Ecophysiology, Morphology and Phylogeography of Insects in the Scotia Arc

Felipe Lorenz Simões

Robinson College

Department of Zoology

University of Cambridge

A thesis submitted for the degree of Doctor of Philosophy

September 2019

Intentionally blank page

Declaration

This dissertation is the result of my own work and includes nothing which is the outcome of work done in collaboration except as declared in the Preface and specified in the text. It is not substantially the same as any that I have submitted, or, is being concurrently submitted for a degree or diploma or other qualification at the University of Cambridge or any other University or similar institution except as declared in the Preface and specified in the text. I further state that no substantial part of my dissertation has already been submitted, or, is being concurrently submitted for any such degree, diploma or other qualification at the University of Cambridge or any other University or similar institution except as declared in the Preface and specified in the text.

It does not exceed the prescribed word limit (60,000), in accordance with the Department of Zoology guidelines.

Felipe Lorenz Simões, MSc.
Cambridge

Intentionally blank page

Summary

Ecophysiology, Morphology and Phylogeography of Insects in the Scotia Arc

Author: Felipe Lorenz Simões

The Scotia Arc, comprising southern South America, South Georgia and the South Orkney Islands and the Antarctic Peninsula, is home to a range of understudied insect species which are constantly exposed to extreme environmental conditions. To help reduce the amount of uncertainty surrounding insect taxa evolution in the region, we aimed to elucidate the evolutionary relationships and divergence times of non-biting midges (Diptera) and beetles (Coleoptera) naturally occurring in the lands around the Scotia Arc. The main objectives here were to learn how the evolution of select species of these two orders of insects is linked to the region's geographical history, through the use of phylogeography, and what kind of adaptations (morphological and physiological) they have developed to deal with the environmental conditions and changes, such as osmotic stress and desiccation tolerance. There was also an intrinsic objective to ascertain the taxonomy of the midge *Telmatogeton magellanicus*, which potentially belongs to the genera *Belgica* or *Halirytus.* The individual studies in this thesis were carried out in the British Antarctic Survey (BAS) and in field stations in Navarino Island (Chile) and the South Shetland Islands (Antarctica), with additional field work in South Georgia Island.

Habitat Characterisation and Ecophysiology: As a first step to enable the understanding of the physiological adaptations of the brachypterous midge *T. magellanicus* we first had to describe, in detail, its habitat. To that end, we made use of Permutational MANOVA and Similarity Percentages, through which we were able to identify the mid-tidal zone of the intertidal as its favoured habitat, while also providing details on their abundance and the fact they mostly need filamentous algae to thrive. Subsequentially**,** we exposed larvae of *T. magellanicus* to different physiological treatments and showed that they are very resistant to osmotic stress and temperature extremes, but that exposure to desiccation are one of the main dangers to their survival.

In the meantime, larvae of *Eretmoptera murphyi* were also exposed to osmotic stress treatments, but were shown to struggle much more to deal with saline water.

Morphology: We hypothesised that the South Georgian isolate of the diving beetle *Lancetes angusticollis*, could have changes to its hind wing morphology, potentially causing a loss of the ability to fly. However, the wings bear only minute changes that did not enable us to correlate that to specific environmental conditions.

Phylogeography: By means of two genetic markers (COX1 and 28S) we were able to assess the phylogeographic structure of the winged Antarctic midge *Parochlus steinenii*, who is spread out across the Scotia Arc, with a clear split of its South American population.

This thesis shows how insects can help us understand the development of a specific region in the globe, but also shows us how much more there is left to explore in terms of the biology and evolution of insect taxa in the Scotia Arc, specially in light of the current international debates on climate change, as these are among the organisms that are the most susceptible to sudden alteration of their habitat composition.

This PhD studentship is shared between the University of Cambridge (UCam) and the British Antarctic Survey (BAS).

Supervisors:
Prof. P. Convey (BAS)
Dr. E. Turner (UCam)

Co-Supervisors:
Chester Sands (BAS)
Dr. J. Jackson (BAS)

Advisors:
Dr. T. Contador (UMAG – Chile)
Dr. S. A. L. Hayward (University of Birmingham)

This study has been funded through a PhD studentship by the *Conselho Nacional de Desenvolvimento Científico e Tecnológico* (CNPq) and the Cambridge TRUST under the Science Without Borders program (ref no. 233923/2014-4). Additional financial support for fieldwork on Navarino Island and the South Shetland Islands was provided by INACH under project no. RT_48-16 (*Dípteros en regiones subantárticas y antárticas: ¿Están listos para los cambios?*).

Intentionally blank page

“All of old. Nothing else ever. Ever tried. Ever failed. No matter. Try again. Fail again. Fail better.”

Samuel Beckett
Worstward Ho
(First published in 1983)

Intentionally blank page

Preface - Statement of Contributions

Peter Convey commented on all chapters, contributed to the overall original study proposal and to the experimental designs of Chapters 2–4 and 6. Edgar Turner commented on all chapters and contributed in result analyses for Chapters 2–4 and 6. Jennifer Jackson and Chester Sands advised on molecular data acquisition, result analyses and commented on Chapter 6. Tamara Contador contributed to the experimental designs of Chapters 2–4 and with expert taxonomic knowledge for Chapters 2, 3 and 5. Javier Rendoll contributed to the experimental design of Chapter 3 and with expert taxonomic knowledge for Chapter 6. Scott Hayward contributed to the experimental designs of Chapters 3 and 4. I designed the experiments, and collected and analysed the data for all chapters. I would like to acknowledge the very helpful comments provided by three anonymous reviewers in Chapter 2. Any mistakes in this dissertation are my own.

My PhD grant was funded by the *Conselho Nacional de Desenvolvimento Científico e Tecnológico* (CNPq - Brazil) (Process no. 233923/2014-4) in collaboration with the Cambridge Trust.

Intentionally blank page

Acknowledgements

I would like to thank my supervisors Prof. Peter Convey, Dr. Edgar Turner, Chester Sands, Dr. Jennifer Jackson, Dr. Tamara Contador and Dr. Scott Hayward for giving me the opportunity to carry out this PhD project, and for their invaluable advice, guidance, support and encouragement throughout the project. A very special thanks to Chester Sands, for his incredible supervision and guidance in the lab, the analyses, and the amazing scientific lessons; and to both Pete Convey and Ed Turner for their endless support, advice, rapid responses to emails, and above all else, patience; I honestly could not have asked for better supervisors. I must also thank the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and the Cambridge TRUST for my PhD studentship. Additional thanks to my advisors Dr. Will Goodall-Copestake and Prof. Richard Preece for their very useful comments and inputs through my yearly reports, and to my college tutor Dr. Brian McCabe, with whom I had some very enlightening and productive chats.

To all the colleagues and friends that I made at the British Antarctic Survey and in the Department of Zoology: I have thoroughly enjoyed my experience here and I am very glad to have had the chance to get to know each and every single one of you. I am a strong believer that the sharing of experiences with people from so many different backgrounds and skills not only enhances your professional skills, but makes you a better human being. Special thanks to both cricket teams, who made for an amazing time discovering this very British sport and which I've come to really enjoy playing.

I am also grateful to all the people who have helped in the gathering and processing of specimens, in particular Elise Biersma, Katherine Short, Jesamine Bartlett, Claudia Maturana, Javier Rendoll, Peter Convey and Tamara Contador. I would also like to thank Christine Taylor from the Natural History Museum for lending very interesting and useful specimens from their historic beetle collection. Particular thanks also go to Dr. Tamara Contador and INACH for funding and supporting the field season in Navarino Island (Chile) and the South Shetlands Islands (Antarctica) in 2017, and to the Swiss Polar

Institute and everyone on Leg 3 of the Antarctic Circumnavigation Expedition for a once in a lifetime opportunity to cross the distance between South America and Africa by sea while working and being able to set foot in amazing and remote places like the island of Bouvet.

I cannot forget to thank Alison Teague for all the help she provides students at BAS from the very first day we arrive there (and way before that as well). I would also like to thank the laboratory staff (Dr. Elaine Fitzcharles, Michelle King, Guy Hillyard and Becs Smith) at BAS for their help and advice. Special thanks to Paul Geissler for his help with the collections at BAS, Laura Gerrish (MAGIC) for her help with preparing the several map figures for the different chapters, and to Dr. David Barnes for his friendship and frequent chats about work over coffee. To the staff of Robinson College, specially Helen Grattidge, I also extend my thanks for all the lunches, formal dinners and for taking care of Gabi.

Finally, I am extremely thankful to my family for always providing me with love and support throughout my time in Cambridge, specially my parents, Jefferson and Ingrid for everything they have always provided me and for being big inspirations for my whole life. Last, and definitely not least, a very special thanks goes to my wife Gabriela, without whom I would have definitely not been able to finish this thesis, and who has also inspired me through her unconditional love and sheer strength through a very tasking and demanding time of her life.

Table of Contents

Intentionally blank page

List of Figures

Intentionally blank page

List of Tables

Intentionally blank page

CHAPTER 1

General Introduction

1. Introduction

1.1 Regional context and evolution of Antarctic insects

The relationship between South America, the Scotia Arc and Antarctica (Figure 1.1) has long been established from the point of view of geography, geology and glaciology (Eagles et al., 2005, 2006, Linse et al. 2006, Livermore et al. 2007, Convey et al. 2009, Griffiths et al. 2009). However, the biological relationships of terrestrial organisms, apart from a few studies on more well-known taxa, is still barely known, partly as a result of the difficulties of working in one of the most inhospitable places on Earth (Convey et al. 2018). Over the last few decades, researchers have begun to fill the gaps in this knowledge using a variety of different methods, including evolutionary reconstructions, morphological and physiological comparisons, and niche modelling (Gressitt 1964, 1970a, 1970b, 1971, Chown 1997, Allegrucci et al. 2006, Convey et al. 2009, Lee et al. 2012, Everatt et al. 2014). Even though the number of studies on the region's invertebrate fauna have increased significantly over the past decades, the few occurring insect species have only recently been considered as the main target for research

The current geography and environment of the Antarctic continent (and South America) and its surrounding islands can largely be linked to consequences stemming from the fragmentation and subsequent movement of the elements of the supercontinent Gondwana (Lawver & Gahagan 2003, Scher & Martin 2006, Torsvik et al. 2008) (which started about 170 Mya). In particular, the isolation of Antarctica resulted in a fundamental cooling in its climate, which was intensified by the opening of the Tasmanian Gateway and the Drake Passage (45–30 mya) and formation of the Antarctic Circumpolar Current (ACC, which initiated around 20 mya) (Hambrey & Barrett 1993, Birkenmajer et al. 2005, Pful & McCave 2005, Whitehead et al. 2006, Livermore et al. 2007), so that 99.7% of the continental land mass is now covered by ice (Burton-Johnson et al. 2016). Since its isolation, the continent has undergone repeated cycles of ice expansion and contraction that are thought to have led to the almost complete extinction of terrestrial life on the continent (see Convey et al. 2008, 2009 for review).

Although not currently present in Continental (East) Antarctica, insects have been found in the Antarctic region from, at least, the early Palaeocene (~70mya) (Allegrucci et al. 2006, Convey et al. 2008, Chown & Convey 2016). Nowadays, insects can be found in the maritime Antarctic (Antarctic Peninsula and associated Scotia Arc archipelagos) and the sub-Antarctic Islands (Fig. 1.1) (Convey & Block 1996, Chown & Convey 2016). Knowledge of their diversity, evolution and ecology, mostly results from studies carried out over the past 50 years, catalysed by the works by J. L. Gressitt (1964, 1970a, 1970b, 1971) and most recently reviewed by Chown & Convey (2016). However, even with this increase in scientific effort, much remains unknown about the evolution and adaptations of insects from this region.

The fauna of the Atlantic sector of the Antarctic region is closely related to South America, with intimate geographical, biological, geological and glaciological histories (Mercer 1976, Clapperton & Sugden 1988, Clapperton et al. 1989, Rodbell et al. 2009, Fernandez et al. 2011), such as seen in the naturally occurring midges (Diptera: Chironomidae) and beetles (Coleoptera) of the region (Chown & Convey 2016). Even though much still remains to be resolved in clarifying the relationship of sub-Antarctic taxa to their sister-groups in South America, the evidence already available (Chown et al. 1998, Chown & Convey 2006, 2007, Convey 2007) allows development of hypotheses and predictions relating to the temporal scale of species' divergences and subsequent colonisation of the Antarctic region. Recent key findings have evidence demonstrating the long-term presence of Antarctic biota, perhaps through persistence of populations in ice-free areas (Convey et al. 2008), and the importance of dispersal in determining patterns of species distribution, as has been shown in *Belgica antarctica* (Allegrucci et al. 2012).

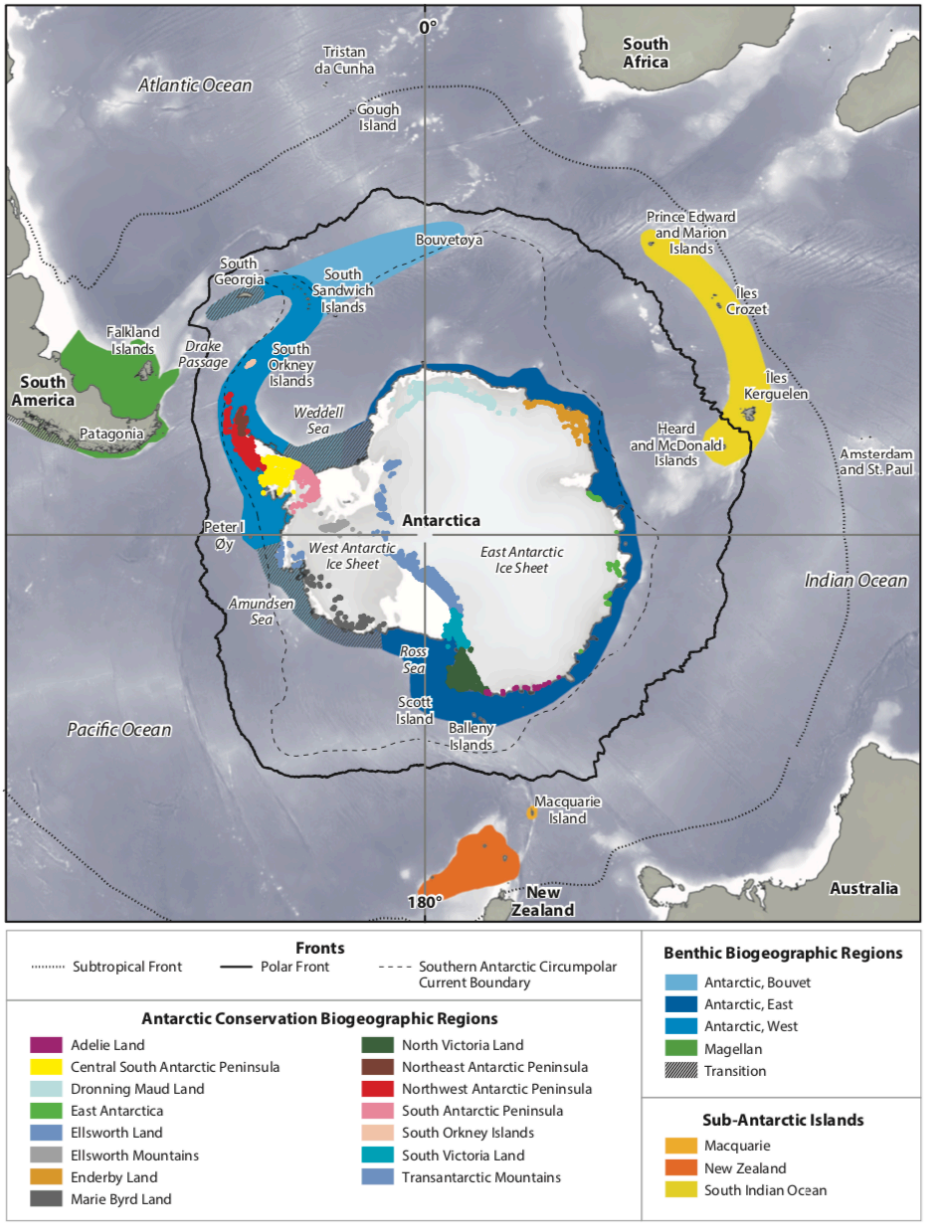


Figure 1.1 Map of the biogeographic regions of Antarctica and the sub- and peri-Antarctic (reproduced from Chown & Convey 2016).

More recently, studies have examined the phylogeography and ecophysiology of some species of Antarctic and sub-Antarctic hexapods. Phylogeographical studies have concentrated on understanding the history and regional biogeographic structure of insect populations. For example, Allegrucci et al (2006, 2012), studying the midge *Belgica antarctica* Jacobs, 1900 and closely related species found a strong genetic structure in

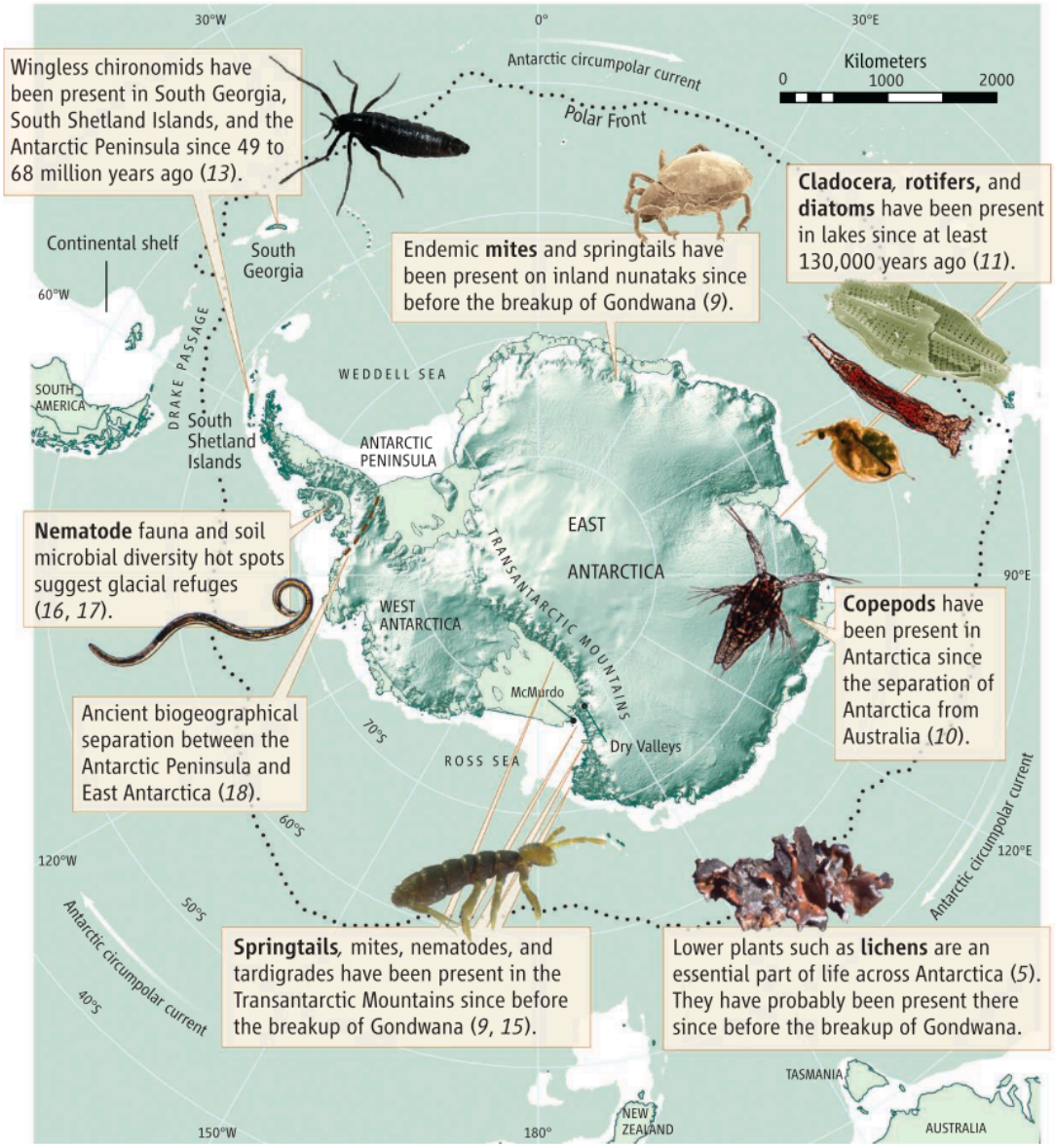
the populations, suggesting long isolation of different populations. Collins et al. (2019), studying Antarctic springtails, found a strong impact of time since dispersal and isolation on distribution patterns. Papadopoulou et al. (2009), studying beetles in the families Carabidae and Curculionidae in the Falkland Islands, found that distribution patterns were related to a range of environmental factors, but that these differed from those in climatically similar areas of the Northern Hemisphere. In the meantime, ecophysiological studies have concentrated on the tolerance of Antarctic species to extreme conditions. For example, Everatt et al. (2012), studying the midge, *Eretmoptera murphyi* Schaeffer, 1914, found that the species was pre-adapted to harsh conditions. This tolerance and potential pre-adaptation to extremes has also been found in other species, such as *B. antarctica* (Lopez-Martinez et al. 2009, Teets et al. 2011) and the promecheilid beetle *Hydromedion sparsutum* (Müller, 1844) (Bale et al. 2000).

Studies carried out over the last 30 years, have demonstrated the power of physiology and DNA-based studies in providing a better understanding of adaptations (Convey 1996, 2010, Lee Jr. et al. 2006, Everatt et al. 2013a, 2013b, Bartlett et al. 2018a, b) and the evolution of insects and other terrestrial invertebrates. Molecular approaches in particular, have allowed an estimation of the timing of species' divergences and colonisation on different land-masses (e.g. Allegrucci et al., 2006, 2012, McGaughran et al., 2010b, Collins et al. 2019). Taken together, these bits of information are particularly pertinent for assessing the biogeography (Convey 2011) of these taxa, through an assessment of the impacts of underlying ecological constraints on dispersal. A related aspect that has received much less research attention is an examination of the relationships between genetic differentiation and ecophysiological specialisation in independently evolving lineages (McGaughran et al. 2010a, b), especially when they occur across different environments, which could provide a setting to assess local adaptation (Moritz et al. 2012, Taylor et al. 2013).

1.2 Polar Entomology

Even though other invertebrates, including nematodes, tardigrades, mites and springtails are present in Antarctica (Convey 2017) (Fig. 1.2), there are currently only two species of insects native to the continent, and neither are found beyond the Gressitt Line (Chown & Convey 2007, 2016, Convey & Stevens 2007), which marks a major biogeographic discontinuity that separates the Antarctic Peninsula and continental Antarctica. A few non-native insects have also found their way into the region, mainly living within and around scientific stations (Hughes & Convey 2014, Bartlett et al. 2018a). However, these have not established beyond human occupation and have, so far, been unable to fully settle in the region.

The two native insects (both midges of the family Chironomidae) have been present within the Antarctic for many millions of years, and in the case of the endemic and wingless *Belgica antarctica* for perhaps up to ~55 mya (Figs 1.2 and 1.4; Allegrucci et al. 2006). To date, there's little evidence on how long the second chironomid, the winged *Parochlus steinenii*, has been established in its current Antarctic distribution with a single contribution being found in Allegrucci et al. (2006), where the authors found that the South American populations have been separate from the Maritime Antarctic ones for at least some millions of years. Both species, however, have been studied quite extensively, with research addressing aspects of their phenology, physiology, metabolomics and molecular biology (Sugg et al. 1983, Edwards & Usher 1985, Shimada et al. 1991, Hahn & Reinhardt 2006, Michaud et al. 2008). In the case of *B. antarctica*, it has been demonstrated that the species possesses one of the smallest insect genomes known (Kelley et al. 2014), potentially related to their adaptation to the harsh conditions of this region (Kelley et al. 2014, Cornette et al. 2015).



Ancient origins. Many organisms have persisted in Antarctica since well before the Last Glacial Maximum.

Figure 1.2 Summary of the presence and evolution of invertebrate taxa in the Antarctic Continent and South Georgia. The Gressitt line can be seen as a dashed brown line between the Antarctic Peninsula and West Antarctica (reproduced from Convey & Stevens 2007).

Considering a wider area than the Antarctic Peninsula alone, much greater insect diversity is apparent in the sub-Antarctic region. This region mostly includes islands with more benign, though chronically cool climates (Convey 1996b) and relatively high

coverage of vegetation, thus creating a more suitable environment for invertebrates in general, and especially for insects. Species richness in some of these islands can be as high as 230 species [such is the case in the Auckland Islands (Chown & Convey 2016)], a number that is likely to be an underestimate given difficulties in accessing such areas for study and the increasing realization of the presence of cryptic species.

Navarino Island, located at the very south of South America, where the Beagle Channel exits into the Atlantic Ocean, is part of the Magellanic sub-Antarctic ecoregion, hosting a wide variety of habitats and microhabitats over a short elevational range (Pisano 1977, Contador et al. 2015a). Its insect fauna is relatively poorly studied, apart from a recent increase in studies of aquatic species (Contador et al. 2015a, b, Gañán-Mora et al. 2015, Rendoll et al. 2019). The island is home to the dytiscid diving beetle, *Lancetes angusticollis*, which also occurs in South Georgia (Nicolai & Droste 1984), raising the question as to how long it has been present in these two relatively distant and isolated areas. Additionally, two species of Chironomidae are found in this region and live in two very distinct habitats on Navarino: *Parochlus steinenii* is restricted to high altitude lakes in the island's mountain range, and the recently rediscovered intertidal *Telmatogeton magellanicus* is found in intertidal zones. The latter species was first described (as a member of the genus *Belgica*) early in the 20th century, but was subsequently not studied or collected again. Although currently thought to be endemic to Tierra del Fuego, no specific surveys have ever been conducted. The species is congeneric with two other species (*T. amphibius* and *T. macquariensis*) found on the remote sub-Antarctic Marion and Macquarie Islands (Gressit 1970, Nondula et al. 2004), raising intriguing questions about their evolutionary history and dispersal.

Further along the Scotia Arc is the sub-Antarctic island of South Georgia, which is considered to be the connecting point between South America and Antarctica (Gressitt 1970). The island is home to several insect species, with South American-derived species reaching their southern limits at this point, and Antarctic species their northern limits. Inhabitants include *Parochlus steinenii* and *Lancetes angusticollis*, and the endemic promecheilid beetles *Hydromedion sparsutum* and *Perimylops antarcticus* (Chown &

Convey 2016). There are also introduced and predatory carabid beetles, *Trechisibus antarcticus* (Dejean, 1831) and *Merizodus soledadinus* (Guérin-Meneville, 1830) which are potentially causing heavy declines in the populations of native insects (Laparie et al. 2010, Chown & Convey 2016, Ouisse et al. 2017).

In the northern maritime Antarctic, between South Georgia and the Antarctic Peninsula, lies the South Orkney Islands archipelago. Within this, Signy Island is a small but paradigmatic example of a maritime Antarctic terrestrial ecosystem (Smith 1990). It hosts a typical microarthropod and microinvertebrate fauna for the region (Convey 2017), but has no native insects. However, it is home to a single non-native insect species,the chironomid *Eretmoptera murphyi* Schaeffer, 1914, that was accidentally transported from its native habitat of South Georgia through plant transplant experiments in the 1960s (Block et al. 1984, Convey 1992, Convey and Block 1996, Bartlett et al. 2018a, b). Despite its generic positioning, this species has long been considered a sister species to the endemic Antarctic midge *Belgica antarctica*, with more recent genetic studies supporting this hypothesis (Cranston 1985; Allegrucci et al. 2006, 2012).

1.3 Aims of the study and thesis outline

1.3.1 Main objectives

To clarify processes surrounding the evolution and adaptation of insects in the Scotia Arc region, this thesis uses a range of methodologies to investigate the evolutionary relationships and divergence times of non-biting midges (Diptera) and beetles (Coleoptera) naturally occurring in southern South America, South Georgia and the South Shetland Islands. It also investigates how their evolution is linked to the region's geographical history, and the adaptations (morphological and physiological) that they have evolved in response to the environmental conditions and changes experienced across this region.

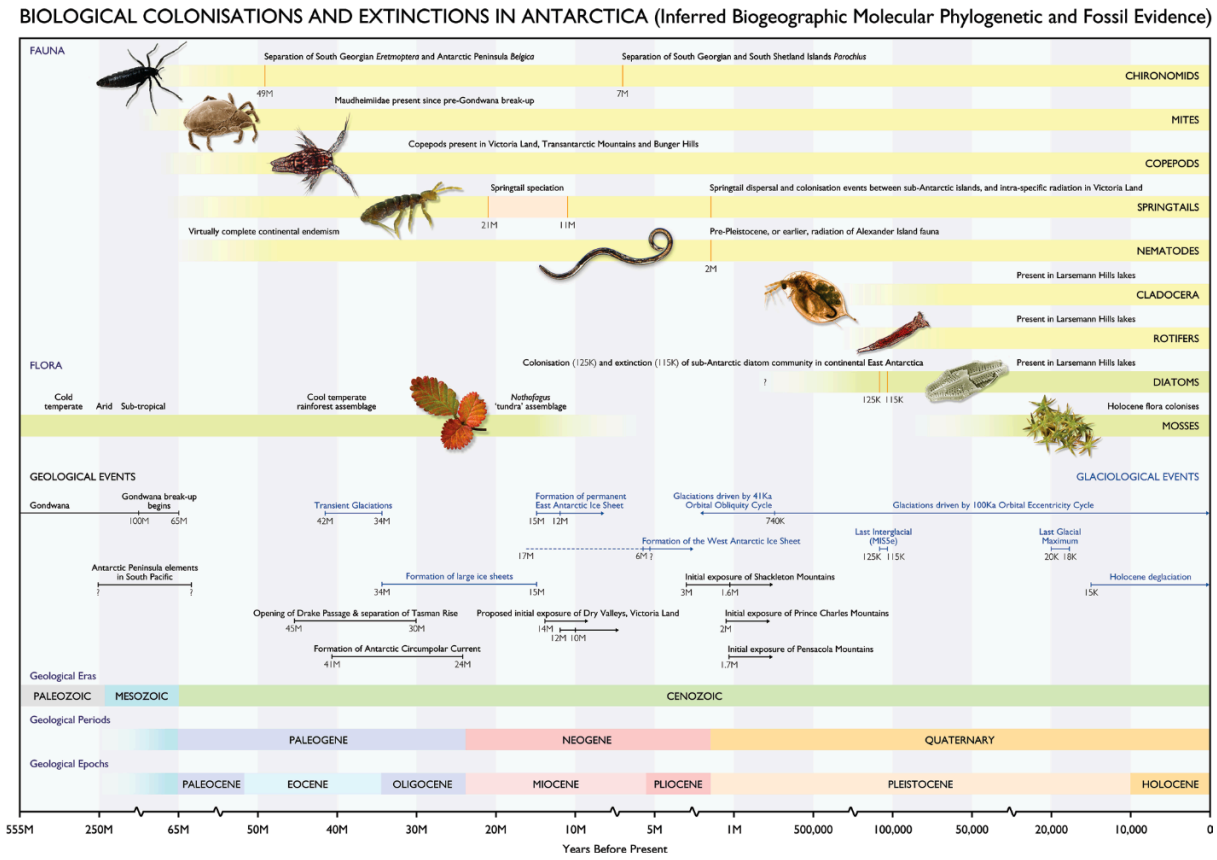


Figure 1.3 Reconstruction of biological colonisations and extinctions in Antarctica. The top row highlights evidence for the separation between South Georgian and Antarctic wingless chironomids in the Eocene and Miocene (reproduced from Convey et al. 2008).

1.3.2 Outline and specific objectives

This thesis makes use of a range of taxa to study the history and evolution of insects in the Antarctic region. By focusing on more than one species and carrying out surveys over a wide area, I aim to determine overarching patterns in evolutionary processes, physiological adaptations and biogeographic patterns. In particular, I will address the following key objectives:

a) characterise the habitat of *T. magellanicus*;
b) determine tolerance limits of *T. magellanicus* to temperature, salinity and desiccation extremes, comparing it to previously studied Antarctic and sub-Antarctic insects;

c) assess the salinity tolerance limits of *Eretmoptera* murphyi, expanding the available literature on its ecophysiological adaptations;
d) identify phylogeographic patterns present in the contemporary distribution of *Parochlus steinenii*;
e) assess potential specific morphological changes influenced by the environment through the comparison of wing morphology in 'island' and 'continental' populations of *Lancetes angusticollis*.

Chapters 2 and 3 address the South American midge *Telmatogeton magellanicus*, and describe its habitat preferences and its physiological tolerances. Chapter 4 further develops the theme of ecophysiological adaptation in southern midges, complementing and expanding recent ecophysiological studies of *Eretmoptera murphyi* to consider its salinity tolerance. Chapter 5 presents a phylogeographic analysis of the Antarctic midge *Parochlus steinenii*, based on material obtained across its South American, sub- and maritime Antarctic distribution. Chapter 6 examines the possible consequences of the long-term isolation of two populations of the diving beetle *Lancetes angusticollis*, found in Tierra del Fuego and South Georgia, on detailed morphological adaptations. Finally, Chapter 7 integrates data from across the thesis with previously published data, to identify advances in the knowledge of polar entomology and future research directions.

In addition to the chapters presented in this thesis, I have co-authored a book chapter on the interactions of Antarctic fungi and invertebrates (Appendix IV) and a scientific paper on the distribution shifts and ecophysiological characteristics of a winged midge (Appendix V), which have also led to a work-in-progress collaboration to study the fungal communities in the Antarctic midge *Parochlus steinenii* from King George Island, Antarctica.

Intentionally blank page

CHAPTER 2

Distribution and habitat of *Telmatogeton magellanicus*

2. Distribution and habitat preferences of *Telmatogeton magellanicus* (Diptera: Chironomidae) on Navarino Island, Chile

2.1 Abstract

The habitat of the intertidal flightless midge *Telmatogeton magellanicus* (Jacobs, 1900) is described for the first time from the northern coast of Navarino Island, Tierra del Fuego, Chile. Additionally, we report the first observations of adult behaviour in the wild. We delineate the species' distribution across three tidal zones (high, mid and low), and identify substrate characteristics that favour the presence of the midge. The mid-tide zone was the key habitat utilized by *T. magellanicus*, with lower densities in the low-tide zone, and no presence in the high-tide zone. There was a strong association between the presence of larvae and filamentous algae, especially *Bostrychia* sp. and, to a lesser extent, *Ulva* spp., and between larvae and the presence of larger, more stable, boulders. As a result, the species' overall distribution was widespread but patchy. We suggest that the main limiting factor was the relative humidity experienced in different habitats. One of the most striking features of behavioural observations during data collection was the extremely active adults, which suggests high energy expenditure over a very short period of time. This may be due to the limited time available to find mates in a single low tide period, when adults have about three hours after emerging from the pupa to complete mating and oviposition before inundation by the tide. The data presented here provide a baseline for future studies on this species' ecology, phenology, physiology and general biology.

2.2 Introduction

2.2.1 Terrestrial invertebrates in the Magellan Strait

The Magellanic region of Tierra del Fuego is not strictly considered to be part of the sub-Antarctic (Selkirk 2007, Chown & Convey 2016), which is defined by the almost complete absence of true terrestrial vertebrates and woody plants. However, because it shares many climatic and environmental features with this region it is often referred to as the Magellanic sub-Antarctic (Rozzi et al. 2012, Contador et al. 2015a, Morrone 2015). The Magellanic region is still largely unexplored, with recorded biodiversity increasing mainly through the discovery of cryptic species and the activity of new research projects working in little studied areas (Chown & Convey 2016). A major component of the plant life is endemic to the region [Rozzi et al. 2012, Contador et al. 2015a, Morrone 2015]), which is likely to have specific suites of associated invertebrates. There are no published syntheses of the invertebrate fauna of the region, either aquatic or terrestrial, although several authors have described endemic species of the latter: Coleoptera (Roig-Juñent 1994, 1995, 2004, Morrone 1992, 1993, Morrone & Anderson 1995, Morrone & Roig-Juñent 1995), Hymenoptera (Durante & Abramovich 2002), Lepidoptera (Angulo 1990) and Neuroptera (Monserrat 1997).

Discovering and characterising invertebrate diversity, distribution and habitat preference is an extremely important part of understanding the ecology of high latitude southern ecosystems. Invertebrates living in these habitats often display adaptations that allow them to tolerate extreme conditions. Therefore, understanding the physiology of resident invertebrate species provides the basis for comparisons across species or environments (Convey 1996a, Gibbs et al. 1997, Gaston & Chown 1999, Hayward et al. 2004, Convey et al. 2014, 2018, Bartlett et al. 2018a, b).

In the case of the Magellanic midge, *Telmatogeton magellanicus* (Jacobs, 1900), early taxonomic studies provided very little information about the species' ecology or habitat requirements. Indeed, since the species' original discovery and description, to our knowledge there have been no published studies addressing any aspect of its biology.

Yet follow-up studies on *T. magellanicus* are extremely timely, as it forms part of a small group of key sub- and maritime Antarctic chironomid midge species that may help to clarify the historical biogeography of this region. This group includes *Belgica antarctica* Jacobs 1900, endemic to the Antarctic Peninsula and South Shetland Islands (Allegrucci et al. 2006, 2012, Lee & Baust 1982), and *Eretmoptera murphyi* Schaeffer, 1914, originally described from and endemic to sub-Antarctic South Georgia and now introduced to maritime Antarctic Signy Island (Convey 1992a, Hughes & Convey 2010, 2012, Hughes & Worland 2010, Worland 2010). Everatt et al. (2012) concluded that the latter species was pre-adapted to harsher conditions than currently prevail on its native South Georgia, where it is now known to be palaeoendemic (Allegrucci et al. 2006, 2012), and therefore has the potential to invade the Antarctic Peninsula region (Pertierra et al. 2019).

2.2.2 *Telmatogeton magellanicus*

The brachypterous midge *Telmatogeton magellanicus* (Figure 2.1) was originally classified by Jacobs (1900), based on material collected on 19 December 1897, in the genus *Belgica*. This is a genus well-known for its type species *Belgica antarctica*, which is one of only two insect species currently native to parts of the Antarctic continent (Convey & Block 1996, Chown & Convey 2016). Rübsaamen (1906) transferred the species to a new genus, *Jacobsiella*, based on examination of the original material, and expanded its description significantly. Edwards (1926) synonymised *Jacobsiella* to *Halirytus* based on features of the tarsi and ovipositor, and raised the possibility of *H. magellanicus* and *H. amphibius* (Eaton, 1875) (a species from the sub-Antarctic Kerguelen archipelago in the Indian Ocean) being the same species; Edwards' comparisons were based on examination of Eaton's specimens of *H. amphibius* along with the description and images of *H. magellanicus*. Edwards (1928) further addressed the taxonomic position of *Halirytus*, placing it in the "*Telmatogeton*" group and commenting that the former is in fact a reduced form of the latter. He considered the status of the two then-described species of *Halirytus* [with the later addition of *H. macquariensis* Brundin (1962)] and concluded that there was no evidence to doubt the validity of *H. magellanicus* as a separate species. There appear to have been no

published reports of new collections of *H. magellanicus* since those originally examined by Jacobs (1900). Wirth (1949) and Sublette & Wirth (1980) further addressed taxonomy in the genus – with the latter considering *Halirytus* to be a brachypterous form of *Telmatogeton*, and synonymising it to the latter. None of these taxonomic studies provided information about the habitat in which the midge was found, although it might be assumed that the species would require similar habitats or conditions to *T. amphibius, T. macquariensis* and other *Telmatogeton* species, which are typically found among filamentous algae attached to hard substrates in the intertidal and supralittoral zones (Tokunaga 1935, Wirth 1947, Brundin 1962, Brodin & Andersson 2009).

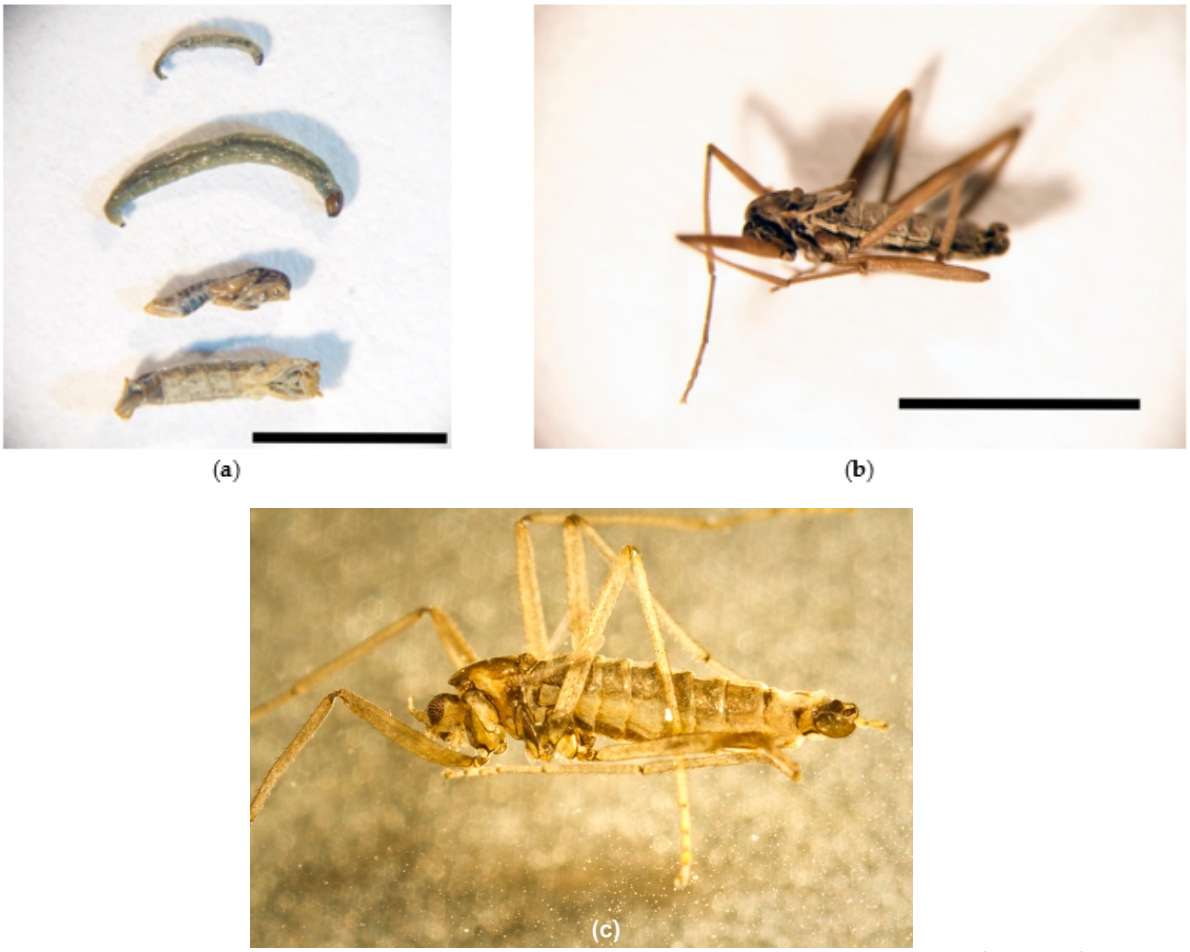


Figure 2.1. Specimens of *Telmatogeton magellanicus*: (**a**) Two larvae (2nd and 4th instars) and two pupae (one larger female and one smaller male); (**b, c**) An adult female [(c) by Gonzalo Arriagada]. (scale bar = 0.5 cm).

Until recently, *T. magellanicus* was known only from the region of the Beagle Channel, and more specifically the type locality on Navarino Island, recorded as the "Grand Glacier Bay, Tierra del Fuego, English Channel, Chile" by Jacobs (1900). In 2015, the species was rediscovered in several intertidal locations across the northern coast of Navarino Island, Chile, and in the Cape Horn Islands; there is also a possibility of it being found along the coastline around Punta Arenas, Chile (S. Rosenfeld pers. comm.). New observations and collections in January 2020 confirmed the species to be present in the same intertidal habitats in Argentinian Tierra del Fuego, in the locality of Puerto Almanza (T. Contador pers. obs.) and Stanley Harbour in the Falkland Islands (P. Convey pers. obs.). However, no formal attempt has been made to characterise the species' preferred habitat or its potential distribution, other than that it is found in the intertidal zone. Other intertidal insects living in similar habitats have been found to have behavioral and physiological rhythms linked to the tidal cycle through either endogenous clock-like mechanisms or direct external factors, which can range from environmental cues (such as temperature or sunlight) to the presence of key habitat features, such as algal food sources and substrate type (e.g. [Neumann 1986, 1988, Soong & Leu 2005, Soong et al. 2006, 2011]).

In this study we document the distribution of *T. magellanicus* on the north coast of Navarino Island and describe aspects of the species' behaviour in its natural habitat. We also categorise the local habitat and microenvironmental conditions where *T. magellanicus* is found. Through this survey we aim to better understand the biotic and abiotic drivers of *T. magellanicus* distribution across tidal zones.

2.3 Materials & Methods

2.3.1 Study site description

Fieldwork took place along the northern coast of Navarino Island (Figure 2.2), Tierra del Fuego, from 23 October to 28 November 2017, and consisted of single day visits to selected bays (Figure 2.2), and several visits to Róbalo Bay (Figure 2.2G, 2.3). Additional monthly visits to the latter were made between late 2016 and early 2018. Air temperatures

in this area range from as low as -12°C in the winter, to 26°C during the summer (means of 6.0°C across the year, 9.6°C during the warmest month and 1.9°C during the coldest [DGAC 2020 – Meteorological Station 550001]). The region is relatively dry, with relative humidity (R.H.) averaging 69.3% but varying between 40.2% and 96.0%. Total precipitation for 2018 was 560.8 mm (a minimum of 23.2 mm in April and maximum of 89.9 mm in June). The climate is heavily influenced by prevailing westerly winds that can reach average speeds of up to 39 kts (in 2018). The average tidal range is 1.40 m, ranging from 0.15 m to 2.51 m (DGAC 2020, SHOA 2020). At each visit, targeted searches were carried out to confirm the presence of adult *T. magellanicus*. We mapped the presence of *T. magellanicus* at each survey location in order to visualise the distribution of the species across Navarino Island. We also made opportunistic notes of any aspects of adult behaviour.

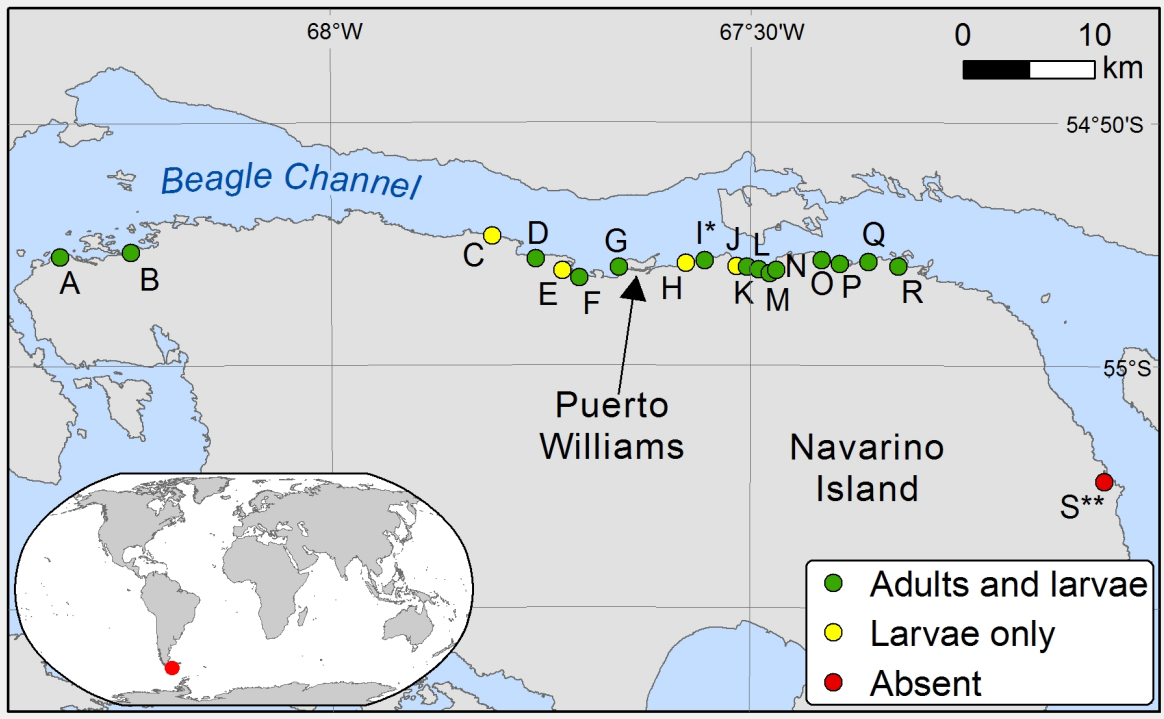


Figure 2.2. Navarino Island with the bays surveyed, colour-coded for presence/absence of adults and larvae of *Telmatogeton magellanicus*. (A, Puerto Navarino; B, Honda Bay; C, 'Bahia Linda'; D, Chicha de Pera; E, 'Second' Bay; F, Los Bronces; G, Róbalo Bay; H, Ukika; I, Puente Guanaco Bay; J, Punta Truco; K, 'Seventh' Bay; L, Amarilla Bay; M, 'Fifth' Bay; N, Corrales Bay; O, 'King Penguin' Bay; P, 'Third' Bay; Q, 'Casita' Bay; R, Eugenia Bay; S, Puerto Toro) (*also *Telmatogeton* sp.; **survey time was very limited).

2.3.2 Habitat characterisation at Róbalo Bay

We selected Róbalo Bay to carry out finer-scale measurements of environmental conditions influencing the abundance of *T. magellanicus*, owing to an abundant population being observed during initial field visits and to the site's ease of access.

Figure 2.3. The location of habitat transects at Róbalo Bay. Each transect included a quadrat (60 x 60 cm) deployed in the high tide, mid tide and low tide zones of the shoreline. (Satellite image edited from Google Earth, earth.google.com/web/)

2.3.3 Environmental variability within low and high tide limits

To describe the habitat of *T. magellanicus* at Róbalo Bay we divided the area into 28 transects (Figure 2.3). Transects were separated by 50 m from each other along the shore, and each ran in a straight line, perpendicular to the coast, from low to high tide limits. At each transect, we randomly selected one area to deploy a 60 x 60 cm quadrat (sub-divided into 5 x 5 sub-quadrats – 25 squares in total) at each of three tidal heights

(low tide, mid tide and high tide) (Figure 2.4). The tide levels were determined by a combination of two factors: i) recording the water level at high and low tide as predicted from average levels taken from tide tables (Benedetti-Cecchi & Cinelli 1997), and ii) biological composition, as defined by Contador et al. (2015a). Thus, we defined the high tide level as the area between 2.5 m down to where molluscs (mainly bivalves) start to occur in abundance (around 1.5 m), the mid tide from ~1.5 m to ~0.8 m, which is where the low tide zone started with the presence of cirriped crustaceans and coralline algae. Upon starting the survey at each station, we measured air temperature, relative humidity (R.H.) and wind speed (1.5 m height) using a thermocouple logger (Hobo® 4-channel UX120-014M) and anemometer (Kestrel 3000 Environmental Meter), respectively. We also recorded substrate surface temperatures and R.H. We then searched the 25 sub-quadrats for 1 minute each, recording, through visual inspection and manipulation of the top layer of the substrate, the presence/absence of *T. magellanicus* larvae of any stage. We chose larvae for this study because they are less mobile than adults and their presence demonstrates that breeding is occurring, making them a more reliable indicator of true habitat requirements for the species.

Each quadrat was photographed for subsequent confirmation of the environmental components, namely, boulders (rocks larger than 26 cm in diameter), stones (rocks between 1 cm and 26 cm), gravel (crushed stone and any clustering of small stones up to 1 cm), sand, bivalves, water, as well as the marine algae: *Bostrychia sp.*, *Ulva lactuca*, *U. intestinalis*, *Adenocystis* sp., *Porphyra* sp., and “other” (including Rhodophyta, *U. prolifera*, *Nothogenia* sp., *Macrocystis* sp., *Scytosiphon lomentaria*, and large patches of mixed dead algae).

2.3.4 Statistical analyses

We performed a Permutational MANOVA (PERMANOVA) with pair-wise tests to test for differences in the habitat composition of the 12 most frequent environmental variables between the three tide levels (low, medium, and high) (Anderson et al. 2008). These variables, acquired through the quadrat methodology, were: Boulder, Stones, Gravel, Sand, Bivalves, Water, *Bostrychia* sp., *Ulva lactuca*, *U. intestinalis*, *Adenocystis* sp.,

Porphyra sp., and Other (which includes other minor features, such as sporadic algal species or clumps of decomposing organic matter). The procedure was performed using normalized data to construct a resemblance matrix based on Euclidean distances with 9999 unique permutations, with "tide" as the only fixed factor included in the model. We calculated the contribution of each environmental variable at each tide level using the Similarity Percentages (SIMPER) procedure. The SIMPER procedure list was cut off when the accumulated contribution of each environmental variable reached 90%. Both analyses, PERMANOVA and SIMPER, were conducted using PRIMER-E v7 with Permanova+ add-on package (Anderson et al. 2008, Clarke et al. 2015).

We also compared the air and substrate temperatures, air and substrate R.H., and average and maximum wind speeds between the three areas using Kruskal-Wallis tests. *Post hoc* paired Wilcoxon tests were used where significant differences were found. These tests and analyses were run with R (version 3.6.0) in RStudio (Version 1.2.1335).

2.3.5 Presence of *T. magellanicus* across the three tidal levels

Proportional occurrence data in quadrats were tested for normality using Kolmogorov-Smirnov tests, but were non-normal, so we used a Kruskal-Wallis test, with *post hoc* paired Wilcoxon tests, to assess whether the occurrence of *T. magellanicus* differed between the tidal zones.

2.3.6 Prediction of presence of *T. magellanicus* within the mid-tide zone

As the vast majority of *T. magellanicus* were found in the mid tide zone, we carried out a more detailed analysis to assess how environmental factors influenced its presence in this zone. Here, we once again ran PRIMER-E v7 with Permanova+ add-on package (Anderson et al. 2008, Clarke et al. 2015), where abundances were transformed to presence/absence data and resemblance matrices created based on Euclidean distances (Clarke et al. 2015). Environmental data were normalized prior to generating Euclidean resemblance matrices. Then, the combined influence of the environmental variables on the habitat preference of *T. magellanicus* was investigated using Distance-based Linear Modelling (DistLM) in Permanova+, using the *Best* selection criterion and *adjusted* R^2

values. The output for the *Best* selection procedure in DistLM aims to provide the best 1-variable model, the best 2-variable model, and so on, on the basis of the chosen selection criteria (Anderson et al. 2008). DistLM seeks the most significant relationships between the similarity matrix and environmental variables by progressively modeling the matrix against the most influential variable, taking the residuals of that relationship, modelling the next most influential variable, and so on (Clarke et al. 2015, Pearson et al. 2019).

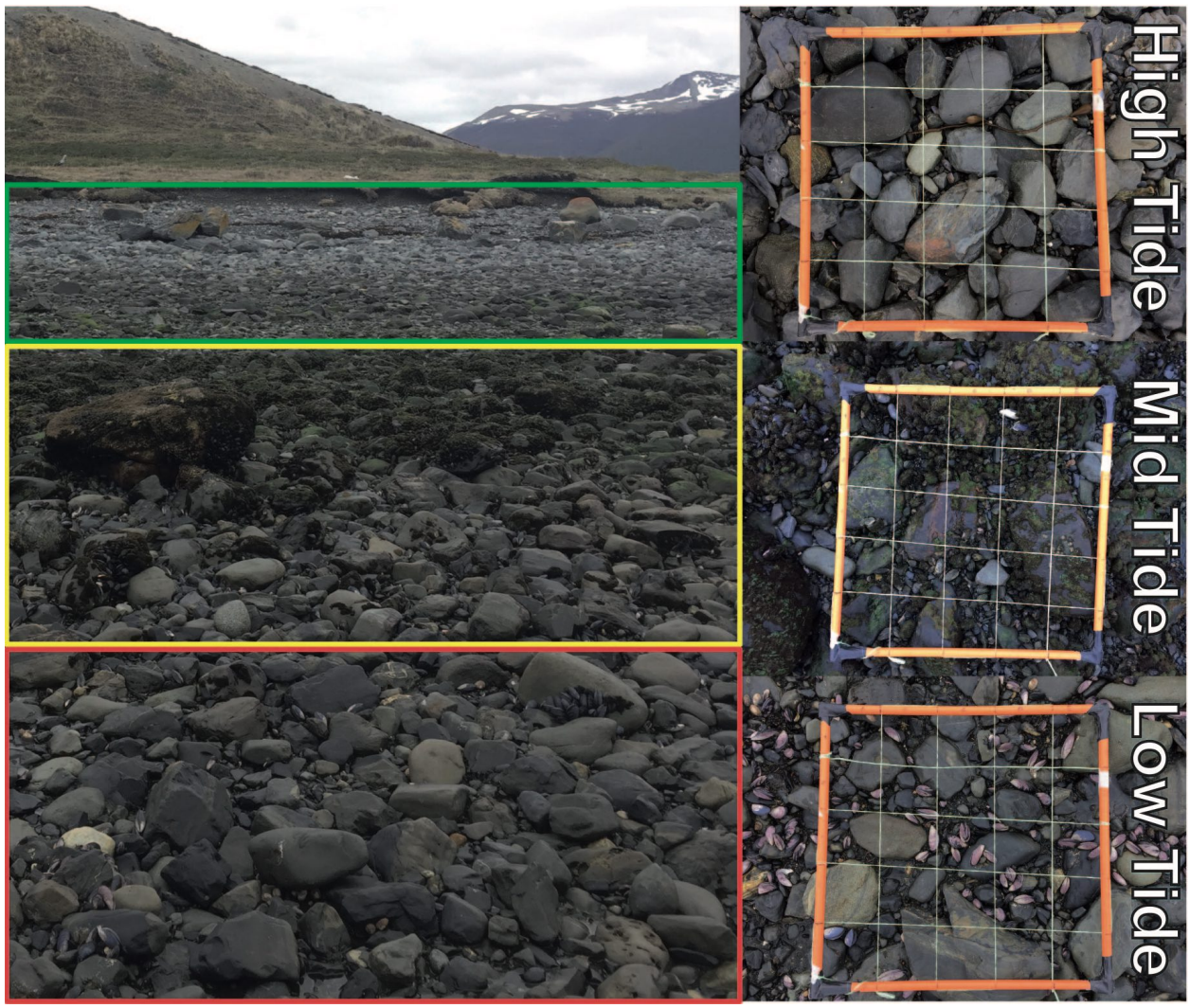


Figure 2.4. The three tidal areas in Róbalo Bay, with representative quadrats (60x60 cm) used for the habitat characterisation.

2.4 Results

2.4.1 Island-wide distribution

Telmatogeton magellanicus was present in most of the bays along the northern coast of Navarino Island (Figure 2.2). In some places, such as the 'Casita' and 'Fifth' Bays (Figure 2.2M and 2.2Q, respectively) there were lower abundances of both adults and larvae. *T. magellanicus* shared the habitat with an unidentified winged species of *Telmatogeton* in Puente Guanaco Bay (Figure 2.2I), but this species was not recorded at any other location. Adults were recorded in almost every month with the exception of July and August, as we could not survey the area during this time. Adults were very active, moving rapidly while searching for mates. While mating was easily observed, we did not observe oviposition in the field, although females would readily oviposit in containers after capture (either on algae or on the surface of the container). Adults actively avoided water, using surface tension to move rapidly across the water surface only when this was unavoidable. Either as a consequence of entrapment in water or as result of post-mating death, the beach became littered with corpses of dead adults at the end of the tidal cycle that were subsequently washed into the sea as the tide rose back again.

2.4.2 Environmental variability within low and high tide limits

There was a fairly even distribution of variables across the tidal zones (Figure 2.5), but the PERMANOVA procedure showed that there were significant differences in habitat composition within the three tide zones (Mean squares = 62.237; Pseudo-F = 5.7927; p = 0.0001). The *post hoc* tests indicated that significant differences existed between the high and low, the high and mid, and the low and mid tidal zones (Table 2.1).

The SIMPER procedure identified the % contribution of the 12 environmental variables that contributed 90% of the habitat composition across the three tidal zones (Table 2.2).

Table 2.1. PERMANOVA *post hoc* tests for environmental conditions between the different tidal zones in Róbalo Bay, Navarino Chile. Asterisks indicate significant differences (* p < 0.05, ** p < 0.01, *** p < 0.001).

Pair Wise test	Environmental conditions (12 variables)		
	t	Unique perms	p (perm)
High vs. Low	2.7567	9939	0.0001***
High vs. Mid	2.8884	9925	0.0001***
Low vs. Mid	1.723	9934	0.0011**

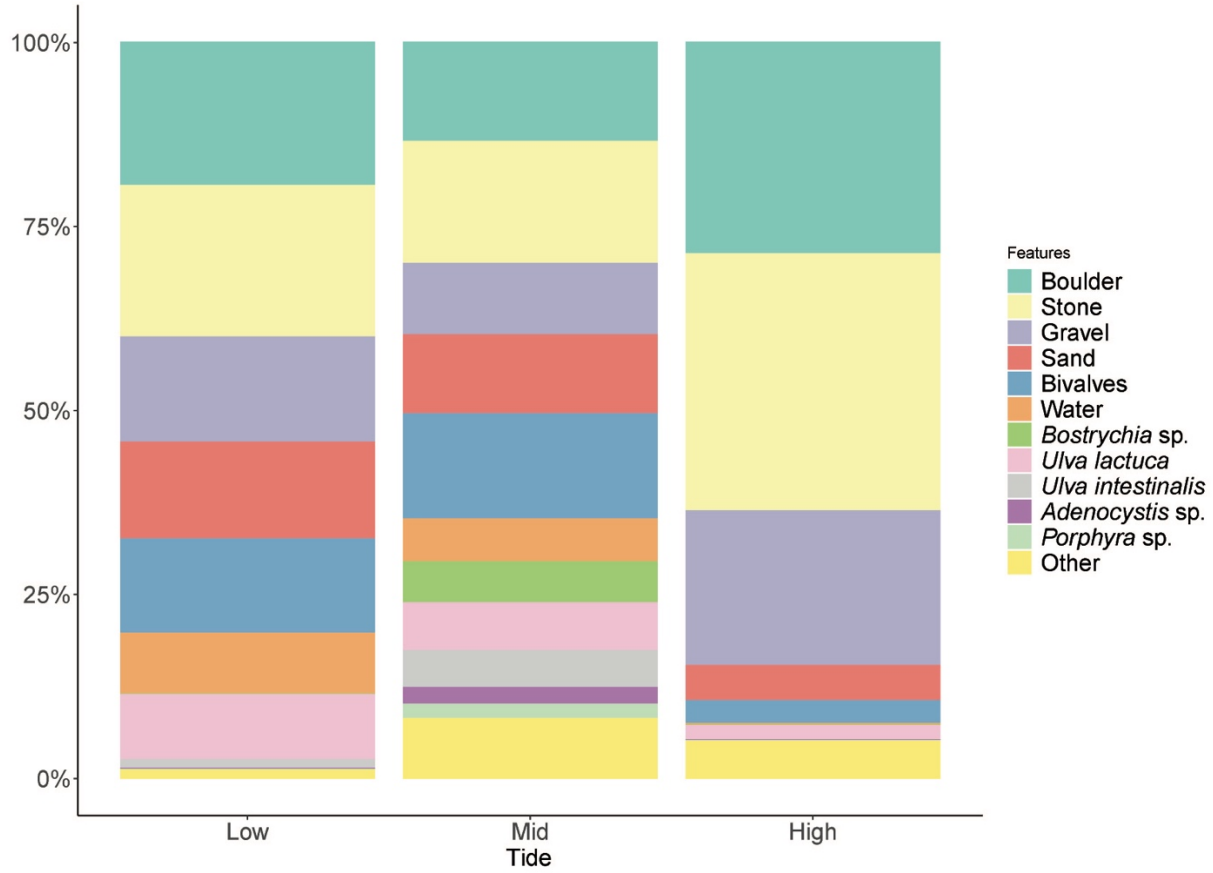


Figure 2.5. Stacked histogram with the distribution of the 12 measured environmental variables across the three tide zones (low, mid and high).

Table 2.2. Similarity Percentages (SIMPER) analysis of environmental variables that contributed 90% of the habitat composition within the three tidal zones studied in Róbalo Bay, Navarino Island, Chile (Contrib. % = percentage of variable contribution; Cum. % = cumulative contribution).

Tide level	Environ. variable	Av. Value	Sq.Dist/SD	Contrib. %	Cum.%
Low	*Bostryichia sp.*	0.04	0.20	0.01	0.01
	Adenocystis sp.	0.04	0.20	0.01	0.01
	Porphyra sp.	0.04	0.20	0.01	0.02
	Other	1.00	0.37	0.54	0.56
	Ulva intestinalis	0.93	0.26	1.19	1.75
	Water	6.26	0.50	7.12	8.87
	Gravel	10.80	0.57	13.95	22.82
	Ulva lactuca	6.63	0.49	14.22	37.04
	Stone	15.6	0.53	14.36	51.40
	Boulder	14.7	0.54	14.70	66.11
	Bivalves	9.7	0.55	15.62	81.73
Mid	*Porphyra sp.*	1.75	0.31	2.97	2.97
	Adenocystis sp.	2.18	0.28	4.45	7.41
	Water	5.43	0.44	5.41	12.82
	Bostryichia sp.	5.25	0.43	6.48	19.30
	Ulva lactuca	6.11	0.45	6.63	25.93
	Ulva intestinalis	4.68	0.42	7.67	33.60
	Stone	15.60	0.51	7.77	41.38
	Boulder	12.60	0.54	8.08	49.46
	Gravel	9.18	0.56	10.67	60.12
	Sand	10.10	0.55	12.42	72.54
	Bivalves	13.5	0.57	13.32	85.86
High	*Porphyra sp.*	0.036	0.19	0.01	0.01
	Bostryichia sp.	0.71	0.27	0.02	0.04
	Adenocystis sp.	0.71	0.19	0.05	0.09
	Water	0.11	0.19	0.12	0.21
	Ulva lactuca	1.18	0.31	3.10	3.31
	Stone	22.20	0.45	4.20	7.51
	Bivalves	2.00	0.26	8.93	16.43
	Sand	3.04	0.40	13.54	29.97
	Other	3.32	0.34	16.60	46.57
	Gravel	13.4	0.54	24.84	71.41

Differences in air temperature were significant between the three zones (χ^2 = 10.814, d.f = 2, p = 0.002), with temperatures in the mid tide being significantly higher than the high tide (p = 0.002). Air R.H. was not significantly different between zones (χ^2 = 4.457, d.f = 2, p = 0.108). Substrate surface R.H. was significantly different (χ^2 = 16.386, d.f = 2, p < 0.001), with R.H. in the high tide zone being significantly lower than the low (p < 0.001) and mid zones (p = 0.020). Average wind speeds were significantly different between zones (χ^2 = 9.301, d.f = 2, p = 0.010), being higher in the high tide zone than in the low zone (p = 0.011) (Figure 2.6).

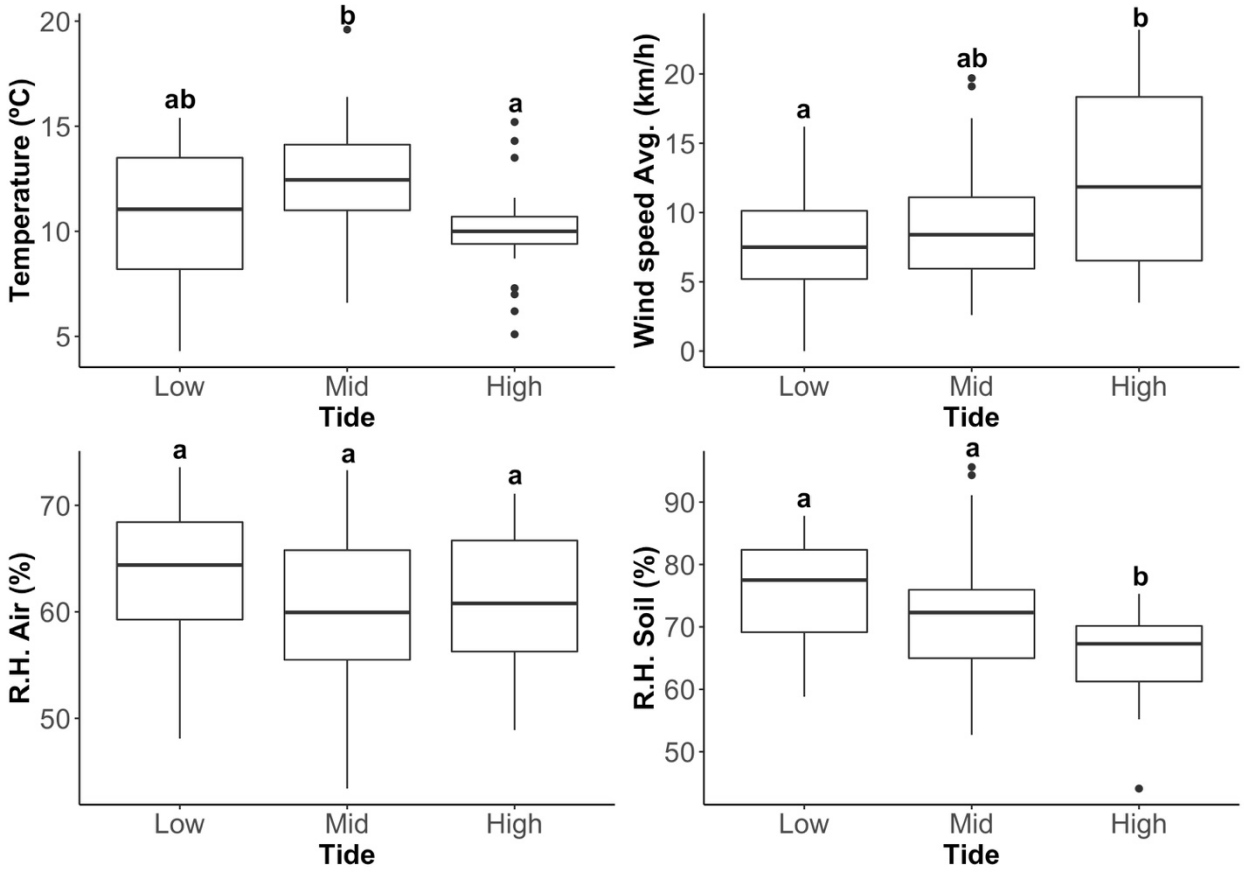


Figure 2.6. Air temperature, average wind speed, and air and substrate R.H. across the three tidal zones (low, mid, high). Means with the same letter are not significantly different at p < 0.05 (Pairwise Wilcoxon Rank Sum Tests).

2.4.3 Difference in presence of *Telmatogeton magellanicus* between the three tidal levels

Larvae of *T. magellanicus* differed significantly in abundance between the three tidal zones (Kruskal-Wallis, $\chi^2 = 23.138$, d.f. = 2, $p < 0.001$), being more common in the mid tide zone (90% of all the sub-quadrats had confirmed presence) than the low tide (9.67%), and the high tide (0.33%) (Figure 2.7).

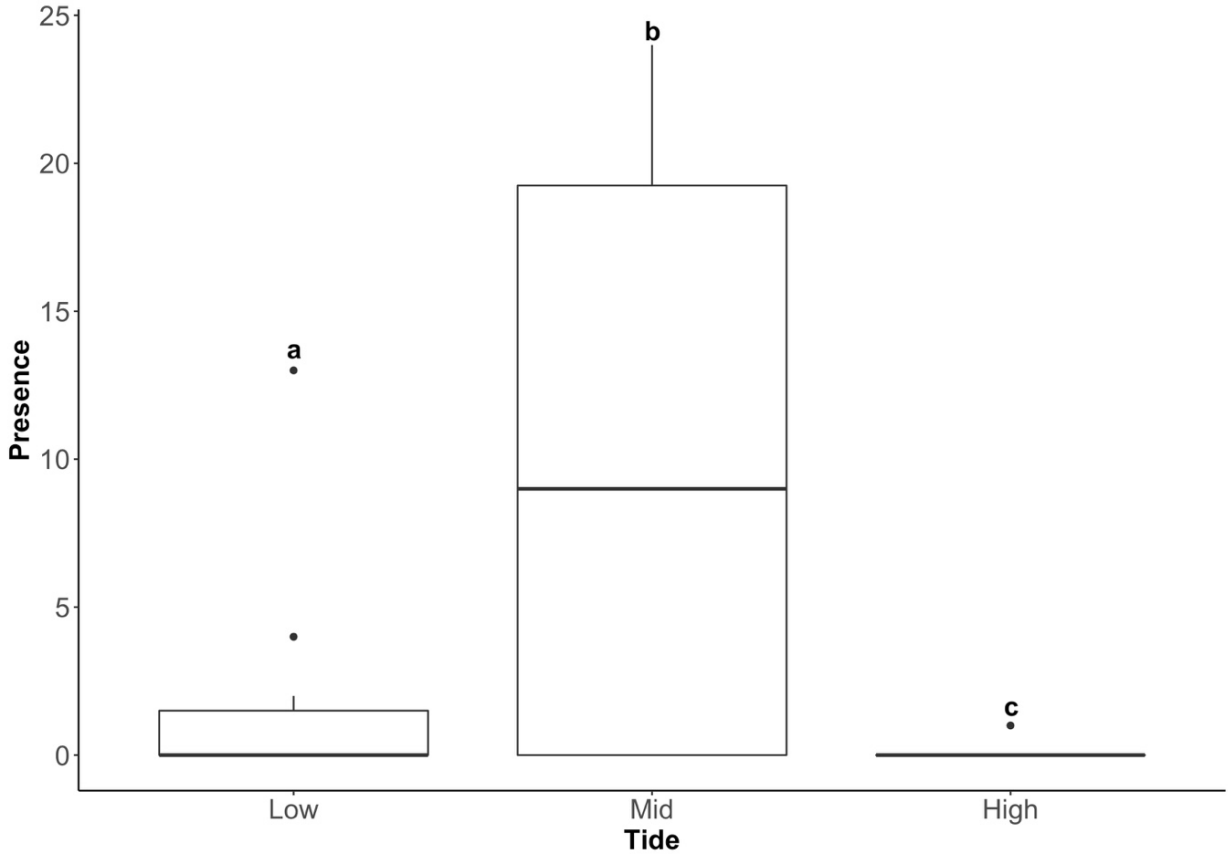


Figure 2.7. Presence of larvae of *T. magellanicus* in quadrats (25 sub-quadrats per quadrat) across the three tidal zones (low, mid, high). Different letters indicate a significant difference at $p < 0.05$ (Pairwise Wilcoxon Rank Sum Tests).

2.4.4 Prediction of presence of *Telmatogeton magellanicus* within the mid-tide zone

Distance-based Linear Modelling (DistLM) analysis of the impact of the 12 environmental variables on the habitat preference of *T. magellanicus* indicated that, considered separately, boulders, sand, bivalves, *Bostryichia* sp., *U. lactuca, U. intestinalis* and

Adenocystis sp. were significantly positively associated with the presence of *T. magellanicus* (Table 2.3). However, when all combinations of variables were considered, the *Best* modelling procedure showed that presence of a combination of boulders (5%), gravel (2%), sand (9%), bivalves (12%), *Adenocystis* sp. (6%), *Bostryichia* sp. (27%), *U. lactuca* (12%), *U. intestinalis* (10%), and *Porphyra* sp. (2%) were the variables that best explained the presence of *T. magellanicus* (adjusted $R^2 = 0.52$).

Table 2.3 Marginal tests obtained from the Distance-based Linear Modelling procedure for the 12 environmental variables measured within the three tidal zones in Róbalo Bay, Navarino Island, Chile. Asterisks indicate significant associations with the presence of *T. magellanicus* (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$), and values in bold the most highly associated variables. (Prop. = proportion of variability)

Variable	Pseudo-F	p	Prop.
Boulder	4.39	0.03*	0.05
Stone	0.02	0.91	0.00
Gravel	1.78	0.16	0.02
Sand	8.17	0.003**	0.09
Bivalves	11.42	0.002**	**0.12**
Water	1.52	0.24	0.02
Bostryichia sp.	30.12	0.001***	**0.27**
Ulva lactuca	10.68	0.004**	**0.12**
Ulva intestinalis	9.48	0.01*	**0.10**
Adenocystis sp.	4.77	0.04*	0.06
Porphyra sp.	1.74	0.13	0.02
Other	0.03	0.86	0.00

2.5 Discussion

Our survey data confirm that *T. magellanicus* is distributed across the northern coast of Navarino Island. Although every bay along the coast could not be examined, our data suggest that the species is present throughout this area, while recent opportunistic observations in Argentinian Tierra del Fuego and the Falkland Islands support an as yet undocumented wider regional distribution. Across the surveyed bays, it was clear that the

favoured microhabitats for the larvae were locations where the algae *Bostrychia* sp. and *Ulva* spp. were present, but also where there was a combination of these algae with *Porphyra* sp., boulders, gravel, sand and bivalves. It is very likely that algae, especially the more filamentous taxa such as *Bostrychia* sp., desiccate more slowly than exposed substrata, maintaining higher R.H. levels during the low tide period. In some of the bays, instead of an abundant presence of *Bostrychia* sp., there was greater presence of *U. intestinalis* or *U. prolifera*, which were also good predictors for the presence of *T. magellanicus*.

One of the most striking features of the adults was their extremely active behaviour, which suggests high energy expenditure in a very short period of time. This is likely driven by the limited amount of time they have to find mates in the period between successive high tides. This is similar to reports of the behavior of *Pontomyia* spp., another intertidal chironomid genus found in the Caribbean, north-eastern Brazil, Japan, south-east Asia and Australia (Soong & Leu 2005, Soong et al. 2006, 2011, Kao et al. 2010, Huang et al. 2014), and which has one of the shortest known insect adult lifespans (maximum of three hours for the winged males and the vermiform females. Even though adults were seen mating in the field, we did not directly observe oviposition but, given the distribution of larvae, this is likely to take place on or within algal mats.

The absence of both adults and larvae in the high intertidal zone is likely associated with the lack of suitable microhabitat to escape from extremes of microclimate in this area. Across all bays, this zone primarily consists of boulders and sand mixed with clay, making the substrate too hard for the larvae to burrow into, and thereby exposing them to dangerous stressors such as sunlight/UV exposure and wind, as well as potential predators (at least one species of insectivorous bird, *Lessonia rufa* (Gmelin, 1789), frequently forages in the intertidal zone of Róbalo Bay, and other bird species including *Vanellus chilensis* (Molina, 1782) and *Xolmis pyrope* (Kittlitz, 1830) are often also present in the area). It was unclear which variables directly restricted the presence of the species in the low tide zone, but this zone is covered by seawater for most of the time, being water-free for only 1–2 hours during low tide. This zone may, therefore, be hostile to the

adults due to the risk of drowning, a hypothesis that is consistent with our casual observations of the high mortality of adults when an area is inundated. A caveat for some of these hypotheses is that our environmental measurements of temperature, R.H. and wind speed were taken at different times of the day and across different days. However, these differences are likely to add noise to the data rather than leading to a systematic difference in readings being recorded between the different zones.

The mid tide zone contained by far the highest numbers and density of *T. magellanicus*, whose larvae were abundant in filamentous algae growing on different substrates, such as bivalves and boulders. Within the mid zone, the variables that were most consistently associated with the presence of *T. magellanicus* were boulders, stones, *Bostrychia* sp. and *U. intestinalis*, consistent with the findings of the more general distribution study (Table 2.3). It is likely that these environmental features provide good shelter from extremes of temperature, irradiation and wind exposure, either by direct physical protection from these factors or by creating microclimates within, thereby reducing desiccation stress.

The intertidal zone is the key habitat type for *T. magellanicus*, but within this zone certain conditions support higher larval population densities, namely large rocks (i.e. boulders) and filamentous algae (particularly *Bostrychia* sp. and *Ulva intestinals*). As a result, the species' overall distribution is widespread but patchy. The data presented lay the foundation for future studies of this unusual insect's distribution, ecology, phenology, physiology and general biology. With improved knowledge of the species' preferred microhabitats we are better able to predict other locations where it may occur, and even to extrapolate the risk of it being transported elsewhere (e.g. in species distribution modelling, which can involve the direct use of GIS data or a combination of biogeographical, physiological and meteorological data (Gañán Mora et al. 2015, Fabri-Ruiz et al. 2018, Pertierra et al. 2019, Vega et al. 2020).

2.6 Acknowledgements

We thank Nadescha Zwerschke for the help with translating Rübsaamen's paper; Javier Rendoll Carcamo and Carolina Perez for the fieldwork assistance; Helen Waters for help analysing the data; and, Laura Gerrish of MAGIC-BAS for the map.

CHAPTER 3

Physiological limits of *Telmatogeton magellanicus*

3. Salinity, temperature and desiccation tolerance limits of *Telmatogeton magellanicus* (Diptera: Chironomidae)

3.1 Abstract

The recent rediscovery of the intertidal flightless midge *Telmatogeton magellanicus* on the shores of Navarino Island, south of the Beagle Channel in Tierra del Fuego, provides the opportunity to explore its biological characteristics and to establish what underpins this species' resilience to the extreme temperatures and generally harsh environmental conditions of the region. We exposed larvae of *T. magellanicus* collected in Róbalo Bay to a series of laboratory treatments in order to assess their tolerance to low and high salinity, a gradient of temperatures ranging from -12ºC to ~38ºC, and exposure to three levels of relative humidity (50, 70 and 90%). The larvae had high resilience to salinity fluctuations and to high temperatures, surviving for at least 10 days in fresh water, seawater and brackish water, as well as at temperatures of up to 34ºC. Additionally, they were relatively resistant to lower temperatures, although survival decreased below -6ºC. It remains to be assessed whether the larvae are freeze-tolerant or freeze-avoiding. However, desiccation appears to be the main threat for the larvae of *T. magellanicus* and the main limiting factor to their distribution, as the larvae are very sensitive to low levels of relative humidity.

3.2 Introduction

Information on Antarctic and sub-Antarctic insect species is limited. However, gaining a better understanding of these species' biology is crucial, especially given their restricted range and potential vulnerability to the impacts of climate change (Chown & Convey 2016). Although not currently present in continental (East) Antarctica, insects have been present in the Antarctic region from at least the early Palaeocene (~70 mya) (Allegrucci et al. 2006, Convey et al. 2008). Nowadays, insects are found in the maritime Antarctic (Antarctic Peninsula and associated Scotia Arc archipelagos) and the sub-Antarctic

Islands (Convey & Block 1996; Chown & Convey 2016). Knowledge of their diversity, evolution and ecology is mostly based on studies undertaken in the past 50 years, catalysed by the works of J. L. Gressitt (Gressitt 1964, 1970a, 1970b, 1971) and most recently reviewed by Chown & Convey (2016). The historical isolation and specificity of insect taxa in the region can provide rich information on how long the fauna and flora of the continents and their related islands have been isolated. They also provide an important test case of how insects can adapt to extreme conditions (Allegrucci et al. 2006, 2012, Convey et al. 2008, 2009).

The terrestrial fauna and flora of the Antarctic Peninsula and Scotia Arc region is related to that of South America, with intimate geographical, biological, geological and glaciological histories (Mercer, 1976, Clapperton & Sugden, 1988, Clapperton et al., 1989, Convey 2007, Rodbell et al. 2009, Fernandez et al., 2011), as seen in the naturally occurring midges (Diptera: Chironomidae) and beetles (Coleoptera) of the region. However, as Chown & Convey (2016) pointed out, the available literature is insufficient to properly determine the factors determining the current distribution and abundance of insect taxa in the region, which has consequences for any attempt to conserve them. In particular, little is known about their tolerance to changing temperature, humidity or salinity, although all of these factors may be influenced by climate change.

Polar and sub-polar regions are constantly exposed to very low temperatures and other extreme conditions, such as low relative air humidity, high radiation, and exposure to strong winds (Walton 1982; Convey 1996; Convey et al. 2014). All of these factors are likely to impose strong selective pressures on the physiological tolerances of species in the region. The physiological tolerance of the brachypterous midge *Telmatogeton magellanicus* has not been studied, although its patchy distribution across the shoreline (Chapter 2) indicates that physiological tolerance to abiotic factors may be key to understanding its distribution. The lack of knowledge about its physiology contrasts with that of *Belgica antarctica* and *Eretmoptera murphyi*, two other brachypterous midges naturally occurring in the Antarctic and South Georgia, respectively (e.g Convey & Block 1996, Worland 2010, Teets et al. 2011, Everatt et al. 2014a, Lee & Denlinger 2015). It is

appropriate to note that *T. magellanicus* was originally described within the genus *Belgica* (Rübsaamen 1906), implicitly sharing important morphological characteristics with this genus.

In this chapter we assess the ability of *T. magellanicus* larvae to tolerate different levels of salinity, temperature and desiccation. In particular we address the following key questions relating to larval survival:

1) What is the impact of changing salinity concentrations?
2) What is the impact of different temperatures?
3) What is the impact of changing relative humidity levels?

3.3 Materials & Methods

3.3.1 Sampling

We manually extracted samples from the intertidal zone of Róbalo Bay (~4 km west of the city of Puerto Williams), Navarino Island, Chile, during October and November 2017 (Fig. 2.2). We first collected adults using an entomological aspirator in order to document their reproductive activity, survival, oviposition pattern and egg batch sizes. Subsequently, substrate (sand + organic matter) was collected and taken to the laboratory at the Omora Field Station in Puerto Williams, whereupon we carefully extracted the larvae. Upon extraction, all larvae were placed in 6-well plates containing sea water [35ppt (parts per thousand) at 4ºC], with a small amount of substrate for food, for 24 h conditioning. In total, we extracted >1200 larvae. First (L1) and fourth instar larvae were not used in the experiments, as numbers were insufficient.

3.3.2 Ecophysiological experiments

3.3.2.1 Salinity tolerance

We assessed survival and wet mass change in larvae under 5 different treatments: fresh water (0 ppt, parts per trillion), brackish water (17.5 ppt), seawater (35 ppt) and two hypersaline concentrations (52.5 and 70 ppt), with each treatment containing 30 larvae in individual tubes containing a small amount of substrate to avoid starvation. Preliminary

observations (not shown here) indicated that larvae tended to cluster together if left in a single tube. These treatments were applied to represent potential conditions that *T. magellanicus* is likely be exposed to in the collection environment, given the proximity of the Róbalo River (fresh water + river delta) on the one hand and the higher salinity concentrations of evaporated seawater during low tide periods on the other. We recorded survival of larvae (defined as spontaneous movement or movement in response to a gentle stimulus) in each sample at the end of the experiment and after a period of 72 h recovery at the end of the experiment in sea water at 4°C. We measured wet mass change by gently drying individuals with a soft tissue and weighing them to the nearest 100 µg (using a Shimadzu AUX220 analytical balance) both before and immediately after treatment and following the recovery period. We obtained fresh water from Róbalo River, which discharges at Róbalo Bay and is one of the purest waters in the world, completely free of chemical contaminants (non-chlorinated) (Hedin, Armesto & Johnson, 1995, Weathers et al. 2000, Contador et al. 2015a, Mach et al. 2016, Rendoll et al. 2019). We prepared brackish water by mixing fresh water with seawater, and the highly saline treatments by reducing seawater through heat evaporation. Salinity was assessed using a Conductivity pH TDS Hanna Tester HI98130 salinity meter.

3.3.2.2 Temperature tolerance

We exposed 30 larvae of *T. magellanicus* in separate tubes to two experiments using heated and cooled test conditions respectively, to assess the upper and lower temperature limits of larvae. First, larvae were warmed at 0.1ºC min^{-1} from 4–37ºC in 1.5 mL tubes containing 1 individual each that were placed in a thermoregulated water bath (Lab. Companion RW-0525G). In pilot experiments, we recorded no mortality until 30ºC. We therefore only began to assess mortality changes after this point, recording survival at 30, 31, 32, 33, 34, 35, 36, and 37 ºC. For the second experiment, larvae were cooled at 0.1ºC min^{-1} from 4ºC until -12ºC was reached (water bath antifreeze liquid limit). In this case, we recorded survival at 0, -3, -6, -9, and -12 ºC. The range of temperatures used in these experiments reflected those experienced in the natural environment at Navarino Island, where temperatures range from as low as -12ºC in the winter, up to 26ºC during the summer (yearly average of 6.0ºC; 9.6ºC during the warmest month and 1.9ºC during

the coldest) (DGAC 2019). We checked survival as described above. We also reassessed survival at 24 h and 48 h after recovery. Wet mass was not measured in this experiment.

3.3.2.3 Desiccation tolerance

We exposed individual larvae (n = 10) of *T. magellanicus* to a 50% relative humidity (R.H.) (~4ºC) treatment for 160 min to test their baseline tolerance to desiccation stress; larvae were spread individually on 2 cm^2 aluminum foil tins. At 10 min intervals throughout the experiment, larvae were checked for survival and wet mass measured as described above. We followed up this low-humidity experiment with another experiment where individual larvae were exposed to 70 or 90% R.H. (n = 30 larvae for each humidity and time period) for 16 h and 84 h respectively. The differing lengths of times used across the different R.H. levels reflect the relatively longer survival times of larvae in high-humidity conditions. Across all treatments, desired R.H. levels were established using an electric humidifier and checked with a Kestrel 3000 anemometer in a closed temperature-controlled vivarium. The R.H. levels chosen reflect the low but variable humidity conditions experienced on Navarino Island, where R.H. averages 69.3% but drop as low as 40.2% or increase to 96.0% (Meteorological Station 550001).

3.3.3 Statistical analyses

To test for normality in all data, Shapiro-Wilk tests were used. Data that were normally distributed underwent an analysis of variance (ANOVA), with Tukey's *post-hoc* tests applied where an initial significant difference was found. Non-normally distributed data were analysed using Kruskal-Wallis (with pairwise Wilcoxon *post-hoc*) or Aligned Rank Transform (ARTool R package; Wobbrock et al. 2011, Harrar et al. 2019) tests. We tested for differences in percentage survival and mass of larvae between the five salinity concentrations and across five time periods (6 h, 1, 2, 5 and 10 d). We also specifically assessed the impact of being alive or dead on mass of larvae at 2 d and 10 d (survival gauged from after recovery period) across the five different salinity concentrations. We tested for differences in percentage survival across different temperature treatments at 30, 31, 32, 33, 34, 35, 36, and 37 ºC for the heating experiment and at 0, -3, -6, -9, and -12 ºC for the cooling experiment. Further we tested whether survival differed across time

periods separately between the three different relative humidity levels. Finally we assessed whether mass of dead and living larvae differed across the different time periods for each of the three relative humidity conditions (50% R.H.: 60, 80, 100, 120, 140, and 160 min; 70% R.H.: 4, 8, 12, and 16 h; 90% R.H.: 12, 24, 48, 72, and 84 h). See Table 3.1 for a summary of statistical methods used and analyses conducted.

Table 3.1. Summary of statistical analyses carried out across three ecophysiological experiments (ART, Aligned Rank Transform).

Experiment	Dataset	Method	Post-hoc	Explanatory variables
Salinity	% Survival	ART	ART	Concentration, time period
	Mass Change	ANOVA	Tukey	Concentration, time period
	Alive/dead x Mass Change (2 d)	ART	ART	Alive/dead, concentration
	Alive/dead x Mass Change (10 d)	ART	ART	Alive/dead, concentration
Temperature	% Survival Heat % Survival Cold	Kruskal-Wallis	Wilcoxon	Temperature
Relative humidity	Mass (R.H. 50%)	ART	ART	Alive/dead, R.H.
	Mass (R.H. 70%)	ART	ART	Alive/dead, R.H.
	Mass (R.H. 90%)	ART	ART	Alive/dead, R.H.

3.4 Results

3.4.1 Reproduction and oviposition

Telmatogeton magellanicus reproduces sexually and, in laboratory conditions, lays egg batches (without egg sacs) containing 1–40 eggs, often in a chain or in a spherical 'globule' shape, but also in random patterns. Eggs are yellow when deposited, transitioning to a brown or dark brown colour as they develop, hatching after ~14 d at 4ºC in seawater.

3.4.2 Salinity tolerance

Survival did not significantly differ across the different salinity concentrations, nor was there a significant interaction between treatment and time (Table 3.2). However, survival decreased over time in all treatments, except ambient seawater (Table 3.2; Fig. 3.1). Larval mass varied significantly across salinity concentrations and with time (Table 3.2). There was also a significant interaction between salinity and time, with inspection of the plots showing that this was driven by a sharper loss of weight in the 70 ppt treatment over time (Table 3.2, Figure 3.2). Mass loss did not differ significantly between living and dead larvae or across concentrations for samples exposed to the different salinity concentrations after 2 d (Table 3.2; Fig. 3.3). However, there was a significant interaction between living/dead larvae and concentration for samples exposed to concentration gradients for 10 d, with inspection of the data showing that this was driven by a lower mass in dead insects than living insects at 70 ppt (Table 3.2; Fig. 3.4).

Table 3.2. Summary of analyses for water salinity tolerance across five different concentration treatments from 6 h to 10 d (ART, Aligned Rank Transform)

	Method	Explanatory variable	F	Df	Chi-squared	DF.res	Pr(>F)
Survival	ART	Time	18.794	4		98.760	<0.001
		Concentration	0.706	4		79.387	0.590
		Time:Concentration	0.515	16		223.047	0.938
Mass Change	ANOVA	Time	7.115	4	8663	2165.7	<0.001
		Concentration	5.280	4	6429	1607.3	<0.001
		Time:Concentration	1.869	16	9103	568.9	0.020
Alive/dead x Mass Change (2 days)	ART	Concentration	0.634	4		549.78	0.638
		Alive/dead	0.505	1		140.00	0.478
		Concentration:Alive/dead	0.158	4		140.00	0.959
Alive/dead x Mass Change (10 days)	ART	Concentration	0.358	4		19664	0.838
		Alive/dead	0.061	1		138	0.805
		Concentration:Alive/dead	4.552	4		138	0.002

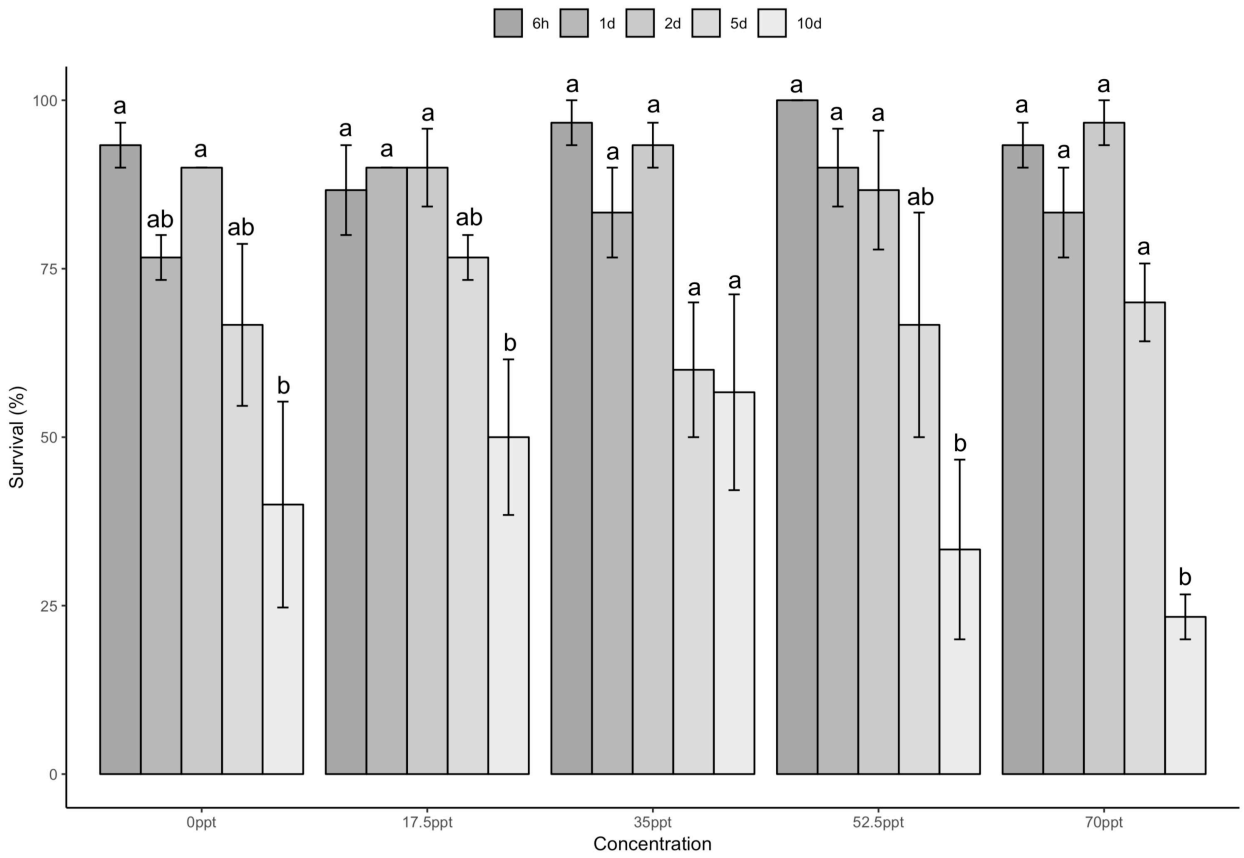


Figure 3.1. Survival (%) of *Telmatogeton magellanicus* 72 h after recovery from exposure to five water salinity concentrations (0 ppt, 17.5 ppt, 35 ppt, 52.5 ppt and 70 ppt) for a range of time periods (6 h, and 1, 2, 5 and 10 d) at 4°C. Means±SEM are presented for three replicates of 10 individuals. Means with the same letter are not significantly different within each concentration group at $p < 0.05$ (Tukey's multiple range test).

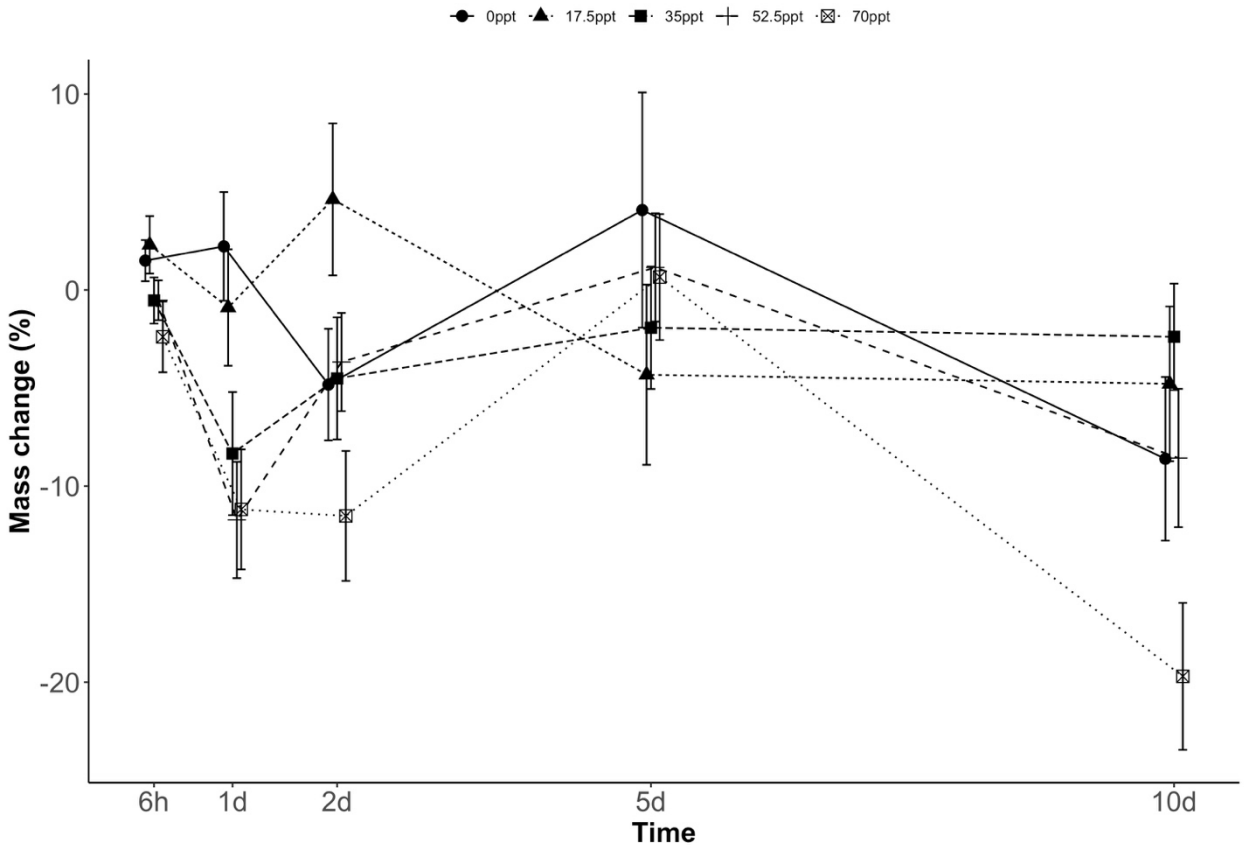


Figure 3.2. Percentage wet mass loss or gain of *Telmatogeton magellanicus* after exposure to one of five water salinity treatments (0 ppt, 17.5 ppt, 35 ppt, 52.5 ppt and 70 ppt) for a range of time periods (6 h and 1, 2, 5 and 10 d). The mean±SEM is presented for three replicates of 10 individuals.

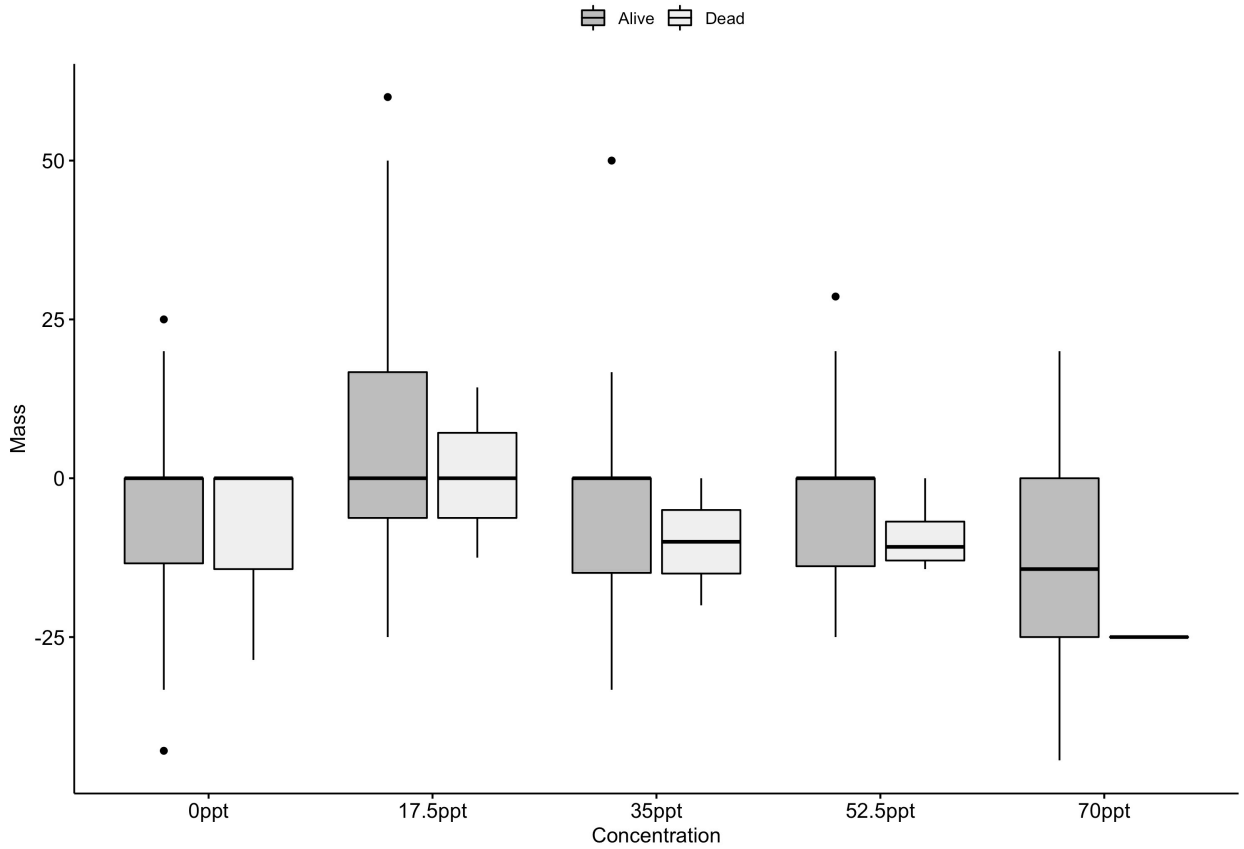


Figure 3.3. Mass loss or gain of living and dead *Telmatogeton magellanicus* larvae 72 h after recovery from exposure to five water salinity concentrations (0 ppt, 17.5 ppt, 35 ppt, 52.5 ppt and 70 ppt) for 2 d at 4°C. Means±SEM are presented for three replicates of 10 individuals.

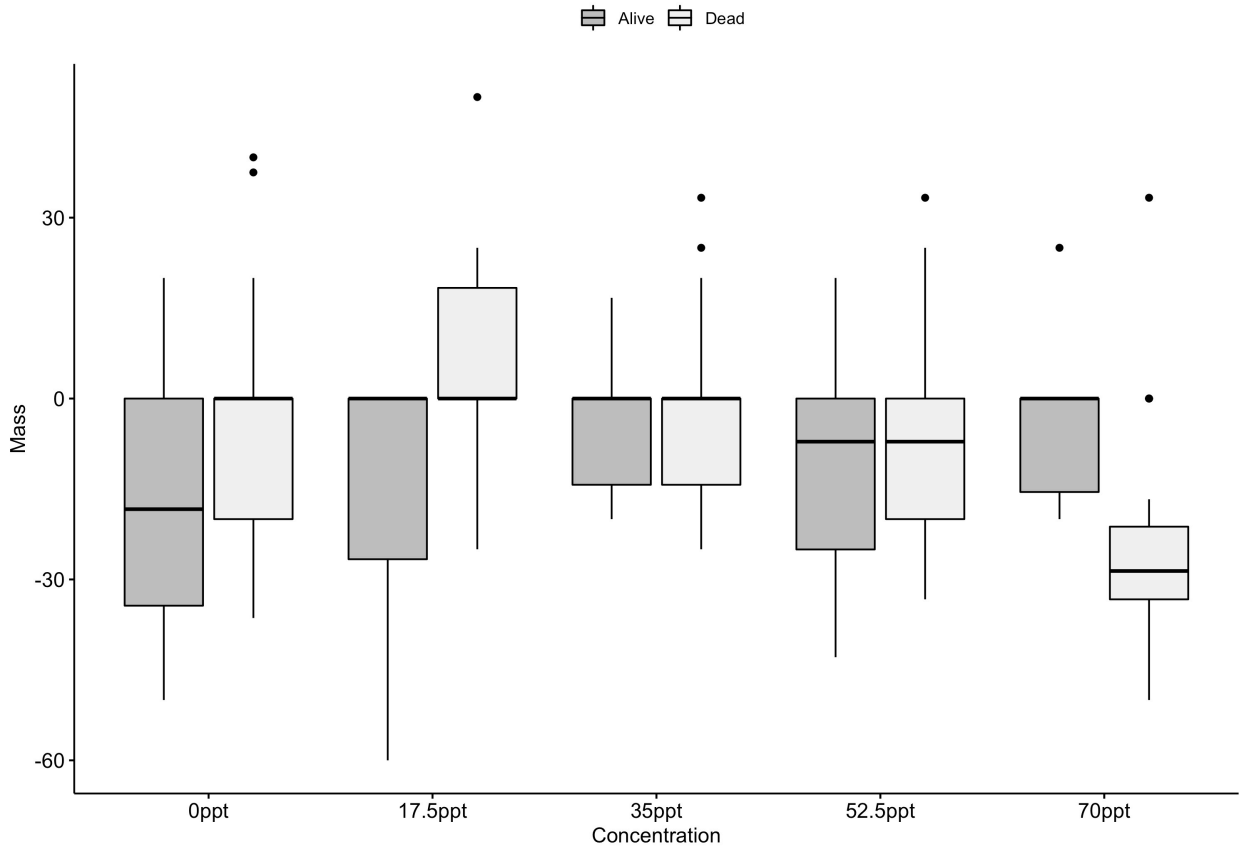


Figure 3.4. Mass loss or gain of living and dead *Telmatogeton magellanicus* larvae 72 h after recovery from exposure to five water salinity concentrations (0 ppt, 17.5 ppt, 35 ppt, 52.5 ppt and 70 ppt) for 10 d at 4°C. Means±SEM are presented for three replicates of 10 individuals.

3.4.3 Temperature tolerance

In the heating experiment (Table 3.3; Fig. 3.5B) larval mortality increased significantly at higher temperatures, with *post hoc* tests showing significant reduction in survival between all temperatures in the range 33–37ºC, at which point all larvae were dead. Similarly in the cooling experiment, larval survival decreased significantly with progression between -3, -6, -9, and -12ºC, when only 20% of larvae were still alive (Table 3.3; Fig. 3.5A), and only one larva recovered from the treatment.

Table 3.3. Analyses of survival against temperature

Experiment	Method	Test	Df	chi-squared	Pr(>F)
Heated	Kruskal-Wallis	Temperature	7	158.48	< 0.001
Cooled		Temperature	4	71.652	< 0.001

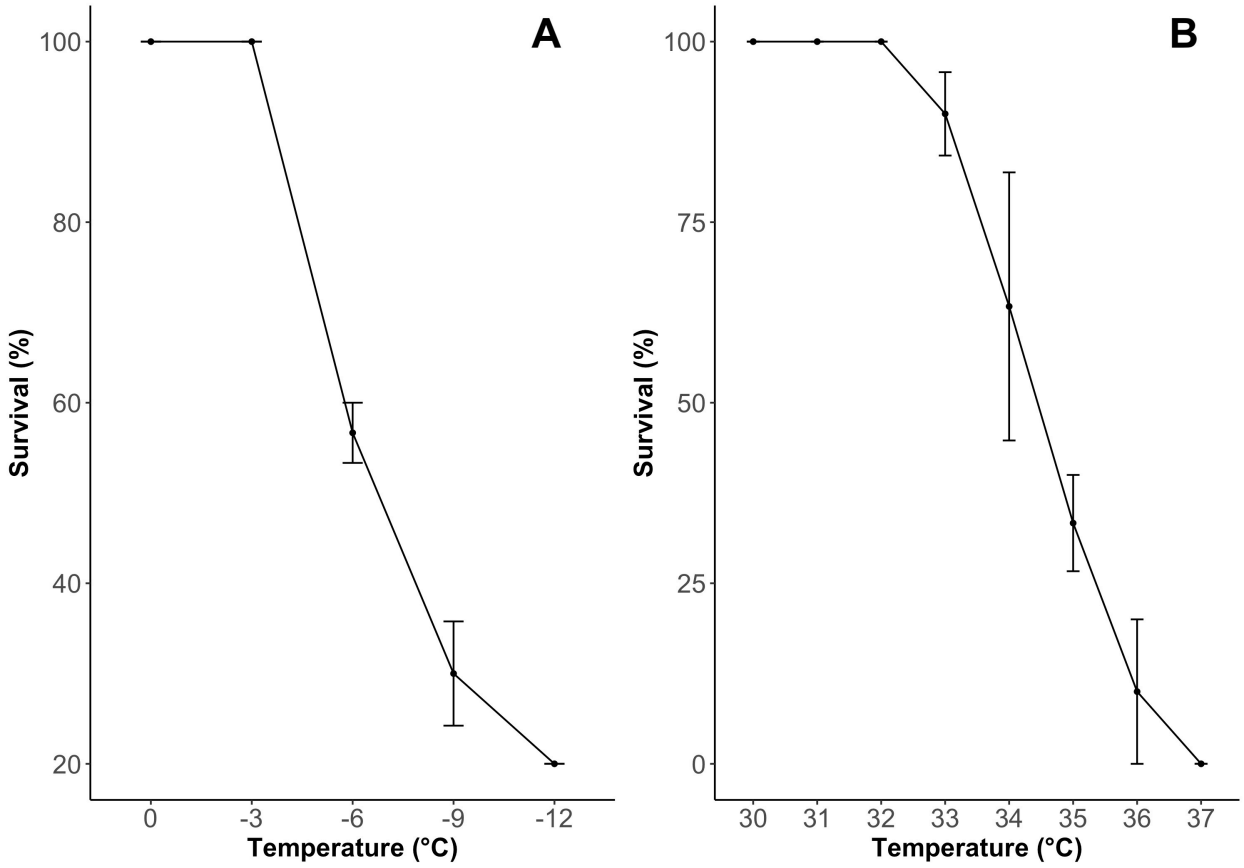


Figure 3.5. Survival of *Telmatogeton magellanicus* larvae 48 h after exposure to two temperature treatments in sea water (35 ppt). A: Progressively cooling temperatures; B: Progressively heating temperatures. Means±SEM are presented for 30 individuals.

3.4.4 Desiccation tolerance

Percentage survival differed significantly (Table 3.4) between the different time-periods at 50% relative humidity (time periods: 60, 80, 100, 120, 140, 160 min; Kruskal-Wallis, Chi-square = 26.818, d.f. = 5, $p < 0.001$), 70% humidity (time periods: 4, 8, 12, 16 h;

Kruskal-Wallis, Chi-square = 35.810, d.f. = 3, p < 0.001), but not 90% humidity (time periods: 12, 24, 48, 72, 84 h; Kruskal-Wallis, Chi-square = 9.321, d.f. = 4, p = 0.054) (Fig. 3.6). At 50% relative humidity (R.H.), mass change did not vary significantly between time periods or between living and dead individuals (Fig. 3.7). However, at both 70% (Fig. 3.8) and 90% (Fig. 3.9) humidity mass was significantly lower in dead individuals than live individuals, but this did not vary with time and there was no interaction between time and living/dead status.

Table 3.4. Summary of analyses investigating the impact of time and living/dead status on mass across three different relative humidity (%) treatments at 4ºC (ART, Aligned Rank Transform)

Experiment	Method	Explanatory variable	Df	DF.res	F value	Pr(>F)
Desiccation 50%	**ART**	Time	5	2656.5	0.118	0.988
		Alive/dead	1	49.0	4.020	0.051
		Alive/dead:Time	4	49.0	0.199	0.938
Desiccation 70%	**ART**	Time	3	5105.2	1.121	0.339
		Alive/dead	1	112.0	51.336	<0.001
		Alive/dead:Time	3	112.0	1.803	0.151
Desiccation 90%	**ART**	Time	4	38161.0	1.082	0.364
		Alive/dead	1	139.0	43.621	<0.001
		Alive/dead:Time	4	139.0	1.432	0.227

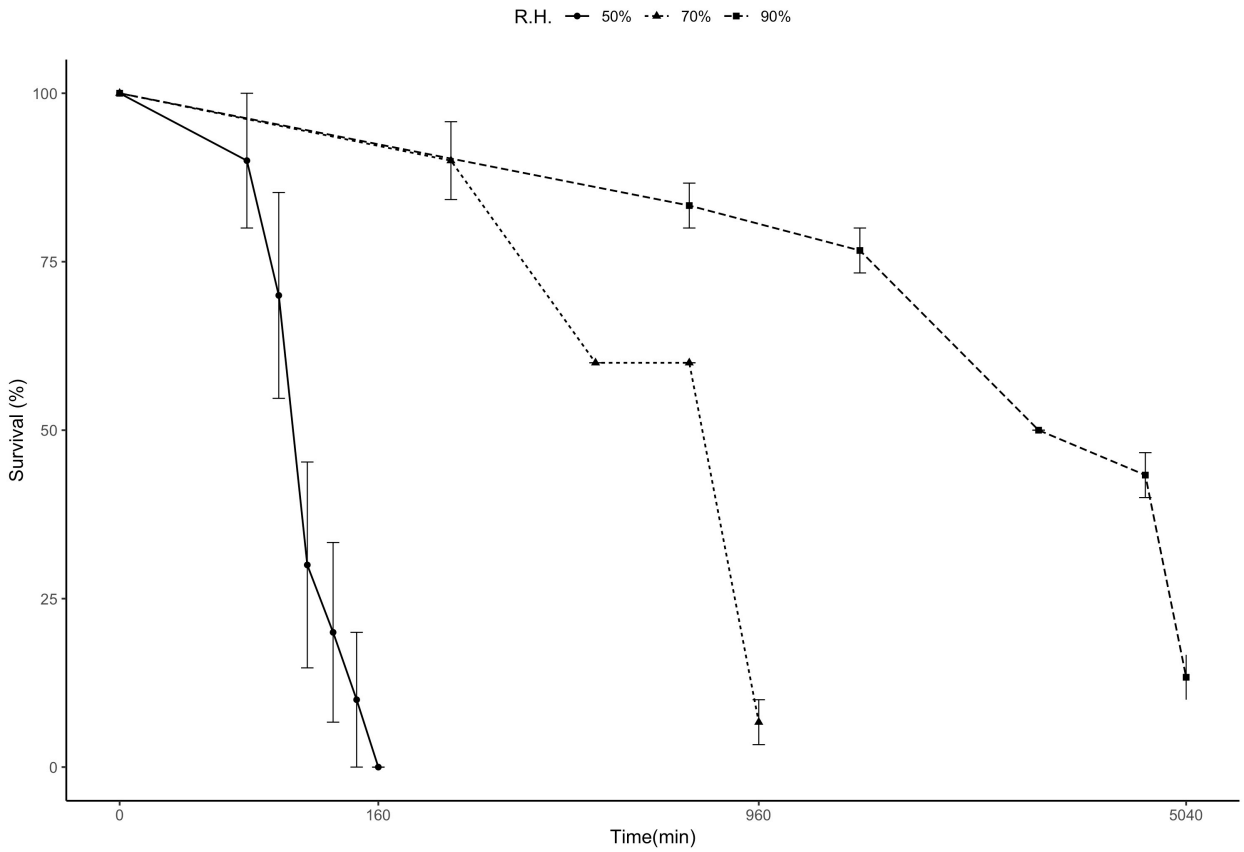


Figure 3.6. Survival of *Telmatogeton magellanicus* larvae after exposure to three relative humidity (R.H.) treatments at 4ºC: 50%, 70% and 90%. Means±SEM are presented for 10 individuals for the 50% treatment, and three replicates of 10 individuals for the 70% and 90% treatments. Data were log-transformed for visualisation purposes.

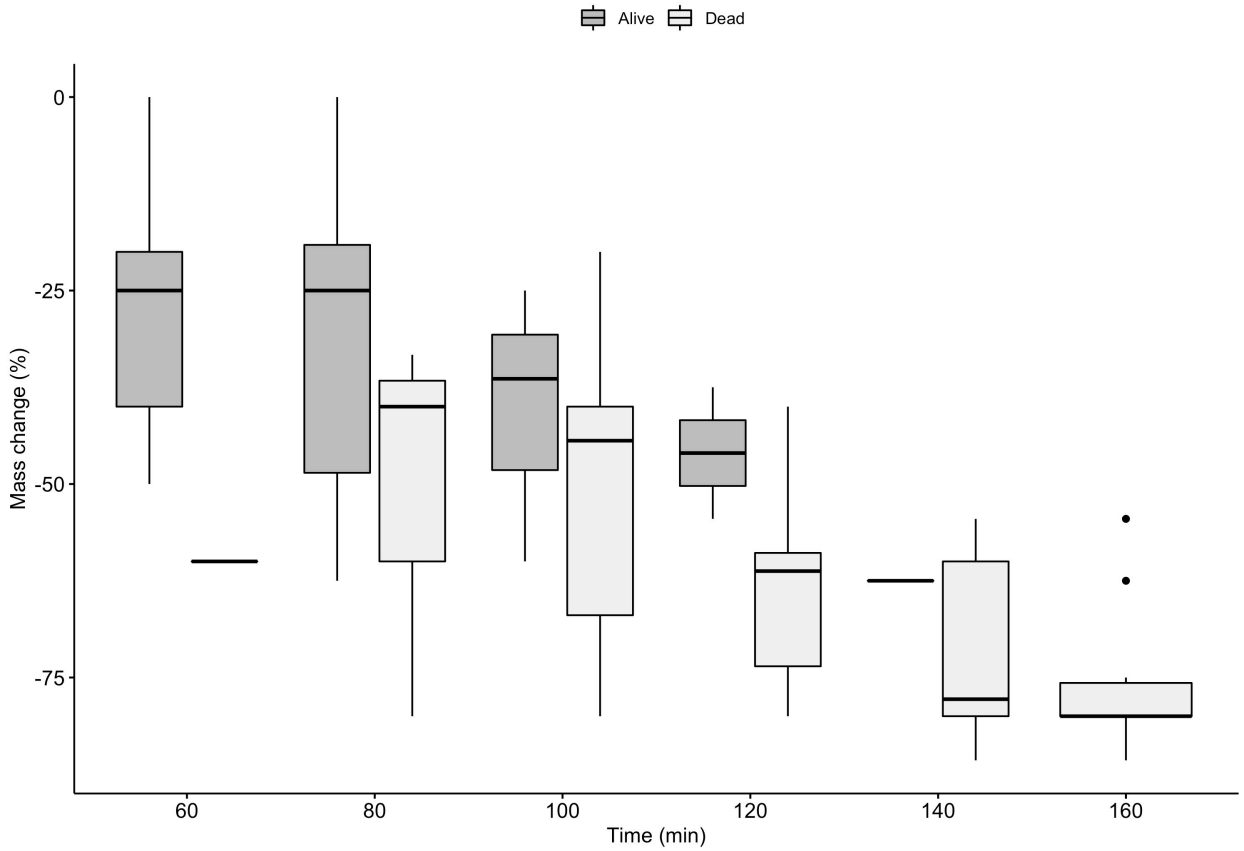


Figure 3.7. Wet mass loss and survival of *Telmatogeton magellanicus* larvae after exposure to 50% relative humidity (R.H.) at 4ºC. Means±SEM are presented for 10 individuals.

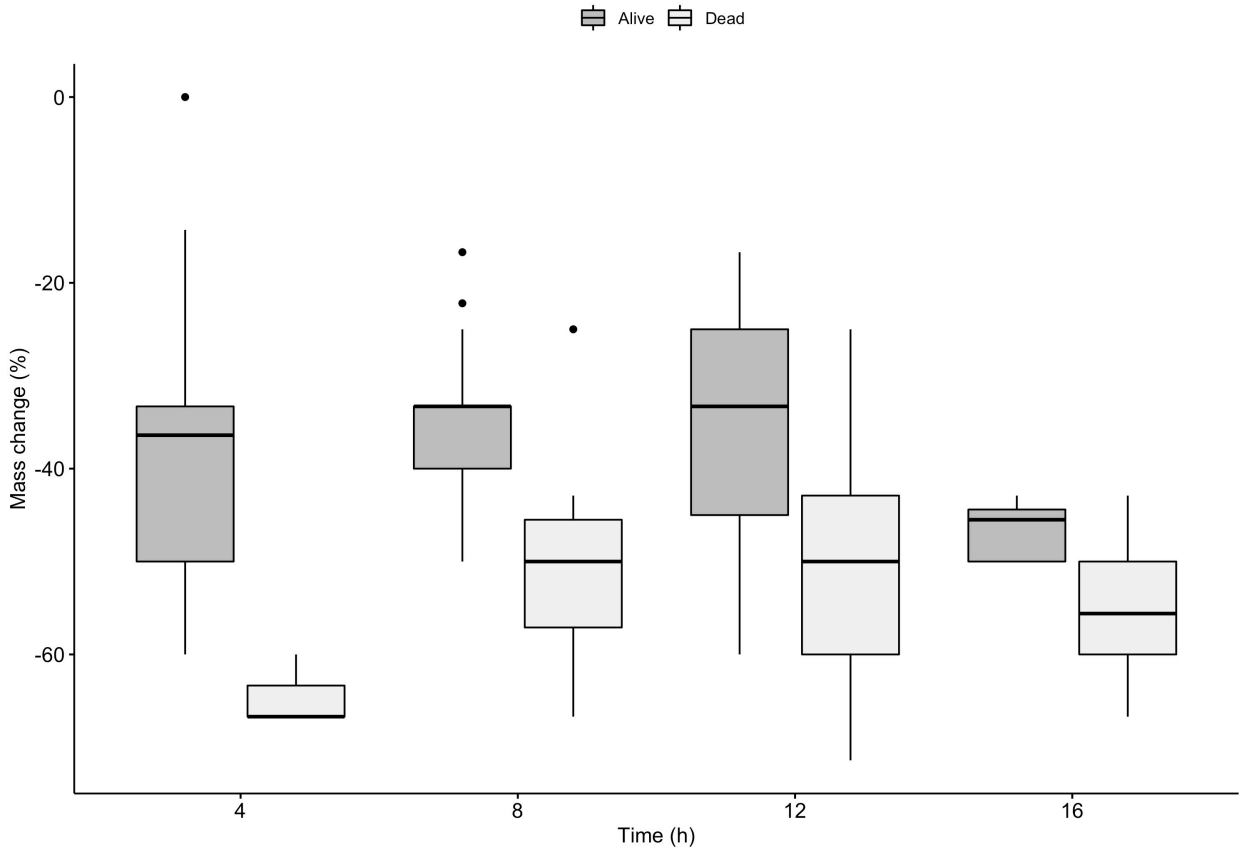


Figure 3.8. Wet mass and survival of *Telmatogeton magellanicus* larvae after exposure to 70% relative humidity (R.H.) at 4ºC. Means±SEM are presented for 30 individuals.

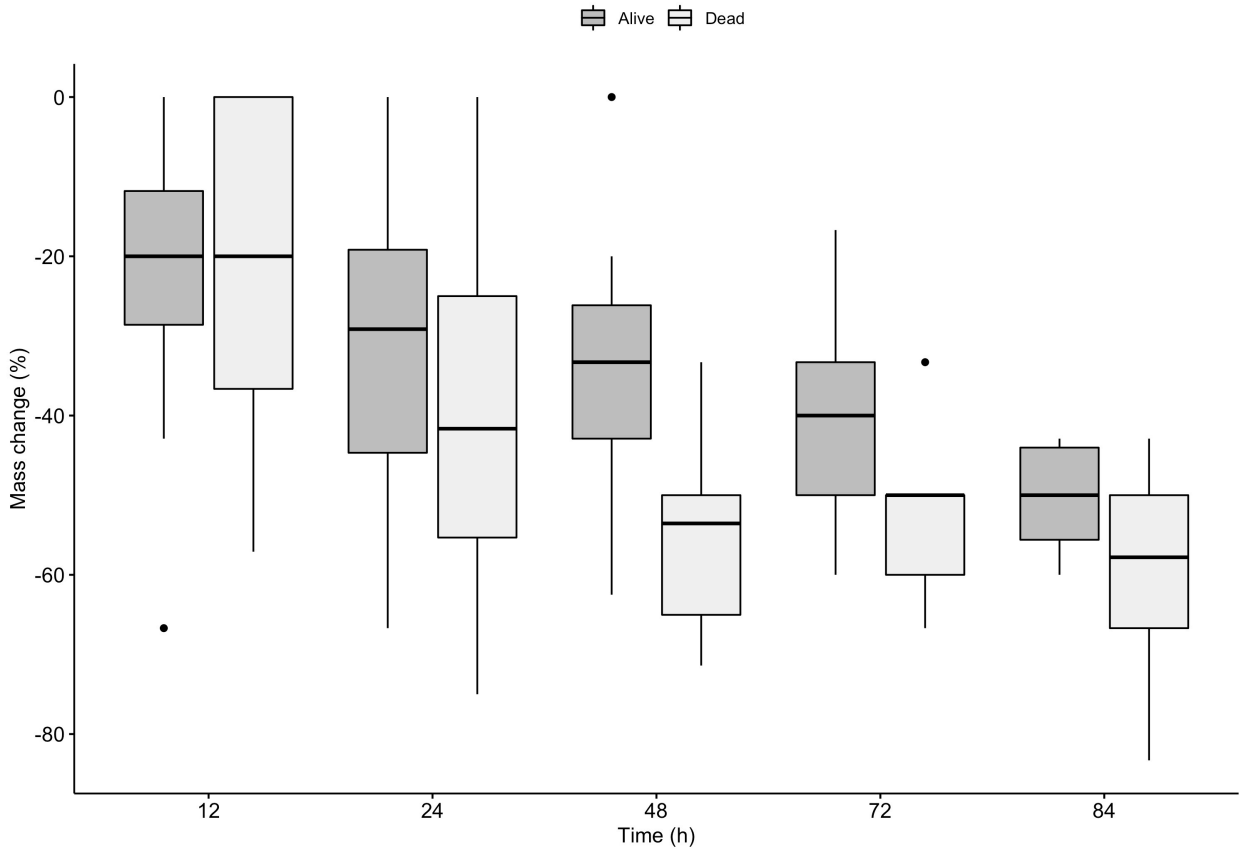


Figure 3.9. Wet mass and survival of *Telmatogeton magellanicus* larvae after exposure to 90% relative humidity (R.H.) at 4ºC. Means±SEM are presented for 30 individuals.

3.5 Discussion

The patterns of oviposition and egg development recorded here are the first for the sub-Antarctic species of *Telmatogeton*. When the adults were put together in the collection tubs, they immediately started to mate and the females would oviposit as soon as possible in any available surface, even though they seem to favour algae, when available. We hypothesise that the random patterns observed are mainly a result of the stress caused by the collection procedures and being kept outside their natural habitat. Egg development time (~14 days) is not that different from *Belgica antarctica* (16 days) (Harada et al. 2014) and *Eremoptera murphyi* (30 days) (Bartlett 2019), but the absence of egg sacs, exposing the eggs to harsher conditions, is a possible limiting factor if *T.*

magellanicus were to be transported (artificially or naturally) to a more extreme location. Additionally, we understand that, being an intertidal animal, the speed with which the eggs develop is heavily affected by the tidal cycle, which we could not replicate in the available laboratory conditions.

We recorded only limited effects of salinity on larval survival, with no differences in survival recorded across treatments, but a significant increase in mortality over time in all treatments but that of sea water (35 ppt). This higher mortality is an indicator of low or high salinity stress. Mass loss was higher in the 70 ppt treatment, but only after ten days. This is possibly driven by the higher mass loss recorded in dead versus live individuals at this time period. In contrast to salinity, temperature had a marked effect on mortality, with % mortality increasing with each temperature increment over 32ºC and all individuals being dead by 37ºC. Similarly, % mortality also increased as temperature was decreased below -3ºC, with 90% of individuals being dead by -12ºC. Finally, humidity also had a marked effect on survival, with mortality occurring much faster at 50% R.H. than at 70% or 90%. There was a general reduction in body mass through time, at 50% and 90% humidity, that was not statistically related to mortality. In contrast, mass did not vary significantly between each time period in any R.H., but was lower in dead than living individuals at both 70% and 90% R.H. These data demonstrate that *T. magellanicus* larvae are tolerant to changes in salinity, but in contrast are sensitive to changes in temperatures (both at high and low temperatures) and relative humidity levels.

It may not be surprising to see larvae thriving in salinity conditions of 17.5 ppt or 52.5 ppt, as their intertidal habitat will experience natural fluctuations in exposure to sunlight and temperature, creating varied degrees of water evaporation, hence affecting salinity levels (Soong & Leu 2005). It is also worth noting that larvae are normally only exposed to these conditions over a limited time period within the tidal cycle, and in particular that the longer time intervals they were submitted to in this experiment would not normally occur and therefore represent extreme conditions. As can be seen by their wet mass changes, the larvae do not seem to be osmotically affected for up to five days, which is a long time period compared to exposure times likely to be experienced in the wild (Fig. 3). This

striking ability to withstand, for relatively long periods, exposure to both fresh water and more hypersaline solutions (70 ppt) also raises the question of whether the species could actively survive in a fluvial system or in briny pools, although we did not locate them in these habitats in our surveys (Chapter 2). Given their tolerance to salinity changes, it is more likely that other life cycle characteristics may be a more significant barrier to them invading these different types of environment than physiological constraints alone.

To a greater degree than salinity, temperatures vary significantly over a full year at Navarino Island, to the point that the bay shorelines may become frozen during the winter, but reach 26°C during the summer. Our data suggest that *T. magellanicus* larvae are pre-adapted to even more extreme temperatures than those already experienced in its current habitat. However, it is also possible that the relatively rapid changes in temperature imposed in this experiment were not reflective of longer-term changes in the wild and therefore did not allow larvae to acclimate. For example, in some trial experiments, we have found that larvae can survive freezing for at least 24 hours, which opens up a window that can be explored in future studies, to test what cellular or extra-cellular strategies they employ. One such strategy might be freeze-avoidance as noted by its very patchy distribution in the wild, which suggests that the areas where they are found provide shelter from extreme conditions, such as where algae (mainly *Bostrychia* sp.) is present (Chapter 2).

Desiccation seems to be, above all else, the main danger for the larvae of *T. magellanicus,* and we found much higher rates of mortality when the larvae were exposed to lower humidity conditions. The absence of the species on the high tide zone identified from our field surveys (Chapter 2) further suggests that desiccation could be a limiting factor. As well as being relatively drier, the high tidal zone also lacks major shelter spots, such as filamentous algae or even sand where the larvae could escape the low humidity conditions.

Overall, the data discussed here suggest that temperature and humidity, but not salinity, are important factors in determining the local-scale distribution of *T. magellanicus* on

Navarino Island. Given that both of these factors are likely to change with climate warming, with the region predicted to become 1.5–2º C warmer over the next 80 years (Collins et al. 2013; Turner et al. 2014), it is likely that local distributions may alter in the future. Again, in this case the main barrier for potential transportation/migration to other areas would be desiccation. A profitable future area of research would be to investigate the natural ability of the species to disperse and also the potential of *T. magellanicus* to deal with colder temperatures and whether they could live in an even more extreme environment such as Antarctica.

3.6 Acknowledgements

We thank Javier Rendoll Carcamo and Carolina Perez for the fieldwork and laboratory assistance; and, Laura Gerrish of MAGIC-BAS for the map.

CHAPTER 4

Salinity tolerance of *Eretmoptera murphyi*

4. Salinity tolerance of the flightless midge *Eretmoptera murphyi* (Diptera: Chironomidae) on Signy Island

4.1 Abstract

Eretmoptera murphyi is a flightless midge native and endemic to South Georgia, but that has been introduced to Signy Island, in the maritime Antarctic South Orkney Islands. The species is known to be pre-adapted to extreme climatic conditions, but its tolerance to varying levels of salinity in water has not been studied. Here, we tested the survival limits of *E. murphyi* larvae to exposure to five different levels of salinity [from fresh water (0ppt) to briny water (70ppt)] over a 10 d period. The larvae were extremely tolerant to exposure to brackish water, with 50% surviving for 10 days, and, to a lesser degree, to freshwater and seawater submersion (falling below 50% survival after 2 days). The high tolerance to brackish conditions can be regarded as a further pre-adaptation to the naturally high salinity levels that may be experienced close to the coast on Signy Island, associated with seaspray and the presence of large numbers of marine vertebrates (seals) on land. Survival differed significantly between fresh or seawater and the two treatments led to very different mass change trends, likely a direct consequence of osmotic imbalance, with fresh water increasing the intake of water in larvae, and the opposite happening in seawater, but with both resulting in increased stress and decreased survival in the larvae. The data obtained further corroborate the pre-adaptation of *E. murphyi* to the harsh conditions of the maritime Antarctic, enabling it to colonise new habitats.

4.2 Introduction

The flightless midge *Eretmoptera murphyi* Schaeffer, 1914 (Chironomidae: Orthocladiinae) is palaeoendemic to sub-Antarctic South Georgia (Allegrucci et al. 2006, 2012) and is thought to have been accidentally introduced in the 1960s to Signy Island, one of the South Orkney Islands of Antarctica, during a series of plant transplant experiments (Block et al. 1984, Convey and Block 1996, Bartlett et al. 2018a, b). Although

not formally confirmed, it has been suggested based on recent molecular phylogenetic studies that the species correctly belongs in the genus *Belgica* Jacobs, 1900 [Allegrucci et al. 2012], a genus that contains *Belgica antarctica* Jacobs, 1900, the only insect endemic to the Antarctic continent, and *B. albipes*, endemic to the sub-Antarctic Crozet archipelago. The species spends most of its two year life cycle as a larva living in and feeding on organic-rich peat soil, with a very short period as an adult, when it reproduces parthenogenetically (Cranston 1985, Convey 1992, Bartlett et al. 2018a).

At Signy Island, *E. murphyi* is mostly found in peat soil, which also acts as its food source. Historically, the species was first introduced close to the coast on Signy island, but recent documentation shows the species' distribution has been expanding away from the coast and to higher altitudes (Hughes & Worland 2010, Bartlett 2019). Hughes et al. (2013) and Bartlett (2019) suggest that the species is capable of considerable further range expansion even under contemporary conditions. It is therefore important to better understand the species' physiological limits in order to allow better prediction of its future expansion. Furthermore, as an invasive species on the island, *E. murphyi* can be considered as a model for other invasive insect and invertebrate taxa both in the Antarctic and sub-Antarctic, with a greater understanding of their physiological limits perhaps leading to a better understanding of the limits to invasion in other species.

Hayward et al. (2007), Worland (2010) and Everatt et al. (2014a) quantified desiccation tolerance in larvae of *E. murphyi*, with the latter contrasting it to the Arctic dipteran *Heleomyza borealis* (Boheman, 1865). Everatt et al. (2014a, b) have also determined its positive and negative temperature limits (Worland & Convey 2001, Worland & Block 2003, Worland 2010). However, to date no studies have assessed larval tolerance to salinity, although Bartlett (2019) measured egg sac survival rates under these conditions. Additionally, Convey (1992) and Bartlett et al. (2018b) investigated the impacts of heat and desiccation stresses on *E. murphyi* egg sac survival and its implications for the species' reproductive success.

In this chapter we assess the ability of the larvae of *Eretmoptera murphyi* to tolerate exposure to different levels of salinity. In particular we assess the impact of salinity on larval survival and wet mass.

4.3 Materials & Methods

4.3.1 Sampling

Substrate samples were obtained close to Signy Research Station, Factory Cove, Borge Bay, Signy Island in the austral summers of 2016 and 2017 (Figure 4.1). The substrate, which is mostly peat and is exposed to sea spray, was then stored in plastic tubs and taken back to the British Antarctic Survey (Cambridge, United Kingdom), whereupon larvae were extracted and immediately submitted to their respective treatments. In total, we extracted 150 larvae. First and fourth instar larvae were not used in the experiments, as the former are very difficult to handle and likely to be extremely susceptible to stress (Bartlett et al. 2018a), while the latter were only present in small numbers.

The Signy field site was generally very stable throughout the 2016/2017 season (Bartlett et al. 2018a, b). The mean pH in the vegetation layer was 5.3 ± 0.13 SE, and underlying soil pH was 5.5 ± 0.11 SE (n = 7 in each layer). Salinity was also largely stable in the vegetation and soil, with only one spike during a week of high storm activity detected in the vegetation layer, when it rose to 27 ppt, from an average of 10 ± 2 µS SE. Salinity in the soil layer remained close to 4 ± 0.5 ppt (Bartlett et al. 2018). Soil temperatures on the island can fall below -10 ºC, but also frequently rise above air temperature, with records of moss or soil surface temperatures peaking as high as 38.5 °C (Walton 1982, Davey et al. 1992, Bokhorst et al., 2008, Bartlett 2019, Convey et al. 2018).

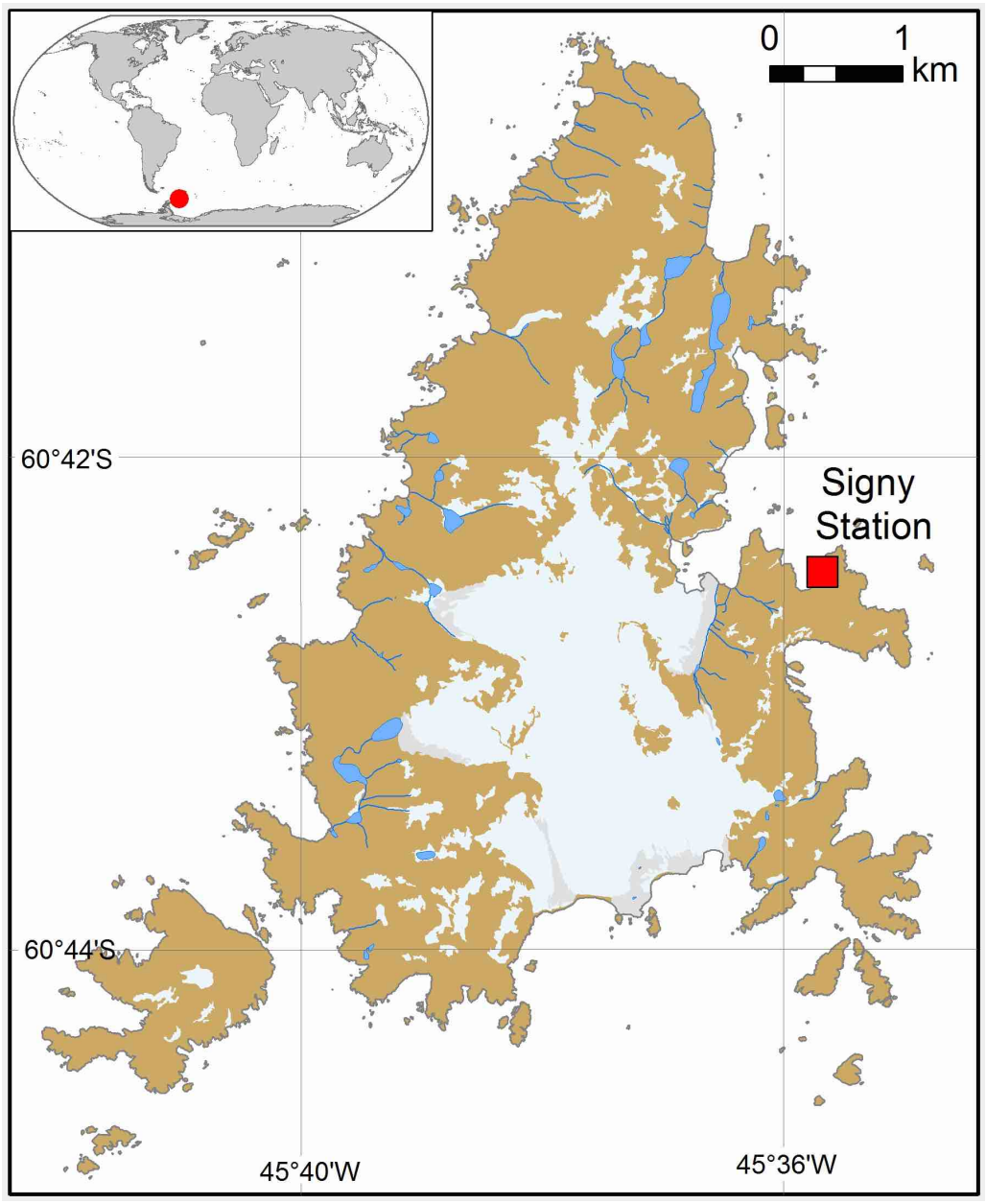


Figure 4.1. Map of Signy Island. In red is Signy Research Station, where the larvae of *Eretmoptera murphyi* were collected.

4.3.2 Salinity tolerance

We assessed the survival and wet mass change of larvae under 5 different salinity exposure treatments over 10 d (in five time steps: 6 h, 1 d, 2 d, 5 d and 10 d): fresh 'field water' (0 ppt), brackish water (17.5 ppt), seawater (35 ppt) and two hypersaline concentrations (52.5 and 70 ppt), with each treatment including 30 larvae in individual

tubes, as well as a small amount of substrate to avoid starvation. ‘Field water’ was created by adding local substrate (peat) to deionised water at a 1:3 ratio. This mixture was then left for 1 week at 5°C in the dark, to replicate the organic matter composition of the environment as per Bartlett et al. (2018b). Brackish water was prepared by mixing fresh ‘field’ water with seawater to the required concentration, and the highly saline treatments by reducing seawater through heat evaporation. Salinity was assessed using a Conductivity pH TDS Hanna Tester HI98130 salinity meter. We recorded survival of larvae (defined as spontaneous movement or movement in response to a gentle stimulus) in each sample at each recording period and, for non-responsive larvae, after a further period of 72 h in wet substrate. The same larvae were used to measure wet mass change, by gently drying individuals with a soft tissue and weighing them to the nearest 100 µg (using a Shimadzu AUX220 analytical balance) both before and immediately after treatment and following the recovery period. Because of the limited number of specimens, and as dead larvae were removed from their respective treatments, numbers present in each treatment reduced over time. All experiments were carried out at a constant temperature of 4˚C.

4.3.3 Statistical analyses

To check for normality in all data, Shapiro-Wilk tests were used. Data that were normally distributed underwent an Analysis of Variance (ANOVA) with Tukey HSD post-hoc tests, while non-normally distributed data were analysed using Kruskal-Wallis tests, with pairwise Wilcoxon post-hoc tests. We tested for differences in % survival (including the 72-h recovery period) and wet mass change of larvae between the five salinity concentrations and across the five time periods (6 h, 1 d, 2 d, 5 d, 10 d). Groups were included in statistical tests for wet mass change when there were at least 10 individuals present, reducing the number of comparisons at later time periods.

4.4 Results

4.4.1 Salinity tolerance

Survival did not differ between salinity treatments after 6 h exposure (x^2 = 1.3263, d.f = 4, p = 0.857), but was significantly different after 1 d (x^2 = 22.4, d.f = 4, p < 0.001), 2 d (x^2 = 31.475, d.f = 4, p < 0.001), 5 d (x^2 = 42.086, d.f = 4, p < 0.001) and 10 d (x^2 = 46.726, d.f = 4, p < 0.001) (Figure 4.2; post-hoc outputs in Table 4.1). Survival differed significantly between time steps for 0 ppt (x^2 = 30.405, d.f = 4, p < 0.001), 35 ppt (x^2 = 47.475, d.f = 4, p < 0.001), 52.5 ppt (x^2 = 67.59, d.f = 4, p < 0.001) and 70 ppt (x^2 = 105.59, d.f = 4, p < 0.001), but not at 17.5ppt (x^2 = 8.2493, d.f = 4, p = 0.083) (post-hoc outputs in Table 4.2).

Table 4.1. Post-hoc test outputs for % survival between different salinity exposures at each sampled time step. Numbers in bold are statistically significant (p < 0.05).

		Salinity			
		0 ppt	17.5 ppt	35 ppt	52.5 ppt
1 d	17.5 ppt	0.499	-	-	-
	35 ppt	0.611	0.286	-	-
	52.5 ppt	0.428	0.123	0.611	-
	70 ppt	**0.002**	**<0.001**	**0.006**	**0.02**
2 d	17.5 ppt	0.339	-	-	-
	35 ppt	0.445	0.106	-	-
	52.5 ppt	0.057	**0.005**	0.215	-
	70 ppt	**<0.001**	**<0.001**	**<0.001**	**0.011**
5 d	17.5 ppt	**0.024**	-	-	-
	35 ppt	0.569	**0.007**	-	-
	52.5 ppt	**0.003**	**<0.001**	**0.007**	-
	70 ppt	**0.003**	**<0.001**	**0.007**	-
10 d	17.5 ppt	**0.048**	-	-	-
	35 ppt	**0.019**	**<0.001**	-	-
	52.5 ppt	**0.005**	**<0.001**	0.334	-
	70 ppt	**0.005**	**<0.001**	0.334	-

Table 4.2. Post-hoc test results for % survival at successive sampling times for each salinity exposure. Numbers in bold are statistically significant ($p < 0.05$). There was no significant difference through time at 17.5 ppt so Post-hoc tests were not conducted at this salinity.

		Sampling time			
		6 h	1 d	2 d	5 d
0 ppt	1 d	0.101	-	-	-
	2 d	**0.006**	0.219	-	-
	5 d	**<0.001**	**0.010**	0.149	-
	10 d	**<0.001**	**0.006**	0.101	0.784
35 ppt	1 d	0.060	-	-	-
	2 d	**0.001**	0.141	-	-
	5 d	**<0.001**	**0.007**	0.172	-
	10 d	**<0.001**	**<0.001**	**0.001**	**0.035**
52.5 ppt	1 d	**0.031**	-	-	-
	2 d	**<0.001**	**0.021**	-	-
	5 d	**<0.001**	**<0.001**	**0.007**	-
	10 d	**<0.001**	**<0.001**	**0.007**	-
70 ppt	1 d	**<0.001**	-	-	-
	2 d	**<0.001**	**0.011**	-	-
	5 d	**<0.001**	**0.011**	-	-
	10 d	**<0.001**	**0.011**	-	-

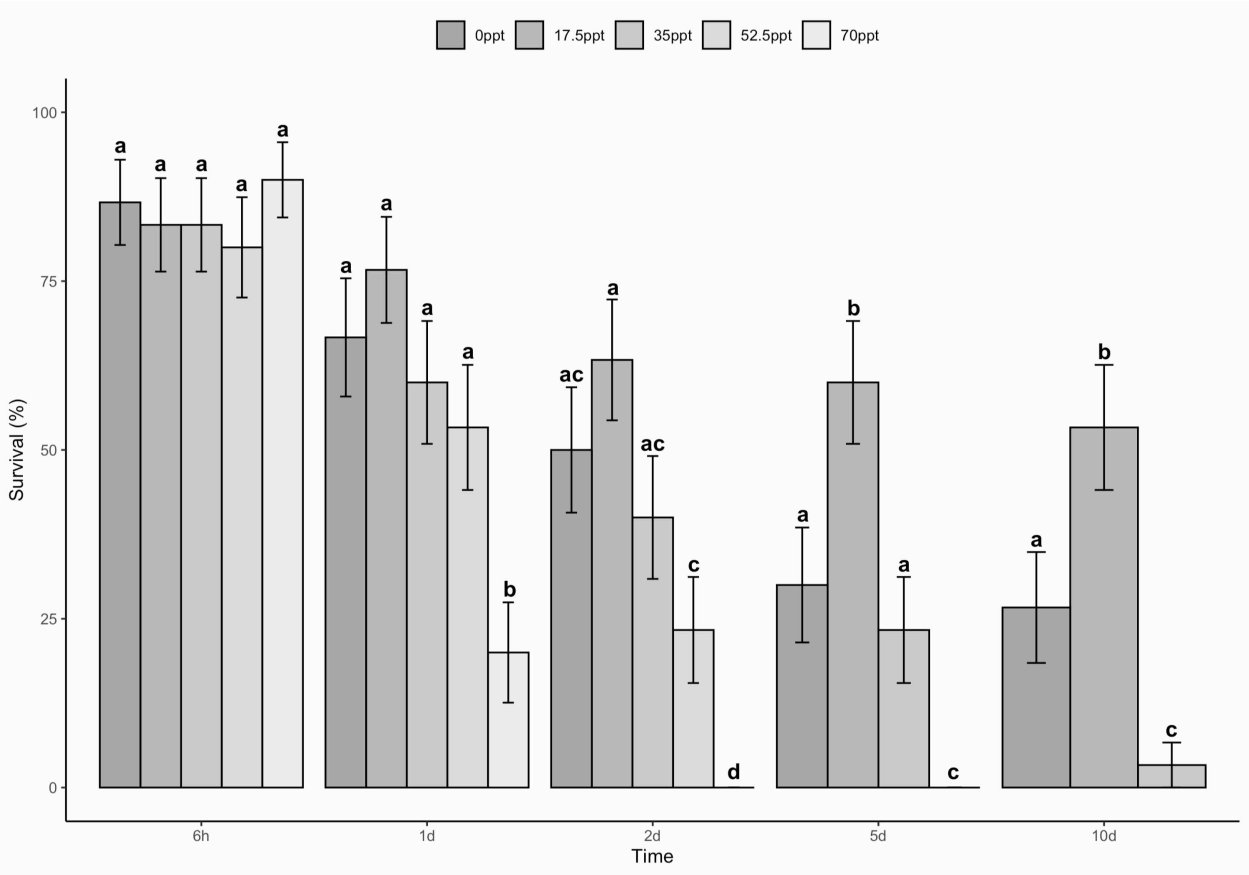


Figure 4.2. Survival (%) of *Eretmoptera murphyi* larvae 72 h after recovery from exposure to one of five salinity exposures (0 ppt, 17.5 ppt, 35 ppt, 52.5 ppt, and 70 ppt) for a range of time periods (6 h, 1, 2, 5, 10 d) at 4°C. Means ±SEM are presented for 30 individuals. Means with the same letter are not significantly different within each sampling time group (Wilcoxon post-hoc test).

Wet mass (Figure 4.3) varied significantly between salinity exposures at all sampling times: 6 h (x^2 = 97.413, d.f = 4, p < 0.001), 1 d (F = 206.5, d.f = 4, p < 0.001), 2 d (F = 67.05, d.f = 2, p < 0.001), 5 d (x^2 = 14.156, d.f = 1, p < 0.001) (post-hoc outputs in Table 4.3). Survival also differed significantly between sampling times at all salinity exposures, 0ppt (chi-squared = 9.2568, d.f = 3, p = 0.026), 17.5ppt (F = 4.804, d.f = 4, p = 0.001), 35ppt (chi-squared = 45.921, d.f = 2, p < 0.001), 52.5ppt (chi-squared = 31.88, d.f = 1, p < 0.001) and 70ppt (chi-squared = 37.136, d.f = 1, p < 0.001) (post-hoc outputs in Table 4.4) (Figure 4.3).

Table 4.3. Post-hoc test results for wet mass change between salinity exposures for each sampling time. Numbers in bold are statistically significant ($p < 0.05$). N.b. number of comparisons declines over time, owing to larval mortality.

		Concentration			
		0ppt	17.5ppt	35ppt	52.5ppt
6 h	17.5ppt	**<0.001**	-	-	-
	35ppt	**<0.001**	**<0.001**	-	-
	52.5ppt	**<0.001**	**<0.001**	**0.006**	-
	70ppt	**<0.001**	**<0.001**	**<0.001**	**<0.001**
1 d	17.5ppt	**<0.001**	-	-	-
	35ppt	**<0.001**	**<0.001**	-	-
	52.5ppt	**<0.001**	**<0.001**	**0.040**	-
	70ppt	**<0.001**	**<0.001**	**<0.001**	**<0.001**
2 d	17.5ppt	**<0.001**	-	-	-
	35ppt	**<0.001**	**<0.001**	**-**	**-**
5 d	17.5ppt	**<0.001**	-	-	-

Table 4.4. Post-hoc test results for wet mass change at successive sampling times for each salinity exposure. Numbers in bold are statistically significant ($p < 0.05$). N.b. number of comparisons declines over time owing to larval mortality.

		Concentration			
		6h	1d	2d	5d
0ppt	1d	0.080	-	-	-
	2d	0.350	0.750	-	-
	5d	**0.030**	0.240	0.240	-
17.5ppt	1d	0.300	-	-	-
	2d	**0.008**	0.580	-	-
	5d	0.064	0.922	0.974	-
	10d	0.994	0.251	**0.011**	0.062
35ppt	1d	**<0.001**	-	-	-
	2d	**<0.001**	**0.001**	-	-
52.5ppt	1d	**<0.001**	-	-	-
70ppt	1d	**<0.001**	-	-	-

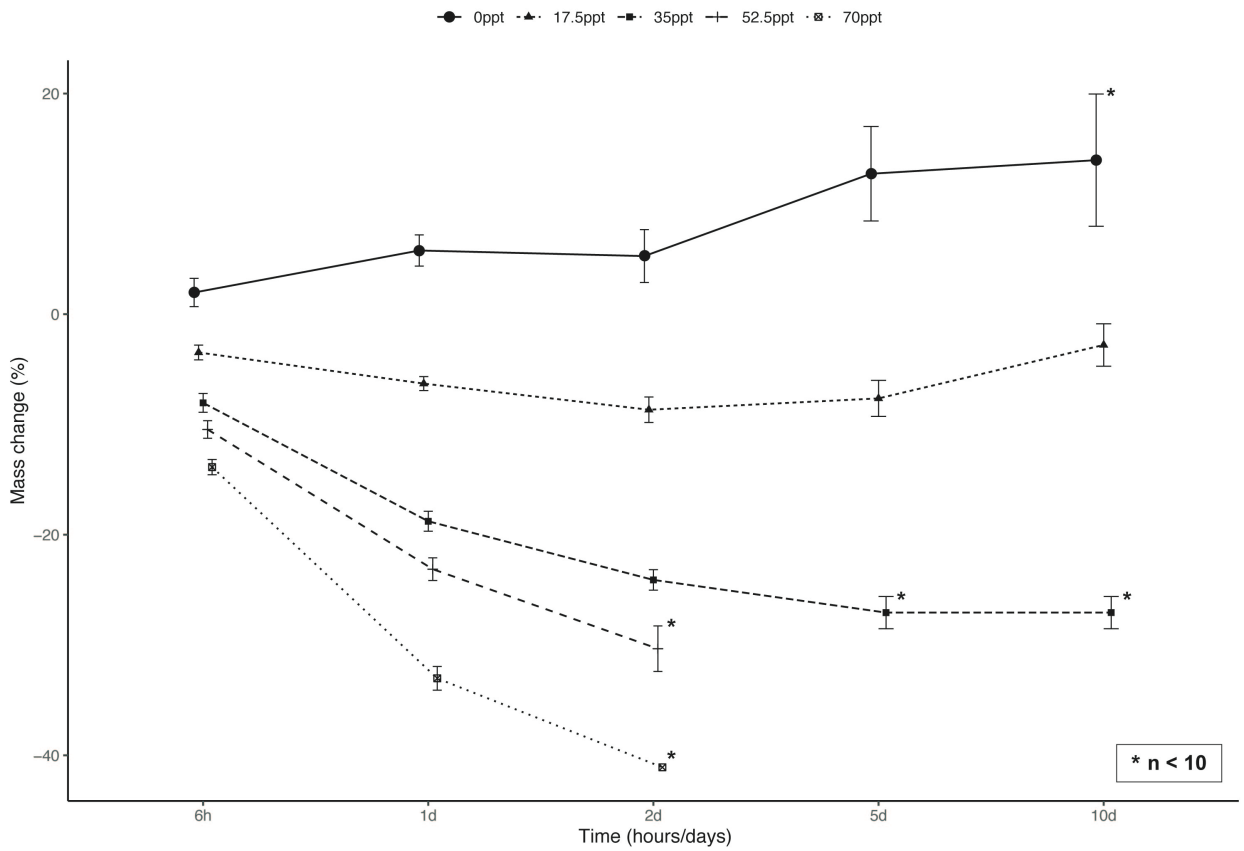


Figure 4.3. Mean percentage wet mass loss or gain of *Eretmoptera murphyi* larvae under exposure to one of five salinity treatments (0 ppt, 17.5 ppt, 35 ppt, 52.5 ppt, 70 ppt) over time (6 h, 1, 2, 5, 10 d). Means ±SEM are presented for 30 individuals at the start of the experiment, with numbers declining over time as the experiment progressed, owing to mortality (* = n < 10 specimens).

Over the first six hours of experimental exposures, there was no difference in larval survival across treatments, but there was a noticeble difference in larval behaviour, with those exposed to higher saline concentrations being extremely active, but those in lower concentrations (especially fresh water) being largely inactive. However, at all other time points survival was different between treatments, with survival declining more rapidly in freshwater and higher salinity concentrations than in brackish water. Wet mass was significantly different between treatments at all time-points, with the three most saline treatments having a lower mass and freshwater having a higher mass. These patterns

became more pronounced over time, with freshwater samples gradually gaining mass and higher saline concentrations losing mass.

4.5 Discussion

It is clear that survival was highest in brackish water, with only limited changes in mortality and wet mass over the entire experiment. This high tolerance to brackish conditions is most likely linked to the natural salinity levels that occur close to the coast at Signy Island [~4ppt to ~27ppt (Bartlett 2019)], associated with seaspray and also the presence of large numbers of marine vertebrates (seals) on land. Survival did differ in exposure to 'field' or seawater, and both treatments had very different mass change trends. The most immediate conclusion would be that this is a direct consequence of osmotic imbalance, with fresh water increasing the intake of water in larvae, with the opposite happening in seawater, resulting in increased stress and decreased survival in the larvae. However, with the limited data at hand we cannot know whether *E. murphyi* is an osmoconformer or osmoregulator and, therefore, exactly what is causing these patterns. To investigate this further, we would need to more deeply analyse other factors, such as haemolymph composition, respiration, water potential and use of metabolic water.

Eretmoptera murphyi has already shown its potential to invade new habitats, being pre-adapted to cold conditions (Worland 2010) and tolerant to desiccation (Everatt et al. 2014a). In this study we have demonstrated that larvae of the species show some sensitivity to both high and low salinity exposures, at least over longer time-periods. This suggests that the potential of this species to invade more coastal or more inland areas may be limited, but not impossible. Assessing these physiological limits in more detail and correlating these data with available habitats will provide a greater insight into which areas the species may ultimately be able to occupy, including nearby regions. This includes the Antarctic Peninsula, where increasing temperatures and ice-free areas, provide new potential colonisation grounds and opportunities for the species (Hughes et al. 2005, Hughes & Convey 2014, Bartlett 2019).

4.6 Acknowledgements

We thank Jesamine Bartlett and Matt Jobson for collecting the substrate at Signy Island, and the University of Birmingham for providing the facilities for the trial experiments.

CHAPTER 5

Phylogeography of *Parochlus steinenii*

5. Parochlus phylogeography

5.1 Abstract

The chironomid midge *Parochlus steinenii*, one of only two holometabolous insects occurring in Antarctica, is also found around lakes in southern South America and in South Georgia. Previously published evidence, based on a small number of sequences of the 28S rDNA gene, inferred separation between South American populations and those in South Georgia and Antarctica, suggesting they diverged around 7 my. To further test the divergence hypothesis, we extracted the DNA from over 150 specimens of *P. steinenii* from 13 different lakes spread through most of the species known range. In addition to obtaining further 28S sequences, we expanded the analysis to include the mitochondrial COX1 gene. No variation was present among the new 28S sequences, in contrast to the previous study. However, sufficient variation was present amongst the COX1 sequences to permit phylogeographic analysis and the generation of a haplotype network and molecular phylogeny. These analyses corroborate the deep genetic separation of South American from South Georgian and Antarctic clades. A subsequent divergence was also apparent between the latter two clades, consistent with a later split, likely through a vicariance event.

5.2 Introduction

Insects have been linked to the Antarctic region from, at least, the early Palaeocene (~70 Mya) (Allegrucci et al. 2006, Convey et al. 2008). Nowadays, insects can be found in the maritime Antarctic (Antarctic Peninsula and associated Scotia Arc archipelagos) and the sub-Antarctic islands (Chown & Convey 2016). Knowledge of their diversity, evolution and ecology mostly stems from studies in the past 50 years, catalysed by the works of J. L. Gressitt (1964, 1970a, 1970b, 1971) and most recently reviewed by Chown & Convey (2016). However, even with the increasing scientific efforts in the area, much remains unknown about the evolution and adaptations of the insect taxa currently found in the region.

The fauna of the Antarctic region is closely related to that of South America, with intimate geographical, biological, geological and glaciological histories (Mercer, 1976; Clapperton & Sugden, 1988; Clapperton et al., 1989; Rodbell et al. 2009; Fernandez et al., 2011). This can be seen in the naturally occurring midges (Diptera: Chironomidae) and beetles (Coleoptera) of the region. Even though much still remains to be resolved in clarifying the relationships of sub-Antarctic and Antarctic taxa to their sister-groups in South America, the evidence already available allows development of hypotheses and predictions relating to the temporal scale of species divergence and subsequent colonisation of the Antarctic region, such as the long-term presence of Antarctic biota and persistence of populations in ice-free areas (Convey et al. 2008), or the dispersal patterns in insect species such as *Belgica antarctica* (Allegrucci et al. 2012).

One of the most tractable modern tools used to investigate species divergence in both micro- and macroevolutionary processes is phylogeography based on mitochondrial gene sequence (Avise 2000). Through its use, it is possible to infer regional patterns of biodiversity by analysing the genetic structure of populations. The Scotia Arc, the geological region extending from southern South America, through South Georgia, the South Sandwich Islands, South Orkney Islands and South Shetland Islands to the Antarctic Peninsula, has been the focus of many studies addressing the geographic evolution of the region through its terrestrial and marine flora and fauna (e.g. Bermingham & Moritz 1998, Linse et al. 2007, Sérsic et al. 2011, Gonzáles-Wevar et al. 2012, Sands et al. 2015, Levy et al. 2016, Biersma et al. 2018). However, many groups of microbes, plants and animals have yet to be studied in this context.

Such work has yielded important insights into the long-term history of the region. For example, recent molecular phylogeographic studies, supported by classical biogeography have demonstrated that the fauna of the region may be of much longer persistence than previously thought. In particular, persistence times for many taxa, including microbial groups, has been estimated to range from hundreds of thousands to multi-million year timescales (e.g., Stevens et al. 2006, Convey and Stevens 2007, Convey et al. 2008,

2009a, De Wever et al. 2009, McGaughran et al. 2010a, Vyverman et al. 2010, Fraser et al. 2014, Pisa et al. 2014, Chong et al. 2015, Iakovenko et al. 2015). For some terrestrial invertebrates, this has resulted in deep phylogeographic population structure even across small spatial scales [Avise 2000 (p. 202), Collins et al. 2019). However, while some taxa have been well studied, little is yet known about many others in this region, making it difficult to establish an overall picture of evolutionary patterns in the region.

One such poorly-studied taxon is *Parochlus steinenii* Gerke, 1889, known commonly as the Antarctic midge, and one of only two insect species native to the Antarctic. Unlike the other native species, *Belgica antarctica*, which is flightless and found on the Antarctic Peninsula and South Shetland Islands, *P. steinenii* is a winged midge which also occurs naturally in southern South America, the Falkland Islands and South Georgia. It was first found in the latter between 1882 and 1883 during the German Polar Expedition (1882/3) and later described by Gerke (1889). Later, Brundin (1966) redescribed the species from adults and pupae collected in Tierra del Fuego, along with the first description of *Parochlus steinenii brevipennis*, a sub-species found in the Andes of Central Chile, south of the Argentinian city of Bariloche (34ºS). Various aspects of the species' biology have been researched, including its morphology, phenology and physiology (Edwards & Usher 1985, Rauschert 1985, Shimada et al. 1991, Richard et al. 1994, Convey & Block 1996), but its genetic diversity has not yet been assessed (Convey & Block 1996) other than in the very preliminary data presented by Allegrucci et al. (2006). Very recently, however, the complete mitochondrial genome of the species (Kim et al. 2016, Shin et al. 2019) has been sequenced, as well as a draft for the complete genome (Kim et al. 2017, Shin et al. 2019).

The wide distribution of *P. steinenii* in the region, and its apparently highly conserved morphology, raises questions about how long regional populations have been isolated (if at all) from each other, and whether they are cryptically divergent. Thus, in this chapter, we use two genetic markers, mitochondrial Cytochrome c Oxidase subunit 1 (COX1) and nuclear ribosomal large subunit 28S, to test the following hypotheses: (1) that regional populations of *P. steinenii* are genetically isolated; and, (2) if so, whether levels of

divergence are sufficient to be regarded as cryptic speciation. The results of this work are important for understanding the long-term evolution of the species, and contribute to the debate surrounding evolutionary patterns in the sub-Antarctic fauna.

5.3 Materials & Methods

5.3.1 Sampling

We collected specimens of *P. steinenii* in the Austral summer of 2017 (except Bird Island, which are from 2018) (Figs. 5.1 and 5.2). The adults were sampled from their natural habitats around lakes in the South Shetland Islands (Antarctica), South Georgia and Bird Island (Fig. 5.3), with the use of an entomological aspirator, while larvae were manually extracted from mosses in a lake near the summit of Cerro Bandera, Navarino Island, Chile (Table 5.1; Fig. 5.4). Unfortunately, it was not possible to obtain specimens of the Andean subspecies.

Table 5.1. List of sites where adults or larvae of *Parochlus steinenii* were collected.

Location	Island	Lake	Latitude	Longitude
Chile	Navarino	Parochlus	-54°58.42333'	-067°38.72633'
South Georgia	South Georgia	Lancetes	-54°15.71417'	-036°30.26567'
	Bird		-54°00.33333’	-038°03.00000
South Shetland Islands	Deception	Crater	-62°59.02500'	-060°40.35833'
		Zapatilla	-62°59.02500'	-060°40.49667'
	King George	Arctowsky	-62°09.93333'	-058°27.75667'
		Glubokoe	-62°11.04333'	-058°54.56833'
		Kitiesh	-62°11.69500'	-058°57.71167'
		Langer	-62°12.24500'	-058°58.15167'
		Las Estrellas	-62°12.02000'	-058°58.39500'
		Tern	-62°13.26000'	-058°57.44333'
		Unidad	-62º11.57500’	-058º57.22300’
	Livingston	Limnopolar	-62°38.74266'	-061°05.81004'

Figure 5.1. Swarms of adults of *Parochlus steinenii* at Lake Kitiesh, Fildes Peninsula, King George Island, Antarctic (arrows indicate adults).

Figure 5.2. Adult of *Parochlus steinenii* in an entomological aspirator (or pooter), Deception Island (arrows and elipse indicate adults). (Photo: Harry Díaz)

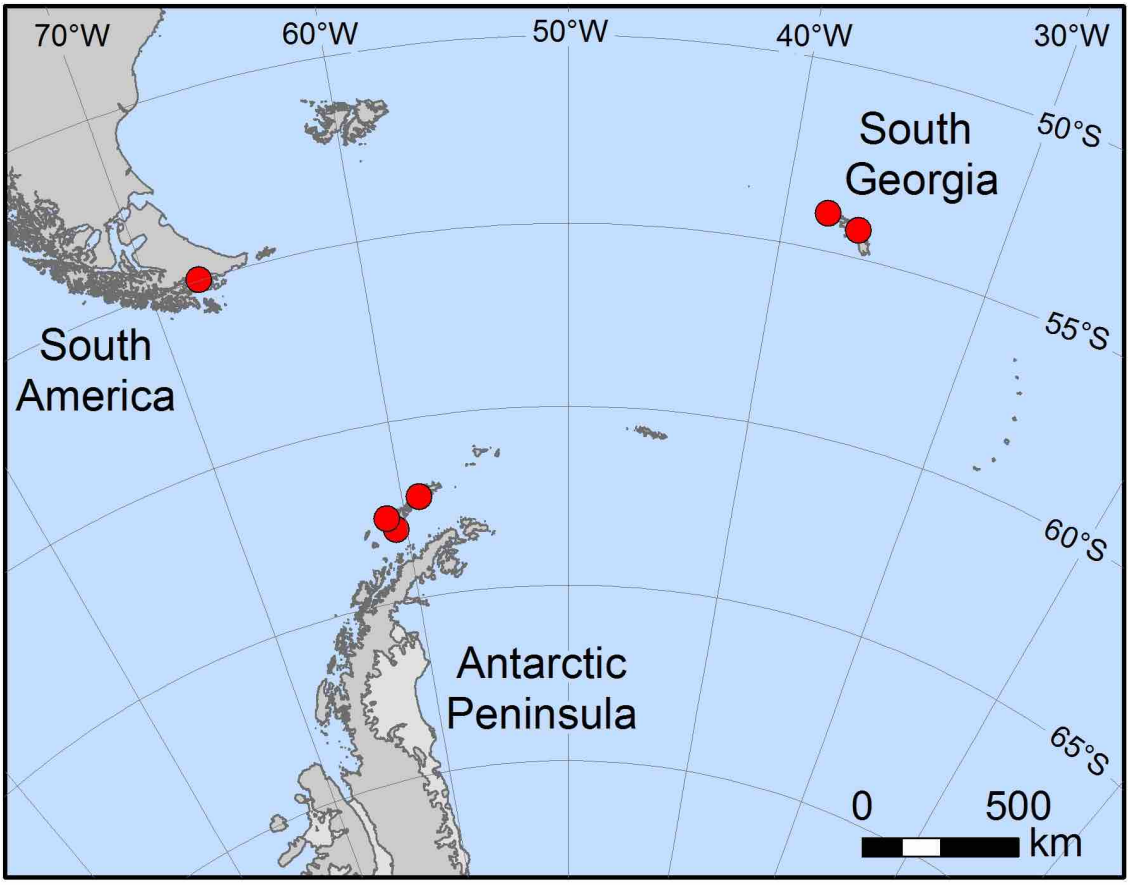


Figure 5.3. Collection sites of *Parochlus steinenii* across South America, South Georgia and the South Shetland Islands (Antarctica).

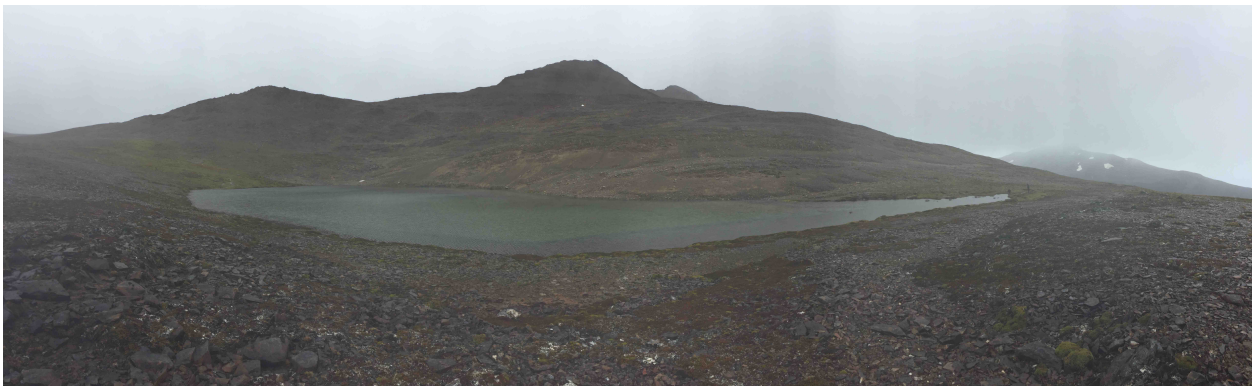


Figure 5.4. Panoramic view of “Parochlus” lake near the summit of Cerro Bandera, Navarino Island, Chile, the only lake with confirmed presence of *Parochlus steinenii* in the island.

5.3.2 DNA Extraction and Sequencing

DNA was extracted from collected individuals using the QIAGEN DNEasy Blood & Tissue and QiAMP Extraction Kits. Individuals were fully submerged into the proteinase K+ATL buffer solution for 4h at 56 ºC or overnight at 40 ºC; adults were not crushed in order to keep them as whole as possible (however, there was some loss in pigmentation on the abdomen), while the larvae were partially or fully crushed before extraction; the remaining steps were followed as per the manufacturer's instructions.

Table 5.2. Primers used for the Polymerase Chain Reaction. Novel primers designed with Geneious (28S).

Gene	Primer Name	Sequence (5'-3')	Reference
COX1	**LCO1490**	GGTCAACAAATCATAAAGATATTGG	Folmer et al. 1994
	HCO2198	TAAACTTCAGGGTGACCAAAAAATCA	Folmer et al. 1994
	UEA5	AGTTTTAGCAGGAGCAATTACTAT	Lunt et al. 1996
	UEA10	TCCAATGCACTAATCTGCCATATTA	Lunt et al. 1996
28S	**rD1.2a**	CCCSSGTAATTTAAGCATATTA	Whiting 2002
	MK_3F	TTTTGGTAAGCAGAACTGGYG	Machida & Knowlton 2012
	28S_1609F	ACCATGAAAGGTGTTGATTGCTG	NOVEL
	28S_1955R	ACCATGAAAGGTGTTGATTGCTG	NOVEL
	rD7b1	GACTTCCCTTACCTACAT	Whiting 2002

Amplifications for the COX1 and 28S genes were carried out with the Qiagen PCR Core Kit with added Ultrapure Bovine Serum Albumine (BSA) [Cat #AM2616 50 mg/mL Lot #0911017] using a combination of forward and reverse primers (Table 5.2). For a more in-depth description of the protocol and techniques used in the Polymerase Chain Reaction, please refer to Appendix I. Finally, products were sent to LGC Genomics

(Germany) and Macrogen (South Korea) for Sanger sequencing. Outgroup sequences were retrieved from GenBank (Table 5.3). We selected *Belgica antarctica* as the primary outgroup, followed by *Podonomus pepinelli* and *Podonums rivolorum*, and stabilised the more basal nodes with more closely related species (*Parochlus* spp.) (see Felsenstein, 1981).

Table 5.3. Outgroup sequences obtained from GenBank.

Gene	Accession no.	Species	Reference
COX1	**JQ672717**	*Belgica antarctica*	Allegrucci et al. (2012)
	FJ570668	*Parochlus araucanus*	Cranston et al. (2010)
	FJ570677	*Parochlus chiloensis*	Cranston et al. (2010)
	FJ570680	*Parochlus kiefferi*	Cranston et al. (2010)
	FJ570682	*Parochlus kiefferi*	Cranston et al. (2010)
	HQ105303	*Parochlus kiefferi*	Ekrem et al. (2010)
	HQ105307	*Parochlus kiefferi*	Ekrem et al. (2010)
	FJ570689	*Parochlus trigonocerus*	Cranston et al. (2010)
	FJ570690	*Parochlus villarricensis*	Cranston et al. (2010)
	JX860263	*Podonomus pepinellii*	Trivinho-Strixino et al. (2012)
	KT633471	*Podonomus rivulorum*	Cranston & Krosch (2015)

5.3.3 Data preparation and genetic analyses

All sequences were manually examined, with forward and reverse sequences assembled, trimmed and aligned in Geneious 9.1.8 (Biomatters, LTD, Auckland, NZ). The alignment of COX1 sequences was carried out with the MUSCLE process (Edgar, 2004). Short, partially incomplete, sections at the ends of each alignment were excluded.

We first tested for genetic structure in our samples, through the use of phylogenetic methods and associated coalescent techniques. Thus, descriptive statistics (Table 5.4) were produced with DnaSP v5.0 (Librado and Rozas, 2009) — Tajima's D, Fu's Fs and RamosOnsins and Rozas' R2 neutrality tests to distinguish randomly evolved sequences — significance was then assessed from 1000 coalescent simulations. Additionally, we used TRACER v1.6.0 (Rambaut et al. 2014) to check for the effective sample sizes of parameters.

Because not all available outgroup sequences encompassed the entire length of the COX1 gene, we opted to use two different alignments with two non-overlapping partitions. The optimal model of nucleotide substitution for COX1 was determined with jModelTest 2 (Darriba & Posada, 2016). Selection was based on the Akaike Information Criterion (AIC) and resulted in selection of the GTR+G model for the first partition and TIM2+I+G for the second partition. Both models have the same substitution parameters (nst = 6). Phylogenetic analysis was performed using MrBayes 3.2 (Ronquist et al. 2012), with 20 million generations, and with RAxML v8.0.0 (Stamatakis 2014), where bootstrap values were acquired through the construction of a Maximum Likelihood tree.

Finally, the phylogeographic structure within ingroup specimens for COX1 was examined with TCS networks (Clement et al. 2000) in the program PopART (Leigh and Bryant 2015), using default settings.

5.4 Results

A total of 165 sequences for COX1 (summary in Table 5.4) and 34 sequences for 28S were obtained. However, there was no variation in the 28S sequences, so no further analyses were carried out using this locus.

Table 5.4. Summary statistics of molecular diversity for populations of *Parochlus steinenii* (n, Number of individuals; Prob, Probablility of having captured the deepest coalescent event; No. haplotypes, Number of haplotypes; H_D, Haplotype diversity; S, Number of segregating sites; π, Nucleotide diversity; D_T, Tajima's D; Fs, Fu's Fs statistic; R_2, RamosOnsins and Rozas' R2 statistic; Max K, Maximum number of nucleotide differences between any two sequences within the population). (*p < 0.05, **p < 0.01)

Population	n	Prob	No. haplotypes	H_D	S	pi	D_T	Fs	R_2	Max. K
Navarino Island (Chile)	18	0.8947	9	0.804	26	0.00437	-0.97220	0.001	0.1040	16
South Georgia	36	0.9459	21	0.870	26	0.00177	-2.17686*	-18.380**	0.0356**	7
Bird Island	7	0.7500	7	1.000	11	0.00312	-0.47530	-3.518	0.1174*	7
Lancetes lake	29	0.9333	14	0.798	16	0.00127	-2.02208*	-10.217**	0.0485**	5
South Shetland Islands	111	0.9821	25	0.662	27	0.00081	-2.36839**	-29.062**	0.0209*	5
Deception Island	31	0.9375	3	0.127	2	0.00010	-1.50558	-2.397	0.1228	2
Crater lake	27	0.9286	1	NA	NA	NA	NA	NA	NA	NA
Zapatilla lake	4	0.6000	3	0.833	2	0.00076	-0.70990	-0.887	0.2500	2
King George Island	53	0.9630	14	0.789	16	0.00112	-1.80826*	-8.205*	0.0448**	5
Arctowsky lake	5	0.6667	2	0.400	2	0.00061	-1.98018*	1.040	0.4000	2
Glubokoe lake	4	0.6000	2	0.500	1	0.00038	-0.61237	0.172	0.4330	1
Kitiesh lake	24	0.9200	5	0.710	16	0.00112	-0.30320	0.074	0.1183	4
Langer lake	5	0.6667	3	0.700	2	0.00061	-0.97256	-0.829	0.2449	2
Las Estrellas lake	5	0.6667	3	0.700	3	0.00092	-1.04849	-0.186	0.2667	3
Tern lake	4	0.6000	2	0.500	1	0.00038	-0.61237	0.172	0.4330	1
Unidad lake	5	0.6667	3	0.700	2	0.00076	0.24314	-0.475	0.2500	2
Livingston Island	27	0.9286	11	0.729	11	0.00083	-2.04482*	-8.554*	0.0532**	4
Total	165	0.9880	52	0.838	73	0.00370	-1.98018*	-32.953**	0.0305	24

Haplotype diversity (H_D) was high in Chile, South Georgia (especially in Bird Island (H_D = 1) and in King George Island, and was markedly lower in Deception Island, as Crater Lake had a single haplotype for 27 individuals (Table 5.4). Tajima's D (D_T) and Fu's Fs were negative and significantly different from zero in South Georgia (and Lancetes Lake), the South Shetland Islands (and in King George and Livingston Islands), however only D_T was significant in Arctowsky. RamosOnsins and Rozas R_2 was significant in South Georgia (and Bird Island and Lancetes Lake), the South Shetland Islands (and in King

George and Livingston Islands). The statistical parsimony haplotype network (Fig. 5.5) showed three main splits in the population, with the Navarino Island population, being the most distinct, and the Antarctic population, also being distinct from the South Georgia and Bird Island populations.

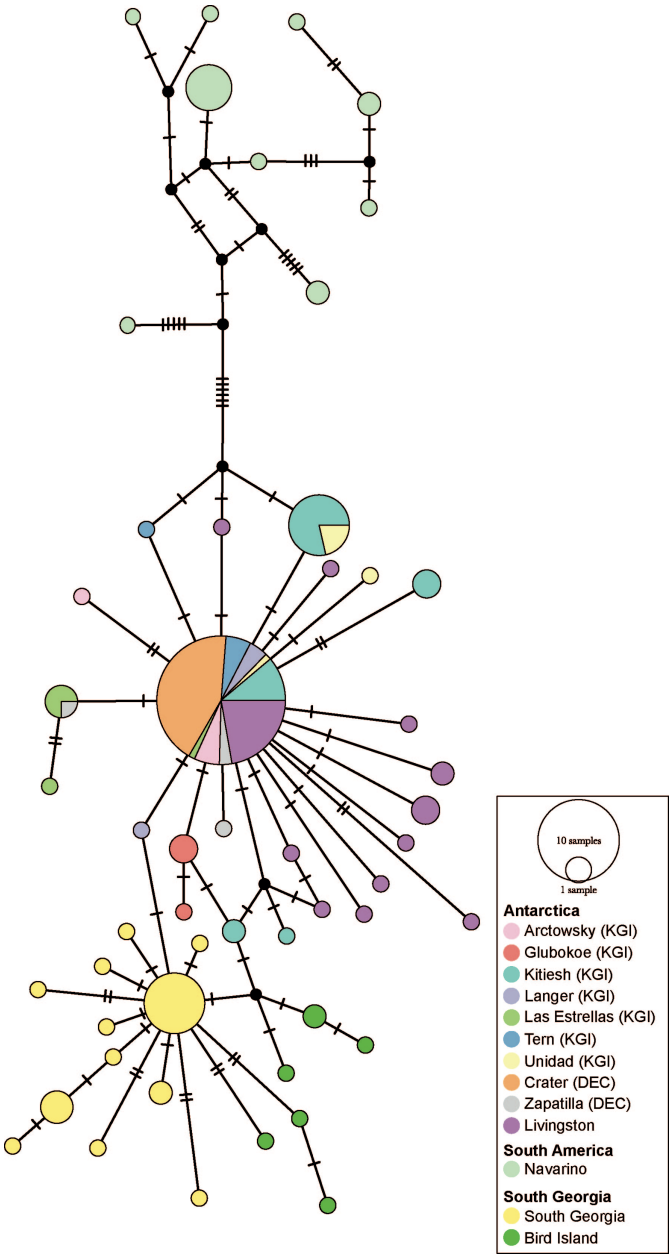


Figure 5.5. Haplotype network from COX1 sequences in populations of *Parochlus steinenii* across 13 localities in Antarctica, Chile, South Georgia and Bird Island. (DEC = Deception Island; KGI = King George Island)

Within the ingroup, the Bayesian phylogenetic reconstructions identified the Navarino component as a single strongly supported clade of geographically defined populations of *P. steinenii* in both fragments of the COX1 gene (Fig. 5.6–5.8). The analysis of the first fragment (barcoding region) identified a low-support clade for the South Georgian component (South Georgia + Bird Island); however, the same was not true in the analysis of the second fragment. Even so, visual inspection of pairings in the Bayesian consensus phylogeny suggests that such a distinction may exist (Fig. 5.6–5.8).

5.5 Discussion

Parochlus steinenii shows a high diversity of haplotypes across its populations for COX1, which is to be expected from a highly mutable gene. In contrast there was almost no variation for 28S; indeed we could not find any variation in the locus across all our samples collected from across the Scotia Arc. However, when we aligned our sequences to the four other available sequences (AY820932–35) from Allegrucci et al. 2006), we found some variation in five sites and also an insertion in two sequences (AY820932 and AY820933). The lack of presence of these variations was very surprising after comparing our own data, and this means that a deeper investigation on the matter is needed, but to do that we would need to obtain many more 28S sequences.

The clear differences we identified between populations of *Parochlus steinenii* at the COX1 locus is a contrast to morphological studies that have found no clear differences between populations (Convey & Block 1996). This finding is intriguing and may indicate conserved selective pressure on populations, reducing morphological divergence between populations. The clear genetic differences on the other hand suggest that populations have been isolated for a long time (particularly the South America and the Antarctic + South Georgian group) and that speciation may be in the process of occurring. The lack of any difference in the nuclear 28S gene versus the mithocondrial COX1 gene is likely to reflect lower mutation rates in the nuclear gene. It is intriguing to consider whether the divergence we have recorded in this study indicates that different sub-

populations identified may be better considered as separate species. More work investigating whether these groups are reproductively isolated or differ in core aspects of their ecology would help to confirm this.

The observations from the network are further supported by the phylogenetic analysis, which heavily supports the South American clade, and to a lesser extent a South Georgian clade. This most likely means that the population from Chile has been isolated from the others for a significantly longer time. Nevertheless, our data still shows that they have not diverged enough to be considered a completely different species.

This study is the first one to identify the intriguing evolutionary history of *P. steinenii* through a comprehensive analysis of the genetic structure of the species since Allegrucci et al. (2006). Nevertheless, this is just the first step into fully understanding how the taxon has evolved, a question that can feasibly be tackled by increasing the number of loci analysed or by taking a look at microsatellites. Also, it would be interesting to learn how the Andean populations fit within the evolutionary context.

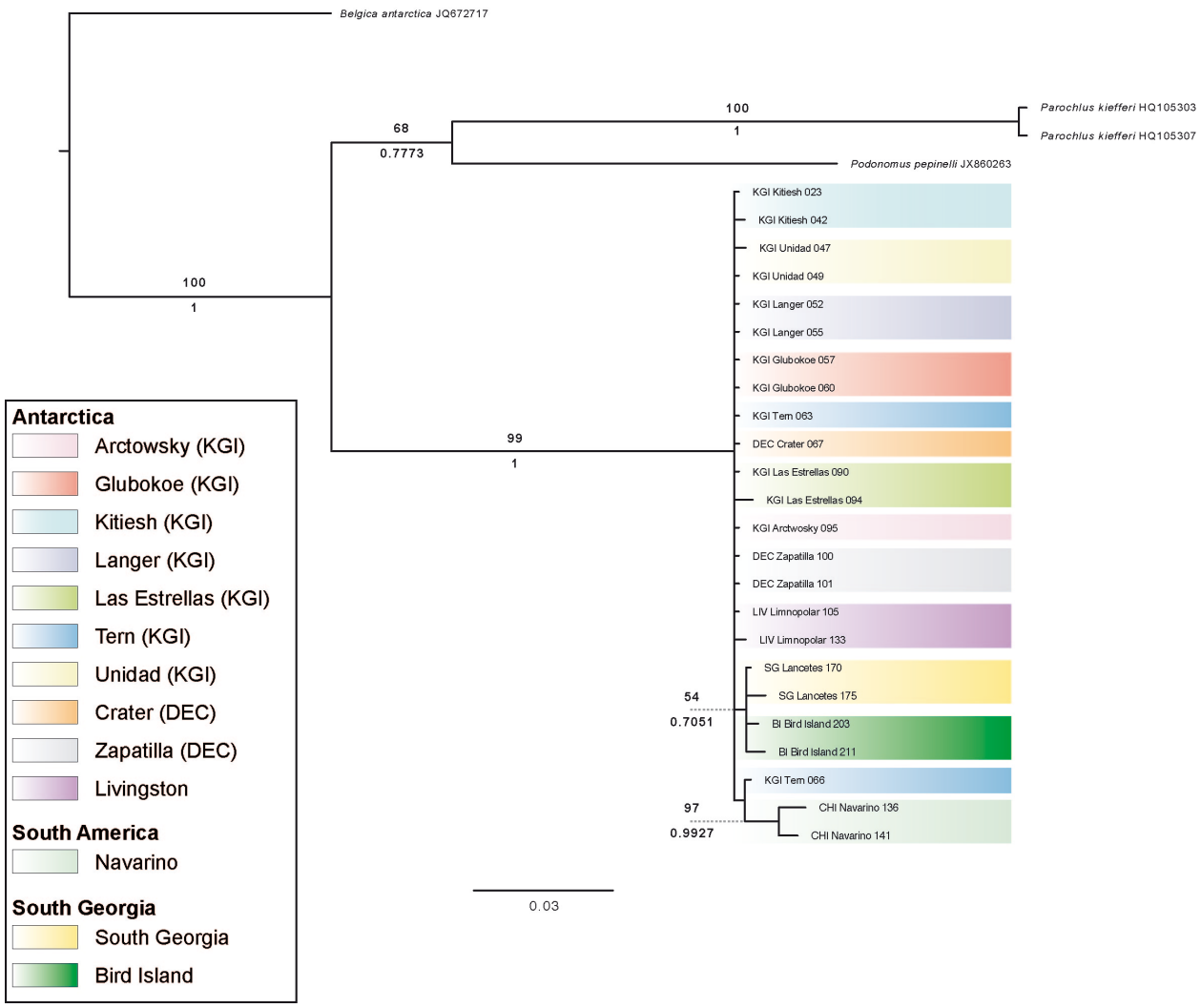


Figure 5.6. Bayesian consensus phylogeny of the first fragment of partitioned COX1 sequences derived from populations of *Parochlus steinenii* from Chile, South Georgia and Antarctica (South Shetland Islands). Posterior probabilities are given under the line by the associated adjacent node and bootstrap values from the Maximum likelihood tree are given above the line (when nodes are exactly the same from the Bayesian analysis). No value is given if the bootstrap value was < 50%. Outgroups are not coloured, and the identifiers on their tips are GenBank system accession numbers for the individual sequences.

COX1 – 2nd Fragment

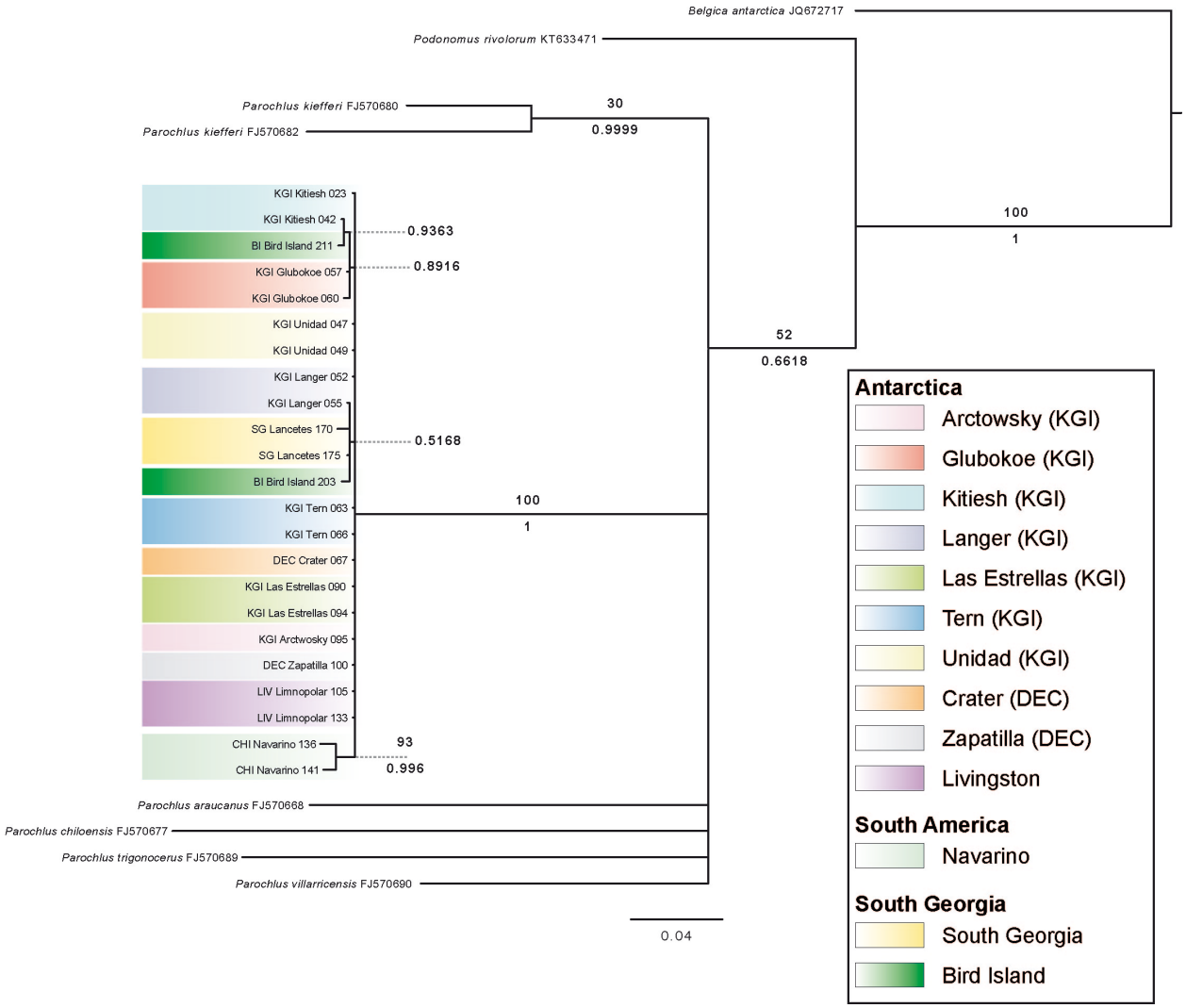
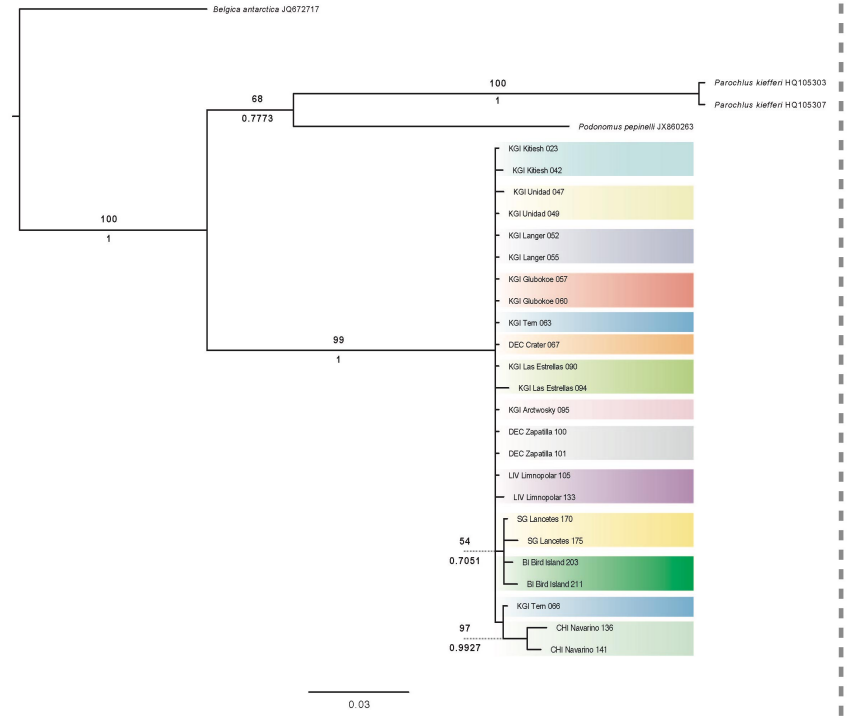


Figure 5.7. Bayesian consensus phylogeny of the second fragment of partitioned COX1 sequences derived from populations of *Parochlus steinenii* from Chile, South Georgia and Antarctica (South Shetland Islands). Posterior probabilities are given under the line by the associated adjacent node and bootstrap values from the Maximum likelihood tree are given above the line (when nodes are exactly the same from the Bayesian analysis). No value is given if the bootstrap value was < 50%. Outgroups are not coloured, and the identifiers on their tips are GenBank system accession numbers for the individual sequences.

COX1

1st Fragment



2nd Fragment

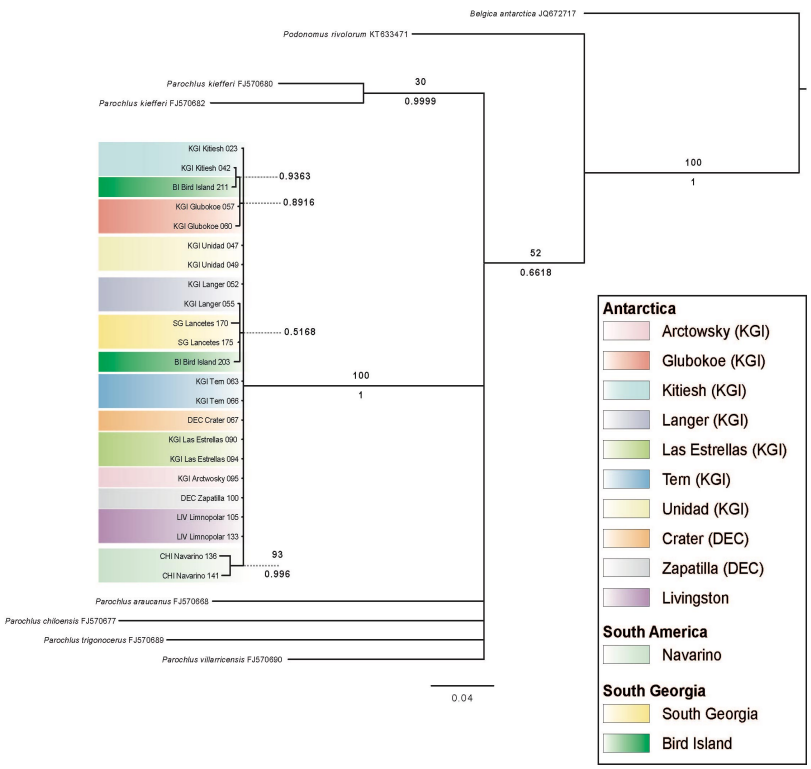


Figure 5.8. (previous page) Combined Bayesian consensus phylogenies of partitioned COX1 sequences derived from populations of *Parochlus steinenii* from Chile, South Georgia and Antarctica (South Shetland Islands). Posterior probabilities are given under the line by the associated adjacent node and bootstrap values from the Maximum likelihood tree are given above the line (when nodes are exactly the same from the Bayesian analysis). No value is given if the bootstrap value was < 50%. Outgroups are not coloured, and the identifiers on their tips are GenBank system accession numbers for the individual sequences.

5.6 Acknowledgements

We would like to thank Claudia Maturana and Javier Rendoll Carcamo for help in collecting the specimens in King George Island and Navarino, respectively.

CHAPTER 6

Hind-wing morphology of *Lancetes angusticollis*

6. Does island-isolation cause a reduction in hind wing size in *Lancetes angusticollis* (Coleoptera: Dytiscidae)?

6.1 Abstract

The diving beetle *Lancetes angusticollis* is found in lakes in southern South America and in South Georgia. The geographical isolation of these populations suggests they have been separated on sufficiently long timescales to permit the evolution of adaptive changes in morphology. One of the more commonly reported morphological variations associated with island isolation is the reduction of wings, generally linked to different requirements in terms of energy reserves and lower predation pressure. Here, we tested the hypothesis that the South Georgian populations of *L. angusticollis* would have reduced hind wings in comparison with their mainland counterparts. To test this, we documented geometric morphometrics, analyzing the data with Principal Component Analyses, to assess body and wing size and shape. Beetles from South Georgia had significantly longer heads, elytra and hind leg lengths, and shorter pronotum length, although they did not differ in overall body length. The centroid size did not vary, meaning that the overall size of the wings was not different, but the calculated wing loads showed that hind wings were of different shapes, with the main differences being in the costal, jugal and posterior margins of the wings along with the cubital cells. However, all of these observed differences in wing shape were subtle and do not clearly link with the hypothesis being tested. Based on this study, we suggest the most likely reasons for the slight differences in morphology were due to founder effects and genetic drift, although more detailed studies of environmental factors and other selective factors driving differences, such as temperature and wind speeds, as well as of beetle behavior (particularly use of flight) would be required to confirm these interpretations.

6.2 Introduction

6.2.1 Flightlessness in insects

The evolution of flight is perhaps the single largest innovation that has enabled insects to become arguably the most successful higher taxon from the lower Carboniferous onwards (Wagner & Liebherr 1992). In particular, flight increased the extent to which insects can reach new habitats, food resources and breeding grounds (Dickinson, Lehmann & Sane, 1999, Dudley, 2002), enabling them to disperse over large areas, migrate and colonise new habitats, while also avoiding inclement conditions.

However, paradoxically, insects on islands commonly show reduced wings and the evolution of flightlessness (Danks 1990, Wagner & Liebherr 1992, Laparie et al. 2016). The incidence of flightlessness is particularly high in some areas of the world, such as the sub-Antarctic Islands (Gressitt & Weber 1959, Chevrier 1996, Vernon 1981, Chown & Convey 2016). For example, on the Kerguelen Islands in the Southern Ocean, 19 out of 23 native insect species are wingless. The loss of wings has been hypothesized to be linked to specific environment characteristics, such as wind exposure and speed (Taylor 1963) and low temperatures, that may limit wing development or provide an evolutionary disadvantage for insects with wings, such as being blown away from islands when in flight and therefore dying out at sea, or the high energetic costs that are involved in the development and maintenance of the related muscles (Laparie et al. 2016). For example, Vernon (1981, 1986) suggests that a reduction of wings and associated muscles may allow the expansion of energy reserves in the body which then facilitate higher starvation resistance. An additional factor favouring loss of wings on islands may be the relatively low levels of predation pressure on terrestrial invertebrates in these systems (Convey 1996, Chevrier et al. 1997, Laparie et al. 2010, 2016, Lebouvier et al., 2011), reducing the evolutionary advantage of maintaining wings for escaping predators. Further to that, in flightless beetles, such as in the case of the promecheilid *Perimylops antarcticus*, also found in South Georgia, the elytra have a tendency to also be shortened, as they lose their function of protecting the hind wings, but in the specific case of diving beetles the elytra can still play an important role in the hydrodynamics of swimming.

Recently, Laparie et al. (2016) have tested if any particular evolution could be detected over time in the size and shape of the wings of *Calliphora vicina*, a cosmopolitan blowfly that invaded the Kerguelen Islands in the 1970s. By approximately knowing when the species arrived in the island, they were able to investigate real-time changes in ecological and evolutionary processes under novel selection regimes (Chevrier et al. 1997, Huey et al. 2005, Sax et al. 2007, Schermann-Legionnet et al. 2007, Lebouvier et al. 2011, Laparie et al. 2016). They questioned whether the flight characteristics of the species had changed over time, and found fingerprints of local adaptation in the invasive population, although they could not affirm if the changes had already incurred aerodynamic consequences. Though non-conclusive, this quick divergence in a relatively short period suggests that even bigger changes would happen in a sub-Antarctic species that has been isolated for a longer period.

6.2.2 *Lancetes angusticollis*

The diving beetle *Lancetes angusticollis* (Curtis, 1839) has long been recorded from the South American (Curtis 1839, Řiha 1961, Brancucci & Ruhnau 1985) mainland and remote islands such as South Georgia (Müller 1884, Brancucci & Ruhnau 1985, Arnold & Convey 1998). Most specimens described from South Georgia appear to have normal wing formation and associated musculature, although they have rarely been observed in flight (Arnold & Convey 1998). However, wing characteristics have not been mapped in South American specimens, so it is not yet known whether there are any differences in wing development between continental and island populations of this species.

In this chapter, we aim to test the hypothesis that a long-term isolation has caused a reduction in the size of hind wings, comparing two different populations of *Lancetes angusticollis* in the southermost region of South America and South Georgia. We hypothesise that strong selective pressure from environmental conditions, coupled with a smaller presence of predators on the South Georgian population will have resulted in morphological differences in individuals compared to the mainland population.

6.3 Materials & Methods

6.3.1 Sampling

Adults (n = 96) of *Lancetes angusticollis* were collected using entomological nets from two lakes at Navarino (n = 65) (Laguna 'Mejillones' [-54°53.92417', -067°57.96600'] and Laguna Zañartu [-54º55.93410', -067°38.78700']), a Chilean island only 50 km away from the Beagle Channel's exit at the Atlantic Ocean, and one lake in South Georgia (n = 31) (Lancetes Lake [-54°15.71417', -036°30.26567']; Fig. 6.1), a remote sub-Antarctic island on the Scotia Arc and around 1,700 km away from the South American coast. All specimens were stored in 96% ethanol.

6.3.3 Morphological measurements

Wing size and shape must be analysed in comparison with the total body size and other body parts if flight ability is being tested (Laparie et al. 2016). To that end, we measured the following in all specimens for posterior wing load comparisons: maximum body length, maximum head length, maximum pronotum length, maximum elytrum length, and maximum hind leg length, the latter playing an important role in the biology of diving beetles, as it is heavily used in swimming.

6.3.3 Wing mounting and landmark acquisition

Left hind-wings were mounted in Chick's Fluid Mountant on microscope slides, which were then sealed with glycerin. Photographs of the ventral side of each wing were captured using a Leica M165-C stereomicroscope with a Leica DMC5400 attached camera. Twenty-one landmarks were selected (Fig. 6.2) for subsequent digitisation with ImageJ. We excluded 11 specimens from Navarino Island as their wings were partially destroyed before or during mounting.

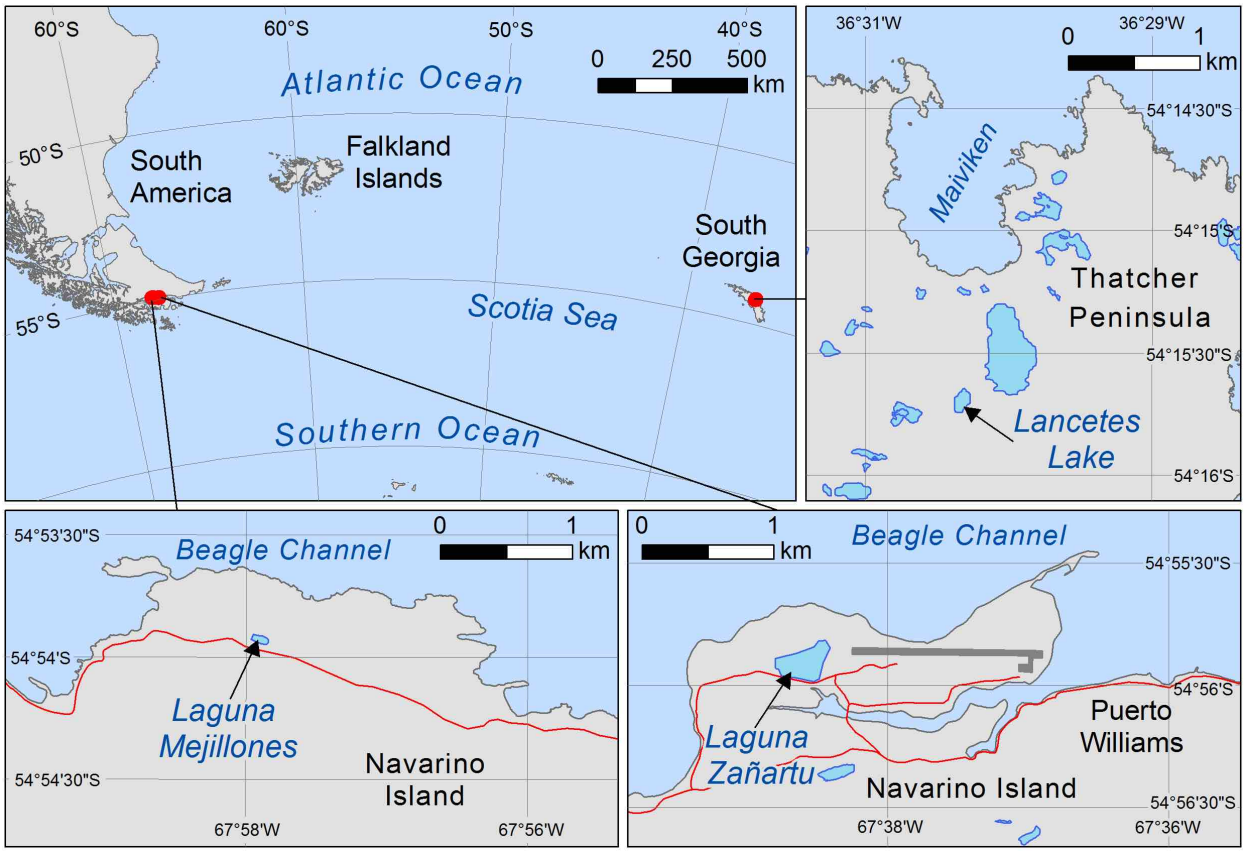


Figure 6.1. Sampling sites for adults of *Lancetes angusticollis* (in red on the top left map and indicated by arrows on the remaining maps). Red lines indicate main roads.

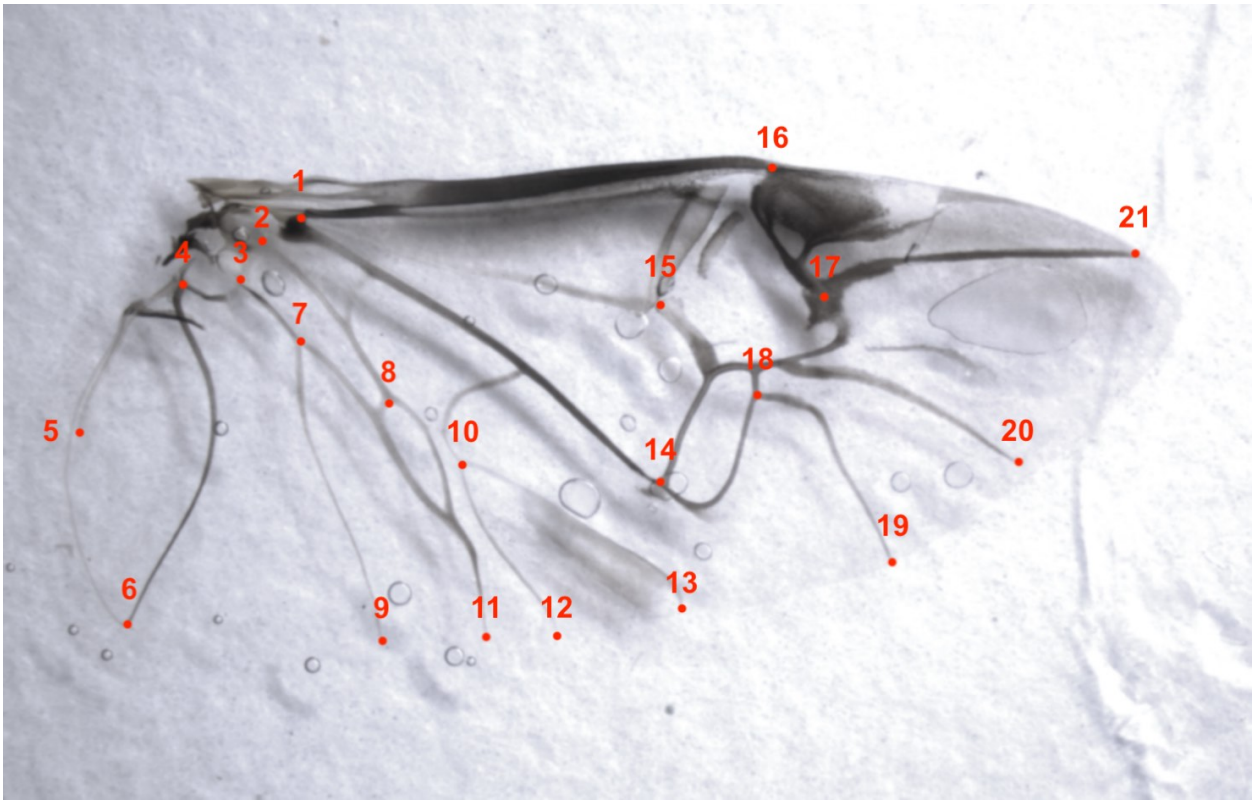


Figure 6.2. The 21 landmarks chosen for the left hind wing (ventral view) morphometric Procrustes Coordinate Analysis.

6.3.4 Statistical analyses and morphometrics

To test for normality in all data, Shapiro-Wilk tests were used. Body size data that were normally distributed were compared between Navarino Island and South Georgia using two-way t-tests, while data that were non-normally distributed were analysed using Mann-Whitney U tests. We also visualized any differences in the overall body shape of individuals including all measurements, using a Principle Component Analysis (PCA).

Hind wing landmark configurations were analysed in MorphoJ (Klingenberg, 2011), where we performed a generalized Procrustes analysis to remove variation in scale, position, and orientation, as well as to obtain the matrix of shape variables (Procrustes coordinates). Subsequently, a PCA was conducted on the Procrustes coordinates to explore the variation in wing shape and visualise shape changes associated with the principal components, which was then followed by a Canonical Variate Analysis (CVA),

used to discriminate shape differences between individuals from Navarino Island and South Georgia. The statistical significance of pairwise differences in mean shapes was tested using permutation tests (10,000 iterations) with Mahalanobis distances and Procrustes distances. We compared the centroid wing sizes between the two populations using Mann-Whitney U tests.

Finally, we computed the ratio of centroid sizes to maximum body length and elytra length measurements (in mm) for comparison of wing loads, i.e. the inverse of wing load (Yeap et al., 2013, Laparie et al. 2016) and compared these between the two populations using Mann-Whitney U tests.

6.4 Results

Body length was not significantly different between the two populations ($W = 1302.5$, $p = 0.061$). However, head ($W = 714.5$, $p = 0.010$), pronotum ($W = 1787.0$, $p < 0.001$), elytrum ($W = 559.5$, $p < 0.001$), and hind leg length ($t = -9.1242$, d.f = 65.395, < 0.001) were all significantly different between the two populations, with South Georgia specimens having shorter pronotum lengths, but longer head, elytrum, and hind leg lengths than specimens from Navarino (Fig. 6.3 and 6.4).

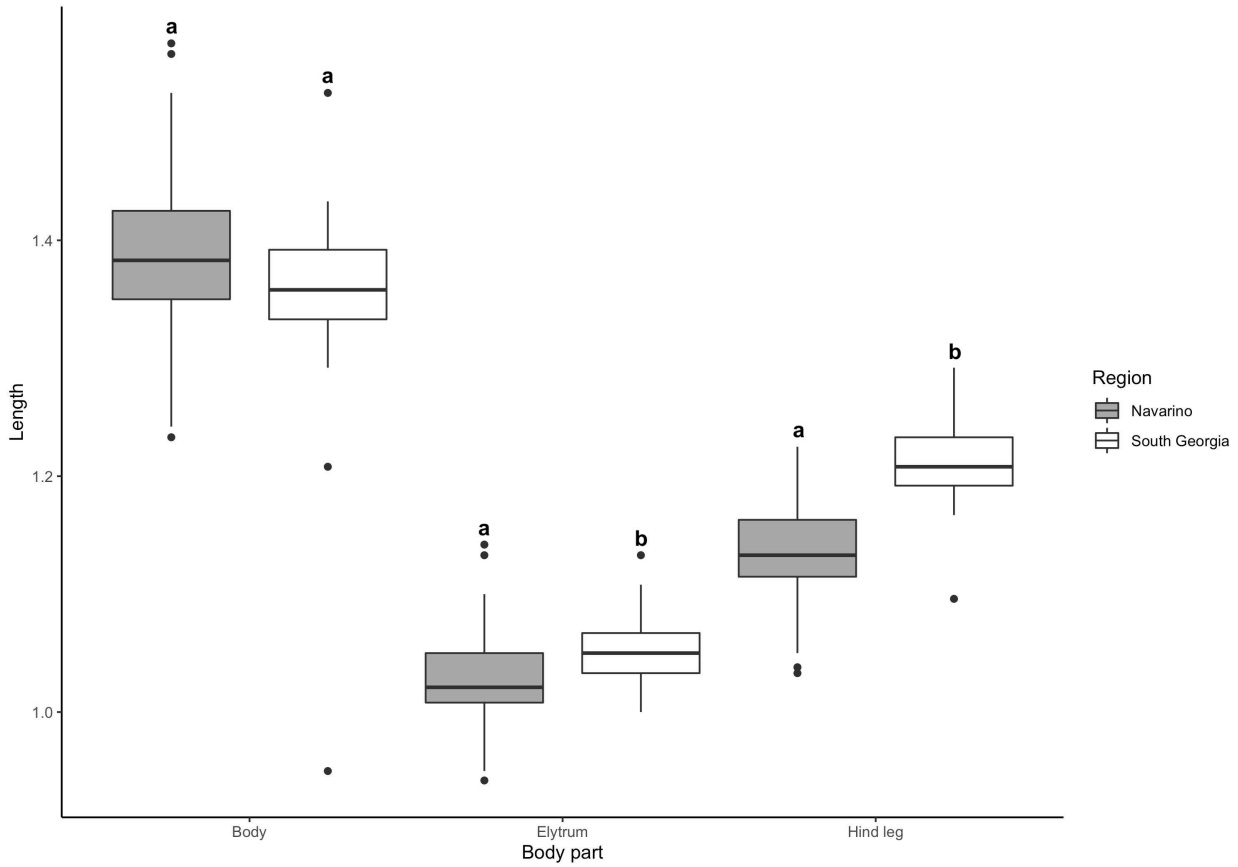


Figure 6.3. Body, elytrum and hind leg lengths (in cm) in populations of *Lancetes angusticollis* from Navarino Island and South Georgia. For each body part, means with the same letter are not significantly different at $p < 0.05$ (t and Mann-Whitney U tests).

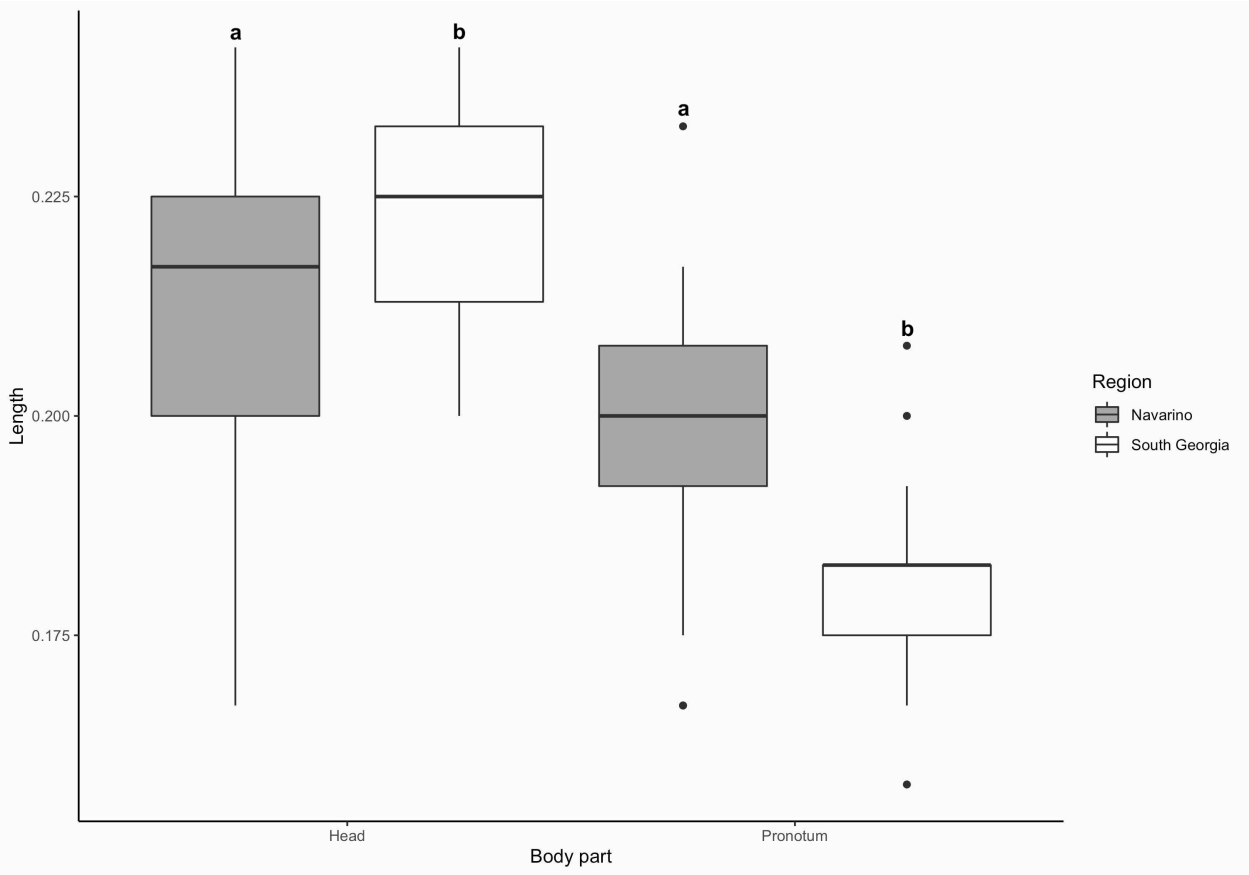


Figure 6.4. Maximum head and pronotum lengths (in cm) in populations of *Lancetes angusticollis* from Navarino Island and South Georgia. For each body part, means with the same letter are not significantly different at $p < 0.05$ (Mann-Whitney U test).

In the Principal Component Analysis (PCA) for body part measurements, PC1 (accounting for 39.1% of variation) was positively associated with all five measurements. PC2 (27.2%) was positively associated with body and pronotum lengths, but was negatively associated with hind leg. PC3 (15.2%) was positively associated with head and pronotum, but negatively with body. PC4 (13.5%) was positively associated with body and head, but negatively with pronotum (Tables 6.1 and 6.2). There was a clear shift in the morphospace occupied by the two populations, with the population from South Georgia having generally higher PCA1 scores, but lower PCA 2 scores (Fig. 6.5).

Table 6.1. Summary of results from a Principal Component Analysis of body part measurements between the two study regions (Navarino and South Georgia).

	PC1	PC2	PC3	PC4
Standard deviation	1.398	1.166	0.873	0.822
Proportion of Variance	0.391	0.272	0.152	0.135
Cumulative Proportion	0.391	0.663	0.815	0.950

Table 6.2. Scores for each variable from the Principal Component Analysis of body part measurements applied to the two study regions (Navarino and South Georgia). Numbers in bold are the three most relevant scores for each PCA axis.

Length	PC1	PC2	PC3	PC4
Body	0.187	**0.626**	**-0.435**	**0.611**
Head	**0.479**	-0.110	**0.689**	**0.491**
Pronotum	0.121	**0.690**	**0.450**	**-0.471**
Elytrum	**0.606**	0.086	-0.311	-0.395
Hind leg	**0.595**	**-0.337**	-0.193	-0.089

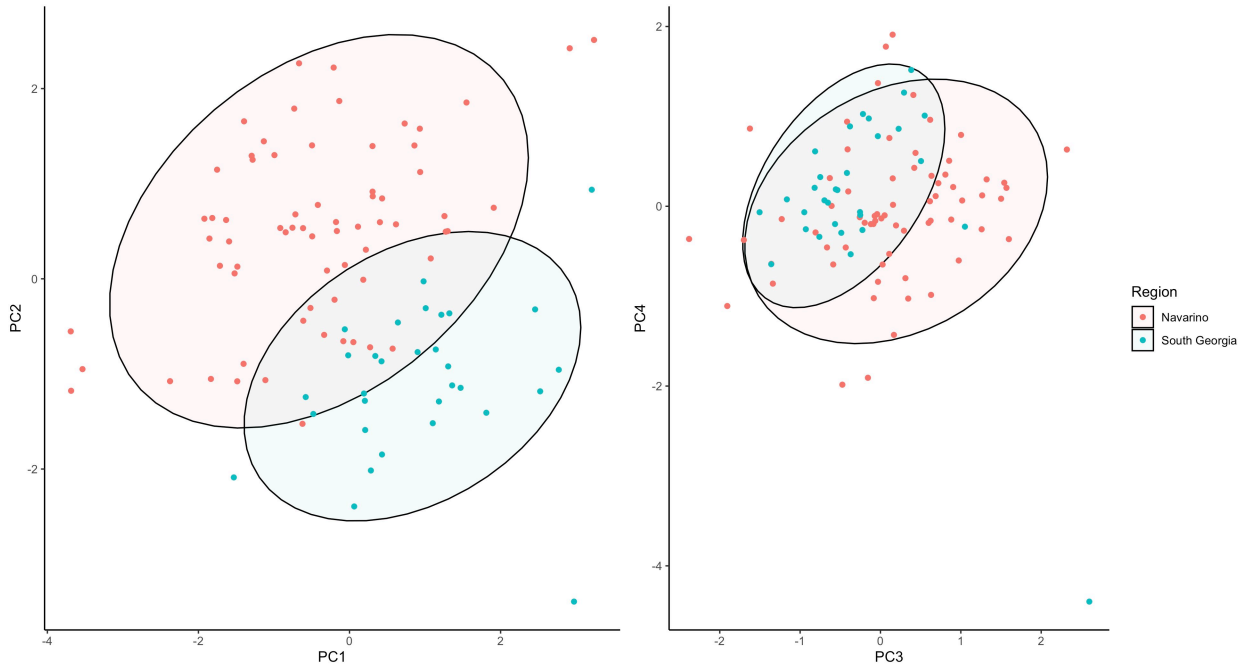


Figure 6.5. Principal Component Analysis output of body measurement variables from four axes in the two regions (Navarino Island and South Georgia). Ellipses refer to each region: red, Navarino; and, green, South Georgia. Dots refer to each specimen and follow the same colour pattern of their respective ellipses.

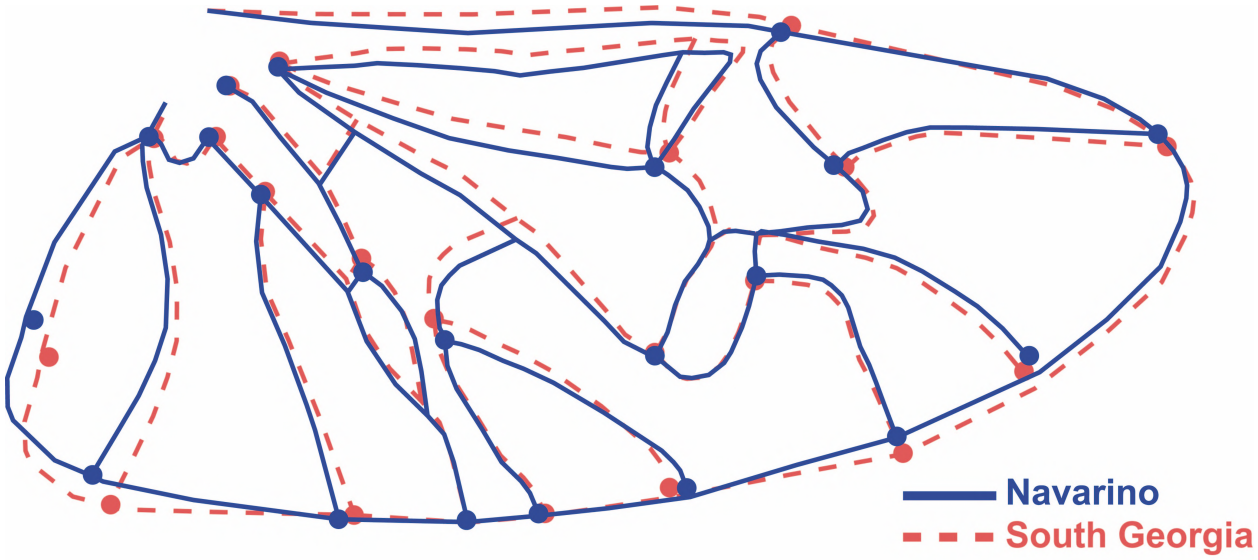


Figure 6.6. Hind-wing shape difference between two populations of *Lancetes angusticollis* based on Procrustes coordinates from 21 landmarks.

In the PCA for hind wing landmarks, PC1 was positively associated with y5, x6, y6 and x9, and y10, and negatively with x13. PC2 was positively associated with y6, x9 and y21, negatively with x5, x6 and y13. PC3 was positively associated with x3 and y11, negatively with x11, x13, y15 and y16. PC4 was positively associated with x6, y9 and x12, negatively with y1, y13 and x19. PC5 was positively associated with x5, x9, x19 and y19, negatively with y5 and y10. PC6 was positively associated with x9, y10 and x21, negatively with y9, y11 and x12 (Table 6.3 and 6.4; Fig. 6.7).

Table 6.3. Summary of results from a Principal Component Analysis of hind wing landmark coordinates between two regions (Navarino Island and South Georgia).

	PC1	PC2	PC3	PC4	PC5	PC6
Eigenvalues	0.0008	0.0003	0.0002	0.0001	0.0001	0.0001
Proportion of Variance %	36.774	14.582	10.172	5.643	5.248	3.365
Cumulative Proportion	36.774	51.356	61.528	67.171	72.419	75.784

Table 6.4. Scores for each variable from the Principal Component Analysis (PCA) from 21 coordinates applied to the two study regions (Navarino Island and South Georgia). Numbers in bold are the six most relevant scores for each PCA axis (three per coordinate axis).

Coordinate axis	PC1	PC2	PC3	PC4	PC5	PC6
x1	-0.063	0.041	0.092	-0.214	-0.051	0.041
y1	-0.110	-0.032	-0.126	**-0.321**	0.109	0.072
x2	-0.068	0.040	0.247	-0.012	-0.033	-0.014
y2	-0.097	-0.037	0.036	-0.098	0.125	0.096
x3	-0.038	0.083	**0.284**	-0.024	-0.106	-0.048
y3	-0.087	0.034	0.036	-0.028	0.093	0.107
x4	-0.047	0.062	0.275	-0.059	-0.053	-0.107

y4	-0.039	0.040	0.016	-0.027	0.084	0.158
x5	0.149	**-0.379**	0.028	0.048	**0.317**	-0.133
y5	**0.541**	0.107	0.053	-0.130	**-0.521**	-0.161
x6	**0.340**	**-0.573**	-0.193	**0.309**	-0.080	0.058
y6	**0.333**	**0.234**	0.206	-0.036	0.046	0.156
x7	-0.065	0.090	0.213	-0.034	-0.091	-0.040
y7	-0.140	-0.028	0.100	0.050	0.038	0.132
x8	-0.077	0.148	0.048	0.047	-0.139	-0.046
y8	-0.219	-0.032	0.098	0.135	-0.101	0.149
x9	**0.167**	**0.276**	-0.151	-0.009	**0.346**	**0.301**
y9	-0.103	0.027	0.112	**0.289**	0.064	**-0.386**
x10	-0.154	0.057	-0.104	0.034	-0.180	0.047
y10	**-0.255**	-0.161	0.064	0.172	**-0.262**	**0.240**
x11	-0.009	0.241	**-0.281**	0.103	0.084	-0.189
y11	-0.086	-0.073	**0.212**	0.239	0.093	**-0.268**
x12	-0.014	0.236	-0.076	**0.237**	0.159	**-0.306**
y12	-0.084	-0.117	0.167	0.083	0.078	-0.152
x13	**-0.214**	0.135	**-0.335**	0.213	-0.076	0.115
y13	-0.032	**-0.224**	0.091	**-0.246**	-0.015	0.050
x14	-0.065	-0.024	-0.051	-0.143	-0.031	0.050
y14	-0.026	-0.082	-0.177	-0.061	0.017	0.049
x15	0.080	0.032	0.061	-0.082	0.012	0.056
y15	-0.139	-0.030	**-0.253**	-0.054	-0.075	-0.032
x16	0.083	-0.000	-0.009	0.066	-0.103	0.089
y16	-0.060	0.018	**-0.210**	-0.114	-0.097	-0.129
x17	0.054	-0.041	0.095	0.015	0.007	0.187
y17	0.037	0.051	-0.174	-0.048	-0.080	-0.095
x18	-0.065	-0.057	-0.027	-0.058	-0.090	0.096

y18	0.054	0.023	-0.176	-0.066	0.002	-0.139
x19	-0.007	-0.112	-0.017	**-0.405**	**0.182**	-0.217
y19	0.180	0.019	0.058	-0.058	**0.357**	-0.008
x20	-0.061	-0.170	-0.161	-0.187	-0.097	-0.200
y20	0.167	0.095	0.026	0.214	0.143	0.138
x21	0.074	-0.085	0.062	0.153	0.023	**0.259**
y21	0.167	**0.168**	-0.157	0.106	-0.098	0.022

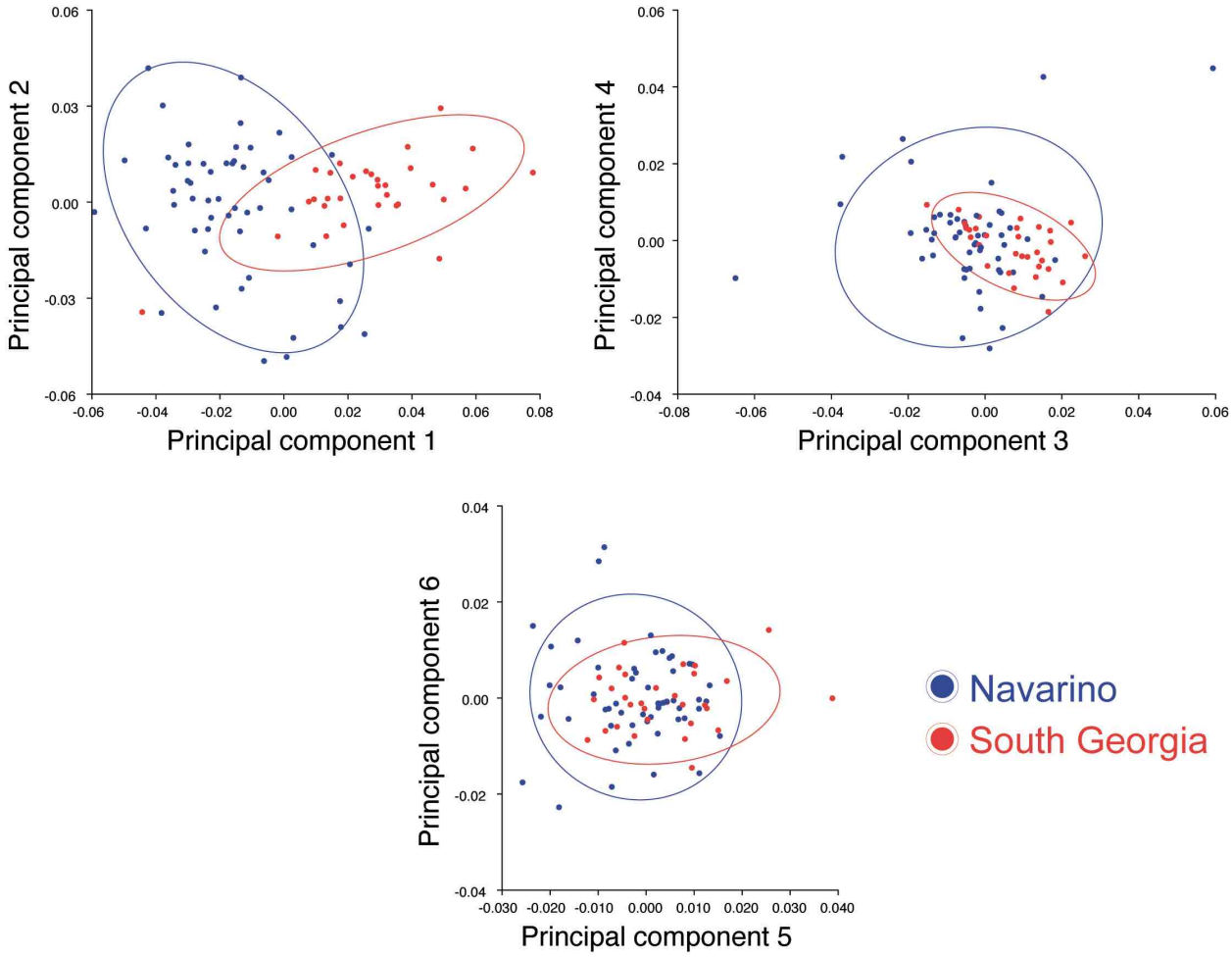


Figure 6.7. Principal Component Analysis output of 21 landmarks on the hind-wing of *Lancetes angusticollis* from six axes from two regions (Navarino Island and South Georgia). Ellipses refer to each region: blue, Navarino, and, red, South Georgia. Dots refer to each specimen and follow the same colour pattern of their respective ellipses.

In the Canonical Variate Analysis (Fig. 6.8), Mahalanobis distances among groups (8.0667) and the Procrustes distances (0.0458) were both significant (p-values from permutation tests < 0.001), indicating that the two populations differed in wing morphology. Inspection of images of the Procrustes coordinates from 21 landmarks suggested that this was due to the population in South Georgia having wider wings (albeit very slightly) than the Navarino population (Fig. 6.6).

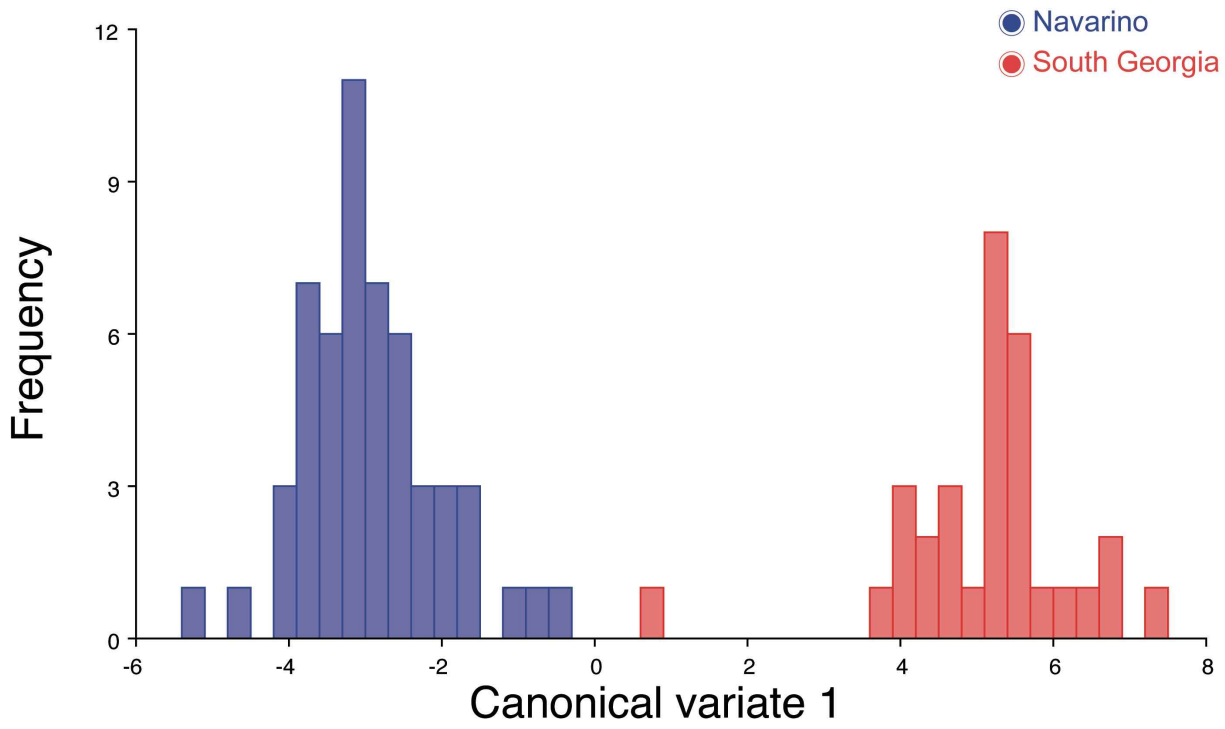


Figure 6.8. Canonical Variate Analysis frequency histogram of *Lancetes angusticollis* wing landmark configurations from canonical variate analysis separating the Navarino and South Georgia populations based on wing shape (variation among groups = 100%).

Centroid size did not differ significantly between the two populations (W = 749, p = 0.4244). The mean±SE of centroid size were 1735.06±9.138 (Navarino) and 1746.55±7.253 (South Georgia) (Fig. 6.9).

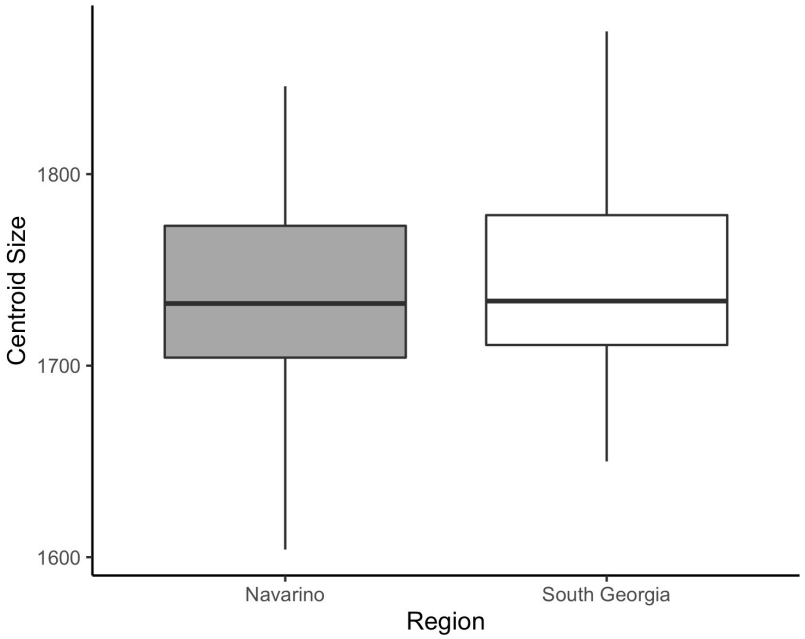


Figure 6.9. Boxplot showing mean hind-wing centroid sizes in two populations of *Lancetes angusticollis*. Centroid sizes did not differ significantly (Mann-Whitney U test).

Wing load based on maximum body length (W = 636.0, p = 0.067), did not differ between the two populations (Fig. 6.10), although wing load based on maximum elytrum length (t = 4.102, d.f = 64.6, p = < 0.001) did, with South Georgia having a higher wing load than Navarino (Fig. 6.11).

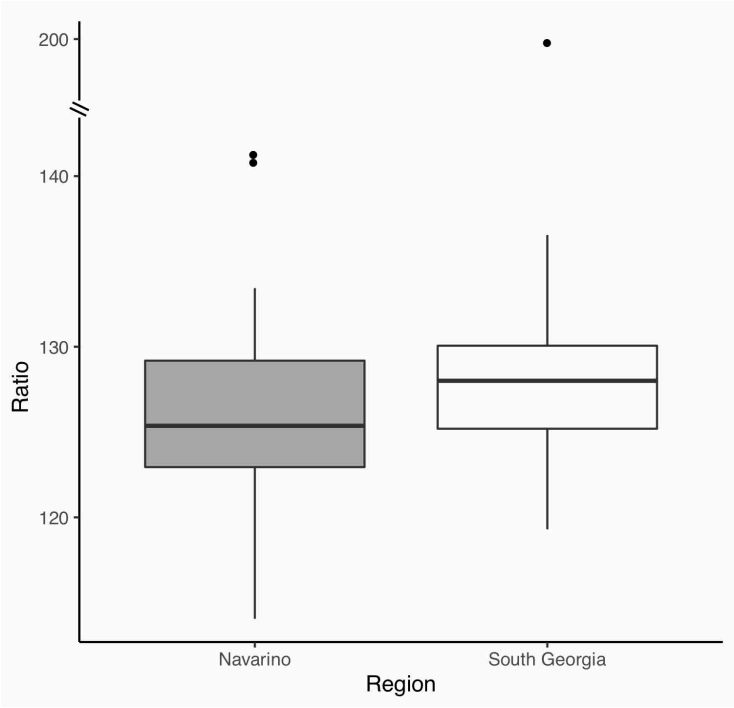


Figure 6.10. Boxplot showing mean wing loads (from maximum body length-centroid size ratio) in two populations of *Lancetes angusticollis*. Wing loads did not differ significantly (Mann-Whitney U test).

6.5 Discussion

We found clear differences in the morphology of *Lancetes angusticollis* between Navarino and South Georgia. In particular, beetles from South Georgia had significantly longer heads, elytra and hind leg lengths, and shorter pronota, although they did not differ in overall body length. Hind wing shape also differed between the two populations, with the South Georgia population having slightly wider wings. However, overall wing centroid size did not differ between the populations. Finally, wing loading based on maximum body length also did not differ significantly between the two populations, but loading based on maximum elytrum length was significantly different, with South Georgia populations having a higher wing-loading.

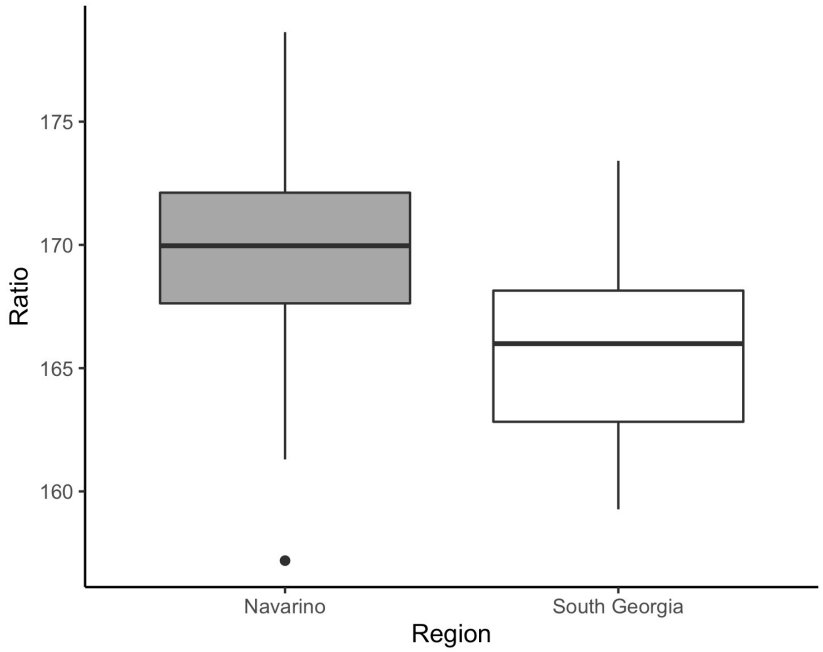


Figure 6.11. Boxplot showing mean wing loads (from maximum elytrum length-centroid size ratio) in two populations of *Lancetes angusticollis*. Wing loads differed significantly (Mann-Whitney U test). As this represents an inverse difference, this indicates that the South Georgia population has higher wing loading.

Even though there was no significant difference in body length between populations, specimens from South Georgia had longer heads and elytra, but shorter pronota, which helps explain why there was no difference in total body length. However, this does not pinpoint exactly what causes these differences. For wings, although centroid size did not vary, meaning the overall size of the wings was not different, the results of the Canonical Variate Analysis (Fig. 6.8) and the wing loads (Figs. 6.10 and 6.11) showed that hind wings were of different shapes in Navarino and South Georgia. The main differences were in the coastal, jugal and posterior margins of the wings along with the cubital cells. However, all of these observed differences in wing shape were quite subtle and not sufficient to support our initial hypotheses that island isolation has caused a shortening of wings and consequential increase in flightlessness. Instead, the higher wing load present in South Georgian is suggestive of weaker selective pressures that may enable the beetles to make better use of their flight ability. We consider the most likely cause for the slight difference in shape and wing load to be from founder effect and genetic drift,

although environmental factors and selective factors driving differences, such as temperature and wind speeds, cannot be discounted without further study.

It is clear there are differences in the two populations, however, we would need to compare genetic components or even ecological factors if we are to make any firmer assumption on the taxon's evolution. Consequently, whether these morphological differences are a consequence of founder-effect, environmental factors or a combination of both, remains to be investigated. This can be achieved by undertaking further morphometric analyses on individual body parts, along with observations and experiments on their general biology, physiology and habitat characteristics, to fully understand the factors in play and determine whether these differences are functionally significant. It would also be interesting to investigate the beetle's larval stages in detail to check for potential morphological and life history differences. A final detail that can be factored in future analyses, provided there is a sufficient sample size, is the effects of potential sexual dimorphism on body shape and size, as seen in other diving beetles (Hájek & Fikáček 2010), which we were not able to take into account in this study.

6.6 Acknowledgements

We would like to thank Dr. Elisabeth Biersma, Javier Rendoll and Javiera Malebrán for the help collecting the specimens; and, Dr. Henry Disney for providing the mounting fluid used in the preparation of the wing slides.

CHAPTER 7

Final Discussion

7. Discussion and concluding remarks

7.1 Summary

Drawing on field studies, lab experiments and genetic analyses, this thesis provides novel information about the ecology, ecophysiology and phylogeography of Antarctic and Sub-Antarctic insects. We present new data on the distribution of *Telmatogeton magellanicus*, showing that it is restricted to the mid and low tidal zones, where there is high coverage of filamentous algae (mainly *Bostrychia* sp.), which provides their larvae with cover from harsh environmental factors, such as low levels of humidity. In the lab, the same species are shown to be very sensitive to desiccation, but also have a robust tolerance to both low and high temperature and, especially, salinity variations. Additionally, larvae of another chironomid species, *Eretmoptera murphyi,* were found to be very resistant to brackish water, but not to heavy saline concentrations. In the meantime, we found a clear genetic split between South American *Parochlus steinenii* and populations of the species in South Georgia and Antarctica, indicating long isolation of different populations and significant genetic divergence. Finally, we also found no significant difference in wing size and loading in *Lancetes angusticollis*, although the populations have slight, thus not confirming the hypothesis of decreased flightlessness, even though we found significant differences in the size of specific body parts.

7.2 Discussion

7.2.1 Ecophysiology tracks with environmental distribution

The distribution of *T. magellanicus* matches closely with its physiological tolerance as measured under laboratory conditions. The lack of resistance to desiccation underlies the species' association with filamentous algae in the wild, which protect the insects from wind and have microhabitats within them that hold water, reducing evaporation and effectively buffering the insects from desiccation, which would be further exacerbated by the wind. It is possible that the patchy distribution of such microhabitat in other areas

hinders the species' ability to disperse on a relatively local scale, and hence expand to other environments, especially given the strong winds and seasonally dry conditions that are typical of this region.

7.2.2 Robust tolerance to variable conditions characteristic of Antarctic fauna

The physiological characteristics and tolerance to salinity and temperature variation seen in *T. magellanicus* are not unlike those recorded in the more studied Antarctic insects (i.e. *Belgica antarctica*, *Eretmoptera murphyi* and *Parochlus steinenii*), even though they do not occupy the same type of habitat. Evidently, *E. murphyi* is less tolerant to higher saline concentrations, consistent with it not being an intertidal animal, although it is strongly resistant to lower (brackish water) salinity conditions. We can thus conclude that *T. magellanicus* is another chironomid species pre-adapted to variable conditions, a characteristic commonly seen among Antarctic insects and micro-arthropods (Convey 1996, Peck et al. 2006).

Intolerance of environmental variability is consistent with the previously reported pre-adaptation of organisms in extreme conditions (Bergstrom et al. 2006, Worland 2010, Everatt et al. 2012), something which is yet to be fully understood. One possible explanation for pre-adaptations may relate to how long an organism has been present in a given region (McGaughran et al. 2010b, 2011). For instance, considering the midge *E. murphyi*, the species is thought to be paleoendemic to South Georgia, separating from its sister species *B. antarctica* (which is, as I previously stated, itself endemic to the Antarctic Peninsula and South Shetland Islands) ~45 mya (Allegrucci et al. 2006). Hence, over their evolutionary histories since divergence, both species have survived through multiple glacial cycles and, thus, periods of more extreme conditions than they currently experience. This evolutionary history may underlie the apparent pre-adaptation of *E. murphyi* in particular to the more extreme conditions experienced at its introduced location in the South Orkney Islands (Worland 2010, Everatt et al. 2012). Additionally, the current habitat of a species may have had the same conditions now present in a different geographical location, which could lead them to having kept the natural fit for both conditions. As noted above, many terrestrial invertebrates in polar regions, especially,

have a broader range of resilience than those found at lower latitudes. They experience different selective pressures, with biotic pressures such as competition and predation being more important at lower latitudes, while the primary selection pressures facing polar organisms are the abiotic features of their extreme environmental conditions (Convey 1996, Hogg et al. 2006).

7.2.3 Deep divergence between populations of *Parochlus steinenii*

Allegrucci et al. (2006) presented a preliminary analysis proposing divergence between southern South American populations of *P. steinenii* and those in South Georgia and Antarctica that dated to around 7.6 mya, and a much closer relationship between the latter two locations, though no specific geological time was provided. The data presented in this thesis, resulting from analyses of COX1 gene sequences, confirmed these deep divergences, although further work is required in order to provide an estimate of the divergence times. However, our 28S data do not completely conform with the data presented by Allegrucci et al. (2006), as the sequences obtained here do not show any of the variations previously reported, and hence are not useful in advancing the discussion of the timing of population divergence. This adds further support to Allegrucci et al.'s (2006) conclusion that the species' presence in the maritime Antarctic South Shetland Islands is not the result of a much more recent anthropogenic introduction associated with the sealing and whaling industries, which had been suggested as a possibility by Convey & Block (1996).

Even though Chapter 5 provides a robust groundwork for genetic analyses with *P. steinenii*, we have barely scratched the surface of the taxon's evolutionary history. However, in order to expand to further analysis, more resources will need to be obtained, which involves significantly higher costs, but then we would be able to make a clear assessment of potential glacial refugia and whether there are any traces of cryptic speciation (Ellis-Evans & Walton 1990, Carapelli et al. 2020, Convey et al. 2020). A good and very recent example of the potential detailed molecular studies have is the one by McGaughran et al. (2019) where they assessed population diversity and differentiation of the Antarctic springtail *Cryptopigus antarcticus* across the Antarctic Peninsula and some

Antarctic islands (spread across 1900 km). Through the use genome-wide single nucleotide polymorphisms (known as SNPs) data, they were able to infer that wind-carried colonisation events, coupled with glacial refugia areas were most likely what allowed the species to thrive for a long period; a deeper look at the genetics of *P. steinenii* may provide a similar answer as highlighted in Contador et al. (2020).

Beyond these more specific studies and analyses, there is an overarching question raised by Kelley et al. (2014) for *Belgica antarctica* regarding the impact of genome sizes on the evolution of insects in extreme regions, which was further acknowledged in Cornette et al. (2014) and Alfsnes et al. (2017). In this latter study, the authors highlight the broad range of fitness-related parameters which are affected by genome sizes in arthropods, such as growth and life history traits, though the latter is even more important among insects than crusteaceans (the other taxonomic group they used in their comparative study). The impacts of the reduction of the genome size in *B. antarctica* (this taxon has the smallest genome for an insect) can be seen in their suggested loss of sensory perception, which may be a reflection of the short-distance mating behaviour and the limited food availability in their environment (Kelley et al. 2014). Finding out if the pattern seen in this few studied organisms repeats in closely related species is one of the main questions for the future of genetics in the Antarctic.

7.2.4 Morphological similarities suggest recent divergence times or similar selective pressures

We found that South Georgian specimens of *Lancetes angusticollis* have slightly, but significantly, different body plans to those from Navarino Island in southern South America, and that their wings were slightly but consistently different in shape and wing loading. These relatively small differences do not permit the proposal of any specific selective factors, but suggest that both populations have been under relatively similar selective pressure or have not been isolated long enough for any major morphological change to take place. Such type of change is evidenced by other taxa such as the fast allochronic changes in invasive blowflies at the Kerguelen Islands (the invasion occurred

in the 1970s) (Laparie et al. 2016). The intriguing question of the timing of separation or isolation of the South Georgian population will require further genetic analyses to clarify.

7.2.5 Work in progress and additional publications

Both *Parochlus steinenii* and *Lancetes angusticollis*, though belonging to different insect orders, share a common distribution in southern South America and South Georgia. Although not included in this thesis, we have completed DNA extraction and collated sequence data that will permit a parallel analysis of COX1 in *Lancetes angusticollis* to that presented here for *Parochlus steinenii*, and will allow us to test the hypothesis that South Georgian populations of both species diverged from those in South America on a similar timescale. Similarly, we have also initiated a study on the phylogeny of *Telmatogeton magellanicus* (presented as unfinished work in Appendix II), in part in order to further confirm its true phylogenetic position. Furthermore, the very recent (February 2020) discovery and collection of *T. magellanicus* on the oceanic cool temperate Falkland Islands raises the exciting possibilty of initiating regional phylogeographic studies of this species, which may help in clarifying its history in southern South America.

7.3 Future directions

There are still many gaps in our knowledge of Antarctic and sub-Antarctic insects, but there is good potential to address at least some of these in the near future. In this section I explore some clear and exciting opportunities relating to species that have potential to link studies of evolutionary history in souther South America, the wider sub-Antarctic and the Scotia Arc, both focusing on and expanding those studied in this thesis.

As mentioned above, the taxonomic identity of *Telmatogeton magellanicus* is not fully resolved, and the same can also be said of the other species in the genus that occupy isolated island locations around the Southern Ocean. These include *T. amphibius* (Kerguelen/Crozet) and *T. macquariensis* (Macquarie), as well others, such as the winged *Telmatogeton* species recorded in the current study co-occurring in one location with *T. magellanicus*. The flightless sub-Antarctic species have been synonymised to their

current genus, but are endemic to very remote islands, suggesting a deep evolutionary divergence from their continental relatives. Specifically focusing on the identity of *T. magellanicus*, the first requirement is to compile genetic, morphological and ecological data on other species of *Telmatogeton*, in particular relating to shared traits, comparing their behaviour, physiological adaptations, morphology and genetic relatedness. Such a study would provide an important advance in understanding of the history and evolutionary relationships between the biota of the remote sub-Antarctica islands. An analogous question relates to the taxonomic status of *Eretmotpera murphyi.* Despite its original description in the genus *Eretmoptera*, both recent molecular studies and earlier morphological and ecological inferences strongly suggest that it should correctly be placed in *Belgica*, but to confirm the claims samples of *E. browni* (the type species of the genus) from California, USA, need to be obtained; however, as with *T. magellanicus*, no studies with *E. browni* appear to have been published since its original descripition. Acquiring sufficient genetic evidence from all these species will allow their relationship to be properly determined, facilitating later comparative studies.

There is much further opportunity to investigate the ecology and ecophysiology of insects in the Antarctic region. Work in this thesis has shown that combining ecological and ecospysiological studies can lead to insights into factors influencing species' distributions. Particularly for *T. magellanicus*, we have barely scratched the surface of understanding the species' physiological adaptations, and further experiments investigating their ability to tolerate (or avoid) freezing conditions, as well as the limits to which they can deal with changes in pH (especially in the context of the growing threat of global ocean acidification) would help explain more about how these remarkable species can live in such harsh environments. By compiling baseline data on the habitat and behaviour of the insects such as those examined here we will also be able to more easily predict the patterns and impacts of future colonisation/invasion events through techniques such as species/niche modelling (Chown et al. 2009, Pertierra et al. 2019, Bartlett et al. 2020, Contador et al. 2020).

This thesis intentionally set out to cover and integrate a broad range of subjects, rather than following a more traditional approach of focusing on the exploration of a single taxon (or group of taxa) or even a given methodology/technique. In part, this was to demonstrate the potential of combining studies and showing how much we can be learnt from approaching taxa or biogeographical regions from different angles. Historically, science has shown us that following a single thread may lead us to incomplete or unintentionally biased answers to our questions (Macleod et al. 2015, Rocca & Andersen 2017, Andersen et al. 2019). Above all, biology is characterised by a very complex network of interactions that are very hard to tease apart without taking into consideration particular areas of knowledge; by having a more thorough view of an area of the globe or a particular habitat we can have a more accurate understanding of how it has developed to its current situation and what may happen to it over different timescales into the future. (Contador et al. (2020) [shown here as Appendix V])

The main connecting factor between the geographic regions and taxa studied in this thesis is the geological continuity between the Andes of South America, the Scotia Arc, and the Antarctic Peninsula, an area also heavily influenced by contemporary climatic changes. As highlighted by Maldonado et al. (2015), the Drake Passage which it crosses is one of Earth's major ocean gateways and extremely important for understanding the behaviour of the Antarctic Circumpolar Current (ACC) (see also Maldonado et al. 2003, Livermore et al. 2004, Scher and Martin 2006, and other references therein). Thus, understanding multiple features of the biology of organisms across this region (and how specifically they are affected by its conditions) will also help to connect the region to the others similarly affected by the ACC, such as Australia/New Zealand, southern Africa, and the sub-Antarctic and Antarctic islands. Taxa like the copepod *Boeckella*, which is recorded all around the Antarctic Circle (Bayly 1992, Pugh et al. 2002, Maturana et al. 2019, 2020; and other references therein), will be key in filling the gaps of this field of research.

In conclusion, this theses highlights once again how remarkably resistant and resourceful these small Antarctic and sub-Antarctic insects are. The wide variety of biological tools

employed by these animals to deal with extreme conditions itself generates the possibility of using more integrative research approaches and methods. While there is justifiable concern how these exceptional insects will cope with the predicted climatic and other environmental changes they may face over next century or so, but judging from what we have learned so far, it is quite likely they will be very capable of rising to the challenge!

8. References

Alfsnes, K.; Leinaas, H. P. & Hessen D. O. (2017) Genome size in arthropods; different roles of phylogeny, habitat and life history in insects and crusteceans. **Ecology and Evolution 7**, 5939–5947.

Allegrucci, G.; Carchini, G.; Todisco, V.; Convey, P. & Sbordoni, V. (2006) A molecular phylogeny of antarctic chironomidae and its implications for biogeographical history. **Polar Biology 29**, 320–326.

Allegrucci G.; Carchini, G.; Convey, P. & Sbordoni, V. (2012) Evolutionary geographic relationships among orthocladine chironomid midges from maritime Antarctic and sub-Antarctic islands. **Biological Journal of the Linnean Society 106**(2), 258–274.

Anderson, M.; Gorley, R.N.; Clarke, K.R. (2008) PERMANOVA + for PRIMER user manual, Vol. 1.

Andersen, F.; Anjum, R. L. & Rocca, E. (2019) Philosophical bias is the one bias that science cannot avoid. **eLife 8**, e44929.

Angulo, A. (1990) Paraeuxoa Forbes, 1933, versus Caphornia Koehler, 1958 (Lepidoptera: Noctuidae): Sinonimia de dos géneros andino-patagónicos. **Revista Chilena de Entomología 18**, 13–17.

Arnold, R. J. & Convey, P. (1998). The life history of the diving beetle, Lancetes angusticollis (Curtis) (Coleoptera: Dytiscidae), on sub-Antarctic South Georgia. ***Polar Biology 20***, 153–160.

Avise, J. C. (2000) **Phylogeography: The history and formation of species**. Harvard University Press, Cambridge, USA. 447 pp.

Bale, J. S.; Block, W. & Worland, M. R. (2000) Thermal tolerance and acclimation response of larvae of the sub-antarctic beetle *Hydromedion sparsutum* (Coleoptera: Perimylopidae). **Polar Biology 23**, 77–84.

Bale, J. S.; Worland, M. R. & Block, W. (2001) Effects of summer frost exposures on the cold tolerance strategy of a sub-Antarctic beetle. **Journal of Insect Physiology 47**, 1161–1167.

Bartlett, J. C. (2019) PhD Thesis. University of Birmingham. pp.

Bartlett, J. C.; Convey, P. & Hayward, S. A. L. (2018a) Life cycle and phenology of an Antarctic invader: the flightless chironomid midge, *Eretmoptera murphyi*. **Polar Biology 42**(1), 115–130.

Bartlett, J. C.; Convey, P. & Hayward, S. A. L. (2018b) Not so free range? Oviposition microhabitat and egg clustering affects *Eretmoptera muprhyi* (Diptera: Chironomidae) reproductive success. **Polar Biology 42**, 271–284.

Bartlett, J. C.; Convey, P. & Hayward, S. A. L. (2020) Surviving the Antarctic winter–Life Stage Cold Tolerance and Ice Entrapment Survival in The Invasive Chironomid Midge *Eretmoptera muprhyi*. **Insects 11**(3): 147.

Bayly, I. A. E. (1992) Fusion of the genera *Boeckella* and *Pseudoboeckella* (Copepoda) and revision of their species from South America and sub-Antarctic islands. **Revista Chilena de Historia Natural 65**, 17–63.

Benedetti-Cecchi, L. & Cinelli, F. (1997) Spatial distribution of algae and invertebrates in the rocky intertidal zone of the Strait of Magellan: are patterns general? **Polar Biology 18**, 337–343.

Bergstrom, D. M.; Hodgson, D. A. & Convey, P. (2006) The Physical Setting of the Antarctic. *In*: Bergstrom, D. M.; Convey P.; Huiskes, A.H.L. **Trends in Antarctic Terrestrial and Limnetic Ecosystems**. Springer, Dordrecht, The Netherlands, 15–35.

Bermingham, E. & Moritz, C. (1998) Comparative phylogeography: concepts and applications. **Molecular Ecology 7**, 367–369.

Biersma, E. M., Jackson, J. A., Bracegirdle, T. J., Griffiths, H., Linse, K., & Convey, P. (2018). Low genetic variation between South American and Antarctic populations of the bank-forming moss Chorisodontium aciphyllum (Dicranaceae). **Polar Biology 41**(4), 599–610.

Birkenmajer, K.; Gaździcki, A.; Krajewski, K. P. ; Przybycin, A.; Solecki, A. & Tatur, A. (2005) First Cenozoic glaciers in West Antarctica. **Polish Polar Research 26**, 3–12.

Block, W.; Burn, A. J. & Richard K. J. (1984) An insect introduction to the maritime Antarctic. **Biological Journal of the Linnean Society 2**3, 33–39.

Bokhorst, S.; Huiskes, A.; Convey, P.; van Bodegom, P. M. & Aerts, R. (2008) Climate change effects on soil arthropod communities from the Falkland Islands and the Maritime Antarctic. **Soil Biology and Biochemistry 40**, 1547–1556.

Brancucci, M. & Ruhnau, S. (1985). Studies On The Genus Lancetes. 1. Additional Notes On *Lancetes angusticollis* (Curtis) And Description Of The Pupa (Coleoptera, Dytiscidae). **Proceedings Of The Academy Of Natural Sciences Of Philadelphia 137**, 46–52.

Brodin, Y., & Andersson, M. H. (2009) The marine splash midge Telmatogon japonicus (Diptera; Chironomidae) - extreme and alien? **Biological Invasions 11**, 1311–1317.

Brundin, L. (1962) Insects of Macquarie Island. Diptera: Chironomidae. **Pacific Insects 4**(4), 945–954.

Brundin, L. (1966) Transantarctic relationships and their significance, as evidenced by chironomid midges with a monograph of the subfamilies Podonominae and Aphroteniinae and the austral Heptagyiae. **Kunglica Svenska Vetenskapsakademiens Handlingar 11**, 1–472.

Burton-Johnson A.; Black, M.; Fretwell, P. T. & Kaluza-Gilbert, J. (2016) An automated methodology for differentiating rock from snow, clouds and sea in Antarctica from Landsat 8 imagery: a new rock outcrop map and area estimation for the entire Antarctic continent. **The Cryosphere 10**, 1665–1677.

Carapelli, A.; Greenslade, P.; Nardi, F.; Leo, C.; Convey, P.; Frati, F. & Fanciulli, P. P. (2020) Evidence for Cryptic Diversity in the “Pan-Antarctic” Springtail *Friesea antarctica* and the Description of Two New Species. **Insects 11**, 141.

Chevrier, M. (1996) **Introduction de deux espèces d’insectes aux Îles Kerguelen: processus de colonisation et exemples d’interactions**. University of Rennes, Dissertation 1.

Chevrier, M.; Vernon, P. & Frenot, Y. (1997) Potential effects of two alien insects on a sub-Antarctic wingless fly in the Kerguelen Islands. *In*: Battaglia, B.; Valencia, J. & Walton, D. W. H. (Eds.), **Antarctic Communities: Species, Structure and Survival.** Cambridge University Press, Cambridge, pp. 424–431.

Chong, C. W.; Pearce, D. A. & Convey, P. (2015) Emerging spatial patterns in Antarctic prokaryotes. **Frontiers in Microbiology 6**, 1058.

Chown, S. L. & Convey, P. (2006) Biogeography. *In*: Bergstrom, D. M.; Convey, P. & Huiskes, A. H. L. (Eds.) **Trends in Antarctic Terrestrial and Limnetic Ecosystems**. Springer. 369 pp.

Chown, S. L. & Convey, P. (2007) Spatial and temporal variability across life's hierarchies in the terrestrial Antarctic. **Philosophical Transactions of the Royal Society B: Biological Sciences 362**, 2307–2331.

Chown, S. L. & Convey, P. (2016) Antarctic Entomology. **Annual Review of Entomology 61**, 119–137.

Chown, S. L. (1997) Thermal sensitivity of oxygen uptake of Diptera from sub-Antarctic South Georgia and Marion Island. **Polar Biology 17**, 81–86.

Chown, S. L.; Gremmen, N. J. M. & Gaston, K. J. (1998) Ecological biogeography of southern ocean islands: Species–area relationships, human impacts, and conservation. **American Naturalist 152**, 562–575.

Chown, S. L.; Spear, D.; Lee, J. E. & Shaw, J. D. (2009) Animal Introductions to Southern Systems: Lessons for Ecology and for Policy. **African Zoology 44**(2): 248–262.

Clapperton, C. & Sugden, D. (1988) Holocene glacier fluctuations in South America and Antarctica. **Quaternary Science Reviews 7(1987)**, 185–198.

Clapperton, C. M.; Sugden, D. E.;, Birnie, J. & Wilson, M. J. (1989) Late-Glacial and Holocene Glacier Fluctuations and Environmental Change on South Georgia, Southern Ocean. **Quaternary Research 31**, 210–228.

Clarke, K.R.; Gorley, R.N. (2015) PRIMER v7: User Manual/Tutorial; First edition. Plymouth, Auckland, New Zealand.

Clement, M.; Posada, D. & Crandall, K. A. (2000) TCS: a computer program to estimate gene genealogies. **Molecular Ecology 9**, 1657–1659.

Collins, M.; Knutti, R.; Arblaster, J.; Dufresne, J. L.; Fichefet, T.; Friedlingstein, P.; Gao, X.; Gutowski, W. J.; Johns, T.; Krinner, G.; Shongwe, M.; Tebaldi, C.; Weaver, A. J. & Wehner, M. (2013) Long-term climate change: projections, commitments and irreversibility. *In*: Stocker, T. F.; Qin, D.; Plattner, G. K.; Tignor, M.; Allen, S. K.; Boschung, J.; Nauels, A.; Xia, Y.; Bex, V. & Midgley, P. (Eds.) **Climate change 2013: the physical science basis. Contribution of Working Group I to the Fifth**

Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, United Kingdom and New York, NY

Collins, G. E.; Hogg, I. D.; Convey, P.; Barnes, A. D. & McDonald, I. R. (2019) Spatial and Temporal Scales Matter When Assessing the Species and Genetic Diversity of Springtails (Collembola) in Antarctica. **Frontiers in Ecology and Evolution 7**, 76.

Contador, T.; Rosenfeld, S.; Ojeda, J. & Kennedy, J. (2015a) **Historia natural de los invertebrados acuáticos del Cabo de Hornos**. LOM Impresores, Chile. 201 pp.

Contador, T.; Kennedy, J. H.; Rozzi, R. & Villarroel, J. O. (2015b) Sharp altitudinal gradients in Magellanic Sub-Antarctic streams: patterns along a fluvial system in the Cape Horn Biosphere Reserve (55°S). **Polar Biology 38**, 1853–1866.

Contador, T.; Gañan, M.; Bizama, G.; Fuentes-Jaque, G.; Morales, L.; Rendoll, J.; Simões, F.; Kennedy, J.; Rozzi, R. & Convey, P. (2020) Assessing distribution shifts and ecophysiological characteristics of the only Antarctic winged midge under climate change scenarios. **Scientific Reports 10**, 9087.

Convey, P. (1992) Aspects of the biology of the midge, *Eretmoptera murphyi* Schaeffer (Diptera: Chironomidae), introduced to Signy Island, maritime Antarctic. **Polar Biology 12**, 653–657.

Convey, P. (1996a) The influence of environmental characteristics on the life history attributes of Antarctic terrestrial biota. **Biological Reviews 71**, 191–225.

Convey, P. (1996b) Overwintering strategies of terrestrial invertebrates in Antarctica - The significance of flexibility in extremely seasonal environments. **European Journal of Entomology 93**, 489–505.

Convey, P. (2007) Influences on and origins of terrestrial biodiversity of the sub-Antarctic islands. **Papers and Proceedings of the Royal Society of Tasmania** 141, 83–93.

Convey, P. (2010) Terrestrial biodiversity in Antarctica – Recent advances and future challenges. **Polar Science 4**(2), 135–147.

Convey, P. (2011) Antarctic terrestrial biodiversity in a changing world. **Polar Biology 34**, 1629–1641.

Convey, P. (2017) Antarctic Ecosystems. *In*: *Reference Module in Life Sciences* (Vol. 229, pp. 278–279). Elsevier.

Convey, P.; Biersma, E. M.; Casanova-Katny, A. & Maturana, C. S. (2020) Refuges of Antarctic Diversity. *In*: Oliva, M. & Ruiz-Fernández (Eds.) **Past Antarctica**. Academic Press, 181–200.

Convey, P. & Block, W. (1996) Antarctic Diptera: Ecology, physiology and distribution. **European Journal of Entomology 93**, 1–13.

Convey, P.; Bowman, V.; Chown, S. L.; Francis, J.; Fraser, C.; Smellie, J. L.; Storey, B. & Terauds, A. (2018) Ice-Bound Antarctica: Biotic Consequences of the Shift from a Temperate to a Polar Climate. *In*: Hoorn, C.; Perrigo, A. & Antonelli A (Eds.) **Mountains, Climate and Biodiversity** (pp. 355–373). John Wiley & Sons, Oxford, UK.

Convey, P.; Chown, S. L.; Clarke, A.; Barnes, D. K. A.; Bokhorst, S.; Cummings, V.; Ducklow, H. W.; Frati, F.; Allan Green, T. G.; Gordon, S.; Griffiths, H. J.; Howard-Williams, C.; Huiskes, A. H. L.; Laybourn-Parry, J.; Berry Lyons, W.; McMinn A.; Morley, S. A.; Peck, L. S.; Quesada, A.; Robinson, S. A.; Schiaparaelli, S. & Wall, D. H. (2014) The spatial structure of Antarctic biodiversity. **Ecological Monographs 84(2)**, 203–244.

Convey, P.; Gibson, J. A. E.; Hillenbrand, C. D.; Hodgson, D. A.; Pugh, P. J. A; Smellie, J. L. & Stevens, M. I. (2008) Antarctic terrestrial life - Challenging the history of the frozen continent? **Biological Reviews 83(2)**, 103–117.

Convey, P. & Stevens, M. I. (2007) Antarctic Biodiversity. **Science 317**, 1877–1878.

Convey, P.; Stevens, M. I.; Hodgson, D. A.; Smellie, J. L.; Hillenbrand, C.-D.; Barnes, D.; Clarke, A.; Pugh, P. J. A.; Linse, K. & Craig Cary, S. (2009) Exploring biological constraints on the glacial history of Antarctica. **Quaternary Science Reviews 28**, 3035–3048.

Cornette, R.; Gusev, O.; Nakahara, Y.; Shimura, S.; Kikawada, T. & Okuda, T. (2015) Chironomid Midges (Diptera, Chironomidae) Show Extremely Small Genome Sizes. **Zoological Science 32**(3), 248–254.

Cranston, P. S. (1985) *Eretmoptera murphyi* Schaeffer (Diptera: Chironomidae), an apparently parthenogenetic Antarctic midge. **British Antarctic Survey Bulletin** 66, 35–45.

Curtis, J. (1839) Descriptions, etc. of the insects collected by Captain P. P. King, R.N.F.R.S & L.S. in the survey of the Straits of Magellan. **Transactions of the Linnean Society of London 18**: 181-205.

Danks, H. V. (1990) **Arctic insects: instructive diversity**. In: Harrington, C. R. (ed) Canada's missing dimension: science and history in the Canadian Arctic Islands. Canadian Museum of Nature, pp 444–470.

Darriba, D.; Taboada, G. L.; Doallo, R. & Posada, D. (2012) jModelTest 2: more models, new heuristics and parallel computing. **Nature Methods 9**, 772.

Davey, M. C.; Pickup, J. & Block, W. (1992) Temperature variation and its biological significance in fellfield habitats on a maritime Antarctic island. **Antarctic Science 4**, 383–388.

De Wever, A.; Leliaert, F.; Verleyen, E.; Vanormelingen, P.; Van der Gucht, K.; Hodgson, D. A.; Sabbe, K. & Vyverman, W. (2009) Hidden levels of phylodiversity in Antarctic green algae: further evidence for the existence of glacial refugia. **Proceedings of the Royal Society B 276**, 3591–3599.

DGAC – Dirección General de Aeronáutica Civil, Dirección Meterológica de Chile (2020) Meteorological Station 550001. Available online: https://climatologia.meteochile.gob.cl/application/index/menuTematicoEmas (accessed on 31 January 2020)

Dickinson, M. H.; Lehmann, F. O. & Sane, S. P. (1999) Wing rotation and the aerodynamic basis of insect flight. **Science 284**, 1954–1960.

Dudley, R. (2002) The biomechanics of insect flight: form, function, evolution. Princeton, NJ: Princeton University Press. 496 pp.

Durante, S.P. & Abrahamovich, A. (2002) New leafcutting bee species of the subgenus *Megachile* (*Dasymegachile*) (Hymenoptera, Megachilidae) from Magellanic Forest province, in Patagonia Argentina. **Transactions of the American Entomological Society 128**, 361–366.

Eagles, G.; Livermore, R. A.; Fairhead, J. D. & Morris P. (2005) Tectonic evolution of the west Scotia Sea. **Journal of Geophysical Research 110**, B02401.

Eagles, G.; Livermore, R. A. & Morris P. (2006) Small basins in the Scotia Sea: The Eocene Drake Passage. **Earth and Planetary Science Letters 242**, 343–353.

Edwards, F. W. (1926) 38. On Marine Chironomidae (Diptera); with Descriptions of a New Genus and four New Species from Samoa. **Proceedings of the Zoological Society of London 96**(3), 779–806.

Edwards, F. W. (1928) A note on *Telmatogeton* Schin. and related genera (Diptera, Chironomidae). **Konowia 7**, 234–237.

Edwards, M. & Usher, M. B. (1985) The winged Antarctic midge *Parochlus steinenii* (Gerke) (Diptera : Chironomidae) in the South Shetland Islands. **Biological Journal of the Linnean Society 26**, 83–93.

Ellis-Evans, J. C. & Walton, D. (1990) The Process of Colonization in Antarctic Terrestrial and Freshwater Ecosystems. **Proceedings of the NIPR Symposium on Polar Biology 3**, 151–163.

Everatt, M. J.; Bale, J. S.; Convey, P.; Worland, M. R. & Hayward, S. A. L. (2013a) The effect of acclimation temperatures on thermal activity thresholds in polar terrestrial invertebrates. **Journal of Insect Physiology 59**, 1057–1064.

Everatt, M. J.; Convey, P.; Worland, M. R.; Bale, J. S. & Hayward, S. A. L. (2013b) Heat tolerance and physiological plasticity in the Antarctic collembolan, *Cryptopygus antarcticus*, and mite, *Alaskozetes antarcticus*. **Journal of Thermal Biology 38**, 264–271.

Everatt, M. J.; Worland, M. R.; Bale, J. S.; Convey, P. & Hayward, S. A. L. (2012) Pre-adapted to the maritime Antarctic? - Rapid cold hardening of the midge, *Eretmoptera murphyi*. **Journal of Insect Physiology 58(8)**, 1104–1111.

Everatt, M. J.; Convey, P.; Worland, M. R.; Bale, J. S. & Hayward, S. A. L. (2014a) Are the Antarctic dipteran, *Eretmoptera murphyi*, and Arctic collembolan, *Megaphorura arctica*, vulnerable to rising temperatures? **Bulletin of Entomological Research 104**, 494–503.

Everatt, M. J.; Convey, P.; Bale, J. S.; Worland, M. R. & Hayward, S. A. L. (2014b) Responses of invertebrates to temperature and water stress: A polar perspective. **Journal of Thermal Biology 54**, 118–132.

Fabri-Ruiz, S.; Danis, B; David, B. & Saucède, T. (2018) Can we generate robust species distribution models at the scale of the Southern Ocean? **Diversity and Distributions 25**, 21–37.

Felsenstein, J. (1981) A likelihood approach to character weighting and what it tells us about parsimony and compatibility. **Biological Journal of the Linnean Society 16**, 183–196.

Fernandez, R. A.; Anderson, J. B.; Wellner, J. S. & Hallet, B. (2011) Timescale dependence of glacial erosion rates: A case study of Marinelli Glacier, Cordillera Darwin, southern Patagonia. **Journal of Geophysical Research: Earth Surface 116** (March 2010), 1–18.

Folmer, O.; Black, M.; Hoeh, W.; Lutz, R. & Vrijenhoek, R. (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. **Molecular Marine Biology and Biotechnology 3**, 294–299.

Fraser, C. I.; Terauds, A.; Smellie, J.; Convey, P. & Chown, S. L. (2014) Geothermal activity helps life survive glacial cycles. *P***roceedings of the National Academy of Sciences 111**(15), 5634–5639.

Gañán Mora, M.; Contador, T. A. & Kennedy, J. H. (2015) La vida en los extremos: el uso de SIG para estudiar la distribución dela mosca antártica alada, *Parochlus* steinenii (Diptera: Chironomidae), en las Islas Shetland del Sur (Antártica marítima). *In*: de la Riva, J.; Ibarra, P.; Montorio, R. & Rodrigues M. (Eds.), *Análisis espacial y representación geográfica: innovación y aplicación* (pp. 1599–1608). Universidad de Zaragozza-AGE.

Gardiner, M. J.; Ellis-Evans, J. C.; Anderson, G. & Tranter, M. (1998) Snowmelt modelling on Signy Island South Orkney Islands. **Annual Review of Glaciology 26**, 161–166.

Gaston, K. J. & Chown, S. L. (1999) Geographic range size and speciation. *In:* Magurran, A. E. & May, R. M. (Eds.) **Evolution of biological diversity**. Oxford University Press, Oxford. pp. 236–259

Gerke, G. (1889) Vorlaufige Nachricht über die Fliegen Süd-Georgiens, nach der Ausbeute der Deutschen Station 1882-83. **Jahrbuch der Hamburgischen Wissenchaftlichen Anstaften 6**, 153–154.

Gibbs, A. G.; Chippindale, A. K. & Rose, M. R. (1997) Physiological mechanisms of evolved desiccation resistance in *Drosophila melanogaster*. **Journal of Experimental Biology 200**, 1821–1832 .

Gonzáles-Wevar, C. A.; Hüne, M.; Cañete, J. I.; Mansilla, A.; Nakano, T. & Poulin, E. (2012) Towards a model of postglacial biogeography in shallow marine species along the Patagonian Province: lessons from the limpet *Nacella magellanica* (Gmelin, 1791). **BMC Evolutionary Biology 12**, 139.

Gressitt, J. L. (1964) Insects of Campbell Island. **Pacific Insects Monograph 7**, 1–663.

Gressitt, J. L. (1970a) Subantarctic Entomology and Biogeography. **Pacific Insects Monograph 23**, 295–374.

Gressitt, J. L. (1970b) Coleoptera: Dytiscidae and Lathridiidae of South Georgia. **Pacific Insects Monograph 23**, 235–239.

Gressitt, J. L. (1971) Antarctic entomology with emphasis on biogeographical aspects. **Pacific Insects Monograph 25**, 167–178.

Gressitt , J. L. & Weber, N. A. (1959) Bibliographic introduction to Antarctic and Subantarctic Entomology. **Pacific Insects 1**(4), 441-480.

Griffiths, H. J.; Barnes, D. K. A. & Linse, K. (2009) Towards a generalized biogeography of the Southern Ocean benthos. **Journal of Biogeography 36**, 162–177.

Hahn, S. & Reinhardt, K. (2006) Habitat preference and reproductive traits in the Antarctic midge *Parochlus steinenii* (Diptera: Chironomidae). **Antarctic Science 18**(2), 175–181.

Hájek, J. & Fikáček, M. (2010) Taxonomic revision of the *Hydroporus bodemeyeri* species complex (Coleoptera: Dytiscidae) with a geometric morphometric analysis of body shape within the group. **Journal of Natural History 44**, 1631–1657.

Hambrey, M. J. & Barrett, P. J. (1993) Cenozoic sedimentary and climatic record, Ross Sea region, Antarctica. **Antarctic Research Series 60**, 91–124.

Harada, E.; Lee, R.; Denlinger, D. & Goto, S. (2014) Life history traits of adults and embryos of the Antarctic midge *Belgica antarctica*. **Polar Biology 37**, 1213–1217

Harrar, S. W.; Ronchi, F. & Salmaso, L. (2019) A comparison of recent nonparametric methods for testing effects in two-by-two factorial designs. **Journal of Applied Statistics 46**(9), 1649–1670.

Hayward, S. A. L.; Rinehart, J. P.; Sandro, L. H.; Lee, R. E. & Denlinger, D. L. (2007) Slow dehydration promotes desiccation and freeze tolerance in the Antarctic midge *Belgica antarctica*. **Journal of Experimental Biology 210**, 836–844.

Hayward, S. A. L.; Worland, M. R.; Convey, P. & Bale, J. S. (2004) Habitat moisture availability and the local distribution of the Antarctic Collembola *Cryptopygus antarcticus* and *Friesea grisea*. **Soil Biology and Biochemistry 36**, 927–934.

Hogg, I. D.; Cary, S. C.; Convey, P.; Newsham, K. K.; O'Donnell, A. G.; Adams, B. J.; Aislabie, J.; Frati, F.; Stevens, M. I. & Wall, D. H. (2006) Biotic interactions in Antarctic terrestrial ecosystems: are they a factor? **Soil Biology and Biochemistry 38**(10), 3035–3040.

Huang, D.; Cranston, P. S. & Cheng, L. (2014) A complete species phylogeny of the marine midge *Pontomyia* (Diptera: Chironomidae) reveals a cosmopolitan species and a new synonym. **Invertebrate Systematics 28**, 277–286.

Huey, R. B.; Gilchrist, G. W. & Hendry, A. P. (2005) Using invasive species to study evolution. *In*: Sax, D. F.; Gaines, S. D. & Stacowicz, J. J. (Eds.) **Species invasions: insights to ecology, evolution and biogeography**. Sunderland, MA: Sinauer Associates, 139–164.

Hughes, K. A. & Convey, P. (2010) The protection of Antarctic terrestrial ecosystems from inter- and intra-continental transfer of non-indigenous species by human activities: A review of current systems and practices. **Global Environmental Change 20**, 96–112.

Hughes, K. A. & Convey, P. (2012) Determining the native/non-native status of newly discovered terrestrial and freshwater species in Antarctica – Current knowledge, methodology and management action. ***J*ournal of Environmental Management 93**, 52–66.

Hughes, K. A., & Convey, P. (2014) Alien invasions in Antarctica is-anyone liable? **Polar Research 33**, 22103.

Hughes, K. A.; Walsh, S.; Convey, P.; Richards, S. & Bergstrom, D. M. (2005) Alien fly populations established at two Antarctic research stations. **Polar Biology 28**, 568–570.

Hughes, K. A. & Worland, M. R. (2010) Spatial distribution, habitat preference and colonization status of two alien terrestrial Invertebrate species in Antarctica. Antarctic Science 22, 221–231.

Hughes, K. A.; Worland, M. R.; Thorne, M. A. S. & Convey, P. (2013) The non-native chironomid *Eretmoptera murphyi* in Antarctica: erosion of the barriers to invasion. **Biological Invasions 15**, 269–281.

Iakovenko, N. S.; Smykla, J.; Convey, P.; Kašparová, E.; Kozeretska, I. A.; Trokhymets, V.; Dykyy, I.; Plewka, M.; Devetter, M.; Duriš, Z. & Janko, K. (2015) Antarctic bdelloid rotifers: diversity, endemism and evolution. **Hydrobiologia 761**, 5–43.

Jacobs, C. (1900) Diagnoses d'insectes recueillis par l'Expédition ant- arctique Belge. Diptera. **Annales de la Société Entomologique de Belgique 44**, 106–107.

Kao, C-C.; Chen, J.; Chen, G-F. & Soong, K. (2010) Variable swarming time of an intertidal midge (*Pontomyia oceana* Tokunaga, 1964) controlled by a circadian clock and temperature. **Marine and Freshwater Behaviour and Physiology 43(1)**, 1–9.

Kawarasaki, Y.; Teets, N. M.; Philip, B. N.; Potts, L. J.; Gantz, J. D.; Denlinger, D. L. & Lee Jr., R. E. (2019) Characterization of drought-induced rapid cold-hardening in the Antarctic midge, *Belgica antarctica*. **Polar Biology 42**, 1147–1156.

Kelley, J. L.; Peyton, J. T.; Fiston-Lavier, A-S.; Teets, N. M.; Yee, M-C.; Johnston, J. S.; Bustamante, C. D.; Lee, R. E. & Denlinger, D. L. (2014) Compact genome of the Antarctic midge is likely an adaptation to an extreme environment. **Nature Communications 5**, 4611.

Kim, S.; Kim, H. & Shin, S. C. (2016) Complete mitochondrial genome of the Antarctic midge *Parochlus steinenii* (Diptera: Chironomidae). **Mitochondrial DNA Parta A: DNA Mapping, Sequencing, and Analysis 27**(5), 3475–3476.

Kim, S.; Oh, M.; Jung, W.; Park, J.; Choi, H.-G. & Shin, S. C. (2017) Genome sequencing of the winged midge, *Parochlus steinenii*, from the Antarctic Peninsula. **GigaScience 6**(3), giw009.

Klingenberg, C. (2011) MorphoJ: An Integrated Software Package for Geometric Morphometrics. **Molecular Ecology Resources 11**(2), 353–357.

Laparie, M.; Lebouvier, M.; Lalouette, L. & Renault, D. (2010) Variation of morphometric traits in populations of an invasive carabid predator (*Merizodus soledadinus*) within a sub-Antarctic island. **Biological Invasions 12**(10), 3405–3417.

Laparie, M.; Vernon, P.; Cozic, Y.; Frenot, Y.; Renault, D. & Debat, V. (2016) Wing morphology of the active flyer *Calliphora vicina* (Diptera: Calliphoridae) during its

invasion of a sub-Antarctic archipelago where insect flightlessness is the rule. **Biological Journal of the Linnean Society 119**, 179–193.

Lawver, L. A. & Gahagan, L. M. (2003) Evolution of Cenozoic seaways in the circum-Antarctic region. **Palaeogeography, Palaeoclimatology, Palaeoecology 198**, 11–37.

Lebouvier, M.; Laparie, M.; Hullé, M.; Marais, A.; Cozic, Y.; Lalouette, L.; Vernon, P.; Candresse, T.; Frenot, Y. & Renault, D. (2011) The significance of the sub-Antarctic Kerguelen Islands for the assessment of the vulnerability of native communities to climate change, alien insect invasions and plant viruses. **Biological Invasions 13**, 1195–1208.

Lee, R. E. & Baust, J. G. (1982) Absence of metabolic cold adaptation and compensatory acclimation in the Antarctic fly, *Belgica antarctica*. **Journal of Insect Physiology 28**(9), 725–729.

Lee, R. E. & Denlinger, D. L. (2015) Stress tolerance in a polyextremophile: the southernmost insect. **Canadian Journal of Zoology 93**, 679–686.

Lee, C. K.; Barbier, B. A.; Bottos, E. M.; McDonald, I. R. & Cary, S. C. (2012) The inter-valley soil comparative Survey: the ecology of Dry Valley edaphic microbial communities. **ISME Journal 6**, 1046–1057.

Lee, R. E., Jr.; Elnitsky, M. A.; Rinehart, J. P; Hayward, S. A. L.; Sandro, L. H. & Denlinger, D. L. (2006) Rapid cold-hardening increases the freezing tolerance of the Antarctic midge *Belgica antarctica*. **The Journal of Experimental Biology 209**, 399–406.

Leigh, J. W. & Bryant, D. (2015) PopART: full-feature software for haplotype network construction. **Methods in Ecology and Evolution 6**, 1110–1116.

Levy, H.; Clucas, G. V.; Rogers, A. D.; Leaché, A. D.; Ciborowski, K. L.; Polito, M. J.; Lynch, H. J.; Dunn, M. J. & Hart, T. (2016) Population structure and phylogeography of the Gentoo Penguin (*Pygoscelis papua*) across the Scotia Arc. **Ecology and Evolution 6**, 1834–1853.

Librado, P. & Rozas, J. (2009). DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. **Bioinformatics 25**, 1451–1452.

Linse, K.; Griffiths, H. J.; Barnes, D. K. A. & Clarke, A. (2006) Biodiversity and biogeography of Antarctic and sub- Antarctic mollusca. **Deep Sea Research Part II: Topics in Oceanography 53**, 985–1008.

Linse, K.; Brandt, A.; Bohn, J. M.; Danis, B.; De Broyer, C.; Ebbe, B.; Heterier, V.; Janussen, D.; López-González, P. J.; Schüller, M.; Schwabe, E. & Thomson M. R. A. (2007) Macro- and megabenthic assemblages in the bathyal and abyssal Weddell Sea (Southern Ocean). **Deep Sea Research Part II 54**, 1848–1863.

Livermore, R.; Eagles, G.; Morris, P. & Maldonado A. (2004) Shackleton Fracture Zone: No barrier to early circumpolar ocean circulation. **Geology 32**, 797–800.

Livermore, R.; Hillenbrand, C.-D.; Meredith, M. & Eagles, G. (2007) Drake Passage and Cenozoic climate: An open and shut case? **Geochemistry Geophysics Geosystems 8**, 1–11.

Lopez-Martinez, G.; Benoit, J. B.; Rinehart, J. P.; Elnitsky, M. A.; Lee, R. E. & Denlinger, D. L. (2009) Dehydration, rehydration, and overhydration alter patterns of gene expression in the Antarctic midge, *Belgica antarctica*. **Journal of Comparative Physiology B 179**, 481–491.

Macleod, M. R.; McLean, A. L.; Kyriakopoulou, A.; Serghiou, S.; Wilde, A.; Sherratt, N.; Hirst, T.; Hemblade, R.; Bahor, Z.; Nunes-Fonseca, C.; Potluru, A.; Thomson, A.; Baginskitae, J.; Egan, K.; Vesterinen, H.; Currie, G. L.; Churilov, L.; Howells, D. W. & Sena, E. S. (2015) Risk of Bias in Repots of In Vivo Research: A Focus for Improvement. **PLoS Biology 13**(10), e1002273.

Maldonado, A.; Barnolas, A.; Bohoyo, F.; Galindo-Zaldivar, J.; Hernández-Molina, J.; Lobo, F.; Rodríguez-Fernández, J.; Somoza, L. & Vázquez, J. T. (2003) Contourite deposits in the central Scotia Sea: the importance of the Antarctic Circumpolar Current and the Weddell Gyre flows. **Palaeogeography, Palaeoclimatology, Palaeoecology 198**, 187–221.

Maldonado, A.; Dalziel, I. W. D. & Leat, P. T. (2014) The global relevance of the Scotia Arc: An introduction. **Global and Planetary Change 125**, A1–A8.

Maturana, C. S.; Rosenfeld, S.; Naretto, J.; Convey, P. & Poulin E. (2019) Distribution of the genus *Boeckella* (Crustacea, Copepoda, Calanoida, Centropagidae) at high

latitudes in South America and the main Antarctic biogeographic regions. **ZooKeys 854**, 1–15.

Maturana, C. S.; Segovia, N. I.; González-Wevar, C. A.; Díaz, A.; Rosenfeld, S.; Poulin, E.; Jackson, J. A. & Convey, P. (2020) Evidence of strong small-scale population structure in the Antarctic freshwater copepod *Boeckella poppei* in lakes on Signy Island, South Orkney Islands. **Limnology and Oceanography**, 1–17.

McGaughran, A.; Convey, P.; Stevens, M. I. & Chown, S. L. (2010a) Metabolic rate, genetic and microclimate variation among springtail populations from sub-Antarctic Marion Island. **Polar Biology 33**, 909–918.

McGaughran, A.; Torricelli, G.; Carapelli, A.; Frati, F.; Stevens, M. I.; Convey, P. & Hogg, I. D. (2010b) Contrasting phylogeographical patterns for springtails reflect different evolutionary histories between the Antarctic Peninsula and continental Antarctica. **Journal of Biogeography 37**, 103–119.

McGaughran, A.; Stevens, M. I.; Hogg, I. D. & Carapelli (2011) Extreme Glacial Legacies: A Synthesis of the Antarctic Springtail Phylogeographic Record. **Insects 2**, 62–82.

McGaughran, A.; Terauds, A.; Convey, P. & Fraser, C. I. (2019) Genome-wide SNP data reveal improved evidence for Antarctic glacial refugia and dispersal of terrestrial invertebrates. **Molecular Ecology 28**, 4941–4957.

Mercer, J. H. (1976) Glacial history of southernmost South America. **Quaternary Research 6(2)**, 125–166.

Michaud, M. R.; Benoit, J. B.; López-Martinez, G.; Elnitsky, M. A.; Lee, R. E. & Denlinger, D. L. (2008) Metabolomics reveals unique and shared metabolic changes in response to heat shock, freezing and desiccation in the Antarctic midge, *Belgica antarctica*. **Journal of Insect Physiology 54**, 645–655.

Monserrat, V. J. (1997) Revisión del genero *Megalomus* de Latinoamerica (Neuroptera, Hemerobiidae). **Fragmenta Entomologica 29**, 123–206.

Moritz, C.; Langham, G.; Kearney, M.; Krockenberger, A.; VanDerWal, J. & Williams S. (2012) Integrating phylogeography and physiology reveals divergence of thermal traits between central and peripheral lineages of tropical rainforest lizards. **Phylosophical Transactions of the Royal Society B 367**, 1680-1687.

Morrone, J. J. (1992) Revisión sistemática, análisis cladístico y biogeografía histórica de los géneros *Falklandius* Enderlein y *Lanteriella* gen. nov. (Coleoptera: Curculionidae). **Acta Entomológica Chilena 17**, 157–174.

Morrone, J.J. (1993) Cladistic and biogeographic analyses of the weevil genus *Listroderes* Schoenherr (Coleoptera: Curculionidae). **Cladistics 9**(4), 397–411.

Morrone, J. J. (2015) Biogeographical regionalisation of the Andean region. **Zootaxa 3936**(2), 207–236.

Morrone, J. J. & Anderson, R. S. (1995) The *Falklandius* generic group: Cladistic analysis with description of new taxa (Coleoptera: Curculionidae: Rhytirrhinini). **American Museum Novitates 3121**, 1–14.

Morrone, J. J. & Roig-Juñent, S. (1995) **The diversity of Patagonian weevils: An illustrated checklist of the Patagonian Curculionoidea (Insecta: Coleoptera)**. L.O.L.A., Buenos Aires, 189 pp.

Müller, C. (1884) Käfer von Süd-Georgien. **Deutsche entomologische Zeitschrift 28**(2), 417–420.

Neumann, D. (1986) Diel eclosion rhythm of a sublittoral population of the marine insect *Pontomyia pacifica.* **Marine Biology 90**, 461–465.

Neumann, D. (1988) Temperature compensation of circa-semilunar timing in the intertidal insect *Clunio*. **Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioral Physiology 163**, 671–676.

Nicolai, V. & Droste, M. (1984) The ecology of *Lancetes claussi* (Müller) (Coleoptera, Dytiscidae), the Subantarctic water beetle of South Georgia. **Polar Biology 3**, 39–44.

Nondula, N.; Marshall, D. J.; Baxter, R.; Sinclair, B. J. & Chown, S. L. (2004) Life history and osmoregulatory ability of *Telmatogeton amphibius* (Diptera, Chironomidae) at Marion Island. **Polar Biology 27**, 629–635.

Ouisse, T.; Laparie, M.; Lebouvier, M. & Renault, D. (2017) New insights into the ecology of *Merizodus soledadinus*, a predatory carabid beetle invading the sub-Antarctic Kerguelen Islands. **Polar Biology 40**(11), 2201–2209.

Papadopoulou, A.; Jones, A. G.; Hammond, P. M. & Vogler, A. P. (2009) DNA taxonomy and phylogeography of beetles of the Falkland Islands (Islas Malvinas). **Molecular Phylogenetics and Evolution 53**, 935–947.

Pearson, R.G.; Connolly, N.; Benson, L.J.; Cairns, A.; Clayton, P.; Crossland, M.; Hortle, K.G.; Leonard, K.; Nolen, J. (2019) Invertebrate responses to land use in tropical streams: Discrimination of impacts enhanced by analysis of discrete areas. **Marine and Freshwater Research 70**, 563–575.

Peck, L. S.; Convey, P. & Barnes, D. K. A. (2006) Environmental constraints on life histories in Antarctic ecosystems: tempos, timings and predictability. **Biological Reviews 81**, 75–109.

Peckham, V. (1971) Notes on the chironomid midge *Belgica antarctica* Jacobs at Anvers Island in the maritime Antarctic. **Pacific Insect Monographies 25**, 145–166.

Pertierra, L.R.; Bartlett, J.C.; Duffy, G.A.; Vega, G.C.; Hughes, K.A.; Hayward, S.A.L.; Convey, P.; Olalla-Tarraga, M.A. and Aragon P. (2019) Combining correlative and mechanistic niche models with human activity data to elucidate the invasive potential of a sub-Antarctic insect. **Journal of Biogeography 47**(3), 658–673.

Pful, H. A. & McCave, N. I. (2005) Evidence for late Oligocene establishment of the Antarctic Circumpolar Current. **Earth and Planetary Science Letter 235**, 715–728.

Pisa, S.; Biersma, E. M.; Convey, P.; Patiño, J.; Vanderpoorten, A.; Werner, O. & Ros, R. M. (2014) The cosmopolitan moss *Bryum argenteum* in Antarctica: recent colonisation or in situ survival? **Polar Biology 37**, 1469–1477.

Pisano E. (1977) Fitogeografía de Fuego-Patagonia Chilena I. Comunidades vegetales entre las latitudes 52 y 56S. **Anales del Instituto de la Patagonia 8**, 121–250.

Pugh, P. J. A.; Dartnall, H. J. G.; McInnes, S. J. (2002) The non-marine Crustacea of Antarctica and the Islands of the Southern Ocean: biodiversity and biogeography. **Journal of Natural History 36**, 1047–1103.

Rambaut, A.; Suchard, M. A.; Xie, D. & Drummond, A. J. (2014) **Tracer v1.6.0** http://beast.bio.ed.ac.uk/Tracer

Rauschert, M. (1985) Beobachtungen an der Chironomide *Parochlus steineni* auf der Insel King George (Südshetlandinseln, Antarktis). **Deutsche Entomologische Zeitschrift 32**(1–3), 183–188.

Rendoll Cárcamo, J.; Contador, T.; Gañan, M.; Pérez Troncoso, C.; Maldonado Márquez, A.; Convey, P.; Kennedy, J. & Rozzi, R. (2019) Altitudinal gradients in Magellanic sub-Antarctic lagoons: the effect of elevation on freshwater macroinvertebrate diversity and distribution. **PeerJ 7**, e7128.

Richard, K. J.; Convey, P. & Block, W. (1994) The Terrestrial Arthropod Fauna of the Byers Peninsula, Livingston Island, South-Shetland-Islands. **Polar Biology 14**, 371–379.

Řiha P. (1961) Die Gattung *Lancetes* (Col., Dytiscidae). **Acta Entomologica Musei Nationalis Pragae 34**, 121–154.

Rocca, E. & Andersen, F. (2017) How biological background assumptions influence scientific risk evaluation of stacked genetically modified plants: an analysis of research hypotheses and argumentations. **Life Sciences, Society and Policy 13**, 11.

Rodbell, D. T.; Smith, J. A. & Mark, B. G. (2009) Glaciation in the Andes during the Late glacial and Holocene. **Quaternary Science Reviews 28(21-22)**, 2165–2212.

Roig-Juñent, S. (1994) Historia biogeográfica de América del Sur austral. **Multequina (Mendoza) 3**, 167–203.

Roig-Juñent, S. (1995) Revisión sistemática de los Creobina de América del Sur (Coleoptera: Carabidae: Broscini). **Acta Entomológica Chilena 19**, 51–74.

Roig-Juñent, S. (2004) Los Migadopini (Coleoptera: Carabidae) de América del Sur: Descripción de las estructuras genitales Masculinas y femeninas y consideraciones filogenéticas y biogeográficas. **Acta Entomológica Chilena 28**, 7–29.

Ronquist, F.; Teslenko, M.; van der Mark, P.; Ayres, D. L.; Darling, A.; Höhna, S.; Larget, B.; Liu, L.; Suchard, M. A. & Huelsenbeck, J. P. (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. **Systematic Biology 61**, 539–542.

Rozzi, R.; Armesto, J. J.; Gutiérrez, J.; Massardo, F.; Likens, G.; Anderson, C. B.; Poole, A.; Moses, K.; Hargrove, G.; Mansilla, A.; Kennedy, J. H.; Wilsson, M.; Jax, K.; Jones, C.; Callicott, J. B. & Kalin, M. T. (2012) Integrating ecology and environmental ethics: earth stewardship in the southern end of the Americas. **BioScience 62**(3), 226–236.

Rübsaamen, E. H. (1906) Diptera, Chironomidae. *In*: Severin, Expédition Antarctique Belge. **Rapp. Sci. Zool. Ins 8**, 75–85.

Sands, C.; O'Hara, T. D.; Barnes, D. K. A. & Martín-Ledo, R. (2015) Against the flow: evidence of multiple recent invasions of warmer continental shelf waters by a Southern Ocean brittle star. **Frontiers in Ecology and Evolution 3**, 63.

Sax, D.; Stachowicz, J.; Brown, J.; Bruno, J.; Dawson, M.; Gaines, S.; Grosberg, R.; Hastings, A.; Holt, R.; Mayfield, M.; O'Connor, M. & Rice, W. (2007) Ecological and evolution- ary insights from species invasions. **Trends in Ecology & Evolution 22**: 465–471.

Schaeffer, C. (1914) Collembola, Siphonaptera, Diptera and Coleoptera of the South Georgia Expedition. **The Bulletin of the Brooklyn Institute of Arts and Sciences 2**, 90–94.

Scher, H. D. & Martin, E. E. (2006) Timing and climatic consequences of the opening of Drake Passage. **Science 312**, 428–430.

Schermann-Legionnet, A.; Hennion, F.; Vernon, P. & Atlan, A. (2007) Breeding system of the subantarctic plant species Pringlea antiscorbutica R. Br. and search for potential insect pollinators in the Kerguelen Islands. **Polar Biology 30**, 1183–1193.

Selkirk, P. M. (2007) The nature and importance of the Sub-Antarctic. **Papers and Proceedings of the Royal Society of Tasmania 141**(1), 1–6.

Sérsic, A. N.; Cosacov, A.; Cocucci, A. A.; Johnson, L. A.; Pozner, R.; Avila, L. J.; Sites, J. W. Jr & Morando, M. (2011) Emerging phylogeographical patterns of plants and terrestrial vertebrates from Patagonia. **Biological Journal of the Linnean Society 103**, 475–494.

Shimada, K.; Ohyama, Y. & Pan, C. X. (1991) Cold-hardiness of the Antarctic winged midge *Parochlus steinenii* during the active season at King George Island. **Polar Biology 11**, 311–314.

Shin, S. C.; Kim, H.; Lee, J. H.; Kim, H.-W.; Park, J.; Choi, B.-S.; Lee, S.-C.; Kim, J. H.; Lee, H. & Kim, S. (2019) Nanopore sequencing reads improve assembly and gene annotation of the *Parochlus steinenii* genome. **Scientific Reports 9**, 5095.

SHOA – Servicio Hidrográfico y Oceanográfico de la Armada (2020) Available online: http://www.shoa.cl/php/inicio (accessed on 31 January 2020)

Smith, R. I. L. (1990) Signy Island as a paradigm of biological and environmental change in Antarctic terrestrial ecosystems. *In*: Kerry, K. R. & Hempel, G., (Eds.) **Antarctic ecosystems, ecological change and conservation**. Springer, Berlin, Germany. pp. 32–50.

Soong, K. & Leu, Y. (2005) Adaptive mechanism of the bimodal emergence dates in the intertidal midge *Pontomyia oceana*. **Marine Ecology Progress Series 286**, 107–114.

Soong, K.; Chen, J. & Tsao, C-H. (2006) Adaptation for accuracy or for precision? Diel emergence timing of the intertidal insect *Pontomyia oceana* (Chironomidae). **Marine Biology 150**, 173–181.

Soong, K.; Lee, Y-J. & Chang I-H. (2011) Short-lived intertidal midge Pontomyia oceana have semilunar eclosion rhythm entrained by night light. **Marine Ecology Progress Series 433**, 121–130.

Stamatakis, A. (2014) RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. **Bioinformatics 30**, 1312–1313.

Stevens, M. I.; Greenslade, P.; Hogg, I. D. & Sunnucks, P. (2006) Southern Hemisphere Springtails: Could Any Have Survived Glaciation of Antarctica? **Molecular Biology and Evolution 23**(5), 874–882.

Sublette, J. E. & Wirth, W. W. (1980) The Chironomidae and Ceratopogonidae (Diptera) of New Zealand's subantarctic islands. **New Zealand Journal of Zoology 7(3)**, 299–378.

Sugg, P.; Edwards, J. S. & Baust, J. (1983) Phenology and life history of *Belgica antarctica*, an Antarctic midge (Diptera: Chironomidae). **Ecological Entomology 8**, 105–113.

Taylor, L. R. (1963) Analysis of the Effect of Temperature on Insects in Flight. **Journal of Animal Ecology 32**(1), 99–117.

Taylor, E. B.; Darveau, C.-A. & Schulte, P. M. (2013) Setting Conservation Priorities in a Widespread Species: Phylogeographic and Physiological Variation in the Lake Club, *Couesius plumbeus* (Pisces: Cyprinidae). **Diversity 5**, 149–165.

Teets, N. M.; Kawarasaki, Y.; Lee, R. E. & Denlinger, D. L. (2011) Survival and energetic costs of repeated cold exposure in the Antarctic midge, *Belgica antarctica*: a

comparison between frozen and supercooled larvae. **The Journal of Experimental Biology 214**, 806–814.

Tokunaga, M. (1935) Chironomidae from Japan (Diptera), IV. The early stages of a marine midge, *Telmatogeton japonicus* Tokunaga. **Philippine Journal of Science 57**, 491–511.

Torsvik, T. H.; Gaina, C. & Redfield, T. F. (2008) Antarctica and global paleogeography: From Rodinia, through Gondwanaland and Pangea, to the birth of the Southern Ocean and the opening of gateways. Em: Cooper, A. K.; Barret, P. J.; Stagg, H.; Storey, B.; Stump, E.; Wise, W. & the 10th ISAES editorial team (eds.) **Antarctica: A keystone in a changing world**. Proceedings of the 10th International Symposium on Antarctic Earth Sciences. Washington, DC: The National Academic Press, 125–140.

Turner, J.; Barrand, N. E.; Bracegirdle, T. J.; Convey, P.; Hodgson, D. A.; Jarvis, M.; Jenkins, A.; Marshall, G.; Meredith, M. P.; Roscoe, H.; Shanklin, J.; French, J.; Goosse, H.; Guglielmin, M; Gutt, J.; Jacobs, S.; Kennicutt, M. C.; Masson-Delmotte, V.; Mayewski, P.; Navarro, F.; Robinson, S.; Scambos, T.; Sparrow, M.; Summerhayes, C.; Speer, K. & Klepikov, A. (2014) Antarctic climate change and the environment: An update. **Polar Record 50**(3), 237–259.

Vega, G. C.; Convey, P.; FRES; Hughes, K. A. & Olalla-Tárraga, M. A. (2019) Humans and wind, shaping Antarctic soil arthropod biodiversity. **Insect Conservation and Diversity**, early access.

Vernon, P. (1981) Peuplement diptérologique des substrats enrichis en milieu insulaire subantarctique (Îles Crozet) — Étude des Sphaeroceridae du genre Anatalanta. PhD Thesis, University of Rennes 1, Rennes, France.

Vernon P (1986) Evolution des réserves lipidiques en fonction de l'état physiologique des adultes dans une population expérimentale d'un Diptère subantarctique: *Anatalanta aptera* Eaton. **Bulletin de la Société d'Ecophysiologie 11**, 95–116.

Vyverman, W.; Verleyen, E.; Wilmotte, A.; Hodgson, D. A.; Willems, A.; Peeters, K.; Van de Vijver, B.; De Wever, A.; Leliaert, F. & Sabbe, K. (2010) Evidence for widespread endemism among Antarctic microorganisms. **Polar Science 4**, 103–113.

Wagner, D. L. & Liebherr, J. K. (1992) Flightlessness in insects. **Trends in Ecology & Evolution 7**, 216–220.

Walton, D. (1982) The Signy Island terrestrial reference sites. XV Micro-climate monitoring 1972–74. **British Antarctic Survey Bulletin 55**, 111–126.

Whitehead, J. M.; Quilty, P. G.; McKelvey, B. C. & O'Brien, P. E. (2006) A review of the Cenozoic stratigraphy and glacial history of the Lambert Graben – Prydz Bay region, East Antarctica. **Antarctic Science 18**, 83–99.

Wirth, W. W. (1947) A review of the genus *Telmatogeton* Schiner, with descriptions of three new Hawaiian species (Diptera: Tendipedidae). **Proceedings of the Hawaiian Entomological Society 13**, 143–191.

Wirth, W. W. (1949) A revision of the Clunionine midges with descriptions of a new genus and four new species (Diptera: Tendipedidae). **University of California Publications in Entomology 8**, 151–182.

Wirth, W. W. & Gressitt, J. L. (1967) Diptera: Chironomidae (Midges). **Antarctic Research Series 10**, 197–203.

Wobbrock, J. O., Findlater, L., Gergle, D., and Higgins, J. J. (2011). The Aligned Rank Transform for nonparametric factorial analyses using only ANOVA procedures. *Proceedings of the ACM Conference on Human Factors in Computing Systems (CHI '11)*. Vancouver, British Columbia (May 7-12, 2011). New York: ACM Press, pp. 143-146.

Worland, M. R. (2010) *Eretmoptera murphyi*: pre-adapted to survive a colder climate. **Physiological Entomology 35**, 140–147.

Worland, M. R. & Block, W. (2003) Desiccation stress at sub-zero temperatures in polar terrestrial arthropods. **Journal of Insect Physiology 49**, 193–203.

Worland, M. R. & Convey, P. (2001) Rapid cold hardening in Antarctic microarthropods. **Functional Ecology 15**, 515–524.

Yeap, H. L.; Endersby, N. M.; Johnson, P. H.; Ritchie, S. A. & Hoffmann, A. A. (2013) Body size and wing shape measurements as quality indicators of Aedes aegypti mosquitoes destined for field release. **American Journal of Tropical Medicine and Hygiene 89**(1), 78–92.

9. Appendices

9.1 Appendix I

9.1.1 PCR protocol

PCR amplification were performed using the Taq PCR Core Kit (Qiagen GmbH, Hilden, Germany) with addition of $MgCl_2$ provided with the kit. In most reactions bovine serum albumin (BSA) was also added (Cat #AM2616 50mg 50mg/ml Lot #0911017). An overview of the quantities and concentrations used in the PCR reactions is given in Table 9.1.1.1. All reactions were prepared on a cool-block and quickly spun down using a centrifuge before the reaction.

Table 9.1.1.1. Volumes and concentrations of components used per PCR reaction

Component	Quantity (µL)
Q solution	4
dNTP (10 mM)	1.2
Forward primer (10 mM)	1
Reverse primer (10 mM)	1
10x PCR Buffer	2
Taq DNA Polymerase (5U/µL)	0.125
ddH20 (nuclease-free water)	5.775
MgCl2 (25 mM)	2.4
BSA (10 mM)	1
Template DNA (concentration variable)	1.5
Total	20

Primers were first tested using a gradient PCR, after which an optimal annealing temperature was chosen for each gene. All PCR cycles used variations of the Thermal Cycler conditions listed in Table 9.1.1.2 PCR products were inspected using gel electrophoresis (see next section). Forward and reverse sequencing was performed by

LGC Genomics (Berlin, Germany) and Macrogen (Seoul, South Korea), using the same primers as used for PCR.

Table 9.1.1.2. Volumes and concentrations of components used per PCR reaction

Process	Time	Temperature (º C)
Initial denaturation	3 min	95
3-step cycle (35 cycles)		
Denaturation	30 sec	95
Annealing	40 sec	60
Extension	3 min	72
Final elongation	10 min	72

9.1.2 Gel electrophoresis

Agarose gels (1.5%) were made by adding 0.75 g agarose (Bioline, London, UK) to 47.5 ml of 1x TBE buffer (Tris Borate EDTA (TBE) or Sodium Borate buffer (SB) — SB was favoured in latter gels because is significantly cheaper and provides better results — made up of 20x solution, in an Erlenmeyer (eventual smaller and bigger gels with the same ratio). The mixture was heated in a microwave and then whirled until completely mixed and dissolved. It was then cooled down slightly and ~5 ml of GelRed™ (Biotium, Inc., Fremont, USA) was added to the mixture, which was again mixed well by whirling. The mixture was then poored into a gel holder and cooled down for ~45 minutes until it was polymerized to a matrix. The gel was put in a gel tank, covered by 1x TBE buffer or 1x SB buffer, after which the PCR products could be added. Between 1.5–4 µl of each DNA sample was loaded with a total concentration of ca 40-100 ng. The sample was mixed with a small drop (1 µl) of loading dye (CoralLoad PCR Buffer; part of the Taq PCR Core Kit; Qiagen GmbH, Hilden, Germany) before being loaded into the gel. Before running the gel two ladders were added, typically HyperLadder™ I and II (Bioline, London, UK). The common running settings were 90V for 40–45 min with TBE and 200V for 25–30 min with SB.

9.2 Appendix II

The following is a work-in-progress manuscript dealing with the taxonomic identity of *Telmatogeton magellanicus*. With this study, we aim to define whether *T. magellanicus* belongs in *Telmatogeton*, *Belgica* (it's original genus) or *Halirytus*.

9.2.1 *Telmatogeton magellanicus* phylogeny

9.2.2.1 Materials & Methods

Sampling

Adult specimens of *Telmatogeton magellanicus* were sampled by Prof. Peter Convey with an entomological aspirator, in the Austral summer of 2016–2017 at the Róbalo (-54.93337º, -67.65699º) and Honda (-54.92262º, -68.2382945º) Bays, Navarino Island, Chile; subsequently they were stored in ethanol 96% and kept at 4ºC. Additionally, three specimens of *Telmatogeton macquariensis* were obtained from Macquarie Island (Garden Cove; -54.49863, 158.94092), collected by Melissa Houghton on 11.II.2018.

Extraction and Sequencing

DNA was extracted using the QIAGEN DNEasy Blood & Tissue and QiAMP Extraction Kits. Adults were fully submerged into the proteinase K+ATL buffer solution for 4h under 56º C or overnight at 40º C; we didn't crush them so that the insects were kept as whole as possible (there was some loss in pigmentation on the abdomen though); the remaining steps were followed as per the manufacturer's instructions.

Amplification for the COX1 and 28S genes was done using the Qiagen PCR Core Kit with added Ultrapure Bovine Serum Albumine (BSA) [Cat #AM2616 50mg 50mg/ml Lot #0911017]. Remaining outgroup sequences were retrieved from GenBank (Table 9.2.2.1.1).

Table 9.2.2.1.1. Primers used. Novel primers designed with Geneious (28S)

Gene	Primer Name	Sequence (5'-3')	Reference
COX1	**LCO1490**	GGTCAACAAATCATAAAGATATTGG	Folmer et al. 1994
	HCO2198	TAAACTTCAGGGTGACCAAAAAATCA	Folmer et al. 1994
	UEA5	AGTTTTAGCAGGAGCAATTACTAT	Lunt et al. 1996
	UEA10	TCCAATGCACTAATCTGCCATATTA	Lunt et al. 1996
28S	**rD1.2a**	CCCSSGTAATTTAAGCATATTA	Whiting 2002
	MK_3F	TTTTGGTAAGCAGAACTGGYG	Machida & Knowlton 2012
	28S_1609F	ACCATGAAAGGTGTTGATTGCTG	NOVEL
	28S_1955R	ACCATGAAAGGTGTTGATTGCTG	NOVEL
	rD7b1	GACTTCCCTTACCTACAT	Whiting 2002

All sequences were manually examined, with forward and reverse sequences assembled, trimmed and aligned with Geneious 9.1.8 (Biomatters, LTD, Auckland, NZ). The alignment of COX1 sequences was carried out with the MUSCLE process (Edgar, 2004), while the 28S sequences required the use of the MAFFT process (Katoh et al. 2002). Short, partially incomplete sections at the ends of each alignment were excluded.

Extraction and Sequencing

DNA was extracted using the QIAGEN DNEasy Blood & Tissue and QiAMP Extraction Kits. Adults were fully submerged into the proteinase K+ATL buffer solution for 4h under 56º C or overnight at 40º C; adults were not crushed in order to keep them as whole as possible (however, there was some loss in pigmentation on the abdomen), while the larvae where partially or fully crushed before extraction; the remaining steps were followed as per the manufacturer's instructions.

Amplifications for the COX1 and 28S genes were carried out with the Qiagen PCR Core Kit with added Ultrapure Bovine Serum Albumine (BSA) [Cat #AM2616 50mg 50mg/ml Lot #0911017] using a combination of primers (Table 2). Finally, products were sent to LGC Genomics (Germany) and Macrogen (South Korea) for Sanger sequencing. Outgroup sequences were retrieved from GenBank (Table 3). We selected *Belgica antarctica* as the most reasonable outgroup, followed by *Podonomus pepinelli* and *Podonums rivolorum*, and stabilised the more basal nodes with more closely related species (*Parochlus* spp.) (see Felsenstein, 1981).

Data preparation and genetic analyses

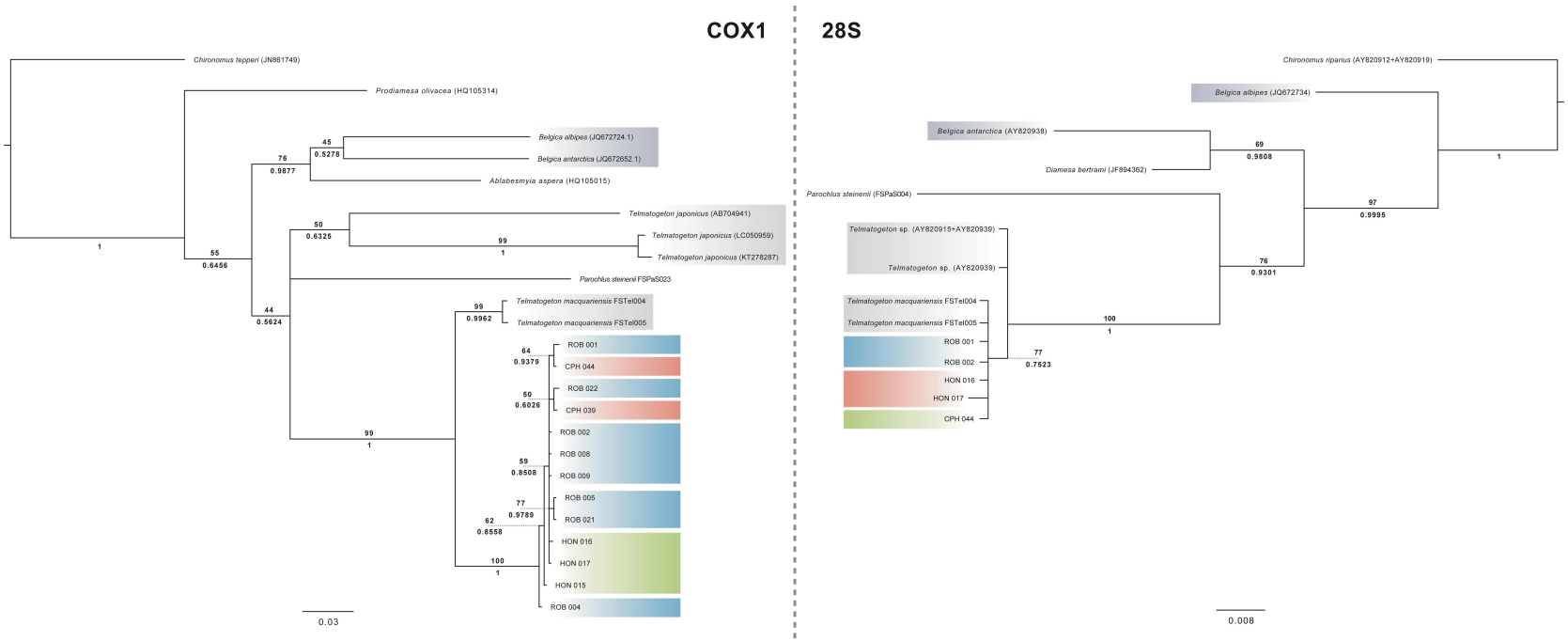
All sequences were manually examined, with forward and reverse sequences assembled, trimmed and aligned with Geneious 9.1.8 (Biomatters, LTD, Auckland, NZ). The alignment of COX1 sequences was carried out with the MUSCLE process (Edgar, 2004). Short, partially incomplete sections at the ends of each alignment were excluded.

To deal with the acquired data, we first had to test for the genetic structure in our samples, through the use of phylogenetic methods and associated coalescent techniques. Thus, descriptive statistics (Table X) where produced with DnaSP v5.0 (Librado and Rozas, 2009); significance was then assessed from 1000 coalescent simulations. Additionally, we used TRACER v1.6.0 (Rambaut et al. 2014) to check for effective sample sizes of parameters.

Because not all available outgroup sequences encompassing the entire length of the COX1 gene, we opted to have two different alignments with two non-overlapping partitions. The optimal model of nucleotide substitution for COX1 was determined with jModelTest 2 (Darriba & Posada, 2016). Selection was based on the Akaike Information Criterion (AIC) and resulted in selection of the GTR+G model for the first partition and TIM2+I+G for the second partition. Both models have the same substitution parameters (nst = 6). Phylogenetic analysis was performed using MrBayes 3.2 (Ronquist et al. 2012), with 20 million generations, and boostrap values were acquired through a a Maximum Likelihood reconstruction with RAxML v8.0.0 (Stamatakis 2014).

9.2.2.2 Results

The analyses (Fig. 9.2.2.2.1, next page) show a strongly supported clade for *Telmatogeton magellanicus* and *Telmatogeton macquariensis* for both genes. However, the relationship of both species with the remaining *Telmatogeton* cannot be confirmed with the available dataset.


COX1
Chironomus tepperi (JN861749)
Prodiamesa olivacea (HQ105314)
Belgica albipes (JQ672724.1)
Belgica antarctica (JQ672652.1)
Ablabesmyia aspera (HQ105015)
Telmatogeton japonicus (AB704941)
Telmatogeton japonicus (LC050959)
Telmatogeton japonicus (KT278287)
Parochlus steinenii FSPaS023
Telmatogeton macquariensis FSTel004
Telmatogeton macquariensis FSTel005
ROB 001
CPH 044
ROB 022
CPH 039
ROB 002
ROB 008
ROB 009
ROB 005
ROB 021
HON 016
HON 017
HON 015
ROB 004
0.03
28S
Chironomus riparius (AY820912+AY820919)
Belgica albipes (JQ672734)
Belgica antarctica (AY820938)
Diamesa bertrami (JF894362)
Parochlus steinenii (FSPaS004)
Telmatogeton sp. (AY820915+AY820939)
Telmatogeton sp. (AY820939)
Telmatogeton macquariensis FSTel004
Telmatogeton macquariensis FSTel005
ROB 001
ROB 002
HON 016
HON 017
CPH 044
0.008

9.3 Appendix III

9.3.1 *Belgica antarctica*

Taxonomy: Diptera – Chironomidae – Orthocladiinae

The most famous of the two antarctic midges, *Belgica antarctica*, has been heavily studied over the last four decades (Convey & Block 1996, Allegrucci et al. 2006, 2012, Hayward et al. 2007, Kelley et al. 2014, Cornette et al. 2015, Kawarasaki et al. 2019; among many others). This brachypterous insect is the largest free-living land animal in the Antarctic Continent; their adult bodies, which range from 4 to 5 mm, are of a very dark tint, with slightly larger females, with a thicker abdomen as the most distinguishable characteristic (Jacobs 1900, Sugg et al. 1983).

Belgica antarctica is a biennial insect, meaning that it takes two full years to complete its life cycle. The first comprehensive description of its phenology and life history dating back to the 1980's (Sugg et al. 1983), even though Peckham (1971) made initial remarks about the taxon in that regard. From these studies, we know that copulation and oviposition are carried out through the first half of a summer, with most specimens reaching their second larval instar before overwintering in this stage (Figure 9.3.1 and 9.3.2). During the second summer, the larvae keep feeding and reach the 4th instar, whereupon they need to overwinter once again, before morphing into pupae and then adults in the third summer, when the cycle restarts (Figure 9.3.2).

Figure 9.3.1 Larvae and mating adults of *B. antarctica* (photos: Richard E. Lee Jr.).

Figure 9.3.2 Adult of *B. antarctica* on moss (photo: Elise Biersma).

9.3.2 *Eretmoptera murphyi*

Taxonomy: Diptera – Chironomidae – Orthocladiinae

Until very recently, the phenology and life history of *E. murphyi* (Figure 9.3.3) was known only from sporadic studies, with research done with the full cycle. Publications such as Block et al. (1984), Cranston (1985), Convey (1992), Gardiner et al. (1998), Worland

(2010), and Hughes et al. (2013) amassed data on egg and egg sac development times, and/or discussed specific larval stages, and the possible parthenogenecity of the species.

Bartlett et al. (2018a) confirmed the latter and fully described the phenology of *E. murphyi*. What is now known is summarised in Figure 9.3.4, showing that eggs develop for around 30 days, and akin to *Belgica antarctica*, larvae develop until they reach the second instar shortly before overwintering in this stage. In the following year, they complete their larval development through the fourth instar, when they need to overwinter once again before spending a 3-week period as pupae. Adults survive for around a week, but not all specimens completely transition from pupae though, as they sometimes oviposit from within their pupae.

Larvae, pupae and adults (known only from females) of *E. murphyi* can reach up to 5 mm long. While the larvae have a yellow to light dark yellow body, adults have light brown colour, similar to that of the pupae. Though brachypterous as in *B. antarctica*, the remaining wings are longer than the Antarctic midge (Schaeffer 1914, Cranston 1985).

Figure 9.3.3 Adult of *E. murphyi* (photo: Robert S. Key).

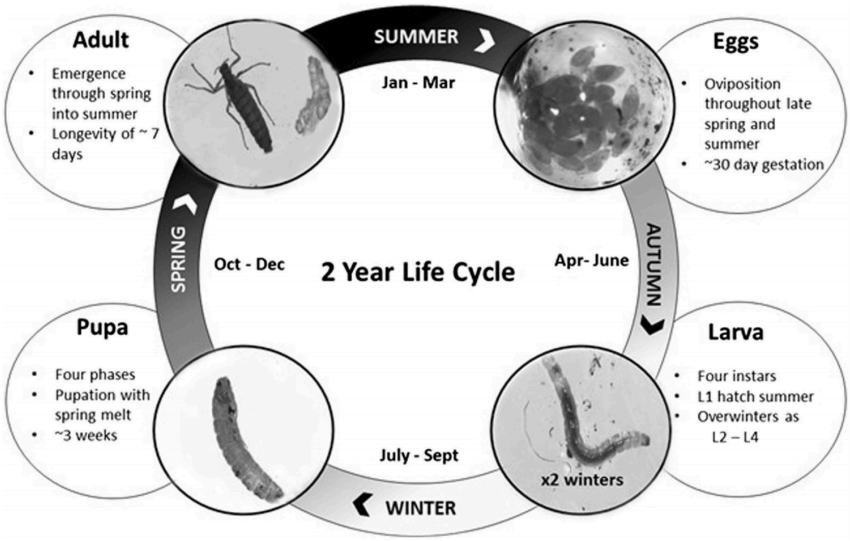


Figure 9.3.4 Life cycle of *E. murphyi* (Bartlett et al. 2018a).

9.3.3 *Parochlus steinenii*

Taxonomy: Diptera – Chironomidae – Podonominae

The first, and most obvious, difference between *Parochlus steinenii* and the other midges studied here is the presence of functional and fully developed wings. Additionally, they have a thinner body and adults can be found mostly on the ground in clusters, but also flying or freely walking around. Edwards & Usher (1985) hinted that the apparent morphological flexibility of *P. steinenii*, through clear variations between populations in the same island, were evidence of a more flexible life history strategy, commonly seen in polar organisms (in comparison with temperate and, even more so, tropical species).

Their bodies are a pitchy brown tint, extending up to 3 mm, with wings almost as long as their bodies (up to 2.63 mm); pupae are slightly longer, around 3.5 mm, with larvae (L4s) extending up to 6 mm, and both are of a very dark tint. One of the main characteristics of the Podonominae is the very thin and long abdomen, also seen *P. steinenii* (Wirth & Gressitt 1967).

Figure 9.3.5 Adult of *P. steinenii* (photo: Gonzalo Arriágada).

Figure 9.3.6 Collection of *P. steinenii* with an entomological aspirator, King George Island.

9.3.4 *Telmatogeton magellanicus*

Taxonomy: Diptera – Chironomidae – Telmatogetoninae

Also a brachypterous species, *T. magellanicus* is slightly larger than *B. antarctica* and *E. murphyi*, with a gray body. Adult males are thinner and longer than the females, ranging from 0.4 to 0.6 mm (4th instar larvae can be up to 0.3 mm longer than that).

As stated in Chapters 2 and 3, little was known about *T. magellanicus* until its very recent rediscovery and the first visual records are shown in Chapter 2, as well as partially reproduced in Figures 9.3.7 and 9.3.8. Here we can see part of the insect's life cycle, including its 2nd and 4th larval instars, as well as two pupae and an adult. As of now, the only specific information we could acquire regarding the development of this species is that they take around 14 days to hatch from their eggs. A study with a full description of the species' life cycle should be fairly straightforward to complete by taxonomists and phenologists along the shores of Navarino Island, Chile, where the species can be abundant.

Figure 9.3.7 Specimens of *T. magellanicus*. From top to bottom: second larval instar, fourth larval instar, two pupae. (black bar = 0.5 cm) (photo: F. L. Simões)

Figure 9.3.8 Adult male of *T. magellanicus* (photo: Gustavo Arriágada).

9.3.5 *Lancetes angusticollis*

Taxonomy: Coleoptera – Dytiscidae

Akin to other Antarctic or sub-Antarctic species, the diving beetle *Lancetes angusticollis* has a biennial cycle (Nicolai & Droste 1984, Arnold & Convey 1998). However, what is known about its life history is almost completely based on the South Georgian populations, and those from the South American mainland still need to be properly reared and studied.

From eggs hatching, which takes around 21 days, larvae develop through to the third or fourth instars (depending on how late the eggs hatched during the first summer of development). This is when specimens of *L. angusticollis* spend their first inactive period for the winter, which is then followed by a second summer where they will complete morphing into pupae, or even all the way to adults in case of early-laid eggs, before

proceeding to a second, temperature-cued, overwintering phase (Arnold & Convey 1998). The cycle starts anew during the third summer.

As with other dytiscids, the body of *L. angusticollis* is distinctively longer than wide (extending up to ~15 mm), mostly ochreous with inconsistent black stripes along their elytra (though the head is of a very dark brown) (Curtis 1839). Though there is no proper description of the larvae, Brancucci & Ruhnau (1985) fully described the pupae, which are 12–12.3 mm long and of a creamy-white colour.

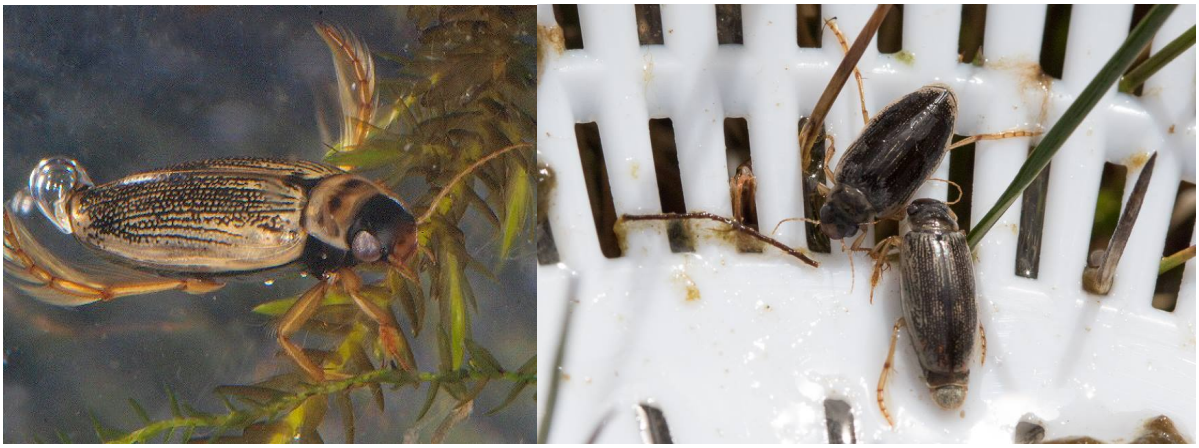


Figure 9.3.9 Adults of the diving beetle *L. angusticollis* (photos: Robert S. Key and Elise Biersma).

Figure 9.3.10 Collection of *L. angusticollis* at Lancetes Lake, Maiviken, South Georgia (photo: Elise Biersma).

9.4 Appendix IV

9.4.1 Fungus-Invertebrate Interactions in Antarctica

Chapter 9 (p. 201–219) in Rosa, L. H. (2019) Fungi of Antarctica: Diversity, Ecology and Biotechnological Applications. Springer, Cham, Switzerland. 345 pp.

Chapter 9
Fungus-Invertebrate Interactions in Antarctica

Felipe Lorenz Simões, Peter Convey, Láuren Drumond, Lívia da Costa Coelho, and Luiz Henrique Rosa

9.1 Introduction

Antarctica (Fig. 9.1) is characterised by its extreme isolation from other continents and lack of ice-free areas, which have a clear influence on its biodiversity. The intense glaciation and glacial cycling from the Miocene to Pleistocene time periods, combined with the isolated nature of this continent, reduce the possibility of refugia, contributing to the reduced diversity and distribution of terrestrial and freshwater organisms (microbial, plant and animal life alike) (Convey 2017; Convey et al. 2018). This is a major contrast with the Arctic, which had easier routes of terrestrial recolonisation due to its continuous contact with the landmasses of North America and Eurasia. Antarctic marine habitats, while much more extensive and biodiverse, have been isolated from lower latitudes over multimillion-year timescales, since the formation of the Antarctic Circumpolar Current (Clarke et al. 2005; Barnes et al. 2006; Fraser et al. 2017).

The marine diversity of the Southern Ocean that surrounds the continent of Antarctica and its outlying sub-Antarctic island groups has received considerable research attention (CAML 2005–2010; De Broyer et al. 2014). However, even though often perceived simply as 'white rocks' or frozen lakes/rivers devoid of life, the terrestrial habitats of the Antarctic also hide a complex network of biogeographic domains and unique and often endemic biodiversity, spread out across three main areas, the sub-, maritime, and continental (or frigid) Antarctic, and currently 16 'Antarctic Conservation Biogeographic Regions' (ACBRs) (Pugh and Convey

F. L. Simões (✉) · P. Convey (✉)
British Antarctic Survey, NERC, Cambridge, UK
e-mail: felsim@bas.ac.uk; pcon@bas.ac.uk

L. Drumond · L. da Costa Coelho · L. H. Rosa
Departamento de Microbiologia, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil

L. H. Rosa (ed.), *Fungi of Antarctica*,
https://doi.org/10.1007/978-3-030-18367-7_9

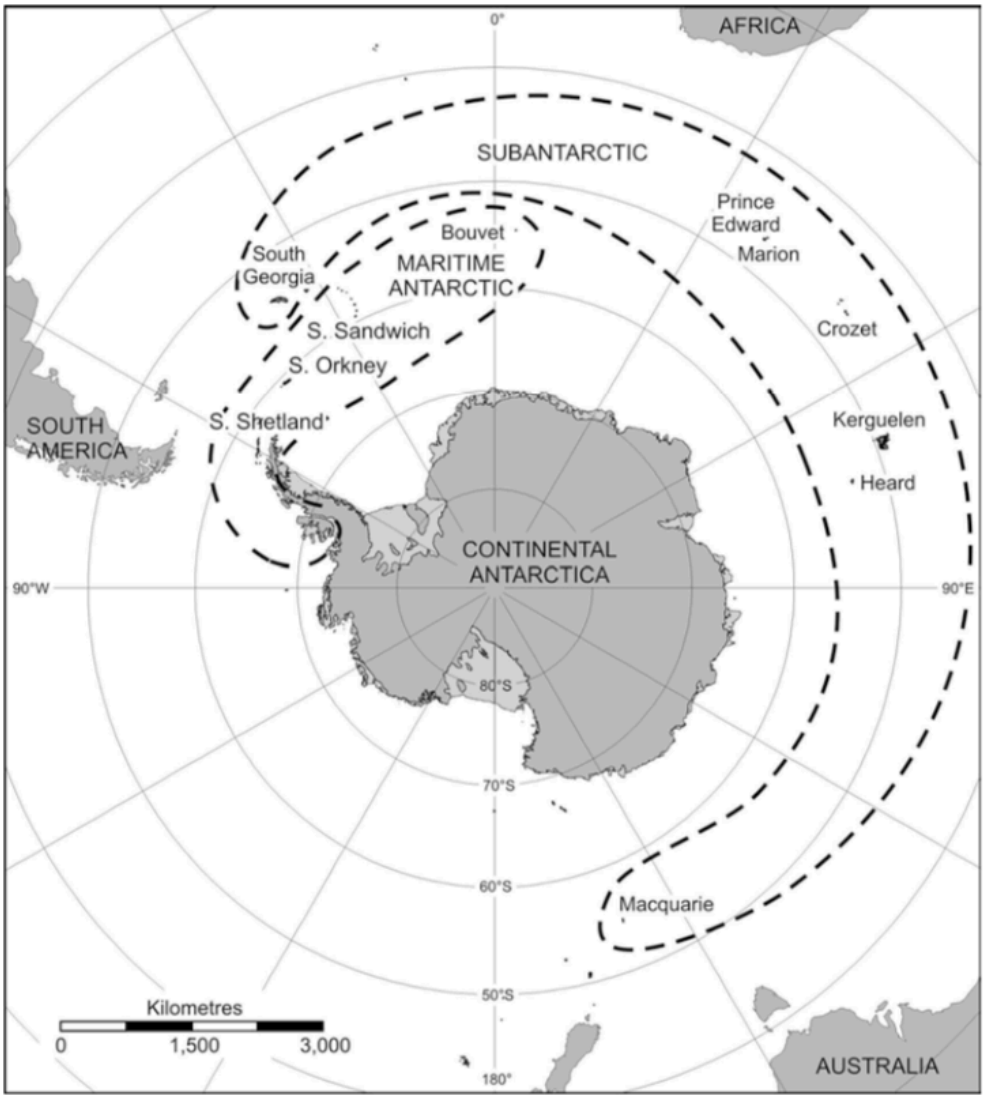


Fig. 9.1 The biogeographic regions of Antarctica (Convey 2017)

2008; Convey et al. 2008; Terauds et al. 2012; Chown and Convey 2016; Terauds and Lee 2016; Convey 2017). Most of the known Antarctic terrestrial biodiversity is found in fragmented and island-like areas of seasonally ice- and snow-free habitats in coastal regions, especially along the Antarctic Peninsula and continental coastline, and in the major mountain ranges inland (although the individual largest ice-free areas are formed by the McMurdo Dry Valleys of southern Victoria Land). Convey (2017) provided an overview of the terrestrial biogeography and biodiversity of the Antarctic and the natural and human-induced processes affecting its ecosystems.

Although it mostly reflects a history of long-term isolation, the invertebrate fauna of the Antarctic is also limited by the continent's extreme environmental conditions

(Convey 2017), and contemporary diversity is limited to extremophiles with the appropriate ecophysiological adaptations. The continent's current terrestrial fauna consists of nematodes, insects (only two species of dipterans on the continent), springtails, tardigrades, mites, and rotifers; other taxa such as molluscs, spiders and earthworms and a wider diversity of insect groups can be found in the less extreme sub-Antarctic regions (see Convey and Lebouvier 2009, Chown and Convey 2016 and Convey 2017, for reviews). Antarctic marine organisms are far more diverse (De Broyer et al. 2014); however, far less is known about marine fungi and their interactions with other taxa.

A wide variety of invertebrates has been studied in detail (terrestrial microarthropods, the krill *Euphausia superba* (Fig. 9.2c), the flightless midge *Belgica antarctica* (Fig. 9.3d) and various marine invertebrates being amongst the best-known examples (see, e.g. Block et al. 2009; Chown and Convey 2016; Cui et al. 2016; Everatt et al. 2014; Peck 2018) in terms of their physiology, adaptation, evolution and ecology. However, very few studies have addressed the interactions between fungi and Antarctic invertebrates. Indeed, Cui et al. (2016) highlighted the complete lack of previous studies regarding the diversity and ecological function of microorganisms associated with *E. superba*; this has resulted in a striking gap of knowledge given that krill are widely known as a key species in the Antarctic marine trophic web.

In this chapter, we review the limited available literature on the associations of invertebrates and fungi across the different environments of Antarctica and the

Fig. 9.2 Examples of Antarctic marine invertebrates associated with fungi. (**a**) *Ophiuroidea* sp., (**b**) Echinodermata, (**c**) *Euphausia superba*, (**d**) *Glyptonotus antarcticus*, and (**e**) *Nacella concinna*. (Photos credits: FM Pellizari)

Fig. 9.3 (**a**) Example of one of the few habitats of terrestrial invertebrates in Antarctica, with mosses and Acari (black spots) (King George Island, South Shetland Islands), (**b**) two Acari species: on the right (brown) *Gamasellus racovitzai* and on the left (black) *Alaskozetes antarcticus*, (**c**) *Cryptopygus antarcticus*, and (**d**) *Belgica antarctica*. (Photos credits: EM Biersma)

diversity involved in these associations, and discuss how interactions may be affected by impacts such as climate change, invasive species and increasing human presence and activity in the region. Research to date has largely focused on terrestrial ecosystems, and hence most examples described in this chapter are drawn from terrestrial studies, although we provide links to marine studies where possible.

9.1.1 Antarctic Terrestrial Biota

In the maritime and continental Antarctic, with the exceptions of steep cliffs and exposed mountain ridges, most terrestrial habitats are covered seasonally by snow and/or ice, providing organisms protection from extreme temperature variations and wind abrasion (Convey et al. 2018). While habitats in the maritime and continental zones may be free of seasonal snow cover for periods ranging from only days or weeks to even 5 months, many sub-Antarctic islands experience only intermittent snow cover, which is often restricted to higher altitudes. Even on the coldest sub-Antarctic islands, subnivean microhabitat temperatures are often sufficient to allow year-round biological activity (Convey 1996a). This is not the case in the maritime and continental zones, where biological processes are arrested by low winter temperatures (Walton 1984).

Antarctic soils are typically poorly developed, with low organic content (Beyer and Bölter, 2002). Formation, development and stability of soils are heavily influenced by cryoturbation (Thomas et al. 2008). There is a clear dichotomy between the sub-Antarctic and the other two zones, with only the latter possessing a widespread permafrost layer. Brown soils are associated only with the larger stands of flowering plants in the maritime Antarctic but are more widespread in the sub-Antarctic. Deep peat deposits have developed since the last glaciation under extensive valley bog communities in the sub-Antarctic. Significant deep moss banks are much more restricted (Fig. 9.3a) in the maritime Antarctic and not found in the continental Antarctic (Fenton 1980, 1982; Fenton and Smith 1981; Royles and Griffiths 2014) and differ from those of the sub-Antarctic in being well preserved by inclusion in permafrost, with little or no decay, and even retaining viability over at least 1500 years of preservation (Roads et al. 2014).

Terrestrial vertebrates are mostly absent in the Antarctic and are limited to a single endemic insectivorous passerine and two species of freshwater ducks in South Georgia and Îles Kerguelen, and two scavenging sheathbills, one each present along the Antarctic Peninsula, Scotia Arc islands, South Georgia and on Marion Island. There are no native mammals, reptiles, amphibians or freshwater fish. Nutrient availability in terrestrial habitats is strongly dependent on the Antarctic marine vertebrate fauna (Bokhorst and Convey 2016). Most such habitats are coastal, but some birds also breed on nunataks several hundred kilometres inland. Antarctic terrestrial fauna, therefore, consist almost entirely of invertebrates.

The milder sub-Antarctic islands host a range of 'higher' insects and other arthropod groups. The most diverse groups are Diptera and Coleoptera although these, and all other groups (e.g. Araneae, Isopoda, Lepidoptera, Hymenoptera, Hemiptera) are represented by very few species in terms of absolute numbers. Terrestrial diversity also includes molluscs and annelid worms, as well as diverse communities of micro-arthropods [Acari (Fig. 9.3b), Collembola (Fig. 9.3c)] and micro-invertebrates (Nematoda, Tardigrada, Rotifera). Overall diversity is lower in the maritime Antarctic, with only two chironomid midges (Diptera) present. Micro-arthropods and other micro-invertebrates are well represented. Although, again, species diversity is low, population densities are often very high, and comparable with or greater than many temperate and even tropical ecosystems. The continental Antarctic fauna includes no insects, and is restricted to micro-arthropods and micro-invertebrates. Though they are of similar diversity overall to the maritime Antarctic, they are much more limited in their spatial distribution. This zone includes the simplest faunal ecosystems on the planet, where even nematodes are apparently absent (Convey and McInnes, 2005; Hodgson et al. 2010). In all three regions, the fauna present includes taxa with characteristic trophic preferences (e.g. algivory, bacterivory, fungivory, predation); however, little detailed autecological work has been attempted, and the specific diets of Antarctic taxa are virtually unknown (Hogg et al. 2006).

9.2 Fungi-Invertebrate Interactions

Interactions between fungi and invertebrates in the Antarctic (both on land and in water) have seldom been studied. This is partly because many taxa have only been found recently and also is a consequence of the known hardships of working with extremophiles. However, there is clearly an assortment of species that actively interact with each other (Bridge and Worland 2008; Bridge and Spooner 2012). Fungal species have been found in the carcasses of dead animals (e.g. Bridge et al. 2005, 2008), predating on micro-invertebrates (e.g. Onofri and Tosi 1992; McInnes 2003) or being utilised as a source of food (e.g. Bokhorst et al. 2007). In the following sections, we present an overview of the interactions known to date across some major invertebrate groups.

9.2.1 Fungal Isolation from Invertebrates

Various methods to recover fungi from invertebrates have been described. Small invertebrates can be surface sterilised by washing in 70% ethanol for 30–60 s and after that transferring to Petri dishes containing culture media (Bridge and Worland 2004; Bridge et al. 2005, 2008; Bridge and Denton 2007). From the marine environment, Henríquez et al. (2014) obtained approximately 1 cm^3 pieces from the inner tissues of invertebrates, which were excised under sterile conditions using a scalpel and forceps, and directly spread them onto Petri dishes containing different culture media. Godinho et al. (2019) collected invertebrates, which were washed twice in a sterile solution 0.9% NaCl (for those sampled in land ecosystems) or seawater (for those from marine ecosystems) for 2–4 min. After that, the invertebrates were ground and placed in Petri dishes containing media for fungal growth. Different media were used for fungal development, such as Sabouraud agar, potato dextrose agar, malt extract agar, marine agar and others, which were supplemented with antibacterial antibiotics (usually chloramphenicol) for the inhibition of bacterial contamination. Table 9.1 summarises the fungi isolated from invertebrates in Antarctica.

9.2.2 Marine Invertebrates

Relative to their abundance, the mycological associations of marine invertebrates are very poorly studied, and the literature is largely restricted to the studies reported by Duarte et al. (2013), who first reported the occurrence of species of yeasts isolated from Antarctic marine invertebrates, including gastropods, tunicates, and isopods (Table 9.1). Cui et al. (2016) recently reported 42 taxa of fungi from a single species of crustacean (the very abundant krill, *Euphasia superba*). Godinho et al. (2019) studied the distribution and diversity of fungi associated with 10 species of

Table 9.1 Fungi isolated from terrestrial and marine invertebrates of Antarctica

Island/ Region	Invertebrate	Proposed taxa	References
Signy Island	*Eretmoptera murphyi* (larvae)	*Alternaria* sp.	Bridge and Denton (2007)
		Arthroderma sp.	
		Acremonium strictum	
		Antarctomyces psychrotrophicus	
		Mortierella gamsii	
		Pythium sp.	
Unknown	*Tetranychus urticae*	*Acremonium implicatum*	Bridge and Worland (2008)
		Cladosporium sp.	
		Lecanicillium lecanii	
	Polyphagotarsonemus latus	*Beauveria bassiana*	
	Dinothrombium giganteum	*Aspergillus flavus*	
	Eotranchyus sp.	*Cladosporium cladosporoides*	
	Thrombidium gigas	*Aspergillus flavus*	
	Oribatid sp.	*Lecanicillium lecanii*	
		Simplicillium lamellicola	
	Abacarus hystrix	*Lecanicillium lecanii*	
Bird Island	*Hydromedion sparsutum*	*Pirella circinans*	Bridge et al. (2008)
Nelson Island	*Alaskozetes antarcticus*	*Neozygites acaridis*	Bridge and Worland (2004)
Adelaide Island	*Cryptopygus antarcticus*	*Paecilomyces antarcticus*	Bridge et al. (2005)
King George Island	Unidentified sea squirt	*Candida sake*	Duarte et al. (2013)
		Wickerhamomyces anomalus	
		Rhodotorula mucilaginosa	
	Unidentified sea sponge	*Debaryomyces hansenii*	
		Bullera pseudoalba	
		Cryptococcus laurentii	
		Rhodotorula mucilaginosa	
	Salpa sp.	*Metschnikowia australis*	
		Cryptococcus victoriae	
		Cystofilobasidium capitatum	
		Cystofilobasidium infirmominiatum	

(continued)

Table 9.1 (continued)

Island/ Region	Invertebrate	Proposed taxa	References
		Rhodotorula mucilaginosa	
	Unidentified sea star	*Meyerozyma guilliermondii*	
		Cryptococcus adeliensis	
		Cryptococcus albidosimilis	
		Cystofilobasidium infirmominiatum	
		Guehomyces pullulans	
	Unidentified sea isopod	*Meyerozyma guilliermondii*	
	Unidentified sea snail	*Meyerozyma guilliermondii*	
	Nacella concinna (Fig. 9.2e)	*Wickerhamomyces anomalus*	
		Cryptococcus laurentii	
		Rhodotorula mucilaginosa	
	Unidentified sea urchin	*Cryptococcus laurentii*	
		Rhodotorula laryngis	
		Rhodotorula mucilaginosa	
	Marine sponge	*Acremonium* sp.	Henríquez et al. (2014)
		Aspergillus versicolor	
		Aureobasidium pullulans	
		Cladosporium cladosporioides	
		Cladosporium sp.	
		Pseudogymnoascus pannorum	
		Penicillium commune	
		Cladosporium sp.	
		Pseudogymnoascus sp.	
		Penicillium polonicum	
		Penicillium solitum	
		Phoma herbarum	
		Phoma sp.	
		Pseudeurotium sp.	
		Pseudogymnoascus pannorum	
		Pseudogymnoascus sp.	

(continued)

Table 9.1 (continued)

Island/ Region	Invertebrate	Proposed taxa	References
		Thelebolus sp.	
		Pseudogymnoascus sp.	
		Thelebolus sp.	
	Tedania sp.	*Cystofilobasidium infirmominiatum*	Vaca et al. (2013)
		Metschnikowia australis	
	Leucosporidiella sp.	*Cystofilobasidium infirmominiatum*	
	Dendrilla sp., *Hymeniacidon* sp., *Poecilosclerida* sp.	*Metschnikowia australis*	
Antarctic Peninsula	*Laevilacunaria antarctica*	*Antarctomyces psychrotrophicus*	Godinho et al. (2019)
		Metschnikowia australis	
		Pseudogymnoascus destructans	
		Pseudogymnoascus verrucosus	
		Vishniacozyma victoriae	
		Pseudogymnoascus cf. *destructans*	
	Antarctonemertes valida	*Cladosporium* sp. 1	
		Didymella longicolla	
		Glaciozyma martinii	
		Metschnikowia sp. 1	
		Mollisia sp.	
		Mortierella sp. 1	
		Mrakia sp.	
		Penicillium brevicompactum	
		Penicillium sp. 1	
		Penicillium sp. 2	
		Penicillium sp. 3	
		Pestalotiopsis kenyana	
	Ascidia sp.	*Antarctomyces psychrotrophicus*	
	Halyclystus antarticus	*Metschnikowia* sp. 1	
		Metschnikowia sp. 2	
	Lumbricillus sp.	*Aspergillus* sp. 1	
		Aspergillus sp. 2	
		Didymella coffeae-arabicae	
		Letendraea sp.	

(continued)

Table 9.1 (continued)

Island/ Region	Invertebrate	Proposed taxa	References
		Metschnikowia australis	
		Nothophoma macrospora	
		Penicillium sp. 1	
		Penicillium swiecickii	
		Penicillium sp. 2	
		Pestalotiopsis kenyana	
		Phoma sp.	
		Pseudogymnoascus cf. *destructans*	
		Thelebolus cf. *globosus*	
	Magelonidae sp.	*Metschnikowia* sp. 2	
		Mrakia frigida	
	Nacella concinna	*Antarctomyces psychrotrophicus*	
		Aspergillus sp. 1	
		Aspergillus sp. 2	
		Candida sp.	
		Candida spencermartinsiae	
		Candida zeylanoides	
		Cladosporium halotolerans	
		Cladosporium sp. 1	
		Clavispora lusitaniae	
		Debaryomyces hansenii	
		Pseudogymnoascus destructans	
		Geotrichum sp.	
		Glaciozyma martinii	
		Holtermanniella festucosa	
		Metschnikowia sp. 2	
		Meyerozyma guilliermondii	
		Mortierella sp. 1	
		Mortierella sp. 2	
		Mortierella sp. 3	
		Mrakia frigida	
		Penicillium sp. 1	
		Penicillium sp. 2	
		Penicillium sp. 3	
		Penicillium sp. 4	

(continued)

Table 9.1 (continued)

Island/ Region	Invertebrate	Proposed taxa	References
		Polypaecilum botryoides	
		Pseudogymnoascus cf. *destructans*	
		Pseudogymnoascus pannorum	
		Pseudogymnoascus verrucosus	
		Rhodotorula mucilaginosa	
		Thelebolus balaustiformis	
		Thelebolus cf. *globosus*	
		Tolypocladium tundrense	
	Ophiuroidea (Fig. 9.2a)	*Pseudogymnoascus* cf. *destructans*	
		Penicillium sp. 5	
		Metschnikowia sp. 2	
	Tigriopus kingsejongensis	*Debaryomyces hansenii*	
		Pseudogymnoascus pannorum	
		Penicillium sp. 3	
		Penicillium sp. 5	
		Pseudogymnoascus appendiculatus	
		Pseudogymnoascus cf. *destructans*	
		Pseudogymnoascus verrucosus	
		Septoria chromolaenae	
	Trepaxonemata sp.	*Cladosporium* sp. 1	
		Debaryomyces hansenii	
		Penicillium sp. 3	

invertebrates from Antarctica and recovered a rich and diverse community with 83 taxa from 27 distinct genera. The most abundant fungi associated with the Antarctic invertebrates were *Cladosporium* sp., *Debaryomyces hansenii*, *Glaciozyma martinii*, *Metschnikowia australis*, *Pseudogymnoascus destructans*, *Thelebolus* cf. *globosus*, *Pseudogymnoascus pannorum*, *Tolypocladium tundrense*, and different *Penicillium* and *Metschnikowia* species. Godinho et al. (2019) showed that the cryptic fungi recovered from Antarctic invertebrates displayed phylogenetic relationships with species that occurred in other cold, temperate and tropical regions of the world, including endemic and cosmopolitan cold-adapted taxa.

9.2.3 Nematodes

Nematodes are among the most abundant and widespread invertebrates occurring on land in Antarctica, only being absent from some of the most isolated inland regions of the continent (Convey and McInnes 2005; Hodgson et al. 2010). Nematophagous (or 'nematode-trapping') fungi, which are globally widespread and occur naturally in habitats with organic detritus (Gray et al. 1982), are also present in Antarctica, with several taxa reported from locations in the maritime Antarctic (Duddington et al. 1973; Spaull 1973; Gray et al. 1982; Gray and Smith 1984; Gray 1985; Velázquez et al. 2017). As noted by Gray et al. (1982), these predacious fungi have a role in the transfer of energy through the food chain, while also potentially controlling the population levels of their prey. More generally, however, Nielsen et al. (2011) suggested that nematodes, which are mostly bacterial grazers, could help the growth of fungi by reducing the ecological dominance of bacteria in the habitat.

9.2.4 Tardigrada and Rotifera

To date, the only study specifically mentioning fungal interactions with tardigrades (water bears) and rotifers is that of McInnes (2003), who reported a new fungal species (*Lecophagus antarcticus*) which was found attacking both invertebrates by means of trapping with adhesive pegs arising from vegetative hyphae (a behaviour previously reported in other members of the genus *Lecophagus*; Vechhi et al. 2016).

9.2.5 Collembola, Arachnida and Insecta

The first published record of interaction between fungi and terrestrial Arthropoda in Antarctica is that of Onofri and Tosi (1992), who reported *Arthrobotrys ferox* predating on the springtail *Gressittacantha terranova* in Kay Island, Edmonson Point and Baker Rocks (Wood Bay, Victoria Land, Antarctica), by means of what they described as 'organs consisting of ovoidal cells surrounded by an adhesive secretion (sic) and supported by a 2-celled stalk'. According to these authors, this species was the first sample of predaceous hyphomycetes collected in continental Antarctica and also the first recorded to predate on springtails in the Antarctic continent. The 2000s saw an increased number of studies, mostly from Bridge and collaborators (Bridge and Worland 2004, 2008; Bridge et al. 2005, 2008; Bridge and Denton 2007; Bridge and Spooner 2012).

Bridge and Worland (2004) discovered *Neozygites*, an entomophthoralean fungus, on living mites at Nelson Island, South Shetland Islands, off the north-west coast of the Antarctic Peninsula. Subsequently, Bridge et al. (2005) isolated a new

species *Paecilomyces antarcticus* from the carapace of dead springtails collected near Rothera Research Station, Adelaide Island, although they could not identify a precise role of the fungus. *Paecilomyces* spp. have been previously reported to grow on wintering insect larvae (ARSEF 2018); however, in the study by Bridge et al. (2005), there was no visible fungal growth before the fungus was cultured in the laboratory. Bokhorst et al. (2007) suggested that the springtail *Cryptopygus antarcticus* feeds facultatively on fungi (among other organic material, such as algae and dead matter) (see also Tilbrook 1970; Broady 1979; Burn 1984; Block 1985; Cannon 1986), suggesting a closer relationship between the findings reported separately in these studies. However, *C. antarcticus* is thought to primarily be an algivore, preferentially grazing on certain microalgal species, rather than being a generalist microbivore (Worland and Lukešová, 2000). Members of the springtail genus *Friesea* are often considered to be fungivorous, but no autoecological studies have been carried out on any of the several Antarctic species to confirm this (Greenslade 2018a, b). Likewise, the oribatid mite *Halozetes belgicae* has been observed grazing on both microalgae and fungal hyphae within the thalli of supralittoral lichens at locations in the South Shetland Islands and along the Antarctic Peninsula (P. Convey, pers. obs.). The sub-Antarctic snail, *Notodiscus hookeri*, obtains specific micronutrients by grazing on lichens (Gadea et al. 2017, 2018).

Bridge and Denton (2007) isolated viable propagules of *Ascomycetes*, *Zygomycetes* and *Oomycetes* from the intestinal tract of *Eretmoptera murphyi*, a chironomid midge native to sub-Antarctic South Georgia, which was introduced accidentally by humans to Signy Island in the maritime Antarctic through plant transplant experiments (Block et al. 1984; Convey and Block 1996). This is a rare example of a study dealing directly with fungi found inside a living host in Antarctica. Normally, fungal growth only becomes apparent in dead animals, as reported by Bridge et al. (2008), who found an association between *Pirella circinans*, a coprophilous fungus, and the South Georgian endemic beetle *Hydromedion sparsutum*. This type of fungus is normally reported from the dung of small mammals, which are absent in South Georgia, and was the only fungus recovered in the insect cadavers, indicating a probable close association between the species.

The presence of entomopathogenic fungi gives potential for examination of their applicability as biological controls (see below). The studies of Bridge and Spooner (2012) and Velázquez et al. (2017) highlighted the importance of identifying and quantifying trophic interactions within the habitats where fungi and invertebrates are found. Bridge and Spooner (2012) noted that it is harder to determine the roles of fungi associated with invertebrates in Antarctica, mainly because the very specific life cycle and adaptive features required by these animals to survive in the extreme environmental conditions of the Antarctic influence the formation of fungal epizootics (see also Bridge and Worland 2008).

9.2.6 *Entomopathogenic Fungi*

No studies have yet formally documented the entomopathogenic potential of fungi in the Antarctic. As noted above, Bridge and Denton (2007) documented the microfungal composition of the intestinal tract of the South Georgian flightless midge *E. murphyi*, using materials collected from the invasive population on Signy Island, and suggested the possibility of the insect larvae working as vectors for fungal introductions. However, it was not possible to ascertain whether the fungal species identified were present on Signy Island before the fly's introduction, since all of them had previously been reported from Antarctica. Bridge and Worland (2008) found *Neozygites*, another pathogenic fungus, associated with the mite *Alaskozetes antarcticus*, and reviewed knowledge of fungal pathogens across Antarctica, highlighting the increasing number of studies on nematodes, flowering plants and mosses (Pegler et al. 1980; Gray and Smith 1984; Bridge et al. 2008) in comparison to those involving arthropods.

Bridge and Denton (2007) also considered the possibility of microfungi being found in hosts elsewhere in the world, such as *Lecanicillium lecanii*, and being able to adapt to the Antarctic if they were introduced, since they generally tolerate wide temperature ranges (Brasier et al. 1999; Nikoh and Fukatsu 2000; Hughes and Lawley 2003). Bridge et al. (2014) highlighted the potential of entomopathogenic fungi to make temperature-resistant mycoinsecticides. Edgington et al. (2014) studied the insecticidal potential of two fungi (*Pseudogymnoascus* and *Mortierella*) found around Rothera Research Station, Adelaide Island, and on Signy Island, finding two species of the latter (*M. alpina* and *M. sygniensis*) to cause significant mortality in larvae and adults of the tested insect species, suggesting a potential to be used elsewhere as pest control. Such pesticides may eventually play a role in combating biological invasions.

9.3 Invasive Species

The introduction of alien species is a matter of concern in any environment in the world, as it can have direct (e.g. predation of bird eggs and terrestrial invertebrates, trampling and grazing of plants) and indirect (e.g. alteration of habitat structure leading to changes in species dominance or behaviour) repercussions on native species and the stability of the local ecology. This is particularly the case in extreme environments where life has adapted very specifically to the driving physical environmental stressors and generally has very little ability to compete effectively with new arriving species (Convey 1996b). One of the main sources of biological invasions in the Antarctic, as globally, is human activity (e.g. Smith 1996; Azmi and Seppelt 1998; Frenot et al. 2005; Chwedorzewska 2009; Lee and Chown 2009; Convey 2010; Lityńska-Zając et al. 2012; Chwedorzewska et al. 2013; Galera et al. 2018). Accidental introductions and deliberate transplant experiments have shown

that a wide range of flora, fauna and microbes are capable of surviving and establishing viable populations, while an even greater number have been recorded on a transient or synanthropic basis (Frenot et al. 2005; Greenslade 2006; Convey 2017).

Whilst survey data documenting the introduction of invertebrates, and their ecological impacts, remain limited in Antarctica, the subject has received increasing attention in recent years (see, e.g. Lee and Chown 2009; Lityńska-Zając et al. 2012; Bartlett 2018a, b; Hughes et al. 2015, 2018; Gonçalves et al. 2017a, b). It is not uncommon for invasive species to be restricted to areas where human occupation is continuous, disappearing as soon as humans leave (Convey 2017; Potocka and Krzemińska 2018). However, some non-native species do become established in the natural environment, where they can cause competitive displacement and local extinction of native species and add new trophic links in terrestrial ecosystems.

Introduction of flowering plants, bryophytes and microbes has accompanied human activity. Given the possible evolutionary isolation of Antarctic microbes, the introduction of fungal strains from outside Antarctica (or even between regions within Antarctica) and the consequential potential for damage to this unique biological resource should not be underestimated (Smith 1996; Wynn-Williams 1996a, b; Frenot et al. 2005; Chown and Convey, 2007; Bridge and Hughes 2010; Cowan et al. 2011; Augustyniuk-Kram et al. 2013; Hughes et al. 2015, 2018).

Finally, Gonçalves et al. (2017a) suggested that some continental Antarctic fungi may be pathogenic to humans and, through humans who come in contact with them, they could possibly spread to other parts of the world. The same can be said about the possibility of the major migrating vertebrates of the Antarctic (de Sousa et al. 2017; Gonçalves et al. 2017b), which can carry pathogens to other parts of the world.

9.4 Conclusion and Perspectives

As a demonstration of the untapped potential for discovery of fungi associated with invertebrates of Antarctica, Cui et al. (2016) reported 42 types of fungi isolated from a single crustacean species (*E. superba*). Some of these produce cytotoxic compounds that may help protect the crustacean against mammalian predators and pathogenic bacteria. The potential, for example, for the discovery of cytotoxic and entomopathogenic compounds from Antarctic fungi is likely to lead to an upsurge in bioprospecting studies. Clearly, much future effort is required to isolate fungi from marine invertebrate taxa, a virtually unexplored field at present. There is also an urgent need for the improved survey and monitoring of microbial – including fungal – diversity across Antarctica, including the assessment of the native or invasive status of isolates. Studies on the impacts of future climate changes must be extended to include microbial groups, and in the context of the current chapter, focus in particular on potential changes in the interactions between fungi and invertebrates (e.g. see Bridge and Spooner 2012).

References

ARSEF (2018) Current catalog. https://data.nal.usda.gov/dataset/ars-collection-entomopathogenic-fungal-cultures-arsef

Augustyniuk-Kram A, Chwedorzewska KJ, Korczak-Abshire M, Olech M, Lityńska-Zając M (2013) An analysis of fungal propagules transported to the Henryk Arctowski Station. Pol Polar Res 34:269–278

Azmi OR, Seppelt RD (1998) The broad-scale distribution of microfungi in the Windmill Islands region, Antarctica. Polar Biol 19:92–100

Barnes DKA, Hodgson DA, Convey P, Allen CS, Clarke A (2006) Incursion and excursion of Antarctic biota: past, present and future. Glob Ecol Biogeogr 15:121–142

Bartlett JC, Convey P, Hayward SAL (2018a) Life cycle and phenology of an Antarctic invader: the flightless chironomid midge, *Eretmoptera murphyi*. Polar Biol 42:115–130

Bartlett JC, Convey P, Hayward SAL (2018b) Not so free range? Oviposition microhabitat and egg clustering affects *Eretmoptera muprhyi* (Diptera: Chironomidae) reproductive success. Polar Biol. Early access: doi: https://doi.org/10.1007/s00300-018-2420-4

Beyer L, Bölter M (2002) Geoecology of Antarctic ice free coastal landscapes. Springer, Berlin, p 429. https://doi.org/10.1007/978-3-642-56318-8

Block W (1985) Arthropod interactions in an Antarctic terrestrial community. In: Siegfried WR, Condy PR, Laws RM (eds) Antarctic nutrient cycles and food webs. Springer, Berlin, Heidelberg. 614–619. https://doi.org/10.1007/978-3-642-82275-9_83

Block W, Burn AJ, Richard KJ (1984) An insect introduction to the maritime Antarctic. Biological J Linn Soc 23:3339

Block W, Lewis Smith RI, Kennedy AD (2009) Strategies of survival and resource exploitation in the Antarctic fellfield ecosystem. Biol Rev 84:449–484

Bokhorst et al (2007) Food choice of Antarctic soil arthropods clarified by stable isotope signatures. Polar Biol 30:983–990

Bokhorst S, Convey P (2016) Impact of marine vertebrates on Antarctic terrestrial microarthropods. Antarct Sci 28:175–186

Brasier CM, Cooke DEL, Duncan JM (1999) Origin of a new *Phytophthora* pathogen through interspecific hybridization. Proc Natl Acad Sci U S A 96:5878–5883

Bridge PD, Denton GJ (2007) Isolation of diverse viable fungi from the larvae of the introduced chironomid *Eretmoptera murphyi* on Signy Island. Polar Biol 30:935–937

Bridge PD, Hughes KA (2010) Conservation issues for Antarctic fungi. Mycol Balcanica 7:11–14

Bridge PD, Spooner BM (2012) Non-lichenized Antarctic fungi: transient visitors or members of a cryptic ecosystem? Fungal Ecol 5:381–394

Bridge PD, Worland MR (2004) First report of an entomophthoralean fungus on an arthropod host in Antarctica. Polar Biol 27:190–192

Bridge PD, Worland MR (2008) An association between the Antarctic mite *Alaskozetes antarcticus* and an entomophthoralean fungus of the genus *Neozygite*. Exp Appl Acarol 46:43–52

Bridge PD, Clark MS, Pearce DA (2005) A new species of *Paecilomyces* isolated from the Antarctic springtail Cryptopigus antarcticus. Mycotaxon 92:213–222

Bridge PD, Hughes KA, Denton JO (2008) Association of the coprophilous fungus *Pirella circinans* with an indigenous beetle on the sub-Antarctic Bird Island. Polar Biol 31:657–661

Bridge PD, Edgington S, Hughes KA (2014) Antarctic fungi: potential novel mycoinsecticides? Outlooks Pest Manag 25:340–343

Broady PA (1979) Feeding studies on the collembolan *Cryptopygus antarcticus* Willem at Signy Island, South Orkney Islands. British Antarc Sur Bull 48:37–46

Burn AJ (1984) Feeding and growth in the Antarctic Collembolan *Cryptopygus antarcticus*. Oikos 36:59–64

Cannon RJC (1986) Diet and acclimation on the cold tolerance and survival of an Antarctic springtail. British Antarc Sur Bull 71:19–30

Census of Antarctic Marine Life [CAML] (2005–2010) Census of marine life. http://www.coml.org/census-antarctic-marine-life-caml/

Chown SL, Convey P (2007) Spatial and temporal variability across life's hierarchies in the terrestrial Antarctic. Phil Trans Royal Soc B 362:2307–2331
Chown SL, Convey P (2016) Antarctic entomology. Annu Rev Entomol 61:119–137
Chwedorzewska KJ (2009) Terrestrial Antarctic ecosystems at the changing world — an overview. Polish Polar Res 30:263–276
Chwedorzewska KJ, Korczak-Abshire M, Olech M, Lityńska-Zając M, Augustyniuk-Kram A (2013) Alien invertebrates transported accidentally to the Polish Antarctic Station in cargo and on fresh foods. Polish Polar Res 34:55–66
Clarke A, Barnes DKA, Hodgson DA (2005) How isolated is Antarctica? Trends Ecol Evol 20:1–3
Convey P (1996a) Overwintering strategies of terrestrial invertebrates in Antarctica – the significance of flexibility in extremely seasonal environments. Eur J Entomol 93:489–505
Convey P (1996b) The influence of environmental characteristics on life history attributes of Antarctic terrestrial biota. Biol Rev 71:191–225
Convey P (2010) Terrestrial biodiversity in Antarctica — recent advances and future challenges. Pol Sci 4:135147
Convey P (2017) Antarctic ecosystems. Encyclopedia of Biodiversity vol. 1:179–188 https://doi.org/10.1016/B978-0-12-809633-8.02182-8
Convey P, Block W (1996) Antarctic Diptera: ecology, physiology and distribution. Eur J Entomol 93:1–13
Convey P, Lebouvier M (2009) Environmental change and human impacts on terrestrial ecosystems of the sub-Antarctic islands between their discovery and the mid-twentieth century. Papers Proc Royal Soc Tasm 143:33–44
Convey P, McInnes SJ (2005) Exceptional tardigrade-dominated ecosystems in Ellsworth Land, Antarctica. Ecology 86:519–527
Convey P, Gibson JAE, Hillenbrand C-D, Hodgson DA, Pugh PJA, Smellie JL, Stevens MI (2008) Antarctic terrestrial life – challenging the history of the frozen continent? Biol Rev 83:103–117
Convey P, Coulson SJ, Worland MR, Sjöblom A (2018) The importance of understanding annual and shorter-term temperature patterns and variation in the surface levels of polar soils for terrestrial biota. Polar Biol 41:1587–1605
Cowan DA, Chown SL, Convey P, Tuffin M, Hughes K, Pointing S, Vincent WF (2011) Non-indigenous microorganisms in the Antarctic: assessing the risks. Trends Microbiol 19:540–548
Cui X, Zhu G, Liu H, Jiang G, Wang Y, Zhu W (2016) Diversity and function of the Antarctic krill microorganisms from *Euphausia superba*. Sci Rep 6:36496
De Broyer C, Koubbi P, Griffiths HJ, Raymond B, Udekem d'Acoz Cd', Van de Putte AP, David B, Grant S, Gutt J, Held C, Hosie G, Huettmann F, Post A, Ropert-Coudert Y (eds) (2014) Biogeographic Atlas of the Southern Ocean. Scientific Committee on Antarctic Research, Cambridge, XII + 498pp
Duarte AWF, Dayo-Owoyemi I, Nobre FS, Pagnocca FC, Chaud LCS, Pessoa A, Felipe MGA, Sette LD (2013) Taxonomic assessment and enzymes production by yeasts isolated from marine and terrestrial Antarctic samples. Extremophiles 17:1023–1035
Duddington CL, Wyborn CHE, Smith RIL (1973) Predacious fungi from the Antarctic. Bull British Antarctic Sur 35:87–90
Edgington S, Thompson E, Moore D, Hughes KA, Bridge P (2014) Investigating the insecticidal potential of *Geomyces* (Myxotrichaceae: Helotiales) and *Mortierella* (Mortierellacea: Mortierellales) isolated from Antarctica. Springerplus 3:289
Everatt MJ, Convey P, Worland MR, Bale JS, Hayward SAL (2014) Are the Antarctic dipteran, *Eretmoptera murphyi*, and Arctic collembolan, *Megaphorura arctica*, vulnerable to rising temperatures? Bull Entomol Res 104:494–503
Fenton JHC (1980) The rate of peat accumulation in Antarctic Moss Banks. J Ecol 68:211–228
Fenton JHC (1982) The formation of vertical edges on Antarctic Moss Peat Banks. Arctic Alpine Res 14:21–26
Fenton JHC, Smith RIL (1981) Distribution, composition and general characteristics of the moss banks of the maritime Antarctic. British Antarc Sur Bull 51:215–236
Fraser CI, Kay GM, du Plessis M, Ryan PG (2017) Breaking down the barrier: dispersal across the Antarctic Polar Front. Ecography 40:235–237

Frenot Y, Chown SL, Whinam J, Selkirk PM, Convey P, Skotnicki M, Bergstrom DM (2005) Biol Rev 80:34–72

Gadea A, Le Pogam P, Biver G, Boustie J, Le Lamer A-C, Le Dévéhat F, Charrier M (2017) Which specialized metabolites does the native Subantarctic gastropod *Notodiscus hookeri* extract from the consumption of the lichens *Usnea taylorii* and *Pseudocyphellaria crocata*? Molecules 22:425

Gadea A, Le Lamer A-C, Le Gall S, Jonard C, Ferron S, Catheline D, Ertz D, Le Pogam P, Boustie J, Le Devehat FL, Charrier M (2018) Intrathalline metabolite profiles in the Lichen *Argopsis friesiana* shape gastropod grazing patterns. doi: https://doi.org/10.1007/s10886-018-0953-1

Galera H, Chwedorzewska KJ, Korczak-Abshire M, Wódkiewicz M (2018) What affects the probability of biological invasions in Antarctica? Using an expanded conceptual framework to anticipate the risk of alien species expansion. Biodivers Conserv 27:1789–1809

Godinho VM, de Paula MTR, Silva DAS, Paresque K, Martins AP, Colepicolo P, Rosa CA, Rosa LH (2019) Diversity and distribution of cryptic cultivable fungi associated with marine animals of Antarctica. Fungal Biol. https://doi.org/10.1016/j.funbio.2019.05.001

Gonçalves VN, Oliveira FS, Carvalho CR, Schaefer CEGR, Rosa CA, Rosa LH (2017a) Antarctic rocks from continental Antarctica as source of potential human opportunistic fungi. Extremophiles 21:851–860

Gonçalves VN, Vitoreli GA, de Menezes GCA, Mendes CRB, Secchi ER, Rosa CA (2017b) Taxonomy, phylogeny and ecology of cultivable fungi present in seawater gradients across the Northern Antarctica Peninsula. Extremophiles 21:1005–1015

Gray NF (1985) Nematophagous fungi from the maritime Antarctic: factors affecting distribution. Mycopathologia 90:165–176

Gray NF, Smith RIL (1984) The distribution of nematophagous fungi in the maritime Antarctic. Mycopathologia 85:81–92

Gray NF, Wyborn CHE, Smith RIL (1982) Nematophagous fungi from the maritime Antarctic. Oikos 38:194–201

Greenslade P (2006) Macquarie Island. Australian Antarctic Division, Hobart

Greenslade P (2018a) An antarctic biogeographical anomaly resolved: the true identity of a widespread species of Collembola. Polar Biol 41:969–981

Greenslade P (2018b) A new species of *Friesea* (Collembola: Neanuridae) from the Antarctic Continent. J Nat Hist 52:2197–2207

Henríquez M, Vergara K, Norambuena J, Beiza A, Maza F, Ubilla P, Araya I, Chávez R, San-Martín A, Darias J, Darias MJ, Vaca I (2014) Diversity of cultivable fungi associated with Antarctic marine sponges and screening for their antimicrobial, antitumoral and antioxidant potential. World J Microbiol Biotechnol 30:65–76

Hodgson DA, Convey P, Verleyen E, Vyverman W, McInnes SJ, Sands CJ, Fernández-Carazo R, Wilmotte A, De Wever A, Peeters K, Tavernier I, Willems A (2010) The limnology and biology of the Dufek Massif, Transantarctic Mountains 82° South. Pol Sci 4:197–214

Hogg ID, Cary SC, Convey P, Newsham KK, O'Donnell AG, Adams BJ, Aislabie J, Frati F, Stevens MI, Wall DH (2006) Biotic interactions in Antarctic terrestrial ecosystems: are they a factor? Soil Biol Biochem 38:3035–3040

Hughes KA, Lawley B (2003) A novel Antarctic microbial endolithic community within gypsum crusts. Environ Microbiol 5:555–565

Hughes KA, Pertierra LR, Molina-Montenegro MA, Convey P (2015) Biological invasions in terrestrial Antarctica: what is the current status and can we respond? Biodivers Conserv 24:1031–1055

Hughes KA, Misiak M, Ulaganathan Y, Newsham KK (2018) Importation of psychrotolerant fungi to Antarctica associated with wooden cargo packaging. Antarct Sci 30:298–305

Lee JE, Chown SL (2009) Breaching the dispersal barrier to invasion: quantification and management. Ecol Appl 19:944–1959

Lityńska-Zając M, Chwedorzewska K, Olech M, Korczak-Abshire M, Augustyniuk-Kram A (2012) Diaspores and phyto-remais accidentally transported to the Antarctic Station during three expeditions. Biodivers Conserv 21:3411–3421

McInnes SJ (2003) A predatory fungus (Hyphomycetes: *Lecophagus*) attacking Rotifera and Tardigrada in maritime Antarctic lakes. Polar Biol 26:79–82
Nielsen UN, Wall DH, Li G, Toro M, Adam BJ, Virginia RA (2011) Nematode communities of Byers Peninsula, Livingston Island, maritime Antarctica. Antarct Sci 23:349–357
Nikoh N, Fukatsu T (2000) Interkingdom host jumping underground: phylogenetic analysis of entomoparasitic fungi of the genus *Cordyceps*. Mol Biol Evol 17:629–638
Onofri S, Tosi S (1992) *Arthrobotrys ferox* sp. nov., a springtail-capturing hyphymycete from continental Antarctica. Mycotaxon 44:445–451
Peck L (2018) Antarctic marine biodiversity: adaptations, environments and responses to change. Oceanogr Marine Bio: An Annual Review 56:105–236
Pegler DN, Spooner BM, Smith RIL (1980) Higher fungi of Antarctica, the Subantarctic zone and Falkland Islands. Kew Bull 35:499–562
Potocka M, Krzemińska E (2018) *Trichocera maculipennis* (Diptera) — an invasive species in Maritime Antarctica. PeerJ 6:e5408
Pugh PJA, Convey P (2008) Surviving out in the cold: Antarctic endemic invertebrates and their refugia. J Biogeogr 35:2176–2186
Roads E, Longton R, Convey P (2014) Millenial timescale regeneration in a moss from Antarctica. Curr Biol 24:R222–R223
Royles J, Griffiths H (2014) Invited review: climate change impacts in polar regions: lessosn from Antarctic moss bank archives. Glob Chang Biol 21:1041–1057
Smith RIL (1996) Introduced plants in Antarctica: potential impacts and conservation issues. Biol Conserv 76:135–146
de Sousa JRP, Gonçalves VN, de Holanda RA, Santos DA, Bueloni CFLG, Costa AO, Petry MV, Rosa CA, Rosa LH (2017) Pathogenic potential of environmental resident fungi from ornithogenic soils of Antarctica. Fungal Biol 121:991–1000
Spaull VW (1973) The Signy Island terrestrial reference sites: IV. The nematode fauna. Bulletin of the British Antarc Sur 37:94–96
Terauds A, Lee JR (2016) Antarctic biogeography revisited: updating the Antarctic conservation biogeographic regions. Div Distrib 22:836–840
Terauds A, Chown SL, Morgan F, Peat HJ, Watts DJ, Keys H, Convey P, Bergstrom DM (2012) Conservation biogeography of the Antarctic. Div Distrib 18:726–741
Thomas DN, Fogg G, Convey P, Fritsen C, Gilli J-M, Gradinger R, Laybourne-Parry J, Reid K, Walton DWH (2008) The biology of polar regions. Oxford University Press, Oxford. 394pp
Tilbrook PJ (1970) The biology of *Cryptopygus antarcticus*. In: Holdgate MW (ed) Antarctic ecology, vol 2. Academic, London, pp 886–896
Vaca I, Faúndez C, Maza F, Paillavil B, Hernández V, Acosta F, Levicán G, Martínez C, Chávez R (2013) Cultivable psychrotolerant yeasts associated with Antarctic marine sponges. World J Microbiol Biotechnol 29:183–189
Vechhi M, Vicente F, Guidetti R (2016) Interspecific relationships of tardigrades with bacteria, fungi and protozoans, with a focus on the phylogenetic position of *Pyxidium tardigradum* (Ciliophora). Zool J Linnean Soc 178:846–855
Velázquez D, Jungblut AD, Rochera C, Rico E, Camacho A, Quesada A (2017) Trophic interactions in microbial mats on Byers Peninsula, maritime Antarctica. Polar Biol 40:1115–1126
Walton DWH (1984) The terrestrial environment. In: Laws RM (ed) Antarctic ecology. Academic Press, London, pp 1–60
Worland MR, Lukešová A (2000) The effect of feeding on specific soil algae on the cold-hardiness of two Antarctic micro-arthropods (*Alaskozetes antarcticus* and *Cryptopygus antarcticus*). Polar Biol 23:766–774
Wynn-Williams DD (1996a) Antarctic microbial diversity: the basis of polar ecosystem processes. Biodivers Conserv 5:1271–1293
Wynn-Williams DD (1996b) Response of pioneer soil microalgal colonists to environmental change in Antarctica. Microbial Ecol 31:177–188

9.5 Appendix V

9.4.1 Assessing distribution shifts and ecophysiological characteristics of the only Antarctic winged midge under climate change scenarios

Contador, T.; Gañan, M.; Bizama, G.; Fuentes-Jaque, G.; Morales, L.; Rendoll, J.; Simões, F.; Kennedy, J.; Rozzi, R.; Convey, P. (2020) Assessing distribution shifts and ecophysiological characteristics of the only Antarctic winged midge under climate change scenarios. Scientific Reports 10, 98076. DOI: 10.1038/s41598-020-65571-3

SCIENTIFIC REPORTS
nature research

Check for updates

OPEN

Assessing distribution shifts and ecophysiological characteristics of the only Antarctic winged midge under climate change scenarios

Tamara Contador[1,2,3,8✉], Melisa Gañan[1,8✉], Gustavo Bizama[4], Guillermo Fuentes-Jaque[4], Luis Morales[4], Javier Rendoll[1,3], Felipe Simoes[7], James Kennedy[1,5], Ricardo Rozzi[1,3,6] & Peter Convey[7]

Parts of Antarctica were amongst the most rapidly changing regions of the planet during the second half of the Twentieth Century. Even so, today, most of Antarctica remains in the grip of continental ice sheets, with only about 0.2% of its overall area being ice-free. The continent's terrestrial fauna consists only of invertebrates, with just two native species of insects, the chironomid midges *Parochlus steinenii* and *Belgica antarctica*. We integrate ecophysiological information with the development of new high-resolution climatic layers for Antarctica, to better understand how the distribution of *P. steinenii* may respond to change over the next century under different IPCC climate change scenarios. We conclude that the species has the potential to expand its distribution to include parts of the west and east coasts of the Antarctic Peninsula and even coastal ice-free areas in parts of continental Antarctica. We propose *P. steinenii* as an effective native sentinel and indicator species of climate change in the Antarctic.

Antarctica and the sub-Antarctic islands are some of the last wilderness areas remaining on the planet. These remote areas remain, to a great extent, free from direct anthropogenic impacts such as overpopulation and over-exploitation of native ecosystems[1], although they are not immune to wider global anthropogenic processes such as climate change and long-range pollution[2,3]. The high latitude regions of the Antarctic Peninsula, Scotia Arc, and the Magellanic Sub-Antarctic have been amongst the most rapidly warming areas in the world in the second half of the Twentieth Century, showing significant glacier retreat and reduction of snow and ice cover in terrestrial and freshwater ecosystems[3]. While these strong regional warming trends have currently paused, they are predicted to resume through the remainder of the Twenty-first Century[4]. These regions are highly sensitive to environmental change and thus are considered natural laboratories in which to study its effects, at all scales, on their ecosystems and biota[3,5].

Today, Antarctica remains in the grip of continental ice sheets, with only about 0.2% of its overall area being ice-free[6], this proportion is somewhat higher in the Antarctic Peninsula region (~3%; British Antarctic Survey unpublished data, Lee *et al*., 2017). Terrestrial and freshwater ecosystems are generally small and isolated, populated by small invertebrates, lower plants, and microbes[7]. The terrestrial fauna consists only of invertebrates, with just two native species of insects, both chironomid midges (the winged *Parochlus steinenii* Gerke and the brachypterous *Belgica antarctica* Jacobs), and two established invasive species with currently restricted ranges, *Eretmoptera murphyi* (Diptera: Chironomidae) and *Trichocera maculipennis* (Diptera: Trichoceridae)[8].

Climatic gradients have changed over geological time at different spatio-temporal scales in these high latitude southern regions, shaping the composition and distribution of modern landscapes and their biota[3,9]. The Eocene marked the beginning of the cooling of the Southern Ocean (*ca*. 35 Ma). The gradual breaking of the link between

[1]Sub-Antarctic Biocultural Conservation Program, Universidad de Magallanes, Punta Arenas, Chile. [2]Millennium Nucleus of Invasive Salmonids (INVASAL), Concepción, Chile. [3]Institute of Ecology and Biodiversity (IEB-Chile), Santiago de Chile, Chile. [4]Laboratory for Research in Environmental Sciences (LARES), Faculty of Agricultural Sciences, Department of Environmental Sciences and Natural Renewable Resources, University of Chile, Santiago, Chile. [5]Department of Biological Sciences, University of North Texas, Texas, USA. [6]Department of Philosophy and Religion Studies, University of North Texas, Texas, USA. [7]British Antarctic Survey, Cambridge, UK. [8]These authors contributed equally: Tamara Contador and Melisa Gañan. ✉e-mail: tamara.contador@umag.cl; melysa_gm@yahoo.es

southern South America and the Antarctic Peninsula, around the same time, permitted initiation of circumpolar atmospheric and subsequently oceanic circulation patterns and progressively isolated Antarctic terrestrial habitats from potential sources of colonists from lower latitudes[10]. This, combined with continental cooling, led to the extinction of major groups of organisms, as well as evolutionary radiation amongst survivors (see Convey *et al*. (2018) for an overview of the history of the Antarctic terrestrial biota[11]). However, a rapidly growing body of molecular, phylogenetic, and classical biogeographic evidence strongly indicates that representatives of all extant higher taxonomic groups in Antarctica, including the native chironomid midges mentioned above, survived within the Antarctic continent throughout these environmental changes[12]. The isolation and fragmentation of species' populations in ice-free areas are amongst some of the evolutionary mechanisms leading to population structuring of contemporary Antarctic taxa[13]. This mosaic of spatial and temporal settings has led to the persistence of a unique biota with varying degrees of tolerance to environmental stresses.

Global climate change and insects in Antarctica. Climate change is a complex process involving changes in multiple environmental conditions[14], over a range of timescales, and is not simply an increase in temperature. Thus, the factors limiting a species' distribution can vary widely over space and time[5,10]. In this context, understanding how multiple environmental factors, both individually and in combination, influence species is essential for predicting how they will be affected over both contemporary and evolutionary timescales.

Ecological Niche Models (ENM) are increasingly used to evaluate the influence of climate change on distribution patterns[15–17]. These models aim to identify areas that are climatically analogous to those within the existing or realised niche of a species. In this context, the integration of life history information and studies on ecophysiology with the results obtained from ENMs provides an effective tool to better estimate and understand the biological consequences of global climate change. Experimental approaches are key to understanding the underlying processes causing biogeographic changes[18]. Therefore, the combination of spatial and physiological data provides an important tool to help identify sentinel species that may provide alarm indicators.

After the middle of the Twentieth Century, the maritime Antarctic experienced significant warming[3], causing deglaciation[19] and the appearance of new ice-free areas and freshwater habitats[20]. Maritime Antarctic lakes have experienced extremely rapid physical ecosystem change over the latter decades of the Twentieth Century[21], even magnifying the very rapid annual air temperature increases over the same period. The lakes, streams and terrestrial habitats that makeup Antarctica's land-based ecosystems are generally small and isolated, and many of their small invertebrates, lichens, and microbes are found nowhere else on Earth[7]. As Antarctica undergoes some of the most rapid changes worldwide in air temperature, glacial cover, and lake seasonality, these organisms are facing extreme changes in their environments.

Insects are very sensitive to temperature variation, which directly affects their growth rates and ability to survive in a given microhabitat, particularly when temperature variation exceeds their tolerance range[22]. Increasing temperatures influence many elements of insect life histories, from physiology, through development and voltinism, to population dynamics and range[23,24]. The phenology, voltinism patterns, and stress tolerances of insects are critical elements in assessing and predicting the consequences of environmental change in freshwater ecosystems, as well as for their surroundings[25].

Of the two native Antarctic species of holometabolous insects, the wingless *B. antarctica*, is endemic and widely distributed along the coast of the western Antarctic Peninsula and its offshore islands northwards from the northern tip of Alexander Island to the South Shetland Islands. In contrast the winged *P. steinenii*, while having limited Antarctic occurrence where it is only found on the South Shetland Islands in the maritime Antarctic, is also found on sub-Antarctic South Georgia, and through the Andes of southern South America to 41°S[26]. The larvae and pupae of *P. steinenii* are aquatic, and inhabit permanent lakes in the maritime Antarctic[27], while the winged adults are terrestrial.

Through this study we aim to integrate new and high-resolution modelling approaches with information on the ecology and physiology of *P. steinenii* to predict shifts in its distribution over the next century. To achieve this objective, we (a) characterize and analyze its present-day distribution across the South Shetland Islands, as well as assessing its ecophysiological characteristics in the laboratory, and (b) create specific climatic variables for the Antarctic at a fine spatial resolution in order to develop ecological niche models for the species, under different climate change scenarios.

Results

Characterization of present-day distribution and ecophysiological characteristics of *P. steinenii*. Based on our field observations, we confirmed 58 presence locations for the species in the South Shetland Islands and confirmed that the current distribution of *P. steinenii* is limited to the South Shetland Islands in the maritime Antarctic (Fig. 1). Nonetheless, the MaxEnt procedure identified the existence of further potential contemporary distribution areas in the Trinity Peninsula and James Ross Island, based on suitable climatic conditions. The lowest presence threshold (LPT) obtained was 0.25, and therefore the suitable habitat areas were reclassified into the following four levels: 0–0.25 (unsuitable); 0.26–0.50 (low suitability); 0.51–0.75 (moderate suitability); 0.76–1 (high suitability). The spatial point pattern analysis shows that *P. steinenii* has a significantly clustered distribution (nearest neighbor analysis with wrap-around edge correction, mean distance: 1.9 km, expected distance: 4.7 km, R: 0.40, Z: −8.79, $p < 0.0001$) throughout the South Shetland Islands (Supplementary Fig. S1). There is a higher density of presence occurrences in King George, Livingston and Deception Islands, which is confirmed by Ripley's function L(r)-r (Montecarlo Test, $p = 0.0093$) (Supplementary Fig. S2).

In terms of the Critical Thermal Limits of *P. steinenii*, our assessment of its temperature preferences showed that the average CTmin and CTmax were significantly different between each developmental instar (−2.0 °C,

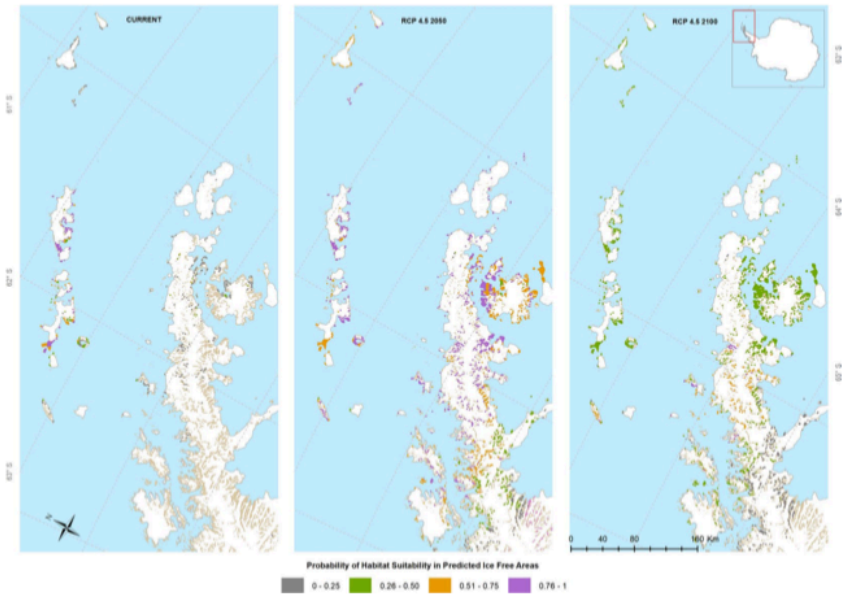


Figure 1. GDLF *Parochlus steinenii* Ecological Niche Model for current and projected distributions for 2050 and 2100 based on RCP 4.5 scenarios, predicted by MaxEnt. This map was created using ArcGIS® software by Esri. ArcGIS® and ArcMap™, ArcGis v.10.1. Base layers: Seamask_high_res_polygon, coastline_high_res_polygon and Rock_outcrop_high_res_polygon available from the Scientific Committee for Antarctic Research (SCAR) Antarctic Digital Database (ADD Version 7; http://www.add.scar.org). These data are licensed according to Creative Commons CC-By – data are free to use, modify and redistribute.

Instar	CT_{Min} (°C)			CT_{Max} (°C)			Thermal range
	Mean ± Std	Lower 95%	Upper 95%	Mean ± Std	Lower 95%	Upper 95%	
Larvae	-2.0 ± 0.8^{A}	−4.3	0.3	33.8 ± 0.6^{A}	30.2	32.7	35.8
Pupae	3.0 ± 1.1^{B}	0.7	5.3	27.5 ± 0.6^{B}	26.6	35.1	24.5
Adult	8.3 ± 1.1^{C}	6.1	10.6	31.4 ± 0.6^{C}	26.3	28.7	23.1

Table 1. Summary statistics of CT_{Min} and CT_{Max} of larvae, pupae and adults of *Parochlus steinenii*. ANOVA: $F = 21.2$, $df = 2$, $p < 0.0001$. Tuckey - Kramer HSD: Adult > pupae > larvae. ANOVA: $F = 27.2$, $df = 2$, $p < 0.0001$. Tuckey - Kramer HSD: Larvae > adult > pupae. $^{A, B, C}$ denote significantly different means (Tukey HSD, $P < 0.001$, α 0.05).

3.0 °C, and 8.3 °C; and 33.8 °C, 27.5 °C, and 31.4 °C, for larvae, pupae, and adults, respectively) (One-way ANOVA, $p < 0.0001$, α 0.05) (Table 1, Fig. 4A).

Ecological Niche Modelling of *P. steinenii* under different climate change scenarios. The results obtained in the null-models indicate that in all cases the previously generated models were significantly different from chance (see Supplementary Materials for Welch two sample T-test). All models had high AUC, Boyce and TSS values, ranging from 0.78–0.99. We selected the GDLF model (AUC 0.99; Boyce 0.91; TSS 0.93) as the best model to predict the potential distribution of *P. steinenii*, based on the present-day occurrence data, and the ecological and physiological information gathered during this study (Supplementary Table S1). Furthermore, the overlapping GCMs provide a good representation of the extent of each GCM and where they intersect, allowing us to better visualize that GDLF is present in all the possible intersections (see Supplementary Materials, Fig. 4 to 8). The smallest AICc value was $\beta = 1$ (see Table S2 in Supplementary Materials). Among the six environmental variables, Temperature Seasonality (Bio4) had the greatest contribution to the distribution model (51.2%) for *P. steinenii*, followed by the Mean Temperature of the Coldest Quarter (36.9%, Bio 11) and the Annual Precipitation (11.5%, Bio12). Together, these three factors explained 96% of the GDLF model distribution.

The Ecological Niche Model (ENM) for *P. steinenii* shows a good match between the species' present-day Antarctic distribution and the current information generated by the model (Fig. 1). The RCP 4.5 scenario,

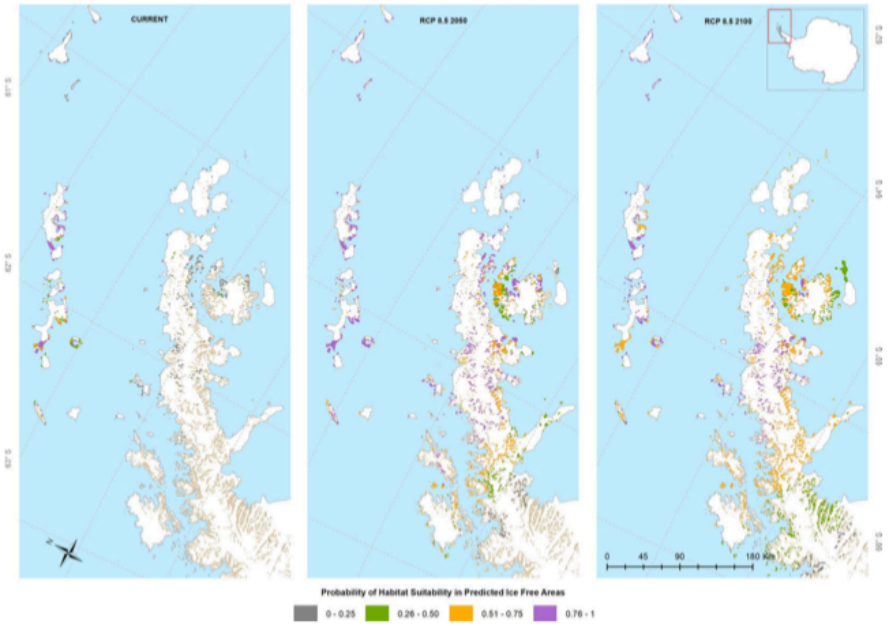


Figure 2. GDLF *Parochlus steinenii* Ecological Niche Model for current and projected distributions for 2050 and 2100 based on RCP 8.5 scenarios, predicted by MaxEnt. This map was created using software ArcGIS® software by Esri. ArcGIS® and ArcMap™, ArcGis v.10.1. Base layers: Seamask_high_res_polygon, coastline_high_res_polygon and Rock_outcrop_high_res_polygon available from the Scientific Committee for Antarctic Research (SCAR) Antarctic Digital Database (ADD Version 7; http://www.add.scar.org). These data are licensed according to Creative Commons CC-By – data are free to use, modify and redistribute.

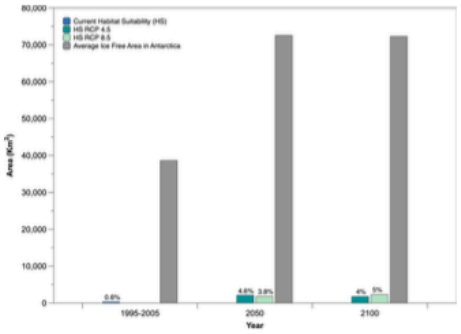


Figure 3. Habitat suitability for *Parochlus steinenii* under IPCC RCP4.5 and 8.5 scenarios in the Antarctic. This graph shows the area suitable for *P. steinenii* in its current distribution (0.6%) in comparison to the area expansion expected under the RCP 4.5 and 8.5 scenarios.

together with the projection of new ice-free areas (as predicted by Lee *et al.*)[28], show that for both 2050 and 2100 there are high probabilities of the midge expanding its distribution within the South Shetland Islands and into the northern Antarctic Peninsula (Fig. 1). Specifically, the model predicts that, by 2050, *P. steinenii* will maintain and increase its distribution range in the South Shetland Islands (current distribution area), but it could potentially be found in highly suitable habitats in Antarctic Conservation Biogeographic Region (ACBR) 3 - North-west Antarctic Peninsula[29]. Gibbs, Clarence and Smith Islands show high habitat suitability, as well as the west coast of James Ross and Vega Islands (0.76–1). Additionally, the east coast of Trinity Peninsula, along with Trinity Island, also present high habitat suitability under this scenario.

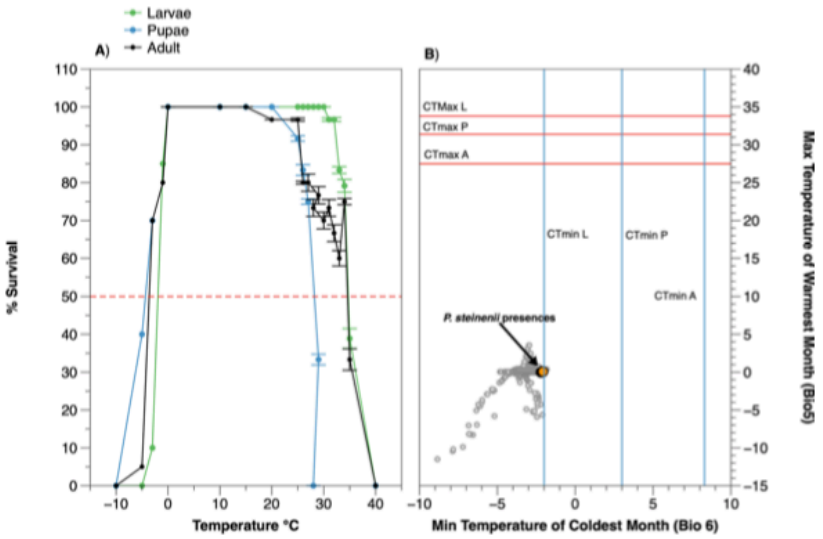


Figure 4. (**A**) Thermal tolerance curves of *Parochlus steinenii* larvae, pupae, and adults. The Critical Thermal Limit at which 50% of the individuals survive is shown by the red-dotted line (CT_{50}). (**B**) Niche space of *P. steinenii* within the minimum temperature of the coldest month (Bio5) and the maximum temperature of the warmest month (Bio6) (gray circles). The temperature envelope in which *P. steinenii* is found is shown (orange circles). The red and blue lines represent the physiological Critical Thermal Maximum (CTmax) and Critical Thermal Minimum (CTmin) for the larvae, pupae, and adult developmental stages of *P. steinenii*.

The model also shows a reduction in probability of suitability in Livingston Island, particularly in Bayer's Bay (0.51–0.75). At the same time Snow Hill Island, the east coast of James Ross Island and the Nordenskjold coast show moderate habitat suitability. The model also predicts expansion to Elephant Island (north-east of the species' current distribution) and to Anvers Island (south-west), with moderate habitat suitability. By 2100 the model predicts a decreased probability of habitat suitability within the South Shetland Islands in the species' current area of distribution, and parts of the Antarctic Peninsula (0.26–0.50). Nonetheless, high or moderate habitat suitability is maintained within Trinity Island and some areas of the west coast of Peninsula (Fig. 1).

The ENM for RCP 8.5 for 2050 shows a higher persistence of high habitat suitability within the South Shetland Islands, expanding to Smith, Clarence, Elephant and Gibbs Islands (Fig. 2). Highly suitable habitats appear on D'Urville, Dundee, and Bransfield Islands, as well as along the coast of Trinity Peninsula, James Ross Island. However, the probabilities of low suitability habitats existing further south around the Foyn Coast increase (to 0.26–0.50) towards 2100 (Fig. 2). It is also notable that, while the area of potential habitat suitability increases, the degree of suitability is predominantly in the moderate range (0.51–0.75), rather than the highly suitable range (0.76–1). Based on the model approach used here and the extent of ice-free areas, we calculated the total area of potential suitable habitat for this midge. The area of currently suitable habitat is 293 km^2, which represents 0.6% of the total ice-free area in the Antarctic. The GDLF ENM predicts that the suitable area will increase by 4.6% and 4% in 2050 and 2100, respectively, under RCP 4.5. Under RCP 8.5, the area will increase by 3.8% and 5% for these time periods (Fig. 3).

The plot of Minimum Temperature of the Coldest Month (Bio6), portrayed against the Maximum Temperature of Warmest Month (Bio5) (Fig. 4B), shows that the tolerance limits of *P. steinenii* lie towards the minimum critical thermal limit for the larvae. But, in general, the species' thermal tolerance range for all developmental stages is much wider and exceeds the higher temperature limits of the environmental temperature predicted by MaxEnt (Fig. 4B).

Methods

Study area and climate. The first stage of this study involved obtaining data across both small-scale microhabitat environmental gradients and larger scale gradients across the South Shetland Islands (63–64°S), the only part of the maritime Antarctic to which *P. steinenii* is native. Fieldwork was conducted during four austral summer seasons (2015/16, 2016/17, 2017/18, 2018/19) during expeditions organized by the Chilean Antarctic Institute (INACH). We surveyed ice-free areas on Deception, Livingston, Greenwich, Robert, Nelson and King George Islands (Fig. 5). These areas are characterized by a geomorphology which includes periglacial landforms, with numerous temporary shallow meltwater ponds and permanent lakes (typically smaller than 100 m^2), which are ice-covered for 9–10 months each year[30]. The highest elevations reach 167 m (Horatio Stump, Fildes Peninsula) and 266 m (Noel Hill, Barton Peninsula)[30]. Terrestrial habitats in the catchments are characterized by the presence of rich herb-moss and fellfield communities, including the grass *Deschampsia antarctica* and a

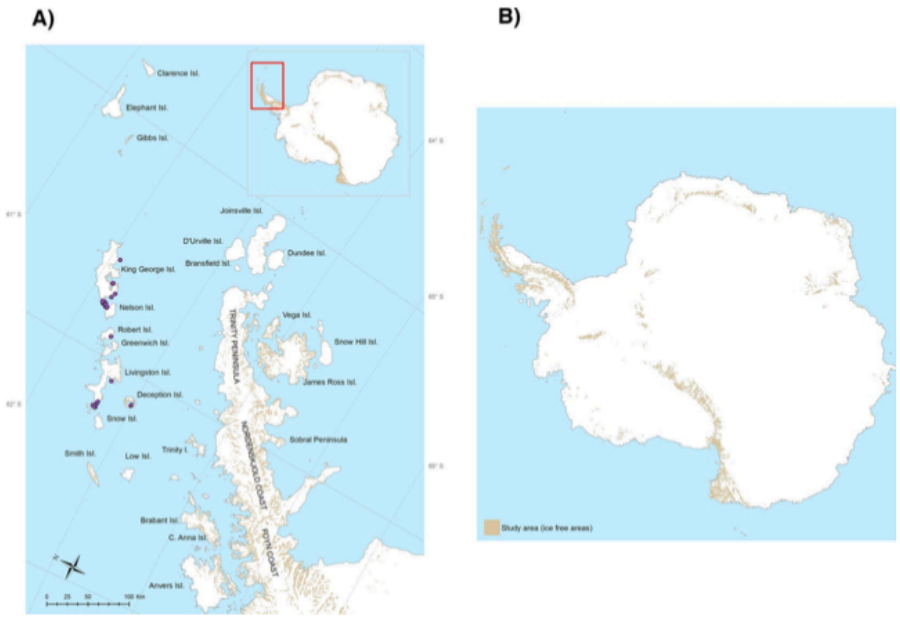


Figure 5. South Shetland Islands in the Maritime Antarctic. Sites assessed with confirmed presences of *Parochlus steinenii* are indicated with purple circles (58 presences in total) (**A**). The total extension of the study area encompasses the ice-free areas of the whole Antarctic continent (**B**). Ice free areas are shown in brown. This map was created using software ArcGIS® software by Esri. ArcGIS® and ArcMap™, ArcGis v.10.1. Base layers: Seamask_high_res_polygon, coastline_high_res_polygon and Rock_outcrop_high_res_polygon available from the Scientific Committee for Antarctic Research (SCAR) Antarctic Digital Database (ADD Version 7; http://www.add.scar.org). These data are licensed according to Creative Commons CC-By – data are free to use, modify and redistribute.

diverse moss and lichen community[31]. Ecophysiological and life history studies were conducted with populations of *P. steinenii* obtained only on King George Island (Fig. 5).

The climate in the South Shetland Islands is typical of the maritime Antarctic[32], and is characterized by average summer monthly air temperatures of 0–2 °C during December-March, annual precipitation of *c*. 460 mm and relative humidity of up to 95%[30]. During the Twentieth Century, the strongest warming extended from the southern part of the western Antarctic Peninsula north to the South Shetland Islands in the Peninsula region[33]. The magnitude decreased northwards, away from Faraday/Vernadsky in the Argentine Islands (ca. 65°S)[33], where the mean annual air temperature rose at a rate of 5.7 ± 2.0 °C per century over this period[34]. The warming trend was not consistent over the annual cycle, with the strongest warming recorded in the winter months, associated with large reductions in winter sea ice extent west of the Antarctic Peninsula, and weaker but still significant trends in the other seasons.

***Parochlus steinenii* present-day distribution and ecophysiology.** To characterize the present-day distribution of *P. steinenii* across the South Shetland Islands, we conducted intensive surveys through the ice-free areas accessed (Fig. 6, Phase 1) and sourced all available information from the existing literature[30,35–37]. All accessible sites were searched for a period of 4 to 6 h, defined by climatic conditions and the availability of logistic support. The fieldwork took place during the active season of the adult flies (austral summer), and we also searched for larvae and pupae in water and around the margins of the lakes accessed. We geo-referenced each location examined with a GPSmap 78sc Garmin© unit. To evaluate the thermal environment in which *P. steinenii* develops from egg to adult, we installed temperature data loggers (HOBO® U22 Water Temp Pro V2) in three lakes located on King George Island. These were installed on 8 February 2014 and continue to operate to the present day. These lakes were selected as they host a high abundance of *P. steinenii* and are easily accessible from the Chilean Estación Professor Julio Escudero.

To better understand the present-day distribution, we analyzed the data obtained using spatial point pattern analyses. Spatial point processes are stochastic models that serve as good tools for the analysis of patterns in populations and communities[38]. We conducted a large-scale spatial analysis of the distribution of *P. steinenii* using univariate spatial point process analyses using PAST software[39]. To evaluate the spatial distribution of *P. steinenii*, we used a Complete Spatial Randomness (CSR) model (H_o: *P. steinenii* has a random spatial distribution in the South Shetland Islands). In this model, spatial points are stochastic and independent, and 'intensity' is interpreted as the average density of points per unit area[40]. We used Ripley's K univariate analysis, with the total area of the

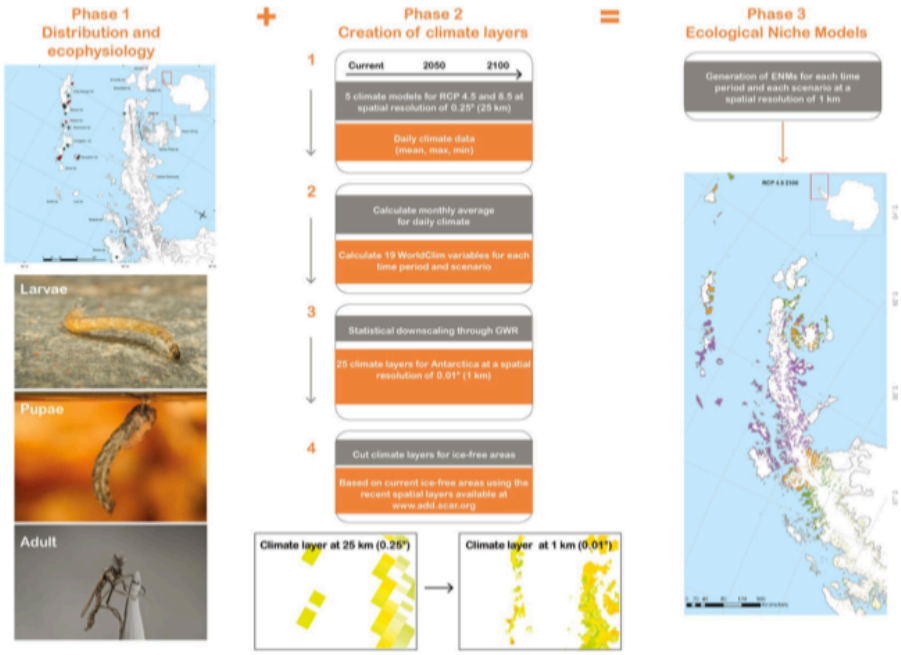


Figure 6. Methodological framework for this study. Phase 1 included assessment of occurrences of *Parochlus steinenii* in its Antarctic distribution, analysis of spatial distribution patterns and thermal ecophysiology of each developmental instar. Phase 2 consisted of the creation of climate layers for the Antarctic, through statistical downscaling, at a spatial resolution of 0.01 (1 km). Phase 3 represents the generation of Ecological Niche Models for each time period and scenario. Photographs of larvae, pupae, and adult Gonzalo Arriagada (CC-BY-4.0). The maps in this figure were created using software ArcGIS® software by Esri. ArcGIS® and ArcMap™, ArcGis v.10.1. Base layers: Seamask_high_res_polygon, coastline_high_res_polygon and Rock_outcrop_high_res_polygon available from the Scientific Committee for Antarctic Research (SCAR) Antarctic Digital Database (ADD Version 7; http://www.add.scar.org). These data are licensed according to Creative Commons CC-By – data are free to use, modify and redistribute.

islands explored. Results were analyzed using the $L(r) - r$ function, which is a transformation of the Poisson K function to a straight line, with a constant value = 0, making it easier to assess the deviation from the theoretical function[41]. Monte Carlo tests were conducted (with a 5% probability level) to compare the empirical and the theoretical functions, constructing envelopes under the CSR null hypothesis. The tests reject H_0 if the observed function lies outside of the critical envelope at any "r" distance value[42].

Ecophysiology: Critical thermal limits. Lower and upper thermal limits of *P. steinenii* were examined using the Critical Thermal Method (CTM), which involves changing temperature at a constant rate until a predefined sub-lethal endpoint (used to estimate lethality) is reached[43]. Larvae, pupae, and adults were collected with an aspirator from Lakes Kitiesh and Langer on King George Island. Live individuals were transported to the laboratory at Estacion Professor Julio Escudero (King George Island) within 2 h of collection. In the laboratory, individuals were acclimated at 8 °C for 24 h in a temperature-controlled cabinet in plastic containers with water, sediment, and small rocks from the collection sites. Larvae (only final instar), pupae and adults were used to conduct experimental assays in the laboratory.

Lower thermal tolerance. Six independent experimental assays for each developmental stage were conducted. Each assay contained 10 individual larvae, pupae, or adults. In the case of larvae and pupae, individuals were placed in plastic vials containing water and were submerged in a programmable, recirculating water bath (Lab Companion RW-0252G, Model AAH57003U, Biotech). Adults were placed in plastic containers, each containing a damp filter paper (to avoid desiccation stress). After a 60 min equilibration period at 0 °C, the specimens were cooled to −1 °C at a rate of 0.1 °C/minute. After 1 hour at this temperature, all individuals were removed from the bath and given 24 h to recover at 8 °C in aged tap water, with the exception of adult individuals, which were kept in damp paper towels. After recovery, the target temperature was subsequently lowered by 1 °C and the temperature again reduced at 0.1 °C/min, and the process repeated until the Critical Thermal Endpoints (CTE) was reached (lack of locomotory response to touch with forceps)[44]. The removal procedure was repeated during each trial (24 h recovery) and each individual was assessed for survival and motor function. Those individuals with full

motor function were retained for the subsequent trial. The lower sub-lethal temperature was considered to be the temperature after which survival was consistently less than 100% (see Klok and Chown, 2000)[45].

Upper thermal tolerance. Six experimental trials were conducted, each again containing 10 larvae, pupae, or adults. Individuals were gradually warmed at 0.1 °C min^{-1} from a starting temperature of 0 °C. This rate of increase needed to be sufficiently rapid to avoid acclimation, but slow enough to ensure that the core temperature reaction to heating was assessed by observing the behavioral response of the test organisms[43]. Individuals were checked for survival and locomotor function after each increase of 5 °C until reaching 25 °C, after which they were checked at every 1 °C interval. At each temperature checkpoint, observations of each organism were made. When an organism exhibited behavioral indications (lack of movement, lack of response to physical stimulation)[44] of reaching the critical thermal point, the temperature was recorded, and the organism was removed from the experimental chamber and placed in an aquarium container at 8 °C. Only organisms that recovered from the experimental exposure were included in the subsequent analyses. Successful recovery was defined as the resumption of normal locomotor functions after 24 h recovery time. Differences between the critical thermal limits of each developmental stage (larvae, pupae, and adults), were analyzed using One-Way ANOVAs using PAST software[39]. All experiments were conducted under permits issued by the Scientific Ethics Committee from Universidad de Magallanes and the Bioethics Committee of the Instituto Antártico Chileno (INACH), for INACH project RT_48_16.

Development of climatic variables for the Antarctic at a fine spatial resolution to develop Ecological Niche Models (ENM) for *Parochlus steinenii*. Following Duffy *et al.*[46], climatic data were downloaded from the NASA Earth Exchange Global Daily Downscaled Projections (NEX-GDDP; https://cds.nccs.nasa.gov/nex-gddp; dataset (Fig. 6, Phase 2). This array of data contains 21 models and climate scenarios at coarse spatial scale globally (spatial resolution of 0.25°, equivalent to 25 × 25 km). This scale is derived from the General Circulation Model (GCM) that is used under Phase 5 of the Coupled Model Intercomparison Project Phase 5 (CMIP5), which was developed with support of the Fifth Assessment Report of the Intergovernmental Panel on Climate Change (IPCC AR5). These 21 climate models include projections for two scenarios of Representative Concentration Pathways (RCP): RCP 4.5 and RCP 8.5. Each of these projections include daily temperature (minimum and maximum) and precipitation data from 1950–2100. RCP4.5 represents a scenario with a median increase of 2.4 °C set at 650 ppm CO_2, while RCP8.5 predicts 5 °C above pre-industrial temperatures, and 1370 ppm CO_2 by 2100[47].

In this study, we selected five of the 21 global climate models (ACCESS1.0, BNU-ESM, CESM1-BGC, CSIRO Mk3.6.0 y GFDL-ESM2M), based on those selected by Duffy *et al.*[46]. Because these five models were originally projected in World Geodetic System 1984 (WGS84), we selected and clipped our study area (the Antarctic Continent, the South Shetland Islands) and re-projected the layers to the Antarctic Polar Stereographic (EPSG 3031) Coordinate System Reference (CRS). Then we calculated monthly values for maximum and minimum temperature and precipitation for each of the scenarios (RCP 4.5 and RCP 8.5), obtaining a total of 25 models, as follows: five models for recent time (1996–2005) and 10 models each for 2050 (2046–2050) and 2100 (2096–2100). For each of the 25 models containing mean maximum, minimum and precipitation data, we built 19 analogous climatic variables derived from the WorldClim dataset[48], using the DISMO package in R[49].

Statistical downscaling from 25 km to 1 km spatial resolution. To increase the spatial resolution of the 19 climatic variables obtained (in each of the 25 models), we conducted a statistical downscaling process (Fig. 6, Phase 2). This process allowed us to increase the spatial resolution from 25 to 1 km, a more appropriate and accurate scale for the evaluation of impacts of climate change on natural habitats and biota[50]. Downscaling was achieved using a multivariable Geographically Weighted Regression (GWR) downscaling method. This type of method is useful in the development of spatio-temporal regressions affected by the phenomenon of parametric instability, providing suitable results to allow the generation of maps with the variables and parameters adjusted to different scales[51]. Other methods use simple regressions to better understand the behavior of spatial variables; nonetheless, the coefficients for such equations do not vary spatially. In this study, we used the Digital Elevation Model (DEM) for the Antarctic at 1 km resolution, which was obtained from the Combined ERS-1 Radar and ICESat laser Satellite Altimetry (available from NISIDC's FTP site: ftp://sidads.colorado.edu/pub/DATASETS/DEM/nsidc0422_antarctic_1km_dem/). The spatial coefficients obtained through the GWR were interpolated using the Inverse Distance Weighted (IDW) interpolation to the 4th power in order to apply them to the DEM predictor. All statistical analyses were conducted using the R packages Hexbin, hydroGOF, Topmodel and GWmodel. For more details on the downscaling methodology and the GWR, see Fotheringham *et al.*[51], and Morales *et al.*[52].

From the distribution data obtained in the field, we obtained a total of 58 confirmed occurrence sites for *P. steinenii* (Fig. 6, Phase 3). Climate suitability for *P. steinenii* was modelled under current and future climates through the application of ENMs. To provide a limit to the true distribution of *P. steinenii*, the spatial background used for this study was the current ice-free areas[28] (Fig. 5A,B), using the recent spatial layers available in the Scientific Committee for Antarctic Research (SCAR) Antarctic Digital Database (ADD Version 7; http://www.add.scar.org). To avoid co-linearity between the 19 WorldClim variables obtained, we ran a correlation test using ENMTools software[53], avoiding the incorporation of pairs of colinear bioclimatic variables (Pearson's $r \geq 0.7$). Using this procedure, the following variables were selected: (1) Annual Mean Temperature (Bio1), (2) Temperature Seasonality (standard deviation *100) (Bio4), (3) Max Temperature of Warmest Month (Bio5), (4) Min Temperature of Coldest Month (Bio6), (5) Mean Temperature of Coldest Quarter (Bio11), and (6) Annual Precipitation (Bio12). The ENMs for *P. steinenii* were calculated in the current period and projected to future scenarios (2050 and 2100 for RCP 4.5 and RCP 8.5). Each model was adjusted using maximum entropy algorithms in MaxEnt 3.33e software[54]. The MaxEnt software has been frequently used to simulate shifts in species ranges under

current and future climate scenarios[55]. It is based on a probabilistic framework, assuming that the incomplete empirical probability distribution (based on species presences), can be approximated by a probability distribution of maximum entropy, which represents a species' potential geographic distribution[56]. The MaxEnt approach has a better performance for datasets based on a limited number of occurrences[57,58], with a combination of high spatio-temporal predictions[59]. The regularization multiplier used for modelling was $\beta = 1$, this was decided after comparing the models obtained with different β values (0.25; 0.5; 0.75; 1.0; 1.25; 1.5; 1.75; 2.0). To achieve this, we used the corrected Akaike information Criterion (AICc) available in the software ENMTOOLS version 1.4.4[53]; and the smallest AICc value was considered[60]. We used 75% of the available data as training data, and the remaining 25% were used to evaluate the model with 50 replicates and 10,000 pseudo-absences. To quantify the predictive performance of presence-only models, we performed null-models to assess if the models developed differ significantly from those that would be expected by chance. To achieve this, we used the methodology proposed by Raes and ter Steege[61]. To choose the most adequate model we used the indices AUC[62], True Skills Statistics (TSS)[63] and Boyce[17,64]. We also used the confirmed contemporary distribution and ecophysiological data obtained in order to select the best ENM for *P. steinenii* in its Antarctic distribution. TSS and Boyce values can range from -1 to $+1$ where the value of $+1$ indicates perfect model performance, a value ~ 0 is not better than random[63], and negative values indicate reverse models. AUC values range from 0 to 1, and a value of 0.5 or below indicates that the model is not better than random[17,64]. Furthermore, we overlapped the GCMs to better visualize the intersections between the models and provide a better view on the effects of the different GCMs. The smallest AICc value was $\beta = 1$ (see Table 2 in Supplementary Materials). MaxEnt also computes response curves showing how the predictions depend on the variables, which greatly facilitates the interpretation of a species' ecological niche and its defining or limiting environmental factors[56]. To assess how the prediction relates to the ecophysiology of the species under the different climate scenarios, we plotted Bio5 and Bio6 with the Critical Thermal Limits obtained for *P. steinenii*. Finally, we assigned habitat suitability levels by choosing the "lowest presence threshold" (LPT). The LPT is conservative, as it identifies a) pixels predicted as being at least as suitable as those where a species' presence has been recorded, and b) the minimum predicted area possible whilst maintaining zero omission error in the training data set[65]. From the LPT, four suitability habitat probability levels were derived: unsuitable, low suitability, moderate suitability, high suitability. Finally, we created maps of the ENMs using ArcMap 10.1 and QGIS v2.18 "Las Palmas" (QGIS Development Team 2016. QGIS Geographic Information System. Open Source Geospatial Foundation Project. http://qgis.osgeo.org).

Discussion

In this study, we aimed to better understand how the native winged midge, *Parochlus steinenii*, may respond to climate change in Antarctica, by combining and integrating physiological and ecological information along with an extensive compilation of the species' current distribution in its native Antarctic range.

First, we identified that the distribution of *P. steinenii* was better explained by temperature seasonality (Bio4) and the mean temperature of the coldest quarter (Bio11). Insects are generally sensitive to thermal variation. Accumulating evidence suggests that metabolism-linked changes in aquatic insect phenology may affect the synchronization between life history stages and the availability of food or habitat, leading to temporal de-coupling that in turn could result in population crashes or extinctions[23]. Bradie and Leung[66] showed that, from 400 distinct environmental variables in 2040 ENMs, the most important variables explaining the distribution of the class Insecta were temperature, precipitation, and habitat patchiness.

The ENM obtained for the current distribution of *P. steinenii* is limited to ACBR 3, and represents well the species' present day Antarctic distribution, as confirmed by our field campaigns and the available literature[29,35,67]. The prediction also includes a small proportion of currently unoccupied suitable habitat in the northern Antarctic Peninsula, particularly on James Ross Island. Nonetheless, there is no evidence from paleolimnological studies of *P. steinenii* occurring in this area, at least since LGM ice retreat[68,69]. From our projections, we can conclude that the area of suitable habitat for *P. steinenii* will increase by 4.6% and 4% (for scenarios RCP4.5 and 8.5 by 2050, respectively, including expansion into ACBR 1. By 2100, our models predict that suitable habitat will increase by 3.8% and 5%, respectively, with a potential expansion in distribution to include ACBRs1, 2 and 3.

How might *P. steinenii* colonize these new suitable habitats in the Antarctic Peninsula and elsewhere in the continent, especially given that paleolimnological evidence suggests that the species has not previously occurred in these areas[68,70]? Dispersal is a major life history trait[71] and is particularly important in changing and extreme environments, and in areas where landscapes are becoming increasingly fragmented, where movement between local populations plays a vital role in the persistence and the dynamics of entire meta-populations[71]. In this context, our study considers a theoretical full-dispersal scenario. In this, dispersal across the Antarctic could be facilitated by either natural or human-induced actions, with species from adjacent areas crossing biogeographic boundaries and establishing in new ecosystems. When long distances have to be covered, as is the case with movement into Antarctica from lower latitudes, and even from the sub-Antarctic islands, such movements are today generally mediated by human activity[72]. *P. steinenii* has a patchy and fragmented distribution and, although it is a flying insect, its dispersal across larger regions may be limited by abiotic factors such as the strong winds and cold temperatures of the Antarctic. In this context, natural dispersal from the South Shetland Islands to other regions may be limited, unless it is facilitated by other vectors such as birds (e.g. skuas) or human transport. The latter in particular may inadvertently move organisms far beyond their natural dispersal ranges[73]. Human activity (i.e. movement by cargo, ship, aircraft and overland travel) in the Antarctic has a substantial potential for transporting species from one biogeographic region to another. Thus, although *P. steinenii* has not been historically found on the mainland of Antarctica, it could potentially expand its distribution to ACBRs located in the Antarctic Peninsula (ACBRs 1, 2, and 3). This becomes particularly plausible for these ACBRs, as they include some of the areas with the highest indices of human footprint and activity/connectivity between each other[73,74].

If such transfer does occur, by whatever means, will *P. steinenii* be able to persist in these new regions? Our data on the species' thermal physiology and habitat preferences, along with the high reproductive output reported by Hahn and Reinhardt[30], and the lack of native potentially competing or predatory species in the new regions, suggest that this species could rapidly colonize habitats that become available. In general, climatic regimes influence species distributions, often through species-specific physiological thresholds of temperature and precipitation tolerance[75]. Here, consistent with the results reported by Shimada *et al.*[76] we found that *P. steinenii* is intolerant to freezing and is currently living near its coldest temperature limit, but otherwise has a wide thermal tolerance range in all of its life stages (Fig. 4B). It is found in terrestrial and aquatic environments, depending on life stage. The larvae and pupae are aquatic and inhabit deeper permanent lakes, while adults are terrestrial and are found in very high abundances and density (~600–800 individuals cm^{-2}) during the Antarctic summer at the edge of lakes and streams[27,77], where copulation and oviposition occur. According to our field observations and to Hahn and Reinhardt[30], *P. steinenii* is likely to mate multiple times. This would suggest that there is a high probability that any dispersing adult females have an adequate sperm supply to found new populations in new suitable habitats. Furthermore, the recent increase in air temperatures in the Maritime Antarctic, especially during winter[34], combined with increasing precipitation[3], will possibly alter the duration and thickness of ice cover on freshwater lakes, as well as water level variability[21]. If the predictions generated by recent climate models are correct, freshwater ecosystems on the Antarctic Peninsula may be harshly affected[21,78], thus affecting the persistence of *P. steinenii* in its current distribution. In this context, *P. steinenii* can be taken as an effective sentinel of climate change in Antarctic terrestrial and aquatic ecosystems, as fluctuations in the thermal environment may significantly impact its current distribution, leading to important ecosystem changes in the Antarctic regions in which it is found.

Data availability

The climate layers generated during the current study are available from the corresponding authors on request. All occurrence data for *Parochlus steinenii* will also be able available at GBIF (www.gbif.org).

Received: 11 April 2019; Accepted: 6 January 2020;
Published online: 03 June 2020

References

1. Mittermeier, R. A. *et al.* Wilderness and biodiversity conservation. *Proc. Natl. Acad. Sci. USA* **100**, 10309–13 (2003).
2. Bargagli, R. *Antarctic Ecosystems Environmental Contamination, Climate Change, and Human Impact*. (2005).
3. Turner, J. *et al.* Antarctic Climate Change and the Environment - A contribution to the international polar year 2007-2008. (Scientific Committee on Antarctic Research (2009).
4. Turner, J. *et al.* Absence of 21st century warming on Antarctic Peninsula consistent with natural variability. *Nature* **535**, 411–415 (2016).
5. Peck, L. S., Convey, P. & Barnes, D. K. Environmental constraints on life histories in Antarctic ecosystems: tempos, timings and predictability. *Biol. Rev. Camb. Philos. Soc.* **81**, 75–109 (2006).
6. Burton-Johnson, A., Black, M., Fretwell, P. T. & Kaluza-Gilbert, J. An automated methodology for differentiating rock from snow, clouds and sea in Antarctica from Landsat 8 imagery: a new rock outcrop map and area estimation for the entire Antarctic continent. *Cryosph.* **10**, 1665–1677 (2016).
7. Convey, P. Antarctic Ecoystems. in *Encyclopedia of Biodiversity* (ed. Levin, S.) 22, 735–740 (Academic Press (2013).
8. Chown, S. L. & Convey, P. Antarctic Entomology. *Annu. Rev. Entomol.* **61**, 119–137 (2016).
9. Norris, R. D. *et al. Marine Ecosystem Responses to Cenozoic Global Change*. **2311**, 2306–2311 (2013).
10. Convey, P. *et al.* The spatial structure of antarctic biodiversity. *Ecol. Monogr.* **84**, 203–244 (2014).
11. Convey, P. *et al.* Ice - Bound Antarctica: Biotic consequences of the shift from a temperate to a polar Climate. in *Mountains, Climate and Biodiversity* (eds. Hoorn, C., Perrigo, A. & Antonelli, A.) 355–374 (John Wiley & Sons Ltd (2018).
12. Allegrucci, G., Carchini, G., Convey, P. & Sbordoni, V. Evolutionary geographic relationships among orthocladine chironomid midges from maritime Antarctic and sub-Antarctic islands. *Biol. J. Linn. Soc.* **106**, 258–274 (2012).
13. Chown, S. L. & Convey, P. Spatial and temporal variability across life's hierarchies in the terrestrial Antarctic. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* **362**, 2307–2331 (2007).
14. Helmuth, B., Kingsolver, J. & Carrington, E. Biophysics, physiological ecology, and climate change: does mechanism matter? *Annu. Rev. Physiol.* **67**, 177–201 (2005).
15. Wang, R. *et al.* Modeling and mapping the current and future distribution of Pseudomonas syringae pv. actinidiae under climate change in China. *PLoS One* 1–21, https://doi.org/10.1371/journal.pone.0192153 (2018).
16. Sillero, N. What does ecological modelling model? A proposed classification of ecological niche models based on their underlying methods. *Ecol. Modell.* **222**, 1343–1346 (2011).
17. Boyce, M., Vernier, P., Nielsen, S. & Schmiegelow, F. Evaluating resource selection functions. *Ecol. Modell.* **157**, 281–300 (2002).
18. Acevedo, P., Jiménez-Valverde, A., Aragón, P. & Niamir, A. New developments in the study of species distribution. in *Current Trends in Wildlife Research. Wildlife Research Monographs* (eds. Mateo, R., Arroyo, B. & Garcia, J.) (Springer, Cham (2016).
19. Cook, A., Poncet, S., Cooper, A. & Herbert, D. Christie Glacier retreat on South Georgia and implications for the spread of rats. *Antarct. Sci.* **22**, 255–263 (2010).
20. Nędzarek, A. & Pociecha, A. Limnological characterization of freshwater systems of the Thomas Point Oasis (Admiralty Bay, King George Island, West Antarctica). *Polar Sci.* **4**, 457–467 (2010).
21. Quayle, W. C. *et al.* Ecological responses of maritime antarctic lakes to regional climate change. *Antarct. Res. Ser.* **76**, 335–347 (2003).
22. Sinclair, B. J., Vernon, P., Klok, C. J. & Chown, S. L. Insects at low temperatures: An ecological perspective. *Trends Ecol. Evol.* **18**, 257–262 (2003).
23. Parmesan, C. & Yohe, G. A globally coherent fingerprint of climate change impacts across natural systems. *Nature* **421**, 37–42 (2003).
24. Wei, J., Zhao, Q., Zhao, W. & Zhang, H. Predicting the potential distributions of the invasive cycad scale Aulacaspis yasumatsui (Hemiptera: Diaspididae) under different climate change scenarios and the implications for management. *PeerJ* **6**, e4832 (2018).
25. Woodward, G., Perkins, D. M. & Brown, L. E. Climate change and freshwater ecosystems: impacts across multiple levels of organization. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* **365**, 2093–106 (2010).
26. Chown, S. L. & Gaston, K. J. Macrophysiology - progress and prospects. *Funct. Ecol.* **30**, 330–344 (2016).
27. Gañán Mora, M., Contador, T. A. & Kennedy, J. H. La vida en los extremos: el uso de SIG para estudiar la distribución dela mosca antártica alada, *Parochlus steinenii* (Diptera: Chironomidae), en las Islas Shetland del Sur (Antártica marítima). *Análisis Espac. y Represent. geográfica innovación y Apl.* 1599–1608 (2015).

28. Lee, J. R. *et al.* Climate change drives expansion of Antarctic ice-free habitat. *Nature* **547**, 49–54 (2017).
29. Terauds, A. & Lee, J. R. Antarctic biogeography revisited: updating the Antarctic Conservation Biogeographic Regions. *Divers. Distrib.* **22**, 836–840 (2016).
30. Hahn, S. & Reinhardt, K. Habitat preference and reproductive traits in the Antarctic midge *Parochlus steinenii* (Diptera: Chironomidae). *Antarct. Sci.* **18**, 175 (2006).
31. Casanova-Katny, M. A. & Cavieres, L. A. Antarctic moss carpets facilitate growth of Deschampsia antarctica but not its survival. *Polar Biol.* **35**, 1869–1878 (2012).
32. Volonterio, O., P de Leon, R., Convey, P. & Krzeminska, E. First record of Trichoceridae (Diptera) in the maritime Antarctic. *Polar Biol.* **36**, 1125–1131 (2013).
33. Turner, J. *et al.* Antarctic climate change during the last 50 years. *Int. J. Climatol.* **25**, 279–294 (2005).
34. Vaughan, D. G. Recent trends in melting conditions on the Antarctic Peninsula and their implications for ice-sheet mass balance and sea level. *Arctic, Antarct. Alp. Res.* **38**, 147–152 (2010).
35. Wirth, W. & Gressitt, J. L. Diptera: Chironomidae (midges). *Antarct. Res. Ser.* **10**, 197–203 (1967).
36. Toro, M. *et al.* Limnological characteristics of the freshwater ecosystems of Byers Peninsula, Livingston Island, in maritime Antarctica. *Polar Biol.* **30**, 635–649 (2006).
37. Rico, E. & Quesada, A. Distribution and ecology of chironomids (Diptera, Chironomidae) on Byers Peninsula, Maritime Antarctica. *Antarct. Sci.* **25**, 288–291 (2013).
38. Baddeley, A. Analysing spatial point patterns in R. *Work. Notes* **12**, 1–199 (2008).
39. Hammer, O., Harper, D. & Ryan, P. PAST: Paleontological Statistics software package for education and data analysis. *Palaeontol. Electron.* **4**, 9 (2001).
40. Baddeley, A. & Turner, R. Modelling spatial point patterns in R. in *Case Studies in Spatial Point Pattern Modelling* (eds. Baddeley, A., Gregory, P., Mateu, M., Stoica, J. & Stoyan, D.) 310 (Springer. https://doi.org/10.1007/0-387-31144-0_2 (2006).
41. Baddeley, A. Handling shapefiles in the spatstat package v1.36-0. CRAN Vignettes 1–9 (2014).
42. Baddeley, A. Practical maximum pseudolikelihood for spatial point patterns. *Aust. New Zeal. J. Stat.* **42**, 283–322 (2000).
43. Dallas, H. F. & Rivers-Moore, N. Critical Thermal Maxima of aquatic macroinvertebrates: towards identifying bioindicators of thermal alteration. *Hydrobiologia* **679**, 61–76 (2011).
44. Ernst, M. R. *et al.* Stream Critical Thermal Maxima of nymphs of three Plecoptera species from an Ozark Foothill Stream. *Freshw. Invertebr. Biol.* **3**, 80–85 (1984).
45. Klok, C. & Chown, S. L. Critical thermal limits, temperature tolerance and water balance of a sub-Antarctic kelp fly, Paractora dreuxi (Diptera: Helcomyzidae). *J. Insect Physiol.* **47**, 95–109 (2001).
46. Duffy, G. A. *et al.* Barriers to globally invasive species are weakening across the Antarctic. *Divers. Distrib.* **23**, 982–996 (2017).
47. Nolan, C. *et al.* Past and future global transformation of terrestrial ecosystems under climate change. *Science (80-.).* **361**, 920–923 (2018).
48. Hijmans, R., Cameron, S., Parra, J., Jones, P. & Jarvis, A. Very high resolution interpolated climate surfaces for global land areas. *Int. J. Climatol.* **25**, 1965–1978 (2005).
49. Hijmans, R., Phillips, S., Leathwick, J. & Elith, J. dismo: Species distribution modeling. R package version 1.0. Retrieved from https:// CRA (2015).
50. Leihy, R., Duffy, G., Nortje, E. & Chown, S. Data descriptor: High resolution temperature data for ecological research and management on the Southern Ocean Islands. *Sci. Data* **5**, 1–13 (2018).
51. Fotheringham, A., Charlton, M. & Brunsdon, C. Two techniques for exploring non-stationarity in geographical data. *Geogr. Syst.* **4**, 59–82 (1997).
52. Morales-Salinas, L. *et al.* A simple method for estimating suitable territory for bioenergy species in Chile. *Cienc. e Investig. Agrar.* **42**, 227–242 (2015).
53. Warren, D. L., Glor, R. E. & Turelli, M. ENMTools: a toolbox for comparative studies of environmental niche models. *Ecography (Cop.).* **1**, 607–611 (2010).
54. Phillips, S., Anderson, R. & Schapire, R. Maximum entropy modeling of species geographic distributions. *Ecol. Modell.* **190**, 231–259 (2006).
55. Wei, J., Zhao, Q., Zhao, W. & Zhang, H. Predicting the potential distributions of the invasive cycad scale Aulacaspis yasumatsui (Hemiptera: Diaspididae) under different climate change scenarios and the implications for management. *PeerJ* https://doi.org/10.7717/peerj.4832 (2018).
56. Buermann, W. *et al.* Predicting species distributions across the Amazonian and Andean regions using remote sensing data. 1160–1176. https://doi.org/10.1111/j.1365-2699.2007.01858.x (2008).
57. Elith, J. *et al.* A statistical explanation of MaxEnt for ecologists. *Divers. Distrib.* **17**, 43–57 (2011).
58. Hernandez, P., Graham, C., Master, L. & Albert, D. The effect of sample size and species characteristics on performance of different species distribution modeling methods. *Ecography (Cop.).* **5**, 773–785 (2006).
59. Heikkinen, R. K., Marmion, M. & Luoto, M. Does the interpolation accuracy of species distribution models come at the expense of transferability? *Ecography (Cop.).* **35**, 276–288 (2012).
60. Morales, N. S., Fernández, I. C. & Baca-González, V. MaxEnt's parameter configuration and small samples: are we paying attention to recommendations? A systematic review. *PeerJ* **5**, e3093 (2017).
61. Raes, N. & Ter Steege, H. A null-model for significance testing of presence-only species distribution models. *Ecography (Cop.).* **30**, 727–736 (2007).
62. Thuiller, W., Lavorel, S. & Araújo, M. B. Niche properties and geographical extent as predictors of species sensitivity to climate change. 347–357. https://doi.org/10.1111/j.1466-822x.2005.00162.x (2005).
63. Allouche, O., Tsoar, A. & Kadmon, R. Assessing the accuracy of species distribution models: prevalence, kappa and the true skill statistic (TSS). *J. Appl. Ecol.* **43**, 1223–1232 (2006).
64. Hirzel, A. H., Le Lay, G., Helfer, V., Randin, C. & Guisan, A. Evaluating the ability of habitat suitability models to predict species presences. *Ecol. Modell.* **9**, 142–152 (2006).
65. Pearson, R., Raxworthy, C., Nakamura, M. & Peterson, T. Predicting species distributions from small numbers of occurrence records: a test case using cryptic geckos in Madagascar. *J. Biogeogr.* **34**, 102–117 (2007).
66. Bradie, J. & Leung, B. A quantitative synthesis of the importance of variables used in MaxEnt species distribution models. 1–18. https://doi.org/10.1111/jbi.12894 (2016).
67. Convey, P. & Block, W. Antarctic Diptera: Ecology. *physiology, and distribution. Eur. J. Entomol.* **93**, 1–13 (1996).
68. Gibson, J. A. E. & Zale, R. Holocene development of the fauna of Lake Boeckella, northern Antarctic Peninsula. *The Holocene* **5**, 625–634 (2006).
69. Gibson, Ja. E., Cromer, L., Agius, J. T., Mcinnes, S. J. & Marley, N. J. Tardigrade eggs and exuviae in Antarctic lake sediments: insights into Holocene dynamics and origins of the fauna. *J. Limnol.* **66**, 65–71 (2007).
70. Hodgson, D. A., Convey, P., Hodgson, D. A. & Convey, P. *A 7000-year Record of Oribatid Mite Communities on a Maritime-Antarctic Island: Responses to Climate Change A 7000-year Record of Oribatid Mite Communities on a Maritime-Antarctic Island: Responses to Climate Change*. **37**, 239–245 (2005).
71. Lakovic, M., Poethke, H. & Hovestadt, T. Dispersal Timing: Emigration of Insects Living in Patchy Environments. *PLoS One* **10**, 1–15 (2015).

72. Frenot, Y. *et al.* Biological invasions in the Antarctic: extent, impacts and implications. *Biol. Rev.* **80**, 45–72 (2005).
73. Hughes, K. A. *et al.* Human-mediated dispersal of terrestrial species between Antarctic biogeographic regions: A preliminary risk assessment. *J. Environ. Manage.* **232**, 73–89 (2019).
74. Pertierra, L., Hughes, K., Vega, G. & Olalla-Tárraga, M. A. High resolution spatial mapping of human footprint across Antarctica and its implications for the strategic conservation of avifauna. *PLoS One* **12**, e0168280, https://doi.org/10.1371/journal.pone.01 (2017).
75. Walther, G. *et al.* Ecological responses to recent climate change. *Nature* **416**, 389–395 (2002).
76. Shimada, K., Ohyama, Y. & Pan, C. Cold-hardiness of the Antarctic winged midge *Parochlus steinenii* during the active season at King George Island. *Polar Biol.* **11**, 311–314 (1991).
77. Rauschert, M. Beobachtungen an der Chironomide Parochlus steineni auf der Insel King George (Sudshetlandinseln, Antarktis). *Dutsch.ent.Z.N.F* **32**, 183–188 (1985).
78. Quayle, W. C. *et al. Extreme Responses to Climate Change in Antarctic Lakes Published by: American Association for the Advancement of Science Linked references are available on JSTOR for this article: Extreme Responses to Climate Change in Antarctic Lakes.* **295**, 645 (2002).

Acknowledgements

This project was funded by Chile's National Fund for Scientific and Technological Development (FONDECYT), the Chilean Antarctic Institute (INACH) and CONICYT PIA Apoyo CCTE (projects 11130451, RT_48_16, and AFB170008, respectively). P.C. was supported by NERC core funding to the British Antarctic Survey (BAS) 'Biodiversity Evolution and Adaptation' Team. F.S. was supported by BAS and the Department of Zoology, University of Cambridge. We thank Simón Castillo, Ramiro Bustamante, Gilliam Graham and Gonzalo Arriagada for their help in the different stages of this project. Gonzalo Arriagada produced the photographs of *Parochlus steinenii* larvae, pupae and adult used in Fig. 6. Climate data were downloaded from the NASA Earth Exchange Global Daily Downscaled Projections (NEX-GDDP; https://cds.nccs.nasa.gov/nex-gddp; Thrasher, Maurer, McKellar, & Duffy, 2012). The rock_outcrop_high_res_polygon, coastline_high_res_line, and seamask_high_res_polygon layers were downloaded from the Antarctic Digital Database (ADD version 7; http://www.add.scar.org). The authors thank the reviewers who helped to greatly improve this manuscript.

Author contributions

T.C., R.R., J.H.K. and P.C., conceived the ideas for this project. T.C., M.G., J.R., F.S., conducted field and laboratory work. M.G., G.B., G.F.-J. and L.M., generated the climate data, and designed and undertook the modelling. T.C. analyzed the data and led the writing with contributions from all authors.

Competing interests

The authors declare no competing interests.

Additional information

Supplementary information is available for this paper at https://doi.org/10.1038/s41598-020-65571-3.

Correspondence and requests for materials should be addressed to T.C. or M.G.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.