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GENETIC EVIDENCES FOR TWO SPECIES TO THE GENUS *ELEDONE* (CEPHALOPODA: OCTOPODIDAE) IN SOUTH BRAZIL

J. A. LEVY,* M. HAIMOVICI† and M. CONCEICÃO*

*Laboratorio de Bioquimica Marinha, Departamento de Quimica and †Departamento de Oceanografia, Fundacão Universidade do Rio Grande, C.P. 474-96200, Rio Grande, RS, Brazil

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Abstract—1. Two Brazilian species of *Eledone* were examined by isoenzyme electrophoresis and isoelectric focusing.

2. The analysis of 24 alleles distributed among 14 enzyme loci exhibited a genetic index identity of 0.73.

3. The loci Xdh, Est, Adh-1, Gpdh-2, Mdh-1, Mdh-2, Sod-1 and Sod-2 are fixed at different isoenzyme for each population.

4. The genetic result and the isoelectric focusing pattern shows that the two taxa are reproductivity isolated and, overall, the evidences indicate that they are separate species.

INTRODUCTION

Six species are described for the genus *Eledone* distributed in the Atlantic Ocean and the Mediterranean Sea: *E. mosclata*, *E. cirrhosa*, *E. thysanohora*, *E. caparti*, *E. massyae* and *E. gaucha*.

In a recent work, Haimovici *et al.* (1986), found two sympatric eledonids: *E. massyae* and *E. gaucha*, are common throughout the year in the south western Atlantic Ocean off southern Brazil, between 60 and 160 m depth. One of them, *E. massyae* is commercially fished off Rio de Janeiro (Costa com. pess.). The differentiation between these species is based mainly on body proportions, spermatophores and arm sizes (Haimovici, 1988). However, their overall similarity suggests that it would be desirable to test whether this putative species is genetically differentiated or not.

Electrophoresis is a powerful tool for the investigation of sibling species (Carter and Thorpe, 1981). The basis of the technique and its uses in genetic studies of morphological populations have been described in detail in many reviews (e.g. Lewontin, 1974; Ayala, 1975; Thorpe, 1979). This technique has been shown to be extremely useful in analysing several marine organisms from a genetic point of view and it has been specially recommended for fisheries management (FAO/UNEP, 1981; Kohen, 1984).

Despite their importance for fisheries, few studies have been published on the electrophoretic analysis of cephalopod populations (Brahma and Lancieri, 1979; Ally and Keck, 1978; Christofferson *et al.*, 1978; Smith *et al.*, 1981).

The aim of this work was to examine through enzyme electrophoresis, the level of genetic identity between the two putative Brazilian species of *Eledone*.

MATERIALS AND METHODS

Ten specimens of each species of *Eledone* were collected in November, 1983 and July, 1984 with bottom trawl net by the O.V. Atlantico Sul, between 30° and 34° S, from 10 to 100 m depth. The individuals could be clearly identified as *E. massyae* of *E. gaucha* based on spermatophore shape and arm length patterns (Haimovici, 1988). The sample sizes were appropriate to this kind of analysis (Nei, 1978; Gorman and Renzi, 1979).

After capture and identification the samples were immediately frozen at −20°C. Tissue samples from the mantle and the cephalic region were removed in the laboratory, minced and extracted according to Christofferson *et al.* (1978) and stored at −20°C for no longer than a month. Horizontal electrophoresis was carried out at 5°C either on 7% polycralamide gels or on cellulose acetate plates (Cellogel–Chemetron, Milan).

Three buffer systems were used: Veronal, pH 8.7 (Brewer, 1970), discontinuous Tris-citrate/borate, pH 8.3 (Poulik, 1957) and Tris-borate, pH 9.0 (Toledo and Magalhaes, 1973).

The system of locus and allele nomenclature suggested by Alendorf and Utter (1978) was used. The enzymes studied, their abbreviations, Enzyme Commission numbers, tissue and supports used are presented in Table 1. Genetic identity between population pairs were estimated using the methods of Nei (1972).

Isoelectric focusing of mantle extracts was performed using polyacrylamide gels containing Ampholine (LKB, Sweden), pH 3.5–10.0. The gels were prepared as described by Levy *et al.* (1983). Five microlitres of each sample were applied on the surface of the gels near the cathode. The gels were run at 4°C for 6 hrs, at a maximum power of 3 W. Fixation, staining and preservation of the gels were performed according to Lundstrom (1980) and Levy *et al.* (1983).

RESULTS AND DISCUSSION

Twenty four alleles, distributed among 14 enzyme loci were detected (Table 2). The loci Aat, Adh-2, Gdh, Gpdh-1 and Hbdh were monomorphic for the sample allele in both putative species. The loci Xdh, Est and Ldh were polymorphic in both and Adh-1, Gpdh-2, Mdh-1, Mdh-2 and Sod-2 were polymorphic in one of the populations only. These and Sod-1 were found to be fixed for apparently different alleles in the two populations analysed.

The isoelectric focusing patterns were very different

Table 1. Electrophoretical conditions for the analysed enzymes

Enzyme		EC.	Support	Tissue	Buffer
Alcohol dehydrogenase	ADH	1.1.1.1	2	M.	III
Aspartate aminotransferase	AAT	2.6.1.1.	1	M.	I
Esterase	EST	3.1.1.1	2	R.	Ii
Glycerophosphate dehydrogenase	GPDH	1.1.1.8	2	M.	III
Glutamate dehydrogenase	GDH	1.4.1.3	1	M.	I
Hydroxybutirate dehydrogenase	HBDH	1.1.1.31	2	R.	III
Lactate dehydrogenase	LDH	1.1.1.27	2	R.	III
Malate dehydrogenase	MDH	1.1.1.37	2	R.	III
Superoxide dismutate	SOD	1.15.1.1	2	R.	II
Xantine dehydrogenase	XDH	1.2.1.37	2	R.	II

EC. number recommended by commission on Biological nomenclature *Enzyme Nomenclature* (Academic Press, New York, 1978).
SUPPORT. 1, Cellulose acetate; 2, polyacrylamide.
TISSUE. M, Mantle; R, Cephalic region.
BUFFER. I, veronal pH 8.7 (Brewer, 1970); II, Tris-citrato-borato pH 8.3 (Poulik, 1957); III, Tris-borate pH 9.0 (Toledo and Magalhães, 1973).

Table 2. Allelic frequencies at 14 loci of Eledone population of South Brazil

Locus	Allele	*E. massyae*	*E. gaucha*
Adh-1	1	1.0	0.5
	2	0.0	0.5
Adh-2	1	1.0	1.0
Aat	1	1.0	1.0
Est	1	0.25	0.25
	2	0.50	0.50
	3	0.25	0.25
Gpdh-1	1	1.0	1.0
Gpdh-2	1	0.0	1.0
	2	1.0	0.0
Gdh	1	1.0	1.0
Hbdh	1	1.0	1.0
Ldh	1	0.5	0.5
	2	0.5	0.5
Mdh-1	1	1.0	0.0
	2	0.0	1.0
Mdh-2	1	0.72	0.0
	2	0.14	1.0
	3	0.14	0.0
Sod-1	1	0.0	1.0
Sod-2	1	0.5	1.0
	2	0.5	0.0
Xdh	1	0.5	0.5
	2	0.5	0.5

for each species (Fig. 1). Thin layer isoelectric focusing of soluble proteins has been extensively used for the identification of industrially processed fish products (Lundstrom, 1980; Levy *et al.*, 1983). Despite the difficulties related to the genetic interpretation of the results produced by this technique, it would appear to have a potential as a tool for the estimation of overall distances between populations (Sarich, 1977; Tegelstrom *et al.*, 1982; Sole-Cava and Levy, 1987).

The two putative species present an overall level of genetic identity (Nei, 1972) of 0.73 and are fixed for different alleles in 6 out of 14 loci. These differences show, therefore, that there is no gene flow between them. For sympatric populations like these, this demonstrates that there is a reproductive barrier between them showing, thus, that they are different biological species. This result is corroborated by the consistent differences found in the isoelectric focusing patterns of both species.

It can be concluded, therefore, that *Eledone messyae* and *E. gaucha* are valid biological species, presenting a level of gene identity of congeneric sibling species.

Further electrophoretic studies comparing all six eledonids could be useful for elucidation of the evolutionary pathways and radiation of this genus.

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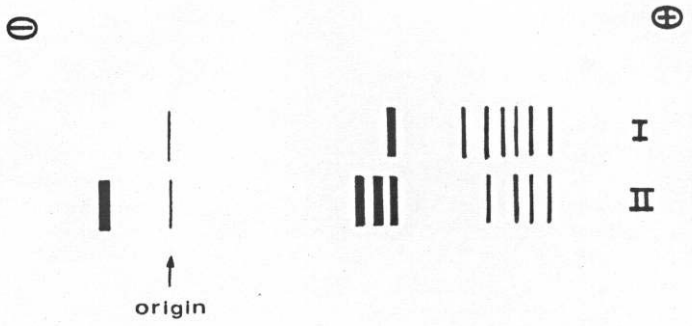

Fig. 1. Characteristic isoelectric focusing patterns of mantle extracts from the two *Eledone* morphotype. I. *E. massyae*; II. *E. Gaucha*.

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