 1).

Table 1. Sampling sites and characteristics of the fishes

Farm	Localities (bay or lake)	Drainage basin	Number of fish examined	Weight, g (mean±SE)	Length (AB), cm
Tulguba	Kondopozhskaya Bay, Lake Onego	Baltic Sea	16	228.7±18.8	29.2±0.7
Raiguba	Sundozero, Lake Onego	Baltic Sea	15	65±6.1	19.8±0.6
Lamberg	Lake Ladoga	Baltic Sea	20	47.6±3.1	16.6±0.3
Rautalahti	Rautalahti, Lake Ladoga	Baltic Sea	15	210.6±9.8	28.5±0.4
Tiksha	Kalmozero	White Sea	18	21.1±1.3	11.9±0.3

Water temperature was measured with a hand-held temperature meter (Polaris) at 1–3 m and 7 m depths.

Partial parasitological dissection was applied to 84 rainbow trout specimens. Parasitological samples were processed by standard techniques (Bykhovskaya-Pavlovskaya, 1985). The fish were immobilized and all fins were examined under LOMO MSP-2 microscope with magnification ranging from ×7 to ×45. When finding helminth-infected fins, they were transferred to and stored in 5 ml Eppendorf tubes with 96° ethanol. When making glycerol-gelatin slides, the cut-off attachment disk was moved from the water drop on the glass slide into a drop of Proteinase K (60 µg/ml) for several minutes to digest the soft tissues and separate the hard parts. Images were taken on Olympus BX-53 microscope with DIC option (KarRC RAS Core Facility, Petrozavodsk, Russia).

Measurements of marginal hooks (µm) from 92 helminth specimens were taken in the Levenhuk ToupView program, V. Levenhuk, Inc., for seven features, according to Shinn et al. (2004): marginal hook sickle length (*MHSL*), marginal hook sickle proximal width (*MHSPW*), marginal hook toe length (*MHSTL*), marginal hook sickle distal width (*MHSDW*), marginal hook aperture (*MHAD*), marginal hook total length (*MHTL*), and marginal hook shaft length (*MHSHL*). The measurements of one marginal hook of a *G. teuchis* specimen were randomly chosen to be used in the statistical analysis.

The distributions of morphological character values were tested for normality using the Shapiro-Wilk test. Two-sample tests were applied for pairwise comparisons between sample sets. The observed frequency distributions were compared by the Kolmogorov–Smirnov test, homogeneity of the variances was assessed by the Fisher's test and significance of the difference in means by the Student's test (basic functions in R Core Team, 2024). The homogeneity of covariance matrices of the characters (multivariate normality) was checked by Box's M-test with approximated Pearson's chi-squared test (da Silva, 2022). The standard $\alpha = 0.05$ level was accepted as a critical value for the significance of differences. The worms were classified by linear discriminant analysis (LDA, Ripley et al., 2024). The predictors, i.e. the morphological characters of greatest discriminatory value, were selected by a stepwise procedure with between-group differences estimated by Wilk's λ value approximated by Fisher's criterion (Roever et al., 2023). Coefficients of the discriminant function and then its values for each specimen were calculated from the predictor datasets. The significance of the resultant equation was measured by the ratio of the between-group covariance matrix discriminant to the within-group covariance matrix discriminant (Wilk's λ test). The value of the discriminant function at the point of intersection of the Gaussian curves for the tested groups was taken as the intergroup separation boundary.

The data were processed in the MS Excel and R 4.4.0 (R Core Team, 2024) environments with the use of basic functions and functions of the packages 'biotools' (da Silva, 2022), 'klaR' (Roever et al., 2023), and 'MASS' (Ripley et al., 2024).

RESULTS AND DISCUSSION

Testing of our helminth samples showed that only a combination of three of them (from "Tulguba" ($n = 21$), "Lamberg" ($n = 29$), and "Tiksha" ($n = 20$)) meets the requirements for discriminant analysis (normal distribution and homogeneous variances for each morphological character). Furthermore, as there were no significant differences between worms from lakes Onego and Ladoga ("Tulguba" and "Lamberg", Baltic Sea basin) in the means of any of the haptoral (marginal hooks) character measurements (Student's test: $t < 1.51$, $p > 0.139$), the subsets could be pooled together. Worms from Lake Kalmozero ("Tiksha", White Sea basin) had smaller hooks on average (Table 2).

Table 2. Statistical parameters of the marginal hook measurements of different samples of *G. teuchis*

Measurements	Parameters	Drainage basin		Two sample tests	
		Baltic Sea	White Sea	Test	*p*
	n	50	20		
Marginal hook sickle length (*MHSL*)	range	7.97–9.10	7.33–8.24	$KS = 0.91$	**<0.001**
	M	8.571	7.733	$t = 12.49$	**<0.001**
	S	0.251	0.260	$F = 1.07$	0.814
	pW	0.927	0.340		
Marginal hook sickle proximal width (*MHSPW*)	range	4.82–6.09	4.67–5.49	$KS = 0.75$	**<0.001**
	M	5.557	4.992	$t = 6.85$	**<0.001**
	S	0.266	0.234	$F = 1.28$	0.563
	pW	0.417	0.748		
Marginal hook toe length (*MHSTL*)	range	1.84–2.56	1.49–2.31	$KS = 0.46$	**0.003**
	M	2.205	2.030	$t = 3.86$	**<0.001**
	S	0.160	0.198	$F = 1.53$	0.234
	pW	0.978	0.147		
Marginal hook sickle distal width (*MHSDW*)	range	6.86–8.35	6.58–7.91	$KS = 0.26$	0.248
	M	7.487	7.345	$t = 1.78$	0.078
	S	0.286	0.341	$F = 1.43$	0.316
	pW	0.310	0.725		

Table 2. *Continuation*

Measurements	Parameters	Drainage basin		Two sample tests	
		Baltic Sea	White Sea	Test	p
	n	50	20		
Marginal hook aperture (*MHAD*)	range	6.39–7.73	6.07–6.88	$KS = 0.88$	**<0.001**
	M	7.111	6.532	$t = 9.24$	**<0.001**
	S	0.252	0.192	$F = 1.73$	0.191
	pW	0.202	0.845		
Marginal hook total length (*MHTL*)	range	36.90–42.05	33.68–37.60	$KS = 0.98$	**<0.001**
	M	39.561	35.197	$t = 16.32$	**<0.001**
	S	0.987	1.072	$F = 1.18$	0.624
	pW	0.664	0.164		
Marginal hook shaft length (*MHSHL*)	range	28.69–34.09	26.55–30.17	$KS = 0.94$	**<0.001**
	M	31.533	27.961	$t = 14.36$	**<0.001**
	S	0.912	1.009	$F = 1.22$	0.555
	pW	0.226	0.071		

N o t e. n – sample size, range – range of variability, M – mean, S – standard deviation, pW – significance of difference from normal distribution (Shapiro–Wilk test), KS – difference of observed distributions (Kolmogorov–Smirnov test), t – difference of means (Student's test), F – difference of variances (Fisher's test), p – significance of the differences.
Significant differences are shown in bold.

Considering that water temperature in Lake Kalmozero during the sampling period was higher (12.9°C at 1 m depth, 8.1°C at 7 m) than in lakes Onego and Ladoga (4.7°C and 5.3°C at 1 m depth, 5°C and 6.5°C at 7 m), then by analogy with, for example, cyprinid-hosted or salmonid-hosted gyrodactylids, for which inverse correlation between the size of attachment organs and the temperature was demonstrated (Ergens, 1976, 1985; Kulemina, 1977; Mo, 1991a, b, c), we can suppose that the intergroup differences observed in our case are due to the different thermal regimes of the lakes.

Generalized distinctions between worms from waters in the Baltic and White Sea drainage basins were measured by linear discriminant analysis (LDA). Selection by a stepwise procedure yielded two meaningful morphological characters, which were used as predictors of specimens' group identity (Table 3).

Table 3. Results of stepwise selection of meaningful characters and the linear discriminant function Z for morphology-based classification of *G. teuchis* from water bodies in the Baltic and White Sea drainage basins

Attribute	Wilk's λ	F	p	Coefficients
Marginal hook total length (*MHTL*)	0.203	266.22	< 0.001	-0.767
Marginal hook sickle length (*MHSL*)	0.185	147.30	< 0.001	-1.462
Linear discriminant function: $Z = -0.767*MHTL - 1.462*MHSL + 41.569$				

N o t e. Wilk's λ – between-group difference estimate, F – approximated Fisher criterion, p – significance of differences.

They are the marginal hook total length (*MHTL*) and marginal hook sickle length (*MHSL*), the transgression of their distributions shown in Fig. 1.

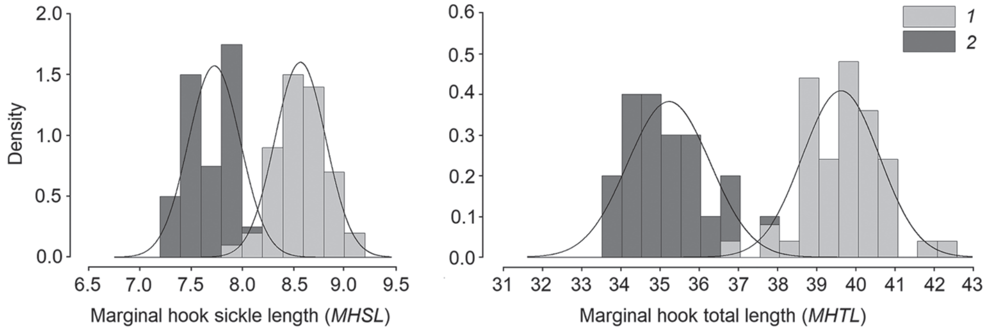

Figure 1. Transgression of the frequency distributions of the morphological characters that predict the discriminant function *Z*: *1* – Baltic Sea basin, *2* – White Sea basin.

The rest of the characters did not improve the discrimination. The between-group variance of the predictors was significantly greater than within-group variance (Wilk's $\lambda = 0.185$, approximate $F = 147.30$, $p < 0.001$), the overall probability of predicting correctly was 98.6%, i.e. only one of 70 specimens was misidentified. The upper boundary for worms from the Baltic Sea basin and the lower boundary for worms from the White Sea basin are aligned in point $Z \sim 0.73$ with $P = 96.4\%$ (Fig. 2), indicating the probability that no more than 4.6% of individuals from the sampled localities can be classified erroneously.

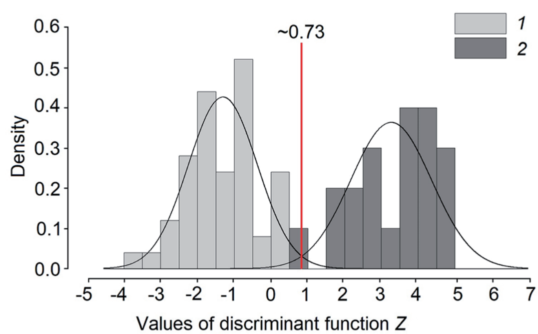

Figure 2. Distribution of the discriminant function *Z* values for *G. teuchis* from water bodies in the Baltic (*1*) and White (*2*) Sea drainage basins. Deviation from normality is insignificant (Shapiro–Wilk test: $W < 0.939$, $p > 0.225$). Red line marks the threshold value (correct prediction probability $P \sim 96.4\%$, see also text).

At the same time, based on the values of the discriminant function *Z* computed for the available 22 specimens of *G. teuchis* from other water bodies in the Baltic Sea drainage basin – "Rautalahti" (Lake Ladoga) and "Raiguba" (Lake Sundozero), at least 73% of the worms were placed into the Baltic group and the rest fell into the White Sea group.

The morphological features of gyrodactylid haptoral parts studied previously by Hahn et al. (2011) exhibited no intraspecies variation where the environmental conditions were the same. In several species examined (*G. teuchis*, *G. salaris* and *G. thymalli*), only 16 of 26 characters tested the normality for further calculations of parametric statistics. Similarly to our results, they found that not a single character can be diagnostic on its own and discrimination between species or, in our case, between geographical groups of populations, requires a combination of characters. Ultimately, Hahn et al. (2011) found that the marginal hook characters that can provide discrimination between samples of *G. teuchis* and *G. thymalli* were *MHTL* and *MHSHL*, while *G. teuchis* was discriminated from *G. salaris* by *MHSDW* and *MHAD*.

Our results provide evidence of a wide intraspecies variability of *G. teuchis* and likely bring out the trend for a smaller size of hooks in dependence with the thermal regime of the water bodies. This will need to be verified using larger samples covering a wide temperature range. The key characters that permitted reliable discrimination between gyrodactylid specimens were the marginal hook total length (*MHTL*) and marginal hook sickle length (*MHSL*). A task that remains relevant is building up data on the haptoral sclerites of gyrodactylids to enable more effective discrimination between the parasites and to expand their applicability for diagnosing quarantine species by veterinary services.

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CONFLICT OF INTEREST

The authors of this work declare that they have no conflicts of interest.

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ИЗМЕНЧИВОСТЬ СТРУКТУР ПРИКРЕПИТЕЛЬНОГО ДИСКА ГЕЛЬМИНТОВ *GYRODACTYLUS TEUCHIS* LAUTRAITE, BLANC, THIERY, DANIEL & VIGNEULLE, 1999 (MONOPISTHOCOTYLA: GYRODACTYLIDAE), ПАРАЗИТИРУЮЩИХ НА САДКОВОЙ РАДУЖНОЙ ФОРЕЛИ В ВОДОЕМАХ КАРЕЛИИ

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Ключевые слова: *Oncorhynchus mykiss*, аквакультура, эктопаразиты, прикрепительные крючья

РЕЗЮМЕ

Изменчивость краевых крючьев червей *Gyrodactylus teuchis,* паразитирующих на радужной форели *Oncorhynchus mykiss* (Walbaum, 1792), изучали в садковых хозяйствах, расположенных в акваториях водоемов бассейнов Балтийского и Белого морей. Установлено, что межгрупповые различия определяются двумя признаками – общей длиной краевого крючка (*MHTL*) и длиной собственно крючка (*MHSL*). Вероятность правильного прогноза принадлежности особи к беломорской или балтийской группе по сконструированной на основе этих предикторов дискриминантной функции составила 98.6%. Между тем апробация функции на паразитах, выборки которых не использовали в дискриминантном анализе, дала правильный прогноз лишь в 73% случаев. Полученные результаты указывают на существенную внутривидовую изменчивость изученных признаков у *G. teuchis*.