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Genetic evidence for two sibling species within *Polydora* cf. *ciliata* (Polychaeta: Spionidae) from the Sea of Japan

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Abstract Two morphologically indistinguishable but genetically very distinct polychaete species of the family Spionidae are found in the Peter the Great Bay of the Sea of Japan. Both species bore into molluscan shells and occur sympatrically. They are close in morphology to *Polydora ciliata* (Johnston, 1838) and some other members of the redefined *P. ciliata* species complex. The systematics of the two species is still under consideration by one of us (V.I.R.). A total of nine enzyme systems comprising 12 isozyme loci were examined in both the species by means of starch gel electrophoresis. Nei's genetic distance between them proved surprisingly high ($D = 1.942$) being the highest among estimates so far obtained for pairs of polydorid species. The levels of intraspecific genetic variation in both species (*Polydora* sp. 1, $H_e = 0.287 \pm 0.064$; *Polydora* sp. 2, $H_e = 0.111 \pm 0.045$) are comparable to those of other electrophoretically studied polydorids. The problem of taxonomic integrity of cosmopolitan marine invertebrate species is briefly discussed as well as the usefulness of isozymes as species-specific characters suitable for unequivocal discrimination between sibling polydorid species. A list of enzymes characterized by interspecifically variable numbers of expressed isozymes within polydorids is given and recommended for such a discrimination.

Introduction

A spionid polychaete, *Polydora ciliata*, was originally described by Johnston (1838) (as *Leucodore ciliatus*) from "between the seams of slaty rocks near low-water mark, burrowing in the fine soft mud which lines the fissures" in England. Later, the species was reported to bore into mussel, oyster and scallop shells and other calcareous substrates from all around the world, and for a long time was known as a common cosmopolitan borer and a tube-dwelling inhabitant of soft sediments (McIntosh 1915; Söderström 1920; Fauvel 1927, 1953; Uschakov 1955; Imajima and Hartman 1964; Hartmann-Schröder 1971; Blake 1983). A number of subspecies of *P. ciliata* were described from different regions of the world (e.g. *P. ciliata brevipalpa* Zachs, *P. ciliata limicola* Annenkova, *P. ciliata spongicola* Berkeley and Berkeley, etc.), but most of them proved to be separate species when examined closely (for review see Radashevsky 1993). Detailed morphological examination of European *P. ciliata* and two other *Polydora* species, *P. cornuta* Bosc (= *P. ligni* Webster) and *P. limicola* Annenkova, revealed considerable variability and overlapping of features of these species (Rasmussen 1973; Michaelis 1978; Kendall 1980; Ramberg and Schram 1983). These three species were considered members of a *P. ciliata* species complex. Boring and non-boring forms of *P. ciliata* were found to be identical with regard to morphology by some authors (Hempel 1957; Ramberg and Schram 1983) and slightly different by others (Mustaquim 1986). Using enzyme electrophoresis, Mustaquim (1988) demonstrated that boring and non-boring forms of *P. ciliata* from England are different species. Despite the extensive literature on *P. ciliata*, the systematics of the species remains uncertain and requires further investigation. This is impeded by the fact that the type material has been lost and life style (boring or non-boring) was not specified in detail in the original description.

Another spionid, *Polydora websteri*, was described by Hartman (1943) as an oyster shell borer from the Atlantic coast of Connecticut, North America. This species is very close in morphology to *P. ciliata* and differs from the latter only in the shape of the accessory structure of the major spines of Setiger 5. This character is not diagnostic for separation of these highly variable and widespread species (V.I.R. unpublished data). Never-

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theless, since Hartman's description, earlier records of *P. ciliata* from North and South America, from Australia and other regions were referred to as *P. websteri* (Hartman 1945; Blake 1971, 1983; Blake and Kudenov 1978).

It has been suggested that *Polydora ciliata* may prove to be limited to the Atlantic Ocean (Blake 1983). Along the Asian coast, *P. ciliata* has been reported from Kamchatka to Vietnam and from India as a common borer of molluscan shells (Monro 1934; Okuda 1937; Annenkova 1938; Fauvel 1939, 1953; Uschakov 1955; Chlebovitsch 1961; Imajima and Hartman 1964; Gallardo 1968; Mori et al. 1985; Radashevsky 1986). These identifications have never been reevaluated, and it is possible that several closely related species are included among these records. Kojima and Imajima (1982) reported *P. websteri* for the first time from the western Pacific, Japan, but did not include any morphological or systematic remarks in their report.

Enzyme electrophoresis was successfully used for discrimination between sympatric and allopatric sibling *Polydora* species (Rice and Simon 1980; Mustaquim 1988; Manchenko and Radashevsky 1993, 1994).

During a comparative electrophoretic survey of polydorids from the Sea of Japan, we found that within the samples tentatively identified by us as *Polydora* cf. *ciliata*, some individuals occasionally demonstrated allozymic patterns sharply different from the others. An explanation for these data was that we were dealing with mixed samples consisting of two sibling species. Additional morphological and electrophoretic investigations were conducted in order to test this hypothesis. The only morphological difference between the two sorts of individuals was in the form of the anterior margin of the prostomium. The typical incised prostomium of *P. ciliata* was discerned in most individuals treated here as *Polydora* sp. 1, while electrophoretically different individuals, treated here as *Polydora* sp. 2, always possessed an entire prostomium. High variability of the shape of prostomium was found to occur within electrophoretically similar *Polydora* sp. 1 samples, including transitions from deeply incised to almost entire prostomia. This variability obscures the proper morphological delineation of the two electrophoretically distinct species. This paper presents electrophoretic data demonstrating the existence of two sibling species within *P.* cf. *ciliata* from the Sea of Japan.

Materials and methods

Sample collection

Samples were collected from Peter the Great Bay of the Sea of Japan off the "Vostok" Marine Biological Station of the Institute of Marine Biology (132°45′E; 42°53′N) in August 1996. Field collections were made in a localized area, subtidally from the depth of 3 to 10 m using SCUBA equipment. Boring polychaetes were removed from the left (upper) valves of the scallop *Mizuhopecten yessoensis* (Jay), and from empty shells of the gastropod *Cryptonatica janthostoma* (Deshayes) inhabited by the hermit crabs *Pagurus capillatus* (Benedict) and *P. brachiomastus* (Thallwitz). Worms were tentatively identified as *Polydora* cf. *ciliata* using Johnston's (1838) description and Uschakov's (1955) identification key. The morphological characters of taxonomic importance (which were found to be identical in our specimens, in *P. ciliata*, and in other species of the *Polydora* complex listed in Table 4) include: diffuse black pigmentation on palps and anterior setigers present or absent; caruncle without occipital tentacle, extending back to end of Setiger 2; Setiger 1 without notosetae; Setiger 5 with dorsal capillaries; modified spines absent in posterior notopodia; neuropodial hooded hooks and branchiae beginning from Setiger 7; pygidium disc- or cup-shaped with dorsal gap or incision. The other characters which were found to be variable or different in these species are listed in Table 4. The names *Polydora* sp. 1 and *Polydora* sp. 2 used in the present paper refer to enzyme electrophoretic but not to morphological patterns.

Enzyme electrophoresis

Preparations for starch gel electrophoresis were obtained by homogenization of whole worms in two volumes of 0.01 *M* tris-HCl buffer (pH 7.0), containing 0.5 mg ml^{-1} of bovine serum albumin. Electrophoresis was carried out as previously described by Manchenko and Radashevsky (1987, 1993). Two continuous buffer systems were used to resolve ten enzymes: (I) TEB (tris-EDTA-boric acid, pH 8.5), for alanopine dehydrogenase (ALPDH, EC 1.5.1.17), α-amylase (α-AMY, EC 3.2.1.1), peptidase (PEP, 3.4.11.- or 13.- detected using leu-gly-gly tripeptid as substrate), phosphoglucomutase (PGM, EC 5.4.2.2) and formaldehyde dehydrogenase (FDH, EC 1.2.1.1); (II) TC (tris-citric acid, pH 7.0), for acid phosphatase (ACP, EC 3.1.3.2, detected with α-naphthyl phosphate as substrate), aspartate transaminase (ATA, EC 2.6.1.1), glucose-6-phosphate isomerase (GPI, EC 5.3.1.9), isocitrate dehydrogenase (IDH, EC 1.1.1.42) and malate dehydrogenase (MDH, EC 1.1.1.37).

After electrophoresis, a gel block was cut into 5 or 6 slices which were then stained for specific enzymatic activities using routine histochemical procedures (Manchenko 1994). Zones of starch hydrolysis caused by α-AMY were observed on electrophoretic gels incubated overnight in 0.1 *M* tris-HCl (pH 7.0) buffer at room temperature.

Genetic interpretations of electrophoretic data were inferred from the specific patterns of enzyme-activity bands displayed on zymograms (Harris and Hopkinson 1976; Buth 1990). The locus and allele designations follow the standardized genetic nomenclature for protein-coding loci (Shaklee et al. 1990).

Data analysis

Genotype frequencies were obtained by direct count from the enzyme electrophoretic patterns on zymograms. Allele frequencies, estimates of observed (H_o) and expected (H_e) heterozygosities, and Nei's (1978) unbiased genetic identity (I) and genetic distance (D) coefficients were calculated from genotype frequencies using the program BIOSYS-1 (Swofford and Selander 1981).

Results

Three out of ten electrophoretically studied enzyme systems displayed banding patterns which were qualitatively distinct within *Polydora* cf. *ciliata* samples. Electrophoretic patterns of these enzyme systems were used for unequivocal identification of two sibling species, *P.* sp. 1 and *P.* sp. 2, during different electrophoretic runs of mixed samples. These enzyme systems are ACP, α-AMY and MDH (see below for details). Genetic interpretations of electrophoretic variations observed in

the ten enzyme systems studied are described below in terms of number of expressed isozyme loci and number of allozymes displayed by presumed homozygotes and heterozygotes.

ACP. Only one isozyme locus (*Acp-1**) was found to be expressed in *P.* sp. 2 while three loci (*Acp-1**, *2**, *3**) were expressed in *P.* sp. 1. Allozyme variants with one-banded homozygotes and two-banded heterozygotes characteristic of monomeric enzymes were observed at the *Acp-1** locus in *P.* sp. 1. Two other ACP loci were monomorphic in *P.* sp. 1, as well as the *Acp-1** locus in *P.* sp. 2.

ALPDH. A single variable locus was found to be expressed in both species. Allozyme variants with one-banded homozygotes and two-banded heterozygotes were observed, providing evidence that the enzyme molecules are monomers.

α-AMY. One monomorphic band of α-AMY was observed in 20 electrophoretically studied individuals of *P.* sp. 1, while no starch-hydrolyzing activity was detected in 21 examined individuals of *P.* sp. 2. This enzyme is useful for qualitative discrimination between species, but it is not taken into account in comparative analysis of electrophoretic data.

ATA. Two loci coding for this enzyme were found to be expressed in both species but only one of them (*Ata-1**) proved to be scorable under the electrophoretic conditions used. Allozyme variants with one-banded homozygotes and three-banded heterozygotes were observed in *P.* sp. 1.

FDH. A single variable locus was found to be expressed in both species. Allozyme variants with one-banded homozygotes and three-banded heterozygotes were observed, providing evidence that the enzyme molecules are dimers.

GPI. Allozyme variants with one-banded homozygotes and three-banded heterozygotes were observed in anodally migrating GPI bands in both species. Some individuals of both species displayed an additional cathodally migrating GPI band. In *P.* sp. 1, this band (expressed in 7 out of 44 individuals examined) demonstrated allozymic variation independent of that displayed by the anodally migrating GPI band, thus providing strong evidence that two loci (*Gpi-1** and *Gpi-2**) are coding for the enzyme in this species. It should be pointed out that the *Gpi-2** locus was shown to be expressed only in mature males. No interlocus hybrid isozyme expected for this dimeric enzyme was observed, suggesting that different GPI loci are expressed in different tissues in this species. In *P.* sp. 2, the cathodally migrating GPI band was also expressed only by mature males. Because of the small size of the *P.* sp. 2 individuals studied, this band was displayed only by 3 out of 21 individuals examined. The cathodally migrating GPI band proved invariable in these individuals as well as the anodally migrating GPI band. It remains unclear therefore whether two true GPI isozymes are expressed in *P.* sp. 2 or one of these is a secondary isozyme (i.e. non-genetic molecular form or post-translational modification). It should be stressed here that most polydorid

Table 1 *Polydora* spp. Allele frequencies, and per locus observed (H_o) and unbiased (Nei 1978) expected (H_e) heterozygosity estimates over 12 isozyme loci in two sibling species, *P.* sp. 1 and *P.* sp. 2, from the Sea of Japan

Locus	*Polydora* species	Sample size	Allele							H_o	H_e
			a	*b*	*c*	*d*	*e*	*f*	*g*		
*Acp-1**	sp. 1	7	0.786	0.214						0.143	0.363
	sp. 2	2		1.000						0.000	0.000
*Alpdh**	sp. 1	23		0.065	0.761	0.152	0.022			0.304	0.402
	sp. 2	11	0.045	0.955						0.091	0.091
*Ata-1**	sp. 1	32	0.016	0.031	0.953					0.094	0.092
	sp. 2	23				1.000				0.000	0.000
*Fdh**	sp. 1	32	0.766	0.203	0.031					0.313	0.377
	sp. 2	13				0.385	0.615			0.462	0.492
*Gpi-1**	sp. 1	44	0.034		0.705		0.250	0.011		0.409	0.445
	sp. 2	21		0.071		0.929				0.143	0.136
*Idh-1**	sp. 1	21				0.071	0.833	0.095		0.333	0.298
	sp. 2	12	0.042	0.875	0.083					0.250	0.236
*Idh-2**	sp. 1	11	0.091	0.864	0.045					0.279	0.255
	sp. 2	13			1.000					0.000	0.000
*Mdh-1**	sp. 1	26	1.000							0.000	0.000
	sp. 2	11	1.000							0.000	0.000
*Mdh-2**	sp. 1	26		1.000						0.000	0.000
	sp. 2	11	0.955	0.045						0.091	0.091
*Pep-1**	sp. 1	25	1.000							0.000	0.000
	sp. 2	21		1.000						0.000	0.000
*Pep-2**	sp. 1	25		0.080	0.440	0.460	0.020			0.200	0.600
	sp. 2	20	1.000							0.000	0.000
*Pgm**	sp. 1	27	0.037	0.278	0.056	0.556	0.074			0.704	0.616
	sp. 2	21					0.024	0.833	0.143	0.333	0.292

species studied electrophoretically display a secondary GPI isozyme detectable only in about a half of the individuals examined, perhaps only in males with mature gonads (G.P.M. and V.I.R unpublished data). Because of uncertainties concerning the genetic basis of the cathodal GPI band in *P.* sp. 2 and restricted data on the *Gpi-2** locus in *P.* sp. 1, only the *Gpi-1** locus coding for the anodally migrating GPI isozyme was taken into account in our further quantitative genetic comparison of these species.

IDH. Two loci, *Idh-1** and *Idh-2**, presumably coding for cytosolic and mitochondrial isozymes of the enzyme were expressed in both species. In both species the slowly moving isozyme (IDH-2) was stained more lightly than the fast-moving one (IDH-1). About a half of the examined *P.* sp. 1 individuals did not display the slow IDH-2 isozyme at all. Allozyme variants with heterozygotes, displayed on zymograms as broad bands which were not resolved into separate allozymes, were observed at both IDH loci of *P.* sp. 1 and at the *Idh-1** of *P.* sp. 2.

MDH. Two bands of MDH activity were detected in *P.* sp. 1, while only one intensely stained band was displayed by *P.* sp. 2. We assume that there are two MDH isozymes in both species, and that the electrophoretic mobility of these isozymes is different in *P.* sp. 1 but coincident in *P.* sp. 2. In the latter species, a single three-banded heterozygote was observed with a skewed binomial ratio of allozyme activities; this situation is to be expected when two electrophoretically coincident isozymes are encoded by two isoloci. We arbitrarily attributed this variant to the *Mdh-2** locus. Both MDH isozymes proved invariant in 26 examined individuals of *P.* sp. 1.

PEP. Two loci, *Pep-1** and *Pep-2**, were found to be expressed in both species. Allozyme variants with heterozygotes, displayed on zymograms as broad bands which were not resolved into separate allozymes, were observed at the *Pep-2** locus of *P.* sp. 1. No variants were revealed at *Pep-1** of *P.* sp. 1 or at *Pep-1** and *Pep-2** of *P.* sp. 2.

PGM. A single polymorphic locus was found to be active in both species. Heterozygotes are two-banded, indicating that the enzyme molecules are monomers.

In total, 12 isozyme loci were detectable in both sibling species. The allele frequencies and estimates of observed (H_o) and expected (H_e) heterozygosities for these loci are given in Table 1. It is evident from these data that the compared species have no common alleles at 6 out of 12 isozyme loci examined. Estimates of Nei's (1978) unbiased genetic identity (I) and genetic distance (D) calculated from allele frequency data are 0.143 and 1.942, respectively (see Table 2).

Discussion

Nei's genetic distance (D) between the morphologically indistinguishable *Polydora* sp. 1 and *P.* sp. 2 proved surprisingly high. Values of this estimate obtained to date for electrophoretically studied *Polydora* species vary from 0.075 to 1.942, with the highest value obtained in the present study (Table 3). We failed to find any qualitative morphological character suitable for unequivocal discrimination between *P.* sp. 1 and *P.* sp. 2 (Table 4). A single ecological difference was the occurrence of *P.* sp. 2 only in shells of the gastropod *Cryptonatica janthostoma* inhabited by hermit crabs. *P.* sp. 1 was found in shells of *C. janthostoma* but also in the shells of the scallop *Mizuhopecten yessoensis*. Out of 30 *Polydora* individuals collected from *C. janthostoma* shells and used in electrophoretic analysis, 23 were identified as *P.* sp. 2, and 7 as *P.* sp. 1. All 44 *Polydora* individuals collected from the scallop shells were electrophoretically identified as *P.* sp. 1. However, because both species co-occur in the gastropod shells, the unequivocal discrimination between them is possible only based on electrophoretic data.

Our data on the existence of two sibling species morphologically indistinguishable from *Polydora ciliata* support the view that *P. ciliata* is not a widespread species. A complex including several morphologically cryptic species may be erroneously considered a single cosmopolitan species. Using methods of molecular systematics, similar situations have recently been revealed among marine invertebrates (Solé-Cava et al. 1991; Todaro et al. 1996). These, as well as our present data, suggest that the taxonomic integrity of cosmopolitan marine invertebrates is open to doubt and, thus, should be considered with caution.

The widespread occurrence of morphologically indistinguishable species among invertebrates is a well-documented phenomenon (for review see Thorpe and Solé-Cava 1994). A satisfactory explanation of the

Table 2 *Polydora* spp. Estimates of average heterozygosity (observed: H_o, expected: H_e) and genetic differentiation (Nei's I and D indices) of two sibling species, *P.* sp. 1 and *P.* sp. 2, from the Sea of Japan. Estimates are calculated from the data presented in Table 1

Species	Per locus sample size (±SE)	H_o (±SE)	H_e (±SE)	I	D
Polydora sp. 1	24.9 (±2.8)	0.231 (±0.050)	0.287 (±0.064)	0.143	1.942
Polydora sp. 2	14.9 (±1.8)	0.144 (±0.045)	0.111 (±0.045)		

Table 3 *Polydora* spp. Genetic identity (*I*) and genetic distance (*D*) between pairs of electrophoretically studied species (*P. ligni* = *P. cornuta*)

Species pair	Number of loci studied	*I*	*D*	Reference
P. ligni vs *P. websteri*	8	0.284	1.259	Rice and Simon (1980)
P. ligni (Fort DeSoto) vs *P. ligni* (Cockroach Bay)	10	0.588	0.531	Rice and Simon (1980)
P. ciliata (boring) vs *P. ciliata* (non-boring) (= *P. ligni*)	13	0.697	0.361	Mustaquim (1988)
P. ciliata (boring) vs *P. limicola*	13	0.786	0.241	Mustaquim (1988)
P. ligni vs *P. limicola*	13	0.928	0.075	Mustaquim (1988)
P. cf. *ciliata* (= *P.* sp. 1) vs *P. limicola*	27	0.317	1.149	Manchenko and Radashevsky (1993)
P. sp. 1 vs *P.* sp. 2	12	0.143	1.942	Present study

phenomenon is commonly based on the relative simplicity of invertebrates, which possess a small number of morphological structures that are easily quantified and compared. A number of sibling species among lower marine invertebrates are characterized by a strikingly high degree of genetic divergence (e.g. Manchenko and Kulikova 1988, 1996; Solé-Cava et al. 1991; present study). The most plausible explanation for this fact is that the rate of morphological evolution in these species is much lower than the rate of their molecular evolution (Manchenko and Kulikova 1988; Palumbi and Benzie 1991; Rogers et al. 1995). Such a scenario was thought to be mainly caused by the stabilizing effect of selection acting on morphological and ecological traits of the species (especially when an adaptive peak is reached), while molecular differences are accumulated at a relatively constant rate (Palumbi and Benzie 1991; Todaro et al. 1996).

The systematic status of *Polydora* sp. 1 and *P.* sp. 2, as treated in the present study, is unclear. Comparison of the present data with those of Mustaquim (1988) provides evidence that *P.* sp. 1 is most similar to *P. ciliata* (the boring form) from England. Indeed, these are the only boring species that express three gene loci coding for ACP (Table 4). Both these species demonstrate variation at the *Acp-1** locus and have invariable *Acp-2** and *Acp-3** loci. The failure of Mustaquim (1988) to detect the cathodally migrating *Gpi-2** locus in *P. ciliata*, which we found in *P.* sp. 1, can be explained by that author's use of polyacrylamide gel electrophoresis for the electrophoretic separation of enzymes. In routine use, this method only allows analysis of anodally migrating isozymes.

The conspecificity of boring and non-boring *Polydora ciliata* forms has not been proved, and the ecology of the true *P. ciliata* is still not clear. The questions put forward by Manchenko and Radashevsky (1993) with regard to the *P. ciliata–limicola* problem are still unsolved, and new questions have arisen with regard to the *P. ciliata–websteri* species (V.I.R. unpublished data). We propose, therefore, to redefine the *P. ciliata* complex of species including *Polydora* sp. 1 and *Polydora* sp. 2 from the Sea of Japan, *P. websteri*, and *P. aggregata* Blake. The last species is close in morphology and ecology to *P. limicola*. We also propose to exclude *P. ligni* (= *P. cornuta*) from the complex because this species seems to be clearly different from the others in the morphology of the companion setae on Setiger 5 and regarding the presence of an occipital tentacle on the caruncle (Blake and Maciolek 1987).

Because considerable confusion exists among species of the redefined *Polydora ciliata* complex, unequivocal species identification is essential when studying members of the complex. Our multilocus enzyme electrophoretic survey of the polydorids showed that isozyme patterns often allow species differentiation on the basis of distinct duplications of isozyme loci among members of the group (Manchenko 1995). The taxonomically most important characteristic of the isozyme pattern is the number of isozymes of the same enzyme expressed by conspecific individuals. The determined intersample difference in the number of expressed isozymes may serve as a strong indicator that the sample in question belongs to a separate species. Such a situation has recently been described for two allopatric sibling species (Manchenko and Radashevsky 1994), for which samples were not available for side-by-side electrophoretic comparison in the same gel. Samples of these species have been analyzed separately but under the same electrophoretic conditions. These samples were shown to express different numbers of isozyme loci coding for the same enzymes. It was concluded therefore that they represent separate species. Similar situations are expected to be very common when studying the taxonomic integrity of any widespread polydorid species or when clarifying the systematics of geographically distant populations of any polydorid species complex. Based on our previous electrophoretic data (Manchenko and Radashevsky 1987, 1993, 1994), on data in the present study, and on our unpublished results, we recommend the following enzymes, characterized by interspecifically variable numbers of expressed isozymes, for discrimination between sibling polydorid species: acid phosphatase (ACP; EC 3.1.3.2), alanopine dehydrogenase

Table 4 *Polydora ciliata* species complex (redefined). Morphological and ecological characters and numbers of isozyme loci in members of the species complex (*, enzyme not studied; **, enzyme studied but number of expressed isozyme loci ambiguous; enzyme abbreviations see "Materials and methods")

	Species					
	P. ciliata	*P.* sp. 1	*P.* sp. 2	*P. websteri*	*P. limicola*	*P. aggregata*
Morphological and ecological characters						
Prostomium	incised to entire	incised to entire	entire	incised to entire	incised	incised
Accessory structure	tooth	tooth or flange	tooth or flange	flange	small tooth	small tooth
of major spines						
Habitat	tube-dweller and/ or borer	shell-borer	shell-borer	shell-borer	tube-dweller	tube-dweller
Reference	Johnston (1838)	Present study	Present study	Blake (1971)	Annenkova (1934)	Blake (1971)
Numbers of isozyme loci						
ACP	3	3	1	*	1	*
ALPDH	*	1	1	*	1	*
α-AMY	*	1	0	*	*	*
GPI	1	2	1 or 2	* or **	2	*
IDDH	*	1	*	*	1	*
MDH	2	2	2 isoloci	* or **	2	*
Reference	Mustaquim (1988) (boring form)	Present study; Manchenko and Radashevsky (1993)	Present study	Rice and Simon (1980)	Manchenko and Radashevsky (1993)	

(ALPDH; EC 1.5.1.17), α-amylase (AMY; EC 3.2.1.1), glucose-6-phosphate isomerase (GPI; EC 5.3.1.9), L-iditol 2-dehydrogenase (IDDH; EC 1.1.1.14), lactoylglutathione lyase (LGL; EC 4.4.1.5), malate dehydrogenase (MDH; EC 1.1.1.37), phosphogluconate dehydrogenase (PGD; EC 1.1.1.44), superoxide dismutase (SOD; EC 1.15.1.1) and xanthine dehydrogenase (XDH; EC 1.1.1.204).

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