Genetic variation and phylogenetic relationships of seven oreo species (Teleostei, Oreosomatidae) inferred from allozyme analysis

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Abstract.—Fishing pressure on deepwater oreosomatids has increased recently in Australian and New Zealand waters, and yet little is known about these fish. Genetic variation and phylogenetic relationships among Australian species was examined. Allozyme variation at 26 loci was examined in seven species: six from Australasia (*Allocyttus niger*, black oreo; *A. verrucosus*, warty oreo; *Neocyttus rhomboidalis*, spiky oreo; *Oreosoma atlanticum*, oxeye oreo; *Pseudocyttus maculatus*, smooth oreo; and a new species *Neocyttus* sp., rough oreo, infrequently captured with the smooth oreo and black oreo) and one from the North Atlantic (*N. helgae*). Two phenetic trees were constructed: an unweighted pair-group method with arithmetic averaging (UPGMA) tree derived from Nei's unbiased genetic distances and a distance-Wagner tree derived from Rogers' distances. A maximum parsimony cladistic analysis, with loci as characters and alleles as unordered states, was also performed. Outgroup species came from three related families: Acanthuridae, Berycidae, and Zeidae.

Mean heterozygosity per locus for the seven oreo species was relatively high for teleosts (11.8%), with *O. atlanticum* having the lowest value (8.3%) and *N.* sp. having the highest value (18.1%). *Oreosoma atlanticum* was the most divergent, with a mean genetic identity (I) of 0.371. The two most closely related species—*N. rhomboidalis* and *N. helgae* (I=0.973)—did not have any diagnostic allozyme loci, although the muscle protein patterns, after Coomassie blue staining, were distinctive. There was little evidence to support the inclusion of *A. niger* and *A. verrucosus* in the same genus; these two species had a genetic identity of 0.695. *Allocyttus niger* appeared to be more closely related to members of the genus *Neocyttus* than to *A. verrucosus*. Phenetic analyses revealed only minor differences in the Oreosomatidae grouping with respect to the three outgroups, whereas cladistic analyses revealed the Zeidae as the most closely related family.

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Oreos are laterally compressed, deep-bodied fish with large heads and large eyes. They are found in deepwater (below 500 m) over the continental slopes of most temperate, and some tropical and subtropical, regions. They appear to be more common in the Southern Hemisphere, but this may reflect a greater deepwater trawling effort in such regions as New Zealand and Australia.

The family Oreosomatidae (order Zeiformes) contains four genera. In a revision of oreos from the southern oceans, James et al. (1988) reported that, although the family is well defined and recognizable, its generic relationships are less clear: the genera *Allocyttus*, *Neocyttus*, and *Oreosoma* need redefining. The fourth genus, *Pseudocyttus*, is well defined and distinguishable.

Oreos are among the most abundant benthopelagic fishes on the continental slope of southern Australia, yet little is known of their biology, stock structure, or phylogeny. In New Zealand waters, oreos have been fished commercially since the late 1970's. A peak catch of 26,500 metric tons (t) was taken in 1981–82; the fishery has since moderated to around 19,000 t per year (Lyle et al., 1992). The New Zealand fishery comprises two main species: the smooth oreo (*Pseudocyttus maculatus* Gilchrist, 1906) and the black oreo (*Allocyttus niger* James et al., 1988). In Australian waters oreos have been caught largely as a bycatch of the deepwater fisheries for blue grenadier (*Macruronus novaezelandiae* (Hector, 1871)) and orange roughy (*Hoplostethus atlanticus* Collett, 1889) and were generally discarded. However, recent drastic reductions in orange roughy catch limits, the development of new deepwater fishing grounds off southern Tasmania, and growing market awareness have resulted in increased targeting of species aggregations and a rapid growth and retention of Australian catches of oreos (Lyle et al., 1992). The retained catch of oreo from the southeast fishery (the main deepwater trawl fishery in Australia) was less than 100 metric tons (t) per annum before 1987, around 2,000 t in 1990 and 1991, over 3,000 t in 1992, and over 1,000 t in 1993 and 1994 (Australian Fisheries Management Authority[1]). Actual catches are prob-

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[1] Australian Fisheries Management Authority. 1995. Burns Centre, 28 National Circuit, Forrest Act 2603, Australia. Unpubl. data.

ably higher because some are not reported and others discarded (Lyle et al., 1992). The recorded tonnages of individual species are unreliable owing to confusion over species identification in the catch log books. As in New Zealand, the smooth and black oreos dominate the Australian catch, whereas the spiky oreo (*Neocyttus rhomboidalis* Gilchrist, 1906) and the warty oreo (*Allocyttus verrucosus* (Gilchrist, 1906)) are also important. A fifth species, the oxeye oreo (*Oreosoma atlanticum* Cuvier, 1829), is commonly caught but discarded because of its small size and low commercial value.

This paper presents the results of an allozyme survey of the five described Australasian species (*Allocyttus niger*, *A. verrucosus*, *Neocyttus rhomboidalis*, *Oreosoma atlanticum*, and *Pseudocyttus maculatus*) and a new species (the rough oreo, *Neocyttus* sp., Yearsley and Last[2]) often captured with *A. niger* and *P. maculatus*. A third *Neocyttus* species, *N. helgae* (Holt and Byrne, 1908), from the North Atlantic, was also examined.

Oreosomatids not included in this study are the North Pacific *Allocyttus folletti* Myers, 1960, the southern Atlantic and Indian Ocean *Allocyttus guineensis* Trunov and Kukuev in Trunov, 1982, and the Indian Ocean *Neocyttus acanthorhyncus* (Regan, 1908). Another member of the family, the Southern Ocean *Pseudocyttus nemotoi* (Abe, 1957), was recently resurrected by Miller (1993).

The intrarelationships of zeiforms have not been discussed in the literature; thus outgroup selection for this phylogenetic study is difficult. Many authors consider the beryciforms to be more primitive than the zeiforms but closely related to them (e.g. Greenwood et al., 1966). Zehren (1979) found the Berycidae to be more primitive than the remaining beryciform families and, thus, a berycid may be a suitable outgroup. However, Rosen (1984) dramatically changed the placement of the zeiforms, including them in the order Tetraodontiformes, with the Caproidae as the sister group to all other tetraodontiforms (the caproids' placement within the Zeiformes was questioned by others [Tighe and Keene, 1984]). A caproid may therefore be a suitable outgroup. Furthermore, Rosen placed the zeids immediately before the oreosomatids in his new division Zeomorphi. He used "acanthurids plus chaetodontids" to establish character polarities. Consequently, a zeid or an acanthurid are also possible outgroups. In the absence of caproid specimens, three outgroups were selected for analysis: the berycid *Beryx splendens* Lowe, 1833 (alfonsino), the zeid *Cyttus australis* (Richardson, 1843) (silver dory), and the acanthurid *Naso tuberosus* Lacepède, 1802 (humphead unicornfish).

Genetic variation present in the Australasian oreosomatids and diagnostic allozyme loci for each species are presented in this paper to assist in future management plans for the developing deepwater fishery. The phylogenetic relationships of previously known species, as well as a new species from Australia and a species from the North Atlantic, are discussed in an effort to understand more fully the systematics of the family Oreosomatidae.

Materials and methods

Samples of muscle and liver tissue were collected from seven oreosomatids (*Allocyttus niger*, *Allocyttus verrucosus*, *Neocyttus* sp. (voucher specimen: CSIRO H2865.01), *Neocyttus helgae*, *Neocyttus rhomboidalis*, *Oreosoma atlanticum* and *Pseudocyttus maculatus*), and from the three outgroup species (*Beryx splendens*, *Cyttus australis*, and *Naso tuberosus*). Sample details and species abbreviations are given in Table 1.

Whole fish were frozen after capture and transported frozen to the laboratory, where tissues were dissected and held at –80°C. Small pieces of tissue were placed in 1.5-mL microcentrifuge tubes, homogenized manually with a few drops of distilled water, and spun at 11,000 rpm in a microcentrifuge for 2 minutes. The supernatant was used for electrophoresis.

Allozyme variation was examined with three gel systems: gel system A—Helena Titan III cellulose acetate plates run at 200 V with a Tris-glycine buffer (0.020 M tris and 0.192 M glycine, Hebert and Beaton[3]); gel system B—Helena Titan III cellulose acetate plates run at 150 V with a Tris-citrate buffer (0.075 M tris and 0.025 M citric acid, pH 7.0); gel system C—8% Connaught starch gels with a histidine/citrate buffer (gel buffer: 0.005 M histidine HCl, pH 7.0; electrode buffer: 0.41 M trisodium citrate, pH 7.0). Standard staining procedures were followed (Richardson et al., 1986; Hebert and Beaton[3]).

In all, 19 enzymes, representing 27 loci, were examined (Table 2) and allele frequencies determined (Table 3). However, the locus *GPI-A** was not included in the phylogenetic analyses because of poor resolution in three species (*A. niger*, *N.* sp., and *P. maculatus*). Loci and alleles are designated by the nomenclature system outlined in Shaklee et al. (1990), except that peptidase loci were identified as *PEP1** and

[2] Yearsley, G. K., and P. R. Last. 1995. CSIRO Division of Fisheries, Castray Esplanade, Hobart, Tasmania 7000, Australia.

[3] Hebert, P. D. N., and M. J. Beaton. 1989. Methodologies for allozyme analysis using cellulose acetate electrophoresis: a practical handbook. Helena Laboratories, Beaumont, Texas.

Table 1

Collection details for specimens analyzed in this study and species abbreviations adopted in this paper.

Species	Abbrev.	Collection area	No. of fish	Collection date
Allocytus niger	AN	Tasmania	202	May 1993
		South Tasman Rise	41	January/June 1992
		New Zealand	100	January 1994
			Total = 343	
Allocytus verrucosus	AV	Western Australia	336	January 1991/March 1992/August 1993
		Great Australian Bight	134	March–June 1992
		Tasmania	126	February 1992/November 1992/April 1994
		New South Wales	32	April 1994
		Lord Howe Rise	83	June 1992
		South Africa	11	September 1993
			Total = 722	
Neocyttus sp.	NA	Southern Tasmania	Total = 16	May 1993
Neocyttus helgae	NH	North Atlantic (ca. 61°39'N, 13°11'W)	Total = 35	February 1993
Neocyttus rhomboidalis	NR	Western Australia	145	October 1993
		Great Australian Bight	31	March 1992
		South Australia	114	October 1993
		Tasmania	451	May 1993–April 1994
		New South Wales	59	February/April 1994
		Lord Howe Rise	98	August 1993
		New Zealand	101	January 1994
			Total = 999	
Oreosoma atlanticum	OA	Southern Tasmania	19	October 1994
		Great Australian Bight	6	March 1992
			Total = 25	
Pseudocyttus maculatus	PM	Western Australia	99	October 1993
		Tasmania	200	May 1993
		New Zealand	100	January 1994
			Total = 399	
Beryx splendens	BS	Western Australia	Total = 6	June 1992
Cyttus australis	CA	Gabo Island, Bass Strait	Total = 6	September 1994
Naso tuberosus	NT	Queensland	Total = 2	March 1994

*PEP2**. Multiple loci encoding the same enzyme were designated by consecutive numbers, with '1' denoting the fastest migrating system. Alleles within each locus were identified by the anodal electrophoretic mobility (rounded to nearest 5%, except for *FH*113*) of their product relative to that of the most common allele observed in the spiky oreo, *N. rhomboidalis*, which was designated '100' (cathodal migration being designated negative). In addition, muscle protein patterns were examined after Coomassie Blue staining. These results are not included in the phylogenetic analyses because of uncertain homologies between species, but patterns were species-specific. The *CK-A** product was one of several protein products visualized by this general protein stain. The mean sample sizes per locus for the seven oreo species had a wide range (from 14 to 598, Table 4) because polymorphic loci for the four main commercial species (*A. niger*, *A. verrucosus*, *N. rhomboidalis*, and *P. maculatus*) were examined in large numbers for stock delineation studies (unpubl. data).

Species relationships were analyzed with BIOSYS-1 software (Swofford and Selander, 1981) and PAUP (phylogenetic analysis using parsimony) 3.0s soft-

Table 2

Details of enzymes used in this study. EC = Enzyme Committee; Tissue: l = liver, m = muscle; Gel: A = cellulose acetate with a Tris-glycine buffer, B = cellulose acetate with a Tris-citrate buffer, C = starch (see text). Multiple loci encoding for the same enzyme are designated by consecutive numbers, with '1' denoting the fastest migrating system. # = loci not included in analyses (see text).

Enzyme	EC number	Locus abbrev.	Locus no.	Tissue	Gel
Aspartate aminotransferase	2.6.1.1	*sAAT-1**	1	m	A
		*sAAT-2**	2	l	A
		*mAAT**	3	m/l	A
Alcohol dehydrogenase	1.1.1.1	*ADH**	4	l	A
Adenylate kinase	2.7.4.3	*AK**	5	m	B
Creatine kinase	2.7.3.2	*CK-A**	6	m	A
Esterase-D (UV, umb. acetate)	3.1.-.-	*ESTD**	7	m/l	A
Fumarate hydratase	4.2.1.2	*FH**	8	m	A
Glyceraldehyde-3-phosphate dehydrogenase	1.2.1.12	*GAPDH-1**	9	m	B
		*GAPDH-2**	10	m	B
Glucose-6-phosphate isomerase	5.3.1.9	*GPI-A**	#	m	A
		*GPI-B**	11	m	A
Glycerol-3-phosphate dehydrogenase	1.1.1.8	*G3PDH-2**	12	m	B/C
Isocitrate dehydrogenase	1.1.1.42	*sIDHP**	13	m	C
		*mIDHP**	14	l	B
l-Lactate dehydrogenase	1.1.1.27	*LDH-C**	15	l	A
		*LDH-1**	16	l	A
		*LDH-2**	17	m	A
Malate dehydrogenase	1.1.1.37	*sMDH-1**	18	m	C
		*sMDH-2**	19	m	C
Mannose-6-phosphate isomerase	5.3.1.8	*MPI**	20	l	A
Peptidase (l-leucyl-l-tyrosine)	3.4.-.-	*PEP1-1**	21	l	A
Peptidase (leu-leu-leu)	3.4.-.-	*PEP2**	22	l	A
Phosphogluconate dehydrogenase	1.1.1.44	*PGDH**	23	m	B/C
Phosphoglucomutase	5.4.2.2	*PGM-1**	24	l	A
		*PGM-2**	25	m/l	A
Superoxide dismutase	1.15.1.1	*sSOD**	26	l	A
General protein		*PROT*	#	m	A

ware (Swofford, 1991). Two phenetic methods of analysis of genetic distance obtained from the allele frequency data were examined with BIOSYS-1. First, Nei's (1978) unbiased genetic distance measure between each species was calculated, and trees were constructed by cluster analysis and the unweighted pair-group method with arithmetic averaging (UPGMA). Second, Rogers' (1972) distance measures were calculated and trees constructed by the distance-Wagner procedure (Farris, 1972) with outgroup rooting. The Wagner procedure, unlike the UPGMA analysis, does not assume a constant rate of evolution. For the cladistic maximum-parsimony analysis (PAUP), the loci were coded as characters and the most common alleles as unordered character states. When two common alleles were at equal frequencies (0.5), they were treated as multiple states and interpreted in the analysis as a polymorphism. The "branch and bound" and "exhaustive" routines were applied to search for the most parsimonious tree.

Results

Several measures of genetic variation were examined (Table 4). Four of the seven oreo species had average sample sizes per locus exceeding 200, but the average sample size for three species was less than 30. Two of the genetic variation parameters, mean number of alleles per locus and percentage of variable loci, are clearly dependent on sample size: both will increase as increasingly rare alleles are detected. Percentage of polymorphic loci, especially with use of the 0.95 rather than 0.99 criterion, will be less sample-size dependent, and mean heterozygosity per locus is little affected by rare alleles. In fact, estimates of heterozygosity (and genetic distance) are influenced more by numbers of loci than by numbers of individuals (Nei, 1978; Gorman and Renzi, 1979), and all estimates of heterozygosity given here are based on the same 26 loci. All oreos showed high levels of variation, with average

Table 3

Allele frequencies. Alleles are presented as allozyme electrophoretic mobilities relative to the most common allele recorded from spiky oreo (NR), *Neocyttus rhomboidalis*. Locus details are presented in Table 2 and species abbreviations are presented in Table 1. *n* = number of individuals scored, — = allele not detected, # = locus not scored due to poor resolution.

			Oreosomatids							Outgroup		
Locus		Allele	AN	AV	NA	NH	NR	OA	PM	BS	CA	NT
1	*sAAT-1**	115	—	—	—	—	—	1.000	—	—	—	—
		110	—	1.000	0.192	0.121	0.246	—	—	—	—	—
		100	1.000	—	0.808	0.879	0.754	—	0.962	—	—	—
		80	—	—	—	—	—	—	0.038	—	—	—
		75	—	—	—	—	—	—	—	—	1.00	—
		65	—	—	—	—	—	—	—	1.00	—	—
		35	—	—	—	—	—	—	—	—	—	1.00
		n	24	24	13	33	778	21	395	6	6	2
2	*sAAT-2**	125	—	—	0.125	0.014	0.001	—	—		—	
		120	—	—	—	—	0.001	—	—		—	
		100	1.000	0.994	0.875	0.986	0.996	—	0.988		—	
		90	—	—	—	—	—	1.000	—		—	
		80	—	0.003	—	—	0.002	—	0.008		0.83	
		60	—	0.003	—	—	—	—	0.004		—	
		55	—	—	—	—	—	—	—		0.17	
		n	226	178	16	35	422	21	248	#	6	#
3	*mAAT**	100	—	—	—	—	0.001	—	—	—	—	—
		0	—	—	—	—	0.007	—	—	—	—	—
		–100	1.000	1.000	0.962	0.958	0.884	1.000	0.959	—	—	—
		–110	—	—	—	—	—	—	—	—	—	1.00
		–150	—	—	—	—	—	—	—	1.00	—	—
		–200	—	—	0.038	0.042	0.107	—	0.041	—	1.00	—
		–250	—	—	—	—	<0.001	—	—	—	—	—
		n	226	178	13	36	971	25	413	6	6	2
4	*ADH**	125	—	—	—	—	—	—	1.000	—	—	—
		100	1.000	1.000	1.000	1.000	1.000	—	—	—	—	—
		35	—	—	—	—	—	0.024	—	—	—	—
		30	—	—	—	—	—	—	—	1.00	—	—
		10	—	—	—	—	—	0.976	—	—	1.00	—
		–30	—	—	—	—	—	—	—	—	—	1.00
		n	226	178	16	35	422	21	248	6	6	2
5	*AK**	100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.00	—	1.00
		80	—	—	—	—	—	—	—	—	1.00	—
		n	226	24	13	23	24	25	248	6	6	2
6	*CK-A**	110	—	0.845	0.269	—	<0.001	—	—	—	—	—
		100	0.872	0.155	0.731	1.000	0.952	1.000	—	—	—	—
		90	0.128	—	—	—	0.047	—	1.000	—	—	—
		80	—	—	—	—	<0.001	—	—	—	—	—
		75	—	—	—	—	—	—	—	—	—	1.00
		60	—	—	—	—	—	—	—	1.00	—	—
		50	—	—	—	—	—	—	—	—	1.00	—
		n	337	700	13	35	987	25	248	6	6	2
7	*ESTD**	135	—	—	—	—	—	—	—	—	1.00	—
		120	—	—	—	—	—	—	—	1.00	—	—
		115	1.000	1.000	0.846	—	—	—	1.000	—	—	1.00
		100	—	—	0.154	1.000	0.998	1.000	—	—	—	—
		85	—	—	—	—	0.002	—	—	—	—	—
		n	226	178	13	23	422	22	248	4	2	2

Table 3 (continued)

Locus		Allele	Oreosomatids							Outgroup		
			AN	AV	NA	NH	NR	OA	PM	BS	CA	NT
8	*FH**	120	0.003	—	—	—	—	—	—	—	—	—
		115	—	0.002	—	—	—	—	0.007	—	—	—
		113	—	—	—	—	0.006	—	—	—	—	—
		110	—	—	—	0.030	—	—	0.027	—	—	—
		100	0.990	0.747	0.962	0.940	0.982	—	0.838	—	—	—
		95	—	—	—	—	—	—	—	—	—	1.00
		80	0.007	0.247	0.038	0.030	0.011	1.000	0.128	1.00	—	—
		65	—	0.004	—	—	—	—	—	—	1.00	—
		n	294	672	13	33	935	23	413	6	6	2
9	*GAPDH-1**	105	—	—	—	—	—	—	—	1.00	—	—
		100	1.000	1.000	1.000	1.000	1.000	—	1.000	—	1.00	—
		90	—	—	—	—	—	1.000	—	—	—	1.00
		n	24	24	13	23	24	22	24	6	6	2
10	*GAPDH-2**	100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	—	—	—
		75	—	—	—	—	—	—	—	1.00	1.00	—
		30	—	—	—	—	—	—	—	—	—	1.00
		n	24	24	16	23	24	25	24	6	6	2
11	*GPI-B**	140	—	0.001	—	—	0.004	—	—	—	—	—
		125	—	—	—	—	—	—	—	0.50	—	0.50
		120	—	0.071	—	—	—	—	—	—	—	—
		115	—	—	—	0.030	—	—	—	—	—	—
		110	—	—	—	—	—	—	—	0.42	—	0.50
		100	1.000	0.584	0.692	0.849	0.550	1.000	1.000	—	—	—
		95	—	—	—	—	—	—	—	0.08	—	—
		85	—	—	—	—	—	—	—	—	0.33	—
		80	—	0.343	0.269	0.121	0.446	—	—	—	—	—
		65	—	—	—	—	—	—	—	—	0.67	—
		60	—	0.001	0.039	—	<0.001	—	—	—	—	—
		n	226	694	13	33	983	25	248	6	6	2
12	*G3PDH-2**	165	—	—	—	—	0.002	—	—	—	—	—
		160	—	—	—	0.080	—	—	—	—	—	—
		145	—	—	—	—	—	1.000	—	—	—	—
		130	0.118	0.007	—	—	0.052	—	—	—	—	—
		100	0.561	0.526	0.846	0.355	0.900	—	—	—	—	—
		75	0.321	0.459	0.154	0.565	0.046	—	—	1.00	—	—
		70	—	—	—	—	—	—	1.000	—	—	—
		65	—	0.008	—	—	—	—	—	—	—	—
		25	—	—	—	—	—	—	—	—	1.00	—
		15	—	—	—	—	—	—	—	—	—	1.00
		n	279	291	13	31	918	18	248	6	6	2
13	*sIDHP**	120	—	—	—	—	—	1.000	—	—	—	—
		110	—	—	—	—	—	—	—	—	1.00	—
		105	—	—	—	—	—	—	—	1.00	—	—
		100	1.000	1.000	1.000	1.000	0.998	—	1.000	—	—	—
		65	—	—	—	—	—	—	—	—	—	1.00
		50	—	—	—	—	0.002	—	—	—	—	—
		n	226	178	13	18	494	21	248	6	6	2
14	*mIDHP**	105	—	—	—	—	—	1.000	—	—	—	—
		100	1.000	1.000	1.000	1.000	1.000	—	1.000	—	—	—
		95	—	—	—	—	—	—	—	0.33	0.08	—
		85	—	—	—	—	—	—	—	0.42	0.92	1.00
		75	—	—	—	—	—	—	—	0.25	—	—
		n	226	178	15	22	422	24	248	6	6	2

Table 3 (continued)

Locus		Allele	Oreosomatids							Outgroup		
			AN	AV	NA	NH	NR	OA	PM	BS	CA	NT
15	*LDH-C**	100	1.000	1.000	1.000	1.000	0.994	—	0.802	—	0.90	
		85	—	—	—	—	0.006	1.000	0.198	—	0.10	
		80	—	—	—	—	—	—	—	1.00	—	
		n	226	178	16	35	494	24	413	4	5	#
16	*LDH-1**	100	1.000	1.000	0.594	1.000	0.999	—	0.776	—	—	—
		40	—	—	0.406	—	0.001	—	0.224	1.00	—	—
		–15	—	—	—	—	—	1.000	—	—	1.00	—
		–35	—	—	—	—	—	—	—	—	—	1.00
		n	226	178	16	35	422	25	415	6	6	2
17	*LDH-2**	100	0.052	1.000	1.000	1.000	1.000	1.000	1.000	1.00	—	—
		50	0.948	—	—	—	—	—	—	—	—	—
		30	—	—	—	—	—	—	—	—	—	1.00
		–35	—	—	—	—	—	—	—	—	1.00	—
		n	336	178	13	23	422	25	248	6	6	2
18	*sMDH-1**	130	—	—	—	—	—	—	—	1.00	—	—
		125	—	—	—	—	—	1.000	—	—	—	—
		110	—	1.000	—	—	—	—	—	—	—	1.00
		100	1.000	—	1.000	1.000	1.000	—	1.000	—	—	—
		50	—	—	—	—	—	—	—	—	1.00	—
		n	226	178	13	23	422	22	248	6	6	2
19	*sMDH-2**	160	—	—	—	—	—	1.000	—	—	—	—
		100	1.000	1.000	1.000	1.000	1.000	—	1.000	—	1.00	—
		95	—	—	—	—	—	—	—	—	—	1.00
		80	—	—	—	—	—	—	—	1.00	—	—
		n	226	178	16	23	422	25	248	6	6	2
20	*MPI**	110	—	0.219	—	0.071	0.049	—	—		—	—
		100	—	0.765	0.318	0.804	0.779	0.476	0.122		—	1.00
		90	1.000	0.016	0.682	0.125	0.170	0.524	0.878		1.00	—
		80	—	—	—	—	0.002	—	—		—	—
		n	226	276	11	28	903	21	388	#	6	2
21	*PEP1-1**	100	1.000	1.000	1.000	1.000	1.000	1.000	—	1.00	—	—
		90	—	—	—	—	—	—	0.998	—	—	—
		85	—	—	—	—	—	—	—	—	1.00	—
		80	—	—	—	—	—	—	0.002	—	—	1.00
		n	226	178	13	23	422	25	248	6	6	2
22	*PEP2**	105	—	1.000	—	—	—	—	—		—	
		100	1.000	—	1.000	1.000	0.999	1.000	0.507		—	
		85	—	—	—	—	0.001	—	0.493		—	
		80	—	—	—	—	—	—	—		1.00	
		n	226	24	13	23	422	21	402	#	6	#
23	*PGDH**	160	—	—	0.750	—	—	—	—	—	—	—
		150	—	—	0.208	—	—	—	—	—	—	—
		130	—	—	0.042	—	<0.001	—	—	—	—	—
		120	—	—	—	—	0.005	—	—	—	—	—
		115	—	—	—	—	—	1.000	—	—	—	—
		110	0.644	—	—	—	0.184	—	—	—	—	—
		105	—	—	—	—	—	—	—	1.00	—	—
		100	0.286	1.000	—	1.000	0.803	—	0.934	—	0.83	—
		90	—	—	—	—	0.007	—	0.033	—	0.17	—

Table 3 (continued)

Locus		Allele	Oreosomatids							Outgroup		
			AN	AV	NA	NH	NR	OA	PM	BS	CA	NT
		85	—	—	—	—	—	—	0.033	—	—	—
		75	0.070	—	—	—	—	—	—	—	—	—
		60	—	—	—	—	—	—	—	—	—	1.00
		n	287	178	13	31	916	14	412	6	6	2
24	*PGM-1**	115	—	—	—	—	<0.001	—	—		—	—
		110	—	—	—	—	0.004	—	—		—	—
		105	—	0.137	—	—	0.099	—	—		—	—
		100	0.011	0.624	—	0.517	0.609	—	—		0.08	—
		95	0.696	0.222	0.433	0.450	0.242	—	0.017		0.83	—
		90	0.273	0.017	0.500	0.033	0.043	—	0.623		0.08	—
		85	0.020	—	0.067	—	0.003	0.250	0.025		—	—
		80	—	—	—	—	—	0.500	0.335		—	—
		75	—	—	—	—	—	0.250	—		—	1.00
		n	276	259	15	30	911	2	395	#	6	2
25	*PGM-2**	130	—	0.005	—	—	—	—	—	—	—	—
		125	—	—	—	—	0.002	—	—	—	—	—
		120	0.006	0.220	0.077	0.043	0.029	0.060	0.002	—	—	—
		100	0.591	0.749	0.923	0.943	0.925	0.840	0.874	—	—	—
		95	—	—	—	—	—	—	—	1.00	—	—
		80	0.282	0.016	—	0.014	0.040	0.060	0.123	—	—	—
		65	0.111	0.009	—	—	0.004	0.040	—	—	—	—
		60	—	—	—	—	—	—	—	—	1.00	—
		50	0.011	—	—	—	—	—	—	—	—	—
		40	—	—	—	—	—	—	—	—	—	0.75
		20	—	—	—	—	—	—	—	—	—	0.25
		n	330	676	13	35	972	25	414	6	6	2
26	*SOD**	180	—	—	—	0.014	—	0.021	0.374	—	—	—
		140	0.204	1.000	0.500	0.057	0.487	0.333	0.626	—	—	—
		130	—	—	—	—	—	—	—	—	1.00	—
		100	0.796	—	0.500	0.929	0.513	—	—	—	—	—
		60	—	—	—	—	—	0.646	—	1.00	—	—
		0	—	—	—	—	—	—	—	—	—	1.00
		n	329	178	19	35	983	24	412	6	6	2
Locus not included in analyses (see text):												
*GPI-A**		150		0.012		—	—	—		—	—	—
		140		0.002		—	—	—		—	—	—
		130		0.203		0.016	0.007	—		—	—	—
		125		0.005		—	0.014	—		—	—	—
		115		0.753		0.031	0.338	—		—		—
		110		—		0.484	—	—		—	—	—
		100		0.017		0.469	0.610	1.000		—	—	—
		95		0.007		—	—	—		—	—	—
		90		0.002		—	0.014	—		—	—	—
		85		—		—	0.016	—		—	—	—
		75		—		—	0.001	—		—	—	—
		45		—		—	—	—		1.00	—	—
		20		—		—	—	—		—	—	1.00
		10		—		—	—	—		—	1.00	—
		N	#	711	#	32	987	25	#	6	6	6

Table 4

Comparison of genetic statistical information for 26 loci scored in each oreosomatid species. A locus is considered variable when more than one allele is present, and polymorphic when the frequency of the most common allele is 0.95 or less. Mean and standard errors are presented. Mean heterozygosity per locus is Nei's (1978) unbiased estimate. Species abbreviations are defined in Table 1.

Species	Mean sample size per locus	Mean no. alleles per locus	Percentage of loci		Mean heterozygosity
			Variable	Polymorphic	
AN	228.1 ±16.7	1.6 ±0.2	30.8	26.9	0.105 ±0.038
AV	237.7 ±40.9	1.8 ±0.3	30.8	26.9	0.116 ±0.039
NA	14.0 ±0.3	1.7 ±0.1	53.8	46.2	0.181 ±0.041
NH	28.7 ±1.1	1.7 ±0.2	38.5	30.8	0.093 ±0.032
NR	597.6 ±63.1	2.5 ±0.3	65.4	34.6	0.127 ±0.037
OA	22.0 ±1.0	1.3 ±0.2	19.2	15.4	0.083 ±0.041
PM	297.8 ±22.0	1.8 ±0.2	50.0	34.6	0.121 ±0.034

heterozygosities ranging from 0.083 in *O. atlanticum* to 0.181 in *N.* sp.: the four commercial species (*A. niger*, *A. verrucosus*, *N. rhomboidalis*, and *P. maculatus*) had values ranging from 0.105 to 0.127. Despite the lowest mean sample size, *N.* sp. showed the highest proportion of polymorphic loci (46.2%) and the highest average heterozygosity.

Oreosoma atlanticum was the most divergent of the oreosomatids (Table 5). Its average genetic identity (Nei, 1978) (0 indicates complete dissimilarity and 1 complete similarity) with the other species was 0.371 (range 0.313 to 0.426 for 26 loci). The two most similar species were *N. rhomboidalis* and *N. helgae*, with a high genetic identity of 0.973. The third *Neocyttus* species, *N.* sp., had a relatively lower identity with the other two *Neocyttus* species: 0.903 with *N. rhomboidalis* and 0.884 with *N. helgae*. The two *Allocyttus* species had a genetic identity of only 0.695.

The three outgroup species were very divergent from both the oreosomatids and each other (Table 5). The acanthurid *N. tuberosus* diverged most from the oreosomatids, with an average genetic identity of 0.112 (range 0.085 to 0.180, from 23 loci). *Cyttus australis* had a mean identity with the oreosomatids of 0.171 (range 0.108 to 0.199, 26 loci) and *B. splendens* a mean identity of 0.164 (range 0.115 to 0.222, 22 loci).

In the acanthurid *N. tuberosus*, 16 of the 23 scorable loci were diagnostic (no shared alleles with any oreosomatid); in the zeid *C. australis*, 15 of 26 loci; and in the berycid *B. splendens*, 15 of 22 loci were diagnostic (Table 3). In a comparison of oreosomatids with one another, *O. atlanticum* had eleven diagnostic loci, *P. maculatus*, three, *A. verrucosus*, two, whereas the other four species revealed that only the muscle protein patterns were diagnostic (Table 3; Fig. 1). However, when the seven oreosomatids were compared pair-wise, each pair (except *N. rhomboidalis* with either *N.* sp. or *N. helgae*) had at least one and up to fourteen diagnostic allozyme loci, other than the general protein difference (Table 6). With the addition of between 4 and 13 loci showing significant allele frequency differentiation ($P<0.05$, with Bonferroni adjustment for multiple tests) (Table 6), even the closely related *N. rhomboidalis* and *N. helgae* were found to differ at five loci (*FH**, *GPI-A**, *GPI-B**, *G3PDH-2**, and *sSOD**) and *N. rhomboidalis* and *N.* sp. at eight loci (*sAAT-2**, *CK-A**, *ESTD**, *GPI-B**, *LDH-1**, *MPI**, *PGDH**, and *PGM-1**). The locus *PGDH** was diagnostic between *N.* sp. and *N. helgae*, with significant allele frequency differences at a further seven loci (*CK-A**, *ESTD**, *G3PDH-2**, *LDH-1**, *MPI**, *PGM-1**, and *sSOD**). *Oreosoma atlanticum* differed from

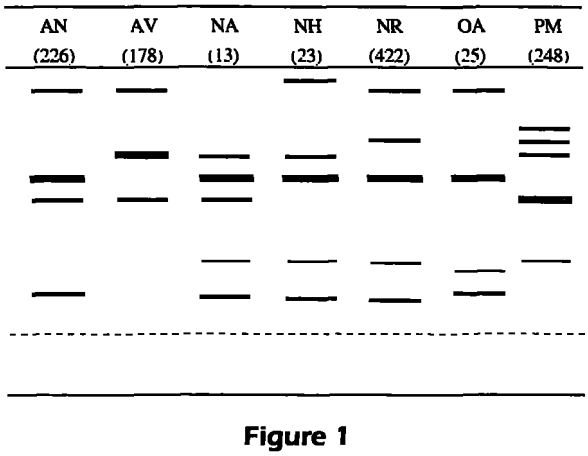

Figure 1

Banding pattern observed for each oreosomatid species with a general protein Coomassie blue stain (*PROT**). Dashed line indicates sample origin. Boldest band represents most common *CK-A** product in each species. Species abbreviations are defined in Table 1. Numbers in parentheses are number of individuals scored.

Table 5

Pairwise comparison of Nei's (1978) unbiased genetic identity (above diagonal) and genetic distance (below diagonal) between species for 26 loci, except for BS (22 loci) and NT (23 loci). Species abbreviations are defined in Table 1.

Species	AN	AV	NA	NH	NR	OA	PM	BS	CA	NT
AN	—	0.695	0.903	0.852	0.841	0.342	0.738	0.118	0.199	0.095
AV	0.364	—	0.773	0.761	0.788	0.313	0.659	0.183	0.169	0.180
NA	0.102	0.257	—	0.884	0.903	0.402	0.764	0.184	0.177	0.108
NH	0.160	0.273	0.123	—	0.973	0.426	0.711	0.174	0.180	0.085
NR	0.173	0.238	0.102	0.027	—	0.422	0.711	0.152	0.174	0.086
OA	1.073	1.161	0.913	0.853	0.864	—	0.319	0.222	0.108	0.128
PM	0.304	0.417	0.269	0.341	0.342	1.143	—	0.115	0.191	0.100
BS	2.138	1.700	1.694	1.749	1.885	1.505	2.159	—	0.068	0.095[1]
CA	1.614	1.780	1.734	1.714	1.748	2.227	1.653	2.689	—	0.042
NT	2.353	1.714	2.229	2.465	2.456	2.057	2.303	2.355[1]	3.168	—

[1] 21 loci common to both species.

the other six species at 17 to 22 loci, in addition to the general protein difference.

Two loci—*AK** and *GAPDH-2**—were invariant across all seven oreosomatid species, but only *GAPDH-2** differed in all three outgroup species; *AK** was different only in *C. australis*. These loci are consistent with a monophyletic origin of the oreosomatids. Three loci (*GAPDH-1**, *mIDHP**, and *sMDH-2**) were monomorphic for the same allele in six of the seven oreo species, with *O. atlanticum* fixed for alternative alleles; these putative synapomorphies indicate that these six species are probably monophyletic.

It is therefore not surprising to find that *O. atlanticum* was clearly separated from the other oreosomatid species on the phenogram constructed by the UPGMA method from Nei's (1978) unbiased genetic distances (Fig. 2). Branching order and significance of the branching nodes did not differ with the choice of outgroup. The three *Neocyttus* species and *A. niger* formed a distinct cluster. There was a very close association of the Southern Hemisphere *N. rhomboidalis* and the Northern Hemisphere *N. helgae*.

The phylogenetic tree constructed by the distance-Wagner procedure from Rogers' (1972) distances, rooted by the outgroup *C. australis*, also showed the divergence of *O. atlanticum* from the other oreosomatids (Fig. 3). Although the closeness of *N. rhomboidalis* and *N. helgae* was maintained, and again the two *Allocyttus* species were not grouped together, *P. maculatus* was found to be grouped with *A. niger* and *N.* sp. A similar tree was produced, by this procedure, with *N. tuberosus* as the outgroup, whereas the tree produced with *B. splendens* as the outgroup resembled those from the UPGMA cluster analyses (with *P. maculatus* divergent from the *Neocyttus* and *Allocyttus* species); a similar tree was produced when all three outgroups were applied together.

Cladistic analysis (PAUP) with all three outgroup species together produced 55 most parsimonious trees, all of which showed the divergence of *O. atlanticum* from other members of the family, but failed to define any structure for the other six species. Analysis with *C. australis* as the outgroup produced eleven most parsimonious trees of 52 units in

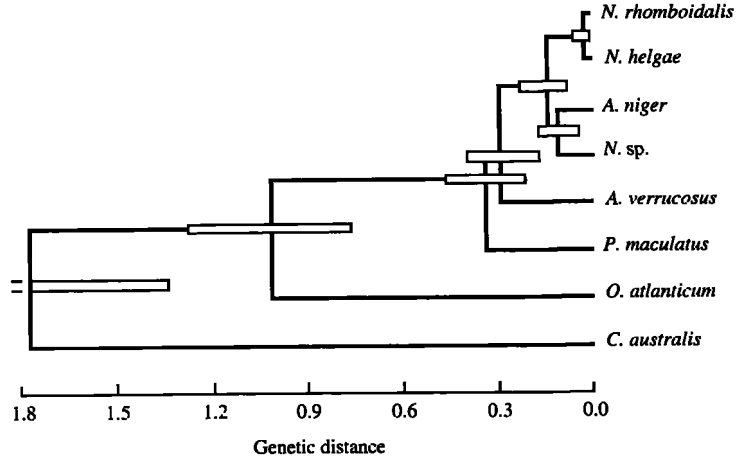

Figure 2

Unweighted pair-group method with arithmetic averaging (UPGMA) phenogram constructed from Nei's (1978) unbiased genetic distance. Open boxes represent standard errors (Nei, 1987) of the branch nodes.

Table 6

Allozyme differences between pairs of oreo species. Above the diagonal: diagnostic loci (no shared alleles); below the diagonal: loci at which significant differences (χ^2 test, $P<0.05$ with Bonferroni adjustment) exist in allele frequencies at loci where alleles are shared, including the GPI-A* locus. Note that the general protein (PROT) staining patterns (not included here but see Fig. 1) are diagnostic for all species. Total numbers are shown in bold and each locus is identified by the reference number shown in Tables 2 and 3. Species abbreviations are defined in Table 1.

	AN	AV	NA	NH	NR	OA	PM
AN		**3** 1,18,22	**1** 23	**1** 7	**1** 7	**13** 1,2,4, 7,9 12,13,14,15 16,18,19,23	**3** 4,12,21
AV	**10** 6,8,11,12 17,20,23 24,25,26		**3** 18,22,23	**3** 7,18,22	**3** 7,18,22	**14** 1,2,4,7,9,12 13,14,15,16 18,19,22,23	**7** 1,4,6,12 18,21,22
NA	**9** 2,6,7,11 16,17,20 25,26	**8** 1,2,6,7 16,20,24 26		**1** 23	**0**	**12** 1,2,4,9,12,13 14,15,16,18, 19,23,24	**5** 4,6,12,21 23
NH	**9** 3,11,12 17,20,23 24,25,26	**9** 1,6,8,11 12,20,24 26,*GPI-A**	**7** 6,7,12,16 20,24,26		**0**	**13** 1,2,4,9,12,13, 14,15,16,18, 19,23,24	**5** 4,6,7,12 21
NR	**11** 1,3,6,11 12,17,20 23,24,25 26	**11** 1,3,6,8 11,12,20 23,25,26 *GPI-A**	**8** 2,6,7,11, 16,20,23 24	**5** 8,11,12,26 *GPI-A**		**11** 1,2,4,9,12,13, 14,16,18,19, 23	**4** 4,7,12,21
OA	**6** 8,17,20 24,25,26	**8** 6,8,11,20 24,25,26 *GPI-A**	**6** 6,7,8,11, 24,26	**4** 8,20,26 *GPI-A**	**8** 8,11,15,20 24,25,26 *GPI-A**		**14** 1,2,4,6,7,9 12,13,14,16 18,19,21,23
PM	**12** 3,6,8,15 16,17,20 22,23,24 25,26	**10** 3,8,11,15 16,20,23 24,25,26	**8** 1,2,7,11 15,22,24 26	**9** 1,11,15 16,20,22 24,25,26	**13** 1,3,6,8 11,15,16 20,22,23 24,25,26	**7** 8,15,20,22, 24,25,26	

length, all again showing the divergence of *O. atlanticum* , but this time defining some structure to the other species. The 50% majority-rule consensus tree is shown in Figure 4. The most significant difference in this analysis from the two phenetic analyses is the reversal of the positions of the two *Allocyttus* species. In the cladistic analysis *A. niger* is separated from the other species, whereas *A. verrucosus* was grouped with either *P. maculatus* and *N.* sp. or the other two *Neocyttus* species. Applying a topological constraint to the search (enforcing predetermined groupings and keeping only those trees that satisfy the constraints) for the three *Neocyttus* species or the two *Allocyttus* species produced the shortest trees only one step longer than the most parsimonious under no-constraint searches. A search for near-optimal trees with lengths of 53 units produced 56 trees, with 98% confirming the branch separation of *O. atlanticum*, and 71% supporting the separation of *N. rhomboidalis* and *N. helgae* from the other four species, among which the branching points could not be resolved.

The zeids appear to be the most likely sister family to the oreosomatids after the cladistic analyses.

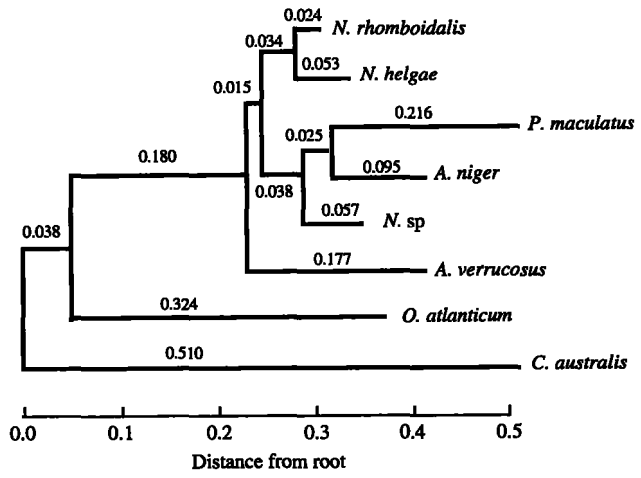

Figure 3

Tree constructed from Rogers' distance by the distance-Wagner procedure. Numbers represent relative branch lengths.

Neither analysis with the berycid or the acanthurid produced the shortest trees that resembled in any way the trees produced from the phenetic analyses or the cladistic analyses with either all three outgroups or *C. australis* alone. *Naso tuberosus* as the outgroup resulted in a single shortest tree (49 units) with *A. verrucosus* as the most divergent oreosomatid and *O. atlanticum* grouped next to the *N. rhomboidalis* and *N. helgae* cluster. Four shortest trees (42 units) resulted from the analysis with *B. splendens*, all of which, although confirming the divergence of *O. atlanticum*, resulted in *N. rhomboidalis* and *N. helgae* diverging independently from the other four species.

Discussion

The mean heterozygosity per locus for the seven oreosomatid species ranged from 8.3% to 18.1%, with an overall mean of 11.8%. These figures are considerably higher than the mean of 5.1% for 195 species of marine and freshwater fish (Ward et al., 1992) and 5.5% for 106 species of marine teleosts (Smith and Fujio, 1982). Three of the seven oreosomatid species had mean heterozygosity values (12.1% to 18.1%) which exceeded the highest value of 11.7% reported by Ward et al. (1994) from comparisons of genetic diversity among populations of 57 species of marine fish. This value was shown by two species, *Fundulus heteroclitus* (Linnaeus, 1766) (15 loci, Ropson et al., 1990) and *Hoplostethus atlanticus* (22 loci, Smith, 1986). Subsequent studies of additional loci in the orange roughy, *H. atlanticus*, raised the estimate of its mean heterozygosity to 13.0% (Elliott and Ward, 1992).

Two or more samples contributed to these heterozygosity estimates for five of the oreo species (Table 1). These estimates of heterozygosity are Hardy-Weinberg expected heterozygosities based on pooled allele frequencies. Had there been substantial differentiation of allele frequencies among samples, such estimates of heterozygosity would have been higher than average sample heterozygosities. In fact, the degree of inter-sample differentiation was, with a single exception, very limited (Lowry et al., unpubl. data). Thus these estimates of total heterozygosity will be very similar to estimates of sample heterozygosity. The one exception was the locus *sSOD** in *N. rhomboidalis*. Variation at this locus was found to be depth-related; samples with a high frequency (>0.6 [cf. <0.2]) of *sSOD**140 came from deeper water (>700 m). The total heterozygosity for this one locus was 0.500, whereas its average sample heterozygosity was 0.285. Use of sample heterozygosity rather than total heterozygosity would effect a small reduction in the overall heterozygosity estimate for this species (from 0.127 to 0.119). Note also that the species with the highest degree of variation, *N.* sp., came from a single sample. The high variability seen in the oreos cannot be attributed to inter-sample differentiation.

It is clear that oreosomatids have higher heterozygosities than most species of teleosts. Interestingly, both oreosomatids and the similarly variable orange roughy occupy deepwater habitats. As speculated by

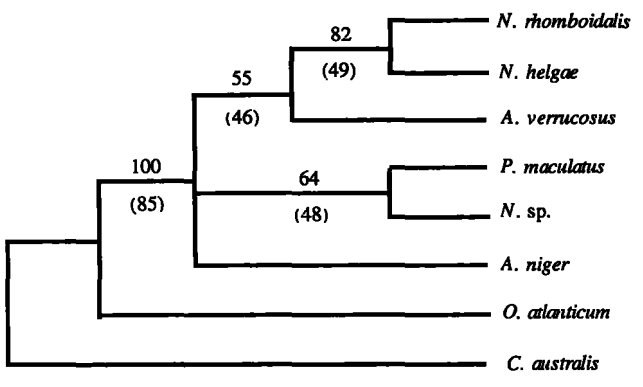

Figure 4

The 50% majority-rule consensus tree of the eleven shortest trees produced by PAUP analysis, with numbers representing the percentage consensus measures for the eleven trees. Numbers in brackets are bootstrapped values (100 replicates).

Elliott and Ward (1992) for the orange roughy, the high heterozygosity shown by these deepwater (500–1200 m) species may reflect their large (prior to exploitation) population sizes and (assuming that deepwater species have been less severely affected by glaciations than shallow water species) a lack of severe bottlenecks in their recent evolutionary past.

As adults, *Pseudocyttus* is the most morphologically distinct oreosomatid genus (James et al., 1988). Its distinguishing characters include the first dorsal-fin spine being longer than the second (vice versa in other species), a pelvic fin with only five rays (usually six or seven in other species), and 40–43 vertebrae (34–41 in other species). However, as juveniles, the genus *Oreosoma* is the most distinctive with prominent cones over the body. Other juvenile oreosomatids have "warts" or protuberances (such structures are absent in at least *N. rhomboidalis)*, but none are quite so pronounced or bizarre as in *O. atlanticum*. Our genetic study confirms the uniqueness of *O. atlanticum*, which has a very low genetic identity (0.371) with the other oreosomatid species—substantially less than the corresponding mean identity (0.650) of *P. maculatus* with other oreosomatids. Morphologically, *O. atlanticum* can be distinguished as an adult by a very large eye (eye diameter 52–60% of head length) and by a prominent horizontal ridge on the operculum.

James et al. (1988) suggested that further study may synonymize the Northern Hemisphere *N. helgae* with the Southern Hemisphere *N. rhomboidalis*. Our allozyme data suggest that, although these two taxa are indeed very closely related (genetic identity I=0.973 for 26 loci and I=0.966 for 27 loci including *GPI-1**), their distinctive muscle protein patterns, not included in the genetic identity values, are consistent with their being separate species. Of the four non-*CK-A** protein bands, two appear to be fixed differently for the two species. However, the amount of genetic differentiation between these two species is only a little greater than that between samples of *H. atlanticus* taken from the same two areas (North Atlantic and off southern Australia) (Elliott et al., 1994). Eleven polymorphic loci were screened in the *H. atlanticus* comparison and just three loci showed significant heterogeneity and gave a genetic identity of 0.990 (N.G.E.'s unpubl. data). Thus the genetic data do not unequivocally validate the recognition of *N. rhomboidalis* and *N. helgae* as distinct species. Morphologically they are also very similar, although there are some differences (Yearsley and Last[2]).

As mentioned earlier, there is depth-related variation in the *sSOD** polymorphism in *N. rhomboidalis*, with samples derived from deeper water having a high frequency of *sSOD**140. We are uncertain as yet whether this indicates reproductive isolation of two forms or selection acting on *sSOD*, although the lack of detectable mitochondrial DNA differentiation (Grewe, Innes, and Evans[4]) suggests that if reproductive isolation is responsible, it is likely to be recent in origin. These data and analyses will be presented in full elsewhere.

The new species *N.* sp., infrequently captured with *P. maculatus* and *A. niger* in southern Australian waters and morphologically similar to *N. rhomboidalis*, showed quite a high degree of genetic similarity to the other two *Neocyttus* species (I=0.903 with *N. rhomboidalis* and 0.884 with *N. helgae*). However, it was genetically distinct from them at several loci (Table 6), and numerous meristic and morphological characters (Yearsley and Last[2]) confirm that it is a separate species. Although it clustered with *A. niger* (I=0.903) in the two phenetic trees constructed from the genetic distance data, in the cladistic analyses it grouped more often with *P. maculatus*. However, classical taxonomic techniques suggest a close association with *Neocyttus* species, particularly the western Indian Ocean *N. acanthorhynchus* (Yearsley and Last[2]).

The two *Allocyttus* species were found to be genetically quite distinct from one another (I=0.695); there was no evidence from either phenetic or cladistic analyses that they made up an exclusive monophyletic group. James et al. (1988) gave no justification for placing *A. niger* in *Allocyttus*. However, they drew attention to problems with generic diagnoses of the oreosomatids. *Allocyttus*, as it currently stands, but excluding *A. niger* (i.e. *A. verrucosus*, *A. guineensis*, and *A. folletti*), may be a natural grouping, with *A. niger* more akin to, but probably not congeneric with, *Neocyttus*. Ongoing morphological work should elucidate these problems.

Whereas the branch node for *O. atlanticum* is clearly resolved to be ancestral to the remaining oreosomatids, the phenetic and cladistic analyses could not resolve unambiguously all the internal nodes for the remaining species. There is strong evidence for a branch node separating *N. rhomboidalis* and *N. helgae* from the other four species, but there is no evidence supporting two species in *Allocyttus*.

Several problems in the family Oreosomatidae remain to be resolved. They include the formal description of the rough oreo, *N.* sp. (Yearsley and Last[2]), a reassessment of the generic affinities of *A. niger* and *A. verrucosus*, and further examination of the two depth-related *sSOD** forms of *N. rhomboidalis* and their relationship to *N. helgae*. As intimated by

[4] Grewe, P. M., B. H. Innes, and B. S. Evans. 1995. CSIRO Division of Fisheries, Castray Esplanade, Hobart, Tasmania 7000, Australia.

James et al. (1988), there remains a clear need for a full and thorough revision of the family Oreosomatidae.

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