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Allozyme differentiation of sixteen species of ommastrephid squid (Mollusca, Cephalopoda)

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Abstract: Allozyme differentiation was investigated at 23 putative enzyme coding loci in 16 ommastrephid squids to identify species and to assess genetic relationships. The species examined were *Illex illecebrosus*, *I. coindetii*, *I. argentinus*, *Todaropsis eblanae*, *Todarodes sagittatus*, *T. angolensis*, *T. filippovae*, *T. pacificus pacificus*, *Nototodarus sloanii*, *N. gouldi*, *Martialia hyadesi*, *Ommastrephes bartramii*, *Sthenoteuthis pteropus*, *S. oualaniensis*, *Eucleoteuthis luminosa*, and *Dosidicus gigas*. A dendrogram based on Nei's genetic distance between the species closely approximates to the latest systematics based on morphological characters, but the positions of *M. hyadesi* and *T. eblanae* were considerably distant from all other species. The results demonstrate the benefits of further biochemical analysis to an understanding of the systematics of the ommastrephid squids.

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Introduction

The existing systematics and inferred phylogenetics of the squid family Ommastrephidae, based on morphological characters are not all in agreement. For instance, Nesis (1987) considers *Todarodes filippovae* a junior synonym of *T. angolensis*. But Roper *et al.* (1984) and Okutani (1991) give, as an identifying feature, only two pairs of carpal suckers at the base of the club and up to 11 teeth on the largest manal sucker of *T. filippovae*. *Todaropsis eblanae* is generally considered to be in the subfamily Illicinae (Roper *et al.* 1984, Nesis 1987, Okutani 1991) but Roeleveld (1988) suggests that *T. eblanae* belongs to the subfamily Todarodinae on the basis of the consistent distal modification of the hectocotylus which they share. The difficulty of specific diagnosis of cephalopods are partly attributable to insufficient meristic characters and body colouration, and to their soft body. The identification of young and juvenile forms of the Ommastrephidae using only morphological characters can be difficult. In the genus *Illex*, for example, very few morphological characters for species identification of the young stages have been reported (Wormuth *et al.* 1992).

Allozyme electrophoresis has been used in the systematics and inferred phylogenetics of marine fishes (e.g. Shaklee *et al.* 1982), but only a small number of studies have adopted this technique for examining squid interspecific relationships (Smith *et al.* 1987, Augustyn & Grant 1988, Garthwaite *et al.* 1989, Brierley *et al.* 1993). The present study reports the results of allozyme electrophoresis of 16 species of ommastrephid squids to identify species and examine genetic relationships.

Materials and methods

Sixteen squid species currently assigned to nine genera and three subfamilies of the family Ommastrephidae were collected (Table I). All but three species (*Todarodes saggittatus*, *Illex coindetii*, and *Todaropsis eblanae*) were collected by jigging from sites in four areas of the Pacific Ocean and three areas of the Atlantic Ocean. These specimens were frozen immediately at -30°C, and kept frozen at -20°C during transit. On arrival at the National Research Institute of Far Seas Fisheries, they were stored at -80°C individually. The specimens of the remaining three species were collected from sites in western French waters by the Institute Français de Recherche pour l'Exploitation de La Mer, France (Table I). These specimens were packed in dry ice and transported by air to Japan.

For allozyme analysis, 1 g tissue was minced, to which 0.2 ml distilled water was added. Tissue was kept at 4°C for 10 min and the drip was used as a crude enzyme extract. Standard horizontal starch gel electrophoresis was performed for 5–7 h at 5 mA/cm^2 at 4°C. The staining procedures were those described by Wada (1991) except for arginine kinase which followed Harris & Hopkinson (1976). For weakly stained NAD and NADP-dependent enzymes, NAD or NADP were added to the gel and electrode buffer (10mg/200ml). Terminology and notation for allozymes are based on the recent recommendations of Shaklee *et al.* (1990). Alleles are given as their anodal mobility relative to the mobility of the allele of *Illex argentinus* which is set at 100 units. Expression and activity of enzymes were compared between mantle muscle, kidney, digestive gland, buccal complex and eyes (eye lens and retina) under the different buffer systems using specimens of *I. argentinus*, *Todarodes pacificus pacificus*, and *Sthenoteuthis oualaniensis*. Of 25 enzymes and five buffer systems initially tested, 14 enzymes and four buffer systems were chosen (Table II).

Calculations of genetic distance (D) and genetic identity (I) followed the method for a small number of individuals (Nei 1978). A dendrogram based on estimates of genetic distance was constructed using the unweighted pair-group method with arithmetic means (UPGMA) of Sokal & Sneath (1963).

Table I. The ommastrephid squid specimens used in the study.

Species	Date of collection	*n*	Area	Locality
Illicinae				
Illex illecebrosus (Lesueur, 1821)	25.05.91	2	Off Nova Scotia	42°57'N 62°9'W
Illex coindetii (Verany, 1839)	21.09.92	2	Bay of Biscay	46°46'N 4°44'W
Illex argentinus (Castanellos, 1960)	07.02.92	2	Off Argentina	46°34'S 60°35'W
Todaropsis eblanae (Ball, 1841)	12.10.92	2	English Channel	48°46'N 4°4'W
Todarodinae				
Todarodes sagittatus (Lamarck, 1799)	22.09.92	1	Bay of Biscay	46°36'N 4°56'W
Todarodes angolensis Adam, 1962	14.05.91	2	Off Namibia	24°0'S 13°0'
Todarodes fillippovae Adam, 1975	12.04.91	2	Off Argentina	47°52'S 57°48'W
Todarodes pacificus pacificus Steenstrup, 1880	12.09.92	2	Off Japan	42°48'N 144°10'E
Nototodarus sloanii (Gray, 1849)	29.01.92	2	Off New Zealand	40°22'S 172°21'E
Nototodarus gouldi (McCoy, 1888)	29.01.92	2	Off New Zealand	40°22'S 172°21'E
Martialia hyadesi Rochebrune & Mabille, 1889	04.07.91	2	Off Argentina	48°4'S 56°32'W
Ommastrephinae				
Ommastrephes bartramii (Lesueur, 1821)	06.08.92	2	North Pacific	43°3'N 176°17'W
Dosidicus gigas (Orbigny, 1835)	09.11.91	2	Central east Pacific	1°15'N 110°6'W
Sthenoteuthis pteropus (Steenstrup, 1855)	17.06.91	2	Off Namibia	21°47'S 11°27'E
Sthenoteuthis oualaniensis (Lesson, 1831)	10.09.91	2	Off Japan	34°13'N 138°32'E
Eucleoteuthis luminosa (Sasaki, 1915)	09.05.91	2	Off Japan	33°52'N 138°52'E

Note: *n* = number of specimens

Table II. Enzymes, loci, buffer systems and tissues analyzed in the study.

Enzymes and abbreviation	E.C. number	Locus	Buffer[4)]	Tissue
Adenylate kinase (AK)	2.7.4.3	*AK*	I,II	Muscle
Amino peptidase (PEP)	3.4.-.-	*PEP-1*[1)]	III	Muscle
		PEP-2[2)]	III,IV	Digestive gland
		PEP-3[3)]	III,IV	Digestive gland
		PEP-4[2)]	III,IV	Digestive gland
Arginine kinase (APK)	2.7.3.3	*APK-1*	II	Muscle
		APK-2	II	Buccal complex
Glycerol-3-phosphate dehydrogenase (G3PDH)	1.1.18	*G3PDH*	I	Muscle
Glucose-6-phosphate dehydrogenase (G6PD)	1.1.1.49	*G6PD*	III	Buccal complex
Isocitrate dehydrogenase (IDH)	1.1.1.42	*IDH-1*	II	Muscle
		IDH-2	II	Buccal complex
Malate dehydrogenase (MDH)	1.1.1.37	*MDH-1*	I	Muscle
		MDH-2	I	Muscle
Malic enzyme (ME)	1.1.1.40	*ME-1*	II	Buccal complex
		ME-2	II	Buccal complex
		ME-3	II	Buccal complex
Mannose-6-phosphate isomerase (MPI)	5.3.1.8	*MPI-1*	I,II	Muscle
		MPI-2	I,II	Muscle
Purine nucleoside phosphorylase (NP)	2.4.2.1	*NP*	I	Muscle
6-Phosphogluconate dehydrogenase (6PGD)	1.1.1.44	*6PGD*	I,III	Muscle
Glucosephospate isomerase (GPI)	5.3.1.9	*GPI*	III	Muscle
Phosphoglucose mutase (PGM)	2.7.5.1	*PGM*	III	Buccal complex
Superoxide dismutase (SOD)	1.15.1.1	*SOD*	III	Digestive gland

Substrate: [1)]Phenylalanyl-Proline, [2)]Glycyl-Leucyne, [3)]Leucyl-Glycyl-Glycyne,
[4)]Buffer systems; I: Citrate-N(3-aminopropyl) morpholine buffer at pH 7.0 (CAPM7); II: Tris-citric acid buffer at pH8.0; III: gel; Tris-boric acid buffer at pH 8.5, electrode; Tris citric acid buffer at PH8.4 (TVB-LB); IV: gel; Tris-citric acid buffer at pH 8.5, electrode; Lithium hydroxide-Boric acid buffer at pH 8.1 (Li-B).

Results

Twenty-three putative enzyme-coding loci were examined for each species. Damage from long term storage of frozen specimens was not severe, although there was some blurring at *G6PD*, *PGM*, *GPD* and *GPI*. In 23 putative loci, only *Pep-2* and *Pep-4* fixed for one allele in all species examined.

The calculated values of D and I are given in Table III. The most similar pair of species was *Illex illecebrosus* and *I. coindetii* (D = 0.37), and the most divergent was *Dosidicus gigas* and *Todaropsis eblanae* (D = 2.31).

The mean genetic distance within the subfamily Illicinae, Todarodinae and Ommastrephinae were 1.16, 0.97 and 0.76,

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Table III. Estimates of genetic distance D (above diagonal) and genetic identity I (below diagonal) between all species pairs.

	Illicinae				Todarodinae							Ommastrephinae				
	I.ar	*I.il*	*I.co*	*T.eb*	*M.hy*	*T.pc*	*T.fi*	*T.sa*	*T.an*	*N.sl*	*N.go*	*O.ba*	*S.ou*	*S.pt*	*E.lu*	*D.gi*
I.ar		0.40	0.37	2.02	1.78	1.23	1.09	1.78	1.00	1.09	1.08	1.18	1.18	1.18	1.17	1.20
I.il	0.67	-	0.37	2.02	1.78	1.23	1.09	1.78	1.00	1.09	1.08	1.18	1.18	1.18	1.17	1.20
I.co	0.69	0.69	-	1.80	1.60	1.24	1.05	1.45	0.84	1.10	1.11	1.11	1.11	1.22	1.10	1.11
T.eb	0.13	0.13	0.16	-	1.77	1.46	1.70	1.32	1.32	1.56	1.47	2.03	2.03	2.03	1.79	2.31
M.hy	0.17	0.17	0.20	0.17	-	1.46	1.18	1.35	1.26	1.68	1.70	2.29	2.07	2.29	1.87	2.29
T.pc	0.29	0.29	0.29	0.23	0.23	-	0.83	1.09	0.83	0.68	0.65	1.21	1.21	1.21	1.09	1.32
T.fi	0.34	0.34	0.35	0.18	0.31	0.44	-	0.90	0.44	0.91	0.84	1.32	1.20	1.20	1.09	1.32
T.sa	0.17	0.17	0.23	0.27	0.26	0.34	0.40	-	0.67	0.96	0.96	1.45	1.45	1.61	1.31	1.45
T.an	0.37	0.37	0.43	0.27	0.28	0.44	0.65	0.51	-	0.80	0.70	1.01	1.01	1.10	0.91	1.10
N.sl	0.34	0.34	0.33	0.21	0.19	0.51	0.40	0.38	0.45	-	0.42	1.17	1.10	1.10	1.00	1.10
N.go	0.34	0.34	0.33	0.23	0.18	0.52	0.43	0.38	0.50	0.66	-	1.01	1.01	1.01	0.92	1.22
O.ba	0.31	0.31	0.33	0.13	0.10	0.30	0.27	0.23	0.37	0.31	0.36	-	0.70	0.93	0.76	0.71
S.ou	0.31	0.31	0.33	0.13	0.13	0.30	0.30	0.23	0.37	0.33	0.36	0.49	-	0.47	0.70	0.71
S.pt	0.31	0.31	0.30	0.13	0.10	0.30	0.30	0.20	0.33	0.33	0.36	0.40	0.63	-	0.92	0.93
E.lu	0.31	0.31	0.33	0.17	0.15	0.34	0.34	0.27	0.40	0.37	0.40	0.47	0.50	0.40	-	0.76
D.gi	0.30	0.30	0.33	0.10	0.10	0.27	0.27	0.23	0.33	0.33	0.30	0.49	0.49	0.40	0.47	-

Species abbreviations: *I.ar, Illex argentinus; I.il, Illex illecebrosus; I.co, Illex coindetii; T.eb, Todaropsis eblanae; M.hy, Martialia hyadesi; T.pc, Todarodes pacificus pacificus; T.fi, Todarodes fillippovae; T.sa, Todarodes sagittatus; T.an, Todarodes angolensis; N.sl, Nototodarus sloanii; N.go, Nototodarus gouldi; O.ba, Ommastrephes bartramii; S.ou, Sthenoteuthis oualaniensis; S.pt, Sthenoteuthis pteropus; E.lu, Eucleoteuthis luminosa; D.gi, Dosidicus gigas.*

respectively. The Ds between *M. hyadesi* and other species in the Todarodinae were 1.18–1.70. The Ds between *T. eblanae* and other species in the Illicinae were 1.81–2.02. Average intergeneric difference were *Todaropsis* and *Illex* 1.95, *Martialia* and *Todarodes* 1.31, *Martialia* and *Nototodarus* 1.69, *Todarodes* and *Nototodarus* 0.81, *Sthenoteuthis* and *Ommastrephes* 0.82, *Sthenoteuthis* and *Eucleoteuthis* 0.81, *Sthenoteuthis* and *Dosidicus* 0.82.

The dendrogram based on Nei's genetic distance between species is shown in Fig. 1. As would have been predicted by the existing systematics, three subfamilial groupings of the Ommastrephidae are well distinguished from each other, except for *Martialia hyadesi* and *Todaropsis eblanae* both of which are considerably distant from all other species examined. *Stenotethis pteropus*, once classified into the genus *Ommastrephes* by Wormuth (1976), is clustered with *S. oualaniensis*. *Todarodes pacificus pacificus* is not clustered in the group of the genus *Todarodes* but in the group of the genus *Nototodarus*.

Discussion

The results of the allozyme analysis are consistent with existing morphological systematics to a considerable degree, although the number of specimens is limited, indicating that allozyme analysis is appropriate for systematics of the Ommastrephidae. The relatively large number of gene loci examined overcomes the disadvantage of the limited number of specimens (Nei 1978, Shaklee *et al.* 1982).

Although general agreement is obtained between the present allozyme analysis and morphological systematics, there are some discrepancies. One is the positions of *Martialia hyadesi* and *Todaropsis eblanae* in the dendrogram, which are considerably distant from all other species examined (Fig. 1). Referring to former studies on genetic diversity of squid using electrophoresis, the genetic distance between local races varies from 0.00 to 0.108, D between congeneric species varies from 0.686 to 0.949, and D between species of confamilial genera varies from 0.40 to 3.054 (Augustyn & Grant 1988, Garthwaite *et al.* 1989, Carvalho *et al.* 1992, Brierley *et al.* 1993). The relatively large Ds among *M. hyadesi*, *T. eblanae* and other confamilial species observed in the present study may necessitate the construction of new subfamily(s). The position of *T. eblanae* in the dendrogram does not support the suggestion of Roeleveld (1988) that *T. eblanae* belongs to the Todarodinae. The reason for this is not clear. *Todarodes pacificus pacificus* appears more closely allied to the genus *Nototodarus* than to the genus *Todarodes*. Although there has been no morphological study which supports this result, this seems interesting from the

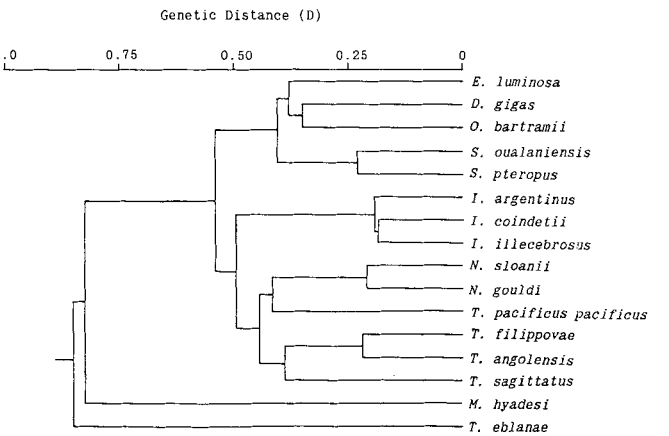

Fig. 1 Cluster analysis by UPGMA using Nei's genetic distance.

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viewpoint of biogeography. In the genus *Todarodes*, *T. pacificus pacificus* and its subspecies *T. pacificus pusillus* are the only species distributed in the Pacific Ocean while in the genus *Nototodarus* all species are distributed in the Pacific Ocean. Further study to clarify the genetic relationships among all species and subspecies of these two genera is necessary.

Okutani (1991) reported that there are 11 genera, 20 species and two subspecies in the family Ommastrephidae. An expanded study which includes the remaining species should be undertaken in future. Data for species of the genus *Hyaloteuthis* and *Ornithoteuthis*, which the present study could not cover, are especially required. Since a limited number of useful morphological characters are available for the systematics of the ommastrephids, the different weighting on each character may considerably affect the inferred phylogenetic relationships between species. Biochemical genetics offers a degree of objectivity and may be useful to support identified morphological differences. A combined study involving both morphological and biochemical analysis would contribute to the refinement of the systematics of the ommastrephids. A study of evolutionary divergence of some of the ommastrephid species such as *T. eblanae*, *O. bartramii* and *Hyaloteuthis pelagica*, which have conspecific populations distributed in several separated areas (Lu & Dunning 1982, Roper *et al.* 1984) would be interesting.

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