# Stable isotope analyses of feather amino acids identify penguin migration strategies at ocean basin scales.

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**Abstract:** Identifying the at-sea distribution of wide ranging marine predators is critical to understanding their ecology. Advances in electronic tracking devices and intrinsic biogeochemical markers have greatly improved our ability to track animal movements on ocean-wide scales. Here we show that, in combination with direct tracking, stable carbon isotope analysis of essential amino acids in tail feathers provides the ability to track the movement patterns of two, wide-ranging penguin species over ocean basin scales. In addition, we use this isotopic approach across multiple breeding colonies in the Scotia Arc to evaluate migration trends at a regional scale that would be logistically challenging using direct tracking alone.

Keywords: migration, geolocation (GLS), seabird, stable isotopes

## 1. Introduction

Identifying the at-sea distribution of wide ranging marine animals is critical to aid in their conservation [1] and advances in electronic tracking devices have revolutionized our ability to track animal movements on ocean-wide scales [2]. However, tracking studies can be limited in scale due to logistical, financial and ethical constraints. Intrinsic biogeochemical markers that retain spatial information, including stable isotope analysis (SIA), have therefore been used to complement direct tracking [3]. SIA can increase the scale of tracking studies by examining a greater number of individuals and/or locations to better generalize population-level movements [4]. However, interpreting bulk tissue SIA can be challenging because it is often difficult to distinguish the influence of a consumer's diet (i.e. what it eats) from geographic differences in isotopic values (i.e. where it is eating) [3, 5].

Compound-specific SIA of amino acids (CSIA-AA) may offer a solution to the bulk SIA problem of distinguishing between diet and geographic differences as some individual amino acids (AAs) faithfully reflect ecosystem baseline isotopic values that can be used to independently evaluate animal movement [5]. However, few studies have applied CSIA-AA at ocean basin scales and most have focused on nitrogen isotopes [5, 6]. Carbon isotope values ($\delta^{13}C$) of essential AA are also likely to be useful for estimating movement patterns of wide-ranging marine species. This is because essential AAs transfer from diet without alteration and reflect primary producer community composition at the base of geographically distinct food webs [7-9]. For example, one recent study found geographic variation in penguin chick AA $\delta^{13}C$ values with latitude, thought at the time they cautioned that using AA $\delta^{13}C$ to track forging locations may not be possible [9].

52 The goal of this research is to test the ability of $\delta^{13}C$ CSIA-AA to discriminate among
53 three migrations strategies identified by archival geolocation tags (GLS) [10] in two wide-
54 ranging species, the Adélie (*Pygoscelis adeliae*) and Chinstrap (*P. antarctica*) penguin. We then
55 use this technique to assign migration strategies to untracked individual Chinstrap penguins from
56 multiple breeding colonies to evaluate regional migration trends at population-level scales.
57
58 **2. Material and methods**
59 Breeding adult Chinstrap and Adélie penguins from Cape Shirreff, Livingston Island and
60 Admiralty Bay, King George Island (Table 1 and 2) were tagged during 2011/12 breeding
61 season with Lotek Nano-Lat 2900-series GLS (Lotek Wireless, Inc.) and recaptured the
62 following year (2012/13). Tags provided daily estimates of latitude and longitude over the
63 austral winter. At recapture a central tail feather was collected, a proximal section of which
64 reflected a late-March to early-June growth period when penguins were migrating to or
65 inhabiting their winter foraging areas [10]. We restricted our spatial analyses to penguins that
66 had GLS data within the window of tail-feather synthesis and isotopic incorporation (i.e. 40-
67 100 days following the onset of molt; Adélie penguins: 25 March - 24 May; chinstrap
68 penguins: 10 April - 9 June). Details on GLS data processing, feather growth rates, and bulk
69 $\delta^{13}C$ values are provided in Hinke et al. [10]. In 2012/13 we collected tail feathers from
70 additional, untracked breeding adult Chinstrap penguins from five breeding sites (Table 2).
71 Tail feather sections (20 mg each) were acid hydrolyzed, derivatized and analyzed for
72 CSIA-AA following the methods outlined McMahon et al. [11]. Samples were analyzed in
73 duplicate with AA and fish muscle standards of known isotopic composition (mean
74 reproducibility: AA standard: ±0.2 ‰; internal fish standard: ±0.6 ‰). We focused on bulk

feather $\delta^{13}C$ and five essential AAs (threonine, isoleucine, valine, phenylalanine, and leucine) and used linear discriminant analyses (LDA) in program R (ver. 2.15.3) [12] with leave-one-out cross-validation to differentiate among the three migration strategies observed by Hinke et al. [10]: Adélie penguins migrating eastward from their breeding sites into the Weddell Sea, Chinstrap penguins migrating eastward into the Scotia Sea, and Chinstrap penguins migrating westward to the Pacific sector of the Southern Ocean (Table 1, Fig. 1). We then used LDA to discriminate between the two Chinstrap penguin migration strategies in isolation and assign untracked individuals to specific migration strategies. We evaluated regional migration trends using only Chinstrap penguins with known migration patterns (GLS) and those that were assigned based on CSIA-AA with ≥ 80% probability of group membership [4, 5].

We also applied a Bayesian mixing-model approach [13] in program R [12] to obtain a probability distribution of migration strategies at the five Chinstrap penguins breeding sites examined. We used essential AAs $\delta^{13}C$ values of GLS tracked Chinstrap penguin as source end-members (eastward vs. westward), and values of all penguins by breeding site regardless of if their migration status was known. We used a small non-zero trophic discrimination factor in the model (0.1 ± 0.1‰) [7] and ran 1 million iterations, thinned by 15, with an initial discard of 40,000 resulting in 64,000 posterior draws.

## 3. Results

LDA classification using AA $\delta^{13}C$ out-performed bulk $\delta^{13}C$ and provided clear separation in canonical multivariate space (Wilk's lambda = 0.16, $P < 0.001$; Table 1, Fig. 1). Individuals misclassified by AA $\delta^{13}C$ were assigned as Chinstrap penguins migrating

eastward. AA $\delta^{13}C$ LDA accuracy was ≥ 89.3% for Chinstrap penguins only (Wilk's lambda = 0.34, $P < 0.001$) and out-performed bulk $\delta^{13}C$ (Table 1).

Migration strategies for 59 of the 66 untracked Chinstrap penguins were assigned with ≥ 80% probability. When combined with individuals of known migration status, a majority of Chinstrap penguins exhibited "Pacific" isotopic signatures, consistent with a westward migration (81.7%). However, we also observed a relatively higher number of individuals exhibiting a "Scotia Sea" signature at sites located farther north and east (Table 2; Fig. 2). This was confirmed by our mixing-model approach, with 95% credibility intervals around the contribution of eastward vs. westward migrants overlapping only at the most northeastern breeding site (Table 2; Fig. 2).

## 4. Discussion

Essential AA $\delta^{13}C$ values in tail feathers successfully discriminated between the winter migrations strategies observed in Adélie and Chinstrap penguins. This approach provided more accurate classifications than bulk $\delta^{13}C$ and successfully differentiated species-specific habitat niches between eastward moving Adélie and Chinstrap penguins (into the ice-covered Weddell Sea vs. ice free Scotia Sea, respectively) [10]. In addition, our results were unaffected by trophic biases [5, 8] as essential AA in penguin tail feathers most likely reflect only the baseline $\delta^{13}C$ values in their specific wintering area [8]. Differences in baseline $\delta^{13}C$ values across wintering areas in this study may be driven by differences in the phytoplankton and/or sea-ice algae community composition and sources of inorganic carbon [14, 15].

Differences in essential AA $\delta^{13}C$ values among eastward vs. westward migrating Chinstrap penguins also provided a basis for assignment of untracked individuals. This allowed

us to expand the overall sample sizes (i.e. number of individuals) and spatial scope (i.e. number and range of breeding sites) of our study to confirm that the dominant migration strategy of chinstrap penguins from the Antarctic Peninsula region and southern Scotia Sea is westward. One possible hypothesis for this trend is competitive avoidance as the Scotia Sea is home to large wintering populations of Macaroni (*Eudyptes chrysolophus*) and southern rockhopper (*E. chrysocome chrysocome*) penguins [16]. In addition, we identified a spatial trend with a relatively higher number of eastward migrating individuals at sites located farther northwards and eastwards (Fig. 2). This may suggest that the location of breeding sites influences migration patterns. Following this trend, one might expect individuals breeding in the South Sandwich Islands to remain in the Scotia Sea during winter, as this archipelago is the farthest northeast and contains the largest Chinstrap penguin breeding population [17]. If so, this might serve as a source of intra-specific competition and further explain dominance of westward migration strategies of Chinstrap penguins from our study sites. An alternate explanation is some individuals from northeastern colonies may obtain a "Scotia Sea" isotopic signature while migrating westward towards the Pacific.

In summary, to our knowledge this research represents the first use of essential AA $\delta^{13}C$ values to track the migration routes and at-sea distribution of a wide-ranging marine predator. While the spatial resolution of essential AA $\delta^{13}C$ is coarse compared to direct tracking, this approach can significantly expand the scope of studies and help facilitate inference about individual and population processes in far-ranging marine species. Future studies that elucidate spatial gradients in oceanic isotopic baselines will further refine our ability to track marine animal movements over ocean basin scales.

**Ethics.** Field work was conducted via an Antarctic Conservation Act permit (ACA 2013-007) and animal use approved by WHOI (27071382) and UCSD (S05480) IACUC.

**Data accessibility.** GLS and isotope data are available online at https://swfsc.noaa.gov/AERD-Data/

**Authors' contributions.** Study design: M.J.P, J.T.H, S.R.T.; Fieldwork: M.J.P, J.T.H, T.H., M.S.; Data analysis: M.J.P, J.T.H, T.H., L.H.; Manuscript: M.J.P; All authors revised and gave final approval for publication and agree to be held accountable for the work performed therein.

**Competing interests.** We have no competing interests.

**Funding.** Funded by the National Marine Sanctuary Foundation (GLS tags), Ocean Life Institute (M.J.P, L.H., S.R.T), Darwin Initiative (T.H.), and SeaWorld Bush Gardens Conservation Fund (M.J.P, S.R.T).

**Acknowledgements.** We thank the US AMLR program, Instituto Antártico Argentino, and Quark Expeditions, K. McMahon, and P. McDowell for field and analysis assistance.

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**Table 1.** Mean±SD $\delta^{13}C$ values and classification accuracies from GLS tracked penguins exhibiting three differing winter migration strategies. Parentheses identify individuals from either Admiralty Bay or Cape Shirreff, and LDA classifications excluding Adélie penguins.

**Table 2.** Mean±SD essential AA $\delta^{13}C$ values and assigned winter migration strategies of Chinstrap penguins from five breeding locations. Parentheses identify GLS tracked individuals at each site.

**Figure 1.** Indices of A) geographic habitat utilization and B) multivariate discrimination based on essential AA $\delta^{13}C$ values of C) Adélie and Chinstrap penguins. Habitat utilization data modified from Hinke et al. [10]. Dotted lines represent 50% probability of assignment.

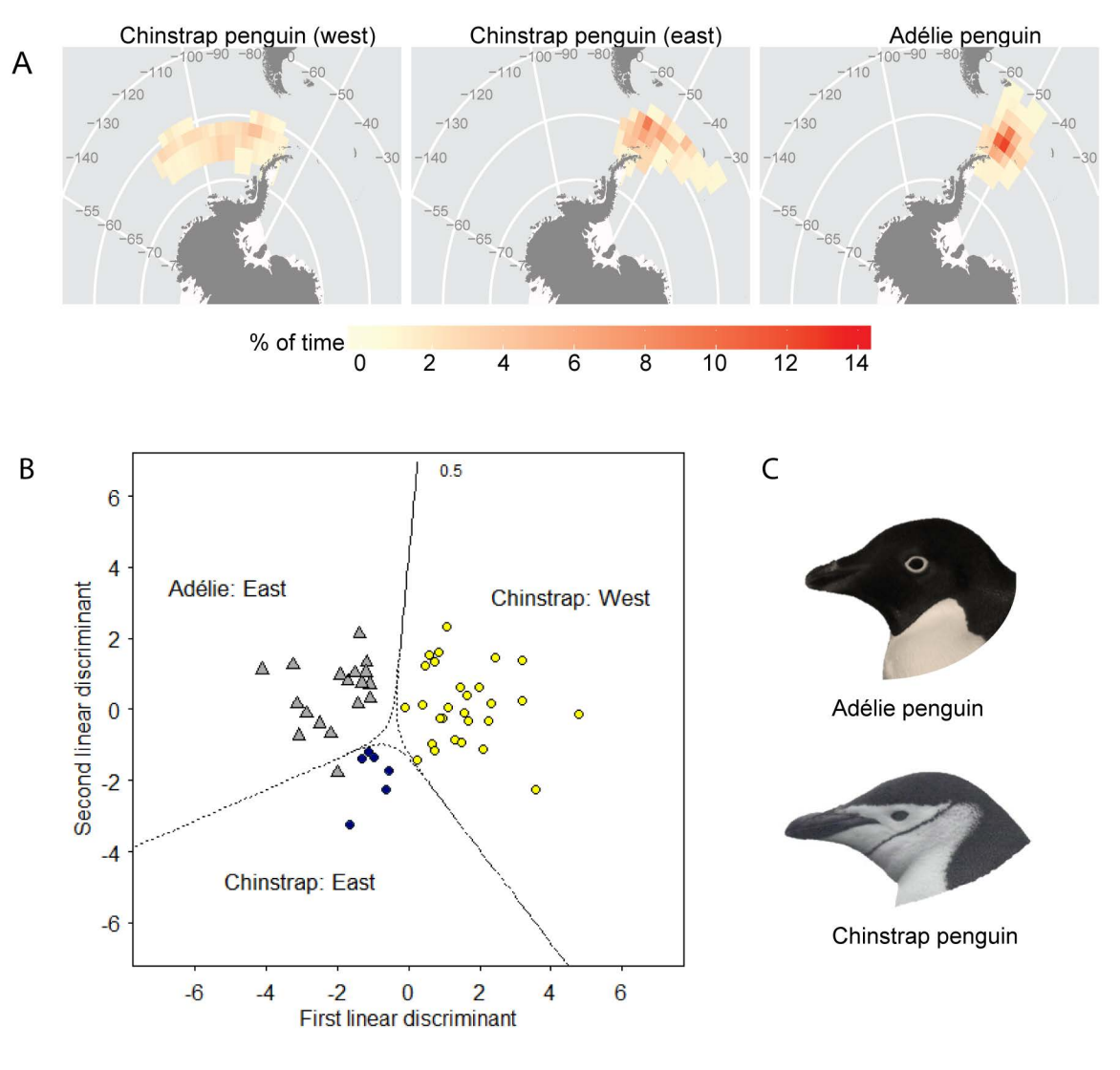
**Figure 2.** A) Multivariate discrimination of tracked (colored points) and untracked (white points) Chinstrap penguins based on essential AA $\delta^{13}C$ values and B) assigned winter migration strategies (eastward or westward) in Chinstrap penguins from five breeding locations using LDA (pie charts) and stable isotope mixing-models (histograms). Dotted line represents 50% probability of assignment.

**Table 1.**

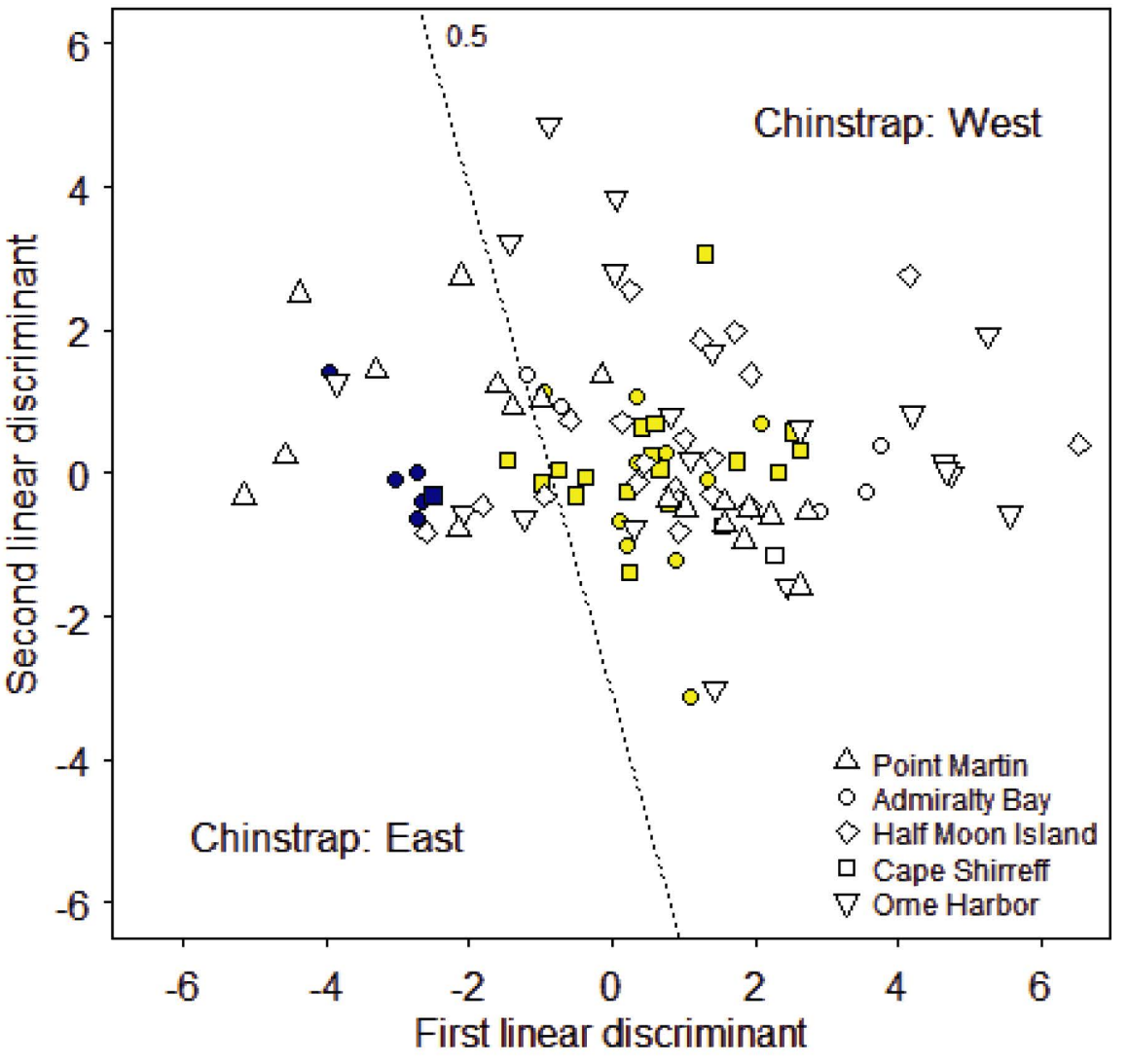
| GLS tracked penguins | Adélie penguin | Chinstrap penguin | |
|---|---|---|---|
| | East, Weddell Sea | East, Scotia Sea | West, Pacific sector |
| *n* | 18 *(18,0)* | 6 *(5,1)* | 28 *(10,18)* |
| $\delta^{13}C$ (‰) | | | |
| *Bulk feather* | -24.3±0.3 | -24.5±0.5 | -22.8±0.6 |
| *Valine* | -30.7±0.7 | -29.7±0.4 | -27.9±0.9 |
| *Isoleucine* | -20.4±2.1 | -17.7±0.9 | -19.4±1.5 |
| *Leucine* | -34.9±0.7 | -33.4±1.7 | -33.4±1.7 |
| *Threonine* | -14.1±1.7 | -11.4±1.5 | -11.7±2.6 |
| *Phenylalanine* | -30.2±0.7 | -30.1±0.4 | -28.7±1.5 |
| LDA (%) | | | |
| *Bulk $\delta^{13}C$* | 66.7 | 33.3 (83.3) | 82.1 (82.1) |
| *Essential AA $\delta^{13}C$* | 94.4 | 100.0 (100.0) | 96.4 (89.3) |

**Table 2.**

| Breeding site | South Orkney Islands | South Shetland Islands | | | Western Antarctic Peninsula |
|---|---|---|---|---|---|
| | Point Martin, Laurie Is. | Admiralty Bay, King George Is. | Cape Shirreff, Livingston Is. | Half Moon Is., Livingston Is. | Orne Harbour, Arctowski Peninsula |
| Lat., Long. | 60.76°S, 44.68°W | 62.17°S, 58.45°W | 62.47°S, 60.78°W | 62.58°S, 62.58°W | 64.62°S, 62.53°W |
| *n* | 20 (0) | 20 (15) | 20 (19) | 20 (0) | 20 (0) |
| $\delta^{13}$C (‰) | | | | | |
| *Valine* | -27.9±1.8 | -28.5±1.1 | -27.8±1.0 | -27.2±1.3 | -27.2±2.1 |
| *Isoleucine* | -18.8±1.9 | -19.5±2.0 | -19.1±1.5 | -19.5±2.6 | -21.0±1.6 |
| *Leucine* | -32.7±2.1 | -33.3±1.7 | -33.5±1.6 | -32.4±1.8 | -33.9±1.6 |
| *Threonine* | -12.1±3.4 | -11.6±2.7 | -11.5±2.2 | -12.1±2.7 | -10.5±4.6 |
| *Phenylalanine* | -30.2±1.9 | -29.1±1.2 | -28.6±1.6 | -30.7±1.6 | -30.5±1.6 |
| LDA (%) | | | | | |
| *East* | 38.9 | 26.3 | 5.0 | 10.5 | 11.8 |
| *West* | 61.1 | 73.7 | 95.0 | 89.5 | 88.2 |
| Mixing-model (%) | | | | | |
| *East* | 32.6 (2.1-58.7) | 23.8 (5.9-41.3) | 9.0 (0.0-19.9) | 10.0 (0.0-28.5) | 11.5 (0.0-32.7) |
| *West* | 67.4 (41.3-97.9) | 76.2 (58.7-94.1) | 91.0 (80.1-100) | 90.0 (71.5-100) | 88.5 (67.3-100) |


A
Chinstrap penguin (west)
Chinstrap penguin (east)
Adélie penguin
% of time
0 2 4 6 8 10 12 14
B
0.5
Adélie: East
Chinstrap: West
Chinstrap: East
Second linear discriminant
First linear discriminant
C
Adélie penguin
Chinstrap penguin

A


0.5
Chinstrap: West
Chinstrap: East
Point Martin
Admiralty Bay
Half Moon Island
Cape Shirreff
Orne Harbor
Second linear discriminant
First linear discriminant
6
4
2
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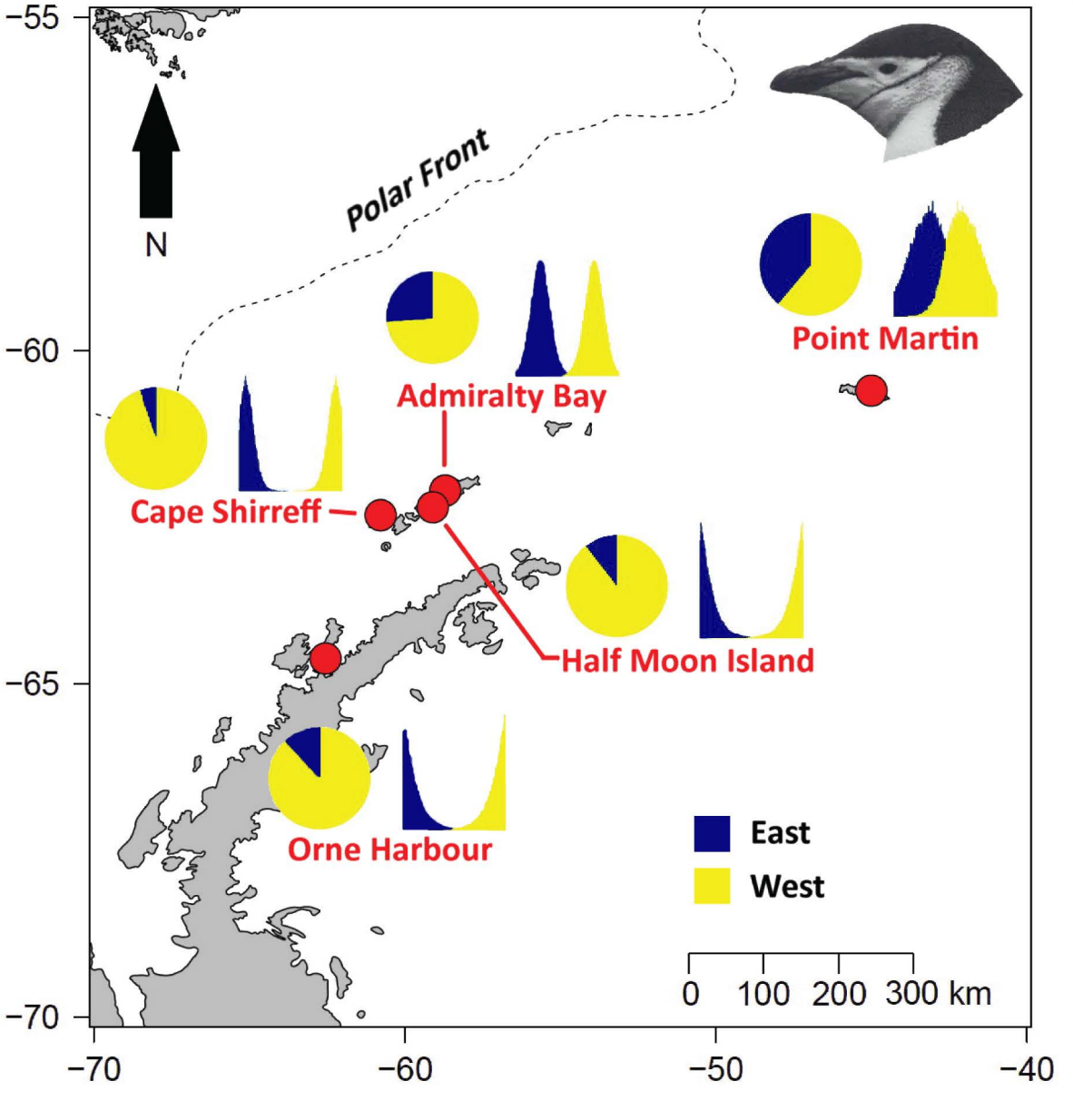
-2
-4
-6

B


Polar Front
N
Point Martin
Admiralty Bay
Cape Shirreff
Half Moon Island
Orne Harbour
East
West
0 100 200 300 km
−55
−60
−65
−70
−70
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−50
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