A review of biopsy sampling experiments and studies of stock structure, phylogeny and taxonomy of large whales based on samples obtained on SOWER cruises

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ABSTRACT

A summary is presented of biopsy sampling conducted during the SOWER programme between 1978/79–2009/10, involving 32 Antarctic cruises and three low latitude cruises, and studies of stock structure, phylogeny and taxonomy of large whales based on the genetic data obtained. While sampling began during the 1988/89 Antarctic cruise, sampling systems have since been developed and improved. The greatest numbers of biopsy samples obtained came from humpback and blue whales. Genetic analysis of these samples has contributed to our understanding of humpback whale stock structure in feeding grounds and the connection between whales in low-latitude breeding grounds and high-latitude feeding grounds. Genetic analysis of blue whale samples from the Antarctic and off Australia, Madagascar and Chile has contributed to our understanding of population genetic structure of pygmy blue whale stocks and between pygmy and Antarctic blue whales. While a relatively large number of samples were obtained from southern right whales, mainly in Area IV, genetic data have yet to be generated. The numbers obtained from other species, such as fin, sei, Bryde's, Antarctic minke and sperm whales, were small. However, in some cases, they have been used to complement population genetic studies based on larger sample sizes from other sources. The samples are an important legacy of the SOWER programme and provide a valuable archive of genetic diversity and population structure of great whales in the Southern Ocean. These efforts should continue because: (a) the Antarctic is an area where large-whale recovery has occurred after severe human exploitation; (b) the area is highly inaccessible without this kind of collaborative platform; and (c) biopsy samples are potentially useful for other studies, such as analysis of pollutants, stable isotopes, fatty acids and epigenetic ageing. In addition to the biological information obtained, the cruises provided important platforms for the development of biopsy sampling systems in the Antarctic and low latitudes of the Southern Hemisphere, and as a means of training scientists in the application of these systems.

KEYWORDS: BIOPSY SAMPLING; BREEDING GROUNDS; FEEDING GROUNDS; STOCK STRUCTURE; SURVEY-VESSEL; TAXONOMY

INTRODUCTION

The International Whaling Commission's (IWC) Scientific Committee undertook a series of Antarctic sighting cruises for assessment of Antarctic minke whales (*Balaenoptera bonaerensis*) as part of the International Decade of Cetacean Research (IDCR) during the austral summer seasons between 1978/79–1995/96. From 1995/96, the

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programme was renamed the Southern Ocean Whale and Ecosystem Research (SOWER) and continued until 2009/10 (Matsuoka *et al*., 2003).

At the start of the SOWER programme, a blue whale (Antarctic blue whale, *B. musculus intermedia*; pygmy blue whale, *B. m. brevicauda*) research component was added at lower latitudes. Three low-latitude blue whale cruises were conducted: (a) during the 1995/96 season off the southern coast of Australia (Kato *et al*., 1996); (b) during the 1996/97 season south of Madagascar (Tsurui *et al*., 1997); and (c) during the 1997/98 season in the southeastern Pacific Ocean off the coast of Chile (Findlay *et al*., 1998). The main aim of these SOWER cruises was to obtain sightings data to estimate large whale abundance (Branch and Butterworth, 2001; IWC, 2013), but several other research activities were also conducted (Matsuoka *et al*., 2003), including biopsy sampling of cetaceans, particularly large whales. Genetic analyses based on biopsy samples have the potential to provide information on stock structure which is important for interpretation of abundance and trend estimates. Genetic analysis of samples can also contribute to the clarification of taxonomy of some large whales, such as blue and fin whales. These genetic data can also be used for various other applications, including estimation of effective population size and directional gene flow. Other studies are potentially possible, such as diet studies based on analysis of stable isotopes and pollutants. This paper summarises: (a) the results of biopsy sampling conducted during SOWER cruises; and (b) the results from genetic analysis of stock structure, phylogeny and taxonomy.

SAMPLING PROCEDURES

Biopsy sampling systems

The development and use of biopsy systems during the SOWER cruises took advantage of several pioneering studies in other oceanic basins (Brown *et al*., 1991; Lambertsen, 1987; Palsboll *et al*., 1991; Weinrich *et al*., 1991). Four main biopsy sampling systems were employed. Fig. 1 shows biopsy sampling activities by a SOWER research team in the Antarctic.

Fig. 1. Biopsy sampling of blue whales from the research vessel *Shonan Maru No. 2* during the 2005/06 SOWER cruise in the Antarctic. Photograph credit: The Institute of Cetacean Research.

System 1 (Fig. 2a)

The 'Japanese air-gun' was developed by the Institute of Cetacean Research (ICR) and consisted of a biopsy dart rifle (a modified air-powered rescue-line gun), biopsy darts and a retrieving system (Kasamatsu *et al.*, 1991; Nishiwaki *et al.*, 1990). Specifications as follows: 40mm calibre; 85cm total length; 45cm stainless steel barrel; 4kg weight. The air pressure was set at 90–150kgf/cm2. The dart comprised a tip and an arrow. The tip was 60mm long, with a 14mm diameter and internal diameter of 11mm. It weighed 100g and was made of stainless steel with a 1mm diameter air exit at the rear. The arrow (220g) was made of Alumite in the form of a tube to reduce total weight. It was 180mm long, with an 18mm diameter and 40mm nylon rings at the tip to allow fit to the barrel. The sample was retained in the dart by an internal hook. An 80m nylon rope with a 3mm diameter

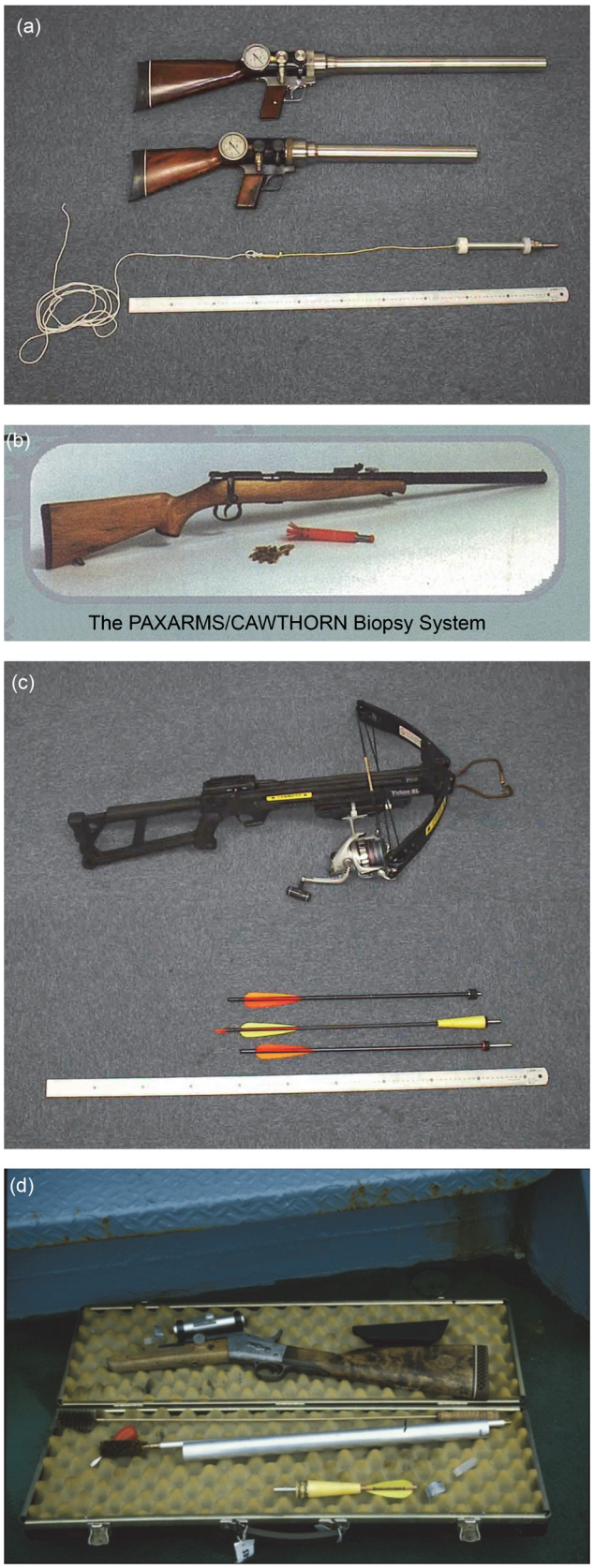

Fig. 2. Main biopsy sampling systems for large whales developed and used during the SOWER cruises: (a) Japanese air-gun; (b) Paxarm/Cawthorn biopsy system; (c) Crossbow biopsy system; (d) Larsen gun. Photographs (a), (c) and (d) were taken by K. Matsuoka (ICR). Photograph (b) was taken from an instruction pamphlet for the Paxarm/Cawthorn system.

was attached to the rear of the arrow. The gun was also tested on other research platforms, both in the western North Pacific and Antarctic (Nishiwaki, 2000).

System 2 (Fig. 2b)

Known as the 'Paxarms/Cawthorn gun', this consisted of a Syringe Projection Rifle of .745 calibre for whales and .509 for seals and land mammals with integrated open sights (0–60m). Darts combined four components: (a) interchangeable stainless steel biopsy head; (b) polycarbonate body or flotation chamber; (c) flooding dye compartment (optional); (d) removable flight. A sample-retaining hook was mounted inside the cutting head. A barb-resetting tool ensured continued retention of samples so that the head could be used multiple times. The cutting heads were screwed directly into the body or flotation chamber. After striking the whale, the biopsy-cutting head cut out a small 8mm-wide skin plug of varying length, depending on which length was selected. The dart would then float vertically in the water where the dye pellet would begin to dissolve, releasing a brilliant stain, which allowed the dart to be recovered with a dip net. A modified Paxarms system for small cetaceans was described by Krützen *et al.* (2002) and consisted of a modified .22 calibre rifle with polycarbonate biopsy darts and stainless-steel biopsy tips.

System 3 (Fig. 2c)

First developed and described by Lambertsen (1987), this system has three components: a biopsy dart, a projection unit and retrieval unit. The biopsy dart consisted of a hollow stainless-steel shaft fitted at one end with a stainless-steel insert, threaded to secure a biopsy punch. The biopsy punch was stainless steel, turned out of solid rod and flanged to limit penetration. The projection unit was a commercially available 68kg draw crossbow, modified to accept the retrieval unit – a hollow fibreglass spinning rod and reel (Lambertsen, 1987). The sample was retained in the dart by internal barbs. Both standard and compound crossbow types were used during the SOWER cruises, with the latter being of higher power (Table 1).

System 4 (Fig. 2d)

An 'improved' Larsen gun was described by Larsen (1998), using as its base a Remington Rolling Block System rifle (model 1867). The chamber was modified to 9mm, allowing the use of 9mm .380 and .357 blank ammunition

Table 1
Availability of biopsy sampling systems during the SOWER cruises

Austral summer season	Antarctic cruises	Low latitude blue whale cruises
1988/89	'Thunderbolt' crossbow	
1989/90	Japanese air-gun	
1990/91	Japanese air-gun	
1991/92	Japanese air-gun	
1992/93	Japanese air-gun	
1993/94	Japanese air-gun	
1994/95	Japanese air-gun, Paxarm gun	
1995/96	Japanese air-gun, Paxarm gun, compound crossbow	Japanese air-gun, Paxarm gun, Danish-modified Paxarm gun, standard crossbow (Kato *et al*., 1996)
1996/97	Japanese air-gun, Paxarm gun, compound crossbow, Pole System	Japanese air-gun, Paxarm gun, standard crossbow (Turui *et al*., 1997)
1997/98	Japanese air-gun, Paxarm gun, crossbow (compound and standard)	Paxarm, compound crossbow, standard crossbow (Findlay *et al*., 1998)
1998/99	Japanese air-gun, Paxarm gun, crossbow (compound and standard), Larsen gun	
1999/00	Japanese air-gun, Paxarm gun, crossbow (compound and standard), Larsen gun	
2000/01	Japanese air-gun, Paxarm gun, crossbow (compound and standard), Larsen gun	
2001/02	Larsen gun, compound crossbow	
2002/03	Larsen gun	
2003/04	Larsen gun	
2004/05	Larsen gun	
2005/06	Larsen gun, Paxarm gun	
2006/07	Larsen gun, Paxarm gun	
2007/08	Larsen gun, compound crossbow	
2008/09	Larsen gun, compound crossbow	
2009/10	Larsen gun, Paxarm gun, compound crossbow	

and fitted with a valve for adjusting gas pressure entering the barrel. The barrel consisted of a 48.5cm aluminium cylinder which allowed use of darts with 27mm-diameter floats. The open sight was replaced with an electronic aiming device (red-dot sight) to enable faster aiming and shooting. The biopsy darts consisted of a carbon-fibre shaft with a high-pressure moulded polyethylene float. The float also functioned as a stop to limit tissue penetration. At the float-end, a threaded insert allowed attachment of a screw-on sampling tip. The tip was a stainless-steel cylinder with a 9mm diameter, 7mm internal diameter and three internal barbs for sample retention. The tip was normally between 20–50mm, depending on the target species, with 40mm most commonly used for *balaenopterids*. The weight of the dart minus sampling tip was 32g (Larsen, 1998).

Use of biopsy systems on the cruises

Table 1 summarises the systems used during the Antarctic and low-latitude cruises. Sampling feasibility experiments in the Antarctic began during the 1988/89 cruise in response to recommendations from the IWC Scientific Committee that the effectiveness of biopsy darts to collect samples from Antarctic minke whales should be investigated (Kasamatsu *et al.*, 1989a; 1989b; IWC, 1989). No biopsy samples from minke whales were obtained, in part due to the limited number of trials and insufficient power of the crossbow (Kasamatsu *et al*., 1989a).

The first biopsy samples were obtained during the 1989/90 Antarctic cruise using the Japanese air-gun from one blue, one fin (*B. physalus*), one sei (*B. borealis*), one humpback (*Megaptera novaeangliae*) and four Antarctic minke whales (Joyce *et al*., 1990). Factors such as the sampling platform and whale species should be considered when discussing the advantages/disadvantages of biopsy systems. During the SOWER Antarctic cruises, biopsy sampling occurred from the forecastle deck of large vessels (c.900 tons, 70m long with forecastle height of 6.5m). Vessel speed, wind speed and direction, in addition to the individual's behaviour and movement patterns, were all important factors. Sufficient system power, shooting range and ease of handling were also necessary for success. Most systems had a range of approximately 30m, but the Japanese air-gun and Larsen gun had more power and were easier to handle. As a result, these systems were used most during the cruises (Table 1).

The Japanese air-gun was available from 1989/90–2000/01 with various modifications to dart design. The Larsen gun was added from 1998/99 and used until the end of the SOWER cruises in 2009/10 as it was lighter and easier to handle for fast-moving whale species.

The systems available on the 1995/96 and 1996/97 low-latitude cruises were the Japanese air-gun, Paxarms gun (standard and modified) and a standard crossbow (Kato *et al*., 1996). Crossbows were used exclusively from an inflatable craft as the sea conditions during low-latitude operations allowed for their safe use (Tsurui *et al*., 1997). On the 1997/98 low-latitude cruise, three systems were available: Paxarms gun, compound crossbow (Barnett RC300) and standard crossbow (Findlay *et al*., 1998). The most efficient system on the Antarctic cruises (Larsen gun) was not available for low-latitude cruises because it was only fully developed by 1998, after completion of the low-latitude cruises (Larsen, 1998).

Biopsy sample preservation

Half of each sample collected was stored in Japan and administered by Japanese scientists. The other half was exported and stored at the US Southwest Fisheries Science Center (SWFSC), National Marine Fisheries Service, where it was administered by the IWC. In the field, the Japanese biopsy samples were preserved at –20°C in salt/DMSO solution (Amos and Hoelzel, 1991), while the IWC samples were preserved in 70% ethanol. Access to these samples can be obtained by submitting a proposal to the Scientific Committee through the IWC Secretariat portal.[2]

GEOGRAPHICAL AND TEMPORAL DISTRIBUTION OF SAMPLES

During the SOWER Antarctic cruises, 191 samples were obtained from Antarctic blue whales, four from pygmy blue whales, 46 from fin whales, five from sei whales, 406 from humpback whales, 60 from southern right whales (*Eubalaena australis*), 19 from Antarctic minke whales, and six from sperm whales (*Physeter macrocephalus*) (Table 2).

[2] *https://portal.iwc.int*

Table 2

Summary of the biopsy samples obtained during the SOWER Antarctic cruises. Biopsy samples obtained during transit to the Antarctic are also listed. The list includes cases of re-sampling (more than one biopsy sample obtained from the same individual).

Species	Austral summer season	No. of biopsy samples
Blue whale (Antarctic)	1989/90	1
	1992/93	2
	1994/95	2
	1995/96	1
	1996/97	4
	1997/98	3
	1998/99	7
	2000/01	20
	2001/02	14
	2002/03	4
	2003/04	14
	2004/05	5
	2005/06	36
	2006/07	72
	2008/09	6
Blue whale (pygmy)	1996/97	1
	1997/98	3
Fin whale	1989/90	1
	2005/06	26
	2006/07	16
	2007/08	3
Sei whale	1989/90	1
	1997/98	3
	1999/2000	1
Humpback whale	1989/90	1
	1990/91	2
	1991/92	2
	1992/93	4
	1993/94	3
	1994/95	2
	1995/96	3
	1996/97	23
	1997/98	10
	1998/99	82
	1999/2000	38
	2000/01	35
	2001/02	1
	2003/04	2
	2004/05	6
	2005/06	70
	2006/07	71
	2007/08	7
	2008/09	23
	2009/10	21
Antarctic minke whale	1989/90	4
	1991/92	1
	2000/01	9
	2003/04	1
	2008/09	4
Southern right whale	1992/93	1
	1996/97	2
	1997/98	5
	1998/99	18
	2004/05	1
	2005/06	2
	2007/08	9
	2009/10	22
Sperm whale	1992/93	1
	2000/01	4
	2002/03	1
Southern bottlenose whale	2003/04	1
Arnoux's beaked whale	2000/01	1
Long-finned pilot whale	1998/99	1
Killer whale	1997/98	3
	1998/99	9
	1999/2000	5
	2000/01	2
	2003/04	2
	2005/06	7
	2006/07	1
	2008/09	1
Hourglass dolphin	1996/97	2
Common dolphin	1997/98	3

*Total 775 biopsy samples from 14 species.

Table 3

Summary of the biopsy samples obtained during the low latitude blue whale cruises. The list includes cases of re-sampling (more than one biopsy sample obtained from the same individual).

Species	Austral summer season	No. of biopsy samples
Blue whale (pygmy)	1995/96	8
	1996/97	8
	1997/98	16
Humpback whale	1996/97	7
	1997/98	2
Bryde's whale	1997/98	9
Antarctic minke whale	1996/97	1
Southern right whale	1995/96	5
Sperm whale	1997/98	3
Pygmy sperm whale	1997/98	1
Bottlenose dolphin	1996/97	1
	1997/98	6
Common dolphin	1996/97	1
Pantropical spotted dolphin	1996/97	1
Striped dolphin	1996/97	1
Southern right whale dolphin	1997/98	1

*Total 71 biopsy samples from 12 species.

On the low-latitude cruises, 32 samples were obtained from pygmy blue whales, nine from Bryde's whales (*B. edeni*), nine from humpback whales, five from southern right whales, one from an Antarctic minke whale and three from sperm whales (Table 3).

Figs. 3–6 show the geographical distribution of large whales sampled during the Antarctic cruises. Some were taken north of 60°S when the vessels were in transit to the research area. Figs. 7, 8 and 9 show the geographical distribution of samples by species collected during the low-latitude cruises in 1995/96, 1996/97 and 1997/98.

STUDIES OF STOCK STRUCTURE, PHYLOGENY AND TAXONOMY BASED ON THE SAMPLES COLLECTED

Many of the studies below make reference to the IWC Management Areas. Their geographical boundaries are shown in Figs. 3–6.

Blue whales

Genetic analysis of blue whale samples obtained in the Antarctic and in low latitudes off Australia, Madagascar and Chile has improved our understanding of the genetic structure of pygmy blue whale stocks and between pygmy and Antarctic blue whales, currently assigned as subspecies. Key studies are summarised below.

LeDuc *et al*. (2007) used samples from the Antarctic (*n* = 30 from SOWER cruises, Fig. 3; *n* = 17 from the Japanese Whale Research Programme under Special Permit in the Antarctic), Chile (*n* = 16 from SOWER, Fig. 9), the southern and western coasts of Australia (*n* = 28 from SOWER, Fig. 7), the Maldives (*n* = 6 from SOWER, Fig. 8) and Peruvian and Ecuadorian waters (*n* = 12 from US SWFSC research cruises). Analysis focused on investigating the pattern of genetic variation in Southern Hemisphere blue whales and the use of an assignment test to detect mixing on the feeding grounds. Genetic markers used were mtDNA control region sequences and seven microsatellite loci. Strong genetic differences were found among samples from the southeast Pacific Ocean, Indian Ocean and around the Antarctic continent. Genetic differentiation between the geographical ranges of the nominal subspecies (i.e., Antarctic vs pygmy blues in the Pacific and Indian Oceans) was not markedly greater than between populations of pygmy blue whales.

Attard *et al*. (2012a) conducted a genetic analysis of samples collected during the feeding season to report on several cases of hybridisation between the two recognised blue whale Southern Hemisphere subspecies in a previously unconfirmed sympatric area off Antarctic. The analysis was based on 21 microsatellite loci and biopsy samples obtained by SOWER cruises in the Antarctic between 1990–2009 (*n* = 186, Fig. 3) and Australian blue

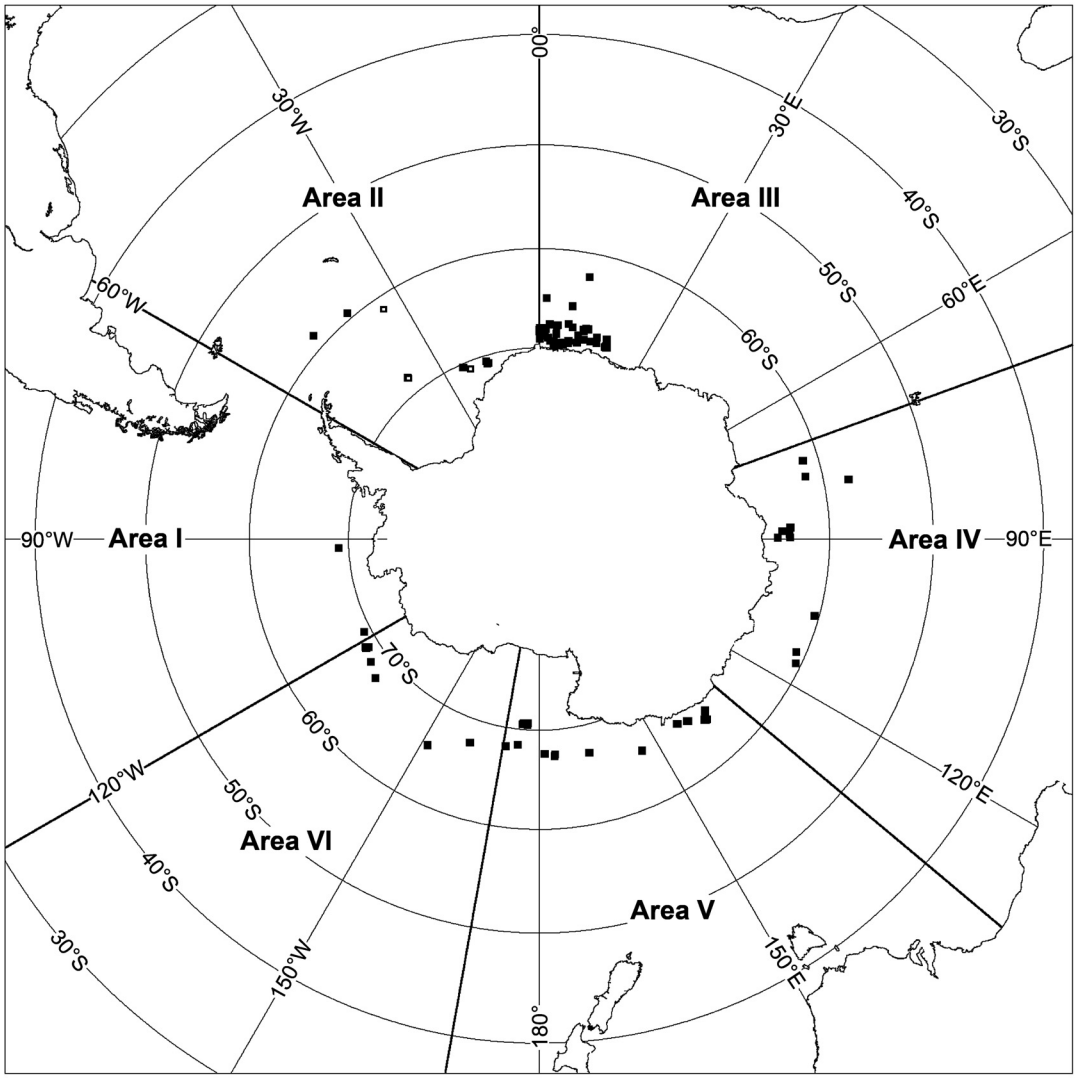

Fig. 3. Geographical distribution of biopsy samples of Antarctic (blue closed square) and pygmy (light blue open square) blue whales obtained during the SOWER Antarctic cruises. A few samples were taken north of 60°S when the vessels were in transit to the research area. Positions are based on the first sighting position. On some occasions, samples were obtained from more than one individual from the same school. In these cases, a single symbol may represent more than one sample.

whale biopsy, stranding and sloughed skin samples collected from the Perth Canyon and Bonney Upwelling feeding aggregations (*n* = 113).

Attard *et al*. (2012b) investigated the genetic identity of blue whales in Geographe Bay, Western Australia, using mtDNA control region sequences and 20 microsatellite loci. The analysis was based on 13 biopsy samples, which were compared with Australian pygmy blue whale feeding aggregations (*n* = 109) and Antarctic samples collected through SOWER (*n* = 152, Fig. 3). Preliminary results indicated that the Geographe Bay blue whales were all pygmy blue whales.

Sremba *et al*. (2012) studied circumpolar diversity and geographic differentiation of mtDNA in Antarctic blue whales. SOWER samples south of 60°S from 1990 and 2009 were used (Fig. 3). Samples included SOWER samples used in LeDuc *et al*. (2007) (*n* = 26) and samples obtained by JARPA and used in LeDuc *et al*. (2007) (*n* = 17) (218 total samples). Analysis was also undertaken with 16 microsatellite loci. A rarefaction analysis predicted that only 72 haplotypes had survived in the contemporary population, but the haplotype diversity was relatively high. Low but significant differentiation in mtDNA diversity among the six IWC Antarctic Management Areas was found (FST = 0.032, $p < 0.005$ for mtDNA, and FST = 0.005, $p < 0.05$ for microsatellites among the six Management Areas).

Torres-Florez *et al*. (2014) used samples from the Antarctic (*n* = 96 from SOWER, Fig. 3), southern Chile

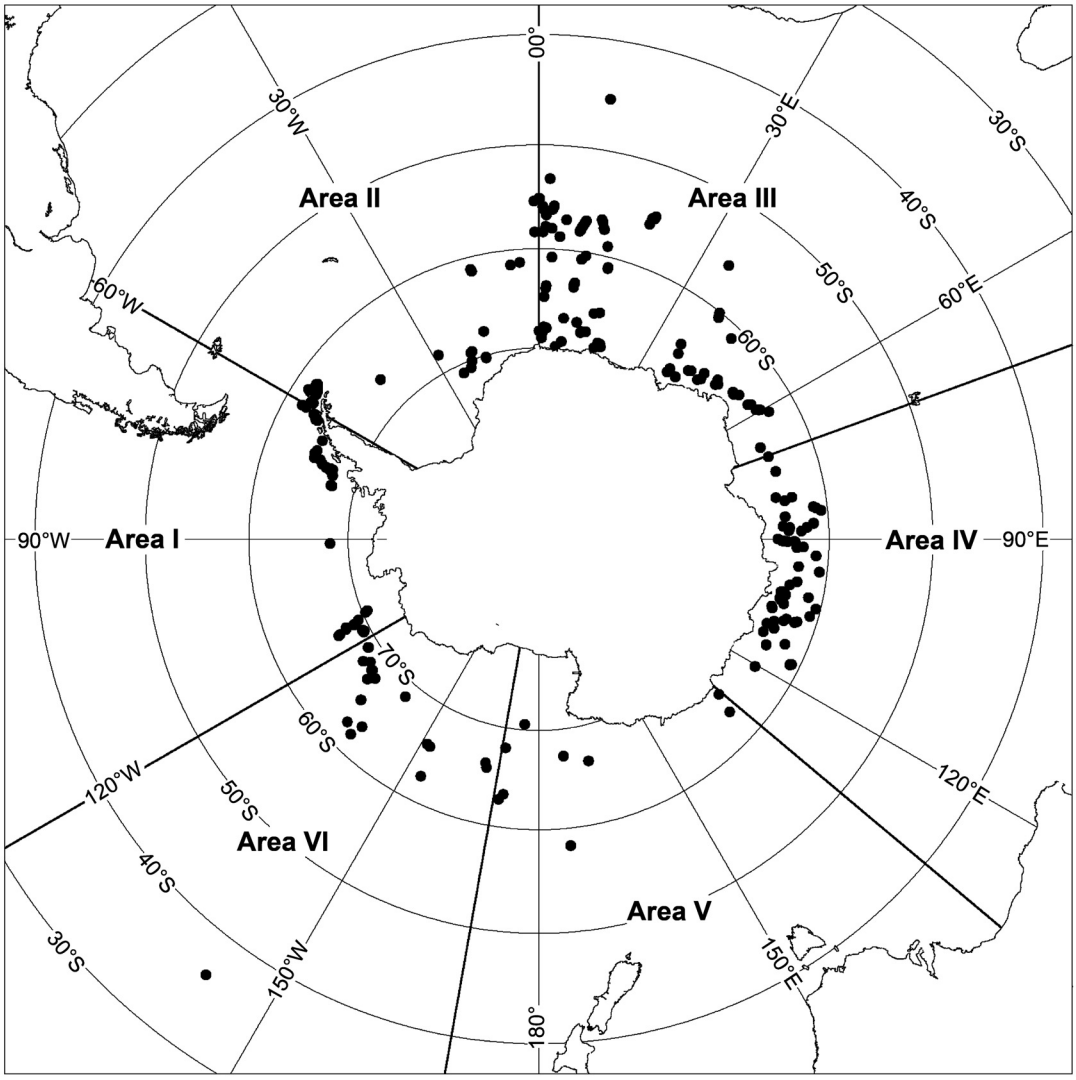

Fig. 4. Geographical distribution of humpback whale samples from SOWER Antarctic cruises. A few samples were taken north of 60°S when the vessels were in transit to the research area. Positions are based on the first sighting position. On some occasions, samples were obtained from more than one individual from the same school. In these cases, a single symbol may represent more than one sample.

(*n* = 60 from Chilean research programme), northern Chile (*n* = 19 from SOWER and Chilean research programme, Fig. 9), and eastern tropical Pacific (ETP) (*n* = 31 from US research programme), to investigate population/subspecies genetic structure in the eastern South Pacific. The genetic markers used were mtDNA control region sequences and seven microsatellite loci. Results suggested that at least two breeding population units or subspecies exist. The lack of difference detected between southern Chile, northern Chile and ETP areas supported the hypothesis that eastern South Pacific blue whales use the ETP area as a breeding area.

Attard *et al*. (2016) investigated population structure of the Antarctic blue whales based on 142 individual samples collected in the Antarctic from 1990–2009 by SOWER cruises. The samples excluded resamples, migrant pygmy blue whales and subspecies hybrids found off the Antarctic in previous studies (Attard *et al*., 2012a). The genetic analyses were based on 20 microsatellite loci and a 414bp-fragment of the mtDNA control region. The study found evidence of three populations that are sympatric in the Antarctic feeding grounds and likely occupy separate breeding grounds.

LeDuc *et al*. (2017) conducted an analysis based on mtDNA control region sequencing (400bp-fragment) and microsatellite DNA at seven loci to investigate the genetic variation of blue whales in the eastern Pacific. Data were the same used in LeDuc *et al*. (2007) and Torres-Flores *et al*. (2014). The study suggested that the eastern Tropical Pacific (ETP) is differentially used by blue whales from the north- and south-eastern Pacific, with the former showing stronger affinity to the region off Central America (Costa Rican Dome), and the latter favouring

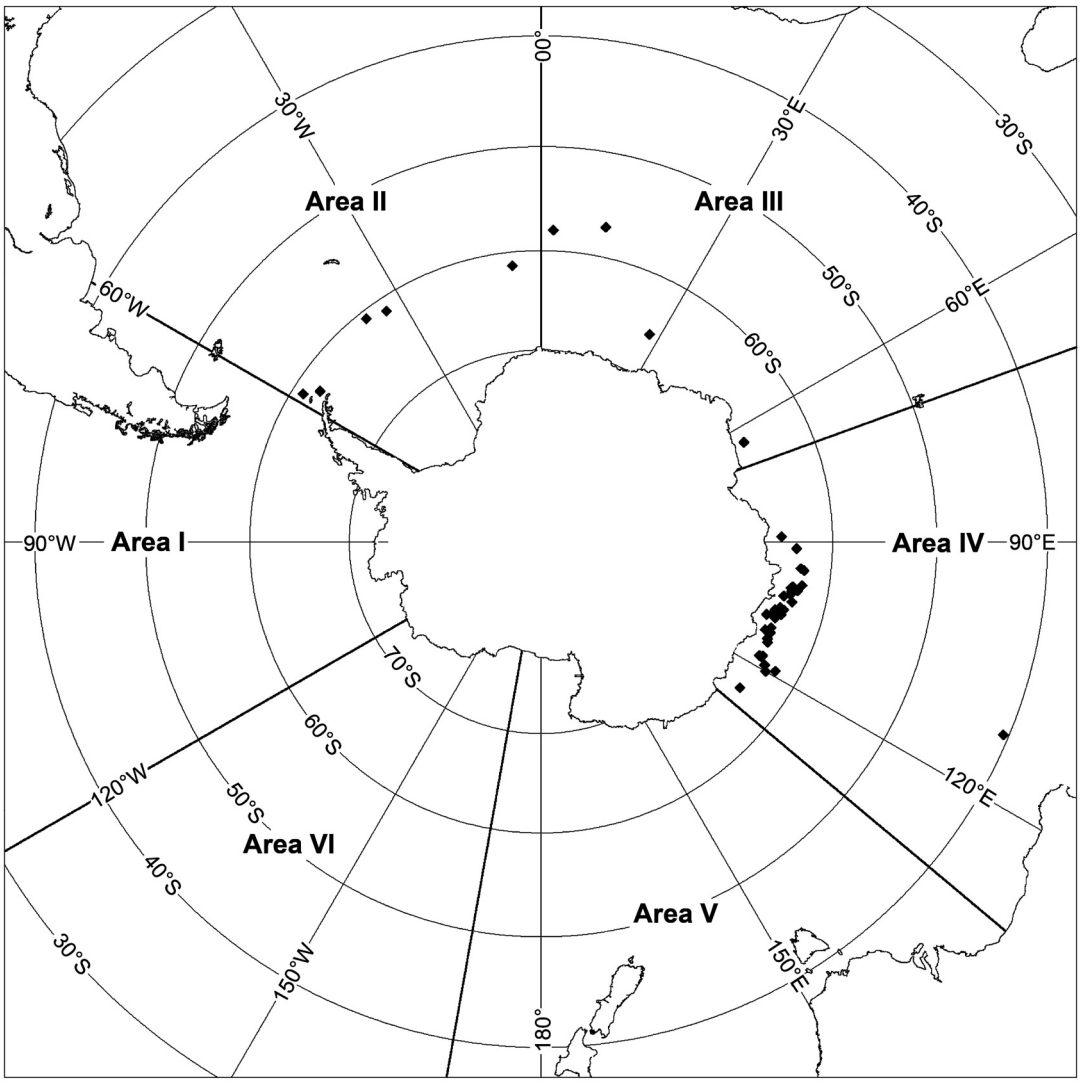

Fig. 5. Geographical distribution of samples of southern right whales from SOWER Antarctic cruises. A few samples were taken north of 60°S when the vessels were in transit to the research area. Positions are based on the first sighting position. On some occasions, samples were obtained from more than one individual from the same school. In these cases, a single symbol may represent more than one sample.

the waters of Peru and Ecuador.

Humpback whales

The IWC Scientific Committee has described hypothetical stock structure and migratory corridors for Southern Hemisphere humpback whales based on information such as Discovery marks, photo-ID, genetics and satellite tracks (IWC, 2005). Seven Breeding Stocks (BS) are recognised: A–G. Some (B, C, E and F) were further subdivided into sub-stocks. The Scientific Committee completed the assessment of most stocks at its 2014 meeting (IWC, 2015). For this assessment, the information on stock structure summarised below was important for interpreting stock abundance and trend, and for allocating historical catches to the various stocks.

Specifically, genetic analyses of biopsy samples obtained during SOWER cruises contributed to our understanding of stock structure at feeding grounds, and the relationship between humpback whales at their Antarctic feeding grounds, in low-latitude migratory corridors and at their breeding grounds.

Analyses among high latitude areas

The most extensive analyses within the feeding grounds were conducted by Pastene *et al*. (2006), Loo *et al*. (2007) and Kanda *et al*. (2014a). Pastene *et al*. (2006) examined population genetic structure in IWC Management Areas IIIE, IV, V and VI based on mtDNA control region sequences and six microsatellite loci. The analysis was based on

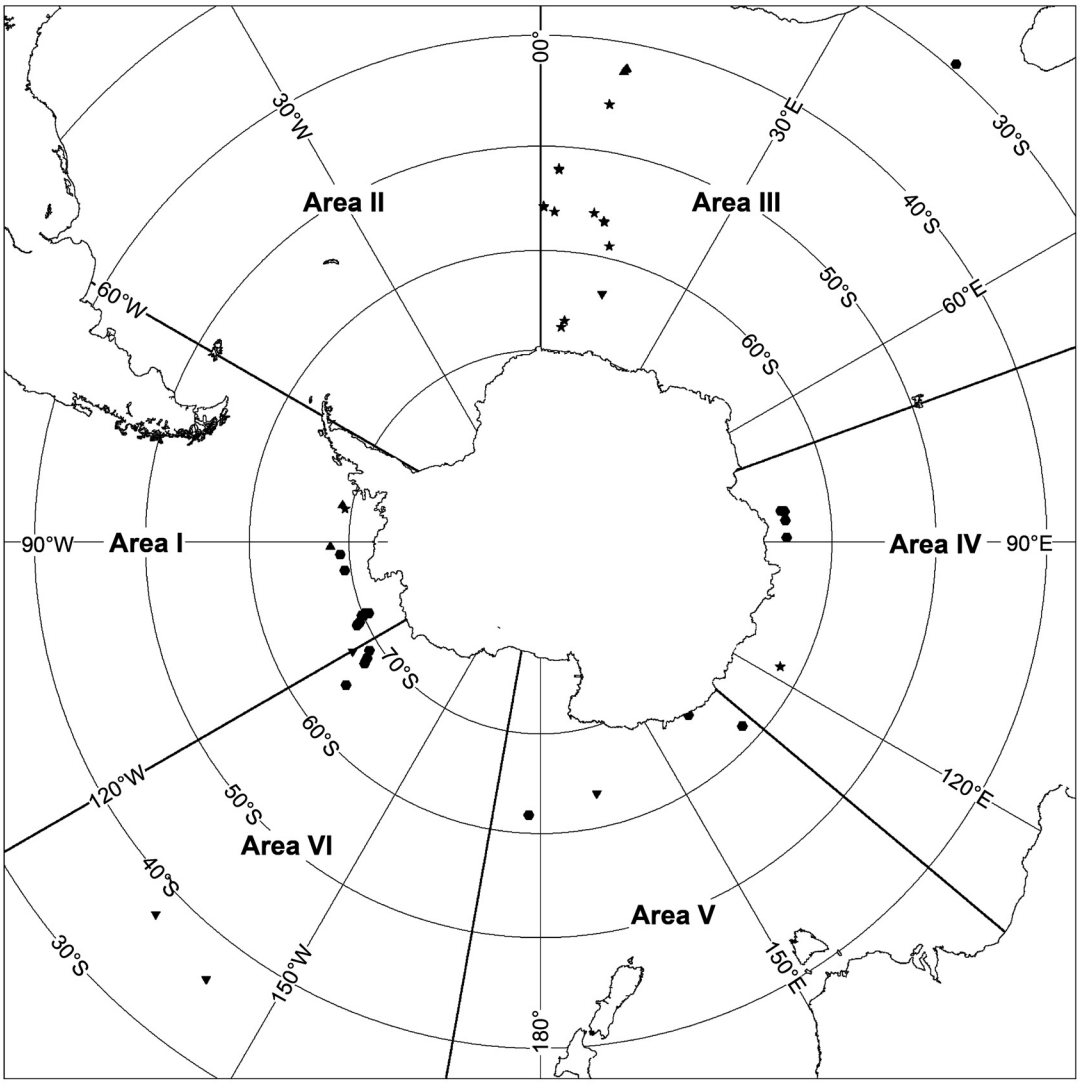

Fig. 6. Geographical distribution of biopsy samples of fin (blue star), sei (orange triangle), Antarctic minke (red hexagon) and sperm (purple inverted triangle) whales from SOWER Antarctic cruises. A few samples were taken north of 60°S when the vessels were in transit to the research area. Positions are based on the first sighting position. On some occasions, samples were obtained from more than one individual from the same school. In these cases, a single symbol may represent more than one sample.

biopsy samples collected by the JARPA and SOWER cruises: *n* = 81 for Area IIIE (JARPA: 50; SOWER: 31); *n* = 172 for Area IV (JARPA: 126; SOWER: 46); *n* = 97 for Area V (JARPA: 90; SOWER: 7); and *n* = 61 for Area VI (JARPA: 44; SOWER: 17) (Fig. 4). The analysis confirmed the high level of genetic diversity for mtDNA and microsatellites. Both genetic markers suggested differentiation among these four areas, with stronger differences between females than males. The authors did not reject the possibility of some population mixing at the borders of these Management Areas.

Loo *et al*. (2007) examined population genetic structure in IWC Management Areas I–VI based on mtDNA control region sequences and 10 microsatellite loci. The analysis was based on samples from SOWER cruises and national research programmes in Chile and the US (Fig. 4). Sample sizes were *n* = 138 for Area I (samples from SOWER, Chile and US research programmes); *n* = 26 for Area II (SOWER); *n* = 112 for Area III (SOWER); *n* = 53 for Area IV (SOWER); *n* = 7 for Area V (SOWER); and *n* = 36 for Area VI (SOWER). In general, the analysis found genetic differences between these areas, but some pairwise comparisons did not show significant differences, which the authors attribute to potential overlap between breeding stocks in some areas. The study was updated by Loo *et al.* (2010) (see below).

Kanda *et al.* (2014a) examined population genetic structure in Areas IIIE, IV, V and VI based on genotypes from 14 microsatellite loci. The analysis was based on JARPA and SOWER cruise samples: *n* = 93 for Area IIIE (JARPA: 62; SOWER: 31); *n* = 218 for Area IV (JARPA: 172; SOWER: 46); *n* = 153 for Area V (JARPA: 146; SOWER:

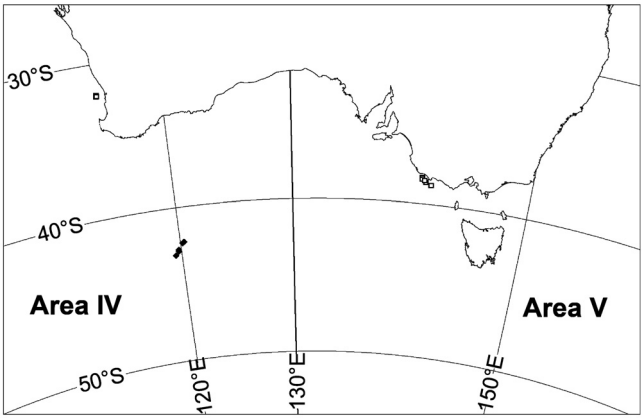

Fig. 7. Geographical distribution of biopsy samples of pygmy blue (light blue open square) and southern right (orange diamond) whales from the 1995/96 SOWER cruise (low-latitude blue whale cruise). Positions are based on the first sighting position. On some occasions, samples were obtained from more than one individual from the same school. In these cases, a single symbol may represent more than one sample.

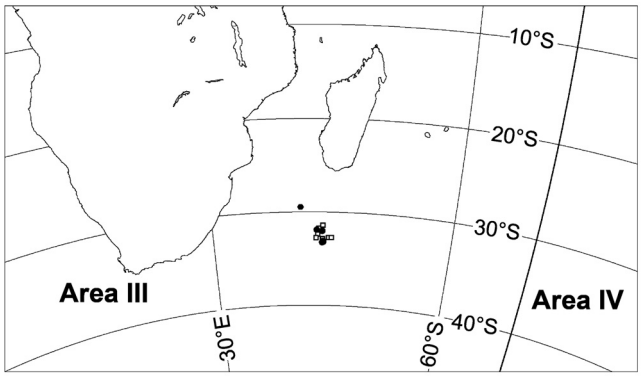

Fig. 8. Geographical distribution of biopsy samples of pygmy blue (light blue open square), humpback (purple circle) and Antarctic minke (red hexagon) whales from the 1996/97 SOWER cruise (low-latitude blue whale cruise). Positions are based on the first sighting position. On some occasions, samples were obtained from more than one individual from the same school. In these cases, a single symbol may represent more than one sample.

7); and *n* = 64 for Area VI (JARPA: 47; SOWER: 17) (Fig. 4). Major genetic differences were attributed to samples from different areas. Stronger differentiation was seen between females than males. Despite the increase of loci from six in the previous analysis to 14, the level of stock differentiation was still too low for analysis at the individual level.

Comparison between feeding and breeding grounds

Steel *et al*. (2008) investigated migratory connections between humpbacks from South Pacific breeding grounds and Antarctic feeding areas based on genotype matching (17 microsatellite loci, sex and mtDNA). The study followed a comprehensive genetic analysis of humpback whales in low latitudes (Olavarría *et al*., 2007). Breeding ground samples from New Caledonia (*n* = 581), Tonga (*n* = 496), Samoa (*n* = 2), the Cook Islands (*n* = 206), French Polynesia (*n* = 176) and Colombia (*n* = 140) were obtained by several groups and research institutions. Feeding ground samples were from IWC Management Areas I (*n* = 98, Chile programme, SO-GLOBEC, SOWER); II (*n* = 1, SOWER); III (*n* = 13, SOWER); IV (*n* = 51, SOWER); V (*n* = 9, SOWER); and VI (*n* = 22, SOWER) (Fig. 4). Comparison of genotypes revealed five matches representing migratory connections: one between New Caledonia and Area

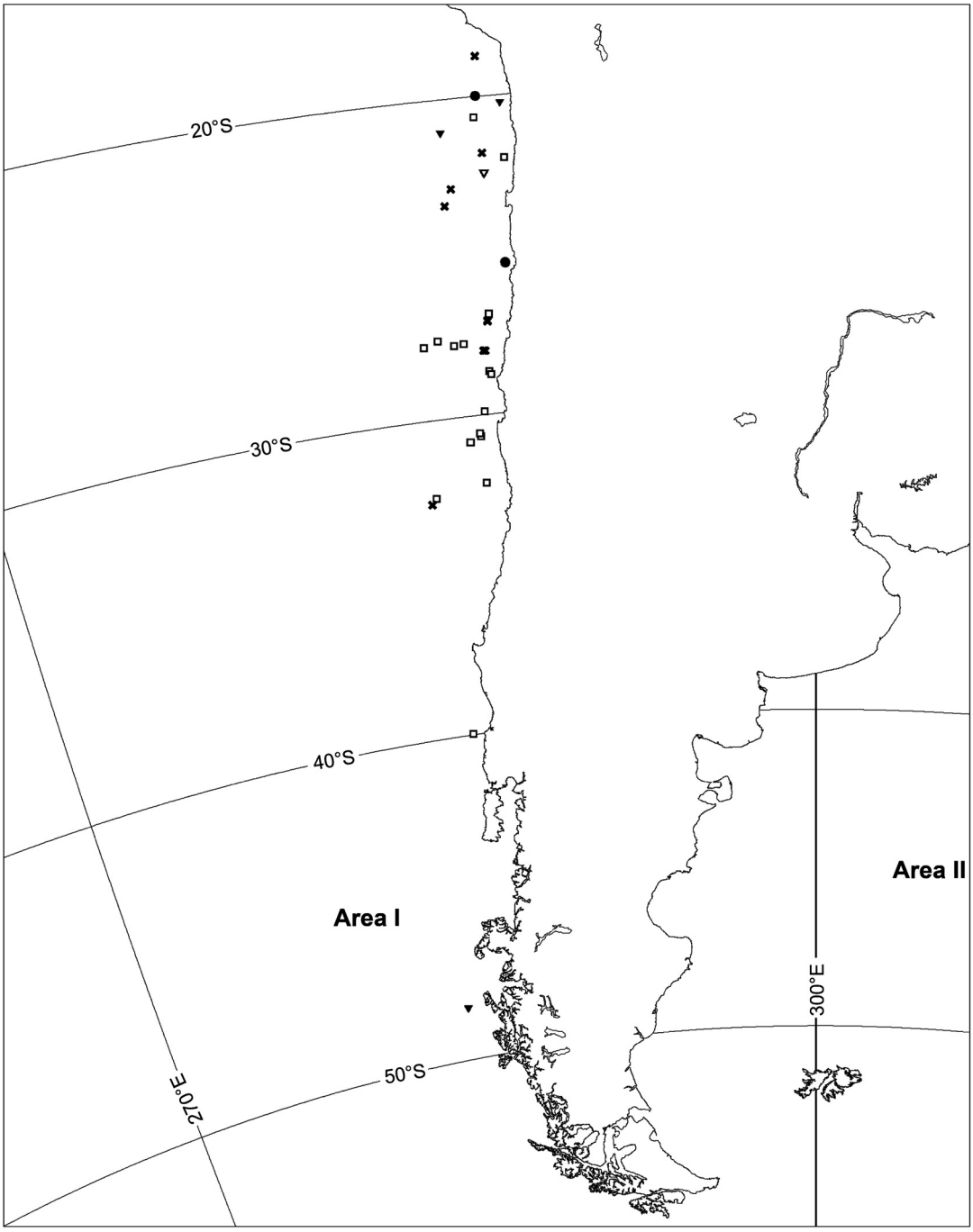

Fig. 9. Geographical distribution of biopsy samples of pygmy blue (light blue open square), humpback (purple circle), Bryde's (green cross), sperm (purple inverted triangle) and pygmy sperm (purple open inverted triangle) whales from the 1997/98 SOWER cruise (low-latitude blue whale cruise). Positions are based on the first sighting position. On some occasions, samples were obtained from more than one individual from the same school. In these cases, a single symbol may represent more than one sample.

V; one between Tonga and Area VI; two between Tonga and Area I (western edge); and one between Colombia and Area I (the Antarctic Peninsula).

Albertson-Gibb *et al*. (2008) estimated migratory allocation of humpback whales from Antarctic feeding areas to South Pacific breeding grounds using mtDNA control region haplotype frequencies. Breeding ground samples from Western Australia (*n* = 132), New Caledonia (*n* = 368), Tonga (*n* = 354), the Cook Islands (*n* = 105), French Polynesia (*n* = 113) and Colombia (*n* = 97) were collected by several groups and research institutions. Feeding ground samples came from IWC Management Areas I (*n* = 77), IV (*n* = 39) and VI (*n* = 28), all from SOWER cruises (Fig. 4). Area I was allocated primarily to Colombia (78.3% SE: 0.057). Area IV was allocated in nearly equal

proportions to Western Australia (33.1% SE: 0.083) and New Caledonia (31.0% SE: 0.126). Area VI whales were allocated primarily to Tonga (78.9% SE: 0.187).

Loo *et al*. (2010) used an increased number of samples in comparison with the previous study (Loo *et al*., 2007) to further examine genetic structure among high-latitude feeding grounds and assess connectivity with BSA, BSB and BSC. The main conclusion was significant differentiation between Gabon and the core feeding region of BSB. The authors suggested that the majority of Gabon animals may travel beyond the core area to feed, despite other evidence of exchange. In contrast, the general lack of differentiation between BSA and Antarctic feeding areas suggested connectivity with the BSB core feeding region and some mixing between BSA and BSC.

Anderson *et al*. (2010) used a total of 13 microsatellite loci to examine genotype matching among humpback whales from eastern Australia, breeding grounds in Oceania and feeding grounds in Antarctic Areas I–VI. The number and source of samples from the breeding and feeding grounds were similar to Steel *et al*. (2008). Samples from the feeding grounds came from the SOWER surveys (Fig. 4). A total of 172 unique individuals were identified among samples from Areas I (n = 86), II (n = 1), III (n = 11), IV (n = 46), V (n = 9) and VI (n = 19). A further 734 were identified from Eastern Australia and 1,086 from Oceania. Migratory connection was detected between eastern Australia and Antarctic Area V (n = 3). No whales from eastern Australia were found to move outside the boundaries of Area V.

Pastene *et al*. (2013) used biopsy samples obtained by JARPA/JARPAII and SOWER surveys and mtDNA control region sequences to study the distribution and mixing rates of breeding stocks BSD, BSE and BSF in Antarctic Areas IIIE–VI. Breeding ground samples from Western Australia (n = 185), eastern Australia (n = 104), New Caledonia (n = 243), Tonga (n = 240), the Cook Islands (n = 56) and French Polynesia (n = 62) were collected by several groups and research institutions. They were provided for analysis under the IWC Scientific Committee Data Availability Protocol (Procedure B) (IWC, 2004). SOWER survey samples were provided as outlined above (Pastene *et al*., 2006). According to one hypothesis on baseline populations, western Australian whales were distributed mainly in Management Areas IVW (84.5% SE: 0.043; FST: 0.0015) and IVE (75.9% SE: 0.092; *FST*: –0.0015); whales from Eastern Australia in Area VW (64.7% SE: 0.074; *FST*: 0.0013); whales from New Caledonia in Area VE (83.9% SE: 0.011; *FST*: 0.0062); and whales from Tonga in Area VI (43.3% SE: 0.015; *FST*: 0.0010). Whales from the Cook Islands and French Polynesia were not represented in feeding ground Areas IIIE–VI.

Southern right whales

Baker *et al*. (1999) examined the distribution and diversity of mtDNA lineages among southern right whales from Australia and New Zealand. Specifically, they examined genetic diversity among two wintering grounds (southwestern Australia (n = 20) and the Auckland Islands (n = 20)) and one offshore feeding ground south of western Australia (n = 5). The latter samples were obtained during the 1995/96 SOWER low-latitude cruise (Fig. 7). Significant genetic differentiation was found between the two wintering grounds.

Patenaude *et al*. (2007) studied mtDNA diversity and population structure among southern right whales from four wintering grounds (Argentina (n = 20), South Africa (n = 41), western Australia (n = 20) and the New Zealand sub-Antarctic (n = 42)) and two summer feeding grounds (South Georgia (n = 8) and southwestern Australia (n = 5)). As above, the latter were obtained during the 1995/96 SOWER low-latitude cruise (Fig. 7). Significant genetic differentiation was found among the four wintering grounds (FST: 0.159; PHIST: 0.238, $p < 0.001$).

Pastene *et al*. (2021) conducted genetic analyses to investigate the individual identification (and matching) of southern right whales from samples collected in the austral summer in the Indian Ocean sector of the Antarctic (between 80°–135°E, south of 60°S). In total, 157 skin biopsy samples were collected from free-ranging whales along the sighting surveys of JARPA/JARPAII (n = 108) and SOWER (n = 49) from the 1993/94 to 2015/16 austral summer seasons. Eight matches were detected (four males and four females) using individual matching by multi-locus genotypes (14 microsatellite DNA loci) supported by mtDNA haplotype and sex determination. These eight resamples show that at least some males and females returned to the same feeding grounds across years. The time spans ranged from 3–13 years, with an average of 6.7 and 7.8 years for males and females, respectively. Sampling and matching occurred in an area where visual surveys showed aggregations of southern right whales associated with high krill concentration.

Fin whales

Three subspecies of fin whales have been described: *Balaenoptera physalus* in the Northern Hemisphere; *B. p. quoyi* in the Southern Hemisphere; and a pygmy form, *B. p. patachonica* (Committee on Taxonomy, 2023). Archer *et al*. (2013) investigated the phylogenetic relationship between fin whales from the North Pacific, North Atlantic and Southern Hemisphere, based on the complete mitogenome from 154 animals, including 43 from the Southern Hemisphere, mainly from SOWER Antarctic cruises (Fig. 6). A Bayesian tree of 136 haplotypes revealed several well-supported clades representing each ocean basin, with no haplotypes shared between oceans. The North Atlantic haplotypes (n = 12) formed a sister clade from the Southern Hemisphere (n = 42). The estimated time to the most recent ancestor for the Atlantic/Southern Hemisphere clade and 81 of the 97 samples from the North Pacific was approximately 2Ma. Fourteen of the remaining North Pacific samples formed a well-supported clade within the Southern Hemisphere. The authors believed these results provide support for the view that North Pacific and North Atlantic fin whales should not be considered the same subspecies and further suggested the need for revision of the global taxonomy of the species.

In a subsequent revision of fin whale subspecies based on genetics, Archer *et al*. (2019) suggested that North Pacific fin whales should be recognised as a separate subspecies.

Bryde's whales

Pastene *et al*. (2015) examined the degree of population genetic structure of South American Bryde's whales, analysis being based upon mtDNA control region sequences and samples from Chile (n = 10), Peru (n = 24) and Brazil (n = 8). Samples from Java (n = 23), Fiji (n = 24) and the western North Pacific (n = 401) were used for comparative purposes. The Chilean samples included eight obtained during an SOWER low-latitude cruise in 1997/98 (Fig. 9). Results of the phylogenetic analysis identified the whales in Chilean waters as Bryde's whale (*Balaenoptera brydei*), as described by Wada *et al*. (2003). It should be noted that the taxonomy of Bryde's whales remains unresolved. The Committee of Taxonomy of the Society for Marine Mammalogy (last updated June 2023) supports the subspecies definition *B. edeni brydei* for the larger offshore Bryde's whale. No statistically significant genetic differentiation was found between Bryde's whales in Chile and Peru (KST: –0.0163, p = 0.9601). Striking genetic differences were found between whales from Peru and Brazil (KST: 0.2236, p = 0.0001) and between Chile and Brazil (KST: 0.3323, p = 0.0001). Peru/Chile and Brazil samples were significantly different from those of the western North Pacific, Fiji and Java (Pastene *et al*., 2015).

CONCLUSIONS

The largest numbers of biopsy samples obtained during the SOWER cruises were from humpback and blue whales. Their genetic analysis has contributed greatly to our understanding of the stock structure of humpback whales in the feeding grounds and the connection between whales in low-latitude breeding grounds and high-latitude feeding grounds. The information has been essential for successful completion of humpback assessments by the Scientific Committee.

For blue whales, genetic analysis of samples obtained on the feeding grounds (mainly Antarctic blue whales) and those from low latitudes off Australia, Madagascar and Chile (pygmy blue whales) contributed to our understanding of population genetic structure among pygmy blue whale stocks and between pygmy and Antarctic blue whales.

While a relatively large number of biopsy samples were obtained from southern right whales, mainly in Area IV, the samples have yet to undergo genetic analysis. It is recommended that analyses similar to those conducted on this species in Area IV are conducted (Kanda *et al*., 2014b). The number of biopsy samples from other species, such as fin, sei, Bryde's and sperm whales, obtained during the SOWER cruises is small. In some cases, they have been used to complement studies based on larger sample sizes from other sources (Archer *et al*., 2013; Pastene *et al*., 2015). A number of biopsy samples were taken from Antarctic minke whales. While the number of samples is small, genetic analysis of samples from areas not covered by previous genetic surveys is recommended (e.g., Area I).

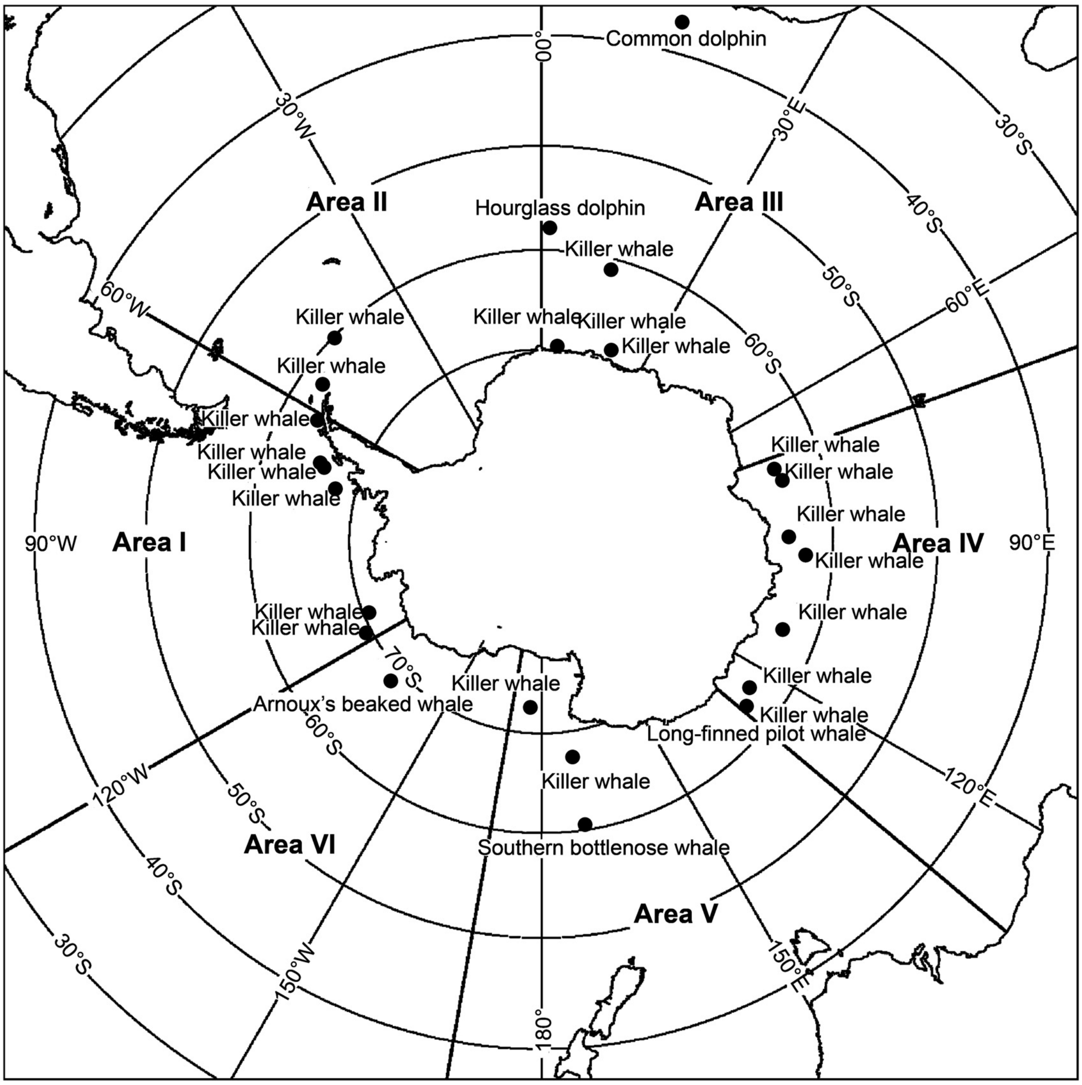

Fig. 10. Geographical distribution of biopsy samples of small whale and dolphin species collected during the SOWER Antarctic cruises. Positions are based on the first sighting position. On some occasions, samples were obtained from more than one individual from the same school. In these cases, a single symbol may represent more than one sample.

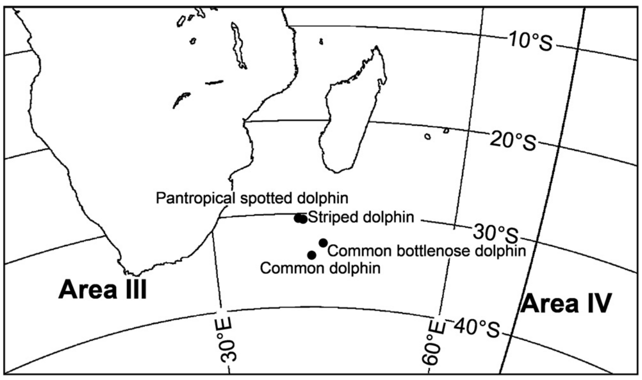

Fig. 11. Geographical distribution of biopsy samples of small whale and dolphin species from the 1996/97 SOWER cruise (low-latitude blue whale cruise). Positions are based on the first sighting position. On some occasions, samples were obtained from more than one individual from the same school. In these cases, a single symbol may represent more than one sample.

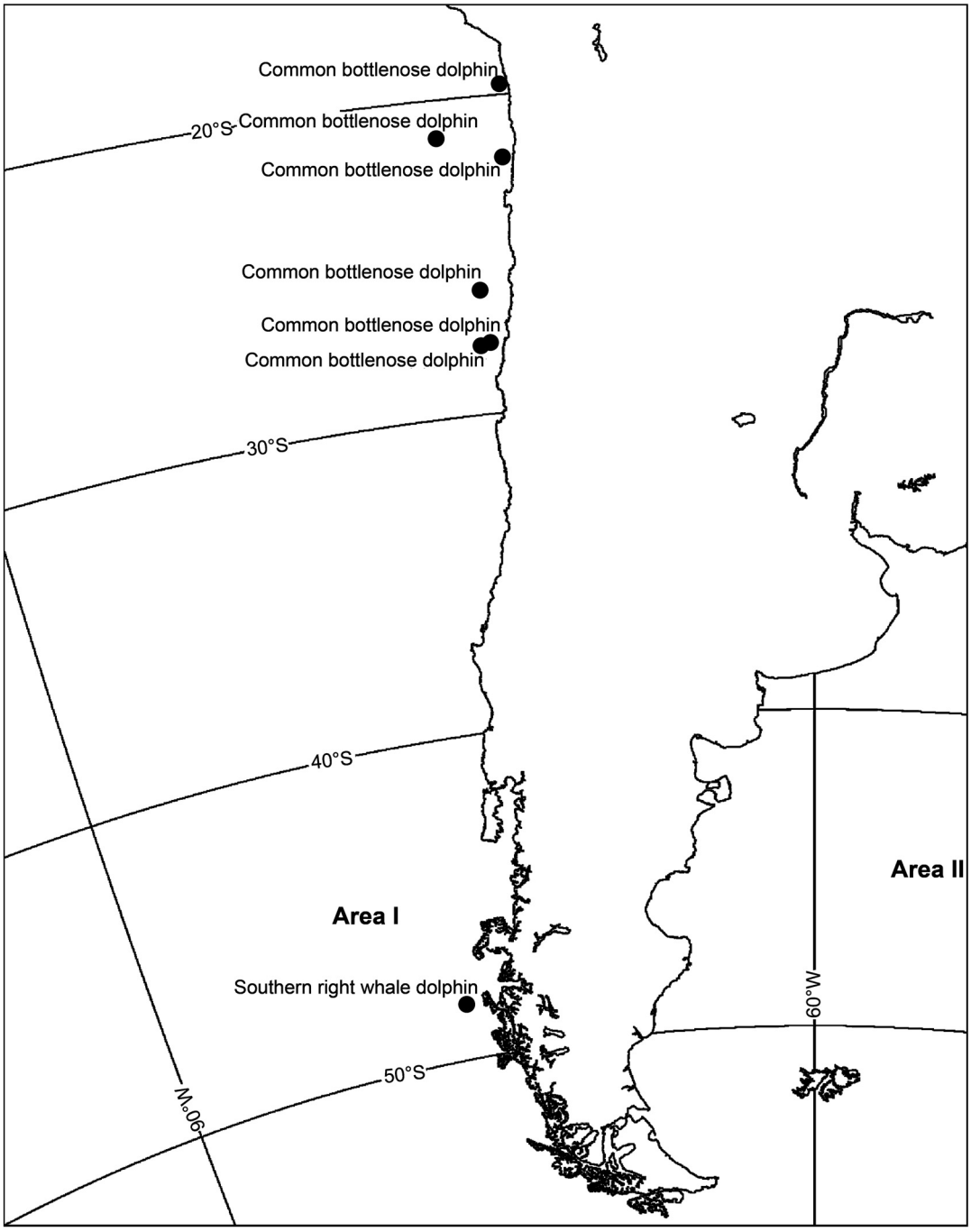

Fig. 12. Geographical distribution of biopsy samples of small whale and dolphin species from the 1997/98 SOWER cruise (low-latitude blue whale cruise). Positions are based on the first sighting position. On some occasions, samples were obtained from more than one individual from the same school. In these cases, a single symbol may represent more than one sample.

This study has focused on biopsy sampling and genetic analyses of large whales. The SOWER cruises also obtained biopsy samples from smaller cetaceans in the Antarctic, south of Madagascar and in Chilean waters (Figs. 10–12). Some analyses of these samples have already been published (Sanino *et al*., 2005).

The samples are an important legacy of the SOWER programme and form a valuable archive of genetic diversity and population structure of great whales in the Southern Ocean. There are several important reasons for recommending that this effort be continued in the future: (a) the Antarctic is a key area for large-whale recovery after severe human exploitation; (b) the Antarctic is highly inaccessible without this kind of collaborative platform; (c) biopsy samples are not only useful for genetic analyses but also potentially for other studies, such

as analyses of pollutants, stable isotopes, fatty acids and epigenetic aging. In addition to the biological information obtained from genetic analysis of the samples, SOWER cruises were important platforms for the development of biopsy sampling systems in the Antarctic and low-latitude waters of the Southern Hemisphere, and as a means of training scientists in the application of these systems.

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