

Short communication

Genetic differentiation between the New Zealand and Falkland Islands populations of southern blue whiting *Micromesistius australis*

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Abstract The southern blue whiting *Micromesistius australis* (Norman, 1937) is found in two geographically distinct areas, the South Atlantic and south-west Pacific Oceans. To date there has been no appraisal of the genetic relationships between the populations in these two areas. Here, we present analyses of New Zealand and Falkland southern blue whiting using mini- and microsatellite loci. Two out of six loci show highly significant allele frequency heterogeneities between the two areas, strongly suggesting genetically distinct populations.

Keywords *Micromesistius australis*; minisatellite DNA; microsatellite DNA; New Zealand; Falkland Islands

INTRODUCTION

Several commercially important Southern Hemisphere marine fishes have disjunct distributions with major populations in the South Atlantic and south-west Pacific Ocean. The relationships among these geographically isolated populations are poorly known and in some cases application of different approaches has led to different conclusions. For example, Grant & Leslie (2001) recognised the South American hake *Merluccius polylepis* and the Australian hake *M. australis* as subspecies, based on allozyme data and geographic isolation, whereas Inada (1981a) found no morphometric or meristic differences between samples from Patagonia and New Zealand, and considered *M. polylepis* to be a junior synonym of *M. australis*.

The southern blue whiting *Micromesistius australis* (Norman, 1937) has disjunct populations, one around South America (on the Falkland (Malvinas) Islands–Patagonia shelf, and off Chile), and one in subantarctic waters around New Zealand in the south-west Pacific Ocean. *M. australis* is a mid water pelagic species supporting large fisheries in both regions. Catches in New Zealand peaked at 76 000 t in the early 1990s but are now controlled by catch limits, set at 35 000 t in 2000–01, although catches have not reached the limits in recent years, largely because of economic factors other than low stock sizes (Annala et al. 2001). Catches of southern blue whiting in the Falkland Islands fishery were 23 000 t in 1999 (Falkland Islands Fishery Statistics, <http://fis/falklandfish>) and c. 100 000 t over all the South Atlantic (Wohler 1999).

The South American and New Zealand populations of southern blue whiting have been considered distinct subspecies, namely *M. australis pallidus* and *M. australis australis* respectively, based on geographic isolation and morphometric and meristic characters (Inada & Nakamura 1975; Shust 1978). Subsequent morphometric analyses have shown considerable within region variation (Wieczaszek 1988; Hanchet 1999), but analysis of 17 characters found no subspecific differentiation

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between populations of southern blue whiting from the Falkland Islands and New Zealand (Trella 1999).

Here we apply five microsatellite markers, developed for northern blue whiting *M. poutassou*

(Moran et al. 1999) and whiting *Merlangius merlangus* (Rico et al. 1997), and one minisatellite marker (McGregor et al. 1996) to test the genetic relationships between Atlantic and Pacific populations of *M. australis*.

Table 1 Genetic variability at six DNA loci in *Micromesistius australis* from New Zealand and the Falkland Islands. Core repeat sequences are given for each locus. (No. of fish, number of fish scored; no. of alleles, number of alleles observed; allele size, size range of alleles in base pairs; H_{obs} , number of heterozygotes observed; H_{exp} , number of heterozygotes expected assuming Hardy-Weinberg equilibrium; P , probability for conformity to Hardy-Weinberg equilibrium; *, significant at the 5% level with a Bonferroni modified P .)

Locus parameter (repeat sequence)	New Zealand	Falkland Islands
<i>MmerAmp1B</i> (AAAGGGTTAGAGGTGGGGTC)		
No. of fish	50	25
No. of alleles	8	5
Allele size	308–476	340–476
H_{obs}	21	14
H_{exp}	25.6	13.71
P	0.060	0.139
<i>MpouBW07</i> (GT) ₁₃		
No. of fish	49	25
No. of alleles	13	8
Allele size	229–259	233–249
H_{obs}	42	18
H_{exp}	40.29	20.08
P	0.700	0.027
<i>MpouBW08</i> (GT) ₁₂		
No. of fish	50	25
No. of alleles	13	12
Allele size	267–295	269–295
H_{obs}	36	20
H_{exp}	35.97	20.06
P	0.268	0.489
<i>MpouBW09</i> (GT) ₅₈		
No. of fish	49	24
No. of alleles	13	10
Allele size	175–205	183–200
H_{obs}	36	20
H_{exp}	42.45	19.44
P	0.028	0.829
<i>MpouBW13</i> (GT) ₁₃		
No. of fish	36	21
No. of alleles	15	12
Allele size	167–198	171–202
H_{obs}	20	15
H_{exp}	33.13	18.66
P	<0.0001*	0.101
<i>MmerUEAW01</i> (AC) ₆ (N) ₅ (AC) ₄ (N) ₃₂ (AGG) ₁₂ (N) ₃ (AGG) ₁₃		
No. of fish	46	24
No. of alleles	8	8
Allele size	205–222	207–222
H_{obs}	32	18
H_{exp}	37.40	17.51
P	0.053	0.404

MATERIALS AND METHODS

Sample collection

Gill tissue samples and sex data were collected from 50 adult southern blue whiting from the Bounty Platform (48°00'S, 17°90'E), in the New Zealand Exclusive Economic Zone (EEZ), aboard the RV *Tangaroa*, in September 1997 and from 25 adult fish south of the Falkland Islands (52°07'S, 63°26'W) aboard the EHUT *Piscator* in May 1997. Gill tissues were fixed in 70% ethanol at sea.

DNA amplification and analyses

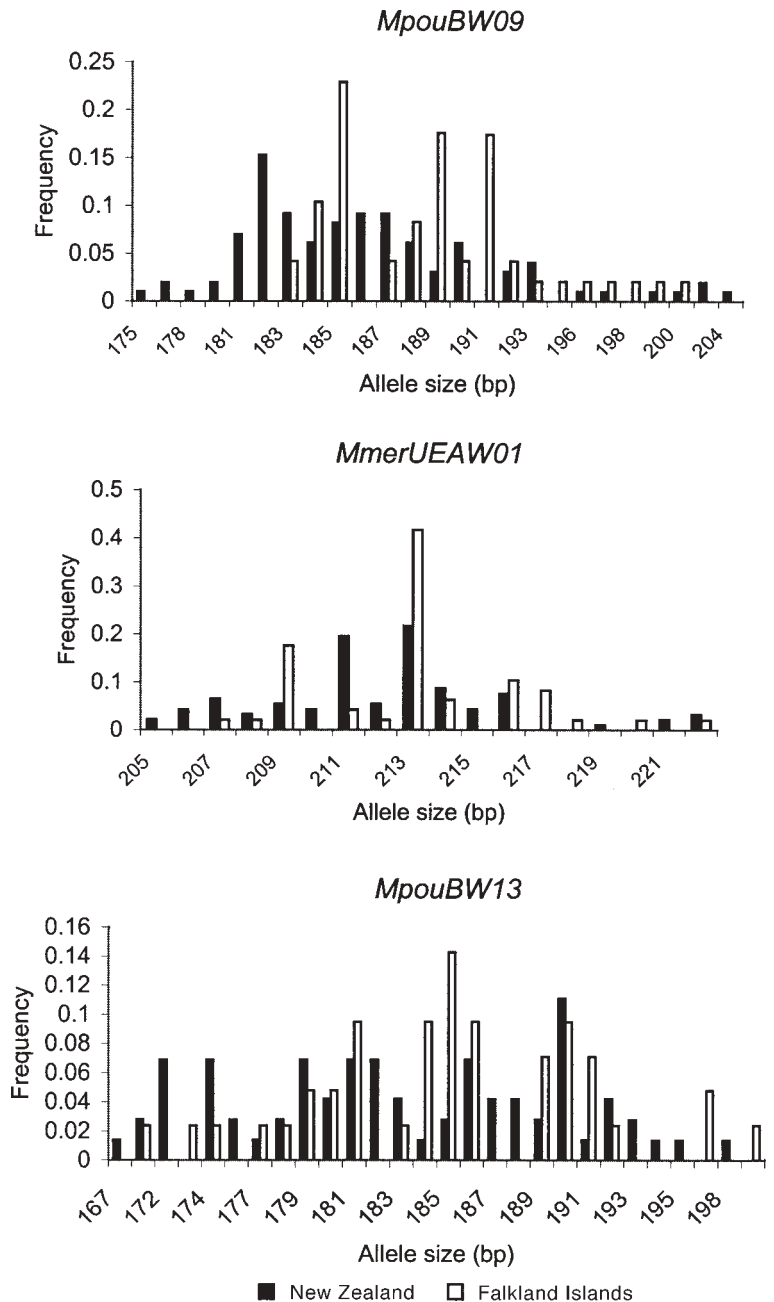
DNA was prepared from gill tissues by standard phenol/chloroform extraction and precipitation in ethanol (Sambrook et al. 1989). Laboratory analyses followed those described by Moran et al. (1999) for five microsatellite loci (*MpouBW07*, *MpouBW08*, *MpouBW09*, *MpouBW13*, and *MmerUEAW01*) and Mattiangeli et al. (in press) for the minisatellite locus (*MmerAmp1B*). Four of the microsatellite loci were di-nucleotide repeats and one, *MmerUEAW01*, had a complex repeat sequence (Table 1). PCR fragments were diluted (1:10 to 1:30, experimentally determined to give optimal resolution) and loaded onto a Li-cor automated sequencer with formamide loading dye (formamide and bromophenol blue) in the ratio 1:2 PCR dilution to loading dye. Molecular weight ladder (Li-cor) and locus-specific standard amplification products (individuals of known genotype) were loaded onto every gel.

Allele frequencies were determined from direct counts of homozygous and heterozygous individuals. At three loci, *MpouBW09*, *MpouBW13*, and *MmerUEAW01*, alleles were binned into alleles differing in size by two nucleotides, following gaps in the observed size distribution (Fig. 1). Tests for departure from Hardy-Weinberg equilibrium were carried out using an exact test with the Markov-Chain method (Guo & Thompson 1992) in the GENEPOP program (Raymond & Rousset 1995). Tests for heterogeneity in allele frequencies among samples, and sexes, were carried out using the χ^2 pseudo-probability programme CHIRXC (Zaykin & Pudovkin 1993), which uses a randomisation procedure to estimate the significance of the χ^2 test (1000 randomisations were used to estimate probability values). Probability levels were modified by the Bonferroni procedure for multiple tests after Rice (1989). The proportion of variation due to differentiation among samples was estimated with Nei's gene-diversity statistic, G_{ST} (Nei 1973).

Table 2 Allele frequencies at six DNA loci in *Micromesistius australis* from New Zealand and the Falkland Is. Private alleles (in two or more fish in one region) are shown in bold. (–, zero values; * no alleles at that particular locus.)

Locus	Sample	No. of alleles	Allele frequencies															
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
<i>MmerAmp1B</i>	New Zealand	100	0.010	0.020	0.010	0.010	0.200	0.670	0.050	0.030	*	*	*	*	*	*	*	*
	Falkland Is	50	–	0.020	–	0.020	0.140	0.640	0.180	0.020	*	*	*	*	*	*	*	*
<i>MpouBW07</i>	New Zealand	98	0.010	0.082	0.031	0.327	0.112	0.051	0.041	0.224	0.051	0.010	0.041	0.010	0.010	*	*	*
	Falkland Is	50	–	0.040	–	0.360	0.060	0.120	0.060	0.220	0.100	0.040	–	–	–	*	*	*
<i>MpouBW08</i>	New Zealand	100	0.010	–	0.020	0.010	0.020	0.010	0.020	0.020	0.440	0.290	0.070	0.040	0.040	*	*	*
	Falkland Is	50	–	0.020	–	0.040	0.020	0.020	–	0.040	0.340	0.280	0.100	0.040	0.020	0.040	0.040	*
<i>MpouBW09</i>	New Zealand	98	0.031	0.010	0.092	0.245	0.143	0.184	0.092	0.061	0.071	–	0.020	0.010	0.010	0.031	*	*
	Falkland Is	48	–	–	–	0.042	0.333	0.042	0.250	0.145	0.062	0.021	0.042	0.042	0.021	–	*	*
<i>MpouBW13</i>	New Zealand	72	0.014	0.028	0.069	0.097	0.042	0.111	0.139	0.056	0.097	0.083	0.139	0.056	0.042	0.014	0.014	–
	Falkland Is	42	–	0.024	0.024	0.024	0.048	0.095	0.095	0.119	0.238	–	0.166	0.095	–	0.048	–	0.024
<i>MmerUEAW01</i>	New Zealand	92	0.065	0.098	0.098	0.250	0.304	0.120	–	0.011	0.054	*	*	*	*	*	*	*
	Falkland Is	48	–	0.042	0.167	0.062	0.479	0.104	0.104	0.021	0.021	*	*	*	*	*	*	*

Fig. 1 Allele frequencies at the *MpouBW09*, *MmerUEAW01*, and *MpouBW13* loci in samples of *Micromesistius australis* from New Zealand and the Falkland Islands.



RESULTS

The six loci were strongly polymorphic with 5–13 alleles sample⁻¹ and heterozygosities >50% in all but one locus-sample, *MpouBW13*, in New Zealand (Tables 1 and 2). One out of 12 loci, *MpouBW13* in the New Zealand sample, showed a significant

departure from Hardy-Weinberg equilibrium with an excess of homozygotes. Other loci were in Hardy-Weinberg equilibrium when the overall data were tested with a Bonferroni procedure for multiple tests (Table 1).

Pseudo-probability χ^2 tests of homogeneity showed a significant allelic heterogeneity at two of

the six loci and in the overall data (Table 3), and estimates of between sample genetic variance (G_{ST}) revealed heterogeneity at the same two loci, *MpouBW09* and *MmerUEAW01* (Table 3). The overall G_{ST} estimate was 0.018; this is highly significant ($P < 0.001$), but indicates that <2% of the genetic diversity was the result of among population differentiation. All loci showed private alleles, that occurred in two or more fish in one region (Table 2; Fig. 1). The private alleles were restricted to the larger New Zealand sample, and the between population differentiation was only significant for *MpouBW09* and *MmerUEAW01*. There was no significant allele frequency difference between sexes for any locus in the two samples.

DISCUSSION

The question of whether geographically disjunct populations of marine fishes are genetically differentiated is central to understanding the biology of the species and for developing management regulations. The level of differentiation may vary from zero (detected) to the level of full species. The DNA data presented here suggest that New Zealand and Falkland Islands *M. australis* are reproductively isolated conspecific populations, which differ in frequencies of shared alleles at two out of six loci. The same two loci, *MpouBW09* and *MmerUEAW01*, also have private alleles as do the other loci (Table 2), supporting the population differentiation, although these might be the result of sampling error at highly variable markers, and need to be confirmed in larger sample sizes from the South Atlantic. Overall, the DNA data indicate significant but shallow genetic differentiation between the Atlantic and Pacific populations with a G_{ST} of only 0.018

(Table 3), and support Trella's (1999) interpretation of conspecific populations of *M. australis* in the South Atlantic and south-west Pacific Oceans. In the northern sister taxa *M. poutassou*, allozyme markers have shown a similar G_{ST} value (0.01) within the north-east Atlantic Ocean, with independent reproductive units at the latitudinal extremes of the range (Mork & Giaever 1995; Giaever & Stien 1998).

The southern hake *Merluccius australis* has a similar distribution to *Micromesistius australis*, with populations reported from southern Argentina, Chile, and New Zealand (Inada 1981b; Ho 1990). Mitochondrial DNA analyses indicate that the Atlantic and Pacific populations of *Merluccius australis* are one species, but no oceanic differences have been noted (Quinteiro et al. 2000). The disjunct populations show similar values in morphometric and meristic characters (Inada 1981a), and carry the same species of copepod parasite (Kabata & Ho 1981). However, Grant & Leslie (2001) reported significant allele frequency differences at two of 10 allozyme loci, and recognised the South American *M. polylepis* and Australian *M. australis* populations as subspecies, based on their genetic data and on geographic isolation. The relatively small genetic distance implies a recent dispersal of *M. australis* across the southern ocean to New Zealand and Australia (Grant & Leslie 2001). The pattern of inter-ocean genetic differentiation is similar in both *Merluccius* and *Micromesistius*. In the circumpolar toothfish *Dissostichus eleginoides* the greatest difference in microsatellite DNA frequencies was found between samples from the Falkland Islands and sites in the southern Indian and Pacific Oceans (Smith & McVeagh 2000), suggesting genetic isolation among Atlantic and Pacific populations, despite the existence of geographically intermediate populations in the Southern Ocean.

Table 3 Heterogeneity χ^2 tests and genetic diversity (G_{ST}) at six loci in population samples of *Micromesistius australis* from New Zealand and the Falkland Islands. (*, significant at the 5% level with a Bonferroni modified P .)

Locus	χ^2	d.f.	P	G_{ST}	P
<i>MmerAmp1B</i>	18.25	7	0.218	0.011	0.117
<i>MpouBW07</i>	24.04	12	0.505	0.005	0.642
<i>MpouBW08</i>	27.71	14	0.466	0.006	0.567
<i>MpouBW09</i>	76.73	13	<0.001*	0.041	<0.001*
<i>MpouBW13</i>	37.00	15	0.233	0.013	0.196
<i>MmerUEAW01</i>	49.88	8	<0.001*	0.029	0.007*
Combined	41.71	12	<0.001*	0.018	<0.001*

In other fish genera, species level differentiation appears to occur between South Atlantic and south-west Pacific sister taxa, e.g., the New Zealand hoki *Macruronus novaezealandiae* and the Patagonian whiptail hake *M. magellanicus*. The ling *Genypterus blacodes* (Daley et al. 2000) and the macrourid *Coelorinchus kaiyomaru* (Arai & Iwamoto 1979) both occur in the south Atlantic Ocean and the south-west Pacific Ocean, but the relationships among the geographically isolated populations are unknown.

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REFERENCES

- Annala, J. H.; Sullivan, K. J.; O'Brien, C. J.; Smith, N. W. 2001: Report from the Fishery Assessment Plenary, May 2001: stock assessments and yield estimates. Unpublished report held in NIWA Library, Wellington, New Zealand. 515 p.
- Arai, T.; Iwamoto, T. 1979: A new species of the macrourid fish genus *Coelorinchus* from off Tasmania, New Zealand and the Falkland Islands. *Japanese Journal of Ichthyology* 26: 238–246.
- Daley, R. K.; Ward, R. D.; Last, P. R.; Reilly, A.; Appleyard, S. A.; Gledhill, D. C. 2000: Stock delineation of the pink ling (*Genypterus blacodes*) in Australian waters using genetic and morphometric techniques. Fisheries Research and Development Corporation, Australia, Project 97/117.
- Giaever, M.; Stien, J. 1998: Population genetic substructure in blue whiting based on allozyme data. *Journal of Fish Biology* 52: 782–795.
- Grant, W. S.; Leslie, R. W. 2001: Inter-ocean dispersal is an important mechanism in the zoogeography of hakes (Pisces: *Merluccius* spp.). *Journal of Biogeography* 28: 699–722.
- Guo, S. W.; Thompson, E. A. 1992: Performing the exact test of Hardy-Weinberg proportions for multiple alleles. *Biometrics* 48: 361–372.
- Hanchet, S. 1999: Stock structure of southern blue whiting *Micromesistius australis* in New Zealand waters. *New Zealand Journal of Marine and Freshwater Research* 33: 599–609.
- Ho, J. S. 1990: Phylogeny and biogeography of hakes (*Merluccius*; Teleostei): a cladistic analysis. *Fishery Bulletin* 88: 95–104.
- Inada, T.; Nakamura, I. 1975: A comparative study of two populations of the gadoid fish *Micromesistius australis* from the New Zealand and Patagonian–Falkland regions. *Bulletin Far Sea Fisheries Research Laboratory* 13: 1–26.
- Inada, T. 1981a: Two nominal species of *Merluccius* from New Zealand and southern South America. *Japanese Journal of Ichthyology* 28: 31–36.
- Inada, T. 1981b: Studies on the Merlucciid fishes. *Bulletin Far Sea Fisheries Research Laboratory* 18: 1–172.
- Kabata, Z.; Ho, J. S. 1981: The origin and dispersal of hake (genus *Merluccius*; Pisces: Teleostei) as indicated by its copepod parasites. *Oceanography and Marine Biology Annual Review* 19: 381–404.
- McGregor, D.; Galvin, P.; Sadusky, T.; Cross, T. 1996: PCR amplification of a polymorphic minisatellite VNTR locus in whiting (*Merlangius merlangius* L.). *Animal Genetics* 27: 49–51.
- Mattiangeli, V.; Galvin, P. T.; Ryan, A. W.; Mork, J.; Cross, T. F. 2002: VNTR variability in Atlantic poor cod (*Trisopterus minutus minutus*) throughout its range: single locus minisatellite data suggest reproductive isolation for the Faroe Bank. *Fisheries Research*: in press.
- Moran, P.; Ryan, A. W.; Rico, C.; Hewitt, G. M. 1999: Four microsatellite loci in the gadoid fish, blue whiting *Micromesistius poutassou* (Risso 1826). *Animal Genetics* 30: 463–464.
- Mork, J.; Giaever, M. 1995: Genetic variation at isozyme loci in blue whiting from the north-east Atlantic. *Journal of Fish Biology* 46: 462–468.
- Nei, M. 1973: Analysis of gene diversity in subdivided populations. *Proceedings of the National Academy of Sciences USA* 70: 3321–3323.
- Quinteiro, J.; Vidal, R.; Rey-Mendez, M. 2000: Phylogeny and biogeographic history of hake (genus *Merluccius*) inferred from mitochondrial DNA control-region sequences. *Marine Biology* 136: 63–174.
- Raymond, M.; Rousset, F. 1995: GENEPOP (version 2.1): population genetic software for exact tests and ecumenicism. *Journal of Heredity* 86: 248–249.
- Rice, W. R. 1989: Analyzing tables of statistical tests. *Evolution* 43: 223–225.

- Rico, C.; Ibrahim, K. M.; Rico, I.; Hewitt, G. M. 1997: Stock composition in North Atlantic populations of whiting using microsatellite markers. *Journal of Fish Biology* 51: 462–475.
- Sambrook, J.; Fritsch, E. F.; Maniatis, T. 1989: Molecular cloning: a laboratory manual. 2nd ed. New York, Cold Spring Harbour Laboratory Press.
- Shust, K. V. 1978: On the distribution and biology of members of the genus *Micromesistius* (family Gadidae). *Journal of Ichthyology* 18: 490–493.
- Smith, P. J.; McVeagh, S. M. 2000: Allozyme and microsatellite DNA markers of toothfish *Dissostichus eleginoides* population structure in the Southern Ocean. *Journal of Fish Biology* 57: 72–83.
- Trella, K. 1999: A comparative study of populations of southern blue whiting (*Micromesistius australis* Norman, 1937) from the Falkland and New Zealand fishing grounds using selected taxonomic characters. *Bulletin of the Sea Fisheries Institute Gdynia* 147: 37–50.
- Wieczaszek, B. 1988: Morphometry of southern blue whiting *Micromesistius australis* (Norman, 1937)—from the region of Burwood bank. *Acta Ichthyologica et Piscatoria* 18: 3–18.
- Wohler, O. C. 1999: Stock assessment of the main austral demersal fish resources of Argentina: southern blue whiting (*Micromesistius australis*) and long tail hake (*Macruronus magellanicus*). In: *Avances en Métodos y Tecnología Aplicados a la Investigación Pesquera. Seminario final del Proyecto INIDEP–JICA sobre Evaluación y Monitoreo de Recursos Pesqueros 1994–1999*. Mar del Plata, Argentina, Instituto Nacional de Investigación y Desarrollo Pesquero (INIDEP). Pp. 177–180.
- Zaykin, D. V.; Pudovkin, A. I. 1993: Two programs to estimate significance of χ^2 values using pseudo-probability tests. *Journal of Heredity* 84: 152–153.

