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Molecular analysis of Southern Ocean skates (*Bathyraja*) reveals a new species of Antarctic skate

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Two regions of mtDNA, cytochrome *b* and cytochrome *c* oxidase subunit 1, were sequenced in nine species of *Bathyraja* from the Southern Ocean and New Zealand. Based on sequence divergence, the species that has been referred to as *Bathyraja eatonii* from the Antarctic continental shelf and slope is a species distinct from *B. eatonii* from the Kerguelen Plateau (the type locality) and is a new and undescribed species *Bathyraja* sp. (*cf. eatonii*). There was no sequence divergence among samples of *Bathyraja* sp. (dwarf) from the Ross Sea and the South Atlantic. However, for both *Bathyraja* sp. (*cf. eatonii*) and *Bathyraja maccaini* in the Ross Sea and the South Atlantic Ocean, the DNA sequence divergences indicate differentiation among ocean basins and within *Bathyraja* sp. (*cf. eatonii*) divergences are similar to those among recognized species of *Bathyraja* in the North Pacific Ocean.

Key words: cytochrome *b*; cytochrome *c* oxidase 1; marine fish; mtDNA.

INTRODUCTION

In Antarctic waters, skates (Rajiidae) are the dominant component of the chondrichthyan fauna (Long, 1994) but have not been well described in part because of the limited number of specimens available for scientific study, making identification difficult for fisheries observers and taxonomists alike (Stehmann & Bürkel, 1990). The development of the toothfish (*Dissostichus eleginoides* Smitt and *Dissostichus mawsoni* Norman) fisheries, and subsequent observer collecting programmes, has provided more skate specimens, leading to better descriptions of the Antarctic chondrichthyan fauna. For example, an unknown species of

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Amblyraja, previously classified as the starry skate *Amblyraja georgiana* (Norman), was recently recognized (but not formally named) from the toothfish (*D. eleginoides*) fishery around South Georgia (Endicott *et al.*, 2002).

Six species of *Bathyraja* have been described from Antarctic and sub-Antarctic waters where they represent the dominant rajid group (Stehmann & Bürkel, 1990), *Bathyraja eatonii* (Günther); *Bathyraja maccaini* Springer; *Bathyraja meridionalis* Stehmann, 1987; *Bathyraja murrayi* (Günther); *Bathyraja irrasa* Hureau & Ozouf-Costaz and *Bathyraja* n. sp., referred to hereafter as *Bathyraja* sp. (dwarf) of Stehmann & Bürkel (1990: 88). Three of these species appear to have restricted distributions, *B. murrayi* around the Kerguelen and Herd Islands, *B. irrasa* on the Kerguelen Plateau and *B. meridionalis* in the South Atlantic Ocean, while the other three appear to be widely distributed, *B. eatonii* on the Kerguelen Plateau (type locality) and in the South Atlantic Ocean and Ross Sea, *B. maccaini* in the South Atlantic and Pacific Oceans and *Bathyraja* sp. (dwarf) in the Atlantic Ocean and Ross Sea. For the three widely distributed species, the intraspecific relationships among regional populations are unknown.

A dominant feature in the Southern Ocean is the Antarctic Circumpolar Current (ACC), which forms a physical barrier to the northward dispersal of Antarctic species and acts as a west-east transport system (Eastman, 1993; DeVries & Steffensen, 2005). Much of the Southern Ocean lies between 3000 and 5000 m in depth with isolated plateaus, ridges, banks and islands; the continental shelf area is relatively narrow except for the large embayments in the Ross and the Weddell Seas, which are characterized by clockwise gyres. The physical heterogeneity, when considered with the age of the bathyal habitats, may have promoted evolutionary processes in this unique environment (Moller *et al.*, 2005). Habitat fragmentation can restrict dispersal among populations, limit gene flow and lead to allopatric speciation (Avise, 2000). Skates are absent from deep ocean basins (Long, 1994, and species of *Bathyraja* may consist of one genetic population with gene flow *via* the ACC or isolated populations contained within gyres in the Ross and the Weddell Seas and to the east of the Kerguelen Plateau (Orsi *et al.*, 1995).

Molecular techniques are increasingly being used to resolve taxonomic-population relationships in fishes. Studies employing DNA markers have reported restricted gene flow in some Antarctic fishes (Smith & Gaffney, 2000, 2005; Smith & McVeagh, 2000; Appleyard *et al.*, 2002; Parker *et al.*, 2002; Zane *et al.*, 2006) and even cryptic species in the squid *Martialia hyadesi* Rochebrune & Mabille (Brierley *et al.*, 1993). There have been few molecular phylogenetic studies of skates and rays in general (Rasmussen & Arnasson, 1999; Sezaki *et al.*, 1999; Valsecchi *et al.*, 2005). A recent study of cytochrome *c* oxidase subunit I (COI) variability among North Pacific *Bathyraja* found shallow divergences (average 0·036) among 10 of 12 species (Spies *et al.*, 2006). The COI marker has also been used to identify juvenile skates in the North Atlantic Ocean (Alvarado Bremer *et al.*, 2005), while partial sequences of part of cytochrome *b* (Cyt *b*) have been used in population studies of North Atlantic skates (Chevolot *et al.*, 2006; Chevolot *et al.*, 2007). Here, two regions of mtDNA (COI and Cyt *b*) are used to determine relationships among Antarctic *Bathyraja* and to provide evidence for a new species.

MATERIALS AND METHODS

TISSUE COLLECTION

Muscle tissue samples were taken from frozen Antarctic skate specimens (Fig. 1), which were preserved and registered in the National Fish Collection (NFC) at the Museum of New Zealand Te Papa Tongarewa: *B. maccaini* from the Ross Sea and the South Atlantic Ocean; *Bathyraja* sp. (dwarf) from the Ross Sea and South Atlantic Ocean; *B. eatonii* from the Ross Sea and South Atlantic Ocean, and hereafter referred to as *Bathyraja* sp. (*cf. eatonii*); and *B. meridionalis* from the South Atlantic Ocean (Fig. 1 and Table I). Tissue samples from the underside of the right fin were taken from freshly thawed specimens prior to specimen fixation in formalin, stored in 90% ethanol and cross-referenced to the unique registration number of the whole specimen in the NFC. Muscle tissue samples were collected by fishery observers at sea from *B. murrayi*, *B. irrasa* and *B. eatonii* [distinguished from *Bathyraja* sp. (*cf. eatonii*) below] on the Kerguelen Plateau and *B. maccaini* and *Bathyraja* sp. (*cf. eatonii*) in the Cooperation Sea (Table I) and stored frozen at −20° C. Additional tissue samples from *Bathyraja* caught in the New Zealand Exclusive Economic Zone (EEZ) were selected for DNA analyses

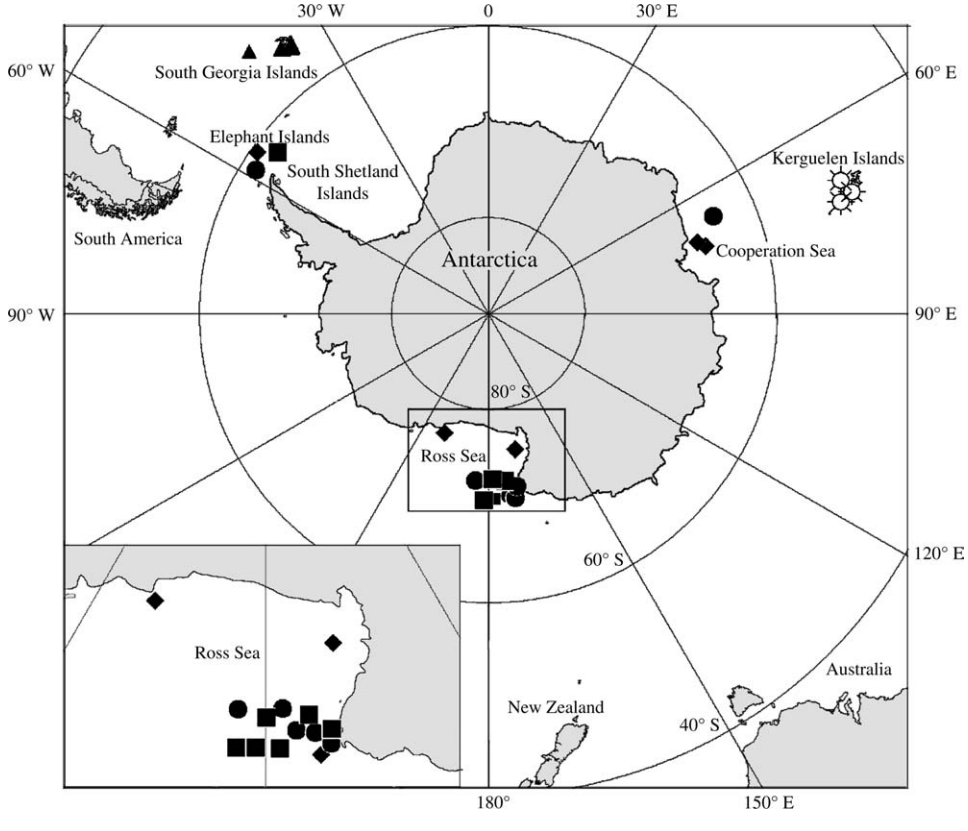


FIG. 1. Capture locations of Antarctic skates. *Bathyraja eatonii* ☼; *Bathyraja* sp. (*cf. eatonii*) ●; *Bathyraja maccaini* ◆; *Bathyraja meridionalis* ▲; *Bathyraja* n. sp. (dwarf) ■. *Bathyraja irrasa* and *Bathyraja murrayi* were taken around the Kerguelen Islands. *Bathyraja shuntovi*, *Bathyraja richardsoni* and *Bathyraja* sp. (blond) were taken around southern New Zealand. Inset: Ross Sea capture locations.

TABLE I. Summary data on Southern Ocean *Bathyraja* spp. used for DNA analyses

Species	Location	Size range L_T (mm)	N sequenced
Bathyraja sp. (*cf. eatonii*)	Ross Sea	561–1129	10
Bathyraja sp. (*cf. eatonii*)	Atlantic	245–637	7
Bathyraja sp. (*cf. eatonii*)	Cooperation Sea	737	1
B. eatonii	Kerguelen (L)	782–871*	5
B. irrasa	Kerguelen (L)	1010–1420	3
B. murrayi	Kerguelen (L)	364–453	4
B. maccaini	Ross Sea	463–882	10
B. maccaini	Cooperation Sea	502–710	5
B. maccaini	South Atlantic (L)	282–586	3
B. meridionalis	South Atlantic (L)	629–1410	7
Bathyraja sp. (dwarf)	Ross Sea	449–564	10
Bathyraja sp. (dwarf)	South Atlantic	494–548	6
Bathyraja sp. (blond)	NZ EEZ	1040–1070	2
B. richardsoni	NZ EEZ	1567	1
B. shuntovi	NZ EEZ	912–1087	2

L_T, total length; L, type locality of species; NZ EEZ, New Zealand exclusive economic zone.
*Not all specimens measured.

(Table I) to provide further estimates of sequence divergence among southern hemisphere *Bathyraja* spp.: *Bathyraja shuntovi* Dolganov, 1985, from the type locality Lord Howe Rise; *Bathyraja richardsoni* (Garrick), from the type locality Cook Strait and *Bathyraja* sp. (blond) from the east Campbell Plateau.

DNA ANALYSES

Total genomic DNA was extracted from small pieces (200–500 mg) of white muscle following standard techniques. Two regions of the mitochondrial genome were amplified using the polymerase chain reaction (PCR) in 50 μl volumes in a Cetus 9600 DNA thermo-cycler (Perkin-Elmer Corporation, Waltham, MA, U.S.A.). The primer pair Cyb 2 and tGludg (Palumbi *et al.*, 1991), which amplify *c.* 400 base pair region of the Cyt *b* gene in fishes (Baker *et al.*, 1995) and the *Raja*-specific COI primers COI_RajaF and COI_RajaR (Spies *et al.*, 2006) were used with all samples. Both the Cyt *b* and the COI regions of mtDNA have been used as markers to identify and distinguish intra-generic skate species: Cyt *b* for *Leucoraja* spp. (Alvarado Bremer *et al.*, 2005) and COI for *Bathyraja* spp. and *Raja* spp. (Spies *et al.*, 2006). In this latter example, 10 of 12 North Pacific *Bathyraja* spp. had unique COI sequences.

Amplifications for Cyt *b* were carried out using an initial denaturation of 94° C for 2 min; 34 cycles of 92° C for 60 s, 54° C for 60 s and 72° C for 90 s, followed by an extension at 72° C for 8 min. Amplifications for COI were carried out using an initial denaturation of 94° C for 1 min; 35 cycles of 94° C for 60 s, 57° C for 90 s and 72° C for 60 s, followed by an extension at 72° C for 5 min. PCR products were purified using the QIAquick gel extraction kit (Qiagen, Valencia, CA, U.S.A.). Sequences were determined using the ABI Taq DyeDeoxy™ Terminator Cycle Sequencing kit according to the manufacture's directions (Applied Biosystems Inc., Foster City, CA, U.S.A.) and run on an ABI prism autosequencer.

DNA sequences were edited in CHROMAS (Technelysium, Queensland, Australia), aligned in CLUSTAL in MEGA 3 (Kumar *et al.*, 2004) and stored in BIOEDIT (Hall,

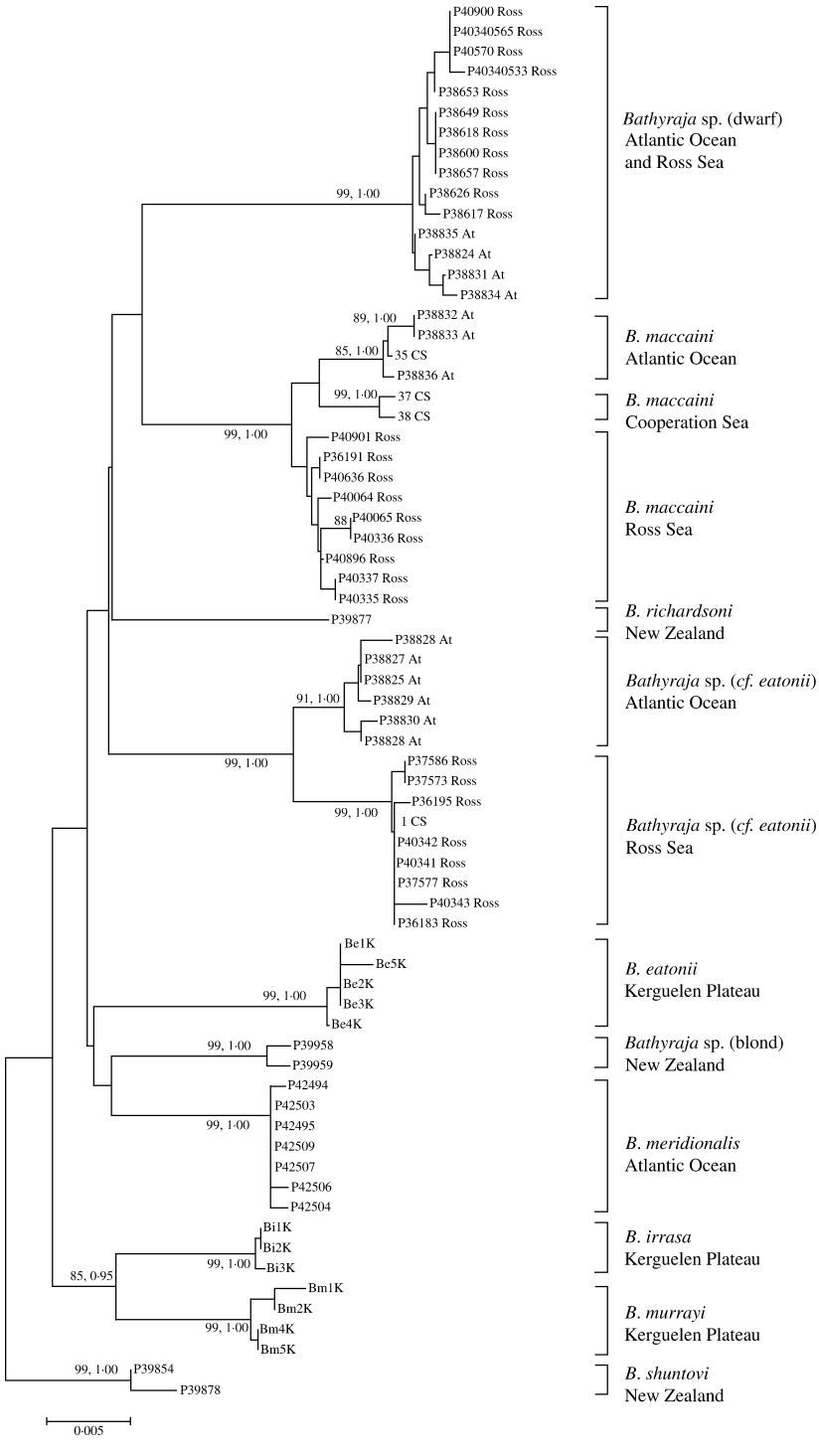
1999). Phylogenetic congruence of the two data sets was tested with a partition homogeneity test (Farris *et al.*, 1994). Five hundred partition replicates were analysed under maximum parsimony using heuristic searches in PAUP 4.0b10 (Swofford, 2003). Phylogenies were explored with neighbour-joining (NJ) and maximum likelihood (ML) methods using MEGA 3.1 and PAUP (Swofford, 2003). MODELTEST 3.7 (Posada & Crandall, 1988) was used to determine the best-fit model using likelihood ratio tests for distance and ML methods, and the HKY+I+G model (Hasegawa *et al.*, 1985) was selected for the pooled Cyt *b* and COI sequence data set. Support for each internode was evaluated by bootstrap replications (Felsenstein, 1985) with 1000 pseudoreplicates for NJ but not for ML trees because of large computational requirements of ML bootstrap replications. Bayesian phylogenetic trees were estimated with MRBAYES 3.0 (Huelsenbeck & Ronquist, 2001) for the pooled Cyt *b*–COI sequence data set, using the codon model established above. Four simultaneous Monte Carlo chains were run for 1×10^6 generations, saving the current tree every 100 generations. A consensus tree with posterior probabilities was created with a burn-in value equal to 5000 (the first 5000 trees were discarded). The consensus phylogram of the remaining trees was viewed in TREEVIEW (Page, 1996). The pooled Cyt *b* and COI trees were rooted with *B. shuntovi*. The Cyt *b* sequences were deposited in GenBank accession numbers (EU070939–071005) and the COI sequences in the Barcode Of Life Database (accession numbers: FNZC001-07 to FNZC072-07).

RESULTS

Unambiguously aligned sequences were obtained for 392 bp of Cyt *b* sequence from 76 tissue samples of *Bathyraja* spp. Sixty-three nucleotide sites were variable and 57 were parsimoniously informative in the total data set. Most substitutions occurred in the third nucleotide position within codons (76%). There were 16 amino acid substitutions of which 14 were phylogenetically informative. Unambiguously aligned sequences were obtained for 691 bp of COI sequence from the same 76 tissue samples of *Bathyraja* spp. Sixty nucleotide sites were variable and 52 were parsimoniously informative in the total data set. The majority of substitutions occurred in the third nucleotide position within codons (96%). There were only four amino acid substitutions of which two were phylogenetically informative (*Bathyraja* n. sp. amino acid substitution F > L, base positions 172–173 and *B. maccaini*, *B. shuntovi* and *B. richardsoni* amino acid substitution V > M, base positions 4–6).

Partition homogeneity tests did not allow rejection of phylogenetic congruence ($P = 0{\cdot}064$), justifying the combination of the Cyt *b* and COI sequence data sets. One hundred and twenty-five nucleotide positions were variable, and 110 were parsimoniously informative in the total 1083 bp data set. All three phylogenetic analyses (NJ, ML and Bayesian) produced trees with similar topologies supported by high bootstrap values (NJ only) and high posterior probabilities (Fig. 2).

Three recognized species, for which specimens were each sampled from one geographic region, *B. irrasa* (Kerguelen Plateau), *B. murrayi* (Kerguelen Plateau) and *B. meridionalis* (South Georgia), appeared as well-supported clades (Fig. 2) with moderate to high relative sequence divergences (Table II). Likewise, the three species sampled in the New Zealand EEZ, *B. richardsoni*, *B. shuntovi* and *Bathyraja* sp. (blond), appeared as well-supported clades (Fig. 2) with moderate to high relative sequence divergences (Table II, sequences for the single tissue sample from *B. richardsoni* were excluded from Table II).


P40900 Ross
P40340565 Ross
P40570 Ross
P40340533 Ross
P38653 Ross
P38649 Ross
P38618 Ross
P38600 Ross
P38657 Ross
99, 1·00
P38626 Ross
P38617 Ross
P38835 At
P38824 At
P38831 At
P38834 At
Bathyraja sp. (dwarf)
Atlantic Ocean
and Ross Sea
89, 1·00
P38832 At
P38833 At
85, 1·00
35 CS
P38836 At
B. maccaini
Atlantic Ocean
99, 1·00
37 CS
38 CS
B. maccaini
Cooperation Sea
99, 1·00
P40901 Ross
P36191 Ross
P40636 Ross
P40064 Ross
88
P40065 Ross
P40336 Ross
P40896 Ross
P40337 Ross
P40335 Ross
B. maccaini
Ross Sea
P39877
B. richardsoni
New Zealand
P38828 At
P38827 At
P38825 At
91, 1·00
P38829 At
P38830 At
P38828 At
Bathyraja sp. (cf. eatonii)
Atlantic Ocean
99, 1·00
P37586 Ross
P37573 Ross
P36195 Ross
99, 1·00
1 CS
P40342 Ross
P40341 Ross
P37577 Ross
P40343 Ross
P36183 Ross
Bathyraja sp. (cf. eatonii)
Ross Sea
Be1K
Be5K
99, 1·00
Be2K
Be3K
Be4K
B. eatonii
Kerguelen Plateau
99, 1·00
P39958
P39959
Bathyraja sp. (blond)
New Zealand
P42494
P42503
99, 1·00
P42495
P42509
P42507
P42506
P42504
B. meridionalis
Atlantic Ocean
Bi1K
Bi2K
99, 1·00
Bi3K
B. irrasa
Kerguelen Plateau
85, 0·95
Bm1K
Bm2K
99, 1·00
Bm4K
Bm5K
B. murrayi
Kerguelen Plateau
99, 1·00
P39854
P39878
B. shuntovi
New Zealand
0·005

Excluding *Bathyraja* sp. (*cf. eatonii*), the Cyt *b* sequence divergences among species ranged from 0·025 to 0·057 and COI from 0·014 to 0·028 (Table II). Specimens of *Bathyraja* sp. (dwarf) from the Ross Sea and the South Atlantic Ocean had a shallow net divergence (Cyt *b* and COI = 0·002; Table II), as did specimens of *B. maccaini* from the Cooperation Sea, Ross Sea and South Atlantic Ocean (Cyt *b* = 0·003–0·006; COI = 0·002–0·009; Table II). The tissue sample from one specimen of *B. maccaini* from the Cooperation Sea captured at 61° E clustered with the Atlantic specimens, while tissue samples from four other specimens captured at 72° E formed a discrete cluster (Fig. 2).

There was a relatively moderate divergence among the South Atlantic Ocean and Ross Sea samples of *Bathyraja* sp. (*cf. eatonii*) for Cyt *b* (0·017) and low COI divergence (0·005; Table II), but a high divergence among *Bathyraja* sp. (*cf. eatonii*) for the South Atlantic Ocean–Ross Sea and *B. eatonii* from the Kerguelen Plateau (Cyt *b* = 0·050 and 0·057, COI = 0·016 and 0·019; Table II).

Among the *B. eatonii* and *Bathyraja* sp. (*cf. eatonii*) specimens sequenced for Cyt *b*, 29/392 bases were variable and 25/392 were phylogenetically informative. Among the South Atlantic Ocean and Ross Sea *Bathyraja* sp. (*cf. eatonii*), only 11/392 bases were variable and 9/392 were phylogenetically informative, leading to 5/130 variable amino acids and 4/130 phylogenetically informative amino acids. There were three amino acid substitutions (A > G, base positions 25–27; M > I, base positions 34–36 and V > I, base positions 220–222) among the South Atlantic Ocean and Ross Sea *Bathyraja* sp. (*cf. eatonii*).

Among the *B. eatonii* and *Bathyraja* sp. (*cf. eatonii*) specimens sequenced for COI, 25/691 bases were variable and 15/691 were phylogenetically informative. Among the South Atlantic Ocean and Ross Sea *Bathyraja* sp. (*cf. eatonii*), 20/691 COI bases were variable and 5/691 were phylogenetically informative, leading to 2/230 variable amino acids, but 0/230 phylogenetically informative amino acids. The shallow divergences and lack of fossil specimens precluded a realistic estimate of the time of divergence among the *Bathyraja* spp.

DISCUSSION

Skates are unique among the Chondrichthyes and noted for their high species diversity but morphological conservatism (McEachran & Dunn, 1998). Certainly, *Bathyraja* in the Southern Ocean are morphologically similar, and it is possible that the generalist morphologies have been advantageous in the cold and deep benthic environment (Long, 1994). The limited morphological divergence among *Bathyraja* spp. in Antarctic waters has made identification difficult for field workers and taxonomists alike (Stehmann & Bürkel, 1990).

FIG. 2. Phylogenetic relationships of *Bathyraja* spp. specimens for combined cytochrome *b* and COI sequences. The scale bar represents an interval of Hasegawa's genetic distance (Hasegawa *et al.*, 1985). Numbers at nodes are bootstrap percentages (>85%) based on distance (after 1000 replicates) and Bayesian inference posterior probability values (>0·90). The tree has been rooted with the temperate Tasman Sea–New Zealand *Bathyraja shuntovi*. P numbers are National Fish Collection (Te Papa) specimen registration numbers; all other sequences were from unregistered tissue samples held at National Institute of Water & Atmospheric Research Ltd. At, South Atlantic Ocean; CS, Cooperation Sea; Ross, Ross Sea.

TABLE II. Net sequence divergence for mtDNA cytochrome *b* (below diagonal) and COI (above diagonal) among species and, or populations of *Bathyraja* spp.

	BmC	BmR	BmA	BeA	BeR	BeK	BnA	BnR	Bmer	Bir	Bmu	Bsh	Bbl
BmC		**0·005**	**0·002**	0·026	0·029	0·023	0·017	0·018	0·018	0·024	0·021	0·021	0·023
BmR	**0·006**		**0·009**	0·025	0·028	0·024	0·016	0·018	0·018	0·024	0·020	0·024	0·020
BmA	**0·003**	**0·005**		0·031	0·034	0·027	0·022	0·024	0·023	0·026	0·026	0·027	0·028
BeA	0·030	0·028	0·030		**0·005**	0·016	0·024	0·025	0·019	0·022	0·019	0·022	0·024
BeR	0·034	0·029	0·032	0·017		0·019	0·027	0·028	0·022	0·025	0·022	0·025	0·027
BeK	0·055	0·053	0·043	0·050	0·057		0·022	0·021	0·018	0·021	0·016	0·022	0·022
BnA	0·051	0·048	0·049	0·050	0·052	0·058		**0·002**	0·019	0·025	0·022	0·021	0·023
BnR	0·053	0·045	0·052	0·052	0·053	0·059	**0·002**		0·021	0·027	0·023	0·022	0·025
Bmer	0·037	0·035	0·036	0·041	0·045	0·038	0·052	0·053		0·024	0·020	0·021	0·016
Bir	0·044	0·043	0·044	0·043	0·042	0·043	0·052	0·054	0·033		0·012	0·018	0·021
Bmu	0·054	0·052	0·053	0·053	0·051	0·043	0·055	0·057	0·034	0·025		0·014	0·017
Bsh	0·043	0·041	0·042	0·040	0·045	0·043	0·053	0·054	0·037	0·031	0·039		0·019
Bbl	0·031	0·030	0·031	0·027	0·037	0·030	0·044	0·045	0·023	0·025	0·032	0·029	

BmC, *B. maccaini* Cooperation Sea; BmR, *B. maccaini* Ross Sea; BmA, *B. maccaini* S. Atlantic Ocean; BeA, *Bathyraja* sp. (*cf. eatonii*) S. Atlantic Ocean; BeR, *Bathyraja* sp. (*cf. eatonii*) Ross Sea; BeK, *B. eatonii* Kerguelen; BnA, *Bathyraja* sp. (dwarf) S. Atlantic Ocean; BnR, *Bathyraja* sp. (dwarf) Ross Sea; Bmer, *B. meridionalis* S. Atlantic Ocean; Bir, *B. irrasa* Kerguelen; Bmu, *B. murrayi* Kerguelen; Bsh, *B. shuntovi* New Zealand EEZ; Bbl, *Bathyraja* sp. (blond) NZ EEZ. Note the shallow divergences (<0·01) among BmC/BmA/BmR and BnA/BnR, and BeA/BeR shown in bold.

Based on the sequence divergences observed among the *Bathyraja* spp. (Table II and Fig. 2), the large sequence divergences among *B. eatonii* from the Kerguelen Plateau and from the South Atlantic–Ross Sea provide evidence for two species in these regions. The species, referred to as *B. eatonii* from the Antarctic continental shelf and slope, is a species distinct from *B. eatonii* from the Kerguelen Plateau (the type locality). Therefore, it is a new and undescribed species and should be referred to as *Bathyraja* sp. (*cf. eatonii*), until it is formally described and named. Preliminary biological data indicate a difference in length–mass relationships between the two species (Francis, 2006), but a formal taxonomic description awaits examination of the Kerguelen type specimens of *B. eatonii* held in the Muséum National d'Histoire Naturelle, Paris (pers. comm.).

The COI sequence divergences observed among the *Bathyraja* spp. were low relative to values reported among other chordates (Hebert *et al.*, 2003) but appear typical of other Chondrichthyes, including skates. A recent report has shown similar low sequence divergences among 12 *Bathyraja* species in the North Pacific with COI divergences ranging from 0·005 to 0·049, average 0·036 (Spies *et al.*, 2006). Rates of mtDNA evolution may be lower in skates than in other chordates; nucleotide substitution rates in the Cyt *b* and COI genes in sharks are seven- to eight-fold lower than in primates or ungulates (Martin *et al.*, 1992; Martin & Palumbi, 1993), and nucleotide substitution rates in Cyt *b* in poikilothermic vertebrates are several-fold lower than rates in mammals and birds (Johns & Avise, 1998).

The species identified from DNA sequences in the Ross Sea comprise *B. maccaini*, *Bathyraja* sp. (dwarf) and *Bathyraja* sp. (*cf. eatonii*), and all three were sampled and sequenced from two or more areas: *B. maccaini* in the Cooperation Sea, Ross Sea and South Atlantic Ocean; *Bathyraja* sp. (*cf. eatonii*) and *Bathyraja* sp. (dwarf) in the Ross Sea and South Atlantic Ocean. For *Bathyraja* sp. (dwarf), the low sequence divergences and single clade (Fig. 2) provided no evidence for significant divergence among the South Atlantic and Ross Sea populations.

In contrast, there was a significant divergence among specimens of *Bathyraja* sp. (*cf. eatonii*) from the Ross Sea and South Atlantic Ocean, with two well-supported clades (Fig. 2), that might be indicative of two cryptic species isolated in these Ocean basins. There were six fixed nucleotide differences and three amino acid substitutions among the Ross Sea and the South Atlantic Ocean specimens for Cyt *b* and four fixed nucleotide differences (sequence divergence 0·017) but no amino acid substitutions for COI (sequence divergence 0·005). The sequence from the one specimen collected in the Cooperation Sea clustered with specimens from the Ross Sea (Fig. 2) and not with specimens from the geographically closer Kerguelen Plateau (Fig. 1), which may be isolated by the sub-Antarctic Convergence and a deep ocean basin (3500–4000 m). Skates in general are absent from deep ocean basins (Long, 1994), which may act as barriers to gene flow and promote regional speciation among isolated plateaus, although *Bathyraja* spp. are known to extend to *c.* 3000 m (Last & Yearsley, 2002). Two morphologically recognized species in the North Pacific Ocean, *Bathyraja lindbergi* Ishiyama & Ishihara and *Bathyraja maculata* Ishiyama & Ishihara, had an equally low COI sequence divergence of 0·005, with no fixed nucleotide differences, while two other morphologically recognized species: *Bathyraja*

abyssicola (Gilbert) and *Bathyraja aleutica* (Gilbert) had a low COI sequence divergence (0·008) with four fixed nucleotide differences between the two species (Spies *et al.*, 2006). Analysis of Cyt *b* sequences in the thornback ray *Raja clavata* L. revealed a nucleotide divergence of 0·006 among European and Mediterranean populations (Chevolot *et al.*, 2006). A similar study of Cyt *b* sequences in the thorny ray *Amblyraja radiata* (Donovan) showed no significant genetic differentiation among samples from Newfoundland, Iceland and the North Sea (Chevolot *et al.*, 2007).

The results for *B. maccaini* appear more complex, with three well-supported clades in the pooled Cyt *b*–COI tree (Fig. 2): Ross Sea; Atlantic Ocean, including one specimen of *B. maccaini* from the Cooperation Sea, captured at 61° E and four specimens from the Cooperation Sea, captured at 72° E. However, the sequence divergences among the three areas are shallow, even when re-calculated based on clustering rather than location. Grouping one specimen from the Cooperation Sea (at 61° E) with the South Atlantic Ocean group, the Cooperation Sea–Atlantic divergences increased (Cyt *b* from 0·003 to 0·011 and COI from 0·002 to 0·007). For Cyt *b*, there were 13 variable and eight phylogenetically informative nucleotide substitutions (5/8 were third nucleotide positions within codons), but only three were geographically consistent. For COI, there were 10 variable and nine phylogenetically informative nucleotide substitutions (7/9 were third nucleotide positions within codons), but only one resulted in an amino acid change (V > M, base positions 4–6; Ross Sea = V, Atlantic Ocean and Cooperation Sea = M).

Unlike many teleosts, skates appear to be weak dispersers. Skates have internal fertilization, and the lack of a pelagic egg and juvenile stage restricts juvenile dispersal potential. Tagging experiments with adult skates in the North Atlantic Ocean indicate limited dispersal <50 nautical miles in the North Sea (Walker *et al.*, 1997; Buckley & Metcalfe, 2002) and typically <60 nautical miles in the northwest Atlantic Ocean (Templeman, 1984), while preliminary tagging results for *A. georgiana* in the Ross Sea found dispersal up to 74 km over 733 days (Francis & Smith, 2002). The two major subpolar cyclonic circulations, the Weddell Sea gyre and the Ross Sea gyre, and a smaller gyre to the east of the Kerguelen Plateau (Orsi *et al.*, 1995) may isolate skates in ocean basins, separated by areas of deepwater (Long, 1994). Chevolot *et al.* (2007) noted that in the North Atlantic Ocean, the continental shelf edge appears to act as a barrier to migration in *R. clavata* but not *A. radiata* inspite of the two species having similar life histories and overlapping distributions.

The taxonomic status of the populations or species of *Bathyraja* sp. (*cf. eatonii*) and of *B. maccaini* in the Ross Sea and the South Atlantic Ocean will be resolved only with the collection of additional specimens from these sea areas and geographically intermediate areas, as opportunities arise. For *Bathyraja* sp. (*cf. eatonii*), the observed COI sequence divergences, although shallow, are typical of *Bathyraja* species in the North Pacific Ocean (Spies *et al.*, 2006), while the Cyt *b* divergences are greater than those reported among Atlantic and Mediterranean populations of *R. clavata* (Chevolot *et al.*, 2006) and among eastern and western Atlantic populations of *A. radiata* (Chevolot *et al.*, 2007) and are indicative of major sub-division among ocean basins. These results have implications for the conservation and management of skates in the Southern Ocean.

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