BIODIVERSITY ASSESSMENT OF FISHES AND INVERTEBRATES IN MERSIN BAY, THE EASTERN MEDITERRANEAN SEA, BY USING DNA BARCODING

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ABSTRACT

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Cataloguing the biodiversity of marine ecosystems is critical for several implications: e.g. protecting species under threat, detecting alien species or ecosystem based management etc. The eastern Mediterranean Sea is a hot spot for bioinvasion, however its biodiversity had been poorly studied. In the view of the ongoing changes in the Mediterranean, fish and invertebrate biodiversity of Mersin Bay were evaluated in this study by using DNA barcoding techniques, based on the mitochondrial cytochrome oxidase subunit I (COI) gene, coupled with morphological identifications of specimens. Sampling was performed by trawl surveys in Mersin Bay between May 2014 and June 2015. All fish specimens were identified to species level by morphological examination and invertebrate species were categorized initially and later identified by molecular analyses. As a result, 186 marine specimens, 101 of which are fish, 29 arthropods, 35 mollusks, 6 annelids, 2 polychaetes, 9 echinoderms and 4 ascidians were analyzed using both methods. Out of 36 fish species analyzed, 14 were Lessepsian migrants. An Indo-Pacific anchovy species, *Encrasicholina punctifer*, is recorded for the first time in the Mediterranean ichthyofauna. Barcode records of 23 fish and 18 invertebrate species for Turkey and of 6 invertebrate species for the Mediterranean Sea were provided for the first time with this study. The sequence data, trace files and specimen details were submitted to the Barcode of Life Data System (BOLD; Ratnasingham and Hebert, 2007). Genetic divergence increased with higher taxonomic level. Conspecific and congeneric distances were 0.66% and 14.84% for fish species, while 0.99% and 13.58% for invertebrate species, respectively. In general, specimen identifications and biodiversity measures were consistent with taxonomic status and earlier studies demonstrating the usefulness and efficiency of the method.

Keywords: Biodiversity, COI, DNA Barcoding, Mersin Bay

ÖZ

DOĞU AKDENİZ, MERSİN KÖRFEZİ' NDEKİ BALIK VE OMURGASIZ BİYOÇEŞİTLİLİĞİNİN DNA BARKODLAMA TEKNİKLERİ KULLANILARAK ARAŞTIRILMASI

Çiftçi, Ozan

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Denizel biyoçeşitliliğin kayıt altına alınması birçok açıdan önemli bir konudur: örn. tehlike altındaki türlerin korunması, istilacı türlerin tespiti veya ekosistem temelli yönetim stratejilerinin belirlenmesi. Doğu Akdeniz, biyoçeşitlilik konusunda az çalışmış bir bölge olmakla birlikte biyo-istila konusunda da bir "hotspot" konumundadır. Bu çalışmada, Mersin Körfezi'nin balık ve omurgasız biyoçeşitliliği morfolojik tanımlamalar ve mitokondriyal sitokrom oksidaz altunite I (COI) genine dayanan DNA barkodlama teknikleri kullanılarak araştırılmıştır. Örneklemeler Mersin Körfezi'nde Mayıs 2014 ve Haziran 2015 tarihleri arasındaki trol seferleri ile yapılmıştır. Balık örneklerinin tümü morfolojik inceleme ile tür seviyesinde tanımlanırken, omurgasız türleri öncelikle morfolojik inceleme ile gruplandırılmış, ardından moleküler analizler ile de tanımlanmıştır. Sonuç olarak, 101 balık, 29 eklem bacaklı, 35 yumuşakça, 6 halkalı ve 2 fıstığımsı solucan, 9 denizyıldızı ve 4 tulumlu olmak üzere toplam 186 denizel örnek bu metodlar kullanılarak analiz edilmiştir. Analiz edilen 36 balık türünden 14'ünün Lessepsiyen türler olduğu tespit edilmiştir. Ayrıca, Hint-Pasifik kökenli bir hamsi türü, *Encrasicholina punctifer,* Akdeniz ihtiyofaunasında ilk defa kayıt altına alınmıştır. 23 balık ve 18 omurgasız türünün Türkiye kıyıları için ve 6 omurgasız türünün Akdeniz için ilk barkod kayıtları veritabanına yüklenmiştir. Bütün örneklerin DNA dizi verileri, iz dosyaları ve örnekleme bilgileri BOLD Sistemi'ne (Ratnasingham and Hebert, 2007) yüklenmiştir. Genetik farklılığın daha yüksek taksonomik seviyelerde arttığı görülmüştür. Ortalama tür içi uzaklıkların ve aynı cinsin farklı türleri arasındaki uzaklıkların balık türleri için %0.66 ve %14.84, omurgasız türleri için ise sırasıyla %0.99 ve %13.58 olduğu tespit edilmiştir. Tür tanımlamaları ve biyoçeşitlilik sonuçları açısından önceki çalışmalar ile uyumlu sonuçlar elde edilmiştir.

Anahtar Kelimeler: Biyoçeşitlilik, DNA Barkodlama, COI, Mersin Körfezi

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1 INTRODUCTION

1.1 Eastern Mediterranean Biodiversity and Lessepsian Migration

Levantine Sea is the easternmost part of the Mediterranean Sea and covers an area of 320.000 km² in total. Human assisted changes in the area roots back to Neolithic with the onset of farming and husbandry (Goren and Galil, 2005). However the greatest change in the marine environment took place by the opening of the Suez Canal in 1869 which provided the first direct link between the Red Sea and the Mediterranean Sea. Initially, the rate of migration was limited due to hyper-salinity of the Great Bitter Lake which forms a part of the canal. However, as the salinity of the lake and the Red-Sea were gradually equalized, the species have begun to colonize the Eastern Mediterranean. Temperature, on the other hand, is a major factor influencing the settlement of tropical alien species (Ben Rais Lasram and Mouillot, 2009). Specifically after 1998, a 150% increase in the rate of alien species introduction was observed, depending on a sudden shift in regional and global temperatures linked to the global climate change (Raitsos et al., 2010). Today, there are nearly four times more alien species along Levantine coasts compared with the western coasts of the Mediterranean (Coll et al., 2010). The studies point up to 1,000 Lessepsian species in the Levantine basin including 96 alien fish species (Golani, 2010; Golani and Bogorodsky, 2010; Zenetos et al., 2012; Fricke et al., 2015).

Marine invasive species are responsible for local population loss, and their effect is so severe that the phenomenon is regarded as the second biggest cause of biodiversity loss after habitat destruction (Breithaupt, 2003). They can disturb competitive interactions and predation regimes, alter basic ecosystem processes and may introduce new pathogens to indigenous populations (Ben Rais Lasram and Mouillot, 2009). The native biota of the Eastern Mediterranean is mostly composed of taxa better adapted to colder and less haline waters while many of them are presumably present at the limit of their ecological tolerance (Galil, 1993). Thus, this part of the Mediterranean is more vulnerable to invasion. Compounded effect of Lessepsian migration, climate warming and habitat loss may have critical and irreversible consequences for native communities and biodiversity of the Eastern Mediterranean. Such a dramatic prediction for whole Mediterranean Sea indicates that by 2060, 25 endemic Mediterranean fish species will qualify to IUCN Red List and six will become extinct due to the combined effects of climate change and invasion (Ben Rais Lasram et al. 2010). Thus, investigating and monitoring the related alterations in the Eastern Mediterranean ecosystem is a matter of urgency.

Mersin Bay is located in the northern coasts of Levantine basin and contains a large international harbor. Thus, ship ballast waters are another possible source of introduction for the region. However, among all Lessepsian fishes only two have likely been imported by this way: *Abudefduf vaigiensis* in the Ligurian Sea (Vacchi and Chiantore 2000), and *Epinephelus coioides* in the Adriatic Sea (Parenti and Bressi 2001). Also, the continental shelf in the region extends wider than the most areas of the northeastern Mediterranean and there is high amount of river discharge resulting in higher eutrophy and productivity (Gücü and Bingel, 1994c). This conditions may favor establishment of invasive species in the region. Gücü et al. (1994b) reported occurence of 20 Lessepsian fishes in Mersin Bay and this number increased to 52 by 2010 (Gücü et al., 2010). On the other hand, benthic habitats of southern coasts of Turkey have also been densely colonized by Lessepsian invertebrate species specifically around harbor environments, depending primarily on transport by ballast waters (Çınar et al., 2012). In Mersin Bay, almost 105 alien invertebrate species have been reported where most of them were mollusks (Çınar et al., 2011). Also, the Levantine Sea is considered as one of the most oligotrophic waters of the world's oceans, so the amount of organic material production in the bottom sediment could be expected to be relatively low (Ediger and YIlmaz 1996; Karakassis and Eleftheriou, 1997). Benthic biodiversity is found to be linked with ecosystem functioning, suggesting that a reduction in the benthic biodiversity might be associated with an exponential decline of ecosystem processes (Danovaro et al., 2008). Therefore, invasion by alien species might have more pronounced effects on the ecosystem of the Mersin Bay. Furthermore, Mersin Bay is located relatively proximal to the source of introduction (Suez Canal), so the region might provide suitable grounds for studies on the dispersal, establishment and monitoring of Lessepsian species.

DNA barcoding is the most widely used molecular technique for exotic species recognition and prevents problems like cryptic morphology or identification of larval forms (Azzuro et al. 2015; Bariche et al. 2015). Bariche et al. (2015) demonstrated the implications by identifying 153 specimens corresponding to 43 alien species from coasts of Lebanon and reported possible cases of unrecognized or cryptic species invasions. Barcoding studies including marine fish species from Eastern Mediterranean and Turkey date back to the studies of Kochzius et al (2008) and M.A. Smith et al. (2008). Until today, DNA barcodes of many other marine species from Turkey including fishes, amphipods, arthropods and mollusks have been generated and uploaded to public databases (Costa et al., 2009; Kochzius et al. 2010; Keskin and Atar, 2013b; Landi et al., 2014; Seyhan and Turan 2016). In the most comprehensive barcoding study in the coasts around Turkey, Keskin and Atar

(2013a) barcoded 1765 specimens of 89 commercially important fish species and demonstrated that DNA barcoding technique can be a highly valuable tool for stock assessment and identification of specimens. Thus, updating the public barcode inventory of the area for researchers might be highly useful for conservation and monitoring efforts.

1.2 What is DNA Barcoding

Cataloging the biodiversity of life on earth is not a simple task. Even considering eukaryotic species only, estimates range from the most conservative 3.6 million up to more than 100 million, with 10 million favored by most analysts. We still know only a minor fraction (10-15%) of the immensity of life's diversity. This percentage is expected to be lower especially for marine taxa regarding the low rate of species discovery in marine habitats. About 10,000 new species are described per year and considering the increasing rate of the global biodiversity loss in all habitats, current taxonomic methods may be inadequate or too slow to capture and manage biodiversity. Moreover, no more than 5% of the named organisms are known in biological detail (Costa and Carvalho, 2007) and the characteristics that separate close species are so complex that most taxonomists can specialize on a small number of taxa. Current taxonomic protocols rely heavily on phenotypic characters, and often require detailed inspection of the specimens. There is no master key for different groups of taxa or different life stages of a single species. The compounded outcome of these difficulties impose a taxonomic obstacle to understanding, utilizing and conserving biological diversity for the whole scientific community and society in general (Costa and Carvalho, 2007).

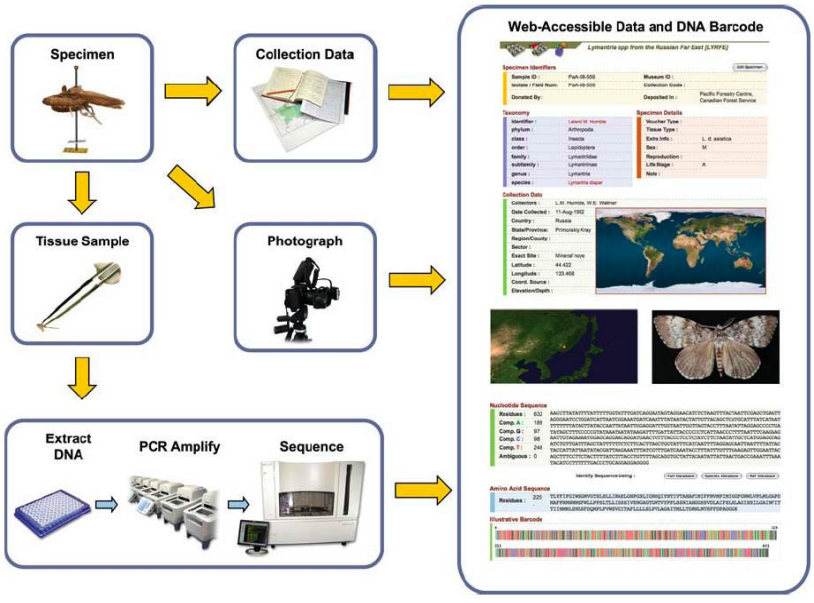

Figure 1 The workflow of DNA barcoding (Floyd et al., 2010)

DNA barcoding is a new diagnostic tool for rapid, accurate and automatable species identification by using one or more short (<700 bp) and standardized genetic markers. The choice of the genetic marker must meet some basic specifications. At first it must be present in all barcoded species. Then, it must be as short as possible for rapid amplification however still contain sufficient diversity to differentiate all species. Also it is desirable to be able to amplify the barcode sequence with a broad-range of primers for universal application. Mitochondrial DNA is clearly a better target because of its lack of introns, haploid mode of inheritance, limited exposure to recombination and high copy numbers in every cell (Saccone et al., 1999). Furthermore, Hebert et al. (2003) have demonstrated that COI region of the mitochondrial genome is appropriate for discriminating between closely related animal species for a diverse phyla and this marker have been effectively used for identification of various species (Hajibabaei et al., 2007; Lakra et al., 2011; Ward et al., 2009; Ribeiro et al., 2012; Landi et al., 2014; Bariche et al., 2015). For plants, on the other hand, no single locus have been found effective for species discrimination and The Consortium for the Barcode of Life (CBOL) has proposed two regions of DNA to be used as a dual-locus barcode.

Power of the resolution of DNA barcoding relies on the possible number of alternative sequences for the chosen genetic marker. A 600 base segment of a protein coding gene, for example, contains enough information to resolve millions of species. This number is not an arbitrary guess. Third nucleotide positions within codons are usually selectively neutral and mutations accumulate by random genetic drift at these sites. This neutrality is an anticipated feature as codon specificity is mostly determined by the first two bases, as stated by Wobble hypothesis (Crick, 1966). So even if there is a bias for any two bases out of four in a group of organisms in this third position, there would still be 2^{200} or 10^{60} possible sequences based on alternative third-position nucleotides.

Accuracy is a critical issue in DNA barcoding which depends on the separation between intraspecific variation and interspecific divergence in the marker region. The extent of this separation is referred as barcoding gap by Meyer and Paulay (2005) and can represent two different cases: one for specimen identification where an individual is closer to a member of its own species than a different species (‘local’ barcoding gap), and one for species discovery where the extent of the gap is compared to a predefined threshold for all species (‘global’ barcoding gap) (Collins and Cruickshank, 2013). However, implementation of such universal thresholds have been controversial. On the other hand, identification through DNA-based methods has several advantages, as diagnosis in all life history stages and

identification of morphologically similar species. These issues will be discussed further in detail in Section 1.7 and 1.8.

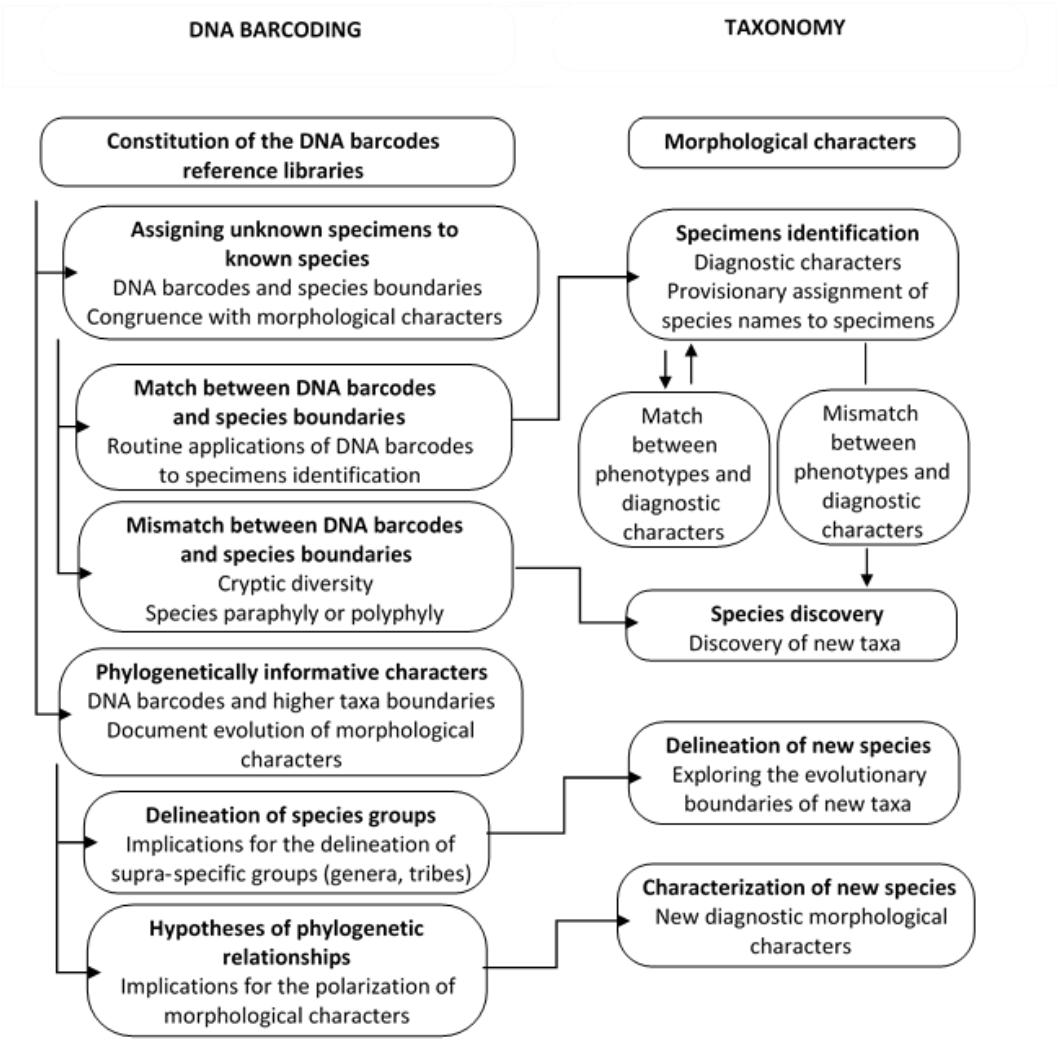

Figure 2 Conceptual links between DNA barcoding and taxonomy (Hubert and Hanner, 2015).

The overall purpose of DNA Barcoding is to build an alliance with morphological taxonomists for rapid species identification rather than proposing a new method based entirely on molecular identification. It promises to solve the problems and controversies related to biodiversity assessment and taxon identification which arise from different leading scientific approaches like internal anatomy, physiology, behavior, genes and isozymes. When addressing potential new species, those approaches should also be tested by a taxonomic expert, however routine identifications and detailed examination of the specimens can be avoided, providing a fast means of screening for large number of samples. Pilot projects have proven the effectiveness of the approach in several groups of animals as birds, fish, cowries, spiders, and several arrays of Lepidoptera, in addition for other groups including plants, macroalgea, fungi, protists and bacteria (Hajibabaei et al., 2007). Integrative approaches are demonstrated to be highly useful to speed the species discovery and identification, and the term turbo-taxonomy has been even applied recently to the procedures including DNA barcoding as a first step, before morphological identifications by an expert taxonomist and high-resolution digital imaging (Butcher et al., 2012).

In addition to the usage of DNA barcoding in research, various socioeconomically important applications are being developed including agricultural pest identification (e.g. Wang et al., 2015); endangered species laws (Holmes et al., 2009); sea-food product authentication (e.g. Hanner et al., 2011), herbal medicines (e.g. Newmaster et al., 2013), and pathogen–vector–host species associations (e.g. Brugman et al., 2015).

1.3 Global Barcoding Efforts and Databases

Only one year after Hebert's proposal on using COI region as a universal marker, an international initiative was established entitled CBOL (The Consortium for the Barcode of Life), devoted to exploring and developing the potential of DNA barcoding for research and as a practical tool for species identification. Members of the consortium include museums, herbaria, zoos, biodiversity research institutes, universities, conservation organizations, government agencies and private companies (Costa and Carvalho, 2007). iBOL (The International Barcode of Life Project) is dedicated to assembling the sequence library and technology research to identify organisms rapidly and inexpensively. However, the proposed adoption of COI for DNA barcoding requires more stringent data quality and standards (Lorenz et al., 2005). CBOL Database Working Group addressed this challenge and together with GenBank and other members of the International Nucleotide Sequence Database Collaboration (INSDC) a reserved keyword, BARCODE, was established for sequences that meet an emerging community data standard (Walters and Hanner, 2006). In

GenBank, data are of limited utility for molecular applications, because the raw sequence data are rarely archived preventing any critical evaluation of the sequence data. BOLD (Ratnasingham and Hebert, 2007) is developed to overcome this challenge and provide a reliable basis for the identification of unknown samples. It is the most convenient and well developed database for DNA barcoding purposes where analytical tools are also integrated. All necessary information is stored in the database including voucher, specimen, collection and identification info and experimental data as trace files and specimen images. Various options exist in BOLD for generating genetic distance estimates and neighbour-joining phenograms from barcode sequences. Currently around 5 million specimen barcodes for 250.000 species are present in this database.

FISH-BOL (The Fish Barcode of Life Initiative) functions as a portal to BOLD and dedicated to coordinating an assembly of a standardized reference sequence library for all fish species derived from voucher specimens with authoritative taxonomic identifications. It is creating a valuable public resource in the form of an electronic database that contains DNA barcodes, images and geospatial co-ordinates for the analyzed specimens. This information is initially organized and analyzed using the BOLD. The information is then delivered via a data feed to the FISH-BOL website to monitor progress in barcode species coverage, which uses a taxonomic authority file derived from FishBase (http://www.fishbase.org), the Catalog of Fishes (CoF; Eschmeyer, 2003) and the Integrated Taxonomic Information System (ITIS, see http:// www.itis.gov). In this respect, FISH-BOL complements and enhances existing genomics and fisheries databases (Ward et al., 2009). FISH-BOL also promises to be a powerful tool for increasing the knowledge and information on the natural history and ecological interactions of fish species (Ward et al., 2009).

iBOL Working Group has several other barcoding campaigns including Marine Barcode of Life (MarBOL), The Mammal Barcode of Life, The Lepidoptera Barcode of Life, All Birds Barcoding Initiative (ABBI) and Formicidae Barcode of Life.

1.4 DNA Barcoding Fishes

Currently fishes represent the most comprehensively sampled and studied group of marine metazoans. However, there are still problems on identifying fish species during various developmental stages or in the absence of intact specimens when primarily relying on morphology. DNA barcoding can identify fish species from fillets, fins, fragments, larvae, and eggs. Examples include identification of northern Australian sharks from fins (Holmes et al., 2009); and Great Barrier Reef and Caribbean fishes from larvae (Pegg et al., 2006).

Another problem for morphological identification is its complex structure which can be a challenge even for the expert taxonomist as it requires accessing existing literature and assessing the validity and priority of various taxon names through global fish fauna. With the development of DNA barcode libraries starting from the first fish barcode study (Ward et al., 2005) now most species are predominantly separable by their barcodes and some have subsequently been named. Results indicate that barcodes seperate 98% and 93% of already described marine and freshwater fishes, respectively (Ward et al., 2009). An intergrated approach to taxonomy using DNA barcoding have proved its power among fishes. The newly named fish species by using DNA barcoding include a goby (Victor, 2008), Antarctic ray *Bathyraja* (P.J. Smith et al., 2008), handfish *Brachionichthys australis* (Last et al., 2007), and five new species of damselfish *Chromis* (Pyle et al., 2008). Considering the current species discovery rate using DNA barcoding, we can expect up to 600 overlooked or cryptic fish species awaiting discovery through similar studies (Bucklin et al., 2011).

As stated by iBOL Working Group: "Access to DNA sequences derived from expert-identified voucher specimens can be used to better characterize and broadly identify species". In the presence of vouchers and photographs, suspect identifications can be re-examined by a taxonomic expert. When both are inadequate for identification, accumulated evidence from multiple specimens might lead to a tentative resolution. On the other hand, generating a critical mass of BARCODE compliant specimen records and the development of an error-free searchable database remain critically important issues for FISH-BOL to tackle (Ward et al., 2009).

1.5 DNA Barcoding Invertebrates

Invertebrates comprise approximately 34 phyla which encompass almost all the animal diversity. So the challenge of barcoding invertebrates is actually the challenge of barcoding all animal species. Also depending on the insufficient characterization of many clades and incomplete genetic data, resolving the taxonomy of invertebrates is difficult. The resolution provided by the COI region differs among taxa, and relevant information and previous studies on different groups of invertebrate species analyzed in this study are listed below:

Mollusks: Problems associated with limited sampling in delineating closely related gastropod species have been reported by Meyer and Paulay (2005). Although the barcode data is sparse, Puillandre et al. (2009) have successfully identified gastropod larvae with DNA barcoding. Johnson et al. (2008) found numerous cryptic species of deep-sea limpets at hydrothermal vents. Limpets of the southeast Africa, on the other hand, lacked barcode

differences indicating morphotypes of a single species (Teske et al., 2007). Similarly, two clams of the *Donax* genus were found to represent one species (Carstensen et al., 2009). Other mollusks studied by DNA barcoding include chitons (Kelly et al., 2007), bivalves (Feng et al., 2010), and cephalopods (Allcock et al., 2010).

Amphipods: Problems between molecular phylogeny and morphological classification, indicating cryptic speciation or monophyly have been revealed, based on COI barcode region for *Gammarus* genus, and Antarctic and northwest Atlantic amphipods (Hou et al. 2007; Costa et al. 2009)

Annelids: Majority of marine annelids belong to the class Polychaeta and the group is diverse with many thousands of more species awaiting discovery and description. DNA barcode studies revealed closely related sympatric species of the genera *Arenicola* and *Tubificoides* in northeast Atlantic and Scandinavia (Luttikhuizen and Dekker, 2010; Ers´eus and Kvist, 2007). Also cryptic species have been found within several annelid species (Barroso et al., 2010; Rice et al., 2008).

Nematodes: Depending on the low abundance and lack of taxonomic expertise, morphological identification of nematodes is often not possible. Marine sediments are estimated to contain a huge diversity of nematode species. Recent studies with COI region revealed cryptic diversity for *Thoracostoma trachygaster* and genetic differentiation between Atlantic and Mediterranean *Monocelis lineata* species (Derycke et al., 2010; Sanna et al., 2009)

Decapods: Identification of decapods in larval and juvenile forms is very difficult. However, species of crabs *Uca* and *Clibanarius* from Indian Ocean and Japan, respectively, can be successfully discriminated by their barcodes (Shih et al., 2009; Hirose et al., 2010). Cryptic species have been found for *Perisesarma guttatum* of eastern coast of Africa by phylogeographic analysis using COI region (Silva et al., 2010). Diagnostic morphological characters have been revised for larvae of *Cancer* crabs of southeastern Pacific Ocean, presenting a good example to the integrative approaches to taxonomy (Pardo et al., 2009).

1.6 DNA Barcoding for Biodiversity Assessment

1.6.1 Biodiversity, phylogenetics and DNA barcoding

The information gathered from DNA barcoding is not sufficient to address extensive population level questions, however it can provide an early insight into the genomic structure and diversity. COI region is a poor target for population genetics studies, as it is uniparentally inherited and more sensitive to single-locus biases which may not truly reflect

population histories. Hence, it has been widely suggested to use multiple loci from different compartments (i.e. mitochondria, chloroplast and nucleus) to overcome this problem and enhance the resolution in different taxonomic ranks. Also, incongruence between different markers is a well recognized problem for multi-locus approaches and the advantages of combining data are still debatable (Giribet, 2002). On the other hand, increasing the number of taxa is recognized to be highly valuable as it aids in recovering the correct phylogeny by reducing the branch lengths and homoplasy (Hajibabaei et al., 2007). As barcoding studies generally target a large number of species, DNA barcoding can be a very powerful tool to conduct phylogenetic studies. There are several examples of studies on DNA barcoding which help to resolve the relationships among closely related taxa (i.e. cryptic species) and nodes between major groups of animals (Fukami et al. 2004; Browne et al. 2007).

Phylogenetics is a critical component of biodiversity which can be measured by various tools as morphology, ecology, adaptive differences and genetic data. However these tools can result in very different assessments of biodiversity, so they should be combined for having a complete perspective of the ecosystem or community. DNA barcoding is highly useful in this sense which may help ecologists and conservation and evolutionary biologists in understanding the biological diversity as well as ecological and evolutionary mechanisms that promote and maintain species diversity. However when using DNA barcoding as a biodiversity assessment tool, taxonomic identification should be done separately and compared to genetic estimates a posteriori (Collins and Cruickshank, 2013).

1.6.2 Phylogenetic and statistical classification methods for DNA Barcoding

The routine data analysis procedure of DNA barcoding basically involves matching the query data with unknown taxonomic status to a preconstructed reference dataset from the same group of organisms belonging to described species. In this context, barcoding methods can be divided into four categories as stated by Austerlitz et al. (2009):

(i) similarity methods based on the match between the query sequence and the reference sequences (alignment algorithms);
(ii) classical phylogenetic approaches like neighbour-joining (NJ) or maximum likelihood (ML) / Bayesian algorithms;
(iii) k-nearest neighbor based on the K2P distance (k - NN) and statistical approaches based on classification algorithms with no underlying biological models;
(iv) genealogical methods based on coalescent theory using maximum likelihood / Bayesian algorithms based on Monte Carlo Markov Chains (MCMC).

Researchers found out that the success rates of these several methods for a simulated dataset were very close. However this was not true when the genetic diversity was high. In that case, distance methods (NJ, k-NN) performed better, except for large sample sizes. (Austerlitz et al., 2009). Also, despite the popularity of NJ trees, it has been suggested that tree-free techniques should be used which really improves the identification success. So the quality of the DNA barcoding analysis can be influenced by choosing the method or methods best-adapted to the configuration of the sample.

BOLD combines similarity methods with distance tree construction. It uses a global alignment system through a Hidden Markov Model (HMM) profile of COI protein and linearly searches the reference library. After selecting the top 100 hits, it constructs a NJ tree based on K2P distances in order to assess the relationship of the query and closest reference sequences. BOLD is an extensive database which should allow reliable analysis of small datasets as well. So by combining different statistical methods it can access the best information provided by the data and prevent methodological problems as mentioned above.

Apart from specimen identification and species discovery purposes, DNA barcoding can be a really powerful tool for biodiversity assessment. For this purpose, single linkage clustering algorithms have been widely used for Operational Taxonomic Units (OTU) recognition by DNA barcode data (Blaxter et al., 2005; Jones et al., 2011; Puillandre et al., 2012; Hao et al., 2011; Pons et al.,2006). In a study by Ratnasingham and Hebert (2013), five single linkage clustering algorithms had been evaluated for their performance and effectiveness in recovering species boundaries. Based on its speed and taxonomic performance, RESL was adopted as the algorithmic approach of BOLD Barcode Index Number System (Ratnasingham and Hebert, 2013), which is defined as:

"… an online framework that clusters barcode sequences algorithmically, generating a web page for each cluster. Since clusters show high concordance with species, this system can be used to verify species identifications as well as document diversity when taxonomic information is lacking. This system consists of three parts:

1) A clustering algorithm employing graph theoretic methods to generate operational taxonomic units (OTUs) and putative species from sequence data without prior taxonomic information.

2) A curated registry of barcode clusters integrated with an online database of specimen and taxonomic data with support for community annotations.

3) An annotation framework that allows researchers to review and critique the taxonomic identifications associated with each BIN and notify data owners of errors."

There is criticism on DNA barcoding depending on the incompatibility of distance based methods and the diagnostic, character-based techniques used by traditional taxonomists. Although, some statistically sophisticated methods of species identification have been developed recently (Matz and Nielsen, 2005; Ross et al., 2008), most are of limited use for DNA barcoding depending on various limitations. Bayesian method currently implemented (Nielsen and Matz, 2006), for example, can not handle more than two species at one time, which is a fatal limitation for its use in DNA barcoding. Alternative approaches to these distance-based methods are character-based approaches. Character-based methods basically identify classification rules depending on the existing taxonomic status and then classify the unknown data (De Salle et al., 2005; Sarkar et al., 2008). Characteristic Attributes Organization System (CAOS) is an algorithm developed for this procedure, however has not been tested on large datasets as BOLD or GenBank. Another character-based approach developed by Bertolazzi et al. (2009), uses a logic mining method based on two optimization models.

1.7 Shortcomings and Limitations of DNA Barcoding

There is a list of shortcomings and limitations of the DNA barcoding analysis in terms of its experimental and methodological design. As mentioned above in Section 1.6.2, the fundamental criticism on the methodological component is the implementation of distance based methods which are incompatible with the classical taxonomic methods relying on morphology. It has been widely recognized that inferring a phylogeny from a single locus DNA sequence would be too naive. Hence majority of the most recent discussions present DNA barcoding as a fast and easy method for initial examination of the specimens.

Shortcomings of the experimental design can be listed as:

- Archived samples which are mostly identified by expert taxonomists and preserved in museums are a perfect source for DNA barcode libraries. Most of these samples are preserved in formalin, however recovering DNA from formalin preserved tissues is difficult as it requires extra procedures. Nevertheless, barcodes for fish (Zhang, 2010) and zooplankton (Kirby and Lindley, 2005) species have been obtained from formalin-preserved tissues.
- The specimens uploaded to databases influence the intra- and interspecific variations and as more individuals accumulate for a given species, the intraspecific

variation will decrease. Thus, the well sampled genera have lower rates of divergence than less extensively studied genera. This difference will become less pronounced as more genera are sampled (Ward et al., 2009).

The methodological constraints include:

- Divergence rates of COI in some taxa may not be sufficient to resolve all phylogeny as revealed for the Anthozoa (i.e. corals and sea anemons) and Porifera (sponges) living on coral reefs (Neigel et al. 2007) and for Cnidaria (Shearer and Coffroth 2008). Species level diagnosis is not possible in this case and hybrids can not be distinguished from maternal species by DNA barcoding. A similar problem occurs when studying on recently diverged species, where the polymorphic sites are mostly ancestral and time is required for those sites to be fixed and distinguished within marker of choice (Austerlitz et al., 2009).
- As mitochondria is a maternally inherited organelle, specimens may be wrongly diagnosed as its maternal species in cases of hybridization and introgression, which are rare outside the groups mentioned above (Anthozoa, Porifera, Cnidaria). In such cases an accurate diagnosis based on the taxonomic status is vital. As an example, there are reported cases of mitochondrial introgression on tuna species, so markers from other compartments (nuclear or ribosomal) should be used for this genus (Chow et al., 2006).
- It has also been suggested that nuclear mitochondrial pseudogenes (NUMTS) may provoke misidentifications as the substitution rates are expected to be lower for nuclear pseudogenes (Thalmann et al., 2004; Sword et al., 2007). However most NUMT's are less than 200 bp (Richly and Leister, 2004) and unlikely to be amplified by barcoding primers.
- Heteroplasmy resulting from somatic mutation (Moum and Bakke, 2001) or cross-species transfer (Barbara et al., 2007) may also cause problems because the rate of divergence will be different than the one inferred by phylogeny.
- COI region is recognized as a selectively neutral marker, however it's diversity is found to be also related with selection (Meiklejohn et al., 2007). For example, it has been found by Foltz et al. (2004) that breeding related differences may influence the COI sequence diversity. Also, selective sweeps (reduction or elimination of sequence variation as the result of recent and strong positive natural selection) are found to be evident for mtDNA (Galtier et al., 2009), so the divergence rates may reflect primarily the time elapsed since the last selective sweep. On the other hand, selective sweeps increase the intraspecific variation and making barcoding gap more pronounced which in turn increases the chance of recovering correct phylogeny (Bucklin et al., 2011).

- Barcoding gap is a measure of the difference between intra- and interspecific variation and shows the power of the marker in choice in distinguishing species. However if a certain threshold value is applied, the specimen differing from existing sequences by more than this value will be assumed to represent a new taxon. This method is vulnerable to both false positives (misidentification depending on presence of species with low intraspecific variation) and false negatives (misidentication depending on presence of species with high interspecific variation). Hence, DNA barcoding for species discovery is a highly debatable issue and it is evident that there is no a priori reason to assume that such a threshold is applicable to all animals, as various factors are effective in coalescent depths including population size and mutation rate (Monaghan et al. 2009; Fujita et al. 2012). As expected, this gap is demonstrated to be evident in a variety of studies (Ward et al., 2005; Hajibabaei et al., 2006; Kerr et al., 2007), however young species or certain taxa may lack a distinctive barcode gap (Ferguson, 2002; Meyer and Paulay 2005; Whitworth et al., 2007).
- Incongruence of COI with other genes is also a highly reported case. A study based on whole mitochondrial genomes of neogastropods, for example, revealed that COI shows the lowest phylogenetic performance (Cunha et al., 2009). In such a case, combining COI with another marker may improve the results as Nichols (2005) demonstrated by using 28S rRNA and COI which yielded highly supported nodes within the sponge class Demospongia. On the other hand, there are also reported cases of COI congruence with other gene regions including 18S and 28S rRNA as for the isopod family Munnopsidae (Osborn, 2009) and deep-sea mysid genus *Pseudomma* (Meland and Willassen, 2004).

1.8 Advantages and Applications of DNA Barcoding

Incorrect identifications are also a problem for traditional taxonomy as a fundamental complexity arises considering different life stages of organisms or in the absence of intact organisms. The ability of barcoding to identify the specimen from whole or part organism and egg or larvea has important implications for various fields of study.

- Identification of an organism from its egg has critical importance for understanding dispersal patterns, spawning and nursery grounds or geographic distributions specifically for marine taxa (Costa and Carvalho, 2007). Those topics are all critical in the context of environmental change caused by global warming. Previous studies demonstrated that over 60% of fish eggs are phenotypically misidentified and specifically for Irish fish stocks this mistake caused an inflation of stock assessments for other fishes (Fox et al., 2005)

- Barcoding is frequently used in studies on exotic species, especially for fishes. It supports the monitoring of exotic species in environmental samples, assessing the invasion potential, localizing the sources of introduction, distinguishing between single and multiple introductions, assessing propagule pressure and recognizing multiple species in complex samples (Bariche et al., 2015).
- Identification of prey-remains from stomach contents is a valuable application for DNA barcoding. This information can be highly beneficial for understanding the trophic chain relationships which may contribute in fisheries management and conservation studies.
- DNA barcoding is specifically proposed as a first step in periodical monitoring of exploited fish stocks in fisheries (Collins and Cruickshank, 2013). This can be useful in identifying the changes in population genetic structure and if needed, further studies as population genomic approaches and other biological tools may be employed for a deeper investigation.
- Food security is another topic where barcoding techniques can be usefully implemented, as even processed food products can be used. Species authenticity along the commercial chain is critical for consumer's security and health (Costa and Carvalho, 2007).

2 AIM OF THIS STUDY

Mitochondrial cytochrome oxidase subunit I (COI) gene region was used to investigate fish, invertebrate and ascidian specimens collected from Mersin Bay. The primary goal of the study is to assess fish and invertebrate biodiversity of Mersin Bay, including invasive and native species, by using DNA barcoding method coupled with morphological identifications. OTU-based approaches were implemented to get biodiversity estimates from the barcode sequences. Another aim of the study is to provide DNA barcode inventory of Mersin Bay and all successful DNA barcodes were uploaded to BOLD database. The efficiency of different primers were evaluated for various taxa sampled in the study. Also, the performance and effectiveness of DNA Barcoding as a method for specimen identification will be evaluated by using the results provided by BOLD tools.

3 MATERIALS and METHODS

3.1 Sample Collection and Study Area

Sampling of fishes and invertebrates was performed during the trawl surveys of the Institute of Marine Sciences of the Middle East Technical University (IMS-METU) in Mersin Bay located in the Levantine basin of the eastern Mediterranean (Figure 3). All samples were

identified based on morphometric and meristic characteristics, photographed and preserved in 70% ethanol. Sample ID's, collection dates, coordinates and depths of trawl surveys are given in Table 1. Trawl surveys were conducted between 50-250 meters to cover the entire bathymetric extent of the continental shelf. In all trawl surveys, the same design of trawl net (locally called Ottoman) was used and the duration of the survey was 30 min.

Table 1 Sample collection information

Sample ID	Date	Latitude (DD)	Longitude (DD)	Depth (m)
IMSA1-IMSA114	22-May-2014	36.532	34.417	100-250
IMSB1-IMSB42	07-Aug-2014	36.492	34.509	100-200
IMSC1-IMSC80	07-Aug-2014	36.492	34.509	100-200
IMSMb1-IMSMb71	07-May-2015	36.595	34.594	150
IMSUm1-IMSUm2	03-Jun-2015	36.492	34.509	200
IMSH1-IMSH4	07-Aug-2014	36.573	34.427	100
IMSO1-IMSO6	07-May-2015	36.595	34.594	150
IMSUp1-IMSUp5	16-May-2015	36.593	34.412	75

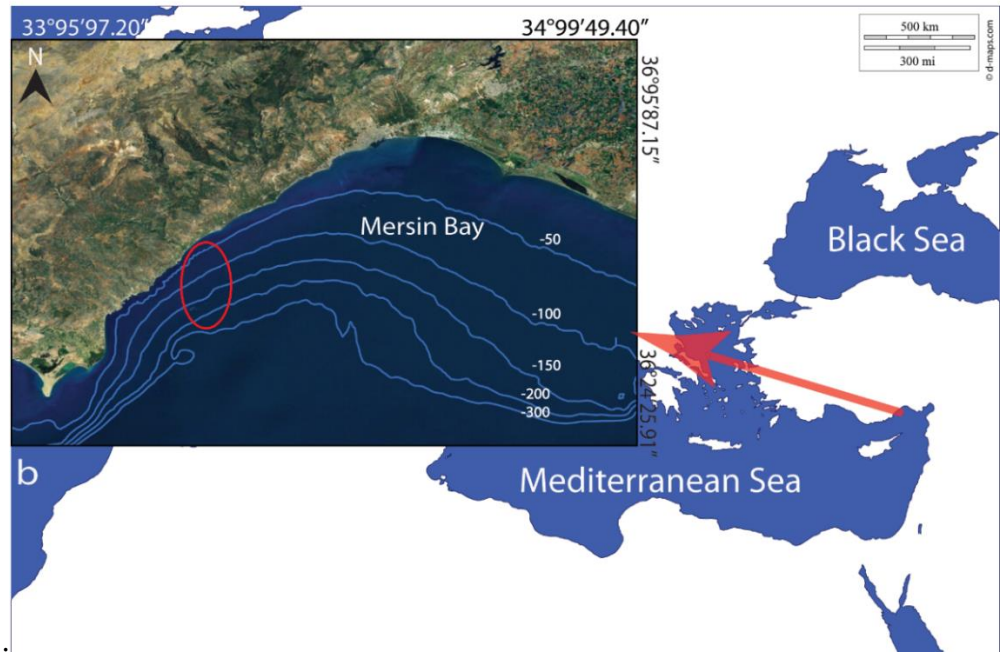

Figure 3 Sampling region

3.2 Morphological Identification of Specimens

All fish specimens were identified by morphological examination according to Whitehead et al. (1985) and Wrongtana et al (1995) by Dr. Yeşim Ak Örek, Dr. Serdar Sakınan and Dr. Meltem Ok. (METU, Institute of Marine Sciences). Morphological identification of invertebrates was not possible due to restrictions on time and availability of taxonomic experts for the diverse group of taxa sampled in this study. Thus, for invertebrate specimens BOLD identifications with higher than 98% coverage were used and OTU (Operational Taxonomic Units) based approaches were followed to assess the number of taxonomic clusters based on barcode data. These methods allow quantification of ecological features such as richness, diversity, and similarity which is also in consistence with the aim of this study.

3.3 DNA Isolation and PCR Amplification

3.3.1 Fishes

Genomic DNA was extracted from 50-100 mg muscle tissue using the CTAB protocol (Stewart and Via, 1993) from all specimens. DNA samples were diluted 2:100 in sterile double distilled water (DDW) and kept at 4°C.

The cytochrome oxidase subunit I (COI) gene (~650 bp) was amplified following Ward et al. (2005) by using primers Fish F1 and Fish R1 (Table 2) . The samples which can not be amplified by this primer pair were amplified with a different primer pair (Fish F2 and Fish R2). Primers used for each fish species are given in Appendix A. Following an activation step of 2 min at 95˚C for the enzyme, the PCR mixture underwent 38 cycles of 30 s at 94˚C, 30 s at 54˚C and 1 min at 72˚C, and a final incubation step of 72˚C for 10 min on a T-100 Thermal Cycler (Bio-Rad). Average DNA concentrations were measured by Inovia MSP-100 Microspectrophotometer and values ranged between 100-1500 ng/ml. Second wash or ethanol precipitation was performed for DNA purification when necessary. The PCR outcomes were screened on 1.3% agarose gel to check the quality of PCR products (Appendix C for sample photo). Sequencing processes were performed by Macrogen Inc. (The Netherlands) for both directions.

3.3.2 Invertebrates and ascidians

Genomic DNA was extracted from 100 mg muscle tissue using the CTAB protocol (Stewart and Via, 1993) from all specimens. DNA samples were diluted 2:100 in sterile double distilled water (DDW) and kept at 4°C.

The cytochrome oxidase I (COI) gene (~650 bp) was amplified following Folmer et al. (1994) by using universal invertebrate primers (HC02198 and LCO1490) (Table 2). Following an activation step of 5 min at 95˚C for the enzyme, the PCR mixture underwent 35 cycles of 1 min at 95˚C, 1 min at 45˚C and 1,5 min at 72˚C, and a final incubation step of 72˚C for 10 min on a T-100 Thermal Cycler (Bio-Rad).

For echinoderms, the cytochrome oxidase I (COI) gene (~600 bp) was amplified following Heimeier et al. (2010) by using primer pair Echino COI-F and Echino COI-R (Table 2). Following an activation step of 3 min at 94˚C for the enzyme, the PCR mixture underwent 35 cycles of 30 sec at 94˚C, 1 min at 52C and 1 min at 72˚C, and a final incubation step of 72˚C for 3 min on a T-100 Thermal Cycler (Bio-Rad).

Primers used for each invertebrate taxon are given in Appendix B. Average DNA concentrations were measured by Inovia MSP-100 Microspectrophotometer and values ranged between 100-1000 ng/ml. Second wash or ethanol precipitation was performed for DNA purification when necessary. PCR conditions were changed for the samples that can not be amplified by the given parameters. The PCR outcomes were screened on 1.3% agarose gel to check the quality of PCR products (Appendix C for sample photo). Sequencing processes were performed by Macrogen Inc. (The Netherlands) for both directions.

3.3.3 Primers

All primers used in the study are given in Table 2.

Table 2 Names and sequences of COI primers for each marker region and group

Taxa	Primer Name	Sequence (5'-3')
Fishes	FishF1 (1)	TCAACCAACCACAAAGACATTGGCAC
	FishR1 (1)	TAGACTTCTGGGTGGCCAAAGAATCA
	FishF2 (1)	TCGACTAATCATAAAGATATCGGCAC
	FishR2 (1)	CTTCAGGGTGACCGAAGAATCAGAA
Invertebrates and Ascidians	LCO1490 (2)	GGTCAACAAATCATAAAGATATTGG
	HC02198 (2)	TAAACTTCAGGGTGACCAAAAAATCA
Echinoderms	Echino COI-F (3)	TTTCYACYAAACACAAGGAYATTGG
	Echino COI-R (3)	TAAACTTCHGGRTGDCCAAARAATCA

(1) Ward et al., 2005; (2) Folmer et al., 1994; (3) Heimeier et al. 2010

3.4 Bioinformatic Tools

3.4.1 Sequencing, alignment and editing

During sequencing process, fluorescently labelled DNA fragments generated during dye terminator cycle sequencing migrate sequentially through a capillary according to their size. Different types of emitted light from the base at the end of each fragment is recorded and represented as a series of colored peaks in a trace file (chromatogram). The shape and resolution of the peak gives information about the quality of each read and by using related parameters, algorithms compute a quality score for each base call depending on the equation:

$Q = -10 \log10 P;$

where "P" represents the probability of an incorrect base call. This data is mostly stored in a separate ".phd" file, where the abbreviation refers to the first program doing this computation, called "PHRED". BOLD uses this quality scores when determining the consensus sequences by taking the base with higher quality score from forward or reverse traces:

"A quality score of 20 indicates that the probability of an incorrect base call is 1 in 1,000, whereas a quality score of 40 indicates that the probability of an incorrect base call is 1 in 10,000. Generally speaking, quality scores less than 20 are considered unacceptable and must be edited."

BOLD also assigns an average quality degree for each trace file as high, medium, low or failed.

"High quality trace files have a mean quality score >40, medium quality trace files have a mean quality score between 30 and 40, and low quality trace files have a mean quality score <30. Trace files with fewer than 10 base calls are designated as failed."

Global alignment algorithms are the most informative and reliable technique and BOLD uses an algorithm aligning the protein translation through profile to a Hidden Markov Model of the COI protein. Then the sequences are edited in "Online Sequence Editor" tool implemented by BOLD by taking the base with higher quality score form forward or reverse trace files for consensus determination.

3.4.2 BOLD Identification Engine

BOLD Identification Engine uses all sequences uploaded to BOLD database from public and private projects to locate the closest match of query records. For animal identification,

it accepts sequences from the 5' region of the mitochondrial cytochrome oxidase subunit I gene (COI). Searching the database by using only species level barcode records or by using all barcode records with interim taxonomy are optionally available. Former option is employed for fish dataset and latter option for invertebrate dataset to obtain the nearest placement to a taxon.

3.4.3 BOLD analyses

Consortium for the Barcode of Life (CBOL) specifies a list of standards for barcode compliance, including a minimum sequence length of 500 bp; less than 1% ambiguous bases; the presence of two trace files; a minimum of low trace quality status; and the presence of a country specification in the record. BOLD systems flags the uploaded records as barcode compliant based on these standards.

As the sequence data will be primarily used for barcoding purposes, the majority of the analysis was performed using the following BOLD v3 and v4(beta) tools:

- *Taxon ID Tree*: Allows for the generation of dendrograms (phylogenetic trees) from sequencing using the neighbor joining algorithm. The BOLD Neighbor Joining (NJ) taxon ID tree was created using K2P method and filtered against contaminants and stop codons.
- *Distance Summary Analysis*: Sequence divergence between barcode sequences at the conspecific and congeneric levels are given. The comparisons done at the level of species, genus and family are available in the results page and also contrasts the distribution of within species divergence to within genus divergence.
- *Barcode Gap Analysis*: The distribution of distances within each species and the distance to the nearest neighbor of each species are investigated to test for the presence of the Barcode Gap.
- *BIN Discordance Report*: Analyzes new COI sequences and assigns them to an existing or a new BIN (Barcode Index Number System) (exp. in Section 1.6.2).
- *Cluster Sequences Analysis*: Generate OTUs from identified and unidentified sequences using the REfined Single Linkage algorithm (RESL – Ratnasingham and Hebert, 2013). Sequences are assigned to OTUs independent of the BIN registry.

BOLD can handle Complete or Partial deletion options in ambiguous base or gap handling during alignment. Results of BOLD analyses will be covered for two different groups, namely as fishes and invertebrates for comparison with other results and evaluation of BOLD tools.

3.4.4 OTU-based approaches: Barcode Index Number System (BIN's) and RESL algorithm

BIN system clusters sequences using RESL algorithm as explained in Section 1.6.2. However this system performs clustering by using all sequences in the database. In BOLD Systems v4 (beta), another option for clustering selected records with RESL algorithm is available (Cluster Sequences Analysis) which will be used for comparison of the OTU-based results based on the current dataset and BOLD database.

4 RESULTS

4.1 Morphological Identifications

4.1.1 Fishes

In total, there were 112 fish specimens from 38 species based on morphological identifications (Table 3). Mean number of specimens per species is 3, and there was a single species with more than 10 specimens in the fish dataset (*Mullus barbatus*). If we exclude them from the calculations, the mean number decreases to 2.54 specimens per species, which better represents this dataset as there were 12 species (31.6%) with single specimens and more than half of the species had only one or two specimens.

Table 3 Number of specimens for each fish species based on morphological identifications

Species	**Order**	**Family**	**Number of specimens**
Argentina sphyraena	Argentiniformes	Argentinidae	2
Arnoglossus laterna	Pleuronectiformes	Bothidae	3
Boobs boobs	Perciformes	Sparidae	4
Bothus podas	Pleuronectiformes	Bothidae	1
Callionymus filamentosus	Perciformes	Callionymidae	4
Capros aper	Perciformes	Caproidae	4
Chlorophtalmus agassizi	Aulopiformes	Chlorophthalmidae	5
Citharus linguatula	Pleuronectiformes	Citharidae	3
Conger conger	Anguilliformes	Congridae	2
Cynoglossus sinusarabici	Pleuronectiformes	Cynoglossidae	4
Dasyatis sp.	Rajiformes	Dasyatidae	1
Dussumieria elopsoides	Clupeiformes	Clupeidae	1
Encrasicholina punctifer	Clupeiformes	Engraulidae	2
Engraulis encrasicolus	Clupeiformes	Engraulidae	2
Equulites klunzingeri	Perciformes	Leiognathidae	6

Table 3 (continued)

Helicolenus dactylopterus	Scorpaeniformes	Sebastidae	2
Hippocampus hippocampus	Syngnathiformes	Syngnathidae	1
Lagocephalus spadiceus	Tetraodontiformes	Tetraodontidae	1
Lagocephalus suezensis	Tetraodontiformes	Tetraodontidae	1
Lepidopus caudatus	Perciformes	Trichiuridae	2
Leptidotrigla cavillone	Scorpaeniformes	Triglidae	5
Lesueurigobius friesii	Perciformes	Gobiidae	3
Mullus barbatus	Perciformes	Mullidae	18
Mullus surmuletus	Perciformes	Mullidae	2
Nemipterus randalli	Perciformes	Nemipteridae	2
Ostorhinchus fasciatus	Perciformes	Apogonidae	5
Oxyurichthys papuensis	Perciformes	Gobiidae	1
Sargocentron rubrum	Beryciformes	Holocentridae	1
Scorpaena notata	Scorpaeniformes	Scorpaenidae	2
Scorpaena scrofa	Scorpaeniformes	Scorpaenidae	1
Serranus cabrilla	Perciformes	Serranidae	2
Siganus luridus	Perciformes	Siganidae	1
Trachurus mediterraneus	Perciformes	Carangidae	3
Trachurus trachurus	Perciformes	Carangidae	1
Trigloporus lastoviza	Scorpaeniformes	Triglidae	1
Upeneus moluccensis	Perciformes	Mullidae	6
Upeneus pori	Perciformes	Mullidae	5
Zeus faber	Zeiformes	Zeidae	2
Total			112

Perciformes was the most abundant fish order and represented with 17 species, while Scorpaeniformes was the second and represented with 5 different species. In total, there were 14 Lessepsian fish species out of 38 based on morphological identifications.

4.1.2 Invertebrates and ascidians

Morphological identification of invertebrates requires detailed examination of the samples. Depending on the limitations on time and availability of experts, all samples were grouped into higher taxonomic ranks. In total, there were 117 invertebrate specimens based on initial morphological examination grouped in phylum and class levels as given in Figure 4 and Figure 5.

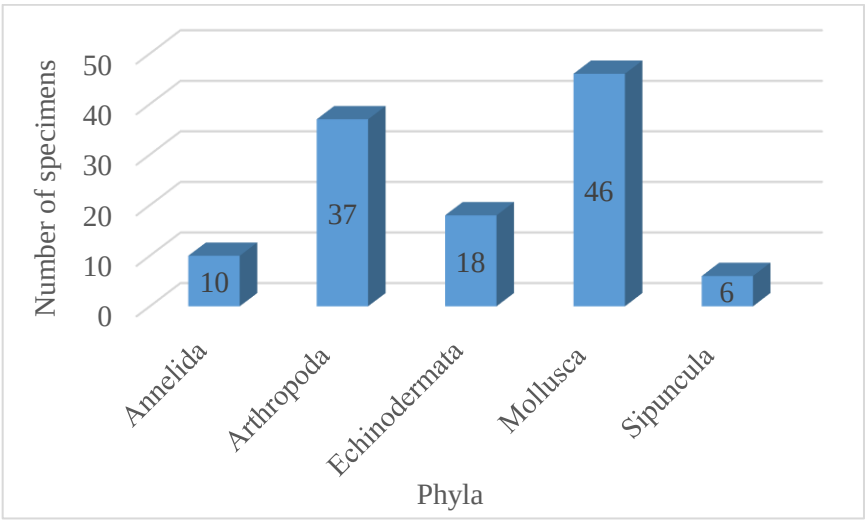

Figure 4 Number of invertebrate specimens for each phylum based on morphological identifications.

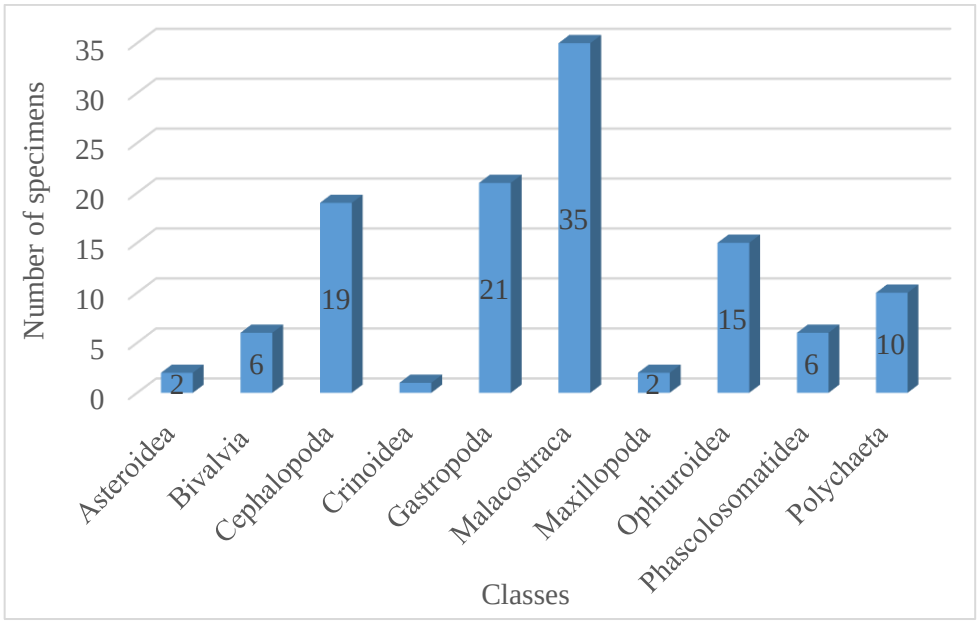

Figure 5 Number of invertebrate specimens for each class based on morphological identifications.

Mollusca was the most abundant group with 39.3% of total invertebrate catch and majority of the group was from Cephalopoda and Gastropoda classes. Arthropoda was the second

most abundat phylum (31.6%) and almost all individuals were from Malacostraca order. Echinoderms, on the other hand, were represented with 3 different classes where Ophiuroidea was the most abundant one.

In addition to fish and invertebrates, there were four ascidian (Tunicata, Chordata) samples in the dataset, making a total of 233 specimens in the study.

4.2 Amplification Success

PCR products were checked by UV screening on 1.3% agarose gel (Appendix C for sample photos) and labelled as high-quality or low-quality. In addition to the products with low signal, other problematic samples (with smears or non-specific products) were also labelled as low-quality in gel electrophoresis step, if the amount of the product corresponding to the target fragment length is considered sufficient for sequencing. 8

Out of 112 fish DNA samples analyzed, 97 (86.6%) were amplified by using primers FishF1 and FishR1, while 13 (11.6%) samples, which can not be amplified by this primer pair, were amplified with primers FishF2 and FishR2. Two DNA samples (1.8%) could not be amplified with neither primer pair and those samples were all *Mullus surmuletus* specimens based on morphological identification. Thus, this species was excluded from the further analysis. PCR amplifications of 100 (89.3%) DNA samples provided high-quality products, while 10 (8.9%) samples provided low-quality products. In total, 110 (98.2%) fish DNA samples were sent to sequencing.

Eighteen echinoderm DNA samples were amplified using primer pair Echino COI-F and Echino COI-R, because they could not be amplified with universal invertebrate primers. PCR amplifications of seven (38.9%) DNA samples provided high-quality PCR products, four (22.2%) samples provided low-quality PCR products and seven (38.9%) samples could not be amplified. There were only two specimens in the dataset belonging to Asteroida class and they could not be amplified with this primer pair. Thus, they were excluded from the further analysis. Remaining five samples with unsuccessful amplifications were from Ophiuroidea class samples. In total, 11 (61.1%) echinoderm DNA samples were sent to sequencing.

Remaining 99 invertebrate DNA samples were amplified with universal primer pair HC02198 and LCO1490. Out of those, 74 (74.8%) samples provided high-quality PCR products, 14 (14.1%) provided low-quality PCR products and 11 (11.1%) could not be amplified with this primer pair. Amplification success of the universal invertebrate primers

for each phylum and class based on initial morphological categorization are given in Figure 6 and Figure 7. In total 88 (88.9%) invertebrate DNA samples were sent to sequencing.

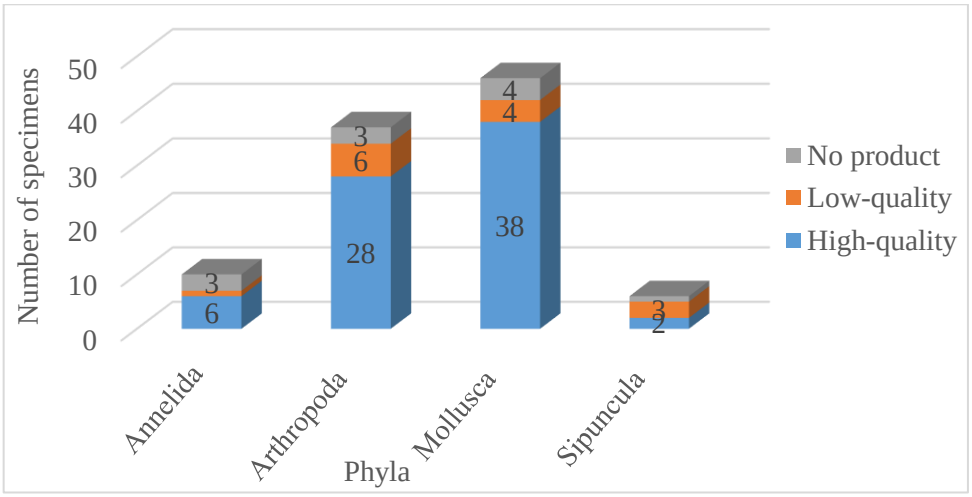

Figure 6 Amplification success of universal invertebrate primers (HC02198-LCO1490) for each phylum.

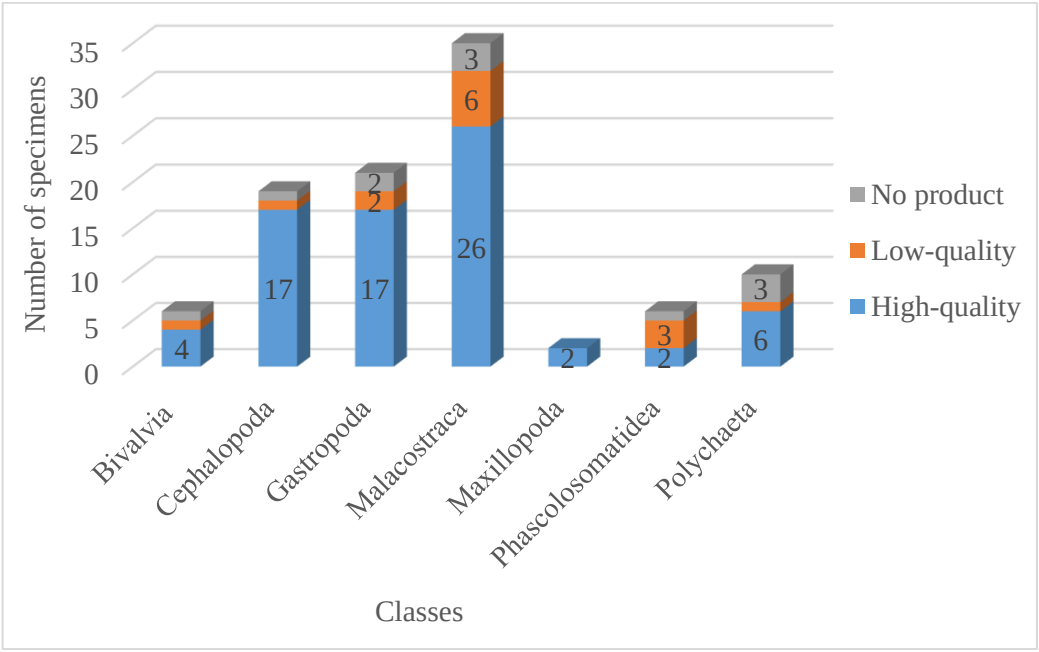

Figure 7 Amplification success of universal invertebrate primers (HC02198-LCO1490) for each class.

Universal invertebrate primers performed better for Mollusca, Arthropoda and Annelida phyla with 82.6%, 75.7% and 60.0% high quality products, respectively. However, they performed very poorly for Sipuncula phylum with only 33.3% high quality products. Annelida and Sipuncula phyla samples in the dataset were comprising Polychaeta and Phascolosomatidea classes, respectively. Arthropoda phylum comprised Maxillopoda and Malacostraca classes in the dataset. There were only two specimens for Maxillopoda class and both gave high-quality PCR products. Considering Mollusca phylum, universal invertebrate primers performed best for Cephalopoda class with 89.5% and worst for Bivalvia class with 66.7% high quality products, while Gastropoda class samples were amplified with 81.0% high quality PCR products.

Four ascidian samples were also amplified with universal invertebrate primers (HC02198 and LCO1490) and all provided high-quality products and sent to sequencing.

4.3 Sequencing Success

In total, 18 trace files belonging to 9 samples were in poor quality in the fish dataset. Those include the single sample for *Scorpaena notata* species. Thus, this species was excluded from the further analysis leaving 36 fish species together with the exclusion of *Mullus surmuletus* in earlier procedures (Section 4.2). In total there were 101 (91.8%) high-quality sequences out of 110 fish DNA samples which were sequenced.

For invertebrates, 28 trace files belonging to 14 samples were in poor quality leaving 74 (84.1%) out of 88 invertebrate samples. For echinoderms, two specimens were excluded leaving nine (81.8%) out of 11 echinoderm samples and all four ascidian PCR products gave high-quality sequences. For the further procedures, invertebrate dataset was expanded by addition of echinoderm and ascidian samples making a total of 87 samples.

4.4 Molecular Analyses

4.4.1 Fish dataset

4.4.1.1 Molecular identifications by BOLD ID Engine

In total, 101 sequences which were aligned and trimmed to 645 bp of length in Bioedit were searched with BOLD ID Engine by using species level barcode records (Section 3.4.2) for comparison with morphological identifications (Appendix D). Taxonomic information of top matches for all specimens were extracted, grouped in species level and compared with morphological identifications. There was a single conflicting identification for *Oxyurichthys papuensis* sample which was identified as *Oxyurichthys petersii* species by

BOLD ID Engine. The numbers are in consistence with a total of 36 fish species in the dataset. Photos and sequences belonging to 101 fish specimens were uploaded to BOLD database (Appendix E and Appendix J).

4.4.1.2 Barcode compliance results

Out of 101 fish sequences, one *Lesueurigobius friesii* (IMSC70) and one *Upeneus moluccensis* (IMSMs1) sequences were containing stop codons and excluded from the analysis leaving 99 fish samples in dataset belonging to 36 morphological species. 93 (93.9%) of remaining 99 sequences were flagged as barcode compliant. Four of the unflagged sequences had poor quality traces depending on BOLD assignment, and two other sequences was shorter than the minimum length required for barcode compliance (Section 3.4.3 for barcode compliance standards). These six problematic sequences were from *Callionymus filamentosus* (IMSC36), *Dasyatis pastinaca* (IMSC73), *Equulites klunzingeri* (IMSA111), *Lesueurogobius friesii* (IMSC72) and *Serranus cabrilla* (IMSC9 and IMSC19) species.

4.4.1.3 BIN Discordance Analysis

Out of 99 samples, four sequences could not be assigned to any BIN including one sample each of *Equulites klunzingeri* (IMSA111), *Chlorophthalmus agassizi* (IMSC37), *Dasyatis pastinaca* (IMSC73) and *Lesueurigobius friesii* (IMSC72) species. Those include the single samples in the dataset for *Lesueurigobius friesii* and *Dasyatis pastinaca* species, hence they were excluded from this analysis leaving 34 morphological species. Remaining 95 records were represented by 35 BIN's while two of them were labelled as singletons which indicates that these samples were assigned to BIN's which contain no other sequences. Those include one *Boops boops* (IMSA31) sequence and one *Callionymus filamentosus* (IMSC36) sequence. 19 of the remaining 33 BIN's were taxonomically discordant while 14 of them were concordant. Three of the discordant BIN's had family level conflicts, two of them had genus level conflicts and remaning 14 BIN's had species level conflicts (Appendix F).

Depending on the BIN analysis, 34 morphological fish species were represented by 33 BIN's excluding singletons. This inconsistency in numbers results from assignment of two *Trachurus* species to the same BIN. This BIN includes 13 different morphological species from this genus and within the cluster, barcoding gap is not pronounced well with an average distance of 1.58% and maximum distance of 5.41%. Further discussion on the molecular and morphological taxonomic status of the problematic *Trachurus* genus will be covered in Discussion (Section 5.65).

4.4.1.4 Distance Summary Analysis

Distance summary analysis is performed to investigate the sequence divergences between 99 sequences which were uploaded to database, excluding sequences containing stop codons. The analysis was performed by using Kimura 2 parameter (K2P) distance model with BOLD alignment algorithm (Amino Acid based HMM).

In the dataset, there were 19 species which were represented by more than single sample, three genera (*Lagocephalus, Trachurus, Upeneus*) which comprise more than single species and five families (Bothidae, Engraulidae, Gobiidae, Mullidae, Triglidae) which comprise more than single genus. Thus, below table and graph were constructed using remaining samples for calculating within species, genus and family distances, respectively.

Table 4 Within species, genus and family distances in percentages of divergence with the number of samples (n) and taxa included in the fish dataset.

Within	**n**	**Taxa**	**Comp.**	**Min Dist (%)**	**Mean Dist (%)**	**Max Dist (%)**	**SE Dist (%)**
Species	82	19	239	0	0.66	5.77	0
Genus	17	3	34	2.08	14.84	23.54	0.12
Family	42	5	197	12.06	18.17	25.87	0.01

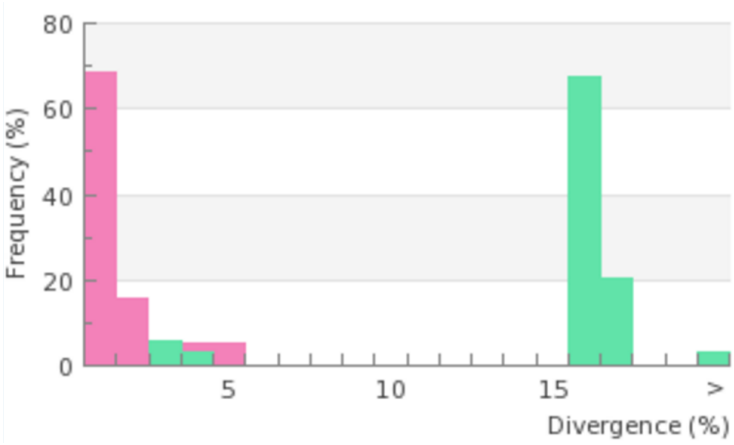

Figure 8 Normalized within species (pink) and within genus (green) distance frequencies against divergence in percentages for the fish dataset.

Maximum within species distance was for *Callionymus filamentosus* with a 5.77% divergence, and minimum within genus distance was between *Trachurus meditteraneus* and *Trachurus trachurus* species with a 2.08% divergence. Mean within species distance was 0.94% (S.E.:0.06) after normalization of within species distribution to reduce sampling bias.

4.4.1.5 Barcoding Gap Analysis

Barcoding Gap Analysis is performed to investigate the sequence divergences between 99 sequences which were uploaded to database, excluding sequences containing stop codons. The analysis was performed by using Kimura 2 parameter (K2P) distance model with BOLD alignment algorithm (Amino Acid based HMM).

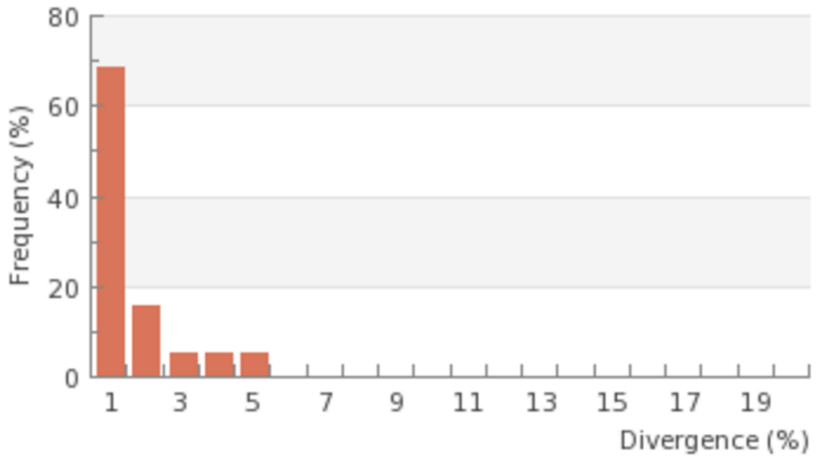

Figure 9 Histogram for mean intraspecific distances for the fish dataset.

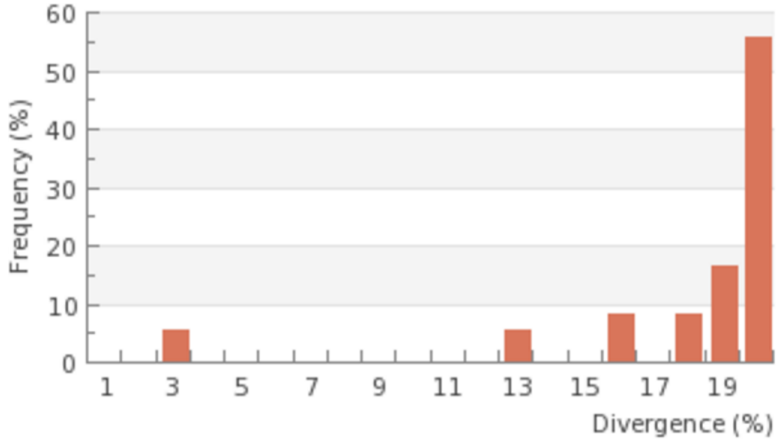

Figure 10 Histogram for distances to the nearest neighbors for the fish dataset.

Mean intraspecific distance was 0.94% (S.E.: 0.06) and the mean distance to the nearest neighbor was 18.61% (S.E: 0.14) with the given frequencies in Figure 9 and Figure 10. *Boops boops, Callionymus filamentosus* and *Serranus cabrilla* species had mean intraspecific distances higher than 2% divergence which corresponds to the highest values in Figure 9. One sample of each species were problematic either in BIN Discordance or Barcode Compliance results. On the other hand, *Trachurus mediterraneus* species distance to its nearest neighbor (2.08% to *Trachurus trachurus*), which corresponds to the smallest value in Figure 10, was less than the maximum intraspecific distance (2.87%) of these species.

4.4.1.6 Cluster Sequences Analysis

99 sequences belonging to 36 morphological fish species were separated into 39 OTU's by using RESL algorithm. In total there were 21 singleton OTU's, and four of them were containing sequences represented by other clusters. Those include one sample each of *Boops boops* (IMSA31), *Callionymus filamentosus* (IMSC36) and *Chlorophthalmus agassizi* (IMSC37) species. Also two *Serranus cabrilla* sequences were clustered in separate OTU's. Exclusion of these four problematic singleton clusters remains 35 OTU's in the dataset, while there are still 36 morphological species in the dataset. This inconsistency again results from clustering two different *Trachurus* species to the same OTU (OTU-3) similar with BIN Discordance Analysis. All other OTU's were containing sequences of single species in consistence with morphological identifications.

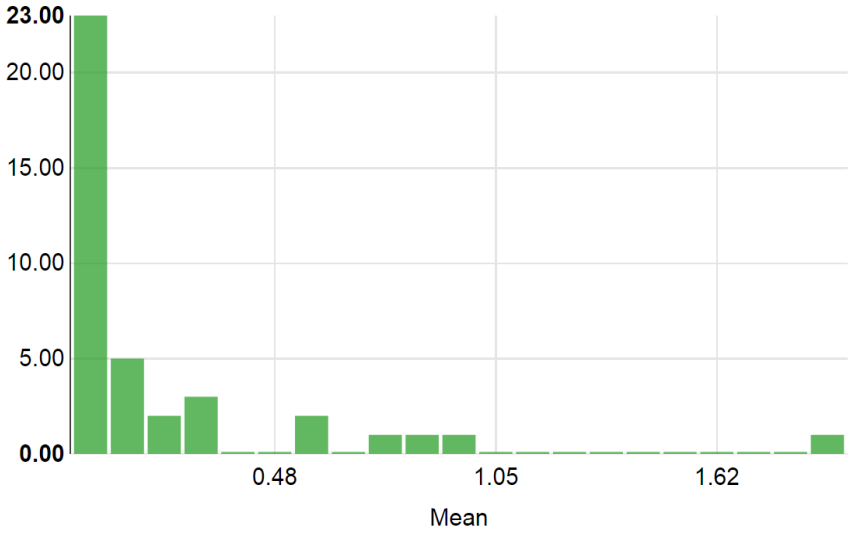

Figure 11 Histogram for mean within OTU distances for the fish dataset.

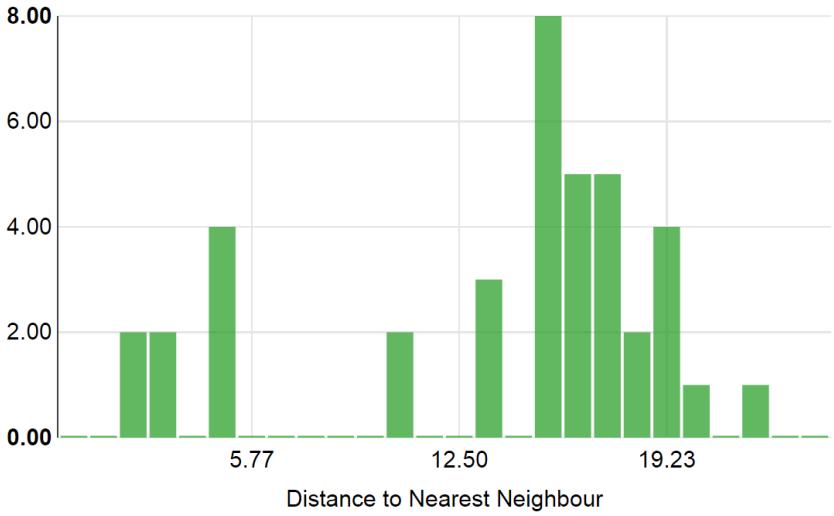

Figure 12 Histogram for distances to nearest neigbor OTU's for the fish dataset.

Highest within OTU distance in Figure 11 was for OTU-3 with a 1.91% divergence which contains two different *Trachurus* species. All other intraspecific distances were lower than 1% divergence. Distances to the nearest neighbours lower than 10% in Figure 12 were all corresponding to the singleton clusters mentioned above which contain sequences represented by other clusters based on morphological identifications.

4.4.1.7 Taxon ID Tree

Neighbor joining tree for 99 fish samples was constructed by using Kimura 2 parameter (K2P) distance model with BOLD alignment algorithm (Amino Acid based HMM).

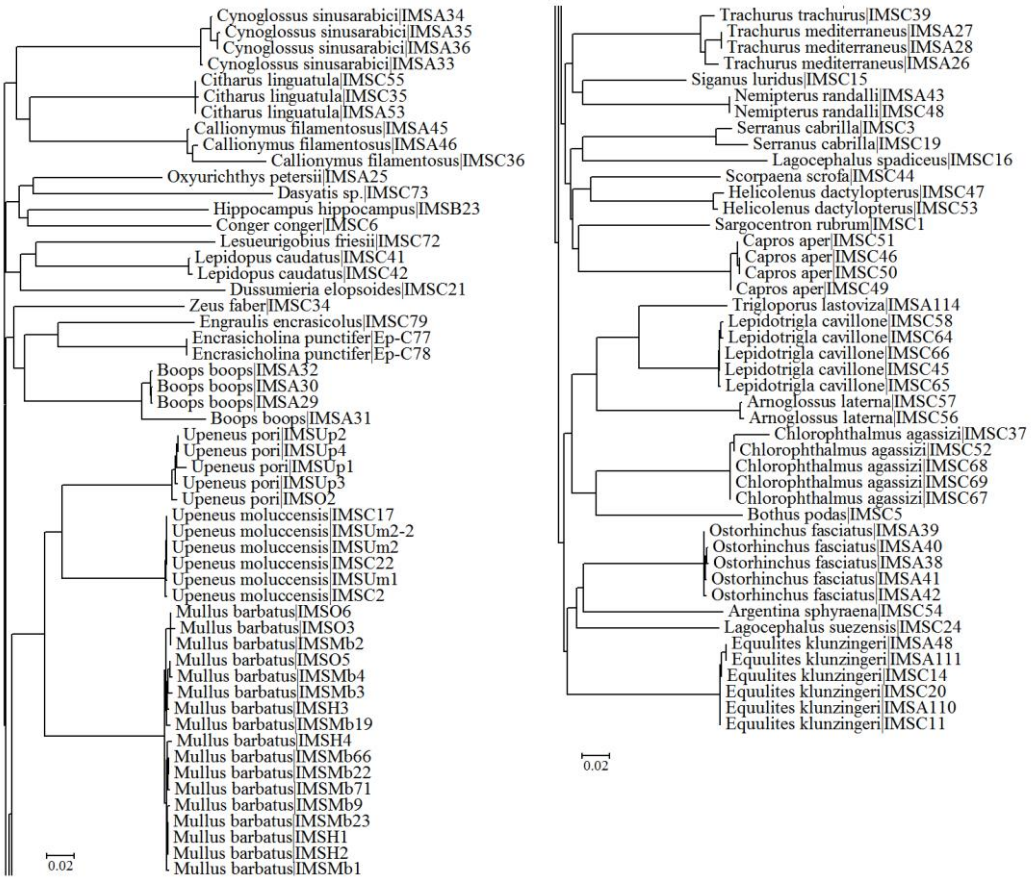

Figure 13 Neighbor joining tree including all haplotypes of 36 fish species from Mersin Bay given with sample ID's.

All species formed monophyletic branches in consistence with morphological identifications. However, there were some deviations in the family level. Although both *Lagocephalus suezensis* and *Lagocephalus spadiceus* species belong to Tetraodontidae family, the former was clustered with species from Argentinidae and Apogonidae families, while the latter was clustered with *Serranus cabrilla* (Family: Serranidae) species. Two Bothidae species, *Bothus podas* and *Arnoglossus laterna,* were clustered with species from Chlorophthalmidae and Triglidae families, respectively. Also two Gobiidae species, *Lesueurigobius friesii* and *Oxyurichthys papuensis*, were clustered in separate branches. Nevertheless, separation was clear at the species level in all of these cases.

4.4.2 Invertebrate and ascidian dataset

4.4.2.1 Molecular identifications by BOLD ID Engine

In total 87 sequences, which were aligned and trimmed to 645 bp of length in Bioedit, were searched with BOLD ID Engine by using all barcode records (Section 3.4.2) for molecular identifications (Appendix G). Two samples belonging to Cephalopoda class (Phylum: Mollusca) indicated contamination by matching with the bacterial *Shewanella livingstonensis* species. Taxonomic information of all remaining 85 top matches in phylum and class levels were extracted and compared with morphological categorization (Figure 14 and Figure 15). There were not any conflicting identifications. All samples had similarity percentages higher than 98% for the identified taxa. Photos and sequences belonging to 85 invertebrate and ascidian specimens were uploaded to BOLD database (Appendix H and Appendix K) .

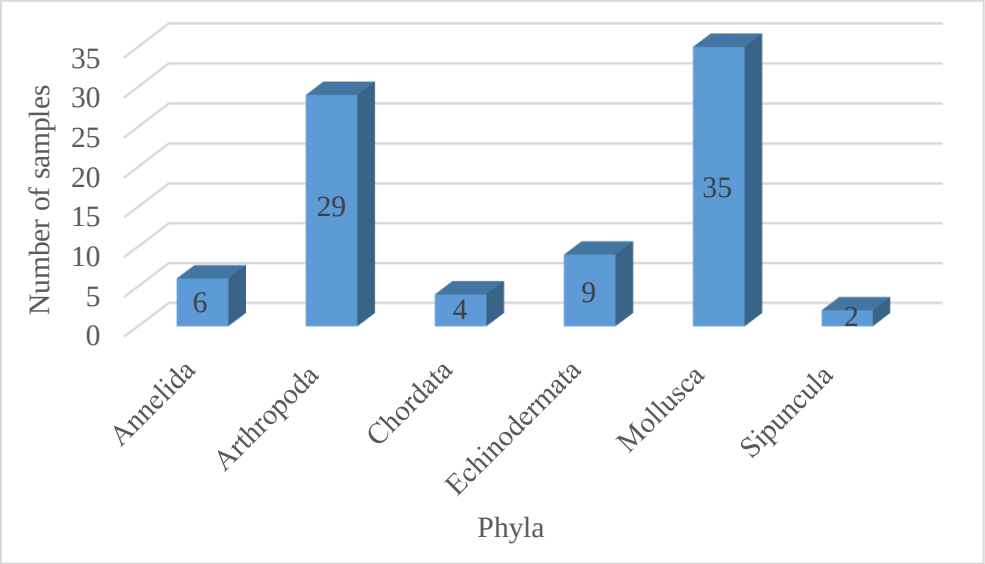

Figure 14 Number of invertebrate and asicidian samples grouped in phylum rank based on molecular identifications.

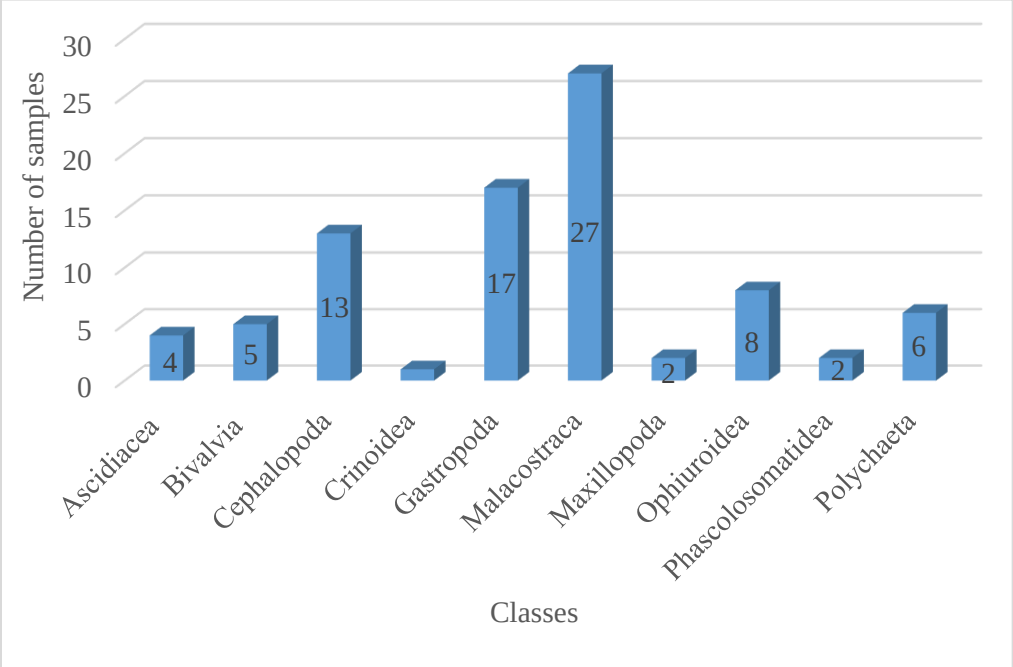

Figure 15 Number of invertebrate and asicidian samples grouped in class rank based on molecular identifications.

BOLD identified 49 sequences to species level with similarity scores higher than 98%. Remaining 36 sequences were identified with interim taxonomy and the number of total species were evaluated by using OTU-based approaches in further procedures. Numbers of identified samples and taxa for different taxonomic ranks based on molecular identifications are given in Table 5 and the names of species and taxa are given in Figure 16 and Figure 17.

Table 5 Number of samples and taxa in invertebrate and asicidian dataset for different taxonomic ranks based on molecular identifications.

Taxonomic rank	**Number of samples**	**Number of taxa**
Species	49	22
Genus	11	7
Family	14	8
Order	9	5
Class	2	2
Total	85	44

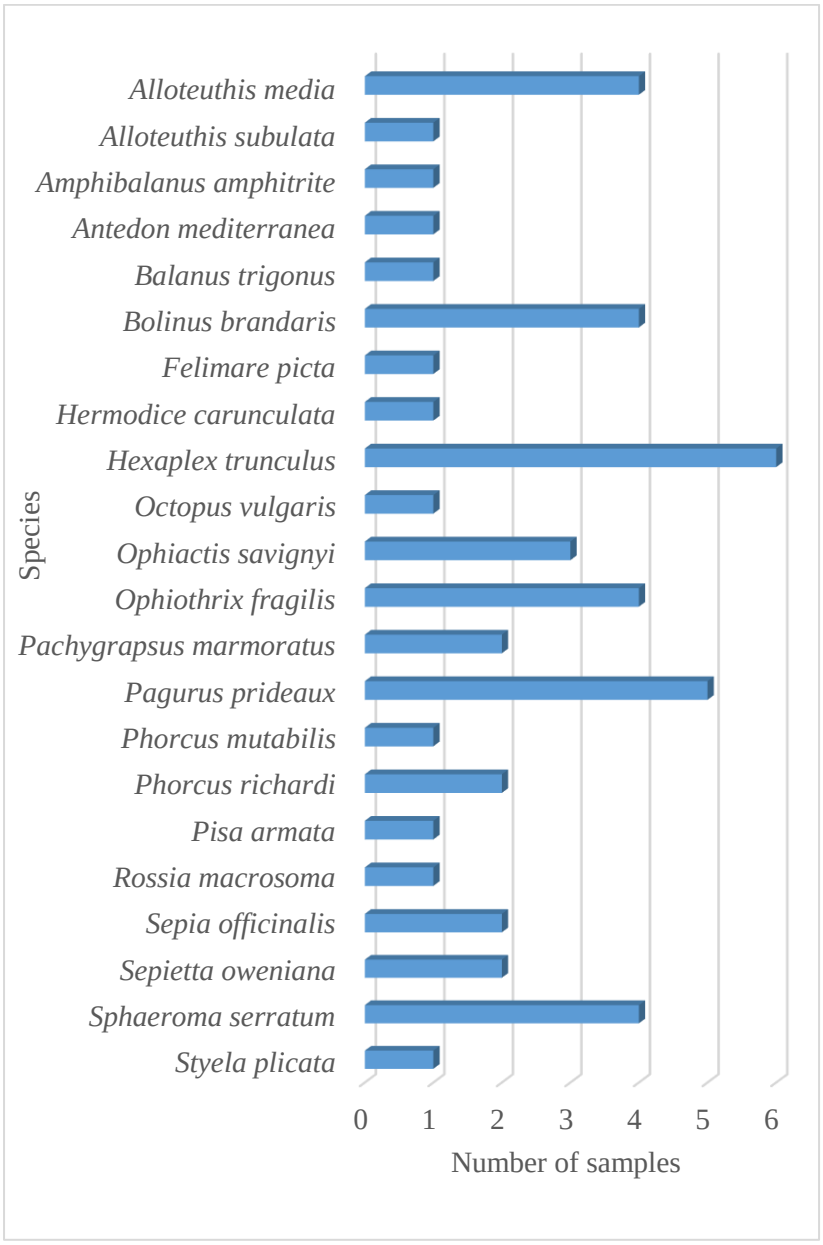

Figure 16 Number of samples and name of species for invertebrate and ascidian dataset based on species level molecular identifications.

Most abundant and diverse phylum in invertebrate samples was Mollusca which comprises 12 different orders. Also, Decapoda order samples (Arthropoda, Malacostraca) were highly diverse and abundant in the total catch with 20 specimens from 9 different species.

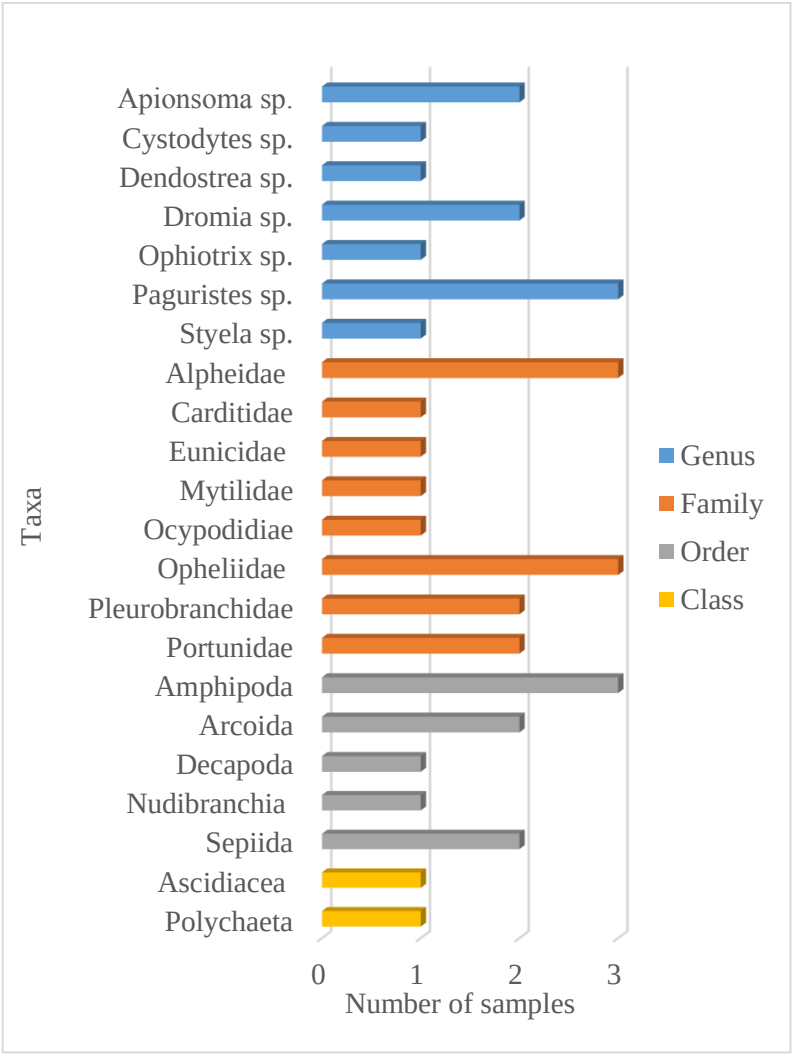

Figure 17 Number of samples and name of taxa for invertebrate and ascidian dataset based on molecular identifications with interim taxonomy.

4.4.2.2 Barcode compliance results

Out of 85 invertebrate and ascidian sequences, five were excluded depending on the presence of stop codons leaving 80 samples in the dataset. Two of these sequences were identified to *Sphaeroma serratum* species and remaining three of them were identified to Carditidae, Ocypodidae and Pleurobranchidae families. Those were the single samples of Carditidae and Ocypodidae families, thus those taxa were excluded from the further analysis leaving 42 taxa in the dataset based on molecular identifications. 65 (74.7%) of the remaining 80 sequences were flagged as barcode compliant. 14 of the unflagged sequences had poor quality traces depending on BOLD assignment, and one other sequence

was shorter than the minimum length required for barcode compliance (Section 3.4.3 for barcode compliance standards).

4.4.2.3 BIN Discordance Analysis

Four sequences could not be assigned to any BIN and remaining 76 records were represented by 43 BIN's. Sequences without BIN assignments include one *Pisa armata* (IMSA75) and one *Phorcus richardi* species (IMSF10), one Apionsoma (IMSA23) genus and one Arcoida order (IMSA19) samples. That was the only *Pisa armata* sample in the dataset, thus 41 taxa were left in the invertebrate dataset based on molecular identifications. Samples belonging to those 41 taxa were assigned to 43 BIN's and this inconsistency in numbers resulted from one Alpheidae family (IMSA12) and one *Sphaeroma serratum* species (IMSF9) samples which were assigned to singleton BIN's. In total, there were 12 singleton BIN's, while 10 BIN's were taxonomically discordant and 21 BIN's were concordant. One of the discordant BIN's had family level conflict and two of them had genus level conflicts and remaining 7 BIN's had species level conflicts (Appendix I).

4.4.2.4 Distance Summary Analysis

Distance summary analysis is performed to investigate the sequence divergences between 80 sequences which were uploaded to database, excluding sequences containing stop codons. The analysis was performed by using Kimura 2 parameter (K2P) distance model with BOLD alignment algorithm (Amino Acid based HMM).

In the dataset, there were 14 species which were represented by more than single sample, four genera (*Alloteuthis, Ophiothrix, Phorcus, Styela*) which comprise more than single species and three families (Balanidae, Muricidae, Sepiolidae) which comprise more than single genus. Thus, below table and graph were constructed using remaining samples for calculating within species, genus and family distances, respectively.

Table 6 Within species, genus and family distances in percentages of divergence with the number of samples (n) and taxa included in the invertebrate and ascidian dataset.

Within	**n**	**Taxa**	**Comp.**	**Min Dist (%)**	**Mean Dist (%)**	**Max Dist (%)**	**SE Dist (%)**
Species	43	14	56	0	0.99	4.82	0.02
Genus	15	4	11	4.64	13.58	23.4	0.62
Family	15	3	27	11.84	13.23	17.74	0.05

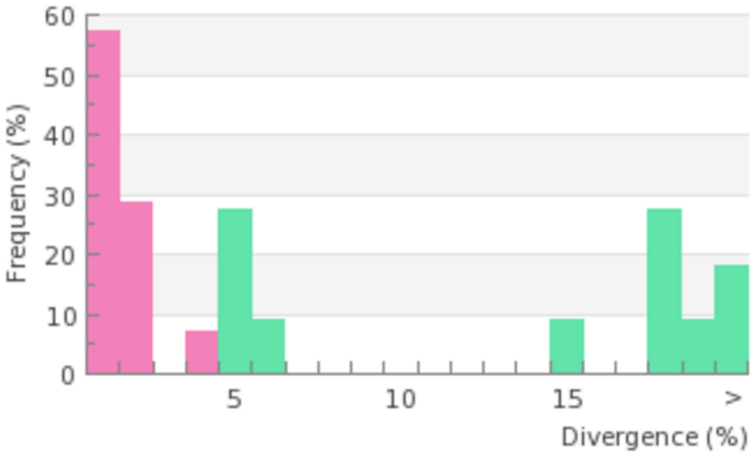

Figure 18 Normalized within species (pink) and within genus (green) distance frequencies against divergence in percentages for the invertebrate and asicidian dataset.

Maximum within species distance was for *Phorcus richardi* with a 4.82% divergence, and minimum within genus distance was between *Alloteuthis media* and *Alloteuthis subulata* species with a 4.64% divergence. Mean within species distance was 1.28% (S.E.:0.09) after normalization of within species distribution to reduce sampling bias.

4.4.2.5 Barcoding Gap Analysis

Barcoding Gap Analysis is performed to investigate the sequence divergences between 80 sequences which were uploaded to database, excluding sequences containing stop codons. The analysis was performed by using Kimura 2 parameter (K2P) distance model with BOLD alignment algorithm (Amino Acid based HMM).

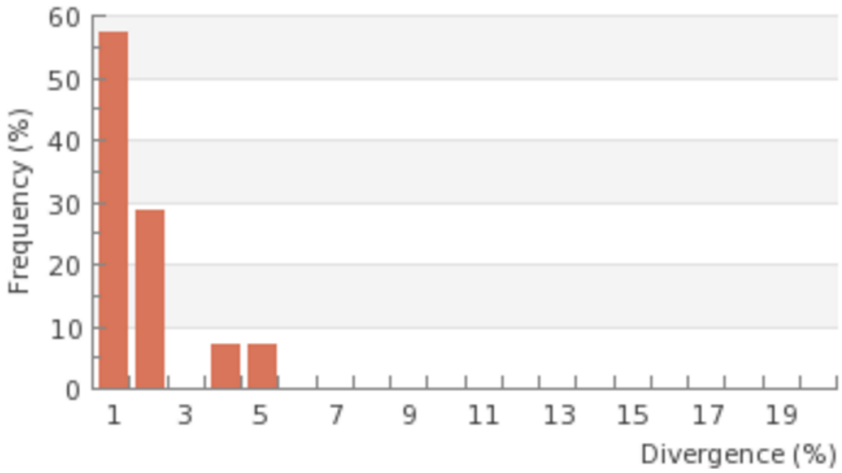

Figure 19 Histogram for mean intraspecific distances for the invertebrate and asicidian dataset.

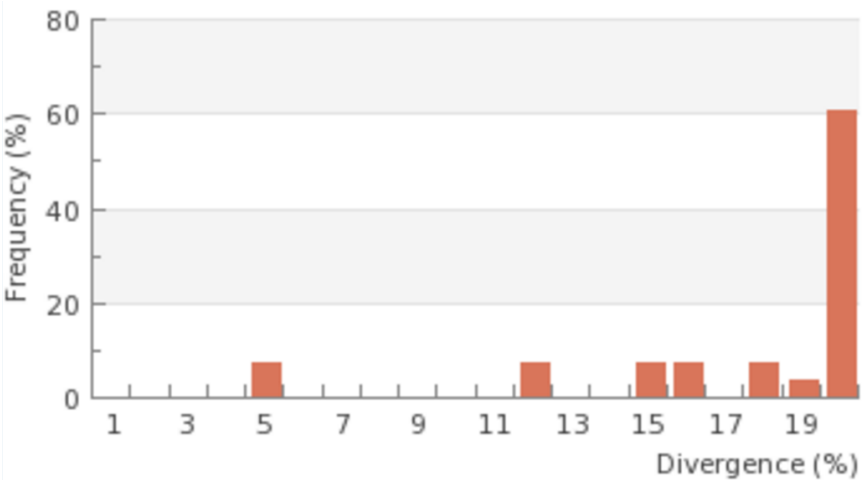

Figure 20 Histogram for distances to the nearest neighbors for the invertebrate and asicidian dataset.

Mean intraspecific distance was 1.28% (S.E.: 0.09) and the mean distance to the nearest neighbor was 22.83% (S.E. : 0.36) with the given frequencies in Figure 19 and Figure 20. Species which had mean intraspecific distances higher than 2% divergence include *Phorcus richardi* (4.82%) and *Sphaeroma serratum* species (3.05%) which corresponds to the highest values in Figure 19. One sample of the former species (IMSF10) could not be assigned to any BIN, and one sample of the latter species (IMSF9) was a singleton BIN in

BIN Discordance Analysis. However, both of these species had distances to their nearest neighbours higher than 15% divergence. On the other hand, *Alloteuthis media* and *Alloteuthis subulata* species had a distance of 4.64% divergence which corresponds to the lowest value in Figure 20.

4.4.2.6 Cluster Sequences Analysis

80 sequences belonging to 42 taxa were separated into 48 OTU's by using RESL algorithm. In total, there were 31 singleton clusters and six of them were from taxa represented by other clusters. Those include one Alpheidae family (IMSA12), one Amphipoda order (IMSA22) and one Opheliidae family (IMSF5) samples which were clustered apart from their contaxal groups. Also two samples each of Arcoida order, *Phorcus richardi* species and *Sphaeroma serratum* species were in separate clusters.

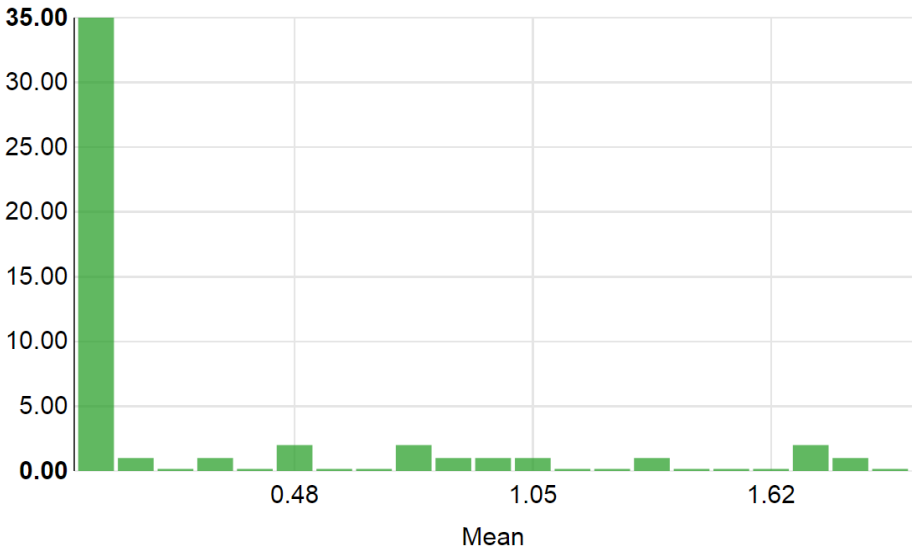

Figure 21 Histogram for mean within OTU distances for the invertebrate and asicidian dataset.

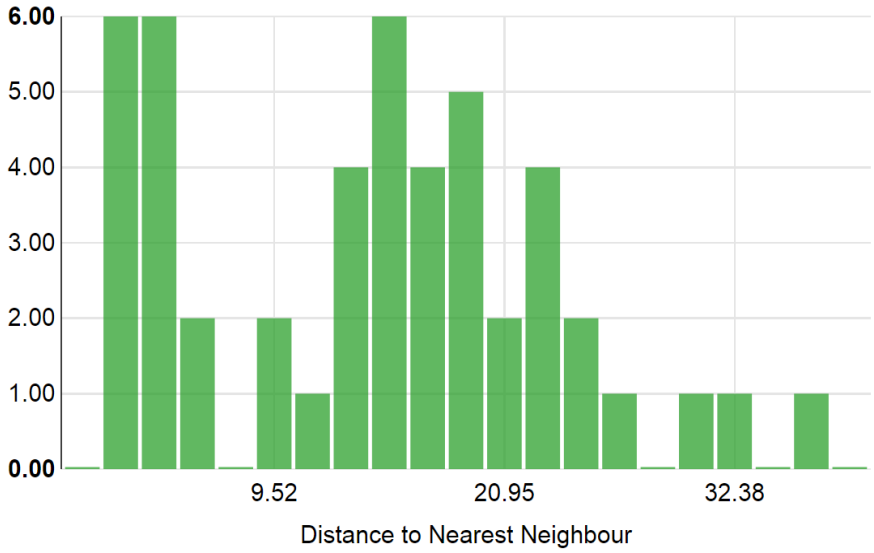

Figure 22 Histogram for distances to nearest neigbour OTU's for the invertebrate and asicidian dataset.

Highest within OTU distance in Figure 21 was for OTU-44 with a 1.86% divergence which contains *Ophiothrix fragilis* samples. Distances to the nearest neighbours lower than 10% in Figure 21 were corresponding to the problematic clusters mentioned above, which contain sequences represented by other clusters based on molecular identifications, except the one between *Alloteuthis media* and *Alloteuthis subulata* species with a 4.64% divergence.

4.4.2.7 Taxon ID Tree

Neighbor joining tree for 85 invertebrate and ascidian samples was constructed by using Kimura 2 parameter (K2P) distance model with BOLD alignment algorithm (Amino Acid based HMM).

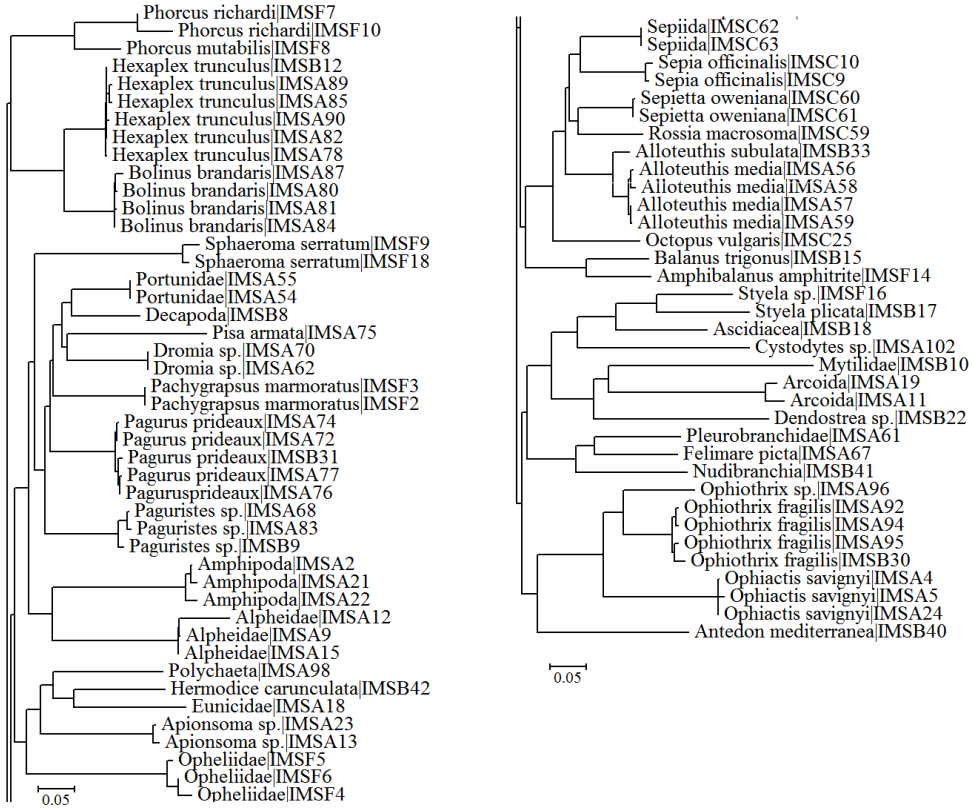

Figure 23 Neighbor joining tree including all haplotypes of 42 taxa from invertebrates and ascidians given with sample ID's.

Samples from Bivalvia class (Phylum: Mollusca) formed a monophyletic sister group to all other groups and Mytilida and Arcoida orders were found to be more related than Ostroida order within the class. Ascidians (Phylum: Chordata) was the sister group to all invertebrate samples except bivalves and the sample identified to class level (Ascidiacea) was clustered with Stolidobranchia samples.

Echinoderm samples from Crinoidea and Ophiuroidea classes formed monophyletic sister clusters and there were no taxonomic deviations at any level. The sample identified to genus level (*Ophiotrix sp.*) was clustered with samples from the same genus in consistence with molecular identifications.

Samples from Maxillopoda (Phylum: Arthropoda) class are found to be more related with Cephalopoda (Phylum: Mollusca) samples. Similarly segmented (Annelida) and sipunculid

(Sipuncula) worms were clustered as a sister group to Malacostraca class (Phylum: Arthropoda). However within the worms, Sipuncula samples were clustered within Annelid group in a separate branch.

Gastropod samples were from four different orders. Nudibranchia and Pleurobranchomorpha orders formed a monophyletic sister cluster with Neogastropoda and Vetigastropoda orders in NJ tree. One deviation was for *Felimare picta* sample (Order: Nudibranchia), which was found to be more related with samples from Pleurobranchidae order.

Samples from Cephalopoda (Phylum: Mollusca) class formed monophyletic clusters in all taxonomic levels. Two samples, which were identified to order level (Sepiida), were clustered with Sepiidae family samples in consistence with molecular identifications.

Considering the samples from Malacostraca order, all taxa formed monophyletic clusters in consistence with morphological identifications. However, samples identified to family level (Alpheidae) were clustered with Amphipoda order samples, although this family belongs to Decapoda order which formed another monophyletic group. Isopods and decapods are found to be more related than amphipods within the order.

5 DISCUSSION

5.1 Specimen Identifications

There was a single conflicting morphological and molecular identification for fish species; *Oxyurichthys papuensis* by morphological identification, but *Oxyurichthys petersii* by molecular analysis. Thus, 99% of the fish samples were identified by molecular analysis in concordance with morphological identifications and all species formed monophyletic clusters in NJ tree. Invertebrate and ascidian samples were grouped a priori into higher taxonomic ranks. There were no conflicting molecular identification with this initial categorization and all taxa formed monophyletic clusters in NJ tree. However 42.4% of the samples could not be identified to species level. This mainly results from the diverse group of taxa sampled in the study and the lack of species level barcode records in the database for these taxa.

5.2 Barcode Records

All fish species sampled in the scope of this study have previous records in BOLD database. However an Indo-Pacific anchovy species, *Encrasicholina punctifer* (Fowler, 1938), is recorded for the first time in the Mediterranean ichthyofauna. Morphological characters were examined in detail and the identification was later approved by morphological

identification according to Wongratana et al. 1995. Out of 38 fish species analyzed 14 were Lessepsian migrants (Table 7), 12 of which had previous barcode records from the Mediterranean Sea. This high percentage (34.2%) of Lessepsian migrants demonstrates the importance of the Eastern Levant as a biodiversity and invasion hotspot (Section 5.7 for further discussion). Also barcode records of 23 fish species from Turkey are provided for the first time with this study.

As of May 2014, Turkish marine fish fauna comprises 512 species where majority of them belong to the class Actinopterygii (446 sp.) (Bilecenoğlu et al., 2014). BOLD database contains 2901 published barcode records of 310 species from Turkey belonging to this class. Elasmobranchii class, on the other hand, is represented by 64 species in Turkey, while 30 published records for 10 of those species had been uploaded to the database (Ratnasingham and Hebert, 2003; Bilecenoğlu et al., 2014). Thus, currently almost 40% of the fish species in Turkish marine fauna do not have DNA barcode records in public databases and with this study alone, barcode inventory of the fauna increases by more than 5% with respect to the number of species. As mentioned earlier, 49 invertebrate samples belonging to 22 species could be identified to species level by molecular analysis. Barcode records of 6 invertebrate species in the Mediterranean Sea and 18 species in Turkish coasts are provided for the first time with this study.

Table 7 List of Lessepsian migrant fish species barcoded in this study.

Species
Callionymus filamentosus
Cynoglossus sinusarabici
Dussumieria elopsoides
Encrasicholina punctifer
Equulites klunzingeri
Lagocephalus spadiceus
Lagocephalus suezensis
Nemipterus randalli
Ostorhinchus fasciatus
Oxyurichthys papuensis
Sargocentron rubrum
Siganus luridus
Upeneus moluccensis
Upeneus pori

5.3 COI Divergence Assessment and Phylogenetic Results

As expected, genetic divergence increased with higher taxonomic rank. For fish species mean K2P distance was 0.66% between individuals within species, 14.84% between species within genera, and 18.17% between genera within family. Congeneric species were 22 times more divergent than conspecific individuals at COI locus, in consistence with earlier findings for fish species (Lakra et al., 2011; Landi et al., 2015). This ratio was found to be higher by Ward et al. (2005) and Keskin and Atar (2013a) with 25.5 and 30 times more divergence, respectively. Barcoding gap analysis, on the other hand, revealed 20 times more divergence between nearest neigbor species compared with conspecific individuals. Mean intraspecific distances calculated in the earlier studies were between 0.30% and 0.50%, which is slightly lower than the current result. The distance analyses were performed by using all sequences including the problematic ones as barcode incompliant sequences and singletons. Exclusion of these samples reduces the mean intraspecific distance to 0.52%, thus the reason of high intraspecific distances calculated for fish species can be the low quality of raw data. Also, 12 out of 38 fish species had single specimens and less than 50% of them had more than two specimens. This small samples sizes may result in removal or underestimation of the intraspecific divergence for more than half of the dataset.

Mean confamilial distance was lower than the congeneric distance for invertebrate species with 0.99%, 13.58% and 13.23% divergence, respectively for conspecific, congeneric and confamilial distances. There were 13 times more divergence between congeneric species than conspecific individuals. When comparing the intraspecific distances with distances to the nearest neighbor species, a higher ratio is found with 18 times more divergence. Exclusion of problematic samples reduces the mean intraspecific distance to 0.59%.

Divergence between conspecific individuals was very low compared with divergences within higher taxonomic ranks both for fishes and invertebrates. Also both NJ trees revealed a clear separation at species level, while there were inconsistent taxonomic classifications in family level. Thus, these results support an obvious increase in genetic divergence at species boundaries, which enables the use of COI locus for species identification purposes for the various taxa included in this study (Hubert et al., 2008; Lakra et al., 2011).

5.4 Cases of relatively high intraspecific distances

In total, there were three fish species (C*allionymus filamentosus, Boops boops and Serranus cabrilla)* which had higher mean intraspecific distances than 2%. However, all of

these species had problematic sequences as indicated by Barcode Compliance or BIN Discordance results (Sections 4.4.1.2 and 4.4.1.3). Exclusion of these problematic samples reduces the mean intraspecific distances to lower than 1% divergence for these species. Thus, all fish species had mean intraspecific distances lower than 2%. The highest intraspecific distance in fish species was for *Trachurus mediterraneus* with a 1.39% divergence. Earlier studies indicate a higher nucleotide diversity within *Trachurus trachurus* species for several loci including COI (Karaiskou et al., 2003; Cardenas et al., 2005; Bektas and Belduz, 2008), however there was a single sample for this species in our dataset and comparison was not possible.

In invertebrates, there were two species with mean intraspecific distances higher than 2% divergence (*Phorcus richardi* and *Sphaeroma serratum*) however those had problematic sequences as indicated by Barcode Compliance or BIN Discordance results (Sections 4.4.2.2 and 4.4.2.3). Exclusion of these samples remains single samples for each species, thus intraspecific distances can not be calculated. All other species had mean intraspecific distances lower than 2%. Samples from Paguristes sp. and *Ophiotrix fragilis* species had relatively high mean intraspecific distances of 1.81% and 1.89%, respectively.

5.5 Cases of relatively low interspecific distances

Lowest interspecific distance observed for fish species was between *Trachurus meditteraneus* and *Trachurus trachurus* species with a 2.08% divergence which is lower than the maximum intraspecific distance of the former species. Both our analyses and earlier studies reveal the low interspecific genetic distances within the genus, not only for COI (Landi et al., 2014) but also for cytochrome b and D-loop regions (Karaiskou et al., 2003; Cardenas et al., 2005; Bektas and Belduz, 2008), which supports the hypothesis of Cardenas (2005) that the low levels of divergence between species might be a common characteristic for the mtDNA in *Trachurus* (Cardenas et al., 2005). Despite this low distances, each species formed an independent branch in NJ tree with unique haplotypes in consistence with earlier studies (Landi et al., 2014).

Lowest interspecific distance for the invertebrate taxa was between two common squid species, *Alloteuthis media* and *Alloteuthis subulata*, with a 4.64% divergence. The former species is thought to prefer warm waters like Mediterranean, while the latter prefer cold waters like North Sea. Their morphological differentiation is highly difficult and even it has been proposed that they represent different ecological forms or ontogenetic stages of a single species (Laptikhovsky et al., 2002; Anderson et al., 2008). Although their interspecific distance is low compared to other results, they were assigned to two separate

BIN's and OTU's demonstrating a barcoding gap useful for discrimination of these two species, in consistence with earlier studies (Gebhardt and Knebelsberger, 2015).

5.6 Cases of Taxonomic Discordance

Based on the morphological identifications there were 38 fish species in the initial dataset. However due to amplification and sequencing failures, two of these species were excluded from the molecular analyses. Traditional taxonomic status and biological information of the species are evaluated by using WORMS (World Register of Marine Species), FishBase, GBIF (Global Biodiversity Information Facility), ITIS (Integrated Taxonomic Information System), FAO (Food and Agriculture Organization of the United Nations) databases and Marine Species Identification Portal together with the most recently updated checklist of marine fishes of Turkey (Bilecenoğlu et al., 2014). Molecular status, on the other hand, were evaluated by BOLD tools analyses and further literature was referred when necessary. For evaluation of the discordant BIN's, majority rule is suggested as a useful way to gauge the validity of identifications, as these identifications derive from several taxonomists and they are more likely to be correct than any outlier identification (Ratnasingham and Hebert, 2013).

19 of the BIN's assigned to fish samples had records from different species (Appendix F). The conflicting cases were in family level for three of them, in genus level for two of them and in species level for the remaining 14 cases. All BIN's which had family and genus level conflicts had majority of the records from taxa concordant with morphological identifications. Considering the discordant BIN's with species level conflicts, there were only two cases where the majority of the records within the BIN were from a different species than morphological identification. Morphological *Dussumieria elopsoides* (Bleeker, 1849) sample (IMSC21, Mersin Bay) was assigned to a discordant BIN (BOLD:AAE0678) which contains primarily *Dussumieria acuta* (Valenciennes, 1847) samples. Earlier barcode records of *D. elopsoides* from Turkey constitute a separate concordant BIN (Keskin and Atar, 2013a). Network analysis, performed by mining the barcode records in these two BIN's, reveal that the current study sample have at least 24 base pair (bp) differences with the other *D. elopsoides* records from Turkey, while less differences was observed with the *D.acuta* samples (Figure 24), indicating a possible incorrect identification. Both species are Indo-Pacific origin and inhabited Mediterranean waters through Lessepsian migration. They also closely resemble each other and earlier records of the species are mixed until Wrongtana (1980) demonstrated the differences between these species. *D. acuta* is listed as a rare migrant in the Mediterranean and it is not

present in the marine fauna of Turkey, while *D. elopsoides* does occur in both (Bilecenoğlu et al., 2014; Whitehead, 1985). Considering the ambiguities in both molecular and morphological status of the species a further investigation is required.

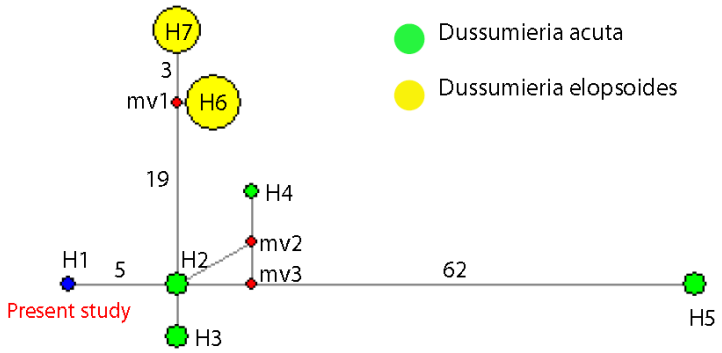

Figure 24 Network analysis for *Dussumieria* species including the barcode records in BOLD database together with the present study samples.

Similarly, *Oxyurichthys papuensis* (Valenciennes, 1837) sample (IMSA25) was assigned to a discordant BIN (BOLD:AAK4732) which contains primarily *Oxyurichthys petersii* (Klunzinger, 1871) samples. The latter species is not listed in ITIS database and given as an unaccepted or doubtful species in GBIF and WORMS databases with *O. papuensis* as the accepted name. However FishBase lists *O. petersii* as a "provisionally accepted species". Although there are earlier records of this species in Turkey (Kaya et al., 1992; Akyol et al., 2006) it is not listed in the latest checklist of the marine fishes of Turkey (Bilecenoğlu et al., 2014). Thus, further investigation specifically on morphological taxonomy of the species is required.

Another critical case of discordance was observed for three *Trachurus mediterraneus* (Steindachner, 1868) samples (IMSA26-28) and one *Trachurus trachurus* (Linnaeus, 1758) sample (IMSC39), which were all assigned to a BIN (BOLD:AAA8614) containing 422 records from 13 different morphological species of this genus. Both species does occur in the Mediterranean Sea and the species can be differentiated by obvious morphological and morphometric characters (i.e. Karaoglu and Belduz, 2011). However, as mentioned earlier low levels of divergence between species might be a common characteristic for the mtDNA

in *Trachurus* (Cardenas et al., 2005) and using different mitochondrial markers already increased the resolution within the genus (i.e. Bektaş and Belduz, 2008).

All 10 discordant BIN's assigned to invertebrate samples had majority of the records from taxa concordant with molecular identifications. Morphological identifications at species level is necessary for invertebrate taxa to evaluate the BIN results properly.

5.7 Biodiversity of Mersin Bay

Around 35% of both total fish catch and total number of fish species were Lessepsian migrants in this study. This percentages are in consistence with earlier findings for Mersin Bay (Gücü and Bingel, 1994a; Çiçek and Avşar, 2015). Also, majority of the fish invaders are spawning, shallow water, benthic carnivores which is an expected result as majority of the fish species that occur in the Red Sea are benthic (80%) and/or carnivores (79%) (Goren, 1993), and also as spawners usually have longer dispersal capabilities from an ecological point of view. The overall species richness in the eastern Mediterranean is known to be relatively low (Rilov and Galil, 2009) leaving plenty of niches open for invasion. In 1980's, the biomass and abundance of Lessepsian fishes were found to be declining at depths deeper than 50 meters in Mersin Bay, however we observed them in all depths in consistence with more recent surveys (Gücü et al., 2010), suggesting that Lessepsian invaders are establishing viable populations in a broader part of the biota. Also, the highest number of individuals for invasive species was observed for *Equulites klunzingeri* in consistence with earlier findings (Gücü and Bingel, 1994b; Gökçe et al., 2016). This species is known to be an opportunistic species with and r-selection strategy (Gücü and Bingel, 1994a)

Most abundant invertebrate group in this study was molluscs with 40% of the total catch. Together with decapods, they represent the invertebrate groups with the highest species richness and abundance in total catch. In a similar finding for the Mersin Bay, Çınar et al. (2012) reported that Mollusca were represented by the highest number of individuals in the area (65%). This had been attributed to the dense settlement of alien gastropod species in the region. Low percentage of species level identification in this study for invertebrates does not allow reliable conclusions on the specific status of alien invertebrate species in the region. However, our results are highly consistent with earlier studies in the region. For example, low abundance and diversity conditions of marine invertebrates had been reported in the Eastern Mediterranean (Danovaro et al., 2010), and Galil and Zenetos (2002) reported that the invertebrate groups with the ighest percentage of invaders in the eastern Mediterranean are decapod crustaceans (87%) and molluscs (88%). Thus, the high

abundance of these groups in this study presumably depends on the settlement of Lessepsian invasives in the region as Çınar et al. (2012) suggested. Furthermore, the rate of migration of Lessepsian molluscs is known to be almost three times slower in 1960's compared with the current rate (Galil and Zenetos, 2009).

6 CONCLUSION

Mersin Bay is characterized in the northeastern Mediterranean with its broad coastal plain which is enough to facilitate the establishment of a relatively larger biota. One of the side effects of this geographical characteristic is its suitability for trawl fisheries and consequently the resources in the area had been exploited for decades (Gücü and Bingel, 1994c). Moreover, the region is much warmer and saltier than the surrounding waters, as an extension of the hydrographical conditions in the northeastern Levant, and the effect of global warming might easily accelerate the tropicalization of the region (Gücü et al., 2010). Thus, fishing pressure, global warming and low indigenous biodiversity in the region provides a distinct advantage over the native fauna for opportunistic lessepsian species with their tropical advantages (Galil and Zenetos, 2010). Monitoring the related changes in the area and investigating the characteristics of "Lessepsian migration" has its unique challenges. In this sense, DNA barcoding might provide a highly useful tool for assessment of biodiversity and monitoring the ongoing invasions in the area. The consistence of findings with earlier studies primarily regarding specimen identification, phylogeny and biodiversity measures demonstrates the usefulness of the method. One of the most critical advantages is the possibility of studying a diverse group of taxa which requires a great effort and expertise with current taxonomic approaches. The identification of new invaders can be done much more quickly and effectively as demonstrated by the first record of *Encrasicholina punctifer* provided in this study. In addition to its implication as a stock assessment and specimen identification tool, it can provide an early insight to the evolutionary history of the species as discussed for *Trachurus* species. Also with a wider sampling area and a greater sample size, information on the food web structure and predator-prey interactions can be gathered by employing DNA barcoding methods from stomach content. Further implications may also include environmental DNA analysis for investigation of community structures.

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APPENDIX A

PRIMER PAIRS USED FOR EACH FISH SPECIES

Primers	Species				
	Argentina sphyraena	*Arnoglossus laterna*	*Boobs boobs*	*Bothus podas*	*Capros aper*
	Chlorophtalmus agassizi	*Citharus linguatula*	*Conger conger*	*Cynoglossus sinusarabici*	*Dasyatis sp.*
FISHF1-	*Dussumieria elopsoides*	*Encrasicholina punctifer*	*Engraulis encrasicolus*	*Equulites klunzingeri*	*Helicolenus dactylopterus*
FISHR1	*Hippocampus hippocampus*	*Lagocephalus spadiceus*	*Lagocephalus suezensis*	*Lepidopus caudatus*	*Lesueurigobius friesii*
	Mullus barbatus	*Nemipterus randalli*	*Ostorhinchus fasciatus*	*Sargocentron rubrum*	*Serranus cabrilla*
	Trachurus mediterraneus	*Trachurus trachurus*	*Upeneus moluccensis*	*Upeneus pori*	*Zeus faber*
FISHF2-	*Callionymus filamentosus*	*Leptidotrigla cavillone*	*Oxyurichthys papuensis*	*Scorpaena scrofa*	*Siganus luridus*
FISHR2	*Trigloporus lastoviza*				

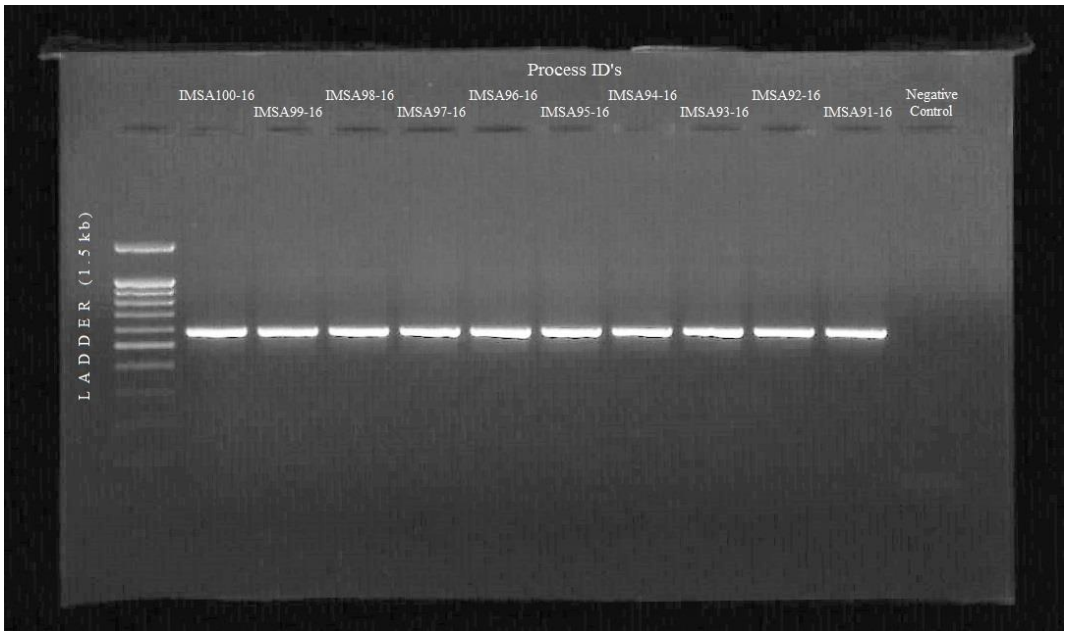
APPENDIX B

PRIMER PAIRS USED FOR EACH INVERTEBRATE TAXON

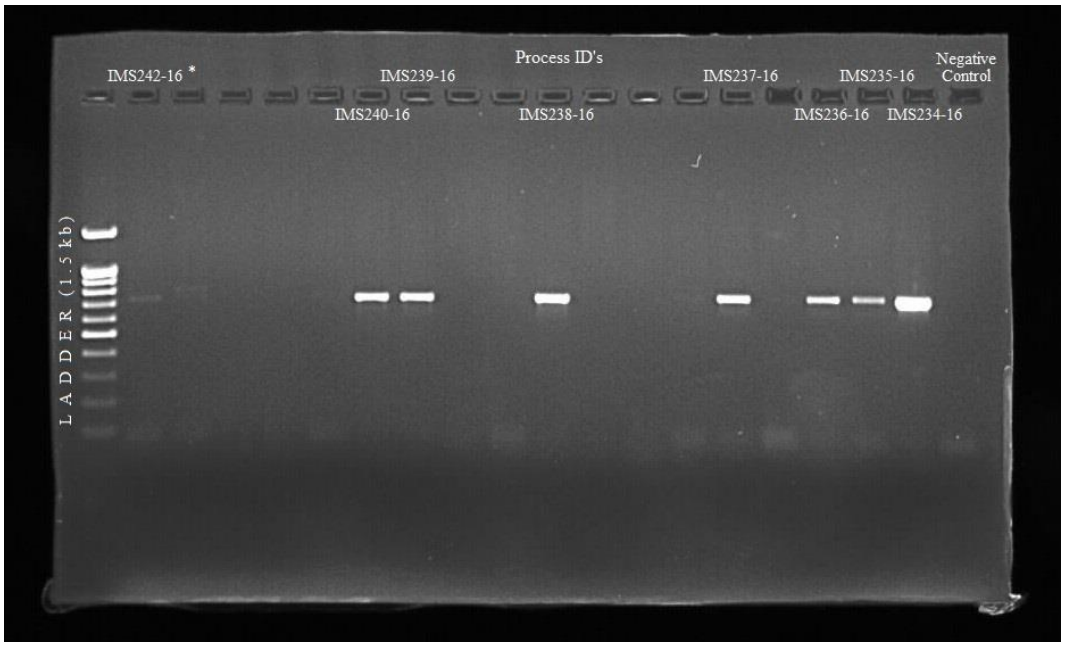
Primers	Taxon				
HC02198-LCO1490	*Alloteuthis media*	*Alloteuthis subulata*	Alpheidae	*Amphibalanus amphitrite*	Amphipoda
	Apionsoma sp.	Arcoida	Ascidiacea	*Balanus trigonus*	*Bolinus brandaris*
	Carditidae	*Cystodytes sp.*	Decapoda	*Dendostrea sp.*	*Dromia sp.*
	Eunicidae	*Felimare picta*	*Hermodice carunculata*	*Hexaplex trunculus*	Mytilidae
	Nudibranchia	*Octopus vulgaris*	Ocypodididae	Opheliidae	*Ophiothrix sp.*
	Pachygrapsus marmoratus	*Paguristes sp.*	*Pagurus prideaux*	*Phorcus mutabilis*	*Phorcus richardi*
	Pisa armata	Pleurobranchidae	Polychaeta	Portunidae	*Rossia macrosoma*
	Sepia officinalis	Sepiida	*Sepietta oweniana*	*Sphaeroma serratum*	*Styela plicata*
	Styela sp.				
ECHINO COI-F **ECHINO COI-R**	*Antedon mediterranea*	*Ophiactis savignyi*	*Ophiothrix fragilis*		

APPENDIX C

SAMPLE PCR PRODUCT CHECK FOR FISH DNA SAMPLES



SAMPLE PCR PRODUCT CHECK FOR INVERTEBRATE DNA SAMPLES



* PCR amplifications were performed again until a product with high quality was obtained.

APPENDIX D

BOLD ID ENGINE RESULTS FOR FISH SAMPLES

Sample ID	Top Hit
IMSA25	Chordata - Perciformes - *Oxyurichthys petersii* (100%)
IMSA26	Chordata - Perciformes - *Trachurus mediterraneus* (100%)
IMSA27	Chordata - Perciformes - *Trachurus mediterraneus* (100%)
IMSA28	Chordata - Perciformes - *Trachurus mediterraneus* (100%)
IMSA29	Chordata - Perciformes - *Boops boops* (100%)
IMSA30	Chordata - Perciformes - *Boops boops* (100%)
IMSA31	Chordata - Perciformes - *Boops boops* (100%)
IMSA32	Chordata - Perciformes - *Boops boop*s (100%)
IMSA33	Chordata - Pleuronectiformes - *Cynoglossus sinusarabici* (100%)
IMSA34	Chordata - Pleuronectiformes - *Cynoglossus sinusarabici* (100%)
IMSA35	Chordata - Pleuronectiformes - *Cynoglossus sinusarabici* (99.69%)
IMSA36	Chordata - Pleuronectiformes - *Cynoglossus sinusarabici* (99.69%)
IMSA38	Chordata - Perciformes - *Ostorhinchus fasciatus* (100%)
IMSA39	Chordata - Perciformes - *Ostorhinchus fasciatus* (100%)
IMSA40	Chordata - Perciformes - *Ostorhinchus fasciatus* (100%)
IMSA41	Chordata - Perciformes - *Ostorhinchus fasciatus* (100%)
IMSA42	Chordata - Perciformes - *Ostorhinchus fasciatus* (100%)
IMSA43	Chordata - Perciformes - *Nemipterus randalli* (100%)
IMSA45	Chordata - Perciformes - *Callionymus filamentosus* (99.84%)
IMSA46	Chordata - Perciformes - *Callionymus filamentosus* (99.84%)
IMSA48	Chordata - Perciformes - *Equulites klunzingeri* (100%)
IMSA53	Chordata - Pleuronectiformes - *Citharus linguatula* (100%)
IMSA110	Chordata - Perciformes - *Equulites klunzingeri* (100%)
IMSA111	Chordata - Perciformes - *Equulites klunzingeri* (100%)
IMSA114	Chordata - Scorpaeniformes - *Trigloporus lastoviza* (100%)
IMSB23	Chordata - Syngnathiformes - *Hippocampus hippocampus* (100%)
IMSC1	Chordata - Beryciformes - *Sargocentron rubrum* (100%)
IMSC2	Chordata - Perciformes - *Upeneus moluccensis* (100%)
IMSC3	Chordata - Perciformes - *Serranus cabrilla* (99.84%)
IMSC5	Chordata - Pleuronectiformes - *Bothus podas* (100%)
IMSC6	Chordata - Anguilliformes - *Conger conger* (100%)
IMSC11	Chordata - Perciformes - *Equulites klunzingeri* (100%)
IMSC14	Chordata - Perciformes - *Equulites klunzingeri* (100%)
IMSC15	Chordata - Perciformes - *Siganus luridus* (100%)
IMSC16	Chordata - Tetraodontiformes - *Lagocephalus spadiceus* (100%)
IMSC17	Chordata - Perciformes - *Upeneus moluccensis* (100%)
IMSC19	Chordata - Perciformes - *Serranus cabrilla* (99.53%)
IMSC20	Chordata - Perciformes - *Equulites klunzingeri* (100%)
IMSC21	Chordata - Clupeiformes - *Dussumieria elopsoides* (100%)
IMSC22	Chordata - Perciformes - *Upeneus moluccensis* (100%)

Appendix D (continued)

IMSC24	Chordata - Tetraodontiformes - *Lagocephalus suezensis* (100%)
IMSC34	Chordata - Zeiformes - *Zeus faber* (100%)
IMSC35	Chordata - Pleuronectiformes - *Citharus linguatula* (100%)
IMSC36	Chordata - Perciformes - *Callionymus filamentosus* (99.84%)
IMSC37	Chordata - Aulopiformes - *Chlorophthalmus agassizi* (100%)
IMSC39	Chordata - Perciformes - *Trachurus trachurus* (99.84%)
IMSC41	Chordata - Perciformes - *Lepidopus caudatus* (100%)
IMSC42	Chordata - Perciformes - *Lepidopus caudatus* (100%)
IMSC44	Chordata - Scorpaeniformes - *Scorpaena scrofa* (100%)
IMSC45	Chordata - Scorpaeniformes - *Lepidotrigla cavillone* (100%)
IMSC46	Chordata - Perciformes - *Capros aper* (100%)
IMSC47	Chordata - Scorpaeniformes - *Helicolenus dactylopterus* (99.84%)
IMSC48	Chordata - Perciformes - *Nemipterus randalli* (100%)
IMSC49	Chordata - Perciformes - *Capros aper* (100%)
IMSC50	Chordata - Perciformes - *Capros aper* (100%)
IMSC51	Chordata - Perciformes - *Capros aper* (100%)
IMSC52	Chordata - Aulopiformes - *Chlorophthalmus agassizi* (100%)
IMSC53	Chordata - Scorpaeniformes - *Helicolenus dactylopterus* (100%)
IMSC54	Chordata - Osmeriformes - *Argentina sphyraena* (100%)
IMSC55	Chordata - Pleuronectiformes - *Citharus linguatula* (100%)
IMSC56	Chordata - Pleuronectiformes - *Arnoglossus laterna* (99.84%)
IMSC57	Chordata - Pleuronectiformes - *Arnoglossus laterna* (100%)
IMSC58	Chordata - Scorpaeniformes - *Lepidotrigla cavillone* (100%)
IMSC64	Chordata - Scorpaeniformes - *Lepidotrigla cavillone* (100%)
IMSC65	Chordata - Scorpaeniformes - *Lepidotrigla cavillone* (99.84%)
IMSC66	Chordata - Scorpaeniformes - *Lepidotrigla cavillone* (100%)
IMSC67	Chordata - Aulopiformes - *Chlorophthalmus agassizi* (100%)
IMSC68	Chordata - Aulopiformes - *Chlorophthalmus agassizi* (100%)
IMSC69	Chordata - Aulopiformes - *Chlorophthalmus agassizi* (100%)
IMSC70	Chordata - Perciformes - *Lesueurigobius friesii* (99.22%)
IMSC72	Chordata - Perciformes - *Lesueurigobius friesii* (99.53%)
IMSC73	Chordata - Perciformes - *Dasyatis sp* (99.53%)
IMSC77	Chordata - Clupeiformes - *Encrasicholina punctifer* (100%)
IMSC78	Chordata - Clupeiformes - *Encrasicholina punctifer* (100%)
IMSC79	Chordata - Clupeiformes - *Engraulis encrasicolus* (100%)
IMSH1	Chordata - Perciformes - *Mullus barbatus* (100%)
IMSH2	Chordata - Perciformes - *Mullus barbatus* (100%)
IMSH3	Chordata - Perciformes - *Mullus barbatus* (100%)
IMSH4	Chordata - Perciformes - *Mullus barbatus* (100%)
IMSMb1	Chordata - Perciformes - *Mullus barbatus* (100%)
IMSMb2	Chordata - Perciformes - *Mullus barbatus* (100%)
IMSMb3	Chordata - Perciformes - *Mullus barbatus* (100%)
IMSMb4	Chordata - Perciformes - *Mullus barbatus* (100%)
IMSMb9	Chordata - Perciformes - *Mullus barbatus* (100%)
IMSMb19	Chordata - Perciformes - *Mullus barbatus* (100%)
IMSMb22	Chordata - Perciformes - *Mullus barbatus* (100%)
IMSMb23	Chordata - Perciformes - *Mullus barbatus* (100%)
IMSMb66	Chordata - Perciformes - *Mullus barbatus* (100%)
IMSMb71	Chordata - Perciformes - *Mullus barbatus* (100%)

Appendix D (continued)

IMSMs1	Chordata - Perciformes - *Upeneus moluccensis* (100%)
IMSO2	Chordata - Perciformes - *Upeneus pori* (100%)
IMSO3	Chordata - Perciformes - *Mullus barbatus* (100%)
IMSO5	Chordata - Perciformes - *Mullus barbatus* (100%)
IMSO6	Chordata - Perciformes - *Mullus barbatus* (100%)
IMSUm1	Chordata - Perciformes - *Upeneus moluccensis* (100%)
IMSUm2-2	Chordata - Perciformes - *Upeneus moluccensis* (100%)
IMSUm2	Chordata - Perciformes - *Upeneus moluccensis* (100%)
IMSUp1	Chordata - Perciformes - *Upeneus pori* (100%)
IMSUp2	Chordata - Perciformes - *Upeneus pori* (100%)
IMSUp3	Chordata - Perciformes - *Upeneus pori* (100%)
IMSUp4	Chordata - Perciformes - *Upeneus pori* (100%)

APPENDIX E

BOLD REGISTRY INFORMATION FOR FISH SAMPLES

Sample ID	Species	Process ID	BIN
IMSC54	*Argentina sphyraena*	IMS071-16	BOLD:AAB6547
IMSC56	*Arnoglossus laterna*	IMS073-16	BOLD:AAB5950
IMSC57	*Arnoglossus laterna*	IMS074-16	BOLD:AAB5950
IMSA29	*Boops boops*	IMS025-16	BOLD:AAB7806
IMSA31	*Boops boops*	IMS027-16	BOLD:ACZ0394
IMSA30	*Boops boops*	IMS026-16	BOLD:AAB7806
IMSA32	*Boops boops*	IMS028-16	BOLD:AAB7806
IMSC5	*Bothus podas*	IMS043-16	BOLD:AAE8135
IMSA46	*Callionymus filamentosus*	IMS036-16	BOLD:ACG9162
IMSA45	*Callionymus filamentosus*	IMS035-16	BOLD:ACG9162
IMSC36	*Callionymus filamentosus*	IMS057-16	BOLD:ACZ0579
IMSC50	*Capros aper*	IMS067-16	BOLD:AAB2992
IMSC46	*Capros aper*	IMS063-16	BOLD:AAB2992
IMSC49	*Capros aper*	IMS066-16	BOLD:AAB2992
IMSC51	*Capros aper*	IMS068-16	BOLD:AAB2992
IMSC52	*Chlorophthalmus agassizi*	IMS069-16	BOLD:AAB2600
IMSC68	*Chlorophthalmus agassizi*	IMS080-16	BOLD:AAB2600
IMSC69	*Chlorophthalmus agassizi*	IMS081-16	BOLD:AAB2600
IMSC67	*Chlorophthalmus agassizi*	IMS079-16	BOLD:AAB2600
IMSC37	*Chlorophthalmus agassizi*	IMS118-16	
IMSC35	*Citharus linguatula*	IMS056-16	BOLD:AAC0204
IMSA53	*Citharus linguatula*	IMS038-16	BOLD:AAC0204
IMSC55	*Citharus linguatula*	IMS072-16	BOLD:AAC0204
IMSC6	*Conger conger*	IMS044-16	BOLD:AAB6795
IMSA33	*Cynoglossus sinusarabici*	IMS029-16	BOLD:ACG6778
IMSA36	*Cynoglossus sinusarabici*	IMS032-16	BOLD:ACG6778
IMSA35	*Cynoglossus sinusarabici*	IMS031-16	BOLD:ACG6778
IMSA34	*Cynoglossus sinusarabici*	IMS030-16	BOLD:ACG6778
IMSC73	*Dasyatis sp.*	IMS179-16	
IMSC21	*Dussumieria elopsoides*	IMS052-16	BOLD:AAE0678
Ep-C78	*Encrasicholina punctifer*	IMS020-15	BOLD:AAF8837
Ep-C77	*Encrasicholina punctifer*	IMS019-15	BOLD:AAF8837
IMSC79	*Engraulis encrasicolus*	IMS084-16	BOLD:AAB2317
IMSC20	*Equulites klunzingeri*	IMS051-16	BOLD:ACG8806
IMSA110	*Equulites klunzingeri*	IMS085-16	BOLD:ACG8806
IMSA111	*Equulites klunzingeri*	IMS086-16	
IMSC11	*Equulites klunzingeri*	IMS045-16	BOLD:ACG8806
IMSA48	*Equulites klunzingeri*	IMS037-16	BOLD:ACG8806
IMSC14	*Equulites klunzingeri*	IMS046-16	BOLD:ACG8806

Appendix E (continued)

IMSC47	*Helicolenus dactylopterus*	IMS064-16	BOLD:AAA9248
IMSC53	*Helicolenus dactylopterus*	IMS070-16	BOLD:AAA9248
IMSB23	*Hippocampus hippocampus*	IMS039-16	BOLD:AAZ6366
IMSC16	*Lagocephalus spadiceus*	IMS048-16	BOLD:AAB5967
IMSC24	*Lagocephalus suezensis*	IMS054-16	BOLD:ACG7296
IMSC42	*Lepidopus caudatus*	IMS060-16	BOLD:AAB3173
IMSC41	*Lepidopus caudatus*	IMS059-16	BOLD:AAB3173
IMSC64	*Lepidotrigla cavillone*	IMS076-16	BOLD:AAB7558
IMSC65	*Lepidotrigla cavillone*	IMS077-16	BOLD:AAB7558
IMSC45	*Lepidotrigla cavillone*	IMS062-16	BOLD:AAB7559
IMSC66	*Lepidotrigla cavillone*	IMS078-16	BOLD:AAB7558
IMSC58	*Lepidotrigla cavillone*	IMS075-16	BOLD:AAB7558
IMSC70	*Lesueurigobius friesii*	IMS082-16	
IMSC72	*Lesueurigobius friesii*	IMS083-16	
IMSH2	*Mullus barbatus*	IMS111-16	BOLD:AAD7866
IMSH1	*Mullus barbatus*	IMS110-16	BOLD:AAD7866
IMSMb4	*Mullus barbatus*	IMS101-16	BOLD:AAD7866
IMSMb3	*Mullus barbatus*	IMS100-16	BOLD:AAD7866
IMSMb2	*Mullus barbatus*	IMS099-16	BOLD:AAD7866
IMSMb1	*Mullus barbatus*	IMS098-16	BOLD:AAD7866
IMSMb23	*Mullus barbatus*	IMS097-16	BOLD:AAD7866
IMSMb22	*Mullus barbatus*	IMS096-16	BOLD:AAD7866
IMSMb19	*Mullus barbatus*	IMS095-16	BOLD:AAD7866
IMSMb9	*Mullus barbatus*	IMS094-16	BOLD:AAD7866
IMSMb71	*Mullus barbatus*	IMS093-16	BOLD:AAD7866
IMSMb66	*Mullus barbatus*	IMS092-16	BOLD:AAD7866
IMSH3	*Mullus barbatus*	IMS112-16	BOLD:AAD7866
IMSH4	*Mullus barbatus*	IMS113-16	BOLD:AAD7866
IMSO3	*Mullus barbatus*	IMS115-16	BOLD:AAD7866
IMSO5	*Mullus barbatus*	IMS116-16	BOLD:AAD7866
IMSO6	*Mullus barbatus*	IMS117-16	BOLD:AAD7866
IMSC48	*Nemipterus randalli*	IMS065-16	BOLD:AAE3907
IMSA43	*Nemipterus randalli*	IMS034-16	BOLD:AAE3907
IMSA38	*Ostorhinchus fasciatus*	IMS088-16	BOLD:AAC1243
IMSA41	*Ostorhinchus fasciatus*	IMS090-16	BOLD:AAC1243
IMSA42	*Ostorhinchus fasciatus*	IMS091-16	BOLD:AAC1243
IMSA40	*Ostorhinchus fasciatus*	IMS033-16	BOLD:AAC1243
IMSA39	*Ostorhinchus fasciatus*	IMS089-16	BOLD:AAC1243
IMSA25	*Oxyurichthys petersii*	IMS021-16	BOLD:AAK4732
IMSC1	*Sargocentron rubrum*	IMS040-16	BOLD:ACG8706
IMSC44	*Scorpaena scrofa*	IMS061-16	BOLD:AAD0014
IMSC3	*Serranus cabrilla*	IMS042-16	BOLD:AAD1027
IMSC19	*Serranus cabrilla*	IMS050-16	BOLD:AAD1027
IMSC15	*Siganus luridus*	IMS047-16	BOLD:AAL9467
IMSA26	*Trachurus mediterraneus*	IMS022-16	BOLD:AAA8614
IMSA28	*Trachurus mediterraneus*	IMS024-16	BOLD:AAA8614
IMSA27	*Trachurus mediterraneus*	IMS023-16	BOLD:AAA8614
IMSC39	*Trachurus trachurus*	IMS058-16	BOLD:AAA8614

Appendix E (continued)

IMSA114	*Trigloporus lastoviza*	IMS087-16	BOLD:AAB8166
IMSC2	*Upeneus moluccensis*	IMS041-16	BOLD:AAB6469
IMSC22	*Upeneus moluccensis*	IMS053-16	BOLD:AAB6469
IMSUm1	*Upeneus moluccensis*	IMS102-16	BOLD:AAB6469
IMSUm2	*Upeneus moluccensis*	IMS103-16	BOLD:AAB6469
IMSUm2-2	*Upeneus moluccensis*	IMS104-16	BOLD:AAB6469
IMSMs1	*Upeneus moluccensis*	IMS105-16	
IMSC17	*Upeneus moluccensis*	IMS049-16	BOLD:AAB6469
IMSUp3	*Upeneus pori*	IMS108-16	BOLD:AAC1406
IMSUp4	*Upeneus pori*	IMS109-16	BOLD:AAC1406
IMSO2	*Upeneus pori*	IMS114-16	BOLD:AAC1406
IMSUp1	*Upeneus pori*	IMS106-16	BOLD:AAC1406
IMSUp2	*Upeneus pori*	IMS107-16	BOLD:AAC1406
IMSC34	*Zeus faber*	IMS055-16	BOLD:AAA7905

APPENDIX F

BIN DISCORDANCE ANALYSIS RESULTS FOR FISH SAMPLES

DISCORDANT BIN'S

Process ID	Identification	Conflicting Taxon in BIN	Rank of Conflict	BIN Number	BIN Total Members	BIN Tax Variation
IMS019-15	*Encrasicholina punctifer*	Engraulidae	Family	BOLD:AAF8837	42	Engraulidae[41], Clupeidae[1]
IMS020-15	*Encrasicholina punctifer*	Engraulidae				
IMS084-16	*Engraulis encrasicolus*	Engraulidae	Family	BOLD:AAB2317	178	Engraulidae[189], Clupeidae[1]
IMS064-16	*Helicolenus dactylopterus*	Sebastidae	Family	BOLD:AAA9248	148	Sebastidae[143], Scorpaenidae[3]
IMS070-16	*Helicolenus dactylopterus*	Sebastidae				
IMS028-16	*Boops boops*	*Boops*	Genus	BOLD:AAB7806	81	*Boops*[87], *Oblada*[2]
IMS026-16	*Boops boops*	*Boops*				
IMS025-16	*Boops boops*	*Boops*				
IMS080-16	*Chlorophthalmus agassizi*	*Chlorophthalmus*	Genus	BOLD:AAB2600	60	*Chlorophthalmus*[52], *Parasudis*[5]
IMS069-16	*Chlorophthalmus agassizi*	*Chlorophthalmus*				
IMS079-16	*Chlorophthalmus agassizi*	*Chlorophthalmus*				
IMS081-16	*Chlorophthalmus agassizi*	*Chlorophthalmus*				
IMS043-16	*Bothus podas*	*Bothus podas*	Species	BOLD:AAE8135	33	*Bothus podas*[30], *Bothus podas podas*[3]
IMS052-16	*Dussumieria elopsoides*	*Dussumieria elopsoides*	Species	BOLD:AAE0678	7	*Dussumieria acuta*[5], *Dussumieria elopsoides*[2]

Appendix F (continued)

IMS045-16	*Equulites klunzingeri*	*Equulites klunzingeri*	Species	BOLD:ACG8806	31	*Equulites klunzingeri*[30], *Equulites leuciscus*[1]
IMS085-16	*Equulites klunzingeri*	*Equulites klunzingeri*				
IMS037-16	*Equulites klunzingeri*	*Equulites klunzingeri*				
IMS051-16	*Equulites klunzingeri*	*Equulites klunzingeri*				
IMS046-16	*Equulites klunzingeri*	*Equulites klunzingeri*				
IMS039-16	*Hippocampus hippocampus*	*Hippocampus hippocampus*	Species	BOLD:AAZ6366	3	*Hippocampus hippocampus*[2], *Hippocampus ramulosus*[1]
IMS048-16	*Lagocephalus spadiceus*	*Lagocephalus spadiceus*	Species	BOLD:AAB5967	56	*Lagocephalus guentheri*[33], *Lagocephalus spadiceus*[20], *Lagocephalus cf. lunaris*[1]
IMS099-16	*Mullus barbatus*	*Mullus barbatus*	Species	BOLD:AAD7866	71	*Mullus barbatus*[77], *Mullus barbatus barbatus*[4]
IMS097-16	*Mullus barbatus*	*Mullus barbatus*				
IMS117-16	*Mullus barbatus*	*Mullus barbatus*				
IMS096-16	*Mullus barbatus*	*Mullus barbatus*				
IMS112-16	*Mullus barbatus*	*Mullus barbatus*				
IMS115-16	*Mullus barbatus*	*Mullus barbatus*				
IMS110-16	*Mullus barbatus*	*Mullus barbatus*				
IMS116-16	*Mullus barbatus*	*Mullus barbatus*				
IMS100-16	*Mullus barbatus*	*Mullus barbatus*				
IMS095-16	*Mullus barbatus*	*Mullus barbatus*				
IMS093-16	*Mullus barbatus*	*Mullus barbatus*				
IMS113-16	*Mullus barbatus*	*Mullus barbatus*				
IMS111-16	*Mullus barbatus*	*Mullus barbatus*				
IMS101-16	*Mullus barbatus*	*Mullus barbatus*				
IMS098-16	*Mullus barbatus*	*Mullus barbatus*				
IMS092-16	*Mullus barbatus*	*Mullus barbatus*				
IMS094-16	*Mullus barbatus*	*Mullus barbatus*				

Appendix F (continued)

IMS034-16	*Nemipterus randalli*	*Nemipterus randalli*	Species	BOLD:AAE3907	39	*Nemipterus randalli*[28], *Nemipterus mesoprion*[10], *Nemipterus zysron*[1]
IMS065-16	*Nemipterus randalli*	*Nemipterus randalli*				
IMS021-16	*Oxyurichthys petersii*	*Oxyurichthys petersii*	Species	BOLD:AAK4732	9	*Oxyurichthys petersii*[6], *Oxyurichthys papuensis*[2], *Oxyurichthys auchenolepis*[1]
IMS061-16	*Scorpaena scrofa*	*Scorpaena scrofa*	Species	BOLD:AAD0014	7	*Scorpaena scrofa*[4], *Scorpaena cf. izensis*[2], *Scorpaena elongata*[1]
IMS042-16	*Serranus cabrilla*	*Serranus cabrilla*	Species	BOLD:AAD1027	81	*Serranus cabrilla*[60], *Serranus knysnaensis*[15], *Serranus novemcinctus*[5], *Serranus sp.*[1]
IMS050-16	*Serranus cabrilla*	*Serranus cabrilla*				
IMS047-16	*Siganus luridus*	*Siganus luridus*	Species	BOLD:AAL9467	14	*Siganus luridus*[12], *Siganus sutor*[2]
IMS022-16	*Trachurus mediterraneus*	*Trachurus mediterraneus*	Species	BOLD:AAA8614	418	*Trachurus trachurus*[126], *Trachurus mediterraneus*[59], *Trachurus delagoa*[53], *Trachurus novaezelandiae*[49], *Trachurus declivis*[36], *Trachurus picturatus*[25], *Trachurus murphyi*[22], *Trachurus japonicus*[17], *Trachurus capensis*[10], *Trachurus lathami*[10], *Trachurus symmetricus*[5], *Trachurus trecae*[5], *Trachurus picturatus urphyi*[3]
IMS024-16	*Trachurus mediterraneus*	*Trachurus mediterraneus*				
IMS023-16	*Trachurus mediterraneus*	*Trachurus mediterraneus*				
IMS058-16	*Trachurus trachurus*	*Trachurus trachurus*				

Appendix F (continued)

IMS104-16	*Upeneus moluccensis*	*Upeneus moluccensis*	Species	BOLD:AAB6469	62	*Upeneus moluccensis*[60], *Upeneus sulphureus*[1]
IMS103-16	*Upeneus moluccensis*	*Upeneus moluccensis*				
IMS041-16	*Upeneus moluccensis*	*Upeneus moluccensis*				
IMS049-16	*Upeneus moluccensis*	*Upeneus moluccensis*				
IMS053-16	*Upeneus moluccensis*	*Upeneus moluccensis*				
IMS102-16	*Upeneus moluccensis*	*Upeneus moluccensis*				
IMS109-16	*Upeneus pori*	*Upeneus pori*	Species	BOLD:AAC1406	43	*Upeneus pori*[32], *Upeneus guttatus*[8]
IMS106-16	*Upeneus pori*	*Upeneus pori*				
IMS108-16	*Upeneus pori*	*Upeneus pori*				
IMS107-16	*Upeneus pori*	*Upeneus pori*				
IMS114-16	*Upeneus pori*	*Upeneus pori*				

APPENDIX F (continued)

CONCORDANT BIN'S

Process ID	Identification	BIN	BIN Total Members
IMS071-16	*Argentina sphyraena*	BOLD:AAB6547	45
IMS074-16	*Arnoglossus laterna*	BOLD:AAB5950	47
IMS073-16	*Arnoglossus laterna*		
IMS036-16	*Callionymus filamentosus*	BOLD:ACG9162	92
IMS035-16	*Callionymus filamentosus*		
IMS067-16	*Capros aper*		
IMS066-16	*Capros aper*	BOLD:AAB2992	35
IMS063-16	*Capros aper*		
IMS068-16	*Capros aper*		
IMS072-16	*Citharus linguatula*		
IMS056-16	*Citharus linguatula*	BOLD:AAC0204	63
IMS038-16	*Citharus linguatula*		
IMS044-16	*Conger conger*	BOLD:AAB6795	30
IMS031-16	*Cynoglossus sinusarabici*		
IMS030-16	*Cynoglossus sinusarabici*	BOLD:ACG6778	27
IMS032-16	*Cynoglossus sinusarabici*		
IMS029-16	*Cynoglossus sinusarabici*		
IMS054-16	*Lagocephalus suezensis*	BOLD:ACG7296	31
IMS059-16	*Lepidopus caudatus*	BOLD:AAB3173	52
IMS060-16	*Lepidopus caudatus*		
IMS078-16	*Lepidotrigla cavillone*		
IMS076-16	*Lepidotrigla cavillone*		
IMS062-16	*Lepidotrigla cavillone*	BOLD:AAB7558	19
IMS077-16	*Lepidotrigla cavillone*		
IMS075-16	*Lepidotrigla cavillone*		
IMS090-16	*Ostorhinchus fasciatus*		
IMS033-16	*Ostorhinchus fasciatus*		
IMS089-16	*Ostorhinchus fasciatus*	BOLD:AAC1243	31
IMS088-16	*Ostorhinchus fasciatus*		
IMS091-16	*Ostorhinchus fasciatus*		
IMS040-16	*Sargocentron rubrum*	BOLD:ACG8706	6
IMS087-16	*Trigloporus lastoviza*	BOLD:AAB8166	15
IMS055-16	*Zeus faber*	BOLD:AAA7905	41

SINGLETONS

Process ID	Identification	BIN
IMS027-16	*Boops boops*	BOLD:ACZ0394
IMS057-16	*Callionymus filamentosus*	BOLD:ACZ0579

APPENDIX G

BOLD ID ENGINE RESULTS FOR INVERTEBRATE SAMPLES

Sample ID	Top Hit
IMSA2	Arthropoda - Amphipoda (99.01%)
IMSA4	Echinodermata - Ophiurida - *Ophiactis savignyi* (99.84%)
IMSA5	Echinodermata - Ophiurida - *Ophiactis savignyi* (100%)
IMSA9	Arthropoda - Decapoda - Alpheidae (99.83%)
IMSA11	Mollusca - Arcoida (98.49%)
IMSA12	Arthropoda - Decapoda - Alpheidae (99.83%)
IMSA13	Sipuncula - Phascolosomatida - *Apionsoma sp.* (100%)
IMSA15	Arthropoda - Decapoda (100%)
IMSA18	Annelida - Eunicidae (97.4%)
IMSA19	Mollusca - Arcoida (98.49%)
IMSA21	Arthropoda - Amphipoda (99.01%)
IMSA22	Arthropoda - Amphipoda (99.01%)
IMSA23	Sipuncula - Phascolosomatida - *Apionsoma sp.* (98.98%)
IMSA24	Echinodermata - Ophiurida - Ophiactis savignyi (99.84%)
IMSA54	Arthropoda - Decapoda - Portunidae (100%)
IMSA55	Arthropoda - Decapoda - Portunidae (100%)
IMSA56	Mollusca - Myopsida - *Alloteuthis media* (100%)
IMSA57	Mollusca - Myopsida - *Alloteuthis media* (100%)
IMSA58	Mollusca - Myopsida - *Alloteuthis media* (100%)
IMSA59	Mollusca - Myopsida - *Alloteuthis media* (100%)
IMSA61	Mollusca - Pleurobranchidae (98.14%)
IMSA62	Arthropoda - Decapoda - *Dromia personata* (99.84%)
IMSA63	Mollusca - Pleurobranchidae (98.14%)
IMSA67	Mollusca - Nudibranchia - *Felimare picta* (99.36%)
IMSA68	Arthropoda - Decapoda - *Paguristes sp.* (99.84%)
IMSA69	Mollusca - Carditidae (99.24%)
IMSA70	Arthropoda - Decapoda - *Dromia personata* (99.84%)
IMSA72	Arthropoda - Decapoda - *Pagurus prideaux* (100%)
IMSA74	Arthropoda - Decapoda - *Pagurus prideaux* (99.84%)
IMSA75	Arthropoda - Decapoda - *Pisa armata* (100%)
IMSA76	Arthropoda - Decapoda - *Pagurus prideaux* (100%)
IMSA77	Arthropoda - Decapoda - *Pagurus prideaux* (100%)
IMSA78	Mollusca - Neogastropoda - *Hexaplex trunculus* (100%)
IMSA80	Mollusca - Neogastropoda - *Bolinus brandaris* (99.84%)
IMSA81	Mollusca - Neogastropoda - *Bolinus brandaris* (99.84%)
IMSA82	Mollusca - Neogastropoda - *Hexaplex trunculus* (100%)
IMSA83	Arthropoda - Decapoda - *Paguristes sp.* (100%)
IMSA84	Mollusca - Neogastropoda - *Bolinus brandaris* (99.83%)
IMSA85	Mollusca - Neogastropoda - *Hexaplex trunculus* (100%)

Appendix G (continued)

IMSA87	Mollusca - Neogastropoda - *Bolinus brandaris* (99.83%)
IMSA89	Mollusca - Neogastropoda - *Hexaplex trunculus* (100%)
IMSA90	Mollusca - Neogastropoda - *Hexaplex trunculus* (99.68%)
IMSA92	Echinodermata - Ophiurida - *Ophiothrix fragilis* (100%)
IMSA94	Echinodermata - Ophiurida - *Ophiothrix fragilis* (100%)
IMSA95	Echinodermata - Ophiurida - *Ophiothrix fragilis* (100%)
IMSA96	Echinodermata - Ophiurida - *Ophiothrix sp.* (100%)
IMSA98	Annelida - Polychaeta (97.6%)
IMSA102	Chordata - Enterogona - *Cystodytes sp.* (99.68%)
IMSB8	Arthropoda - Decapoda (99.68%)
IMSB9	Arthropoda - Decapoda - *Paguristes sp.* (99.12%)
IMSB10	Mollusca - Mytilidae (99.24%)
IMSB12	Mollusca - Neogastropoda - *Hexaplex trunculus* (100%)
IMSB15	Arthropoda - Sessilia - *Balanus trigonus* (99.04%)
IMSB17	Chordata - Stolidobranchia - *Styela plicata* (99.69%)
IMSB18	Chordata - Ascidiacea (100%)
IMSB22	Mollusca - Ostreoida - *Dendostrea sp.* (99.24%)
IMSB30	Echinodermata - Ophiurida - *Ophiothrix fragilis* (99.63%)
IMSB31	Arthropoda - Decapoda - *Pagurus prideaux* (100%)
IMSB33	Mollusca - Myopsida - *Alloteuthis subulata* (100%)
IMSB40	Echinodermata - Comatulida - *Antedon mediterranea* (100%)
IMSB41	Mollusca - Nudibranchia (98.63%)
IMSB42	Annelida - Amphinomida - *Hermodice carunculata* (99.5%)
IMSC9	Mollusca - Sepiida - *Sepia officinalis* (99.84%)
IMSC10	Mollusca - Sepiida - *Sepia officinalis* (99.84%)
IMSC25	Mollusca - Octopoda - *Octopus vulgaris* (100%)
IMSC59	Mollusca - Sepiolida - *Rossia macrosoma* (99.53%)
IMSC60	Mollusca - Sepiolida - *Sepietta oweniana* (100%)
IMSC61	Mollusca - Sepiolida - *Sepietta oweniana* (100%)
IMSC62	Mollusca - Sepiida (100%)
IMSC63	Mollusca - Sepiida (100%)
IMSF1	Arthropoda - Decapoda - Ocypodidae (99.12%)
IMSF2	Arthropoda - Decapoda - *Pachygrapsus marmoratus* (100%)
IMSF3	Arthropoda - Decapoda - *Pachygrapsus marmoratus* (100%)
IMSF4	Annelida - Opheliidae (98.53%)
IMSF5	Annelida - Opheliidae (98.86%)
IMSF6	Annelida - Opheliidae (98.86%)
IMSF7	Mollusca - Archaeogastropoda - *Phorcus richardi* (99.69%)
IMSF8	Mollusca - Archaeogastropoda - *Phorcus mutabilis* (99.68%)
IMSF9	Arthropoda - Isopoda-Sphaeroma serratum (99.84%)
IMSF10	Mollusca - Archaeogastropoda - *Phorcus richardi* (99.69%)
IMSF11	Arthropoda - Isopoda-*Sphaeroma serratum* (99.84%)
IMSF12	Arthropoda - Isopoda-*Sphaeroma serratum* (99.84%)
IMSF14	Arthropoda - Sessilia - *Amphibalanus amphitrite* (100%)
IMSF16	Chordata - Stolidobranchia - *Styela sp.* (99.21%)
IMSF18	Arthropoda - Isopoda - *Sphaeroma serratum* (100%)

APPENDIX H

BOLD REGISTRY INFORMATION FOR INVERTEBRATE SAMPLES

Sample ID	Morphological Identification	Process ID	BIN
IMSA59	*Alloteuthis media*	IMS135-16	BOLD:AAB2767
IMSA57	*Alloteuthis media*	IMS133-16	BOLD:AAB2767
IMSA56	*Alloteuthis media*	IMS132-16	BOLD:AAB2767
IMSA58	*Alloteuthis media*	IMS132-16	BOLD:AAB2767
IMSB33	*Alloteuthis subulata*	IMS167-16	BOLD:AAE5562
IMSA15	Alpheidae	IMS123-16	BOLD:ACZ9063
IMSA9	Alpheidae	IMS157-16	BOLD:ACZ9063
IMSA12	Alpheidae	IMS121-16	BOLD:ACZ9064
IMSF14	*Amphibalanus amphitrite*	IMS186-16	BOLD:AAO4493
IMSA21	Amphipoda	IMS127-16	BOLD:ADA0996
IMSA22	Amphipoda	IMS128-16	BOLD:ADA0996
IMSA2	Amphipoda	IMS126-16	BOLD:ADA0996
IMSB40	*Antedon mediterranea*	IMS241-16	BOLD:ADA9883
IMSA13	*Apionsoma sp.*	IMS122-16	BOLD:ACH2635
IMSA23	*Apionsoma sp.*	IMS129-16	
IMSA11	Arcoida	IMS120-16	BOLD:ACZ8051
IMSA19	Arcoida	IMS125-16	
IMSB18	Ascidiacea	IMS164-16	BOLD:ACZ8491
IMSB15	*Balanus trigonus*	IMS162-16	BOLD:AAI7707
IMSA81	*Bolinus brandaris*	IMS150-16	BOLD:ADA0660
IMSA80	*Bolinus brandaris*	IMS149-16	BOLD:ADA0660
IMSA84	*Bolinus brandaris*	IMS153-16	BOLD:ADA0660
IMSA87	*Bolinus brandaris*	IMS155-16	BOLD:ADA0660
IMSA69	Carditidae	IMS141-16	
IMSA102	*Cystodytes sp.*	IMS119-16	BOLD:ACZ8855
IMSB8	Decapoda	IMS170-16	BOLD:ACX0604
IMSB22	*Dendostrea sp.*	IMS165-16	BOLD:ADA0155
IMSA62	*Dromia sp.*	IMS137-16	BOLD:ADA0350
IMSA70	*Dromia sp.*	IMS142-16	BOLD:ADA0350
IMSA18	Eunicidae	IMS124-16	BOLD:ACZ9855
IMSA67	*Felimare picta*	IMS139-16	BOLD:ACV7506
IMSB42	*Hermodice carunculata*	IMS169-16	BOLD:AAB3315
IMSA82	*Hexaplex trunculus*	IMS151-16	BOLD:ACG9353
IMSA78	*Hexaplex trunculus*	IMS148-16	BOLD:ACG9353
IMSA85	*Hexaplex trunculus*	IMS154-16	BOLD:ACG9353
IMSA89	*Hexaplex trunculus*	IMS156-16	BOLD:ACG9353
IMSA90	*Hexaplex trunculus*	IMS158-16	BOLD:ACG9353
IMSB12	*Hexaplex trunculus*	IMS161-16	BOLD:ACG9353
IMSB10	Mytilidae	IMS160-16	BOLD:ACG9004

Appendix H (continued)

IMSB41	Nudibranchia	IMS168-16	BOLD:ACZ7842
IMSC25	*Octopus vulgaris*	IMS173-16	BOLD:AAB0290
IMSF1	Ocypodidae	IMS181-16	
IMSF4	Opheliidae	IMS191-16	BOLD:ACZ7830
IMSF6	Opheliidae	IMS193-16	BOLD:ACZ7830
IMSF5	Opheliidae	IMS192-16	BOLD:ACZ7830
IMSA24	*Ophiactis savignyi*	IMS236-16	BOLD:ABA2117
IMSA5	*Ophiactis savignyi*	IMS235-16	BOLD:ABA2117
IMSA4	*Ophiactis savignyi*	IMS234-16	BOLD:ABA2117
IMSA94	*Ophiothrix fragilis*	IMS238-16	BOLD:AAW5572
IMSB30	*Ophiothrix fragilis*	IMS242-16	BOLD:AAW5572
IMSA95	*Ophiothrix fragilis*	IMS239-16	BOLD:AAW5572
IMSA92	*Ophiothrix fragilis*	IMS237-16	BOLD:AAW5572
IMSA96	*Ophiothrix sp.*	IMS240-16	BOLD:ACA4134
IMSF2	*Pachygrapsus marmoratus*	IMS189-16	BOLD:AAE4277
IMSF3	*Pachygrapsus marmoratus*	IMS190-16	BOLD:AAE4277
IMSB9	*Paguristes sp.*	IMS171-16	BOLD:ACZ8112
IMSA83	*Paguristes sp.*	IMS152-16	BOLD:ACZ8112
IMSA68	*Paguristes sp.*	IMS140-16	BOLD:ACZ8112
IMSA72	*Pagurus prideaux*	IMS143-16	BOLD:AAB0339
IMSA76	*Pagurus prideaux*	IMS146-16	BOLD:AAB0339
IMSA77	*Pagurus prideaux*	IMS147-16	BOLD:AAB0339
IMSB31	*Pagurus prideaux*	IMS166-16	BOLD:AAB0339
IMSA74	*Pagurus prideaux*	IMS144-16	BOLD:AAB0339
IMSF8	*Phorcus mutabilis*	IMS195-16	BOLD:ACA1721
IMSF10	*Phorcus richardi*	IMS182-16	
IMSF7	*Phorcus richardi*	IMS194-16	BOLD:ACA1540
IMSA75	*Pisa armata*	IMS145-16	
IMSA61	Pleurobranchidae	IMS136-16	BOLD:ACZ8325
IMSA63	Pleurobranchidae	IMS138-16	
IMSA98	Polychaeta	IMS159-16	BOLD:ACZ7668
IMSA54	Portunidae	IMS130-16	BOLD:ADA0410
IMSA55	Portunidae	IMS131-16	BOLD:ADA0410
IMSC59	*Rossia macrosoma*	IMS174-16	BOLD:ACI9643
IMSC9	*Sepia officinalis*	IMS180-16	BOLD:AAA1559
IMSC10	*Sepia officinalis*	IMS172-16	BOLD:AAA1559
IMSC61	*Sepietta oweniana*	IMS176-16	BOLD:AAH9800
IMSC60	*Sepietta oweniana*	IMS175-16	BOLD:AAH9800
IMSC63	Sepiida	IMS178-16	BOLD:ACZ9292
IMSC62	Sepiida	IMS177-16	BOLD:ACZ9292
IMSF18	*Sphaeroma serratum*	IMS188-16	BOLD:ACZ9522
IMSF11	*Sphaeroma serratum*	IMS183-16	
IMSF12	*Sphaeroma serratum*	IMS184-16	
IMSF9	*Sphaeroma serratum*	IMS196-16	BOLD:ACZ9509
IMSB17	*Styela plicata*	IMS163-16	BOLD:AAC0645
IMSF16	*Styela sp.*	IMS187-16	BOLD:ACZ8324

APPENDIX I

BIN DISCORDANCE ANALYSIS RESULTS FOR INVERTEBRATE SAMPLES

DISCORDANT BIN'S

Process ID	Identification	Conflicting Taxon in BIN	Rank of Conflict	BIN	BIN Total Members	BIN Tax Variation
IMS235-16	*Ophiactis savignyi*	Ophiactidae	Family	BOLD: ABA2117	40	Ophiactidae[6], Ophiocomidae[1]
IMS234-16	*Ophiactis savignyi*	Ophiactidae				
IMS236-16	*Ophiactis savignyi*	Ophiactidae				
IMS186-16	*Amphibalanus amphitrite*	*Amphibalanus*	Genus	BOLD:AAO44	116	*Amphibalanus*[110], *Balanus*[4]
IMS169-16	*Hermodice carunculata*	*Hermodice*	Genus	BOLD:AAB331	223	*Hermodice*[222], *Eurythoe*[1]
IMS135-16	*Alloteuthis media*	*Alloteuthis media*	Genus	BOLD: AAB2767	170	*Alloteuthis media*[141], *Alloteuthis sp*.[28], *Alloteuthis subulata*[1]
IMS134-16	*Alloteuthis media*	*Alloteuthis media*				
IMS132-16	*Alloteuthis media*	*Alloteuthis media*				
IMS133-16	*Alloteuthis media*	*Alloteuthis media*				
IMS122-16	*Apionsoma sp.*	*Apionsoma sp.*	Species	BOLD: ACH2635	2	*Apionsoma sp*.[1], *Apionsoma misakianum*[1]
IMS162-16	*Balanus trigonus*	*Balanus trigonus*	Species	BOLD: AAI7707	25	*Balanus trigonus*[12], *Balanus amphitrite*[2]
IMS137-16	*Dromia sp.*	*Dromia sp.*	Species	BOLD: ADA0350	3	*Dromia sp*.[2], *Dromia personata*[1]
IMS142-16	*Dromia sp.*	*Dromia sp.*				
IMS172-16	*Sepia officinalis*	*Sepia officinalis*	Species	BOLD: AAA1559	317	*Sepia officinalis*[314], *Sepia tenuipes*[1]
IMS180-16	*Sepia officinalis*	*Sepia officinalis*				

Appendix I (continued)

IMS176-16	*Sepietta oweniana*	*Sepietta oweniana*	Species	BOLD: AAH9800	11	*Sepietta oweniana*[10], *Sepietta neglecta*[1]
IMS175-16	*Sepietta oweniana*	*Sepietta oweniana*				
IMS163-16	*Styela plicata*	*Styela plicata*	Species	BOLD: AAC0645	40	*Styela plicata*[37], *Styela partita*[1]

APPENDIX I (continued)

CONCORDANT BIN'S

Process ID	Identification	BIN	BIN Total Members
IMS128-16	Amphipoda		
IMS126-16	Amphipoda	BOLD:ADA0996	3
IMS127-16	Amphipoda		
IMS170-16	Decapoda	BOLD:ACX0604	2
IMS177-16	Sepiida	BOLD:ACZ9292	2
IMS178-16	Sepiida		
IMS123-16	Alpheidae	BOLD:ACZ9063	2
IMS157-16	Alpheidae		
IMS193-16	Opheliidae		
IMS191-16	Opheliidae	BOLD:ACZ7830	3
IMS192-16	Opheliidae		
IMS167-16	*Alloteuthis subulata*	BOLD:AAE5562	22
IMS155-16	*Bolinus brandaris*		
IMS153-16	*Bolinus brandaris*	BOLD:ADA0660	6
IMS149-16	*Bolinus brandaris*		
IMS150-16	*Bolinus brandaris*		
IMS139-16	*Felimare picta*	BOLD:ACV7506	2
IMS148-16	*Hexaplex trunculus*		
IMS151-16	*Hexaplex trunculus*		
IMS154-16	*Hexaplex trunculus*	BOLD:ACG9353	46
IMS156-16	*Hexaplex trunculus*		
IMS158-16	*Hexaplex trunculus*		
IMS161-16	*Hexaplex trunculus*		
IMS160-16	Mytilidae	BOLD:ACG9004	2
IMS173-16	*Octopus vulgaris*	BOLD:AAB0290	243
IMS240-16	*Ophiothrix sp.*	BOLD:ACA4134	128
IMS238-16	*Ophiothrix fragilis*		
IMS242-16	*Ophiothrix fragilis*	BOLD:AAW5572	42
IMS239-16	*Ophiothrix fragilis*		
IMS237-16	*Ophiothrix fragilis*		
IMS189-16	*Pachygrapsus marmoratus*	BOLD:AAE4277	14
IMS190-16	*Pachygrapsus marmoratus*		
IMS152-16	*Paguristes sp.*		
IMS140-16	*Paguristes sp.*	BOLD:ACZ8112	3
IMS171-16	*Paguristes sp.*		
IMS147-16	*Pagurus prideaux*		
IMS143-16	*Pagurus prideaux*		
IMS144-16	*Pagurus prideaux*	BOLD:AAB0339	59
IMS146-16	*Pagurus prideaux*		
IMS166-16	*Pagurus prideaux*		

Appendix I (continued)

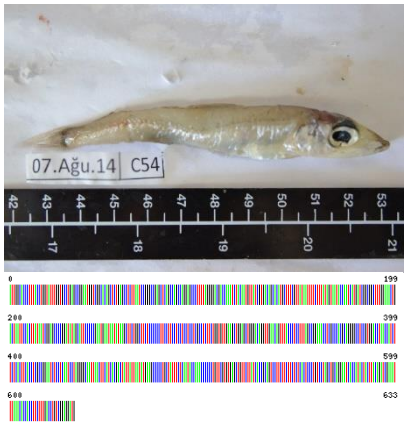
IMS195-16	*Phorcus mutabilis*	BOLD:ACA1721	5
IMS194-16	*Phorcus richardi*	BOLD:ACA1540	5
IMS131-16	Portunidae	BOLD:ADA0410	3
IMS130-16	Portunidae		
IMS174-16	*Rossia macrosoma*	BOLD:ACI9643	9
IMS188-16	*Sphaeroma serratum*	BOLD:ACZ9522	12

SINGLETONS

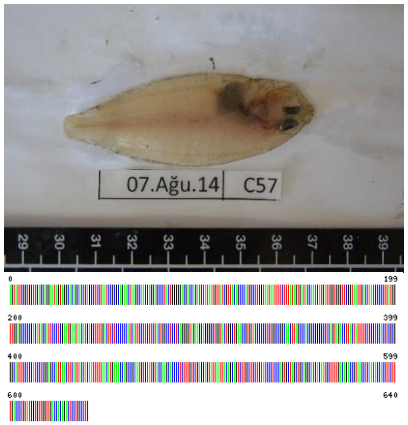
Process ID	Identification	BIN
IMS164-16	Ascidiacea	BOLD:ACZ8491
IMS159-16	Polychaeta	BOLD:ACZ7668
IMS120-16	Arcoida	BOLD:ACZ8051
IMS168-16	Nudibranchia	BOLD:ACZ7842
IMS121-16	Alpheidae	BOLD:ACZ9064
IMS124-16	Eunicidae	BOLD:ACZ9855
IMS136-16	Pleurobranchidae	BOLD:ACZ8325
IMS241-16	*Antedon mediterranea*	BOLD:ADA9883
IMS119-16	*Cystodytes sp.*	BOLD:ACZ8855
IMS165-16	*Dendostrea sp.*	BOLD:ADA0155
IMS196-16	*Sphaeroma serratum*	BOLD:ACZ9509
IMS187-16	*Styela sp.*	BOLD:ACZ8324

APPENDIX J

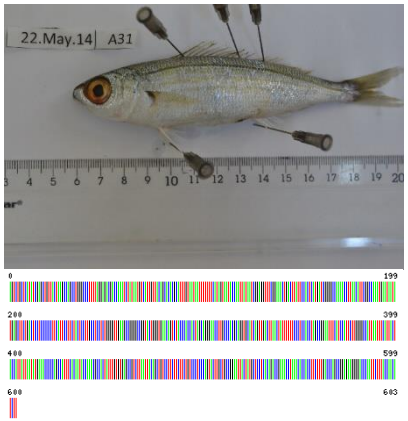
PHOTOS AND DNA BARCODES OF FISH SPECIMENS



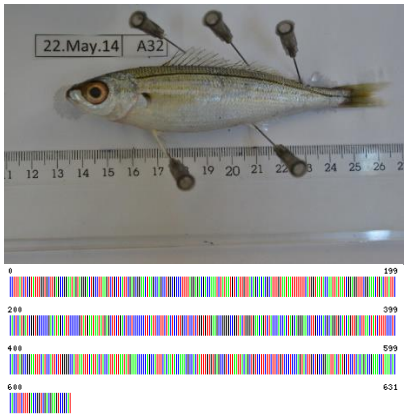
Argentina sphyraena
(IMS071-16)



Arnoglossus laterna
(IMS074-16)



Boops boops
(IMS027-16)



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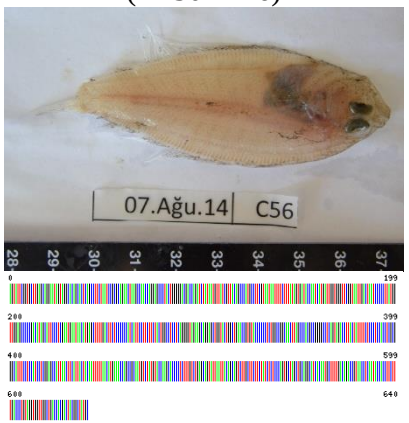
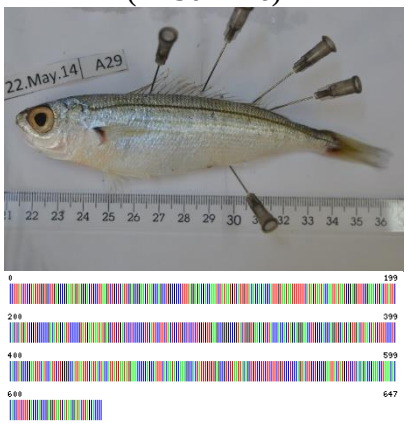
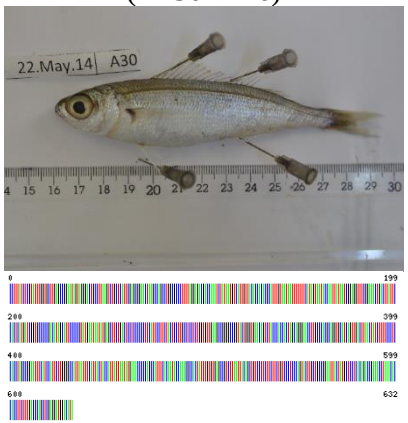
(IMS028-16)



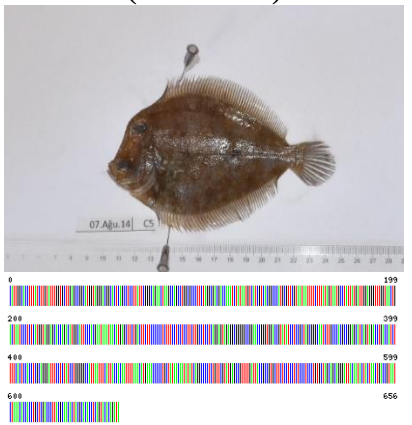
Arnoglossus laterna
(IMS073-16)



Boops boops
(IMS025-16)

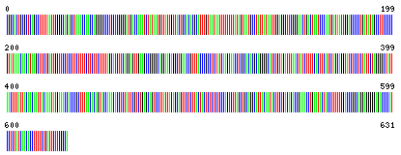


Boops boops
(IMS026-16)



Bothus podas
(IMS043-16)

Callionymus filamentosus
(IMS057-16)



Callionymus filamentosus
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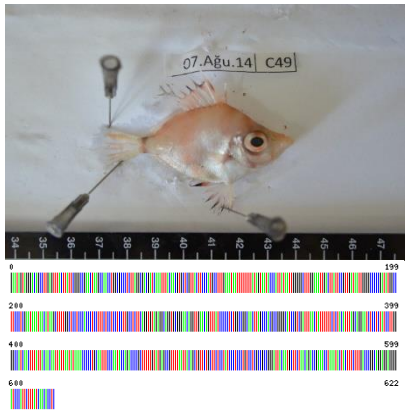
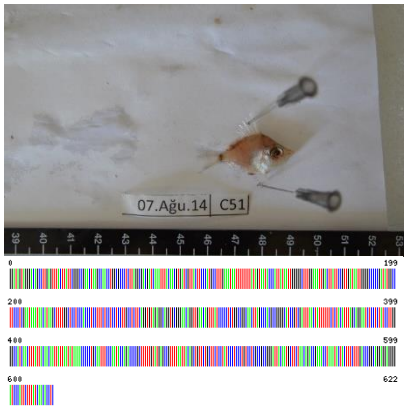
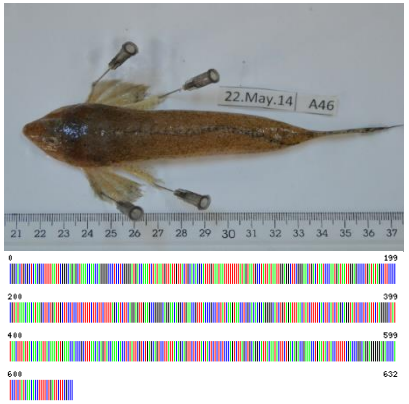


Capros aper
(IMS063-16)



Capros aper
(IMS068-16)



Callionymus filamentosus
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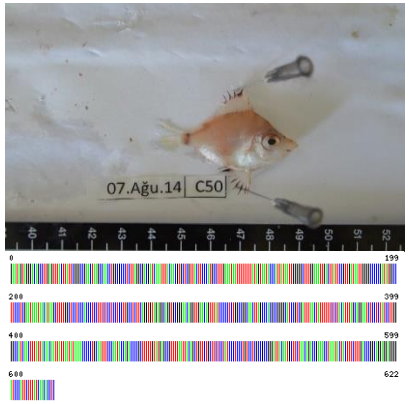
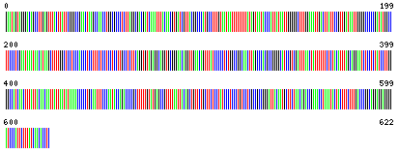
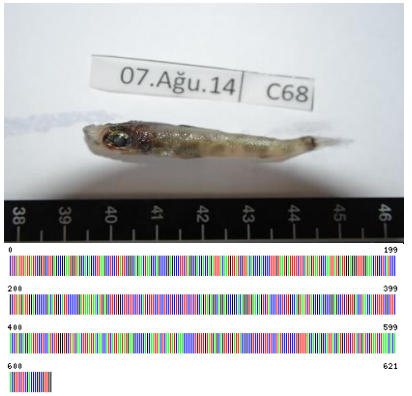


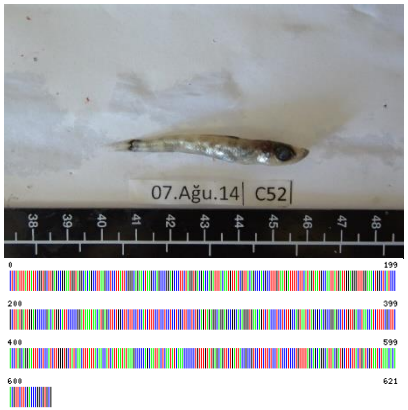
Capros aper
(IMS067-16)



Capros aper
(IMS066-16)

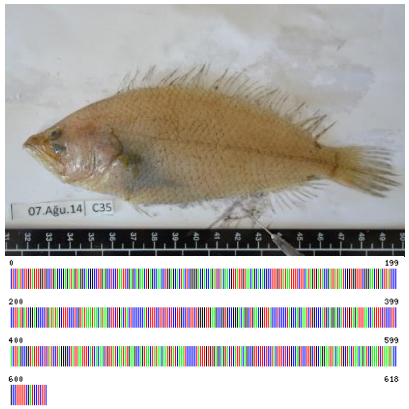


Chlorophthalmus agassizi
(IMS080-16)

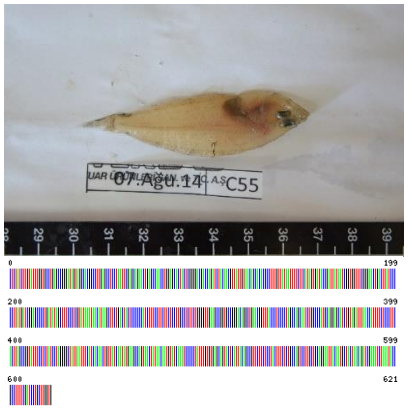


Chlorophthalmus agassizi
(IMS069-16)

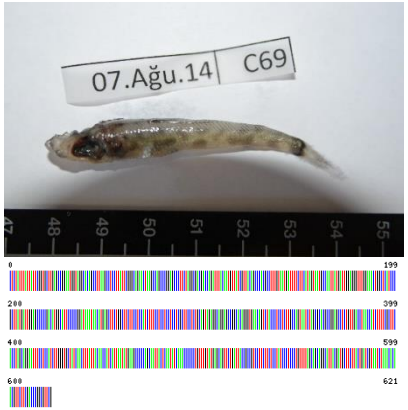
Chlorophthalmus agassizi
(IMS079-16)



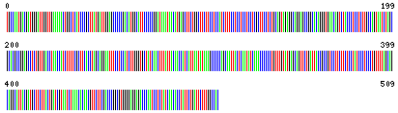
Citharus linguatula
(IMS056-16)



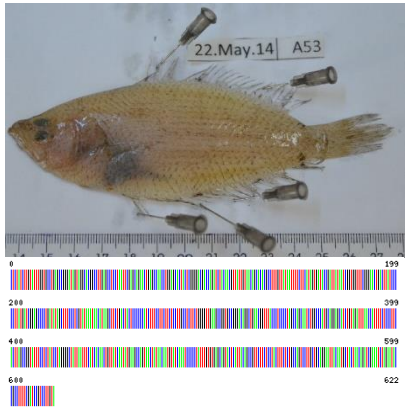
Citharus linguatula
(IMS072-16)



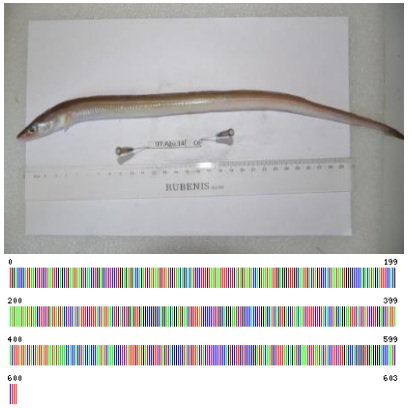
Chlorophthalmus agassizi
(IMS081-16)



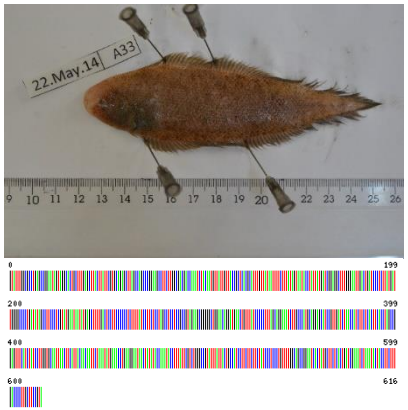
Chlorophthalmus agassizi
(IMS118-16)



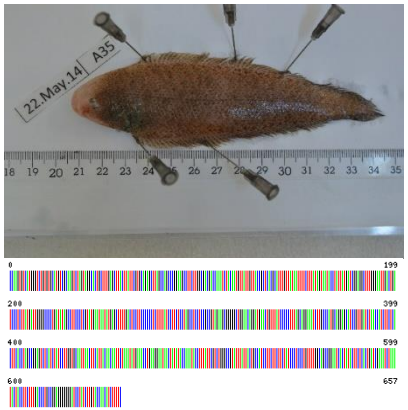
Citharus linguatula
(IMS038-16)



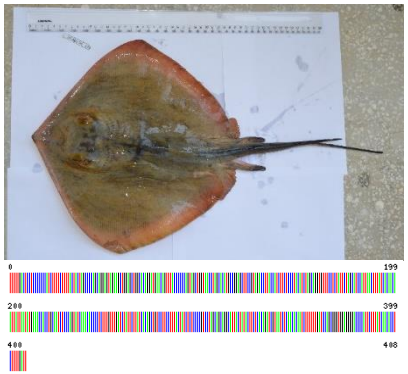
Conger conger
(IMS044-16)



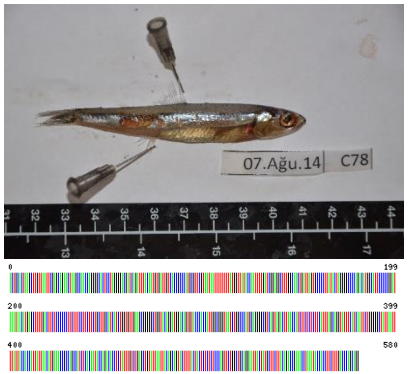
Cynoglossus sinusarabici
(IMS029-16)



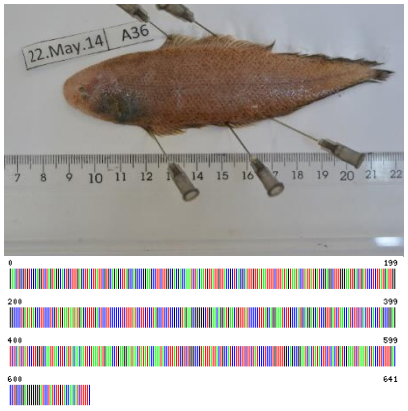
Cynoglossus sinusarabici
(IMS031-16)



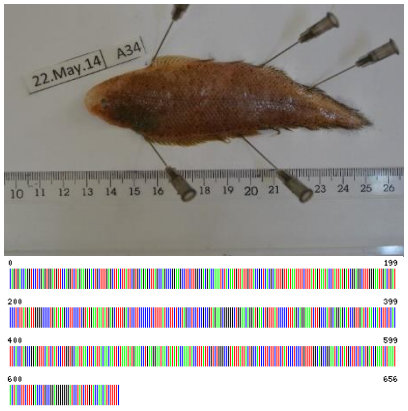
Dasyatis sp.
(IMS179-16)



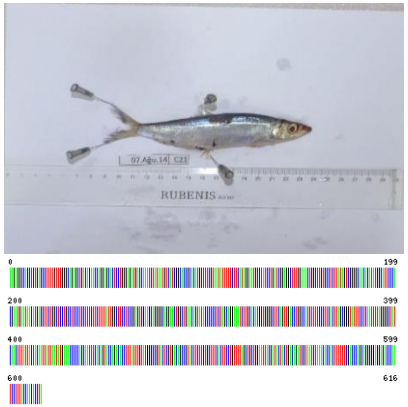
Encrasicholina punctifer
(IMS020-15)



Cynoglossus sinusarabici
(IMS032-16)



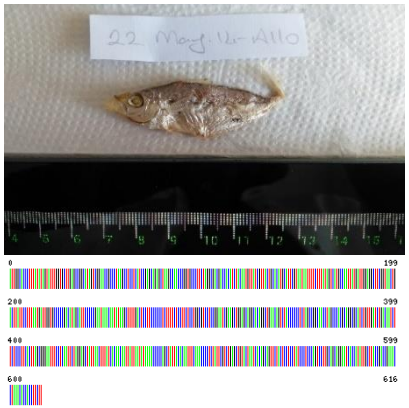
Cynoglossus sinusarabici
(IMS030-16)



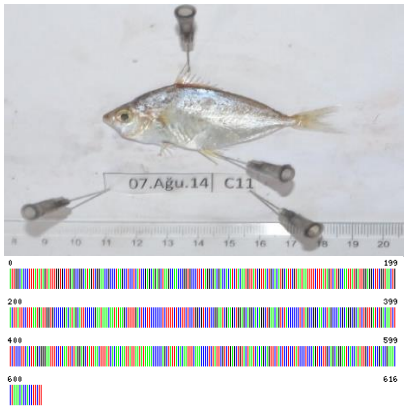
Dussumieria elopsoides
(IMS052-16)

Encrasicholina punctifer
(IMS019-15)

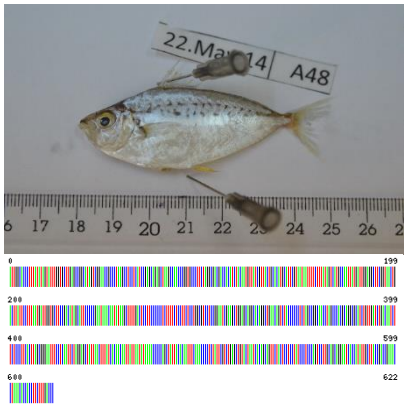
Engraulis encrasicolus
(IMS084-16)



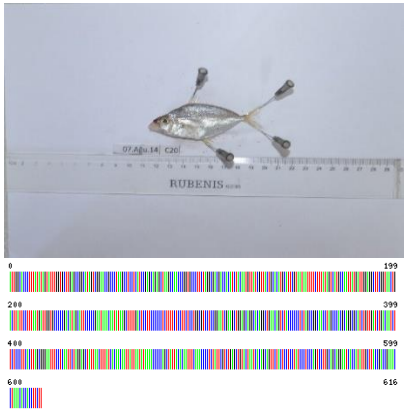
Equulites klunzingeri
(IMS085-16)



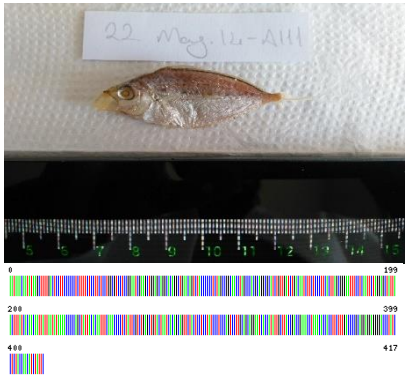
Equulites klunzingeri
(IMS045-16)



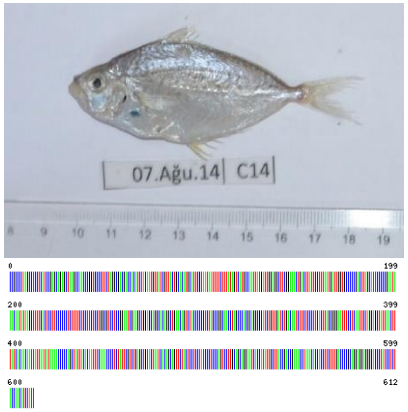
Equulites klunzingeri
(IMS037-16)



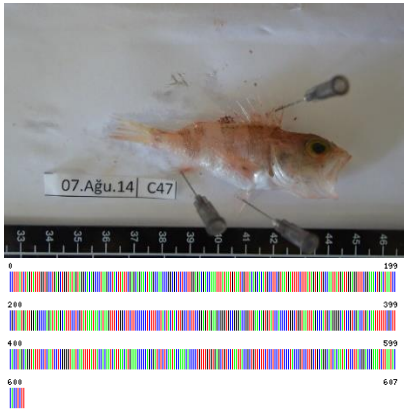
Equulites klunzingeri
(IMS051-16)



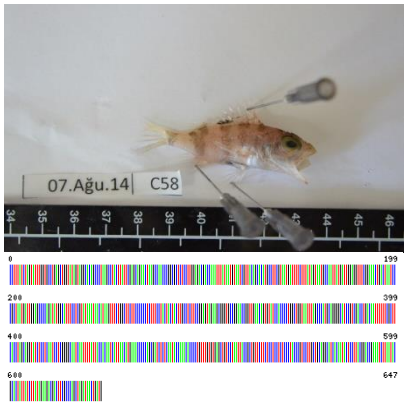
Equulites klunzingeri
(IMS086-16)



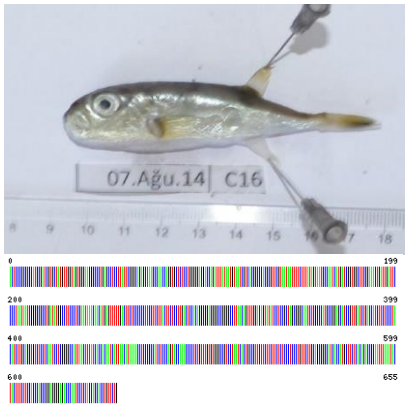
Equulites klunzingeri
(IMS046-16)



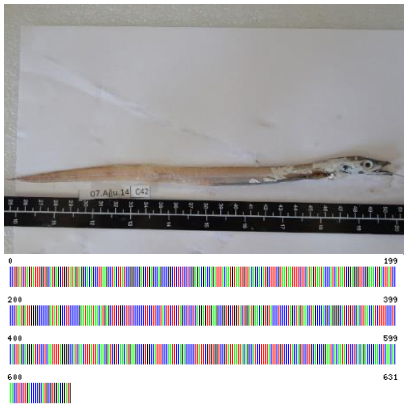
Helicolenus dactylopterus
(IMS064-16)



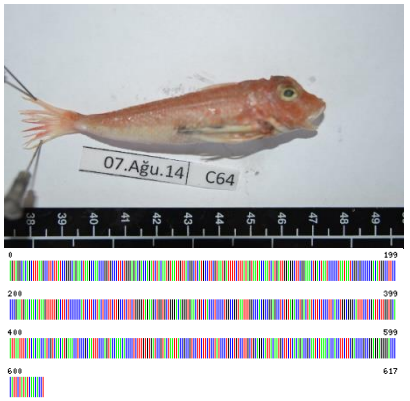
Helicolenus dactylopterus
(IMS070-16)



Lagocephalus spadiceus
(IMS048-16)

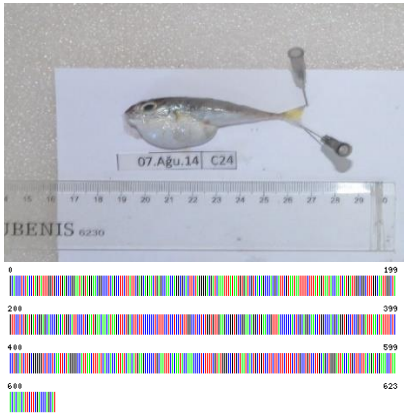


Lepidopus caudatus
(IMS060-16)

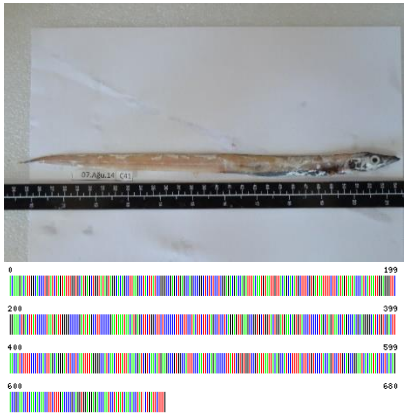


Lepidotrigla cavillone
(IMS076-16)

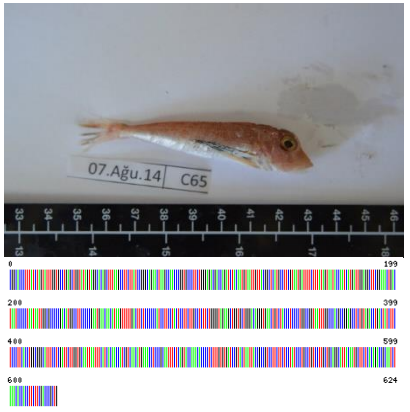
Hippocampus hippocampus
(IMS039-16)



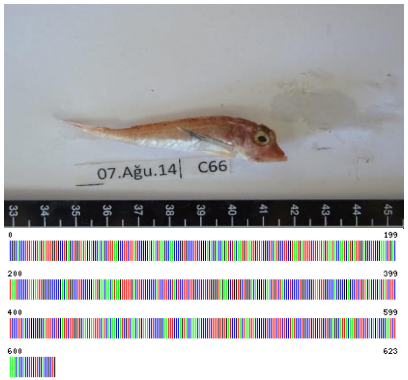
Lagocephalus suezensis
(IMS054-16)



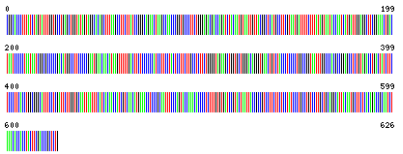
Lepidopus caudatus
(IMS059-16)



Lepidotrigla cavillone
(IMS077-16)



Lepidotrigla cavillone
(IMS078-16)

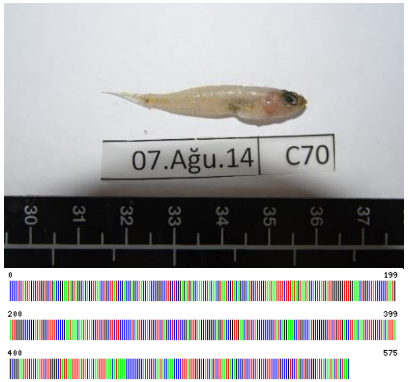


Lepidotrigla cavillone
(IMS075-16)

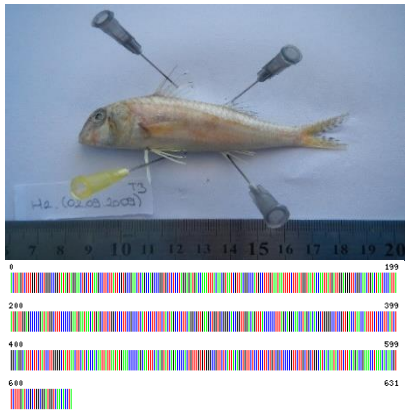
Lesueurigobius friesii
(IMS083-16)

Mullus barbatus
(IMS110-16)

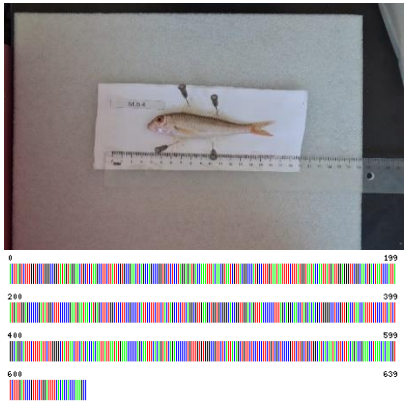
Lepidotrigla cavillone
(IMS062-16)



Lesueurigobius friesii
(IMS082-16) *



Mullus barbatus
(IMS111-16)



Mullus barbatus
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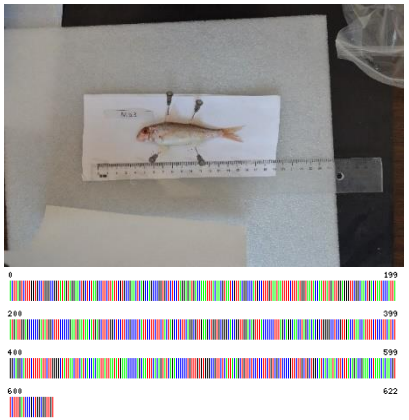
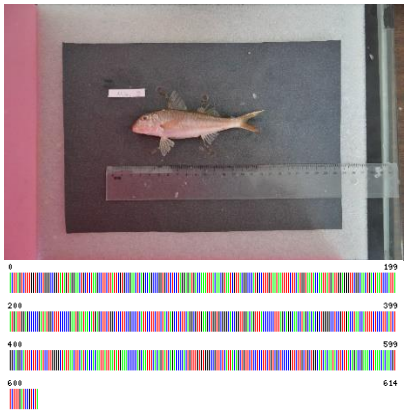


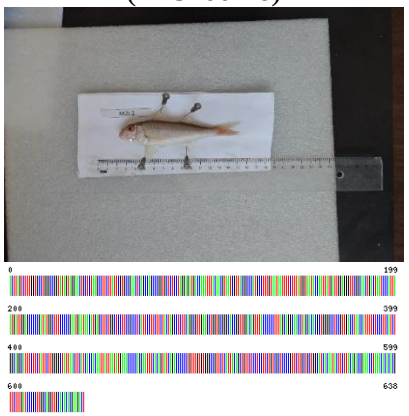
Mullus barbatus
(IMS100-16)

Mullus barbatus
(IMS098-16)

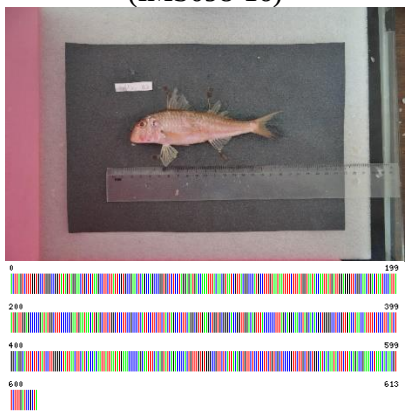
Mullus barbatus
(IMS096-16)



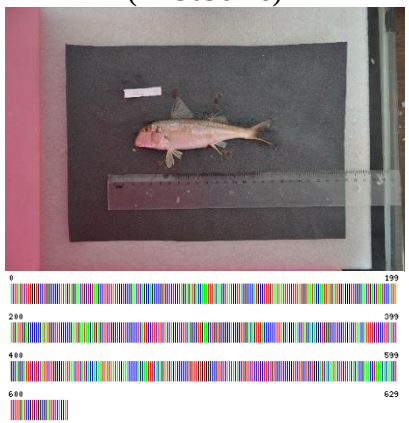
Mullus barbatus
(IMS094-16)



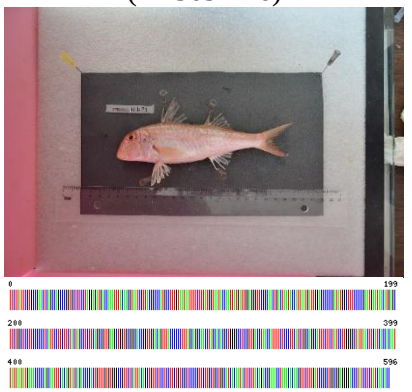
Mullus barbatus
(IMS099-16)



Mullus barbatus
(IMS097-16)

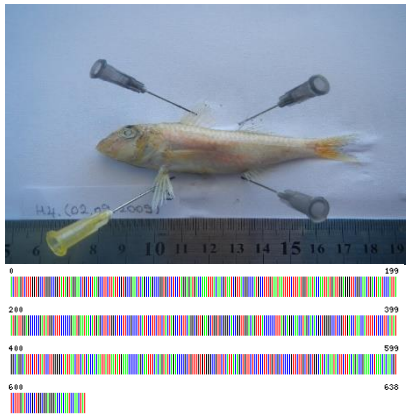


Mullus barbatus
(IMS095-16)

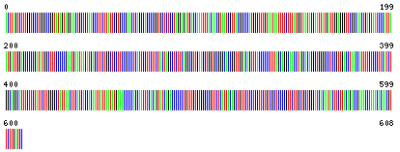


Mullus barbatus
(IMS093-16)

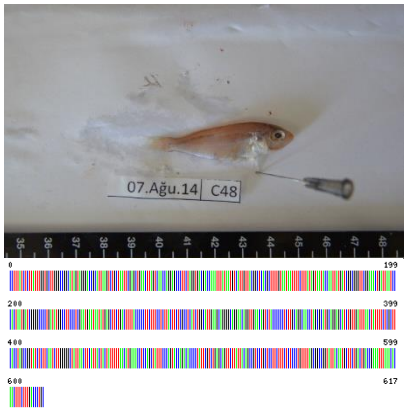
Mullus barbatus
(IMS092-16)



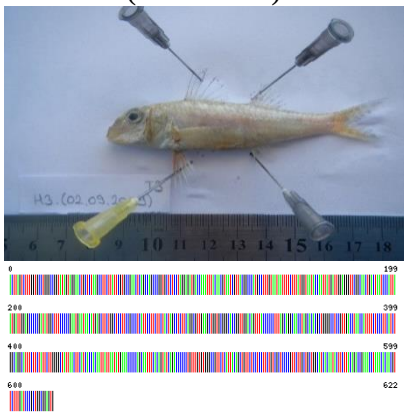
Mullus barbatus
(IMS113-16)



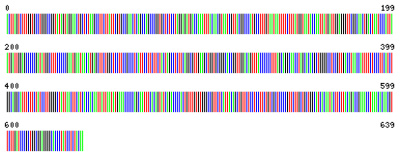
Mullus barbatus
(IMS116-16)



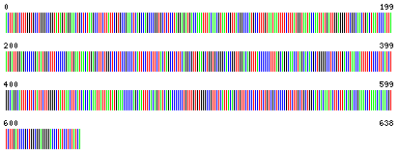
Nemipterus randalli
(IMS065-16)



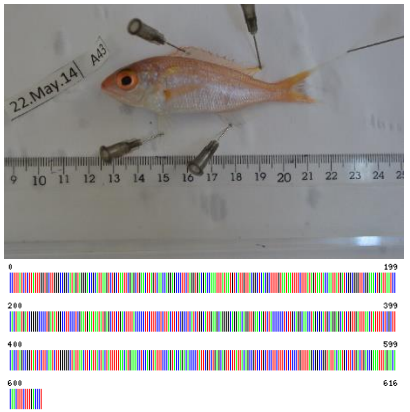
Mullus barbatus
(IMS112-16)



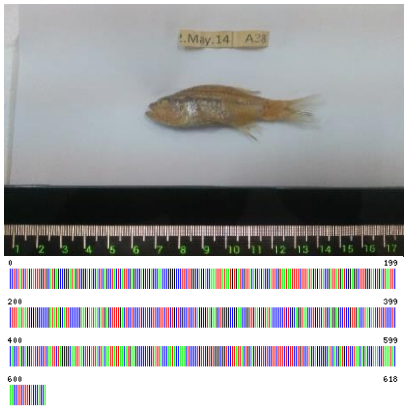
Mullus barbatus
(IMS115-16)



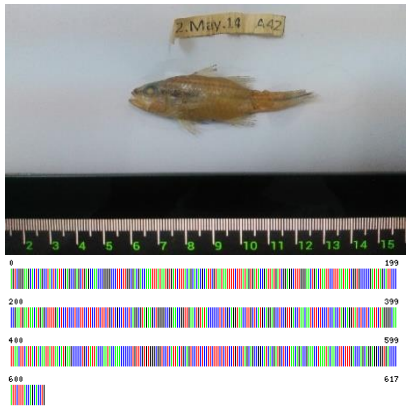
Mullus barbatus
(IMS117-16)



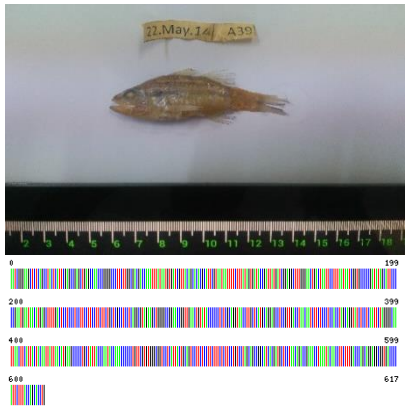
Nemipterus randalli
(IMS034-16)



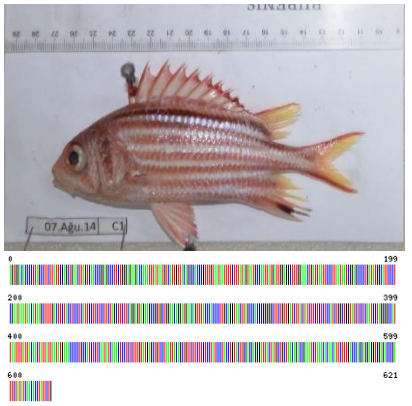
Ostorhinchus fasciatus
(IMS088-16)



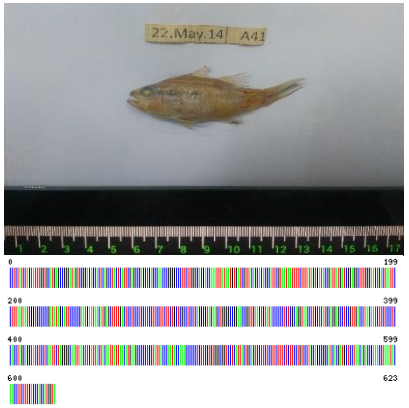
Ostorhinchus fasciatus
(IMS091-16)



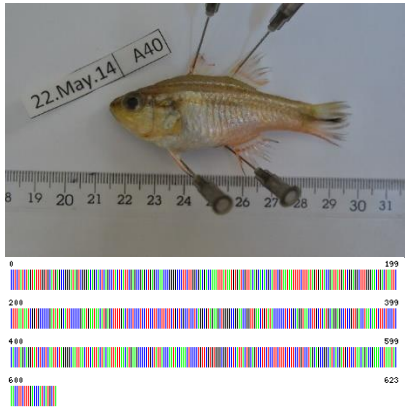
Ostorhinchus fasciatus
(IMS089-16)



Sargocentron rubrum
(IMS040-16)

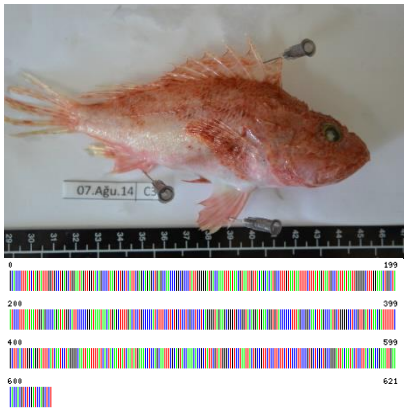


Ostorhinchus fasciatus
(IMS090-16)

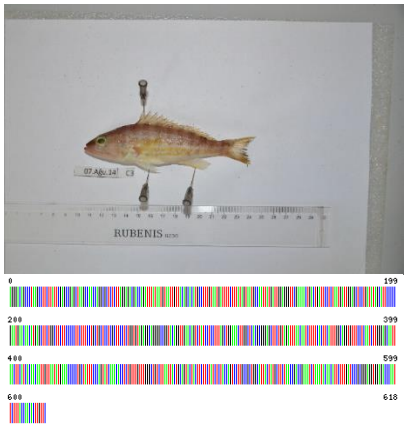


Ostorhinchus fasciatus
(IMS033-16)

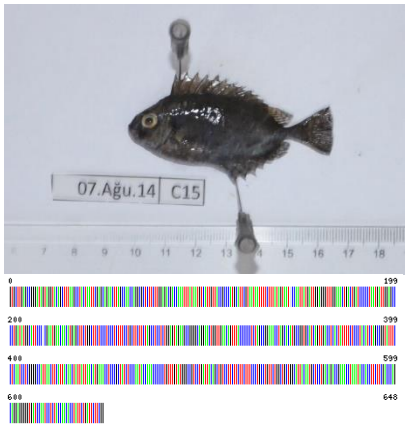
Oxyurichthys petersii
(IMS021-16)



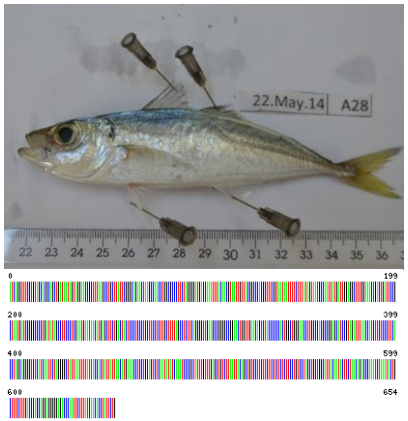
Scorpaena scrofa
(IMS061-16)



Serranus cabrilla
(IMS042-16)

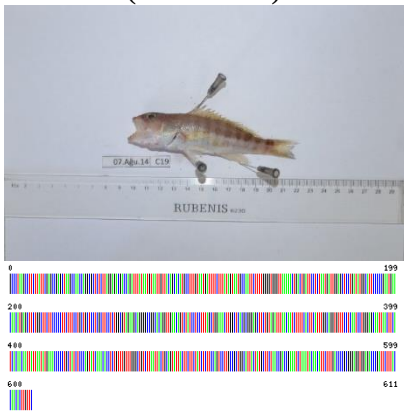


Siganus luridus
(IMS047-16)

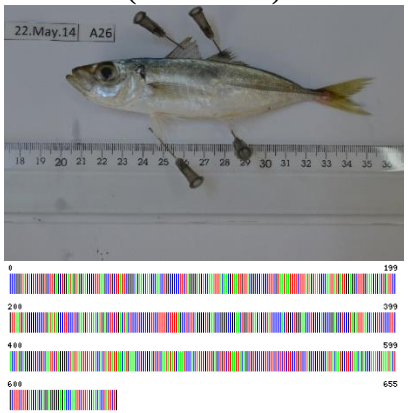


Trachurus mediterraneus
(IMS024-16)

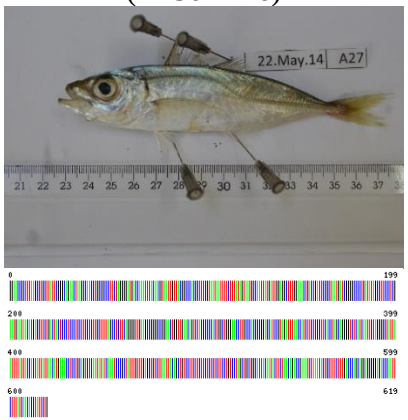
Trachurus trachurus
(IMS058-16)



Serranus cabrilla
(IMS050-16)

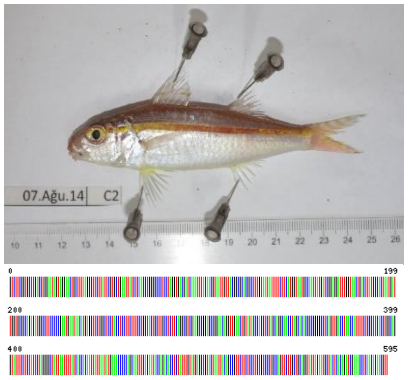


Trachurus mediterraneus
(IMS022-16)



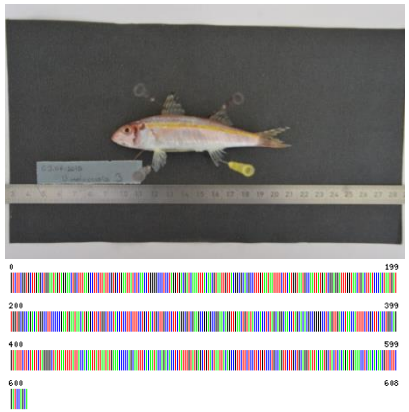
Trachurus mediterraneus
(IMS023-16)

Trigloporus lastoviza
(IMS087-16)

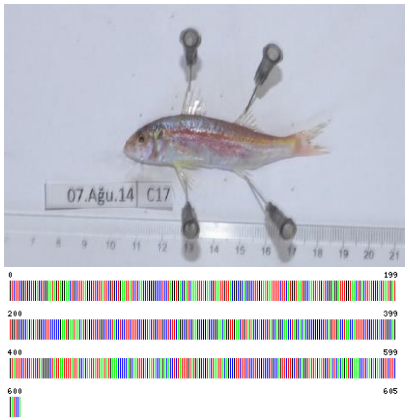


Upeneus moluccensis
(IMS041-16)

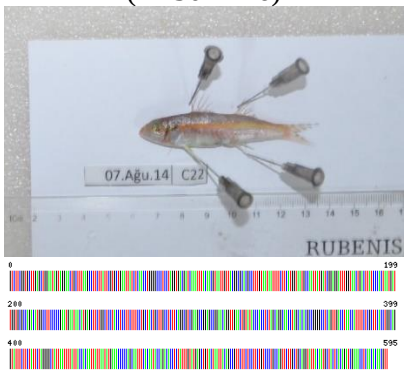
Upeneus moluccensis
(IMS102-16)



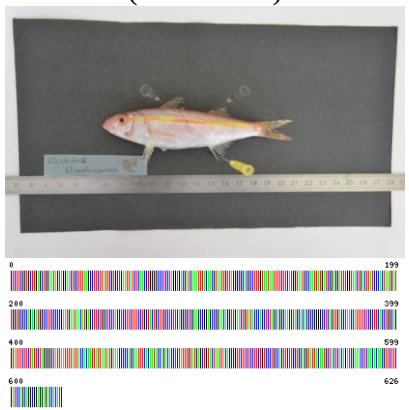
Upeneus moluccensis
(IMS104-16)



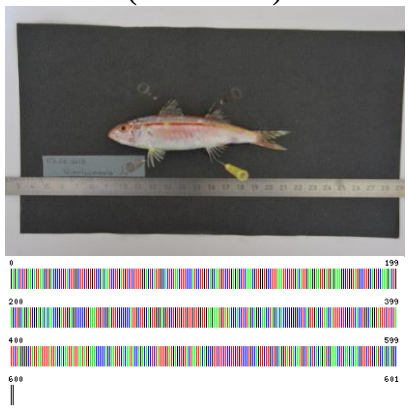
Upeneus moluccensis
(IMS049-16)



Upeneus moluccensis
(IMS053-16)

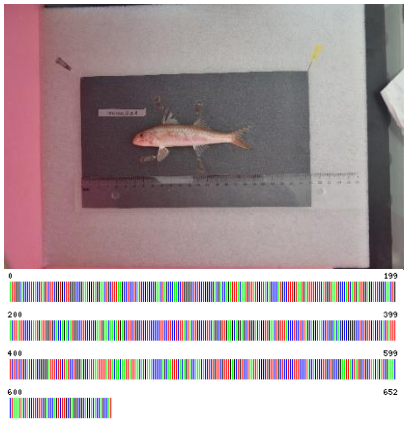


Upeneus moluccensis
(IMS103-16)



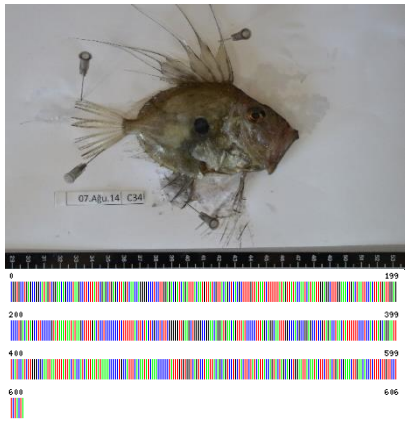
Upeneus moluccensis
(IMS105-16) *

Upeneus pori
(IMS108-16)

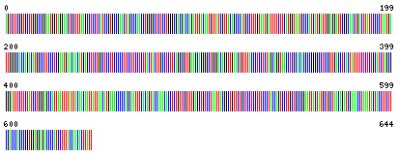


Upeneus pori
(IMS109-16)

Upeneus pori
(IMS106-16)



Zeus faber
(IMS055-16)

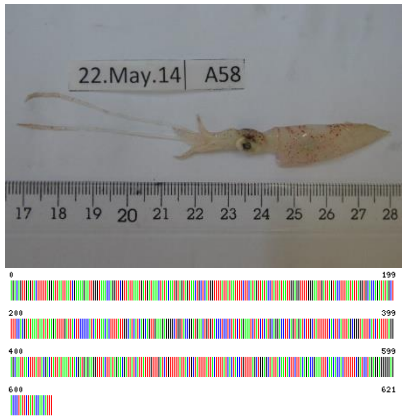


Upeneus pori
(IMS114-16)

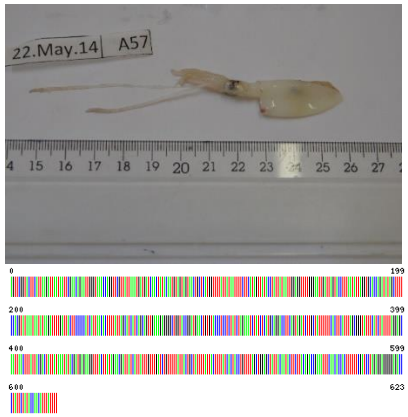
Upeneus pori
(IMS107-16)

APPENDIX K

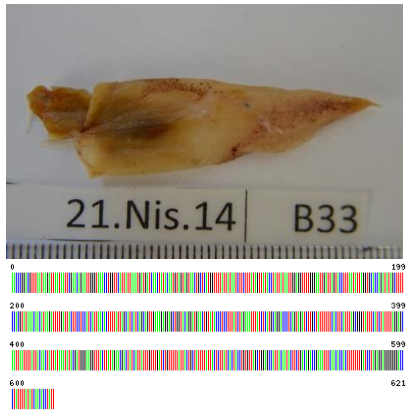
PHOTOS AND DNA BARCODES OF INVERTEBRATE AND ASCIDIAN SPECIMENS



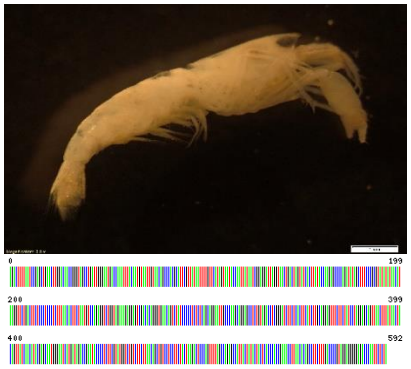
Alloteuthis media
(IMS134-16)



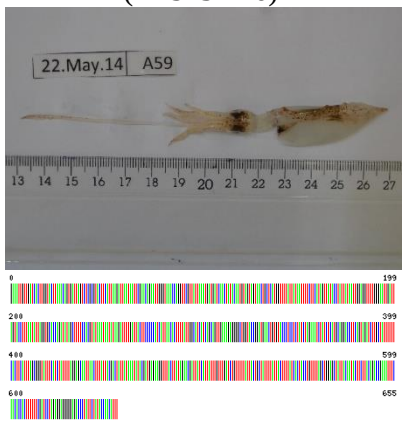
Alloteuthis media
(IMS133-16)



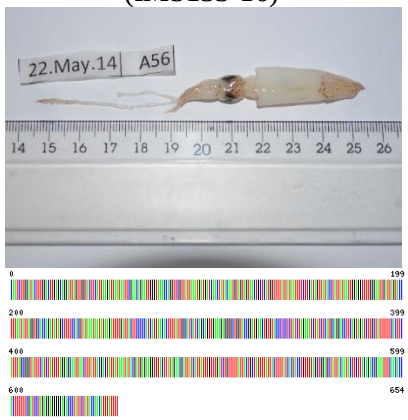
Alloteuthis subulata
(IMS167-16)



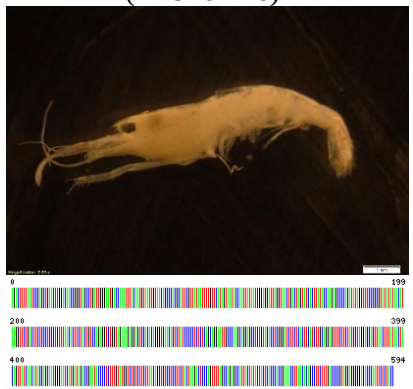
Alpheidae
(IMS157-16)



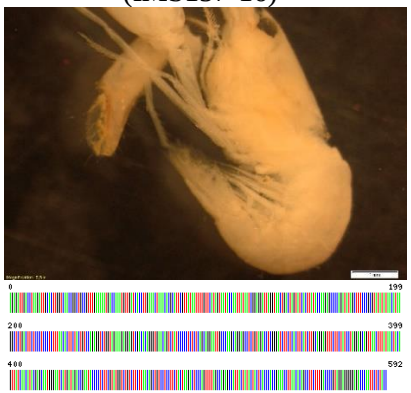
Alloteuthis media
(IMS135-16)



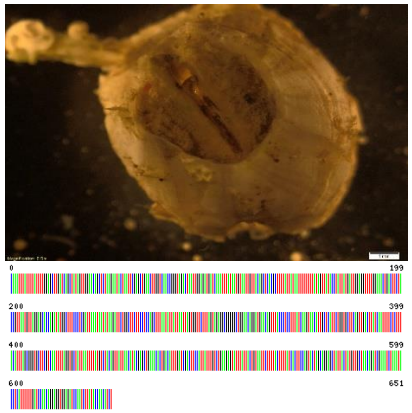
Alloteuthis media
(IMS132-16)



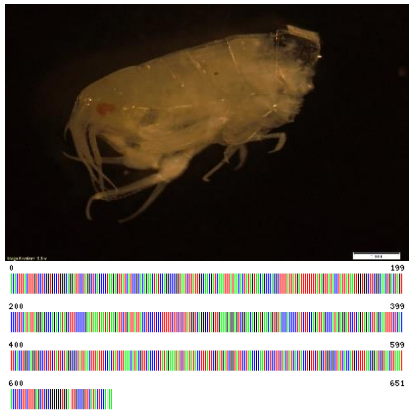
Alpheidae
(IMS123-16)



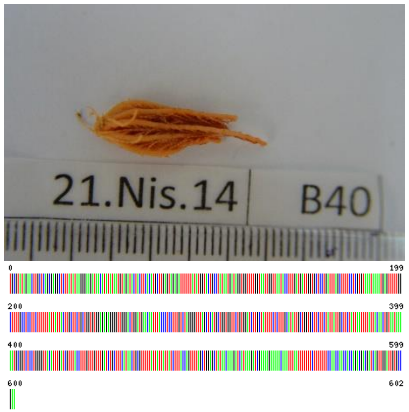
Alpheidae
(IMS121-16)



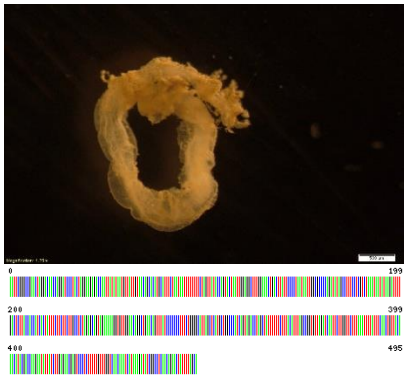
Amphibalanus amphitrite
(IMS186-16)



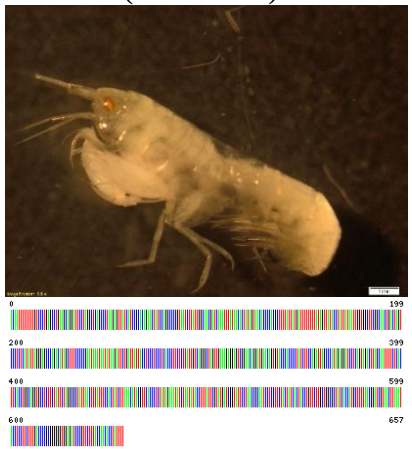
Amphipoda
(IMS128-16)



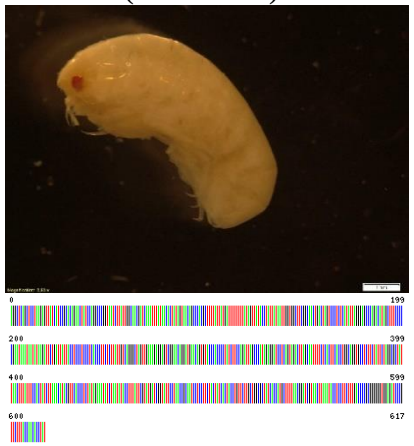
Antedon mediterranea
(IMS241-16)



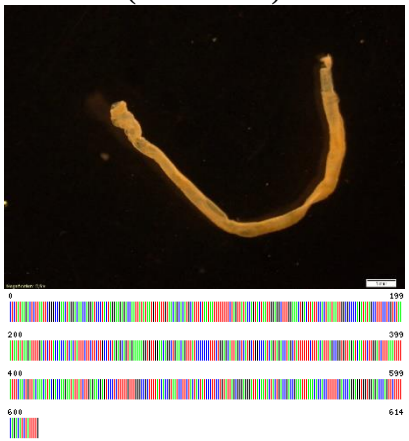
Apionsoma sp.
(IMS129-16)



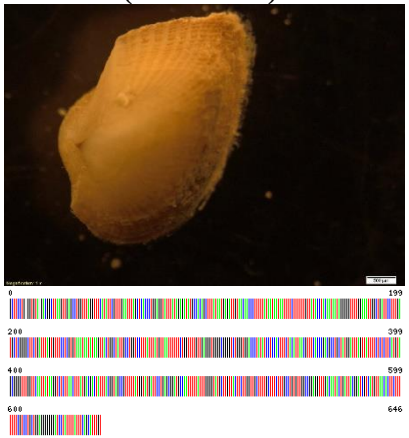
Amphipoda (IMS127-16)



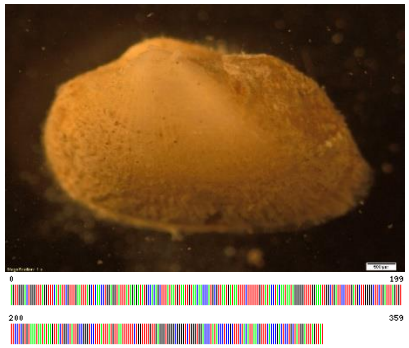
Amphipoda
(IMS126-16)



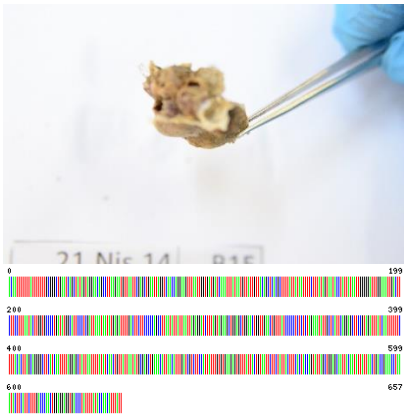
Apionsoma sp.
(IMS122-16)



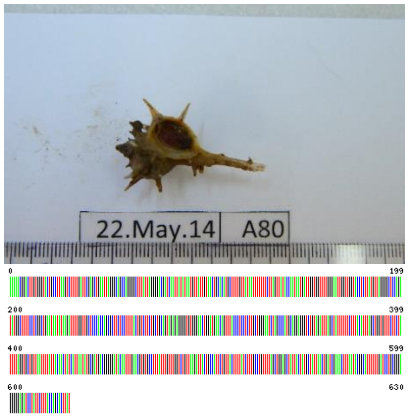
Arcoida
(IMS120-16)



Arcoida
(IMS125-16)



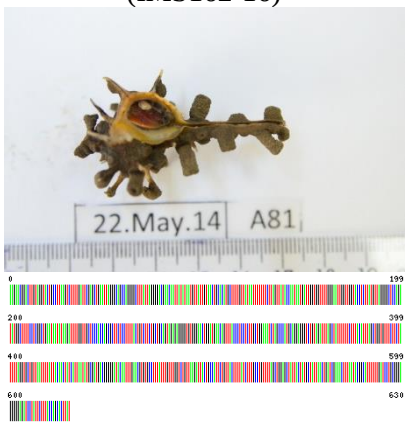
Balanus trigonus
(IMS162-16)



Bolinus brandaris
(IMS149-16)

Bolinus brandaris
(IMS155-16)

Ascidiacea
(IMS164-16)

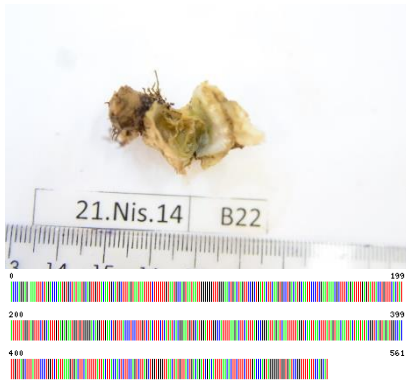


Bolinus brandaris
(IMS150-16)

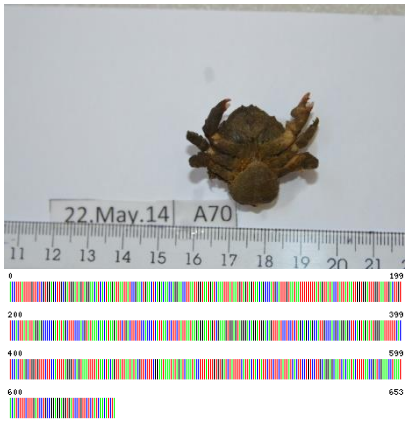
Bolinus brandaris
(IMS153-16)

Carditidae
(IMS141-16) *

Cystodytes sp.
(IMS119-16)

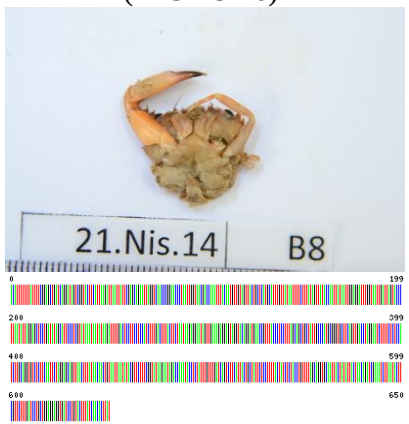


Dendostrea sp.
(IMS165-16)

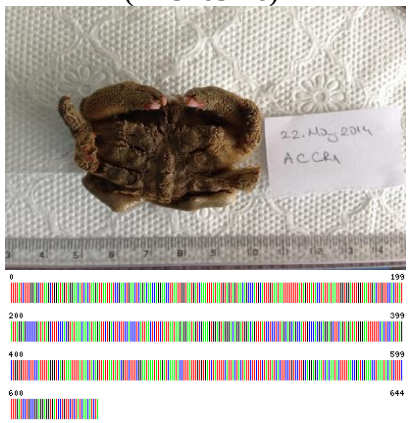


Dromia sp.
(IMS142-16)

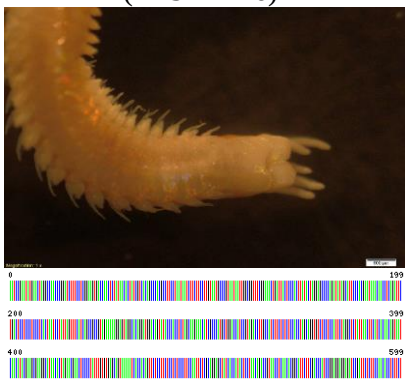
Felimare picta
(IMS139-16)



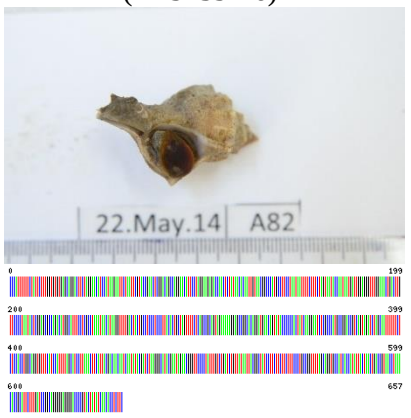
Decapoda
(IMS170-16)



Dromia sp.
(IMS137-16)



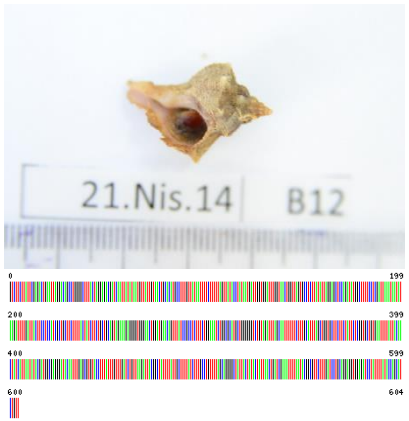
Eunicidae
(IMS124-16)



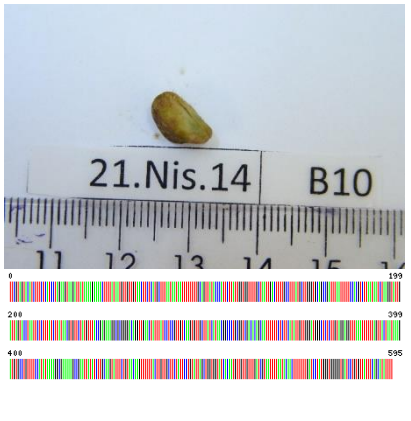
Hexaplex trunculus
(IMS151-16)

Hexaplex trunculus
(IMS151-16)

Hexaplex trunculus
(IMS156-16)



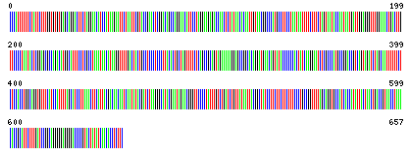
Hexaplex trunculus
(IMS161-16)



Mytilidae
(IMS160-16)

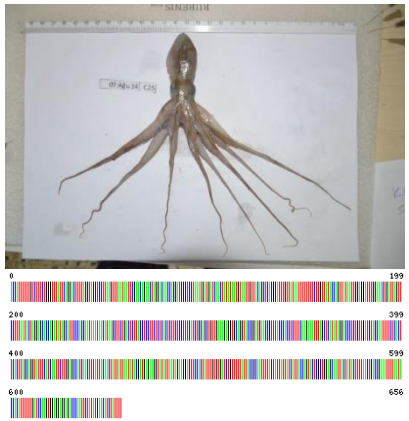
Hexaplex trunculus
(IMS154-16)

Hexaplex trunculus
(IMS158-16)

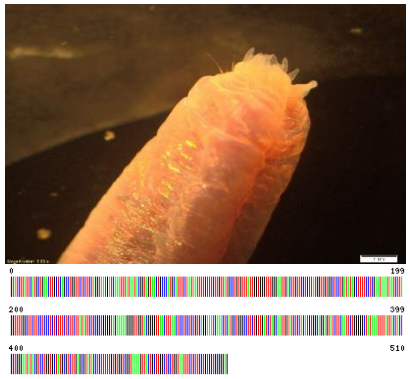


Hermodice carunculata
(IMS169-16)

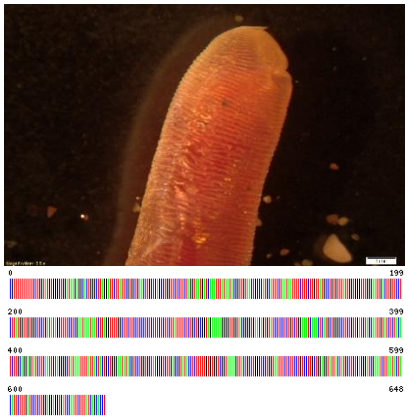
Nudibranchia
(IMS168-16)



Octopus vulgaris
(IMS173-16)

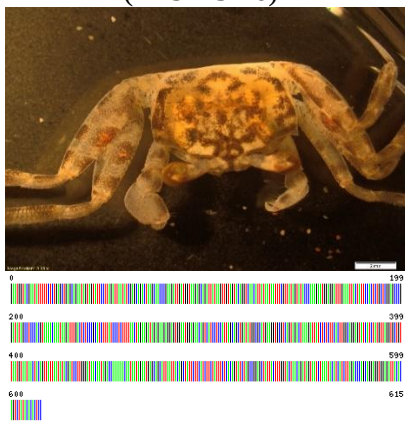


Opheliidae
(IMS191-16)



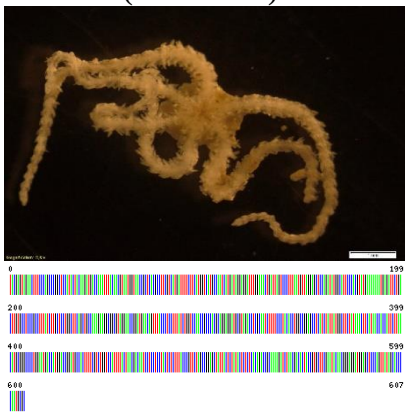
Opheliidae
(IMS192-16)

Ophiactis savignyi
(IMS235-16)

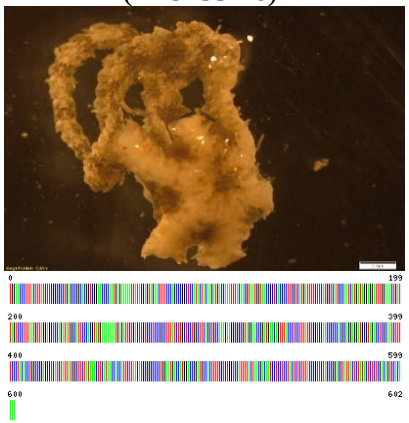


Ocypodidae
(IMS181-16) *

Opheliidae
(IMS193-16)

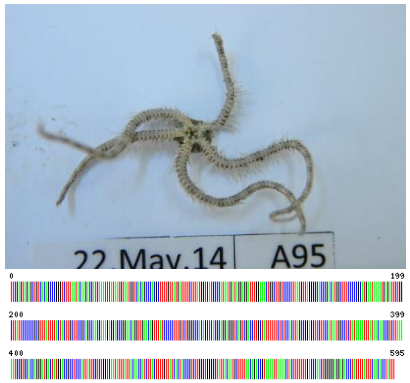


Ophiactis savignyi
(IMS236-16)



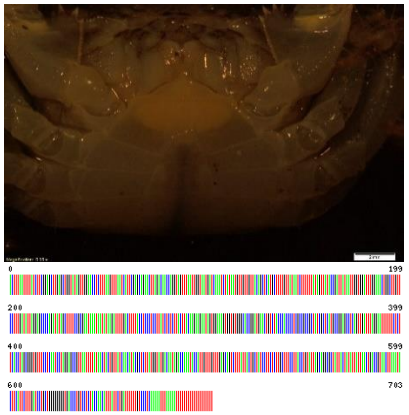
Ophiactis savignyi
(IMS234-16)

Ophiothrix fragilis
(IMS238-16)



Ophiothrix fragilis
(IMS239-16)

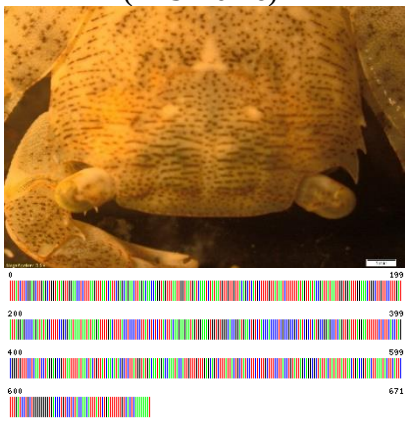
Ophiothrix sp.
(IMS240-16)



Pachygrapsus marmoratus
(IMS190-16)

Ophiothrix fragilis
(IMS242-16)

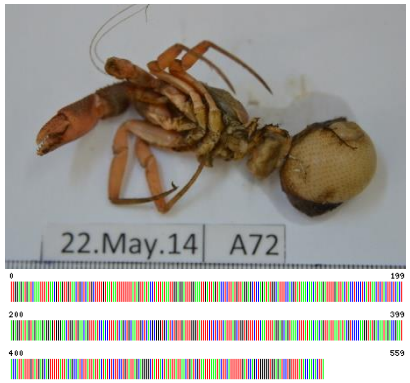
Ophiothrix fragilis
(IMS237-16)



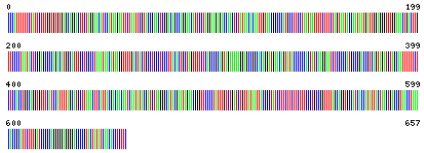
Pachygrapsus marmoratus
(IMS189-16)

Paguristes sp.
(IMS171-16)

Paguristes sp.
(IMS152-16)



Pagurus prideaux
(IMS143-16)

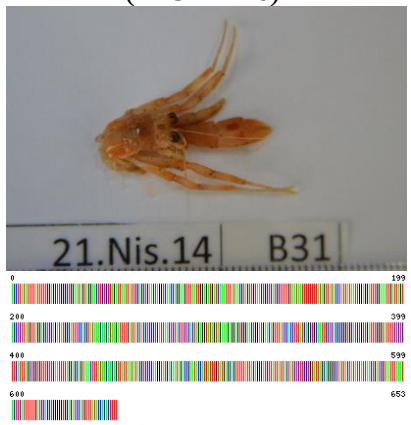


Pagurus prideaux
(IMS147-16)

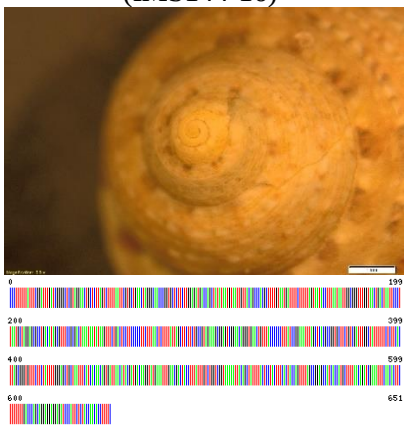
Pagurus prideaux
(IMS144-16)

Paguristes sp. (IMS140-16)

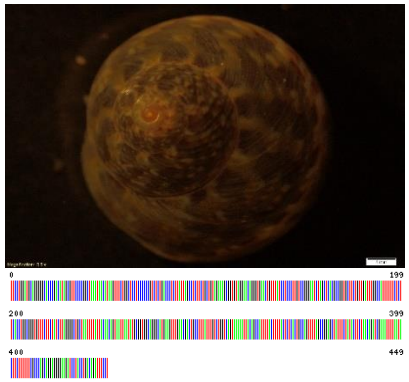
Pagurus prideaux
(IMS146-16)



Pagurus prideaux
(IMS166-16)

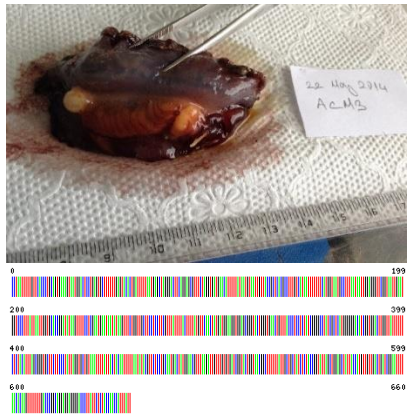


Phorcus mutabilis
(IMS195-16)

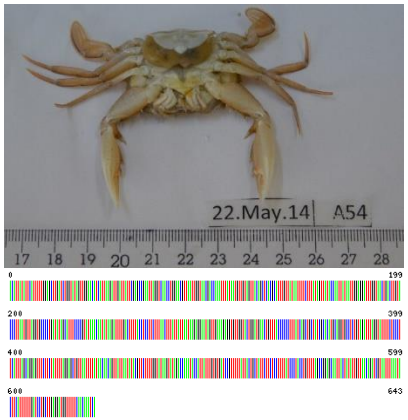


Phorcus richardi
(IMS182-16)

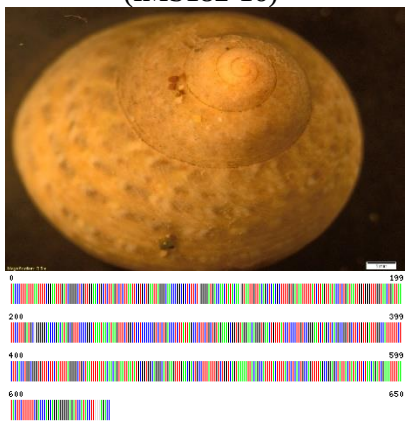
Pisa armata
(IMS145-16)



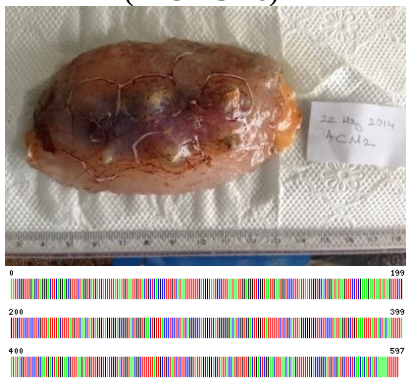
Pleurobranchidae
(IMS136-16)



Portunidae
(IMS130-16)

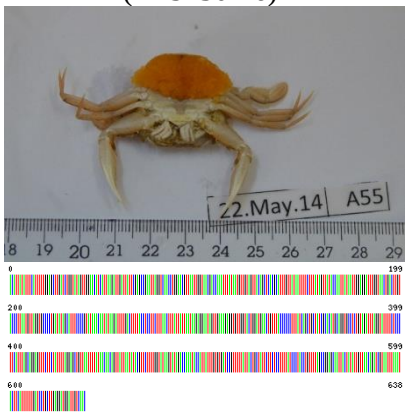


Phorcus richardi
(IMS194-16)

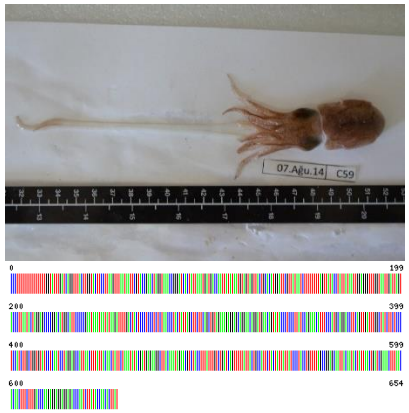


Pleurobranchidae
(IMS138-16) *

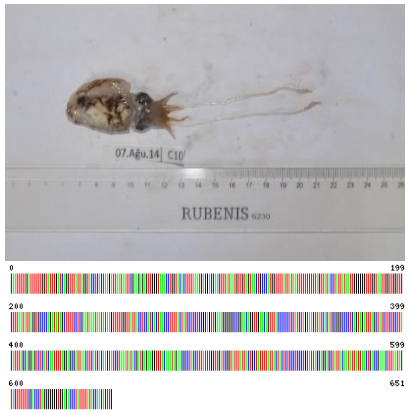
Polychaeta
(IMS159-16)



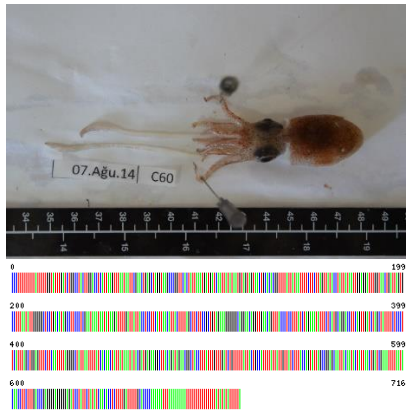
Portunidae
(IMS131-16)



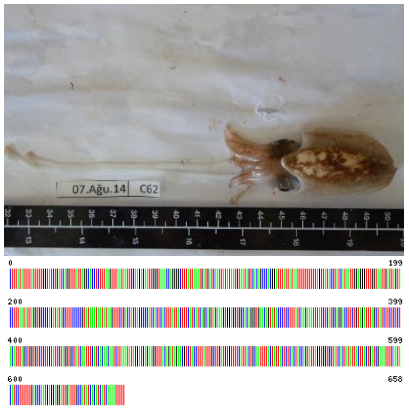
Rossia macrosoma
(IMS174-16)



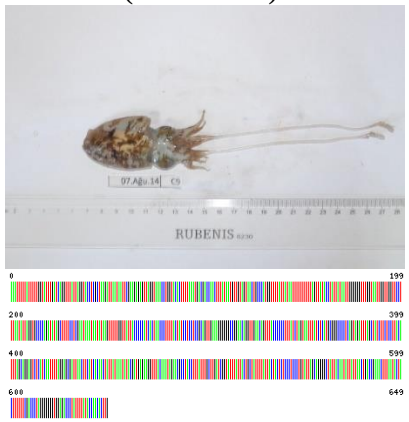
Sepia officinalis
(IMS172-16)



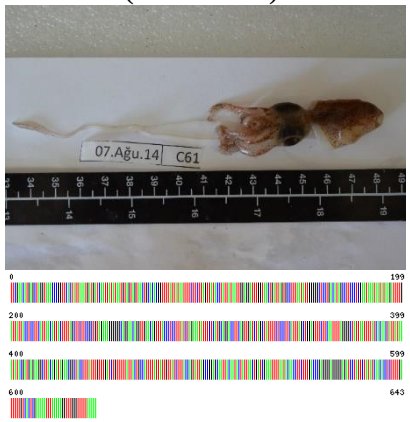
Sepietta oweniana
(IMS175-16)



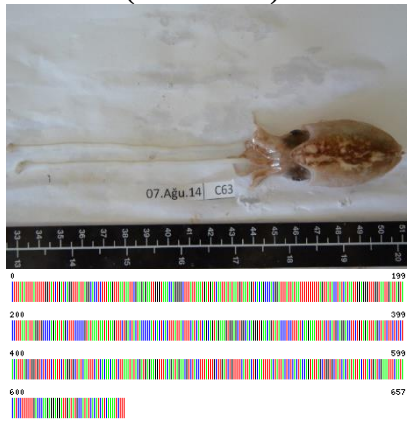
Sepiida
(IMS177-16)



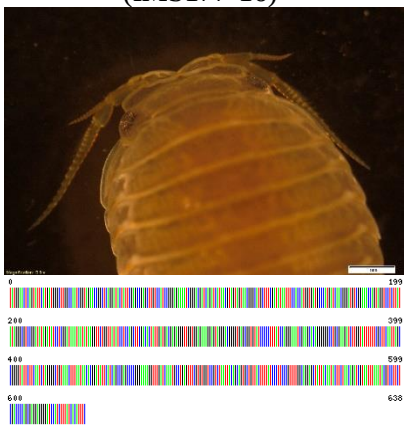
Sepia officinalis
(IMS180-16)



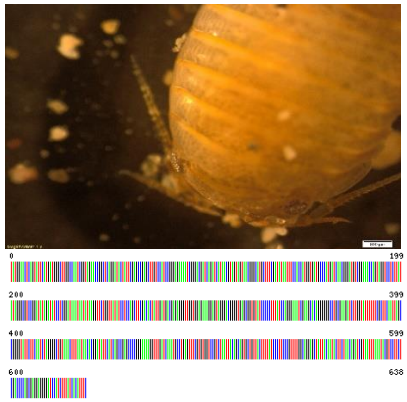
Sepietta oweniana
(IMS176-16)



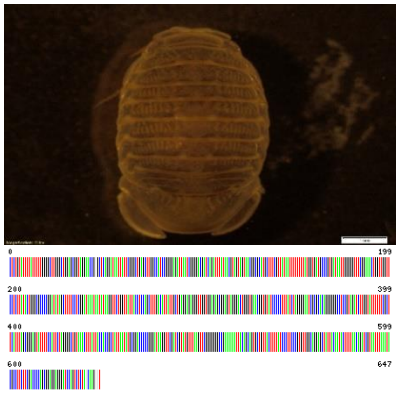
Sepiida
(IMS178-16)



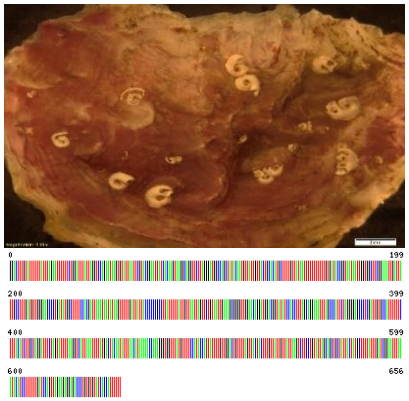
Sphaeroma serratum
(IMS183-16) *



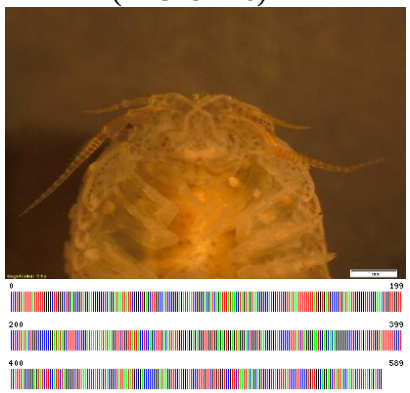
Sphaeroma serratum
(IMS184-16) *



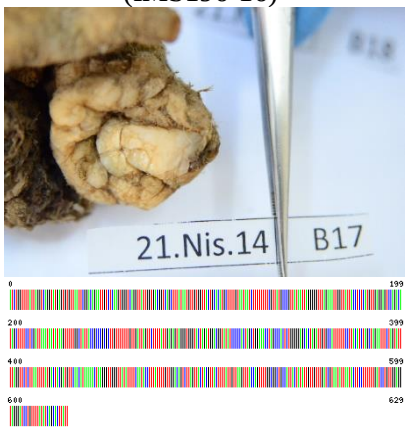
Sphaeroma serratum
(IMS196-16)



Styela sp.
(IMS187-16)



Sphaeroma serratum
(IMS188-16)



Styela plicata
(IMS163-16)