Environmental Research 242 (2024) 117636

Contents lists available at ScienceDirect

Environmental Research

journal homepage: www.elsevier.com/locate/envres

Molecular evidence for multiple origins and high genetic differentiation of non-native winter crane fly, *Trichocera maculipennis* (Diptera: Trichoceridae), in the maritime Antarctic

Seunghyun Kang [a], Sanghee Kim [a], Kye Chung Park [b], Andrius Petrašiūnas [c], Hyung Chul Shin [a], Euna Jo [a], Sung Mi Cho [a], Ji Hee Kim [a,*]

[a] Korea Polar Research Institute, Incheon, 21990, South Korea
[b] The New Zealand Institute for Plant and Food Research Ltd., Christchurch, 8140, New Zealand
[c] Department of Zoology, Institute of Biosciences, Vilnius University Life Sciences Center, LT 1022, Vilnius, Lithuania

ARTICLE INFO

Keywords:
Antarctica
Biological invasion
Winter crane fly
King George Island
Microsatellite analysis
mtDNA COI

ABSTRACT

Native biodiversity and ecosystems of Antarctica safeguarded from biological invasion face recent threats from non-native species, accelerated by increasing human activities and climate changes. Over two decades ago, the winter crane fly, *Trichocera maculipennis*, was first detected on King George Island. It has now successfully colonized several research stations across King George Island. To understand the origin, genetic diversity, and population structure of this Holarctic species, we conducted mitochondrial DNA cytochrome *c* oxidase subunit I (COI) sequence analysis across both its native and invasive ranges. In parallel, we performed microsatellite loci analysis within the invasive ranges, utilizing 12 polymorphic microsatellite markers. Furthermore, we compared body sizes among adult males and females collected from three different locations of King George Island. Our COI sequence analysis exhibited two different lineages present on King George Island. Lineage I was linked to Arctic Svalbard and Polish cave populations and Lineage II was related to Canadian Terra Nova National Park populations, implying multiple origins. Microsatellite analysis further exhibited high levels of genetic diversity and significant levels of genetic differentiation among invasive populations. Body sizes of adult *T. maculipennis* were significantly different among invasive populations but were not attributed to genetics. This significant genetic diversity likely facilitated the rapid colonization and establishment of *T. maculipennis* on King George Island, contributing to their successful invasion. Molecular analysis results revealed a substantial amount of genetic variation within invasive populations, which can serve as management units for invasive species control. Furthermore, the genetic markers we developed in the study will be invaluable tools for tracking impending invasion events and the travel routes of new individuals. Taken together, these findings illustrate the highly invasive and adaptable characteristics of *T. maculipennis*. Therefore, immediate action is necessary to mitigate their ongoing invasion and facilitate their eradication.

1. Introduction

Until about the midpoint of the last century, the native biodiversity and ecosystems of Antarctica were believed to be safeguarded from biological invasion due to the Antarctic circumpolar current and extreme weather. However, indigenous terrestrial and marine ecosystems in Antarctica are threatened by the introduction of non-native species due to rapid global climate change in addition to the impact of species that have already become invasive. Risks of invasion are getting higher due to rising levels of human activities in Antarctic regions (Frenot et al., 2005; Chown et al., 2012; Hughes et al., 2019, 2020; Bartlett et al., 2021; Chwedorzewska et al., 2020). Numerous invasive species have extensive geographic ranges and wide climatic tolerances, which may influence how they react to climate change (Hellmann et al., 2008).

Antarctica, particularly Antarctic Peninsula region including the maritime Antarctic Islands, has seen the largest temperature increase almost 3 °C (5.4 °F) since the middle of the 20th century, causing

* Corresponding author. Korea Polar Research Institute, 26 Songdomirae-ro, Yeonsu-gu, Incheon, South Korea.
E-mail address: jhalgae@kopri.re.kr (J.H. Kim).

https://doi.org/10.1016/j.envres.2023.117636
Received 15 September 2023; Received in revised form 7 November 2023; Accepted 8 November 2023
Available online 11 November 2023
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significant glacier retreat and reduction of ice cover (Cook et al., 2005; Thomas et al., 2009). This region has relatively higher ice-free area (~3 %) than the whole continent (ca. 0.2 %). It supports the most abundant terrestrial and freshwater ecosystems in Antarctica (Contador et al., 2020). Invertebrates, which have a relatively low species richness and restricted distribution to the ice-free area, account for the majority of the terrestrial fauna (Block, 1984). They have narrow habitat ranges, simple community structure, and stereotypic life history. These characteristics make the communities of terrestrial invertebrate vulnerable to invasion (Hughes and Convey, 2012; Houghton et al., 2016).

Within the Antarctic Treaty area (the area south of latitude 60° S), intentional introduction of non-native species without a permit from a Treaty Party's governmental authority under Annex II of the Protocol on Environmental Protection to the Treaty is prohibited. The issue of inadvertent non-native species introduction has been the biggest concern for Antarctic conservation since the protocol's implementation in 1998 (Hughes et al., 2019, 2020). Currently, the cause of biological invasion has mostly been related to human activities such as research programs and tourism in Antarctica (McGeoch et al., 2015). There are some detailed investigations on pathways of unintentional introduction of alien invertebrates connected with national Antarctic programs (Chwedorzewska et al., 2013; Houghton et al., 2016). A substantial proportion of inadvertent introductions are insects, many of which have established successfully, with subsequent impacts on the Antarctic biota (Chown and Convey, 2016). About one-third of non-native species (up to 200 species) known to be established in Antarctica and Sub-Antarctic islands are insects (Frenot et al., 2005; Chown and Convey, 2016).

In Antarctic Peninsula regions, two species of macro-arthropods live in natural environment or in synanthropic habitats. A wingless midge, *Ereptmoptera murphyi* (Diptera: Chironomidae), was introduced from Sub-Antarctic Islands. It has already become invasive in the region (Hughes et al., 2013; Chown and Convey, 2016; Bartlett et al., 2021). The other is a winged winter crane fly, *Trichocera maculipennis* (Diptera: Trichoceridae) (Supplementary Fig. S1). Known as a Holarctic species, it has a wide natural occurrence across Europe, North America, Asia, and India, ranging from temperate regions to Arctic regions (Dahl, 1970a; Petrašiūnas and Podenas, 2017; Potocka and Krzmińska, 2018).

The initial detections of the non-native winter crane fly in King George Island, the maritime South Shetland Islands, were reported about 15–20 years ago (Volonterio et al., 2013; Potocka and Krzmińska, 2018), whereas its introduction to the Sub-Antarctic Kerguelen Island took place much earlier about 80 years ago (Dahl, 1970b). The majority of non-native fly populations on King George Island at various points have colonized sewage systems of the seven Antarctic stations where they are currently established (Potocka and Krzmińska, 2018; León et al., 2021). Some infected stations (Uruguay and Republic of Korea) attempted separately to eliminate these flies from their facilities. However, these flies were found again from the facilities, indicating that either the eradication process was not completed or that individuals from the natural ecosystem or local synanthropic population had recolonized the stations (Volonterio et al., 2013; León et al., 2021). A new introduction from the outside of Antarctica is also conceivable. The majority of Antarctic Treaty parties operating stations on the island are taking part in a joint program and working together to identify appropriate management techniques in order to provide effective monitoring and control of non-native flies.

The populations of recently introduced invasive species often show low genetic diversity than native populations during founding events (Puillandre et al., 2008). This reduced genetic diversity observed in newly colonized ranges is likely to be the result of a 'founder effect' as new habitats are colonized typically by a few individuals carry only a small portion of allelic diversity of source populations (Nei et al., 1975; Tsutsui and Suarez, 2003; Lockwood et al., 2013). When the size of founder populations remains small over many generations, they may lose most of their genetic variations due to genetic drift, leading to a high inbreeding rate, a process known as a genetic bottleneck (Nei et al., 1975). On the other hand, when founding individuals are from multiple origins, founding populations might show relatively high genetic diversity (Davis, 2009). This may facilitate local adaptation to new environments and increase new trait diversity which can lead to successful invasion (Facon et al., 2006). Besides, when introduction involves many founder individuals, they may also show large fraction of genetic diversity (Roman, 2006). To ensure that appropriate management and biosecurity measures can be put in place, it is essential to understand the routes for introduction and later dispersal within the region (Bartlett et al., 2021). The degree of genetic variation is a significant factor influencing the longevity and adaptive potential of founder individuals of alien species (Lee, 2002; Facon et al., 2006).

DNA markers have been used to study the genetic diversity and how different populations are structured in various species. Each molecular marker represents different points in time in the historical demography of species. Mitochondrial DNA (mtDNA) is more suited to detect genetic differentiation of larger geographic scale than nuclear DNA because its maternal inheritance requires one quarter of the effective population size (Chen et al., 2004; Jung et al., 2007; O'Loughlin et al., 2007). Particularly, the number of incursions can be inferred using mtDNA which can estimate the number of genetically unique female founders (Vincek et al., 1997; Ficetola et al., 2008; Dickey et al., 2013; Tay et al., 2016). In general, each unique mtDNA haplotype observed in invading sites is considered as a separate and independent incursion event (Ascunce et al., 2011; Goldstien et al., 2011) if introduced populations are found in distant locations and each population has a unique mtDNA haplotypic lineage (Castalanelli et al., 2011; Goldstien et al., 2011). On the other hand, microsatellite, which is co-dominantly inherited and highly polymorphic (Zong et al., 2015), has been used to detect contemporary gene flow among populations (Chase et al., 1996) and genetic structure of a fine geographic scale (Ma et al., 2001).

Trichocera maculipennis population established in 2017 at the Arctowski Polish Antarctic Station in Admiralty Bay, King George Island, was found to have European ancestry according to molecular analysis by Polish researchers (Potocka et al., 2020). However, the information about its origin appeared to be insufficient to understand its populations that have persisted in stations of Maxwell Bay of the island. In addition, although it has not been explicitly confirmed that this non-native fly has established in the natural environment, its life-history characteristics and thermal tolerance make it likely (León et al., 2021; Pertierra et al., 2021). It becomes more difficult to remove or control a non-native fly if it can reproduce in both natural and anthropogenic environments of Antarctica. Thus, it is urgent to determine the potential influence of this non-native fly on native fauna and its potential for outdoor reproduction.

This study aimed to determine possible origins (source locality), population genetic structure, and levels of genetic diversity within populations of the non-native winter crane fly, *T. maculipennis*, recently settled in Antarctic research stations of King George Island.

2. Materials and methods

2.1. Sample collection

Specimens for mtDNA COI analysis were collected from four different locations (Chilean Frei Base, Russian Bellingshausen Station, Uruguayan Artigas Base and Korean King Sejong Station) (Fig. 1) in King George Island. A total of 14 sequences were used to analyze, consisting of 10 sequences of individuals taken from the four stations during the 2017/2018, 2018/2019, and 2019/2020 Antarctic summer seasons (Frei: five indv., Bellingshausen: one indv., Artigas: two indv., and King Sejong: two indv.) and one sequence of an individual captured from Artic (Svalbard).

Population-level microsatellite variation was investigated based on 118 specimens from five populations at Antarctic stations during the 2017/2018, 2018/2019, and 2019/2020 Antarctic summer seasons

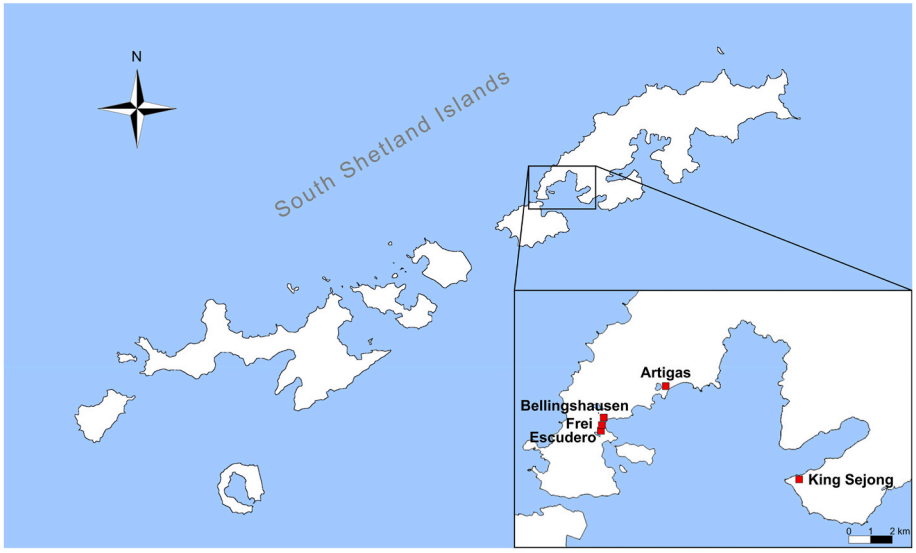

Fig. 1. Geographic locations of the five invasive *T. maculipennis* populations sampled from King George Island in Antarctica.

(Frei: five indv., Bellingshausen: 23 indv., Escudero: 30 indv., King Sejong: 30 indv., and Artigas: 30 indv.).

2.2. Mitochondrial DNA gene sequencing

Total DNA was extracted from the entire body following a standard protocol of a DNeasy Blood and Tissue kit (Qiagen, Valencia, CA, USA). Polymerase chain reaction (PCR) was carried out using an EmeraldAmp® GT PCR Master Mix (TaKaRa Biotechnology, Dalian, China). PCR mixtures were prepared in a total volume of 25 μl containing 12.5 μl of 2X premix, 11.5 μl of sterilized distilled water, 0.25 μl of each primer (0.2 μM), and 0.5 μl of template DNA. To amplify 658-bp COI gene fragments of mtDNA, LCO1490 and HCO2198 primers (Folmer et al., 1994) were used. PCR conditions were as follows: initial denaturation at 94 °C for 3 min, 35 cycles of denaturation at 95 °C for 15 s, annealing at 45 °C for 30 s, elongation at 72 °C for 1 min, and final extension at 72 °C for 7 min. PCR products were sequenced by Macrogen Inc. (Seoul, Republic of Korea) on an ABI 3730xl DNA Analyzer (Applied Biosystems, Waltham, MA, USA).

2.3. Microsatellite marker development and genotyping

We developed 12 polymorphic microsatellite loci (Supplementary Table S1) using the Illumina Miseq platform (Illumina, San Diego, CA, USA). DNA samples from multiple individuals were collected in 2016 from King Sejong Station in King George Island, Antarctica. Total DNA was extracted from the entire body using a DNeasy Blood and Tissue kit (Qiagen). Microsatellite sequence containing regions were identified using a QDD3 software (Meglécz et al., 2014). Variability of each locus was screened by genotyping 30 individuals from the same collection site. Primers used for PCR were designed using Primer3 software (Rozen and Skaletsky, 2000). Forward primers including M13 tails were attached at the 5′ end (FAM: TTTCCCAGTCACGACGTTG, VIC: TAAAACGACGGCCAGTGC and PET: GCGGATAACAATTTCACACAGG). Reverse primers were pigtailed at the 5' end (GTTTCTT). A multiplex PCR reaction with a final volume of 16 μl was performed in a mixture containing 8 μl of 2 × Multiplex PCR master mix (Qiagen), 0.5 μl of DNA template, 0.05 μM of each M13-tailed forward primer, 0.5 μM of each pigtailed reverse primer, and 0.1 μM of each fluorescently-labeled M13 primer. PCR cycle conditions were as follows: initial denaturation at 95 °C for 15 min, 41 cycles of denaturation at 95 °C for 30 s, annealing at a specific temperature for 90 s (seven cycles at 65 °C, seven cycles at 61 °C, seven cycles at 58 °C and 21 cycles at 55 °C), elongation at 72 °C for 30 s, and a final extension at 72 °C for 20 min using a Mastercycler (Eppendorf, Hamburg, Germany). QIAxcel (Qiagen) with a QIAxcel DNA Screening Kit (Qiagen) was used for PCR amplification and visualizing sizes of amplified fragments. Obtained fragments were run on an ABI 3730 DNA Analyzer with a GeneScan 500 LIZ Size Standard (Applied Biosystems, Foster City, CA, USA) and analyzed with GeneMarker 1.85 (SoftGenetics, State College, PA, USA).

2.4. Data analysis of COI sequences

Three COI sequences including the sequence from Arctowski Polish Antarctic Station uploaded at NCBI (MH378440.1), one Canadian Terra Nova National Park sequence (KR386810.1), and one Poland Jaskinia (cave) Pod Sokola sequence (MK517414.1) were also analyzed together. Two sequences of *Trichocera parva* (MK517412.1) and *T. saltator* (MN868729.1) species were used as an outgroup. These COI sequences were aligned with CLUSTAL X version 2.0 (Larkin et al., 2007) and transformed to NEXUS and PHYLIP formats for data analyses. The frequency of each haplotype and number of haplotypes were calculated using DnaSP version 5 (Librado and Rozas, 2009). A haplotype network was depicted using PopART version 1.7 to visualize relationships among haplotypes of COI sequences (Leigh and Bryant, 2015). To examine the phylogenetic relationship among sequences, Neighbor-joining method with Kimura 2 parameter and bootstrap iteration 1,000 were used with a MEGA X software (Tamura et al., 2011).

2.5. Data analysis of microsatellites

Summary statistics of microsatellite loci such as the number of alleles (*Na*), the number of effective alleles (*Ne*), Shannon's information index (*I*),polymorphism information content (PIC), observed (*Ho*) and expected (*He*) heterozygosity, analysis of molecular variance (AMOVA), principal coordinates analysis (PCoA), and the inbreeding coefficient (*Fis*) were calculated using GenAlEx 6.5 software (Peakall and Smouse, 2012). Deviation from Hardy–Weinberg equilibrium (HWE) for all populations was tested using a GENEPOP ver. 4.2 software (Rousset, 2008) as implemented for online use (http://genepop.curtin.edu.au/genepop_op1.html). The occurrence and frequency of null alleles were calculated with a Micro-Checker 2.2.3 software (Van Oosterhout et al., 2004). The distance matrix of pairwise *Fst* (a measure of alternative fixation of alleles) and *Rst* (analogous to *Fst* but using allele size) with permutations of 1,000 was evaluated by ARLEQUIN 3.5.2.2 (Excoffier and Lischer, 2010). Population structure patterns were inferred using a

Bayesian model-based clustering algorithm implemented in STRUCTURE version 2.3.4 (Pritchard et al., 2003). The program was run in ten iterations for each K from 1 to 10, with a burn-in period of 100,000 and subsequent 100,000 iterations. The optimum value of K was predicted and CLUMPP input files were prepared using a web-based software STRUCTURE HARVESTER version 0.6.92 (Earl and VonHoldt, 2012). CLUMPP v.1.1.2 (Jakobsson and Rosenberg, 2007) was used to align the ten repetitions with optimal K. Graphically displayed results of CLUMPP were depicted using Distruct v.1.1 (Rosenberg, 2004).

2.6. Comparison of body size among different populations of *T. maculipennis* in King George Island

The size of body and wings of adult males and females were compared among different populations of *T. maculipennis* collected from three different locations in King George Island. Adults of *T. maculipennis* were collected either by hand or using UV traps around their breeding sites at Korean King Sejong Station, Uruguayan Artigas Base and Chilean Frei Base, respectively, during the years 2018–2020. They were sexed and three morphometric parameters, body length, wing length and wing width, were measured, respectively, from digital images taken from each individual using ImageJ software (Schneider et al., 2012). Their mean values were then compared among locations through analysis of variance (ANOVA) followed by Tukey's honestly significant difference (HSD) test.

3. Results

3.1. mtDNA COI phylogenetic tree and haplotype network diagram

Our mtDNA analysis showed the presence of two distinct lineages (Lineages I and II) suggesting separate source regions for King George Island (Figs. 2 and 3) populations. The genetic variation between these two lineages was 3 % (14 mutation steps). A total of five haplotypes were observed with distribution different between stations. Lineage I (Hap_1 and Hap_3) was nested with the native Artic Svalbard and Polish cave sequences, while Lineage II (Hap_2, Hap_4, and Hap_5) was associated with native Canadian Terra Nova National Park sequence. The most frequent haplotype was Hap_2 shared by Chilean Frei Base, Korean King Sejong Station, and Uruguayan Artigas Base. The second most shared haplotype was Hap_1. It was shared by Chilean Frei Base, native Poland cave, and Arctic Svalbard. Noticeably, both lineages occupied Chilean Frei Base.

3.2. Microsatellite polymorphism and diversity

Microsatellite variation analysis using 12 microsatellite markers showed that there were 134 polymorphic alleles among the five populations. Mean values of PIC, *I*, and *H*e were 0.7291, 1.7527, and 0.7629, respectively (Supplementary Table S2). These results indicate that newly introduced populations have a high genetic diversity. The value of PIC ranged from 0.5130 (Tricho3_08) to 0.8910 (Tricho3_07) with a mean of 0.7291 per locus. The *N*a varied from 5 (Tricho1_02) to 27 (Tricho3_07) with a mean of 11.2 per locus. The *N*e varied from 2.3507 (Tricho3_08) to 9.8979 (Tricho3_07) with a mean of 4.7869 per locus. The *I* ranged from 1.1225 (Tricho3_08) to 2.6620 (Tricho3_07) with a mean of 1.7527 per locus. The *H*o ranged from 0.0784 (Tricho3_08) to 0.6496 (Tricho2_25) with a mean of 0.4162 per locus. The *H*e varied from 0.5774 (Tricho3_08) to 0.9029 (Tricho3_07) with a mean of 0.7629. Overall, Tricho3_08 loci showed the lowest polymorphism, whereas Tricho3_07 loci showed the highest polymorphism value. HWE deviation test within-sample using the Markov chain algorithm (10,000 steps) was performed and significant deviation from the expected value ($p < 0.05$) was observed in 32 (53.33 %) of 60 tests. Loci Tricho3_08 of King Sejong Station population was monomorphic (Supplementary Table S3). Genotypic linkage disequilibrium (LD) test between all pairs of alleles across 12 loci and five populations using 10,000 permutation tests revealed a significant LD ($p < 0.05$) in 64 (19.39 %) of 330 tests.

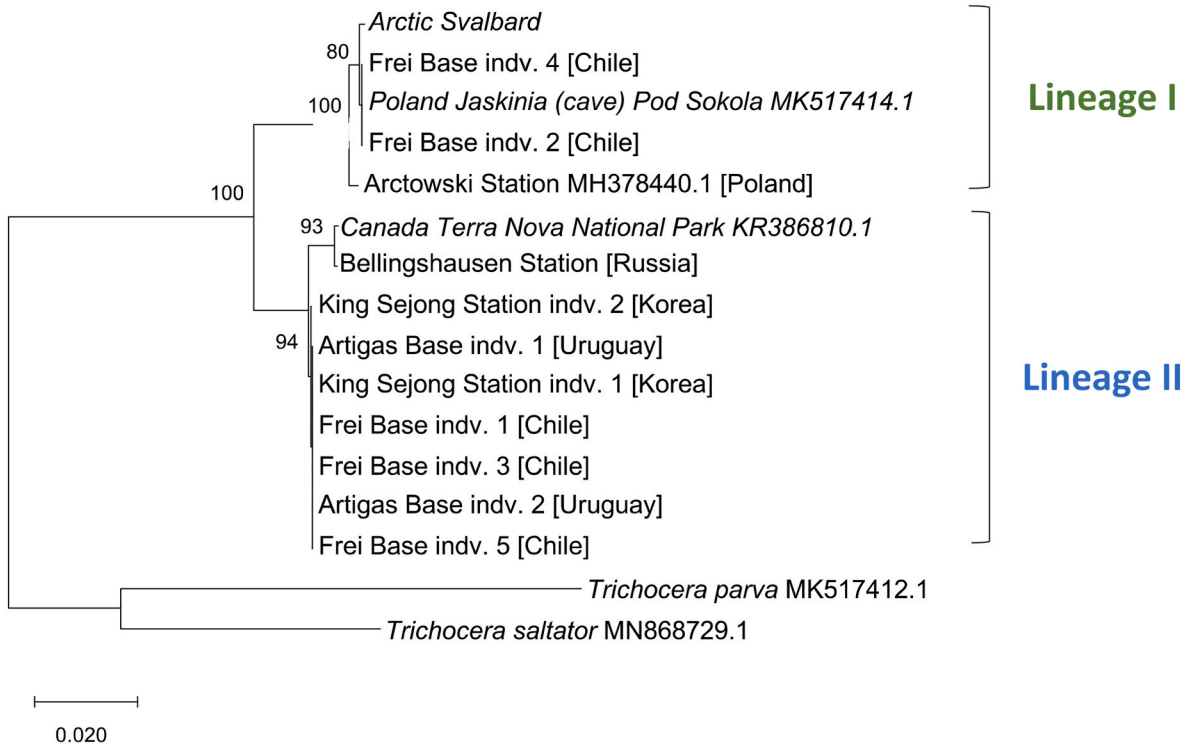

Fig. 2. Phylogenetic tree of mtDNA COI sequences based on Neighbor-joining tree with Kimura 2 parameter and bootstrap iteration 1,000. Italic sequence names represent sequences belong to native sites. Scale bar shows the number of substitutions per site.

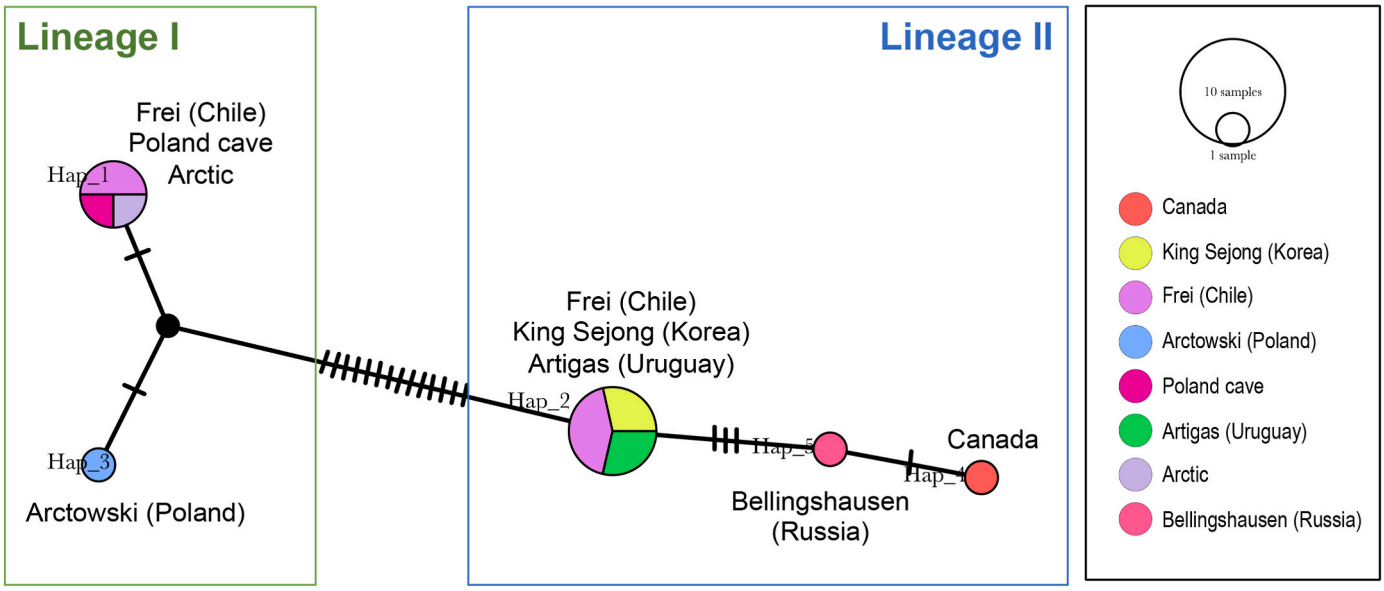

Fig. 3. Haplotype network of the five haplotypes. Colors depict different sample collection sites where each haplotype is observed. Each line represents a single nucleotide mutational change. Number of individuals per haplotype is relative to circle size.

3.3. Population genetic diversity

Microsatellite genetic diversity parameters among five Antarctic station populations were moderate to moderately high (mean *Na*, 4.267; mean *I*, 1.081, mean *Ho*, 0.440; Table 1). Chilean Escudero Base population showed the highest *Na* (6.333), *I* (1.360), *He* (0.672), and *Fis* (0.411) values. As the sample size of Chilean Frei Base was the smallest (N = 5) among stations, the diversity parameters were the lowest for *Na* (2.583), *Ne* (1.736), *I* (0.622), *He* (0.391), and *Fis* (−0.003). The second lowest parameters were *Na* (3.000), *Ne* (2.627), *I* (0.985), *He* (0.597), and *Fis* (0.150). They were observed from Artigas Base population.

3.4. Genetic relationships and population structure analysis

Fst values per locus ranged from 0.107 (Tricho1_02) to 0.415 (Tricho1_04), with an average of 0.232. *Fis* values per locus ranged from −0.013 (Tricho3_01) to 0.719 (Tricho3_08), with an average of 0.253. *Nm* values ranged from 0.353 (Tricho1_04) to 2.095 (Tricho1_02), with a mean of 0.826 (Supplementary Table S4). Pairwise *Fst* and *Rst* values and *P*-values calculated from permutation test showed significant differences among populations (Table 2). Pairwise *Fst* values ranged from 0.130 to 0.383 ($p < 0.001$). This suggests that significant genetic differentiation exists among populations from different stations. Noticeably, the largest genetic differentiation occurred between Chilean Frei Base and Korean King Sejong Station populations (*Fst* = 0.383, $p < 0.001$). The smallest genetic differentiation was observed between Chilean Frei Base and Russian Bellingshausen Station populations (*Fst* = 0.130, $p < 0.001$).

The Bayesian analysis of population structure indicated that the most likely number of clusters was four when evaluated based on Delta *K* (Fig. 4A and B). Among these four clusters, the Chilean Frei Base population was clustered with Russian Bellingshausen Station population. The other three clusters represented each of the rest stations. The discriminant analysis of principal components (DAPC) plot of microsatellites also showed four clearly distinct clusters (Supplementary Fig. S2), as did the Bayesian analysis. For the DAPC, 25 principal components (83 % of total variance) and four discriminant functions were retained. Population genetic variance analysis through AMOVA analysis showed that most of the genetic variance existed within populations (75.90 %, $p < 0.001$) and that the second largest genetic variance among groups was not statistically significant (12.45 %, $p = 0.094$)

Table 2
A matrix of pairwise *Fst* (upper diagonal) and *Rst* (lower diagonal) based on microsatellites.

Population	Frei	Bellingshausen	Escudero	King Sejong	Artigas
Frei	–	0.130 ***	0.170 ***	0.383 ***	0.191 ***
Bellingshausen	0.211 *	–	0.151 ***	0.361 ***	0.194 ***
Escudero	0.072 NS	−0.003 NS	–	0.347 ***	0.142 ***
King Sejong	0.270 **	0.352 ***	0.207 ***	–	0.265 ***
Artigas	0.366 ***	0.258 ***	0.291 ***	0.455 ***	–

P-values: $p > 0.05$ (NS), $0.01 < p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***).

Table 1
Genetic diversity of *T. maculipennis* populations across 12 microsatellite loci.

Population	Sample size	*Na*	*Ne*	*I*	*Ho*	*He*	*Fis*
Frei	5	2.583	1.736	0.622	0.389	0.391	−0.003
Bellingshausen	23	4.083	2.872	1.140	0.379	0.625	0.371
Escudero	30	6.333	3.337	1.360	0.397	0.672	0.411
King Sejong	30	5.333	3.491	1.300	0.458	0.658	0.324
Artigas	30	3.000	2.627	0.985	0.579	0.597	0.150
Mean	118	4.267	2.812	1.081	0.440	0.589	–

Number of alleles (*Na*), number of effective alleles (*Ne*), information index (*I*), observed heterozygosity (*Ho*), expected heterozygosity (*He*), and inbreeding coefficient (*Fis*).

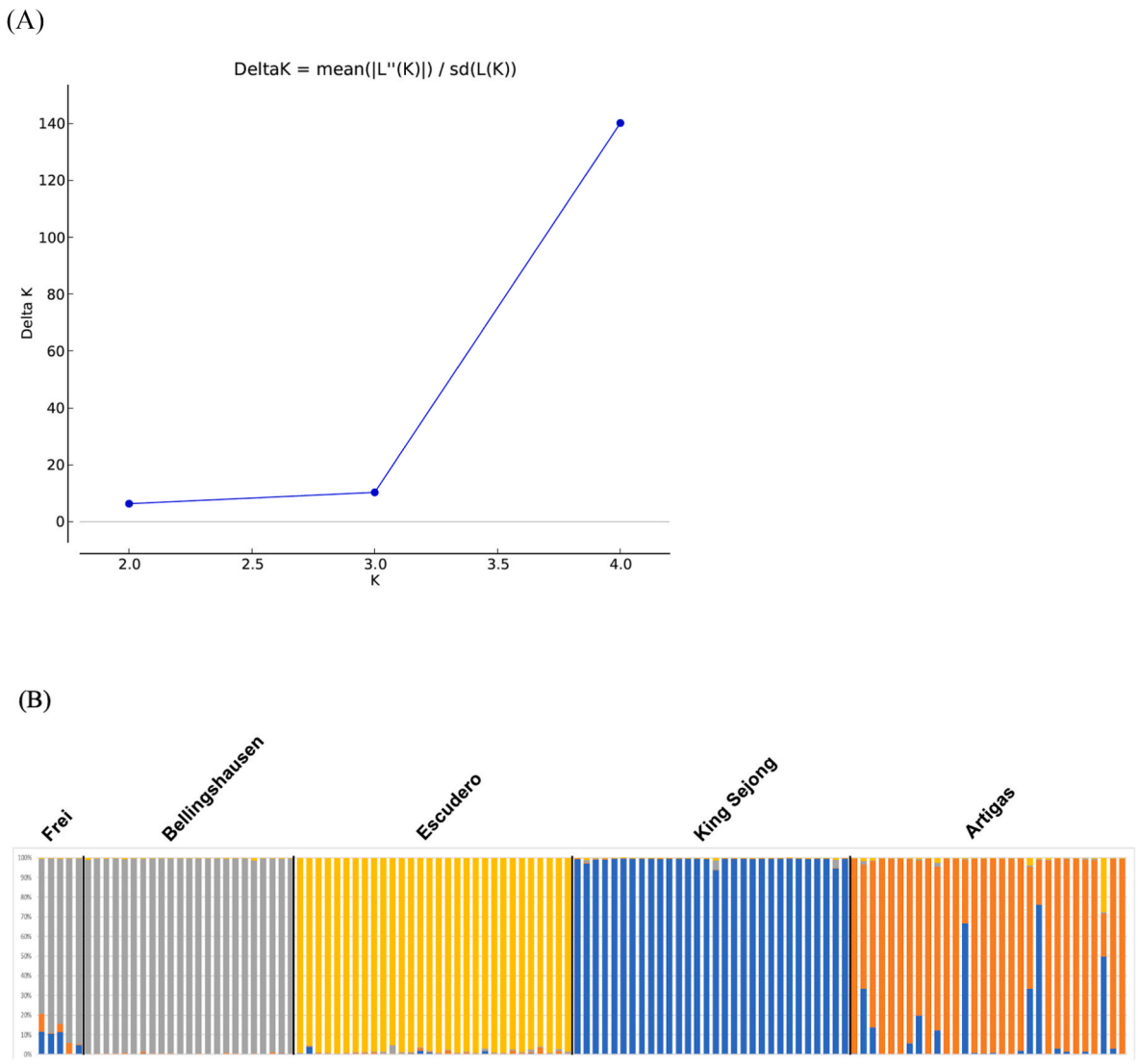

Fig. 4. Clustering analysis of microsatellite data performed using STRUCTURE and STRUCTURE HARVESTER software. (A) Plot of values for Delta *K*; (B) Bar plot of estimated clustering group of each individual in $K = 4$ clusters.

(Supplementary Table S5).

3.5. Comparison of body size among different populations of *T. maculipennis* in King George Island

Significant differences were found in all three morphometric parameters among different populations of *T. maculipennis* collected from Korean King Sejong Station, Uruguayan Artigas Base and Chilean Frei Base (Supplementary Table S6). Body length, wing length, and wing width were significantly larger in females than in males for all populations of *T. maculipennis* examined (Student *t*-test, $p = 0.01$). In gross comparison, body sizes of both sexes of *T. maculipennis* adults were the largest among those collected from King Sejong Station and the smallest among those collected from Artigas Base. Adults of both sexes collected from King Sejong Station were significantly larger for all three parameters (body length, wing length, and wing width) than those collected from Artigas Base. However, such morphometrical difference was less conspicuous between Frei Base and King Sejong Station or between Frei Base and Artigas Base populations. Body sizes of adult *T. maculipennis* were similar between Frei Base and Artigas Base populations, showing significant differences between these two populations only in body length and wing length of males (Supplementary Table S6).

4. Discussion

In the present study, we established phylogenetic origin of invasive populations of *T. maculipennis* on King George Island and assessed the levels of genetic diversity and populational genetic differentiation in the invasive range. Based on the phylogenetic analysis and haplotype similarity of mtDNA COI sequences, our findings support at least two geographic origins of invasive populations, one from Europe and Artic region and the other from North America. There was a 3 % genetic variation between these two lineages. This may imply highly structured population structure in native ranges. Among the five invasive populations, only the population from Chilean Frei Base contained both lineages. Our analysis with 12 microsatellite loci also indicated a high genetic diversity among *T. maculipennis* populations across various stations of King George Island.

Moreover, despite the geographical proximity of these stations (e.g., Chilean Frei Base is only a few hundred meters apart from Chilean Escudero Base), our results suggest significant levels of genetic differentiation (pairwise *Fst* values = 0.130–0.383, $p < 0.001$) among invasive populations. AMOVA showed that most of the genetic variance existed within populations (75.90 %, $p < 0.001$), whereas the among-group variance was insignificant (12.45 %, $p = 0.094$). Meanwhile,

both Bayesian analysis STRUCTURE ($K = 4$) and DAPC plot support the presence of four distinct clusters. Likewise, our morphometric study also exhibited significant differences in sizes of adults across various populations of *T. maculipennis*. However, evidence from this study appears to be insufficient to establish clear association of genetic origins with observed genetic differentiation and variations in body size. In this context, diverse sampling dates further complicated the correlation analysis.

The apparent population differentiation is probably a consequence of rapid evolutionary changes occurring for adapting the new environment. Invasive species have the potential to undergo rapid evolutionary changes due to selection pressures induced by new habitats (Sakai et al., 2001; Lee, 2002; Ochocki and Miller, 2017). This swift genetic adaptation of invasive species could play a crucial role in promoting their fitness and enhancing their success of colonization (Suarez and Tsutsui, 2008; Le Roux and Wieczorek, 2009; Shine et al., 2011). Considering the relatively short time period (ca. 20 years) since their introduction, the high level of genetic diversity in *T. maculipennis* might have played a significant role in their successful establishment across the majority of Antarctic stations in King George Island. These results suggest that genetic variation can be delineated as a management unit at the population level.

Moreover, the presence of considerable genetic diversity within an invasive species can be attributed to either multiple introductions or the establishment of large founding population. It has been postulated that the occurrence of multiple introductions is correlated with increased diversity owing to the provision of increased variation and the establishment of novel genetic communities (Dlugosch and Parker, 2008). Multiple introductions can help invasive populations exhibit significantly greater genetic diversity than those originating from a single source population (Sakai et al., 2001). Considering its relatively brief history in King George Island, the high genetic diversity and successful colonization of *T. maculipennis* might also be associated with the multiple invasive events. Therefore, the high genetic variation might have facilitated the rapid establishment of *T. maculipennis* in King George Island by providing rich genetic resources for early adaptation. The high genetic variation of *T. maculipennis* in King George Island is further supported by our DAPC plot and Bayesian analyses showing that populations can be classified into four distinct clusters without apparent association with geographical distribution, although AMOVA analysis did not support variance among groups. Furthermore, phylogenetic analysis of mtDNA haplotypes indicated at least two geographic sources, implying that multiple introduction events might have occurred in King George Island.

King George Island is a part of a logistical supply network that links multiple locations in the South America and Antarctic Peninsula, even Eastern Antarctica (Hughes et al., 2019). Winged insects disperse more easily than flightless ones into other Antarctic Conservation Biogeographic Regions (ACBRs, Terauds and Lee, 2016) because they can fly wharf to ship and ship to shore without hitching into cargo (Houghton et al., 2016; Hughes et al., 2019). In addition, typical north-easterly prevailing wind in the region can facilitate the movement of insects from Fildes peninsula, the main traffic hub of King George Island, to other southerly peninsulas. The distribution range of the alien winter crane fly, *T. maculipennis*, could be expanded together with the only indigenous winged midge (*Parochlus steinenii*) by human-mediated dispersal to other ACBRs and established in suitable environments under climate change scenarios (Contador et al., 2020; Hughes et al., 2019; Pertierra et al., 2021).

To date, larvae and pupae of *T. maculipennis* have not yet been reported in the natural environment of Antarctica, with only flying adults of *T. maculipennis* observed (Volonterio et al., 2013; Potocka and Krzmińska, 2018; León et al., 2021). However, our recent environmental DNA analysis revealed a positive indication of DNA fragments for the presence of *T. maculipennis* in the outdoors of Fildes and Barton Peninsula (data not published), suggesting the likelihood of natural establishment of the non-native fly in King George Island. Recent experimental evidence has shown that *T. mculipennis* has cold tolerance comparable to a native winged chironomid, *P. steinenii* (Pertierra et al., 2021). Co-existence of both species in the same region in the South Shetland Islands (Chown and Convey, 2016; Pertierra et al., 2021) also implies the possibility of sharing habitats between *T. maculipennis* and *P. steinenii*. If the non-native fly establishes in the natural ecosystem, it is highly likely to become invasive, just like *E. murphyi* in Signy Island, South Orkney Islands (Hughes et al., 2013). Considering that *E. murphyi* is a detritivore without competitors or predators, accelerating nutrient cycling, its natural establishment have broader impacts on all levels of indigenous biodiversity (Hughes et al., 2013; Bartlett et al., 2020). The same is probably true for *T. maculipennis* which is detritivorous or saprophagous in both its natural distribution range as well as in introduced maritime Antarctic region (Karandikar, 1931; Dahl, 1970b; Volonterio et al., 2013; Potocka and Krzmińska, 2018). The region has relatively rich biomass with many sources of suitable food such as decaying organic matter and vegetation including microbial mats. The non-native fly can seek refuge in a synanthropic environment to survive. It can even complete its life cycle during harsh season.

Females of *T. maculipennis* have larger body sizes than males. This is common for many other insects since adult females carry large ovary. However, it is unclear why body sizes of *T. maculipennis* adults are significantly different among those from different bases in King George Island. Considering the small amount of time (20 years or so) since their introduction into the region and the proximity of bases, it is less likely that a genetic change is directly involved. Nevertheless, it is possible that the morphometric difference between populations of *T. maculipennis* is driven by high genetic diversity and genetic differentiation due to multiple introductions. This was supported by our DNA analysis indicating that Korean King Sejong Station and Uruguayan Artigas Base were occupied only by Lineage II, whereas Chilean Frei Base was occupied by both Lineage I and Lineage II.

However, our study is yet to provide further evidences for a conclusive role of genetic origins in shaping the observed genetic differentiation and body size differences among populations of *T. maculipennis*. As their main habitats in King George Island are closely associated with human activities, the main driver for the morphological difference could also be nutritional difference in their larval habitats due to differences in the food consumed by people in each region (Banerjee et al., 2015).

This study revealed the rapid adaptations of *T. maculipennis* to Antarctic environments, emphasizing the need for implementing strict biosecurity measures to prevent entry and establishment of alien species into Antarctica. At present, King George Island appears to have suboptimal conditions for the survival of *T. maculipennis* in an outdoor environment. Considering cold tolerance of this species, increasing human traffic in this island, and global warming, however, expansion of this species and invasion of additional alien species in King George Island seem likely. Our study provides valuable insights into the origin, differentiation, and adaptation of *T. maculipennis* in King George Island. While the precise invasion pathway of *T. maculipennis* remains unclear, our study demonstrates the separate origins of *T. maculipennis* and its rapid adaptation to a new environment, which suggests a strong invasiveness potential for this species. Careful intervention of their re-entry and further expansion in King George Island would be needed in addition to our current effort to eradicate this species from the invasive range.

Credit author statement

Seunghyun Kang: Investigation, Writing- Original draft, **Kye Chung Park**: Investigation, Writing- Original draft, **Andrius Petrašiūnas**: Investigation, **Euna Jo**: Investigation, **Sung Mi Cho**: Investigation, **Sanghee Kim**: Investigation, Conceptualization, **Hyung Chul Shin**: Conceptualization, **Ji Hee Kim**: Supervision, Funding acquisition,

Writing- Original draft, reviewing and editing.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) used GPT-3.5 in order to review grammar and suggests replacements for the identified errors. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

We thank Frei Base, Escudero Base, Bellingshausen Station and Artigas Base for accommodating sampling of test insects. This research benefited from the joint monitoring program of non-native flies in King George Island and was supported by the Korea Polar Research Institute (KOPRI, PE23170 and PE23140).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2023.117636.

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