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The use of isozymes to identify specimens of *Pomphorhynchus* (Acanthocephala) in flounder, *Platichthys flesus* from the Baltic Sea

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Specimens of flounder *Platichthys flesus* were randomly sampled from three regions of the southern Baltic Sea: the Gulf of Gdańsk, the Pomeranian Bay, the open sea off Łeba and from the northern Øresund, Kattegat. The collected parasites were morphologically identified and their genotypes at seven loci coding for enzymes were checked. Wide ranges in both the numbers of rows of rostellar hooks and the numbers of rostellar hooks per row were detected, indicating the presence of two species in the material: *Pomphorhynchus kostylewi* and *Pomphorhynchus laevis*. However, electrophoretic data does not support occurrence of two independent gene pools.

INTRODUCTION

The genus *Pomphorhynchus* Monticelli, 1906 has been rather poorly explored as far as individual variability is concerned, and this often creates difficulties in determining species. Two species belonging to the genus *Pomphorhynchus* have been reported in the intestine of the flounder *Platichthys flesus* (L.) from the Baltic Sea. Fagerholm & Køie (1994) reported *Pomphorhynchus laevis* (Müller, 1776). However Rokicki (1975) has stated that representatives of the genus *Pomphorhynchus* from *Platichthys flesus* from the Gulf of Gdańsk was *Pomphorhynchus kostylewi* Petrotsčhenko, 1956. Grabda-Kazubska (1997) determined specimens from flounder *Platichthys flesus* from the Gulf of Gdańsk as *Pomphorhynchus kostylewi* and *P. laevis*. Meanwhile in the Baltic Sea Kennedy (1984) reported it as *Pomphorhynchus* sp. and he suggested that in marine flounders, whether in the Baltic or the North Sea, *Pomphorhynchus* sp. belong to a different strain according the development and life cycle. The aim of this study was to compare the morphological and genetic variability within the genus *Pomphorhynchus* in flounder *Platichthys flesus* (L.) from the Baltic Sea.

MATERIALS AND METHODS

Pomphorhynchus parasites were collected from specimens of flounder *Platichthys flesus* (L.) which were randomly sampled from nets of local fishermen from three regions of the southern Baltic Sea: the Gulf of Gdańsk (G), Pomeranian Bay (P), open sea off Łeba (L) and from the northern Øresund, Kattegat (K). Some of the collected parasites were cleaned of mucus in physiological saline (0.9%) and prepared for hook counting by washing in distilled water followed by clearing in Berland's fluid then in lactic acid and mounted in glycerol–gelatine: 42 individuals of parasite from the Gulf of Gdańsk, 31 from the open sea off Łeba, 48 from Pomeranian Bay, and 21 from the northern Øresund, Kattegat. A total of 410 parasites (details in Table 1) were washed in distilled water and frozen at −70°C for horizontal cellulose acetate electrophoresis.

The activity of 16 enzymes was tested. Only six of them (GOT, GPI, LDH, LAP, MDH and PGM) were chosen for systematic examination. The remaining enzymes were eliminated from the investigation due to lack of activity on the gels i.e. ACON, ADH, EST, FUM and IDH or a very weak activity i. e. G-6PDH, G-3-PDH, GPDH, ME and 6PGDH.

Standard horizontal cellulose acetate electrophoresis followed by staining for specific enzyme activities was applied to the homogenized parasite samples with respect to the selected enzymes. A BIOSYS-2 computer program was used for statistical analysis of the electrophoretic data, including Nei's measures of unbiased genetic identity and distance.

RESULTS AND DISCUSSION

The parasites from the three regions of the southern Baltic Sea showed 12 and 17 rows as the minimum and the maximum number of rows of rostellar hooks respectively, with a mean of 14.5 rows. The minimum and the maximum number of rostellar hooks per row were 9 and 15 hooks respectively, with mean of 12 hooks per row. The parasites from northern Øresund, Kattegat showed 14 and 20 rows as the minimum and maximum number of rows of rostellar hooks respectively, with mean of 17.5 rows. The minimum and maximum number of rostellar hooks per row were 10 and 13 hooks respectively, with mean of 11.5 hooks per row. These data showed wide ranges in both the numbers of rows of rostellar hooks and the numbers of rostellar hooks per row.

The LAP and LDH electrophoretic patterns obtained for *Pomphorhynchus* were in accordance with the model predicted for a monomeric protein coded by a single locus

Table 1. *Allelic frequencies observed at seven loci in four local populations.*

	Sampling localities			
Alleles	Gulf of Gdańsk (G)	Open sea of Łeba (L)	Pomeranian Bay (P)	Kattegat (K)
Got^a	0.000	0.005	0.004	0.107
Got^b	0.266	0.113	0.099	0.164
Got^c	0.734	0.882	0.897	0.729
n	64	93	131	61
H	0.313	0.215	0.176	0.344
χ^2	2.534	0.900	0.331	2.710
P	0.111	0.765	0.565	0.100
Gpi^a	0.006	0.006	0.011	0.000
Gpi^b	0.994	0.994	0.985	0.986
Gpi^c	0.000	0.000	0.004	0.014
n	78	84	134	72
H	0.013	0.012	0.030	0.028
χ^2			0.031	0.001
P			0.861	0.992
Lap^a	0.227	0.293	0.216	0.289
Lap^b	0.539	0.537	0.590	0.514
Lap^c	0.234	0.170	0.194	0.197
n	64	94	116	71
H	0.516	0.574	0.534	0.648
χ^2	1.461	0.602	0.410	0.013
P	0.227	0.438	0.833	0.911
Ldh^a	1.000	0.995	0.996	0.993
Ldh^b	0.000	0.005	0.004	0.007
n	88	97	138	72
H		0.010	0.007	0.014
Mdh-1^a	0.016	0.010	0.000	0.000
Mdh-1^b	0.903	0.936	0.948	0.910
Mdh-1^c	0.081	0.054	0.052	0.090
n	93	101	134	67
H	0.151	0.129	0.090	0.119
χ^2	2.534	0.418	0.030	0.186
P	0.111	0.489	0.863	0.666
Mdh-2^a	0.000	0.000	0.008	0.000
Mdh-2^b	0.912	0.933	0.961	0.806
Mdh-2^c	0.088	0.067	0.031	0.194
n	74	90	130	54
H	0.122	0.089	0.062	0.278
χ^2	0.164	0.221	0.067	0.693
P	0.685	0.639	0.796	0.405
Pgm^a	1.000	1.000	1.000	1.000
n	41	67	41	49
$\overline{H}$	0.159	0.147	0.128	0.204

n, sample size; H, observed heterozygosity; $\overline{H}$, average heterozygosity over loci; χ^2, values calculated for quality of fit to Hardy–Weinberg expectations of genotype frequencies; P, probability connected with given χ^2 value.

Table 2. *Estimates of genetic distance D (above diagonal) and genetic identity I (below diagonal) between four local populations.*

	Estimates of genetic distance D			
Populations	G	L	P	K
G	—	0.006	0.006	0.004
L	0.996	—	0.001	0.006
P	0.996	1.000	—	0.006
K	0.998	0.996	0.996	—

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with three and two co-dominant alleles, respectively. The GOT, GPI and MDH phenotypes corresponded to the model predicted for a dimeric protein coded by a single locus (GOT, GPI) or two loci (MDH), with three codominant alleles. The LDH and PGM were found to be monomorphic in all samples however at the LDH-coding locus a rare allele was ascertained. Loci and alleles were designed according to the electrophoretic mobility of the corresponding proteins, i.e. the fastest one as '1' or 'a', respectively.

The allele frequencies, data on heterozygosity and χ^2 values connected with Hardy–Weinberg expectations are presented in Table 1. Not all of electromorphs could be interpreted genetically in some individuals and therefore in Table 1 different numbers of individuals analysed are given with reference to the particular loci. All polymorphic loci proved to be in Hardy–Weinberg equilibrium. The occurrence of rare alleles in Gpi (a, c), Mdh-1 (a), Mdh-2 (a) loci was recorded. Among them only Mdh-2^a was restricted to the one population. Wright's F_{ST} as a measure of interpopulation differentiation, calculated for the highly polymorphic loci, amounted to 0.0395 for *Got*, 0.0042 for *Lap*, 0.0045 for *Mdh-1* and 0.0411 for *Mdh-2*. The genetic identity and genetic distance of the four local populations, estimated from the data on all loci studied, are presented in Table 2.

Although the number of parasites used for counting the hooks were relatively small it was still possible to speculate the results. The data of the present study showed wide ranges in both the numbers of rows of rostellar hooks and the number of rostellar hooks per row. Comparing the morphological data of the present study with previous ones on different populations described by Rokicki (1975) for *P. kostylewi* from the Gulf of Gdańsk and by Petročenko (1956) for both *P. laevis* and *P. kostylewi* from Russia (Sevan Lake), the parasites from the three regions of southern Baltic Sea were identified as *P. kostylewi*, while the parasites from northern Øresund, Kattegat were considered as *P. laevis*. Unequivocal identification of species within the genus *Pomphorhynchus* is currently still very difficult so therefore isozyme electrophoresis was performed for decisive verification of the species.

Morphological variability is not reflected at the enzyme markers level. Electrophoretic data indicate a very high similarity among the local populations analysed (Table 1). High values of genetic identity index I (0.996–1.0) (Table 2) and low values of F_{ST} (0.0042–0.0411) support the hypothesis that these four local populations belong to the same undivided population. On the base of this statement *P. kostylewi* could be recognized as a new junior synonym of *P. laevis*.

Because of the lack of comparative results on the allozyme variability in *Pomphorhynchus* spp. comparison is made with data observed for other Acanthocephala. Väinölä et al. (1994) reported I =0.97 and F_{ST} =0.23 for the lakes (Keitele and Pulmankijärvi, Finland) and Baltic population of *Echinorhynchus bothniensis*, F_{ST} =0.27 for Baltic and Atlantic populations of *Echinorhynchus gadi*, $\overline{H}$=0.08 and 0.16 for lakes and Baltic populations of *Echinorhynchus bothniensis*, respectively and 0.04 and 0.09 for Baltic and Atlantic populations of *Echinorhynchus gadi*, respectively. Our data show that interpopulation variability in *P. laevis* is considerably lower than for species of *Echinorhynchus* while intrapopulation variability is considerably higher. Relatively high intrapopulation variation ($\overline{H}$=0.128–0.204) (Table 1) corresponds to high morphological variability in *P. laevis* reported by Brown (1987). The statement, concerning the very low level of geographic differentiation of *Pomphorhynchus laevis* in the Baltic Sea and Kattegat is in accordance with results by Munro et al. (1990) for English freshwater and marine strains of this species at the total repetitive DNA polymorphism level.

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