DNA BARCODING OF FIFTEEN SHRIMP AND PRAWN SPECIES (DECAPODA, CRUSTACEA), LIVING IN TURKISH COASTAL WATERS WITH PHYLOGEOGRAPHIC COMPARISONS

by

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ABSTRACT

DNA barcoding is useful for the quick identification and potential discovery of species. Systematic DNA barcoding on decapods has not been done in Turkey previously, and this thesis will be a first in this regard. In this study, DNA barcoding technique is used for the determination of genetic diversity of the shrimp and prawn species in Turkish waters. Through this approach the genetic diversity of fifteen shrimp and prawn species living in Turkey was evaluated and when possible the genetic data were compared with those available from different parts of the Mediterranean and eastern Atlantic. 51 COI sequences from 12 different species, and 64 16S sequences from six different species are generated for this study. The barcoding gap using CO1 seemed to work well with these species. Out of the new sequences generated, five species had unique barcode sequences that are distinct from those found in any other species in BOLD and four 16S sequences are new (based on a comparison with GenBank). In nine species two intraspecific clades were retrieved after analyses. In eight species for which CO1 data was produced, the tree identification method worked accurately. Finally, as a part of the barcoding approach, genetic material from the studied species is now stored at Institute of Environmental Sciences Laboratories, comprising a first step towards formation of a DNA Barcoding bank for these species.

ÖZET

DNA barkodlama tekniği genetik analiz ve tür tanımlamada oldukça çabuk ve yararlı bir yöntemdir. Bu çalışma ile Türkiye sularında bulunan karideslerin genetik barkodlaması sistematik olarak ilk kez yapılmıştır. Bu çalışma Türkiye sularında bulunan karideslerin genetik çeşitliliğini DNA barkodlama tekniği ile belirlemeyi hedeflemektedir. Bu yöntem ile Türkiye sularından on beş farklı karides türünün genetik çeşitliliği belirlenmiş ve mümkün olduğunda diziler kullanılarak Akdeniz ve doğu Atlantik'teki diğer dizilerle filocoğrafi karşılaştırmalar da yapılmıştır. 12 farklı türden 51 mitokondriyal CO1 bölgesi ve altı farklı türden 64 16S dizisi elde edilmiştir. BOLD veritabanından 12 farklı bireye ait CO1 bölgesi, GenBank veritabanından ise dört farklı bireye ait 16S ve beş farklı bireye ait CO1 bölgeleri incelenmiştir. Çalışmada elde edilen dizilerde barkodlama aralığının iyi çalıştığı gözlemlenmiştir. İncelenen 12 yeni CO1 dizisinden beşinin, altı 16S dizisinden ise dördünün kendi türüne özgü bir barkoda sahip olduğu gözlemlenmiştir (BOLD ve GenBank ile karşılaştırılmıştır). Dokuz türde iki tür içi klad gözlenlenmiştir. CO1 dizisi elde edilen sekiz türde ağaç tanımlanması tam olarak çalışmıştır. Son olarak, kataloglamanın temel adımlarından olan türlerden çıkarılan DNA'nın uzun süre Enstitü'nün Çevre Bilimleri Laboratuvarlarında saklanması ile DNA barkodlama bankasının oluşturulması için ilk adım atılmıştır.

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LIST OF SYMBOLS/ABBREVIATIONS

BOLD	Barcode of Life Database
bp	Base pair
CBOL	Consortium for the Barcode of Life
Cytochrome c oxidase I	COI, CO1, cox1
DNA	Deoxyribonucleic Acid
DNTP	Deoxyribonucleotide triphosphate
EDTA	Ethylenediaminetetraacetic acid
H	Haplotype
mtDNA	Mitochondrial deoxyribonucleic acid
PCR	Polymerase chain reaction
RNA	Ribonucleic acid
rRNA	Ribosomal ribonucleic acid
TBE	Tris base boric acid, EDTA

1. INTRODUCTION

Categorizing biological diversity requires defining species in order to understand the human impact on terrestrial and marine ecosystems, and for exploring the continuous change in biodiversity, at any geographical scale. The binomial identification system that was outlined by Caroli Linnaeous around 1750s has paved the way for the accumulation of current taxonomic knowledge over 250 years (Tautz et al., 2003). It is estimated that there are between 10-100 million species of organisms on our planet. However, despite 250 years of morphological work, only 1.7 million of these species have been formally described. Determination of morphological differentiation can be hard and impractical by existing approaches. It is obvious that the motivation for a new approach to species recognition is necessary.

As an alternative to morphological approaches, microgenomic systems can help categorize diversity of life through the analysis of small segments of the genome, and they represent a promising approach to the analysis of biological diversity. DNA Barcoding is a recent method, first proposed in 2003 by the Canadian geneticist Paul Hebert (Hebert et al., 2003). A short region of mitochondrial DNA (mtDNA) can be sequenced and used as a standard region. This region can be used as an identifier for species, which is referred to as a "DNA barcode". DNA barcode sequences are very short relative to the entire genome and they can be obtained practically quickly and cheaply, using basic sequencing methods. Subsequently, the barcode from unknown specimens are compared with a library of reference barcodes to identify them. The methodology includes genetic analyses of known species to create reference databases, photographing of the species investigated for visual identification and cataloging, and long-term storage of the DNA extracted from them. This approach helped to discover new species and characterize the taxonomic and genetic diversity of different geographic regions. In 2004, after Hebert's pioneering work, the Alfred P. Sloan Foundation funded the 160-member, 50-country Consortium for the Barcode of Life (CBOL). CBOL is currently organizing efforts around the world to accumulate a global reference library of DNA barcodes.

During the last 30 years, DNA sequence analysis has been used to help in identification of biological species. In several studies, different DNA data sequence sets were used for different taxonomic groups (Ward et al., 2005). Hebert et al. (2003) suggested that only one gene sequence, COI (cytochrome *c* oxidase subunit 1) mitochondrial DNA region, could be enough to identify the animal species and to differentiate them. According to Hebert et al. (2003), genetic material of each species would not cause any taxonomic confusion in identification if it is supported with photographic material, which shows morphological characters. Nowadays genetic barcoding helps to reinforce classical taxonomy, and it is one of the most helping agents of the taxonomic process (Costa et al., 2007).

Past phylogenetic work has heavily focused on mitochondrial genes encoding ribosomal (12S, 16S) DNA. However their utility in taxonomic analyses can be compromised by the wide extension of insertions and deletions (indels) that complicate sequence alignments (Doyle and Gaut, 2000). The 13 protein-coding genes in the animal mitochondrial genome are better for such comparative analyses as indels, which can lead to shift in the reading frames, are rare. There is no obvious reason to focus on a specific gene for DNA barcoding, however the COI gene has two important advantages. Firstly, the universal primers for this gene are very strong, enabling recovery of its 5' end from the representatives of most animal phyla (Folmer et al., 1994). Secondly, Hebert et al. (2003) showed that COI likely has a greater range in phylogenetic signal than any other mitochondrial gene. Its third position nucleotides show a high frequency of base substitutions, however, changes in its amino acid sequence occur more slowly than some of the other mitochondrial genes.

For the identification of the marine invertebrate species, DNA barcoding may be a very useful tool. Many decapods are clearly distinguishable as adults but their larval and juvenile forms are difficult to identify as species. The larvae are rarely distinguishable, and they are not usually matched easily to the correct adult form (Bucklin, 2010). Jones and Macpherson (2007) examined new species of the East Pacific galatheid squat lobsters of the genus *Munidopsis* using morphological characters and mitochondrial COI sequences. Seven new *Munidopsis* species from the East Pasific were described revealing high gene flow among distant conspecific populations, and by differentiation of the closely related

species, the existence of sibling species in the genus *Munidopsis* was confirmed. In another study by Shih et al. (2009), the barcodes were used to distinguish six species of the fiddler crab *Uca* in the Indian Ocean. Another barcoding study by Pardo et al. (2009) showed the diagnostic morphological characters for species identification. In this study, Megalopae larvae of three sympatric crab species of the genus *Cancer* were collected to identify visible diagnostic characters. COI gene sequences of larvae were compared with adult *Cancer* samples, identified by morphology, and used to confirm the utility of diagnostic characters. The study indicated that simultaneous use of morphological and molecular tools is useful in identifying cryptic decapods species. The phylogeographic analysis of decapods also identified the cryptic species within the crab *Perisesarma guttatum* along an eastern African latitudinal gradient, again using COI (Silva et al., 2010).

1.1. Marine Decapod Crustaceans

This thesis focuses on the marine decapod crustaceans, specifically shrimp fauna in the Turkish Seas. The Crustacea include a wide range of animals, mostly aquatic, showing great variation in form, but all with the dorsal body surface covered by a shield-like carapace. Crustaceans (Crustacea) are a large group of arthropods, comprising almost 52,000 described species (Budd et al., 2001). This phylum is also economically important. Many crustacean species are consumed by humans in large quantities, and nearly 10,000,000 tons were harvested in 2005 (Fast and Lester, 1998). The vast majority of this output is of decapod crustaceans: crabs, lobsters, shrimp and prawns. Over 70% of all crustaceans by weight caught for consumption are shrimp and prawns, and over 80% are produced in Asia, with China alone producing nearly half the world's total. Non-decapod crustaceans are not widely consumed, with only 130,000 tons of krill being caught, despite krill having one of the greatest biomasses on the planet (Fast and Lester, 1998).

The most detailed species list prepared on decapods reported from Turkish seas, consists of 244 species (Ateş et al., 2010). Even if numerous studies have been made in Turkish seas on the taxonomy of decapod species and their distribution (Kocataş and Katağan, 2003), genetic data on decapod species that focus on Turkish seas are lacking in the recent literature.

1.2. Importance of Shrimp and Prawn Species

One of the most important groups of decapods includes shrimps and prawns. The Food and Agriculture Organization of the United Nation (FAO) lists 343 shrimp species that are actually or potentially commercially important (Fast and Lester, 1998). Of these, 110 species belong to the family Penaeidae and account for about 80% of the world's wild-caught shrimp. It contains many species of economic importance, such as the tiger prawn (*Penaeus monodon*), whiteleg shrimp (*Litopenaeus vannamei*), Atlantic white shrimp (*Penaeus setiferus*) and Indian prawn (*Fenneropenaeus indicus*). Eight more species have significant importance in aquaculture. These species include: *Penaeus chinensis, P. indicus, P. merguiensis, P. monodon, P. stylirrostris, P. vannamei* and *Metapenaeus ensis*. According to a global FAO study of shrimp fisheries, global shrimp catch is about three million tones *per* year. However, shrimp are strongly affected by illegal trawling, habitat destruction and climatic factors, which is reflected on the decline in global shrimp imports in recent years (Güngör et al., 2007).

1.3. The Shrimp of Turkey

The number of known decapod crustaceans on the Turkish Seas is 244. Among these, 88 are Natantia, 17 are Macrura Reprantia, 37 are Anomura, and 102 are Brachyura (Ateş et al., 2010). The shrimp fauna in Turkish seas consists of a total of 84 species (Ateş et al. 2010). More recent publications indicate the presence of several new species and new records for the shrimp fauna of Turkish Mediterranean shores. Among these, the Mediterranean caridean shrimp *Processa acutirostris* Nouvel and Holthuis, 1957 was collected recently from the Mediterranean, Turkey, being a new record for Turkey(Ateş et al., 2010). It is reported that a total of eight species belonging to the genus *Processa* are found in the Turkish seas. In Turkey, shrimp fishing and catch increased to a maximum of 8380 t in 1989 (Zengin et al., 2007). Shrimp are the most abundant species in the benthic fauna of the Sea of Marmara (Zengin et al., 2007) and currently, 72% of the shrimp production in Turkey is from the stocks in the Sea of Marmara.

1.4. Species Investigated in this Study

In this project, 15 species are investigated. These species are described below, with a discussion of their distribution in Turkey and basic taxonomy.

1.4.1. *Processa edulis* (Risso, 1816)

The common name of *Processa edulis* is Nika shrimp (FAO, 2011) and it is found in the Turkish Strait Systems, the Aegean, and the Mediterranean (Ateş et al., 2010). There are three recognized subspecies of *Processa edulis: Processa edulis arcassonensis* (Nouvel & Holthuis, 1957), *Processa edulis crassipes* (Nouvel & Holthuis, 1957) and *Processa edulis edulis* (Risso, 1816). The subspecies *Processa edulis edulis* is recorded in the Mediterranean and the Atlantic. The other subspecies *P. e. arcassonensis* is only known from France and *P. e. crassipes* is found in the rest of the European Atlantic coast (FAO, 2011).

1.4.2. *Processa acutirostris* (Nouvel and Holthuis, 1957)

The common name of *Processa acutirostris* is the Mediterranean shrimp and it is found only in the Mediterranean (Ateş et al., 2010). This species is endemic to the Mediterranean and it lives at a depth range between 0 and 25 m in the Adriatic Sea (Ateş et al., 2010). It does not have any recognized subspecies (FAO, 2011).

1.4.3. *Palaemon elegans* (Rathke, 1837)

The common name of *Palaemon elegans* is rockpool prawn and it is found along the coasts of the Mediterranean, the Aegean, the Bosphorus and Dardanelles straits, and the Black Sea (Özcan, 2007). It does not have any recognized subspecies (FAO, 2011). *Palaemon elegans* is very similar to *Palaemon serratus, Palaemon longirostris* and *Palaemon adspersus*, but can be separated from these species by the number of rostral teeth and the mandibular palp, which is composed of two segments (The Marine Life Information Network, 2011).

1.4.4. *Palaemon adspersus* (Rathke, 1837)

The common name of *Palaemon adspersus* is Baltic Prawn (FAO, 2011) and it is found in the Turkish Strait Systems, the Aegean, the Black Sea and the Mediterranean (Ateş et al., 2010). *Palaemon adspersus* is a species that is found frequently in the Baltic Sea, and is highly valuable for fisheries in Denmark. Characteristic red pigment spots on the lower half of rostrum help to distinguish it from other *Palaemon* species (Marine Species Identification Portal, 2011). No subspecies are recognized within *Palaemon adspersus* (FAO, 2011).

1.4.5. *Palaemon serratus (Pennant, 1777)*

The common name of *Palaemon serratus* is common prawn (FAO, 2011) and it is found in the Turkish Strait Systems, the Aegean, the Black Sea and the Mediterranean (Ateş et al., 2010). It does not have any recognized subspecies (FAO, 2011). *Palaemon serratus* can sometimes be misidentified as congener species such as *Palaemon elegans, Palaemon longirostris* and *Palaemon adspersus* (Marine Species Identification Portal, 2011). It can be diagnosed by the shape of the rostrum and the number of dorsal and ventral rostral teeth (The Marine Life Information Network, 2011).

1.4.6. *Sicyonia carinata* (Brünnich, 1768)

The common name of *Sicyonia carinata* is Mediterranean rock shrimp and it is found in the Mediterranean and the Aegean (Özcan, 2007). It does not have any recognized subspecies (FAO, 2011).

1.4.7. *Lysmata seticaudata* (Risso, 1816)

The common name of *Lysmata seticaudata* is Monaco shrimp (FAO, 2011) and it is found in the Mediterranean, the Aegean, the Black Sea and the straits of Turkey (Ateş et al., 2010). It does not have any recognized subspecies (FAO, 2011).

1.4.8. *Stenopus spinosus* (Risso, 1826)

The common name of *Stenopus spinosus* is golden coral shrimp and it is found in the Aegean, and the Mediterranean (Ateş et al., 2010). The species can be consumed, but is not very popular. It does not have any recognized subspecies (FAO, 2011).

1.4.9. *Synalpheus gambarelloides* (Nardo, 1847)

The common name of *Synalpheus gambarelloides* is sponge shrimp and it is found in the Mediterranean and the Aegean (Özcan, 2007). It does not have any recognized subspecies (FAO, 2011).

1.4.10. *Gnathophyllum elegans* (Risso, 1816)

Gnathophyllum elegans is recorded in the coasts of the Aegean (Ateş et al., 2010). It does not have any recognized subspecies (FAO, 2011).

1.4.11. *Hippolyte sapphica* (D'Udekem d'Acoz, 1993)

Hippolyte sapphica is an endemic Mediterranean and Black Sea species, which has been reported from the Adriatic, the Ionian, the Aegean and Black Seas. It does not have any recognized subspecies (Ntakis et al., 2010). It is not recorded in the Turkish Seas (Ateş et al., 2010).

1.4.12. *Parapenaeus longirostris* (Lucas, 1846)

The common name of *Parapenaeus longirostris* is deep water rose shrimp (FAO, 2011) and it is found in the Mediterranean, the Aegean and the straits of Turkey (Ateş et al., 2010). It does not have any recognized subspecies (FAO, 2011). The total catch reported for this species to FAO for 1999 was 19 056 t. The countries with the largest catches were Italy (4 631 t) and Spain (4 237 t). It is the most important commercial species of the Mediterranean coasts of Spain, France and Italy. Also although it is

uncommon in Algeria, Tunisia, Greece and Turkey, the species is still commercially valuable in these countries (FAO, 2011).

1.4.13. *Eualus cranchii* (Leach, 1817)

Eualus cranchii was formerly recognized as *Thoralus cranchii*, however this species definition is not accepted anymore (The Marine Life Information Network, 2011). The species is found in the Mediterranean, the Aegean and the straits of Turkey (Ateş et al., 2010). It does not have any recognized subspecies (FAO, 2011).

1.4.14. *Crangon crangon* (Linnaeus, 1758)

The common name of *Crangon crangon* is brown shrimp (FAO, 2011) and it is found in the Mediterranean, the Aegean, the Black Sea and the straits of Turkey (Ateş et al., 2010). It has cryptic coloration (a form of camouflage) and its colour can be changed by chromatophores. Normally it has a sandy brown colour, but this can be changed to match the environment. It is commercially caught by nets or trawls fishery for human consumption in Europe and the Mediterranean (Bilgin and Samsun, 2006). The total catch reported for this species to FAO in 1999 was 37 223 t. The countries with the largest catches were Germany (17 457 t) and Netherlands (13 772 t) (FAO, 2011). No subspecies are recognized within *Crangon crangon* (FAO, 2011).

1.4.15. *Athanas nitescens* (Leach, 1814)

The common name of *Athanas nitescens* is hooded shrimp and it is found in the coasts of the Mediterranean, the Aegean, Black Sea and the straits of Turkey (Özcan, 2007). It does not have any recognized subspecies (FAO, 2011). This species is often found in groups of adults and juveniles.

1.5. Literature Review

The use of DNA sequences as taxonomic tags can improve our recognition of species and verify phylogenetic relationships. DNA barcoding data provide a useful tool for species identification, and the sequences can be analyzed to determine phylogenetic relationships (Hirose et al., 2010). Recent studies showed that DNA barcoding can indeed be a powerful tool in describing biological diversity (Hebert et al., 2003).

Genetic studies of shrimp populations have been conducted using many methods such as isozyme protein electrophoresis, microsatellite and allozyme analysis (Zitari-Chatti et al., 2008). Nuclear genes such as the 28S ribosomal RNA (rRNA), 18S rRNA, 5.8S rRNA, and internal transcribed spacer 1 (ITS1) have been used for the study of phylogenetic relationships among shrimps (Porter et al., 2005). For phylogenetic analyses mitochondrial DNA is useful for its unique characteristics, such as an evolutionary rate 10 times faster than that of nuclear DNA. The 16S rRNA, 12S rRNA, and COI genes, the control region, and the cytochrome b gene (cytb) are the most used molecular markers. Both natural shrimp populations and captive populations can be classified by such mtDNA markers (Bouchon et al., 1994).

The DNA barcoding approach has been used for marine invertebrates. In Bucklin et al. (2007), for many species of euphausiids (Euphausiacea, Crustacea) which were classified by morphological characters, DNA barcodes were used and correct species classification was done by a detailed DNA barcode database. Moreover, identification of cryptic species (the species that are morphologically indistinguishable, but genetically distinct) was also done by DNA barcoding. For instance, *Euphausia brevis*, showed great difference in COI between Atlantic and Pacific populations. Finally DNA barcoding was used to evaluate taxonomically important geographic variation (Bucklin et al., 2007).

Among decapods, Wang et al. (2008) studied brine shrimp (*Artemia*) species by using DNA barcoding procedures. They have reported phylogenetic relationships of *Artemia* species. Using COI the study concluded apparent distinctions among *Artemia* populations in different parts of China.

In another study, Calo-Mata et al (2009) showed phylogenetic relationships among shrimp species by using the mtDNA markers; 16S rRNA and COI. Studies indicate that the 16S rRNA region exhibits a low rate of evolution. This means that it is better for examination of interspecific, rather than intraspecific, differentiation of species. Accordingly, certain studies based on the 16S rRNA gene showed relationships among shrimp species at different taxonomic levels (Lavery et al., 2004). Conversely, COI is a strong marker for determining both intraspecific and interspecific relationships. Recent studies showed these relationships in a variety of marine mollusks. Baldwin et al. (1996), revealed that within some shrimp species, there was a big difference in the mitochondrial COI gene. As a result, the genetic divergences studied in the COI gene among different shrimp species were higher than that seen in 16S rRNA. COI gene also showed higher nucleotide diversity to allow the discovery of different haplotypes (de Francisco and Galetti, 2005).

The common or brown shrimp *Crangon crangon* Linnaeus, 1758 (Crustacea: Caridea: Crangonidae) is a highly abundant species in its range. It is important both ecologically and commercially. But knowledge on its population structure and historical biogeography is limited. Luttikhuizen et al. (2008) examined population genetic structure of this species using the COI gene. The study showed powerful population structuring and high levels of genetic diversity. In the study, four groups have been recognized for the *C. crangon*. These groups were found in the Atlantic, the Mediterranean, the Adriatic Sea and the Black Sea. Gene flow across known oceanographical barriers of these shrimp was limited and the high level of variation was most marked in the western Mediterranean. This can be explained as evidence of a long and stable population history.

The common European littoral prawn *Palaemon elegans* Rathke, 1837 (Crustacea: Caridea: Palaemonidae) is adapted to live in variable environmental conditions. It tolerates a wide range of salinities, temperatures and oxygen (Berglund and Bengston, 1981). Recent studies indicate that *Palaemon elegans* was also observed in the Baltic Sea. *Palaemon elegans* is an important species because it has a large ecological niche (Grabowski, 2006). Reuschel et al. (2010) investigated the degree of genetic variation within broadly distributed *Palaemon elegans*, using DNA-sequences of two mitochondrial genes, 16S rRNA and CO1. Mitochondrial sequences were obtained from the Atlantic, the

Baltic, the Mediterranean, the Black and the Caspian Seas. Their study showed a complex population structure. Three main haplogroups were separated: one from the Atlantic (Type I) and two from the Mediterranean (Types II and III). Mediterranean types were sympatric. Among type I group a phylogeographic break was observed. This can be explained by genetic isolation of Atlantic and Mediterranean populations. Type III is the most different haplogroup with high levels of nucleotide divergence. This fact indicates the possible occurrence of a cryptic species. According to the study, the colonization of the southeastern Baltic Sea could be due to the human activity.

1.6. The Thesis Objective

The genetic cataloging of the shrimp and prawn diversity in Turkish waters is the main objective of this study. Systematic DNA barcoding on decapods has not been done in Turkey previously, and this thesis will be a first in this regard. With DNA barcoding, the genetic diversity of fifteen shrimp and prawn species living in Turkey was evaluated and when possible the genetic data were compared with those available from different parts of the Mediterranean and eastern Atlantic. Finally, again, as a part of the barcoding approach, genetic material from the studied species is now stored at Institute of Environmental Sciences Laboratories, comprising a first step towards formation of a DNA Barcoding bank for these species.

2. MATERIALS AND METHODS

2.1. Field Methods

Within this project, collection of samples was done from the Sea of Marmara, the Black Sea, the Aegean and the Mediterranean. The sample collection was done between June 2009 and October 2010. A total of 94 shrimp and prawn samples from 15 species were collected and analyzed from 12 locations including five from the Black Sea (Ordu, Kemalpaşa-Hopa, Kilyos, Akçakoca and Gideros), four from the Aegean (Ayvalik, Dardanelles Strait, Bodrum and Seferihisar), one from the Sea of Marmara (Burgazada), and two from the Mediterranean (Kaş and Kekova). (Table 2.1, Figure 2.1). Representative images for the studied species are given in Appendix A.

Table 2.1. List of sampling location, sample size and geographic coordinates of the sites.

Code	Sampling site	N	Geographic coordinates (N, E)
1	Sea of Marmara–Burgaz Island	14	Transect between 40.51770 N, 29.04676 E and 40.52354 N, 29.03256 E
2	Black Sea–Kilyos	5	Transect between 41.15839 N, 29.01005 E and 41.16180 N, 28.58993 E
3	Black Sea–Gideros	8	41.8599 N and 32.85697 E
4	Black Sea–Akçakoca	7	41.12 N and 31.16 E
5	Black Sea–Kemalpaşa, Hopa	8	41.57 N and 41.57 E
6	Black Sea–Ordu, Perşembe	1	41.13338 N and 37.68163 E
7	Aegean Sea–Ayvalık, Cunda Island	25	39.00572 N and 26.011402 E
8	Aegean Sea–Dardanelles Strait-Yapıldak DAR01	7	40.23151 N and 26.53682 E
9	Aegean Sea–Bodrum, Sea Garden EGE06	1	36.99430 N and 27.53322 E
10	Aegean Sea–Seferihisar, close to Akkum Beach	1	38.190214 N and 26.770133 E
11	Mediterranean Sea–Hidayet Bay, Kaş	15	36.189606 N and 29.609511 E
12	Mediterranean Sea–Kekova, Üçağız	2	36.195425 N and 29.845381 E

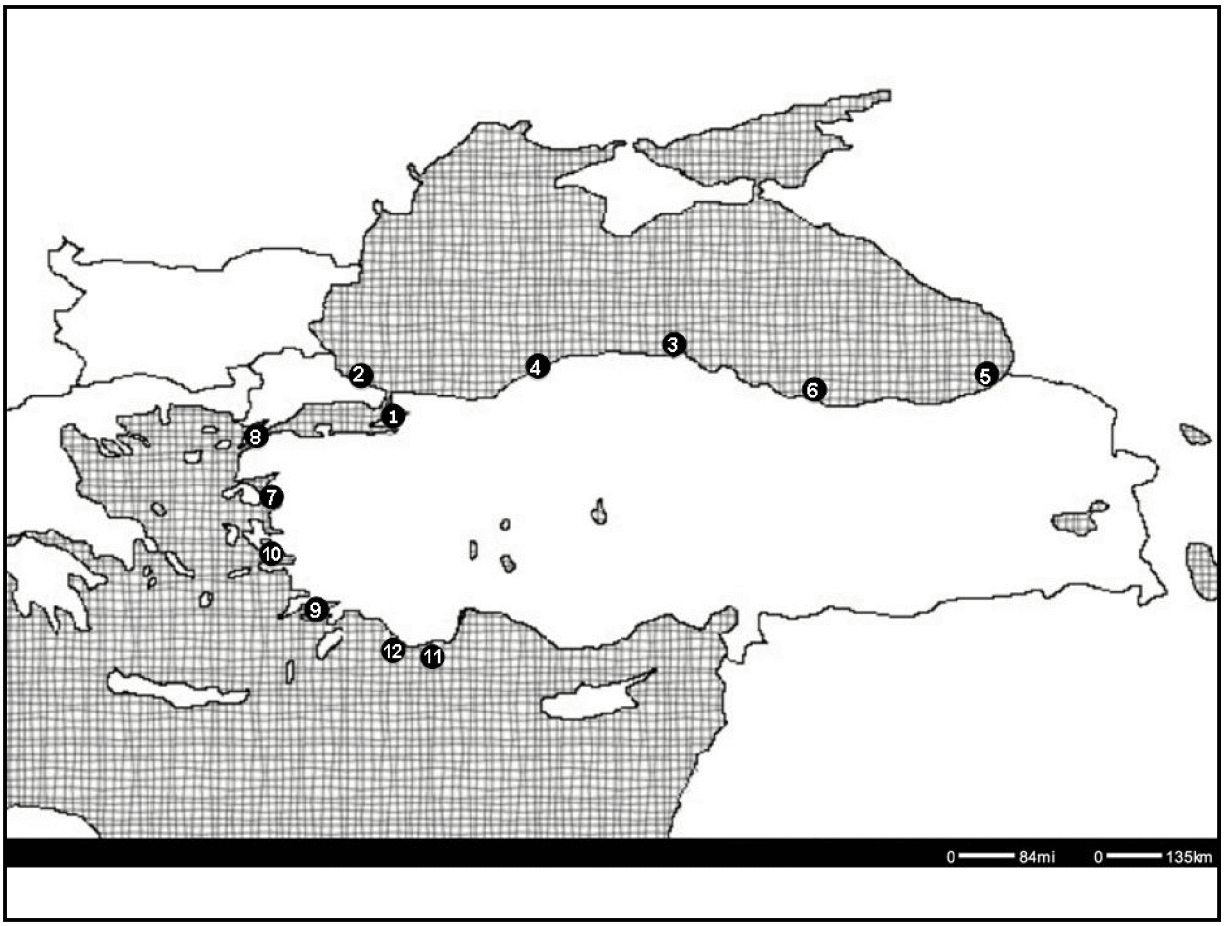


Figure 2.1. The map shows sampling locations. Numbers indicate the codes of the sampling sites as given in Table 4.1.

2.2. Laboratory Protocols

2.2.1. DNA Extraction

For the DNA extraction of shrimp tissue samples, Roche DNA Extraction Kit (Mannheim, Germany) was used, following the manufacturer's protocol with one modification. This included the first step of the protocol, where the incubation time of sample tissue with Lysis Buffer and Proteinase K was increased up to 48 hours, to enhance the lyses of the cell membranes.

The isolated DNA was checked on 1% agarose gels, prepared in 1X TBE (Tris base, boric acid, EDTA) buffer with Ethidium bromide. For each reaction, 3 µl of the

isolated DNA was loaded on the gel, mixed with 3 µl of 2X loading dye (Fermantas). Samples were run at 90 V for 45 minutes. The band images were taken under ultraviolet light by Biorad Gel Doc Imaging System (Figure 4.2).

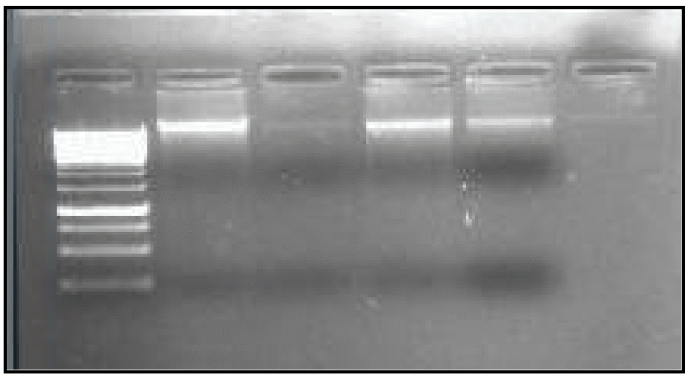


Figure 2.2. The agarose gel photograph of DNA samples after electrophoresis at 90 V for 45 minutes.

2.2.2. PCR Amplification

Approximately 310–685 bp was amplified from the 5' region of the CO1 gene from mitochondrial DNA depending on the primer combination. Primer sets used were as follows: Forward primer LCO1490: 5'- GGTCAACAAATCATAAAGATATTGG-3';
Reverse primer HCO2198: 5'-TAAACTTCAGGGTGACCAAAAAATCA-3' for COI (Folmer et al., 1994). A slightly different protocol was used in the amplification of COI barcode using HCO2198 as reverse primer combined with one of the two forward primers:
CrustF1: 5'-TTTTCTACAAATCATAAAGACATTGG-3', and
CrustF2: 5'-GGTTCTTCTCCACCAACCACAARGAYATHGG-3' (Costa et al., 2007). Another primer set for COI amplification is as follows:
COL6: 5'-TYTCHACAAAYCATAAAGAYATYGG-3' (new, substitute COL1490)
COH6: 5'-TADACTTCDGGRTGDCCAAARAAYCA-3' (Schubart and Huber, 2006, substitute HCO2198).

Also, we amplified mitochondrial DNA from the large subunit rRNA (16S) gene. For these purposes the following primer combinations were used:
16L29: 5'–YGCCTGTTTATCAAAAACAT–3' (Schubart et al., 2002 as "16L2"),

16H11: 5'–AGATAGAAACCRACCTGG–3' (Schubart, 2009).

For COF-CO1R primer set 1 µl of DNA was added to a 49 µl reaction mixture, containing 5 µl 10x high fidelity buffer, 5 µl $MgCl_2$ (25 mM), 1 µl of 10 mM deoxyribonucleotide triphosphate mix (dNTPs), 1.5 µl of each primer (20µm), 34.7 µl H_2O and 0.3 µl Taq DNA polymerase. Cycling parameters consisted of an initial denaturation step of 5 min at 94°C, followed by 35 cycles of 1 min at 95°C, 1 min at 45°C and 1.5 min at 72°C, with a final extension step of 7 min at 72°C. For 16L29 -16H11, CO1F1-CO1R, CO1F2-CO1R and COL6-COH6 primer sets, the amount of DNA, $MgCl_2$ and primers were changed as follows: 1.5 µl of DNA, 4,4 µl $MgCl_2$ (25 mM), 0.5 µl of each primer (20µm) and 36.8 µl water. For CO1F1-CO1R, CO1F2-CO1R and COL6-COH6 primer sets, cycling parameters consisted of an initial denaturation step of 5 min at 94°C followed by 35 cycles of 1 min at 95°C, 1 min at 43°C and 1.5 min at 72°C, with a final extension step of 7 min at 72°C. For 16L29 -16H11 primer set, cycling parameters consisted of an initial denaturation step of 2 min at 94°C, followed by 40 cycles of 45 sec at 94°C, 60 sec at 48°C and 60 sec at 72°C, with a final extension step of 5 min at 72°C.

Some samples either failed to amplify or produced multiple bands that were not sequenced. For these samples several different optimization schemes were tried out. An alternative cycling regime with a touchdown PCR for *Palaemon elegans*, gradient PCR (different temperatures) and different amounts of chemicals and different amounts of DNA among all different primers were tried. Some modifications to the protocols improved amplification. The cause of these failures (for example, primer incompatibility or DNA degradation) is not known. Congeners of some of these species produced good sequences. Accordingly, the primers that worked best for specific species are listed in Table 2.2.

Table 2.2. List of primers and the region sequences were obtained from, for which PCRs worked, in a particular species.

Species	Region	Primers
Processa edulis	CO1	CO1F2-CO1R
Processa acutirostris	16S	16L29-16H11
Sicyonia carinata	CO1	COF-CO1R
Palaemon elegans	16S, CO1	16L29-16H11, CO1F2-CO1R, COF-CO1R
Athanas nitescens	16S	16L29-16H11
Crangon crangon	CO1	COF-CO1R, CO1F2-CO1R, CO1F1-CO1R
Palaemon adspersus	16S, CO1	16L29-16H11, COF-CO1R, CO1F1-CO1R
Palaemon serratus	CO1	COF-CO1R
Hippolyte sapphica	16S	16L29-16H11
Synalpheus gambarelloides	CO1	COF-CO1R, CO1F1-CO1R
Stenopus spinosus	CO1	COF-CO1R, CO1F1-CO1R
Eualus cranchii	CO1	COF-CO1R
Lysmata seticaudata	16S	16L29-16H11
Gnathophyllum elegans	16S, CO1	16L29-16H11, COF-CO1R
Parapenaeus longirostris	CO1	CO1F1-CO1R

2.2.3. Gel Electrophoresis

The PCR products were checked on 1% agarose gels, prepared in 1X TBE (Tris base, boric acid, EDTA) buffer with Ethidium bromide. For each reaction, 3 µl of the PCR product was loaded on the gel, mixed with 3 µl of 2X loading dye (Fermantas). Samples were run at 95 V for 45 minutes and finally, the band images were taken under ultraviolet light by Biorad Gel Doc Imaging System. Specimens giving the most intense product were selected for sequencing. An example of a PCR gel is given in Figure 2.3.

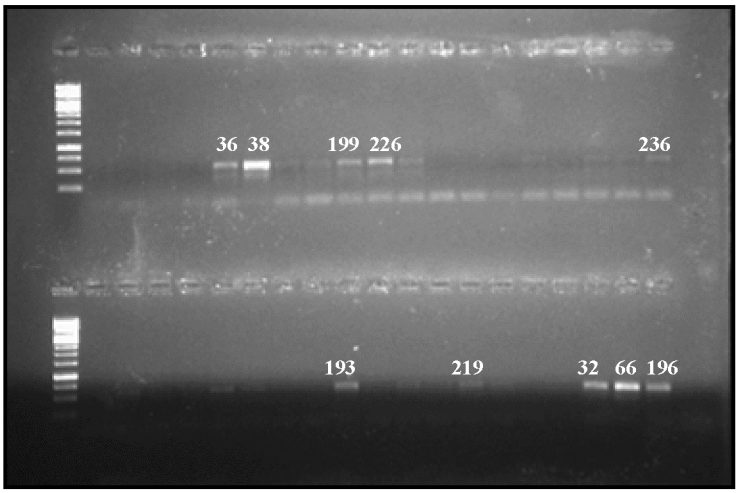


Figure 2.3. Band image of PCR products that worked with the 16L29 -16H11 primer set after electrophoresis at 90 V for 50 minutes.

2.2.4. PCR Clean-up and Sequencing

The PCR products were purified by using Roche PCR cleanup kit. PCR products were sent to the Macrogen Inc. in South Korea for sequencing. The obtained sequences were cleaned manually with Sequencher v. 4.1 (Gene Codes Corp.), and also aligned using the same program. These sequences will be deposited to GenBank and Consortium for the Barcode of Life databases (BOLD).

2.3. Analytical methods

2.3.1. Species Identification:

Taxonomic keys were used for species identification (Holthuis, 1980, 1987, Zariquiey, 1968, Ngoc-Ho, 2003), using a stereomicroscope (Olympus, SZ61).

2.3.2. Sequence Analysis

With the sequences obtained, phylogenetic trees were constructed using neighbor-joining method. This method has been observed to be appropriate when using CO1 data (Hebert et al., 2003). The fundamental aspect of the method is to find pairs of taxonomic

units that reduce the total branch length. The method requires knowledge of the distance between each pair of taxa in the tree. The MEGA program (Tamura, 2007) was used for constructing the tree. A neighbor joining tree for each species, when possible, was prepared using Kimura 2-parameter distances and boostrap method with 1000 bootstrap replications. The samples were combined with sequences downloaded from BOLD and GenBank, when available, for a comprehensive analysis. Intraspecific and interspecific distances were also calculated by MEGA using Kimura 2-parameter distances. DnaSP (Rozas et al., 2003) was used to build haplotype data file and TCS 1.21 (Clement et al., 2000) was used to prepare haplotype networks. The obtained sequences were analyzed by using BOLD. The specimens were identified by comparing the sequences in BOLD under the "identify specimen" option of BOLD. Only one individual was compared for species that formed a single clade, whereas one barcode from each clade were compared for the species in which the generated barcodes formed two or more different clades.

3. RESULTS

51 COI sequences from 12 different species were generated for this study. 12 CO1 sequences were added from BOLD and five sequences are added from GenBank. In addition, 64 16S sequences from six different species were also generated during the course of this study. Four 16S sequences were added from GenBank. It should be noted that the sequences that we obtained do not represent pseudogenes as there were no double peaks in the chromotograms, no stop-codons in the amino acid translation, and no indels in the alignments.

The mean intraspecific divergence for the CO1 sequences was 1.3%. The mean interspecific distance was 21.03%. A plot of the frequency of intraspecific and interspecific distances is given in Fig. 3.1. The minumum intraspecific distance was 0.73% for *Palaemon adspersus*, and the maximum intraspecific distance was 2.75% for *Crangon crangon*. The minumum interspecific distance was 12.6% for *Palaemon serratus* and *Palaemon adspersus,* and the maximum interspecific distance was 26.8% for *Synalpheus gambarelloides* and again for *Palaemon adspersus.*

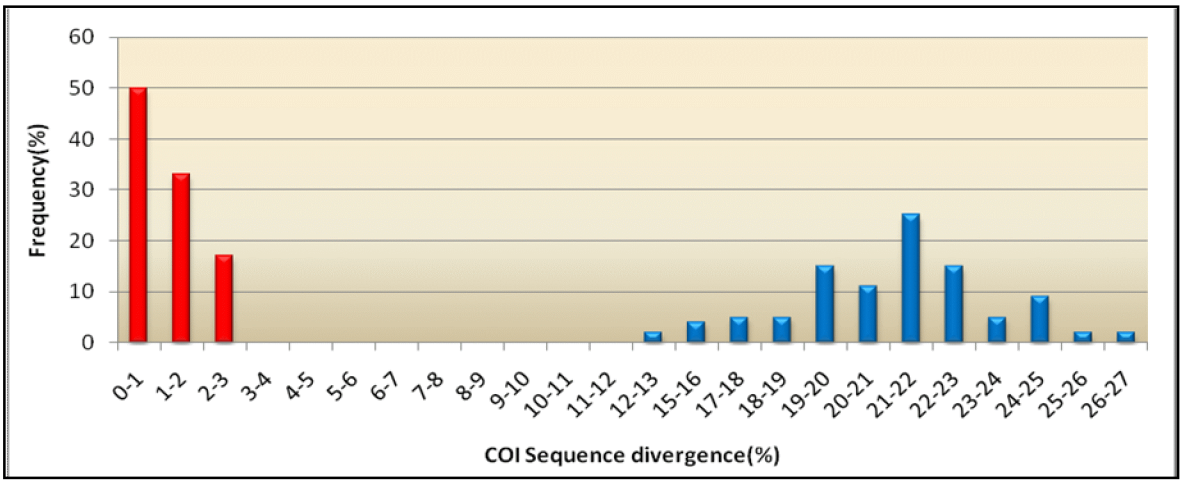


Figure 3.1. Frequency distribution of mean divergences for CO1 sequences for 51 samples, calculated by the Kimura 2- parameter model. Two taxonomic levels are represented: within species (red bars) and between species (blue bars).

For each species a map that shows the locations of samples, an intraspecific tree, a haplotype network, a table of the haplotypes, a similarity table and a neighbor-joining tree for that species are prepared. In the haplotype network, different colors which are used to indicate different regions are shown in Table 3.1.

Table 3.1. Colors that are used to indicate different regions in the haplotype networks.

Region	**Color**
Black Sea	Green
Aegean Sea	Pink
Mediterranean Sea	Blue
The Sea of Marmara	Yellow
Israel	Gray
Italy	Purple
Poland	Orange

3.1. *Processa edulis* (Risso, 1816)

One COI sequence from Turkey was analyzed for *Processa edulis*. The sample was collected from Kaş, the Mediterranean (Figure 3.2). The haplotype network and the intraspecific tree could not be prepared as there was only one sample for this species.



Figure 3.2. The sampling location for *Processa edulis*. The pink circle indicates sampling location Kaş, the Mediterranean that the sample was collected from for this project. CO1 sequence data were not available in BOLD or GenBank for this species.

Comparing with the five matches that are taken from BOLD, first closest match to this sample is *Acanthephyra purpurea* with a specimen similarity range of 83.75-84.25%, the second is *Acanthephyra quadrispinosa* with a specimen similarity range of 83.42-83.58% (Table 3.2).

Table 3.2. Specimen similarity table for *Processa edulis*.

Species	Specimen Similarity range (%)
Acanthephyra purpurea	83.75-84.25
Acanthephyra quadrispinosa	83.42- 83.58
Processa modica	82.09-82.75
Acanthephyra pelagica	81.99
Neoliomera richtersi	81.92

These results indicate that our sequence is the first DNA barcode in BOLD for this species. The neighbor-joining tree for *Processa edulis*, constructed *via* BOLD is shown in Fig 3.3. The sample that is barcoded from Turkey (shown in red) falls within the *Processa* clade and is sister to the lineage that contains *Periclimenes schmitti*. It is possible that *Periclimenes schmitti* is paraphyletic in this case.

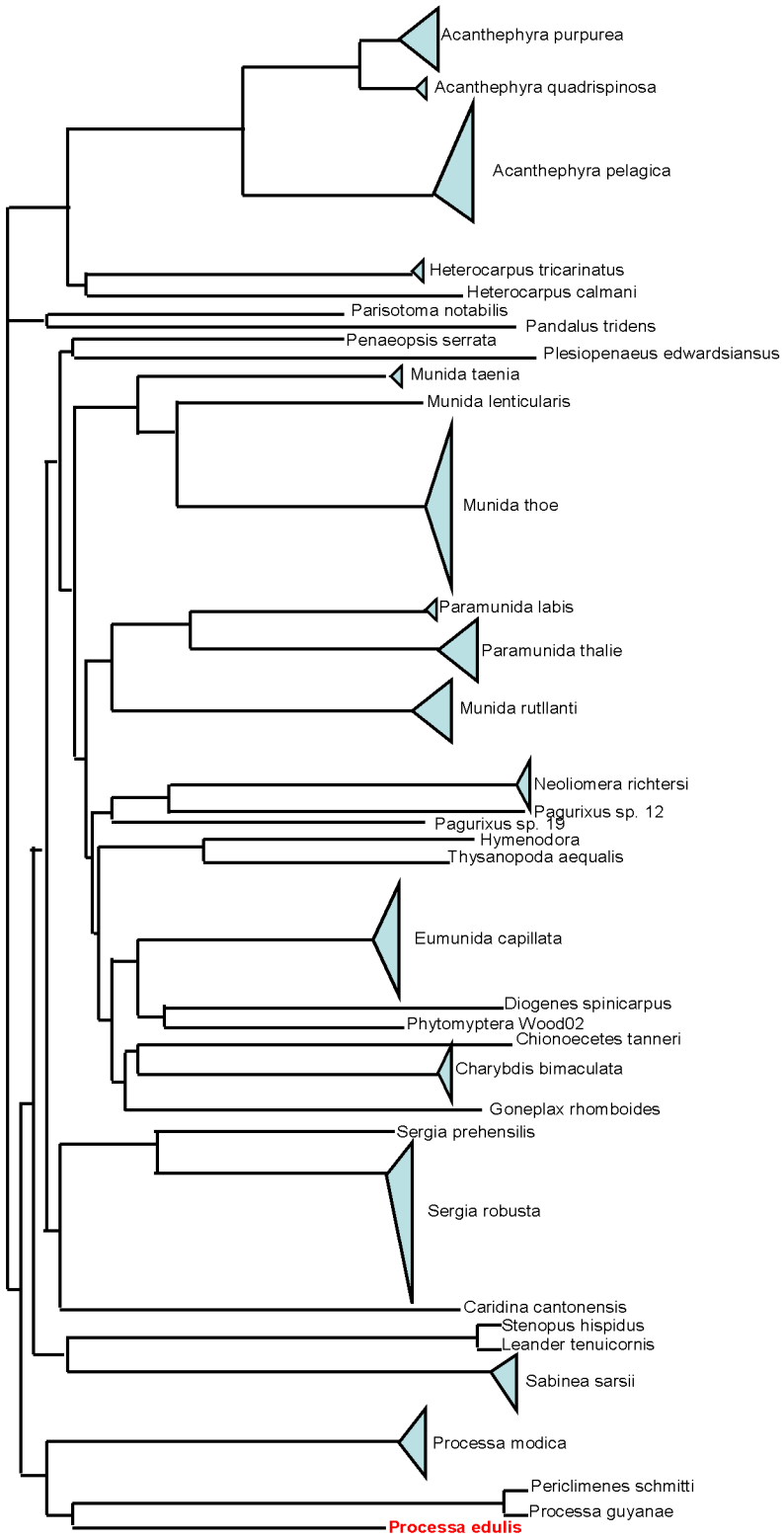


Figure 3.3. Neighbor-joining tree for our *Processa edulis* sequence (shown in red) constructed based on BOLD. The size of the triangles are proportional to the number of sequences in BOLD for that species.

3.2. *Processa acutirostris* (Nouvel and Holthuis, 1957)

One COI sequence from Turkey was analyzed for *Processa acutirostris*. The sample was collected from Hidayet Bay, Kaş, the Mediterranean (Fig. 3.4). The haplotype network and the intraspecific tree could not be prepared as there was only one sample for this species.



Figure 3.4. The location for *Processa acutirostris*. The pink circle indicates sampling location Kaş, the Mediterranean that the sample was collected from for this project. CO1 sequence data were not available in BOLD or GenBank for this species.

Comparing with the two matches that are taken from BOLD, first closest match to this sample is *Processa modica* with a specimen similarity range of 82.72–83.03%, and second one is *Macrobrachium asperulum* with a specimen similarity range of 82.11–82.57%. This is also the first DNA barcode in BOLD for this species. The neighbor-joining tree for this species, constructed *via* BOLD is shown in Fig. 3.5. The sample that is barcoded from Turkey (shown in red) does not fall within any *Processa* clade.

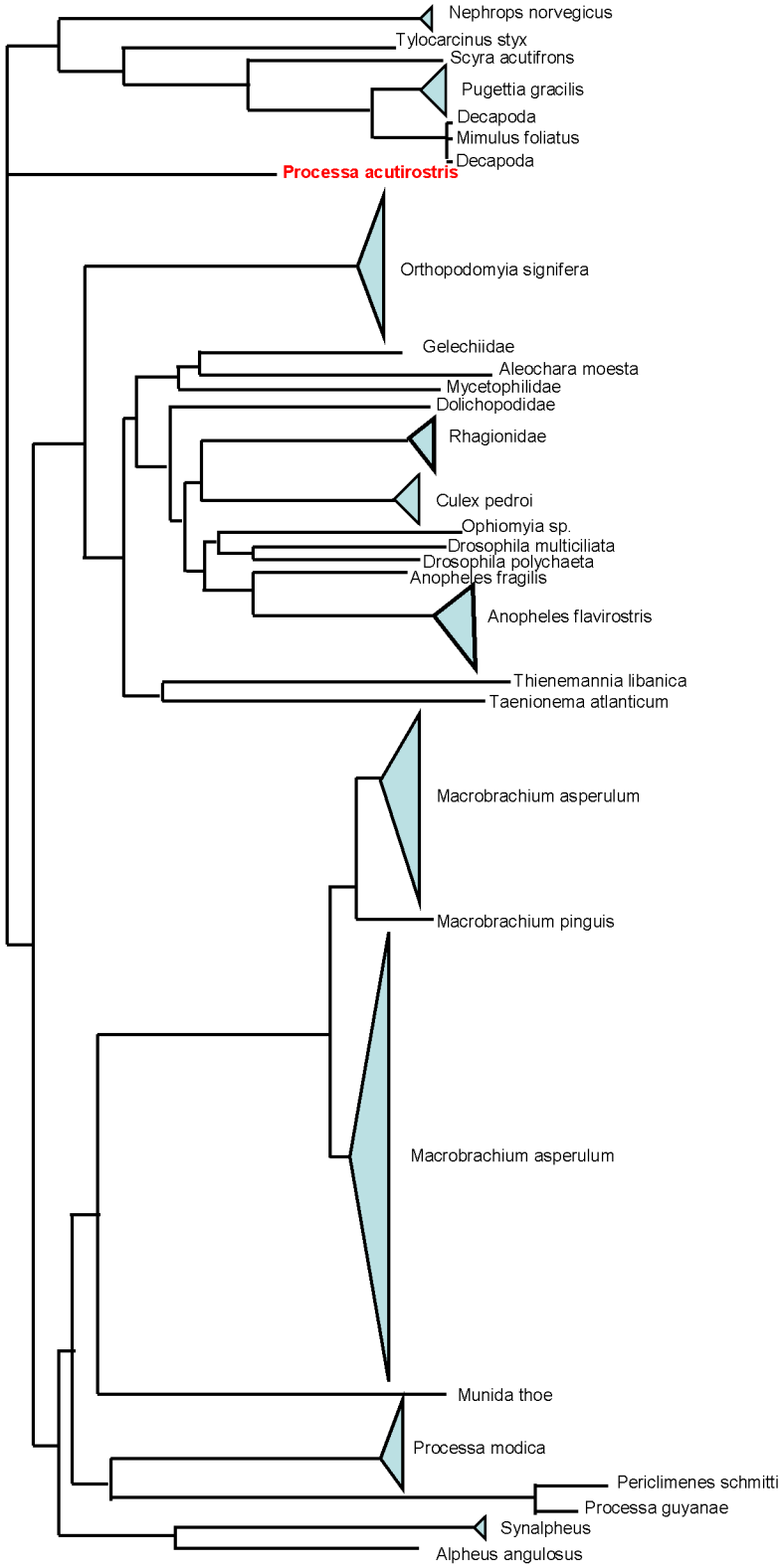


Figure 3.5. Neighbor-joining tree for our *Processa acutirostris* sequence (shown in red) constructed based on BOLD. The size of the triangles are proportional to the number of sequences in BOLD for that species.

3.3. *Palaemon elegans* (Rathke, 1837)

38 16S sequences and 21 COI sequences were analyzed for *Palaemon elegans*.

3.3.1. 16S Sequences

The samples were collected from eight locations including three (Hopa, Akçakoca and Gideros) from the Black Sea, two (Ayvalik and Dardanelles Strait) from the Aegean, one (Burgazada) from the Sea of Marmara, and two (Kaş and Kekova) from the Mediterranean (Fig. 3.6). Also three sequences were added from GenBank.

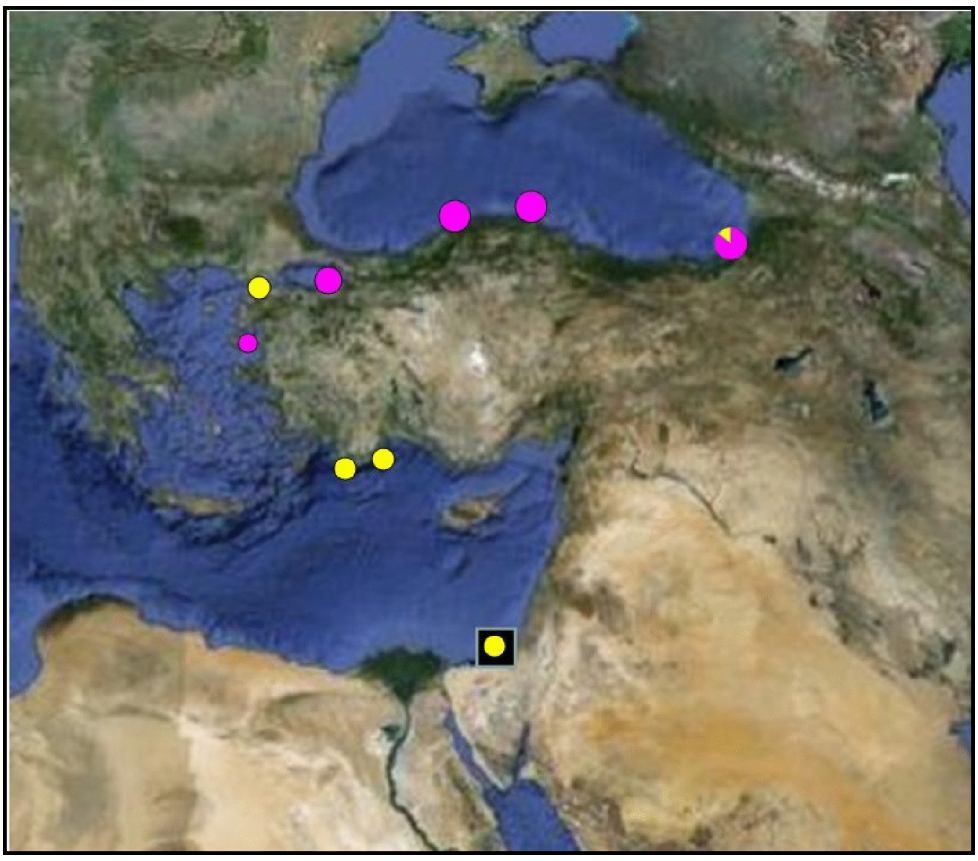


Figure 3.6. The sampling locations for *Palaemon elegans* (16S). The circles are proportional to the number of individuals. The pink color indicates sampling locations for the first group and the yellow color indicates sampling locations for the second group in the intraspecific tree (Fig. 5.7). The yellow circle within a black square indicates a sampling location (Israel) for sequences that were retrieved from GenBank.

Intraspecific tree for *Palaemon elegans* (16S) is shown in Fig. 3.7. All 16S sequences generate two clades. The bootstrap values support the division of the tree into two clades. In the first clade there are sequences from the Black Sea, the Aegean and the Sea of Marmara, and in the second clade there are sequences from the Mediterrenean, Black Sea, the Aegean, and those from Israel (GenBank). Also two other sequences from GenBank, DQ07972 and EU86869, whose locality information is not available, are seen to form a separate clade within group 2.

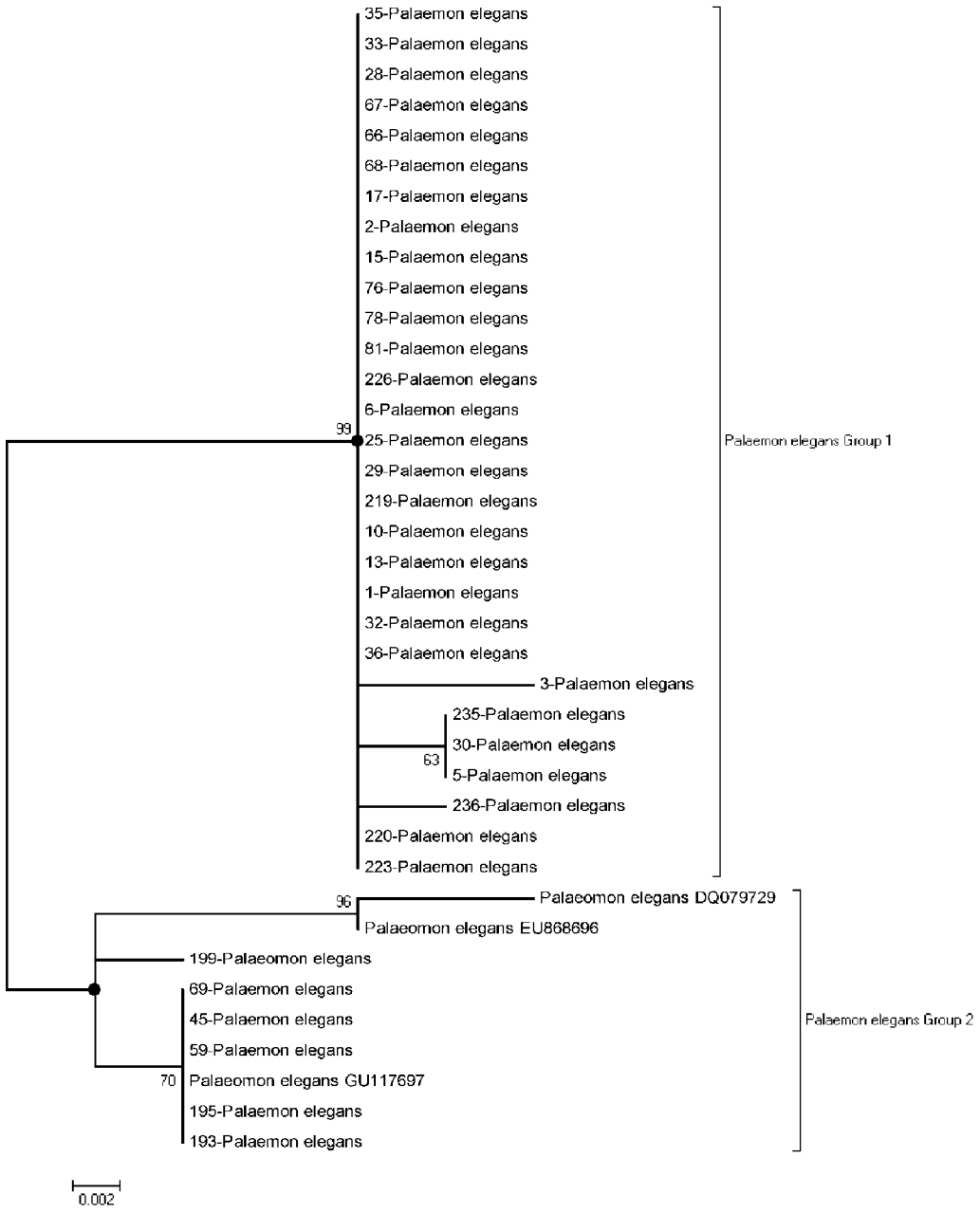


Figure 3.7. Intraspecific tree for *Palaemon elegans*(16S).

19 different haplotypes were observed in this species in the haplotype network (Fig. 3.8). The haplotypes within the pink area make up the first group, and the haplotypes within the yellow area make up the second group, in a similar manner to that in the intraspecific tree. Hence the intraspecific tree and the haplotype network are consistent with each other. Haplotypes in the two groups are differentiated by a minimum of eight base pairs. A haplotype network table for this species, showing the corresponding codes in the intraspecific tree is shown in Table 3.4. In terms of individual haplotypes, H5 was observed in the Mediterranean, the Aegean and the Sea of the Marmara. H7 was observed in Black Sea and the Sea of the Marmara, H6 in the Sea of the Marmara, H1 in the Black Sea and Mediterranean, and H10 and H11 were observed in the Aegean. H2, H3 and H9 were observed in Israel. Remaining nine haplotypes were only found in one location, Black Sea. It should be noted that H5 was the most common haplotype (observed in 17 individuals), and was predominantly observed in the Mediterranean.

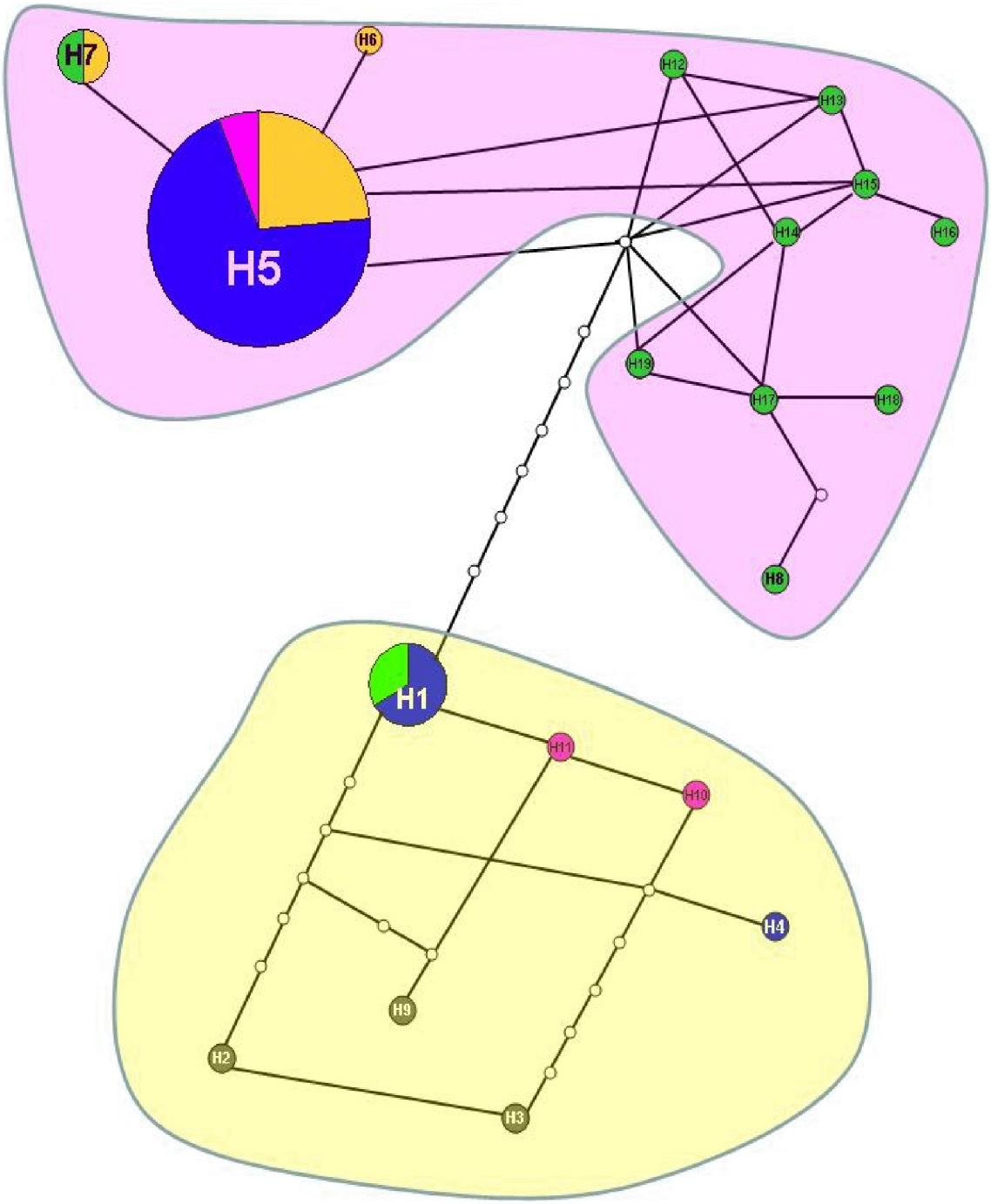


Figure 3.8. Haplotype network for *Palaemon elegans* (16S). The size of circles is proportional to the number of individuals. See Table 3.1 for the key to the color-codes of the geographic positioning of the haplotypes.

Table 3.3. Haplotype network table for *Palaemon elegans* (16S).

Haplotype	Number of individuals	Codes for the individuals as shown in the intraspecific tree (Figure 3.7)
H1	3	45-59-69
H2	1	DQ07972
H3	1	EU86869
H4	1	199
H5	17	2-6-17-25-28-33-35-36-66-67-68-76-78- 219-220-223-226
H6	1	236
H7	2	5-235
H8	1	3
H9	1	GU11769
H10	1	195
H11	1	193
H12	1	32
H13	1	81
H14	1	15
H15	1	13
H16	1	10
H17	1	29
H18	1	30
H19	1	1

3.3.2. CO1 Sequences

The sequences were obtained from six locations including two (Hopa and Ordu) from the Black Sea, two (Ayvalik and Dardanelles Strait) from the Aegean, one (Burgazada) from the Sea of Marmara, and one (Kaş) from the Mediterranean. Also four sequences from GenBank (Poland) and four sequences from BOLD (Poland) were included in the analysis (Fig. 3.9).

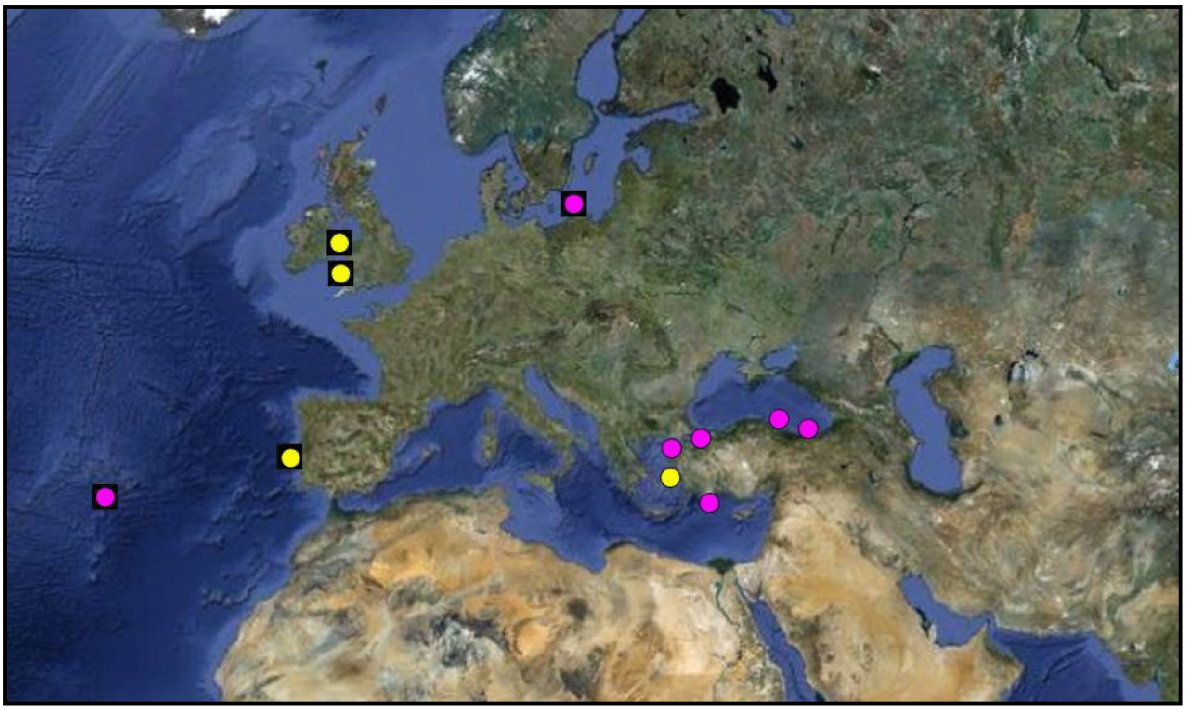


Figure 3.9. The sampling locations for *Palaemon elegans* (CO1). The circles are proportional to the number of species. The pink color indicates sampling locations for the first group; the yellow color indicates sampling locations for the second group, based on BOLD trees (Fig 3.12 and 3.13). The pink circles within a black square indicate localities for which collection site information was available from BOLD courtesy of Joana Matzen da Silva (North Atlantic, Poland, Portugal and United Kingdom). The site information are as follows: U.K. Wales, Anglesey: 53.22000 N , 4.51999 W; Portugal, Azores, Sao Miguel: 37.7400 N, 25.60000 W.

Intraspecific tree for *Palaemon elegans* (COI) is shown in Figure 3.10. All COI sequences generate two clades. Group 1 is seen in the Black Sea, the Sea of Marmara, the Aegean, and the Mediterrenean. K6 from Ordu, Black Sea falls within same clade, Group 1, but on a different branch. This is due to that haplotype (H2) being different from the rest by three bases (see haplotype network below, Figure 3.11). Moreover, the individual that is coded as sample 38 forms its own clade, which is shown with a red branch in Figure 3.10, and was collected from Ayvalik in the Aegean.

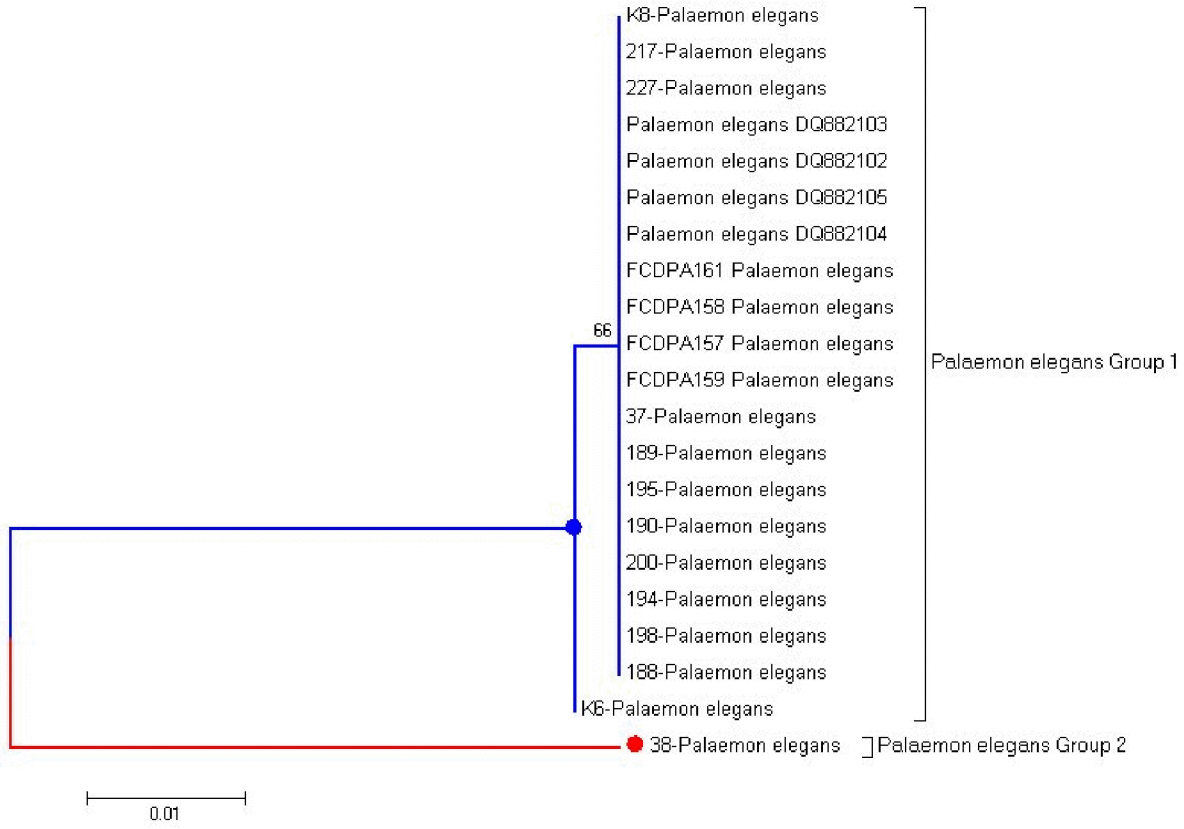


Figure 3.10. Intraspecific tree for *Palaemon elegans* (COI). The codes that begin with DQ are taken from GenBank and are collected from Poland. The codes that begin with FCDPA are taken from BOLD and they are collected from Poland Polish Baltic Sea.

There are four different haplotypes in the haplotype network (Fig. 3.11). H1 is the most common haplotype (found in 18 individuals), and is found in the Black Sea, the Aegean, the Mediterranean, Italy, and has its highest frequency in Poland within the data set. Haplotypes H1, H2 and H4 form one group, which is separated by 45 base pairs from H3 (which corresponds to sample *Palaemon elegans*-38 in the tree). H3 is found exclusively in the Aegean and was separated from the rest of the samples in the intraspecific tree.

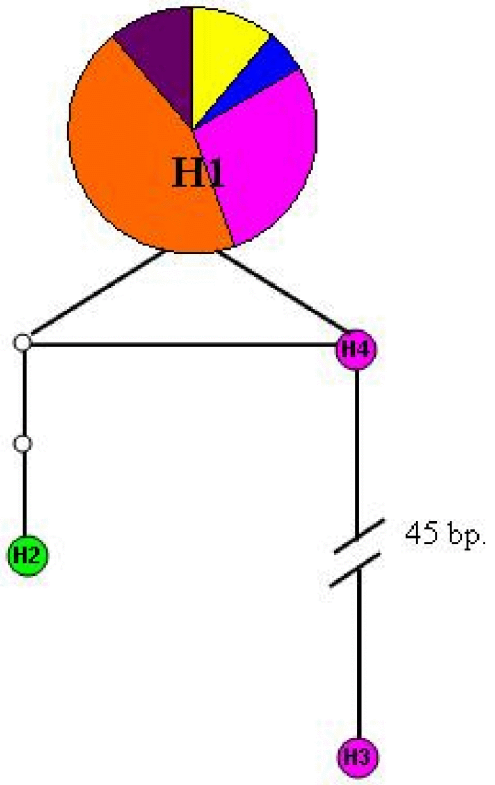


Figure 3.11. Haplotype network for *Palaemon elegans* (COI). The size of circles are proportional to the number of individuals. See Table 3.1 for the key to the color-codes of the geographic positioning of the haplotypes.

Table 3.4. Haplotype network table for *Palaemon elegans* (COI).

Haplotype	Number of individuals	Code for the Individuals as shown in the intraspecific tree (Figure 3.10)
H1	18	DQ882102-DQ882103-DQ882104-DQ882105-FCDPA157-FCDPA158-FCDPA159- FCDPA161-K8-37-188-189-190-194-198-200-217-227-
H2	1	K6
H3	1	38
H4	1	195

As a next step, we wanted to compare the individuals from each clade to those available in BOLD. From group 1 we selected one representative individual, 217, and comparing with data from BOLD, first closest match to this sample is *Palaemon elegans* with a specimen similarity range of 91.26-100%, second is *Palaemon longirostris* with a specimen similarity range of 83.62-84.23% and third is *Palaemon serratus* with a specimen similarity range of 83-83.31 %. Comparing an individual from group 2, *Palaemon elegans*-38, with the two matches that taken from BOLD, first closest match of *Palaemon elegans*-38 is *Palaemon elegans* with a specimen similarity range of 91.98-98.97%, and second is *Palaemon longirostris* with a specimen similarity range of 80.86-81.28%.

The neighbor-joining tree for *Palaemon elegans*, created *via* BOLD, using one individual belonging to Group 1, *Palaemon elegans*-217 is shown in Fig. 3.12. In the tree, the barcoded individuals from Turkey clustered closely with barcodes in BOLD, from Poland. The neighbor-joining tree from BOLD for *Palaemon elegans*-38 is also shown in Fig 3.13. It should again be noted that *Palaemon elegans*-38 falls within the opposite branch (with BOLD barcodes from United Kingdom and Portugal), but into the same general *Palaemon elegans* clade with all other *Palaemon elegans* COI sequences. Hence the two clades retrieved for this species in our intraspecific tree, and the two clades observed in the BOLD tree are in conformation.

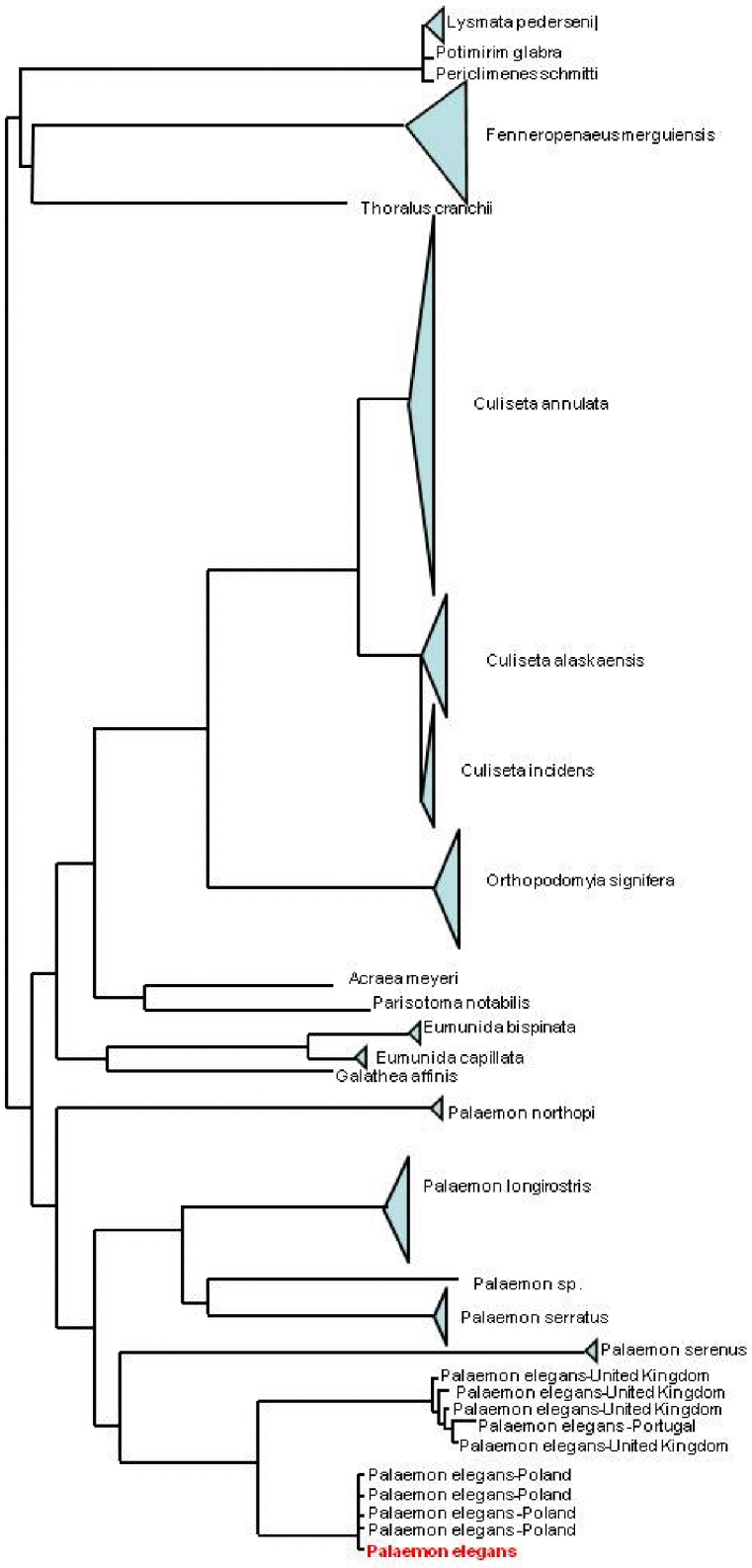


Figure 3.12. Neighbor-joining tree for one of our *Palaemon elegans* (COI) sequences, sample 217, (shown in red) constructed based on BOLD. The size of the triangles are proportional to the number of sequences in BOLD for that species.

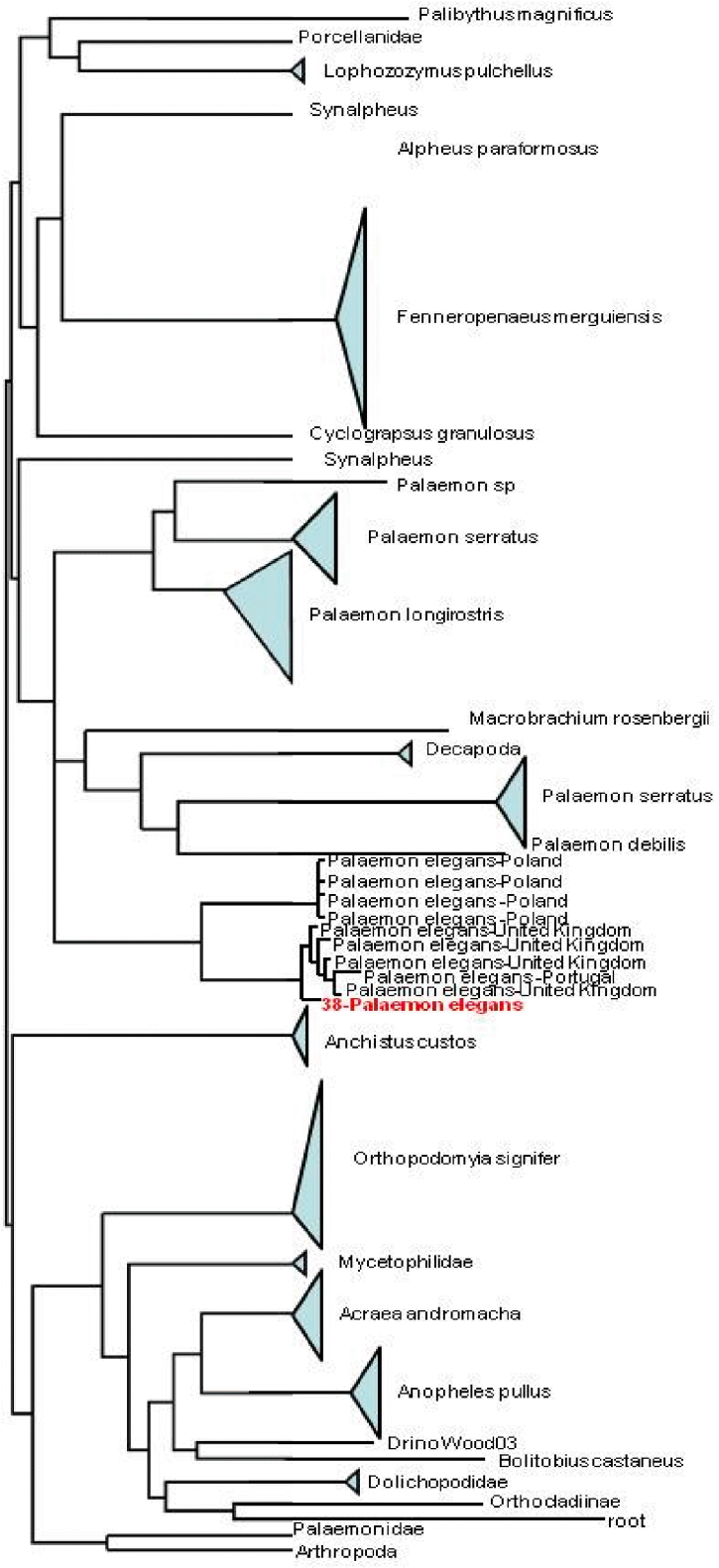


Figure 3.13. Neighbor-joining tree for our *Palaemon elegans*-38 (COI) sequence (shown in red) constructed based on BOLD. The size of the triangles are proportional to the number of sequences in BOLD for that species.

3.4. *Palaemon adspersus* (Rathke, 1837)

Ten COI sequences and one 16S sequence from Turkey were analyzed for *Palaemon adspersus*. The samples were collected from one location: Lale Island, Ayvalık, the Aegean (Figure 3.14).



Figure 3.14. The locations for *Palaemon adspersus*. The orange circle within a black square indicates the collection site for which sequence data were available from BOLD (Poland), the pink color indicates the sampling location for the first group and the yellow color indicates the sampling location for the second group in the intraspecific tree (see Fig. 3.15).

Intraspecific tree for *Palaemon adspersus* (COI) is shown in Figure 3.15. All COI sequences generate two clades, first clade consists of six individuals and the second clade of four individuals. The second clade consists of two branches. This is because of the dissimilarity between sample 213 *vs.* samples 34, 205, and 209. A more detailed analysis is presented in the haplotype network (Figure 3.16), which shows four different haplotypes. The haplotypes H2 and H3 are separated by seven base pairs from H4. As also seen in the

haplotype network, H4 corresponds to the the first group (pink area), whereas the rest of the haplotypes correspond to the second group (yellow area).

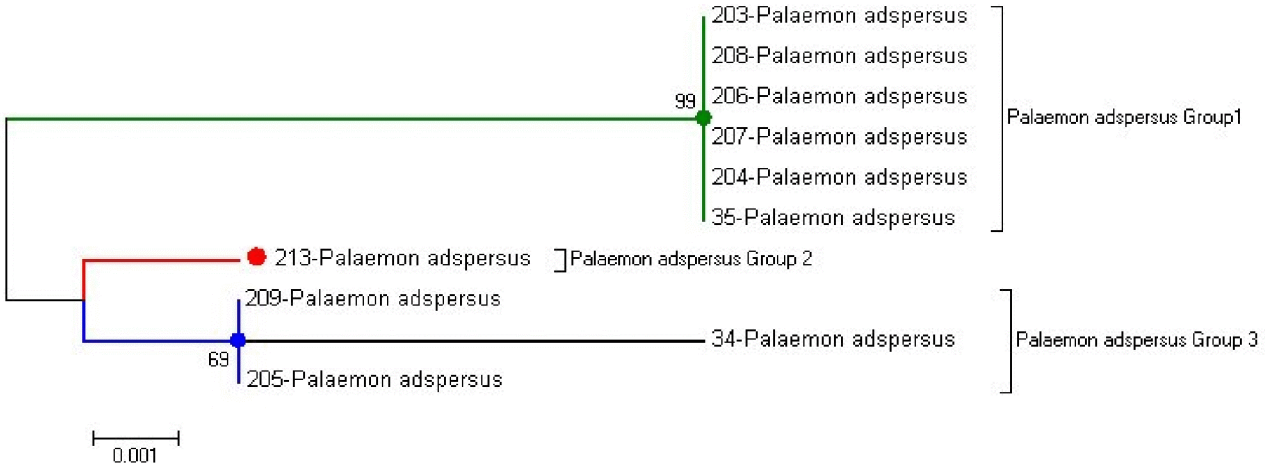


Figure 3.15. Intraspecific tree for *Palaemon adspersus*.

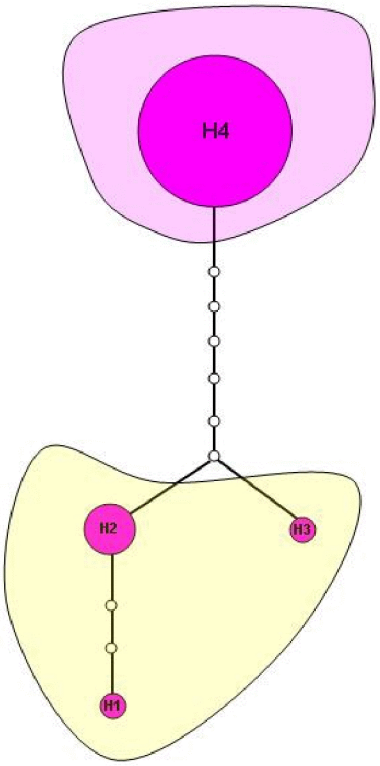


Figure 3.16. Haplotype network for *Palaemon adspersus*. The size of circles are proportional to the number of individuals. See Table 3.1 for the key to the color-codes of the geographic positioning of the haplotypes.

Table 3.5. Haplotype network table for *Palaemon adspersus.*

Haplotype	Number of individuals	Code for the individuals as shown in the intraspecific tree (Figure 3.15)
H1	1	34
H2	2	205-209
H3	1	213
H4	6	35-203-204-206-207-208

Comparing an individual from group 1, sample 206, with BOLD, the first closest match to this sample is *Palaemon sp.* with a specimen similarity of 99.67%, second is *Palaemon longirostris* with a specimen similarity range of 87.38-87.95% and third is *Palaemon serratus* with a specimen similarity range of 86.14-86.47 %. Comparing an individual from group 2, sample 209, with BOLD, the first closest match to this sample is *Palaemon sp.* with a specimen similarity of 98.69%, second is *Palaemon longirostris* with a specimen similarity range of 87.19-87.68% and third is *Palaemon serratus* with a specimen similarity range of 86.7-86.86 %. Comparing an individual from group 2, sample 213, with the BOLD, the first closest match to this sample is *Palaemon sp.* with a specimen similarity of 98.69%, second is *Palaemon longirostris* with a specimen similarity range of 87.52-88.01% and third is *Palaemon serratus* with a specimen similarity range of 82.27-86.7 %.

These results indicate that our sequence is a new barcode in BOLD. Based on these results, it is possible that the specifically unidentified barcode from BOLD, *Palaemon sp.* (Process-id: FC-DPBAS02D from Poland) is actually *Palaemon adserpsus.* The neighbor-joining tree for *Palaemon adspersus*-206 (group 1) constructed *via* BOLD is shown in Figure 3.17. The sample that is barcoded from Turkey (shown in red) falls within the *Palaemon* clade, and forms a clade together with *Palaemon sp.* from BOLD and this clade is sister to *Palaemon serratus. Palaemon adspersus*-209 and 213 (group 2) also fall within the *Palaemon* clade and provide the same sister group relationships as sample 206 (Fig 3.18 and Fig 3.19).

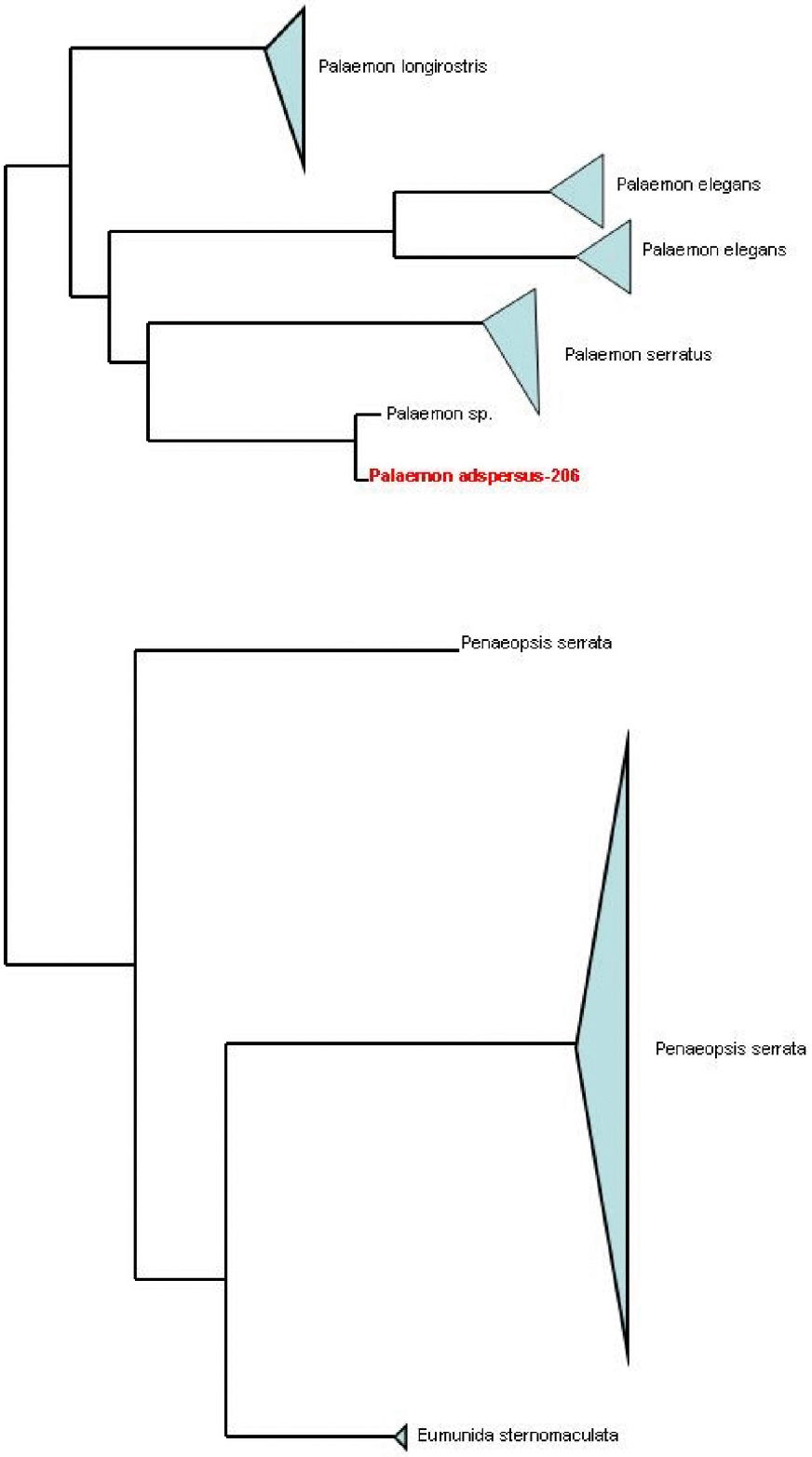


Figure 3.17. Neighbor-joining tree for our *Palaemon adspersus*-206 sequence (shown in red) constructed based on BOLD. The size of the triangles are proportional to the number of sequences in BOLD for that species.

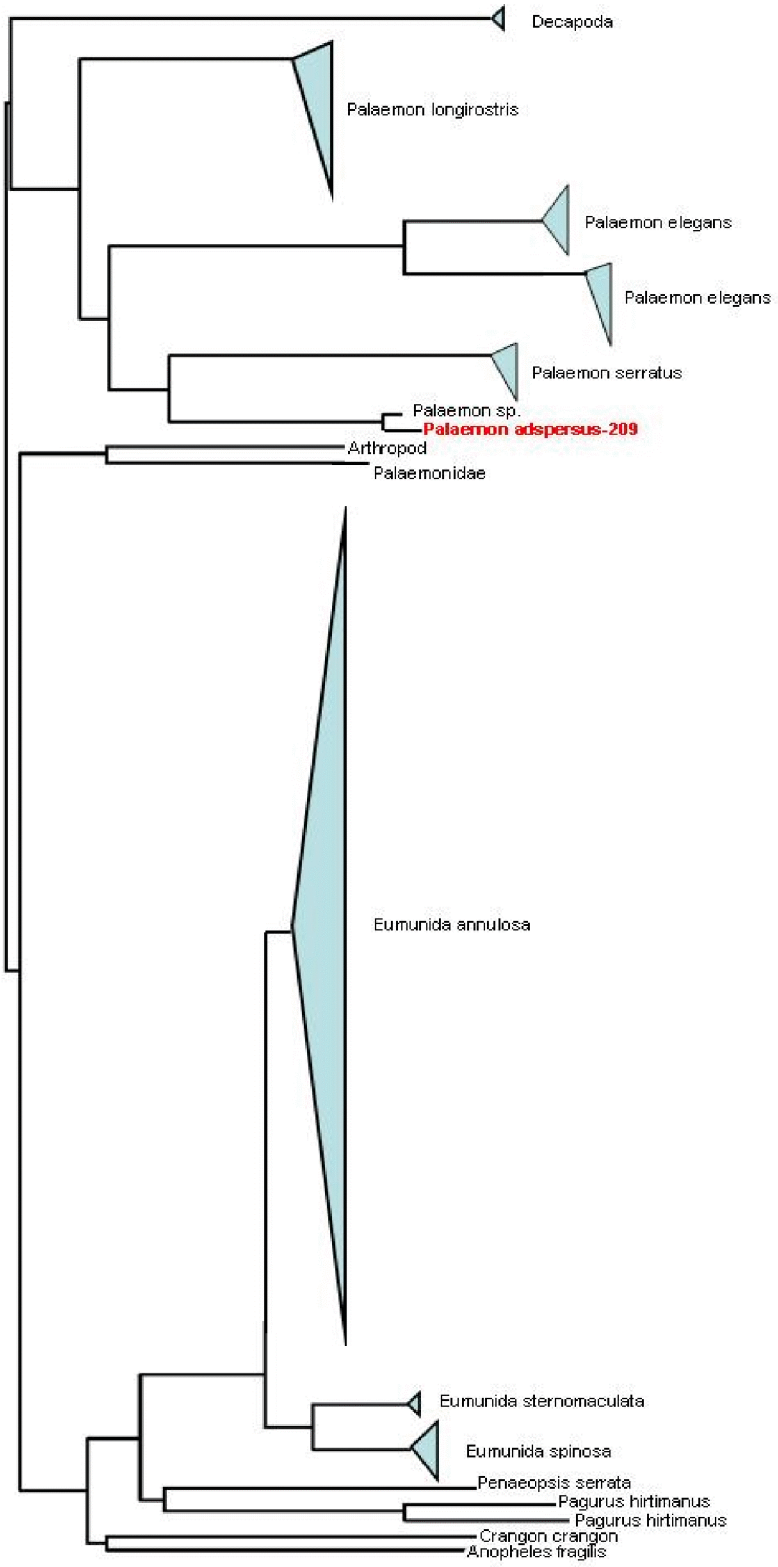


Figure 3.18. Neighbor-joining tree for our *Palaemon adspersus*-209 sequence (shown in red) constructed based on BOLD. The size of the triangles are proportional to the number of sequences in BOLD for that species.

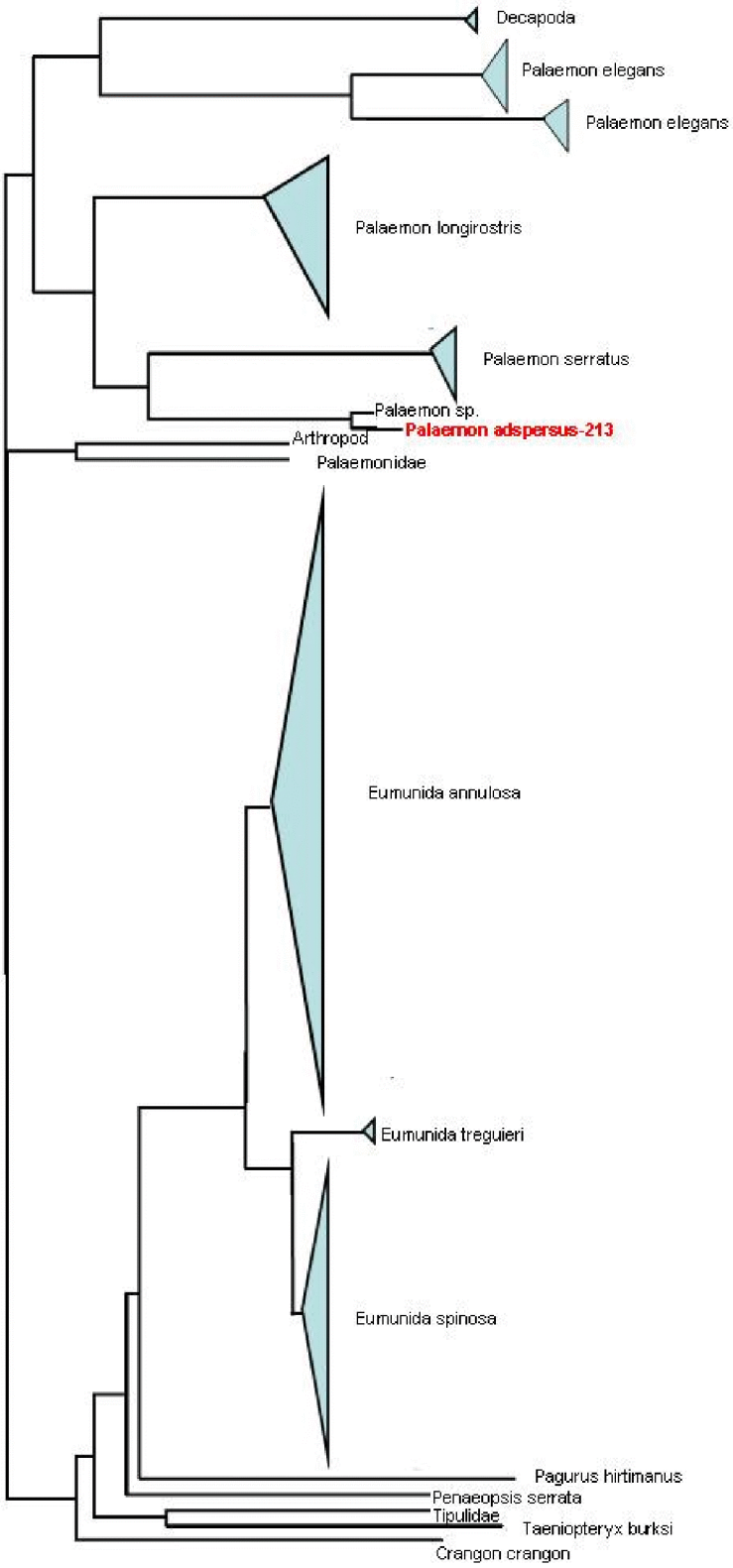


Figure 3.19. Neighbor-joining tree for our *Palaemon adspersus*-213 sequence (shown in red) constructed based on BOLD. The size of the triangles are proportional to the number of sequences in BOLD for that species.

3.5. *Palaemon serratus (Pennant, 1777)*

One sample (COI sequence) from Turkey was analyzed for *Palaemon serratus*. The sample was collected from Lale Island, Ayvalık, the Aegean (Fig. 3.20). A haplotype network and intraspecific tree could not be prepared as there was only one sample for this species.

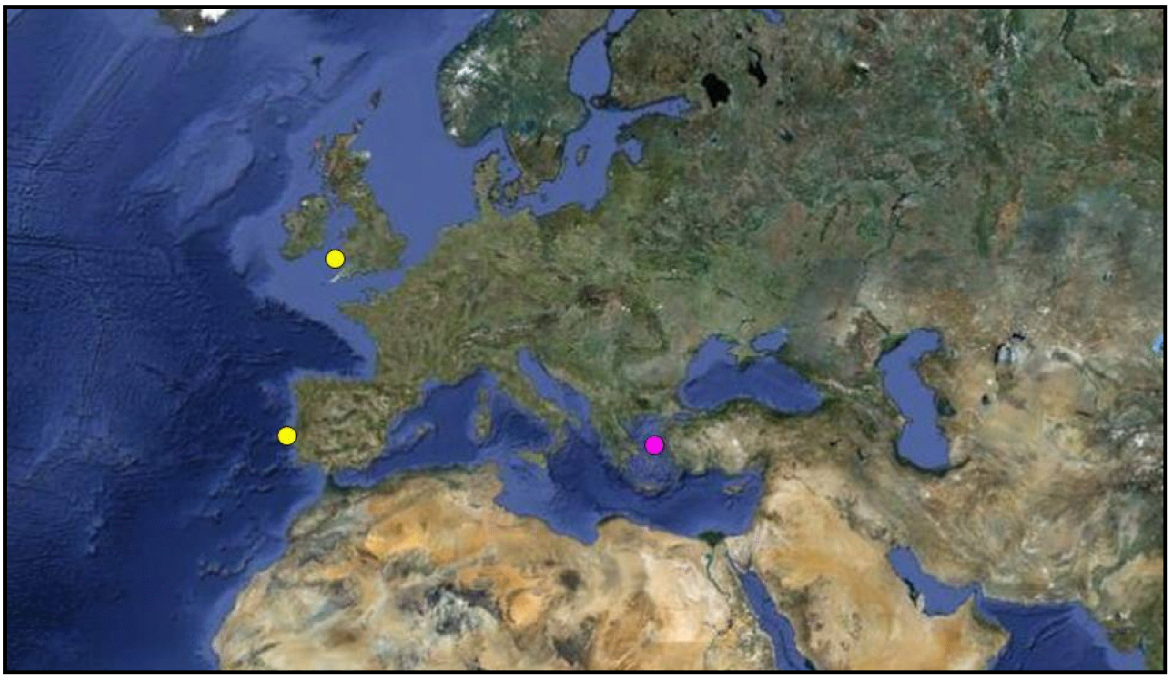


Figure 3.20. The sampling locations for *Palaemon serratus*. The yellow circles indicate localities which collection sites were available from BOLD (United Kingdom and Portugal), the pink circle indicates sampling location that is collected from for this project.

Comparing with the three matches that are taken from BOLD, first closest match to this sample is *Palaemon serratus* with a specimen similarity range of 89.33-89.7%, second *Palaemon longirostris* with a specimen similarity range of 85.02-85.88% and third *Palaemon elegans* with a specimen similarity of 82.58%.

The neighbor-joining tree for *Palaemon serratus* constructed *via* BOLD is shown in Fig 3.21. The sample that is barcoded from Turkey (shown in red) falls within the *Palaemon* clade and is sister to the Palaemon *serratus* clade that includes individuals from United Kingdom and Portugal.

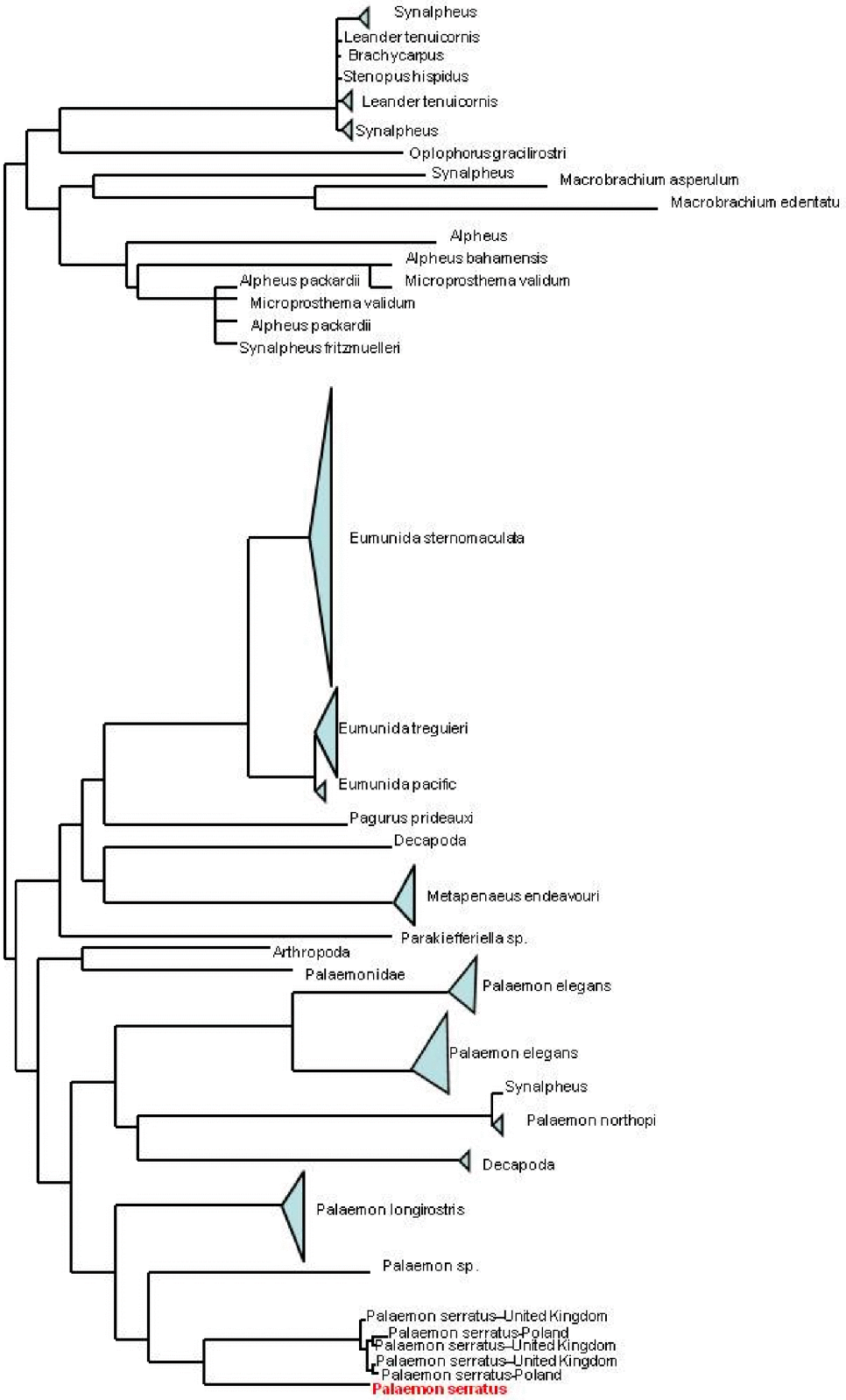


Figure 3.21. Neighbor-joining tree for our *Palaemon serratus* sequence (shown in red) constructed based on BOLD. The size of the triangles are proportional to the number of sequences in BOLD for that species.

3.6. *Sicyonia carinata* (Brünnich, 1768)

Two samples (COI sequences) from Turkey were analyzed for *Sicyonia carinata*. The samples were collected from one point: Hidayet Bay, Kaş, the Mediterranean (Figure 3.22). An intraspecific tree could not be constructed as there were only two sequences. Two different haplotypes are observed for this species (Fig. 3.23), which are separated by four base pairs.



Figure 3.22. The sampling location for *Sicyonia carinata*. The pink circle indicates sampling location Kaş, the Mediterranean that samples were collected from for this project. CO1 sequence data were not available in BOLD or GenBank for this species.



Figure 3.23. Haplotype network for *Sicyonia carinata.* The size of circles are proportional to the number of individuals. See Table 3.1 for the key to the color-codes of the geographic positioning of the haplotypes.

Comparing with the five matches that are taken from BOLD, first closest match to this sample is genus *Stomatopoda* with a specimen similarity of 86.8%, second is *Penaeopsis serrata* with a specimen similarity of 86.61%, and third is *Alpheus beanii* with a specimen similarity of 86.47% (Table 3.6).

Table 3.6. Specimen similarity table for *Sicyonia carinata*.

Species	Specimen Similarity range (%)
Stomatopoda	86.8
Penaeopsis serrata	86.61
Alpheus beanii	86.47
Thor manningi	86.14-86.3
Eumunida minor	85.31-85.64

These results indicate that our sequence is the first DNA barcode in BOLD for this species. The neighbor-joining trees for these two sequences of *Sicyonia carinata* constructed *via* BOLD are shown in Fig 3.24 and Fig 3.25. The sample that is barcoded from Turkey (shown in red) falls within a clade that consists of three different species in other genera.

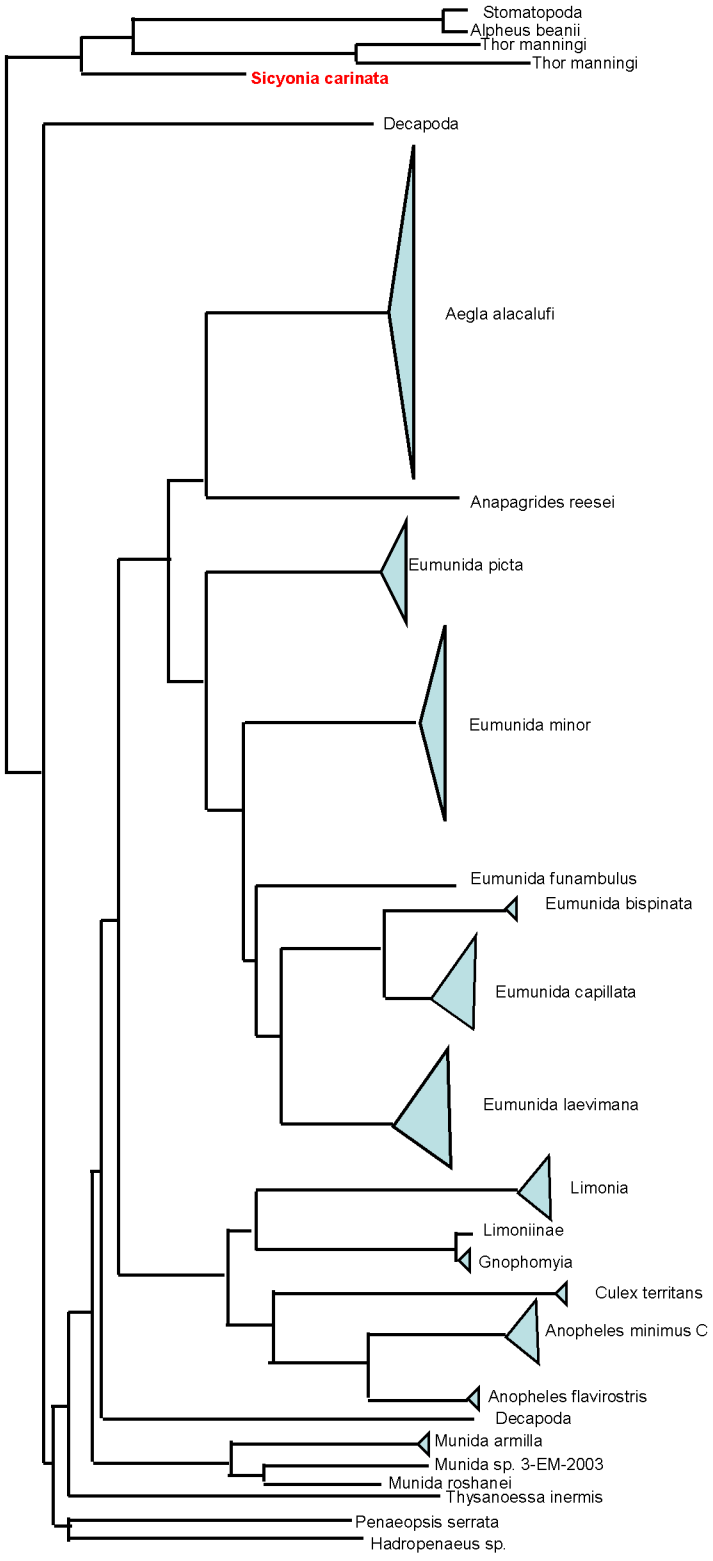


Figure 3.24. Neighbor-joining tree for our *Sicyonia carinata*-8 sequence (shown in red) constructed based on BOLD. The size of the triangles are proportional to the number of sequences in BOLD for that species.

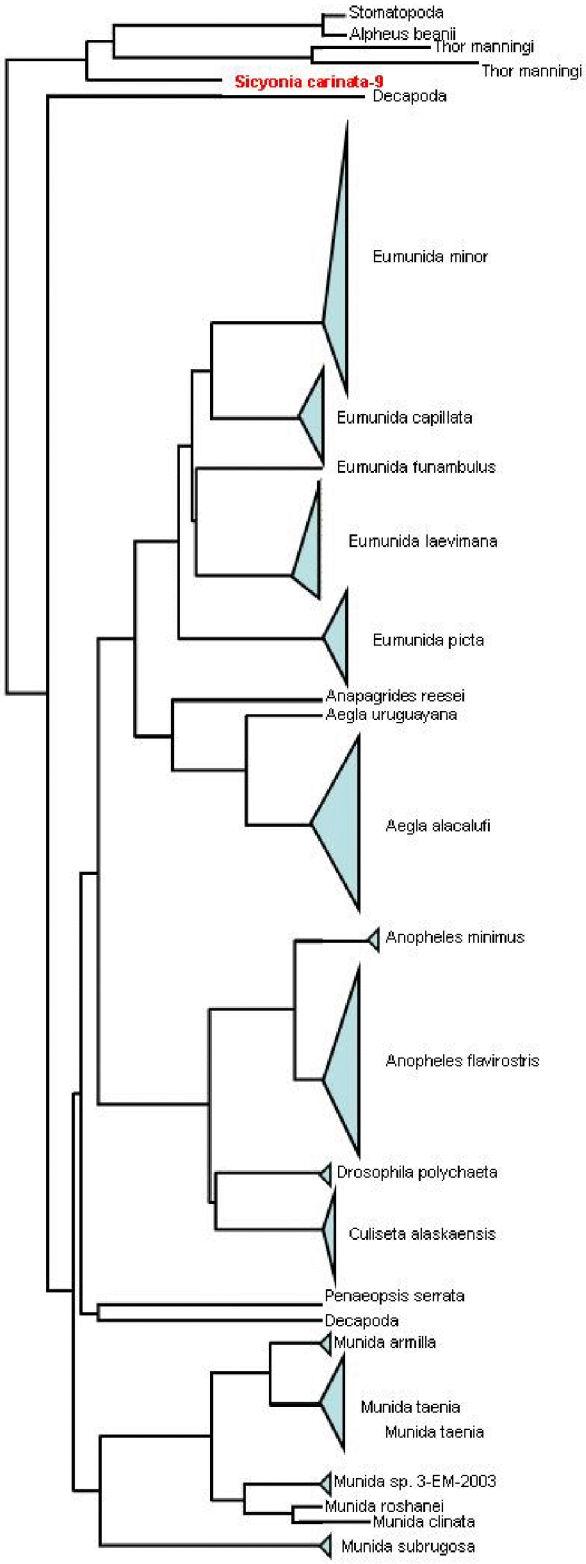


Figure 3.25. Neighbor-joining tree for our *Sicyonia carinata*-9 sequence (shown in red) constructed based on BOLD. The size of the triangles are proportional to the number of sequences in BOLD for that species.

3.7. *Lysmata seticaudata* (Risso, 1816)

One sample (16S sequence) from Turkey was analyzed for *Lysmata seticaudata*. The sample was collected from the Dardanelles Strait (Fig. 3.26). The haplotype network and the intraspecific tree could not be prepared as there was only one sample for this species.



Figure 3.26. The sampling locations for *Lysmata seticaudata*. The pink circle indicates sampling location Dardanelles Strait, the Aegean that the sample was collected from for this project. CO1 sequence data were not available in BOLD or GenBank for this species.

3.8. *Stenopus spinosus* (Risso, 1826)

Two samples (COI sequence) from Turkey were analyzed for *Stenopus spinosus*. The samples were collected from Kaş, the Mediterranean (Fig. 3.27). The intraspecific tree could not be constructed as there were only two sequences. Two different haplotypes are observed for this species (Fig. 3.28), which are separated by 12 base pairs.



Figure 3.27. The sampling location for *Stenopus spinosus*. The pink circle indicates sampling location Kaş, the Mediterranean that the samples were collected from for this project. CO1 sequence data were not available in BOLD or GenBank for this species.

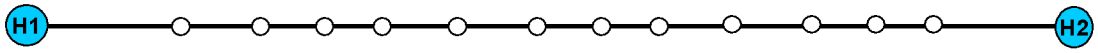


Figure 3.28. Haplotype network for *Stenopus spinosus*. The size of circles are proportional to the number of individuals. See Table 3.1 for the key to the color-codes of the geographic positioning of the haplotypes.

Comparing an individual with haplotype H1, sample 183, with the BOLD, the first closest match to the Mediterranean sample is Alpheidae, *Synalpheus* with a specimen similarity of 88.06%, second is *Stenopus zanzibaricus* with a specimen similarity of 86.73% and third is *Stenopus hispidus* with a specimen similarity range of 84.67-85.24%. Comparing an individual with haplotype H2, sample 201, with the BOLD, the first closest match is Alpheidae, *Synalpheus* with a specimen similarity of 87.6%, second is *Stenopus zanzibaricus* with a specimen similarity of 85.85% and third is *Stenopus hispidus* with a specimen similarity range of 84.8-85.08%.

These results indicate that our sequences are the first DNA barcodes in BOLD for this species. The neighbor-joining trees of both sequences for *Stenopus spinosus* constructed *via* BOLD are shown in Fig. 3.29 and Fig. 3.30. The first sample, 183, clusters with species from six different genera in one clade. The second sample, 201, clusters with species from five different genera in one clade. The sample that is barcoded from Turkey (shown in red) falls within the *Stenopus* clade. As it can be seen, *Synalpheus* is a highly paraphyletic genus; it is seen almost in every clade, clustering with different species.

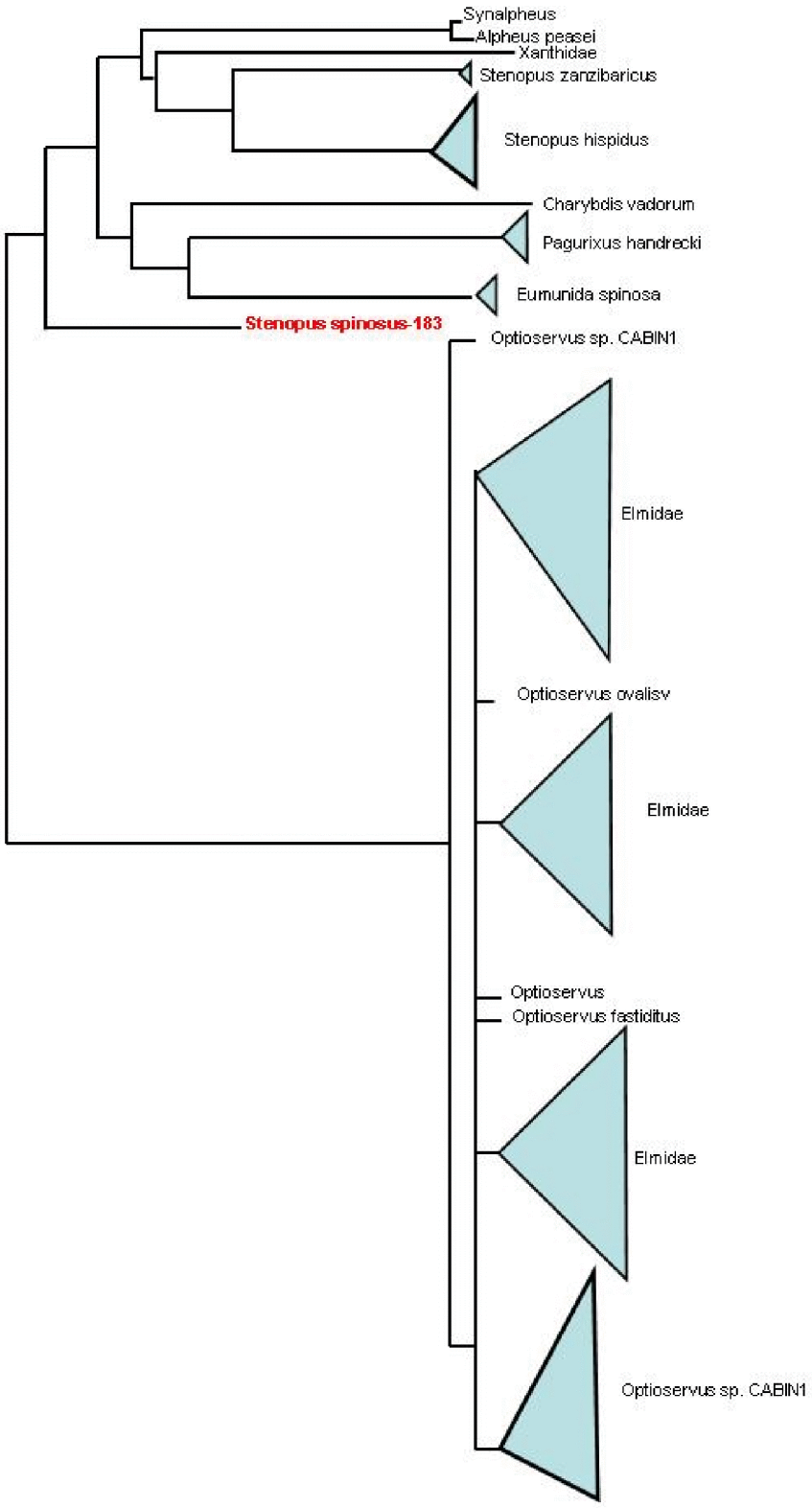


Figure 3.29. Neighbor-joining tree for our *Stenopus spinosus*-183 sequence (shown in red) constructed based on BOLD. The size of the triangles are proportional to the number of sequences in BOLD for that species.

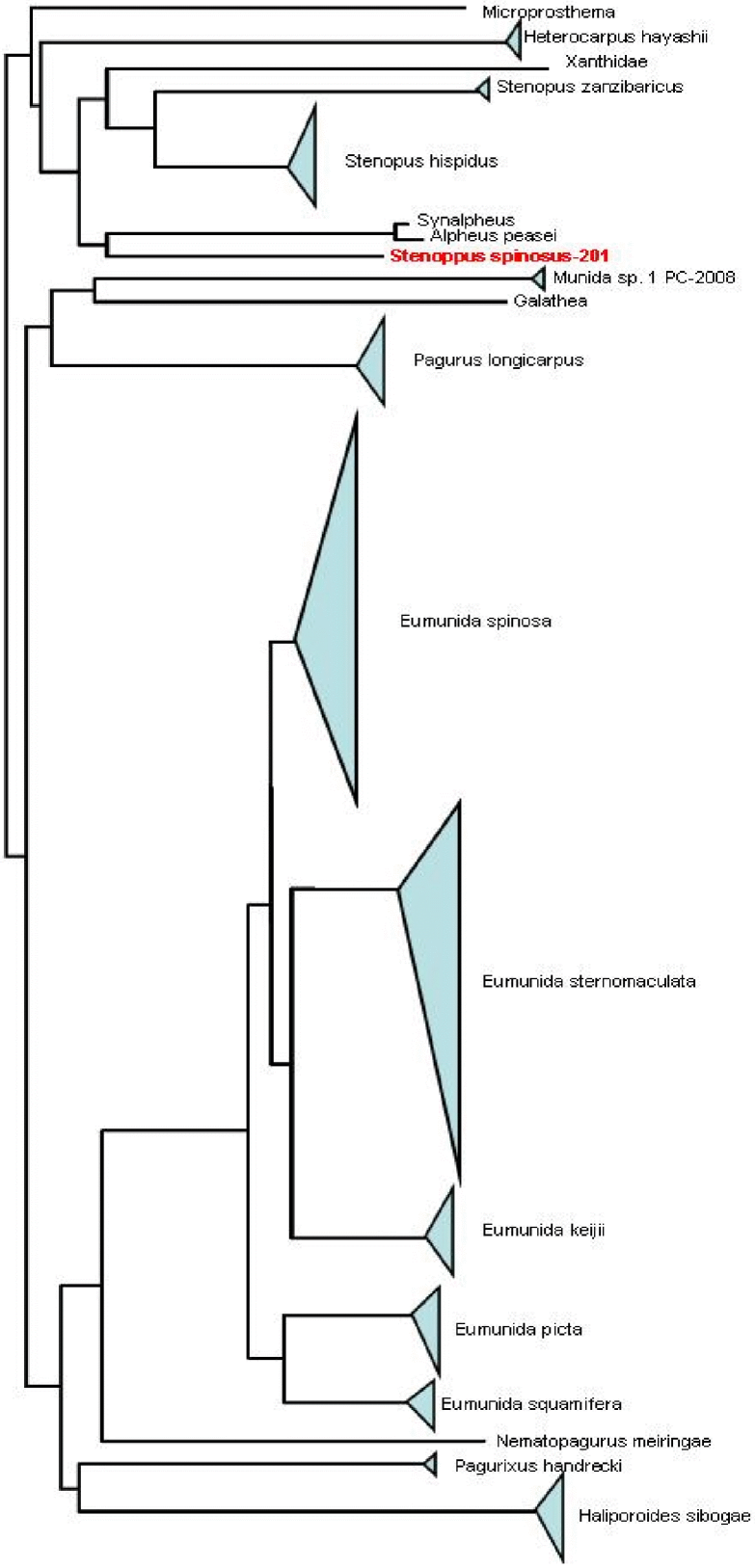


Figure 3.30. Neighbor-joining tree for our *Stenopus spinosus*-201 sequence (shown in red) constructed based on BOLD. The size of the triangles are proportional to the number of sequences in BOLD for that species.

3.9. *Synalpheus gambarelloides* (Nardo, 1847)

One sample (COI) from Turkey was analyzed for *Synalpheus gambarelloides*. The sample was collected from Kaş, the Mediterranean (Fig. 3.31). The haplotype network and the intraspecific tree could not be prepared as there was only one sample for this species.



Figure 3.31. The sampling location for *Synalpheus gambarelloides*. The pink circle indicates sampling location Kaş, the Mediterranean that the sample was collected from for this project. CO1 sequence data were not available in BOLD or GenBank for this species.

Comparing with the BOLD, first closest match to this sample is *Alpheus hippothoe* with a specimen similarity range of 83.63-84.02%, second *Alpheus edwardsii* with a specimen similarity range of 83.4-83.63% and third *Penaeopsis serrata* with a specimen similarity of 83.27% (Table 3.7).

Table 3.7. Specimen similarity table for *Synalpheus gambarelloides*.

Species	Specimen Similarity range (%)
Alpheus hippothoe	83.63-84.02
Alpheus edwardsii	83.4-83.63
Penaeopsis serrata	83.27
Heterocarpus hayashii	82.65-83.12

The neighbor-joining tree for *Synalpheus gambarelloides* constructed *via* BOLD is shown in Fig 3.32. The sample that is barcoded from Turkey (written in red) falls within a complex clade. The neighbor-joining tree consists of a high number species from *Synalpeus* genus, which are seen in almost every clade, hence this genus is highly paraphyletic.

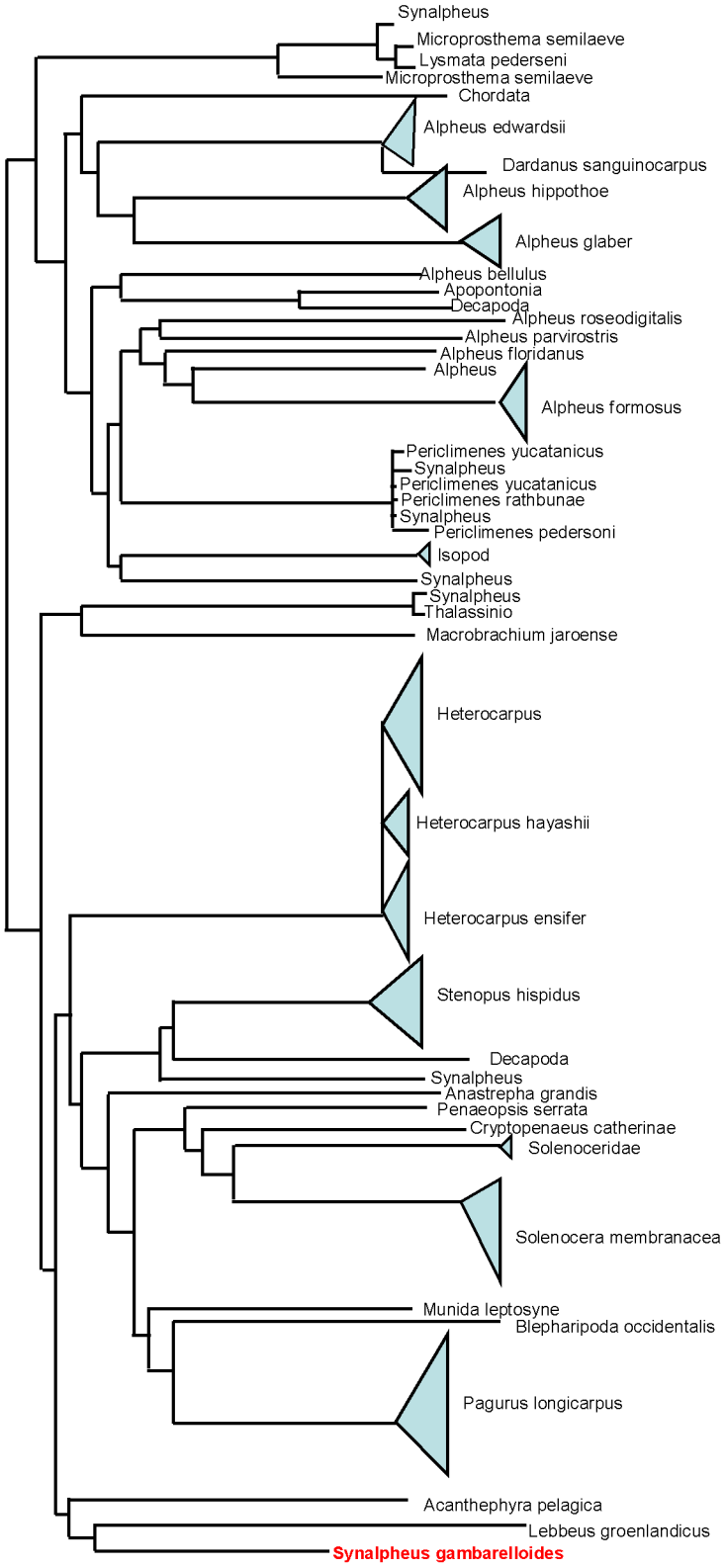


Figure 3.32. Neighbor-joining tree for our *Synalpheus gambarelloides* sequence (shown in red) constructed based on BOLD. The size of the triangles are proportional to the number of sequences in BOLD for that species.

3.10. *Gnathophyllum elegans* (Risso, 1816)

One COI sequence and one 16S sequence from one sample was analyzed from Turkey for *Gnathophyllum elegans*. The sample was collected from Bodrum, the Aegean (Fig. 3.33). The haplotype network and the intraspecific tree could not be prepared as there was only one sample.



Figure 3.33. The sampling location for *Gnathophyllum elegans*. The pink circle indicates sampling location Bodrum, the Aegean that the sample was collected from for this project. CO1 sequence data were not available in BOLD or GenBank for this species.

Comparing with the matches to the BOLD, first closest match to this sample is *Gnathophyllum americanus* with a specimen similarity of 85.64%, the second *Gnathophyllum americanum* with a specimen similarity of 85.64%, and third Alpheidae, *Synalpheus* with a specimen similarity of 83.39% (Table 3.8).

Table 3.8. Specimen similarity table for *Gnathophyllum elegans*.

Species	Specimen Similarity range (%)
Gnathophyllum americanus	85.64
Gnathophyllum americanum	85.64
Alpheidae Synalpheus	83.39
Periclimenes lamellibrachiophilus	82.92-83.09

These results indicate that our sequence is the first DNA barcode in BOLD for this species. The neighbor-joining tree for this species constructed based on BOLD is shown in Fig 3.34. The sample that is barcoded from Turkey (shown in red) falls within the *Gnathophyllum* clade. Being a paraphyletic genus, as mentioned above, *Synalpheus* is also found in this clade.

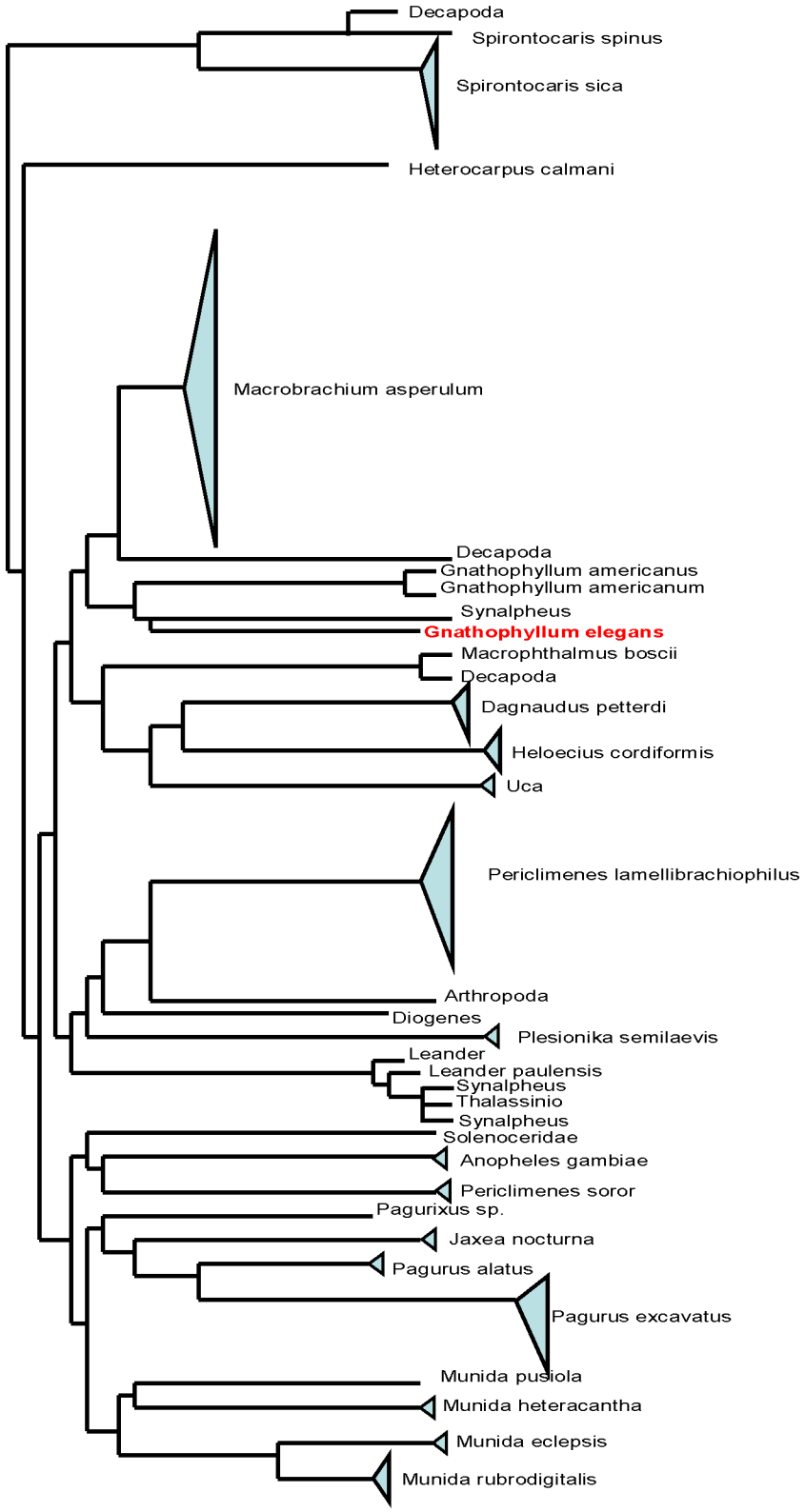


Figure 3.34. Neighbor-joining tree for our *Gnathophyllum elegans* sequence (shown in red) constructed based on BOLD. The size of the triangles are proportional to the number of sequences in BOLD for that species.

3.11. *Hippolyte sapphica* (D'Udekem d'Acoz, 1993)

10 samples (16S sequence) from Turkey were analyzed for *Hippolyte sapphica.* The samples were collected from one location: Lale Island, Ayvalık, the Aegean (Fig. 3.35).



Figure 3.35. The sampling location for *Hippolyte sapphica.* The pink circle indicates sampling location Ayvalık, Aegean Sea that the sample was collected from for this project. CO1 sequence data were not available in BOLD or GenBank for this species.

Intraspecific tree for *Hippolyte sapphica* is shown in Fig. 3.36. All 16S sequences generate two clades. *Hippolyte sapphica*-70 falls within a different branch (shown in red Fig. 3.36) and all other sequences form one clade. It is also shown as H2 in haplotype network which is separated by one base pair from other haplotype (Fig. 3.37 and Table 3.9). This one pair difference, though negligible, is what causes sample 70 to form its own branch in the intraspecific neighbor-joining tree.

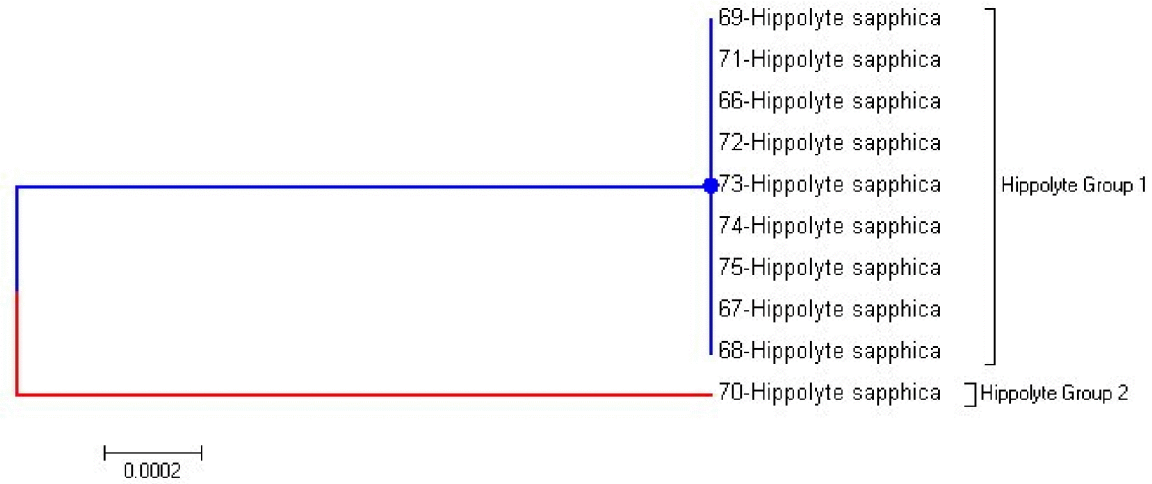


Figure 3.36. Intraspecific tree for *Hippolyte sapphica.*

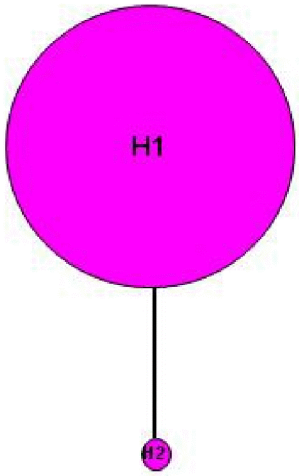


Figure 3.37. Haplotype network for *Hippolyte sapphica.* The size of circles are proportional to the number of individuals. See Table 3.1 for the key to the color-codes of the geographic positioning of the haplotypes.

Table 3.9. Haplotype network table for *Hippolyte sapphica.*

Haplotype	Number of individuals	Codes for the individuals as shown in the intraspecific tree (Figure 3.36)
H1	9	66-67-68-69-71-72-73-74-75
H2	1	70

3.12. *Parapenaeus longirostris* (Lucas, 1846)

Three samples from Turkey and four samples from BOLD (Italy) were analyzed for *Parapenaeus longirostris*. The samples from Turkey were collected from Burgaz Island, Sea of Marmara (Fig. 3.38).

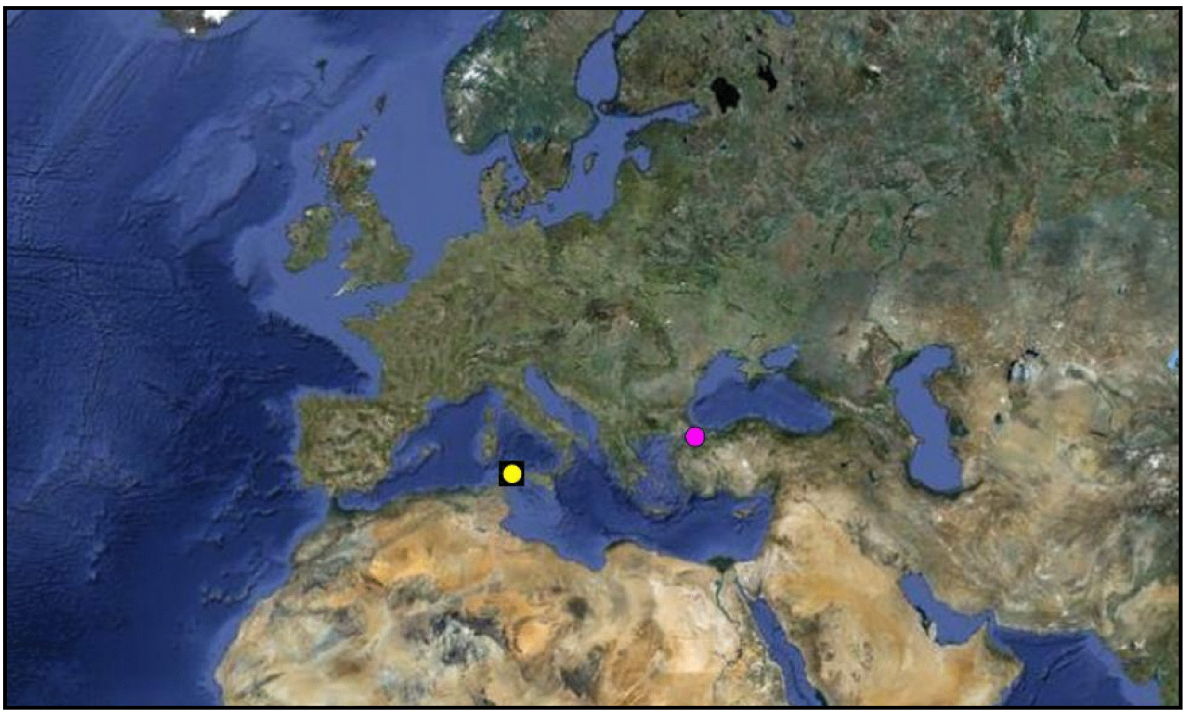


Figure 3.38. The sampling locations for *Parapenaeus longirostris* for which CO1 Barcode Data were available from BOLD. The yellow circle indicates a sequence for which collection site information was available from BOLD (Italy), the pink circle indicates sampling location that samples were collected from for this project.

Intraspecific tree for *Parapenaeus longirostris* is shown in Fig. 3.39. All COI sequences generate two clades. Mainly two groups are seen. All barcoded individuals from the Sea of Marmara (22, 23, 26) cluster closely within the same clade (group 2), whereas those from Italy form another clade (group 1). Two different haplotypes (H1 from Italy and H2 from the Sea of Marmara) are observed for this species (Fig. 3.40), which is also paralleled by the existence of two clades in the tree. These two haplotypes are separated by eight base pairs.

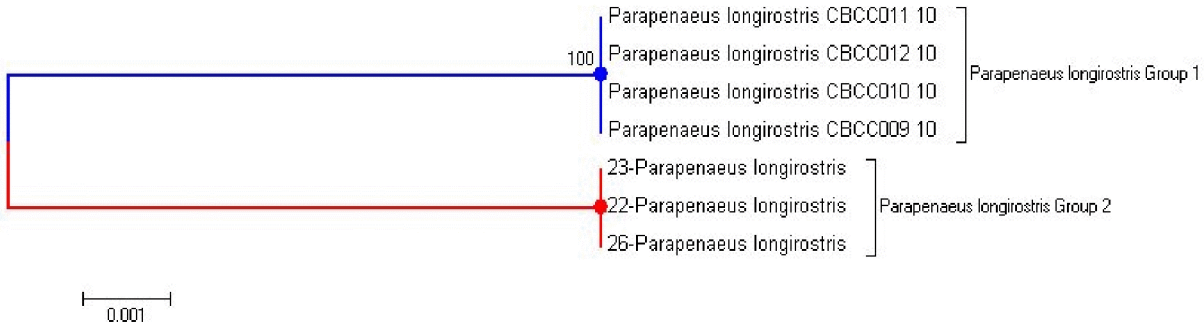


Figure 3.39. Intraspecific tree for *Parapenaeus longirostris*.

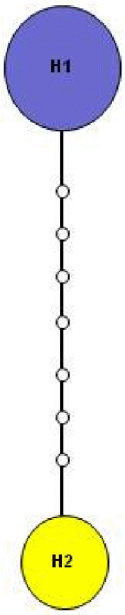


Figure 3.40. Haplotype network for *Parapenaeus longirostris*. The size of circles are proportional to the number of individuals. See Table 3.1 for the key to the color-codes of the geographic positioning of the haplotypes.

Table 3.10. Haplotype network table for *Parapenaeus longirostris*.

Haplotype	Number of individuals	Codes for the individuals as shown in the intraspecific tree (Figure 3.39)
H1	4	CBCC009-CBCC010-CBCC011-CBCC012
H2	3	22-23-26

Comparing with the BOLD, first closest match to the haplotype H2 from Turkey 2 is *Parapenaeus longirostris* with a specimen similarity range of 98.43-98.71%, second is *Penaeopsis serrata* with a specimen similarity of 86.65% and third *Munida militaris* with a specimen similarity of 83.09% (Table 3.11).

Table 3.11. Specimen similarity range table for *Parapenaeus longirostris*.

Species	Specimen Similarity range (%)
Parapenaeus longirostris	98.43-98.71
Penaeopsis serrata	86.65
Munida militaris	83.09
Agononida fortiantennata	83.09
Galathea affinis	83

The neighbor-joining tree for *Parapenaeus longirostris* constructed *via* BOLD is shown in Fig. 3.41. All barcoded individuals from Turkey (haplotype) cluster closely in the tree with other samples from BOLD, from Italy, however the relatively long branch length of the samples from Turkey indicate some differentiation as seen in the intraspecific tree and haplotype network.

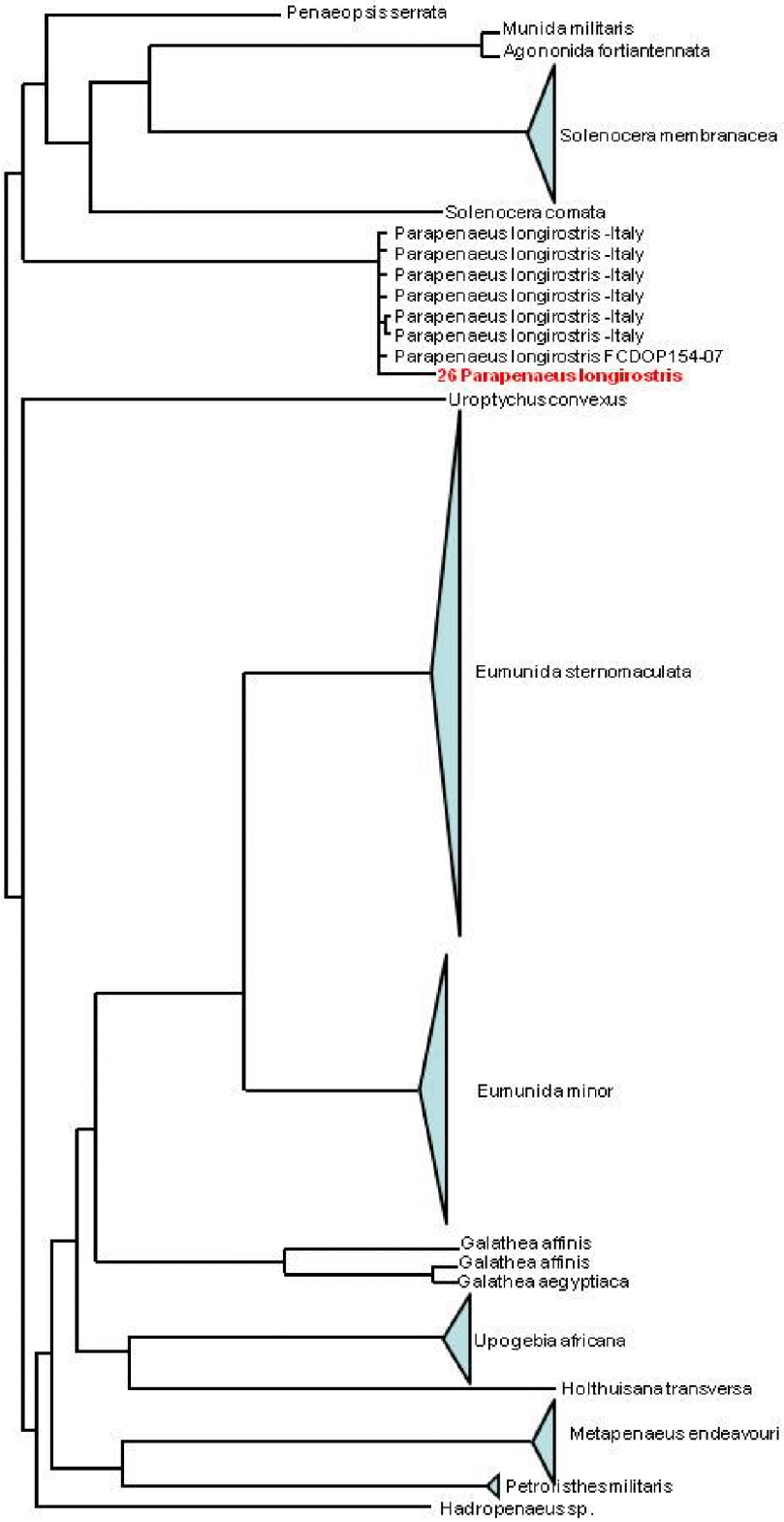


Figure 3.41. Neighbor-joining tree for our *Parapenaeus longirostris* (26) sequence (shown in red) constructed based on BOLD. The size of the triangles are proportional to the number of sequences in BOLD for that species.

3.13. *Eualus cranchii* (Leach, 1817)

One sample (COI sequence) from Turkey was analyzed for *Eualus cranchii*. The sample was collected from Kaş, the Mediterranean. The haplotype network and the intraspecific tree could not be prepared as there was only one sample.



Figure 3.42. The sampling locations for *Eualus cranchii*. The yellow circles indicate localities which collection sites were available from BOLD (United Kingdom), the pink circle indicates sampling location that was collected from for this project.

Although *Thoralus cranchii* has been synonymized for *Eualus cranchii* as mentioned before, *Thoralus cranchii* is still used in BOLD. Therefore please note that when we use *Thoralus* below, it refers to *Eualus*, but with an emphasis that the data were obtained from BOLD. Comparing with BOLD, first closest match to this sample is *Thoralus cranchii* with a specimen similarity range of 84.82-85.3%, second is *Chorilia longipes* with a specimen similarity range of 82.09-82.76% (Table 3.12).

Table 3.12. Specimen similarity table for *Eualus cranchii*.

Species	Specimen Similarity range (%)
Thoralus cranchii	84.82-85.3
Chorilia longipes	82.09-82.76
Cancer magister	82.02
Munida rutllanti	81.92

The neighbor-joining tree for this species constructed with BOLD is shown in Fig. 3.43. The sample that is barcoded from Turkey (shown in red) falls within the *Thoralus cranchii* clade, but on a different branch to those from the United Kingdom. *Acanthephyra pelagica* seems to be a paraphyletic species in this case.

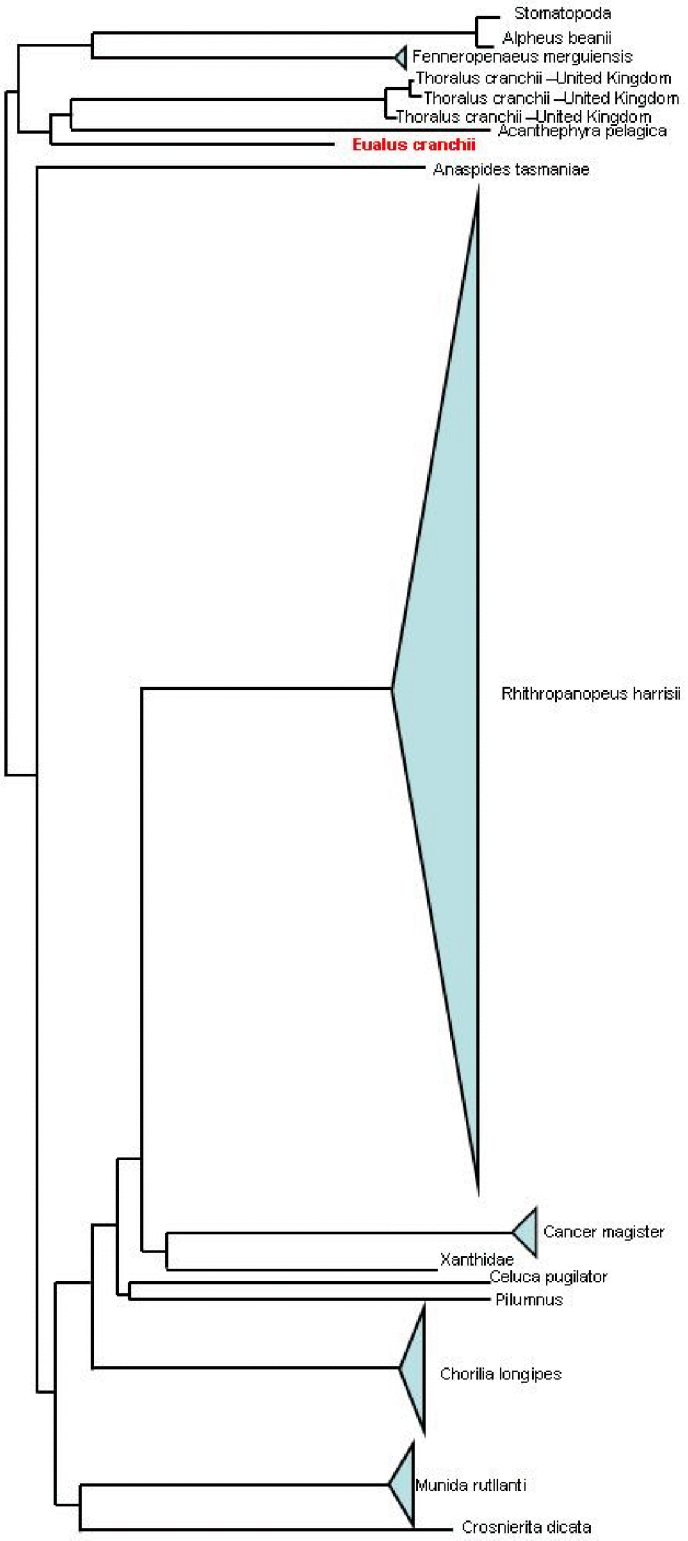


Figure 3.43. Neighbor-joining tree for our *Eualus cranchii* sequence (shown in red) constructed based on BOLD. The size of the triangles are proportional to the number of sequences in BOLD for that species.

3.14. *Crangon crangon* (Linnaeus, 1758)

Four COI sequences from Turkey were analyzed for *Crangon crangon*. The samples were collected from Kilyos, Black Sea (Fig. 3.44).



Figure 3.44. The sampling locations for *Crangon crangon*. The yellow circles indicate localities which collection sites were available from BOLD (Germany and North Atlantic Ocean), the pink circle indicates the sampling location for this project.

Intraspecific tree for *Crangon crangon* is shown in Fig. 3.45. All COI sequences generate two clades, where samples 28 and 29A form one clade, and 29 and 30 form another. All sequences correspond to different haplotypes, as seen in the haplotype network (Fig. 3.46 and table 3.13). H2 is differentiated from H1 by two bases and from H4 by 14 bases. H3 haplotype differs by one base pair from H4. Specimen identification from BOLD is also done for a representative sequence from each clade (28 and 29), to see the differences in the phylogenetic trees, if any.

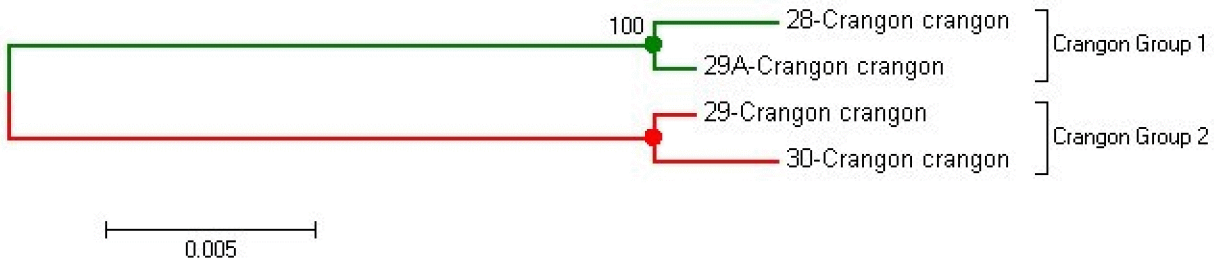


Figure 3.45. Intraspecific tree for *Crangon crangon.*

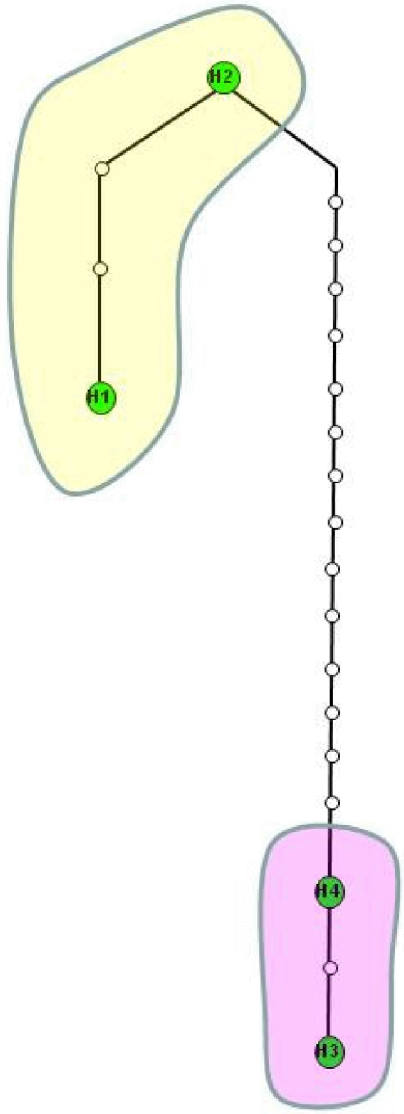


Figure 3.46. Haplotype network for *Crangon crangon.* The size of circles are proportional to the number of individuals. See Table 3.1 for the key to the color-codes of the geographic positioning of the haplotypes.

Table 3.13. Haplotype network table for *Crangon crangon.*

Haplotype	Number of individuals	Codes for the individuals as shown in the intraspecific tree (Figure 3.45)
H1	1	28
H2	1	29A
H3	1	29
H4	1	30

Comparing an individual from group 1, sample 28, with the BOLD, the first closest match to this sample is *Crangon crangon* with a specimen similarity range of 97.99-98.16%, and second is *Crangon septemspinosa* with a specimen similarity range of 92.33-94.83%. Comparing an individual from group 2, sample 29, with the BOLD, the first closest match to this sample is *Crangon crangon* with a specimen similarity range of 95.45-95.62%, and second is *Crangon septemspinosa* with a specimen similarity range of 90.42-93.03%.

The neighbor-joining tree for *Crangon crangon* constructed *via* BOLD is shown in Fig. 3.47 and 3.48. The samples that are barcoded from Turkey (shown in red) fall within the *Crangon crangon* clade and each one forms a sister branch to the two sequences from BOLD. When comparing the two trees, the branch length of sample *Crangon crangon*-28 is longer than the branch length of sample *Crangon crangon*-29, indicative of the differentiation of the two clades we observed in the intraspecific tree and haplotype network.

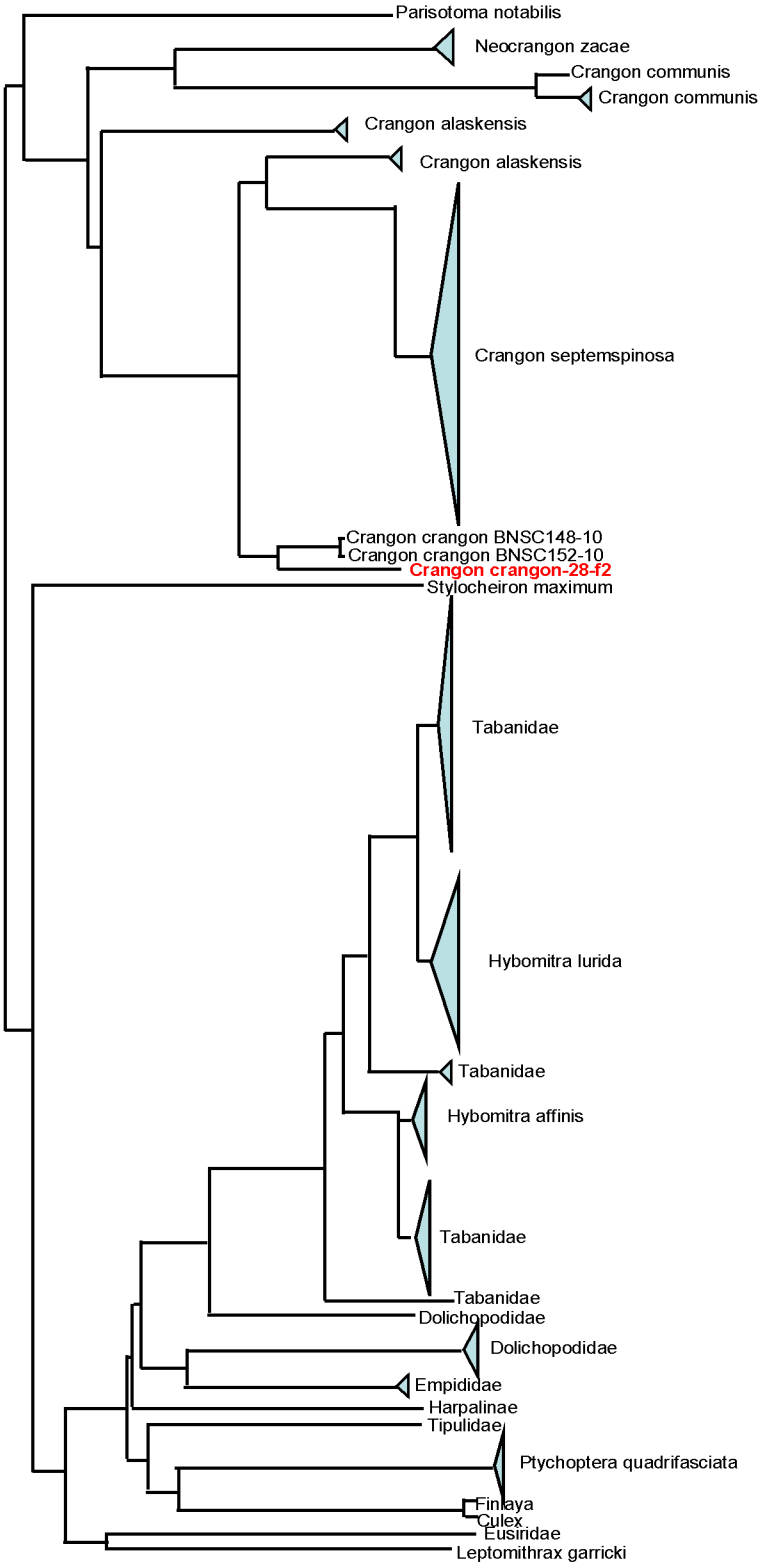


Figure 3.47. Neighbor-joining tree for our *Crangon crangon* (28) sequence (shown in red) constructed based on BOLD. The size of the triangles are proportional to the number of sequences in BOLD for that species.

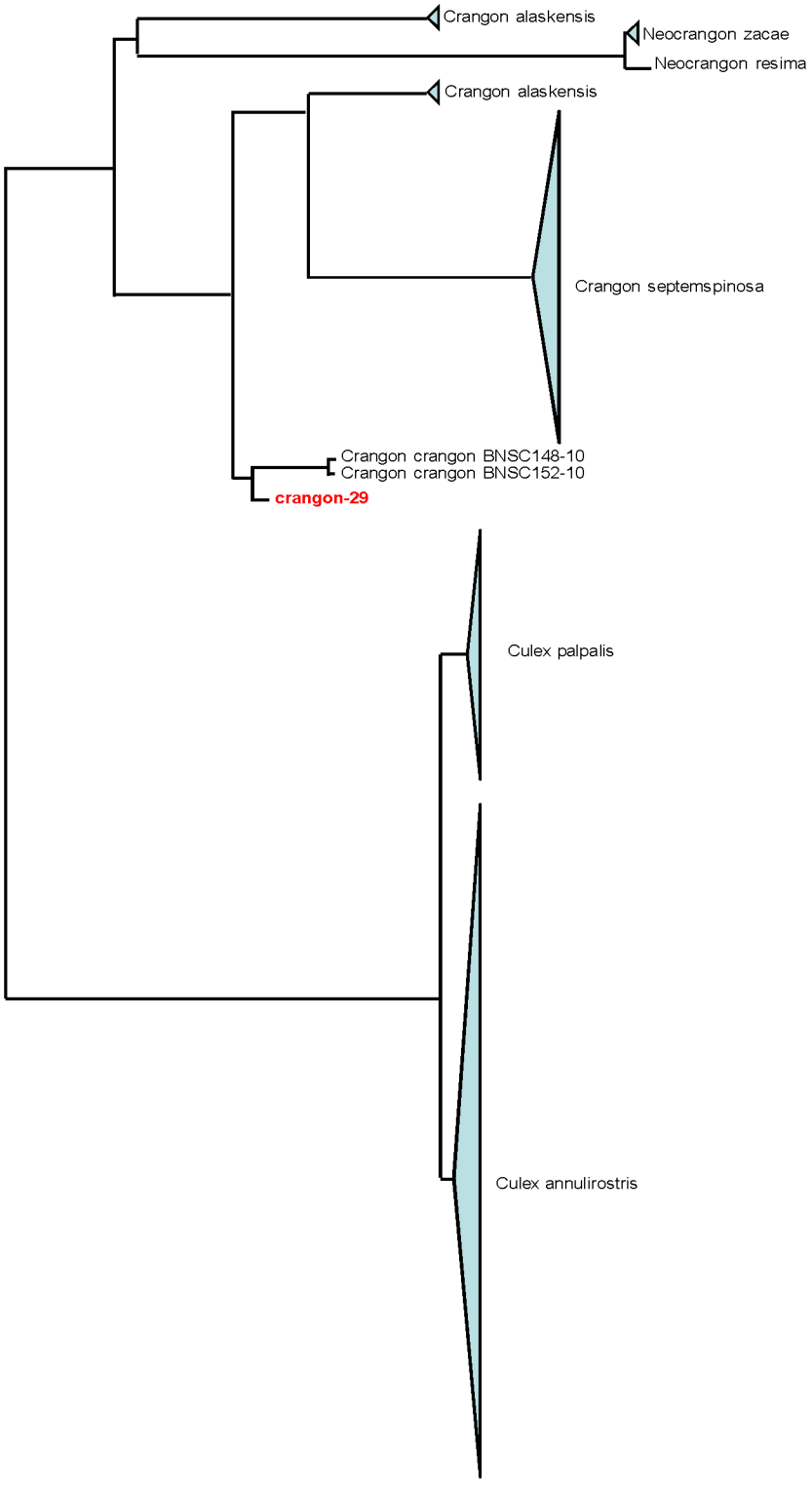


Figure 3.48. Neighbor-joining tree for our *Crangon crangon* (29) sequence (shown in red) constructed based on BOLD. The size of the triangles are proportional to the number of sequences in BOLD for that species.

3.15. *Athanas nitescens* (Leach, 1814)

Two 16S sequences from Turkey were analyzed for *Athanas nitescens.* The samples were collected from Kaş, the Mediterranean and Seferihisar, the Aegean (Fig. 3.49). The intraspecific tree could not be constructed as there were only two sequences.



Figure 3.49. The sampling locations for *Athanas nitescens.* The pink circles indicate sampling locations that were collected for this project.

Two different haplotypes are observed for this species (Fig. 3.50). These two haplotypes are separated by 51 base pairs. H1 is collected from the Mediterranean, whereas H2 is collected from the Aegean.

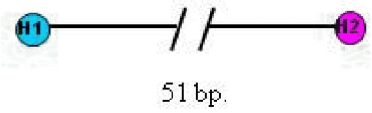


Figure 3.50. Haplotype network for *Athanas nitescens.* The size of circles are proportional to the number of individuals. See Table 3.1 for the key to the color-codes of the geographic positioning of the haplotypes.

Further analysis could not be undertaken on this species because other data were not available from GenBank for this species. Hence we present the first 16S sequences for this species.

4. DISCUSSION

DNA barcoding is useful for the quick identification and potential discovery of species. It is considered to be successful when interspecific variation exceeds intraspecific variation by one order of magnitude, which is called the "barcoding gap" (Wiemers and Fiedler, 2007). In this study, the mean intraspecific divergence is 1.3%. Hence, taking the 'order of magnitude times greater' interspecific differentiation to define the barcoding gap, the value is 13%, which is smaller than the mean interspecific distance of 21%. This result demonstrates that the species that are studied in this project are suitable for DNA barcoding. However it should be noted that the number of species for which intraspecific divergence was calculated for was 12. Out of these 12 species, for nine of them the number of samples that were used was under five. Hence the comparisons mentioned above should be interpreted with caution.

15 species and 115 sequences (94 new, 12 from BOLD and nine from GenBank) are analyzed in this study. Out of the new sequences generated, five species had unique barcode sequences that are distinct from those found in any other species in BOLD. These species are *Processa edulis*, *Processa acutirostris*, *Sicyonia carinata*, *Stenopus spinosus*, and *Gnathophyllum elegans*. There are four species, for which 16S sequences are reported for the first time from this study (based on a comparison with GenBank). These species are *Gnathophyllum elegans*, *Palaemon adspersus*, *Hippolyte sapphica*, and *Athanas nitescens*.

Considering the species investigated in this study, we can observe some general patterns in different species. First group comprises the species that form more than one clade, and the second group comprises those that form only one clade. Nine species showed genetic differentiation, which comprised more than one clade and corresponding haplogroups. Out of these nine species, six of them had data available from BOLD with locality information from the Mediterranean and/or Atlantic, which made it possible to make more global comparisons. These species were *Crangon crangon, Palaemon elegans, Parapenaeus longirostris, Eualus cranchii, Palaemon adspersus* and *Palaemon serratus.* The individual details of these species will be discussed next.

Focusing on *Crangon crangon*, two groups are found in the Aegean in this study. Luttikhuizen et al. (2007) observed four groups in the Atlantic, the Mediterranean, the Adriatic Sea and the Black Sea. The sequences in this study might be expected to correspond to the Mediterranean or the Black Sea groups. However we were not able to make a direct comparison with these sequences, as it was not possible to align these sequences with ours.

In *Palaemon elegans*, both 16S and COI sequences showed the most extensive population structure observed in this study. Reuschel et al. (2010) established the degree of genetic variation within broadly distributed *P. elegans*, using DNA-sequences of the same genes. Mitochondrial sequences were obtained from the Atlantic, the Baltic Sea, the Mediterranean, the Black Sea and the Caspian Sea and three main haplogroups were separated in their study: one from the Atlantic (Type I) and two from the Mediterranean (Types II and III). The authors interpreted these differences as suggesting the presence of cryptic species. The 16S and COI sequences in this study could not be compared with the sequences in that study as the sequences were not available on GenBank and also could not be obtained from the author. Our 16S sequences formed two groups that were differentiated by a minimum of eight base pairs. One group included the most common haplotypes that were predominantly observed in the Mediterranean, whereas the other group was found predominantly in the Black Sea. Our COI sequences also formed two groups, with the second group consisting of only one individual from the Aegean. Comparing group one with BOLD, the barcodes from Turkey clustered closely with barcodes in BOLD from Poland. The individual from group two (38), fell within the opposite branch (with barcodes from United Kingdom and Portugal). Hence our comparative results support the idea of two cryptic species in *Palaemon elegans* in Turkey.

Parapenaeus longirostris is another species for which two groups were observed, and for which comparisons with BOLD were possible. All barcoded individuals from the Sea of Marmara (22, 23, and 26) clustered closely within the same clade, whereas those from Italy formed another clade. The existence of two clades in the tree is paralleled by two different haplotypes that were divergent by eight base pairs. Hence also in this case we see some differentiation between the Italian and Turkish samples.

A different version of the first group can be recognized for *Eualus cranchii*. As mentioned before, *Thoralus cranchii* is not the accepted nomenculature for this species anymore, however in BOLD it is still referred to as *Thoralus cranchii*. This change should be made in BOLD. A comparison of our sample with BOLD showed that the closest match was with *Thoralus cranchii*, however only with 85.3% similarity, with the individuals that were collected from United Kingdom. The sample that is barcoded from the Mediterranean, Turkey falls within the *Thoralus cranchii* clade, but on a different branch. This suggests past isolation for this species as well.

Palaemon serratus is another species in the first group. A comparison with BOLD showed that the closest match was with *Palaemon serratus,* however only with 89.7% similarity, with the individuals that were collected from United Kingdom and Portugal. The sample that is barcoded from the Aegean, Turkey falls within the *Palaemon* clade and is sister to the *Palaemon serratus* clade that includes individuals from United Kingdom and Portugal.

In *Palaemon adspersus* 10 COI sequences were analyzed from one region, the Aegean, and two groups were observed, which were differentiated by seven base pairs. These sequences also were seen to form two clades. The second clade consisted of two branches as a result of the five base pair difference between 213 *versus* samples 34, 205, and 209. Comparing with the BOLD, it is possible that a specifically unidentified barcode from BOLD, *Palaemon sp.* (Process-id: FC-DPBAS02D) is actually *Palaemon adspersus.* The sample that was barcoded from Turkey falls within the *Palaemon* clade, and forms a clade together with this *Palaemon sp.* from Poland, BOLD.

We can also talk about species for which two or more clades were found within Turkey's coastal waters, but without the possibility of global comparisons, as sequences and locality information were not available in BOLD or GenBank. These include *Sicyonia carinata, Stenopus spinosus,* and *Athanas nitescens*. In *Sicyonia carinata* and *Stenopus spinosus*, again two COI clades were observed for each species (differentiated by five and 13 base pairs, respectively), and all of them were found in the Mediterranean. Two 16S sequences of *Athanas nitescens* also formed two groups, being differentiated by 51 base pairs. It should be noted that the samples were collected from only two locations. The

samples for this species were collected from the Mediterranean and the Aegean. Although no subspecies are currently recognized for the species, the differentiation observed in this study suggests that there might be some cryptic speciation, and that an evaluation of subspecific/species taxonomy of these species can be useful and informative. It should be noted that only two specimens were compared for this species and isolation by distance can be the cause of the differentiation we observed. Also, another species from this genus is also recorded in the Aegean, *Athanas amazone* (Holthuis, 1951) (Ateş et al., 2010), and the differences that we observe could be due to a misidentification.

As another category of species, we can mention *Hippolyte sapphica* for which we observed only one clade/haplogroups within. In this species, the 10 16S sequences obtained from the Aegean formed a single clade, although one sample, 70, fell within a different branch. However this haplotype is separated by one base pair from the other haplotype, and this one pair difference is negligible. Our sequence will comprise the first 16S data for this species in Genbank. This species is also recorded for the first time in Turkey (Ateş et al., 2010).

Another category that we outlined in our study is species for which only one sample was collected within Turkey's coastal waters, analyzed by the "identify specimen" option in BOLD, but without our own direct comparison of sequences in BOLD, as these data were not publicly available. These include *Processa edulis* (COI), *Processa acutirostris* (COI), *Synalpheus gambarelloides* (COI), and *Gnathophyllum elegans* (COI and 16S). We discuss the species for which only one COI sequence was available for as such below. For *Lysmata seticaudata*, for which only one 16S sequence data were generated in this study, but no other 16S data were available from GenBank, no other analyses were possible.

Our analysis of *Processa edulis,* collected from the Mediterranean, indicates that our sequence is the first DNA barcode in BOLD. The sample that is barcoded from Turkey falls within the *Processa* clade. Also in *Gnathophyllum elegans,* collected from the Aegean, our sequence is the first DNA barcode in BOLD for this species. The sample that is barcoded from Turkey fell within the *Gnathophyllum* clade. In addition to *Processa edulis* and *Gnathophyllum elegans*, for which the comparison of our sequences with BOLD, and trees generated under the "identify specimen" option of BOLD, placed these

species in their correct respective genera, in six other species the barcodes we generated fell within the correct clade for that species. These species were *Palaemon elegans* (with 100% similarity with BOLD), *Parapenaeus longirostris* (98.72%), *Eualus cranchii* (85.3%), *Palaemon serratus* (89.7%), *Crangon crangon* (98.16%), and *Palaemon adspersus* (99.67%). Hence for these eight species, CO1 seems to be a good marker of choice for DNA barcoding, with minimal species gaps in the database. However, in these species, the rest of the branches in trees did not necessarily show expected phylogenetic relationships, hence the BOLD trees do not seem to have very high reliability.

The trees generated through BOLD also did not give expected results for three species, where our species did not cluster with the sequences of the same species in BOLD. For *Processa acutirostris,* collected from the Mediterranean, we present the first DNA barcode in BOLD. However, the sample that is barcoded from Turkey did not fall within the *Processa* clade, although the species it showed the highest similarity to was *Processa modica* (83.03%). This observation warrants a case for further investigation. Another such species is *Synalpheus gambarelloides.* The sample for this species was collected from the Mediterranean. The sample that was barcoded from Turkey fell within a clade that seems to be extensively paraphyletic, and not clustering with another barcode of the same species from Panama. A general examination of the BOLD trees we generated indicates general paraphyly in the genus *Synalpheus* in the BOLD trees and the same is true for the sequence we generated for our *Synalpheus gambarelloides* samples. The problem associated with *Synalpheus* seemed to extend to other species in different genera as well. For instance *Stenopus spinosus* fell within a clade together with an unidentified species of *Synalpheus* genus, rather than with *Stenopus zanzibaricus* or *Stenopus hispidus*, which were the second and third closest matches to the species, respectively. The *Stenopus zanzibaricus* samples in BOLD being from French Polynesia, and *Stenopus hispidus* being from French Polynesia and the western coast of US, probably also contributed to this discrepancy. Finally *Sicyonia carinata* was another species in which our barcode did not fall into the expected clade in the same genera with the species from BOLD. However, the four species from BOLD were from the New World including the coasts of US and Peru, hence these discrepancies might be expected.

In conclusion, the genetic cataloging of 15 shrimp and prawn species in Turkish waters was done in this study. When possible the genetic data were compared with those available from different parts of the Mediterranean and eastern Atlantic. Systematic DNA barcoding on decapods has not been done in Turkey previously, and this thesis is a first in this regard. The barcoding gap seemed to be working well for these 15 species. Unique CO1 barcodes were seen in five species, and four 16S sequences were generated for the first time from this study (based on a comparison with GenBank). In a phylogeographic perspective, out of the 15 species investigated, nine showed the presence of two clades within. Previous research has shown the presence of more than one clade in Turkish coastal waters in decapods (Luttikhuizen et al., 2008 and Reuschel et al., 2010), bivalves (Kalkan, *in prep.*) and fish (Magoulas et al., 2006), and in nine of the species we investigated, a similar two clade pattern was observed. These nine species are good candidates for discovery of cryptic species or subspecies, and more extensive sampling both in Turkish coastal waters, and when possible from the Mediterranean and the Atlantic will be useful for taxonomic clarification. Genetic barcoding of the shrimp species gave similar results with BOLD for eight species for which CO1 sequences were generated for in this study, showing the effectiveness of COI barcodes as an identification tool for these. In four species the generated CO1 barcodes did not match with others in BOLD. In two of these four species (*Stenopus spinosus* and *Sicyonia carinata*) barcodes from the same genus were not available from the eastern Atlantic, Mediterranean or Turkish coasts, hence the mismatches might be expected. For *Synalpheus gambarelloides*, the only matching sequence in BOLD was from Panama, and the discrepancy in BOLD tree could be reflecting this or the general paraphyletic nature of the genus *Synalpheus.* In *Processa acutirostris* we present the first barcode for this species, however although the closest match for this species in terms of similarity was *Processa modica,* the two species did not form a clade together on the BOLD tree, which indicate a problem with the BOLD identification scheme for this species. Finally, again, as a part of the barcoding approach, genetic material from the studied species is now stored at Institute of Environmental Sciences Laboratories, comprising a first step towards formation of a DNA Barcoding bank for these species. For the future, we recommend, a more detailed investigation of COI sequences of the studied species, both globally and from the remaining parts of Turkey to get a more complete picture of their interspecific and instraspecific genetic differentiation.

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APPENDIX A: THE PHOTOGRAPHS OF SPECIES INVESTIGATED IN THIS STUDY



1. ***Processa edulis***



2. ***Processa acutirostris***



3. *Palaemon elegans*



4. *Palaemon adspersus*



5. ***Palaemon serratus***



6. ***Lysmata seticaudata***



7. *Stenopus spinosus*



8. *Synalpheus gambarelloides*



9. ***Gnathophyllum elegans***



10. ***Hippolyte sapphica***



11. ***Parapenaeus longirostris***



12. ***Eualus cranchii***



13. ***Crangon crangon***



14. ***Athanas nitescens***