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Genetic differentiation in three *Sepia* species (Mollusca: Cephalopoda) from Galician waters (north-west Iberian Peninsula)

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Abstract The systematics of the genus *Sepia* is not yet clear. Morphological evidence has led to *S. officinalis* Linnaeus, 1758 being considered as belonging to the subgenus *Sepia sensu stricto*, and *S. orbignyana* Férussac, 1826 and *S. elegans* Blainville, 1827 as belonging to the subgenus *Rhombosepion*. Samples of 30 individuals of *S. officinalis* and *S. orbignyana* from both sides of an oceanographic boundary off the north-west Iberian Peninsula, and a sample of *S. elegans* from the northern side, were collected in 1993–1994. Allozyme electrophoresis for 32 presumptive loci revealed low levels of genetic variability for the three *Sepia* species (mean expected heterozygosity estimates were < 0.052). No significant differences in allozyme frequencies were detected among populations of either *S. officinalis* or *S. orbignyana*. The genetic identities (I) of *S. officinalis* and *S. orbignyana* ($I = 0.12$) and of *S. elegans* ($I = 0.13$) were significantly different from that of *S. orbignyana* and *S. elegans* ($I = 0.49$). The former are typical of values for confamilial genera, and a new generic status is proposed for the latter two species, which become *Rhombosepion orbignyana* (Férussac, 1826) and *R. elegans* (Blainville, 1827).

Introduction

The commercially exploited *Sepia officinalis*, *S. orbignyana* and *S. elegans* are nektobenthic cephalopods with a limited migratory capacity (Mangold-Wirz 1963; Boucaud-Camou and Boismery 1991; Wurtz et al. 1991; Guerra 1992). The three species exhibit different ecological characteristics, especially in relation to their bathymetric distribution (Mangold-Wirz 1963; von Boletzky 1983; Ward and von Boletzky 1984; Guerra 1985a, 1992; Guerra and Castro 1988, 1989; Castro and Guerra 1989, 1990). *S. officinalis* lives and spawns throughout the Galician rías (50 m maximum depth; north-west Iberian Peninsula), whereas *S. orbignyana* occurs only on the Galician continental shelf and slope between 50 and 450 m depth (Guerra and Castro 1988; Guerra 1992). *S. elegans* inhabits the central and outer parts of the rías and the continental shelf waters between the littoral zone and 430 m depth (Guerra 1985b). An oceanographic boundary of two sub-surface water masses, North Atlantic Central Water and Bay of Biscay Central Water, separates the northern and western sub-surface Galician waters (Fig. 1), which exhibit distinct ecological characteristics (Fraga et al. 1982; Estrada 1984; González-Gurriarán and Olaso 1987). Therefore, some degree of genetic differentiation between northern and western *Sepia* spp. populations could be expected. Moreover, this differentiation may vary between species because of their different habitat preferences.

There are some problems with the systematics of the genus *Sepia*. Khromov (1987), on the basis of its internal cone structure and the shape of its cuttlebone, considered *S. officinalis* to belong to the subgenus *Sepia sensu stricto*, whereas *S. orbignyana* and *S. elegans* were classified in the subgenus *Rhombosepion* Rochebrune, 1884. The validity of these and other subgenera has been discussed by Khromov et al. (1996), who subdivided the genus *Sepia* into six taxa, including *Sepia sensu stricto* and *Rhombosepion*.

Allozyme electrophoresis has several advantages over more conventional systematic criteria (e.g. morphology), because allozymes are primary products of the genome (Avise 1974, 1994; Ayala 1983). Allozyme polymorphisms have proved to be effective

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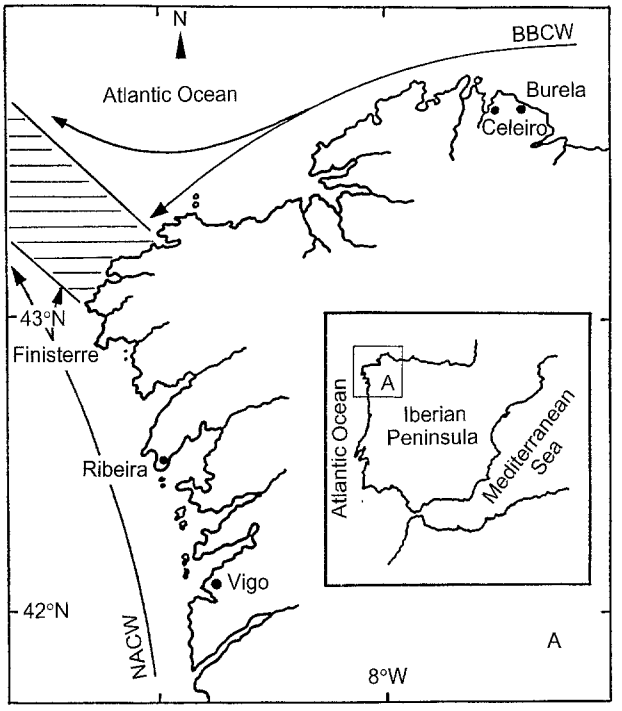

Fig. 1 Map of Galician coast (north-west Iberian Peninsula, *A*) showing northern (Burela and Celeiro) and western (Ribeira and Vigo) fishing ports where samples of *Sepia officinalis*, *S. orbignyana* and *S. elegans* were collected from commercial catches [*Arrows* show general circulation of central waters between 100 and 400 m depth; *hatching* lateral contact between North Atlantic Central Water (*NACW*) and Biscay Bay Central Water (*BBCW*)]. Redrawn from Fraga et al. (1982)

for detecting population differences in commercially exploited fish and cephalopod species (Whitmore 1990; Hatanaka et al. 1993; Carvalho and Hauser 1994; Carvalho and Nigmatullin 1996). There have been approximately twenty such studies on cephalopods, but allozyme electrophoresis has not yet been applied to assess genetic variation in the order Sepioidea (Katugin 1993; Yeatman and Benzie 1994; Yokawa 1994; Brierley et al. 1995; Carvalho and Nigmatullin 1996, and references therein). The aims of the present study were to use allozyme electrophoresis to describe the genetic structure of Galician populations of *Sepia officinalis* and *S. orbignyana*, and to evaluate the systematic relationships among three *Sepia* species.

Materials and methods

Samples of 30 individuals were collected in 1993–1994 from commercial catches in fishing ports of the north-west Iberian Peninsula (Fig. 1): *Sepia officinalis* Linnaeus, 1758 from Burela, Celeiro (northern area, N) and Vigo (western area, W), and *S. orbignyana* Férussac, 1826 from Burela (N) and Ribeira (W), which display large differences in their ecological characteristics, and *S. elegans* Blainville, 1827 from Celeiro (N). Specimens had been caught by fishing boats using bottom trawls, and also traps for *S. officinalis*. The collected specimens were immediately sorted, frozen in dry ice, transported to the laboratory, and stored at $-72\,^{\circ}C$ until required.

Horizontal starch-gel electrophoresis was carried out based on the method of Murphy et al. (1990). Mantle tissue was cleaned by removing the skin and by rinsing off any ink residues, and a piece of mantle ($\simeq 60$ mg) was homogenised with a similar volume of 0.01 *M* dithiothreitol solution. The homogenate was centrifuged at 12 000 $\times g$ for 10 min at 4 °C, and the supernatant was absorbed with Whatman strips. The 12% hydrolysed starch (Sigma S-4501) gels were run at constant voltage at 4 °C. Of a total of 47 assayed enzymes, 25 displayed adequate enzyme activity and good resolution, and were routinely examined (see Pérez-Losada et al. 1996 for details): aspartate transaminase (AAT; E.C. 2.6.1.1), acid phosphatase (ACP; E.C. 3.1.3.2), adenylate kinase (AK; E.C. 2.7.4.3), arginine kinase (ARK; E.C. 2.7.3.3), dihydrolipoamide transaminase (DDH; E.C. 1.8.1.4), carboxylic ester hydrolase (EST; E.C. 3.1.1.-; substrate: α-naphthyl acetate), methylumbelliferyl-acetate deacetylase (ESTD; E.C. 3.1.1.56), fructosebiphosphate aldolase (FBALD; E.C. 4.1.2.13), glycerol-3-phosphate dehydrogenase (NAD^+) (G3PDH; E.C. 1.1.1.8), glutamate dehydrogenase (GLUDH; E.C. 1.4.1.2), glucose-6-phosphate isomerase (GPI; E.C. 5.3.1.9), L-iditol 2-dehydrogenase (IDDH; E.C. 1.1.1.14), isocitrate dehydrogenase ($NADP^+$) (IDHP; E.C. 1.1.1.42), L-leucyl aminopeptidase (LAP; E.C. 3.4.11.1), malate dehydrogenase (MDH; E.C. 1.1.1.37), malate dehydrogenase (oxaloacetate-decarboxylating) ($NADP^+$) (MEP; E.C. 1.1.1.40), mannose-6-phosphate isomerase (MPI; E.C. 5.3.1.8), D-octopine dehydrogenase (OPDH; E.C. 1.5.1.11), cytosol non-specific dipeptidase (PEPA; E.C. 3.4.13.18; substrate: gly-leu), tripeptide aminopeptidase (PEPB; E.C. 3.4.11.4; substrate: leu-gly-gly), X-pro dipeptidase (PEPD; E.C. 3.4.13.9; substrate: phe-pro), phosphogluconate dehydrogenase (decarboxylating) (PGDH; E.C. 1.1.1.44), phosphoglucomutase (PGM; E.C. 5.4.2.2), pyruvate kinase (PK; E.C. 2.7.1.40), superoxide dismutase (SOD; E.C. 1.15.1.1). The Tris-citrate, pH 8.0 buffer system (gel-buffer dilution 1:9) of Ward and Beardmore (1977) was used for ACP and FBALD running at 4.6 V cm^{-1}, and for AAT, AK, ARK, EST, ESTD, IDDH, IDHP, LAP, MDH, PGDH, PGM, PK and SOD at 5.4 V cm^{-1}. The Tris–borate–EDTA, pH 8.7 buffer system (gel-buffer dilution 1:4) of Boyer et al. (1963) was used for DDH at 10 V cm^{-1}, and for G3PDH, GLUDH, GPI, MEP, MPI, OPDH, PEPA, PEPB and PEPD at 12.3 V cm^{-1}. The enzymes were stained according to recipes in Murphy et al. (1990), with the exception of ACP, DDH, MPI and PK (Harris and Hopkinson 1976), ESTD, LAP, PEPA, PEPB and PEPD (Ahmad et al. 1977), and AAT, IDHP, PGDH and PGM (Shaw and Prasad 1970). These 25 enzymes resolved 32 putative enzyme-coding loci (Table 1). Banding patterns of the presumptive loci have been interpreted according to the current subunit structure of each enzyme. Terminology and notation for allozymes are based on recommendations by Shaklee et al. (1990) and IUBMB (1992). Arabic numerical suffixes (*1*, *2*,..) for multiple loci and lower-case letters (**a*, **b*,..) for alleles are presented in order of decreasing and increasing anodal mobility, respectively.

Tests for goodness-of-fit of genotype frequencies to Hardy–Weinberg equilibrium expectations were assessed by means of an *F*-statistic developed by Robertson and Hill (1984). This statistic is unbiased for $F = 0$ and a significance test for $F = 0$ is more powerful than the usual chi-squared test (Robertson and Hill 1984; Sanjuan et al. 1990). Heterogeneity of allele frequencies among samples was tested using a chi-squared test for homogeneity. The probability of the null-hypothesis was estimated using a Monte Carlo simulation (Roff and Bentzen 1989) because expected numbers were small. After establishing that no genetic differentiation occurred within each species, data were pooled and estimates of unbiased mean expected heterozygosity (*H*), mean number of alleles, and proportion of polymorphic loci were calculated for each species (Nei 1987). The number of loci (> 30) and individuals (≥ 30) for each species was sufficient to accurately estimate the levels of genetic variability and divergence (Nei 1978, 1987; Archie 1985). Difference in mean heterozygosity between two species was tested using a Student's *t*-test (Archie 1985; Nei 1987, p. 183). Unbiased genetic identity (*I*) and

Table 1 *Sepia officinalis* (*Sf*), *S. orbignyana* (*So*) and *S. elegans* (*Se*). Allele frequencies for 32 enzyme loci in populations from northern (*N*) and western (*W*) waters off Galician coast (north-west Iberian Peninsula); 30 individuals were assayed for each locus and sample, except *IDDH** in *Se* (N) (19) and *IDHP-1** in *Sf* (W) (10). No activity was detected (*nd*) for *IDHP-1** in *So* and *Se* populations or for *MEP-2** in *Sf* populations

Locus, allele	*S. officinalis*		*S. orbignyana*		*S. elegans*
	N	W	N	W	N
*AAT-1**					
**a*	1	1	0	0	0
**b*	0	0	1	1	0
**c*	0	0	0	0	1
*AAT-2**					
**a*	0	0	0.77	0.78	0
**b*	0	0	0.23	0.22	0.92
**c*	1	1	0	0	0
**d*	0	0	0	0	0.08
*ACP**					
**a*	0	0	0	0	1
**b*	0	0	0.13	0.23	0
**c*	1	1	0	0	0
**d*	0	0	0.87	0.77	0
*AK**					
**a*	1	1	0	0	0
**b*	0	0	1	1	1
*ARK**					
**a*	0	0	1	1	1
**b*	1	1	0	0	0
*DDH**					
**a*	1	1	0	0	0
**b*	0	0	0.85	0.93	0.93
**c*	0	0	0.15	0.07	0.07
*EST**					
**a*	1	1	1	1	1
*ESTD**					
**a*	0	0	0	0	0.03
**b*	0	0	1	1	0.07
**c*	0	0	0	0	0.90
**d*	1	1	0	0	0
*FBALD**					
**a*	1	1	0	0	0
**b*	0	0	1	1	0
**c*	0	0	0	0	1
*G3PDH**					
**a*	1	1	0	0	0
**b*	0	0	1	1	0.98
**c*	0	0	0	0	0.02
*GLUDH**					
**a*	1	1	0	0	0
**b*	0	0	1	1	1
*GPI-1**					
**a*	0	0	0	0	0.98
**b*	0	0	0	0	0.02
**c*	0	0	1	1	0
**d*	1	1	0	0	0
*GPI-2**					
**a*	0	0	0	0	0.97
**b*	0	0	0	0	0.03
**c*	0	0	1	1	0
**d*	1	1	0	0	0
*IDDH**					
**a*	0	0	0	0	0.47
**b*	0.42	0.38	0.03	0.03	0
**c*	0.58	0.62	0.97	0.97	0
**d*	0	0	0	0	0.53
*IDHP-1**					
**a*	0.05	0	nd	nd	nd
**b*	0.95	1	nd	nd	nd
*IDHP-2**					
**a*	1	1	0	0.02	0
**b*	0	0	0.97	0.97	0
**c*	0	0	0	0	1
**d*	0	0	0.03	0.02	0
*LAP**					
**a*	0	0	0	0	1
**b*	0	0	1	1	0
**c*	1	1	0	0	0
*MDH-1**					
**a*	0	0	0	0	1
**b*	1	1	0	0	0
**c*	0	0	1	1	0
*MDH-2**					
**a*	1	1	1	1	1
*MEP-1**					
**a*	1	1	0	0	0
**b*	0	0	1	1	1
*MEP-2**					
**a*	nd	nd	1	1	1
*MPI**					
**a*	0	0	1	1	1
**b*	1	1	0	0	0
*OPDH-1**					
**a*	0	0	0	0	0.83
**b*	0	0	0.83	0.92	0
**c*	0	0	0.17	0.08	0.17
**d*	0.73	0.78	0	0	0
**e*	0.27	0.22	0	0	0
*OPDH-2**					
**a*	0	0	0.02	0	0.98
**b*	0	0	0.98	1	0.02
**c*	1	1	0	0	0
*PEPA**					
**a*	1	1	1	1	1
*PEPB**					
**a*	1	1	0	0	0.07
**b*	0	0	1	1	0.93
*PEPD**					
**a*	0	0	1	1	0
**b*	1	1	0	0	1
*PGDH**					
**a*	0	0	0	0	1
**b*	0	0	0.97	1	0
**c*	1	1	0.03	0	0
*PGM**					
**a*	1	1	0	0	0
**b*	0	0	1	1	0
**c*	0	0	0	0	0.98
**d*	0	0	0	0	0.02
*PK-1**					
**a*	1	1	0	0	0
**b*	0	0	1	1	0.98
**c*	0	0	0	0	0.02
*PK-2**					
**a*	0	0	0	0	1
**b*	0	0	1	1	0
**c*	1	1	0	0	0
*SOD**					
**a*	0	0	1	1	1
**b*	1	1	0	0	0

distance (D) values among species were calculated (Nei 1987). A dendrogram was constructed using the unweighted pair-group method using arithmetic averages (UPGMA: Sneath and Sokal 1973). Most of the genetic statistics were calculated using the GENET2 program (Quesada et al. 1992). Chi-squared statistics using the Monte Carlo procedure were computed using Zaykin and Pudovkin's (1993) programs. Unbiased genetic identities of Nei (1987) and their bootstrap confidence estimates, were carried out with the Dbot program (Zaykin, Tatarenkov and Pudovkin personal communication). The bootstrap method used loci as the units to be resampled, and the 95% bootstrap confidence limit on I was constructed by the percentile method (Felsenstein 1988).

Results

Allele frequencies of the 32 enzyme loci are shown in Table 1. One locus exhibited activity only for *Sepia officinalis* (*IDHP-1**), another only for *S. orbignyana* and *S. elegans* (*MEP-2**). Of the remaining 30 loci, 9 were completely diagnostic of the three *Sepia* species (*AAT-1**, *ACP**, *FBALD**, *GPI-1**, *GPI-2**, *LAP**, *MDH-1**, *PGM**, *PK-2**) and 2 were partially diagnostic (*IDHP-2**, *PGDH**). Moreover, 14 loci distinguished *S. officinalis* from the other two species, whereas a single locus (*IDDH**) distinguished *S. elegans* and a single locus (*PEPD**) *S. orbignyana* from the other two species. No significant deviation from a Hardy–Weinberg distribution was found at any polymorphic locus (F-statistics, Table 2, and chi-squared tests, data not shown). No significant differences in allele frequencies at polymorphic loci were found between the northern and western populations of either *S. officinalis* or *S. orbignyana* (Table 2). Using Monte Carlo simulations, estimated probabilities for rejection of the null-hypothesis were $P > 0.25$ for all comparisons. Low levels of genetic variability were detected overall (Table 2). *S. officinalis* and *S. elegans* exhibited

Table 2 *Sepia officinalis*, *S. orbignyana* and *S. elegans*. Estimates of F-statistics in each sample (after Robertson and Hill 1984), and homogeneity chi-squared values between allele frequencies of northern and western samples of *S. officinalis* and *S. orbignyana*. Estimates of unbiased mean expected heterozygosity and standard error ($H \pm SE$), mean number of alleles and standard error ($Na \pm SE$) and percentage of loci polymorphic by the 95% criterion (P_{95}) and by the 99% (P_{99}) criterion are also shown for each species. No polymorphic locus showed significant differences from the Hardy–Weinberg equilibrium-expected proportions for test of $F = 0$. Estimated probabilities of homogeneity chi-squares after 1000 runs of Monte Carlo simulations were all $P > 0.24$

Locus	*S. officinalis*			*S. orbignyana*			*S. elegans*
	N	W	χ^2	N	W	χ^2	N
*AAT-2**	–	–		0.09	0.14	0.05	– 0.08
*ACP**	–	–		0.15	0.09	2.00	–
*DDH**	–	–		0.10	– 0.06	2.16	– 0.06
*ESTD**	–	–		–	–		– 0.04
*G3PDH**	–	–		–	–		0.00
*GPI-1**	–	–		–	–		0.00
*GPI-2**	–	–		–	–		– 0.02
*IDDH**	– 0.15	– 0.04	0.14	– 0.02	– 0.02	0.00	–
*IDHP-1**	– 0.04	–	1.04	–	–		–
*IDHP-2**	–	–		– 0.02	0.00	1.33	–
*OPDH-1**	0.34	0.14	0.41	0.06	– 0.08	1.91	0.06
*OPDH-2**	–	–		0.00	–	1.01	0.00
*PEPB**	–	–		–	–		– 0.06
*PGDH**	–	–		– 0.02	–	2.03	–
*PGM**	–	–		–	–		0.00
*PK-1**	–	–		–	–		0.00
$H \pm$ SE	0.030 ± 0.019			0.040 ± 0.017			0.052 ± 0.020
$Na \pm$ SE	1.10 ± 0.05			1.29 ± 0.09			1.42 ± 0.10
P_{95}	6.45			12.90			19.35
P_{99}	9.6			22.58			38.71

Table 3 *Sepia officinalis* (*Sf*), *S. orbignyana* (*So*) and *S. elegans* (*Se*). Unbiased genetic identities (I, Nei 1978) and unbiased genetic distances (D, Nei 1978) between *Sf*, *So* and *Se*. 95% confidence interval (95% CI) and bootstrap estimates for Nei's similarities are shown; number of bootstrap samples for each pair was 1000

Species pairs	I	(SE)	D	95% CI	Bootstrap	
					(5%)	(95%)
Sf–So	0.117	(0.057)	2.145	0.006 – 0.228	0.033	0.223
Sf–Se	0.132	(0.061)	2.022	0.012 – 0.253	0.036	0.234
So–Se	0.492	(0.089)	0.708	0.318 – 0.666	0.352	0.649

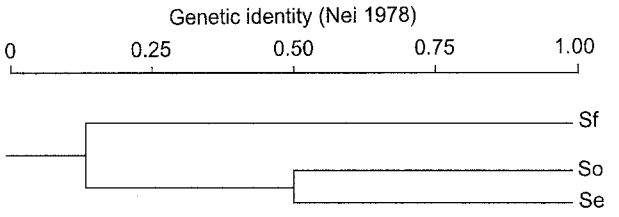

Fig. 2 *Sepia officinalis* (*Sf*), *S. orbignyana* (*So*) and *S. elegans* (*Se*). UPGMA dendrogram plot upon unbiased genetic identities (Nei 1978)

least and most variability, respectively, as indicated by the estimates of unbiased mean expected heterozygosity (0.03 and 0.05), the mean number of alleles (1.1 and 1.4), and the percentage of polymorphic loci (6.5 and 19.4%). Mean heterozygosities, one of the most informative indicators of the genetic variability of populations (Nei 1975), showed no significant differences among the three *Sepia* species [Student's *t*-tests: $t_{(Sf-So)} = 0.392$; $t_{(Sf-Se)} = 0.797$; $t_{(So-Se)} = 0.457$, where *Sf*, *So*, *Se* represent *S. officinalis*, *S. orbignyana*, and *S. legans*, respectively].

Unbiased genetic identities (*I*, Table 3) displayed greater similarity between *Sepia orbignyana* and *S. elegans* ($I = 0.49$) than between *S. officinalis* and either of the other two species ($I \leq 0.13$). Non-overlap of the bootstrap confidence intervals indicated significant differences between the *I* values for *S. orbignyana* and *S. elegans* ($I = 0.49$) and those for *S. officinalis* and *S. orbignyana* ($I = 0.12$) and for *S. officinalis* and *S. elegans* ($I = 0.13$). The UPGMA dendrogram of Nei's *I* summarises these results, grouping *S. orbignyana* and *S. elegans* closer to each other than to *S. officinalis* (Fig. 2).

Discussion

Estimates of genetic variability in the present study (mean heterozygosity < 0.05, Table 2), the first reported for the order Sepioidea, fall below the average for invertebrate species (0.10, Nevo et al. 1984; 0.15, Ward et al. 1992) and within the range for most cephalopods. These results seem to confirm that low levels of genetic variability are apparently characteristic for most cephalopods, with the exception of *Berryteuthis magister*, *Nautilus belauensis* and *Photologilo edulis* (see Carvalho et al. 1992; Brierley et al. 1993a; Katugin 1993; Yeatman and Benzie 1994; Carvalho and Nigmatullin 1996 for discussions of possible causes of low variability for various cephalopods).

Partial geographic isolation of northern and western Galician *Sepia* spp. populations, with restricted gene flow and subsequent genetic divergence, might have been expected on the basis of oceanographic features (present Fig. 1, and Fraga et al. 1982). However, no significant genetic differences between the northern and western Galician populations of either *S. officinalis* or *S. orbignyana* were detected (Tables 1 and 2). Intraspecific allozyme homogeneity is known for many cephalopod species (Ally and Keck 1978; Carvalho and Pitcher 1989; Yeatman and Benzie 1993, 1994). This absence of intraspecific genetic differentiation in cephalopods may possibly be related to low apparent levels of genetic variability detected by allozyme electrophoresis. However, electrophoretic studies on cephalopods have also demonstrated major intraspecific genetic structuring (Garthwaite et al. 1989; Carvalho et al. 1992; Brierley et al. 1993b, 1995; Carvalho and Nigmatullin 1996), as well as cryptic speciation or species' misidentification (Smith et al. 1981; Augustyn and Grant 1988; Carvalho et al. 1992; Brierley et al. 1993a; Yeatman and Benzie 1994). Consequently, more extensive allozyme studies and more samples from distant areas may possibly detect more genetic variability and differentiation within *Sepia* species. Alternatively, the application of DNA techniques could provide genetic markers able to detect intraspecific genetic differentiation (Hatanaka et al. 1993; Carvalho and Hauser 1994; Skibinski 1994).

Genetic identity (*I*) between *Sepia officinalis* and each of *S. orbignyana* and *S. elegans* ($I \leq 0.13$) differed significantly from that between *S. orbignyana* and *S. elegans* ($I = 0.49$) (Table 3). Estimates of genetic divergence based on allozyme polymorphisms are generally well correlated with taxonomic categories based on morphological analyses, as would be expected if both are functions of evolutionary time (Avise 1974, 1994; Thorpe 1982, 1983). Generally, an *I* value of $\simeq 0.35$ distinguishes congeneric species of confamilial genera (Thorpe 1982, 1983). However, the correspondence between the genetic divergence of species and their taxonomic category may vary between animal phyla or classes (Avise and Aquadro 1982; Avise 1983, 1994; Thorpe 1983). Focussing on the Cephalopoda, the similarity of congeneric species usually falls within the range $I = 0.3$ to 0.8, and, with some exceptions, confamilial genera have *I* values of < 0.4 (see Augustyn and Grant 1988; Levy et al. 1988; Garthwaite et al. 1989; Brierley et al. 1993b; Yeatman and Benzie 1993; Brierley and Thorpe 1994; Yokawa 1994). In general, the relationship between genetic divergence and taxonomic categories in cephalopods seems to agree well with those for other invertebrates (Thorpe 1982, 1983; Brierley et al 1993b). Consequently, the *I* values for *S. officinalis* and *S. orbignyana* ($I = 0.12$) and for *S. officinalis* and *S. elegans* ($I = 0.13$) are clearly typical values for distinct genera. These results are in line with morphological (Khromov 1987) and mitochondrial DNA (Bonnaud et al. 1994) differences among *Sepia* spp. On the basis of morphology, Khromov (1987) and Khromov et al. (1996) have suggested that *S. officinalis* belongs to the subgenus *S. sensu stricto*, and *S. orbignyana* and *S. elegans* to the subgenus *Rhombosepion*. It has been suggested that where conventional studies

leave taxonomic status in doubt, an estimate of genetic divergence from allozyme polymorphisms could provide an objective and useful criterion (Thorpe 1983). According to the genetic results presented here and the reported morphological and mtDNA data, we propose that *S. orbignyana* and *S. elegans* should be considered as belonging to a different genus than *S. officinalis*. Consequently, their specific names should be *S. officinalis* Linnaeus, 1758, *Rhombosepion orbignyana* (Férussac, 1826) and *R. elegans* (Blainville, 1827).

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References

Ahmad M, Skibinski DOF, Beardmore JA (1977) An estimate of the amount of genetic variation in the common mussel *Mytilus edulis*. Biochem Genet 15: 833–846

Ally JRR, Keck SC (1978) A biochemical–genetic population structure study of market squid, *Loligo opalescens*, along the California coast. Fish Bull Calif 169: 113–121

Archie JW (1985) Statistical analysis of heterozygosity data: independent sample comparisons. Evolution 39: 623–637

Augustyn CJ, Grant WS (1988) Biochemical and morphological systematics of *Loligo vulgaris vulgaris* Lamarck and *Loligo vulgaris reynaudii* D'Orbigny nov. comb. (Cephalopoda: Myopsida). Malacologia 29: 215–233

Avise JC (1974) Systematic value of electrophoretic data. Syst Zool 23: 465–481

Avise JC (1983) Protein variation and phylogenetic reconstruction. In: Oxford GS, Rollinson D (eds) Protein polymorphism: adaptive and taxonomic significance. Academic Press, London, pp 103–130

Avise JC (1994) Molecular markers, natural history and evolution. Chapman & Hall, New York

Avise JC, Aquadro CF (1982) A comparative summary of genetic distances in the vertebrates: patterns and correlations. Evolutionary Biol 15: 151–184

Ayala FJ (1983) Enzymes as taxonomic characters. In: Oxford GS, Rollinson D (eds) Protein polymorphism: adaptive and taxonomic significance. Academic Press, London, pp 3–26

Boletzky von S (1983) *Sepia officinalis*. In: Boyle PR (ed) Cephalopod life cycles. Vol 1. Academic Press, London, pp 31–52

Bonnaud L, Boucher-Rodoni R, Monnerot M (1994) Phylogeny of cephalopods based on partial 16S rDNA nucleotide sequences. C r Acad Sci hebd Séanc Paris (Sciences Vie) 317: 581–588

Boucaud-Camou E, Boismery J (1991) The migrations of the cuttlefish *Sepia officinalis L.* in the English Channel. In: Boucaud-Camou E (ed) Proceedings of the First International Symposium on the Cuttlefish *Sepia* (Caen, 1–3 June, 1989). Centre de Publications de l'Université de Caen, Caen, France, pp 179–189

Boyer SH, Fainer DC, Naughton MA (1963) Myoglobin: inherited structural variation in man. Science, NY 140: 1228–1231

Brierley AS, Rodhouse PG, Thorpe JP, Clarke MR (1993a) Genetic evidence of population heterogeneity and cryptic speciation in the ommastrephid squid *Martialia hyadesi* from the Patagonian Shelf and Antarctic Polar Frontal Zone. Mar Biol 116: 593–602

Brierley AS, Thorpe JP (1994) Biochemical genetic evidence supporting the taxonomic separation of *Loligo gahi* from the genus *Loligo*. Antarctic Sci 6: 143–148

Brierley AS, Thorpe JP, Clarke MR, Martins HR (1993b) A preliminary biochemical genetic investigation of the population structure of *Loligo forbesi* Steenstrup, 1856 from the British Isles and the Azores. In: Okutani T, O'Dor RK, Kubodera T (eds) Recent advances in cephalopod fisheries biology. Tokai University Press, Tokyo, pp 61–69

Brierley AS, Thorpe JP, Pierce GJ, Clarke MR, Boyle PR (1995) Genetic variation in the neritic squid *Loligo forbesi* (Myopsida: Loliginidae) in the northeast Atlantic Ocean. Mar Biol 122: 79–86

Carvalho GR, Hauser L (1994) Molecular genetics and the stock concept in fisheries. Rev Fish Biol Fish 4: 300–326

Carvalho GR, Nigmatullin ChM (1996) Species identification and stock structure analysis within the genus *Illex*. In: Rodhouse PG, Dawe EG, O'Dor RK (eds) A review of recruitment process in the squid genus *Illex*. FAO, Rome (FAO Fish tech Pap) (In press)

Carvalho GR, Pitcher TJ (1989) Biochemical genetics studies on the Patagonian squid *Loligo gahi* d'Orbigny. II. Population structure in Fakland waters using isozymes, morphometrics and life history data. J exp mar Biol Ecol 126: 243–258

Carvalho GR, Thompson A, Stoner AL (1992) Genetic diversity and population differentiation of the shortfin squid *Illex argentinus* in the south-west Atlantic. J exp mar Biol Ecol 158: 105–121

Castro BG, Guerra A (1989) Feeding pattern of *Sepia officinalis* (Cephalopoda, Sepiodea) in the Ría de Vigo (NW Spain). J mar biol Ass UK 69: 545–553

Castro BG, Guerra A (1990) The diet of *Sepia officinalis* (Linnaeus, 1758) and *Sepia elegans* (Blainville, 1827) (Cephalopoda, Sepioidea) from the Ría de Vigo (NW Spain). Scientia mar 54: 375–388

Estrada M (1984) Phytoplankton distribution and composition off the coast of Galicia (northwest of Spain). J Plankton Res 6: 417–434

Felsenstein J (1988) Phylogenies from molecular sequences: inference and reliability. A Rev Genet 22: 521–565

Fraga F, Mouriño C, Manriquez M (1982) Las masas de agua en la costa de Galicia: junio–octubre. Resultados Exped cient (Supl Investigación pesq) 10: 51–77

Garthwaite RL, Berg Jr CJ, Harrigan J (1989) Population genetics of the common squid *Loligo pealei* LeSueur, 1821, from Cape Cod to Cape Hatteras. Biol Bull mar biol Lab, Woods Hole 177: 287–294

Gonzalez-Gurriarán E, Olaso I (1987) Cambios espaciales y temporales de crustáceos decápodos de la plataforma continental de Galicia (NW de España). Investigación pesq 51: 323–341

Guerra A (1985a) Food of the cuttlefish *Sepia officinalis* and *Sepia elegans* in the Ría de Vigo (NW Spain) (Mollusca: Cephalopoda). J Zool Can. 207: 511–519

Guerra A (1985b) An extension of the known depth range for *Sepia elegans* Blainville, 1827 (Cephalopoda: Sepioidea). Veliger 28: 217–218

Guerra A (1992) Mollusca, Cephalopoda. In: Ramos MA et al. (eds) Fauna ibérica Vol. 1. Museo Nacional de Ciencias Naturales, CSIC, Madrid, pp 1–327

Guerra A, Castro BG (1988) On the life cycle of *Sepia officinalis* (Cephalopoda, Sepioidea) in the Ría de Vigo (NW Spain). Cah Biol mar 29: 395–405

Guerra A, Castro BG (1989) Some aspects of the biology of *Sepia elegans* (Cephalopoda, Sepioidea) from the Ría de Vigo, NW Spain. Vie Milieu 39: 213–218

Harris H, Hopkinson DA (1976) Handbook of enzyme electrophoresis in human genetics. North-Holland, Amsterdam

Hatanaka H, O'Dor RK, Roper CFE (1993) Population structure session. In: Okutani T, O'Dor RK, Kubodera T (eds) Recent advances in cephalopod fisheries biology. Tokai University Press, Tokyo, pp 721–739

IUBMB (International Union of Biochemistry and Molecular Biology) (1992) Enzyme nomenclature 1992. Academic Press, New York

Katugin ON (1993) Genetic variation in the squid *Berryteuthis magister* (Berry, 1913) (Oegopsida: Gonatidae). In: Okutani T, O'Dor RK, Kubodera T (eds) Recent advances in cephalopod fisheries biology, Tokai University Press, Tokyo, pp 201–213

Khromov DV (1987) Systematics and phylogeny of the cuttlefish family Sepiidae (Cephalopoda). [In Russ.] Zool Zh 66: 1164–1176

Khromov DV, Lu CC, Guerra A, Dong Zh, Boletzky SV (1996). A synopsis of Sepiidae outside Australian waters (Cephalopoda: Sepioidea). In: Voss NA, Toll RB, Vecchione M (eds) Systematics and biogeography of cephalopods. Smithsonian Institution, Washington, (in press) Smithson Contr Zool

Levy JA, Haimovici M, Conceicao M (1988) Genetic evidences for two species to the genus *Eledone* (Cephalopoda: Octopodidae) in south Brazil. Comp Biochem Physiol 90B: 275–277

Mangold-Wirz K (1963) Biologie des céphalopodes benthiques et nectoniques de la mer Catalane. Vie Milieu 13 (Suppl): 1–285

Murphy RW, Sites JW Jr, Buth DG, Haufler CH (1990) Proteins. I: Isozyme eletrophoresis. In: Hillis DM, Moritz C (eds) Molecular systematics. Sinauer Associates Inc., Sunderland, Massachusetts, pp 45–126

Nei M (1975) Molecular population genetics and evolution. North-Holland, Amsterdam

Nei M (1978) Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics, Austin, Tex 89: 583–590

Nei M (1987) Molecular evolutionary genetics. Columbia University Press, New York

Nevo E, Beiles A, Ben-Shlomo R (1984) The evolutionary significance of genetic diversity: ecological, demographic and life history correlates In: Mani GS (ed) Evolutionary dynamics of genetic diversity. Springer-Verlag, New York, pp 13–213

Pérez-Losada M, Guerra A, Sanjuan A (1996) Allozyme electrophoretic technique and phylogenetic relationships in three species of *Sepia* (Cephalopoda: Sepiidae) Comp Biochem Physiol B: (In press)

Quesada H, Sanjuan J, Sanjuan A (1992) GENET2: a BASIC program for the analysis of deviations from Hardy–Weinberg law. J Hered 83: 460–461

Robertson A, Hill WG (1984) Deviations from Hardy–Weinberg proportions, sampling variances and use in estimation of inbreeding coefficients. Genetics Austin, Tex 107: 703–718

Roff DA, Bentzen P (1989) The statistical analysis of mitochondrial DNA polymorphisms: χ^2 and the problem of small samples. Molec Biol Evolut 6: 539–545

Sanjuan A, Quesada H, Zapata C, Alvarez G (1990) On the occurrence of *Mytilus galloprovincialis* Lmk. on the N.W. coast of the Iberian Peninsula. J exp mar Biol Ecol 143: 1–14

Shaklee JB, Allendorf FW, Morizot DC, Whitt GS (1990) Gene nomenclature for protein-coding loci in fish. Trans Am Fish Soc 119: 2–15

Shaw CR, Prasad R (1970) Starch gel electrophoresis of enzymes—a compilation of recipes. Biochem Genet 4: 297–320

Skibinski DOF (1994) The potential of DNA techniques in the population and evolutionary genetics of aquatic invertebrates. In: Beaumont AR (ed) Genetics and evolution of aquatic organisms. Chapman & Hall, London, pp 177–199

Smith PJ, Roberts PE, Hurst RJ (1981) Evidence for two species of arrow squid in the New Zealand fishery. NZ Jl mar Freshwat Res 15: 247–253

Sneath PHA, Sokal RR (1973) Numerical taxonomy. W.H. Freeman & Co., San Francisco

Thorpe JP (1982) The molecular clock hypothesis: biochemical evolution, genetic differentiation and systematics. A Rev Ecol Syst 13: 139–168

Thorpe JP (1983) Enzyme variation, genetic distance and evolutionary divergence in relation to levels of taxonomic speciation. In: Oxford GS, Rollinson D (eds) Protein polymorphism: adaptive and taxonomic significance. Academic Press, London, pp 131–152

Ward RD, Beardmore JA (1977) Protein variation in the plaice *Pleuronectes platessa*. Genet Res 30: 45–62

Ward PD, Boletzky von S (1984) Shell implosion depth and implosion morphologies in three species of *Sepia* (Cephalopoda) from the Mediterranean Sea. J mar biol Ass UK 64: 955–966

Ward RD, Skibinski DOF, Woodwark M (1992) Protein heterozygosity, protein structure and taxonomic differentiation. Evolutionary Biol 26: 73–159

Whitmore DH (ed) (1990) Electrophoretic and isoelectric focusing techniques in fisheries management. CRC Press, Boca Raton

Wurtz M, Matricardi G, Rebora F (1991) Abundance estimates and distribution of *Sepia elegans* and *Sepia orbignyana* in two fishing areas of the central Mediterranean; trawl surveys (1985–1987) In: Boucaud-Camou E (ed) Proceedings of the First International Symposium on the Cuttlefish *Sepia* (Caen, 1–3 June 1989). Centre de Publications de l'Université de Caen, Caen, France, pp 301–311

Yeatman J, Benzie JAH (1993) Cryptic speciation in *Loligo* from Northern Australia. In: Okutani T, O'Dor RK, Kubodera T (eds) Recent advances in cephalopod fisheries biology. Tokai University Press, Tokyo, pp 641–652

Yeatman J, Benzie JAH (1994) Genetic structure and distribution of *Photololigo* spp. in Australia. Mar Biol 118: 79–87

Yokawa K (1994) Allozyme differentiation of sixteen species of ommastrephid squid (Mollusca, Cephalopoda). Antarctic Sci 6: 201–204

Zaykin DV, Pudovkin AI (1993) Two programs to estimate significance of χ^2 values using pseudo-probability tests. J Hered 84:152