AN INVESTIGATION ON INTERTIDAL ZONE AMPHIPOD CRUSTACEANS ON THE COASTS OF THE SOUTH SHETLAND ISLANDS AND ANTARCTIC PENINSULA WEST COAST ISLANDS USING DNA BARCODING METHOD

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ABSTRACT

AN INVESTIGATION ON INTERTIDAL ZONE AMPHIPOD CRUSTACEANS ON THE COASTS OF THE SOUTH SHETLAND ISLANDS AND ANTARCTIC PENINSULA WEST COAST ISLANDS USING DNA BARCODING METHOD

After Gondwana's breakup, Antarctica got isolated as a continent resulting in the endemicity of the organisms, well-adapted to their extreme habitats. Antarctic Peninsula is vulnerable to climate change and environmental stressors, including tourism and fisheries, among others. Species that might not adapt to such environmental changes can face extinction. Studies indicate that invertebrates comprise 97% of animal species and are the most vulnerable. They need to be represented more since most studies focused on charismatic species like sea birds, mammals, and fishes. Species identification and distribution are crucial for conservation initiatives. Since traditional taxonomy requires time and expertise, barcoding is utilized to identify species and estimate the species diversity of cryptic and error-prone groups. We precisely utilized the COI barcode for the "reverse taxonomy" approach to identify and estimate phylogeographic relationships of Antarctic amphipods collected during Turkish Antarctic expeditions in 2017, 2018, and 2019. We observed *Bovallia gigantea*, *Vibilia antarctica*, *Eurymera monticulosa*, *Gondogeneia antarctica*, *Cheirimedon femoratus*, *Hippomedon kergueleni*, *Prostebbingia brevicornis*, *Orchomenella rotundifrons,* and *Paramoera walkeri.* A non-target sequence was amplified and found to be related to *Shewanella.* An individual was assigned to the *Paraceradocus* genus based on morphology. We could not identify four OTUs at the species level through DNA barcoding. The next step of this study is to collaborate with Antarctic amphipod taxonomists to determine if these morphotypes are new species or belong to species that have already been defined. Despite ongoing taxonomic research on Antarctic amphipods, more barcodes still need to be uploaded to BOLD and GenBank.

ÖZET

GÜNEY SHETLAND ADALARI VE ANTARKTİKA YARIMADASI BATI KIYISI ADALARINDA GEL-GİT ZONU AMFİPOD KRUSTASE FAUNASININ DNA BARKODLAMA YÖNTEMİ KULLANILARAK ARAŞTIRILMASI

Gondwana'nın ayrılmasından sonra çevresinden izole olan Antarktika, ekstrem habitatlara adapte olabilen endemik organizmaların bu bölgede türleşerek yayılmasına sebep olmuştur. Antarktika Yarımadası, turizm, balıkçılık vb. olmak üzere çevresel stres faktörlerine ve özellikle de hızlı iklim değişikliğine karşı en dayanıksız bölgelerden biridir. Bu değişimlere adapte olamayan türler ise yok olma tehlikesiyle karşı karşıyadır. Hayvan biyoçeşitliliğinin %97'sini oluşturan omurgasızların yok oluş karşında daha savunmasız organizmalar olduğu, biyoçeşitlilik çalışmalarında daha çok temsil edilmesi gerektiği, ancak koruma çalışmalarının karizmatik türlere (deniz kuşları, memeliler ve balıklar gibi) odaklandığı düşünülmektedir. Türlerin tanımlanması ve dağılımlarının tespitinin hızlanarak devam etmesi, koruma girişimleri için çok önemlidir, ancak geleneksel taksonominin zaman ve uzmanlık gerektirmesinden "barkodlama" yaklaşımı benimsenmeye başlanmıştır. Çalışmamızın odak grubu Antarktik amfipodlarda kriptik türleşme yaygın görüldüğünden tür tayini için COI sekansının barkodlanarak veri tabanlarıyla karşılaştırılmasını ve bu eşleşmelerin morfolojik incelemelerle doğrulanmasını içeren "tersine taksonomi" yöntemi kullanılmıştır. Bu çalışmada, Türkiye Ulusal Antarktika Seferleri'nde (2017, 2018, 2019) toplanan amfipod örneklerini tanımlamak için COI dizileri kullanılmıştır. Çalışmanın sonucunda *Bovallia gigantea, Vibilia antarctica, Eurymera monticulosa, Gondogeneia antarctica, Cheirimedon femoratus, Hippomedon kergueleni, Prostebbingia brevicornis, Orchomenella rotundifrons, Paramoera walkeri* gözlemlenmiştir. Bir bireyde Shewanella cinsiyle benzerlik görülmüştür. Operasyonel taksonomi birimlerden (OTU) dört tanesinin tür bazında tayini genetik ya daha morfolojik karşılaştırmalarla yapılamamıştır ancak birinin *Paraceradocus* cinsinden olduğu tespit edilmiştir. Tanımlanamamış örneklerin yeni türler mi yoksa önceden tanımlanmış fakat henüz barkodlanmamış türler mi olduğuna Antarktik ampfipodlar ile çalışmakta olan taksonomistler ile yapılacak iş birliği ile karar verilecektir. Antarktik amfipodlarda taksonomik çalışmalar devam etse de BOLD ve Genbank'ta barkodu yüklenmemiş türler mevcuttur.

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LIST OF SYMBOLS/ABBREVIATIONS

Symbol	**Explanation**
µl	Microliter
π	Nucleotide Diversity
Abbreviation	**Explanation**
ABBED	Admiralty Bay Benthos Diversity Database
ACC	Antarctic Circumpolar Current
ASAP	Assemble Species by Automatic Partitioning
DNA	Deoxyribonucleic Acid
DNTP	Deoxyribonucleotide Triphosphate
A.P.	Antarctic Peninsula
APF	Antarctic Polar Front
MarBOL	Marine Barcode of Life
CAML	The Census of Antarctic Marine Life
COI	Cytochrome oxidase 1
BOLD	Barcode of Life
iTOL	Interactive Tree Of Life
WoRMS	World Register of Marine Species
NCBI	National Center for Biotechnology Information
h	Number of Haplotypes
IUCN	International Union for Conservation of Nature
K	Average Number of Nucleotide Differences
KGI	King George Island
mtDNA	Mitochondrial Deoxyribonucleic Acid
n	Number of Tissue Samples
nM	Nanomolar
µM	Micromolar
PCR	Polymerase Chain Reaction
PopART	Population Analysis with Reticulate Trees
STF	Subtropical Front

1. INTRODUCTION

1.1. Geography and Biodiversity of Antarctica and Antarctic Peninsula

The idea persists that the Antarctic biota has been isolated from the rest of the world ever since Gondwana split. The Southern Ocean, one of the largest marine ecosystems on the globe, surrounding Antarctica is also hydrologically separated due to the deep flow of the Antarctic Circumpolar Current (ACC) from the Atlantic, Pacific, and Indian Oceans (Clarke et al., 2005; Rintoul et al., 2001). According to geological records, ACC is formed following the division of the Gondwana and isolation of the Antarctic Continent from Australia and Antarctic Peninsula South America in the Oligocene period, about 25 million years ago. Circumpolar thermal fronts, such as the Subtropical Front (STF) and Antarctic Polar Front (APF) (Figure 1.1.) (Dunn et al., 2017), delineate the transition zones between Antarctic, sub-Antarctic, and temperate waters, and first appeared with the advent of the Antarctic Circumpolar Current (ACC). Strong, circumpolar winds and currents encourage dispersal to the east, but impedes the north-south movement of water compounding the effects of steep temperature gradients and limited water exchange across these zones as dispersal barriers which causes distinguishing differences in biogeography through vicariance (Chown et al., 2015; Cowman and Bellwood, 2013; Fraser et al., 2012; Leese et al., 2010; Lefebvre et al., 2012) culminating in observation of species-level endemism of marine fauna. The APF is a significant obstacle to faunal interchange, although it is not an such as strong barrier in mid water and deep water. Dispersal over the APF has been postulated for several marine taxa, including molluscs, echinoderms, and crustaceans, according to phylogeographic studies (Chown et al., 2015). Since Antarctica has no landmass connected to it, its massive ice sheet can be preserved because the ACC keeps warm ocean waves away from the continent and under extreme conditions (IPCC, 2022). Scientists and environmentalists are particularly interested in Antarctica; the continent is one of the fastest warming regions on the planet. Global warming causes glacier loss on the continent, driving climatic alterations and acidification of the oceans. This is caused by increased carbon dioxide, another threat to this unique continent and its endemic fauna (IPCC, 2022; McNeil and Matear, 2008).

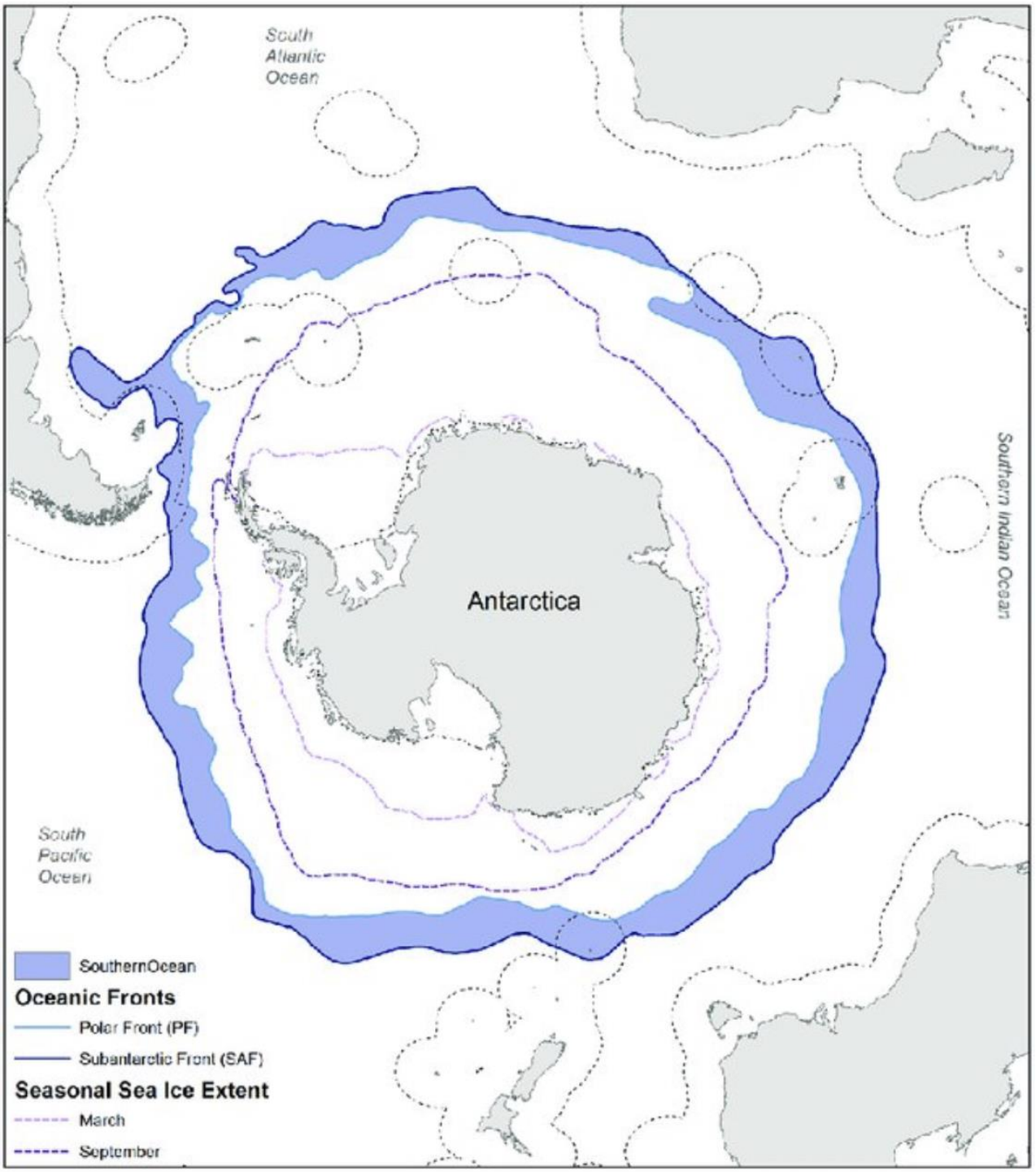


Figure 1.1. The diagram that shows that Antarctic Circumpolar Current is bounded by Polar Front and Subantarctic Front (Dunn et al., 2017).

Due to the harshness of the Antarctic environment, unique characteristics and evolutionary innovations are expected for the organisms to survive and thrive, while those without the adaptations die off (DeVries and Wohlschlag, 1969; Giordano et al., 2012). And indeed, early investigations of biodiversity in the continent revealed a diverse and abundant fauna, contrary to predictions. This diversity does not apply to all taxa, however, with some groups having radiated widely while others are underrepresented or absent entirely (Chown et al., 2015; Janosik and Halanych, 2010). Some taxonomic groups such as bryozoans, sponges, and amphipods are rather high in species richness, while species richness in some other groups such as gastropod and bivalve mollusks, and isopods are lower in the Southern Ocean than in comparable shelf regions elsewhere (Chown et al., 2015). Furthermore, several fish and decapod crustaceans are totally missing, although being known from the Eocene fossil record, they are possibly non-adaptive to this harsh environment (Chown et al., 2015; Grant and Linse, 2009). Although invertebrates have persisted for millions of years in refugia in the harsh Antarctic terrestrial environments, they are now restricted to a small number of ice-free areas. A biodiversity pump was created in the region as a result of repetitive ice scouring on the Antarctic continental shelf throughout glacial maxima which obligate the marine fauna continually adapt to changing habitat (Convey and Peck, 2019; Pugh and Convey, 2008). Needs to adapt specific ecological requirements, and geographic constraints of these invertebrate species cause significantly less gene flow across populations during glacial maxima, resulting in significant genetic differences without a change in morphology and cryptic speciation (Convey and Peck, 2019; Giordano et al., 2012; Grant and Linse, 2009; Kapralova et al., 2011).

The Antarctic Peninsula (AP), Scotia Arc, South Georgia Islands, and the South Sandwich Islands form one of the most productive parts of the Southern Ocean. AP is the continent's northernmost part that stretches approximately 1,500 kilometers toward the tip of South America (National Geographic Society accessed 25-07-2022; Barnes et al., 2009). Due to the strong natural barrier provided by ACC (Barnes et al., 2009), a significant percentage of unique and endemic flora and fauna are found in these waters. AP is also home to a high breeding population of penguins, seals, and whales, and is thought to contain 75% of the krill population that surrounds Antarctica (National Geographic Society accessed 25-07-2022). Antarctica has already a fragile and delicate environment, and besides AP is affected by increasing human activities and global warming more than any other region on the earth (Vaughan et al., 2003). The West AP, which is the geographic region of interest in this study, is undergoing one of the fastest environmental changes currently in the world (Siegert et al., 2019). The most important factors of

this change are temperature increase and its inevitable result of meltdown of the glaciers and decrease of the sea-ice cover.

Species with long generation cycles cannot adapt to rapid climate change because the environment changes faster than their offspring generation. Those species that cannot adapt to environmental changes have one other chance: migration. However, Antarctica is quite isolated from its surroundings; thus, migration from the continent is extremely challenging. To protect marine and terrestrial biodiversity in the region, there is a great need for knowledge about species living in the region (Baird et al., 2011; Bellard et al., 2012; Clarke et al., 2007; Convey and Peck, 2019; Flores et al., 2012). Since the climate is changing day by day the studies should also be undertaken at a high pace. Otherwise, many species might go extinct without their discovery (Peck and Welch, 2004; Wilson et al., 2007).

There are some initiatives to understand marine biodiversity at a global scale. The Census of Marine Life (CoML; www.coml.org) has been a global endeavor that aims to provide a 10-year synthesis of the status of marine biodiversity on the planet from 2000 to 2010. The Census of Antarctic Marine Life (CAML; http://www.caml.aq) is the Antarctic component of CoML and the mission of CAML is to "survey the distribution of Antarctica's rich marine biodiversity in order to develop a baseline for human benefit." In addition, another aim of the CAML initiative was to contribute Barcode of Life (BOLD – www.barcodinglife.org) project, which produces genetic catalogs of species worldwide (Grant et al., 2011; Grant and Linse, 2009).

1.2. DNA Barcoding

Taxonomy is a biological discipline with the aim to identify, describe and name organisms, which provides baseline data essential for many other biological disciplines. In the past centuries, the identification of organisms was only based on their morphological features, such as size, shape anatomy, coloration etc. Traditional taxonomy takes too much time and effort, and it requires expertise, and the number of available taxonomists is not enough to identify new species in a short timespan because there are so many species, but so little time to protect biodiversity. Traditional taxonomy can also fail when it comes to morphologically indistinguishable (cryptic) taxa, quite often identified as the same species. The discovery of molecular techniques helped the taxonomists to identify species faster and help detect and solve cryptic speciation (Hebert et al., 2003; Hupało et al., 2019; Ratnasingham and Hebert, 2013).

DNA barcoding is a technique of identifying species based on a short, standardized piece of the genome as a "biomarker." DNA is used by taxonomists, ecologists, conservation biologists, and regulatory agencies, among others, for helping protect the environment (Baird et al., 2011; Hebert et al., 2003). It is a useful method for assessing undiscovered biodiversity, facilitating population differentiation, speciation, and phylogeographic investigations, as well as elucidating taxonomy and identification of species (Grant et al., 2011; Grant and Linse, 2009). DNA barcoding methods, undertaken either with Sanger sequencing or Next Generation Sequencing approaches, facilitate the cataloging of biodiversity in the world and the discovery of new species. In this approach, one or more DNA markers representing a certain group are usually sequenced and documented as a species barcode (e.g., cytochrome oxidase 1(COI) for animals, 16S for bacteria and archaea, 18S for fungi). As of August 24, 2022, 10.580.183 barcodes were uploaded to The Barcode of Life Data System (BOLD) (Ratnasingham and Hebert, 2013). The barcoding approach aims to help with:

1) Species cataloging: Known type of a specimen's sequence can be entered into BOLD as a new DNA barcode of that species.

2) Species detection: If the sample's phylogenetic category is unknown, this sample's barcode sequence is compared with the other sequences in the BOLD database to ascertain the species.

3) Species discovery: As in the species detection, the sequences of the samples are compared with sequences previously uploaded to BOLD, and if the sequence does not match with any known sequence, the conclusion is that more comprehensive phenotypical investigations are needed to identify possible new species (Hajibabaei et al., 2007, 2006).

DNA barcoding, on the other hand, should not be viewed as a substitute for traditional taxonomy; its primary function is to serve as an accessible label by connecting barcodes to thoroughly defined voucher specimens. Barcoding has its limitations for identification as well: barcoding accuracy appears to be dependent on taxonomic expertise and the sample group's coverage (Havermans et al., 2011; Meyer and Paulay, 2005). Species identification with barcoding has difficulties caused by incomplete lineage sorting, genetic introgressions, pseudogenes, and bacterial infections (Buhay, 2009). In addition, some species have been investigated far more extensively than others. A high number of samples have been obtained of a small number of huge, charismatic megafaunal species, such as sea birds, sea mammals and fishes, or for specific taxa, such as chordates, whereas invertebrates such as ascidians, crustaceans are underrepresented (Griffiths, 2010; Griffiths et al., 2009; Monsarrat and Kerley, 2018).

1.3. Crustaceans (Arthropoda: Crustacea: Amphipoda)

Invertebrates are an animal group that has not developed vertebral columns. They contain around 97% of animal species Invertebrates have major roles on terrestrial and marine environments, and they can be used for monitoring environmental change (Collier et al., 2016; Karam-Gemael et al., 2020; Salvador et al., 2021). The identification of the invertebrates is important for scientific questions in ecology or practical applications such as pest control in farming, prevention of parasitic disease, and conservation biology (Collier et al., 2016). According to several scientists we are in the middle of the sixth extinction caused by anthropogenic activities. The species are being erased day by day, and most of the extinct animals will be invertebrates (Collier et al., 2016; Karam-Gemael et al., 2020). Since the conservation of species is only possible if the species, their amount, and their habitats are known, it is crucial to accelerate the taxonomic studies with the help of molecular methods to identify them before they become extinct.

Crustacea is a subphylum of the invertebrate phylum Arthropoda. The Crustacea has the greatest morphological diversity (with eight classes and 30 orders) found in any subphylum of animals, which comprises motivation for biologists their study as a group. Crustaceans have ecologically important roles, as smaller marine crustacean animals like amphipods serve crucial functions as food for bigger marine species. The majority of crustaceans are either parasites or scavengers (Havermans et al., 2018; Seefeldt et al., 2018). Terrestrial crustaceans frequently reside in wet, humid conditions on the ground, concealed by boulders or debris, and eat decomposing plants (Havermans et al., 2018; Seefeldt et al., 2018).

Amphipoda is one of the most diverse and abundant classes under phylum Arthropoda and subphylum Crustacea, which have approximately 10,000 documented species worldwide. In many freshwater and marine habitats, amphipods are "keystone" species and frequently the most ubiquitous macroinvertebrates. They play a significant role in the waste processing in these habitats, producing significant volumes of excrement and fine organic matter particles that are consumed by other invertebrates (Havermans et al., 2010).

1.4. Importance and DNA Barcoding of Antarctic Amphipods

More than 500 species of amphipod crustaceans (Arthropoda: Crustacea: Peracarida: Amphipoda) are found in Antarctica (Havermans et al., 2011). They are often found in the Southern Ocean's deep and shallow water populations (Havermans et al., 2018). Many fish living in the Antarctic and Subantarctic waters feed primarily on amphipods, which are both benthic and pelagic in nature (Dauby et al., 2003). Pelagic ones are prey also for several seabird species and a few species of squid (Dauby et al., 2003; Elliott et al., 2008; Mehlum and Gabrielsen, 1993).

The Antarctic marine macroinvertebrate fauna has an astonishing high species richness and high rate of endemism due to the Antarctic Circumpolar Current's isolating/insulating influence. Significant number of macroinvertebrates are the amphipods, which have high genetic diversity in Antarctic marine fauna. Lysianassoidea is a superfamily one of the key amphipod taxa in terms of species diversity, abundance, and ecological significance in the area (De Broyer et al., 2004; Havermans et al., 2018; Seefeldt et al., 2018). Many of the Lysianassoidea scavengers are known to aggregate in large numbers on massive corpses and can consume in a short time (hours or days). Meantime they provide a major food source for higher taxa, for instance, birds, fishes, and mammals (Seefeldt et al., 2018)

Species identification of amphipods, especially of lysianassoids is error-prone and their phenotypic taxonomy is remarkably controversial due to their conservative and frequently minor morphological diagnostic features. Meanwhile, correct species identifications are vital for a variety of downstream assessments, including or depending on ecological and biodiversity data (Seefeldt et al., 2018). Therefore, genetic methods such as DNA barcoding are extremely useful in systematics and phylogenetics studies related to this group. Molecular studies on interspecies and within-species variation concentrated on peracarid crustaceans, including the Amphipoda, have stepped up in recent years. While recent studies indicate the presence of cryptic speciation, species complexes and new species have been clarified and identified (D'Udekem d'Acoz and Verheye, 2017; Havermans et al., 2018, 2011, 2010).

Tphe amphipod species that have been barcoded thus far are sublittoral or deep-sea species (Grant et al., 2011) in Antarctica, however, investigations of amphipod species diversity using the DNA barcoding method are rare in the tidal zone (0-3 m depth) habitats in Antarctica. Even though genetic studies have gained pace in Antarctica, the BOLD database indicates that many isopod and amphipod groups in the tidal zone have not been sequenced yet. The current situation strengthens the possibility of discovering new species in this region, which will help highlight and preserve Antarctic biodiversity.

1.5. Aim of the Project

The vast majority of the barcoded amphipod species are sampled from subtidal or deep sea (see e.g., Lörz, 2010; Havermans et al., 2010, 2011, 2018; Baird et al., 2011; d'Udekem d'Acoz and Verheye, 2017, d'Udekem d'Acoz et al., 2018). In this perspective, the proposed study will be the first focused on DNA barcoding of amphipods species diversity in the tidal zone (0-3 m depth) habitats in Antarctica. Moreover, the first findings of the amphipod fauna of the Nansen and Robert Islands will be obtained within the scope of the project. Our project will fill the gap of barcodes of amphipods in many tidal zones that have not been barcoded until now. Among these species, there will probably be new species to be discovered by this project.

2. LITERATURE REVIEW

Genetic methods have provided breakthroughs in research into the biology of many species. Developments in polymerase chain reaction, universal primers, and sequencing technology (Sanger, ddRAD seq, RNA sequencing) provide to answer many questions in biology. The choice of gene locus is essential to the efficacy of DNA barcoding, and analyses of mitochondrial and nuclear DNA facilitated the investigation of subjects such as a study of kinship and species identification and has led to the discovery of phenotypically unnoticed "cryptic" species (Johnson and Cicero, 2004; Lee and Foighil, 2004; Narang et al., 1993; Rocha-Olivares et al., 1999; Wilcox et al., 1997). Barcoding methods have been used in animal behavioral studies, speciation and hybridization, and population genetic structure analysis. Mitochondrial DNA is more advantageous for species identifications since the evolution rate is faster than other genes. COI has been the most extensively used as a universal marker in the taxonomy of the animal kingdom (Hebert et al., 2003)

A reliable reference library of well-known taxa is an essential need for efficient identification by using DNA barcoding. The quality of the reference library and the extensive coverage is important for the accuracy of the detection of the species. The Barcode Index Number (BIN) system (Ratnasingham and Hebert, 2013), which serves as a tool for prospective species detection, complements the Barcode of Life Data System (BOLD, www.boldsystems.org), which aims to provide barcodes for all currently extant organisms. As of September 2022, more than 6000 papers have been published for the discovery and identification of the species, and 10.6 million COI barcodes for animal identification are stored in BOLD System (BOLD, www.boldsystems.org). GenBank® is a main public repository of the genetic sequence of the National Institute of Health as a part of the International Nucleotide Sequence Database Collaboration and has a highly integrated data exchange system with BOLD that allows automatic data submission (Benson et al., 2012).

Since the first identification of an amphipod species in the early 1900s (Coleman, 2015) the rate at which new species have been discovered has gradually increased, with a mean of over one hundred taxa described per annum since the 1960s (Horton et al., 2020; Jażdżewska et al., 2021). If the rate of discovery over the past sixty years keeps up, there could be as many as 8,000 new species documented

by the year 2100. It is estimated by the more cautious sources that will be about 6.100m (Jażdżewska et al., 2021).

In 2009, Grant and Linse published a study highlighting the severe dearth of barcoding research in Antarctica, notably for marine invertebrates, which account for the largest proportion of Antarctic biodiversity. According to the study, there is less than one hundred cytochrome c oxidase (COI) gene sequences for marine invertebrates that are available in the public domain. Only two phyla were represented in this study's barcodes: crustaceans and mollusks, and the majority of the sequences originated from the Weddell Sea and the Antarctic Peninsula. Additionally, the geographic coverage has expanded with the studies of different research groups, and it now encompasses a more significant proportion of the continental shelf that surrounds the Antarctic continent (Grant and Linse, 2009). During International Polar Year 2008/2009, access to an extensive dataset suitable for DNA barcoding has been made possible through the cooperation of the Census of Antarctic Marine Life (CAML), the Marine Barcode of Life, and the Canadian Centre for DNA Barcoding. CAML has made it possible for the scientific community to begin significant DNA barcoding studies to record the genetic diversity of Antarctic marine animals. Grant et al. reports on the existing geographic and taxonomic coverage of DNA barcode data in the Southern Ocean and highlight the remaining gaps in this information (Grant et al., 2011).

Marine organisms were sampled for molecular barcoding at more than 2.000 locations in the Southern Ocean during the sampling program of CAML (The Census of Antarctic Marine Life), which contributed significantly to MarBOL (Marine Barcode of Life) (Grant et al., 2011). The objective of the MarBOL project is sequencing 50.000 marine species. Under this project, 18.270 species out of 192.000 (~ 9.5%) of marine metazoan species, which are already identified, were sequenced. CAML program's contribution to MarBOL was approximately 13% of till ending in December 2010 (Bucklin et al., 2011). The intensive sampling within CAML spans a broad geographic area from Scotia to East Weddell Sea, the Ross Sea, and Dumont D' Urville Sea in the Antarctic. However, many points have not yet been sampled around, particularly East Antarctic Region, the Bellingshausen Sea, and the Amundsen Sea. Although through these initiatives barcoded Antarctic marine species increased from 432 to 20.355 from 2007 to 2011 (Grant et al., 2011; Grant and Linse, 2009), several marine groups do not have barcode sequences: Calcareans (Porifera), Stauromedusae (Cnidaria), Pterobranchia (Hemichordata), Ciliophorans (Alveolate), Dinoflagellata (Myzozoa), Monoplacophora and Aplacophora (Mollusca), Priapulida, Protozoa, Tardigrada, and Zooflagellata (Grant et al., 2011).

The World Amphipoda Database feeds data into the World Register of Marine Species (WoRMS), the global standard in its field as the most extensive reference material for reliable information on marine species. Scientists, consultants, conservationists, journalists, the general public, and others utilize the information frequently accessed via various databases fed by WoRMS. The most comprehensive background study data of the amphipod fauna in Antarctica was obtained by scientists working in the Arctowski Station of Poland and Comandante Ferraz Antarctic Station of Brazil. The Admiralty Bay Benthos Diversity Database website (ABBED, www.abbed.uni.lodz.pl; Seefeldt et al., 2018) lists 172 amphipod species belonging to six families. In the first amphipod inventory for the sublittoral zone of Marian Bay, Antarctica one of the fjord-like coves in Maxwell Bay, 22 amphipod species from 12 families were recorded (Havermans et al., 2010).

Genetic approaches, such as DNA barcoding, are immensely helpful in amphipod taxonomy and phylogeny investigations due to the controversies in amphipod morphological taxonomy (Havermans et al., 2010; Radulovici et al., 2009). Therefore, researchers have been focusing more on the molecular characteristics of peracarid crustaceans, such as the Amphipoda, both at the intra- and inter species levels. Species complexes have been revealed by DNA barcoding, and new species are regularly discovered thanks to these efforts (Havermans et al., 2018; Leese et al., 2010; Lörz et al., 2012; Raupach et al., 2010). The BOLD database reveals that majority of the Antarctic isopods and amphipod species that are the focus of our study have not yet been sequenced, even though genetic studies on peracarid crustaceans have increased in the region recently. Therefore, there is a good chance that new species to science are waiting to be discovered in the Antarctic Peninsula.

In the past years, evidence for cryptic speciation has been recorded in ophiuroids (Hunter and Halanych, 2008), crinoids (Wilson et al., 2007), and pycnogonids (Krabbe et al., 2010) in Antarctica. Peracarid crustaceans, especially amphipods and isopods, are ideal candidates to investigate genetic variation in the Antarctic continental shelf since they are abundant and repeatedly cited as examples for spotlight characteristics such as gigantism and endemism (Havermans et al., 2010). Since they do not have an independent larval stage in their development, they can spread only in a limited manner, and this pattern leads to frequent intraspecific genetic variation in *Peracarida* (Bucklin et al., 2011; Havermans et al., 2011, 2010) a potential group to discover cryptic species in Antarctica.

DNA barcoding is utilized to understand distribution and diversification of the species in addition to identification, and some of the key studies undertaken in Antarctica include those on *E. gryllus* (Havermans et al. 2013), genus *Eurythenes* (Havermans et al., 2016), *E. thurstoni, Epimeria cleo sp. nov*, E. *maldoror, E. gryllus s.s., and E. andhakarae*. The details of these key studies are described below.

Havermans et al. (2013) examined *Eurythenes gryllus* which was suggested as a genetically and morphologically diverse and cosmopolitan species. *E. gryllus* is a widespread amphipod that lives in the bathyal, abyssal, and hadal zones. Large-scale sampling and cutting-edge molecular approaches have only lately been applied to the question of the genetic connectedness of *E. gryllus*, notwithstanding research from two decades ago by France and Kocher (1996a, b) that prompted concerns regarding the global nature of its distribution. The global genetic diversity of the species from the Arctic, Atlantic, Pacific, and Southern Oceans were studied by utilizing nuclear (28S rDNA) and mitochondrial (COI, 16S rDNA) sequence data in a phylogeographic context (Havermans et al., 2013). Previous investigations found genetically and morphologically diverse lineages, disputing its global and eurybathic distribution. Havermans et al. (2013) also examined its genetic diversity at the world scale for the first time (*E. gryllus* has nine potential species-level clades, and bathyal and abyssal samples divide genetically at 3,000 m). Two bathyal and two abyssal lineages were common; and five were ocean basin specific. According to the depth-differentiation hypothesis, the deep sea's bathyal zone offers an exceptional seedbed for new species to emerge. Therefore, genetic differentiation was expected take place across quite small vertical distances, especially in the upper continental slope (200-1000 m), which was contradicted by the results that indicated the abyss's higher diversity. Despite the abyss' uniform habitat and absence of separating impediments, populations were seen to be more likely to diversify and speciate than expected. Bipolar bathyal clade specimens were most like 'genuine' *E. gryllus*, and the study provided first molecular evidence of bipolar distribution in a macrobenthic deep-sea organism.

Another publication of Havermans and collegues in 2016 was about investigation of additional materials from the well-known giant amphipod *Eurythenes*, which included samples from Antartica. Representatives of the genus *Eurythenes* have been discovered recently. Based on morphology and genetics, the genus *Gryllus* has been separated into twelve different species-level lineages, several of which have been designated distinct species. They discovered three new species-level lineages from various sample locations, suggesting that species diversity within *Eurythenes* can still increase with only a small amount of effort put into the sampling process. One species-level lineage was detected in the Indian Ocean and another in the Pacific Ocean; both lineages were later identified as belonging *to E.*

thurstoni. In addition to the three species previously reported from the Southern Ocean, a new bathyal species was discovered in the Weddell Sea of Antarctica (*E. maldoror, E. gryllus s.s., and E. andhakarae)*. The presence of *gryllus* was discovered in freshly tested locations near the Kerguelen Islands, as well as additional samples from the Svalbard Archipelago, confirming its status as an amphitropic species. The study is important since the regional and bathymetric distribution of numerous species were found and supported with molecular methods. It also emphasizes the importance of processing all individuals sampled due to the presence of two or more sympatric species in varying proportions, as well as the fact that results on diversity and distribution can alter dramatically when sampling intensity and coverage are increased.

Epimeria, a monophyletic and diverse group, referred to as a species flock (Lecointre et al., 2013; Lörz & Held, 2004) has also been recorded in the Antarctic. Coleman reclassified Antarctic *Epimeria* (2007), and Lörz (2009) and Lörz et al. (2007, 2009, 2012) described new species. Molecular and morphological investigations into the group revealed overlooked species (Lörz et al., 2012; Verheye et al., 2016). d'Udekem d'Acoz & Verheye (2017) revised Antarctic *Epimeria* using new data, which described 27 new species. Due to the significant number of morphologically similar *Epimeria* species and occasional sampling in the Southern Ocean, other species may yet be discovered. Verheye (2018) added additional *Epimeria* species from the Ross Sea to support this claim. The recent finding of an additional species of *Epimeria* with the discovery of *Epimeria cleo sp. nov.* brought the total number of *Epimeria* species found in the Ross Sea to 11. It is shown to be in the subgenus/clade *Drakepimeria*, together with *E. similis*, *E. macrodonta*, and as well as 11 species that look *like E. similis and E. macrodonta* were most recently outlined in the publication of d'Udekem d'Acoz and Verheye in 2017. d'Udekem d'Acoz and Verheye (2017) showed that *E. schiaparelli and E. reoproi* are also in the same clade *as E. macrodonta and E. similis* (D'Udekem d'Acoz and Verheye, 2017).

In 2018 Seefeldt et al. presented the results of a large-scale, integrative study of lysianassoid amphipods in Potter Cove on King George Island/ Isla 25 de Mayo (KGI) in Antartica, which used a combination of morphological and molecular species identification (COI barcoding) methods to analyze over 41,000 specimens collected in baited traps. Twenty-three hundred and nine specimens from neighboring Marian Cove were analyzed for comparison. Outer Potter Cove was home to ten different lysianassoid species, whereas *Cheirimedon femoratus* Pfeffer, 1888 (99.44% relative abundance) dominated the inner cove (50 m). In this study (Seefeldt et al., 2018). *Orchomenella pinguides* (Walker, 1903) and *Abyssorchomene charcoti* (Chevreux, 1922) were also detected in King George Island waters

for the first time. In addition, the lysianassoid amphipod species *Orchomenella infinita sp.* is reported as a new species (Seefeldt et al., 2017). *Cheirimedon femoratus*, *Hippomedon kergueleni* (Miers, 1875), and *Orchomenella rotundifrons* (K.H. Barnard, 1932) all acquired unique DNA barcodes for the first time.

Havermans et al. (2018) provides a comprehensive inventory of the amphipod scavenging fauna (Lysianassoidea) in the Filchner area, located in the southeastern part of the Weddell Sea. More than 4400 lysianassoids were assessed for species richness, abundance, and distribution at 20 different locations via a range of sample approaches, including the southernmost baited traps placed to this point (76°S). High species diversity was found, with 29 different species, five of which were undiscovered previously. During the course of this study, 109 cytochrome c oxidase I gene (COI) sequences were collected. These sequences were combined with sequences obtained from specimens gathered in other Antarctic regions in order to carry out molecular species delimitation methods. Distance-based analyses (trees and the Automatic Barcode Gap Discovery method) pointed to the existence of 42 lineages; for four of the species, multiple (cryptic) lineages were discovered. The species *Orchomenella pinguides* is accounted for more than 96% of the lysianassoids that were caught using baited traps.

Studies have successfully used DNA barcodes to identify marine amphipods in the Arctic (Tempestini et al., 2018), Antarctic (Havermans et al., 2011) Atlantic (Costa et al., 2009), and Pacific (Jażdżewska and Mamos, 2019). However, despite of all the barcoding efforts there is still enormous barcoding-gap according to Jażdżewska and colleagues (2021), who used amphipods as a model to detect the hiatus of the barcoding data in the two main repository NCBI GenBank and BOLD. The gap analysis performed by Jażdżewska and colleagues (2021) demonstrated that out of 25,702 records, only 10% of amphipod species are represented by barcodes, despite the fact that 3,835 BINs (identifying potential species) were recognized by BOLD. According to the quality control system of BOLD at least three sequences are needed for comparisons, however most BINs are not reliable because they are made up of a limited number of sequences, mostly singletons (Fontes et al., 2021; Jażdżewska et al., 2021; Oliveira et al., 2016; Seefeldt et al., 2018).

3. MATERIALS AND METHODS

3.1. Sample Collection

The amphipod samples to be examined in the scope of the project were collected by three different researchers from the South Shetland Islands and the islands on the west coast of the Antarctic Peninsula between 2017-2019. Some of the samples were collected from Bulgaria St. Kliment Ohridski base, located on Livingston Island in Antarctica, by Dr. Evrim Kalkan as part of the bilateral cooperation between Istanbul Technical University Polar Research Center (PolReC) and the Bulgarian Antarctic Institute (BAI) in January 2017 (Table 3.1., Figure 3.1). Another group of examples was collected under the responsibility of the Ministry of Science, Industry, and Technology and under the coordination of the Istanbul Technical University Polar Research Center (PolReC). They were collected from King George, Robert, Deception, and Nansen Islands by Kübra Karaman, who participated in the National Antarctic Science Expedition II (Table 3.1, Figure 3.1) in 2018. The remaining examples were collected in the National Antarctic Science Expedition III by Dr. S. Ünsal Karhan from King George and Nansen Islands and Ardley and Galíndez Islands in 2019 (Table 3.1, Figure 3.1). The amphipod specimens to be examined were collected directly from the coastal area, 0.5–3 m from the shoreline, by hand, occasionally using a hand scoop, and by free diving at one location (Galíndez Island). The habitat types the amphipod individuals were collected were recorded in detail, specifying the substrate structure, the position of the sampled individual on the ground, and the vegetation, if any, used as a habitat. All specimens were fixed using 96% ethanol. Fixed samples in sealed containers were labeled and brought to the laboratory for analysis without any damage.

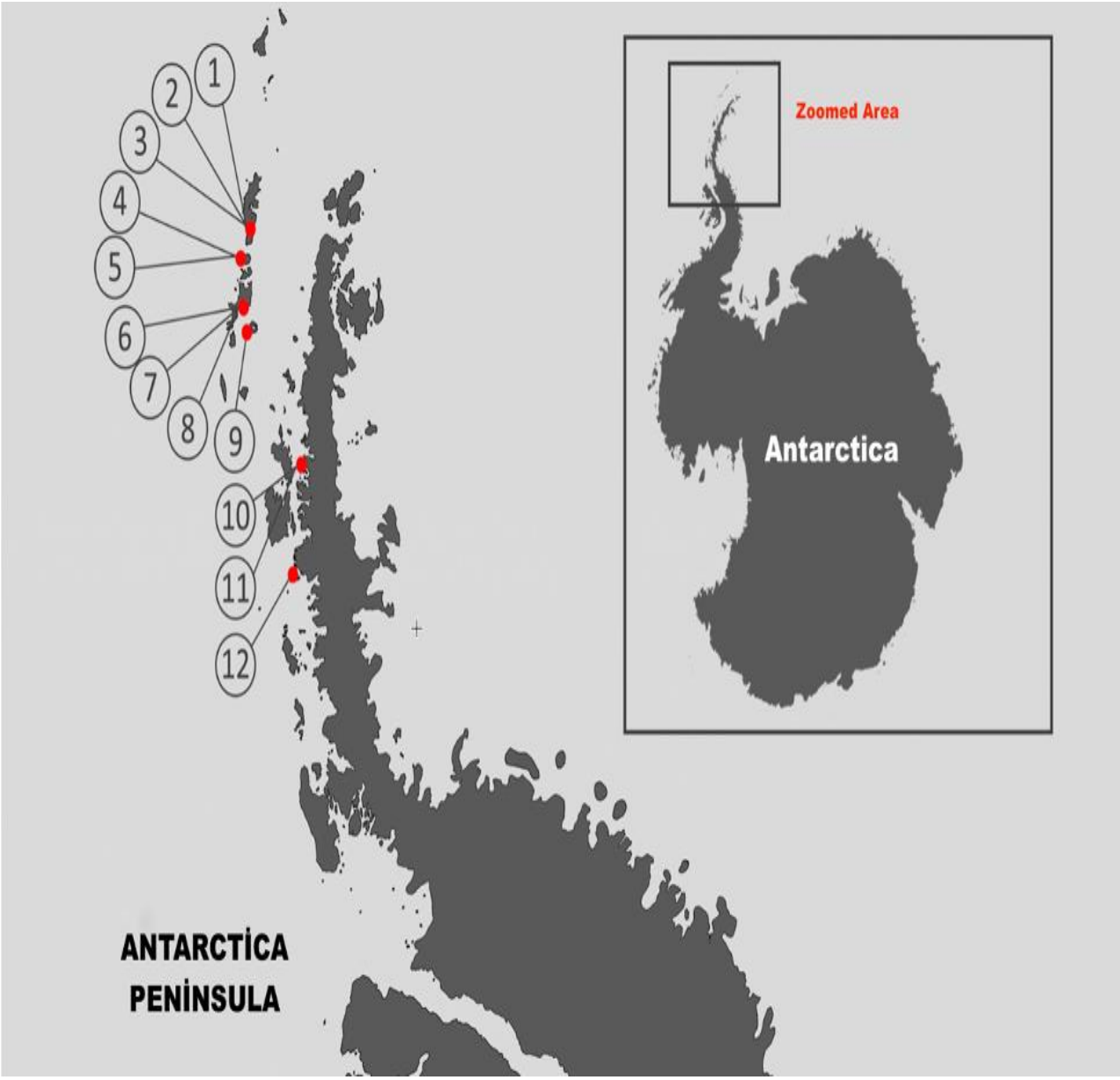


Figure 3.1. Sampling points where amphipod specimens were collected on the South Shetland Islands (1–9) and the islands (10–12) on the west coast of the Antarctic Peninsula.

Table 3.1. Name and map code of the sampling points.

Map Code	Location	Coordinates	Sampling Date	Total number of specimens
1	South Shetland Islands, Fildes P. East C.	62°11'54.31"S 58°57'14.90"W	22.03.2018	42
2	South Shetland Islands, Fildes Peninsula West Coast	62°11'44.96"S 58°59'51.40"W	05.02.2019	23
4	South Shetland Islands, Carlota Cove	62°22'26.34"S 59°40'59.98"W	01.04.2018 02.04.2018	46
6	South Shetland Islands, East of the Bulgaria St. Kliment Ohridski base	62°38'13.03"S 60°21'46.13"W	23.01.2017	97
7	South Shetland Islands, near Hespérides Point	62°38'30.95"S 60°22'15.53"W	25.01.2017	50
8	South Shetland Islands, Hannah Point	62°39'14.68"S 60°37'06.35"W	24.01.2017	43
9	South Shetland Islands, Telephone Bay	62°55'17.56"S 60°38'38.64"W	13.04.2018	8
10	East coast of Nansen Island	64°35'13.99"S 62°03'28.67"W	12.04.2018	24
11	West Coast of the A. P., Nansen Island N.west coast	64°32'38.65"S 62°04'31.18"W	28.02.2019	64
12	Antarctic Peninsula west coast, Galíndez Island- Vernadsky Station	65°14'36.38"S 64°15'41.58"W	26.02.2019	60
TOTAL				=457

3.2. Specimen Sorting

In the scope of this project, amphipods specimens were examined via a stereo microscope (Olympus SZ61). The individuals with the same distinctive properties were grouped by coding but the species identification was not made at this step. The classification was done via series of monograph and identification keys (e.g., De Broy, 1975; Coleman, 2007; Lörz, 2009; Lörz et al., 2012; d'Udeke d'Acoz and Verheye, 2017; d'Udeke d'Acoz et al., 2018), subsequently, following reverse taxonomy approach mentioned below. Each amphipod sample was photographed for documentation.

3.3 Molecular Methods

A reverse taxonomy approach, including species identification based on DNA barcoding followed by morphological analysis, was adopted to speed up species-level identification and uncover any cryptic diversity (Markmann and Tautz, 2005). Initially, amplification of the marker gene cytochrome c oxidase subunit I (COI) was performed on the collected samples, and the resulting sequences were clustered according to their genetic similarity. After species identification by comparing COI sequences of DNA barcodes of samples with the COI barcodes in the databases (BOLD and GenBank), morphological analysis was performed to confirm the DNA-based identification of species and to see if the presumed cryptic species could be distinguished by morphological features that might be overlooked.

The tissues were prepared and stored in 96% ethanol. First of all, the pereopod of each individual was used for DNA extraction with the Microtissue DNA Isolation Kit (AMBRD, İstanbul). However, the extractions were generally not successful. In line with this result, the amount of tissue used was increases. Instead of the leg, a piece from the posterior part of the trunk of the individuals was used along with the and Genomic DNA Extraction kit (AMBRD, İstanbul) instead of the Microtissue DNA Isolation Kit, mentioned above. By increasing the amount of tissue, an optimum method has been established in the tissue shredding process, which includes incubating the tissues at 65 °C for a minimum of 70 minutes and vortexing them at regular intervals during incubation. The initial PCR was established using the samples obtained from the extraction and universal invertebrate primers (LCOI490 and HCO021928) and conditions in Folmer et al. (1994), however the expected bands of 650 bp were not detected in agarose gels. Upon these results, 0.5 µL of 25 mM $MgCl_2$ was added to the 25 µL reaction. However, the expected bands were still not observed. Later, LCOI490 and HCO02198 (Folmer et al., 1994), CrustDF1 and CrusDR1 (Knox et al., 2020), cocktail primers LCO, HCO, COI-E, polyHCO (R) and polyshort COIR (R) (Braiser et al., 2016) and three different PCR amplification reactions were set up (Table 3.2). The

reactions using CrustD and primer cocktail (Table 3.2) on 1.5% TAE agarose gel were found to be successful (See Appendix A). Subsequently, CrustDF1 and CrustDR1 primers were used (Steinke et al., 2016; Knox et al., 2020). The optimum 25 µl PCR reaction consists of 1 µL CrusDF1 (10 µM), 1 µl CrustDR1 (10 µM), 12.5 µL 2X AMBRD PCR B Mix, 0.5 µL 25 mM $MgCl_2$, and 2–5 µL DNA and dH_2O. The optimum PCR protocol involved two PCR steps. The first step involved, initial denaturation at 94 °C for 1 min, followed by 5 cycles of denaturation at 94 °C for 1 min., primer annealing at 45 °C for 1.5 min., and primer elongation at 72 °C for 1.5 min. The second step following this procedure comprised 35 cycles of denaturation at 94 °C for 1 min., primer coupling at 50 °C for 1.5 min., primer elongation at 72 °C for 1 min., followed by 5 min. of elongation at 72 °C. The PCR products were loaded in 1.5% agarose gel prepared with 1X TAE buffer with RedSafe. The products and the 100 bp ladder were run at 95 V for 45 minutes. The gel images were taken under UV via Invitrogen E-Gel Safe Imager. The amplicons were sent to Macrogen Europe for commercial Sanger sequencing in both directions.

Table 3.2. Primers used in this study.

Gene	Name of the primers	Sequence 5'-3'
COI	LCOI490	5' GGTCAACAAATCATAAAGATATTGG-3'
	HCO2198	5'-TAAACTTCAGGGTGACCAAAAAATCA-3'
	CrustDF1	5'GGTCWACAAAYCATAAAGAYATTGG-3'
	CrustDR1	5'TAAACYTCAGGRTGACCRAARAAYCA-3'
	LCOI490-JJ	5′- CHACWAAYCATAAAGATATYGG-3′)
	HCOI490-JJ	5′-AWACTTCVGGRTGVCCAAARAATCA-3′)
	Cp_COIF3	5'-GGTCAYCCAGAAGTRTATATT-3'
	COI2R	5'-GGRTATTCWGARTAWCGNCGWGGTAT-3'
	CO1-E	5'-TATACTTCTGGGTGTCCGAAGAATCA-3'
	PolyLCO (F)	5'GAYTATWTTCAACAAATCATAAAGATATTGG-3'
	polyHCO (R)	5'-TAMACTTCWGGGTGACCAAARAATCA-3'
	polyshortCOIR (R)	5'-CCNCCTCCNGCWGGRTCRAARAA-3'

3.4. Data Analysis: Species Delimitation and Phylogenetic Analyses

Sequencher v.4.1 (Gene Codes Corp.) was used to edit and align the obtained sequences. Sequences with low quality were not included in the downstream analysis. The cleaned and assembled COI sequences were searched in the BOLD and NCBI GenBank databases and were matched with previously identified DNA barcodes to determine the species.

The sequences shorter than 300 base pairs or including stop codons were excluded from further analysis (See Appendix B). The sequences were uploaded to BOLD under the project ANTBU. For the delimitation of species, we did not use a single threshold. Instead, we performed two different analyses via ASAP and BOLD for. We clustered sequences into OTUs and performed RESL cluster analysis by the systems provided by BOLD, which helped to determine the number of possible species for unidentified specimens. The sequences were aligned by BOLD and used to create distance data via MEGA X (Kumar et al., 1993). The distance data was used for species delimitation in ASAP (Assemble Species by Automatic Partitioning), which use pairwise distance and does not require a predetermined cut-off similarity (Puillandre et al., 2020). According to results from ASAP, cluster and barcode gap analysis results from the BOLD and morphological comparison, the undescribed specimens were grouped and prepared for further identification steps. After determining the clusters (OTUs) of COI sequences, for each individual whose species was identified by matches to BOLD or GenBank, morphological identification was made with keys and literature (Andres, 1984; Andres, 1990; Barnard, 1932; Bone, 1972; Branch et al., 1981; Castellanos et al., 1963; Chevreux, 1906a; Chevreux,1906b; Chevreux,1906c; Coleman, 1989; Jażdżewska et al., 2014; Nagata, 1986; Nicholls, 1938; Ren and Huang, 1991; Thurston, 1974; Vinogradov, 1999; Walker, 1903; Zeidler, 2003), to confirm the molecular identification. If required, checks for the indications of probable cryptic speciation was made as well.

Clustal W was used for multiple sequence alignment and Neighbor-Joining tree construction (Larkin et al., 2007). The nwk tree data were downloaded for tree construction. PopART (Leigh and Bryant, 2015) was used to build a minimum-spanning haplotype network. Inkscape was used to edit haplotype networks. Moreover, distribution maps were prepared with Adobe Photoshop and Inkscape.

4. RESULTS AND DISCUSSION

4.1 Species Delimitations and Barcoding Gap

Initially, amplification of a partial fragment of the cytochrome c oxidase subunit I (COI) gene was performed from the collected samples. 97 COI sequences were uploaded to BOLD (http://www.boldsystems.org) under the project ANTBU after editing via Sequencher.

The operational taxonomic units (OTUs) are generated using Refined Single Linkage Analysis using a 2.2% and 4.4 % predefined sequence delimitation threshold (Table 4.1) and are subsequently refined using a graphical Markov clustering algorithm (Ratnasingham and Hebert, 2013; Gibbs, 2017). Thirteen groups (OTUs) were generated from this analysis (Table 4.1). The mean distance (intraspecific divergence) between OTUs was calculated as 0.318%. Therefore, the cut-off point (barcoding gap) was calculated to be 96.82%, where ten times the mean intraspecific divergence in terms of genetic distance (Hebert et al., 2004) is used in the calculation (96.82%=100%-10x0.318%). We also utilized ASAP (Assemble Species by Automatic Partitioning) for species delimitation, which only uses the pairwise distance of single locus data and does not require any previous biological knowledge of intra-specific genetic distance (Puillandre et al., 2020). The first ranked result of ASAP calculated the threshold limit of intraspecific distance as 6.5% (Figure 4.1). The highest-ranked result of ASAP was considered, which clustered sequences into 13 groups (OTUs), in concordance with the previous result from BOLD (Figure 4.2), which supports the notion that each cluster refers to distinct species (Puillandre et al., 2020).

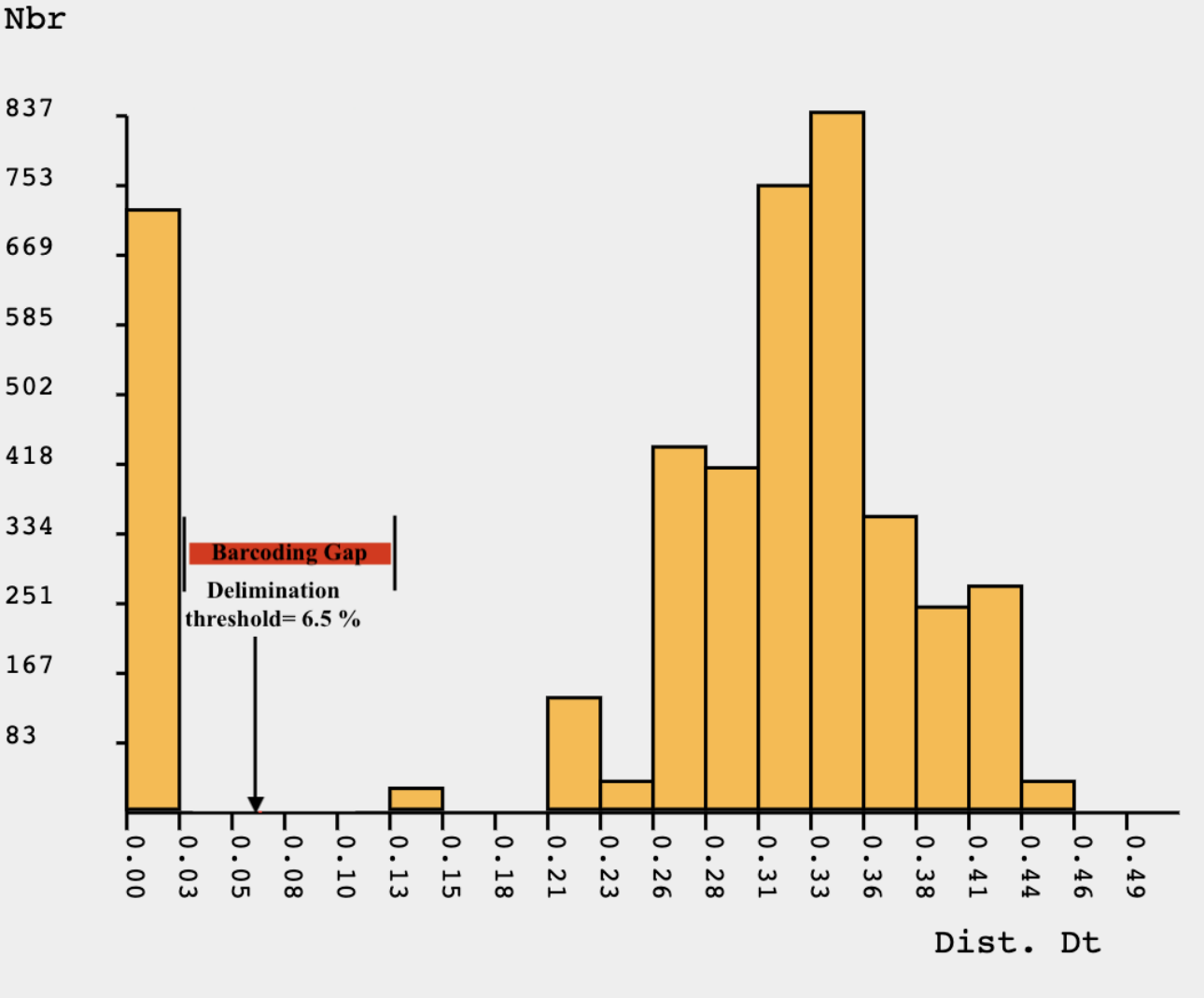


Figure 4.1. Histogram indicating the K2P pairwise distance between the sequences created by ASAP.

Table 4.1. Clustering results and divergence values calculated for all analyzed amphipod sequences. The summary was obtained via Barcode of Life Data System's "cluster sequences" function was used to figure out Kimura 2-parameter distances (BOLD). Align sequencing: BOLD aligner, pairwise deletion for ambiguous bases and gaps. ISD refers to intraspecific distance.

OTU	Mean ISD%	Max ISD %	Taxon	Count	NN Dist %
OTU-1	0.339	0.917	*V.antarctica*	14	22.902
OTU-2	0.376	0.947	*C.femoratus*	29	11.261
OTU-3	0.159	0.430	*G. antarctica*	6	21.442
OTU-4	0.208	0.838	*E.monticulosa*	18	17.816
OTU-5	0.164	0.164	*P.walkeri*	2	22.366
OTU-6	0.688	1.481	*Amphipoda*	6	21.061
OTU-7	0.149	0.641	*P.brevicornis*	9	17.816
OTU-8	0.451	1.351	*Amphipoda*	4	21.061
OTU-9	N/A	N/A	*H. kergueleni*	1	21.324
OTU-10	N/A	N/A	*Amphipoda (ANTBU070-22)*	1	21.242
OTU-11	N/A	N/A	*B. gigantea*	1	21.242
OTU-12	N/A	N/A	*Amphipoda*	1	25.893
OTU-13	N/A	N/A	*O. rotundifrons*	1	11.261

Genetic similarity of the OTUs was inferred using the Neighbor-Joining method. Figure 4.2 indicates the consensus tree. The clusters in the neighbor-joining tree overlap with 13 OTUs created via BOLD and ASAP, as expected (Table 4.1). The categorization of species using the neighbor-joining tree demonstrates that the taxa of the same species clustered together, which in itself is evidence of the usefulness of COI genes for amphipod species delineation. Furthermore, the literature indicates that the threshold similarity for delimitation of marine amphipods ranges from 93% to 97% (Hebert et al., 2003; Knox et al., 2012; Tempestini et al., 2018; Mohrbeck et al., 2021).

Moreover, GenBank and BOLD databases were used for the identification of each specimen by comparison between COI sequences of the specimens and databases. 9 OTUs (consisting of 81 individuals) were identified as nine species-level taxa: *Vibilia antarctica* (Stebbing, 1888), *Cheirimedon femoratus* (Pfeffer, 1888*), Gondogeneia antarctica* (Chevreux, 1906), *Eurymera monticulosa* (Pfeffer, 1888), *Hippomedon kergueleni* (Miers, 1875*), Bovallia gigantea* (Pfeffer, 1888), *Prostebbingia brevicornis* (Chevreux, 1906), *Orchomenella rotundifrons* (Pfeffer, 1888), *Paramoera walkeri* (Stebbing , 1906) discussed below. Following this, the morphological examination was carried out to verify the DNA-based species identification and cluster analysis was performed via BOLD and ASAP. We

observed 13 morphospecies that affirms the previous genetic results. However, 12 individuals in 4 OTUs (OTU-6, OTU-8, OTU-10, OTU-12) that grouped in four morphospecies could not be identified at the species level using genetic data or via morphological keys. Only one individual (OTU-12) was identified to the genus level as *Paraceradocus*, according to keys and monographs (Coleman, 1989). We could not identify the other three morphospecies (OTU-6, OTU-8 and OTU-10) neither genetically nor morphologically, since they did not resemble closest genera in BOLD or GenBank. Hence, we provide the first COI barcode for these four morphospecies. It is also possible that these individuals might be undiscovered species or species without a barcode, and further analyses by specialists are needed to identify their respective taxa.

Moreover, one of the COI sequences of the individual within the same morphogroup as *Cheirimedon femoratus* was closely related to the bacterial genus *Shewanella*, which is discussed in the "Unidentified Samples and COI Barcoding Limitations " Section below.

While molecular techniques are being increasingly used to study the taxonomic group Amphipoda, this large and diversified class of macrofauna remains underrepresented in the BOLD barcode collection. There is ongoing data integration between the BOLD and GenBank. In terms of taxonomy, it is necessary to synchronize the data in these two databases to obtain consistent results.

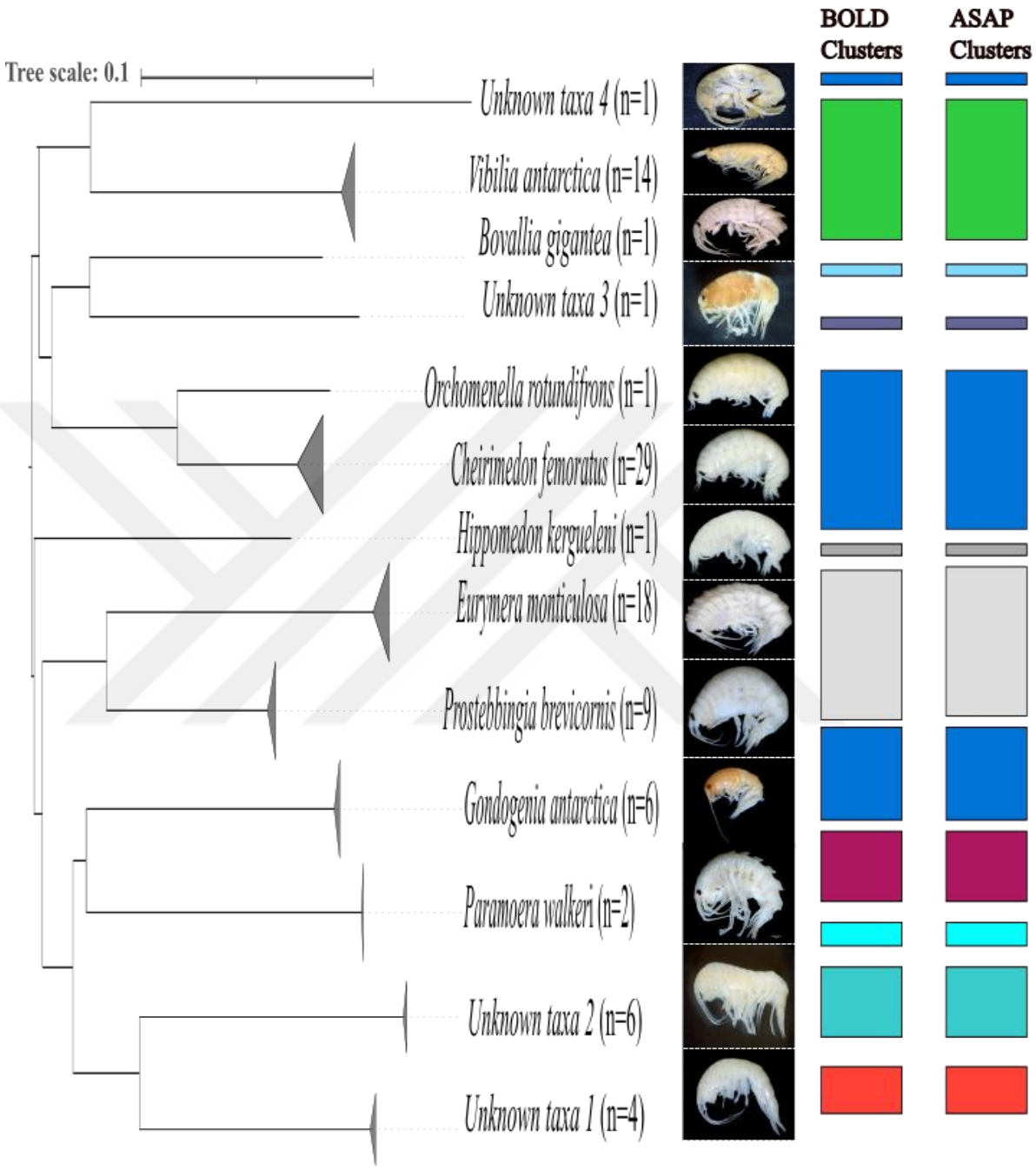


Figure 4.2. The optimal Neighbor-Joining tree is indicated. Clustering results by BOLD and ASAP are indicated next to the N-J tree. Unknown taxa 1, 2, 3, 4 refers to OTU-8, OTU-6, OTU-10 and OTU-12 respectively.

4.2. *Bovallia gigantea* (**Pfeffer, 1888)**

Only one individual (ANTBU069-22) was identified as *Bovallia gigantea* (Figure 4.3) by comparing COI data from BOLD. However, two BOLD data, which were 100% similar to our sequence, identified their individuals under the genus *Epimeria*. Morphological examination was performed for the validation of molecular species identifications was performed under a stereomicroscope using the literature and the individual identified as *Bovallia gigantea.* We suggest that there might be a taxonomic mistake in the BOLD entry needs a revision.

In certain instances, various names were used to describe new species (present in the database under both former and newly established names). The occurrence of many names for a single BIN may also be due to difficulties with the morphological identification of cryptic species and the absence of well-established diagnostic features among closely related species (Jażdżewska et al., 2021). Neighbor-joining tree data was constructed via BOLD to see the location of our sample ANTBU069-22 in a phylogenetic tree (Figure 4.3). The sample fell into the same clade with both Bovallia gigantea from Southern Ocean and Epimeria (as an identification mistake) from Antarctica. The similarity between the closest species level identified neighbor *Sternomoera japonica* is 82.12%, and the second closest neighbor *Leptamphopus sarsi* is 81.75 %, and the third was *Paromoera bousfieldi* with 80.49% similarity according to BOLD identification engine. Additionally, there are genetically closer individuals which assigned only to genus, family or order level. We did not use data that has taxonomically doubtful for further analysis. ANTBU069-22 inhabited Carlota Cove, and INTGS012-22 inhabited Paradise Bay and shared the same haplotype H1 (Figure 4.4, Table 4.2). H2, which has only one individual INTGS013-22 inhabited Bremen Island, separated from them with seven base differences between the haplotype of our sequence.

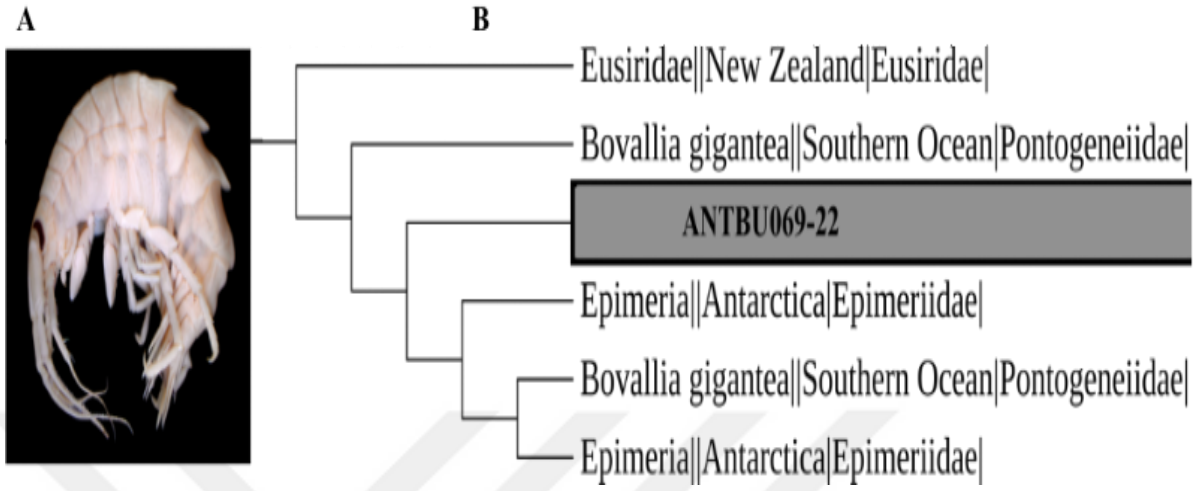


Figure 4.3. A. *Bovallia gigantea* Pfeffer, 1888, Coll. Code B294, Carlota Cove, Robert Island, South Shetland Islands 03.04.2018 (Photo by S.Ü. Karhan). B. Neighbor-Joinining tree constructed using sequence ANTBU069-22 and retrieved published data from BOLD.

Table 4.2. The haplotype network table for *Bovallia gigantea.*

Haplotypes	Locations	Sequences from this study	References from BOLD	No. of specimens
H1	Carlota Cove	ANTBU069-22		1
	Paradise Bay*	X	INTGS013-22	1
H2	Bremen Island**	X	INTGS012-22	2

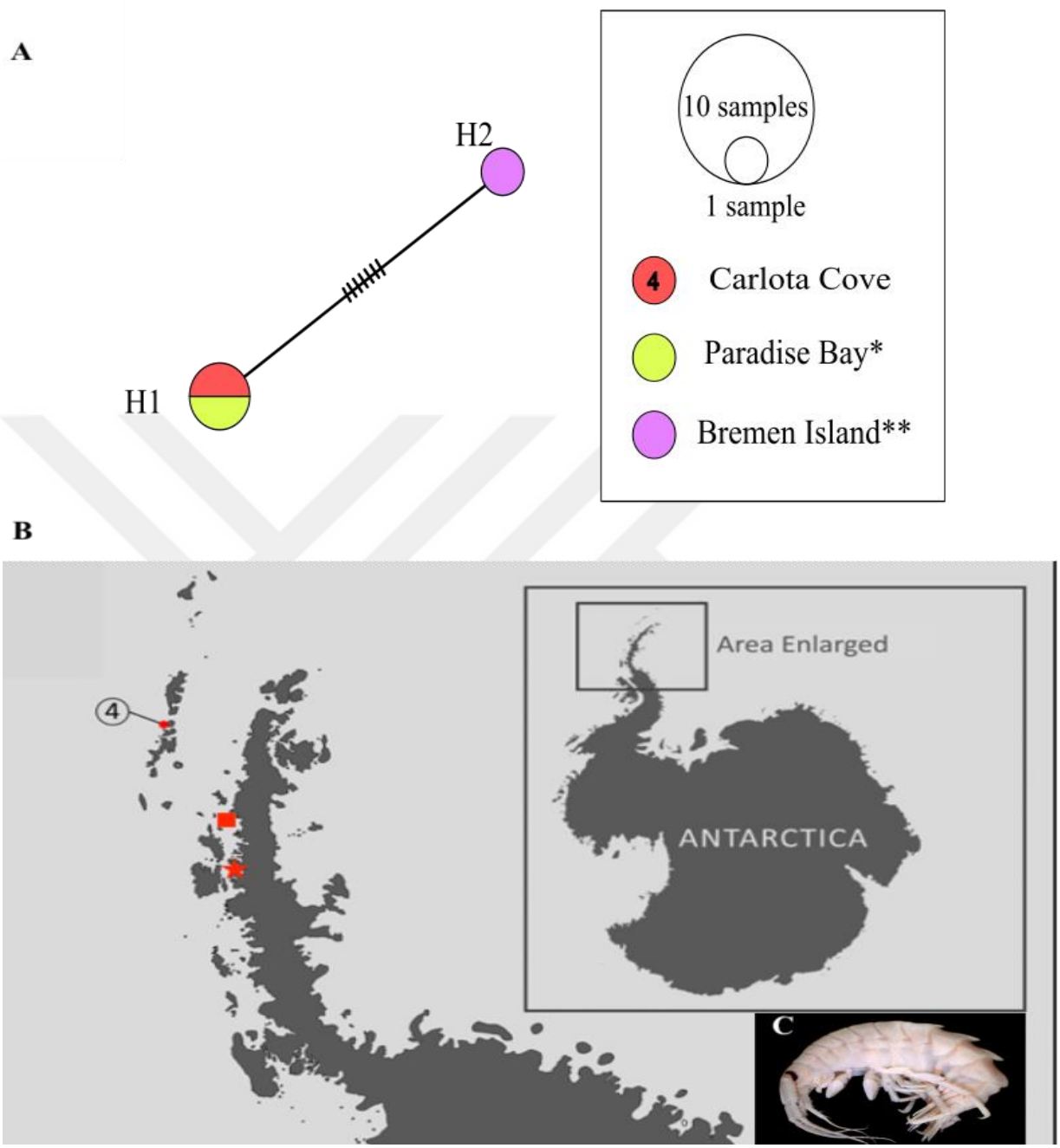


Figure 4.4. A. Haplotype network of *Bovallia gigantea*. Haplotype network colors indicated the locations as seen in the legend. The "*" and "**" designated data retrieved from BOLD/. Each hatch indicates one nucleotide difference. B. Distribution map represents sampling points. Number 4 indicates location (Carlota Cove) of ANTBU069-22. Star and rectangle represent Paradise Bay and Bremen Island respectively. C. *Bovallia gigantea* Pfeffer, 1888, Coll. Code B294, Carlota Cove, Robert Island, South Shetland Islands 03.04.2018 (Photo by S.Ü. Karhan)

4.3. *Eurymera monticulosa*, (Pfeffer, 1888)

Eighteen samples were identified as *Eurymera monticulosa* using the identification tool in the BOLD database. Additionally, each sample was examined morphologically, and genetic identification was confirmed by referencing the literature as a part of reverse taxonomy approach, outlined above.

The maximum similarity was 100%. There were only three COI barcodes for *E. monticulosa* in the BOLD systems; with this study, we will be adding eighteen more COI sequences which enables more reliable comparisons. Specimen ANTBU031-22 was chosen as a representative sequence to build a phylogenetic tree via BOLD to see its location in a phylogenetic tree. The phylogenetic tree indicated that our sample fell into the same clade as *E. monticulosa* (Figure 4.5). The closest neighbor species is *Prostebbingia brevicornis,* with 81.09 % similarity, the second *Pontogeneia rostrata* with 79% similarity and the third neighbor was *Ampithoe lacertosa* according to BOLD identification engine. Additionally, there are closer neighbor that are identified only family or genus level

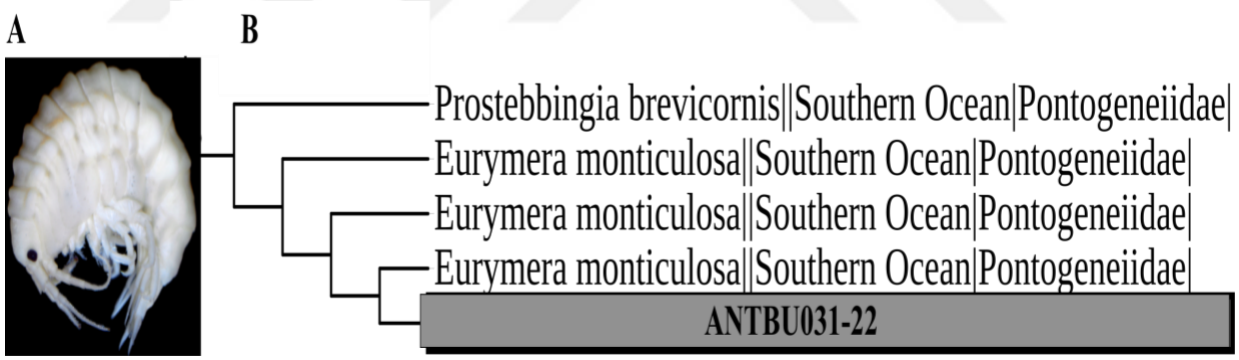


Figure 4.5. A. *Eurymera monticulosa* Pfeffer, 1888, Coll. Code B284, Carlota Cove, Robert Island, South Shetland Islands 03.04.2018 (Photo by S.Ü. Karhan) B. Neighbor-Joining tree constructed using sequence ANTBU031-22 and published data in BOLD. Phylogenetic tree constructed with published data retrieved from BOLD and COI sequence of individual.

The outcomes supported that the individuals in the same species were accurately grouped morphologically, which was undertaken as a first step of the reverse taxonomy approach described above. Subsequently, the morphology of all the specimens was compared to references and the monographs; thus, genetic identification was confirmed via traditional taxonomy. The mean intraspecific and maximum distance was calculated as 0.208% and 0.838%, respectively, and the distance to the closest neighbor in our data set *Prostebbingia brevicornis*, was 17.816%. Since the intraspecific distances are lower than the limit of the cut-off points for the marine amphipods (3%-7%), we do not suspect cryptic speciation in this species. For further comparison, PopART was utilized to build a haplotype network.

As indicated in Figure 4.6, specimens were collected from Carlota Cove and the East Coast of the Fildes Peninsula, close to St. Klement Ohridski Base. The highest similarity percentage was 100%. The COI sequences retrieved from BOLD were added to ours for comprehensive analysis. The haplotype network represented seven different haplotypes for *Eurymera monticulosa*. Seven individuals from Carlota Cove, two individuals collected from East Coast Fildes Peninsula, four individuals collected close to St. Klement Ohridski Base, and reference sequence INTGS023_22 collected from Southeast of the Anvers Island shared share the same haplotype (H1) (Figure 4.6, Table 4.3). Two of seven haplotypes, H5 and H4, were only seen in Carlota Cove and two were on the East Coast of Fildes Peninsula, and two were only seen in the Fildes Peninsula (Figure 4.6, Table 4.3). Only one individual is observed in each haplotype H2, H3, H5, H6, and H7. The most genetically distant haplotypes were H2 and H7; they were separated from each other with five base pair differences (Figure 4.6).

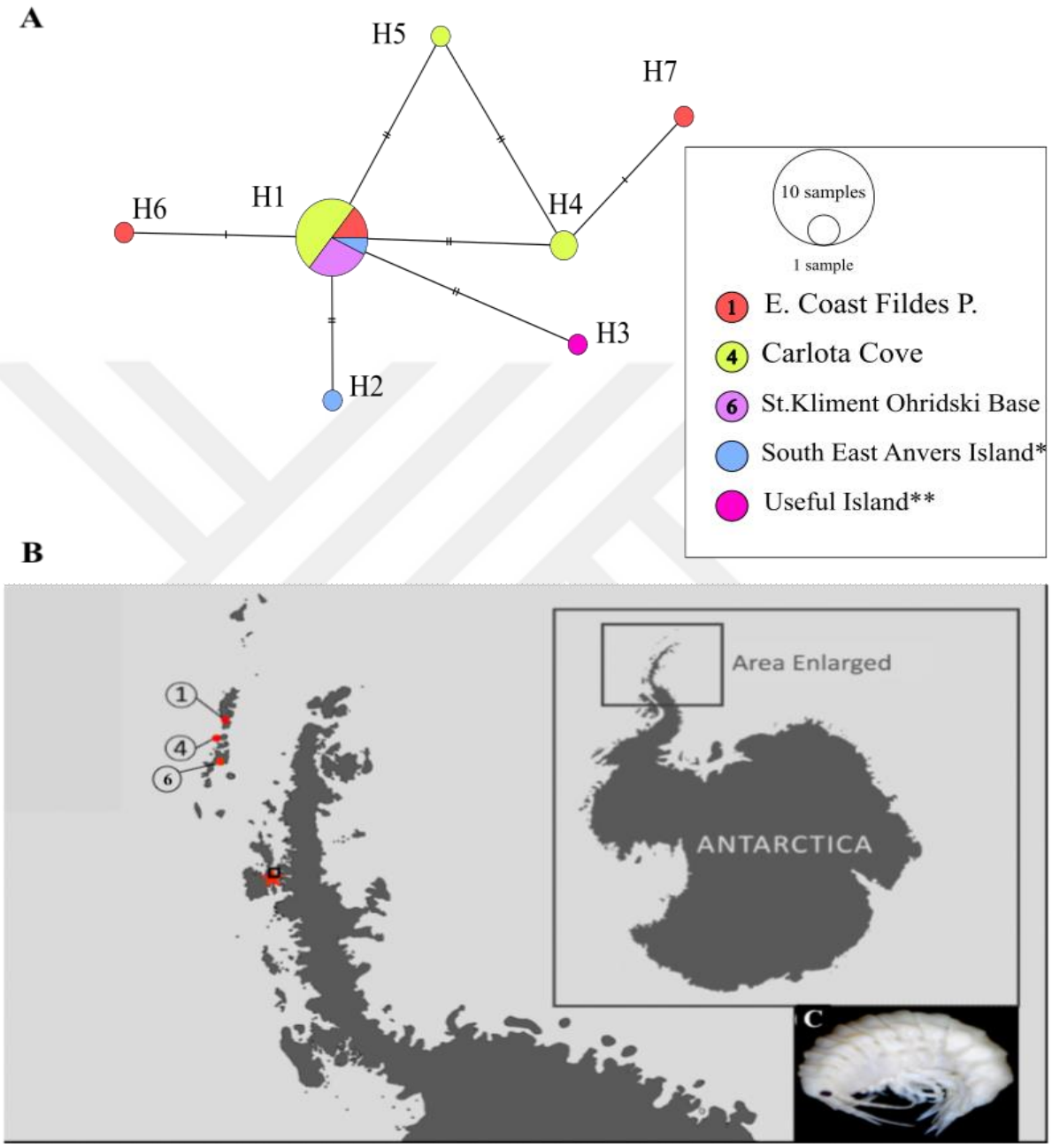


Figure 4.6. A. Haplotype network of the *Eurymera monticulosa.* Haplotype network colors indicate the locations as seen in the legend. The asterisk "*" designated data were retrieved from BOLD/GenBank. Each hatch indicates one nucleotide difference. The colors indicated the locations as seen in the legend. Numbers indicated the locations on the map. B. Distribution map presents sampling points. Red star and black circle represent the locations of the reference sequences, Southeast Anvers Island and Useful Island respectively. C. *Eurymera monticulosa* Pfeffer, 1888, Coll. Code B284, Carlota Cove, Robert Island, South Shetland Islands 03.04.2018 (Photo by S.Ü. Karhan).

Table 4.3. The haplotype network table for *Eurymera monticulosa*

Haplotype	Locations	Sequences from this study	References from BOLD	No. of Individuals
H1	Carlota Cove	ANTBU016-22 ANTBU020-22 ANTBU021-22 ANTBU022-22 ANTBU066-22 ANTBU067-22 ANTBU076-22	X	7
	Southeast Anvers Island	X	INTGS023_22	1
	East Coast Fildes Peninsula	ANTBU030-22 ANTBU031-22	X	2
	St Klement Ohridski Base	ANTBU098-22 ANTBU099-22 ANTBU100-22 ANTBU101-22	X	4
H2		X	INTGS022_22	1
H3		X	INTGS021_22	1
H4		ANTBU017-22, ANTBU018-22	X	2
H5		ANTBU019-22	X	1
H6		ANTBU028-22	X	1
H7		ANTBU029-22	X	1

4.4. *Vibilia antarctica,* (Stebbing, 1888)

Fourteen individuals were identified as *Vibilia antarctica*. Firstly, the identification function of the BOLD was used. The neighbor-joining tree is built via BOLD to see the position of a representative sequence ANTBU075-22 of the species. The phylogenetic tree indicated that a representative of our samples (ANTBU075-22) fell into the same clade as *Vibilia antarctica* (Figure 4.7). After the identification of the individuals by the initial COI sequencing and comparison with the databases, we also confirmed the BOLD results by morphologically examine each specimen (Figure 4.7) considering the literature, such as monographs and identification keys aforementioned in methodology section as a part of reverse taxonomy approach.

Based on identification engine, the maximum sequence similarity was 100% with data not publicly available on BOLD and was identified as *Vibilia antarctica,* and the similarity was 99.83 % with published *Vibilia antarctica* sequences. The closest neighbor was an individual in the genus *Vibilia*, which has not been identified at the species-level with 90.86 similarity, the second closest neighbor was *Vibilia propinqua* with 90.02% similarity, and the third *Vibilia armata* with 89.73% similarity.

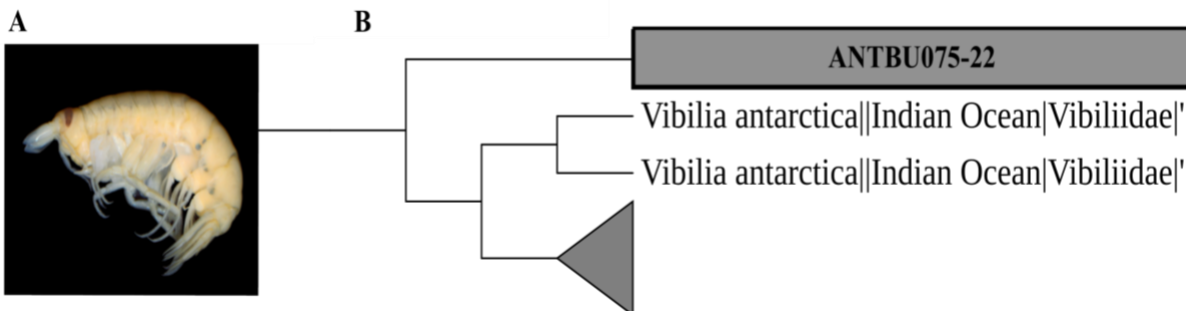


Figure 4.7. A. *Vibilia antarctica* Stebbing, 1888 Coll. Code B002, Hannah Point, Livingston Island, South Shetland Islands 24.01.2017 (Photo by S.Ü. Karhan). B. Neighbor-Joining tree constructed via BOLD.

Table 4.4. The haplotype network table for *Vibilia antarctica.*

Haplotype	Locations	Sequences from this study	References from BOLD	No. of Individuals
H1	Hannah Point	ANTBU087-22	X	1
H2	Hannah Point	ANTBU085-22	X	1
H3	Hannah Point	ANTBU072-22 ANTBU075-22 ANTBU082-22 ANTBU086-22	X	4
	Indian Ocean*	X	KC754385.1 KC754387.1	2
H4	Hannah Point	ANTBU080-22 ANTBU083-22	X	2
H5	Hannah Point	ANTBU071-22	X	1
H6	Hannah Point	ANTBU001-22 ANTBU074-22	X	2
	Weddel Sea**	X	EF989689.1	1
	Indian Ocean*	X	KC754386.1	1
H7	Hannah Point	ANTBU073-22 ANTBU081-22	X	2
H8	Hannah Point	ANTBU084-22	X	1

Four published data out of nineteen reference COI sequences were retrieved for a comprehensive analysis. The other sequences were private (i.e., not publicly available), and therefore we could not use them for comparisons. Our individuals exhibited eight different haplotypes (H1, H2, H3, H4, H5, H6, H7, H8) (Figure 4.8). Two individuals from Hannah Point, one reference individual from Weddel Sea (EF989689.1), and one reference individual from the Indian Ocean (KC754386.1) shared the same haplotype (H6) (Figure 4.8, Table 4.4). Two reference individuals from the Indian Ocean (KC754385.1, KC754387.1) and two of our individuals shared the same haplotype (H3). Haplotypes H1, H2, H4, H5, H7 and H8 were inhabited in Hannah Point.

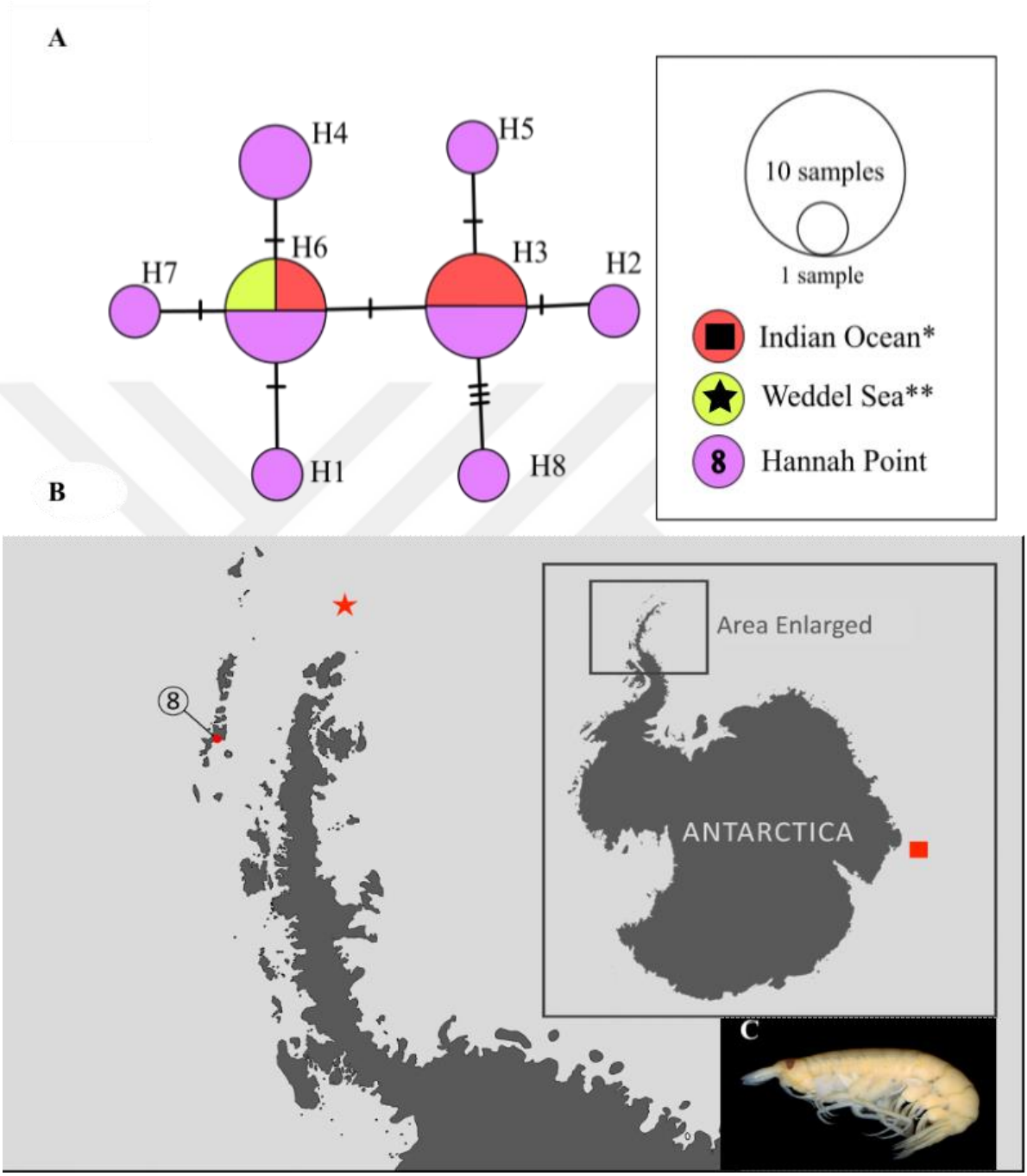


Figure 4.8. A. Haplotype network of the *Vibilia antarctica.* Haplotype network colors indicate the locations as seen in the legend. The asterisks (* and**) designated data were retrieved from BOLD. Each hatch indicates one nucleotide difference. B. Distribution map represents sampling points of the *Vibilia antarctica*. Point 8, Star and Square stand for Hannah Point, Weddell Sea, and Indian Ocean, respectively. C. *Vibilia antarctica* Stebbing, 1888 Coll. Code B002, Hannah Point, Livingston Island, South Shetland Islands 24.01.2017 (Photo by S.Ü. Karhan)

4.5. *Gondogeneia antarctica*, (Chevreux, 1906)

Six individuals were identified *as Gondogeneia antarctica* (Chevreux, 1906) based on three COI records in BOLD. The neighbor-joining tree was constructed from the COI sequence of a representative individual (ANTBU013-22) from our samples to see the position of our sample in a phylogenetic tree. The representative sample fell into the same clade with other individuals of *Gondogenia antarctica* (Figure 4.9). The maximum and minimum hits for the *Gondogeneia antarctica* sequences were 99.78% and 99.65%, respectively. The genetic identification was confirmed with traditional taxonomy. The closest neighbor was identified at the genus level *Eursiridae* and the second closest the was *Gammarus pisinnus*, with similarities of 82.25% and 81.28% respectively, based on BOLD identification engine.

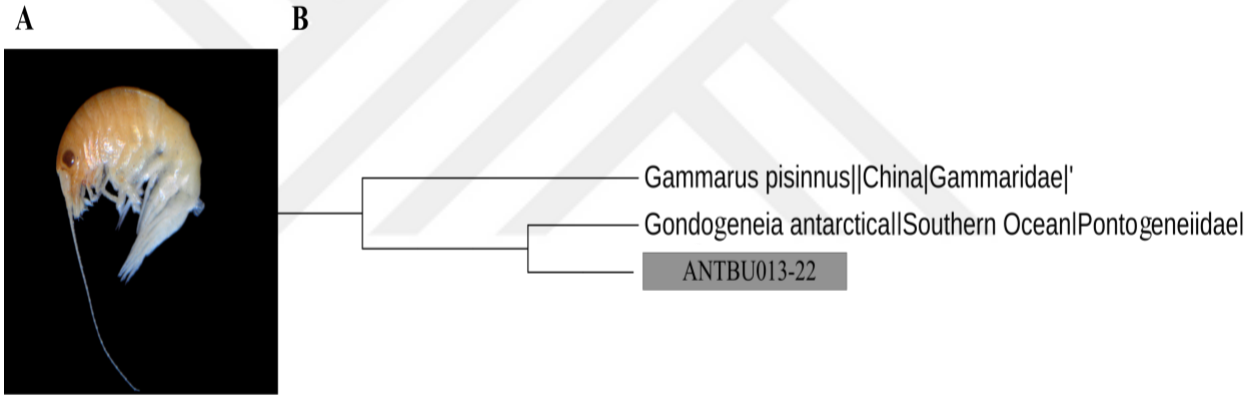


Figure 4.9. A. *Gondogeneia antarctica* Chevreux, 1906, Coll. Code ANTBU013-22, off Vernadsky Station, Galíndez Island, Wilhelm Archipelago, Western Antarctic Peninsula 26.02.2019 (Photo by S.Ü. Karhan) B. Neighbor-Joining tree constructed with data retrieved from BOLD

Table 4.5. The haplotype network table for *Gondogeneia antarctica*.

Haplotype	Locations	Sequences from this study	References from BOLD	No. of Individuals
H1	Enterprise Island	X	INTGS006-22	1
H2	E. Coast of Nansen Island	ANTBU013-22	X	1
	W.Coast of Fildes P.	ANTBU051-22	X	1
	Hesperides	ANTBU063-22	X	1
	Vernadsky S.	ANTBU062-22	X	1
	Paradise Bay		INTGS004_22, INTGS005_22	2
H3	Hesperides P.	ANTBU064-22	X	1
H4	E. Coast Nansen I	ANTBU014-22	X	1

Four haplotypes were observed for *G. antarctica*, as seen in Figure 4.10. One individual was collected from the East Coast of Nansen Island, the West Coast of the Fildes Peninsula, Vernadsky Station, and Hesperides Point, and two individuals from Paradise Island share the same haplotype H2 (Figure 4.10 and Table 4.5). Samples from the East Coast of Nansen Island shared two haplotypes, H2 and H4. Haplotype H4 contains one individual (ANTBU014-22) that inhabited the East Coast of Nansen Island. Individuals that inhabited these five locations (E. Nansen, E. Fildes, Hespérides P., Vernadsky St., and Enterprise Island) shared the same haplotype H2. There was only three *G. antarctica* (Chevreux, 1906) COI sequence before our study; we added six more sequences, which will enable the proper barcode evaluation.

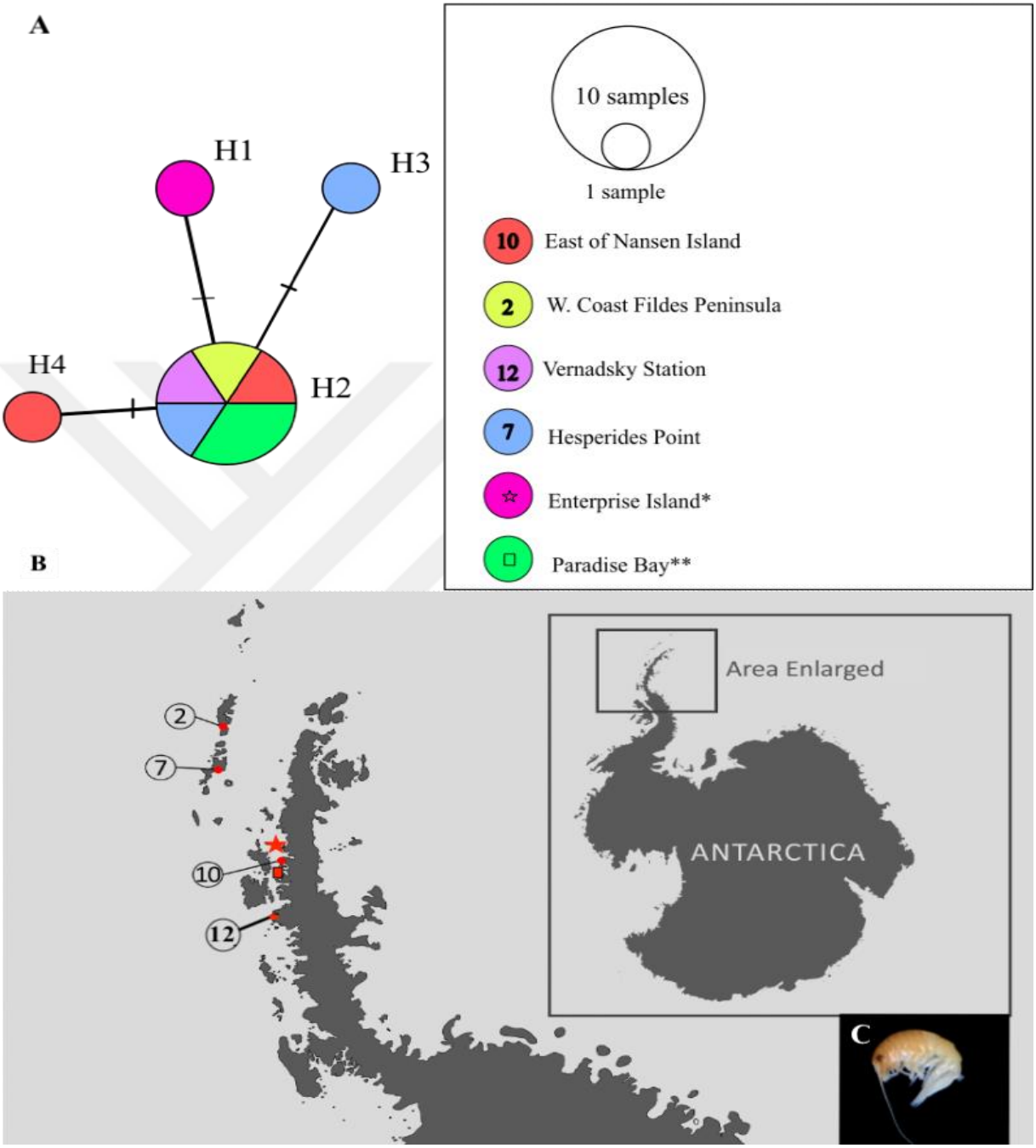


Figure 4.10. A. Haplotype network colors indicated the locations as seen in the legend The asterisk "*" designates data retrieved from BOLD. Each hatch indicates one nucleotide difference. B. Distribution map presents sampling points. Point 2, 7, 10,12, star and square (reference location) stand for West Coast of the Fildes Peninsula, near Hespérides Point, east coast of Nansen Island, Vernadsky Station, Enterprise Island and Paradise Bay. C. *Gondogeneia antarctica* Chevreux, 1906, Coll. Code ANTBU013-22, 26.02.2019 (Photo by S.Ü. Karhan).

4.6. *Prostebbingia brevicornis* (Chevreux, 1906)

Nine individuals were identified as *Prostebbingia brevicornis* according to only one molecular data recorded in BOLD with 99.82% similarity. Morphological examination was performed as a part of the reverse taxonomy approach and to confirm the molecular identification. Next, the neighbor-joining tree indicated that our sample fell into a clade along with *Prostebbingia brevicornis*, and the next closest species being *Eurymere monticulasa* (Figure 4.11). The closest neighbor species was *Sternomoera rhyaca* with 84.32% similarity, the second was *Sternomoera yezoensis* 84.07% similarity and *Awacaris yezoensis* with 83.7 similarity. All the closest species were in the Pontogeneiidae family.

There were three different haplotypes (H1, H2, H3) detected in nine individuals (one retrieved from BOLD). The reference sequence and seven other shares the same haplotype samples (Figure 4.12). We should emphasize that there is only one barcoded individual of *Prostebbingia brevicornis* in BOLD, hence the data are not adequate for the reliability of the barcode; at least three sequences are suggested for proper identification and comparison. (Fontes et al., 2021; Jażdżewska et al., 2021; Oliveira et al., 2016; Seefeldt et al., 2018).

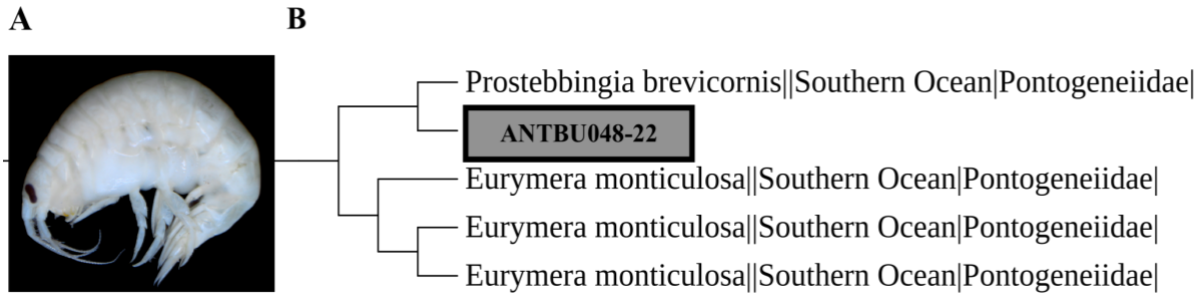


Figure 4.11. A. *Prostebbingia brevicornis* Chevreux, 1906, Coll. Code B090, Fildes Peninsula (eastern coast) (Photo by S.Ü. Karhan). B. Neighbor-Joining tree constructed with published data retrieved from BOLD and COI sequence of individual ANTBU048-22

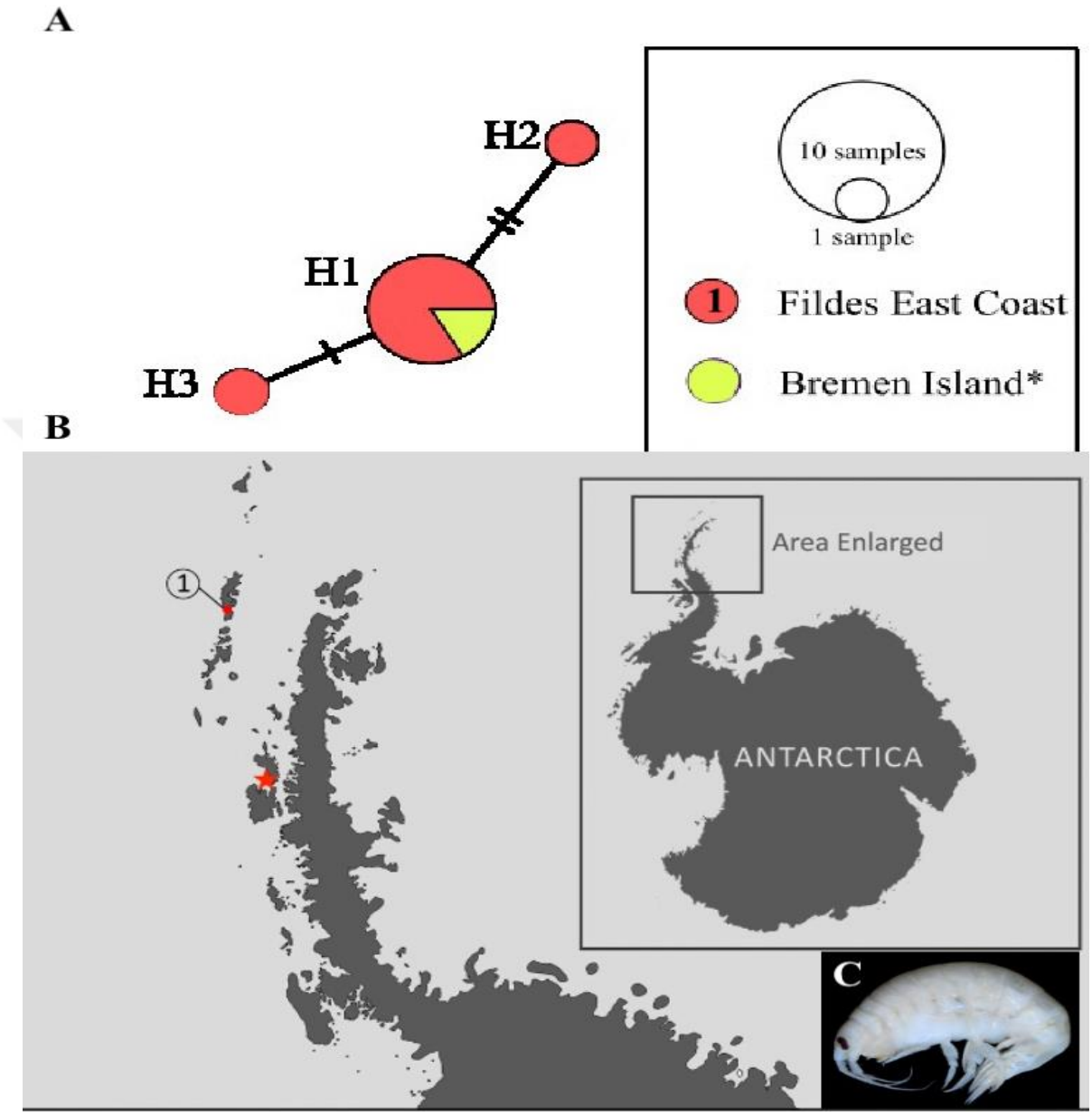


Figure 4.12. A. Haplotype network of the *Prostebbingia brevicornis*. Haplotype network colors indicate the locations as seen in the legend. The asterisk (*) stands for data retrieved from BOLD. Each hatch indicates one nucleotide difference B. Distribution map presents sampling points. Point 1 and star (reference location) stand for East Coast of the Fildes Peninsula and Bremen Island respectively. C. *Prostebbingia brevicornis* Chevreux, 1906, Coll. Code B090, Fildes Peninsula (eastern coast) (Photo by S.Ü. Karhan).

4.7. Lysianassoids: *Cheirimedon femoratus* (Chevreux, 1906), *Orchomenella rotundifrons* (K.H. Barnard, 1932) and *Hippomedon kergueleni* (Miers, 1875)

4.7.1. *Cheirimedon femoratus* (Chevreux, 1906)

Thirty individuals were grouped into the same morphogroup according to their morphological similarity which was undertaken as a first step of the reverse taxonomy approach described above (as in Mohrbeck et al., 2020; Jażdżewska et al., 2021). Then COI sequences of each individual were used for identification by comparison with the databases BOLD and GenBank. Twenty-nine individuals were identified as *Cheirimedon femoratus* (Chevreux, 1906) based on COI barcodes between 99.02-100% similarity and one individual (ANTBU092-22) was identified as *Orchomenella rotundifrons* with 99.46% similarity. At first, we sorted them into same morpho group assuming that they are the same species. The details might have been overlooked in first examination of the samples since the individuals of the family have different coloration and morphology, as a result of convergent evolution. The molecular taxonomy enabled us for re-evaluation to differentiate two Lysianassoid species according to literature, as mentioned in the methodology. It should be noted that convergent evolution is common for amphipods and especially Lysianassoids are error-prone for morphological taxonomy. We strongly recommend the integration of DNA barcoding and traditional taxonomy (Seefeldt et al., 2018) in general for studies of amphipods, and especially for Lysianassoids.

Additionally, fourteen published COI sequences of as *Cheirimedon femoratus* were retrieved from BOLD for a comprehensive analysis including construction of phylogenetic tree (Figure 4.13) and haplotype networks (Figure 4.14). ANTBU035-22 as a representative of our data was selected to build neighbor joining tree to see its position in the phylogenetic tree (Figure 4.13). The BOLD identification results indicated that the closest neighbor was *Abyssochomene charcoti* with 90.69 similarity, the second closest neighbor species was *Orchomenella cavimonus* with % 90.02 similarity the third was individual *Orchomenella acanthurus* with 89.95% from the same family Tryphosidae. Additionally, there were closer neighbors only described order and genus level assigned neighbors which should be revisited by the researchers created the date for more reliable database. The intraspecific diversity sequences of *Cheirimedon femoratus* from our data was between 0.376 %-0.947% (Table 4.1).

Fourteen haplotypes were observed. Our samples share ten different haplotypes (H1, H2, H3, H4, H7, H8, H9, H10, H12, H14). Eleven haplotypes were observed in only one individual each (Figure 4.14, Table 4.6).

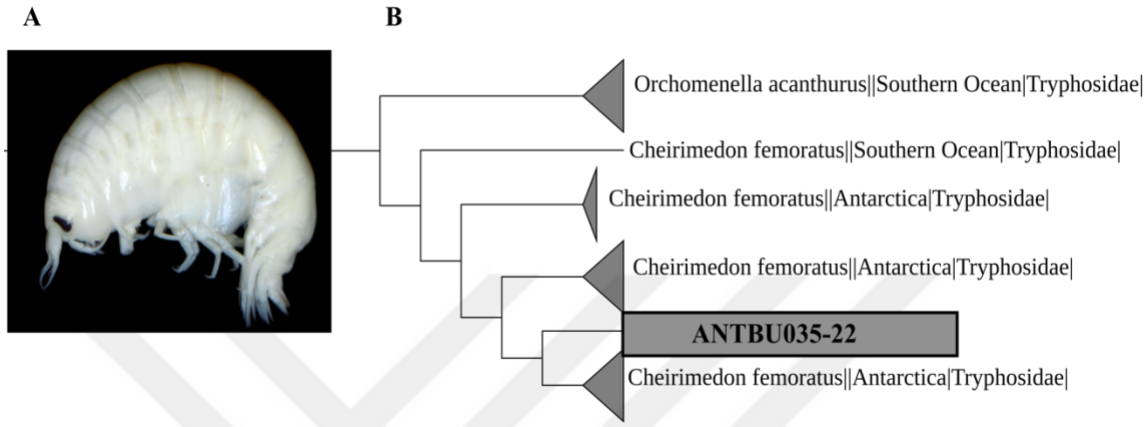


Figure 4.13. A. *Cheirimedon femoratus*, Chevreux, 1906, Coll. Code B018, Fildes Peninsula (western coast), King George Island, South Shetland Islands, 05.02.2019 (Photo by S.Ü. Karhan). B. Neighbor-Joining tree constructed with data retrieved from BOLD.

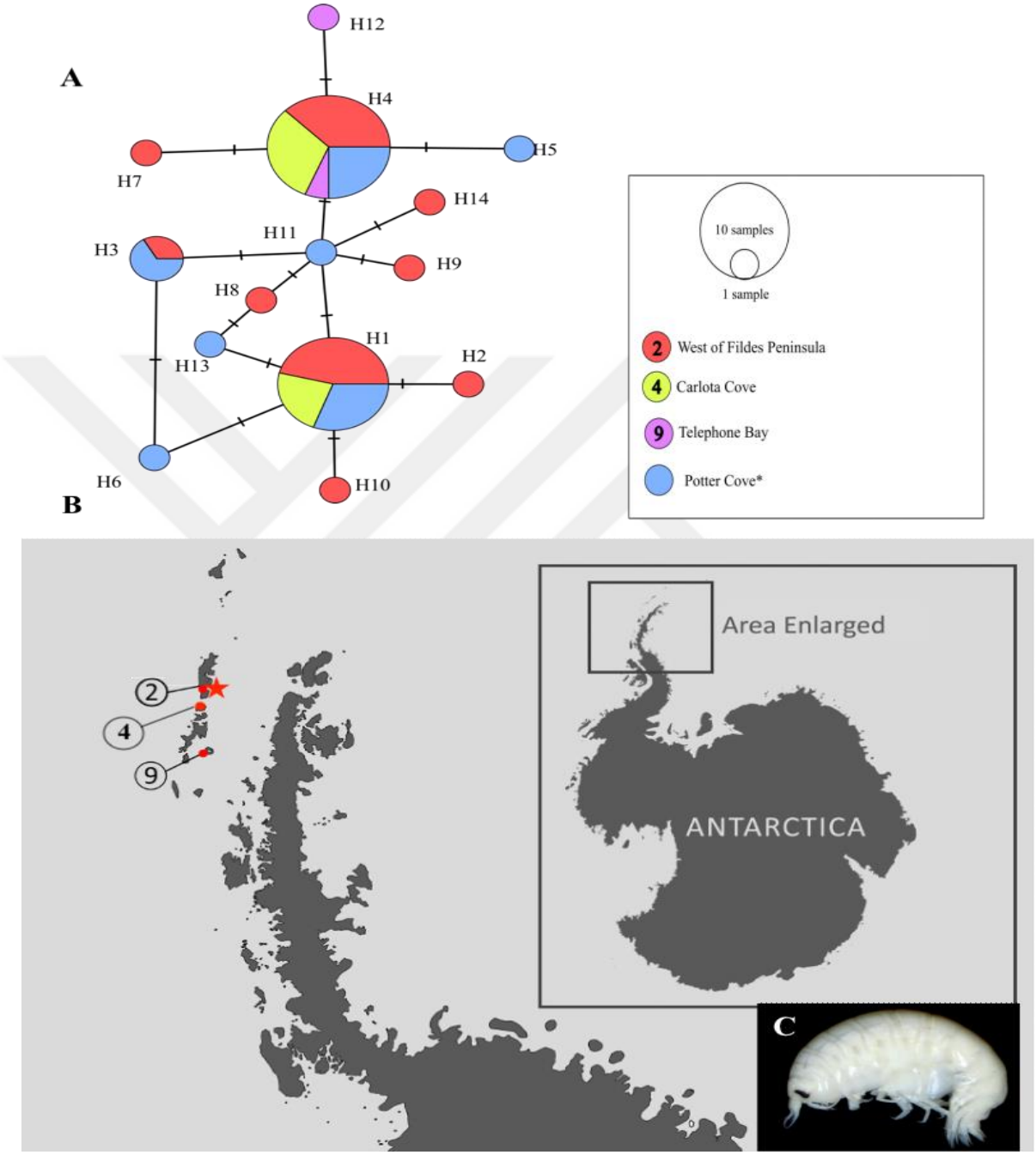


Figure 4.14. A. Haplotype network of the *Cheirimedon femoratus* (Chevreux, 1906). Haplotype network colors indicate the locations as seen in the legend, The asterisk "*" designates data retrieved from GenBank. Each hatch indicates one nucleotide difference. B. Distribution map represents sampling points. Point 2,4,9, and star (reference location) stand for West Coast of the Fildes Peninsula, Carlota Cove, Telephone Bay, and Potter Cove, respectively. C. *Cheirimedon femoratus*, (Chevreux, 1906), Coll. Code B018.

Table 4.6. The distribution of the haplotypes and individuals for *Cheirimedon femoratus*.

Haplotypes	Locations	No.of Individual
H1	West Coast of Fildes P.	6
	Carlota Cove	3
	Potter Cove	4
H2	West Coast of Fildes P.	1
H3	West Coast of Fildes P.	1
	Potter Cove*	2
H4	Carlota Cove	5
	W.Coast of Fildes P.	6
	Telephone Bay	1
	Potter Cove*	4
H5	Potter Cove*	1
H6	Potter Cove*	1
H7	West C. of Fildes P.	1
H8	West C. of Fildes P	1
H9	West C. of Fildes P	1
H10	West C. of Fildes P	1
H11	Potter Cove*	1
H12	Telephone Bay	1
H13	Potter Cove*	1
H14	West C. of Fildes P.	1

4.7.2. *Orchomenella rotundifrons* (K.H. Barnard, 1932*)*

The haplotype network indicated six different haplotypes for *Orchomenella rotundifrons* (Figure 4.16). Individual ANTBU092-22 and twelve individuals shared the most common haplotype (H1). Three individuals shared the same haplotype (H2). Four out of six haplotypes were found in a single individual each. According to WoRMS, *Orchomenella rotundifrons* was observed and recorded in Southern Ocean, South Shetland Island, Weddell Sea, and South Orkney Island.

From the previous studies the Lysianassoids are known to share very similar habitats (Havermans et al., 2018; Seefeldt et al., 2018). *Cheirimedon femoratus* and *Orchomenella rotundifrons* samples in this study were also observed together in west coast of Fildes Peninsula, which emphasize their geographic and bathyal overlap, due to their scavenger style. In addition, the majority of the scavenger amphipods observed in the inner cove in summer were *Cheirimedon femoratus*. It is likely that the glacier retreat triggers the colonization of the algae. Seefeldt et al. (2017) examined the feeding strategies of Antarctic scavenger Lysianassoids to understand and concluded that the nutritional flexibility *of C. femoratus* is a potential ecological driver and crucial to its success in colonizing newly accessible, ice-free Antarctic coastal environments. In our case, *Cheirimedon femoratus* was the most common species in intertidal zone, which may support the notion of their greater ability to live in these areas when compared to other species; however, for this inference more individuals need to be recorded at different time intervals (e.g., seasons).

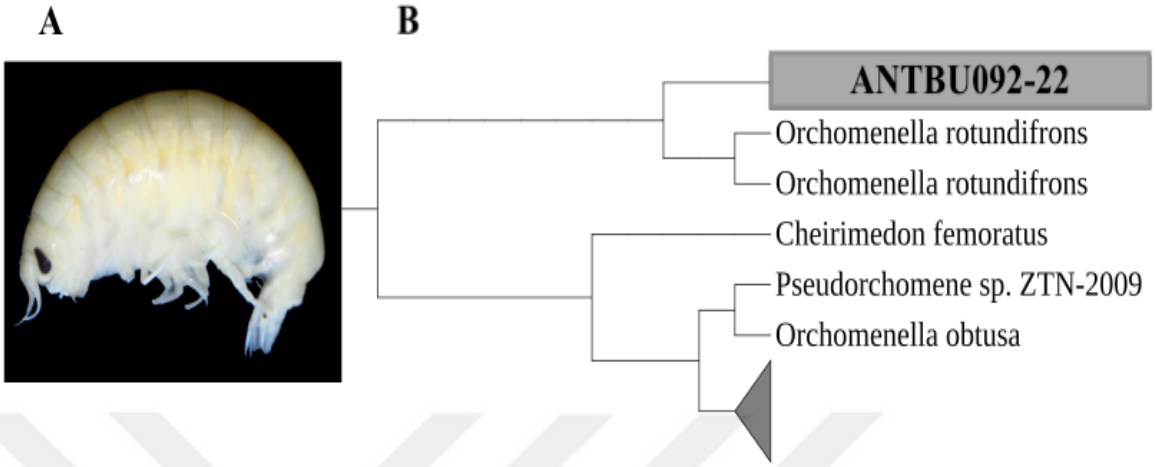


Figure 4.15. A. *Orchomenella rotundifrons*, (K.H. Barnard, 1932), Coll. Code B376, Fildes Peninsula (western coast), King George Island, South Shetland Islands, 05.02.2019 (Photo by S.Ü. Karhan). B. Phylogenetic tree constructed with data retrieved from BOLD. COI sequence of individual ANTBU092-22 was used for phylogenetic tree.

Table 4.7. The distribution of the haplotypes and individuals for *Orchomenella rotundifrons.*

Haplotypes	Locations	No. of individuals
H1	Potter Cove*	12
	W.Coast of Fildes P.	1
H2	Potter Cove*	3
H3	Potter Cove*	1
H4	Potter Cove*	1
H5	Potter Cove*	1
H6	Potter Cove*	1

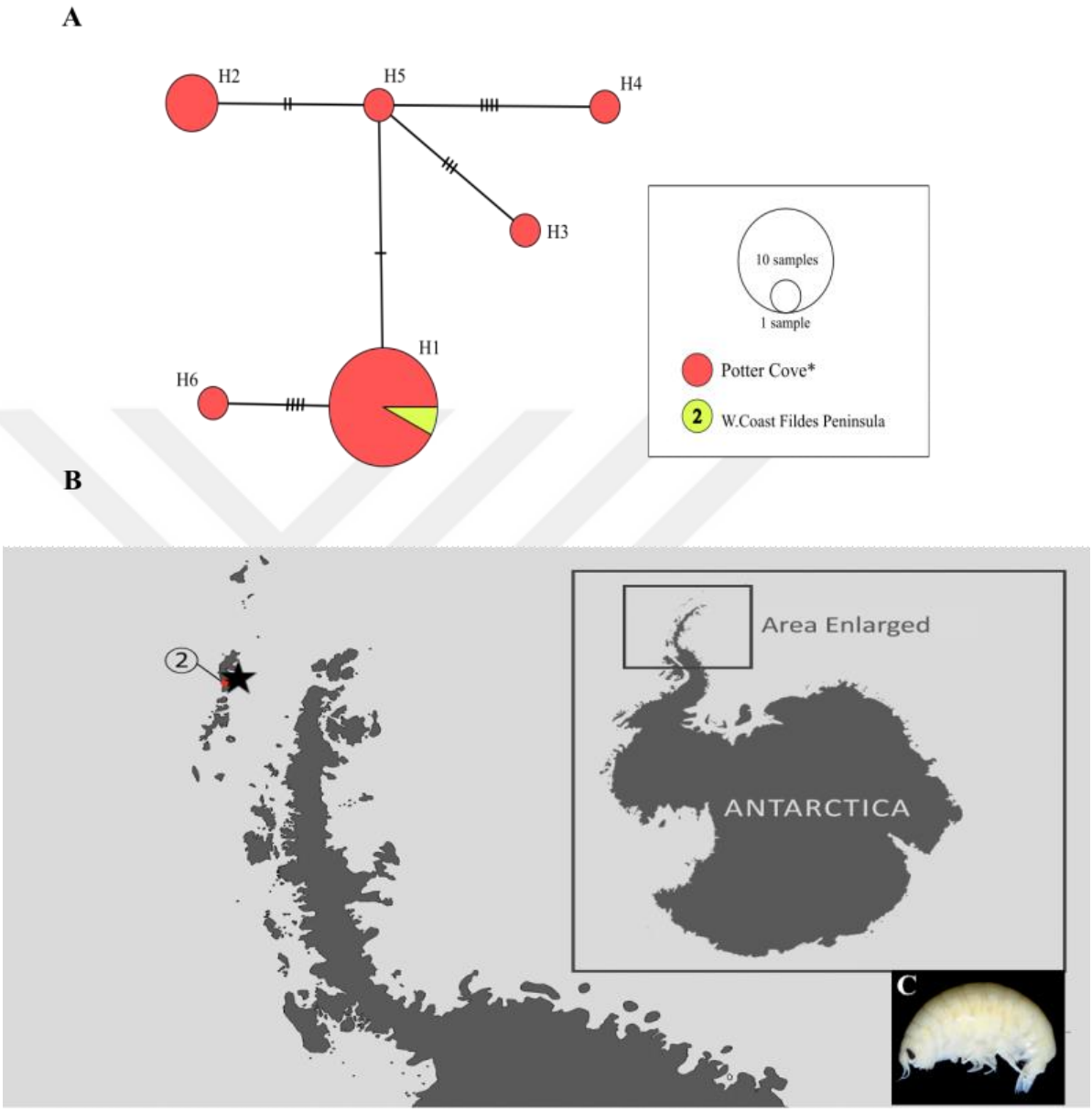


Figure 4.16. A. Haplotype network of the *Orchomenella rotundifrons,* (K.H. Barnard, 1932). The colors in the network indicate the locations as seen in the legend. The asterisk "*" designates data retrieved from GenBank. Each hatch indicates one nucleotide difference. B. Distribution map represents sampling points. Points 2, and star (reference location) stand for West Coast of the Fildes Peninsula and Potter Cove, respectively.

4.7.3. *Hippomedon kergueleni* (Miers, 1875)

Only one individual was identified as *Hippomedon kergueleni* according to comparison between sequences in the GenBank and BOLD databases. The morphology examination was performed to confirm the genetically assigned species as a part of reverse taxonomy approach. Neighbor-joining tree was built via BOLD to see the location of ANTBU058-22 sequence (which was the only representative of *Hippomedon kergueleni* species in our dataset) and its neighbor taxa in a phylogenetic tree (Figure 4.17). Fifteen COI sequences recorded in BOLD and GenBank were retrieved for a comprehensive analysis. Seven haplotypes were observed for sixteen individuals. Individual ANTBU058-22 (Figure 4.18) shared the most common haplotype H1 with other nine reference individuals. The other six haplotypes were detected in a single individual each at the Potter Cove (Figure 4.18).



Figure 4.17. A. *Hippomedon kergueleni* (Miers, 1875), Coll. Code B123, off Vernadsky Station, Galíndez Island, Wilhelm Archipelago, Western Antarctic Peninsula, 26.02.2019 (Photo by S.Ü. Karhan). B. Neighbor-Joining tree constructed with data retrieved from BOLD and COI sequence of individual ANTBU058-22 was used for phylogenetic tree.

Table 4.8. The distribution of the haplotypes and individuals for *Hippomedon kergueleni.*

Haplotypes	Locations	No. of individuals
H1	Potter Cove*	9
	Vernadsky St.	1
H2	Potter Cove*	1
H3	Potter Cove*	1
H4	Potter Cove*	1
H5	Potter Cove*	1
H6	Potter Cove*	1
H7	Potter Cove*	1

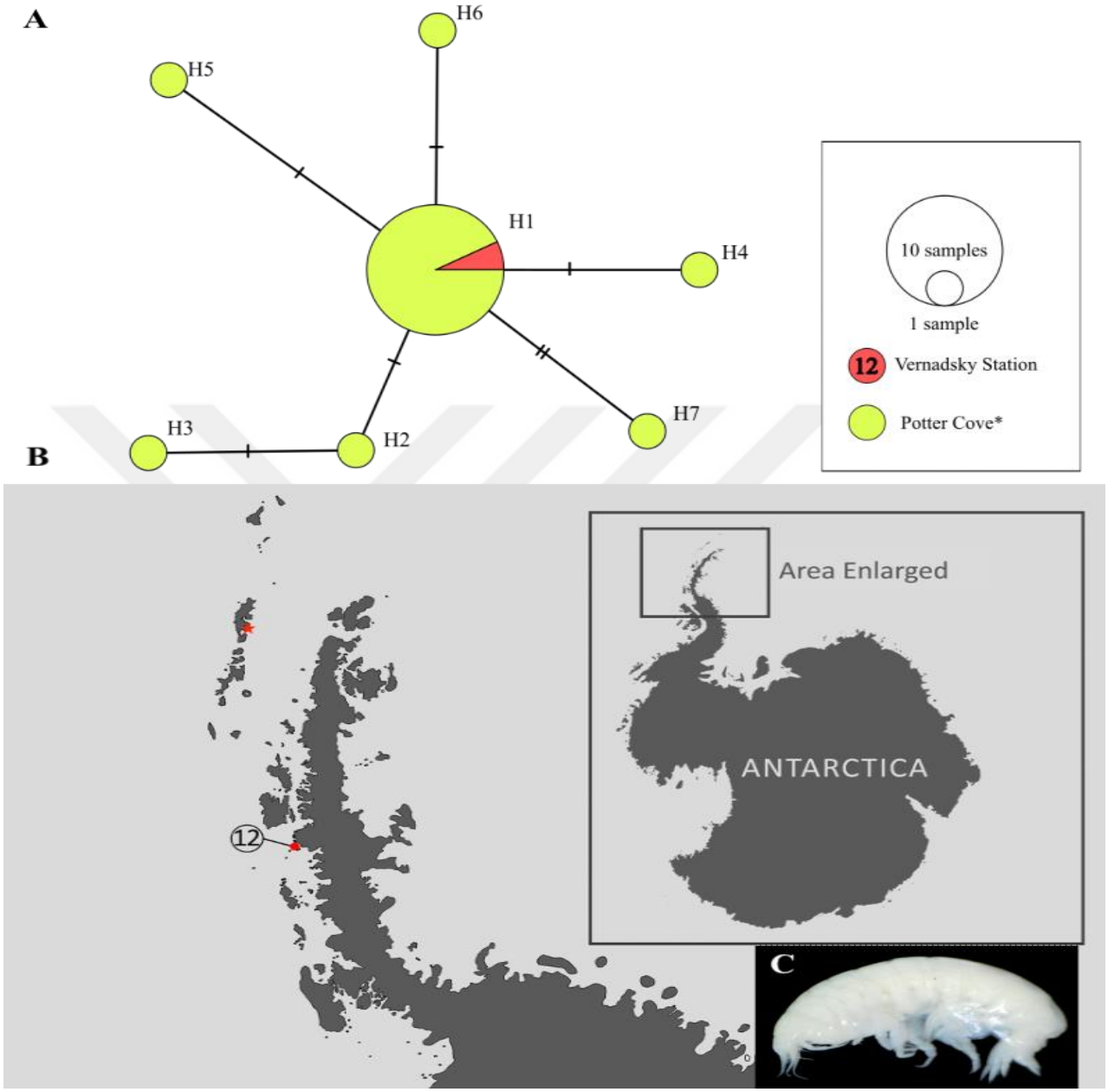


Figure 4.18. A. Haplotype network of the *Hippomedon kergueleni* (Miers, 1875). The colors in the network indicate the locations as seen in the legend. The asterisk "*" designates data retrieved from GenBank. Each hatch indicates one nucleotide difference. B. Distribution map represents sampling points. Point 12 and star (reference location) stand for Vernadsky Station and Potter Cove respectively. C. *Hippomedon kergueleni* Miers, 1875, Coll. Code 123, off Vernadsky Station, Galíndez Island, Wilhelm Archipelago, Western Antarctic Peninsula, 26.02.2019 (Photo by S.Ü. Karhan).

4.8. *Paramoera walkeri* (Stebbing ,1906)

Two individuals located in Telephone Bay, were identified as COI *Paramoera walkeri* with 100% similarity with a private COI data from BOLD. According to literature such as expedition notes, identification keys and monographs the genetic identification confirmed by morphological assessment under stereomicroscope. ANTBU026-22 was selected as a representative of *Paramoera walkeri*, (Stebbing ,1906) to build a phylogenetic tree via BOLD (Figure 4.19). Based on BOLD identification results, the closest neighbor species was *Hirondella dubia* with 79.37% similarity, however there were closer neighbors that were identified only at the genus (e.g., 80.85% similarity with the genus *Arrhis*), family (80.59% with Eusiridae) or even order level (as Amphipoda). Additionally, *P. walkeri* inhabits Antarctic Ocean including South Shetland Islands.

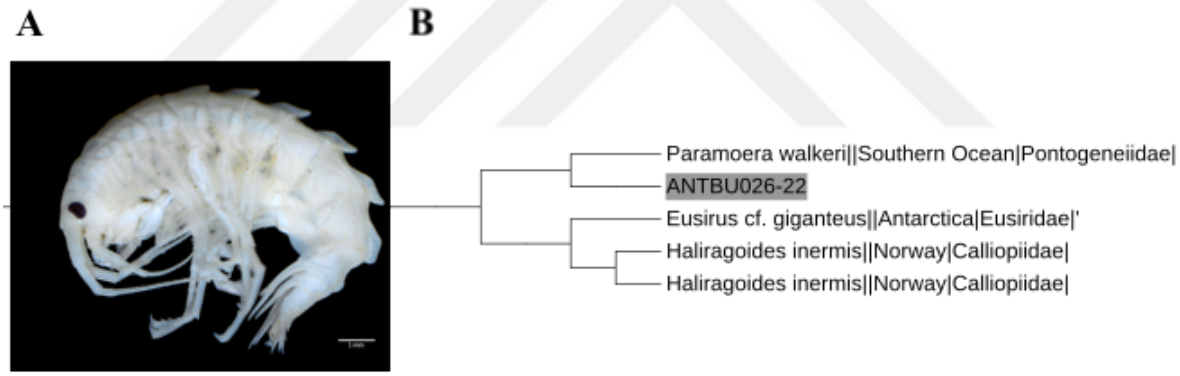


Figure 4.19. A. *Paramoera walkeri* (Stebbing ,1906) Coll. Code 067 (S.Ü. Karhan) B. Phylogenetic tree constructed with data retrieved from bold BOLD. COI sequence of individual ANTBU026-22 was used for phylogenetic tree.

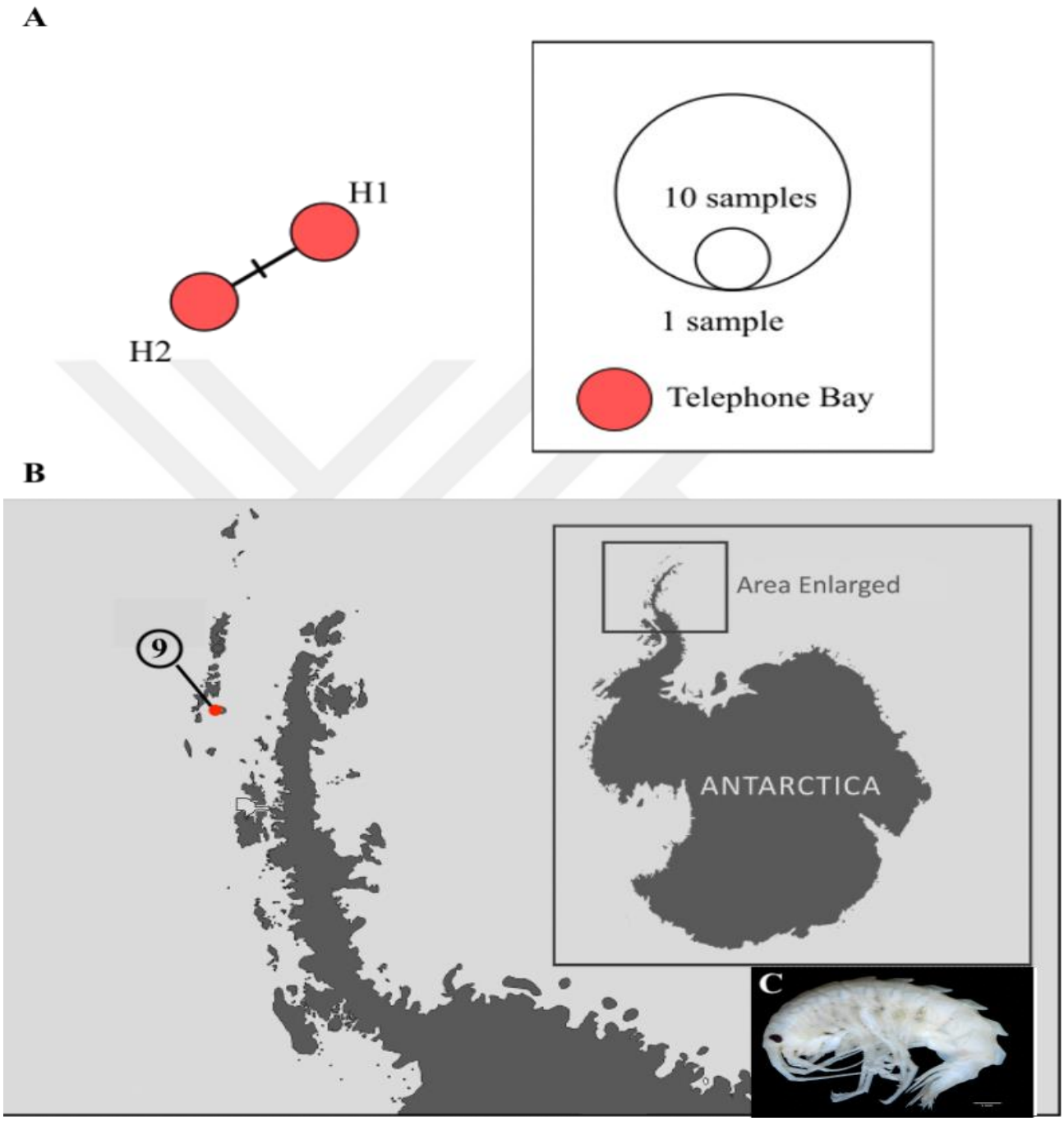


Figure 4.20. A. Haplotype network of the *Paramoera walkeri*. Haplotype network colors indicate the locations as seen in the legend, The asterisk "*" designates data retrieved from BOLD. Each hatch indicates one nucleotide difference. B. The map represents sampling point, Telephone Bay.
C. *Paramoera walkeri* (Stebbing ,1906) Coll. Code 123 (S.Ü. Karhan).

4.9. Unidentified Samples and COI Barcoding Limitations

As mentioned above, the individuals clustered in OTU-6, OTU-8, and OTU-10 (see Figure 23) could not be identified since they do not resemble any of the close genera in either BOLD or GenBank. OTU-12 is assigned to the *Paraceradocus* genus according to morphological features.



Figure 4.21. A. Undescribed individual that is a member of genus *Paraceradocus*. (OTU-12). B. Undescribed individual as a representative of OTU-10. C. Undescribed individual as a representative of OTU-6. D. Undescribed individual as a representative of OTU-8 (All photographed by S.Ü. Karhan)

These four OTUs were clearly separated from each other in the haplotype network (Figure 4.22) of unidentified OTUs, as well as in the neighbor joining tree (Figure 4.23). Each OTU creates a clade, which supports the hypothesis that they are different species, as shown in the ASAP and BOLD clustering results (see Figure 4.2, above). Fourteen haplotypes were observed as seen in Figure 4.24. *Vibilia antarctica* was represented by two haplotypes, but because of a single base pair difference between them, they are considered to be in the same clade.

Table 4.9. Haplotype network table of undescribed OTUs.

Haplotypes	Locations	No. of individuals
H1(OTU-8)	Vernadsky S.	2
	E.Coast Fildes P.	4
H2(OTU-6)	Vernadsky S.	3
	Nansen Island	1
H3(OTU-12)	Vernadsky S.	1
H4(OTU-10)	E.Coast Fildes P.	1

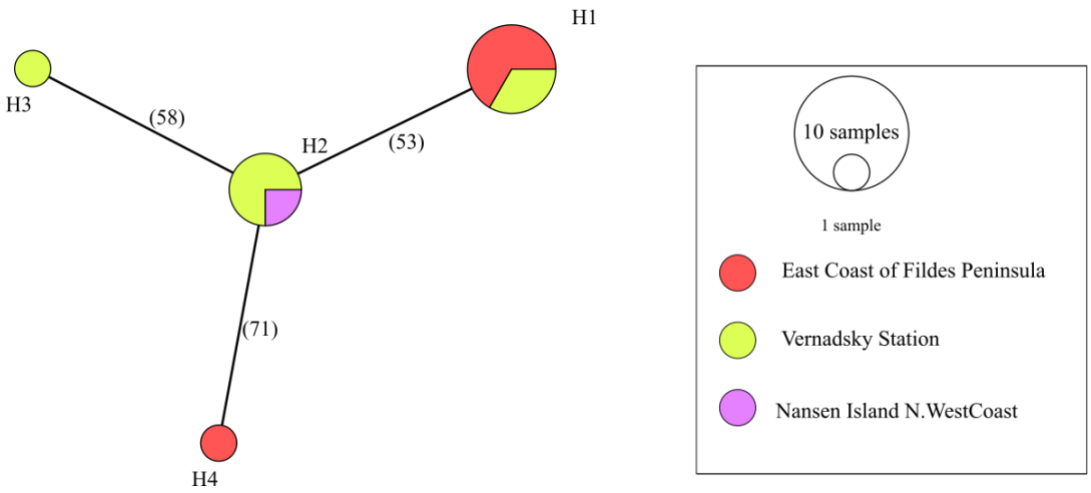


Figure 4.22. Haplotype network of undescribed OTUs. H1, H2, H3, H4 are OTU-8, OTU-6, OTU-12 and OTU-10 respectively.

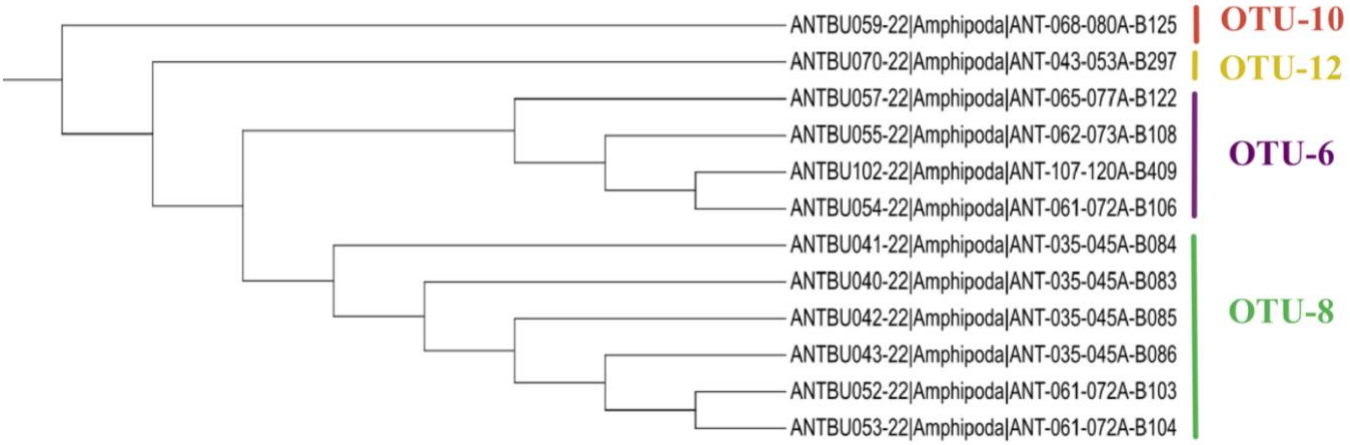


Figure 4.23. Neighbor joining tree built by using the sequences of undescribed OTUs.

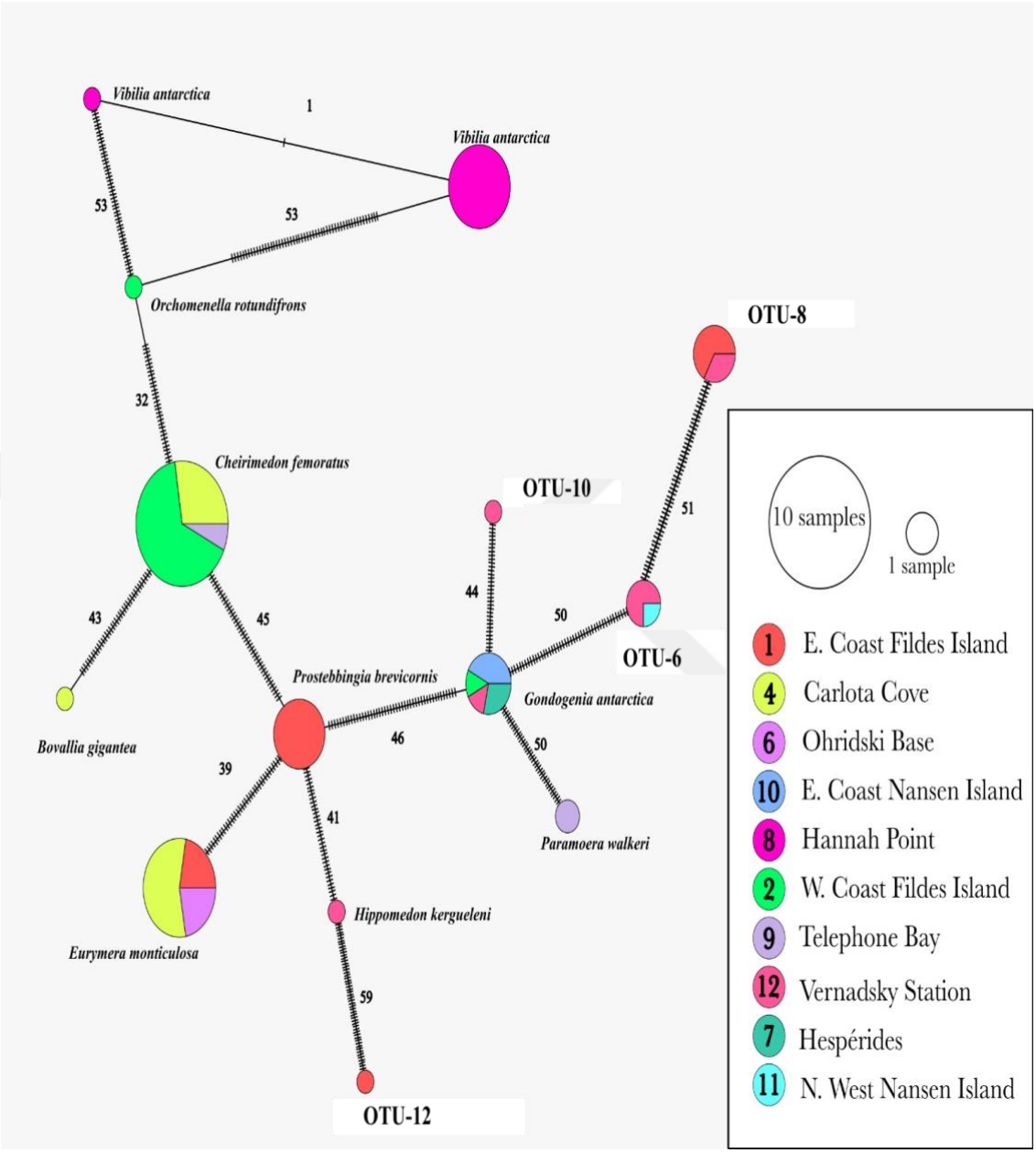


Figure 4.24. Haplotype network that represents all of the samples in a single minimum spanning network.

Three OTUs were unassigned at the species or genus level. Neighbor-joining trees were used to compare ANTBU052-22, an OTU-6 representative, to the BOLD data (Figure 4.25). *Gammarus lacutris* was the closest neighbor with 79.49% similarity, and the second closest individual in family Pontogeneiidae with 78.27% similarity and *Echinogammarus tabu mutus* sub species with 77.78% similarity.

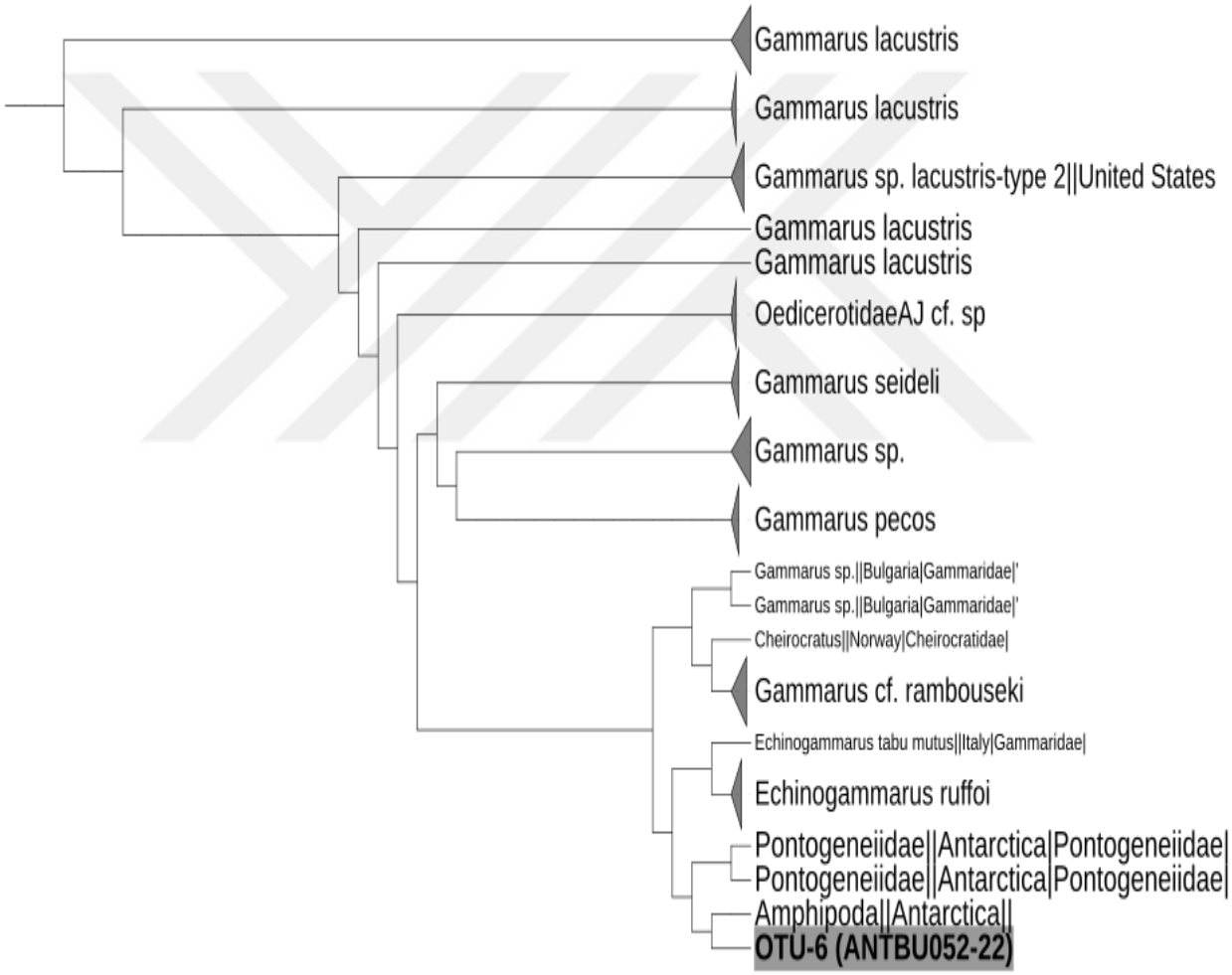


Figure 4.25. Phylogenetic tree constructed with data retrieved from BOLD and COI sequence of individual ANTBU052-22. The sizes of the triangles are proportional to the size of the clades.

ANTBU055-22 was chosen as a representative individual of OTU-8 and has a 100% match with individuals that were identified as “Amphipoda”. The next closest neighbor with 79.15% similarity is in the Eurisidae family; however, the genus and the species were not named. And the third closest individual, with 78.59% similarity, was assigned as *Anonyx cf. laticoxae.* Neighbor-joining tree represents the location of OTU-8 in a phylogenetic tree constructed using all the recorded data in BOLD (Figure 4.26).

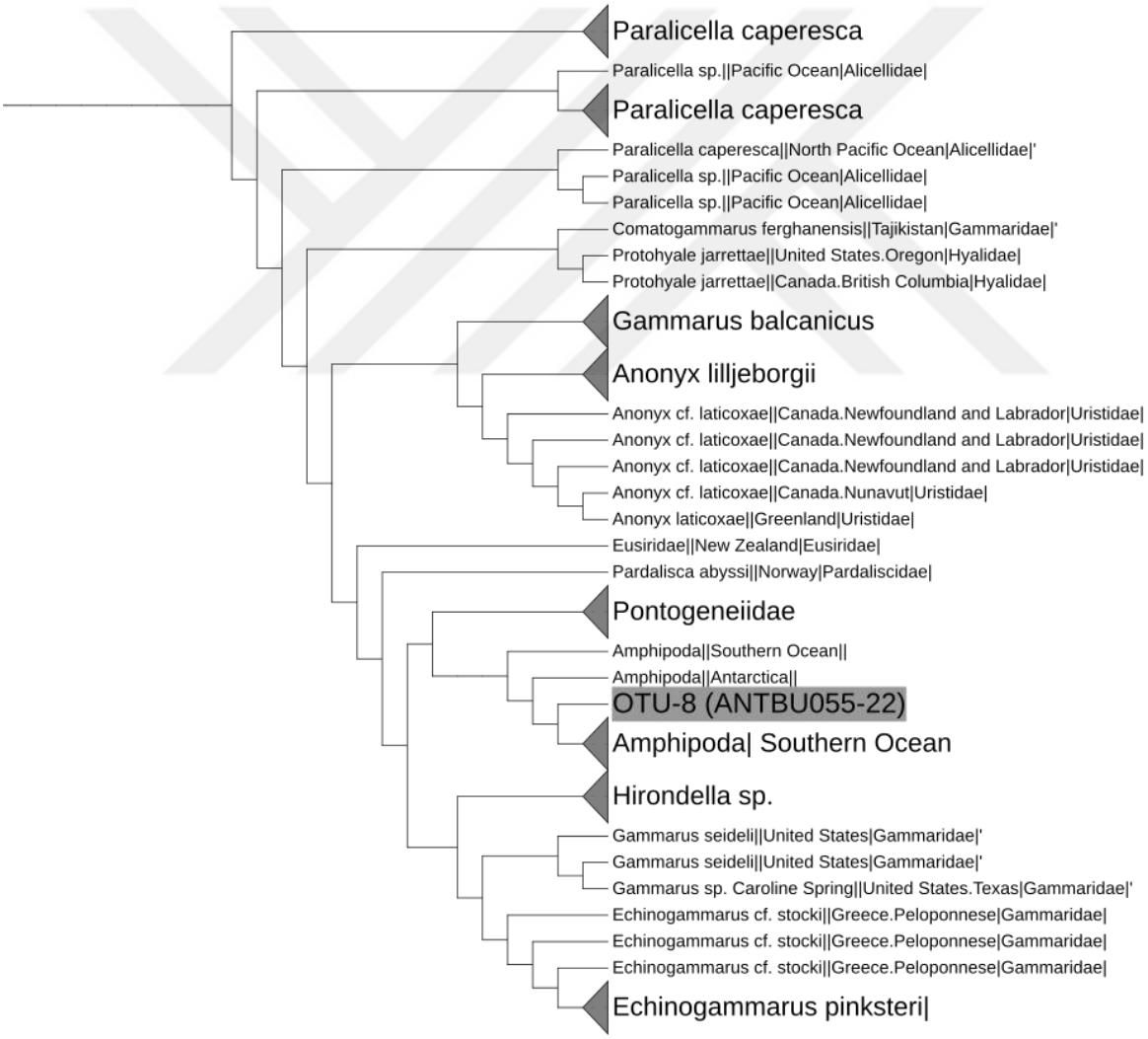


Figure 4.26. Phylogenetic tree constructed with data retrieved from BOLD and COI sequence of individual ANTBU055-22.

Only one OTU-10 individual has a maximum degree of similarity of 83.33% with data assigned to the Eusridae family that is not publicly available in BOLD. The second closest neighbor was assigned to the *Cleanordo* genus but was not identified at the species level. The third closest one was placed in the family Oedicerotidae, where it was 81.05% similar to our sample (see Figure 4.27).

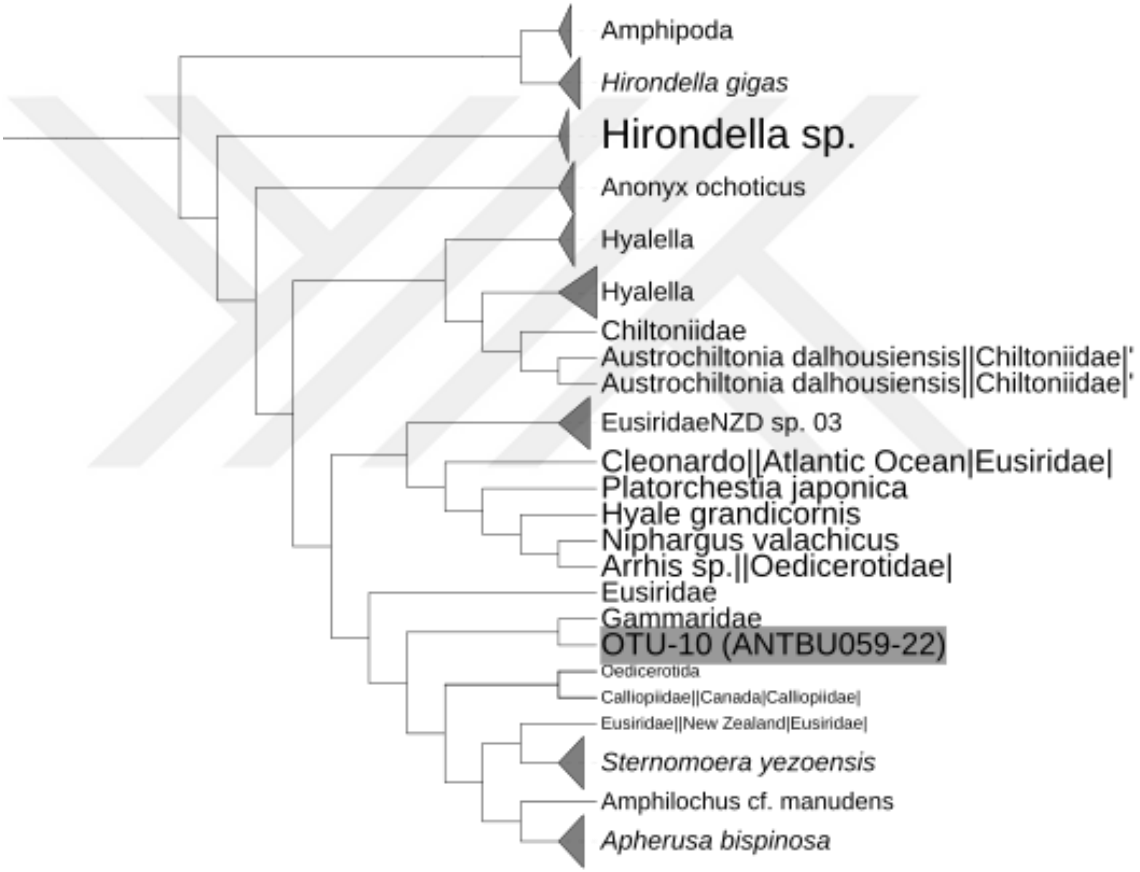


Figure 4.27. Phylogenetic tree constructed with data retrieved from BOLD and COI sequence of the individual ANTBU059-22. The sizes of the triangles are proportional to the size of the clades.

OTU-12, as mentioned before, was assigned to the *Paraceradocus* genus. Further analysis needs to be performed by a specialist for each of the four unidentified OTUs. With 81.19% similarity, the next-closest neighbor was also assigned to the same group (Figure 4.28).

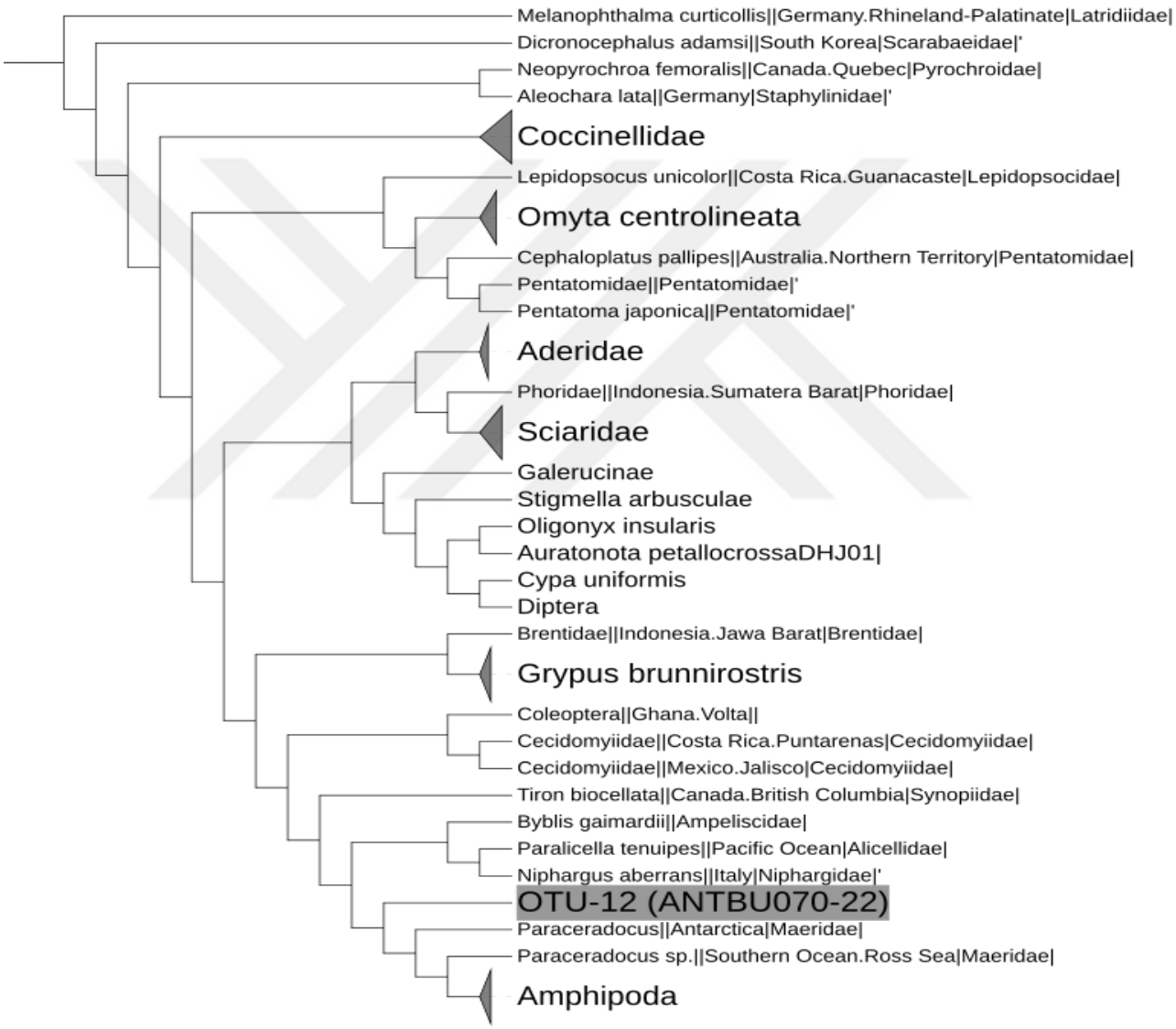


Figure 4.28. Phylogenetic tree constructed with data retrieved from BOLD and COI sequence of individual ANTBU070-22. The sizes of the triangles are proportional to the size of the clades.

In this study, we did not set a certain limit (i.e., a barcoding gap) for delimitation of the species. Instead, multiple analysis was used such as RESL provided by BOLD and ASAP which set the limit between 2.2% and 6.5% respectively. We encountered multiple unidentified taxa that were assigned to an order or genus or species since the barcodes represent only 10% of all identified amphipod species (Jażdżewska et al., 2021). In some cases, we observed that different names were grouped under a single BIN (e.g., see *Bovallia gigantea* section above), The case we encountered with *Bovallia gigantea* was most likely a misidentification of the species; and it is possible to decrease the frequency these problems with well-established diagnostic keys and revision of the data by related researchers (Jażdżewska et al., 2021). Additionally, presence of both former and newly established names of a species in databases is a common problem since there are several ongoing or finished projects uploaded from different researchers and institutions. Keeping the data (including identification of individuals) up-to-date is recommended (Jażdżewska et al., 2021). Due to database inconsistencies, species definitions solely based on genetic data (e.g., COI-barcodes) may be prone to errors, especially if there is a singleton sequence for a species needed to be confirmed by the specialist taxonomist to prevent possible mistakes (Seefeldt et al., 2018). Integrative taxonomy, which takes both genetic and morphological features into consideration, is more likely to produce more reliable results.

Amplification of one COI-like bacterial sequence was observed in the sample B059 which was identified morphologically as *Cheirimedon femoratus*. This sequence does not match and does not have any close neighbors to any of the amphipod data in GenBank or BOLD. The greatest similarity for the sequence from this species was found to be 83.87% with a bacterium, *Shewanella woodyii*. The neighbor-joining tree indicated that all the closest neighbors were in the *Shewanella* genus. Due to obtaining low amounts of DNA by using only legs for DNA extraction, we started to use half of the body of an individual amphipod, including the gut of the species. We suspected this might be the reason for the amplification of the non-target sequence of genome of bacteria from a close neighbor of the *Shewanella* genus (Figure 4.29). However, this might be speculation without the metabarcoding of gut bacteria (Mioduchowska et al., 2018). Based on previous findings amplification of non-target COI-like sequences such as bacterial genome and numts (nuclear mitochondrial pseudogenes) are a common problem for COI barcoding with Folmer primers (Buhay et al., 2009; Folmer et al., 1994; Mioduchowska et al., 2018; Song et al., 2008). In silico analyses of eukaryotic and prokaryotic genomes shows homology with the target of the Folmer primers (Siddal et al., 2009; Mioduchowska et al., 2018). According to previous

studies the reverse Folmer primers tend to match the genomes of some gamma-proteobacteria, rather than metazoan taxa (Mioduchowska et al., 2018).

COI amplification from metazoans is even harder, which encourage researchers to redesign the primers (Geller et al., 2013). Even though more specific primers were suggested and hundreds of modified Folmer primes were redesigned to reduce the amount of non-target region amplification, amplified numts that are as same size as COI and that do not have stop codons in previous studies (Moulton et al., 2010). We were able to obtain high-quality sequences from only 20.3% of the individuals which corresponds to half of the PCR products that were sent to sequencing based on presence of 650 bp bands. The reason for the low-quality sequencing results might be due to the amplification of both target and non-target sequences. Another reason for low yield of high-quality sequence could be DNA degradation since some of the samples collected were non-alive. In addition, it should be noted that there are already inaccurate datasets published and accessible in the databases. For example, there are non-target COI-like sequences from bacterial genomes that have been misidentified as animal sequences (Mioduchowska et al., 2018). This is crucial, as inaccurate datasets can lead to misinterpretations and incorrect conclusions that are based on inaccurate reference data.

To sum up, traditional (morphological) taxonomy, amphipod species distinction necessitates specific expertise and accurate identification keys. Convergent evolution is a common phenomenon among several amphipod species, where these species have similar morphologies that evolved independently, making identification of taxa with comparable morphology challenging. At this time, DNA barcoding provides an alternative for differentiating species based on genetic dissimilarity and identifying individuals based on "barcode" comparisons in databases such as BOLD and NCBI GenBank. Nevertheless, precise data are essential for species identification based on barcode genes, and erroneous data was detected in databases. In addition, there is a barcoding gap because not all species have been barcoded (e.g., only 10% of known Amphipoda species are currently barcoded). The barcoding gap is a challenge because it limits precise identification through barcode comparisons. Further studies would help increase the number of species and diminish this gap. Because of the limitations of identification via DNA barcodes, integration of genetic and morphological features should be considered. In this study we used reverse taxonomy as an example of this integrative taxonomy approach.

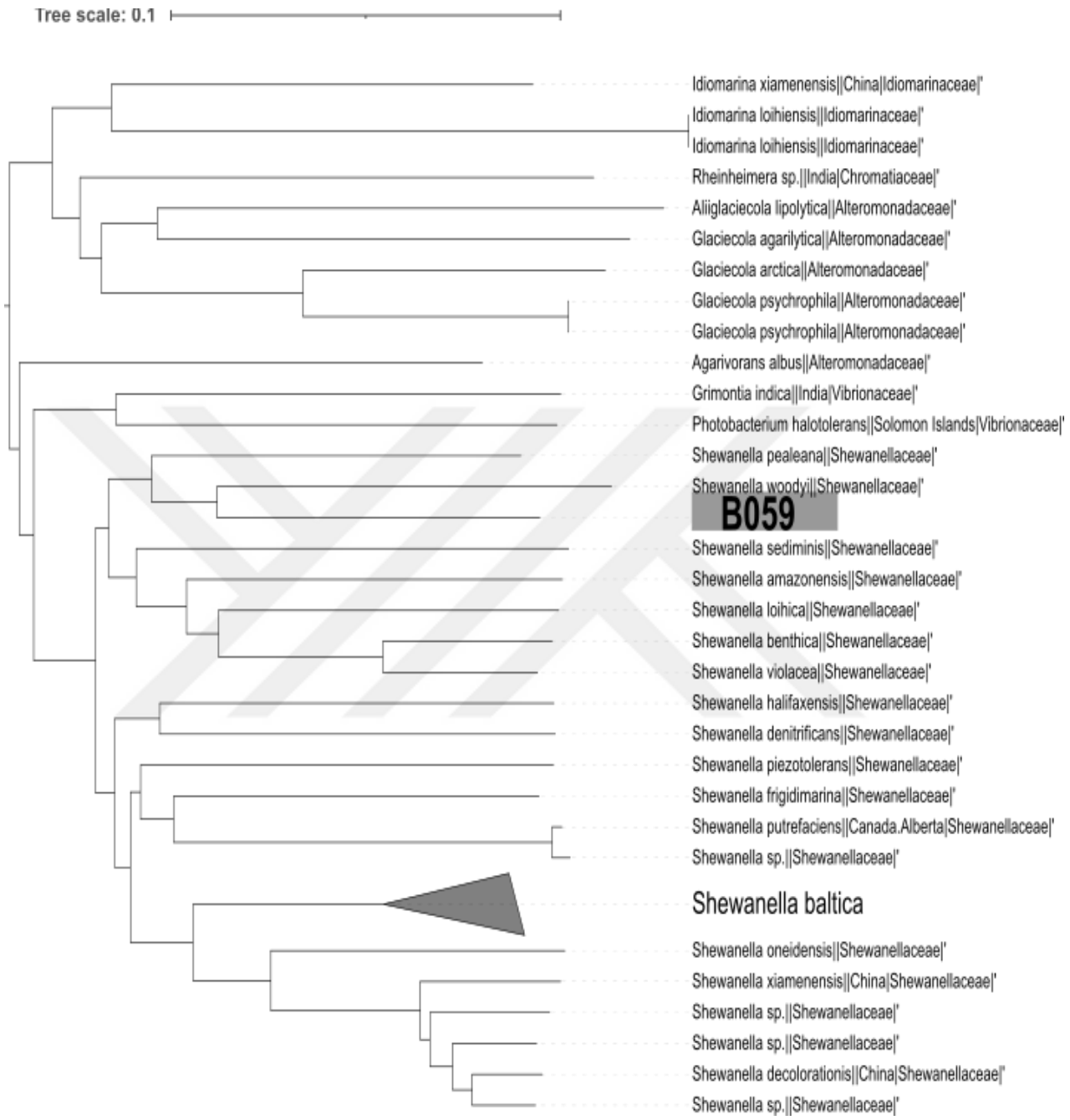


Figure 4.29. Neighbor-joining tree by using individual B059 and BOLD data.

5. CONCLUSION AND RECOMMENDATIONS

In total, 453 individuals were examined and prepared for steps of molecular laboratory analyses, however only 97 amphipod COI sequences were obtained. After filtering them (>300 bp and no stop codon), only 93 individuals were used for further analysis. This low success rate is probably and partially due to DNA degradation since some of the individuals were not alive during their collection. Additionally, the difficulty of COI amplification of metazoans via Folmer primers might cause co-amplification of non-target regions of the genome that might cause heteroplasmy, low quality sequences or non-target region of non-target taxa (e.g., gamma-proteobacteria). We strongly recommend that design of more specific Folmer primers. In addition, for the further analysis, some PCR amplifications could potentially be repeated with different primers aforementioned at methodology section.

Hippomedon kergueleni, Orchomenella rotundifrons, Cheirimedon femoratus, Bovallia gigantea, Vibillia antarctica, Prostebbingia brevicornis, Gondogeneia antarctica and *Eurymera monticulosa* and *Paramoera walkeri* were observed and identified via reverse taxonomy at the rarely studied intertidal zones of the Antarctic Peninsula. In addition, no alien species were observed in this study. Eleven individuals of three haplogroups could not be identified, due to absence of the reference barcode sequences of some species in BOLD and NCBI GenBank. One haplogroup was assigned to genus *Paraceradocus*, however it could not be identified in species level. The undescribed specimens are planned to be sent to a taxonomy specialist on Antarctic amphipods in order to identify their taxon.
We emphasize that revision of the already available data and improvement of the synchrony between the BOLD and NCBI databases could comprise good steps to help alleviate cases of misidentification.

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APPENDIX A: GEL IMAGES OF PCR REACTIONS WITH DIFFERENT PRIMERS

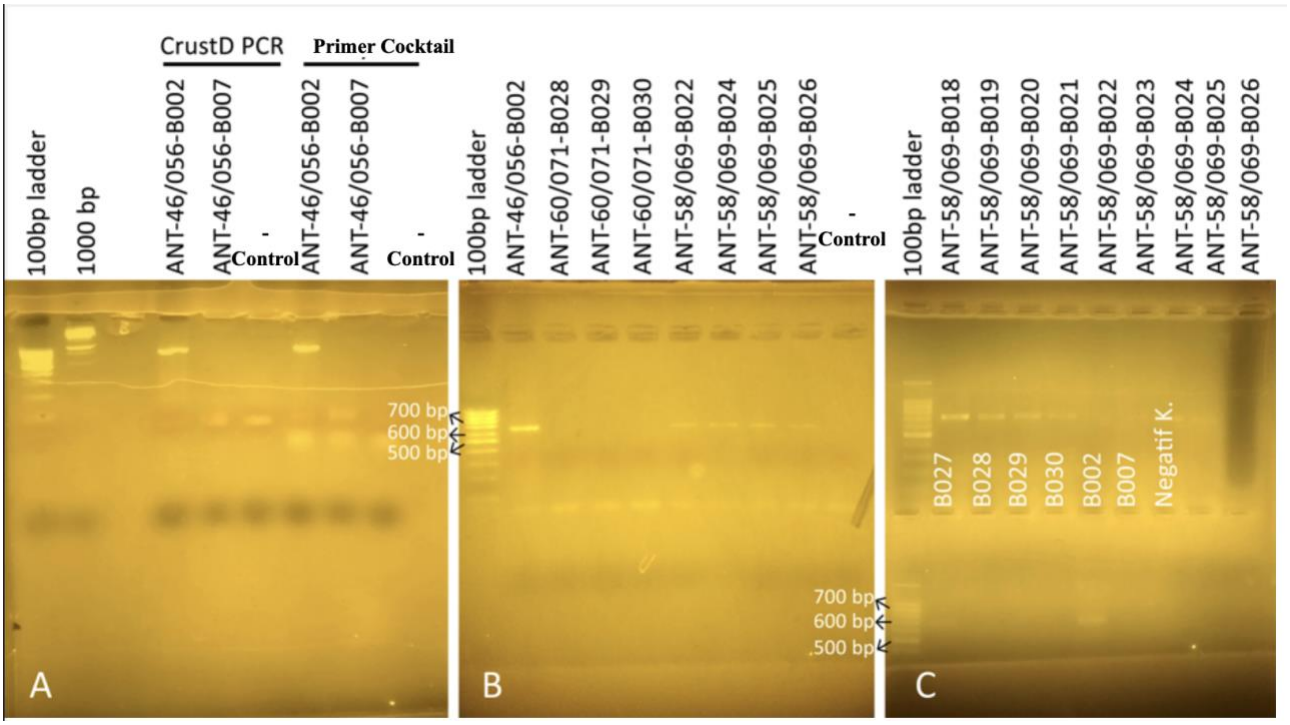


Figure A. Gel images of PCR reactions with different primers

APPENDIX B: OVERVIEW TABLE

Table B. Overview Table

Number of Individuals	Sequenced PCR Products	High Quality Sequence >300	High Quality Sequence >500	Sequences with stop codon	Bacteria Sequence
457	186	93	87	2	1

APPENDIX C: DETAILED HAPLOTYPE TABLE OF *Cheirimedon femoratus.*

Table C. Detailed haplotype table of *Cheirimedon femoratus.*

Haplotype	Locations	Sequences from this study	References from BOLD	No.of Individual
H1	West Coast of Fildes P.	ANTBU002-22, ANTBU004-22, ANTBU011-22, ANTBU088-22, ANTBU095-22, ANTBU097-22		6
	Carlota Cove	ANTBU035-22 ANTBU036-22 ANTBU038-22		3
	Potter Cove		MF124093.1 MF124094.1 MF124095.1 MF124096.1	4
H2	West Coast of Fildes P.	ANTBU007-22		1
H3	West Coast of Fildes P.		ANTBU090-22	1
	Potter Cove*		MF124099 MF124100.1	2

Table C. Detailed haplotype table of *Cheirimedon femoratus.*

Haplotype	Locations	Sequences from this study	References from BOLD	No.of Individual
H4	Carlota Cove	ANTBU032-22 ANTBU033-22 ANTBU034-22 ANTBU037-22 ANTBU039-22		5
	W.Coast of Fildes P.	ANTBU003-22 ANTBU006-22 ANTBU009-22 ANTBU093-22 ANTBU094-22 ANTBU096-22		6
	Telephone Bay	ANTBU025-22		1
	Potter Cove		MF124090.1 MF124091.1 MF124102.1 MF124103.1	4
H5	Potter Cove		MF124101.1	
H6	Potter Cove		MF124098.1	
H7	West C. of Fildes P.	ANTBU005-22		
H8	West C. of Fildes P	ANTBU008-22		
H9	West C. of Fildes P	ANTBU089-22		
H10	West C. of Fildes P	ANTBU091-22		
H11	Potter Cove	MF124092.1		
H12	Telephone Bay	ANTBU024		1
H13	Potter Cove*	MF124097.1		1
H14	West C. of Fildes P.	ANTBU010-22		1

APPENDIX D:

SEQUENCES GENERATED IN THIS STUDY

>ANTBU098-22|Eurymera monticulosa
ATGCCTATTATAATTGGCGGGTTCGGTAATTGACTTGTCCCTCTAATACTAGGTAGACCAG
ATATGGCCTTCCCGCGAATAAATAACATGAGATTTTGACTTCTACCTCCCGCCTTAACTTT
CCTACTTGTTAGGGGATTAATCGAAAGAGGTGTTGGGACAGGCTGAACTGTCTACCCCCC
CTTATCTGCCAATATTGCACATAGTGGGGGTTCAGTCGACACCGCTATCTTCTCCTTACAT
CTGGCAGGAGCAAGCTCTATCTTAGGGGCCATTAATTTTATCTCAACAGTAAACAATATGC
GAGCTCCAAGTATTAGCCCAGACCAGATACCTTTGTTTGTCTGATCCGTTCTTATCACTGCT
ATTTTATTACTTCTATCCCTACCTGTCTTAGCAGGAGCAATCACCATGTTACTTACAGATCG
TAACCTTAATACCTCATTTTTTGATCCAAGAGGTGGCGGCGACCCTATCTTATACCAGCAC
CTATTTTGATTCTTCGGGCATCCTGAAGCA

>ANTBU001-22|Vibilia antarctica
ATTTGATCAGGTTTAGGGGGTATATCCTTAAGTACATTAATTCGTATTGAACTAGCATATA
CAGGTACTATAATTGGTGATGATCAAATTTACAATGTTATAGTTACAGCTCATGCTTTTAT
TATAATTTTTTTTATGGTTATACCTATTATAATTGGTGGTTTTGGTAATTGATTAGTACCTTT
AATACTTGGTGCTGTAGATATATCATTTCCTCGTATAAATAATATAAGATTTTGACTATTA
CCTCCTTCATTAATTTTATTATTTTTAAGAAGACTAGTTGAAAATGGTGTTGGTACTGGATG
AACAGTATACCCTCCTTTAGCAGGTTTAATTAGACATAGAGGTTCTTCAGTTGATATAGCT
ATTTTTTCTTTACATTTAGCAGGTATTTCATCTATTTTAGGAGCTGTAAACTTTATTACTAC
TTGTTTAAATATACGAGGTTGTTTTTCTATAGACCAAATACCCTTATTTGTGTGATCTGTAT
TTATTACTGCTATTTTATTACTATTATCTTTACCTGTTCTAGCAGGAGCTATTACTATACTA
TTAACAGACCGTAATTTAAATACTACTTTTTTTG

>ANTBU002-22|Cheirimedon femoratus
AAAGATATTGGGACATTATATTTTATTTTAGGGGGTTGAGCGAGATTTGTTGGGACTTCTT
TAAGAGTAATTATTCGATCAGAGTTAAGTGGGCCTGGGAGTTTAATTGGAAGGGATCAAA
TTTATAATGTAATAGTAACTGCGCATGCTTTTGTTATAATTTTTTTTATAGTAATGCCTATT
ATAATCGGAGGGTTTGGTAATTGGTTGGTTCCTTTAATATTGGGGAGACCTGATATAGCAT
TCCCTCGTATAAATAATATAAGATTTTGGTTATTACCTCCTTCTTTAATTTTATTGTTAATA
AGGGGTATAGTTGAAACAGGTGTTGGCACGGGGTGAACTGTTTATCCACCCCTAGCTTCA
AGGGTTGCCCATAGGGGGGGTTCAGTAGATATAGCTATTTTTTCTTTGCATTTAGCAGGTG
CTTCTTCTATTTTAGGGGCCATTAACTTTATTTCTAGGGTTATTAATATACGTAGTTATGGG
ATAAGGTTTGATCGGTTATCTTTATTTGTTTGATCTGTATTTATTACGGCTATTTTACTTCTT
TTATCTTTACCTGTGTTAGCTGGGGCTATTACAATATTATTAACAGATCGAAATTTAAATA
CCTCGTTTTTTGATCCTAGGGGTGGGGGGGATCCCATCCTTTATCAACATTTATTTTGATTC
TTCGGTC

>ANTBU003-22|Cheirimedon femoratus
TGAGCGAGATTTGTTGGGACTTCTTTAAGAGTAATTATTCGATCAGAGTTAAGTGGGCCTG
GGAGTTTAATTGGAAGGGATCAAATTTATAATGTAATAGTAACTGCGCATGCTTTTGTTAT
AATTTTTTTTATAGTAATGCCTATTATAATTGGAGGGTTTGGTAATTGGTTGGTTCCTTTAA
TATTGGGGAGACCTGATATAGCATTCCCTCGTATAAATAATATAAGATTTTGGTTACTACC
TCCTTCTTTAATTTTATTGTTAATAAGGGGTATAGTTGAAACAGGTGTTGGCACGGGGTGA

ACTGTTTATCCACCCCTAGCTTCAAGGGTTGCCCATAGGGGGGGTTCAGTAGATATAGCTA
TTTTTTCTTTGCATTTAGCAGGTGCTTCTTCTATTTTAGGGGCCATTAACTTTATTTCTAGGG
TTATTAATATACGTAGTTATGGGATAAGGTTTGATCGGTTATCTTTATTTGTTTGATCTGTA
TTTATTACGGCTATTTTACTTCTTTTATCTTTACCTGTGTTAGCTGGGGCTATTACAATATTA
TTAACAGATCGAAATTTAAATACTTCGTTTTTTGATCCTAGGGGTGGGGGGGATCCTATCC
TTTATCAACATTTATTTTGATTCTTCGGTCACC

>ANTBU004-22|Cheirimedon femoratus
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GGGAGTTTAATTGGAAGGGATCAAATTTATAATGTAATAGTAACTGCGCATGCTTTTGTTA
TAATTTTTTTTATAGTAATGCCTATTATAATCGGAGGGTTTGGTAATTGGTTGGTTCCTTTA
ATATTGGGGAGACCTGATATAGCATTCCCTCGTATAAATAATATAAGATTTTGGTTATTAC
CTCCTTCTTTAATTTTATTGTTAATAAGGGGTATAGTTGAAACAGGTGTTGGCACGGGGTG
AACTGTTTATCCACCCCTAGCTTCAAGGGTTGCCCATAGGGGGGGTTCAGTAGATATAGCT
ATTTTTTCTTTGCATTTAGCAGGTGCTTCTTCTATTTTAGGGGCCATTAACTTTATTTCTAGG
GTTATTAATATACGTAGTTATGGGATAAGGTTTGATCGGTTATCTTTATTTGTTTGATCTGT
ATTTATTACGGCTATTTTACTTCTTTTATCTTTACCTGTGTTAGCTGGGGCTATTACAATATT
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>ANTBU005-22|Cheirimedon femoratus
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>ANTBU006-22|Cheirimedon femoratus
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>ANTBU007-22|Cheirimedon femoratus
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>ANTBU035-22|Cheirimedon femoratus
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>ANTBU036-22|Cheirimedon femoratus
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>ANTBU037-22|Cheirimedon femoratus
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>ANTBU038-22|Cheirimedon femoratus
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>ANTBU039-22|Cheirimedon femoratus
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>ANTBU040-22|Amphipoda
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>ANTBU041-22|Amphipoda
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>ANTBU042-22|Amphipoda
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>ANTBU043-22|Amphipoda
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>ANTBU044-22|Prostebbingia brevicornis
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>ANTBU045-22|Prostebbingia brevicornis
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>ANTBU046-22|Prostebbingia brevicornis
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>ANTBU049-22|Prostebbingia brevicornis
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>ANTBU051-22|Gondogeneia antarctica
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>ANTBU052-22|Amphipoda
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>ANTBU053-22|Amphipoda
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>ANTBU054-22|Amphipoda
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>ANTBU055-22|Amphipoda
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>ANTBU057-22|Amphipoda

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>ANTBU058-22|Hippomedon kergueleni
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>ANTBU059-22|Amphipoda
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>ANTBU062-22|Gondogeneia antarctica
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>ANTBU063-22|Gondogeneia antarctica
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>ANTBU064-22|Gondogeneia antarctica
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>ANTBU066-22|Eurymera monticulosa
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>ANTBU067-22|Eurymera monticulosa
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>ANTBU068-22|Eurymera monticulosa
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>ANTBU069-22|Bovallia gigantea
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>ANTBU070-22|Paraceradocus sp.
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>ANTBU071-22|Vibilia antarctica
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>ANTBU072-22|Vibilia antarctica
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>ANTBU073-22|Vibilia antarctica
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>ANTBU074-22|Vibilia antarctica
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>ANTBU075-22|Vibilia antarctica
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>ANTBU076-22|Eurymera monticulosa
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>ANTBU077-22|Prostebbingia brevicornis
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>ANTBU078-22|Prostebbingia brevicornis
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>ANTBU079-22|Prostebbingia brevicornis
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>ANTBU080-22|Vibilia antarctica
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>ANTBU081-22|Vibilia antarctica
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>ANTBU082-22|Vibilia antarctica
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>ANTBU083-22|Vibilia antarctica
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>ANTBU084-22|Vibilia antarctica
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>ANTBU085-22|Vibilia antarctica
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>ANTBU086-22|Vibilia antarctica
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>ANTBU087-22|Vibilia antarctica
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>ANTBU088-22|Cheirimedon femoratus
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>ANTBU089-22|Cheirimedon femoratus
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>ANTBU090-22|Cheirimedon femoratus
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>ANTBU091-22|Cheirimedon femoratus
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>ANTBU092-22|Orchomenella rotundifrons
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>ANTBU093-22|Cheirimedon femoratus
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>ANTBU094-22|Cheirimedon femoratus
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>ANTBU095-22|Cheirimedon femoratus
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>ANTBU096-22|Cheirimedon femoratus
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>ANTBU097-22|Cheirimedon femoratus
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>ANTBU099-22|Eurymera monticulosa
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>ANTBU100-22|Eurymera monticulosa
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>ANTBU101-22|Eurymera monticulosa
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TGAT

>ANTBU102-22|Amphipoda
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>B059|Bacteria
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