

BIOCHEMICAL DIFFERENTIATION BETWEEN *OPHIDION BARBATUM* AND *O. ROCHEI* (PISCES, *OPHIDIIDAE*)

M. Casadevall¹, J.L. García-Marín², J. Matallanas³ and C. Pla²

1 Unitat Biologia Animal, Departament de Ciències Ambientals, Universitat de Girona, plaça Hospital 6, 17071 Girona.

2 Laboratori d'Ictiologia genètica, Departament de Biologia, Universitat de Girona, plaça Hospital 6, 17071 Girona.

3 Departament Biologia Animal, Biologia Vegetal i Ecologia, Universitat Autònoma de Barcelona, 08193 Bellaterra, Barcelona.

RESUM

El gènere *Ophidion* està representat al Mediterrani per dues espècies, *O. barbatum* L. i *O. rochei* Müller. Són dues espècies molt similars en aspecte i difícils d'identificar només a partir dels seus caràcters morfològics externs. El treball descriu en primer lloc, i a partir de dades obtingudes per tècniques electroforètiques, alguns marcadors bioquímics que permeten diferenciar les dues espècies. En segon lloc, i a partir del càlcul de la "Distància Genètica de Nei", es fa una estimació del temps de divergència entre *O. barbatum* i *O. rochei*.

RESUMEN

El género *Ophidion* está representado en el Mediterráneo por dos especies, *O. barbatum* L. y *O. rochei* Müller. Las dos especies presentan un aspecto muy similar y es difícil identificarlas sólo a partir de sus caracteres morfológicos externos. El presente trabajo describe en primer lugar, y a partir de datos obtenidos por técnicas electroforéticas, algunos marcadores bioquímicos que permiten diferenciar las dos especies. En segundo lugar, y a partir del cálculo de la "Distancia Genética de Nei", se hace una estimación del tiempo de divergencia entre *O. barbatum* y *O. rochei*.

Keywords: biochemical differentiation, genetic distance, Mediterranean, Ophidiidae, *Ophidion*.

INTRODUCTION

Most species of the *Ophidion* genus are distributed along the Atlantic and Pacific coasts of America. *O. barbatum* L. and *O. rochei* Müller, were the only two species of cusk-eels of the genus *Ophidion* assigned to the CLOFNAM area until now (Hureau and Monod, 1973), even though Matallanas (1990) recently described a new species, *O. lozanoi*. *O. barbatum* is distributed in the west Mediterranean (from Gibraltar to the Adriatic) and in the North-East Atlantic ocean (from Southern England to Senegal) (Nielsen, 1986). The distribution of *O. rochei* is exclusively Mediterranean, including the Adriatic and Black sea (Fischer *et al.*, 1987).

Because their external morphology is practically identical, description and differentiation of *Ophidion barbatum* and *O. rochei* in earlier studies was not very clear and often mistaken (Müller, 1845; Fage, 1918; Facciola, 1933; Tortonese, 1955; Lozano Rey, 1960). Svetovidov (1961) was the first to define and identify them more clearly, but reviews of past work have not completely clarified the differentiation; Tortonese (1975) suggested they be considered twin species, and Nielsen (1986), recognised very few distinguishing characters.

The aim of this work is to describe the first biochemical markers for distinguishing these two species of cusk-eels by means of starch-gel electrophoresis. The potential of using biochemical characters in systematics is now fully recognised, since this technology provides a method for examining variation at the protein level where environmental factors have only a very limited effect on the phenotype, and hence variations are mainly genetic (Ferguson, 1980; Buth 1990).

MATERIAL AND METHODS

The specimens, caught by trawling in the North-west Mediterranean (Blanes, Catalan coast), were preserved frozen at -20°C until electrophoresis was carried out.

Even though *O. rochei* is not rare along our coasts it is collected very irregularly, and during a period of three years, only two fresh (and immediately frozen) specimens of *O. rochei* were available for analysis. That is the reason of the limited sample size studied in this paper (5 individuals of *O. barbatum* and 2 of *O. rochei*).

Meristic, morphometric and biological characters have been used to separate the seven individuals of these two species, following criteria used by Svetovidov (1961), Nielsen (1986) and Casadevall (1996).

Standard methods of horizontal starch-gel electrophoresis were used to examine protein coding loci in extracts of skeletal muscle, liver and eye. The electrophoretic buffers were, as described in García-Marín *et al.* (1991): aminocitrate (AC) buffer (pH 7.0); tris-citrate buffer (TC/LB); tris-phosphate (TP) buffer; and tris-borate (TBE) buffer modified by adding 30 mg NAD^+ per 100 ml buffer. All gels were made of 12% starch.

The enzymatic stains (with abbreviation and number of enzymatic commission in parentheses) used were the following: Aspartate aminotransferase (sAAT, 2.6.1.1), Catalase (CAT, 1.11.1.6), Esterase (EST, 3.1.1.1), Acid phosphatase (ACP, 3.1.3.2), Glucose-6-phosphate isomerase (GPI, 5.3.1.9), Phospho-glucomutase (PGM, 5.4.2.2), Glyceraldehyde-3-phosphate dehydrogenase (GAPDH, 1.2.1.12), Glycerol-3-phosphate dehydrogenase (G3PDH, 1.1.1.8), Isocitrate dehydrogenase (sIDHP, 1.1.1.42), Lactate dehydrogenase (LDH, 1.1.1.27), Malate dehydrogenase (sMDH, 1.1.1.37), Malate dehydrogenase (NADP⁻-dependent) or malic enzyme (sMEP, 1.1.1.40), Peptidase substrate phenylalanine-proline (PEPD, 3.4.13.9), Peptidase substrate Leucylglycylglycine (PEPB, 3.4.*.*) and Superoxide dismutase (sSOD,

1.15.1.1). The terminology used in the designation of enzymatic loci follows the recommendations of Shaklee et al. (1990).

The genetic relationship between the two species at a particular locus was numerically expressed using the Sørensen coefficient (García-Marín, 1991): $S=2W/(a+b)$; where *a* is the number of alleles in the locus in one of the two species; *b* is the same for the other species; and *W* is the number of alleles shared at the locus by the two species. From the resulting value for each locus, an average value of this coefficient can be calculated for all detected loci (\bar{S}). This average coefficient summarizes the degree of genetic similarity between the two species.

RESULTS

The 14 enzymatic stains revealed 20 electrophoretically detectable loci which could be reliably screened for genetic variation. Our estimation of the number of protein coding loci is conservative in that they reflect the minimum number of loci involved. For example, a single invariant band found in all individuals examined may represent the product of any number of isoloci fixed for the same allele. In such situations the exact number of loci cannot be determined, but we have decided to treat the protein as coded by only one locus. Table 1 summarizes the results of electrophoretic analysis and the value of *a*, *b*, *W* and *S* for each locus used in the calculation of the Sørensen coefficient average (\bar{S}). For the purpose of description, the 20 loci were classed in three groups:

- (1) Loci with the same pattern in the two species.
- (2) Polymorphic loci with shared alleles.
- (3) Enzymes having at least one locus with fixed allelic differences between the two species.

- (1) Loci with the same pattern in *O. barbatum* and *O. rochei*.

A single invariant band with the same mobility in the two species was detected for EST, ACP, GAPDH, sIDHP and PEPD. Therefore, each of these enzymes was considered the result of the expression of a single locus, which were: *EST-1**, *ACP-1**, *GAPDH-1**, *sIDHP-1** and *PEPD**.

Malate dehydrogenase was represented by three invariant and identical activity zones in the two species. Each one was interpreted as being coded by a different locus and are called *sMDH-1** (slow mobility), *sMDH-2** (medium mobility) and *sMDH-3** (fast mobility). *sMDH-1** and *sMDH-2** were detected in all three analysed tissues, whereas *sMDH-3** was not observed in liver extracts.

Two activity zones were observed in the liver for malic enzyme. These were considered as being coded by two different loci, called *sMEP-1** and *sMEP-2**. Mobility for the two loci was identical in the two species.

- (2) Polymorphic loci with shared alleles.

In the two species, sAAT stain yielded a single activity zone in liver extracts. This was considered the expression of a single locus, *sAAT-1**. One

individual of *O. barbatum* showed a three-banded pattern, characteristic of heterozygotes for dimeric enzymes such as sAAT. This variant allele had a slower mobility than the other one shared by the two species.

For CAT (a tetrameric enzyme), a single activity zone, assigned to the locus *CAT-1**, was observed in the two species. In one of the *O. rochei* specimens, a very wide activity band was observed. This band was considered to be the result of the presence of the intralocus heterotetramers in a heterozygote individual for one slow allele.

PGM is coded by a single locus (*PGM-1**) in the two species. The same allele of *O. rochei* was present in *O. barbatum*, but some individuals of this last species showed a two-banded pattern characteristic of heterozygotes for this monomeric protein. That variant allele had slower mobility than the common one shared by the two species.

Two loci were detected in LDH stains: one in the muscle, *LDH-1**, and other in the eye, *LDH-2**. The eye locus was identical and invariant in the two species. The muscle locus was polymorphic for one allele in *O. rochei*, while the other allele was the same as in *O. barbatum*.

(3) Enzymes having at least one locus with fixed allelic differences between the two species.

In the case of the dimeric enzyme GPI, two activity zones were observed. The most cathodic zone had the same mobility in the two species and was assigned to the locus *GPI-1**. The mobility of the *GPI-2** locus product was faster in *O. barbatum* than in *O. rochei*.

For G3PDH, a single zone with a different mobility in the two species was observed. This was considered to be the expression of a single locus, *G3PDH-1**. Allele mobility in *O. rochei* was slightly slower in comparison to *O. barbatum*.

PEPB stains also showed a single activity zone in the two species. This was considered to be the expression of a single locus, *PEPB**, with faster mobility in *O. barbatum*.

Finally, a single activity zone was observed for superoxide dismutase. This enzyme is considered in both species to be the manifestation of a single locus, *sSOD-1**. Band mobility in *O. rochei* was faster than in *O. barbatum*.

The genetic relationship between the two species was numerically expressed by the average value of the Sørensen index for all the detected loci: $\bar{S} = 0.734$.

Stain	Buffer	Tissue	Loci	species	alleles		alleles		S
					1	2	W	(a+b)	
sAAT	TP	L	<i>sAAT-1*</i>	<i>barbatum</i>	+	+	1	3	0.67
				<i>rochei</i>		+			
CAT	TP	L	<i>CAT-1*</i>	<i>barbatum</i>	+	+	1	3	0.67
				<i>rochei</i>					
EST	TC/LB	L	<i>EST-1*</i>	<i>barbatum</i>	+				
				<i>rochei</i>	+		1	2	1.00
ACP	TP	L	<i>ACP-1*</i>	<i>barbatum</i>	+				
				<i>rochei</i>	+		1	2	1.00
GPI	TC/LB	M	<i>GPI-1*</i>	<i>barbatum</i>	+				
				<i>rochei</i>	+		1	2	1.00
	TC/LB	M	<i>GPI-2*</i>	<i>barbatum</i>	+	+			
				<i>rochei</i>	+		0	2	0.00
PGM	AC	M	<i>PGM-1*</i>	<i>barbatum</i>	+	+			
				<i>rochei</i>	+		1	3	0.67
GAPDH	TBE+NAD	M	<i>GAPDH-1*</i>	<i>barbatum</i>	+				
				<i>rochei</i>	+		1	2	1.00
G3PDH	TC/LB	M	<i>G3PDH-1*</i>	<i>barbatum</i>	+	+			
				<i>rochei</i>	+		0	2	0.00
sIDHP	AC	L	<i>sIDHP-1*</i>	<i>barbatum</i>	+				
				<i>rochei</i>	+		1	2	1.00
LDH	TC/LB	M	<i>LDH-1*</i>	<i>barbatum</i>	+	+			
				<i>rochei</i>	+	+	1	3	0.67
	TC/LB	E	<i>LDH-2*</i>	<i>barbatum</i>	+				
				<i>rochei</i>	+		1	2	1.00
sMDH	AC	M,L,E	<i>sMDH-1*</i>	<i>barbatum</i>	+				
				<i>rochei</i>	+		1	2	1.00
	AC	M,L,E	<i>sMDH-2*</i>	<i>barbatum</i>	+				
				<i>rochei</i>	+		1	2	1.00
	AC	M,E	<i>sMDH-3*</i>	<i>barbatum</i>	+				
				<i>rochei</i>	+		1	2	1.00
sMEP	AC	L	<i>sMEP-1*</i>	<i>barbatum</i>	+				
				<i>rochei</i>	+		1	2	1.00
	AC	L	<i>sMEP-2*</i>	<i>barbatum</i>	+				
				<i>rochei</i>	+		1	2	1.00
PEP	AC	E	<i>PEPB*</i>	<i>barbatum</i>	+				
				<i>rochei</i>	+		0	2	0.00
	AC	E	<i>PEPD*</i>	<i>barbatum</i>	+				
				<i>rochei</i>	+		1	2	1.00
sSOD	TC/LB	L	<i>sSOD-1*</i>	<i>barbatum</i>	+				
				<i>rochei</i>	+	+	0	2	0.00

Table 1. Enzymatic electrophoretic analysis in liver (L), muscle (M) and eye (E) for *Ophidion barbatum* and *O. rochei*. S = Sørensen Index.

DISCUSSION

Biochemical differentiation between O. barbatum and O. rochei.

Four of the 20 protein coding loci described in this paper have shown fixed allelic differences in the two species: *GPI-2**, *G3PDH-1**, *PEPB** and *sSOD-1**. The *O. rochei* alleles observed in these four loci are probably at high frequency or fixed for this species, but, in low frequency or absent in *O. barbatum*, and the opposite situation can be concluded for the alleles only observed in the later species. Therefore, we can use this whole set of 4 loci to identify individuals belonging to either species, and we may consider them as probably diagnostic loci.

Awise (1974) observed that congeneric species often differ in the fixation of distinct alleles at some loci rather than in fluctuation of allelic frequencies. For this reason, and when it is difficult to obtain a large enough sample, it is possible to use alleles as qualitative data, to measure the genetic similarity between species. The Sørensen coefficient is then useful because it can be easily computed (García-Marín, 1991). Our result obtained for the two species of *Ophidion*: $\text{O}(S_i) = 0.734$, is similar to the average calculated between congeneric fish species (0.765 ± 0.115 ; García-Marín, 1991) and we can therefore confirm the genetic individuality of these two *Ophidion* species.

Due to the small number of individuals which represent each taxon, it is possible to infer a false allele relationship between the species, caused by observing the rare allele homozygotes and not the common genotypes for the two species. In such a situation, the value of the Sørensen index obtained for some loci would be highly wrong. However, the effect of this potential misinterpretation would not be very important in the Sørensen index average calculation.

If we consider that a non detected common allele has an intermediate frequency ($p=0.5$), the probability of not detecting the common genotype in an *O. rochei* sample for one locus is $0.0625 (((1-p)^2)^2)$ and 0.00098 for *O. barbatum* ($(((1-p)^2)^2)$) (note that if $p > 0.5$, the probability of the rare genotype decrease and then the number of errors). That implies there may be one locus with the error in *O. rochei* for the 20 studied loci, and none in *O. barbatum*. The worst case would be if the *O. rochei* error occurred in a diagnostic locus. There, the Sørensen index would change from 0 to 0.67, but in the final weighting of this value against the other 19 loci, the Sørensen index average would increase from 0.734 to 0.768 (a change of only 0.034 in value), which is closer to the value observed between congeneric fish species. This evaluation suggests that the likelihood of inferring false descriptions in interspecific differentiation is small despite the reduced small size, a point already noted for other genetic distances based on electrophoretic data (Nei, 1978; Gorman and Renzi, 1979). In fact, the Sørensen index will be overestimated, and then, the biochemical divergence between the two species should be stronger. In this sense, although the two species are externally very similar, a simultaneous study has demonstrated a lot of morphometric, meristic and anatomical differences (Casadevall, 1996).

Sample collection from the same fishing area is another possible source of error. Their genetic differentiation would only reflect the differences between two particular populations, one of each species of *Ophidion*. However, electrophoretic diffe-

rentiation between populations within species is very small in marine fishes (Shaklee et al., 1982; Grant, 1987; Ward et al., 1994).

Evolutionary considerations

The most widely used measure of genetic similarity in systematic electrophoretic studies is Nei's Genetic Identity (I), or its associated measure of Genetic Distance ($D = -\ln I$). These identity and distance coefficients, as outlined by Nei (1972, 1978), are based on biological rather than purely statistical concepts, so estimations of divergence times can be calculated. In fish species, a significant correlation between the Sørensen coefficient and Nei's Genetic Identity has been observed and a Genetic Identity value can be assigned to a specific Sørensen value index (García-Marín, 1991). By calculating I and D on the basis of our Sørensen coefficient result, the following values were obtained: $I = 0.758$, and $D = 0.277$. These values are in the range described for congeneric species (Nei, 1978).

The Nei's Genetic Distance (D) is linearly related to the time of divergence (T) of the two populations: $T = kD$; where k is a proportionality constant related to the average rate of codon substitution that is detectable by electrophoresis (Nei, 1975). In fish species, two values of k have been commonly suggested for the calibration of this clock of molecular evolution: $k = 5 \cdot 10^6$ (Nei, 1975) and $k = 18-19 \cdot 10^6$ (Gorman and Kim, 1977; Vawter et al., 1980; Grant, 1987). For *Ophidion barbatum* and *O. rochei*, the first value gives a time of divergence of 1.4 m.y.a., whereas the second gives a higher period of 5.0 - 5.2 m.y.a.

Otolith fossil registers analysed by Nolf and Steurbaut (1989) allowed dating of the origin of some Ophidiiformes. According to the authors, the earliest *Ophidion* fossil otoliths were found in Pliocene neritic sediments (from approx. 5.2 m.y.a. forward). If we consider that divergence between the species occurred afterwards, our first calculation of 1.4 m.y.a. would fit this estimation. However, we know of no historical event to account for the separation of *O. barbatum* and *O. rochei* at that time.

Lea (1980) believes that the *Ophidion* genus originated in the Tethys Sea and present distribution could be due to the discontinuity of an ancestral transatlantic population. All the species of the genus are concentrated in tropical and temperate regions (from the western coastlines of the Americas to the Mediterranean), following approximately the ancient Tethys area, thereby corroborating his hypothesis. Moreover, the presence of *Ophidion* species along the Pacific and Atlantic coastlines of Central America suggests that the genus already existed at the time when the Panama isthmus closed (two estimations of approx. 3 and 5.7 m.y.a., reviewed by Grant, 1987).

As shown by Maldonado (1985), at the beginning of the Messinian stage (approx. 6 m.y.a.), the Mediterranean openings to the Atlantic in the northern Betic and southern Rif straits were closed. This isolation produced the Messinian "salinity crisis". A number of low salinity lakes, referred to collectively as the "Lago Mare", came into existence in the eastern Mediterranean during this time. The end of that isolation coincided with the creation of the permanent connection between the Atlantic and the Mediterranean Sea, though the Strait of Gibraltar (approx. 5 m.y.a.).

Thus, our second estimation of 5 - 5.2 m.y.a. coincides with this resettlement of marine conditions. If we consider the genus as having originated before the Messinian crisis, our hypothesis is that this phenomenon allowed allopatric conditions for divergence between a more western (or Atlantic) population (the present *O. barbatum* species) and a more eastern population (the present *O. rochei* species). The latter could have been a population which was relegated during the crisis to the eastern Mediterranean basins, or an eastern Atlantic population which may have occupied the Mediterranean more quickly than the other when the Strait was opened. This would also account for their present distribution.

We reconaise that this part of the discussion is quite unreliable, and more loci should give a better estimate, so we are conscious that conclusions should be limited to the biochemical confirmation after increasing the sample size and validation of the statistical results. Nevertheless, we think that the discussion argument, regarding the two different estimates divergence times (based on different "calibrations" of the molecular clock) and possible geological causes, should have its own interest.

References

- AVISE, J.C. 1974. Systematic value of electrophoretic data. *Systematic Zoology* 23: 465-481.
- BUTH, D. 1990. Genetic principles and the interpretation of electrophoretic data. A: D.H. Whitmore (ed.) *Electrophoretic and isoelectric focusing techniques in fisheries management*. CRC Press. Boca Raton.
- CASADEVALL, M. 1996. Morphometric, meristic and anatomical differences between *Ophidion barbatum* and *O. rochei* (Pisces, Ophidiidae). *Proceedings of the VIII Congress of the Societas Europaea Ichthyologorum (SEI)* (En premsa)
- FACCIOLÀ, L. 1933. L'*Ophidion barbatum* Linn. e la sua vescica natatoria. *Atti della Società Italiana delle Scienze naturale* 72: 157.
- FAGE, L. 1918. Shore-fishes: Ophidiidae. *Report of the Danish oceanographical Expeditions* 2: 148-149.
- FISCHER, W., BAUCHOT, M.L. i SCHNEIDER, M. 1987. *Fiches FAO d'identification des espèces pour les besoins de la pêche*. Méditerranée et Mer Noire, vol.II, Vertébrés. FAO, Rome. pp. 761-1530
- GARCIA-MARIN, J.L. 1991. Le coefficient de Sørensen et l'electrophorese enzymatique. *Bulletin de la Société Zoologique de France* 116: 3-14.
- GARCIA-MARIN, J.L., JORDE, P.E., RYMAN, N., UTTER, F. i PLA, C. 1991. Management implications of genetic differentiation between native and hatchery populations of brown trout (*Salmo trutta*) in Spain. *Aquaculture* 95: 235-249.
- GORMAN, G.C. i KIM, Y.J. 1977. Genotypic evolution in the face of phenotypic conservativeness: *Abudefduf* (Pomacentridae) from Atlantic and Pacific sides of Panama. *Copeia* 1977: 694-697.
- GORMAN, G.C. i RENZI, J.Jr. 1979. Genetic distance and heterozygosity estimates in electrophoretic studies: Effects of sample size. *Copeia* 1979: 242-249.
- GRANT, S. 1987. Genetic divergence between congeneric Atlantic and Pacific ocean fishes. A: N.Ryman and F.M. Utter (eds.) *Population genetics and Fishery management*. Washington sea grant program. University Washington Press. Seattle.

- HUREAU, J.C. i MONOD, T.H. (eds.). 1973. CLOFNAM: *The check-list of the fishes of the North-Eastern Atlantic and the Mediterranean*. UNESCO. Paris.
- LEA, R.N. 1980. *Systematics and Zoogeography of cusk-eels of the family Ophidiidae, Subfamily Ophidiinae, from the eastern Pacific Ocean*. Unpublished PhD Dissertation, University of Miami. Coral Gables F.L.
- LOZANO REY, L. 1960. *Peces Fisoclistos*, vol. XIV. Memorias de la Real Academia de Ciencias. Madrid.
- MALDONADO, A. 1985. Evolution of the Mediterranean basins and a detailed reconstruction of the Cenozoic Paleocanography. A: R. Margalef (ed.) *Western Mediterranean*. Pergamon Press Ltd. London.
- MATALLANAS, J. 1990. *Ophidion lozanoi*, (Pisces: Ophidiiformes) an undescribed ophidiid from the north east Atlantic. *Journal of Fish Biology*, 37: 449-453.
- MÜLLER, J. 1845. Untersuchungen über die Eingeweide der fische... *Abhandlungen der Akademie der Eissenchr*. Berlin. pp. 150-154.
- NEI, M. 1972. Genetic distance between populations. *American Naturalist* 106: 283-292.
- NEI, M. 1975. *Molecular population genetics and evolution*. American Elsevier. New York.
- NEI, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* 89: 583-589.
- NIELSEN, J.G. 1986. F.Ophidiidae. A: Whitehead et al. (ed.) *Fishes of the North-Eastern Atlantic and the Mediterranean*. UNESCO. Paris.
- NOLF, D. i STEURBAUT, E. 1989. Importance and restrictions of the otolith-based fossil record of Gadiform and Ophidiiform fishes. A: D.M. Cohen (ed.) *Papers on systematics of Gadiform fishes*. Natural History Museum of Los Angeles County, Science Series 32: 47-59.
- SHAKLEE, J.B., ALLENDORF, F.N., MORIZOT, D.C. i WHITT, G.S. 1990. Gene nomenclature for protein-coding loci in fish. *Transactions of American Fisheries Society* 119: 2-15.
- SHAKLEE, J.B., TAMURA, C.S. i WAPLES, R.S. 1982. Speciation and evolution of marine fishes studied by the electrophoretic analysis of proteins. *Pacific Science* 36: 141-157.
- SVETOVIDOV, A.N. 1961. The European species of the family Ophidiidae. *Vop. Ikhtiol.* (Translation n.41 from Ichthyology Laboratory), U.S. Natural Museum. Washington (D.C.) 17: 3-13.
- TORTONESE, E. 1955. On *Ophidion vassali* Risso, Type of new genus of Ophidiid fishes (*Parophidion*). *Pubblicazione della Stazione Zoologica di Napoli* 25: 372-373.
- TORTONESE, E. 1975. Fauna d'Italia. XI Osteichthyes. Ed. Calderini. Bologna.
- VAWTER, A.T., ROSENBLATT, R. i GORMAN, G. C. 1980. Genetic divergence among fishes of the Eastern Pacific and the Caribbean: Support for the molecular clock. *Evolution* 34: 705-711.
- WARD, R.D., WOODWARK, M. i SKJBINSKI, D. O. 1994. A comparison of genetic diversity levels in marine, freshwater and anadromous fishes. *Journal of Fish Biology* 44: 213-232.