QUALITY OF LIFE AND MANAGEMENT OF LIVING RESOURCES PROGRAMME (1998-2002)

Genetic Catalogue, Biological Reference Collections and Online Database of European Marine Fishes

FINAL REPORT

PROJECT IDENTIFICATION + FINAL SCIENTIFIC REPORT

Contract number: **QLRI-CT-2002-02755**

Project acronym: **FishTrace**

QoL action line: **Area 14.1, Infrastructures**

Reporting period: **01/01/03 - 30/06/06**

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Title of the project Genetic Catalogue, Biological Reference Collections and Online Database of European Marine Fishes	
Acronym of the project FishTrace	
Type of contract Thematic network	**Total project costs** (in euro) 2000000 **€**
Contract number QLRI-CT-2002-02755	**Period covered by the Final Report** (e.g. 1 February 2000 – 31 January 2001) 1 January 2003 – 30 June 2006

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Key words (5 maximum - Please include specific keywords that best describe the project.)
Genetics, Fish, Database

World wide web address (the project's www address)
www. fishtrace.org / www. fishtrace.com

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INDEX

FINAL REPORT

1.- Introduction 6
2.- Objectives 11
3.- Material and Methods 14
3.1.- Standardization of experimental procedures 14
3.2.- Sampling and taxonomic identification of targeted fish species 15
3.3.- Biological collections from target species 26
3.4.- Molecular genetics procedures 28
3.5.- Molecular analysis of fish populations 33
3.6.- Data validation 34
3.7.- Database implementation 37
3.8.- Public web interface 39
4.- Results 40
4.1.- Standardization of experimental procedures 40
4.2.- Sampling and Taxonomy 41
4.3.- Biological Reference Collections 45
4.4.- Genetic Catalogue of European marine fishes 46
4.5.- Population structure of six European marine fish species 54
4.6.- Validation of taxonomic and genetic data 60
4.7.- Database 64
4.8.- Public web interface 74
5.- Discussion 82
6.- Conclusions 101
7.- Exploitation and dissemination of results 103
8.- Policy related benefits 109
9.- Future actions 110
10.- Action requested to the Commission 111
11.- Tables and Figures 112
12.- References 205
13.- World Wide Web Links 223
14.- Abbreviations 224

Annexes

Annex I: 1st FishTrace Plenary Meeting. Madrid, 12-14th February, 2003.

Annex II: FishTrace Database Structuration Meeting. Paris, 13-14th May, 2003.

Annex III: FishTrace Standardization Meeting. Stockholm, 4-7th June, 2003.

Annex IV: 2nd FishTrace Plenary Meeting. Las Palmas de Gran Canaria (Spain), 19-21st November, 2003.

Annex V: FishTrace Meeting on Data Validation. Ispra, Italy. 15-16th April, 2004.

Annex VI: 3rd FishTrace Plenary Meeting. Paris, 25-27th November, 2004.

Annex VII: FishTrace Database & Web Interface Meeting. Madrid, 11th March, 2005.

Annex VIII: 4th FishTrace Plenary Meeting. Funchal (Portugal), 24-26th October, 2005.

Annex IX: FishTrace Concluding Meeting. Kavala (Greece), May 10-12th, 2006.

Annex X: Sampling and Taxonomy (WP2) Protocol.

Annex XI: Results from Molecular Genetic Procedures Standardisation.

Annex XII: Molecular identification and DNA barcoding (WP3). Protocol and PCR conditions.

Annex XIII: Phylogenetic Validation of Sequences. Guidelines.

Annex XIV: Preparing sequence files for the Sequin tool.

Annex XV: Reference Collections (WP5) Protocol, Loan Request Form and Invoice of Specimens.

Annex XVI: Guidelines for Validation purposes (WP7) including a protocol defining format for the Validation tasks.

Annex XVII: Concise Protocol for the online validation process.

Annex XVIII: The Bibliography Module in the FishTrace Database.

Annex XIX: Results from the genetic population structure analysis.

Annex XX: Results from haplotype connectivity network analysis.

Annex XXI: Final Report on Validation and Missing Data.

Annex XXII: Control Data Validation Flow Document.

Annex XXIII: Leaflet presenting the FishTrace project.

1.- Introduction

Taxonomy and systematics of most animal species have been described in sufficient detail to permit the classification of practically any organism and, in particular, may be of use in the identification of the species and subspecies for the fish trade and ecological concerns regarding fisheries. However, before FishTrace started there was a lack of fast reference tools that hindered the efficient identification and differentiation of teleosts required in fisheries management, biological and ecological research, and human consumption. This problem could affect several of the socio-economic activities related to Fisheries Policies and therefore, the constitution of a database for identification of fish species of commercial, ecological and zoological interest for the European countries was clearly necessary.

Thus, the FishTrace network has catalysed during the last years the pooling of biological material and sequence data corresponding to more than 220 European marine fish species with commercial, ecological and zoological interest. These species have been *ad hoc* sampled from most European sea areas as well as from some extra-European areas. Moreover, the sampling of species overlapping different geographical areas has allowed the morphological and genetic comparison of specimens from widespread species across the European seas.

FishTrace has achieved a critical mass of expertise by joining the efforts of sample collectors, ichthyologists, curators, molecular biologists, and database developers. The workplan was designed to directly confront the problem of reliable fish species identification and differentiation through the creation of a public online database containing a genetic catalogue of marine teleosts which could be used for DNA barcoding of fish. The final goal of the database has been to set up the basics for the development of efficient tools for marine fish species identification aimed at establishing standardised authenticity procedures. This genetic catalogue has included a selected group of species of food interest in the EC markets, as well as of ecological and zoological interest across Europe.

The structure of FishTrace is based on five methodological pillars. These five sections allow cross talk activities and interchange of biological materials and biological and commercial information. The five methodological sections include:

- *Taxonomy*
- *Molecular Genetics*
- *Biological Reference Collections*
- *Public Online Database*
- *Technological Development*

Taxonomy

The main tasks to approach fish taxonomy were the sampling of adequate biological material, the precise biometric sampling and, taxonomical identification, and finally, the distribution of samples to research groups participating in the remaining methodological sections.

A representative number and size of samples for each targeted species and subspecies has been guaranteed for the network according to market readiness or abundance. The setting up of an adequate sample size for each species, i.e. the number of fish individuals to deal with (to be sampled, identified, distributed, sequenced, analysed, validated, etc.), has been an important decision took within the network's methodological strategy.

Thus, to elaborate the genetic catalogue, five specimens from each targeted species has been sampled at each geographical area covered. In addition, many of these species overlap through the different geographical areas and therefore there is more than 100 species wich are largely represented by more than ten specimens. For the analysis of European fish populations structures, the number of specimens increased to twenty per each targeted species and geographical area. Species identification was performed by morphological, chromatic and meristic procedures. For this purpose, the main UNESCO catalogues, the FAO guides and the major species identification catalogues have been used. Also, species synopses, living marine resources, local catalogues and specialised bibliography have been extensively used for identification purposes. Authoritative taxonomic fish classification ensured that all information has been assigned to current scientific names even if a publication uses an outdated taxonomy. Special attention has been paid to the traditional problems of differentiating adults of morphologically similar species and immature specimens of sister species within the same family. In the same way, the investigation of immature, female and male specimens of the same species, assigned to another species on the grounds of differences in morphological and chromatic features, has been undertook once completlely filling the database in. Finally, the frequent use of different vernacular names to designate different sex,

groups (juvenile vs. adults) or commercial sizes of the same species for fish food products has been investigated and recorded in the database.

Molecular Genetics

FishTrace uses molecular features (DNA sequences) to determine the differential characteristics of the taxonomically identified species, to build the genetic catalogue. Special attention has been paid to the quality control of the procedures used by all participants to guarantee reliablility in the precise identification and DNA barcoding of the species.

The main objective in this methodological section has been the obtaining of the DNA sequences from two genes, one mitochondrial (cytochrome *b*) and one genomic-chromosomal (rhodopsin) specific to each of the species under study. Species definitions has been based on the estimation of genetic distances between multiple alignments and cladistics analysis from the DNA sequences validated and available for the Consortium through the database.

Within this objective the main goal was obtained through the compilation of a general genetic catalogue of about 220 marine teleost fish species from eight European geographical marine areas and also from Extra-European sea areas where catches enter into European markets. The genetic catalogue available in the FishTrace database contains molecular data (including genetic variability among specimens analysed and polymorphisms) together with detailed information on sampling, taxonomy, geographical origin, use in food industry, position in the food network, fishing activity and commercialisation, in connection with their distribution and ecology. The FishTrace database provides information on the nucleotide sequences of the mitochondrial cytochrome *b* gene and the nuclear rhodopsin gene from the target species. This molecular data constitutes the basics for the validation of taxonomic data and for the development of practical tools for species diagnosis. Given the possible subtle genetic variation in populations at the mtDNA level, the second genetic marker used served as an internal quality control procedure. The nuclear gene coding for rhodopsin shows minimal population variation in fish and is intron-less in all teleost species. This second marker allows to confirm sequence analysis from the mitochondrial sequence and to confer upon them the degree of reliability required to quantify the level of divergence among species, while maintaining homogeneity in the same species. The supply of sequences from two genes with different evolution rate in different species from the same phylum guarantees its application for DNA barcoding and for the development of phylogenetic tools for the precise ascribing of

a given DNA sample. Moreover, the given independent variation rate for each gene has allowed to track basal phylogenetic relationships and identify any rare case of heteroplasmy, paraphyly or hybridization between close species.

Genetic validation of the identity of all the network samples has also been a main task for the correct comparison of the results from the different laboratories participating. This was aimed at confirming the precise species origin, and the transfer of results, thus monitoring the homogeneity of criteria for species identification.

Biological Reference Collections

FishTrace network holds backup biological reference collections including DNA, tissue, voucher specimens, and otoliths from the taxonomically and genetically validated fish species. These collections, deposited in four European natural history museums, constitute a reference infrastructure, unique in Europe, with important applications in fish species authenticity and related biological research. These new collections serve as a reference for applications related to fish species authenticity and associated biological research and socioeconomic interests. Apart from the valuable cultural and scientific contribution, the creation of a biodiversity collection of marine fish has been a conceptual landmark for the network's objectives which provides an indisputable source for the identification of marine fish of market-oriented interest for the food industry, consumers and administrative bodies. Given the excellence and tradition of the host Natural History museums involved in FishTrace, the long-term preservation and maintenance of these collections is guaranteed. FishTrace reference collections have the added advantage of easy access through a interface in the online database for consultation, loan and exchange of material.

Public Online Database

The World Wide Web (WWW) has provided an ideal tool for presenting data for genetic identification of fish species readily accessible to the scientific community. FishTrace has built an open access database on European teleost information. Database development participants, in collaboration with the data pooling centers, have produced a new user-friendly interface (www.fishtrace.org) and a set of tools defined for data processing. The online molecular and morphological identification tools are available from the web interface. Data entered in database has been sistematically validated before to be stored in the central site.

The data structure chosen allows data validation by means of a smooth transition from direct storage to database storage through the database interface.

The online database at www.fishtrace.org actually contains standardised information on taxonomy, DNA sequences and reference collections designed to directly confront the problem of reliable fish species identification and/or the differentiation between closely related species. FishTrace database ensures the highest standards for marine fish identification through the accurate validation of the information compiled in the database.

Technological Development

The genetic and taxonomic information supplied in FishTrace database have also assisted in the design of model tools towards the development of pre-competitive, analytical procedures for unequivocal identification and quality control aimed at end users, mainly producers, and regulatory administrations. User-oriented detection procedures can be directly designed by users from the web interface to enforce regulations concerning fish products.

FishTrace has provided through the database with state-of-the-art technology for the DNA barcoding of fish species and for the assessment of the quality and origin of fish materials, to yield value-added products and fight against fraud, as well as to improve the quality of European fish products.

2.- Objectives

The main aim of this thematic network has been to catalyse the cooperation and pooling of material and data corresponding to the genetic identification and characterisation of more than 200 common European marine fish species to guarantee the source and authenticity of fish products. Achievements in the form of standarised information has been directly focused on safety of raw materials derived from fish and their traceability throughout the food chain to assist public policy decisions to enforce common policies in the fields of fisheries, food safety, labelling and ecology. Further applications of the information released are related to the economic activities of the European fish market.

Earlier, at the beginning of this decade, the FishTrace consortium identified a requirement to promote common protocols, to interconnect expertise and stimulate interoperability between complementary resources with the aim of generating an accessible database to researchers and control laboratories with standardised data of European marine fishes. Faced with these arguments, the general objectives of this network have been the following:

A) To draw up a genetic catalogue of a large, representative number of marine fish species regularly commercialised in the European markets. The catalogue would include gene sequences as a molecular marker related to morphological data as indisputable evidence for the origin of the fish and fish products.

B) To pool reference biological materials, including DNA and tissue samples, and to promote their use for standardisation and cross-referencing with respect to fish DNA barcoding and traceability through European markets.

C) To establish a public accessible database compiling the new standardised data generated in the network (taxonomy, molecular genetics and reference collections) with existing data from other sources.

D) To validate the information compiled in the database to ascertain its applicability for end-users (including biological research laboratories, control laboratories, consumers and regulatory bodies) in terms of cost-effective methodologies for the analysis,

characterisation and commercial diagnosis of marine fish species with regard to fisheries and fish products.

E) To use the collection of standarised information gained in this network to lend support to European policies and to enforce these and national policies regarding fishery stocks, food traceability and environmental protection.

Fundamentally, all these objectives have been gained along the duration of the project through the close interaction of partners belonging to different fields of knowledge, i.e. field taxonomists, natural history museums, molecular biology laboratories and software and database managing experts. This interaction has lead to compile all necessary data and information in a multidisciplinary approach, and to grant the long-term preservation and maintenance of the pooled data and reference materials.

The specific experimental aims of the thematic network have been the following:

1) Sampling of a representative number of specimens from the selected fish species covering, whenever possible, a wide range of sizes, at each European sea areas.

2) Taxonomic identification of species using morphological, chromatic and meristic characters.

3) Coordination, distribution and networking of biological samples from each sampling institution.

4) Creation and long-term preservation of biological reference colections (DNA, tissues, otoliths and vouchers) and compilation of an open inventory for exchange and supply using a common database.

5) Molecular genetic identification by nucleotide sequencing of a mitochondrial (cytochrome *b*) and a nuclear (rhodopsin) gene.

6) Quality control of molecular genetic procedures at participating laboratories and standardisation of analytical procedures for quality management.

7) Identification and registration of genetic variation found in widespread species from several geographical areas.

8) Development and long-term maintenance of a World Wide Web searchable database of the new standardised data generated with links to other major taxonomic, biogeographical, molecular and diagnostic online databases.

9) Designing model laboratory detection methods (DNA barcoding), from the data supplied in the database, for a fast transfer of technology on identification of marine fish species.

10) Promoting the use of the database to potential end-users in the fields of biological research, food technology, ecology and fisheries.

The multidisciplinary nature of this project has included specific aspects of research and technological development in a well-defined innovative networking context designed to yield a high output of transferable results. Thus, among the achievements is included the maintenance of an online database with standardised molecular data to support specific policies and administrative resources, marine ecosystems and certified food products. The DNA barcoding capacity given by FishTrace database provides European fish products with the possibility to grant authenticity labels (green labels) increasing their economic value and offering a guarantee of their origin and biological authenticity. Effective quality control systems are also favoured for fish and derived products consumed in the EU addressing consumer needs regarding food safety, food quality and low environmental impact.

FishTrace provides a new capacity for developing quick and sensitive technology to establish the traceability of fish species and their products, which in turn assist in the identification of food products from non- certified sources (within and outside the EU) thus avoiding the spread of undesirable attributes (e.g. contaminated foodstuffs) in food networks.

3.- Material and Methods

3.1.- Standardization of experimental procedures

This preliminary part of the work accomplished in FishTrace was focused to generate protocols of standardized methodologies for biometric and genetic analysis, as well as for the creation of the biological collections. This task implied the validation of the analytical methods, the establishment of dataset and data structure and the study of legal aspects related with the use of information in a public domain. The whole process, from the generation of the data to its inclusion into the centralized FishTrace database is summarized in Figure 3.1. The standardization of methodologies for the generation of data was carried out within the three main disciplines participating in FishTrace: sampling and taxonomic identification, biological reference collections and molecular genetics. Responsible partners for these disciplines at FishTrace have tested its own methodologies at each respective institutions, based on the previous experience acquired, thus results obtained were compared and subsequently tested by the rest of research groups involved, in order to reach consensus and standardize for the network.

For the standardization of sampling, taxonomy and reference collections procedures, a committee formed by the FishTrace's specialists in these fields reached a general consensus about methodological sampling, regional information on taxonomy, biology, socioeconomics, and reference collections from each species and sea sampling area towards standardization of protocols and development of the database.

Standardization of molecular genetics procedures was carried out by direct analysis of samples at the beginning of the project. Thus, for the standardization of the DNA extractions and PCR methods, tissue samples from six different teleost species were provided to all groups involved in molecular genetic tasks, where DNA was extracted using its own methodology. DNA samples isolated using different methods were analysed to deliver protocols for DNA extraction and PCR amplification of the rhodopsin and cytochrome *b* genes. Alternative strategies were designed to avoid difficulties with the amplifications.

The data set and the definitive structure of the database were established by consensus at several meetings along the project (Annexes I to IX).

Methods and specific protocols delivered from the standardization of technical procedures are given in the following Annexes (Annexes X to XVIII):

- Annex X: Sampling and Taxonomy (WP2). Protocol.
- Annex XI: Results from Molecular Genetic Procedures Standardization.
- Annex XII: Molecular Genetic Identification (WP3). Protocol and PCR conditions.
- Annex XIII: Phylogenetic Validation of Sequences. Guidelines.
- Annex XIV: Preparing sequence files for the Sequin tool.
- Annex XV: Reference Collections (WP5). Protocol and Forms.
- Annex XVI: Guidelines for validation purposes including a protocol defining format for the validation tasks
- Annex XVII: Resumed Protocol for the *online* validation process.
- Annex XVIII: The Bibliography Module in the FishTrace Database.

3.2.- Sampling and taxonomic identification of targeted fish species

Target species

The FishTrace network has compiled essential information (for identification purposes) from main marine teleost species of fisheries, ecological, zoological and biogeographical interest in Europe. A total of 220 species belonging to 75 different families and 17 different higher teleostean orders (Grade Teleostomi; Class Actinopterygii; Subclass Neopterygii; Division Teleostei. Based on review by Nelson, 2006) have been included in this multidisciplinary research. The list of targeted species and their geographical origin are detailed in Table 3.1. Taking into account the overlapping of the species sampled in more than one geographical area, the total number of teleost species taxonomically identified increased up to 514. These species are all marine forms with 9 marine-brackish species and 6 marine-brackish-freshwater species, as specified in Table 3.1. The distribution of species within teleost orders covered by the sampling tried to be representative of species availability and consumer's demand in European markets, including representative number of species from major orders like Perciformes, with 9958 species. Source: FishBase (www.fishbase.org; Froese and Pauly,

2000). The rationale for the selection of sampled species also included their economical value and fisheries abundance.

On the other hand, the six species that were selected for the study of the genetic divergence in the European seas (See Table 3.2) were chosen based on the following requirements:

1) The presence of the target species in at least four geographical areas.

2) Samples of each species are easily accessed, i.e. rare species are excluded.

3) The life histories of the species selected are likely to contribute towards specific population structuring, i.e. migratory species are excluded.

Geographical areas of sampling

Eight European sea areas have been sampled to collect specimens from the targeted marine teleost species. In addition, species and specimens from outside European waters but with interest in the EC markets, have been also sampled. From North to South, the European sea areas covered were: Skagerrak and the Baltic Sea (BS), the North Sea (NS), the English Channel and the Bay of Biscay (CB), the Cantabric Sea and the NW Iberian Peninsula (CS), the Western Mediterranean Sea and Bay of Cadiz (WM), the Eastern Mediterranean Sea, Greek Seas (EM), the Madeira archipelago (MA) and the Canary Islands (CI). Within the Extra-European group (EE), five sea areas have been covered to collect samples: the Northern Atlantic Ocean, the South-Western Atlantic Ocean, South Africa, the Eastern Atlantic Ocean and the Indo-Pacific Ocean. Figure 3.2 shows the geographical distribution of the European and extra-European teleost species collected in FishTrace.

Field sampling protocol

Representative samples of teleost fish species were collected by strategic field sampling from the areas previously defined (Figure. 3.2). The sampling and taxonomic tasks included the identification and validation of specimens using updated fish identification bibliography, specific for each region. The bibliographic references used are specifically given at the FishTrace database.

Fish sampling and data recording from each specimen followed the standardized protocol shown below:

1) Collecting fish specimens: Individual teleost specimens were obtained by collecting on an *ad hoc* basis, from short collecting cruises carried out on board research vessels and also from national commercial fisheries (landings and by-catch species), local fish markets and local companies collaborating as end-users. From five to twenty individuals per targeted teleost species and location were collected, depending on the aim of the collection. Whenever possible five specimens were sampled from the targeted species in any given geographical area, which were the basis for the generation of the reference collections, the database and the genetic catalogue, and up to twenty specimens from each of the species selected for the fish population structure analysis, listed in Table 3.2.

2) Specimen and tissue sample tagging and numbering: Collected fish specimens were tagged and numbered indicating the FishTrace's specimen code. The FishTrace code is constructed from the first three letters of the generic name, the first three letters of the specific name, two letters denoting geographical area, two digits denoting the specimen number (e.g. the second specimen of *Mullus surmuletus* collected in the Western Mediterranean area has the following code: MulSur-WM-02). Species with identical first three letters in generic name and species epithet require an *ad hoc* code, that is constructed based on the first differing letter, since each specimen has been assigned a unique FishTrace code. For Reference Collections, tagging was performed before photography and tissue sampling. All specimens were classified with numbered tags (exclusive tags for each museum/institution involved), and the tag number indicated on the tissue sample and otolith tubes. The tag number differed from the FishTrace's specimen number (exclusive number/reference at each museum/institution involved).

Muscle tissues sampled from specimens at each geographical location numbered 01 and 02 have been used for the genetic analysis, while specimens labelled 03 to 05 were used for back up (for cross-referencing if necessary), and reference collections. For the biogeographical genetic variation analysis performed on certain taxa (See Table 3.2), a total of twenty specimens were required from each geographic area (these specimens have been numbered from 06 to 20).

3) Specimen treatment: Collected fish specimens were processed according to the following standardized specific instructions:

- Specimen #01: Photographed. Muscle tissue removed: three samples, one for analysis, one for backup, one for reference collection. Voucher storage: the whole specimen was sent for reference collection.

- Specimen #02: Photographed. Muscle tissue removed: three samples, one for analysis, one for backup, one for reference collection. Voucher storage: the whole specimen was sent for reference collection. Otoliths: both sagittal otoliths were extracted, photographed right one and it was sent for reference collection. Voucher storage: the whole specimen was sent for reference collection.

- Specimen #03: Photographed. Otoliths: both sagittal otoliths were extracted, photographed right one and it was sent for reference collection. Muscle tissue: two samples kept preserved. Voucher storage: the whole specimen was sent for reference collection.

- Specimen #04: Photographed. Muscle tissue removed: two samples, one for analysis, one for backup. Voucher storage: the whole specimen was sent to the NRM for reference collection.

- Specimen #05: Photographed. Muscle tissue removed: two samples, one for analysis, one for backup. Voucher storage: the whole specimen was sent to the MNHN for reference collection.

- Specimens for biogeographical genetic variation: Specimens numbered 04 to 20: Muscle tissue was taken from every specimen captured for this task. They were used and stored according to preferred procedures by each institution (mainly representative muscle tissue samples stored and preserved in 70% ethanol for further DNA analysis). The treatment given to each specimen sampled is shown in Table 3.3.

4) Specimen quality: Specimens were sampled fresh as far as possible. Specimens collected were whole or gutted, but intact specimens were always preferred. All biological material collected within the project has been kept. For tissue and otolith sampling, as well as for systematic analysis, adult specimens showing diagnostic marks were always preferable. For some taxa, it was more convenient and realistic to sample young specimens, or to use adults

as primary tissue source, and juveniles as backups. When large size, collection space limitation, or fishing/conservation restrictions made it expensive or difficult to preserve available specimens (e.g., of marlins or swordfish), one of the three following strategies applied:

4.1) To take a photograph and basic measurements of the specimen and remove 3 tissue samples. After that, it was agreed to release the fish/return to fisherman.

4.2) To take a photograph and basic measurements of the specimen and remove 3 tissue samples keeping the head (and tail, if possible) as voucher, extracting the otoliths from the head.

4.3) To inquire with the major museums (NRM and MNHN), whether they considered the transportation worthwhile, and if so, performed the usual photography and tissue and otolith sampling.

5) Fish specimen photographs: Colour pictures were taken from all specimens in its freshest state. Occasionally, additional specimens were also photographed to cover sexes, reproductive status and ontogenetic stages displaying the variability within a species. Pictures taken were digital colour images, at a resolution of 1024 x 768 pixels or more. Photos were saved in minimally compressed JPEG format, or in TIFF or similar non-destructive formats. Photographs have been taken from the left side of the specimen, except certain flatfishes where the eyed side was shown with the gill opening down. Photographs have been also taken in lateral aspect, and when relevant of other aspects (e.g. flatfishes: right and left side; anglerfishes: dorsal and ventral aspect). Fins were spread as much as possible using alcohol-soaked cotton swabs and supports when relevant. Photographs were taken in ambient outdoor light, in shadow, and without flash. The background was a neutral grey. In the case of uneven light conditions, e.g., in early morning or evening sun, the fish has been directed so that more light reaches the dorsum than the ventral parts. All photos taken show the specimen plus a label showing the FishTrace's specimen number and a ruler. Image files have been labelled with the specimens FishTrace's code, plus the appropriate file extension (e.g. MulSur-SB-05.jpg).

6) Tissue sampling: Tissue sampling was done on fresh or fresh frozen fish/fish on ice. Handlers/operators always used disposable latex gloves or equivalent. The equipment

(disposable clean gloves, tissue sample tubes, scalpels, tweezers, marking pens and 95% pure not denatured ethanol) was always cleaned before to start the sampling on other specimen. Scalpel blades were changed between each specimen. Chlorine was used to clean instruments away from DNA, but needed careful washing in ethanol. Tissue samples were taken from muscle right behind the right pectoral fin base (muscle from inside the head in case of large fish) scrapping away the scales and cutting a square of muscle (about 5 X 5 mm). Samples were torn into smaller pieces before placing into tissue sample tubes. Tubes were marked with FishTrace code and stored in a cool place until posting. If more than one sample was taken from the same specimen, they were identified as follows: a = sequencing; b = reference collection; c = backup. Sampling of tissue samples for the biogeographical genetic variation analysis followed the same procedure, but vouchers beyond specimen 05 were not required to be saved.

7) Preservation of voucher specimens: Fish specimens for collections were fixed in 10% formalin (one part commercial formalin, 36-40% formaldehyde in solution, into nine parts of distilled or deionized water). When possible, a flat, wide tray with tight lid for initial fixation was used. Preservation procedures starting with fresh fish were as follows:

7.1) A small amount of 10% formalin was injected in the abdominal cavity and in selected thicker muscular parts of the fish. If the fish specimen was larger than 30 cm, also the right side abdominal wall was cut about 5-10 cm, to promote entry of fixation fluid. If the fish specimen was smaller than 10 cm, no injection was needed (except in herbivorous fish). With frozen fish, it was thoroughly thawed before fixation.

7.2) A cotton or cloth swab was dipped in 10% or full strength formalin, and the fin base padded with one hand while raising the each fin with the other hand, to permanently kept the fins erect. Leave so for a few minutes, but keep the whole fish wet/damp during the process.

7.3) Fish were then placed in the fixation container with 10 % formalin, where it was kept there for at least one week (maximum for one month), ensuring that a volume of formalin covered the specimen.

7.4) Fish was rinsed in water for a few hours. Run in graded series of ethanol, 25-40-70%, one day in each.

8) Otoliths: Otoliths were removed before or after fixation. Both otoliths (right side and left side) were obtained from two specimens. This operation was performed under a stereo dissection microscope with low magnification. Otoliths are contained in the prootic bulla and more or less visible from the outside of the cranium in many fish species. To extract them, the gill cover was lifted and the bulla was poked with the scalpel tip, and cut a round hole on the bulla, extracting the sagitta with a fine-tipped tweezers, avoiding scraping it. Otoliths from both preserved and fresh fish were washed lightly in water and blotted dry. With preserved fish it was important to remove all traces of formalin by more intense rinsing in water. Otoliths were kept individually and dry in small tubes, with an inner label stating the FishTrace specimen number, the tag number (optional), and from which side each sagitta was extracted (left or right). Otolith image file name includes the FishTrace number plus Otolith: e.g. MulSur-SB-04-otolith.jpg.

9) Identification: Captured specimens were identified to species level in the field using standard literature references (e.g. Eschmeyer, 1990; Nelson, 2006; The FAO Species Identification and Data Programme, "SIDP" at www.fao.org, etc.). Species names followed FishBase nomenclature even when known to be incorrect (e.g., *Scophthalmus maximus* instead of *Psetta maxima*). Subspecies names were not be used. Field guides and PDA pages (Personal Digital Assistants for taxonomists) from FishBase were used as field reference literature (www.fishbase.org).

10) Decisions on nomenclature and systematics: The experts within the sampling and taxonomy groups from FishTrace took final decisions on scientific name and systematic position of each species as to be shown on the FishTrace website. The decision was made by consensus agreement and cleared through consideration of a most recent systematic revision and the International Code of Zoological Nomenclature (See http://www.iczn.org/).

11) Field Morphometric data: Field morphometric information included the following, when applicable, and was taken from each of the five required specimens:

11.1) Fresh Standard Length (abbreviated SL). From the tip of the snout to the end of the hypural fan. To mm precision.

11.2) Fresh Total Length (abbreviated TL). From the tip of the snout to the end of the caudal fin (if forked, lobes were pressed against each other, and the length took to the tip of the longer lobe). To mm precision.

11.3) Fresh Fork Length (abbreviated FL). From the tip of the snout to the end of the middle caudal fin rays. To mm precision.

11.4) Fresh weight in grams. Noted whether gutted or intact.

12) Laboratory morphometric data: Morphometric information took in the lab included the following (when applicable) and was taken from each specimen preserved for analysis. The data was recorded by the recipient collection.

12.1) Standard Length (as above). To nearest 1/10 mm in fish smaller than 100 mm, otherwise to full mm.

12.2) Head length. From the tip of the snout to the most distant point of the margin of the gill cover (operculum or suboperculum as the case may be). To nearest 1/10 mm.

12.3) Body depth. Depends on systematic group. Usually from the ventral/pelvic fin base to the dorsal midline. To nearest 1/10 mm.

12.4) Dorsal fin rays. Separate into spinous (I, II, III, etc), unbranched (i, ii, iii, etc), and branched (1, 2, 3, etc.), and give separate count for each dorsal fin (usually one, in mugilids and gobies two, in some gadoids, three; occasionally no dorsal fin present). Note: This information was needed to be sampled in the field for large specimens that are not preserved (less Standard Length which was taken as fresh Standard Length).

12.5) Anal fin rays. The same treatment than for dorsal fin.

12.6) Gill rakers. Counted ceratobranchial gill rakers only.

12.7) Pectoral fin rays. Counted.

12.8) Lateral line scales. Counted. (optional).

12.9) Sex determination (when possible).

13) Species data: Regional and/or general information on each species was collected covering the following aspects:

13.1) Basic morphology with emphasis on species diagnostic characters. This information was compiled on a per species basis.

13.2) Biological information relating to habitat, reproduction, feeding, size and geographic distribution. This information was compiled on a per region basis for each species.

13.3) Common names. This information was compiled both from general sources, such as FishBase (www.fishbase.org) and FAO (www.fao.org), and on a per region basis within FishTrace.

13.4) Threat status. This information was compiled on a regional basis.

13.5) Fisheries information. This information was compiled on a regional basis.

13.6) Socioeconomic information. This information was compiled on a regional basis.

13.7) Market appearance of products. This information was compiled on a regional basis.

13.8) Bibliography.

14) Basic morphology: This information was compiled by the Sampling and Taxonomy FishTrace working groups and entered in a free text database field.

15) Biological information: This information was submitted by regional groups, and stated:

a. Habitat
b. Depth range
c. Migratory behaviour

d. Foraging behaviour (prey capture method)
e. Aggregation behaviour (schooling, solitary…)
f. Sexuality (gonochorist, hermaphrodite, sex change…)
g. Spawning period
h. Spawning grounds
i. Spawning depth
j. Size at first maturity
k. Spawning type (scatterer, guarder, livebearing)
l. Litter size/egg number
m. Known average age
n. Known maximum age (per sex if possible)
o. Main prey
p. Known average size in catches
q. Known maximum size
r. Current commercial size
s. Current minimum size (as enforced by local laws)
t. Climate zone
u. North latitude limit
v. South latitude limit
w. General distribution area by geographic descriptors

When information was not applicable it was indicated as N/A; when not known, it was indicated as unknown; when not researched it was indicated as not researched; when not yet considered, it was left blank.

16) Common names: This information was submitted by regional groups. The source of the common name was referenced, since in other databases (e.g. FishBase) common names are compiled without real quality check.

17) Threat status: Regional information on this matter was submitted by regional groups, and stated source of information; since normally it is a national Red List. The Sampling and Taxonomy FishTrace responsible groups supplied global IUCN threat status (www.iucn.org).

18) Fisheries information: This information was submitted by regional groups considering in a synoptic fashion per species:

a. Type of fisheries
b. Fishing methods
c. Capture period
d. Exploitation level
e. Commercial interest

19) Socioeconomic information: This information was submitted by regional groups considering, in a synoptic fashion per species:

a. Forms of use (fresh, frozen, salted, dried, dried and salted, warm smoked, cold smoked, macerated, etc.)
b. Transformed product before commercialization (whole, decapitated, fillet, sliced, roe only, fins, etc.)
c. Cooking options (steamed, fried, deep-fried, grilled, raw, etc.)
d. Typical end-consumer (industry, house-hold, subsistence, restaurants)
e. Consumption site (local, national, exported)
f. Known market substitutions
g. When possible, fish products were photographed as well

20) Bibliography: The Sampling and Taxonomy FishTrace responsible groups selected general bibliography which was added to the public database. Bibliography consulted during the realization of the project is basically scientific books, reviews and articles on these thematic subjects: fish taxonomy, biology, reference collections, fisheries management bibliography and molecular biology (including standard methodologies, DNA barcoding, fish phylogeny) and some other references on different fields (See section 12.- References).

Regional groups provided local references from libraries or official sources. The format for references was provided by the FishTrace Database responsible group (See Annex XVIII). Other relevant sources of information were also used (e.g. FAO, ETI, FishBase and PescaBase [www.pescabase.org] web pages).

3.3.- Biological collections from target species

Creation of the biological reference collections

Four official FishTrace reference collection centres were created within FishTrace at: 1) the French National Museum of Natural History (MNHN), 2) the Swedish Museum of Natural History (NRM), 3) the Tenerife Museum of Natural History (TFMC) and 3) the Institute of Marine Research - Museu Municipal do Funchal (IMAR – MMF). Each Museum designated a curator responsible for these collections. The four different FishTrace reference collections, consolidated at each centre, included: 1) voucher specimens; 2) muscle tissues; 3) otoliths and 4) replicate DNA samples. After sampling, the resulting collections were permanently stored at each FishTrace reference collection centre, according to the standardized protocol (Annex XV).

Building up

All specimen's vouchers, tissue samples, otoliths and DNA sequences were labelled in accordance to the FishTrace's Sampling and Taxonomy protocol (Annex X) prior to its incorporation into the respective reference collection. As an option, each specimen obtained an additional register number and label according to particular procedures in each centre involved. Specimen vouchers were preserved in 70% ethanol or 50% isopropanol, whenever possible. Tissue samples were preserved in 96% (or 70%) ethanol in a dark cool place. Otoliths were photographed (each side separately) and stored dry. Replicate DNA samples were kept frozen at –20ºC to –80ºC. Photographs taken from biological reference collections were uploaded at the FishTrace's database.

Long-term preservation conditions

All four museums involved agreed to incorporate the FishTrace collections in their own ones and therefore keep them well preserved *ad infinitum*. In the case that one of the four Museums would be in need to dispose its FishTrace collections, the other three must be contacted in order to determine the final destination of such collections.

Loan requests by users was fixed by the Consortium. Thus, loan request by users should be addressed to the respective Curator using a specific form provided in the FishTrace web page: *RTF document of loan request form* (Annex XV). Loans were stipulated to be granted to Institutions for periods between three to six months, renewable upon request. Loans would be accompanied by the invoice of costs involved. Voucher treatment have been previously described in Section 3.2 of this report.

Access to FishTrace reference collections

Biological references availability, as well as the policy for sample exchange, loans and gifts are specific for each museum, whereas the four museums agreed to make FishTrace collections fully available for the use of the FishTrace consortium during the project. After one year of the end of FishTrace, each Museum's policy will apply to FishTrace collections. Genetically validated voucher specimens and related samples (otoliths and tissues) shall remain available to members of FishTrace's Consortium on loan. Precautions should be taken in order to avoid damage or loss of specimens during transport.

Specimens numbered 04 and 05 (See section 3.2) are assigned to potential exchange, lent or donation to any FishTrace Museum, upon request, respecting the following conditions:

a) Loans are made between members of FishTrace consortium.

b) Requests shall be directed by the respective Curator, using a standard form (Annex XV).

c) Specimens on loan must be accompanied by an Invoice (See *Invoice of Specimens*, Annex XV).

d) Loans are made for periods of three or six months, renewable upon request.

e) Loans cannot be refused to members of the FishTrace consortium. Loans to third party institutions should only be granted under exceptional conditions, after authorization of the FishTrace Coordinator and assuring the agreement of the partners.

f) The borrower is responsible for the good preservation conditions of the specimens received on loan and cannot change preservation medium without prior consent of the curator of the collection.

g) Dissecting, clearing and staining, cutting or any other intrusive/damaging procedure cannot be done without prior consent of the curator of the collection.

h) The borrower cannot transfer specimens on loan to any other individuals or institutions without prior consent of the curator of the collection. In this case a new loan contract must be done.

i) Each Curator shall decide the best way to send a loan (e.g. courier, air mail or by hand).

The FishTrace consortium will retain exclusive rights over the samples until June 30th, 2007. After that date, each Museum's policy applies to FishTrace collections.

3.4.- Molecular genetics procedures

Tissue sampling

Tissue samples were removed from each specimen sampled from the muscle behind the right pectoral fin base (muscle from inside the head in case of large fish), scrapping away the scales and cutting a square of muscle from about 25 mm^3 (5 x 5 mm). Samples were torn into smaller pieces before placing into tubes containing 95% ethanol. Tubes were marked with FishTrace code and stored in a cool place until its posting to the Molecular Genetic laboratory in charge. Tissue samples were conserved at –20ºC until their use for DNA extraction. All data from tissue samples was transferred to the FishTrace internal database (offline database) including the state of the samples which was notified to the sender. Data files accompanying samples from the sender were stored and classified.

DNA extraction

DNA from each specimen tissue sample numbered 01 and 02 was extracted following different protocols, depending on the laboratory in charge and also on the set and number of

samples treated. Several methodologies and protocols most frequently used by each of the laboratory involved (IFREMER, NAGREF, NRM, RIVO and UCM) were tested during earlier standardization stage (detailed in Section 3.1) with the aim to select the most effective methodologies. The chosen methods to test included the standard phenol/chloroform/isoamyl alcohol using Proteinase-K for tissue digestion (Sambrook *et al.*, 1989), DNA isolation station (ABI PRISM™ 6100 Nucleic Acid PrepStation; Applied Biosystems, Inc.) and commercial column kits (Qiagen Dneasy Kit®, Qiagen Dneasy Tissue Kit® and QiAmp DNA mini kit®; QIAGEN Inc., Valencia, Calif.). The results obtained with all the DNA extraction methods tested (from the purification to the final PCR amplification of both genes) indicated that they satisfied its aim (see Figures in Annex XI). Most used methodologies during the experimental period of the project were the DNA isolation station and commercial column kits. DNA quality was routinely visualized in 0.8% agarose gels. When required, DNA concentration was determined by PicoGreen® DNA quantitation kit (Molecular Probes) in a 96 multiwell microplate fluorometer reader. Details on the DNA extraction method used for each sample was recorded and has been made available in the FishTrace database.

PCR amplification conditions

For the DNA barcoding of the targeted teleost specimens, the mitochondrial cytochrome *b* (cyt*b*) and the nuclear rhodopsin (rhod) gene were PCR amplified. Length of the fragments amplified (1141 bp for cyt*b* and 460 bp for rhod), guaranteed accuracy and efficiency in species identification by this molecular approach (Jérôme *et al.*, 2003; Dettai and Lecointre, 2005). Table 3.4 shows the standardised optimal PCR conditions used in the amplification of the targeted DNA fragments and the corresponding thermocycling programmes. Changes in the annealing temperature and extension time were the most frequent modifications to specifically optimise DNA amplification across different DNA origins.

A total of 57 different conditions of primer pairs combinations, thermocycling parameters and other modifications (alternative strategies) from the standard protocol (Annex XII), were used for the PCR amplification of both target genes. These conditions for PCR amplification (fish versatile and fish specific), which were optimized at each laboratory, were also archived into the FishTrace database.

Direct and nested PCR strategies for the amplification of cyt*b*, and rhod are described in Tables 3.5. and 3.6 respectively. Up to 41 different primers were used in FishTrace (primers

sequence are given in Tables 3.7, 3.8 and 3.9). Among them, a collection of 21 primers was granted to the FishTrace network by a previous research project (See www.pescabase.org). The rest 20 primers were designed within FishTrace.

Cytochrome b amplification

Direct or nested PCR amplification of cyt*b* was carried out using the conditions described in Table 3.5. Nested PCR was used due to the improved efficiency for quick amplification and sequencing. Table 3.7 shows the 12 fish versatile primers used for cyt*b* amplification. These primers were designed in regions of low variability, flanking the areas of PCR amplification of the cyt*b*, as described in Figure 3.3. Other 18 species-specific primers, listed in Table 3.8, were designed to improve specific PCR amplifications from some samples, or specimens, from a given family/order.

In nested PCR, the same product from the first PCR run (~2 µl) was used as a template for the subsequent PCR amplification of both fragments cyt*b*-5' (~750 bp) and cyt*b*-3' (~700 bp). Direct PCR for the complete amplification of the gene (1141 bp) was also carried out following one of the four different amplification conditions described in Table 3.5 (Named A to D). All alternative PCR conditions used to amplify either the complete cyt*b*, or the cyt*b*-5' and cyt*b*-3' fragments separately, which have been used in FishTrace (Table 3.10), are deposited in the database.

Rhodopsin amplification

For the amplification of the targeted 460 bp rhodopsin fragment, only nested PCR was carried out using the conditions detailed in Table 3.6 with the set of nine primers (5 forward and 4 reverse) designed in a previous research project (www.pescabase.org), and granted to FishTrace. Details on these sets of primers are given in Table 3.9. Figure 3.4 shows the rhodopsin amplification scheme and the relative location of the nine primers used, corresponding to the 5' position in the *Astyanax mexicanus* rhodopsin gene (GenBank accession number: U12328). Usually, the product from the first PCR run (~2 µl) was used as a template for a second amplification (nested PCR), obtaining enough amount of DNA for its sequencing.

Sequencing

PCR products expected were fragments of DNA of a defined length. To check the length of the PCR fragments, agarose gel electrophoresis was carried out (Sambrook *et al.*, 1989). Comparison of the DNA band size with known molecular-weight markers allowed to identify the expected molecular weight fragments. DNA concentration at each band is proportional to the band intensity. Thus, PCR products selection for sequencing was done by estimation of band intensity. PCR products were sequenced bidirectionally and most of them using the same PCR primers in an ABI 3730 multicapillary sequencer. Figure 3.5 shows an image taken from the ABI 3730 software, from the sequencing of 96 samples at once giving 100% effectiveness. Sequencing PCR reactions in the presence of the corresponding fluorescent di-deoxynucleotides nucleotides were carried out according to standard protocols (McBride *et al.*, 1989; Bevan *et al.*, 1992; Carrilho *et al.*, 1996; Dovichi, 1997). Given the large amount of sequencing performed in FishTrace, all sequence data, including original electropherograms, and sequencing files are available for inspection at the respective FishTrace Consortium's Institutions.

Curation of sequences obtained

Electropherograms from the automated sequencer were aligned and corrected by the visualization software tool SeqScape v2.5 (Applied Biosystems, Inc.). Figure 3.6 shows representative cyt*b* sequences and electropherograms. Curated sequences obtained were routinely phylogenetically validated, as described in Annex XIII.

Submission of sequences to NCBI-GenBank

Sequences obtained (and used as DNA-barcodes in this study) are being submitted to the NCBI GenBank (www.ncbi.nlm.nih.gov/Genbank). FishTrace has adapted a standard protocol for sequence submission to this major sequence database. This protocol aimed the batch preparation of the whole amount of sequences obtained from both target genes to deposit them at the sequence database. The protocol was placed as downloadable file from the European Commission Project Tracking & Archive web site (http://pta.jrc.cec.eu.int/). Accession numbers of sequences will be listed at the FishTrace web page as soon as they would be included in GenBank.

Sequence alignments

Sequence alignments from both nucleotide data sets were carried out separately using ClustalX (Higgins and Sharp, 1988; Thompson *et al.*, 1997) with practically no need for manual corrections. Visual inspection of the resultant alignments were performed using GeneDoc (Nicholas *et al.*, 1997). Resulting sequence aligments were used to build a single nucleotide matrix for the subsequent phylogenetic analyses, using MEGA 3.1 (Kumar *et al.*, 2001). Cyt*b* and rhod sequences taken from GenBank were also included in the alignments as quality control elements.

Phylogenetic analyses

Different phylogenetic analyses were carried out using MEGA 3.1 with the nucleotide sequences obtained in FishTrace, to: (i) test the potential phylogenetic resolution of FishTrace DNA-barcodes; (ii) validate data; and (iii) identify species.

Distance and parsimony criteria were used to construct Neighbor-Joining (NJ), Minimum Evolution (ME) and Maximum Parsimony (MP) trees (Fitch, 1971; Saitou and Nei, 1987; Rzhetsky and Nei, 1992). Bootstrap analysis of each phylogenetic hypothesis was carried out to determine reliability of the inferred trees (Felsenstein, 1985). The phylogenetic parameters used in the different analysis were the following: (i) the “Pairwise Deletion” option was selected for handling sequence alignment gaps and/or missing data, obtaining by this way the largest possible number of informative sites to be compared among sequences; (ii) unweighted treatment of Transitions and Transversions substitutions; (iii) uniform rate among sites analyzed; (iv) homogeneous pattern among lineages.

Combinations of the mitochondrial and nuclear gene sequences were tested as DNA-barcodes for phylogenetic reconstructions for all teleost taxa. Phylogenetic resolution of DNA-barcodes was performed on sets of sequences (cyt*b*, rhod and cyt*b* + rhod) using MEGA 3.1 phylogenetic software.

Results from these analyses were also compared to published fish phylogenies (Chen *et al.*, 2003; Miya *et al.*, 2003) to determine the resolution of the phylogenetic hypotheses obtained with each gene separately and with both sequences joined in a single matrix.

Cladistic analyses were also performed on representative teleost species for validation purposes. For this, phylogenetic analyses were performed to identify the location of the sequence in the topology pattern of main fish groups. The addition of the newly obtained sequences (cyt*b* and rhod) into this matrix, was used as a "template" for sequence validation. The position of each FishTrace sequence on fish phylogeny was one of the steps for its validation (the complete validation process is described later under Section 3.6). In addition, another phylogenetic analyses were used for the identification of species by DNA barcoding.

3.5.- Molecular analysis of fish populations

Target species

Within the FishTrace scheme of sampling, those widespread distributed species across several geographical areas, could generate information on the degree of sequence variability which could in turn be correlated with potential populations. Thus, a pilot study on population analysis of six species distributed in several sampling areas was carried out with a statistical significant number of specimens and through the sequencing of the complete cyt*b* gene. The chosen species are listed in Table 3.2. Twenty specimens were sampled from each geographical area. Tissue sampling, DNA extraction, PCR and sequencing from these fish specimens were performed as detailed above (See Section 3.4).

Genetic Population Structure Analysis

The collection of a representative number of specimens (twenty) from each of the six targeted species (Table 3.2) was followed with the amplification and sequencing of cyt*b*. Thus, sequence variability analysis provided additional information to the FishTrace database about particular genotypic markers of fish populations.

An initial approach to recognize potential population structures was carried out by calculation of F_{st}[1] and AMOVA molecular variances for population structure based on cyt*b* nucleotide sequence data. These statistical analyses were performed using Arlequin v.2.000 software (Schneider *et al.*, 2000; http://anthro.unige.ch/arlequin).

[1] F_{st} is a measure of population subdivision based on genetic polymorphism data (Hudson, 1992). This statistics compare the genetic variability within and between populations.

Another statistic analysis using the TCS algorithm (TCS software v.1.21; Clement *et al.*, 2000) was performed to detect haplotype connectivity on the targeted fish species populations. A full description of the methods and parameters used in these analysis is indicated in the spreadsheets for each species in the report of results from both analysis, included as Annex XIX and Annex XX.

3.6.- Data validation

Data compiled in the database was generated by different groups of taxonomists, geneticists and curators. Because the high degree of specialized knowledge collected within FishTrace, an exhaustive validation of the data obtained was required. This large amount of information required to be checked for reliability and standardisation among the different data sets and from the various groups generating it. The process of validation was defined through four steps: (i) specific data checking (errors and missing data); (ii) specific data compilation; (iii) specific database field validation; and (iv) data collating and arranging information.

A responsible partner (ICCM) was in charge of coordinating the other partners to follow the complete data validation process. Protocols and guidelines we delivered for the correct validation of the data obtained within FishTrace on the three different sets of data: (i) taxonomic-biological-ecological; (ii) molecular genetic data; and (iii) data from the biological reference collections.

Actions taken for data validation included:

a) Allocation of main validation tasks to the different research groups involved.
b) Assigning species for validation to research groups according to geographic proximity or familiarity criterion.
c) Generation of protocols to define the database format, its contents and the sequential procedures for validation of datasets and the research groups involved. Three main protocols to follow were delivered: 1) *Guidelines for Validation purposes including a protocol defining format for the Validation tasks* (Annex XVI); 2) *Resumed Protocol for the online validation process* (Annex XVII); and 3) *Phylogenetic Validation of Sequences, Guidelines* (Annex XIII).

d) Thematic discussions on validation and meetings to evaluate the data status progress. Meeting agendas are given in Annexes I to IX).
e) Bimonthly and Final Reports on Validation and Missing Data (Annex XXI).

Data validation in database: Validation tools

Through the *offline* database, FishTrace partners had access to all data deposited in the database in form of tables, allowing data to be compared for each information field of the database. Thus, each responsible for the validation of a set of species was able to retrieve the information entered by other groups. Validation responsible completed the validation process following the protocol described in Annexes XIII, XVI and XVII, which included the filling of an Excel file to update periodically the level of completion (*Control Data Validation Flow Document*: Annex XXII). After validation of data entered in the different database fields, each scientist responsible for taxonomy, collections and molecular genetics signed the validation. Also an optional field for remarks after data validation was available to each scientist responsible.

The complete validation process implied the cross-checking of the data obtained from the taxonomic identification of the specimens sampled against the DNA sequences obtained from them. Thus, the whole process was completed only when sequences were obtained and available in the *offline* database.

An *offline* tool conceived for the visualization and comparison of the information entered in the database and assists in the data validation was implemented at the Data Loader Interface through the clickable link “View Data” (Figure 3.7). The access to this tool was restricted to the FishTrace partners, for use in data validation. This tool allows retrieving and comparison of data deposited by other partners by selecting a specific database field. Thus, the tool displays the information requested that should be validated for a given species, as shown in Figure 3.8 (e.g. comparison of all cyt*b* sequences obtained from the *Mullus surmuletus* specimens captured at five different geographical areas). The output for this tool is a table which could be exported to an Excel file.

In addition, an *online* tool for the comparison of all data fields for the different specimens of a given species was implemented in the public web interface since it can be used also for

comparison of data from specimens of different populations. This tool was used for data validation purposes at the second level of the validation (see below).

The specimens data comparison tool has three steps:

1) By searching a target fish species (by scientific or common name) at the FishTrace web interface, the user access to a web page which contains the information related to the chosen species (Figure 3.9).

2) By selecting the "Specimen" tab resulted from the previous species searching, the user access to the specimens information table, where the information from each specimen of the given species is available (Figure 3.10).

3) The "Data comparison" link allow the comparison of the different regional information entered in the database for a chosen species. This page contains a table where all the fields with the same information from fish specimens (from different geographical origin) are displayed, allowing simultaneous comparison of the data deposited (Figure 3.11).

Taxonomic validation of species sampled and deposited in the biological reference collections

The process for the validation of taxonomic data obtained consisted in three levels:

1st Level: Each taxonomic group was responsible for their internal data validation to detect possible errors or missing data.

2nd Level: Once entered in the *offline* database, sampled species were assigned to participating groups to proceed with the validation of the taxonomical, biological ecological, regional parameters and molecular data recorded during the identification of each specimen at the species level.

3rd Level: Sampling-taxonomy-collections data and molecular genetics data was cross-validated along the database.

The data validation from the biological reference collections created within FishTrace was devoted to guarantee that the reference numbers given for the collection voucher, DNA, tissue and otolith at each institution corresponded exactly to the samples and specimens from that species, genetically and taxonomically assigned.

DNA sequence validation

Each laboratory participating was responsible for internal detecting any error related to molecular identification of species. This validation process implied: a) to compare both DNA sequences obtained from specimens 01 and 02 at each geographical locations; b) to annotate every change on the sequence; and c) to verify the position of each sequence on accepted molecular phylogeny of fish (e.g. through BLAST searching, calculating trees, etc. Extended information on the phylogenetic validation of sequences is given in Annex XIII). Once validated at this first level, sequences were deposited in the *offline* database.

Thus, the second level of the genetic data validation included the comparison of all sequences from a given species. Each laboratory involved (Ifremer, NAGREF, NRM, RIVO and UCM) was responsible for the validation of an assigned list of species. This second validation step was carried out following the same steps of the first level. Consistency of phylogenies and gene variability analysis of the set of sequences determined the validation of the species.

The last step of the validation process (third level) included the final data validation by the workpackage responsible partner ICCM that finish the data validation process doing a specific data checking (errors, missing data,…) and a specific data compilation, collating and arranging. Table 3.11 describes the whole flow of procedures for the validation of the genetic data obtained, at the three different levels.

3.7.- Database implementation

System description

The FishTrace database system was designed to comply with two main functions: 1) To collect the information concerning European marine fish species from the FishTrace Consortium; and 2) To display the collected data in a consistent way for end users and general

public. Figure 3.1 shows the different type of information collected within the project by different partners as the catch data (location, depth, collector name), the taxonomic information or the genetic sequences, which are stored in a central system that could be retrieved by the user. The system also displays output information from tools implemented for the analysis of the data selected specifically by users.

For the data collection the main part of the information exchange was based on XML. The collected data was transformed in an XML string and then send to the dataset where they were stored. For data display on the general public FishTrace was based on a 3-tier architecture (e.g. the information is processed in 3 steps: the database interrogation, the JSP and the web pages). This system was convenient to build dynamic web pages because the JSP can treat very complex information using a programming language java before sending them in a web page. In the following lines, an example of XML string describing a sampling environment is shown:

<ID_BIOGEO>ICCM_AkGa_30_04_03</ID_BIOGEO><LOCALITY>ICES VIII C1</LOCALITY><DEPTH>-1</DEPTH><FISHING_METHOD>bottom trawl</FISHING_METHOD><FISHMARKET></FISHMARKET><LON>-2</LON><LAT>-2</LAT><COORD_SOURCE>other</COORD_SOURCE><SAMP_PURCH_DATE>30-4-2003</SAMP_PURCH_DATE><SAMP_COLLECTOR>Arego.S</SAMP_COLLECTOR><CRUISE_NAME>Aketze Gaztelugatze</CRUISE_NAME><LANDING_SITE>Port of Ondarroa</LANDING_SITE><CRUISE_NAME>Aketze Gaztelugatze</CRUISE_NAME><COLLECT_REMARKS></COLLECT_REMARKS>

Main system characteristics

The FishTrace database architecture was based in three components, a database, a web server and a set of intelligent functions that communicates between the database and the Internet pages.

Main system characteristics of the database are listed in Table 3.12: the database was Oracle 8i, choice made to ensure the future extension of the system to include a large amount of data. The server is an open source software Jakarta Tomcat 5.0 and both were installed on a Windows 2000 server.

Tomcat is based on Java servlets and JSP (Java Server Page) technologies used to extract or insert information from the database and to communicate with web pages sent to the user on a

simple web browser as Netscape or Internet Explorer. All these technologies are based on Java and JavaScript.

3.8.- Public web interface

Public web interface implementation

There are two levels in the implementation of the FishTrace web interface. The first level is the web interface describing information about the project, that was written in classic HTML-Javascript. The second level is the interface written in JSP that communicate with the oracle database. The URL www.fishtrace.org, and all files were installed on a domain server (Jakarta Tomcat 5.0.25), so the database can be interrogated using the web interface.

Public web interface design

FishTrace web interface was written using PHP Macromedia® Dreamweaver® 8. For the design of the web interface, particular attention was given to:

- Provide the most user friendly possible interface to allow an easy public access to the database content.

- Allow user to access species information but also to all type of data concerning each individual specimen collected within the project.

4.- Results

4.1.- Standardization of experimental procedures

In order to reach consensus on the different experimental procedures used by each institution, the specific guidelines and methodological protocols for biometric and genetic analysis and for the creation of the biological collections were delivered at the beginning of the project. To promote the use of these guidelines procedures and protocols within the Consortium, they were promptly uploaded on the PTA web site (http://pta.jrc.cec.eu.int/), allowing easy access to FishTrace participating groups.

These standardized procedures delivered within FishTrace have been annexed to this Report as follows:

Sampling and Taxonomic identification

- Annex X: Sampling and Taxonomy (WP2) Protocol.

Molecular identification and DNA barcoding (Genetic Catalogue)

- Annex XI: Results from Molecular Genetic Procedures Standardisation.

- Annex XII: Molecular identification and DNA barcoding (WP3) protocol and PCR conditions.

- Annex XIII: Phylogenetic Validation of Sequences. Guidelines.

- Annex XIV: Preparing sequence files for the Sequin tool.

Biological Reference Collections

- Annex XV: Reference Collections (WP5) protocol, loan request form and invoice of specimens.

Data validation

- Annex XVI: Guidelines for Validation purposes (WP7) including a protocol, defining format for the Validation tasks.

- Annex XVII: Concise Protocol for the online validation process.

Bibliography

- Annex XVIII The Bibliography Module in the FishTrace Database.

4.2.- Sampling and Taxonomic identification

The *ad hoc* sampling of biological material (fresh specimens of European teleost species from strategic geographical sea areas) and its taxonomic identification was a major objective and a milestone to subsequently proceed with the molecular identification of those sampled specimens.

From the original sampling plan, more than 100 % of the total number of targeted species was covered (220 species sampled in front of the original plan of 180). When not taken into account the geographical overlapping of some targeted species, the number of sampled species increased to 514, as detailed in Table 3.1. Only a few of the fish species scheduled were replaced by other available in the area. Comparing the original target list with the final list developed during the course of the project, 14 European and 32 Extra-European taxa were removed mainly because specimens could not be obtained at the required time. Those removed from the list were already rare species in the case of European species, and the market options for extra-European species is dynamic and not always offer the same species, which could not be longer available. The final list of sampled species (Table 3.1) includes 97 taxa not reflected in the original target list, (being 24 of them, Extra-European species). On the other hand, eight taxa that were originally listed as "*species* spp." in the original list (indicating a group of species of the same genus) were subsequently represented with 19 different species, included in those taxa, as follows:

1) *Arnoglossus* spp. (Family Bothidae, Order Pleuronectiformes), was represented by *A. laterna*.
2) *Callionymus* spp. (Family Callionymidae, Order Perciformes), was represented by *C. reticulatus* and *C. maculatus*.
3) *Chelidonichthys* spp. (Family Triglidae, Order Scorpaeniformes), was represented by *C. lastoviza*, *C. lucernus* and *C. obscurus*.
4) *Epinephelus* spp. (Family Serranidae, Order Perciformes), was represented by *E. caninus*, *E. costae*, *E. marginatus* and *E. tauvina*.
5) *Gymnothorax* spp. (Family Muraenidae, Order Angulliformes), was represented by *G. afer*, *G. polygonius* and *G. unicolor*.

6) *Liza* spp. (Family Mugilidae, Order Perciformes), was represented by *L. ramado* and *L. aurata*.

7) *Spicara* spp. (Family Carangidae, Order Perciformes), was represented by *S. flexuosa, S. maena* and *S. smaris*.

8) *Umbrina* spp. (Family Scianidae, Order Perciformes), was represented by *U. canariensis*.

At present, 2461 fish specimens belonging to targeted fish species were sampled within FishTrace. It should be noted that some other extra specimens (all from the Canary Islands sampling area) were obtained increasing the number of species sampled within FishTrace (Table 3.1). Nevertheless, 26 of them were not included in the definitive list since they have not been completely processed at the time of writing this report (Table 4.1). However, the FishTrace Consortium does not wish to waste this extra effort and will finish the whole data obtained and processing to include it in the FishTrace database. Figure 4.1 shows the relationship between planned and completed number of species sampled at each geographical sampling area. Table 4.2 shows the relative contribution of each partner at each geographical area, on six main parameters: number of species sampled, entire fish vouchers included in a reference collection, photographs taken from specimens caught, tissue samples removed from vouchers, otoliths extracted from vouchers and number of photographs taken from the otoliths.

Number of species was relatively similar from each area (48-61 species), except for the Western Mediterranean, which is represented by as many as 91 species. Across the areas, a species may be represented in one to seven areas, and the mean value was 2.3 areas, indicating that some species are particularly abundant in a single area. This also suggests that major diversity was targeted in the sampling, and that less common but regionally significant species were also included. A total of 15 species were collected in six native areas (one of these also Extra-European), but only one species in all seven European areas. Forty of the species were sampled in only one area. Figure 4.2 shows the contribution of the sampling in each area in percentage.

According to the taxonomic identification of the 220 fish species included in FishTrace, teleost diversity was represented by 17 orders, more than 75 different families and 112 genera. Most represented orders (by the number of different families included) were:

Perciformes (with 32 families, 67 genera and a total of 106 species), followed by Scorpaeniformes (10 fam., 17 gen. and 23 sp.), Pleuronectiformes (6 fam., 22 gen. and 26 sp.), Gadiformes (5 fam., 15 gen. and 24 sp.), Tetraodontiformes (4 fam., 4 gen. and 4 sp.), Anguilliformes (3 fam., 5 gen. and 10 sp.), Aulopiformes (3 fam., 3 gen. and 3 sp.), Clupeiformes (2 fam., 6 gen. and 8 sp.), Osmeriformes (2 fam., 3 gen. and 3 sp.), Atheriniformes (1 fam., 1 gen. and 2 sp.), Batrachoidiformes (1 fam., 1 gen. and 1 sp.), Beloniformes (1 fam., 2 gen. and 2 sp.), Beryciformes (1 fam., 1 gen. and 2 sp.), Lophiiformes (1 fam., 1 gen. and 2 sp.), Ophidiiformes (1 fam., 1 gen. and 1 sp.), Salmoniformes (1 fam., 1 gen. and 2 sp.) and Zeiformes (1 fam., 1 gen. and 1 sp.). Detailed information on sampled species and geographical distribution is shown in Table 3.1.

Sampling in FishTrace has been mainly conducted following biodiversity, commercial and ecological criteria. Concerning the order Perciformes, it contains some of the most common and appreciated groups of fishes in the European markets, which are, in turn, under examination of potential depletion. Examples of these species, well represented in FishTrace, belongs to order Perciformes (e.g. fam. Scombridae: *Thunnus* spp.; fam. Serranidae: *Epinephelus* spp.; fam. Sparidae: *Boops boops*; etc.), order Gadiformes (e.g. fam. Merlucciidae: *Merluccius merluccius*; fam. Gadidae: *Gadus morhua*; etc.), order Clupeiformes (e.g. fam. Clupeidae: *Sardina pilchardus*; fam. Engraulidae: *Engraulis encrasicolus*; etc.) and order Pleuronectiformes (e.g. fam. Soleidae: *Solea solea*; etc.). Thus, within FishTrace have been collected crucial fish orders for traceability studies, due to its high commercial and ecological values.

The taxonomic information deposited in the database (www.fishtrace.org) from each targeted fish species includes specific regional data on biological and socioeconomical aspects. Such information has been also enriched with the list main regional publications on taxonomy, distribution and ecology. This is of particular interest for fish identification purposes in European seas.

As example of the applicability of these results we have selected the case of the genus *Trisopterus* that provided new insights into their genetic identification and distribution in Europe.

The gadid fish genus *Trisopterus* includes three well known European species, *T. minutus, T. esmarkii*, and *T. luscus*. Although *T. minutus* is at present not considered to be differentiated

into subspecies, a report based on allozymes (Mattiangeli *et al.*, 2000) suggested that *T. minutus* might be separated into two subspecies, one from the Atlantic Coast of Western Europe (*T. minutus minutus*) and one from the Mediterranean (*T. minutus capelanus*). Molecular genetic analysis in FishTrace confirmed this separation into subspecies given the large interspecific cyt*b* variation (14.5 %) between population in Atlantic and Mediterranean (Table 4.3). In addition, morphological data taken from the collected specimens within FishTrace found at least one marker, the number of gill rakers, which can be used for rapid identification of whole specimens, as well as the number of rays present in the third dorsal fin: 18-22 in *T. minutus* against the 15-17 described for *T. minutus capelanus* (Table 4.4). Meristic characters taken from *Trisopterus minutus auct.*[1] sampled in the NE Atlantic (representing *T. minutus s. str.*[2]) and Mediterranean (*T. minutus capelanus*) are given in Table 4.4. These features support the inclusion of morphological data in FishTrace as a validator and complement to the genetic data. Thus, the phylogenetic analysis based on the FishTrace DNA-barcodes demonstrates diagnosability of the Atlantic and Mediterranean *T. minutus*, supporting even species level status for the former subspecies, which might be referred to as *T. minutus* (Linnaeus, 1758) and *T. capelanus* (La Cèpede, 1800), respectively. Furthermore, cladistic analyses performed showed that *T. capelanus* is more closely related to *T. luscus* (interspecific cyt*b* variation of 4.5 %) than to *T. minutus*, supporting earlier association (Mattiangelli, *et al.*, 2000). Moreover, *T. minutus* (from CB and NS) rendered a monophyletic clade with *T. esmarkii* (interspecific cyt*b* variation of 11 %), indicating a more close association between these two species. Thus, the morphological closeness between the two *Trisopterus minutus* subspecies is probably due to morphological convergence since genetically they are more separated than from the other *Trisopterus* species. This particular result is a clear example of taxonomic identifications assisted and corroborated by DNA-based analysis. These results might have also consequences for regulations, conservation and exploitation programs for the *Trisopterus* species.

A similar case of disagreement between morphological and genetic analyses is in the Ammodytidae specimens: *A. tobianus* and *A. marinus* (sand lacers). Ammodytids were sampled only from Skagerrak (BS). These two species were identified using morphological characters, and subjected to cytochrome *b* and rhodopsin sequencing. Results obtained suggest that the two species are genetically diagnosable, but without enough confidence (low interspecific cyt*b* variation of > 1 %). On the other hand, the morphological identification did

[1] *Auctorum*
[2] *Sensu strictu*

not permit assignation to any of the known species. Ammodytids were therefore dropped from the list (Table 3.1), and will require more extensive taxonomic revision before they can be subjected to bar-coding attempts.

Finally, it should be mentioned to this respect that the Extra-European biological specimens identified as *Solea solea* in markets were subsequently identified by the FishTrace DNA-barcode as *Michrochirus azevia*. This case is a clear example of taxonomic misidentification and market substitution that could be detected by DNA-based analysis (see section 4.4 of this Report).

4.3.- Biological Reference Collections

Four Biological Collections, voucher specimens, total DNA, tissue and otoliths, were created from the target species at the four museums participating in the FishTrace network: Muséum National d'Histoire Naturelle (Paris); Naturhistoriska riksmuseet (Stockholm); Museo de Ciencias Naturales (Tenerife); and Museu Municipal, História Natural (Funchal). For the purposes of the FishTrace project, reference collections were considered completed for the vouchers "01" and "02" sampled. At present, a total of 1468 specimen vouchers (exceeding the 1040 expected) are stored in the collections. At the time of writing this report, some reference collections (mostly fish specimen vouchers and otoliths) still need to be transferred between participating institutions. The DNA samples (>2500), that were extracted from the specimen vouchers have been also stored at each museum. Around 600 pair of otoliths removed from fishes sampled were also added to the reference collections. FishTrace have composed a photo-gallery including more than 4000 photographs directly taken from fish specimens and also more than 650 images from otoliths and fish products. The actual stage of completion of these collections is described for each participant museum in Table 4.5 and Figures 4.3 and 4.4.

These collections of biological material, from taxonomically and genetically identified fish species, serve as a reference infrastructure in Europe providing the potential for future applications related to fish species authenticity and/or associated biological research. The reference collections have the added advantage of easy access, through an interface in the online database, for consultation, loan and exchange of material. Reference numbers are given in the FishTrace database. User requests shall be addressed to the respective curator using a

standard form (Annex XV). Loans could be made to public institutions for periods of three or six months, renewable upon request, and the specimens on loan must be accompanied by an Invoice (Annex XV).

4.4.- Genetic Catalogue of European marine fishes

Species included in the FishTrace Catalogue

The FishTrace Consortium reached its main objective in creating an *online* database with the necessary number of DNA sequences for the DNA barcoding of most commercialized European teleost fish species. The number of targeted species increased along the project, from 180 species planned in the Technical Annex, to 220 species finally sampled, taxonomically and genetically identified and included in Reference Collections (Table 3.1). Taking into account the geographical overlapping of some targeted species, this research handled taxonomic, ecogeographical and genetic data obtained from 514 geographically separated teleost species (e.g. *Boops boops* was captured at five of the areas sampled in this study: MA, EM, WM, CS and CB areas).

The molecular genetics information obtained (DNA sequences from all the species sampled) is the main part of the FishTrace Genetic Catalogue, covering 17 major Actinopterigii orders, 75 different families and 112 genera. Represented orders include Anguilliformes, Clupeiformes, Osmeriformes, Salmoniformes, Aulopiformes, Ophidiiformes, Gadiformes, Batrachoidiformes, Lophiiformes, Atheriniformes, Beloniformes, Beryciformes, Zeiformes, Scorpaeniformes, Perciformes, Pleuronectiformes and Tetraodontiformes. Detailed taxonomic and geographical distribution of targeted species is described in Table 3.1.

Tissue samples processed

According to the list of targeted species (Table 3.1), and taking into account the species overlapping by the geographical area of origin, 1028 tissue samples were initially planned to be processed in order to obtain the sequences for the Genetic Catalogue. By June 2006, the Consortium reached the collection of more than 2500 tissue samples, including samples for backup and cross-referencing purposes). 1028 tissue samples were addressed for molecular identification analyses (DNA barcoding). The rest of samples were set aside and stored to

backup and cross-referencing purposes (Annex X). All of them were included in the FishTrace Biological Reference Collections.

For the creation of the DNA barcoding-based Genetic Catalogue, the step-by-step process included: DNA extractions, PCR amplification and sequencing of both targeted genes, the corresponding genetic sequence analysis for its validation and the subsequent storage of sequences into the public *online* database.

DNA extractions

DNA from fish tissue samples was extracted following the protocols that yielded better DNA at each laboratory. DNA was isolated from all the tissue samples received at the laboratories of the Consortium, following the protocols described in Section 4.1. DNA extractions from fish tissues were independently performed in two batches. One of them was used to carry out the whole standardised process for Molecular Genetic Identification, and the other respective copy was included in the Biological Reference Collections established within FishTrace.

DNA sequences obtained

The complete cyt*b* sequence comprise 1141 bp. Given its mitochondrial origin, with high evolutionary change, its analysis allows sample identification at species level. Thus, PCR primers and protocols described herein (See Section 3.4) provide a powerful tool for the DNA barcoding of practically all teleost fish species. Furthermore, they have been successfully applied in providing fully validated sequence data for the FishTrace genetic catalogue. At the end of the project, more than >90 % of the cyt*b* sequences, corresponding to the 220 species were obtained (514 species, taking into account the geographical overlapping), thus exceeding the 180 species planned in the Technical Annex at the beginning of the project (see Table 3.1 for detailed information on species added and excluded from the original list of the Technical Annex). Curated and validated cyt*b* sequences obtained from fish specimens caught at each geographical area covered are detailed in Figure 4.5.

As for cyt*b*, the DNA barcoding of 220 teleost fish species at the 460 bp PCR fragment from the nuclear gene rhodopsin was successfully achieved, far exceeding the expected number of rhod sequences. By June 2006, the Genetic Catalogue of fish rhodopsin sequences was

completed at 95 %. Curated and validated rhod sequences obtained from fish specimens caught at each geographical area covered within FishTrace are detailed in Figure 4.5.

This large representative number of fish DNA sequences has also allowed the standardisation of genetic methodologies for diagnosis and quality control purposes. Among other main objectives of this research, the developing of faster and more sensitive DNA-based technologies has been designed to assist taxonomists in the identification of teleost fishes and food products and to establish the molecular traceability basis for fish species. These rapid identification tools could also serve to enforce regulations concerning food products and consumer safety.

Genetic Catalogue and teleost species identification by DNA-barcoding

Focused in the proved capacity of DNA barcodes to allow rapid species identifications, the first European DNA-barcodes repository of teleost fish species was created within FishTrace. In addition to the taxonomic data compiled and linked to the reference collections established, the genetic data obtained by the DNA-barcoding of the collected fish specimens provide a key biological information resource to assist in the sound identification of teleost species. Newly determined DNA sequences, obtained from the complete mitochondrial cyt*b* gene and the nuclear rhodopsin fragment, can be directly retrieved from the *online* and open-access Genetic Catalogue created at www.fishtrace.org. Available DNA sequences can be also taken from the GenBank (www.ncbi.nlm.nih.gov/Genbank/index.html). Accession numbers of sequences will be listed at the FishTrace web page as soon as they have been accepted and included in GenBank.

A first approach towards the implementation of efficient DNA-based techniques that can confront the problem of reliable fish species identification implied the design of model tools to facilitate the development of pre-competitive, analytical procedures for unequivocal identification of fish species. To this respect, the following procedures were designed within FishTrace:

a) Three molecular identification tools were *online* implemented at the FishTrace web interface, aimed to assist users in the identification of teleost fish species using the genetic information entered in the Genetic Catalogue (expanded information on the

online Fish Identification Tools is given in Section 4.8. of this Report: *Public web interface*).

b) Standardization of an identification method based on the phylogenetic analysis of targeted DNA-barcodes assembled (cyt*b* and rhod sequences).

c) Collection of robust PCR primers and a set of optimized amplifications conditions developed to obtain the DNA-barcode from practically any teleost fish species at the targeted genes (See Section 3.4 for details). These optimized PCR protocols can be applied in any modest molecular laboratory.

The reliability of the DNA-barcoding for fish species identification is fully practicable from the FishTrace database. Thus, as a way of illustration, details on the results obtained from specific studies are given: **(i)** The DNA-barcoding of 120 species, comprising 102 genera belonging to 16 major teleost orders. **(ii)** The study of the order Clupeiformes (including 59 DNA barcodes from major European species of sardines, anchovies and herrings). **(iii)** The study on the family Scombridae (comprising 71 DNA barcodes from mackerels, bonitos and tunas). **(iv)** The analysis of fourteen genera from gadids, comprising DNA barcodes from 112 specimens.

(i) Different phylogenetic analyses were carried out in order to compare topologies of 120 representative teleost taxa. Tree performance was analysed by bootstrap. The species chosen for these tests comprise major Actinopterygii orders, including most commercialized teleost species in the European markets (120 sequences comprise 102 genera belonging to 16 major teleost orders). Species names are listed in (Table 4.6).

Tree topologies and support values revealed different degree of resolution for species identification among the taxonomic groups analysed. Comparison of the phylogenetic inferences obtained for the three sets of data (1141 bp cyt*b*, 460 bp rhod and 1601 bp cyt*b* + rhod) showed the highest consistency and definition of the clades with the DNA-barcode assembling both sequences (cyt*b* + rhod). Reproducibility and support of major groups (see Figure 4.6 and Table 4.7) followed clades previously defined by molecular and taxonomic studies (Chen *et al.*, 2003; Miya *et al.*, 2003). Thus, this analysis indicated not only the validity of the genetic data but also the accuracy of the methods employed to run the cladistic analyses. Although NJ and ME trees rendered the poorest bootstrap values for basal nodes

(data not shown) the MP consensus tree (derived from the 100 most parsimony trees obtained) present well supported nodes ranging values from 80 % to 100 % occurrences in major defined monophyletic clades like the orders Pleuronectiformes, Gadiformes or Clupeiformes and the family Scombridae (order Perciformes). These clades were also generated in the analyses performed using cyt*b* and rhod sequences separately, as described in Table 4.7. Reproducibility of clades formed from these separated analysis are also indicated with a red dot in Figure 4.6.

The comparative study of trees was also done with regards to the methodology employed to generate them (NJ, ME and MP). Thus, in the topologies adopted by each main clade of interest (e.g. major orders cited above), the MP analysis from the assembled cyt*b* and rhod sequences rendered the most consistent results, resolving phylogenetic relationships and grouping into monophyletic clades the closely related species with high supporting values (Figure 4.6). Consistency and retention indexes obtained from the MP analysis were 0.107943 and 0.442995, respectively. The other two methods employed, NJ and ME under K2P evolutionary model, also rendered well defined major clades from the cited above, and both methods resulted faster than the MP, but support values for these monophyletic clades obtained in NJ and ME analyses were significantly lower than in MP.

Although some basal phylogenetic relationships among teleost taxa analyzed were not optimally resolved with the fast phylogenetic procedures, robustness of the method could be improved with further phylogenetic approaches. The DNA-barcode herein proposed to be used for identifying species through phylogenetic analysis is composed by the assembling of two nucleotide sequences from two protein-coding genes, and therefore, other parameters may be taken into account. Transition *vs.* transversions rate, possible saturation effect at the third codon position and computation of phylogenetic trees using translated protein sequences, would contribute to resolving phylogenetic relationships of major teleost taxa groups. In the following specific studies, the DNA-barcode is applied to resolve at high-resolution fish species identification through cladistic analyses.

(ii) European anchovy (*Engraulis encrasicolus*, Linnaeus, 1758) have become one of the most threatened fish species within the European seas due to the overexploitation suffered during the last decades. Indeed, the Cantrabric stock has been indiscriminately harvested (FAO; Magoulas *et al.*, 2006). The rapid and unequivocal molecular identification of this species based in DNA-barcoding and subsequent genetic population structure analyses could allow to

control anchovy stocks by determining genetic diversity and also quantifying population biodiversity. This approach contributes to reinforce common fishery policies on the control of European anchovy catches.

Herein we have inferred the phylogeny of 51 clupeid specimens collected within FishTrace using different phylogenetic methods and evolutionary models. This study was aimed to demonstrate reliability of the cladistic analysis used to identify clupeids target samples to the species level, using the 1601 bp-length DNA-barcode resulted from the assembling of both, cyt*b* + rhod target sequences.

Cladistic analyses were carried out including these 51 newly determined DNA-barcodes (Taxa analyzed are described in Table 4.8-A). FishTrace DNA barcodes used in this study were obtained from European sardine, anchovy and herring species, following the taxonomic and molecular procedures for reliability and data validation. Other non-FishTrace sequences from sardine, anchovy and herring species were also retrieved from the NCBI-GenBank (www. ncbi.nlm.nih.gov/Genbank/index.html), and included into the data matrix as reference sequences for quality control purposes. In addition, an outgroup formed by two DNA-barcodes from the gadid species *Gadus morhua* and *Merlangius merlangus* was included in the analyses. Accession numbers of sequences used as quality control and outgroup are given in Table 4.8-B.

Trees from ME and MP cladistic analyses revealed clear and well supported distribution for the specimens analyzed, compared to previous studies on Clupeiformes (Jérôme *et al.*, 2003). We excluded the NJ method in this study because in preliminary tests performed, the topologies and support values obtained were similar than those rendered from the ME analysis (data not shown). In order to quantify the accuracy and resolution of the analyses, bootstrap tests were computed. Support values obtained ranged from 56 to 100 % with the MP method rendering highest values. In Figure 4.7, bootstrap values are shown in nodes corresponding to MP/ME methods.

This DNA-based identification system discriminated between clupeid families Engraulidae and Clupeidae with full confidence, rendering bootstrap values of 100 % in both MP/ME analyses. Moreover, all the equivalent DNA-barcodes retrieved from GenBank used as quality control matched the expected phylogeny (Figure 4.7).

The efficiency of this methodology for the precise identification of clupeids to species level was further evaluated using partial sequences of the complete assembled cyt*b* + rhod DNA-barcode. Analysing the ~750 bp of the 5' end from the SarPil-CS-01 cyt*b* sequence, the phylogenetic topologies performed with MP and ME allowed to cluster the sequence with their relatives *Sardina pilchardus*. When this experiment was performed with the SarPil-CS-02, with only the rhod sequence, the taxon was correctly clustered in the trees (Figure 4.7).

(iii) The potential identification of tuna species (*Thunnus* spp.) by phylogenetic analysis with the assembled mitochondrial and nuclear 1601 bp DNA-barcode was also assayed, given their lower genetic intraspecific variation of these species (Lin *et al.*, 2005).

Family Scombridae (Order Perciformes) comprises commercially appreciated teleost species such as mackerels, bonitos and tunas. Complete DNA barcodes (cyt*b* + rhod sequences) from 71 specimens of mackerel, bonito and tuna sampled in FishTrace from seven different European an extra-European sea areas (Table 3.1), were analyzed for its molecular characterization and identification at species level (list of taxa described in Table 4.9-A). Control cyt*b* and rhod sequences from some mackerel and tuna retrieved from GenBank were also included in the nucleotide data matrix (Table 4.9-B). An outgroup of two GenBank DNA-barcodes from the gadid species *Gadus morhua* and *Merlangius merlangus* was used in the analyses (Table 4.9-B).

For this study, the optimal phylogenetic method and evolutionary model was searched. The reconciled phylogenetic tree shown in Figure 4.8 reveals that both, ME (under the K2P model) and MP inferred trees, performed high resolution in topologies and bootstrap values, discriminating among major Scombridae species analyzed. The different clades formed correspond to monophyletic groups including the same species. Bootstrap values ranged from 70 to 100 %, in ME analysis and 100 % in all MP clades performed. This phylogenetic relationship was recovered for all tunas, mackerel and bonito species analyzed.

Subsequently, the second part consisted in the precise identification to the species level through the phylogenetic analysis of 29 *T. alalunga*, *T. thynnus*, *T. obesus* and *T. albacares* DNA-barcodes. The detailed subtree containing four different clades of tuna species, shown in Figure 4.8, is the reconciled from the NJ (K2P) and the MP analysis. NJ method was the fastest method used in this study. Resulting phenograms from the bootstrap tests performed under NJ (K2P), ME (K2P) and MP methods, corroborated the accuracy of the system used

for the identification of tuna species. Bootstrap supports ranged from 83 %, in the node discriminating between *T. albacares* and *T. thynnus* monophyletic clades under the NJ method, to 100 %, in all nodes performed from the MP analysis. Average K2P congeneric (*Thunnus* spp.) sequence divergence found was around 1.7 %, as detailed in (Table 4.9-C).

(iv) A set of 95 FishTrace DNA-barcodes from gadid specimens by MP and ME (K2P) was analyzed. 17 reference sequences from the same FishTrace gadid species represented in the analysis and an outgroup of 2 more target DNA-barcodes from clupeid species, were retrieved from the GenBank and added to the data matrix for quality control purposes (Tables 4.10-A and 4.10-B). Both MP and ME hypothesis revealed an optimal resolution, giving high bootstrap supports for the different clades rendered, being 100 % in all MP clades and ranged from 90 to 100 % in the ME analysis (Figure 4.9). Furthermore, all taxa analyzed matched the expected position in both (ME and MP) phylogenetic reconstructions (Bakke and Johansen, 2005; Teletchea *et al.*, 2006). Repeatability of clades performed supports the robustness of the DNA-barcode as an optimal molecular marker for phylogenetic studies. Such information was considered important for our investigation on developing a powerful and fast molecular tool, which serve to assist precise taxonomic identification of teleost species. In addition, we study in depth robustness of this DNA-based identification method in the particular case of the gadid *T. minutus*.

Cladistic analyses of DNA-barcodes within FishTrace have tested species status of *Trisopterus minutus*. The average genetic K2P divergence detected among the seven FishTrace *Trisopterus* spp. DNA-barcodes analyzed was of 6 %, but this genetic divergence increases to >10 % among *Trisopterus minutus* ("TriMin") specimens from EM/WM and NS/CB areas, demonstrating diagnosability of the Atlantic and Mediterranean forms and supporting the species level status for the former subspecies, which should be referred to as *T. minutus* (Linnaeus, 1758) and *T. capelanus* (La Cèpede, 1800), respectively. This study has also been supported by taxonomic evidences since the taxonomic identification of the FishTrace "TriMin" specimens sampled revealed some morphological differences between both populations, the Atlantic and the Mediterranean. Thus, this find helped in the taxonomic approach exerted for the separation between these species, as explained in Section 4.2.

In conclusion, the DNA-barcoding identification system developed within FishTrace, herein described, provides a powerful tool for the sound identification of practically all teleost fishes to the species level.

4.5.- Population structure of six European marine fish species

Species selected for population structure analysis

In order that meaningful results would be obtained from the population analysis, certain criteria for the selection of the species to be examined were defined at the beginning the project (see Annex I). Criteria for selection included: (i) the presence of the targeted species in at least four geographical areas; (ii) samples of each species easily accessed, (e.g. rare species were excluded); and (iii) life histories of the species selected were likely to contribute towards specific population structuring (e.g. migratory species were excluded).

Thus, finally the following six species were selected according to the criteria set above: *Merluccius merluccius*, *Micromesistius poutassou*, *Mullus surmuletus*, *Pagellus erythrinus*, *Pagrus pagrus* and *Solea solea*. For each of these species, twenty individuals at each geographical area were examined for sequence variation at the cyt*b* locus (Table 3.2).

Two different studies were performed on these six species, a genetic population structure analysis (F_{ST} and AMOVA) and an haplotype connectivity network analysis. Both population studies were based on all available and validated cyt*b* sequences obtained from the chosen species at each geographical area. The final number of complete cyt*b* sequences obtained at each area from the above species and the responsible molecular laboratory in charge is shown in Table 4.11. The specific details on the genetic population structure and the haplotype connectivity network analyses obtained for each of these six species are described below.

Solea solea genetic population structure and haplotype connectivity network

According to the AMOVA results (methods and parameters used in the analysis performed were as indicated in Annexes XIX and XX, this species exhibits a high percentage of variation among populations (55.2%) surpassing the variation within populations. In agreement with that, F_{ST} *P* values indicate significant differences between all samples (populations) examined. The genetic relationship/distance of the different populations examined is depicted in Figure 4.10.

On the other hand, the haplotype connectivity network of Figure 4.11 displays a well defined genetic population structure for *Solea solea* in the different geographical areas. The only exception is that concerning the CB and NS samples where a significant number of individuals share a common haplotype. This suggests potentially important gene flow between these two proximate geographical areas. However, it is of interest to note that the NS sample contains only a four different haplotypes. This low level of genetic variation is indicative of a homogeneous population and therefore implies a possible genetic bottleneck. Additional sampling from this area may be necessary in order to conclude on this issue. This contrasts to the eight haplotypes observed in the BS samples, the eleven of the WM sample, and the twelve of the EM sample. Note also that the eight BS haplotypes are intermediately positioned between the bulk of the NS and WM haplotypes. Therefore, there is no clear haplotype gradient, in terms of accumulated mutations in the cyt*b* sequence, from North to South to East. Furthermore, the network demonstrates that the EM sample is most genetically distant from the outgroup that contains only NS and CB haplotypes.

Important and of interest to traceability purposes is that a single A to G transition, at position 1020 in 21 of the 22 EM individuals examined, is sufficient to genetically distinguish this population from that of WM and in fact from all populations studied.

Detailed results from both analyses on *Solea solea* population are given in Annexes XIX and XX.

Meluccius merluccius genetic population structure and haplotype connectivity network

According to F_{ST} and AMOVA analyses, the samples used can be divided in two groups: those that are genetically related to the CS sample (e.g. CI, CB, BS and WM), and those that are not (EM and NS). Furthermore, the NS sample is genetically distinct from all other samples while the EM sample is not significantly different from that of area CB (P=0.295). The population diagram given as Figure 4.12 explain the results of the F_{ST} analysis for the populations of this species and also demonstrate that the NS sample is more genetically distant from the other samples analyzed. Additional sampling from WM may be necessary in order to clarify the population structure of *Meluccius merluccius* in this area, due to the intriguing relationship found between WM and NS specimens analyzed.

This particular result is both interesting and intriguing. It could possibly be explained by assuming that a single ancient population of the species, related to the present population of CB, colonized the north-eastern part of the Mediterranean, as inferred by the presence of common haplotypes in both the CB and EM samples. More recent arrivals and expansions of *Meluccius merluccius* populations in the central Atlantic area (e.g. a CS-related population), in addition to selection pressure, may have resulted in the loss of the CB haplotypes from the populations of both the BS and WM areas and possibly in other Mediterranean and Atlantic areas that were not considered in the present study. This is consistent with i) the presence of shared haplotypes between the CS, WM, and BS areas, ii) the presence of common haplotypes between the CS, CI, and CB areas and, iii) the absence of CI or CB haplotypes in either the BS or WM areas. According to this hypothesis CS haplotypes should be present in the southern part of the Iberian Peninsula, on both sides of the Gibraltar straights, and possibly also in the central Mediterranean area. Therefore, in order to test this hypothesis additional samples need to be obtained and analyzed from the above mentioned areas. Likewise the analysis will profit from data concerning the spawning behaviour, self-recruitment, and mechanisms of near-shore retention of larvae in this species. Furthermore, environmental factors, including past sea level changes, and present or past physical barriers such as ocean currents, which may have mixed or disrupted the populations of Meluccius merluccius from different geographic locations need also to be considered.

Finally, the genetic heterogeneity of the NS sample from all of the other samples examined suggests that the *Meluccius merluccius* population in this area is genetically isolated and/or is influenced by other populations of the species such as those of the North Atlantic. Additional samples from Norway, Iceland and even Canada could allow conclusions concerning this particular population.

On the other hand, the network of Figure 4.13 (resulted from the haplotype connectivity network analysis) demonstrates that a significant number of CI, CS, CB and BS individuals share a common haplotype (outgroup EM_08). Another significant cluster of a shared haplotype (EM_02) contains mostly EM and CB individuals.

Detailed results from both analyses on *Meluccius merluccius* population are given in Annexes XIX and XX.

Micromesistius poutassou genetic population structure and haplotype connectivity network

As for the case of *Meluccius merluccius* (also of the Gadidae family) this species exhibits an interesting genetic population structure for the areas from which samples were analyzed. However, in contrast to the significant genetic distance between the EM and CS populations of *Meluccius merluccius*, this analysis demonstrates a genetic relationship between the above two populations of absence of *Micromesistius poutassou*. Furthermore, neither of the WM or NS populations seems to be related to either of the CS, CB, or EM populations (Figure 4.14). However, the relatively low variation among populations of the species (12.68% vs. 15.39% in *Meluccius merluccius* and 55.2% in *Solea solea*) must be noted.

Concerning the NS population, the hypothesis put forth for *Meluccius merluccius* above may also be valid for this species as well. However, it is notable that the NS population includes only five different haplotypes compared to 18 in the EM sample, and 15, 14, and 14 in the WM, CB and CS samples, respectively. A similar genetic homogeneity was observed for *Solea solea* in the NS area suggesting a possible genetic bottleneck.

For the WM population it must be assumed that it has been genetically influenced by a more recent invasion of the Mediterranean basin with population(s) of the species originating possibly from the Atlantic coast of Morocco. This hypothesis could be tested by the analysis of additional samples from Morocco as well as from the North African coast within the Mediterranean. Factors that could explain the population structuring of this gregarious and erratic species and which, therefore, must be considered concern the life history of the species and environmental influences as these have been listed for the other species above.

The network of Figure 4.15, resulted from the haplotype connectivity network analysis, demonstrates that the majority of the *Micromesistius poutassou* EM haplotypes contain one or two substitutions as compared to those of the CB and CS samples, explaining, thus, the results of the F_{ST} analysis that did not detect significant differences in the populations of these three geographical areas. The network also demonstrates the high degree of genetic variation within all populations with the exception of that of the NS.

Detailed results from both analyses on *Micromesistius poutassou* population are given in Annexes XIX and XX.

Mullus surmuletus genetic population structure and haplotype connectivity network

As is illustrated in Figure 4.16, all the *Mullus surmuletus* samples examined, with the exception of NS, share a significant number of common haplotypes and thus, structuring of populations from the Atlantic (the Canary Islands) to the North-eastern Mediterranean is not evident. The MA sample appears to be significantly different from both the WM and CB ones. However, this sample contains only eight individuals. Increasing the number of sampled individuals from the MA area is likely to reveal haplotypes common to the WM and CB areas. It should also be noted that this analysis has not included the CS sample, which by virtue of its central geographic location could further support the panmictic nature of the Atlantic-Mediterranean populations.

The haplotype connectivity diagram of Figure 4.17 demonstrates that the results of the F_{ST} analysis are primarily based on the haplotype cluster around the EM_08 outgroup containing individuals mostly from areas EM, WM, CI, MA, and CB. In contrast, the bulk of the NS haplotypes is located around the EM_05 outgroup. The sample with the largest number of unique haplotypes is that of area CB (12 unique haplotypes), followed by the NS sample (8 unique haplotypes). The MA sample with only 8 individuals examined appears to be related to both the CI and EM samples but more genetically distant from either of the WM or the CB samples.

Detailed results from both analyses on *Mullus surmuletus* population are given in Annexes XIX and XX.

Pagrus pagrus genetic population structure and haplotype connectivity network

The distribution of the populations of this species and consequently their structure appears sensitive to physical barriers. Thus, there is a clear separation between EM and MA and each one of these populations is different from those of CI and WM, which share a significant number of common haplotypes (Figure 4.18). The above suggests that gene flow between the CI and WM areas follows the West African coastline through the Gibraltar straights into the Mediterranean. This could be further supported by additional samples from the Moroccan coast and from both sides of the straights. Isolation of the MA population is possibly due to prohibitive depths for the biology of this species, separating the Canaries Islands from Madeira. A possible barrier within the Mediterranean separating the WM and EM populations

could be the Sicelo-Tunisian straight. However, the analysis of additional Mediterranean samples is necessary in order to demonstrate this separation. Note that the CS sample, which could provide important information concerning the genetic relationship of the Atlantic and Mediterranean populations, is missing from this analysis. Furthermore, the EM sample contains only twelve individuals a fact that could bias the results of the analysis.

The haplotype connectivity network of Figure 4.19 demonstrates that areas CI and WM share a significant number of common haplotypes. Specifically the EM_03 haplotype is shared by six CI and six WM individuals and the EM_04 haplotype is shared by six and seven CI and WM individuals, respectively. In contrast of the twelve individuals of the EM sample tested, only 4 share haplotypes with the WM and CI samples (two for each of the EM_03 and EM_04 haplotypes). Furthermore, 19 out of the twenty individuals of the MA sample present are specific haplotypes. The above explain the results of the F_{ST} analysis for the populations of this species, which suggest non-significant differences in the genetic population structure of areas CI and WM and the existence of genetically distinct populations in areas MA and EM.

Detailed results from both analyses on *Pagrus pagrus* population are given in Annexes XIX and XX.

Pagellus erythrinus genetic population structure and haplotype connectivity network

With only three samples of *Pagellus erythrinus* analyzed (from areas CI, WM and EM, see Annexes XIX for details), no obvious structuring of populations was observed (Figure 4.20). In fact for this species the variation within populations was at 100% with essentially each individual representing a distinct haplotype (17 haplotypes in 18 individuals in the EM sample, 18 haplotypes in 19 individuals in the CI sample, and 15 haplotypes in 17 individuals in the WM sample). This situation is indicative of a panmictic population in the areas examined due to high dispersal rates and large effective population size. Further sampling and analysis from areas MA and CS could demonstrate the existence or absence of a genetically distinct population(s) of the species in the Atlantic.

Subsequently, the network diagram of Figure 4.21 resulted from the haplotype connectivity analysis from areas CI, WM and EM (see Annex XX for details), demonstrates the high level of interpopulation genetic variability. A limited but nevertheless significant number of shared

haplotypes, as well as clusters of haplotypes within the three populations examined, with one or two substitutions explain the results of the F_{ST} analysis, i.e. the pammictic nature of the populations of the species. Notable is however, the accumulation of mutations in the individuals placed in the lower part of the network suggesting a large effective population size.

Detailed results from both analyses on *Pagellus erythrinus* population are given in Annexes XIX and XX.

4.6.- Validation of taxonomic and genetic data

A main document, the *Guidelines for Validation Purposes* (Annex XVI) was created to define objectives, expertise involved, protocols and the step-by-step validation process. The validation tasks were allocated to different expert groups according to the type of data to be validated: ICCM-TFMC, IMAR, NAGREF, IFREMER-MNHN and NRM on sampling and taxonomy; UCM, RIVO, NAGREF, IFREMER and NRM on molecular genetics; and TFMC, IMAR, MNHN and NRM on biological reference collections. Thus, the total 220 target species were assigned for validation purposes to these groups.

1st Level of data validation: Each specific specimen was first validated by its responsible scientist following the standardized protocols for data sets of taxonomy and genetics. Almost all data compiled from the taxonomic identification of FishTrace specimens satisfied the validation, except for a few cases where incongruous taxonomic evidences were found. This was the case of *Trisopterus minutus*, resolved after completing the second level of the validation process (see below). On the other hand, from the validation of the FishTrace DNA-barcodes at first the first level, >95% of the sequences obtained satisfied the standardized requirements. Thus, sequence alignments and BLAST searches for sequence validation satisfied the expected result in almost 100 % of cases (data not shown).

At this first validation level, the cyt*b* 3' end sequence fragment obtained (~750 bp) from the *Trachurus trachurus* FishTrace specimens TraTra-WM-01 and TraTra-WM-02, presented 27 changes in the pairwise alignment, indicating potential source of error in the PCR or sequencing processes. This was resolved by repeating the molecular genetics procedures from the DNA extraction to the sequencing of the PCR products obtained from these specimens, so

correct sequences were recovered. In relation to the other target gene, the rhodopsin, only three cases of contamination and/or sample mislabelling were reported. Concretely, an incongruent number of differences were found in the pairwise alignment of the 460 bp rhod sequences obtained from both "01" and "02" specimens belonging to the FishTrace specimens: SalSal-SB (*Salmo salar*) TraTra-WM (*Trachurus trachurus*) and MelAeg-CS (*Melanogrammus aeglephinus*). As for the cyt*b*, it was resolved by repeating molecular genetics procedures from the DNA extraction to the sequencing of the PCR products obtained from these specimens.

2nd Level of data validation: Data validation at this level was performed to verify phylogenetic status of the DNA-barcodes obtained from all specimens obtained. Four examples have been selected to illustrate the output of data validation at this level.

a) The taxonomic identification of the FishTrace "TriMin" specimens needed to be contrasted with the genetic data obtained from them since taxonomic evidences revealed differences between both populations studied from EM/WM and NS/CB areas. Finally, molecular genetic analysis in FishTrace confirmed the separation into two subspecies, *Trisopterus minutus minutus* and *Trisopterus minutus capelanus*, given the large interspecific genetic variation (14.5 %) between both populations DNA-barcodes. The phylogenetic analysis based on the FishTrace DNA-barcodes demonstrated diagnosability of the Atlantic and Mediterranean *T. minutus*, since they were clustered into two distantly separated clades, supporting even species level status for the former subspecies, *T. minutus minutus* (Linnaeus, 1758) and *T. minutus capelanus* (La Cèpede, 1800), and the genetic data generated for the "TriMin" specimens were thus validated.

b) An *Engraulis encrasicolus* specimen sampled at the Cantabric Sea (EngEnc-CS-01) was barcoded at the target nucleotide sequences (cyt*b* and rhod). Subsequent phylogenetic analysis clearly discriminated closely related clupeid species and, in the resulted tree, EngEnc-CS-01 was placed together with the corresponding *Engraulis encrasicolus* reference DNA-barcode, used in the analysis for quality control. Furthermore, topology adopted by representative taxa for families Engraulidae and Clupeidae (Figure 4.22) successfully fit with the previously reported (Jérôme *et al.*, 2003). Thus, target sequences obtained from EngEnc-CS-01 could be safety validated.

c) *Thunnus thynnus*: Target DNA-barcode of a *Thunnus thynnus* specimen caught at the Madeira Archipelago (ThuThy-MA-01) was included into a single cyt*b* + rhod DNA data-matrix for alignment and phylogenetic analysis. Topology obtained from the bootstrap analysis rendered high statistical supports, ranged from 78 to 100 %, discriminating target specimen analyzed among other four congeneric taxa, and placing ThuThy-MA-01 DNA-barcode in the correct position into the *Thunnus thynnus* reference clade resulted in the tree (Figure 4.23). The topology obtained was in accordance with the obtained from the analysis of a 655 bp fragment of the cytochrome oxidase subunit I (COI) from 46 taxa belonging to eight different *Thunnus* species (Ward *et al.*, 2005). Thus, target sequences obtained from ThuThy-MA-01 also safety validated.

d) *Gadus morhua*: The FishTrace DNA-barcode from GadMor-NS-01, the first *Gadus morhua* specimen caught at the North Sea, was validated by phylogenetic analysis. The resulted NJ tree constructed using the K2P model adopted similar evolution pattern than the previously reported (Bakke and Johansen, 2005). In addition, GadMor-NS-01 DNA-barcode clustered together with the *Gadus morhua* reference DNA-barcode used for quality control (Figure 4.24). By this procedure, target sequences obtained from GadMor-NS-01 were validated.

Apart of these successful results above presented, there were also some cases of taxonomic misidentification subsequently confirmed by phylogenetic analysis performed for the validation of the genetic data obtained. Taxonomic misidentification of twenty pleuronectiform fish specimens sampled from the extra-European area was detected at the 2nd validation level. These twenty specimens were commercially identified as *Solea solea* specimens (SolSol-EE-01 to SolSol-EE-20). Phylogenetic analyses performed on cyt*b* and rhod sequences obtained from these target specimens, together with a large number of sequences from other different soleid species obtained within FishTrace and from the GenBank (Table 4.12) revealed, with enough accuracy, that 18 from the twenty target specimens were *Microchirus azevia* specimens instead of *Solea solea* (Figures 4.25 and 4.26). Thus, it was clear that the morphological records taken from these misidentified soleid specimens need to be checked for their precise identification.

In conclusion, these examples demonstrate that phylogenetic analyses performed for the validation of the genetic data obtained from the fish specimens sampled also assists in

taxonomic identifications. On the other hand, missed taxonomic and/or genetic data was handled as data validation failure since a database-sheet can not be completed for a given species. According to this definition, only cyt*b* sequences from the FishTrace *Conger conger* sampled specimens ConCon 01 and 02 from the CB, CS, MA, CI, WM and EM areas, were missed. In fact, the amplification of *Conger conger* cyt*b* gene could not be recovered at any of the involved laboratories. This amplification failure can be explained on the basis of different organization of this gene within the mitochondrial genome of this species, and some other anguilliforms (Inoue *et al.*, 2000). Thus, standardized FishTrace primers did not allow to amplify *Conger conger* cyt*b*. However, FishTrace is still dealing with new primer designing in order to solve this lack of information on these target specimens, since there was not any problem to amplify the cyt*b* from other FishTrace anguilliform species like *Anguilla anguilla*.

3rd Level of data validation: According to the last updated file to control data validation (July 18, 2006 in Annex XXII), the status of taxonomic validation is almost completed, although in some areas needs to be defined and completed. Information on target species validated at each geographical area is summarised in Table 4.13. The reference collections data validation can be also considered completed except for three areas where needs to be calculated. Information on target species validated at each area is shown in Table 4.14. The genetic data validation is completed in a 80%, since some sequences need to be compared from some areas (Table 4.15).

In conclusion, and regarding the three-levels validation process described in Section 3.6 of this Report, all data obtained from FishTrace specimens sampled was subjected to the inter-validation process between genetics and taxonomy/biometrics. The corroboration of taxonomic identifications performed by molecular genetics analyses rendered the same diagnosis in almost 100 % of specimens. After validation of the DNA-barcoding data, approximately 2 % of them did not match the expected phylogeny, mainly due to errors in sampling (misidentified specimens) or in amplification-sequencing. In these cases, repetition of the procedure with newly extracted DNA or new samples, resulted in successful amplification and sequencing of the target genes.

Finally, the status of taxonomic, reference collections and genetic data validation reached by each partner indicates that a high percentage of work was accomplished, but not the 100 % yet, due basically to the complexity of this task. However, FishTrace Consortium agreed and committed to center efforts and still working in close collaboration in order to finish with the

validation of the 100 % from the different batches of data generated during the project life. This last task to be done takes significant relevance since the standardized data validation process of FishTrace guarantees the reliability and the high data quality deposited in the *online* database (www.fishtrace.org).

4.7.- Database

Database architecture

The FishTrace database architecture was deeply evaluated along the project time and the final result is the entity relationship diagram (ER diagram) showed in Figure 4.27, in which all the relations between database tables are described. The database is composed of twelve tables (listed below as 1 to 12) which can be included into three different main components: a) fish name management, b) specimen descriptions and c) species general information.

a) The fish name management (yellow[3] in the ER diagram, Figure 4.27) is also composed itself of three tables:

1) The database table called as "SPECIES_LIST" includes fish scientific (latin) names (genus and species names), the name of family, which the species belongs, and also accepted common names in English. This is a key table because it contains the "Spec_code", a unique species number used as link between FishTrace and FishBase. As the other tables of fish name management the "Spec_code" was directly imported from FishBase, once the commercial European marine species (3884 items) were filtered. There is a field called "INF_SPECIESLIST0305" to flag if a species is described or not in FishTrace (where information on 220 teleost species was loaded). This table also includes other items as fields for describing species habitat (e.g. marine, brackish or freshwater), the relative importance of the species (e.g. high or low economical importance in the frame of European fisheries and fish markets), etc.

2) The table called as "COMNAMES" contains a list of fish common names accepted and used at the geographical region of origin of the specimens collected, in several languages (e.g. French, Greek, Spanish, Portuguese, etc).

[3] If printed in black and white printer, the yellow will appears as light grey.

3) "SYNONYMS". This table of the database contains a list of fish scientific name synonyms, in English.

b) Specimens (red in ER diagram). This main component of the database can be broken down into the following five tables:

4) The main table was called "SPECIMEN". It contains all the taxonomic and genetic information obtained from each fish specimen analyzed within FishTrace. It includes specimen total length, weight, gillrakers, sex, etc. as well as the complete cyt*b* and rhod sequences, PCR conditions used, etc.).

   A new database sheet was created for each fish specimen (information) included in this table. Each specimen was referenced using an unique identification number called "idFishTracecode". The FishTrace code construction has been widely explained in Section 3.2 of this report but basically, it is composed by a contraction of the latin name in the first six letters (e.g.: *Merlucius merlusius* = MerMer), the following two letters are the FishTrace region abbreviated (e.g. Western Mediterranean = WM) and finally the (unique) number of the specimen treated (e.g. 01, 02, etc.).

   DNA sequences were stored following an alphanumeric character string, describing the four DNA bases: A (adenine), C (cytosine), G (guanine) and T (thymine).

5) "TISSUE SAMPLE". This table contains all the information concerning tissues extracted from each fish specimen collected (tissue collection number, FishTrace code of the treated specimen, storage medium used, etc.) and several tissue samples could be taken from a single specimen.

6) The "AMPLIFICATION CONDITION" table was built up to describe the PCR conditions followed to amplify both targeted genes. This table was completed before entering the DNA sequence into the "SPECIMEN" table.

7) "COLLECTING ENV". The information entered in this table describes the procedure followed in the sampling of each fish specimen in the field, including the location of

the capture, the ship name etc. This table also contains the geographical coordinates in decimal degrees, which could allow a link to a GIS tool in the future.

8) The table called "PICTURES" contains the description of the photographs taken from fresh fish specimens, otoliths, fish products, etc., deposited in the database. Photos are not entered themselves in the database but were stored into an associate folder by FTP (File Transfer Protocol) at ftp://infoweb.jrc.it/.

c) Species components in database (dash green[4] in ER diagram, Fig. 4.19) are described in four tables. It was very important to separate this information from specimen descriptions since FishTrace partners used the specimen data loaded (taxonomic, genetic and collections) as basis for filling in corresponding species tables, and completed them with additional specific regional information on other fields of knowledge (e.g. ecology, distribution, behaviour, specific or regional bibliography, etc.).

9) The main table within the species components is called "SPECIES". It contains species general descriptions (taxonomic and ecologic data, photographs, etc.) and the reference DNA sequences for each species. Both reference DNA sequences (cyt*b* and rhod) are the most representative from all specimen sequences (belonging to this species) entered in the database. Reference sequences were selected and subsequently loaded by the partner in charge of species data validation, since a different list of species was assigned to responsible partners for data validation (the whole list of species included in FishTrace was divided into five and each one was assigned to responsible partners for taxonomic- collections data validation and to responsible partners for molecular genetic data validation. See Section 3.6 for further information).

10) "REGIONAL INFO" This table contains the specific information on fish taxonomy and biology, given in a concrete region. There are several regional information database sheets for those species collected in more than one geographical area (See Table 3.1).

11) The table called "BIBLIOGRAPHY" contains information on the bibliographic references used for taxonomic identifications and fish species description purposes.

[4] If printed in black and white printer, the green will appears as light grey.

12) Finally, the table called "HAPLOTYPE" contains information on relative genetic variations found among specimen sequences, to the reference sequences (cyt*b* and rhod) for species.

Database Information Structure

FishTrace database was created to compile and show (in an ordered and rational way) all the information collected from three different levels of knowledge: Sampling and Taxonomy, Reference Collections and Molecular Genetics. This extensive information on 220 European teleost species, contained in this open access database, can be retrieved by users through Internet via www.fishtrace.org. At present, this is the database structure scheme (Figure 4.28):

1. Fish species:

· Morphology
· Scientific photographs
· Biology
· Distribution
· Regional information
· Conservation status
· Bibliography

2. DNA barcoding data:

· DNA sequences from two barcoding genes (cytb and rhod)
· DNA sequence polymorphisms
· Biogeographical genetic variation
· Gene amplification conditions including primers
· Guidelines for phylogenetic validation of the DNA sequences obtained

3. Specimen information:

· Identification details (morphological and DNA sequences)
· Environmental data
· Geographical coordinates of sampling with map included
· Specimen taxonomy information
· Individual pictures

4. Reference Collections information:

- Vouchers
- Tissue and otolith collections
- DNA collection
- Reference collections allocations

5. Other information:

- Bibliographic references
- Statistics
- FAQ
- Control: Data validation flow document
- The database has been implemented with Identification Tools allowing fish identification by DNA sequence or morphological data.

Database Loader Interface

FishTrace database is operative since early 2003, when the first interface system for data loading was designed and promptly implemented (Figure 4.29). This first generation of the database loader interface was allocated at: http://infoweb.jrc.it/fishtrace, but FishTrace Consortium improved its content and appearance and built up the actual web interface at: www.fishtrace.org, which is operative since May, 2005.

Opening the database loader interface (by clicking in the tab "Database Loader", placed in the left bar menu of the main page and included into "The Project" menu; see Figure 4.28), FishTrace partners, after registration using password, were able to fill in one by one the twelve database tables previously described in Section 4.7. They could also correct the data entered, enter modifications after filling (e.g. missing data subsequently obtained), etc. In those cases, changes were taken into account and the new information entered replaced immediately the old one, in order to be shown in the interface. Up to now, only the FishTrace Consortium is allowed to insert data. On the other hand, when a new file is completely filled, users (general public) can run a search at the open-access interface to display and check all the information updated.

The data loader interface contains a list of clickable links to access to the database tables (described above) and some brief guidelines for users.

The actual FishTrace Database Loader Interface is structured as follows:

1) Load specimen taxonomy information:
   - Load environmental table (Figure 4.30)
   - Load environmental table (With map included, Figure 4.31)
   - Load specimen table (Figure 4.32). Add a new tissue sample (Figure 4.33)
   - Load sample table (Figure 4.34)

2) Load genetic specimen information:
   - Load amplification condition table (Figure 4.35)
   - Load genetic information (Figure 4.36)
   - Guidelines for phylogenetic validation of sequences (Figure 4.37)

3) Load species information:
   - Load species table (Figure 4.38)
   - Load regional information table (Figure 4.39)
   - Load haplotyping table (Figure 4.40)
   - Load bibliography table (Figure 4.41)

4) View and delete data:
   - View data (Figure 4.42)
   - Delete data (Figure 4.43)

5) View statistics (Figure 4.44)

6) FAQ (Figure 4.45)

7) Control:
   - Data validation flow document (Figure 4.46)

1) <u>Load specimen taxonomy information</u>:

- Load environmental table: This table was aimed to enter detailed information related to the specimen catches (Figure 4.30).

There is another version of this table, which includes a map where FishTrace partners could select the capture location and coordinates (Figure 4.31).

- Load specimen table (Figure 4.32): This table served to enter all the taxonomic data obtained from fish specimens caught. Data from tissue samples removed was linked to the corresponding specimen by clicking on the button "Add a new tissue sample" (Figure 4.33).

Photos taken from fish specimens, otoliths and fish products could be uploaded and linked to the corresponding specimen database sheet. These three types of information were allocated into three different folders (one for all the photos taken from the voucher, one for all the photos taken from otoliths and a folder for the other kind of photos: fish products, etc.). Thus, there can be several tissue samples/photos linked to one concrete specimen.

Each specimen table must be linked to an "environmental table" (previously filled by partners), so, there are several specimens linked to a single environment data.

- Load sample table (Figure 4.34): This database sheet contained several fields destined to enter data concerning the tissue sample removed from each fish specimen caught. This information could also be loaded using the "specimen table".

2) Load genetic specimen information:

- Load the amplification condition table (Figure 4.35): Aimed to enter information concerning standardized PCR conditions for the amplification of each targeted DNA sample extracted from the tissue samples removed from the chosen specimens (See Section 3.4 for further information).

- Load genetic information (for specimen, Figure 4.36): This database table contained the fields to enter the genetic information (cyt*b* and rhod sequences) obtained. DNA extraction method used at each molecular laboratory of the Consortium could be directly chosen using the scroll down menu implemented. Thus, there can be several genetic information linked to one method.

- Guidelines for phylogenetic validation of Sequences (Figure 4.37): Before entering the sequences, partners in charge carried out the validation procedure according to the protocol described in this downloadable PDF document (Annex XIII).

3) Load species information:

- Load species table (Figure 4.38): This section was aimed to enter all the general information concerning fish species included in FishTrace. Information entered was based on data obtained from each specimen collected belonging to the same species. This information was also linked to the bibliographic resources used.

- Load regional information table (Figure 4.39): This database sheet was designed to enter information collected in a specific region covered in FishTrace.

- Load the haplotyping table (Figure 4.40): This table contains fields to report on fish specimens with particular genetic variations at their DNA sequences compared with reference sequences described (cyt*b* and rhod) for species, and other fields designed and placed at this table in order to describe in detail the genetic variations detected (e.g. "Variation relative to reference for cyt*b*": G270A; T330C; ...).

- Load bibliography table (Figure 4.41): It contains fields aimed to enter information on bibliographic references used to describe fish species within FishTrace (including taxonomic identification keys). This information on fish species entered was immediately linked to the bibliographic resources used. Thus, there are several species linked to one bibliography and several bibliography linked to one species.

4) View and delete data:

- View data (Figure 4.42): This tool was implemented in the *offline* database to assists participating groups in this research. It allows to quickly find sets of information entered in FishTrace database. This is a suitable tool to retrieve all the information entered in database concerning one species sampled from more than one of the geographical areas covered (e.g. to look for all available genetic information on the same species present at Eastern and Western Mediterranean and the Cantabric Sea). Thus, this tool is closely related with the data validation process, as detailed in Section 3.6 of this Report.

- Delete data (Figure 4.43): This tool allows responsible partners for the validation of the data to delete (after validation) erroneous data entered in database.

5) View statistics: *Offline* tool implemented to allow partners to check the progress achieved (showing a bar-graph indicating the stage of completion and the expected goal) in taxonomic identifications, fish specimens collected, DNA sequences obtained and the relative level of completion of the database after the validation of the data entered (Figure 4.44).

6) FAQ: In this section most frequently questions on database asked by participating groups, as well as the respective answers were placed in order to assists all partners to understand the whole process for filling the FishTrace database tables (with data obtained) and the data validation process (Figure 4.45).

7) Control: Since data compiled in the database was generated at different fields of knowledge, such information required to be checked among all partners involved for reliability. An Excel file containing the list of targeted fish species by area was designed in order to control the validation data status summary at each area and to know the real progress of the database content (Figure 4.46). This file was periodically updated by responsible partners and uploaded by the JRC group to a "ftp" folder at the *offline* database. In this section there is a link to the Excel file: "Control Data Validation Flow Document.xls" (See Annex XXII for further information).

Data quality

One of the main challenges of this project was to ensure to the quality, consistency and completion of the database through the data deposited. To reach this goal, FishTrace partners developed standard protocols to harmonize taxonomic identification keys, biological collections procedures and molecular genetic methodologies but this it needed to be completed at the database level by the implementation of reliable systems to avoid/reduce data entry errors or misunderstanding.

A first level of data quality control implied that the required information at each database field has to be entered because the system does not accept incomplete data. Thus, to accept DNA sequences uploaded in the database, the responsible partner had to declare the DNA

sequence length before entering it and automatically, the system compared the length and accepts or rejects the information in case of problems (mismatches). To avoid mistakes concerning the geographical location (related to the specimen catching data), there is an *online* interactive map where the user could point each fish specimens catching location and the system automatically retrieved the geographical coordinates (latitude and longitude).

Finally, there are some other fields in the database for signing by the person involved in data validation responsibilities, placed at the bottom of each data loading table. General data were loaded and reviewed a second time by these verificators and curators (data validation responsible partners). They checked the data before signing in the validation fields.

Database degree of completion

The degree of information deposited in the FishTrace database increased exponentially since the database loader interface is working. This increment were reflected in the Progress Reports periodically sent from all the participants to the project coordinator, as well as in the minutes taken from the Annual Plenary Meetings celebrated from 2003 to 2006 (Annexes I, IV, VIII and IX). At the end of the project, the stage of completion of the project database could be considered as more than the 100%, taking into account the expected results described at the Technical Annex which refers to the taxonomic and genetic cataloguing of 180 European teleost species (See Table 3.1 to compare the original and the final list of species covered by FishTrace). The final number of species included in the database grew until reach a total of 220 fish species, and some extra-species were also treated but excluded from the final list due to the problems/complications described in a previous section of this report (See results from sampling and taxonomic identifications in Section 4.2 of this Report).

Modules location (for programming)

Database code was included in a Jakarta Tomcat, installed on infoweb.jrc.it in *C:/Jakarta_ tomcat*. The database loader code was included in */webapps/examples*, and the interface for the loading pages, was placed in *C:/apache2/htdocs/FishTrace*. The general public interface code was included in */webapps/ FishTrace_int*. Finally, the Java Server Pages (JSP) was deposited in */webapps/FishTrace_int/FishTrace/gb* and HTML pages were stored on the *infoweb.org* server.

Developing the Web site/Database interconnection

This objective was fully completed in 2005. JRC installed a server infrastructure (Tomcat + Ant), which communicates with the database to deliver/load information using loader page in a web interface. Part of the code was written in Java servlets and, for the public interface, JRC built an architecture divided in three levels. The first level consisted to build a series of function written in java (called Java beans) to communicate with the database. The second level was to make corresponding the beans to some functions usable in a web page (called JSP- Java Server Pages) and finally the third level consisted in building a web interface in HTML including the JSP calls.

Communications between the web page and the database were done in XML using specific functions (OracleXML) that directly read or load data in XML from/in the Oracle database. Also the web pages used to load data in the database sent the information to the server in XML.

4.8.- Public web interface

Connecting to www.fishtrace.org

The first version of the FishTrace public web interface is successfully working *online* at www.fishtrace.org (although the first web interface prototype was promptly allocated at the very beginning of the project at http://infoweb.jrc.it/fishtrace/web/, allowing FishTrace partners to start filling in the database). FishTrace interface is an open-access www site to the general public. Opening a web browser and entering the above URL, the FishTrace general interface displays its Main Menu, which shows information on the project, allows searches on the database and gives access to the fish identification tools.

Description

The online database at www.fishtrace.org contains validated information on taxonomy, DNA sequences and reference collections obtained within the FishTrace project. Designed to directly confront the problem of reliable fish species identification, FishTrace web interface

offers biological, ecological and genetic information (DNA sequences) on more than 200 European teleost species. Besides, FishTrace web site supplies users with several *online* molecular and morphological tools for fish species identification by interrogating the database on taxonomic, ecological and genetic homologies between the target fish and the species included in FishTrace. Following there is a detailed description of the structure and appearance of the web interface, including a brief explanation of every web page that forms part of www.fishtrace.org:

1) Cover Page. This page was designed to show three principal frames (Figure 4.47):

i) The FishTrace's logo and the whole title of the project,
ii) a left-bar menu containing basic information on the project and the database, and,
iii) a third frame specifically designed to place the fish species searching and the fish identification tools. Searching and identification tools are further described in Chapters 6 and 7 of this Section, respectively.

From the cover page, the user finds the accession to:

2) Main menu (Figure 4.47). Sited at the left-bar of the cover page, it contains seven different submenus:

a) **Aims**: This section contains general information related to the project aims and objectives (Figures 4.48 and 4.49).

b) **The Consortium**: This page shows information on the Consortium members, including the name of all participating institutions, its URLs, their location in Europe (detailed into an interactive map) and email addresses for contact responsible persons at each institution (Figure 4.50).

c) **Personnel and Expertise**: In this section, a brief description of the personnel involved in the development of this project is given indicating name, professional title, contribution to the project and the duration of its contribution (Figure 4.51).

d) **Database structure**: A scheme of the FishTrace database content is presented here (Figure 4.28).

e) **Database loader**: This page contains links to go to direct accessions for filling in all database tables with the information obtained within this network (database table fields and content have been previously described in Section 4.7 of this Report). The access to

these links to the database tables is restricted to FishTrace partners who have to register themselves typing a private *username* and *password* (Figure 4.29).

f) **Publications**: A list of publications directly emanated from FishTrace is available in this page. Also, these publications can be downloaded by users freely (open access to the public) in PDF format by clicking in the titles (Figure 4.52).

g) **Dissemination and Photos**: This page is divided in two sections:

1) A list of documents related to the dissemination of the results obtained within the network, including the FishTrace dissemination brochure and several interviews published in local and national newspapers as well as in Food and Nutrition Safety URLs. All these documents are freely downloadable for the general public in PDF format by clicking in the titles (Figure 4.53).

2) The photo-gallery is itself divided into four thematic sections including photographs related to FishTrace meetings and workshops, sampling and taxonomic tasks, molecular genetic procedures and biological collections created (Figure 4.53).

3) Sampling and Taxonomy. This menu contains four different submenus:

a) **Aims**: This section includes a brief description of the aims and objectives pursued to achieve this essential part of the project: the collection of representative number of European teleost fish specimens by strategic field sampling. There is also a link in this page to access to the whole list of targeted species in FishTrace (Figure 4.54).

b) **Sampling areas**: A map of Europe is shown in this page pointing the eight European sea areas covered for sampling the biological material used within FishTrace (Figure 4.55). Fish species (specimens) caught outside this areas were catalogued as Extra-European species.

c) **Targeted species**: The list of teleost species included in the FishTrace database can be consulted in this page. The whole has been divided into nine (detached by the geographical area of origin of species). There are two links in this page, one to access to the previous section “Targeted Species” and other link to download a table (a file in Excel format) describing the whole list of species and its geographical area of origin (Figure 4.56).

d) **Standard protocols**: Downloadable documents in PDF formats describing the protocols and procedures followed to accomplish Sampling and Taxonomy tasks are available to the general public in this section (Figure 4.57). Users can retrieve the protocols by clicking titles (links).

4) Reference Collections. This menu contains four different submenus:

a) **Aims**: In this section, general information on the four newly biological reference collections built up within FishTrace is given, describing aims, objectives and future applications of them (Figure 4.58).

b) **Biological Collections**: In this page, general information on the biological reference collections built up within FishTrace and a brief description about its content (available biological material collected) are given (Figure 4.59).

c) **Access to Collections**: This section contains details on biological sample exchange rights agreed by the FishTrace Consortium. There is also a link to freely download the "Loan Request Form" in RTF format (Figure 4.60).

d) **Standard protocols**: This page includes two links to download the protocols (PDF) followed to settle the FishTrace biological collections and the "Loan Request Form" (RTF). Users can retrieve freely (open access) these documents by clicking titles (Figure 4.61).

5) Genetic Catalogue:

a) **Aims**: This section provides general information on the aims and objectives pursued in the creation of the FishTrace Genetic Catalogue. Expected goals for the molecular fish identifications and the detection of biogeographical polymorphisms among the European teleost species covered within the FishTrace project are detailed. Finally, the structure and contents of the Genetic Catalogue is briefly described (Figure 4.62).

b) **Molecular Id. Tools**: Clicking in this tab, the user find a link to each Molecular Id. Tools implemented: BLAST, RFLPs and the Phylogeny tool. Appearance of the interface for these tools and web pages showing results from each tool are given in Figures 4.63, 4.64, 4.65, 4.66, 4.67 and 4.68, respectively).

c) **Standard protocols**: Downloadable PDFs of the protocols followed to accomplish Molecular Genetic tasks (Figure 4.69).

At the bottom of each of the above described web pages there is a link to directly access to the cover page, allowing users to interrogate the database using the three different fish species identification tools implemented. Further information on these tools is given below:

6) Searching and tools (Figure 4.70). Searches of fish species information on FishTrace can be performed interrogating the database through www.fishtrace.org at two levels: i) by scientific and ii) by common names.

i) Searching species "by scientific name": The search of a species is performed simply by selecting the scientific (latin) name of the species in the scroll down menu (Figure 4.71). Species are sorted in alphabetic order (with the possibility to jump directly to letter by typing a character when the user are selecting an item). One selected, a web page describing the species appears automatically (Figure 4.72).

ii) Searching species "by common name": Entering a part of a common name (e.g. "Tuna") the system will propose a list of the species containing your key word (at least English, French, German, Portuguese). Results from the search appear as a list of names of the species proposed. Names in the list are clickable links to directly access and visualize the species information sheet required (Figure 4.73).

FishTrace database contains information by species but also all the data collected for each individual specimen. After interrogating the database by searching, the next page opened contains a table where the available information for each species is detailed (including all different "Regional Information" compiled). In addition, other information related to the species can be displayed using the buttons of the tabulation bar:

a) "Species info": to view the species data. The information displayed here in a table is a summary of the knowledge collected for each species based on the specimen data collected within the project, and completed by other source as bibliography (Figure 4.74).

b) "Genetics": this is used tab to view the DNA data of reference for the chosen species. The genetic sequences presented are the most representative cyt*b* and rhod sequences for this species, chosen (by the responsible partner for validation of this species) within the specimen data entered (Figure 4.75).

c) "Specimens": There are five specimens collected by region (sampling area). At least, two of them were DNA barcoded at the two target genes (so there is available genetic information for them). The specimen data includes a description of:

- the catch environment (location, collector name, depth etc.),
- the taxonomic description (size, taxonomic keys, etc.),
- the tissue sample and collection data (photographs, otoliths, etc.), and,
- the genetic data (rhod and cyt*b* sequences, DNA extraction and amplification conditions, etc.).

FishTrace user description: To visualize the information collected by specimen: 1) Select the tab "specimen" in the bar showed below located in the top of the species file (Figure 4.72). The next page opened contains a table where the available information for each specimen is indicated. In the first column of the table there is the **FishTrace code** of each specimen (Figure 4.74). 2) Click on the blue buttons of the next column "ID details" to get general information related to the collection of the chosen specimen (collector id., fishing method used, etc.). 3) By clicking in the blue buttons at the column "DNA data", the user can retrieve from the database the genetic data available for a chosen specimen, including both target gene sequences (cyt*b* and rhod) as well as the amplification conditions followed to obtain the sequences (Figure 4.76). 4) Finally, there was implemented a tool which allow the comparison of the different regional information entered in the database for a chosen species. To use this tool, click on he blue buttons at the column "Data comparison" and all the fields containing the same information from fish specimens from different geographical origin will be displayed allowing simultaneous comparisons of data, for example, the comparison of all cyt*b* sequences obtained from the ten *Sparus aurata* specimens captured at five different geographical areas (Figure 4.77). This tool, which was mostly used for data validation purposes, was previously described in Section 3.6 of this Report (Data Validation Methods).

d) "Bibliography": This tab serves to display the bibliographic reference used for complete the information related, including taxonomic, ecological and "regional" information on the chosen species (Figure 4.78).

7) Fish identification tools. The implementation of innovative *online* molecular and taxonomic tools for fish species identifications were the most significant achievements of the

project concerning the usefulness and the exploitation of the results obtained by users (general public, researchers, ichthyologists, biologists, etc.).

The first objective reached for the completion of this part of the work was to develop a genetic tool which serve to compare and search homologies (by multiple alignment performing) between a target DNA sequence introduced by users and the rest of sequences deposited in the database (both cyt*b* and rhod sequences obtained within FishTrace from more than 1000 fish specimens treated). This informatics application already exists in the scientific community and all choices were evaluated. It was considered to create an algorithm and implement it within the FishTrace system but finally it was decided to adapt the most employed sequence comparison tool: i) BLAST (Basic Local Alignment Search Tool), from the National Center for Biotechnology Information (NCBI). One problem was that NCBI's BLAST (www.ncbi.nlm.nih.gov/BLAST/) was not designed to work in relation with a database and a web server like in FishTrace system and responsible partners had to adapt it. Java modules were built to establish communication with the database and to display the result in a web page. ii) A second tool aiming to apply in the database the result of the RFLP (Restriction Fragment Length Polymorphism) technique was also developed and it is actually working properly. iii) The third molecular tool was developed to find and format DNA those sequences retrieved from the FishTrace database which share the maximum number of sequence homologies with the target sequence entered by the user. This tool allows to visualize the phylogenetic classification of an unknown (target) fish specimen among the FishTrace database species. iv) The last tool implemented for fish identification is the "Morphological Tool", based in the identification of target specimens by the comparison of morphological characters among the species included in the database. Further information on molecular and morphological identification tools implemented in www.fishtrace.org, including a brief operation description for users is given below:

i) **BLAST** algorithm was developed by the NCBI (National Center for Biotechnology Information, www.ncbi.nlm.nih.gov/BLAST). Aimed to find regions of local similarity between sequences, the program compares nucleotide or protein sequences to sequence databases and calculates the statistical significance of matches. BLAST can be used to infer functional and evolutionary relationships between sequences as well as help identify members of gene families. NCBI propose a web version but only for LINUX and we adapted it for WINDOWS using Java modules. BLAST uses a specific database format and we developed an application to extract the genetic sequence from the FishTrace database and transform

them in BLAST specific database format. We implemented a second module to get the result from the BLAST application link them with the data in the FishTrace database and display them in web pages.

FishTrace user description: 1) To use BLAST in FishTrace, go to the main page and select the icon BLAST in the section "Molecular Identification Tools". 2) Copy and paste the DNA sequence that you want to compare in the text box "Enter sequence below in FASTA format" (Figure 4.63). Choose the options in the other fields, you can click on the links (in blue) to get help for each item. Then click on the button "Search". 3) A new window appears with the list of the specimen (species name and specimen code) having the most similarity with your sequence ordered from up to down to the most to the less accurate (Figure 4.64).You can click on the links to access directly to the specimen data included in FishTrace.

ii) **RFLP**: The RFLP tool developed in FishTrace simulates the RFLP methodology on the sequences included in the database to facilitate fish identification. Restriction Fragment Length Polymorphism is a technique in which organisms may be differentiated by analysis of patterns derived from cleavage of their DNA. If two organisms differ in the distance between sites of cleavage of a particular restriction endonuclease, the length of the fragments produced will differ when the DNA is digested with a restriction enzyme. The similarity of the patterns generated can be used to differentiate species (and even strains) from one another. The objective is to simulate virtual restriction enzyme action on the DNA segment included in the FishTrace database. This is be very useful for DNA sequence comparison without using sequencing.

FishTrace user description: 1) In the main page select the icon "RFLP" in the section "Molecular Identification Tools". 2) Select if you want to do a "search on specimen" or a "search on species" from the FishTrace database. 3) Select a restriction enzyme you want to use by selecting them in the left window, and they will appear on the right window (Figure 4.65). 4) Using the same method select a list of species or specimen from the FishTrace database. 5) Select the sequence type or PCR primer. 6) Click on the button "submit". 7) A new window appears containing a table giving for each enzyme a list of simulated fragment size by specie or specimen (Figure 4.66).

iii) **Phylogenetic Tree**: The phylogenetic tree tool facilitates visualizing in a tree the similar sequences extracted from the database. It has been directly derived from BLAST. As BLAST

enter a reference sequence for comparison but the system delivers a multi-sequence file ready to be used in a phylogenetic tool instead of a list.

FishTrace user description: 1) In the main page select the icon "Tree" in the section "Molecular Identification Tools". 2) Select the maximum number of sequences to be phylogentically analysed. (by default 5) 3) Copy and paste the DNA sequence you want to compare in the text box "Enter sequence below in FASTA format" (Figure 4.67). Select the options in the other fields, click on the links (in blue) to get help for each item. Then click on the "Search" button. A new window appears with in a text box the sequences with the most similarity with your sequence ordered from up to down to the most to the less accurate (Figure 4.68):

iv) **Morphology tool**: The morphology tool aims to identify fish using the taxonomic keys. The user enters taxonomic characteristics that are compared to the database information and the results are the specimens/species corresponding to the keys.

FishTrace user description: 1) In the main page, select "Morphological Tool" of this section (Figure 4.79). 2) Enter a value for each taxonomic key and if needed enter a range (Figure 4.80). The value will be searched between the values of more or less that range. For non-numeric value select one item in the scroll down menu. The system will search for the exact match. The value entered has to be positive. If no value is filled in the table, or if a range is given without a value, before pressing the buttons, an error popup opens. 3) By pressing the button "Search specimens" the user gets the specimens corresponding to the values entered. 4) Pressing the button "Compare to species: Search specimens" after having selected a specie in the scroll down species list, permits to see if the selected specie has the chosen characteristics. 5) A new window appears giving the specimen corresponding to the data you have entered. Buttons allow you to come back to the main menu or the morphological tools.

5.- Discussion

The FishTrace network aimed to establish the first European repository of biological data and reference material on marketed teleost fishes. Thus, FishTrace has catalysed the cooperation and the pooling of data and biological material in pararell to the genetic identification and characterisation of main marine teleost species from European waters and/or marketed in Europe. Results compiled from this project network have generated a searchable *online* database recently launched to the general public via Internet at the URL: www.FishTrace.org.

Current taxonomy and systematics tools permit the classification of practically all fish species. However, its usefulness is hindered by the lack of efficient and fast reference tools (Godfray *et al.*, 2002; Blaxter *et al.*, 2003). This capability is of particular interest to fisheries management, biological and ecological research as well as to issues related to fisheries products for human consumption. Other fish identification databases mainly collect taxonomical and general biological information from worldwide distributed species (e.g. FishBase: www.fishbase.org; The Census of Marine Life: www.coml.org; The FAO Species Identification and Data Programme, SIDP: www.fao.org, and independent Natural History museums databases). FishTrace database covers most teleost fish species of commercial, ecological and zoological interest for the European countries, paying particular emphasis to local data collected in Europe. In addition, FishTrace provides molecular data, detailed protocols and tools for the correct identification of fish species, standardized photographs taken from fish specimens, otoliths and fish products, and also, a large list of relevant technical publications on taxonomy, distribution, ecology and biological parameters. All the information collected in FishTrace is connected to a biological reference collection from the fish specimens, allowing cross-referring analyses. Other biological databases on biodiversity global information such as the Global Biodiversity Information System (GBIF: www.gbif.org), the Barcode of Life Database (BoLD: www.barcodinglife.org) or FishBase, does not provide access to the original samples where data was obtained and therefore, FishTrace has developed a unique infrastructure in Europe, for referencing and comparison of teleost fish information and material.

In FishTrace, a large number of fish specimens (>2500) from 220 targeted teleost fish species has been collected by strategic field sampling, together with their regional data, related to

common names, field marks, biology, size, fisheries and forms of use, transformation, end consumers, ecological and zoological interests, conservation status and genetic markers. All specimens caught within FishTrace were identified to species level using standard morphometric and meristic procedures (e.g. Eschmeyer, 1990; Nelson, 2006; The FAO-SIDP, etc.). The taxonomy of each target species was critically evaluated, with particular emphasis to geographical and regional differences. Biological samples from the same specimens (muscle tissues and otoliths) were obtained by standardized FishTrace protocols and subsequently characterized molecular genetics procedures and voucher specimens deposited into biological reference collections.

Four official biological reference collections has been created within FishTrace at the Natural History museums of MNHN (Paris), NRM (Stockholm), TFMC (Tenerife, Spain) and MMF (Funchal, Portugal). Collections stored in these museums comprise entire fish specimens, muscle tissues otoliths and DNA samples. Specimen vouchers used for these purposes were individually validated following standardized protocols for cross validation of taxonomic and genetic data. Finally, they were tagged and kept to ensure cross-referencing for accurate species identifications at individual level throughout the information contained in the FishTrace database. Ancient DNA from museum specimen vouchers which has been stored for up to 50 years has been reported to be recovered from biological material fixed with formalin and preserved using ethanol (Shiozawa *et al.*, 1992; Sheldlock *et al.*, 1997; Wirgin *et al.*, 1997; Junqueira *et al.*, 2002; Boyle te al., 2004; Chakraborty *et al.*, 2006). Thus, the subsequent use of the preserved FishTrace samples is guaranteed. In FishTrace collaborating museums, voucher specimens and tissue samples extracted have been preserved in 70% ethanol, guaranteeing its long-term conservation for cross-referring and molecular genetics analysis.

The use of old museum specimens has been reported to contribute in molecular approaches to follow species and population genetic frequency changes through time that can be compared to the present genetic status defined (Li *et al.*, 2000; De la Herran *et al.*, 2004). Biodiversity studies used ancient DNA extracted from organisms entrapped in glacial ice have provided information on evolutionary processes and ancient biodiversity (Ma *et al.*, 2000). These applications of biodiversity research based on molecular genetics analysis have recently conferred a new practical function to Natural History museums, converting them into DNA inventories of both extant and extinct species (Rivers and Ardren, 1998). This is also the case in FishTrace with the new collections of fish genetically characterized, that gives new

functionalities to the museums involved. Thus, evolutionary phylogenetic studies performed on molecular data obtained from biological samples stored in museums is assisting biodiversity science in reviewing and addressing the species classification already accepted (Hammond *et al.*, 2001; Roca *et al.*, 2001; Eggert *et al.*, 2001). Also, taxonomic genetics have launched new terms for the biodiversity classification as the "phylotypes" (Moreira and López-García, 2002) and "molecular operational taxonomic units" (Blaxter *et al.*, 2005) that is enriching the long-term debate on the "species concept". Biodiversity taxa identification is now not only defined in morphological terms and comparisons but complemented by DNA sequence data analyses. Thus, beyond the species identification purposes, and since DNA from fixed fish specimens can be obtained from museum voucher specimens without their destruction (Shiozawa *et al.*, 1992; Sheldlock *et al.*, 1997), it would be possible to regain taxonomic capability for groups that currently lack an authority (Herbert *et al.*, 2003), and the reviewing of the present species classification (Blaxter, 2003). In addition to the fish specimens and tissue samples stored, DNA extracted from all these samples have been included into the FishTrace collections. DNA isolated from each specimen has been preserved within the optimal buffer solutions, allowing the use in future molecular analysis (Asahida *et al.*, 1996; Murphy *et al.*, 2002; Gurdebeke and Maelfait, 2002).

The otoliths collected, which are hard structures with distinctive species fingerprint, can be used for future applications related to fish species authenticity and/or associated biological research (Panella, 1971; Campana and Neilson, 1985; Heath, 1992; Lychakov and Rebane, 2000) and serves as additional reference for fish identification and data validation. Otoliths are the most widely used hard structures for fish identification, since they exhibit a high interspecific variability (Pierce and Boyle, 1991) and can be observed and studied from the larva stage of fishes (Brothers *et al.*, 1976). Based on their microstructure-image analysis, their features has been included in dichotomic keys for fish species identification guides (Nolf, 1985; Härkönen, 1986; Smale *et al.*, 1995). Otoliths are used in dietary studies for the identification of predator and prey fishes (Recchia and Read, 1989; Prime and Hammond, 1990). Otolith grow pattern is also used to determine accurately the age of fishes (Panella, 1971). Finally, the otoliths collection represent another source of DNA since it can be easily extracted from this dry tissue (Hutchinson *et al.*, 1999).

In addition to the collection of fish specimens and biological materials, FishTrace has created a large repository of standardized photographs from the collected fish specimens (>4000) and otoliths (>650). This repository of photographs would assist *online* taxonomical fish species

identification since all images deposited were taken following a standardized methodology. In contrast with other fish databases, i.e. FishBase or The Census of Marine Life, the images in FishTrace show the specimen labelled with the identification number and a ruler, for biometric comparisons. Also, images collection cover sex, reproductive status and ontogenetic stages, displaying the variability within a species (e.g. in flatfishes, both sides are shown).

Due to the global trade growth of seafood products, fish species identification through reliable and fast methodologies is required to enable authentication of fish products (Pinoti *et al.*, 2005). However, processed fish do not retain morphological characteristics for identification. Thus, DNA-based identification techniques have proved to be reliable (Bossier, 1999; Lockley and Bardsley 2000a, b; Blaxter *et al.*, 2003; Marko *et al.*, 2004; Trotta *et al.*, 2005[1]). From the protein electrophoresis patterns, performed forty years ago for species identification (Manwell *et al.*, 1963), to the recent potential applications of DNA-chips (EC Fish and Chips Project: www.fish-and-chips.uni-bremen.de) and real-time PCR (Trotta *et al.*, 2005[1]), the genetic diversity is used to identify fish species. Modern developed DNA-based technologies has the potential to greatly simplify methods of food ingredients authentication and many of them could be easily adopted for its use in the marketplace. A range of them are at present available for some group of species, including sequencing of PCR products (Pepe *et al.*, 2005), patterns of restriction digestion of PCR products (PCR-RFLPs) (Hsieh *et al.*, 2002; Hwang *et al.*, 2002; Jérôme *et al.*, 2003a, b), PCR-SSCP (Rehbein *et al.*, 1997), PCR-RAPD (Partis and Wells, 1996) and other emerging technologies such real-time quantitative PCR (Trotta *et al.*, 2005), real-time uniplex and duplex polymerase chain reaction (López-Andreo *et al.*, 2006), DNA hybridization on DNA-chips (microarray technology) are currently available (Fish and Chips Project; Lockley and Bardsley 2000a). In addition, combinations of morphological and several DNA-based techniques are actually being performed for species identification and phylogenetic analysis with "total evidence" (Chen *et al.*, 2003; Lecointre and Deleporte, 2005; Costedoat *et al.*, 2006; Fitzhugh, 2006).

Tauzt *et al.*, (2003) have extensively explored DNA-based taxonomy systems since the utility of DNA sequences for taxonomical-phylogenetic purposes is well established at present, and Herbert *et al.*, (2003) have proposed that a single gene sequence would be sufficient to differenciate, at least, the vast majority of animal species, since congeneric species of animals regularly posses enough divergence between nucleotide sequences to ensure easy specific diagnosis. This concept forms the basis for the implementation of databases for biological

identifications through the DNA analysis, and aimed to develop molecular systems based on DNA species-specific "profiles" or DNA-barcodes, that can be used as unique genetic fingerprint for living beings, allowing further investigation of DNA variation among them. Current studies in this field support the barcoding concept (Hebert *et al.*, 2003; Blaxter *et al.*, 2004; Ward *et al.*, 2005; Schindell and Miller, 2005) although other authors claimed that DNA-barcodes can not replace morphology for identification and classification of species (Will and Rubinoff, 2004; Ebach and Holdrege, 2005; Gregory, 2005).

Comparative genetics of fish species has notably improved due, to a great extent, to the ease PCR amplification of specific DNA sequences (Partis and Well, 1996; Heindel *et al.*, 1998; Rehbein *et al.*, 1999; Ferrari *et al.*, 1999) and the subsequent automated DNA sequencing (McBride *et al.*, 1989). Thus, FishTrace DNA sequencing has proved to be a highly accurate method for the unequivocal identification of fish species, subspecies and populations. Moreover, based on the genetic data entered in the FishTrace database, tailored molecular identification systems for fish teleost species, can be specifically developed (Trotta *et al.*, 2005). FishTrace database also provides the full description of the molecular methods used, and consequently, a collection of robust primers and optimal PCR conditions for the DNA barcoding of almost any teleost species are available (Sevilla *et al.*, manuscript in preparation[2]).

Metazoans mitochondrial genomes (mtDNA) are more suitable for the implementation of a microgenomic identification system than nuclear genomes. The usual limits of intraspecific divergence in mitochondrial genes derived from phylogenetic analyses were established between 1 to 2 % in general animal species (Avise *et al.*, 1987; Avise, 2000). Fish genomes undergo genetic changes rapidly, often due to polyploidiation, gain of spliceosomal introns, speciation and gene duplication phenomenon (Robinson-Rechavi, 2001a, 2001b; Ventakesh, 2003). Initiatives, such as BoLD, which includes The Fish Barcode of Life (Fish-BOL: www.fishbol.org), focus on a DNA-based identification system using a relatively small sequence fragment (~600 bp) from the mitochondrial cytochrome *c* oxidase subunit I (COI). This DNA sequence provides sufficient identification labels in terms of nucleotide positions (Hebert *et al.*, 2003) to discriminate even between congeneric fish species, where a 2% sequence divergence is found in 98% of them (Ward *et al.*, 2005). Cyt*b* contains enough phylogenetic information to discriminate from the intraspecific to the intergeneric level (Kotcher *et al.*, 1989), and possesses a phylogenetic performance equivalent to that of COI (Zardoya and Meyer, 1996). However, this short fragment of the COI sequence proposed as universal DNA-barcode presents low interspecific divergences, or what it the same, low

phylogenetic resolution in particular fish families like tunas. In a recent study, ~1 % average interspecific K2P distance was obtained from the phylogenetic analysis of 46 tuna COI barcodes (Ward *et al.*, 2005), while the average interspecific K2P distance obtained from the analysis of 29 FishTrace tuna DNA-barcodes increased to ~1.7 %, strengthening the efficacy of the FishTrace DNA-barcode for identifying fish species. Therefore, it is clear that longer length DNA barcodes can provide safer identification labels. In addition, DNA-barcoding efficiency can also be further improved by the simultaneous use of two genes with different evolutionary rates and genomic locations. These latter requirements were originally fulfilled in FishTrace by the use of the complete mitochondrial cyt*b* (1141 bp) and a nuclear fragment (460 bp) of the rhod gene, with independent genetic variation rate for each of them (Brown *et al.*, 1979; Vawter and Brown, 1986). In fact, both cyt*b* and rhod genes have been widely used as effective molecular markers for fish species identification and for the establishment of unresolved or unknown fish phylogenies (Zardoya and Doadrio 1999; Farias *et al.*, 2001; Chen *et al.*, 2002; Dettai and Lecointre 2005). The absence of introns in the fish rhodopsin makes it to serve as excellent molecular marker since the four introns found in the ancestral chordate rhod gene were simultaneously lost in a common ancestor of ray-finned fishes, although are actually conserved in chondrichthyes and tetrapods (Venkatesh *et al.*, 1999). The use of two genes has also the advantage of including an internal phylogenetic control for the other, with an increased resolution and guarantee for the sound identification of fishes to the species level. From the phylogenetic analyses performed within FishTrace both mitochondrial and nuclear DNA sequence data formed produced similar phylogenetic tree topologies, and congruency with other taxonomical-based phylogenies (Nelson, 1994; Helfman *et al.*, 1997; Johnson and Patterson, 1993; Stiassny *et al.*, 1997; Inoue and Miya, 2001). Furthermore, phylogenetic analysis of both sequences assembled (cyt*b* + rhod) revealed that most recent evolutionary changes are better resolved by the cyt*b* whereas basal phylogenetic relationships are better defined by the rhod gene, since rhod is higher conserved than cyt*b* (less overall changes between taxa). Indeed, FishTrace DNA-barcode efficiency has been improved by the simultaneous use of these two genes whose combination also allow to identify any rare case of paraphyly or hybridizations between close related species (Avise and Saunders, 1984; Streit *et al.*, 1994).

The FishTrace Genetic Catalogue includes cyt*b* and rhod gene sequences as molecular markers related to morphological validated data as indisputable evidence for the origin of fishes. Supported by the taxonomic-systematic and biological reference collection, FishTrace has standardized and simplify molecular protocols for fish species identification based on the

discriminating capacity of those sequences to establish clear and well supported phylogenetic relationships. Furthermore, the use of two independent genes allows to avoid erroneous ascribing of DNA-barcodes. Thus, cases of contamination or errors occurs during the PCR amplification can be detected since each gene sequence is independently validated and phylogenetically analyzed to finally perform a morphological cross-checking for testing the reliability of the formed clades.

The choice of these two genes as satisfactory identification markers was exhaustively tested in a previous project, PescaBase (www.pescabase.org), and in FishTrace has been confirmed its universal use for fish identification. Systematic resolution of cyt*b* and rhod were settled by phylogenetic analyses including representative sequences from both genes directly retrieved from the FishTrace database and from GenBank. These last sequences were used as quality control in the analyses. Resultant topologies were validated by comparing the cyt*b* and rhod topologies with previous phylogenetic systematics studies on fish biodiversity (Miya *et al.*, 2001; Miya *et al.*, 2003; Chen *et al.*, 2002; Dettai and Lecointre, 2005). From them, it was stated that cyt*b* + rhod DNA barcoding sequences from representative fish taxa generate standard topologies to cluster unknown DNA sequences that are correctly identified.

In general terms, major fish clades matched the expected phylogeny compared with previous results where the whole mitochondrial genome was used to construct the phylogeny of 100 different teleost taxa (Miya *et al.*, 2003), even though that FishTrace DNA-barcode is 1/10 shorter than the whole mitochondrial genome. The main teleost monophyletic groups found in FishTrace have been also previously described for Anguilliformes (Obermiller and Pfeiler, 2003; Inoue *et al.*, 2004; Watanabe *et al.*, 2005); Cypriniformes (Chen and Chen, 2001; Saitoh *et al.*, 2003; He *et al.*, 2004; Amemiya *et al.*, 2006); Clupeiformes (Lavoue *et al.*, 2005); Gadiformes (Bakke and Johansen, 2001; Teletchea *et al.* 2005; Bakke and Johansen, 2005; Akasaki *et al.*, 2006); Pleuronectiformes (Chapelau, 1993; Tinti *et al.*, 1999; Tinti *et al.*, 2000; Pardo *et al.*, 2005); and Salmoniformes (Grande *et al.*, 2004), in both morphological and molecular genetics-based taxonomic studies. Thus, the FishTrace DNA-barcode proved successful for assessing most taxonomic groups.

The order Pleuronectiformes is monophyletic on the basis of three synapomorphies: cranial asymmetry associated with ocular migration, advanced position of the dorsal fin over the cranium and presence of a recessus orbitalis (Chapelau, 1993). Furthermore, species within the order Pleuronectiformes share many other morphological features (BenTuvia, 1990;

Hensley, 1997), thus making that molecular barcoding would become a powerful tool for flatfish species identification and classification (Infante *et al.*, 2004). In FishTrace phylogenetic analysis, the monophyly of the order Pleuronectiformes was recovered and well supported with the cyt*b* and rhod sequences assembled. On the whole, the evolutionary history of pleuronectiform species traced by FishTrace DNA-barcodes fully supports previously proposed taxonomy (BenTuvia, 1990; Chapelau, 1993). FishTrace DNA-barcoding strongly differentiate pleuronectiform taxa, clustering three main families in separated clades: Pleuronectidae, Scophtalmidae and Soleidae, all well supported by high bootstrap values, in agreement with previous molecular analysis with shorter sequences (Sotelo *et al.*, 2001). Commercialized flatfish fillets are often mislabelled, and identification of them as fish products is necessary to prevent frauds and substitutions. To this respect, several molecular techniques have been developed for the authentication of flatfish derived products, among them PCR with species-specific primers (Cespedes *et al.*, 1999), PCR-RFLP (Cespedes *et al.*, 1998; Cespedes *et al.*, 1999a; Sanjuan and Comesana, 2002; Sanjuan *et al.*, 2002), PCR-SSCP (Cespedes *et al.*, 1999), indirect enzyme-linked immunosorbent assays (Cespedes *et al.*, 1999) and RAPD-based techniques to identify microsatellite repeats (Iyengar *et al.*, 2000).

Methods for the authentication of European anchovy (*Engraulis encrasicolus*) and gilt sardine (*Sardinella aurita*) semipreserves are highly required and are a valuable tool for European fisheries, and thus, methods have been developed by DNA-based analysis (Sebastio *et al.*, 2001; Jérôme *et al.*, 2003a; Jérôme *et al.*, 2003b). Phylogenetic trees generated from the cladistic analyses of the FishTrace DNA-barcode on clupeiform taxa adopted similar topology, although with higher supported basal nodes, to previously reported phylogenies with shorter cyt*b* fragments (Jérôme *et al.*, 2003a; Jérôme *et al.*, 2003b). According to the repeatability of clades formed by the clupeiform genera included in the present analyses (ME and MP) and supported by the high bootstrap values obtained, the DNA-barcode used in FishTrace demonstrated high potential to discriminate between Clupeidae and Engraulidae families and also among intrafamilial species.

Regarding Clupeidae species, the FishTrace DNA-barcodes belonging to all genera and those retrieved from GenBank formed a well supported monophyletics clades, indicating a high data quality. *Sprattus* + *Clupea* formed a clear monophyletic clade between them, in agreement with previous RFLP and cyt*b*-based phylogenetic analyses (Jérôme *et al.*, 2003a). As expected by the reported classification based on their morphological closeness (Parrish *et*

al., 1989), both clades including *Sardinella* DNA-barcodes formed a monophyletic clade clearly separated from the other taxa belonging to the family Clupeidae. The association of subfamily *Alosinae* with the subfamily Clupeinae by their DNA-barcodes is not in agreement with the accepted taxonomic classification based on morphological characters (Stedovidov, 1952; Eschmeyer, 1990; Nelson, 2006), and therefore, more representative DNA-barcodes from other species of the genus *Sardina* should be analyzed in order to evaluate the phylogeny of these two subfamilies, given that only *Sardina pilchardus* DNA-barcode is available in FishTrace.

With respect to the family Engraulidae, the ten DNA-barcodes from European anchovy (*Engraulis encrasicolus*) herein analyzed resulted in two different clades grouped together in a monophyletic clade distantly separated from the family Clupeidae. One clade contains the GenBank reference DNA-barcode and the samples collected from the North and Cantabric Seas and the Bay of Biscay areas. The other clade contains all the Mediterranean samples with the exception of one intruder sample from the Bay of Biscay area. These results suggests the presence of two potential different phylogeographycal structures between both Atlantic and Mediterranean population European anchovy, as previously reported from a mtDNA-RFLP analysis of 1238 samples collected along the Atlantic coast of the Iberian Peninsula, the Bay of Biscay and the Mediterranean, Aegean and Black seas (Magoulas *et al.*, 2006). The potential isolation of both European anchovy populations herein reported could be explained by the intervention of the Strait of Gibraltar as a physical barrier between them (Bargeloni *et al.*, 2003).

Family Gadidae includes many well known and commercially important teleost species commonly named as “codfishes”. However, their phylogeny and classification are not firmly established (Weitzman, 1991). A wide number of gadiform taxa covered in FishTrace was studied in depth to confirm the accuracy of the DNA-barcode for phylogenetics and species identification purposes. Taxa included in this phylogenetic study comprise fourteen gadiform genera: *Brosme, Ciliata, Echelyopus*, *Gadiculus*, *Gadus*, *Gaidropsarus, Melanogrammus, Merlangius, Merluccius, Micromesistius, Molva*, *Pollachius, Phycis* and *Trisopterus*. DNA-barcodes from all hese gadids were resolved following a cladistic topology in agreement with previous phylogenetic hypothesis generated from mitochondrial cyt*b* and COI sequences analysis (Teletchea *et al.* 2005), cyt*b* PCR-RFLP analysis (Aranishi *et al.*, 2005; Akasaki *et al.*, 2006), and alignment and sequence characterization of different regions from the small and large subunit ribosomal RNAs (Bakke and Johansen, 2002; Bakke and Johansen, 2005).

In addition, cladistic close associations of several gadid groups resulted in FishTrace have been previously described in morphological and molecular-based classifications.

Family Merluccidae appeared in the DNA-barcoding phylogenenetic inference as the most basal ancestor of the FishTrace gadids analyzed. This phylogenetic pattern has been repeatedly described in previous morphological-based studies on the order Gadiformes (Howes, 1991; Nelson, 1994). However, the clade containing *Merluccius* genus included in *Phycis*, is in disagreement with both, morphological and molecular inferred phylogenies of gadiforms, which classify *Phycis* into a separated family, Phyciidae, together with *Echelyopus*, *Ciliata* and *Gaidropsarus* (Nelson, 1994; Teletchea *et al.* 2006). On the contrary, *Echelyopus*, *Ciliata* and *Gaidropsarus* genera were clustered together in FishTrace, supporting the subfamily status for them (Subfam. Gaidropsarinae), within the family Phyciidae (Nelson, 1994; Teletchea *et al.* 2006). Family Phyciidae resulted nested within the order Gadiformes, flanked by Merluccidae and Gadidae families. Thus, it represents the inmediate gadid clade evolved from Merluccidae, and then, the ancestor group of the family Gadidae, in agreement with current taxonomic classification (Nelson, 2006) and phylogenetic hypothesis (Teletchea *et al.* 2006).

The analysis of FishTrace DNA-barcodes grouped all taxa belonging to the family Gadidae into a single and well supported clade comprising *Brosme*, G*adiculus*, *Gadus*, *Melanogrammus*, *Merlangius*, *Micromesistius*, *Molva*, *Pollachius*, and *Trisopterus* genera. At the same time, into the family Gadidae, two monophyletic clades separating subfamilies Gadinae (*Gadiculus*, *Gadus*, *Melanogrammus*, *Merlangius*, *Micromesistius*, *Pollachius*, and *Trisopterus*) and Lotinae (*Brosme* and *Molva*) were recovered from DNA-barcoding analysis, in agreement with previous morphological classification (Nelson, 1994). Furthermore, the Lotinae group has been previously described with morphological evidences as an independent family, "Lotidae", within the order Gadiformes (Howes, 1991). In addition, this morphological-based classification of the two subfamilies has been also described in a more recent molecular study (Teletchea, 2006). In conclusion, *Molva* and *Brosme* (Lotinae) FishTrace taxa were clustered together, representing the closest ancestor group of the family Gadidae. Moreover, two separated monophyletic clades appeared within the subfamily Gadinae: (i) *Gadiculus*, *Micromesistius* and *Trisopterus* + (ii) *Gadus*, *Melanogrammus*, *Merlangius* and *Pollachius*. Phylogenetic analysis with the FishTrace DNA-barcode revealed that *Gadiculus* is the most basal Gadinae genus, followed by two sister groups: *Micromesistius* and *Trisopterus*, in accordance with other molecular studies (Bakke and

Johansen, 2005). Divergence times calculated from molecular approaches indicates that the most ancient gadid split occurred about 20 million years ago between *Gadiculus* and the remaining Gadinae genera (Bakke and Johansen, 2005). On the other hand, *Gadus*, *Melanogrammus*, *Merlangius* and *Pollachius* resulted together within the other respective monophyletic clade. This last association has been recently recovered from both cyt*b* + COI analysis (Teletchea, 2006). In addition, the phylogenetic analysis performed with gadid species was relevant for *Trisopterus* genera since the topology obtained supported the species status for the former subspecies *Trisopterus minutus minutus* and *T. minutus capelanus* (Mattiangelli, *et al.*, 2000).

In our molecular phylogenetic analyses using the FishTrace DNA-barcode, some teleost orders traditionally grouped based on morphological characters, Beryciformes, Perciformes, Tetraodontiformes and Scorpaeniformes resulted divided into two or three separated clades (e.g. two separated groups of Scorpaeniformes appeared nested with perciform groups). However, this segregation has been previously observed in phylogenies obtained with complete mitochondrial genomes (Miya *et al.*, 2003). Monophyly and taxonomical content of some Acanthomorph groups had never been questioned because of the amount of morphological data supporting them, as in pleuronectiforms. Meanwhile, monophyly of some orders like Tetraodontiformes (Holcroft, 2004), Scorpaeniformes (Stiassny and Moore, 1992; Imamura and Shinohara, 1998; Smith and Wheeler, 2004), and the large order Perciformes has been repeatedly questioned (Johnson and Patterson, 1993; Dettai and Lecointre, 2005) due to the poliphyly of them in molecular and morphological analyses.

The high variety in morphology, biology and genetics of the species within Perciformes suggested to analyze all sequences from this order obtained in FishTrace to contribute to the phylogeny of this large order. Tree topologies from the FishTrace DNA-barcodes and those obtained with other genes, including a 759 bp fragment from the rhodopsin gene (Dettai and Lecointre, 2005) agree in the lack of monophyly in the Perciformes order, that was not recovered in any of the phylogenetic hypothesis generated.

At family level, Serranidae, Scaridae, Sparidae and Scombroidae FishTrace taxa were clustered properly, showing high cohesion in these monophyletic groups, with the exception of the genus *Serranus* in family Serranidae. This last taxonomic genus does not cluster with other serranids like *Epinephelus*, but its cladistic association with scorpaeniform taxa obtained in FishTrace (*Helicolenus, Sebastes and Scorpaena*) has been previously described in both,

morphological and molecular approaches (Chen *et al.*, 2003; Dettai and Lecointre, 2005). The monophyletic group formed by Scaridae with Labridae in FishTrace also coincide with previous described fish phylogenies suggesting that they share a common ancestor (Chen *et al.*, 2003; Dettai and Lecointre, 2005).

Although genus Spicara is traditionally included in Centracanthidae (Eschmeyer, 1990; Nelson, 1994), recent analyses with cyt*b* (Orrell *et al.*, 2002) and cyt*b* + 16S RNA (Orrell and Carpenter, 2004) and the FishTrace DNA-barcoding supported a monophyletic Sparidae + *Spicara* clade. Also, the FishTrace barcodes and other subtelomeric satellite analyses resolved *Pagellus erythrinus, Pagrus* spp. and *Dentex* spp. as monophyletic group (De la Herran, 2001). We concluded that the Sparidae family in FishTrace is composed by two major lineages: one comprising the species of the genera *Sparus*, *Diplodus*, *Lithognathus*, *Boops*, and *Sarpa* and the other lineage is comprised of the species of *Pagrus* and *Dentex*, and one species of *Pagellus* (*P. erythrinus*). A previous large allozyme analysis included *Sparus aurata* and *Pagrus pagrus* in different genera (Reina *et al.*, 1994), in agreement with the molecular phylogenetics from FishTrace but in spite of their high morphological similarity since this classification clearly contradicts previous morphological phylogenies based on fish dentition analysis (Meyer, 1993). Thus, FishTrace DNA-barcodes allow intra-familial sparid relationships and classification.

Recent molecular studies on PCR-SSCP of a 148 bp amplicon of the mitochondrial cyt*b* (Weder *et al.*, 2001) and COI-based DNA-barcoding (Ward *et al.*, 2005; Dalziel *et al.*, 2006) have been used to differentiate among tuna and mackerel species. FishTrace DNA-barcodes also allow the cladistic differentiation among scombrid species, rendering high statistical support in nodes discriminating among both, tuna and mackerel species. In addition, FishTrace phylogenetic analyses of scombrid species are in agreement with morphological taxonomic studies that also recovered Scombroidei taxa as a monophyletic group into the perciform bush, sharing a common ancestor with family Bramidae, (Jhonson, 1986).

The high interest for fisheries to identify Scombridae species is demonstrated by the large number of methods for its molecular characterization. Thus, PCR-RFLP (Ram *et al.*, 1996; Quinteiro *et al.*, 1998; Sebastio *et al.*, 2001; Aranishi, 2005a; Aranishi, 2005b; Lin *et al.*, 2005) , and a PCR- SSCP (Rehbein et. al., 1995) analyses have been largely applied for the precise identification of tuna and mackerel species. The cluster discrimination among the skipjack (*Katsuwonus pelamis*), the yellofin (*Thunnus albacares*) and the bigeye (*T. obesus*)

tunas, forming different clades separated from *Sarda sarda* taxa, as described in previous cyt*b* (Lockley and Bardsley, 2000; Terol *et al.*, 2002) and COI (Dalziel *et al.*, 2006) studies, is reproduced in FishTrace. As for the tuna group, mackerels (*Scomber* spp.) have been properly differentiated through the phylogenetic analysis of their FishTrace DNA-barcodes, that are placed in a basal position, representing an ancestor group for tuna species (Dalziel *et al.*, 2006).

Also in the FishTrace perciform taxa, the clade grouping *Liza* (Family Mugilidae) with Atherinomorpha (orders Atheriniformes + Beloniformes), represented in FishTrace by *Belone, Oryzas and Tylosurus*) was recovered in agreement with previous molecular and morphological phylogenies (Miya *et al.*, 2003; Dettai and Lecointre, 2005). Thus, this monophyly of Atherinomorpha is also supported by the derived morphological characters such as the ethmoid region of the skull, gill arches, pelvic girdle, jaw musculature, olfactory organ, and inferred reductions in the infraorbital series and some other bones (Parenti, 1993). Also, a common ancestor for Atherinomorpha and Mugilidae resulted in our study, as previously reported by morphological (Jhonson and Patterson, 1993) molecular (Miya *et al.*, 2003) and combined analyses (Wiley *et al.*, 2000; Chen *et al.*, 2002; Dettai and Lecointre, 2005).

As detailed above, the new combined DNA-barcode assayed for the covered FishTrace species, generated robust species assignments through cladistic analysis, rendering in practice enough resolution for teleost species and even distinguishing among geographically isolated fish populations when sufficient number of associated sequences are available (see below). This methodology could be applied to fish-products authentication and traceability analyses, as it has been implemented for fish fillets from grouper and common substitute fish species (Trotta *et al.*, 2005) and even the FishTrace cyt*b* barcode locus has been used for designing new primers pairs to barcode species authentication in other food products by real-time PCR systems (López-Andreo, *et al.*, 2005). The different geographical origin of the specimens sampled within FishTrace has detected potential biogeographical genetic divergences. Indeed, information on polymorphisms found has also been included in the FishTrace Genetic Catalogue. Thus, genetic divergence detected within a species could be interpreted as a possible effect of population isolation or as fixed polymorphisms in a population (Billington and Hebert, 1991; Gold *et al.*, 1994; Arnegard *et al.*, 1999; Latch and Rhodes, 2005).

Although phylogeographic studies performed on marine species revealed less population structuring compared to the fresh-water species (Hauser and Ward, 1998), the lack of barriers in the sea could support gene flow among populations, mainly in highly migratory species. Analysis of genetic variability in fish species is being approached by the use of DNA-based methods like RFLPs (Mamuris *et al.*, 1999), allozymes (Avise and Saunders 1984; Streit *et al.*, 1994; Perdices *et al.*, 2001; Schönhuth *et al.*, 2001), microsatellites studies (Hoarau *et al.*, 2002; Mattiangeli *et al.*, 2006) and microarrays (Moriya *et al.*, 2004). Assessment of population structures has been also widely addressed by sequence analysis of mitochondrial DNA genes (Meyer, 1993; Ostellari *et al.*, 1996; Carvalho and Hauser, 1998; Rocha-Olivares, 1999; Tabata and Taniguchi, 2000). Cyt*b* is considered one of the most useful genes for population studies and is also probably the best well known mitochondrial gene with respect to its phylogenetic resolution (Sturmbauer, 1992; Zardoya and Meyer, 1996; Johns and Avise, 1998; Farias *et al.*, 2001) and its structure and function (Esposti *et al.*, 1993; Prusak and Grzybowski, 2004). Accordingly, the cyt*b* locus was chosen in FishTrace for population analysis of six species of wider distribution, which were specifically sampled with a representative number of specimens at each geographical location. It should be pointed out the limited amount of information on population analysis of the six species chosen for haplotype analyses: *Merluccius merluccius*, *Micromesistius poutassou*, *Mullus surmuletus*, *Pagellus erythrinus*, *Pagrus pagrus* and *Solea solea*.

Merluccius merluccius (European hake) populations from seven geographical areas, comprising the Baltic Sea and North Sea, Atlantic (Bay of Biscay, Cantabric Sea, Canary Islands and Madeira Archipelago), and the Mediterranean, were studied in FishTrace, based in the cyt*b* locus, finding that only the North Sea samples were genetically distinct. These results do not exactly agree with analysis by allozymes (Cimmaruta *et al.*, 2005), where results obtained diagnosed two different genetic structure status of the Atlantic and Mediterranean stocks. These independency between Atlantic and Mediterranean populations have been described using five nuclear microsatellites loci (Castillo *et al.*, 2004), that also detected population genetic structures between the Western Mediterranean and the Aegean sea. Other six microsatellite loci were used to study genetic variability and population structure in Atlantic and Mediterranean populations of European hake, detecting significant genetic variability within the Bay of Biscay (Lundy *et al.*, 1999). The diagnosability of both Atlantic and Mediterranean populations was also justified by ecological, behavioural and oceanographic information (Lundy *et al.*, 2000).

The blue whiting (*Micromesistius poutassou*) is found along the continental margin of the Northeast Atlantic, with smaller populations in the Northwest Atlantic and the Mediterranean (Ryan *et al.*, 2005). In FishTrace, this species presented a relative low degree of genetic variation among populations studied: North Sea, Bay of Biscay, Cantabric Sea and Eastern and Western Mediterranean. However, the North Sea population resulted separated in the genetic structure analysis. Albeit, samples studied presented high degree of genetic variation within populations (from 14 to 18 haplotypes in 20 specimens examined), with the exception of the North Sea population, where only five haplotypes were detected. Accordingly, significant geographic heterogeneity in allele frequencies of this species from the British Isles was also demonstrated with enzyme loci IDHP-2 and PGM-I (Mork and Giaever, 1995). This genetic heterogeneity appears to be at the same level determined for the demersal gadoids cod and haddock (Giaever and Stien, 1998). On the other hand, we attributed the genetic isolation of the North Sea population to a potential reproductive bottleneck in the blue whiting of this area. Also, Western and Eastern Mediterranean *M. poutassou* populations resulted separated in FishTrace. It could be assumed that Western Mediterranean has been genetically influenced by an invasion with populations possibly originating from the Atlantic coast of Morocco. This hypothesis could be tested by the analysis of additional samples from Morocco as well as from the North African coast within the Mediterranean. This factor could explain the population structuring of this gregarious and erratic species in the Mediterranean. Life history and environmental influences must be also considered in FishTrace since previous analysis of *M. poutassou* populations from the Northeast Atlantic and the Mediterranean, using one minisatellite and five microsatellite loci, revealed significant geographic heterogeneity and isolated populations at the extremes of the species range in the Barents Sea and the Mediterranean (Ryan *et al.*, 2005).

FishTrace *Mullus surmuletus* (striped red mullet) populations examined (North Sea, Bay of Biscay, Canary Islands, Madeira Archipelago and Western and Eastern Mediterranean) shared a significant number of common cyt*b* haplotypes and thus, structuring of populations from the Atlantic to the Mediterranean was not evident, with the exception of the North Sea population, which appeared separated from the others in the genetic structure analysis. As in the case of FishTrace *M. poutassou* populations, this genetic bottleneck effect could be driven by natural barriers separating the North Sea from the Atlantic. A previous study using joined data from allozyme and random amplified polymorphic DNA-RAPD detected high degree of genetic polymorphism within six striped red mullets Mediterranean populations, revealing longer distance between the French and the Greek populations (Mamuris *et al.*, 1999). A

RFLP-based study with three mitochondrial regions (control region, COI, and 12S-16S ribosomal RNA), has found high interpopulation genetic structuring for Mediterranean populations of *M. surmuletus* (Mamuris *et al.*, 2001). It should also be noted that FishTrace analysis has not included the Cantabric Sea population, which by virtue of its central geographic location could further support the panmictic nature of the Atlantic-Mediterranean populations.

Only four different populations of *Pagrus pagrus* have been examined in FishTrace. The divided genetic distribution of these populations in FishTrace suggests the existence of reproductive barriers. Western and Eastern Mediterranean resulted clearly separated, possibly due to the existing within the Sicelo-Tunisian straight. The isolation of the Madeira population is possibly due to prohibitive depths for the biology of this species, separating the Canaries from Madeira. In addition, the close relation detected between Western and Canary populations suggests that gene flow between both areas follows the West African coastline through the Gibraltar straights into the Mediterranean. However, allozyme data have revealed strong differentiation when comparing Atlantic and Mediterranean samples (Bargelloni *et al.*, 2003). Since the Strait of Gibraltar has been proposed to be a physical barrier between two marine biogeographical regions, the Mediterranean Sea and the Northeast Atlantic, these results provide evidence for a sharp phylogeographical break between the both *Pagrus pagrus* populations. Thus, the study of the Cantabric Sea population, currently missed in FishTrace, could provide important information concerning the genetic relationship of the Atlantic and Mediterranean populations.

For the other sparid (*Pagellus erythrinus*) analyzed in FishTrace, the haplotype connectivity network demonstrates high level of interpopulation genetic variability within them (Canary Islands, Western and Eastern Mediterranean). Thus, no obvious structuring of populations was observed. However, a comparison of available growth data from the Mediterranean and the Atlantic revealed higher lengths-at-age for red pandora in the north-western Mediterranean and the Atlantic than in the central and eastern Mediterranean, implying a common 'growth space' for the populations in these areas (Somarakis and Machias 2002). We can not conclude with genetic data available in FishTrace that this differentiation between populations is induced by genetics, so these differences can be attributed to the synergistic combination of trophic and thermal conditions (Somarakis and Machias 2002).

Solea solea samples studied showed the largest amount of genetic variation, observed in FishTrace, associated to populations. The haplotype connectivity network of *Solea solea* also revealed a well defined genetic population structures in Mediterranean and Atlantic areas (Baltic Sea, Bay of Biscay and North Sea). Thus, a total of four populations can be distinguished: (i) The North Sea and the Bay of Biscay; (ii) the Baltic Sea; (iii) the Western Mediterranean; and (iv) the Western Mediterranean population. Only the North Sea and the Bay of Biscay populations share a significant number of common haplotypes, but both are closer to the Baltic than for the Mediterranean. This suggests some potential gene flow between these two proximate geographical areas since hybrid zones are a common phenomenon for marine fishes in the transition area between the North Sea and the Baltic. These results are in agreement with the obtained for other flatfish population, the turbot (*Scophthalmus maximus*) that showed a clear transition zone between the Baltic Sea and the North Sea and limited or no genetic differentiation was found (Nielsen *et al.*, 2004). It reveals a slight reduction of genetic variability in the North European sea areas. However, despite this high level of gene flow, geographic differentiation were observed in allozyme analysis of a few loci (Exadactylos *et al.*, 1998). The population differentiation of the common sole along the Portuguese coast was also studied using morphological and parasitological data, and some differentiation was found between north-centre and the south Portuguese coast, evidencing the existence of an ecological differentiation of the sole along the Portuguese coast (Marques *et al.*, 2006). Other studies on the genetic population structure of soles indicate that several distinct breeding populations exist within its distributional range in European waters (Imsland *et al.*, 2003). These results indicate the role of both ecological and evolutionary structuring mechanisms in determining the genetic population structure of *S. solea*.

A main interest for the scientific management of European fisheries is the access to reliable scientific information about genetic structure of stocks and populations which is scarcely reported in the literature (Hartley 1995; Nesbo *et al.* 2000; Schonhuth *et al.* 2005; Magoulas *et al.* 2006). Thus, these pilot studies on the population structures across Europe of six species demonstrate the feasibility for identification and potential control of genetic variability of fish stocks and the implementation of technical means for fish and fish-products traceability.

To catalogue present Biodiversity is a scientific priority. Several international projects related to cataloguing the living world has been launched along the last decades since only a small part of the actually extant species on Earth, from more than 100 million existing species (May, 1988), have been described. For this purpose, the Global Biodiversity Information

Facility and The Barcode of Life Data Systems had been launched by the beginning of this 21st Century. To this respect, trustworthiness of global information in Biodiversity databases rely on the reliability of standardized data that could be compared from different sources, and this has been achieved in the FishTrace database. Moreover, patterns in marine fish biodiversity can be further assessed from databases by quantifying temporal variation in the rates of population change, abundance, life history and demography concomitant with long-term reductions in abundance (Hutchings and Baum, 2005). FishTrace, like other databases of fishes such as FishBase, is an *online* open-access database that can be interrogated by users to find fish species general descriptions and biological, taxonomical and ecological information connected with the molecular characterization of the specimens defining a species. FishTrace has applied new protocols for the validation of experimental procedures and data. As an example of the effectiveness of the validation process implemented within FishTrace, those cases of taxonomic misidentification were detected during the data validation in FishTrace. The most remarkable case was those twenty flatfish specimens sampled from the extra-European area that were taxonomically identified as *Solea solea*. After their phylogenetic analysis performed using the FishTrace DNA-barcode, all those specimens were identified as *Microchirus azevia*. It should be pointed out that this species is probably a common substitute of *Solea solea* in the European markets, particularly given the difficulties in distinguish them only from morphological characterization.

Potential application of FishTrace data include the development of tailored diagnostic tools for quality control purposes (Lockley and Bardsley, 2000), for example, the implementation of standardized rapid molecular tests to identify substitution frauds, frequently observed in fish markets (Perez and Garcia-Vazquez, 2004; Trotta *et al.*, 2005). For this purpose, *online* molecular and morphological identification tools are also available from the FishTrace web interface, including (i) a dedicated FishTrace BLAST (Altschul, 1990), which allow accurate DNA-barcode identifications by comparison of target sequences introduced by users); (ii) an *online* RFLPs simulator to tailor diagnostic comparison of species by users; (iii) a Fish Phylogenetic Tree tool, to assign evolutionary history and phylogeny to sequences introduced by users, and (iv) a Morphological tool for an initial classification of fish species based on taxonomical data entered by users against morphological records archived in FishTrace. These practical applications for fish species identification, together with the large amount of available data deposited in the *online* database comprising European, regional and local information on fish species and the standardized images repository allowing taxonomic identifications, stand FishTrace up from other fish databases herein cited.

In conclusion, FishTrace database establish a new concept in fish species identification through the close connection of molecular genetics information obtained from fish species of specific fisheries interest in Europe, distributed among main European sea areas. In addition, FishTrace network holds backup biological reference collections including DNA, tissue, voucher specimens, and otoliths from the taxonomically and genetically validated fish species. These collections, deposited in European natural history museums are public repositories for fish identification as a unique infrastructure in Europe.

6.- Conclusions

1) The critical mass of expertise gathered in the FishTrace network has compiled a large amount of biological data following a strategic sampling of fish specimens. Sampling orientated to obtain representative information on fish species of interest for European markets has been fully achieved.

2) A highly structured database has been *de novo* developed. This new database named "FishTrace database" is the loading and storage system to deposit the data collected by the network. The FishTrace database is also a data retrieval system for analysis and data comparison.

3) The database accomplished, is accessible in the Internet as an open-access web page to the general public at the URL: www.fishtrace.org, which has been designed in a user-friendly environment for searching and comparison of fish biological and genetic data.

4) Taxonomic identification of more than 2500 fish specimens sampled, belonging to 220 different marine teleost species commonly commercialized in the European markets has been completed. Fish sampling coverage comprises main European sea areas (Skagerrak and Baltic Sea, North Sea, English Channel and Bay of Biscay, Cantabric Sea and NW Iberian Peninsula, Western and Eastern Mediterranean, Madeira archipelago and Canary Islands), and also includes extra-European areas. Cataloguing of the taxonomic information obtained has been deposited at the FishTrace database.

5) DNA barcodes from the specimens that stand for the 220 fish species have been obtained. These diagnostic DNA barcoding sequences correspond to mitochondrial cytochrome *b* and nuclear rhodopsin genes. Genetic standardized tools developed permit accurate identification and differentiation of European teleost species at molecular level. Morphological misidentifications can be now detected by the molecular identification systems developed in FishTrace.

6) A pilot study on population genetics with six fish species inhabiting distantly separated sea areas was conducted demonstrating the potential of cyt*b* haplotyping to determine genetic population structures.

7) Biological reference collections emerged from the strategic sampling performed have been deposited at four European Natural History museums (Stockholm, Paris, Funchal and Tenerife). Collections comprise voucher specimens, tissue samples and DNA from vouchers and otoliths that can be accessed for cross-referencing, and as research resources for the identification of European commercial fish species. Data from these collections has been deposited in FishTrace database.

8) Methodological procedures to gain and compile information on taxonomy, biological collections and genetics of fish species have been standardized within FishTrace. This methodological standardization guaranteed reliability of data deposited into the database.

9) Fish species identification tools have been developed within FishTrace and implemented in a web page. These *online* tools comprise molecular and morphological systems for species identification by interrogating the database on taxonomy, ecology and genetics of the target fish and the 220 species included in FishTrace.

10) Results obtained within FishTrace serves to provide authenticity and traceability systems for European fish products, increasing their economic value and offering a guarantee of their biological and also geographical origin. FishTrace information to establish the origin of fish and derived fish products can also assists in the identification of food products from non certified sources.

7.- Exploitation and dissemination of results

The major contribution FishTrace network has been the establishing of the first European online and open access database on global information from most commonly marketed teleost fish species with particular emphasis on molecular genetics characterization by a standardized DNA-barcode and accession to reference biological collection.

Results obtained from this multidisciplinary research network have been widely disseminated through Internet at www.fishtrace.org to the scientific community involved in issues of fish taxonomy, genetics and reference collections, as well as to the general public involved in other fields of knowledge (aquaculture industry, nutrition companies, marketing, etc.).

Through the web site, exploitation of data compiled has been achieved in a first level through model tools tailored for end-users for identification and differentiation of fish species. Three fish identification tools based on DNA have been developed, BLAST, RFLPs and Phylogenetic analysis, and a morphological tool, MORPHO tool.

Selected set of species has been chosen to define tailored systems for molecular identification. Development of these methods has been either published or prepared for submission to scientific journals. At present, the following articles have been published:

- M. Trotta, S. Schönhuth, T. Pepe, M. L. Cortesi, A. Puyet, J. M. Bautista (2005). *Multiplex PCR method for use in Real-Time PCR for identification of fish fillets from grouper (Epinephelus and Mycteroperca species) and common substitute species*. Journal of Agricultural and Food Chemistry. 53 (8). 2039-2045.

- S. Jiménez, S. Schönhuth, I. J. Lozano, J. A. González, R. G. Sevilla, A. Diez, and J. M. Bautista (2007). *Morphological, ecological, and molecular analyses separate Muraena augusti from Muraena helena as a valid species*. Copeia. (1). 101–113.

The FishTrace Consortium agreed to submit to scientific journal a series of manuscripts containing specific results directly emanating from the FishTrace database. The tentative list

of topics covered and the responsible institution is shown in Table 7.1. The following title of manuscript are being submitted for publication to a scientific journal in a near future:

- R. G. Sevilla, A. Diez, M. Norén, O. Mouchel, M. Jérôme, V. Verrez-Bagnis, H. van Pelt, L. Favre-Krey, G. Krey, The FishTrace Consortium and J. M. Bautista (2007). *Barcoding of fish with a mitochondrial and a nuclear gene: A collection of primers and PCR conditions for the amplification of the cytochrome b and rhodopsin genes from teleost fish species*. Molecular Ecology Notes. In process.

Other reports directly emanating from FishTrace:

1.- N. Kourti and P. Carreau (2005). *Genetics and Fisheries*. EC Report.

2.- E. Scanlan and P. Carreau (2005). *Scientific Protocol for Genetic Inspections of Fish, Genins. Using FishTrace for detecting fish fraud*. EC Report.

3.- S. Chardron (2005). *Influence de la géographie sur la divergence génétique d'espèces de poissons d'intérêt commercial: exemple de la sole commune (Solea solea) et du merlu européen (Merluccius merluccius)*. Ifremer. Master of Research.

To promote the access to the *online* database and the use of reference collections, the following disseminations activities have been performed:

1.- Edition of leaflet presenting the FishTrace project, in English and French (Annex XXIII).

2.- Mini CD-ROM presenting the FishTrace project and the web site. Its content is in different European languages. (First Prototype available since September 2006).

3.- FishTrace web site linked to each partner's institution web site and other international institutions as FishBOL, The Natural History Museum of London, FishGen project and FishBase *online* database.

List of Symposiums/Seminars attended, and contributions presenting FishTrace by partners:

1.- 34th Western European Fish Technologist's Association Meeting. Lübeck, Germany. September 12-15th, 2004. V. Verrez-Bagnis (Ifremer): *FishTrace: a DNA database for European marine fish - Genetic catalogue, biological reference collections and online database of European marine fishes (EC project QLRI-CT-2002-02755.)* Invited oral presentation.

2.- XIII Iberian Symposium for Marine Benthos Studies. Las Palmas de Gran Canaria, Canary Islands, Spain. September 21-24th, 2004. S. Jiménez et al. *Murénidos comercializados y protegidos en Canarias (Osteichthyes, Anguilliformes, Muraenidae).* Poster.

3.- XIII Iberian Symposium for Marine Benthos Studies. Las Palmas de Gran Canaria, Canary Islands, Spain. September 21-24th, 2004. M. Gimeno et al. *Identificación y diferenciación de lenguados (Soleidae) y otros peces planos afines (Psettodidae, Cynoglossidae) comercializados en Canarias*. Poster.

4.- XIII Iberian Symposium for Marine Benthos Studies. Las Palmas de Gran Canaria, Canary Islands, Spain. September 21-24th, 2004. M. F. Marrero et al. *Corvinas y corvinatos oeste-africanos comercializados en canarias: Argyrosomus, Atractoscion, Pseudotolithus (Osteichthyes, Sciaenidae)*. Poster.

5.- XIII Iberian Symposium for Marine Benthos Studies. Las Palmas de Gran Canaria, Canary Islands, Spain. September 21-24, 2004th. J. I. Santana et al. *Identificación y Diferenciación de especies del género Seriola y otros Carángidos afines (Caranx, Lichia) (Osteichthyes, Carangidae) presentes en aguas de Canarias*. Poster.

6.- XIII Iberian Symposium for Marine Benthos Studies. Las Palmas de Gran Canaria, Canary Islands, Spain. September 21-24th, 2004. J. I. Santana et al. *Grandes serránidos comercializados en Canarias: Epinephelus, Mycteroperca, Cephalopholis (Osteichthyes, Serranidae)*. Poster.

7.- EFARO (European Fisheries and Aquaculture Research Organisation) Meeting. Lisbon, Portugal. October 28-31st, 2004. J. M. Bautista (UCM): *Using genetic tools for food*

products traceability and security; and *Molecular genetic database for increased traceability in European marine products*. Invited oral presentations.

8.- Aquaculture Europe '04 Meeting. Barcelona, Spain. October 20-23rd, 2004. M. Trotta (UCM): *A Multiplex-PCR method for use in Real-Time PCR for identification of fillets from grouper (Epinephelus spp. and Mycteroperca spp.) and its usual substitution species*. Invited oral presentation.

9.- FAO Meeting on Fishery Utilization and Marketing Service. Bremen, Germany. December 14th, 2004. M. Etienne (Ifremer). Invited oral presentation on FishTrace.

10.- European Bioinformatic Institute (EBI). Hinxton, United Kingdom. February 4th, 2005. P. Carreau (JRC). Invited oral presentation on FishTrace.

11.- University of Bologna. Bologna, Italy. February, 2005. J. M. Bautista (UCM): *Molecular tools based on standarized genetic data for fish-food products traceability and security*. Invited oral presentation.

12.- European Commission DG Fish. Brussels, Belgium. March, 2005. P. Carreau (JRC). Invited oral presentation. Invited oral presentation on FishTrace.

13.- 2005 Glasgow Traceability Seminar. Scottish Exhibition and Conference Centre (SECC). Glasgow, United Kingdom. May 19th, 2005. M. Etienne (Ifremer): *FishTrace project (PF5)* and A. Puyet (UCM): *Molecular tools based on standarized genetic data for fish-food products traceability and security*. Invited oral presentations.

14.- FISH-BOL First Initiative Meeting. University of Guelph Arboretum, Ontario, Canada. June 5-8th, 2005. Michael Norén (NRM). Invited oral presentation on FishTrace.

15.- 1st BIOPRO Project Meeting. Ifremer, Nantes, France. July, 2005. V. Verrez-Bagnis, Marc Jérôme (Ifremer) and Philippe Carreau (JRC). Discussion on the linkage between BIOPRO and FishTrace projects.

16.- Petrus Artedi Tricentennial Symposium on Systematic Ichthyology. The Royal Swedish Academy of Sciences, Stockholm, Sweden. September 13-14th, 2005. Michael Norén (NRM). Invited oral presentation on FishTrace.

17.- European Commission DG Fish. Brussels, Belgium. May, 2006. N. Kourti (JRC). Invited oral presentation on FishTrace.

18.- Data Analysis working group of the DNA Barcoding of Life Project. Paris, France. July 6-8th, 2006. J. M. Bautista (UCM): *The control gene, the data validation analysis and the backup reference biological data.* Invited oral presentation.

19.- 13th World Congress of Food Science and Technology. Nantes, France. September 17-21st, 2006. V. Verrez-Bagnis (Ifremer): *FishTrace: A Tool for Identification of Fish Species and Traceability of Fish Products*. Poster.

20.- ICES (International Council for the Exploitation of the Sea) Annual Science Conference. Maastricht, The Netherlands. September 20-23rd, 2006. H. van Pelt (RIVO): *Development of a genetic catalogue, biological reference collections and online database of European marine fishes (FishTrace)*. Invited oral presentation.

21.- "Fish & Chips" satellite symposium on DNA-based identification of marine organisms at the "Marine Genomics Conference". Sorrento, Italy. October 29th, 2006. J. M. Bautista (UCM): *Results and prospects of FishTrace in relation to Fish & Chips: Fish barcoding from the FishTrace database*. Invited oral presentation.

Besides the above international conferences, FishTrace has been presented in the press for the general public:

1.- "Diario de Noticias". October 26th, 2005. This newspaper from Funchal (Portugal) informed about the FishTrace project during the last Annual Meeting held in Madeira. (Portuguese).

2.- "www.consumaseguridad.com". February 3rd, 2006. This *online* Spanish magazine on Food Security interviewed J. M. Bautista, FishTrace Coordinator. (Spanish).

3.- "El País". February 15th, 2006. This Spanish newspaper interviewed J. M. Bautista, FishTrace Coordinator. (Spanish).

4.- "www.chilepesquero.cl". February 24th, 2006. This *online* Chilean magazine on South Pacific Fish Resources and Fisheries Management presented FishTrace and its applicability. (Spanish).

5.- "Conxemar". No. 25, August-September, 2006. This bi-monthly magazine, edited by the Spanish Association of Wholesalers, Importers, Manufacturers and Exporters of Fish products and Fish farming, interviewed J. M. Bautista, FishTrace Coordinator. (Spanish).

8.- Policy related benefits

FishTrace contributes with three main policy related benefits: (i) Support to EU common fisheries policies, fishing industry and markets; (ii) Infrastructure to assist the traceability of commercial teleost fish species and fish food; and (iii) Providing genetic information for sustainable exploitation of living resources and to establish appropriate marine biodiversity conservation policies.

(i) The objectives originally set in FishTrace have been successfully met by the compilation, curation and validation of biology and genetic data from more than 220 teleost species of particular interest to the European fisheries and fish markets. Via Internet, through www.fishtrace.org, FishTrace provides researchers, industry and authorities with the information resources required for a multidisciplinary approach to main issues on fisheries management: standardized information on exploited fish species and stocks. Hence, DNA-barcodes and haplotypes information, and also biological regional information on taxonomy, distribution and ecology have been collected from main commercialized European fish species.

(ii) FishTrace has settled up a solid infrastructure to seize the potential of newly implemented technologies for the precise identification and reliable labelling of fish and fish products, in the areas of Food Safety legislation and Health and Consumer Protection. This network has exploited new technologies, such as the fish DNA-barcoding analysis, by the designing and subsequent implementation of model tools for accurate fish identifications through an *online* database. Thus, the accessible molecular information deposited in the database and the implemented *online* tools can assist FishTrace end-users in fish species authentications, allowing rapid detection of frauds and species substitutions.

(iii) The further advancement and potential applications of results obtained from this multidisciplinary network could also have significant impact on issues of natural resources conservation since sustaining the components of a fish population and understanding their function is important to avoid over-exploitation of local fish populations and loss of genetic material.

9.- Future actions

As an European infrastructure, the FishTrace database is a major European effort on fish traceability and marine fish identification and consequently, all Consortium members are willing to maintain it at long-term as a public and permanent *online* database. Thus, the future prospects in FishTrace are:

1) To expand FishTrace database by extending cooperation with research institutions and administrative bodies.

2) Long-term preservation and storage of reference biological materials.

3) The building up of a worldwide cyt*b* database (at present, more than 56000 entries for cyt*b* are available in GenBank.

4) Genetic and taxonomic information supplied in the FishTrace database can assist the design of model tools for the technical development of pre-competitive analytical procedures of unequivocal identification and quality control aimed at producers as well as regional, national and European governments.

5) Further developments in species-specific DNA-microarray technology will allow to design innovative fish species identification system, also suitable to resolve most important problems on taxonomic identification of different stages of fish development: egg, larva, etc.

6) It will be now possible to follow species and populations genetic frequency changes through time using both old museum specimen and modern samples included within FishTrace.

7) To participate in further international initiatives, specially under FP7 calls to cover fish food traceability, support to fisheries and biodiversity.

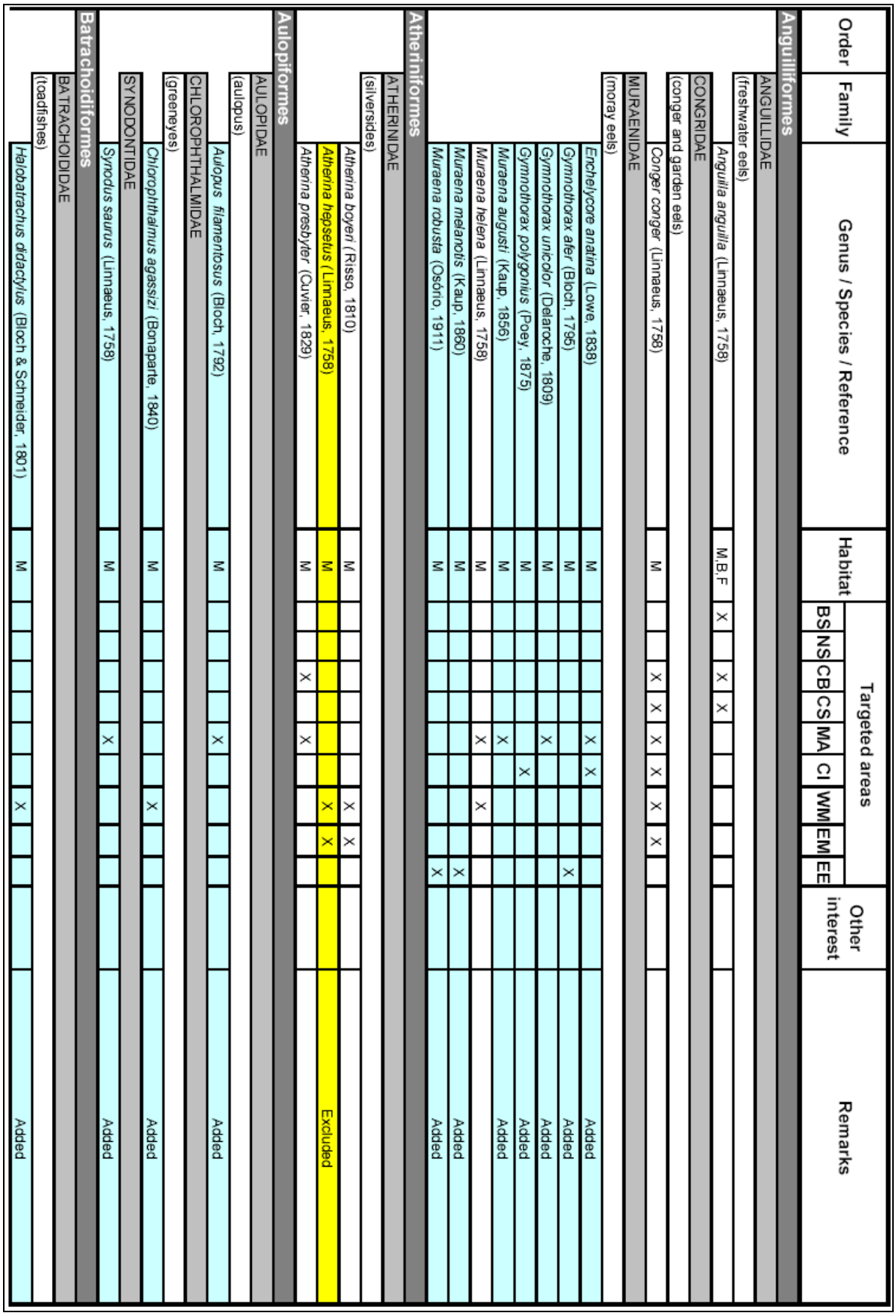
10.- Action requested to the Commission

No actions are requested to the Commission.

11.- Tables and Figures

Tables

Table 3.1.- Target marine teleost species of food, ecological and zoological interest in Europe.



Order	Family	Genus / Species / Reference	Habitat	Targeted areas								Other interest	Remarks
				BS	NS	CB	CS	MA	CI	WM	EM	EE	
Anguilliformes													
	ANGUILLIDAE												
	(freshwater eels)												
		Anguilla anguilla (Linnaeus, 1758)	M,B,F	X		X	X						
	CONGRIDAE												
	(conger and garden eels)												
		Conger conger (Linnaeus, 1758)	M			X	X	X	X	X	X		
	MURAENIDAE												
	(moray eels)												
		Enchelycore anatina (Lowe, 1838)	M					X	X				Added
		Gymnothorax afer (Bloch, 1795)	M								X		Added
		Gymnothorax unicolor (Delaroche, 1809)	M					X					Added
		Gymnothorax polygonius (Poey, 1875)	M						X				Added
		Muraena augusti (Kaup, 1856)	M					X					Added
		Muraena helena (Linnaeus, 1758)	M					X		X			Added
		Muraena melanotis (Kaup, 1860)	M								X		Added
		Muraena robusta (Osório, 1911)	M								X		Added
Atheriniformes													
	ATHERINIDAE												
	(silversides)												
		Atherina boyeri (Risso, 1810)	M						X	X			
		Atherina hepsetus (Linnaeus, 1758)	M						X	X			Excluded
		Atherina presbyter (Cuvier, 1829)	M			X		X					
Aulopiformes													
	AULOPIDAE												
	(aulopus)												
		Aulopus filamentosus (Bloch, 1792)	M					X					Added
	CHLOROPHTHALMIDAE												
	(greeneyes)												
		Chlorophthalmus agassizi (Bonaparte, 1840)	M						X				Added
	SYNODONTIDAE												
		Synodus saurus (Linnaeus, 1758)	M					X					Added
Batrachoidiformes													
	BATRACHOIDIDAE												
	(toadfishes)												
		Halobatrachus didactylus (Bloch & Schneider, 1801)	M						X				Added

Beloniformes												
BELONIDAE												
(needlefishes)												
Belone belone (Linnaeus, 1761)	M	X	X	X					X			
Tylosurus acus (Lacepède, 1803)	M						X	X				Added
Beryciformes												
BERYCIDAE												
(alfonsinos)												
Beryx decadactylus (Cuvier, 1829)	M					X						
Beryx splendens (Lowe, 1834)	M				X							Added
TRACHICHTHYIDAE												
(slimeheads)												
Hoplostethus atlanticus (Collett, 1889)	M		X									Excluded
Clupeiformes												
CLUPEIDAE												
(herrings, shads, sardines and menhadens)												
Alosa alosa (Linnaeus, 1758)	M,B			X								
Alosa fallax (Lacepède, 1803)	M,B	X	X	X								
Clupea harengus (Linnaeus, 1758)	M,B	X	X	X								
Sardina pilchardus (Walbaum, 1792)	M		X	X	X	X	X	X	X		ZI	
Sardinella aurita (Valenciennes, 1847)	M						X	X	X			
Sardinella maderensis (Lowe, 1838)	M					X						
Sardinops sagax (Jenyns, 1842)	M									X		Excluded
Sprattus sprattus (Linnaeus, 1758)	M,B	X	X	X								
ENGRAULIDAE												
(anchovies)												
Engraulis anchoita (Hubbs & Marini, 1935)	M									X		Excluded
Engraulis encrasicolus (Linnaeus, 1758)	M		X	X	X		X	X	X		ZI	
Engraulis ringens (Jenyns, 1842)	M									X		Excluded
Gadiformes												
GADIDAE												
(cods and haddocks)												
Gadiculus argenteus (Guichenot, 1850)	M			X							EI	
Gadus macrocephalus (Telesius, 1810)	M									X		Excluded
Gadus morhua (Linnaeus, 1758)	M	X	X		X					X	EI, ZI	
Melanogrammus aeglephinus (Linnaeus, 1758)	M	X	X	X	X					X		
Merlangius merlangus (Linnaeus, 1758)	M	X	X	X								
Micromesistius poutassou (Risso, 1826)	M	X	X	X	X			X	X			
Pollachius pollachius (Linnaeus, 1758)	M		X	X	X							
Pollachius virens (Linnaeus, 1758)	M	X	X								EI, ZI	

Theragra chalcogramma (Pallas, 1811)	M									X	EI, ZI	Excluded
Trisopterus esmarkii (Nilsson, 1855)	M	X	X									
Trisopterus luscus (Linnaeus, 1758)	M		X	X	X							
Trisopterus minutus (Linnaeus, 1758)	M		X	X				X	X		EI, ZI	
LOTIDAE												
(hakes and burbots)												
Brosme brosme (Ascanius, 1772)	M	X									EI, ZI	
Ciliata septentrionalis (Collett, 1875)	M		X									Added
Enchelyopus cimbrius (Linnaeus, 1766)	M	X	X	X								
Gaidropsarus biscayensis (Collett, 1890)	M				X							Added
Gaidropsarus mediterraneus (Linnaeus, 1758)	M				X							Added
Molva dypterygia (Pennant, 1784)	M				X							
Molva macrophthalma (Rafinesque, 1810)	M							X				Excluded
Molva molva (Linnaeus, 1758)	M	X	X	X	X						EI, ZI	
MACROURIDAE												
(grenadiers or rattails)												
Coryphaenoides rupestris (Gunnerus, 1765)	M	X									EI	
MERLUCCIIDAE												
(merluccid hakes)												
Macruronus magellanicus (Lönnberg, 1907)	M									X		Excluded
Macruronus novaezelandiae (Hector, 1871)	M									X		Excluded
Merluccius australis (Hutton, 1872)	M									X		
Merluccius capensis Castelnau, 1861	M									X		
Merluccius gayi gayi (Guichenot, 1848)	M									X		Excluded
Merluccius hubbsi (Marini, 1933)	M									X		Excluded
Merluccius merluccius (Linnaeus, 1758)	M	X		X	X		X	X	X			
Merluccius polli (Cadenat, 1950)	M									X		Added
Merluccius senegalensis (Linnaeus, 1758)	M									X		Excluded
PHYCIDAE												
(phycid hakes)												
Phycis blennoides (Brünnich, 1768)	M			X	X	X		X	X			
Phycis phycis (Linnaeus, 1766)	M					X		X	X			
Urophycis tenuis (Mitchill, 1814)	M									X	EI, ZI	Excluded
Lampriformes												
LAMPRIDAE												
(opah)												
Lampris guttatus (Brünnich, 1788)	M									X		Excluded
Lophiiformes												
LOPHIIDAE												
(goosefishes)												

Lophius budegassa (Spinola, 1870)	M			X	X			X	X	X		
Lophius piscatorius (Linnaeus, 1758)	M	X	X	X	X			X	X			
Ophidiiformes												
OPHIDIIDAE												
(cusk-eels)												
Brotula barbata (Bloch & Schneider, 1801)	M									X		Added
Genypterus blacodes (Forster, 1801)	M									X		Excluded
Genypterus capensis (Smith, 1847)	M									X		Excluded
Osmeriformes												
ARGENTINIDAE												
(argentines or herring smelts)												
Argentina silus (Ascanius, 1775)	M	X										Excluded
Argentina sphyraena (Linnaeus, 1758)	M	X			X							
Glossanodon leioglossus (Valenciennes, 1848)	M							X				Added
OSMERIDAE												
(smelts)												
Osmerus eperlanus (Linnaeus, 1758)	M,B,F	X										
Perciformes												
AMMODYTIDAE												
(sand lancers)												
Ammodytes marinus (Raitt, 1934)	M	X									EI, ZI	Excluded
Ammodytes tobianus (Linnaeus, 1758)	M,B	X	X								EI, ZI	Excluded
Hyperoplus lanceolatus (Le Sauvage, 1824)	M,B		X								EI, ZI	
ANARHICHADIDAE												
(wolffishes)												
Anarhichas lupus (Linnaeus, 1758)	M	X	X							X		
Anarhichas minor (Olafsen, 1772)	M									X		Added
BRAMIDAE												
(pomfrets)												
Brama brama (Bonnaterre, 1788)	M				X	X		X				
Taractichthys longipinnis (Lowe, 1843)	M						X					Added
CALLIONYMIDAE												
(dragonets)												
Callionymus lyra (Linnaeus, 1758)	M	X	X	X								
Callionymus maculatus (Rafinesque, 1810)	M		X									Added
Callionymus reticulatus (Valenciennes, 1837)	M		X									Added
CARANGIDAE												
(jacks and pompanos)												
Caranx crysos (Mitchill, 1815)	M						X					Added
Lichia amia (Linnaeus, 1758)	M						X	X				Added

Pseudocaranx dentex (Bloch & Schneider, 1801)	M					X	X				ZI	
Seriola carpenteri (Mather, 1971)	M						X					Added
Seriola dumerili (Risso, 1810)	M					X	X	X	X			
Seriola fasciata (Bloch, 1793)	M					X	X					Added
Seriola rivoliana (Cuvier, 1833)	M					X	X				ZI	
Trachurus mediterraneus (Steindachner, 1868)	M			X				X	X			
Trachurus picturatus (T.E. Bowdich, 1825)	M					X	X	X				
Trachurus trachurus (Linnaeus, 1758)	M	X	X	X	X			X	X			
CENTRACANTHIDAE												
(picarels)												
Centracanthus cirrus Rafinesque, 1810	M							X				Excluded
Spicara flexuosa (Rafinesque, 1810)	M							X	X			Added
Spicara maena (Linnaeus, 1758)	M							X	X			Added
Spicara smaris (Linnaeus, 1758)	M							X	X			Added
CENTROLOPHIDAE												
(medusafishes)												
Centrolophus niger (Gmelin, 1789)	M							X				Added
Schedophilus ovalis (Cuvier, 1833)	M						X					Added
Schedophilus velaini (Sauvage, 1879)	M									X		Added
CEPOLIDAE												
(bandfishes)												
Cepola macrophthalma (Linnaeus, 1758)	M							X				Added
CORYPHAENIDAE												
(dolphinfishes)												
Coryphaena equiselis (Linnaeus, 1758)	M					X						Added
Coryphaena hippurus (Linnaeus, 1758)	M					X		X				
EMMELICHTHYIDAE												
(rovers)												
Erythrocles monodi (Poll & Cadenat, 1954)	M									X		Added
GEMPYLIDAE												
(snake mackerels)												
Ruvettus pretiosus (Cocco, 1833)	M						X					Added
HAEMULIDAE												
(grunts)												
Plectorhinchus mediterraneus (Guichenot, 1850)	M									X		Added
Pomadasys incisus (Bowdich, 1825)	M					X		X				Added
Pomadasys perotaei (Cuvier, 1830)	M									X		Added
ISTIOPHORIDAE												
(billfishes)												
Makaira nigricans (Lacepède, 1801)	M						X					

	Tetrapturus albidus (Poey, 1860)	M					X	X					Excluded
LABRIDAE													
(wrasses)													
	Labrus bergylta (Ascanius, 1767)	M	X			X						TS	
	Labrus mixtus (Linnaeus, 1758)	M	X						X				Excluded
	Xyrichthys novacula (Linnaeus, 1758)	M					X						
MORONIDAE													
(temperate basses)													
	Dicentrarchus labrax (Linnaeus, 1758)	M		X	X	X				X			
	Dicentrarchus punctatus (Bloch, 1792)	M				X			X				Excluded
MUGILIDAE													
(mullets)													
	Chelon labrosus (Risso, 1827)	M				X							Added
	Liza aurata (Risso, 1810)	M			X	X			X				Added
	Liza ramado (Risso, 1810)	M			X								Added
	Mugil cephalus (Linnaeus, 1758)	M,B								X			
MULLIDAE													
(goatfishes)													
	Mullus barbatus (Linnaeus, 1758)	M				X			X	X			
	Mullus surmuletus (Linnaeus, 1758)	M		X	X	X	X		X	X			
	Pseudupeneus prayensis (Cuvier, 1829)	M									X		Added
POLYPRIONIDAE													
(wreckfishes)													
	Polyprion americanus (Schneider, 1801)	M					X	X					
POMACENTRIDAE													
(damselfishes)													
	Abudefduf luridus (Cuvier, 1830)	M					X						Added
	Chromis limbata (Valenciennes, 1833)	M					X	X					Added
POMATOMIDAE													
(bluefishes)													
	Pomatomus saltatrix (Linnaeus, 1766)	M							X	X			
PRIACANTHIDAE													
(bigeyes or catalufas)													
	Heteropriacanthus cruentatus (Lacepède, 1801)	M						X					Added
SCARIDAE													
(parrotfishes)													
	Scarus hoefleri (Steindachner, 1881)	M									X		Added
	Sparisoma cretense (Linnaeus, 1758)	M					X						Added
	Sparisoma rubripinne (Valenciennes, 1840)	M									X		Added
SCIAENIDAE													

(drums or croakers)												
	Argyrosomus regius (Asso, 1801)	M								X		
	Pseudotolithus elongatus (Bowdich, 1825)	M								X		Added
	Pseudotolithus senegalensis (Valenciennes, 1833)	M								X		Added
	Pseudotolithus senegallus (Cuvier, 1830)	M								X		Added
	Pseudotolithus typus (Bleeker, 1863)	M								X		Added
	Sciaena umbra (Linnaeus, 1758)	M						X				
	Umbrina canariensis (Valenciennes, 1843)	M					X					Added
SCOMBRIDAE												
(mackerels, tunas and bonitos)												
	Auxis rochei (Risso, 1810)	M					X	X				
	Auxis thazard (Lacepède, 1800)	M								X		Excluded
	Euthynnus affinis (Cantor, 1849)	M								X		Excluded
	Euthynnus alletteratus (Rafinesque, 1810)	M						X				
	Katsuwonus pelamis (Linnaeus, 1758)	M				X	X	X				
	Sarda sarda (Bloch, 1793)	M			X	X	X	X	X			
	Scomber japonicus (Houttuyn, 1782)	M			X	X	X		X	X		(Synonym: *Scomber colias*)
	Scomber scombrus (Linnaeus, 1758)	M	X	X	X	X			X	X		
	Thunnus alalunga (Bonnaterre, 1788)	M			X	X	X	X	X	X		
	Thunnus albacares (Bonnaterre, 1788)	M						X			X	
	Thunnus obesus (Lowe, 1839)	M			X		X	X			X	
	Thunnus maccoyii (Castelnau, 1872)	M									X	TS Excluded
	Thunnus thynnus (Linnaeus, 1758)	M			X	X	X		X	X		
	Thunnus tonggol (Bleeker, 1851)	M									X	Excluded
SERRANIDAE												
(groupers and fairy basslets)												
	Anthias anthias (Linnaeus, 1758)	M							X			Added
	Cephalopholis taeniops (Valenciennes, 1828)	M									X	Added
	Epinephelus caninus (Valenciennes, 1843)	M									X	Added
	Epinephelus costae (Steindachner, 1878)	M							X	X	X	Added
	Epinephelus marginatus (Lowe, 1834)	M						X	X	X	X	Added
	Epinephelus tauvina (Forsskål, 1775)	M									X	Added
	Mycteroperca rubra (Bloch, 1793)	M									X	Excluded
	Serranus atricauda (Günther, 1874)	M						X				Added
	Serranus cabrilla (Linnaeus, 1758)	M							X	X		Added
	Serranus hepatus (Linnaeus, 1758)	M							X	X		Added
	Serranus scriba (Linnaeus, 1758)	M							X			Added
SPARIDAE												
(porgies)												
	Boops boops (Linnaeus, 1758)	M			X	X	X		X	X		

Dentex canariensis (Steindachner, 1881)	M									X		Excluded
Dentex dentex (Linnaeus, 1758)	M						X	X	X			
Dentex gibbosus (Rafinesque, 1810)	M					X						Added
Dentex macrophthalmus (Bloch, 1791)	M						X			X		
Diplodus annularis (Linnaeus, 1758)	M						X	X	X			
Diplodus cervinus (Lowe, 1841)	M					X	X			X		
Diplodus puntazzo (Cetti, 1777)	M						X		X	X		Added
Diplodus sargus (Linnaeus, 1758)	M				X	X		X	X			
Diplodus vulgaris (E. Geoffroy Saint-Hilaire, 1817)	M				X	X		X	X			
Lithognathus mormyrus (Linnaeus, 1758)	M							X	X			Added
Oblada melanura (Linnaeus, 1758)	M							X	X	X		Added
Pagellus acarne (Risso, 1827)	M				X	X		X				Added
Pagellus bellottii (Steindachner, 1882)	M						X			X		Added
Pagellus bogaraveo (Brünnich, 1768)	M			X	X	X		X	X			
Pagellus erythrinus (Linnaeus, 1758)	M			X		X		X	X			
Pagrus auriga (Valenciennes, 1843)	M							X			ZI	Excluded
Pagrus pagrus (Linnaeus, 1758)	M					X		X	X			
Sarpa salpa (Linnaeus, 1758)	M					X		X	X			Added
Sparus aurata (Linnaeus, 1758)	M			X	X			X	X	X		
Spondyliosoma cantharus (Linnaeus, 1758)	M			X	X	X		X	X			
SPHYRAENIDAE												
(barracudas)												
Sphyraena sphyraena (Linnaeus, 1758)	M							X				Added
TRACHINIDAE												
(weeverfishes)												
Echiichthys vipera (Cuvier, 1829)	M		X									
Trachinus draco (Linnaeus, 1758)	M	X						X	X			
Trachinus radiatus (Cuvier, 1829)	M							X				Added
TRICHIURIDAE												
(cutlassfishes)												
Aphanopus carbo (Lowe, 1839)	M					X	X					
Lepidopus caudatus (Euphrasen, 1788)	M						X	X				
Trichiurus lepturus (Linnaeus, 1758)	M				X			X				Excluded
URANOSCOPIDAE												
(stargazers)												
Uranoscopus scaber (Linnaeus, 1758)	M							X	X			
XIPHIIDAE												
(swordfishes)												
Xiphias gladius (Linnaeus, 1758)	M			X			X	X	X			
ZOARCIDAE												

(eelpouts)												
	Zoarces viviparus (Linnaeus, 1758)	M,B	X	X								
Pleuronectiformes												
BOTHIDAE												
(lefteye flounders)												
	Arnoglossus laterna (Walbaum, 1792)	M		X	X							Added
	Bothus podas (Lowe, 1834)	M						X	X			EI Added
CITHARIDAE												
(citharids)												
	Citharus linguatula (Linnaeus, 1758)	M							X			Added
PLEURONECTIDAE												
(righteye flounders)												
	Glyptocephalus cynoglossus (Linnaeus, 1758)	M	X	X								
	Hippoglossoides elassodon (Jordan & Gilbert, 1880)	M								X		Excluded
	Hippoglossoides platessoides (Fabricius, 1780)	M	X	X							EI, ZI	
	Hippoglossus hippoglossus (Linnaeus, 1758)	M	X	X								
	Limanda ferruginea (Storer, 1839)	M								X		Excluded
	Limanda limanda (Linnaeus, 1758)	M	X	X	X							
	Microstomus kitt (Walbaum, 1792)	M	X	X	X							
	Platichthys flesus (Linnaeus, 1758)	M,B,F	X	X	X	X						
	Pleuronectes platessa (Linnaeus, 1758)	M	X	X	X						EI, ZI	
	Reinhardtius hippoglossoides (Walbaum, 1792)	M								X		Excluded
PSETTODIDAE												
(psettodids)												
	Psettodes bennettii (Steindachner, 1870)	M								X		Added
SCOPHTHALMIDAE												
(scophthalmids or turbots)												
	Lepidorhombus boscii (Risso, 1810)	M			X	X			X	X		
	Lepidorhombus whiffiagonis (Walbaum, 1792)	M			X	X				X		
	Phrynorhombus norvegicus (Günther, 1862)	M		X								Added
	Psetta maxima (Linnaeus, 1758)	M	X	X	X	X					EI, ZI,	(Synonym: *Scophthalmus maximus*)
	Scophthalmus rhombus (Linnaeus, 1758)	M	X	X	X	X			X	X		
	Zeugopterus punctatus (Bloch, 1787)	M	X									Excluded
SOLEIDAE												
(soles)												
	Buglossidium luteum (Risso, 1810)	M	X	X								
	Dicologlossa cuneata (Moreau, 1881)	M							X		X	
	Microchirus azevia (Risso, 1810)	M						X				
	Microchirus variegatus (Donovan, 1808)	M		X	X	X			X			
	Pegusa cadenati (Chabanaud, 1954)	M									X	Added

Pegusa lascaris (Risso,1810)	M			X			X	X	X			
Solea senegalensis (Kaup, 1858)	M				X			X				
Solea solea (Linnaeus, 1758)	M	X	X	X	X			X	X	X	EI, ZI	
Synaptura lusitanica (Brito Capello, 1868)	M							X		X		
Synapturichthys kleinii (Risso, 1827)	M						X	X	X			
Salmoniformes												
SALMONIDAE												
(salmonids)												
Oncorhynchus mykiss (Walbaum, 1792)	M,B,F									X		Excluded
Oncorhynchus gorbuscha (Walbaum, 1792)	M,B,F									X		Excluded
Oncorhynchus keta (Walbaum, 1792)	M,B,F									X		Excluded
Oncorhynchus kisutch (Walbaum, 1792)	M,B,F									X		Excluded
Oncorhynchus tschawytscha (Walbaum, 1792)	M,B,F									X		Excluded
Salmo salar (Linnaeus, 1758)	M,B,F	X										
Salmo trutta (Linnaeus, 1758)	M,B,F	X	X								TS	
Scorpaeniformes												
AGONIDAE												
(poachers)												
Agonus cataphractus (Linnaeus, 1758)	M		X									
COTTIDAE												
(sculpins)												
Myoxocephalus scorpius (Linnaeus, 1758)	M,B	X	X									
Taurulus bubalis (Euphrasen, 1786)	M,B	X										
Triglopsis quadricornis (Linnaeus, 1758)	M,B,F	X									TS	
CYCLOPTERIDAE												
(lumpfishes and snailfishes)												
Cyclopterus lumpus (Linnaeus, 1758)	M,B	X	X									
DACTYLOPTERIDAE												
(flying gurnards)												
Dactylopterus volitans (Linnaeus, 1758)	M							X				Added
LIPARIDAE												
(snailfishes)												
Liparis liparis (Linnaeus, 1766)	M		X									
Liparis montagui (Donovan, 1804)	M	X										Added
PERISTEDIIDAE												
(armored searobins or armored gurnards)												
Peristedion cataphractum (Linnaeus, 1758)	M							X				Added
SCORPAENIDAE												
(scorpionfishes or rockfishes)												
Scorpaena elongata (Cadenat, 1943)	M									X		Added

Order	Family	Genus / Species / Reference	Habitat	Targeted areas									Other interest	Remarks
				BS	NS	CB	CS	MA	CI	WM	EM	EE		
Anguilliformes														
	ANGUILLIDAE													
	(freshwater eels)													
		Anguilla anguilla (Linnaeus, 1758)	M,B,F	X		X	X							
	CONGRIDAE													
	(conger and garden eels)													
		Conger conger (Linnaeus, 1758)	M			X	X	X	X	X	X			
	MURAENIDAE													
	(moray eels)													
		Enchelycore anatina (Lowe, 1838)	M					X	X					Added
		Gymnothorax afer (Bloch, 1795)	M									X		Added
		Gymnothorax unicolor (Delaroche, 1809)	M					X						Added
		Gymnothorax polygonius (Poey, 1875)	M						X					Added
		Muraena augusti (Kaup, 1856)	M					X						Added
		Muraena helena (Linnaeus, 1758)	M					X		X				
		Muraena melanotis (Kaup, 1860)	M									X		Added
		Muraena robusta (Osório, 1911)	M									X		Added
Atheriniformes														
	ATHERINIDAE													
	(silversides)													
		Atherina boyeri (Risso, 1810)	M							X	X			
		Atherina hepsetus (Linnaeus, 1758)	M							X	X			Excluded
		Atherina presbyter (Cuvier, 1829)	M			X		X						
Aulopiformes														
	AULOPIDAE													
	(aulopus)													
		Aulopus filamentosus (Bloch, 1792)	M					X						Added
	CHLOROPHTHALMIDAE													
	(greeneyes)													
		Chlorophthalmus agassizi (Bonaparte, 1840)	M							X				Added
	SYNODONTIDAE													
		Synodus saurus (Linnaeus, 1758)	M					X						Added
Batrachoidiformes														
	BATRACHOIDIDAE													
	(toadfishes)													
		Halobatrachus didactylus (Bloch & Schneider, 1801)	M							X				Added

Beloniformes												
BELONIDAE												
(needlefishes)												
Belone belone (Linnaeus, 1761)	M	X	X	X					X			
Tylosurus acus (Lacepède, 1803)	M						X	X				Added
Beryciformes												
BERYCIDAE												
(alfonsinos)												
Beryx decadactylus (Cuvier, 1829)	M					X						
Beryx splendens (Lowe, 1834)	M				X							Added
TRACHICHTHYIDAE												
(slimeheads)												
Hoplostethus atlanticus (Collett, 1889)	M		X									Excluded
Clupeiformes												
CLUPEIDAE												
(herrings, shads, sardines and menhadens)												
Alosa alosa (Linnaeus, 1758)	M,B			X								
Alosa fallax (Lacepède, 1803)	M,B	X	X	X								
Clupea harengus (Linnaeus, 1758)	M,B	X	X	X								
Sardina pilchardus (Walbaum, 1792)	M		X	X	X	X	X	X	X		ZI	
Sardinella aurita (Valenciennes, 1847)	M						X	X	X			
Sardinella maderensis (Lowe, 1838)	M					X						
Sardinops sagax (Jenyns, 1842)	M									X		Excluded
Sprattus sprattus (Linnaeus, 1758)	M,B	X	X	X								
ENGRAULIDAE												
(anchovies)												
Engraulis anchoita (Hubbs & Marini, 1935)	M									X		Excluded
Engraulis encrasicolus (Linnaeus, 1758)	M		X	X	X		X	X	X		ZI	
Engraulis ringens (Jenyns, 1842)	M									X		Excluded
Gadiformes												
GADIDAE												
(cods and haddocks)												
Gadiculus argenteus (Guichenot, 1850)	M			X							EI	
Gadus macrocephalus (Telesius, 1810)	M									X		Excluded
Gadus morhua (Linnaeus, 1758)	M	X	X		X					X	EI, ZI	
Melanogrammus aeglephinus (Linnaeus, 1758)	M	X	X	X	X					X		
Merlangius merlangus (Linnaeus, 1758)	M	X	X	X								
Micromesistius poutassou (Risso, 1826)	M	X	X	X	X			X	X			
Pollachius pollachius (Linnaeus, 1758)	M		X	X	X							
Pollachius virens (Linnaeus, 1758)	M	X	X								EI, ZI	

Theragra chalcogramma (Pallas, 1811)	M									X	EI, ZI	Excluded
Trisopterus esmarkii (Nilsson, 1855)	M	X	X									
Trisopterus luscus (Linnaeus, 1758)	M		X	X	X							
Trisopterus minutus (Linnaeus, 1758)	M		X	X				X	X		EI, ZI	
LOTIDAE												
(hakes and burbots)												
Brosme brosme (Ascanius, 1772)	M	X									EI, ZI	
Ciliata septentrionalis (Collett, 1875)	M		X									Added
Enchelyopus cimbrius (Linnaeus, 1766)	M	X	X	X								
Gaidropsarus biscayensis (Collett, 1890)	M				X							Added
Gaidropsarus mediterraneus (Linnaeus, 1758)	M				X							Added
Molva dypterygia (Pennant, 1784)	M				X							
Molva macrophthalma (Rafinesque, 1810)	M							X				Excluded
Molva molva (Linnaeus, 1758)	M	X	X	X	X						EI, ZI	
MACROURIDAE												
(grenadiers or rattails)												
Coryphaenoides rupestris (Gunnerus, 1765)	M	X									EI	
MERLUCCIIDAE												
(merluccid hakes)												
Macruronus magellanicus (Lönnberg, 1907)	M									X		Excluded
Macruronus novaezelandiae (Hector, 1871)	M									X		Excluded
Merluccius australis (Hutton, 1872)	M									X		
Merluccius capensis Castelnau, 1861	M									X		
Merluccius gayi gayi (Guichenot, 1848)	M									X		Excluded
Merluccius hubbsi (Marini, 1933)	M									X		Excluded
Merluccius merluccius (Linnaeus, 1758)	M	X		X	X		X	X	X			
Merluccius polli (Cadenat, 1950)	M									X		Added
Merluccius senegalensis (Linnaeus, 1758)	M									X		Excluded
PHYCIDAE												
(phycid hakes)												
Phycis blennoides (Brünnich, 1768)	M			X	X	X		X	X			
Phycis phycis (Linnaeus, 1766)	M					X		X	X			
Urophycis tenuis (Mitchill, 1814)	M									X	EI, ZI	Excluded
Lampriformes												
LAMPRIDAE												
(opah)												
Lampris guttatus (Brünnich, 1788)	M									X		Excluded
Lophiiformes												
LOPHIIDAE												
(goosefishes)												

Lophius budegassa (Spinola, 1870)	M			X	X			X	X	X		
Lophius piscatorius (Linnaeus, 1758)	M	X	X	X	X			X	X			
Ophidiiformes												
OPHIDIIDAE												
(cusk-eels)												
Brotula barbata (Bloch & Schneider, 1801)	M									X		Added
Genypterus blacodes (Forster, 1801)	M									X		Excluded
Genypterus capensis (Smith, 1847)	M									X		Excluded
Osmeriformes												
ARGENTINIDAE												
(argentines or herring smelts)												
Argentina silus (Ascanius, 1775)	M	X										Excluded
Argentina sphyraena (Linnaeus, 1758)	M	X			X							
Glossanodon leioglossus (Valenciennes, 1848)	M							X				Added
OSMERIDAE												
(smelts)												
Osmerus eperlanus (Linnaeus, 1758)	M,B,F	X										
Perciformes												
AMMODYTIDAE												
(sand lancers)												
Ammodytes marinus (Raitt, 1934)	M	X									EI, ZI	Excluded
Ammodytes tobianus (Linnaeus, 1758)	M,B	X	X								EI, ZI	Excluded
Hyperoplus lanceolatus (Le Sauvage, 1824)	M,B		X								EI, ZI	
ANARHICHADIDAE												
(wolffishes)												
Anarhichas lupus (Linnaeus, 1758)	M	X	X							X		
Anarhichas minor (Olafsen, 1772)	M									X		Added
BRAMIDAE												
(pomfrets)												
Brama brama (Bonnaterre, 1788)	M				X	X		X				
Taractichthys longipinnis (Lowe, 1843)	M						X					Added
CALLIONYMIDAE												
(dragonets)												
Callionymus lyra (Linnaeus, 1758)	M	X	X	X								
Callionymus maculatus (Rafinesque, 1810)	M		X									Added
Callionymus reticulatus (Valenciennes, 1837)	M		X									Added
CARANGIDAE												
(jacks and pompanos)												
Caranx crysos (Mitchill, 1815)	M						X					Added
Lichia amia (Linnaeus, 1758)	M						X	X				Added

	Pseudocaranx dentex (Bloch & Schneider, 1801)	M					X	X				ZI	
	Seriola carpenteri (Mather, 1971)	M						X					Added
	Seriola dumerili (Risso, 1810)	M					X	X	X	X			
	Seriola fasciata (Bloch, 1793)	M					X	X					Added
	Seriola rivoliana (Cuvier, 1833)	M					X	X				ZI	
	Trachurus mediterraneus (Steindachner, 1868)	M			X				X	X			
	Trachurus picturatus (T.E. Bowdich, 1825)	M					X	X	X				
	Trachurus trachurus (Linnaeus, 1758)	M	X	X	X	X			X	X			
CENTRACANTHIDAE													
(picarels)													
	Centracanthus cirrus Rafinesque, 1810	M							X				Excluded
	Spicara flexuosa (Rafinesque, 1810)	M							X	X			Added
	Spicara maena (Linnaeus, 1758)	M							X	X			Added
	Spicara smaris (Linnaeus, 1758)	M							X	X			Added
CENTROLOPHIDAE													
(medusafishes)													
	Centrolophus niger (Gmelin, 1789)	M							X				Added
	Schedophilus ovalis (Cuvier, 1833)	M						X					Added
	Schedophilus velaini (Sauvage, 1879)	M									X		Added
CEPOLIDAE													
(bandfishes)													
	Cepola macrophthalma (Linnaeus, 1758)	M							X				Added
CORYPHAENIDAE													
(dolphinfishes)													
	Coryphaena equiselis (Linnaeus, 1758)	M					X						Added
	Coryphaena hippurus (Linnaeus, 1758)	M					X		X				
EMMELICHTHYIDAE													
(rovers)													
	Erythrocles monodi (Poll & Cadenat, 1954)	M									X		Added
GEMPYLIDAE													
(snake mackerels)													
	Ruvettus pretiosus (Cocco, 1833)	M						X					Added
HAEMULIDAE													
(grunts)													
	Plectorhinchus mediterraneus (Guichenot, 1850)	M									X		Added
	Pomadasys incisus (Bowdich, 1825)	M					X		X				Added
	Pomadasys perotaei (Cuvier, 1830)	M									X		Added
ISTIOPHORIDAE													
(billfishes)													
	Makaira nigricans (Lacepède, 1801)	M						X					

	Tetrapturus albidus (Poey, 1860)	M					X	X					Excluded
LABRIDAE													
(wrasses)													
	Labrus bergylta (Ascanius, 1767)	M	X			X						TS	
	Labrus mixtus (Linnaeus, 1758)	M	X						X				Excluded
	Xyrichthys novacula (Linnaeus, 1758)	M					X						
MORONIDAE													
(temperate basses)													
	Dicentrarchus labrax (Linnaeus, 1758)	M		X	X	X				X			
	Dicentrarchus punctatus (Bloch, 1792)	M				X			X				Excluded
MUGILIDAE													
(mullets)													
	Chelon labrosus (Risso, 1827)	M				X							Added
	Liza aurata (Risso, 1810)	M			X	X			X				Added
	Liza ramado (Risso, 1810)	M			X								Added
	Mugil cephalus (Linnaeus, 1758)	M,B								X			
MULLIDAE													
(goatfishes)													
	Mullus barbatus (Linnaeus, 1758)	M				X			X	X			
	Mullus surmuletus (Linnaeus, 1758)	M		X	X	X	X		X	X			
	Pseudupeneus prayensis (Cuvier, 1829)	M									X		Added
POLYPRIONIDAE													
(wreckfishes)													
	Polyprion americanus (Schneider, 1801)	M					X	X					
POMACENTRIDAE													
(damselfishes)													
	Abudefduf luridus (Cuvier, 1830)	M					X						Added
	Chromis limbata (Valenciennes, 1833)	M					X	X					Added
POMATOMIDAE													
(bluefishes)													
	Pomatomus saltatrix (Linnaeus, 1766)	M							X	X			
PRIACANTHIDAE													
(bigeyes or catalufas)													
	Heteropriacanthus cruentatus (Lacepède, 1801)	M						X					Added
SCARIDAE													
(parrotfishes)													
	Scarus hoefleri (Steindachner, 1881)	M									X		Added
	Sparisoma cretense (Linnaeus, 1758)	M					X						Added
	Sparisoma rubripinne (Valenciennes, 1840)	M									X		Added
SCIAENIDAE													

(drums or croakers)												
Argyrosomus regius (Asso, 1801)	M									X		
Pseudotolithus elongatus (Bowdich, 1825)	M									X		Added
Pseudotolithus senegalensis (Valenciennes, 1833)	M									X		Added
Pseudotolithus senegallus (Cuvier, 1830)	M									X		Added
Pseudotolithus typus (Bleeker, 1863)	M									X		Added
Sciaena umbra (Linnaeus, 1758)	M							X				
Umbrina canariensis (Valenciennes, 1843)	M						X					Added
SCOMBRIDAE												
(mackerels, tunas and bonitos)												
Auxis rochei (Risso, 1810)	M						X	X				
Auxis thazard (Lacepède, 1800)	M									X		Excluded
Euthynnus affinis (Cantor, 1849)	M									X		Excluded
Euthynnus alletteratus (Rafinesque, 1810)	M							X				
Katsuwonus pelamis (Linnaeus, 1758)	M				X	X	X					
Sarda sarda (Bloch, 1793)	M			X	X	X	X	X	X			
Scomber japonicus (Houttuyn, 1782)	M			X	X	X		X	X			(Synonym: *Scomber colias*)
Scomber scombrus (Linnaeus, 1758)	M	X	X	X	X			X	X			
Thunnus alalunga (Bonnaterre, 1788)	M			X	X	X	X	X	X			
Thunnus albacares (Bonnaterre, 1788)	M						X			X		
Thunnus obesus (Lowe, 1839)	M			X		X	X			X		
Thunnus maccoyii (Castelnau, 1872)	M									X	TS	Excluded
Thunnus thynnus (Linnaeus, 1758)	M			X	X	X		X	X			
Thunnus tonggol (Bleeker, 1851)	M									X		Excluded
SERRANIDAE												
(groupers and fairy basslets)												
Anthias anthias (Linnaeus, 1758)	M							X				Added
Cephalopholis taeniops (Valenciennes, 1828)	M									X		Added
Epinephelus caninus (Valenciennes, 1843)	M									X		Added
Epinephelus costae (Steindachner, 1878)	M							X	X	X		Added
Epinephelus marginatus (Lowe, 1834)	M					X		X	X	X		Added
Epinephelus tauvina (Forsskål, 1775)	M									X		Added
Mycteroperca rubra (Bloch, 1793)	M									X		Excluded
Serranus atricauda (Günther, 1874)	M					X						Added
Serranus cabrilla (Linnaeus, 1758)	M							X	X			Added
Serranus hepatus (Linnaeus, 1758)	M							X	X			Added
Serranus scriba (Linnaeus, 1758)	M							X				Added
SPARIDAE												
(porgies)												
Boops boops (Linnaeus, 1758)	M			X	X	X		X	X			

	Dentex canariensis (Steindachner, 1881)	M									X		Excluded
	Dentex dentex (Linnaeus, 1758)	M						X	X	X			
	Dentex gibbosus (Rafinesque, 1810)	M					X						Added
	Dentex macrophthalmus (Bloch, 1791)	M						X			X		
	Diplodus annularis (Linnaeus, 1758)	M						X	X	X			
	Diplodus cervinus (Lowe, 1841)	M					X	X			X		
	Diplodus puntazzo (Cetti, 1777)	M						X		X	X		Added
	Diplodus sargus (Linnaeus, 1758)	M				X	X		X	X			
	Diplodus vulgaris (E. Geoffroy Saint-Hilaire, 1817)	M				X	X		X	X			
	Lithognathus mormyrus (Linnaeus, 1758)	M							X	X			Added
	Oblada melanura (Linnaeus, 1758)	M							X	X	X		Added
	Pagellus acarne (Risso, 1827)	M				X	X		X				Added
	Pagellus bellottii (Steindachner, 1882)	M						X			X		Added
	Pagellus bogaraveo (Brünnich, 1768)	M			X	X	X		X	X			
	Pagellus erythrinus (Linnaeus, 1758)	M			X		X		X	X			
	Pagrus auriga (Valenciennes, 1843)	M							X			ZI	Excluded
	Pagrus pagrus (Linnaeus, 1758)	M					X		X	X			
	Sarpa salpa (Linnaeus, 1758)	M					X		X	X			Added
	Sparus aurata (Linnaeus, 1758)	M			X	X			X	X	X		
	Spondyliosoma cantharus (Linnaeus, 1758)	M			X	X	X		X	X			
SPHYRAENIDAE													
(barracudas)													
	Sphyraena sphyraena (Linnaeus, 1758)	M							X				Added
TRACHINIDAE													
(weeverfishes)													
	Echiichthys vipera (Cuvier, 1829)	M		X									
	Trachinus draco (Linnaeus, 1758)	M	X						X	X			
	Trachinus radiatus (Cuvier, 1829)	M							X				Added
TRICHIURIDAE													
(cutlassfishes)													
	Aphanopus carbo (Lowe, 1839)	M					X	X					
	Lepidopus caudatus (Euphrasen, 1788)	M						X	X				
	Trichiurus lepturus (Linnaeus, 1758)	M				X			X				Excluded
URANOSCOPIDAE													
(stargazers)													
	Uranoscopus scaber (Linnaeus, 1758)	M							X	X			
XIPHIIDAE													
(swordfishes)													
	Xiphias gladius (Linnaeus, 1758)	M			X			X	X	X			
ZOARCIDAE													

(eelpouts)												
	Zoarces viviparus (Linnaeus, 1758)	M,B	X	X								
Pleuronectiformes												
BOTHIDAE												
(lefteye flounders)												
	Arnoglossus laterna (Walbaum, 1792)	M		X	X							Added
	Bothus podas (Lowe, 1834)	M						X	X			EI Added
CITHARIDAE												
(citharids)												
	Citharus linguatula (Linnaeus, 1758)	M							X			Added
PLEURONECTIDAE												
(righteye flounders)												
	Glyptocephalus cynoglossus (Linnaeus, 1758)	M	X	X								
	Hippoglossoides elassodon (Jordan & Gilbert, 1880)	M									X	Excluded
	Hippoglossoides platessoides (Fabricius, 1780)	M	X	X							EI, ZI	
	Hippoglossus hippoglossus (Linnaeus, 1758)	M	X	X								
	Limanda ferruginea (Storer, 1839)	M									X	Excluded
	Limanda limanda (Linnaeus, 1758)	M	X	X	X							
	Microstomus kitt (Walbaum, 1792)	M	X	X	X							
	Platichthys flesus (Linnaeus, 1758)	M,B,F	X	X	X	X						
	Pleuronectes platessa (Linnaeus, 1758)	M	X	X	X						EI, ZI	
	Reinhardtius hippoglossoides (Walbaum, 1792)	M									X	Excluded
PSETTODIDAE												
(psettodids)												
	Psettodes bennettii (Steindachner, 1870)	M									X	Added
SCOPHTHALMIDAE												
(scophthalmids or turbots)												
	Lepidorhombus boscii (Risso, 1810)	M			X	X			X	X		
	Lepidorhombus whiffiagonis (Walbaum, 1792)	M			X	X				X		
	Phrynorhombus norvegicus (Günther, 1862)	M		X								Added
	Psetta maxima (Linnaeus, 1758)	M	X	X	X	X					EI, ZI,	(Synonym: *Scophthalmus maximus*)
	Scophthalmus rhombus (Linnaeus, 1758)	M	X	X	X	X			X	X		
	Zeugopterus punctatus (Bloch, 1787)	M	X									Excluded
SOLEIDAE												
(soles)												
	Buglossidium luteum (Risso, 1810)	M	X	X								
	Dicologlossa cuneata (Moreau, 1881)	M							X		X	
	Microchirus azevia (Risso, 1810)	M						X				
	Microchirus variegatus (Donovan, 1808)	M		X	X	X			X			
	Pegusa cadenati (Chabanaud, 1954)	M									X	Added

Pegusa lascaris (Risso,1810)	M			X			X	X	X			
Solea senegalensis (Kaup, 1858)	M				X			X				
Solea solea (Linnaeus, 1758)	M	X	X	X	X			X	X	X	EI, ZI	
Synaptura lusitanica (Brito Capello, 1868)	M							X		X		
Synapturichthys kleinii (Risso, 1827)	M						X	X	X			
Salmoniformes												
SALMONIDAE												
(salmonids)												
Oncorhynchus mykiss (Walbaum, 1792)	M,B,F									X		Excluded
Oncorhynchus gorbuscha (Walbaum, 1792)	M,B,F									X		Excluded
Oncorhynchus keta (Walbaum, 1792)	M,B,F									X		Excluded
Oncorhynchus kisutch (Walbaum, 1792)	M,B,F									X		Excluded
Oncorhynchus tschawytscha (Walbaum, 1792)	M,B,F									X		Excluded
Salmo salar (Linnaeus, 1758)	M,B,F	X										
Salmo trutta (Linnaeus, 1758)	M,B,F	X	X								TS	
Scorpaeniformes												
AGONIDAE												
(poachers)												
Agonus cataphractus (Linnaeus, 1758)	M		X									
COTTIDAE												
(sculpins)												
Myoxocephalus scorpius (Linnaeus, 1758)	M,B	X	X									
Taurulus bubalis (Euphrasen, 1786)	M,B	X										
Triglopsis quadricornis (Linnaeus, 1758)	M,B,F	X									TS	
CYCLOPTERIDAE												
(lumpfishes and snailfishes)												
Cyclopterus lumpus (Linnaeus, 1758)	M,B	X	X									
DACTYLOPTERIDAE												
(flying gurnards)												
Dactylopterus volitans (Linnaeus, 1758)	M							X				Added
LIPARIDAE												
(snailfishes)												
Liparis liparis (Linnaeus, 1766)	M		X									
Liparis montagui (Donovan, 1804)	M	X										Added
PERISTEDIIDAE												
(armored searobins or armored gurnards)												
Peristedion cataphractum (Linnaeus, 1758)	M							X				Added
SCORPAENIDAE												
(scorpionfishes or rockfishes)												
Scorpaena elongata (Cadenat, 1943)	M									X		Added

Scorpaena porcus (Linnaeus, 1758)	M				X		X	X	X			
Scorpaena scrofa (Linnaeus, 1758)	M					X	X	X	X	X		
SEBASTIDAE												
(rockfishes, rockcods and thornyheads)												
Helicolenus dactylopterus (Delaroche,1809)	M	X		X	X	X		X	X			
Sebastes marinus (Linnaeus, 1758)	M	X			X					X		Excluded
Sebastes mentella (Travin, 1951)	M									X		Added
Sebastes viviparus (Krøyer, 1845)	M	X										Added
SETARCHIDAE												
Setarches guentheri (Johnson, 1862)	M						X					Added
TRIGLIDAE												
(searobins)												
Aspitrigla cuculus (Linnaeus, 1758)	M		X	X	X			X				Added (Synonym: *Chelidonichthys cuculus*)
Chelidonichthys lastoviza (Bonnaterre, 1788)	M				X			X				Added (Synonym: *C. capensis*)
Chelidonichthys lucernus (Linnaeus, 1758)	M	X	X	X	X	X		X				Added (Synonym: *C. lucerna*)
Chelidonichthys obscurus (Bloch & Schneider, 1801)	M				X			X				Added
Eutrigla gurnardus (Linnaeus, 1758)	M	X	X	X	X							Added (Synonym: *Chelidonichthys gurnardus*)
Lepidotrigla cavillone (Lacepède, 1801)	M							X				Added
Trigla lyra (Linnaeus, 1758)	M							X	X			
Tetraodontiformes												
BALISTIDAE												
(triggerfishes)												
Balistes capriscus (Gmelin, 1789)	M						X					Added
MOLIDAE												
(molas or ocean sunfishes)												
Ranzania laevis (Pennant, 1776)	M						X					Added
MONACANTHIDAE												
(filefishes)												
Aluterus heudelotii (Hollard, 1855)	M									X		Excluded
Aluterus scriptus (Osbeck, 1765)	M						X					Added
TETRAODONTIDAE												
Sphoeroides pachygaster (Müller & Troschel, 1848)	M						X					Added
Zeiformes												
ZEIDAE												
(dories)												
Zeus faber (Linnaeus, 1758)	M			X	X		X	X	X			

Scorpaena porcus (Linnaeus, 1758)	M				X		X	X	X			
Scorpaena scrofa (Linnaeus, 1758)	M					X	X	X	X	X		
SEBASTIDAE												
(rockfishes, rockcods and thornyheads)												
Helicolenus dactylopterus (Delaroche,1809)	M	X		X	X	X		X	X			
Sebastes marinus (Linnaeus, 1758)	M	X			X					X		Excluded
Sebastes mentella (Travin, 1951)	M									X		Added
Sebastes viviparus (Krøyer, 1845)	M	X										Added
SETARCHIDAE												
Setarches guentheri (Johnson, 1862)	M						X					Added
TRIGLIDAE												
(searobins)												
Aspitrigla cuculus (Linnaeus, 1758)	M		X	X	X			X				Added (Synonym: *Chelidonichthys cuculus*)
Chelidonichthys lastoviza (Bonnaterre, 1788)	M				X			X				Added (Synonym: *C. capensis*)
Chelidonichthys lucernus (Linnaeus, 1758)	M	X	X	X	X	X		X				Added (Synonym: *C. lucerna*)
Chelidonichthys obscurus (Bloch & Schneider, 1801)	M				X			X				Added
Eutrigla gurnardus (Linnaeus, 1758)	M	X	X	X	X							Added (Synonym: *Chelidonichthys gurnardus*)
Lepidotrigla cavillone (Lacepède, 1801)	M							X				Added
Trigla lyra (Linnaeus, 1758)	M							X	X			
Tetraodontiformes												
BALISTIDAE												
(triggerfishes)												
Balistes capriscus (Gmelin, 1789)	M						X					Added
MOLIDAE												
(molas or ocean sunfishes)												
Ranzania laevis (Pennant, 1776)	M						X					Added
MONACANTHIDAE												
(filefishes)												
Aluterus heudelotii (Hollard, 1855)	M									X		Excluded
Aluterus scriptus (Osbeck, 1765)	M						X					Added
TETRAODONTIDAE												
Sphoeroides pachygaster (Müller & Troschel, 1848)	M						X					Added
Zeiformes												
ZEIDAE												
(dories)												
Zeus faber (Linnaeus, 1758)	M			X	X		X	X	X			

Table 3.2.- Species chosen for biogeographical genetic variation analysis by cytb sequencing. Geographical area of origin are given as follows: BS: Skagerrak and Baltic Sea; NS: North Sea; CB: English Channel and Bay of Biscay; CS: Cantabric Sea and NW Iberian Peninsula (Galicia and Portugal); MA: Madeira archipelago; CI: Canary Islands; WM: Western Mediterranean and Bay of Cadiz; EM: Eastern Mediterranean (Greek Seas) and EE: extra-European marine teleost species.

Species name	Targeted areas
Merluccius merluccius	BS, NS, CB, CS, CI, WM, EM
Micromesistius poutassou	NS, CB, CS, WM, EM
Mullus surmuletus	NS, MA, CI, WM, EM
Pagellus erythrinus	CI, WM, EM
Pagrus pagrus	MA, CI, WM, EM
Solea solea	BS, NS, CB, WM, EM

Table 3.3.- Treatment distribution to each specimen sampled and collected from each species and geographical area.

Specimen	01	02	03	04	05
Photograph	+	+	+	+	+
Tissue Analysis	+	+	-	+	+
Tissue Backup	+	+	+	+	+
Tissue Voucher	+	+	-	-	-
Otolith	-	+	+	-	-
Otolith photo	-	+	+	-	-
Voucher	+	+	+	+ (NRM)	+ (MNHN)

Table 3.4.- Standardised optimal conditions for PCR master mixtures used in the amplification of FishTrace targeted genes: cytochrome *b* and rhodopsin.

Components	Volume per reaction (µl)		Final Concentration
	Cyt*b*[(C)]	Rhod[(R)]	
10X Reaction Buffer	2.5	2.5	1X
dNTP mix (10mM of each dNTP)	1	1	0.4mM
Taq DNA polymerase (5U/μl)	0.125	0.125	1.25U/reaction
25mM $MgCl_2$	2.5	2.5	2.5mM
Forward primer	0.25	0.5	0.5[(R)] - 0.25[(C)] ng/µl
Reverse primer	0.25	0.5	0.5[(R)] - 0.25[(C)] ng/µl
Water MQ (to a final volume of 25μl)	-	-	-

Table 3.5.- Flow diagram of PCR protocols for the amplification of targeted fragments of the cyt*b* gene in teleost fishes. Nested PCRs are composed of two reactions. The first reaction uses a pair of outer primers (named A to D), which in cyt*b* flank the whole gene. The second reaction has specific inner primers (numbered) for the gene fragment. Protocol efficiency is indicated by a letter followed by a number. Thus, the corresponding protocol A1 for either gene is the first choice and protocol B1 is the third choice.

Cyt*b*: consecutive options 1st reaction[1]			
A	**B**	**C**	**D**
FishcytB-F + TruccytB-R	GluFish-F + TruccytB-R	FishcytB-F + THR-Fish-R	GluFish-F + THR-Fish-R

Cyt*b*-5′: consecutive opt. 2nd reaction[2]	
1	**2**
FishcytB-F + CytBI-5R	FishcytB-F + CytBI-4R

Cyt*b*-3′: consecutive options 2nd reaction [2]			
1	**2**	**3**	**4**
Cytbl-7F + THR-Fish-R	Cytbl-7F + TruccytB-R	Cytbl-6F + THR-Fish-R	Cytbl-6F + TruccytB-R

PCR programmes given in "temperature in ºC – seconds" as follows:

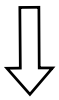
Initial Denaturation / (Denaturation / Annealing / Extension) x Number of Cycles / Final Extension

[1] 95-420 / (94-30 / 55-35 / 72-120) x35 / 72-420

[2] 95-420 / (94-30 / 55-35 / 72-45) x38 / 72-420

Table 3.6.- Flow diagram of PCR protocols for the amplification of targeted fragments of the rhod gene in teleost fishes. Nested PCRs are composed of two reactions. The first reaction uses a pair of outer primers (named A to D). The second reaction has specific inner primers (numbered) for the gene fragment. Protocol efficiency is indicated by a letter followed by a number. Thus, the corresponding protocol A1 for either gene is the first choice and protocol B1 is the third choice.

Rhod: consecutive options 1st reaction(1')			
A	**B**	**C**	**D**
Rod-F2B + Rod-5R	Rod-F2B + Rod-5R	RHO-30F + RHO-319R	Rod-F2B + Rod-5R



Rhod: consecutive opt. 2nd reaction(2')		
1	**2**	**3**
Rod-F2W + Rod-R4n	Rod-F2X + Rod-R4n	Rod-F2W + Rod-R4n

PCR programmes given in "temperature in ºC – seconds" as follows:

Initial Denaturation / (Denaturation / Annealing / Extension) x Number of Cycles / Final Extension

(1') 95-420 / (94-30 / 62-30 / 72-30) x40 / 72-420

(2') 95-420 / (94-30 / 56-30 / 72-30) x40 / 72-420.

Table 3.7.- Fish-versatile primers. (A) Primer pairs for the amplification of mitochondrial cytochrome *b* (1141 bp). (B) Primers for cyt*b* sequencing purposes Fish-seq and 7F-seq were respectively used for sequencing FishcytB-F and CytBI-7F amplifications products.

A)

(*a*)	Name (*b*)	Sequence (5'-3') (*b*)	Location (*c*)	Size (bp)	%GC	*Tm* (ºC) (*d*)
1	GluFish-F	AACCACCGTTGTTATTCAACTACAA	15329	25	36.0	57.7
2	FishcytB-F	ACCACCGTTGTTATTCAACTACAAGAAC	15330	28	39.3	60.7
3	CytBI-6F	TTCTCAGTAGACAACGCCACCCT	15862	23	52.2	61.0
4	CytBI-7F	CTAACCCGATTCTTTGCCTTCCACTTCCT	15883	29	48.3	68.3
5	CytBI-1F	CGATTCTTCGCATTCCACTTCCT	15889	23	47.8	62.5
6	*CytBI-5R*	*GGTCTTTGTAGGAGAAGTATGGGTGGAA*	16018	28	46.4	63.5
7	*CytBI-3R*	*GGGGTAAAGTTGTCTGGGTCTCC*	16111	23	56.5	60.9
8	*CytBI-2R*	*GCGGGGGTAAAGTTGTCTGGGTC*	16114	23	60.9	65.5
9	*CytBI-4R*	*AGGAAGTATCATTCGGGCTTAATATG*	16159	26	38.5	58.9
10	*TruccytB-R*	*CCGACTTCCGGATTACAAGACCG*	16528	23	56.5	64.6
11	*THR-Fish2-R*	*AACCTCCGACATCCGGCTTACAAGACCG*	16528	28	57.1	72.1
12	*THR-Fish-R*	*ACCTCCGATCTTCGGATTACAAGACC*	16529	26	50.0	64.4

B)

(*a*)	Name	Sequence (5'-3')	Location (*c*)	Size (bp)	%GC	*Tm* (ºC) (*d*)
13	Fish-seq	CCACCGTTGTTATTCAACTACAAG	15331	24	41.7	56.6
14	7F-seq	CTAACCCGATTCTTTGCCTTC	15883	21	47.6	56.7

(*a*): Numbers correspond to positions in Figure 3.3.

(*b*): Reverse primers in italics.

(*c*): Nucleotide location corresponding to the 5' position in the *Oncorhynchus mykiss* mitochondrial genome (GenBank accession number: NC_001717). Locations of reverse primers have been given based on the reverse-complementary primer sequence position.

(*d*): *Tm* calculated using PrimerExpress™ 2.0 (Applied Biosystems).

Table 3.8.- Fish species-specific cyt*b* primers.

Species-specific cyt*b* primers	Sequence (5'-3')	Species amplified	Location[(a)]	Size	%GC	Tm[(b)]
15 CytBI-7F-mer	TATTCCCCTTTGTCGTAGCTGCC	Family Merlucciidae	15911	23	52.2	62.3
16 BWF730	TTCTTGGACTAACTTCCCTCGC	Class Actinopterygii	16067	22	50.0	58.5
17 FW1020	TCATTATCGGTCAAGTGGCATC	Class Actinopterygii	16424	22	45.5	58.5
18 Melaeg830	AATTGCTTATGCTATCCTCCG	*Melanogrammus aeglephinus*	16185	21	42.9	55.0
19 MS-CYT3F	GCCGCAATGACAGTGATTC	*Mullus surmuletus*	16473	19	52.6	56.2
20 *MS-CYT3R*	*TACAAGACCGGCGCTCTGG*	*Mullus surmuletus*	16519	19	63.2	60.9
21 MulSur	CTGACCCGCTTCTTTGCATTCCACTTCCTATTCCCC	*Mullus surmuletus*	15883	36	52.8	77.1
22 *Pleuronectif*	*TCTGGACGCTGAGCTACTAGTGCA*	Order Pleuronectiformes	16501	24	54.2	62.3
23 *R75*	*AGGGAACCAAAGTTTCATCATACTGAAAT*	*Scomber scombrus*	15439	29	34.5	62.2
24 *RIVO-1000*	*ATCCGAAGTTTCATCAGACCGA*	*Anarhichas lupus*	15442	22	45.5	59.5
25 *RIVO86*	*AGGCCTAGAAGAGAGCCAAAATTTCA*	*Ciliata septentrionalis*	15451	26	42.3	62.6
26 RIVO-Sol_620	GAAACAGGCTCAAATAACCCCAC	*Solea solea*	15964	23	47.8	59.7
27 ScoSco820	CACCCCTCCCCACATCAAGC	*Scomber scombrus*	16239	20	65.0	63.4
28 Sol-CYTBF1	ACAATGACTAGTCTACGAAAATCCC	*Solea solea*	15358	25	40.0	55.9
29 Sol-CYTBF2	TCTCCCATTTATCTTAGCGGC	*Solea solea*	16012	21	47.6	57.1
30 *Sol-CYTBR1*	*GGCGCTCTAACACTGAGCTAC*	*Solea solea*	16508	21	57.1	55.7
31 TriEsm820	TGTTTGCCTACGCTATTTTACG	*Trisopterus esmarkii*	16184	22	40.9	56.1
32 TriLus800	ATTTGCCTATGCCATCTTACG	*Trisopterus luscus*	16185	21	42.9	55.7

(*a*): Nucleotide location corresponding to the 5' position in the *Oncorhynchus mykiss* mitochondrial genome (GenBank accession number: NC_001717). Locations of reverse primers have been given based on the reverse-complementary primer sequence position.

(*b*): *Tm* calculated using PrimerExpress™ 2.0 (Applied Biosystems).

Table 3.9.- Primers pairs for the amplification of the targeted fragment from the rhodopsin nuclear gene (460 bp).

(*a*)	Name (*b*)	Sequence (5'-3') (*b*)	Location (*c*)	Size (bp)	%GC	*Tm* (ºC) (*d*)
1	RHO-30F:	CCNTAYGAYTAYCCNCARTAYTA	67	23	41.3	53.5
2	Rod-F2B:	GTCTGCAAGCCCATCAGCAACTTCCG	415	26	57.7	71.0
3	Rod-F2w:	AGCAACTTCCGCTTCGGTGAGAA	430	23	52.2	65.1
4	Rod-F2x:	AGCAACTTCCGCTTCGGCGAGAA	430	23	56.5	68.8
5	Rod-F2:	AGCAACTTCCGCTTCGGAGAGAA	430	23	52.2	64.4
6	*Rod-R4n:*	*GGAACTGCTTGTTCATGCAGATGTAGAT*	913	28	42.9	63.6
7	*Rod-4R:*	*CTGCTTGTTCATGCAGATGTAGAT*	913	24	41.7	57.2
8	*Rod-5R:*	*GGTGGTGATCATGCAGTGGCGGAA*	937	24	58.3	70.7
9	*RHO-319R:*	*TTNCCRCARCAYAANGTNGT*	955	20	45.0	66.6

(*a*): Numbers correspond to positions in Figure 3.4.

(*b*): Reverse primers in italics.

(*c*): Nucleotide location corresponding to the 5' position in the *Astyanax mexicanus* rhodopsin genomic gene (GenBank accession number: U12328). Locations of reverse primers have been given based on the reverse-complementary primer sequence position.

(*d*): *Tm* calculated using PrimerExpress™ 2.0 (Applied Biosystems).

Table 3.10.- PCR conditions detailing direct and nested amplifications, and alternative strategies for fish DNA barcoding. (A) Cytochrome *b*. (B) Rhodopsin 460bp-length fragment.

(A)

No.	PCR[1]	Forward + Reverse	PCR cycles[2]	Remarks[3]
1	D cp cyt*b*	FishcytB-F + THR-Fish-R	95-420 / (94-30/50-30/72-30)x40 / 72-420	
2	D cp cyt*b*	FishcytB-F + THR-Fish-R	95-300 / (95-30/50-30/72/45)x40 / 72-420	
3	D cp cyt*b*	FishcytB-F + THR-Fish2-R	95-420 / (94-30/50-35/72-90)x40 / 72-420	***(a)***
4	D cp cyt*b*	FishcytB-F + TruccytB-R	95-420 / (94-30/50-35/72-90)x40 / 72-420	***(a)***
5	D cp cyt*b*	GluFish-F + TruccytB-R	95-420 / (94-30/50-35/72-90)x40 / 72-420	***(a)***
6	D cyt*b*-5'	FishcytB-F + CytBI-5R	95-420 / (94-30/50-35/72-60)x40 / 72-420	***(b)***
7	D cyt*b*-5'	FishcytB-F + CytBI-5R	95-300 / (95-30/50-30/72-45)x40 / 72-420	
8	D cyt*b*-5'	FishcytB-F + CytBI-5R	98-40 / (98-15/60-30/72-30)x45 / 72-420	***(c)***
9	D cyt*b*-5'	FishcytB-F + CytBI-4R	95-420 / (94-30/50-35/72-60)x40 / 72-420	***(d)***
10	D cyt*b*-5'	FishcytB-F + CytBI-3R	95-420 / (94-30/50-35/72-60)x40 / 72-420	***(d)***
11	D cyt*b*-5'	FishcytB-F + TruccytB-R	95-420 / (94-30/50-35/72-90)x40 / 72-420	***(e)***
12	D cyt*b*-3'	CytbI-7F + THR-Fish-R	95-300 / (95-30/52-30/72-30)x40 / 72-420	
13	D cyt*b*-3'	CytbI-7F + THR-Fish2-R	95-420 / (94-30/50-35/72-60)x40 / 72-420	***(d)***
14	D cyt*b*-3'	CytbI-7F + THR-Fish2-R	98-40 / (98-15/60-30/72-30)x45 / 72-420	***(c)***
15	D cyt*b*-3'	CytbI-7F + TruccytB-R	95-420 / (94-30/50-35/72-60)x40 / 72-420	***(d) (f)***
16	D cyt*b*-3'	CytbI-7F + TruccytB-R	95-300 / (95-30/50-30/72-45)x40 / 72-420	
17	D cyt*b*-3'	CytbI-6F + THR-Fish-R	95-360 / (95-55/52-55/72-55)x40 / 72-600	
18	D cyt*b*-3'	CytbI-6F + THR-Fish2-R	95-420 / (94-30/50-35/72-60)x40 / 72-420	***(d)***
19	D cyt*b*-3'	CytbI-6F + TruccytB-R	95-300 / (95-30/52-30/72-45)x40 / 72-400	
20	D cyt*b*-3'	FishcytB-F + TruccytB-R	95-420 / (94-30/50-35/72-90)x40 / 72-420	***(g)***
21	N 1st	FishcytB-F + TruccytB-R	95-420 / (94-30/55-35/72-120)x35 / 72-420	
	N 2nd cyt*b*-5'	FishcytB-F + CytBI-5R	95-420 / (94-30/55-35/72-45)x38 / 72-420	***(h)***
	N 2nd cyt*b*-5'	FishcytB-F + CytBI-5R	95-420 / (94-30/50-35/72-60)x38 / 72-420	
	N 2nd cyt*b*-5'	FishcytB-F + CytBI-4R	95-420 / (94-30/55-35/72-45)x38 / 72-420	***(h)***
	N 2nd cyt*b*-3'	CytbI-7F + THR-Fish-R	95-420 / (94-30/55-35/72-45)x38 / 72-420	***(i)***
	N 2nd cyt*b*-3'	CytbI-7F + TruccytB-R	95-420 / (94-30/55-35/72-45)x38 / 72-420	***(i)***
	N 2nd cyt*b*-3'	CytbI-6F + THR-Fish-R	95-420 / (94-30/55-35/72-45)x38 / 72-420	***(i)***
	N 2nd cyt*b*-3'	CytbI-6F + TruccytB-R	95-420 / (94-30/55-35/72-45)x38 / 72-420	***(i)***
22	N 1st	FishcytB-F + THR-Fish-R	95-420 / (94-30/50-35/72-90)x40 / 72-420	
	N 2nd cyt*b*-5'	FishcytB-F + CytBI-2R	95-420 / (94-30/50-35/72-60)x40 / 72-420	***(d)***
	N 2nd cyt*b*-3'	CytbI-7F + THR-Fish2-R	95-420 / (94-30/50-35/72-60)x40 / 72-420	***(d)***
	N 2nd cyt*b*-3'	CytbI-7F + TruccytB-R	95-420 / (94-30/50-35/72-60)x40 / 72-420	***(j)***
	N 2nd cyt*b*-3'	CytbI-7F + THR-Fish-R	95-420 / (94-30/50-35/72-60)x40 / 72-420	***(d)***
	N 2nd cyt*b*-3'	CytbI-6F + THR-Fish-R	95-420 / (94-30/50-35/72-60)x40 / 72-420	***(k)***
23	N 1st	FishcytB-F + THR-Fish-R	94-240 / (94-30/55-35/72-120)x35 / 72-420	
	N 2nd cyt*b*-5'	FishcytB-F + CytBI-5R	94-240 / (94-30/55-35/72-45)x38 / 72-420	
	N 2nd cyt*b*-3'	CytbI-7F + THR-Fish-R	94-240 / (94-30/55-35/72-45)x38 / 72-420	
	N 2nd cyt*b*-3'	CytbI-7F + TruccytB-R	94-240 / (94-30/55-35/72-45)x38 / 72-420	
	N 2nd cyt*b*-3'	CytbI-6F + TruccytB-R	94-240 / (94-30/55-35/72-45)x38 / 72-420	
22	N 1st	FishcytB-F + TruccytB-R	95-240 / (94-30/55-30/72-30)x40 / 72-420	
	N 2nd cyt*b*-5'	FishcytB-F + CytBI-3R	95-240 / (94-30/55-30/72-30)x35 / 72-420	
	N 2nd cyt*b*-3'	CytbI-7F + TruccytB-R	95-240 / (94-30/55-30/72-30)x35 / 72-420	
26	N 1st	GluFish-F + TruccytB-R	95-420 / (94-30/55-35/72-120)x35 / 72-420	
	N 2nd cyt*b*-5'	FishcytB-F + CytBI-5R	95-420 / (94-30/55-35/72-45)x38 / 72-420	***(h)***
27	N 1st	FishcytB-F + TruccytB-R	95-420 / (94-30/50-35/72-90)x40 / 72-420	
	N 2nd cyt*b*-3'	CytbI-7F + TruccytB-R	95-420 / (94-30/50-35/72-60)x40 / 72-420	
24	N 1st	FishcytB-F + THR-Fish2-R	95-420 / (94-30/50-35/72-90)x40 / 72-420	
	N 2nd cyt*b*-3'	CytbI-7F + THR-Fish2-R	95-420 / (94-30/50-35/72-60)x40 / 72-420	***(l)***

(B)

No.	PCR[1]	Forward + Reverse	PCR cycles[2]	Remarks[3]
25	D	Rod-F2W + Rod-R4n	95-240 / (94-30/60-30/72-45)x40 / 72-490	
26	D	Rod-F2W + Rod-R4n	96-60 / (96-30/50-30/60-240)x25 / 4-∞	
27	N 1st	Rod-F2B + Rod-5R	95-420 / (94-30/62-30/72-30)x40 / 72-420	
	N 2nd	Rod-F2W + Rod-R4n	95-420 / (94-30/56-30/72-30)x40 / 72-420	
	N 2nd	Rod-F2W + Rod-R4n	95-420 / (94-30/54-30/72-30)x40 / 72-420	
	N 2nd	Rod-F2X + Rod-R4n	95-420 / (94-30/56-30/72-30)x40 / 72-420	
28	N 1st	Rod-F2B + Rod-5R	95-420 / (94-30/60-30/72-30)x40 / 72-420	
	N 2nd	Rod-F2W + Rod-R4n	95-420 / (94-30/56-30/72-30)x40 / 72-420	
	N 2nd	Rod-F2X + Rod-R4n	95-420 / (94-30/56-30/72-30)x40 / 72-420	
29	N 1st	RHO-30F + RHO-319R	95-420 / (94-30/62-30/72-30)x40 / 72-420	
	N 2nd	Rod-F2W + Rod-R4n	95-420 / (94-30/56-30/72-30)x40 / 72-420	
30	N 1st	Rod-F2B + Rod-5R	94-240 / (94-30/60-35/72-120)x35 / 72-420	
	N 2nd	Rod-F2 + Rod-4R	94-240 / (94-30/60-35/72-40)x35 / 72-420	
31	N 1st	Rod-F2B + Rod-5R	95-600 / (95-55/54-55/72-55)x40 / 72-600	
	N 2nd	Rod-F2 + Rod-4R	95-600 / (95-55/72-55/72-55)x35 / 72-600	
32	N 1st	Rod-F2B + Rod-5R	95-240 / (94-30/55-30/72-30)x40 / 72-420	
	N 2nd	Rod-F2 + Rod-4R	95-240 / (94-30/55-30/72-30)x40 / 72-420	
33	N 1st	Rod-F2B + Rod-5R	95-600 / (94-55/56-55/72-55)x40 / 72-600	***(m)***
	N 2nd	Rod-F2 + Rod-4R	95-600 / (94-55/56-55/72-55)x40 / 72-600	***(m)***

Notes to Table 3.10:

(1) D, direct amplification; N, nested-PCR; 1st, first reaction of the nested amplification using outer primers; 2nd, second reaction of the nested amplification using inner primers; cp cyt*b*, amplification of the complete length of cyt*b* gene (1141 bp); cyt*b*-5', amplification of the targeted 5' fragment from the cyt*b* gene (~750 bp); cyt*b*-3', amplification of the targeted 3' fragment from the cyt*b* gene (~700 bp).

(2) PCR cycles given in "temperature in ºC – seconds" as follows:

Initial Denaturation / (Denaturation / Annealing / Extension) x Number of Cycles / Final Extension

(3) Remarks:

(*a*) Elongation step could be extended to 120 seconds.

(*b*) FishCytB-F or GluFish-F could be used as forward primer for sequencing.

(*c*) Polymerase used: Phusion High-Fidelity DNA polymerase (Finnzymes).

(*d*) Elongation step could be extended to 90 seconds.

(*e*) FishCytB-F and CytBI-5R are used for sequencing.

(*f*) THR-Fish-R and CytBI-1F could be used for sequencing.

(*g*) CytB-7F and TruccytB-R could be used for sequencing.

(*h*) Fish-seq is used for sequencing (instead of FishcytB-F).

(*i*) 7F-seq is used for sequencing (instead of CytB-7F).

(*j*) CytBI-1F and THR-Fish-R could be used for sequencing.

(*k*) Truccytb-R and THR-Fish2-R could be used for sequencing.

(*l*) GluFish-F or FishcytB-F and CytBI-2R or CytBI-3R could be used for sequencing.

(*m*) Hotstar Qiagen kit (Q-solutions).

Table 3.11.- Flow of procedures for the validation of the genetic data obtained.

1st Level: Internal validation by **each partner:**	**2nd Level:** Validation by **responsible group:**	**3rd Level:** Definitive validation by the **ICCM*:**
1) To compare both sequences obtained from individuals 01 and 02 (Sequence alignment)	1) To align and compare all data submitted by each partner.	1) Specific data checking (errors, missing data,....)
2) To annotate every change on the sequence.	2) To annotate every change on the sequences and methods employed.	2) Specific data compilation.
3) Verifying the position of each sequence on fish phylogeny.	3) Phylogenetics (BLAST, Trees,...)	3) Specific database field validation.
	4) Submit info to the WP7 responsible.	4) Data collating and arranging.

* Responsible partner for the Workpackage 7: *Data Validation in Database* (WP7).

Table 3.12.- FishTrace database main system characteristics.

Database:	Oracle 8i
Web server:	Jakarta Tomcat 5.0.25
Server OS:	Windows 2000
Technologies:	Java servlets, JSP (Java server pages, XML

Table 4.1.- Extra specimens collected. Data from these specimens will be loaded in FishTrace database in a near future.

Species	Specimen code
Bodianus scrofa	BodScr-CI-01 and 02
Dentex gibbosus	DenGib-CI-01 and 02
Diplodus sargus	DipSar-CI-01 and 02
Diplodus vulgaris	DipVul-CI-01 and 02
Helicolenus dactylopterus	HelDac-CI-01 and 02
Lithognathus mormyrus	LitMor-CI-01 and 02
Mycteroperca fusca	MycFus-CI-01 and 02
Pagellus acarne	PagAca-CI-01 and 02
Pagellus bogaraveo	PagBog-CI-01 and 02
Pagellus erythrinus	PagEry-CI-01 and 02
Pomadasys incisus	PomInc-CI-01 and 02
Serranus atricauda	SerAtr-CI-01 and 02
Stephanolepis hispidus	SteHis-CI-01 and 02

Table 4.2.- Sampling at each geographical area, and contribution by participating institutions.

Area	Species	Entire Fish Vouchers	Photos	Tissue	Otoliths	Otolith images	Partner
BS	48	220	304	400	14	1	**NRM**
CB	55*	273	394	NAD	NAD	NAD	**IFREMER**
CB	59*	327	NAD	216	74	NAD	**MNHN**
CI	51	97	296	229	72	7	**ICCM**
CS	56	112	768	281	100	8	**ICCM**
EM	61	113	357	592	54	274	**NAGREF**
MA	51	139	633	435	34	NAD	**IMAR**
NS	52	248	240	NAD	80	NAD	**RIVO**
WM	91	178	786	434	153	18	**ICCM**
EE	45	88	131	186	73	9	**ICCM**

NAD: Not Available Data. *55 Overlapped species between both institutions.

Table 4.3.- Kimura-two-parameters distance matrix computed from "TriMin" FishTrace specimens DNA-barcodes.

	1	2	3	4	5	6	7
1 TriMin-WM-01							
2 TriMin-WM-02	0.002						
3 TriMin-EM-02	0.003	0.002					
4 TriMin-EM-01	0.002	0.001	0.001				
5 TriMin-NS-02	0.110	0.109	0.108	0.108			
6 TriMin-NS-01	0.110	0.109	0.108	0.108	0.000		
7 TriMin-CB-01	0.110	0.110	0.109	0.108	0.008	0.008	
- Average sequence divergence among taxa: **0.064 (~ 6%)**							
- Average sequence divergence among EM-WM / NS-CB groups: **0.104 (> 10 %)**							

Table 4.4.- Meristic characters of *Trisopterus minutus auct.* sampled in the NE Atlantic (representing *T. minutus s.str.*) and Mediterranean (*T. minutus capelanus*), respectively. Data taken from the FishTrace database.

	Trisopterus minutus NE Atlantic **(NS, CB)**	***Trisopterus capelanus*** Mediterranean **(WM, EM)**
Gill rakers on upper part of first gill arch	5-7	2-5
Gill rakers on lower part of first gill arch	**20-22**	**13-17**
Rays in first dorsal fin	11-13	11-14
Rays in second dorsal fin	18-20	18-23
Rays in third dorsal fin	**18-22**	**15-17**
Rays in first anal fin	22-27	25-28
Rays in second dorsal fin	16-23	17-21
Rays in pectoral fin	16-19	12-17

Table 4.5.- Fishtrace Reference Collections: Vouchers and otoliths in the four participant Museums.

Museum	Total species	Species sampled	Sp completed (V01+V02)	Pairs otoliths (V02)	Goal species (%)
MNHN	60	59	49	20	98
TFMC - WM	91	91	87	87	100
TFMC - CS	56	57	55	54	102
TFMC - CI	51	51	46	43	100
TFMC - EE	45	45	43	40	100
MMF	52	50	46	18	96
NRM	52	48	46	12	92

V01,V02 = Specimen vouchers tagged "01" and "02", deposited in Reference Collection.

Table 4.6.- DNA-barcoded teleost species included in the phylogenetic analysis, indicating taxonomic order and family.

Species name {Order (Family)}	Species name {Order (Family)}	Species name {Order (Family)}
Anguilla anguilla {Anguilliformes}	*Seriola rivoliana {Perciformes (Carangidae)}*	*Diplodus vulgaris {Perciformes (Sparidae)}*
Anguilla japonica {Anguilliformes}	*Trachurus trachurus {Perciformes (Carangidae)}*	*Sparus auratus {Perciformes (Sparidae)}*
Gymnothorax afer {Anguilliformes}	*Caranx crysos {Perciformes (Carangidae)}*	*Pagrus pagrus {Perciformes (Sparidae)}*
Muraena robusta {Anguilliformes}	*Seriola fasciata {Perciformes (Carangidae)}*	*Pagellus erythrinus {Perciformes (Sparidae)}*
Atherina presbyter {Atheriniformes}	*Spicara smaris {Perciformes (Centracanthidae)}*	*Dentex dentex {Perciformes (Sparidae)}*
Atherina boyeri {Atheriniformes}	*Spicara maena {Perciformes (Centracanthidae)}*	*Sphyraena sphyraena {Perciformes (Sphyraenidae)}*
Chlorophthalmus agassizi {Aulopiformes}	*Schedophilus ovalis {Perciformes (Centrolophidae)}*	*Trachinus draco {Perciformes (Trachinidae)}*
Belone belone {Beloniformes}	*Pomadasys perotaei {Perciformes (Haemulidae)}*	*Echiichthys vipera {Perciformes (Trachinidae)}*
Tylosurus acus {Beloniformes}	*Pomadasys incisus {Perciformes (Haemulidae)}*	*Xiphias gladius {Perciformes (Xiphiidae)}*
Oryzias latipes {Beloniformes}	*Xyrichthys novacula {Perciformes (Labridae)}*	*Zoarces viviparus {Perciformes (Zoarcidae)}*
Sargocentron sp {Beryciformes}	*Labrus bergylta {Perciformes (Labridae)}*	*Solea solea {Pleuronectiformes}*
Beryx decadactylus {Beryciformes}	*Dicentrarchus labrax {Perciformes (Moronidae)}*	*Microchirus azevia {Pleuronectiformes}*
Myripristis sp {Beryciformes}	*Liza aurata {Perciformes (Mugilidae)}*	*Microstomus kitt {Pleuronectiformes}*
Sardina pilchardus {Clupeiformes}	*Liza ramado {Perciformes (Mugilidae)}*	*Limanda limanda {Pleuronectiformes}*
Engraulis encrasicolus {Clupeiformes}	*Mugil cephalus {Perciformes (Mugilidae)}*	*Buglossidium luteum {Pleuronectiformes}*
Sardinella maderensis {Clupeiformes}	*Chelon labrosus {Perciformes (Mugilidae)}*	*Hippoglossoides platessoides {Pleuronectiformes}*
Alosa fallax {Clupeiformes}	*Mullus barbatus {Perciformes (Mullidae)}*	*Hippoglossus hippoglossus {Pleuronectiformes}*
Alosa alosa {Clupeiformes}	*Mullus surmuletus {Perciformes (Mullidae)}*	*Glyptocephalus cynoglossus {Pleuronectiformes}*
Danio rerio {Cypriniformes}	*Polyprion americanus {Perciformes (Polyprionidae)}*	*Dicologlossa cuneata {Pleuronectiformes}*
Carassius auratus {Cypriniformes}	*Pomatomus saltatrix {Perciformes (Pomatomidae)}*	*Lepidorhombus boscii {Pleuronectiformes}*
Gadiculus argenteus {Gadiformes}	*Sparissoma cretense {Perciformes (Scaridae)}*	*Pegusa lascaris {Pleuronectiformes}*
Phycis phycis {Gadiformes}	*Scarus hoefleri {Perciformes (Scaridae)}*	*Salmo trutta {Salmoniformes}*
Enchelyopus cimbrius {Gadiformes}	*Sparissoma rubripinne {Perciformes (Scaridae)}*	*Salmo salar {Salmoniformes}*
Merlangius merlangus {Gadiformes}	*Umbrina canariensis {Perciformes (Sciaenidae)}*	*Oncorhynchus mykiss {Salmoniformes}*
Brosme brosme {Gadiformes}	*Thunnus thynnus {Perciformes (Scombridae)}*	*Plecoglossus altivelis {Salmoniformes}*
Ciliata septentrionalis {Gadiformes}	*Thunnus obesus {Perciformes (Scombridae)}*	*Scorpaena porcus {Scorpaeniformes}*
Micromesistius poutassou {Gadiformes}	*Scomber japonicus {Perciformes (Scombridae)}*	*Sebastes viviparus {Scorpaeniformes}*
Merluccius merluccius {Gadiformes}	*Scomber scombrus {Perciformes (Scombridae)}*	*Chelidonichthys lucernus {Scorpaeniformes}*
Melanogrammus aeglefinus {Gadiformes}	*Thunnus albacares {Perciformes (Scombridae)}*	*Helicolenus dactylopterus {Scorpaeniformes}*
Molva molva {Gadiformes}	*Katsuwonus pelamis {Perciformes (Scombridae)}*	*Aspitrigla cuculus {Scorpaeniformes}*
Phycis blennoides {Gadiformes}	*Sarda sarda {Perciformes (Scombridae)}*	*Liparis liparis {Scorpaeniformes}*
Gadus morhua {Gadiformes}	*Thunnus alalunga {Perciformes (Scombridae)}*	*Myoxocephalus scorpius {Scorpaeniformes}*
Pollachius virens {Gadiformes}	*Euthynnus alletteratus {Perciformes (Scombridae)}*	*Chelidonichthys gurnardus {Scorpaeniformes}*
Lophius piscatorus {Lophiiformes}	*Serranus cabrilla {Perciformes (Serranidae)}*	*Cyclopterus lumpus {Scorpaeniformes}*
Lophius budegassa {Lophiiformes}	*Serranus hepatus {Perciformes (Serranidae)}*	*Fugu rubripes {Tetraodontiformes}*
Brotula barbata {Ophidiiformes}	*Epinephelus marginatus {Perciformes (Serranidae)}*	*Tetraodon nigroviridis {Tetraodontiformes}*
Argentina sphyraena {Osmeriformes}	*Epinephelus costae {Perciformes (Serranidae)}*	*Balistes capriscus {Tetraodontiformes}*
Osmerus eperlanus {Osmeriformes}	*Boops boops {Perciformes (Sparidae)}*	*Sphoeroides pachygaster {Tetraodontiformes}*
Anarhichas lupus {Perciformes (Anarhichadidae)}	*Sarpa salpa {Perciformes (Sparidae)}*	*Ranzania laevis {Tetraodontiformes}*
Taractichthys longipinnis {Perciformes (Bramidae)}	*Lithognathus mormyrus {Perciformes (Sparidae)}*	*Zeus faber {Zeiformes}*

Table 4.7.- Reproducibility (indicated by an **X**) of the major clades obtained from the phylogenetic inferences performed using three sets of data: cyt*b* sequences (1141 bp), rhod sequences (460 bp) and cyt*b* + rhod sequences (1601 bp). Taxonomical fragmented clades are indicated by a diamond.

Taxa clade	Cytb	Rhod	Cytb + Rhod
Clupeiformes	X	X	X
Angulliformes	X	X	X
Cypriniformes	X	X	X
Gadiformes	X	X	X
Osmeriformes			X
Salmoniformes	X	X	X
Aulopiformes			
Beryciformes 1 ◆			
Perciformes – Sparidae 1 ◆		X	X
Perciformes - Centracanthidae	X	X	X
Perciformes – Sparidae 2 ◆		X	X
Tetraodontiformes 1 ◆	X	X	X
Scopaeniformes 1 ◆		X	X
Lophiiformes		X	X
Perciformes - Zoarcidae	X	X	X
Perciformes - Anarhichadidae	X	X	X
Scopaeniformes 2 ◆	X	X	X
Perciformes - Scombridae	X	X	X
Perciformes - Bramidae	X	X	X
Perciformes - Centrolophidae	X	X	X
Perciformes - Pomatomidae	X	X	X
Perciformes - Mullidae	X	X	X
Ophidiiformes			
Beryciformes 2 ◆			
Perciformes - Labridae		X	X
Perciformes - Scaridae		X	
Atheriniformes	X	X	X
Beloniformes	X	X	X
Perciformes - Mugilidae	X	X	X
Perciformes - Scianidae		X	X
Perciformes - Trachinidae	X	X	X
Perciformes - Serranidae 1 ◆		X	X
Scopaeniformes 3	X	X	X
Perciformes – Serranidae 2		X	
Tetraodontiformes 2 ◆			
Perciformes - Polyprionidae	X		
Perciformes - Haemulidae		X	X
Perciformes - Xiphiidae	X	X	X
Perciformes - Carangidae	X	X	X
Perciformes - Sphyraenidae			X
Perciformes - Moronidae			X
Perciformes - Pleuronectiformes		X	X

Table 4.8.- Clupeid taxa analyzed, indicating FishTrace code. A) FishTrace DNA-barcodes. B) Clupeidae cyt*b* and rhod sequences retrieved from GenBank and included in the phylogenetic analyses for quality control and outgroup.

A)

Family	**Clupeidae**							**Engraulidae**
Genus	***Alosa***		***Clupea***	***Sardina***	***Sardinella***		***Sprattus***	***Engraulis***
Species	*alosa*	*fallax*	*harengus*	*pilchardus*	*aurita*	*maderensis*	*sprattus*	*encrasicolus*
	AloAlo-CB-01	AloFal-SB-01	CluHar-CB-01	SarPil-CB-01	SarAur-CI-01	SarMad-MA-01	SprSpr-CB-01	EngEnc-CB-01
	AloAlo-CB-02	AloFal-SB-02	CluHar-CB-02	SarPil-CB-02	SarAur-CI-02	SarMad-MA-02	SprSpr-CB-02	EngEnc-CB-02
		AloFal NS-01	CluHar-NS-01	SarPil-EM-01	SarAur-WM-01		SprSpr-NS-01	EngEnc-CS-01
		AloFal-NS-02	CluHar-NS-02	SarPil-EM-02	SarAur-WM-02		SprSpr-NS-02	EngEnc-CS-02
		AloFal-CB-01	CluHar-SB-01	SarPil-WM-02	SarAur-EM-01		SprSpr-SB-01	EngEnc-EM-01
		AloFal-CB-02	CluHar-SB-02	SarPil-WM-01	SarAur-EM-02		SprSpr-SB-02	EngEnc-EM-02
				SarPil-NS-01				EngEnc-NS-01
				SarPil-NS-02				EngEnc-NS-02
				SarPil-CS-01				EngEnc-WM-01
				SarPil-CI-02				EngEnc-WM-02
				SarPil-CI-01				
				SarPil-MA-01				
				SarPil-MA-02				

B)

Taxa	cyt*b* **GenBank Acc. No.**	rhod **GenBank Acc. No.**
Alosa alosa	DQ419760	n/a
Alosa fallax	AY937212	n/a
Clupea harengus	AF472580	AF385831
Engraulis encrasicolus	AF472579	AY158051
Sardinella aurita	AF472584	n/a
Sardinella maderensis	AF472583	n/a
Sardina pilchardus	AF472582	Y18677
Sprattus sprattus	AF472581	n/a
Outgroup: *Gadus morhua*	DQ174046	AF137211
Outgroup: *Merlangius merlangus*	DQ174058	AY141260

Table 4.9.- Scombridae taxa analyzed, indicating FishTrace code. A) FishTrace DNA-barcodes. B) Scombridae cyt*b* sequences retrieved from GenBank and included in the phylogenetic analyses for quality control and outgroup. C) Kimura-two-parameters (K2P) distance matrix. Genetic divergence found between Thunnus DNA-barcodes analyzed within FishTrace. Taxa: [1] *Thunnus alalunga*; [2] *Thunnus albacares*; [3] *Thunnus obesus* and [4] *Thunnus thynnus.*

A)

Genus	***Auxis***	***Euthynnus***	***Katsuwonus***	***Sarda***	***Scomber***		***Thunnus***			
Species	*rochei*	*alletteratus*	*pelamis*	*sarda*	*colias*	*scombrus*	*alalunga*	*thynnus*	*albacares*	*obesus*
	AuxRoc-CI-01	EutAll-WM-01	KatPel-CI-01	SarSar-CI-01	ScoCol-CB-01	ScoSco-CB-01	ThuAla-CI-01	ThuThy-WM-01	ThuAlb-CI-01	ThuObe-CI-02
	AuxRoc-CI-02	EutAll-WM-02	KatPel-CI-02	SarSar-CI-02	ScoCol-CB-02	ScoSco-CB-02	ThuAla-CI-02	ThuThy-WM-02	ThuAlb-CI-02	ThuObe-CB-01
	AuxRoc-WM-01		KatPel-CS-01	SarSar-WM-01	ScoCol-CS-01	ScoSco-CS-01	ThuAla-WM-01	ThuThy-CS-01	ThuAlb-EE-01	ThuObe-MA-01
	AuxRoc-WM-02		KatPel-CS-02	SarSar-WM-02	ScoCol-CS-02	ScoSco-CS-02	ThuAla-WM-02	ThuThy-CS-02	ThuAlb-EE-02	ThuObe-MA-02
			KatPel-MA-01	SarSar-CB-01	ScoCol-EM-01	ScoSco-EM-01	ThuAla-CB-01	ThuThy-EM-01		
			KatPel-MA-02	SarSar-CB-02	ScoCol-EM-02	ScoSco-EM-02	ThuAla-CS-01	ThuThy-EM-02		
				SarSar-CS-01	ScoCol-MA-01	ScoSco-NS-01	ThuAla-CS-02	ThuThy-MA-01		
				SarSar-CS-02	ScoCol-MA-02	ScoSco-NS-02	ThuAla-EM-01	ThuThy-MA-02		
				SarSar-EM-01	ScoCol-WM-01	ScoSco-SB-01	ThuAla-EM-02			
				SarSar-EM-02	ScoCol-WM-02	ScoSco-SB-02	ThuAla-MA-01			
						ScoSco-WM-01	ThuAla-MA-02			
						ScoSco-WM-02				

B)

Taxa	GenBank Acc. No.
Thunnus alalunga	NC_005317
Thunnus thynnus thynnus	AY302574
Auxis rochei	NC_005313
Euthynnus alletteratus	AB099716
Katsuwonus pelamis	AB101290
Sarda sarda	X81562
Scomber japonicus	AB032516
Scombers combrus	AB120717
Outgroup: *Gadus morhua*	DQ174046
Outgroup: *Merlangius merlangus*	DQ174058

C)

	1	2	3	4
[1]				
[2]	0.018			
[3]	0.022	0.012		
[4]	0.020	0.008	0.014	-

Average sequence divergence: 1.67 (~1.7%)

Table 4.10.- Gadid taxa analyzed, indicating FishTrace code. A) FishTrace DNA-barcodes. B) Cyt*b* and rhod sequences retrieved from GenBank and included in the phylogenetic analyses for quality control and outgroup.

A)

Family	**Gadidae**								
Genus	***Gadiculus***	***Gadus***	***Melanogrammus***	***Merlangius***	***Micromesistius***	***Pollachius***		***Trisopterus***	
Species	*argenteus*	*morhua*	*aeglefinus*	*merlangus*	*poutassou*	*pollachius*	*virens*	*luscus*	*minutus*
	GadArg-CB-01	GadMor-CS-01	MelAeg-CB-01	MeaMea-NS-01	MicPou-CB-01	PolPol-CB-01	PolVir-NS-01	TriLus-NS-02	TriMin-WM-01
	GadArg-CB-02	GadMor-CS-02	MelAeg-CB-02	MeaMea-NS-02	MicPou-CS-01	PolPol-CB-02	PolVir-NS-02	TriLus-NS-01	TriMin-WM-02
		GadMor-EE-01	MelAeg-CS-01	MeaMea-SB-01	MicPou-CS-02	PolPol-NS-01	PolVir-SB-01	TriLus-CB-02	TriMin-NS-02
		GadMor-NS-01	MelAeg-EE-01	MeaMea-SB-02	MicPou-EM-01	PolPol-NS-02	PolVir-SB-02	TriLus-CB-01	TriMin-NS-01
		GadMor-NS-02	MelAeg-EE-02		MicPou-EM-02	PolPol-CS-01		TriLus-CS-02	TriMin-EM-02
		GadMor-SB-01	MelAeg-NS-01		MicPou-NS-01				TriMin-EM-01
		GadMor-SB-02	MelAeg-NS-02		MicPou-NS-02			*esmarkii*	TriMin-CB-01
			MelAeg-SB-01		MicPou-SB-01			TriEsm-SB-02	
			MelAeg-SB-02		MicPou-SB-02			TriEsm-SB-01	
					MicPou-WM-01			TriEsm-NS-02	
					MicPou-WM-02			TriEsm-NS-01	

Family	**Lotidae**						
Genus	***Brosme***	***Ciliata***	***Enchelyopus***	***Gaidropsarus***		***Molva***	
Species	*brosme*	*septentrionalis*	*cimbrius*	*biscayensis*	*mediterraneus*	*dypterygia*	*molva*
	BroBro-SB-01	CilSep-NS-01	EncCim-CB-01	GaiBis-CS-01	GaiMed-CS-01	MolDyp-CS-01	MolMol-CS-01
	BroBro-SB-02	CilSep-NS-02	EncCim-CB-02		GaiMed-CS-02	MolDyp-CS-02	MolMol-CS-02
			EncCim-NS-01				
			EncCim-NS-02				
			EncCim-SB-01				
			EncCim-SB-02				

Family	**Merluccidae**				**Phycidae**	
Genus	***Merluccius***				***Phycis***	
Species	*australis*	*capensis*	Species	*polli*	*blennoides*	*phycis*
	MerAus-EE-01	MerCap-EE-01	MerMer-WM-01	MerPol-EE-01	PhyBle-CB-01	PhyPhy-EM-01
	MerAus-EE-02		MerMer-WM-02		PhyBle-CB-02	PhyPhy-EM-02
			MerMer-CS-01		PhyBle-EM-01	PhyPhy-WM-01
			MerMer-CS-02		PhyBle-EM-02	PhyPhy-WM-02
			MerMer-CI-01		PhyBle-MA-01	
			MerMer-CI-02			
			MerMer-CB-01			
			MerMer-EM-01			
			MerMer-EM-02			
			MerMer-SB-01			

B)

Taxa	Cyt*b* Acc. No.	Rhod Acc. No.
Brosme brosme	DQ174037	
Ciliata mustela	DQ174039	
Enchelyopus cimbrius	DQ174040	
Gadiculus argenteus	DQ174042	
Gadus morhua	DQ174046	AF137211
Gaidropsarus vulgaris	DQ174050	
Melanogrammus aeglefinus	DQ174054	
Merlangius merlangus	DQ174058	AY141260
Merluccius merluccius	DQ174062	
Micromesistius poutassou	DQ174068	
Molva dypterygia	AJ517493	
Molva molva	DQ174071	
Phycis blennoides	DQ174072	AY368321
Pollachius pollachius	DQ174076	
Pollachius virens	DQ174078	
Trisopterus esmarkii	AF081695	
Trisopterus luscus	DQ174081	
Trisopterus minutus	DQ174083	
Outgroup: *Sardina pilchardus*	AF472582	Y18677
Outgroup: *Clupea harengus*	AF472580	AF385831

Table 4.11.- Population structure analyses: Number of complete cyt*b* sequences from each sampling area.

Species	**Geographical Areas**							
	BS	NS	CB	CS	MA	CI	WM	EM
Merluccius merluccius	20	5	18	19	NA	19	20	18
Micromesistius poutassou	NA	20	20	16	NA	NA	18	20
Mullus surmuletus	NA	20	NA	NA	9	16	18	20
Pagellus erythrinus	NA	NA	NA	NA	NA	20	17	20
Pagrus pagrus	NA	NA	NA	NA	18	20	20	12
Solea solea	20	20	14	NA	NA	NA	15	22
Partner	NRM	RIVO	IFREMER	UCM				NAGREF

NA: Not applicable

Table 4.12.- Flatfish specimens, indicating FishTrace code, included in the phylogenetic analysis performed for the validation of the genetic data collected (cyt*b* and rhod sequences) within FishTrace.

Solea solea			***Solea senegalensis***	***Microchirus azevia***	***Synaptura kleinii***
SolSol-WM-01	SolSol-EE-01	SolSol-CB-01	SolSen-CS-01	MicAze-CI-01 [1]	SynKle-CI-01
SolSol-WM-02	SolSol-EE-02	SolSol-CB-02	SolSen-CS-02	MicAze-CI-02	SynKle-EM-01
SolSol-WM-03	SolSol-EE-03	SolSol-CS-01	SolSen-WM-01	MicThe-CI-00	SynKle-EM-02
SolSol-WM-04	SolSol-EE-04	SolSol-CS-02	SolSen-WM-02	MicThe-CI-01	SynKle-WM-01
SolSol-WM-05	SolSol-EE-05	SolSol-EE-02		MicThe-CI-02	SynKle-WM-02
SolSol-WM-06	SolSol-EE-06	SolSol-EM-01		Maze-327 [2]	
SolSol-WM-07	SolSol-EE-07	SolSol-EM-02	***Solea* (syn. *Pegusa*)[3] *cadenati***	Maze-328	
SolSol-WM-08	SolSol-EE-08	SolSol-NS-01	PegCad-EE-02	Maze-739	
SolSol-WM-09	SolSol-EE-09	SolSol-NS-02		Maze-740	
SolSol-WM-10	SolSol-EE-10	SolSol-SB-01			
SolSol-WM-11	SolSol-EE-11	SolSol-SB-02	***Solea* (syn. *Pegusa*)[3] *lascaris***	***Microchirus variegatus***	***Synaptura lusitanica***
SolSol-WM-12	SolSol-EE-12	SolSol-WM-01	PegLas-CI-01	MicVar-CB-01	SynLus-WM-01
SolSol-WM-13	SolSol-EE-13	SolSol-WM-02	PegLas-CI-02	MicVar-CB-02	
SolSol-WM-14	SolSol-EE-14		PegLas-CB-01	MicVar-CS-01	
SolSol-WM-15	SolSol-EE-15		PegLas-CB-02	MicVar-CS-02	
SolSol-WM-16	SolSol-EE-16		PegLas-EM-01	MicVar-NS-01	
SolSol-WM-17	SolSol-EE-17		PegLas-EM-02	MicVar-NS-02	
SolSol-WM-18	SolSol-EE-18		PegLas-WM-01	MicVar-WM-01	
SolSol-WM-19	SolSol-EE-19		PegLas-WM-02	MicVar-WM-02	
SolSol-WM-20	SolSol-EE-20				

Taxa[4]	**cyt*b*** Acc. No.	**rhod** Acc. No.
Solea solea	AB125327	NA
Synaptura kleinii	AY164474	NA
Synaptura lusitanica	AY164468	NA
Solea senegalensis	AB125326	NA
Solea (syn: *Pegusa) lascaris*	AB125325	NA
Microchirus variegatus	AF113201	AY141284
Microchirus azevia	AB125329	NA
Gadus morhua	DQ174046	AF137211
Merlangius merlangus	DQ174058	AY141260

Notes to table 4.12: [1] **MicThe:** *Microchirus theophila* (synonym of *M. azevia*) specimen sequences granted from previous research project. [2] **Maze:** *Microchirus azevia* specimen sequences granted from www.pescabase.org. [3] Syn. = synonym. [4] Cyt*b* and rhod sequences retrieved from the GenBank (www.ncbi.nlm.nih.gov/Genbank/index.html) included in the anlaysis as quality control, and the corresponding accession numbers.

Table 4.13.- Validation of Taxonomic Data by target species and geographical area sampled.

Area	Partner	Target species	Species validated	% done
BS	**NRM**	48	38	79
NS	**RIVO**	52	52	100
CB	**MNHN**	59	NAD	NAD
CS	**ICCM**	56	56	100
MA	**IMAR**	52	NAD	NAD
CI	**ICCM**	51	51	100
WM	**ICCM**	91	91	100
EM	**NAGREF**	61	61	100
EE	**ICCM**	45	45	100

NAD: Not Available Data.

Table 4.14.- Validation of Reference collections Data by target species and geographical area sampled.

Area	Partner	Target species	Species validated	% done
BS	**NRM**	48	37	77
NS	**MNHN**	52	NAD	NAD
BB	**MNHN**	59	NAD	NAD
CS	**TFMC**	56	56	100
MA	**IMAR**	52	NAD	NAD
CI	**TFMC**	51	51	100
WM	**TFMC**	91	91	100
EM	**MNHN**	61	61	100
EE	**TFMC**	45	45	100

NAD: Not Available Data.

Table 4.15.- Validation of Genetic Data by target species and geographical area sampled.

Area	Partner	Target species	Species validated	% done
BS	**NRM**	48	43	90
NS	**RIVO**	52	51	98
BB	**IFREMER**	59	54	92
CS	**UCM**	56	37	66
MA	**UCM**	52	27	52
CI	**UCM**	51	36	71
WM	**UCM**	91	60	66
EM	**NAGREF**	61	61	100
EE	**UCM**	45	24	53

Table 7.1.- Tentative topics covered by manuscripts to be submitted to scientific journals by the FishTrace Consortium.

Topic / Tentative title	**Responsable partner(s)**
General presentation of the FishTrace Database	UCM
Morphological data and reference collections	IMAR
Database structure and data quality in FishTrace	JRC
General fish phylogeny based on cyt*b* + Rhod vs COI	NRM + Ifremer
Genetic variation in European populations (6 species: 6 papers or just 1?)	NAGREF
Quick method to identify fish species by DNA sequencing	RIVO
New methodology for fish collections	MNHN
On species or group particularities identified in the project:	
Solea group + pleuronectiforms	ICCM + NAGREF
Centracanthidae (Short communication)	NAGREF + ICCM
Trisopterus	NAGREF + NRM
Sarda sarda	UCM + ICCM
Alosa alosa vs *Alosa fallax*	Ifremer + NRM

Figures

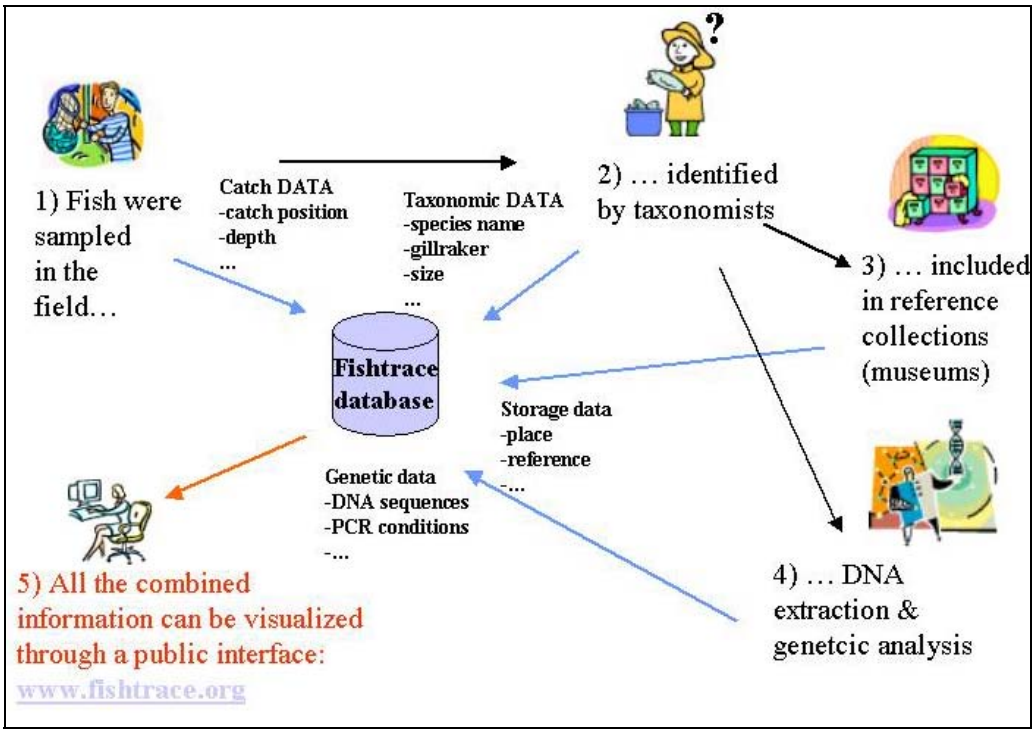


Figure 3.1.- Data collection process before its introduction into the centralized FishTrace database.

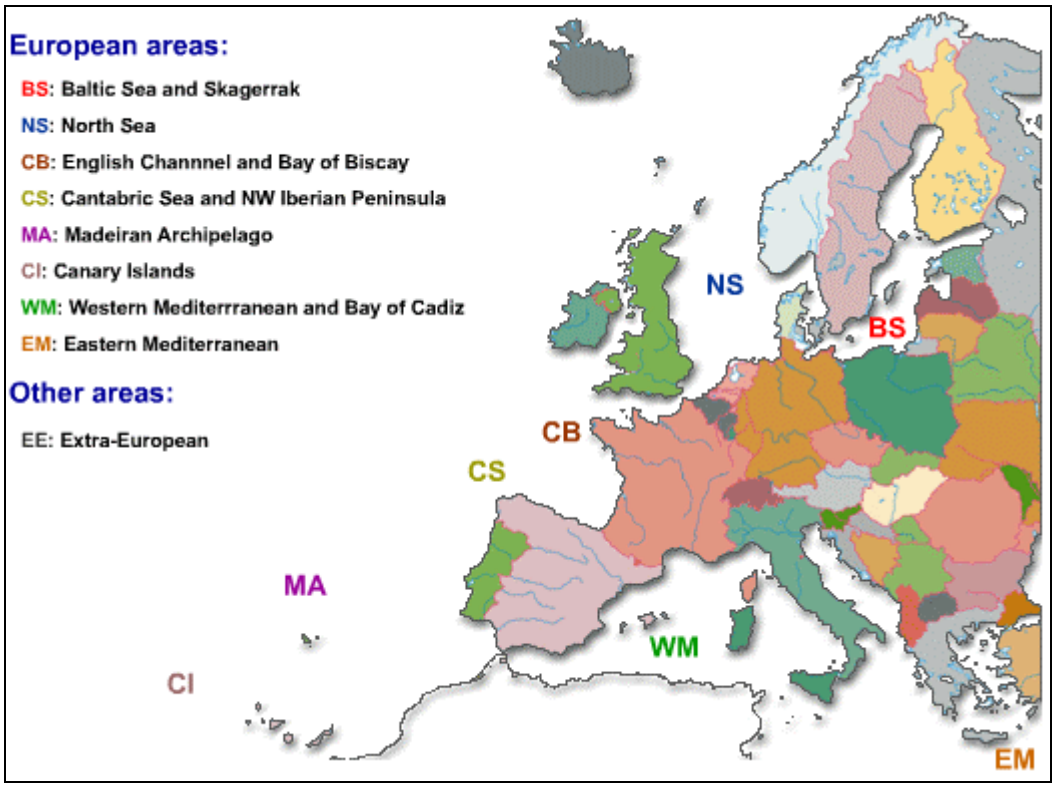


Figure 3.2.- European sea areas of sampling covered by FishTrace.

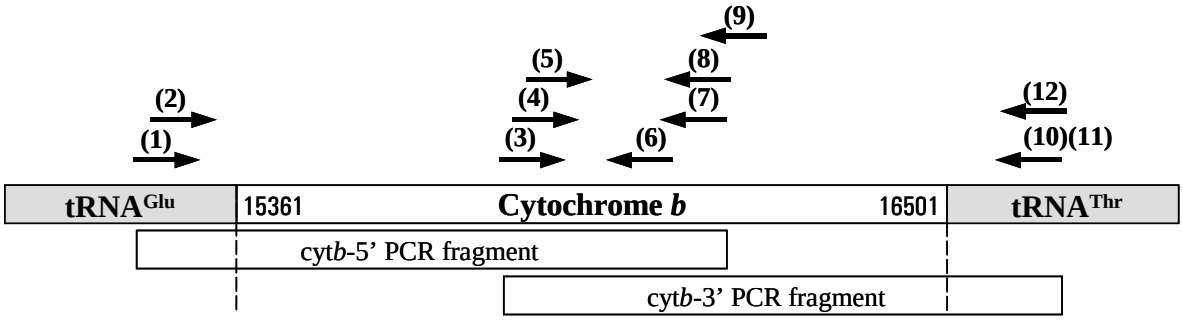


Figure 3.3.- Typical vertebrate cytochrome *b* organization indicating flanking regions (tRNA^{Glu} - cyt*b* - tRNA^{Thr}). Gene position corresponding to the *Oncorhynchus mykiss* mitochondrial genome (GenBank accession number: NC_001717) is indicated: 15361 to 16501. Relative length of targeted cyt*b*-5' and cyt*b*-3' PCR fragments is represented. Detailed information on represented primer pairs is given in Table 3.7.

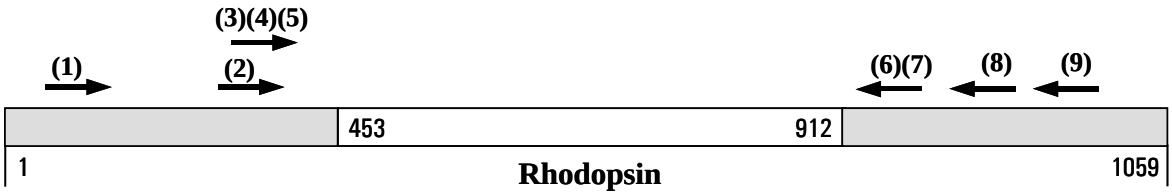


Figure 3.4.- Rhodopsin amplification scheme. Targeted 460bp-length fragment and primer location have been given corresponding to the 5' position in the *Astyanax mexicanus* rhodopsin gene (GenBank accession number: U12328). Detailed information on represented primer pairs is given in Table 3.9.

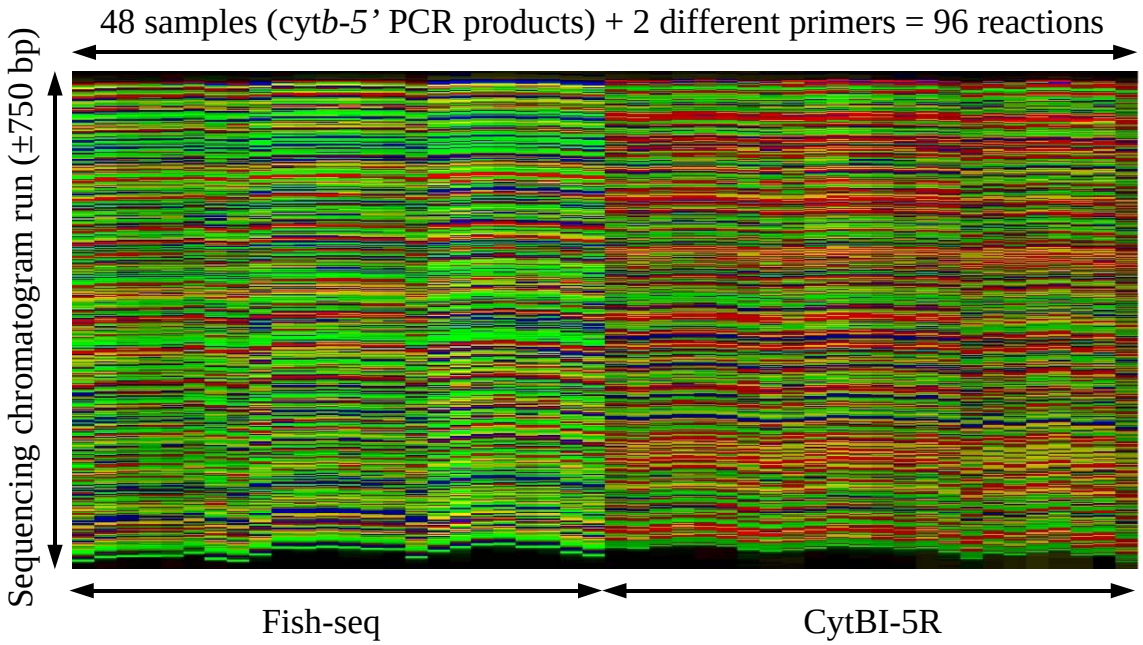


Figure 3.5.- High-throughput automated DNA sequencing. 96 samples (48 PCR products from the second Nested-PCR reaction on cyt*b*-5') were sequenced at once, giving 100% effectiveness. Primers Fish-seq and CytBI-5R (see Tables 3.7A and B) were used for the double stranded sequencing.

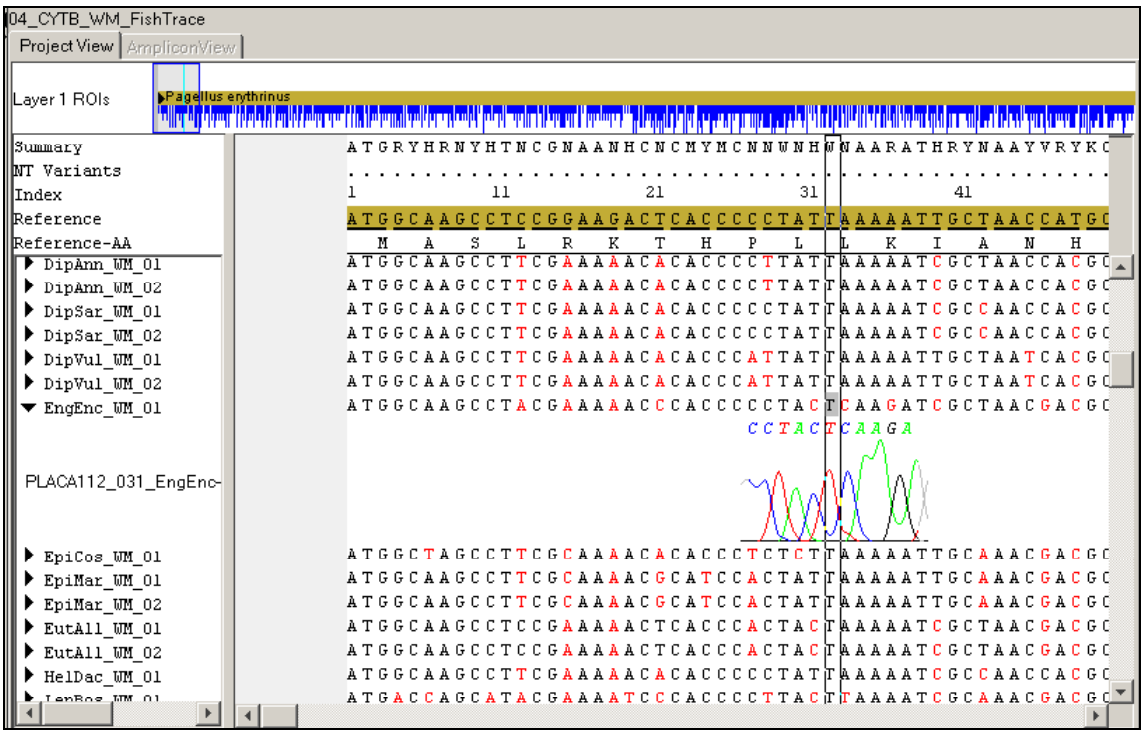


Figure 3.6.- Representative figure from the validation of cyt*b* sequences by the inspection of electropherograms in SeqScape™ software.

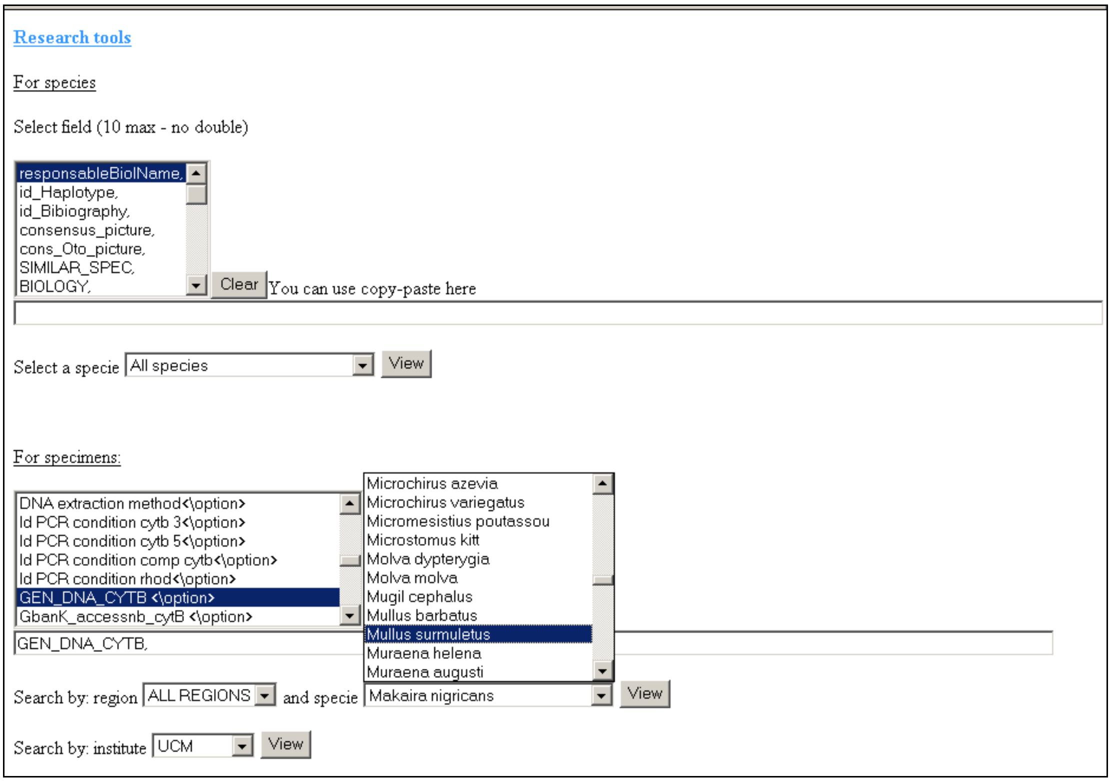
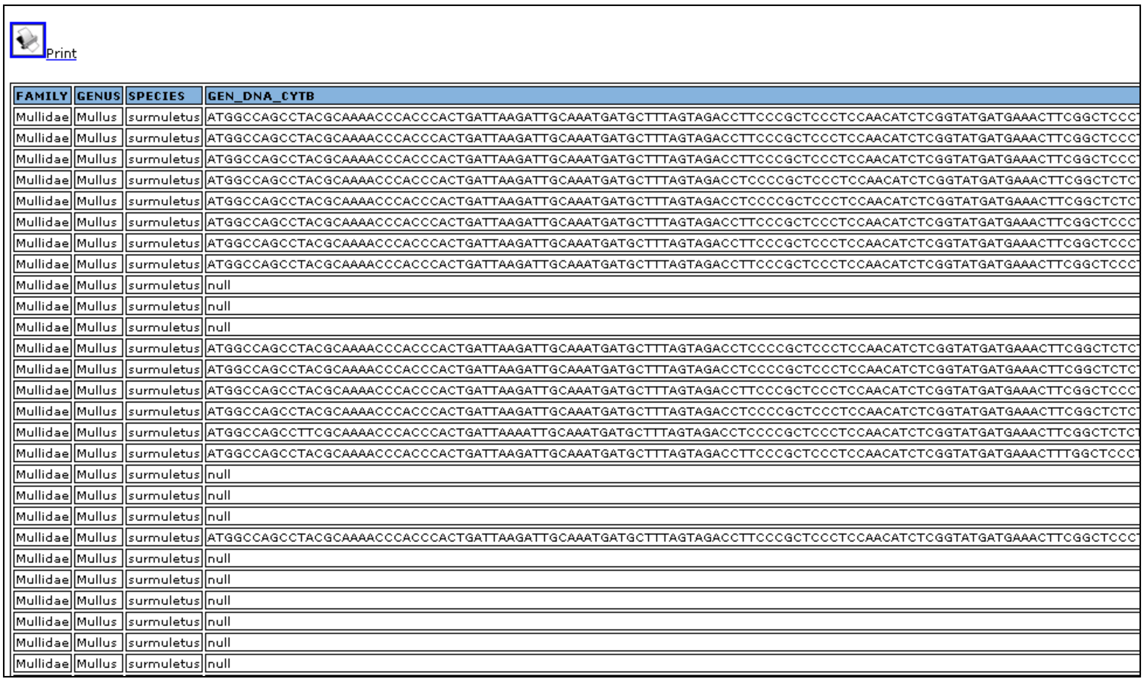


Figure 3.7.- View Data Tool (Interface).



FAMILY	GENUS	SPECIES	GEN_DNA_CYTB
Mullidae	Mullus	surmuletus	ATGGCCAGCCTACGCAAAACCCACCCACTGATTAAGATTGCAAATGATGCTTTAGTAGACCTTCCCGCTCCCTCCAACATCTCGGTATGATGAAACTTCGGCTCCC
Mullidae	Mullus	surmuletus	ATGGCCAGCCTACGCAAAACCCACCCACTGATTAAGATTGCAAATGATGCTTTAGTAGACCTTCCCGCTCCCTCCAACATCTCGGTATGATGAAACTTCGGCTCCC
Mullidae	Mullus	surmuletus	ATGGCCAGCCTACGCAAAACCCACCCACTGATTAAGATTGCAAATGATGCTTTAGTAGACCTTCCCGCTCCCTCCAACATCTCGGTATGATGAAACTTCGGCTCCC
Mullidae	Mullus	surmuletus	ATGGCCAGCCTACGCAAAACCCACCCACTGATTAAGATTGCAAATGATGCTTTAGTAGACCTCCCCGCTCCCTCCAACATCTCGGTATGATGAAACTTCGGCTCTC
Mullidae	Mullus	surmuletus	ATGGCCAGCCTACGCAAAACCCACCCACTGATTAAGATTGCAAATGATGCTTTAGTAGACCTCCCCGCTCCCTCCAACATCTCGGTATGATGAAACTTCGGCTCTC
Mullidae	Mullus	surmuletus	ATGGCCAGCCTACGCAAAACCCACCCACTGATTAAGATTGCAAATGATGCTTTAGTAGACCTTCCCGCTCCCTCCAACATCTCGGTATGATGAAACTTCGGCTCCC
Mullidae	Mullus	surmuletus	ATGGCCAGCCTACGCAAAACCCACCCACTGATTAAGATTGCAAATGATGCTTTAGTAGACCTTCCCGCTCCCTCCAACATCTCGGTATGATGAAACTTCGGCTCCC
Mullidae	Mullus	surmuletus	ATGGCCAGCCTACGCAAAACCCACCCACTGATTAAGATTGCAAATGATGCTTTAGTAGACCTTCCCGCTCCCTCCAACATCTCGGTATGATGAAACTTCGGCTCCC
Mullidae	Mullus	surmuletus	null
Mullidae	Mullus	surmuletus	null
Mullidae	Mullus	surmuletus	null
Mullidae	Mullus	surmuletus	ATGGCCAGCCTACGCAAAACCCACCCACTGATTAAGATTGCAAATGATGCTTTAGTAGACCTCCCCGCTCCCTCCAACATCTCGGTATGATGAAACTTCGGCTCTC
Mullidae	Mullus	surmuletus	ATGGCCAGCCTACGCAAAACCCACCCACTGATTAAGATTGCAAATGATGCTTTAGTAGACCTCCCCGCTCCCTCCAACATCTCGGTATGATGAAACTTCGGCTCTC
Mullidae	Mullus	surmuletus	ATGGCCAGCCTACGCAAAACCCACCCACTGATTAAGATTGCAAATGATGCTTTAGTAGACCTTCCCGCTCCCTCCAACATCTCGGTATGATGAAACTTCGGCTCCC
Mullidae	Mullus	surmuletus	ATGGCCAGCCTACGCAAAACCCACCCACTGATTAAGATTGCAAATGATGCTTTAGTAGACCTCCCCGCTCCCTCCAACATCTCGGTATGATGAAACTTCGGCTCTC
Mullidae	Mullus	surmuletus	ATGGCCAGCCTTCGCAAAACCCACCCACTGATTAAAATTGCAAATGATGCTTTAGTAGACCTCCCCGCTCCCTCCAACATCTCGGTATGATGAAACTTCGGCTCTC
Mullidae	Mullus	surmuletus	ATGGCCAGCCTACGCAAAACCCACCCACTGATTAAGATTGCAAATGATGCTTTAGTAGACCTTCCCGCTCCCTCCAACATCTCGGTATGATGAAACTTTGGCTCCC
Mullidae	Mullus	surmuletus	null
Mullidae	Mullus	surmuletus	null
Mullidae	Mullus	surmuletus	null
Mullidae	Mullus	surmuletus	ATGGCCAGCCTACGCAAAACCCACCCACTGATTAAGATTGCAAATGATGCTTTAGTAGACCTTCCCGCTCCCTCCAACATCTCGGTATGATGAAACTTCGGCTCCC
Mullidae	Mullus	surmuletus	null
Mullidae	Mullus	surmuletus	null
Mullidae	Mullus	surmuletus	null
Mullidae	Mullus	surmuletus	null
Mullidae	Mullus	surmuletus	null
Mullidae	Mullus	surmuletus	null

Figure 3.8.- View Data Tool. Example of Visualization of cyt*b* sequences from each *Mullus surmuletus* specimen within FishTrace database. This tool is the basis of the validation process.

Figure 3.9.- *Online* tool to search species in the FishTrace database.

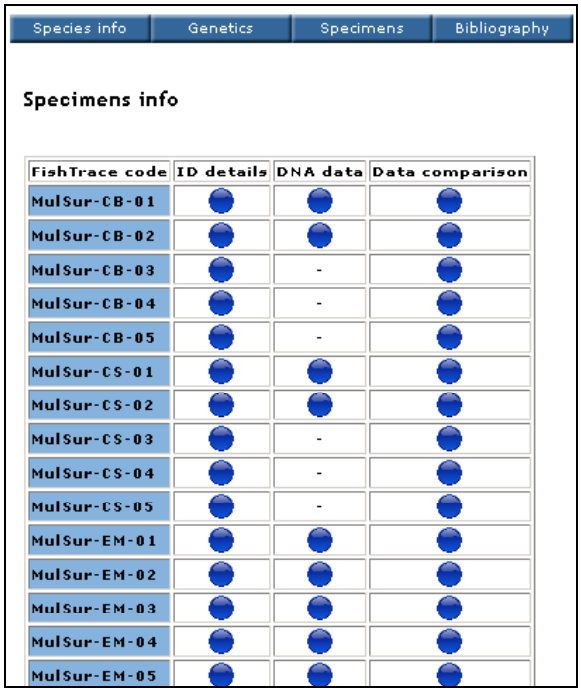
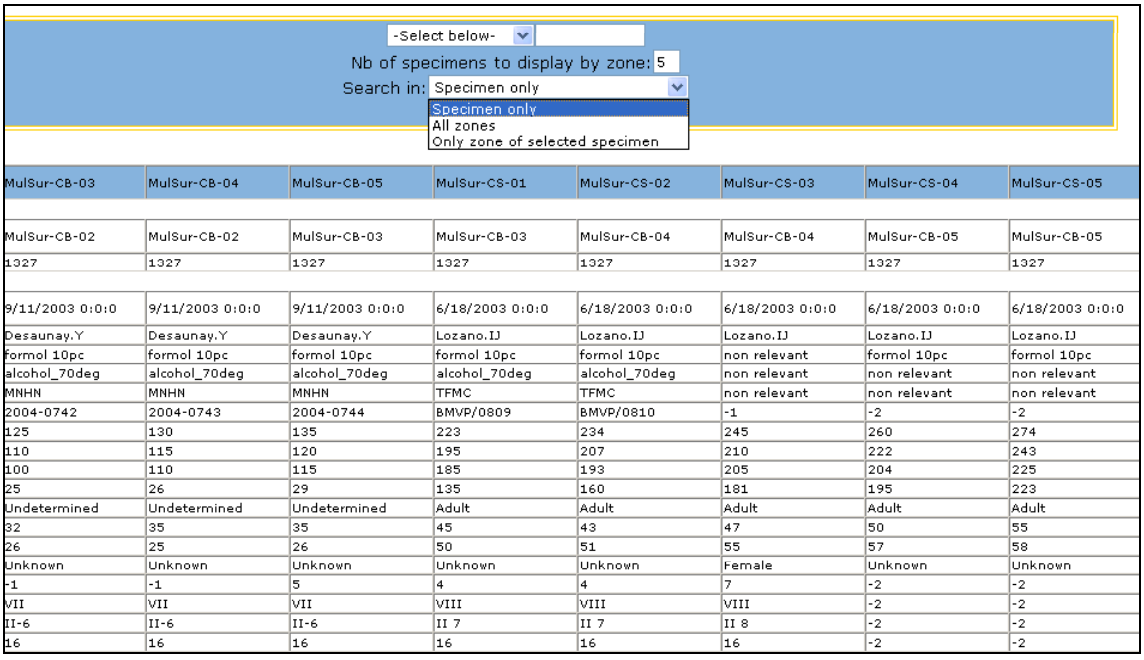


Figure 3.10.- "Specimens info" table, where the information available for each specimen can be selected.



MulSur-CB-03	MulSur-CB-04	MulSur-CB-05	MulSur-CS-01	MulSur-CS-02	MulSur-CS-03	MulSur-CS-04	MulSur-CS-05
MulSur-CB-02	MulSur-CB-02	MulSur-CB-03	MulSur-CB-03	MulSur-CB-04	MulSur-CB-04	MulSur-CB-05	MulSur-CB-05
1327	1327	1327	1327	1327	1327	1327	1327
9/11/2003 0:0:0	9/11/2003 0:0:0	9/11/2003 0:0:0	6/18/2003 0:0:0	6/18/2003 0:0:0	6/18/2003 0:0:0	6/18/2003 0:0:0	6/18/2003 0:0:0
Desaunay.Y	Desaunay.Y	Desaunay.Y	Lozano.IJ	Lozano.IJ	Lozano.IJ	Lozano.IJ	Lozano.IJ
formol 10pc	formol 10pc	formol 10pc	formol 10pc	formol 10pc	non relevant	formol 10pc	formol 10pc
alcohol_70deg	alcohol_70deg	alcohol_70deg	alcohol_70deg	alcohol_70deg	non relevant	non relevant	non relevant
MNHN	MNHN	MNHN	TFMC	TFMC	non relevant	non relevant	non relevant
2004-0742	2004-0743	2004-0744	BMVP/0809	BMVP/0810	-1	-2	-2
125	130	135	223	234	245	260	274
110	115	120	195	207	210	222	243
100	110	115	185	193	205	204	225
25	26	29	135	160	181	195	223
Undetermined	Undetermined	Undetermined	Adult	Adult	Adult	Adult	Adult
32	35	35	45	43	47	50	55
26	25	26	50	51	55	57	58
Unknown	Unknown	Unknown	Unknown	Unknown	Female	Unknown	Unknown
-1	-1	5	4	4	7	-2	-2
VII	VII	VII	VIII	VIII	VIII	-2	-2
II-6	II-6	II-6	II 7	II 7	II 8	-2	-2
16	16	16	16	16	16	-2	-2

Figure 3.11.- *Online* table to compare all data from chosen specimens.

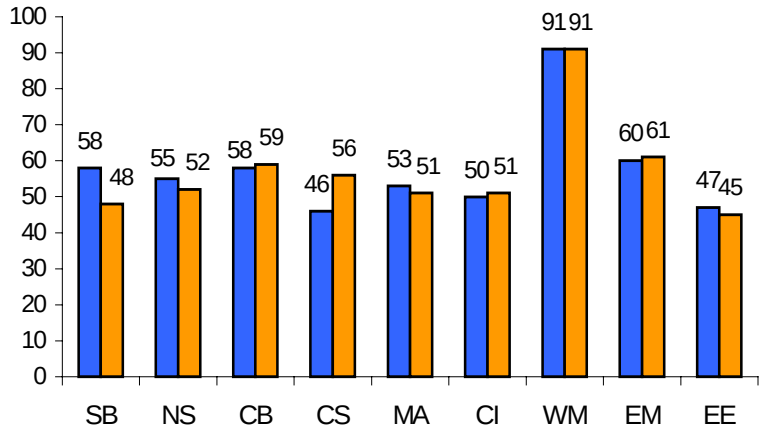


Figure 4.1.- Relationship between planned (in blue)[1] and completed number of species sampled (in orange).

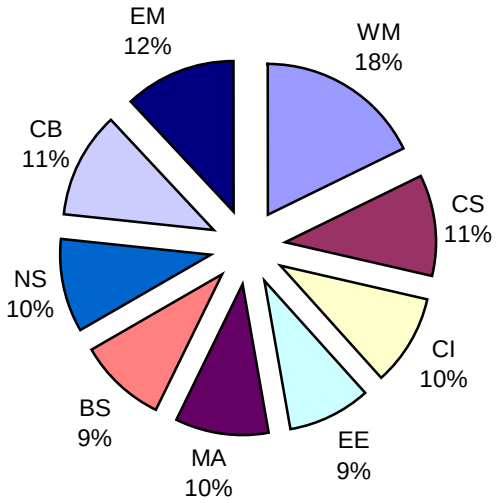


Figure 4.2.- Percentage of species at each geographical area covered by FishTrace.

[1] If printed in black and white printer, orange will appears as light grey and blue will appears as dark grey.

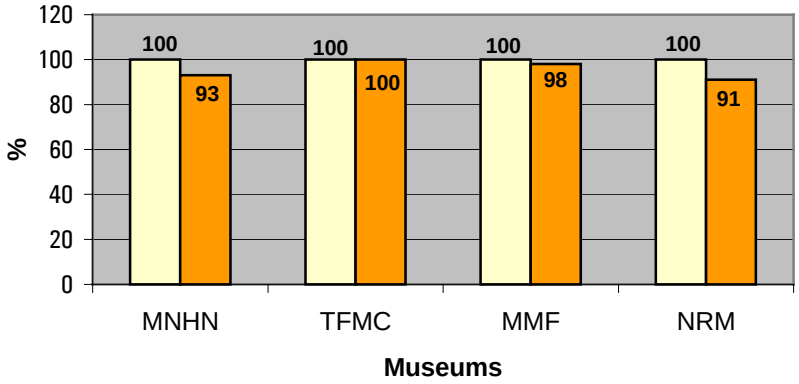


Figure 4.3.- FishTrace Reference Collections (vouchers and otoliths) in each museum at the end of the project. Orange bars represent the percentage of reference collections completed in each museum in June, 2006. Yellow bars are the main goal of FishTrace project.

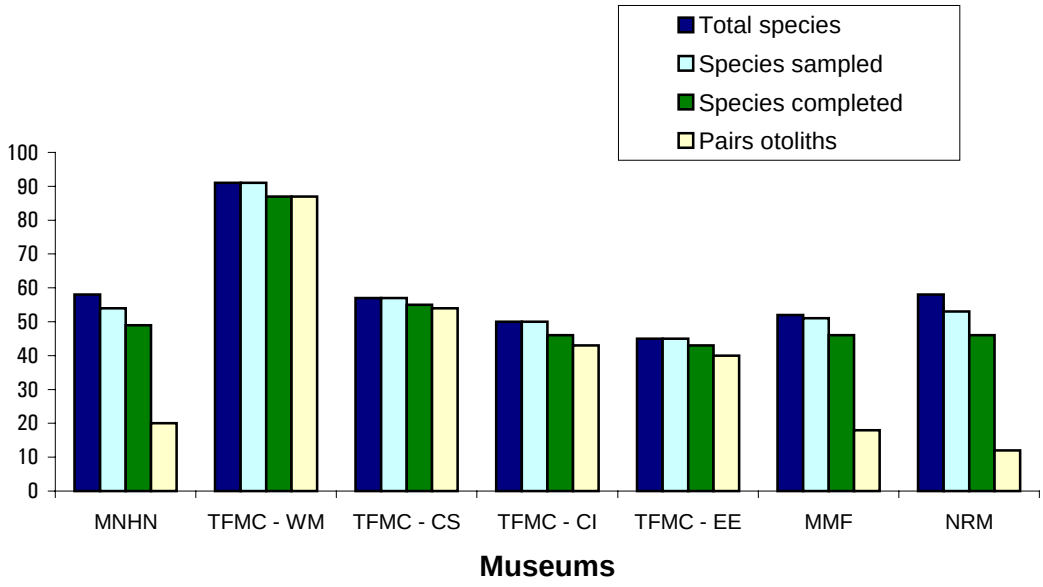


Figure 4.4.- FishTrace reference collections (vouchers and otoliths) in each museum.

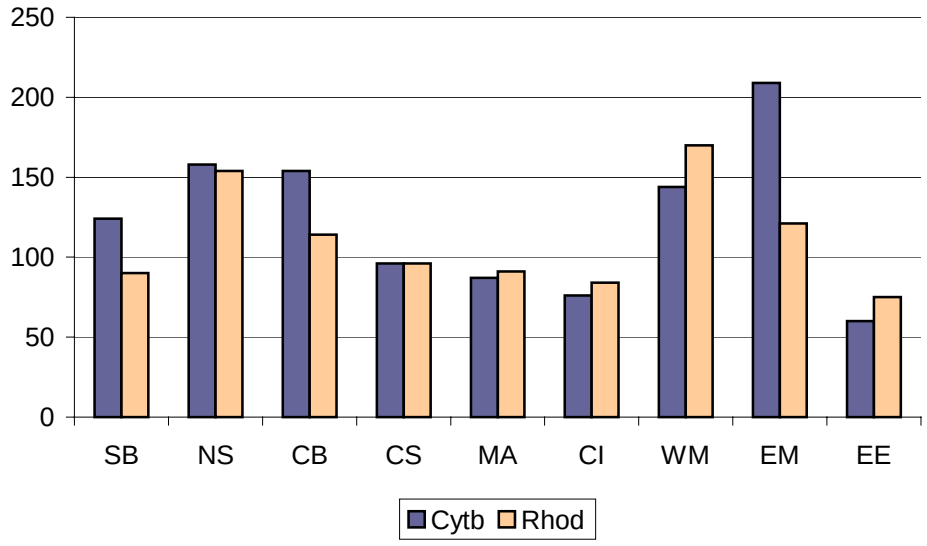


Figure 4.5.- Curated and validated cyt*b* and rhod sequences obtained from fish specimens caught at each geographical area covered within FishTrace .

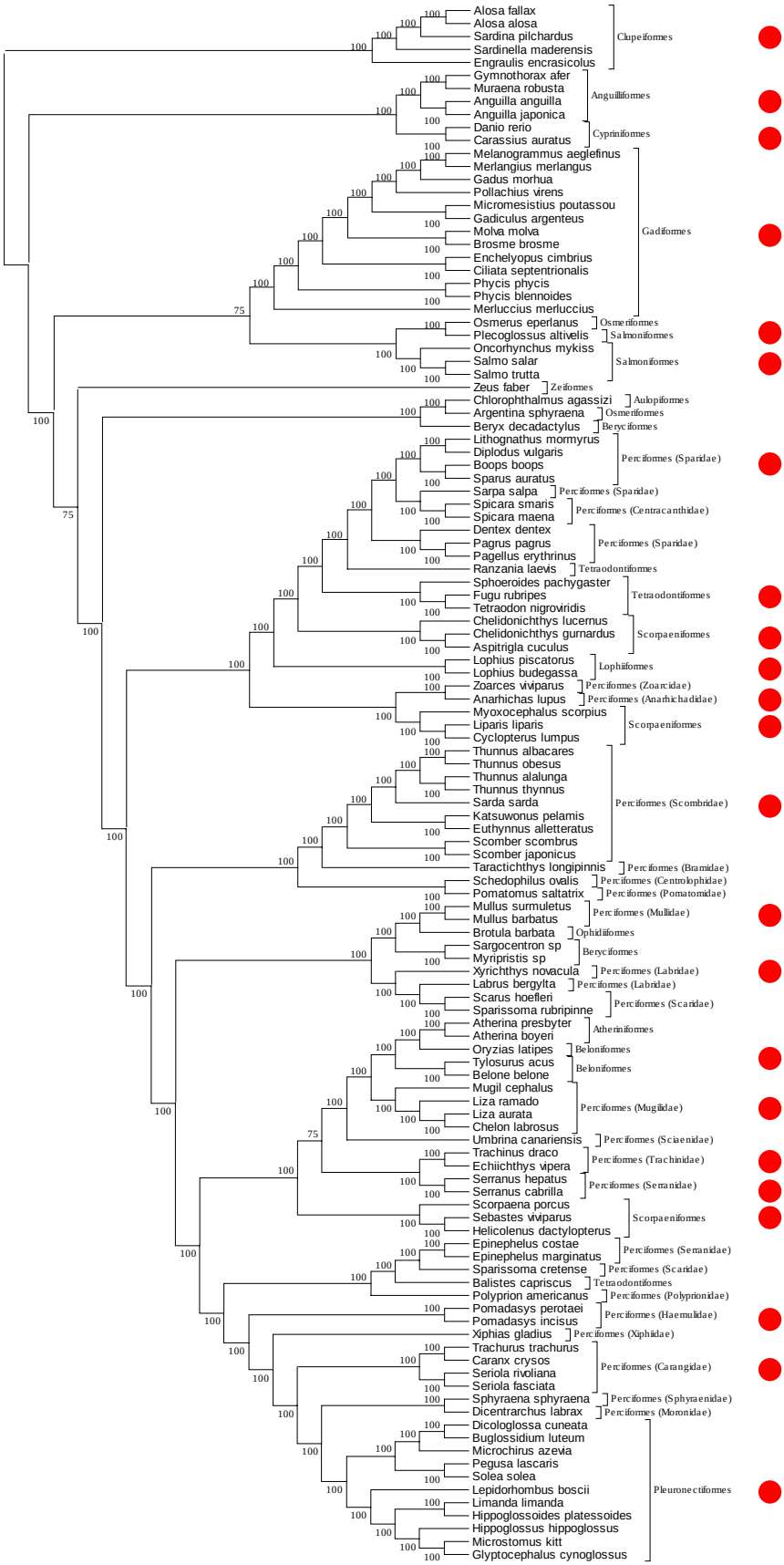


Figure 4.6.- Most parsimonious (MP) tree resulted from the unweighted Ts/Tv analysis of 120 fish DNA-barcodes (Table 4.6). Average bootstrap values from 1000 replicates are given in nodes. Red[1] dots indicate monophyletic clades repeated in the phylogenetic inferences performed using 3 sets of data: cyt*b* (1141 bp), rhod (460 bp) and cyt*b* + rhod (1601 bp) DNA sequences.

[1] If printed in black and white printer, red will appears as light grey.

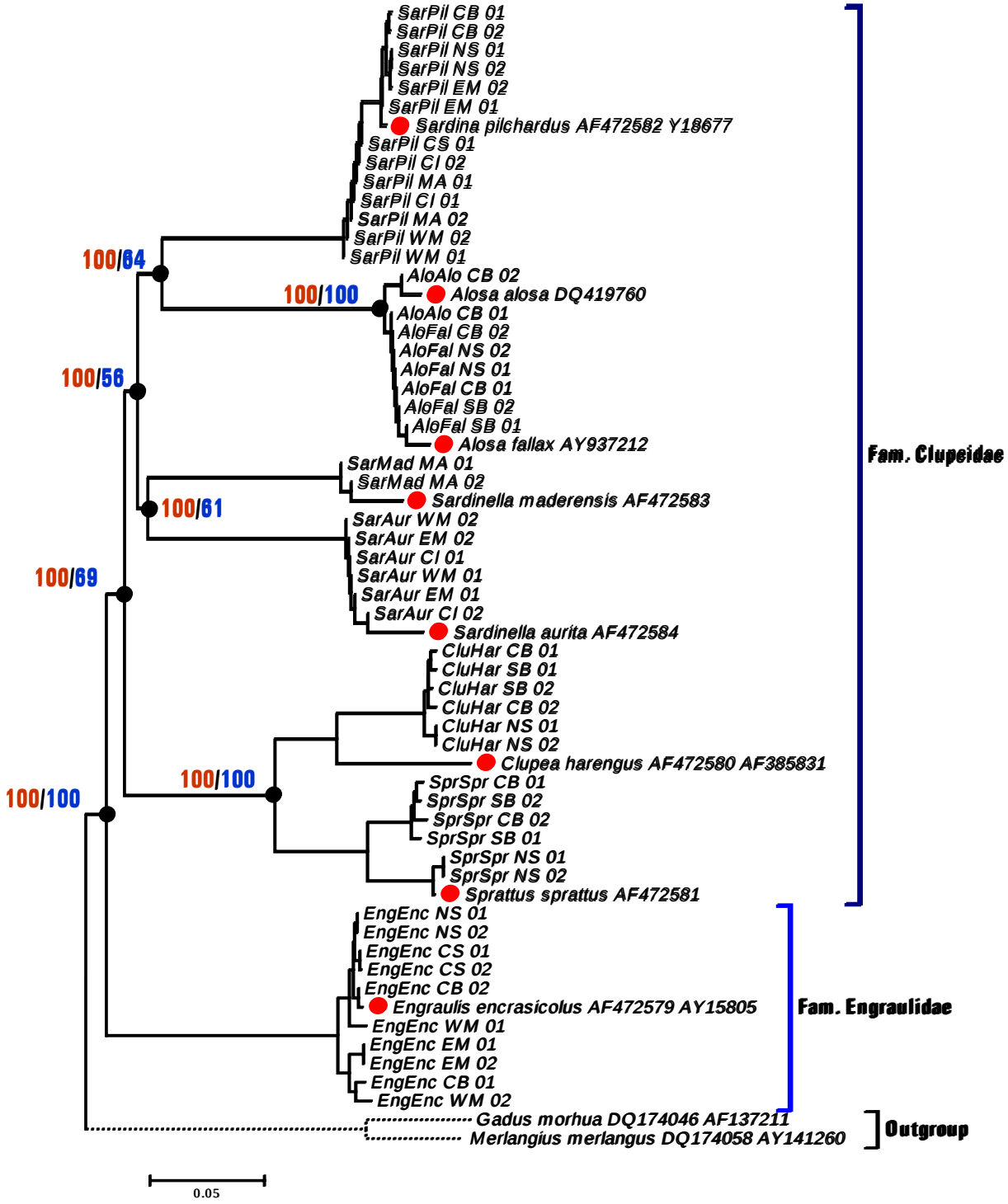


Figure 4.7.- Reconciled phylogenetic tree from the ME and MP analyses performed on 51 FishTrace clupeiform DNA-barcodes. Bootstrap values after 1000 replicates, shown in nodes, correspond to MP/ME values respectively. Sequences taken from GenBank are labelled with a red dot[2].

[2] If printed in black and white printer, red will appears as light grey.

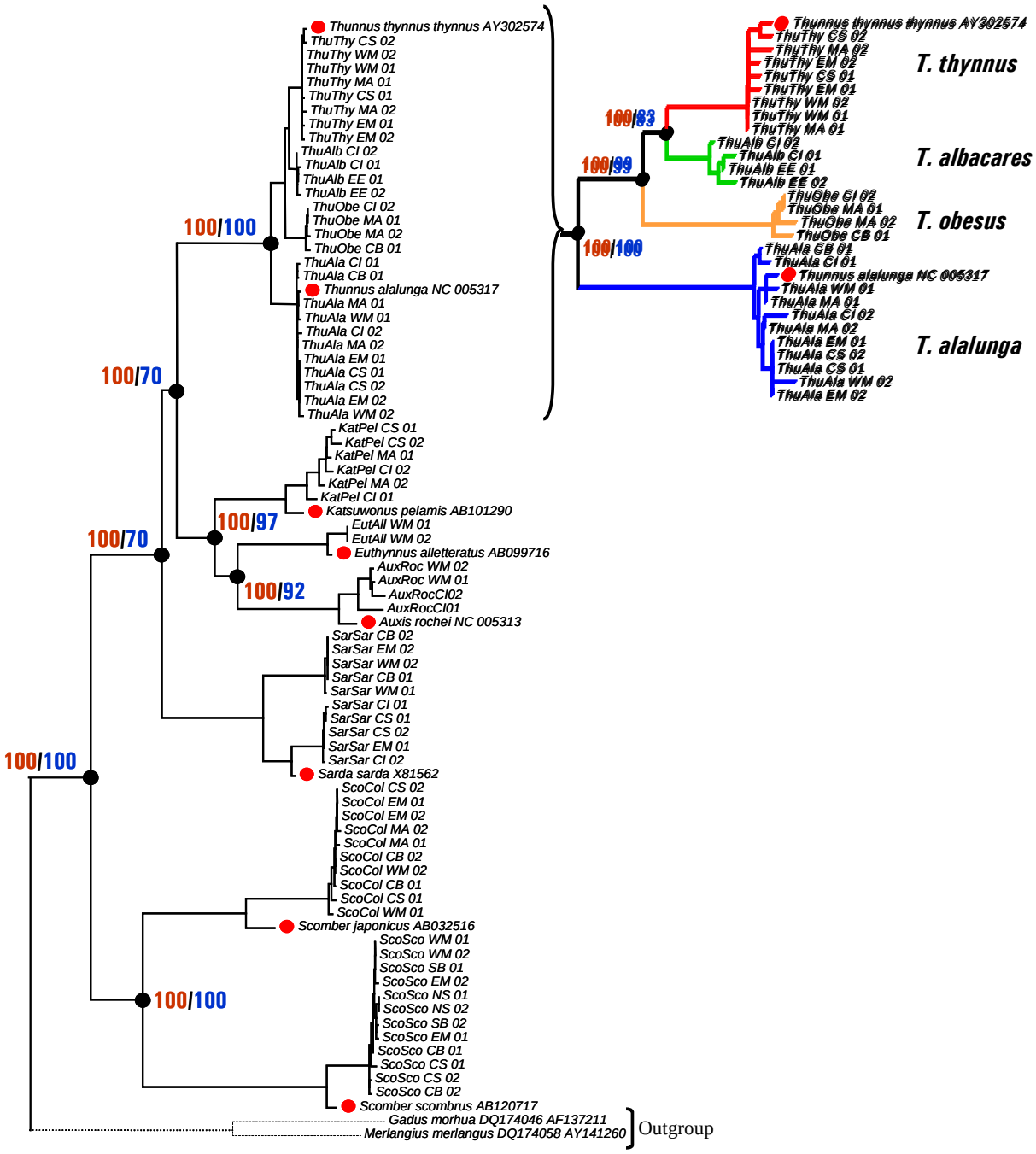


Figure 4.8.- Reconciled phylogenetic tree from the MP and ME analyses performed on 71 FishTrace Scombridae DNA-barcodes. Bootstrap values after 1000 replicates, shown in nodes, correspond to MP/ME values respectively. Sequences taken from GenBank are labelled with a red dot[3].

[3] If printed in black and white printer, red will appears as light grey.

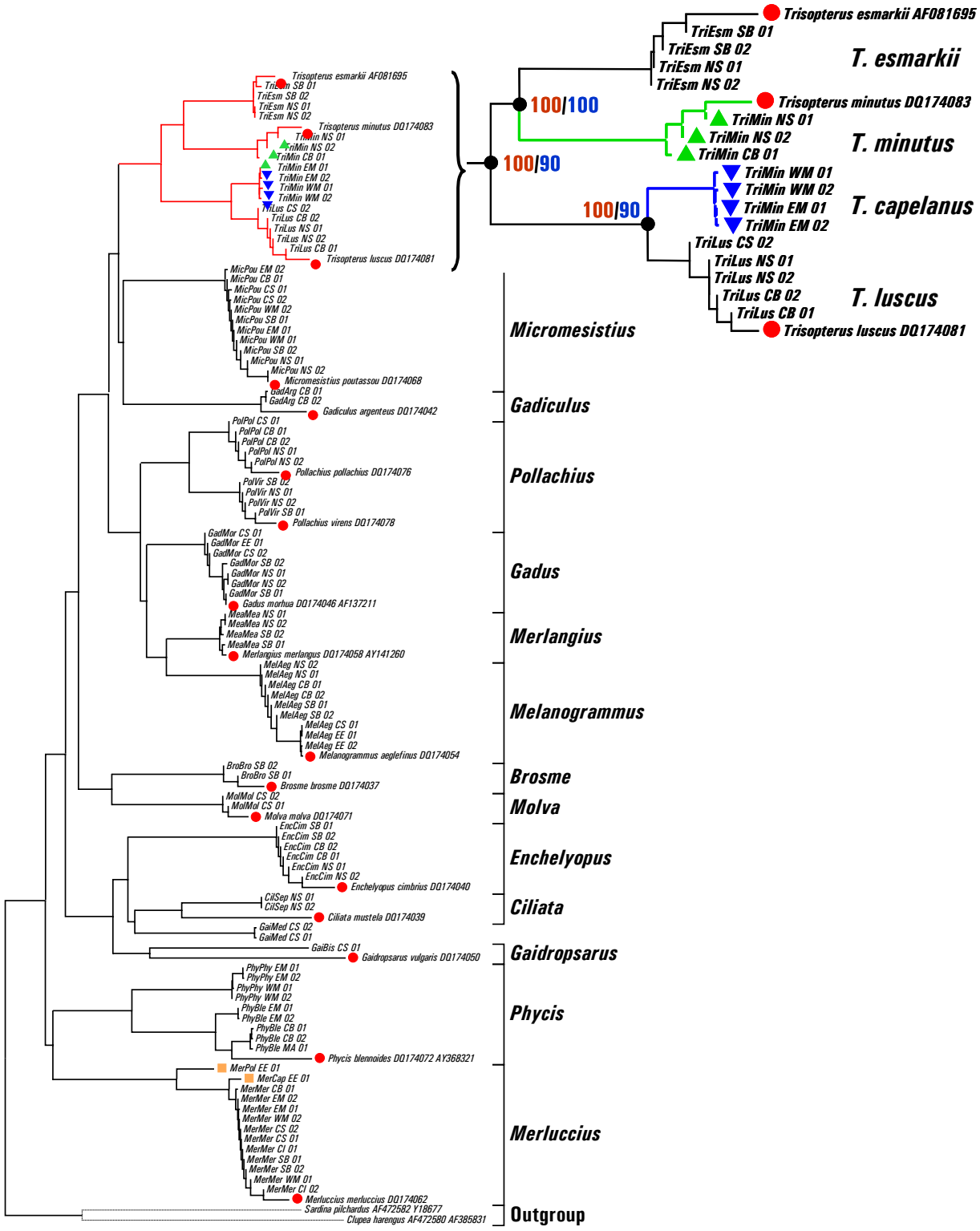


Figure 4.9.- Reconciled phylogenetic tree from the MP and ME analyses performed on 95 gadid taxa for the identification of *Trisopterus spp*. specimens. Bootstrap values after 1000 replicates, shown in nodes, correspond to MP/ME values respectively. Sequences taken from GenBank are labelled with a red dot[4]. Single FishTrace *Merluccius polli* and *M. capensis* included in the analyses are labelled with orange squares. FishTrace *Trisopterus minutus* specimens from NS and CB are labelled with green triangles and *T. minutus capelanus* from both Mediterranean areas sampled within FishTrace (EM/WM) are labelled with blue triangles.

[4] If printed in B&W printer, orange, gren and red will appears as light grey and blue will appears as dark grey.

Figure 4.10.- Genetic relations between distinct populations of *Solea solea*. Each circle represents distinct samples as indicated (n=14, 22, 20, 15, 20 for CB, EM, BS, WM, and NS, respectively). Overlapping circles indicate non-significant differences in genetic population structure, as resulted from population pairwise F_{ST}. This two-dimensional model attempts also to consider and to illustrate the relative genetic distances between populations.

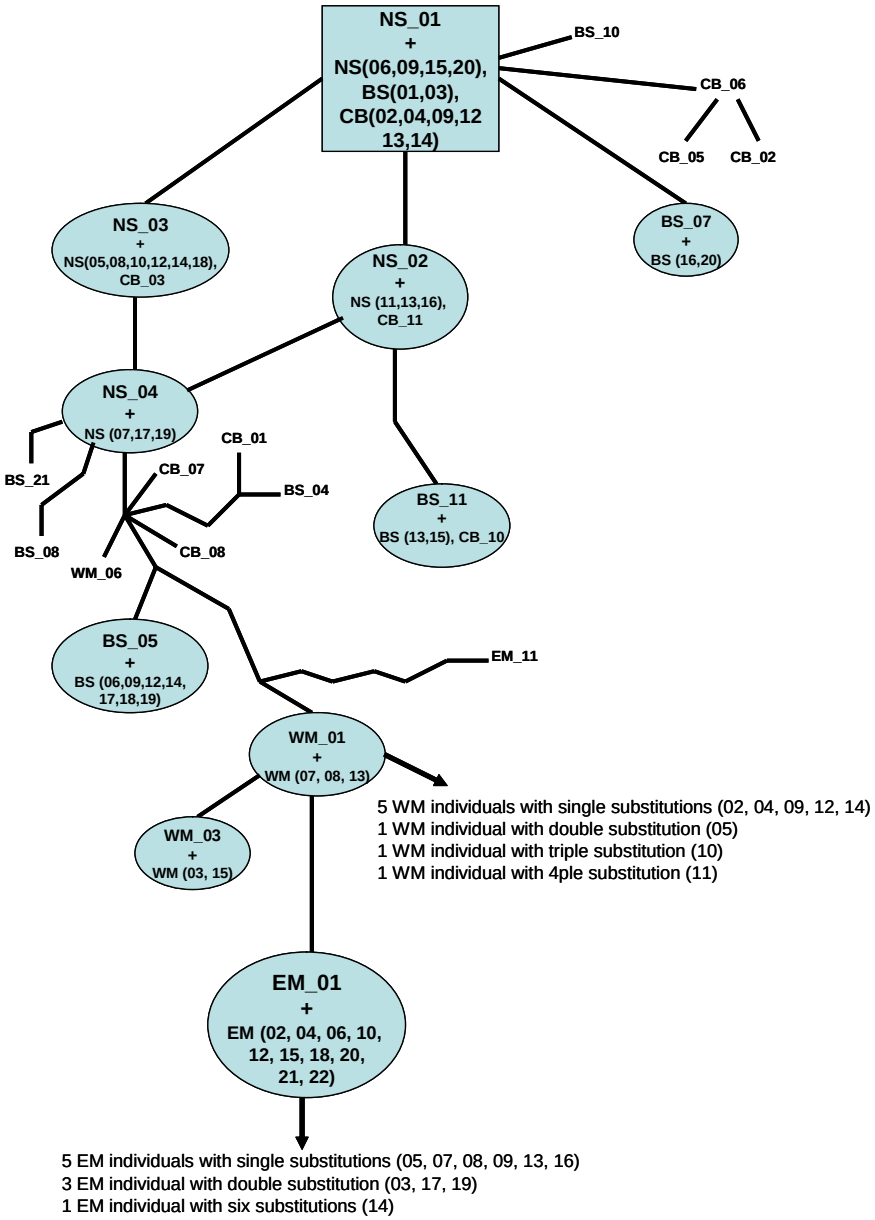


Figure 4.11.- Haplotype connectivity network analysis of *Solea solea* populations with the TCS v1.21 algorithm.

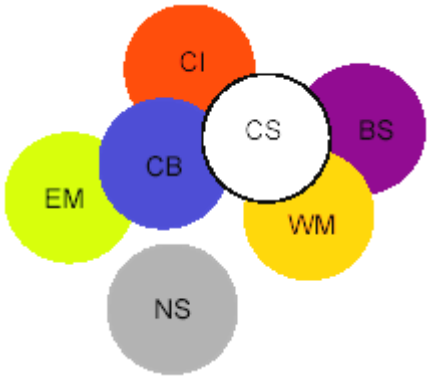


Figure 4.12.- Genetic relations between distinct populations of *Meluccius merluccius*. Each circle represents distinct samples as indicated (n=18, 20, 18, 19, 19, 20,18 for EM, WM, CB, CI, CS, BS, and NS, respectively). Overlapping circles indicate non-significant differences in genetic population structure, as resulted from population pairwise FST. This two-dimensional model attempts also to consider and to illustrate the relative genetic distances between populations.

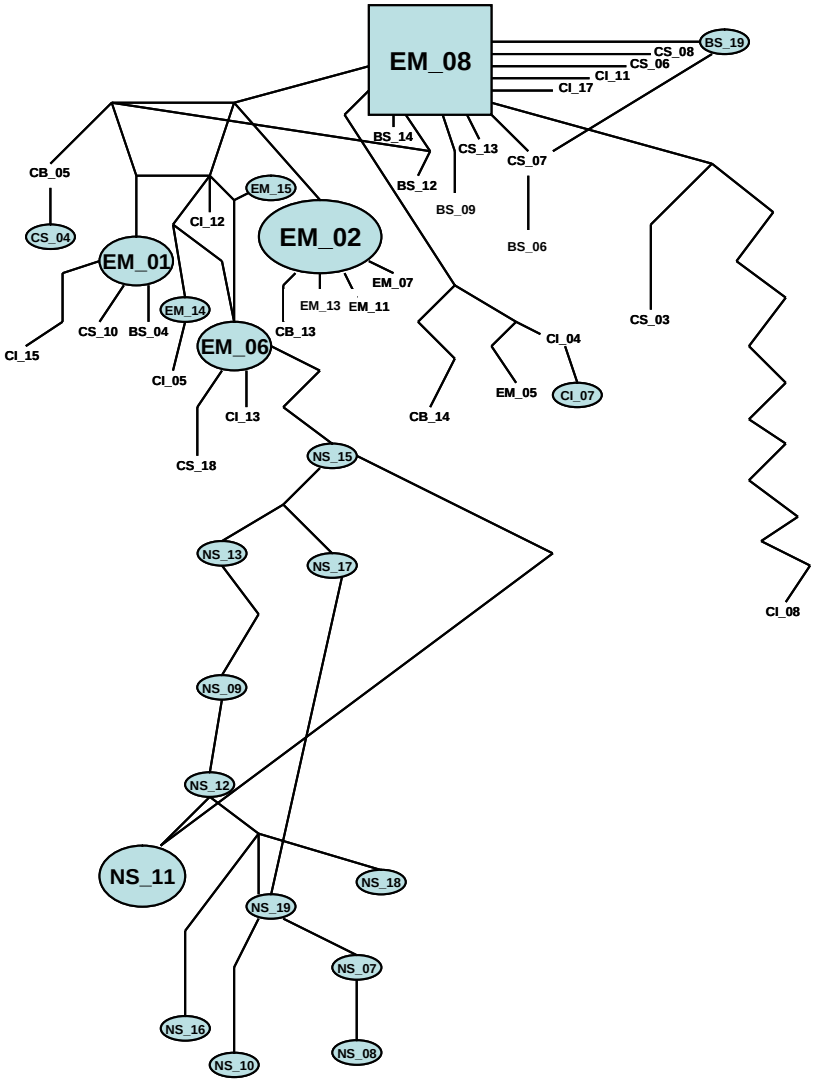


Figure 4.13.- Haplotype connectivity network analysis of *Meluccius merluccius* populations with the TCS v1.21 algorithm.

Figure 4.14.- Genetic relations between distinct populations of *Micromesistius poutassou*. Each circle represents distinct samples as indicated (n=20, 20, 18, 20, 16 for EM, CB, WM, NS, and CS, respectively). Overlapping circles indicate non-significant differences in genetic population structure, as resulted from population pairwise F_{ST}. This two-dimensional model attempts also to consider and to illustrate the relative genetic distances between populations.

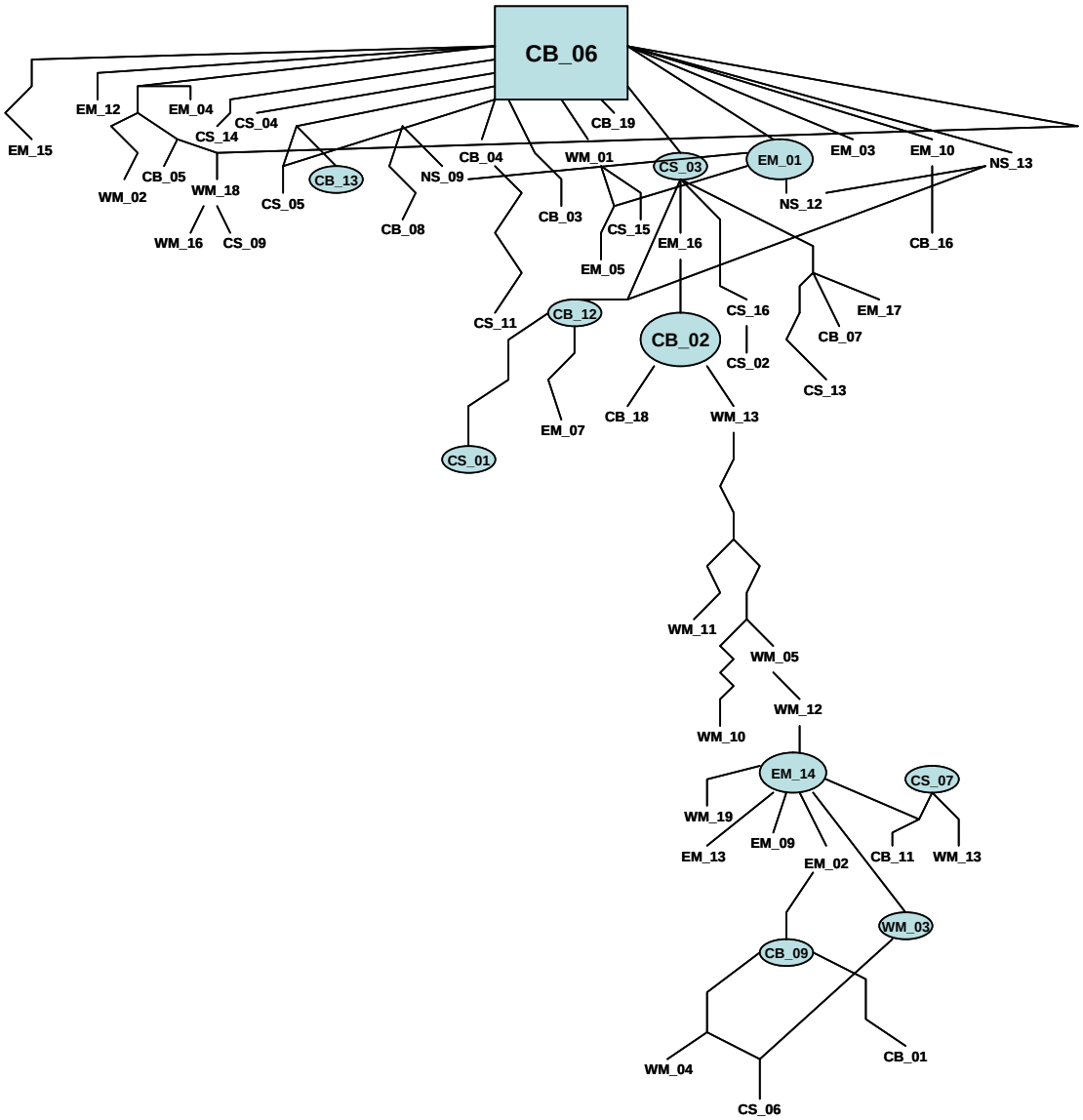


Figure 4.15.- Haplotype connectivity network analysis of *Micromesistius poutassou* populations with the TCS v1.21 algorithm.

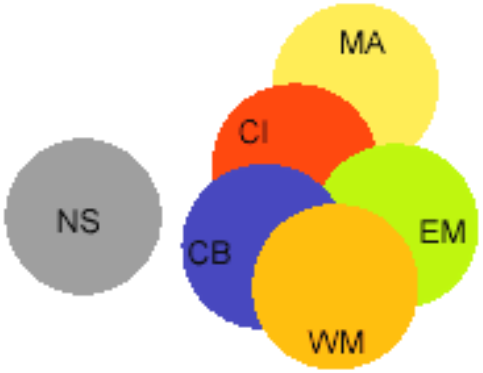


Figure 4.16.- Genetic relations between distinct populations of *Mullus surmuletus*. Each circle represents distinct samples as indicated (n=20, 20, 19, 18, 16, 8 for EM, NS, CB, WM, CI and MA, respectively). Overlapping circles indicate non-significant differences in genetic population structure, as resulted from population pairwise F_{ST}. This two-dimensional model attempts also to consider and to illustrate the relative genetic distances between populations.

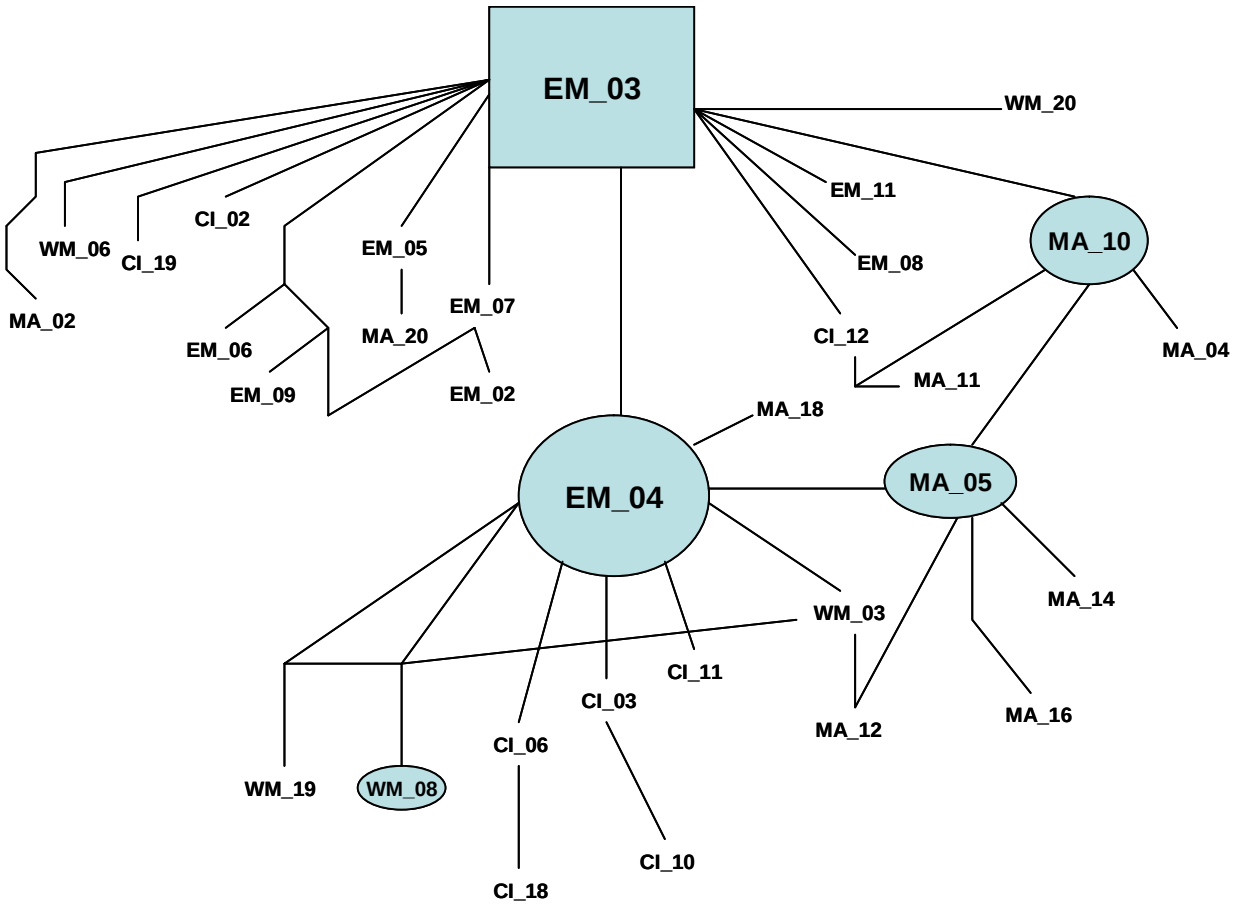


Figure 4.17.- Haplotype connectivity network analysis of *Mullus surmuletus* populations with the TCS v1.21 algorithm.

Figure 4.18.- Genetic relations between distinct populations of *Pagrus pagrus*. Each circle represents distinct samples as indicated (n=20, 20, 20, 12 for CI, WM, MA, and EM, respectively). Overlapping circles indicate non-significant differences in genetic population structure, as resulted from population pairwise FST. This two-dimensional model attempts also to consider and to illustrate the relative genetic distances between populations.

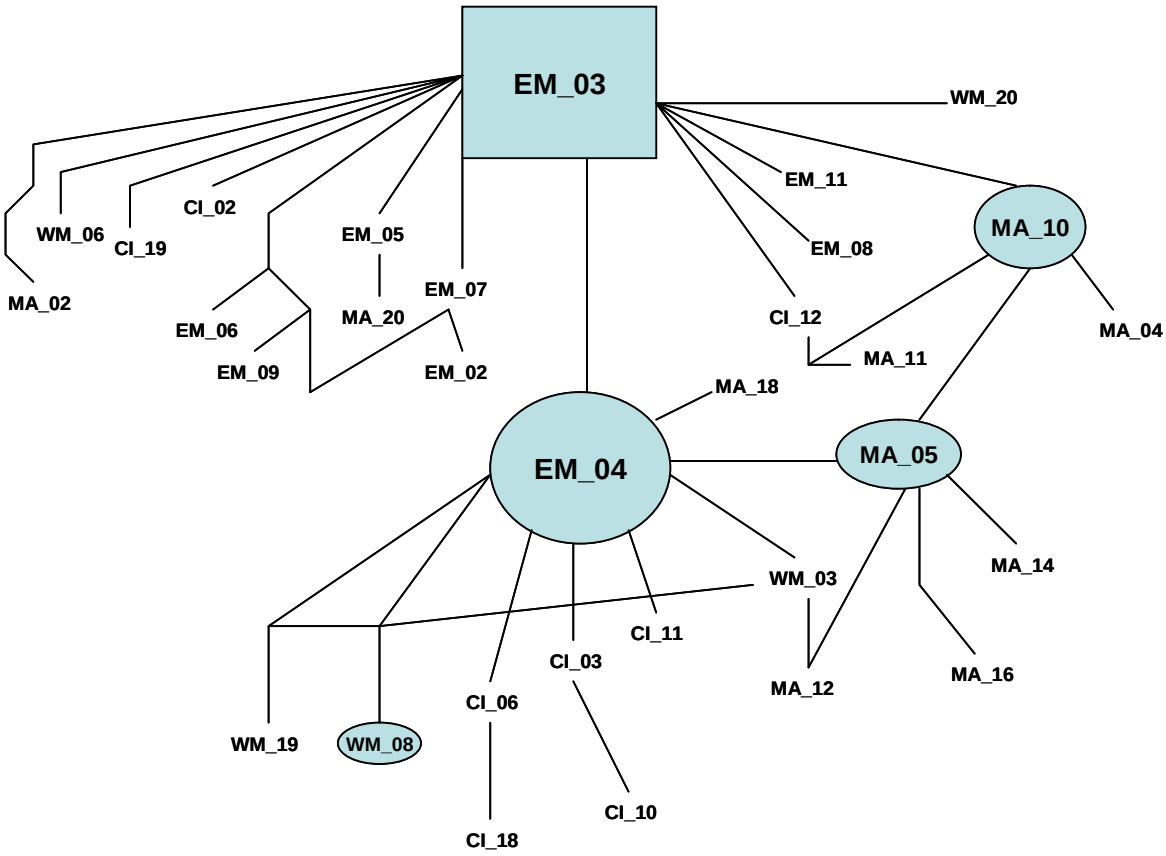


Figure 4.19.- Haplotype connectivity network analysis of *Pagrus pagrus* populations with the TCS v1.21 algorithm.

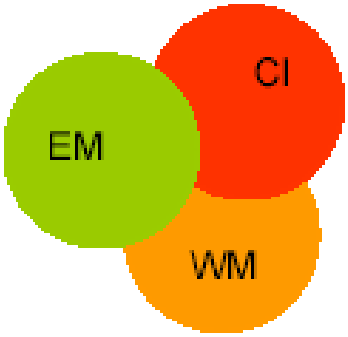


Figure 4.20.- Genetic relations between distinct populations of *Pagellus erythrinus*. Each circle represents distinct samples as indicated (n=19, 18, 17 for CI, EM, and WM, respectively). Overlapping circles indicate non-significant differences in genetic population structure, as resulted from population pairwise F_{ST}.

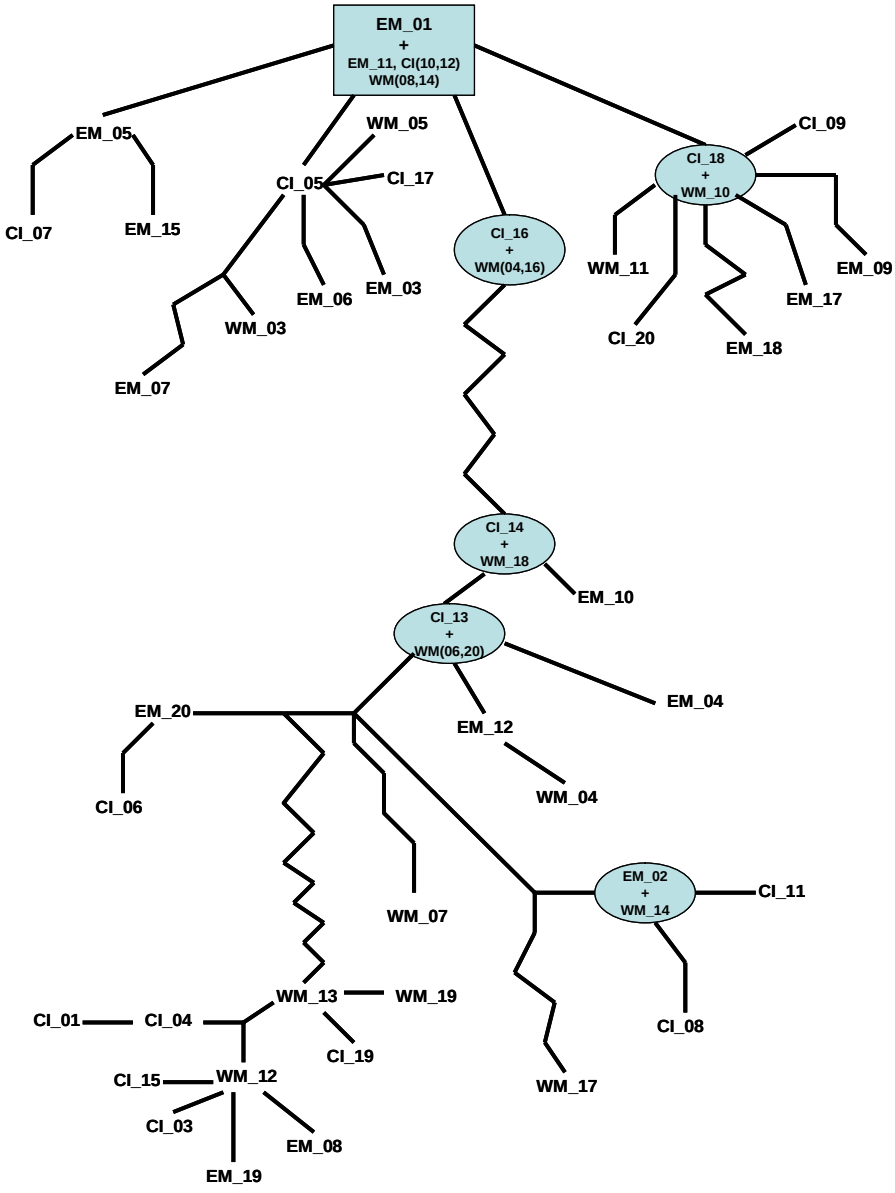


Figure 4.21.- Haplotype connectivity network analysis of *Pagellus erythrinus* populations with the TCS v1.21 algorithm.

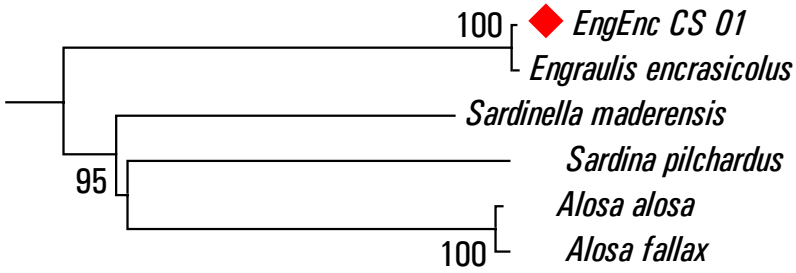


Figure 4.22.- NJ subtree extracted from the unrooted NJ tree containing 121 taxa (Table 4.6), phylogenetically analyzed for the validation of the FishTrace EngEnc-CS-01 DNA-barcode. Target taxon has been labelled with a red diamond. Bootstrap values are given in nodes.

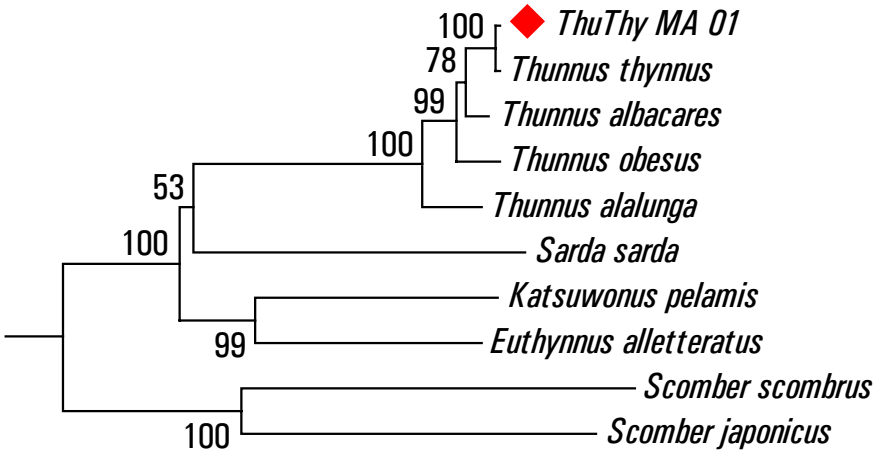


Figure 4.23.- NJ subtree extracted from the unrooted NJ tree containing 121 taxa (Table 4.6), phylogenetically analyzed for the validation of the FishTrace ThuThy-MA-01 DNA-barcode. Target taxon has been labelled with a red diamond. Bootstrap values are given in nodes.

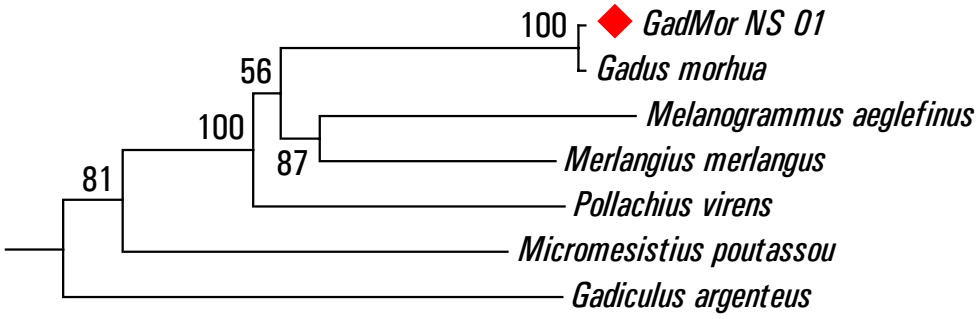


Figure 4.24.- NJ subtree extracted from the unrooted NJ tree containing 121 taxa (Table 4.6), phylogenetically analyzed for the validation of the FishTrace GadMor-NS-01 DNA-barcode. Target taxon has been labelled with a red diamond. Bootstrap values are given in nodes.

Figure 4.25.- Phylogenetic tree resulted from the bootstrap analysis of cyt*b* gene sequences through the ME method under the K2P evolutionay model. 17 target taxa (SolSol-EE-04 to SolSol-EE-20) misidentified as *Solea solea* were phylogenetically identified as *Microchirus azevia*. Bootstrap values are given in nodes.

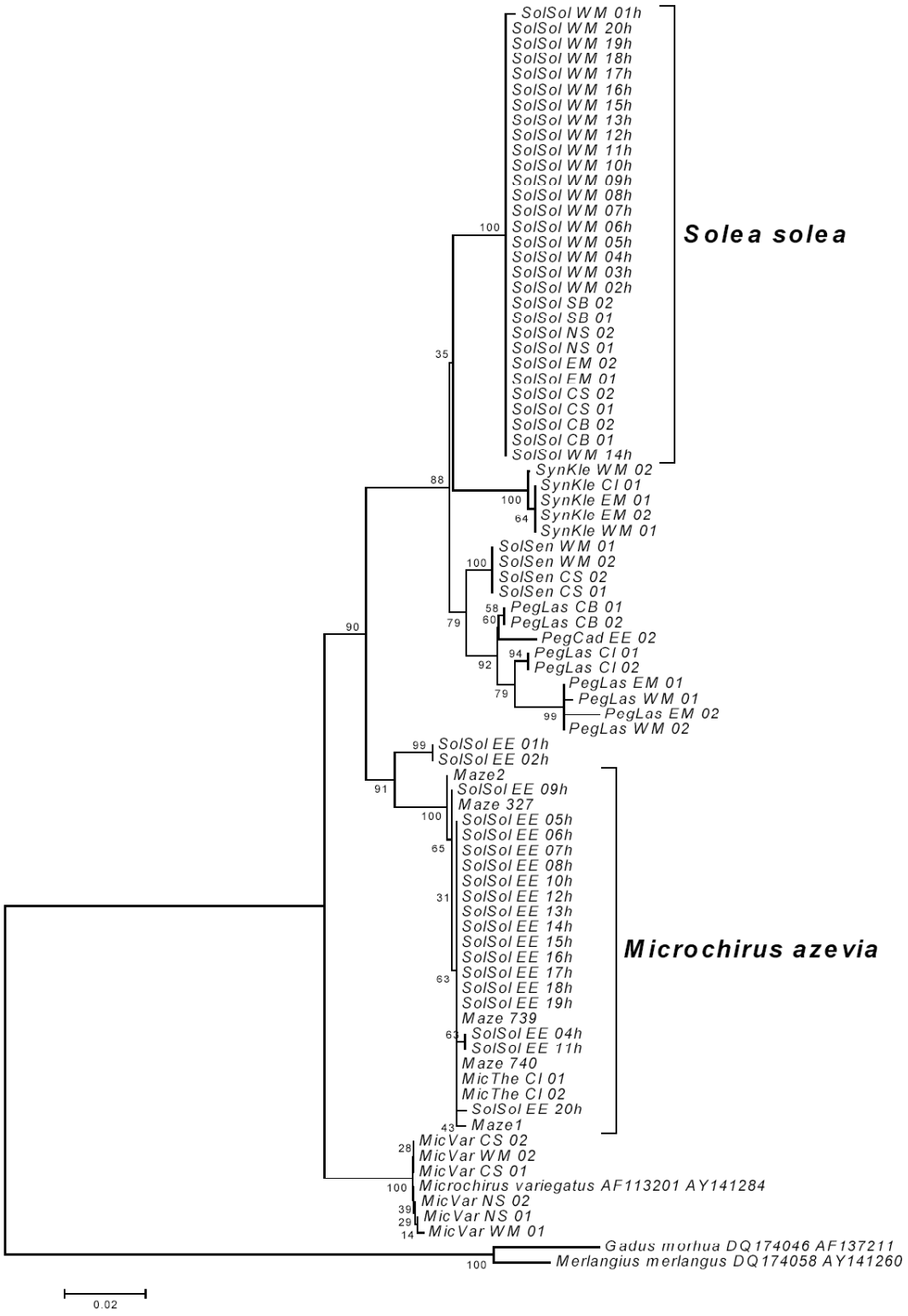


Figure 4.26.- Phylogenetic tree resulted from the bootstrap analysis of rhod gene sequences through the ME method under the K2P evolutionay model. 17 target taxa (SolSol-EE-04 to SolSol-EE-20) misidentified as *Solea solea* were phylogenetically identified as *Microchirus azevia*. Bootstrap values are given in nodes.

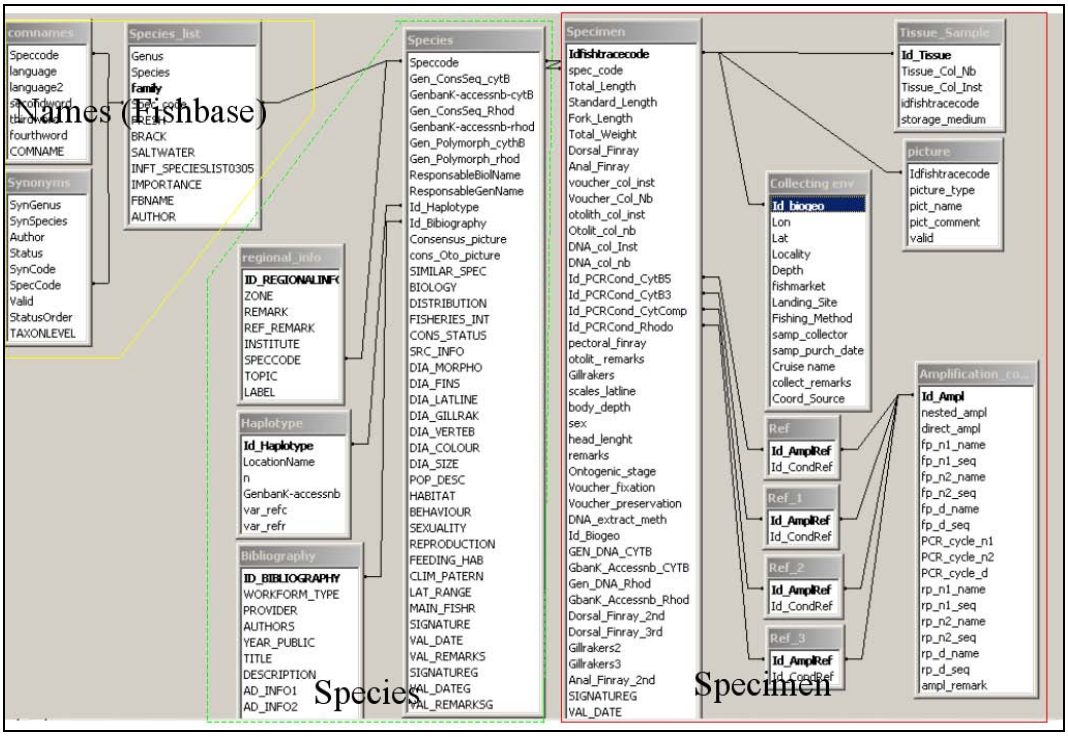


Figure 4.27.- FishTrace database entity relationship diagram (ER).

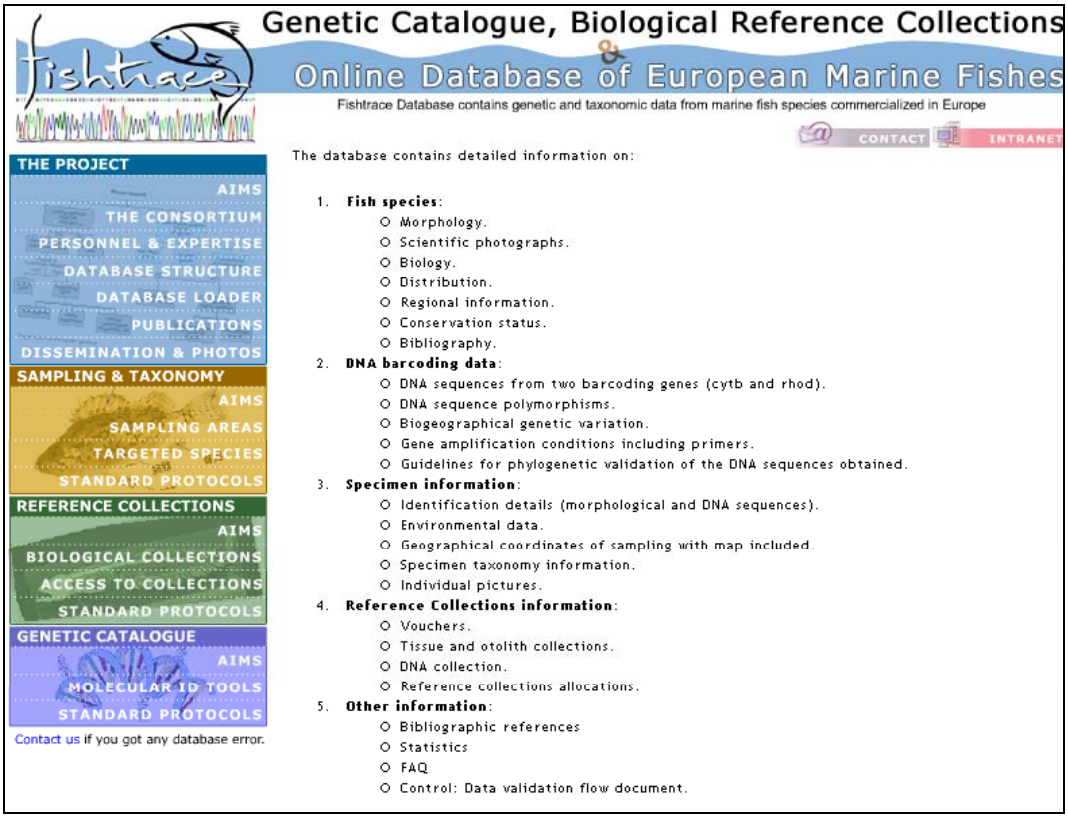


Figure 4.28.- Database Structure.

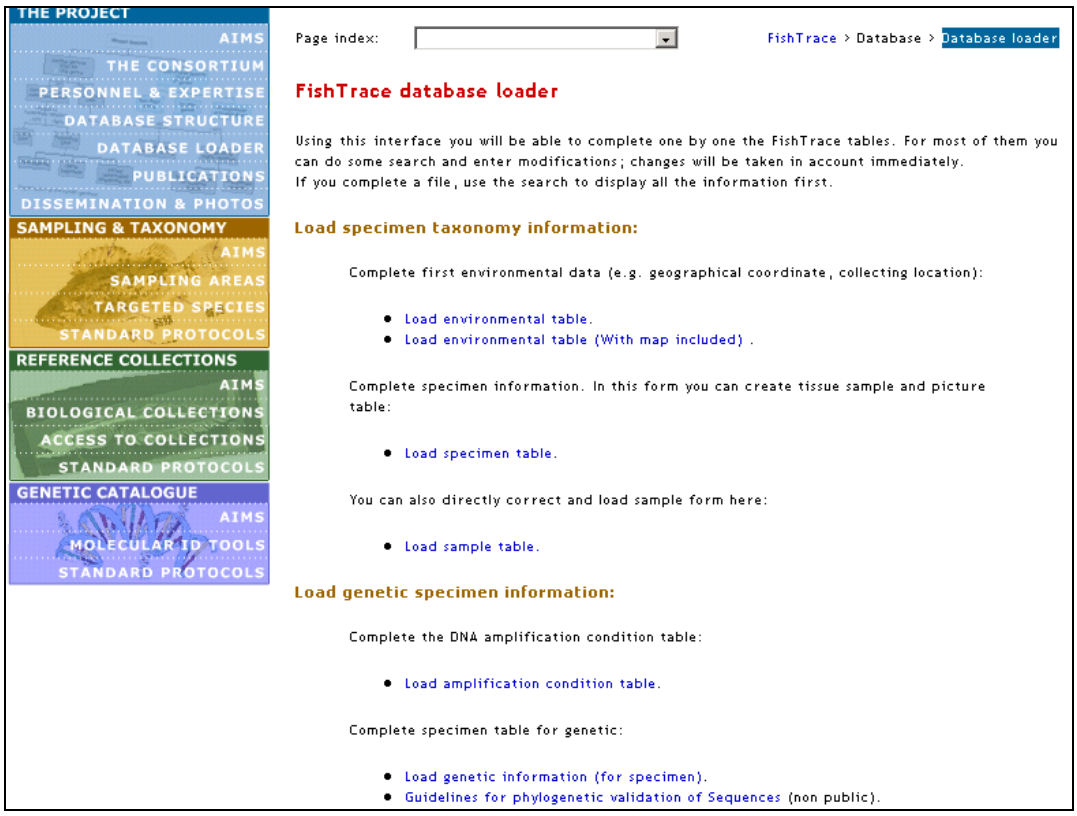


Figure 4.29.- Database Loader Interface.

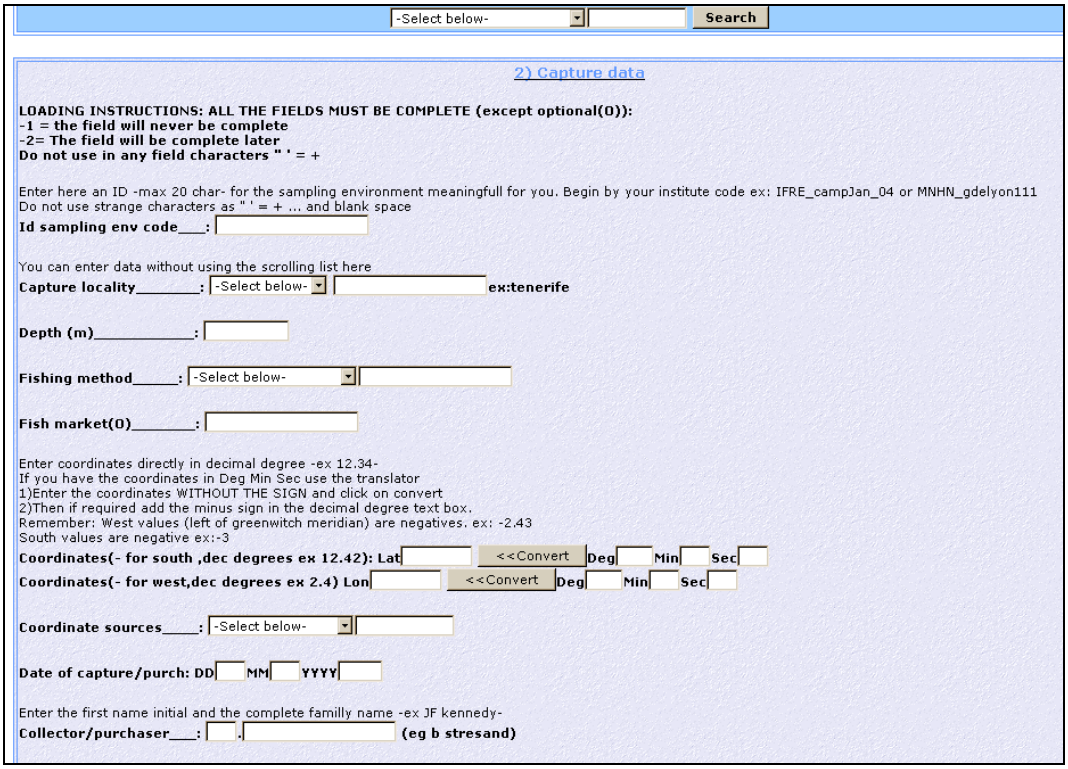


Figure 4.30.- Load Enviromental Table.

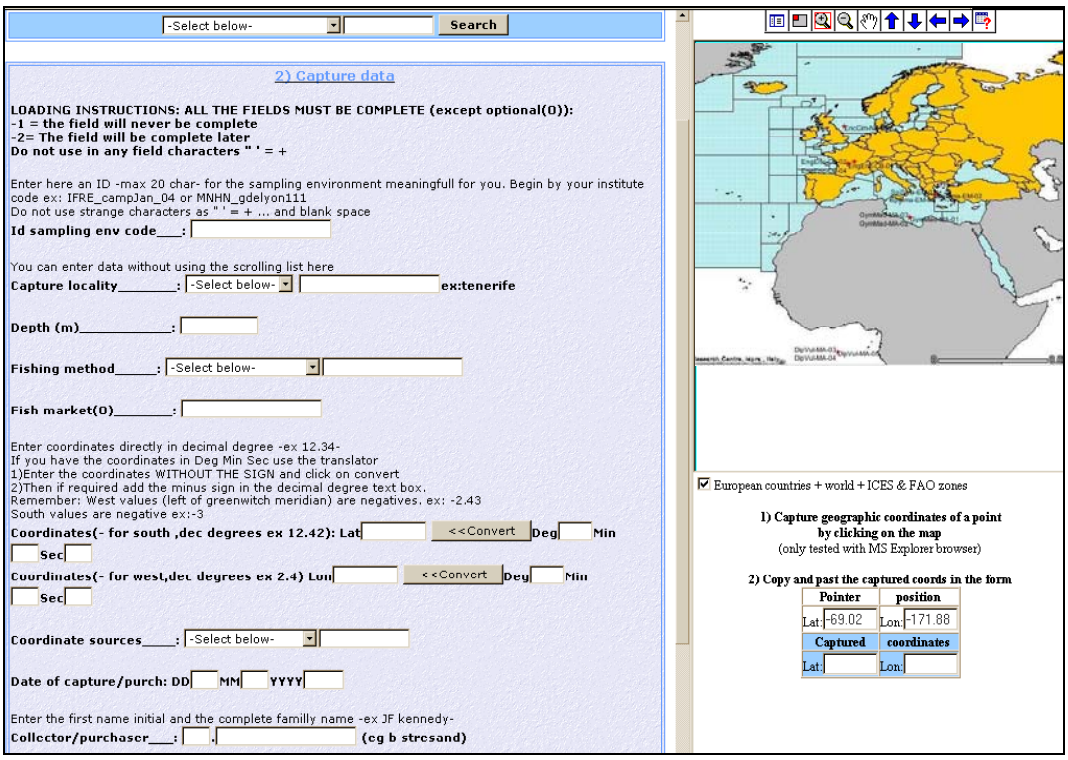


Figure 4.31.- Load Enviromental Table (with map included).

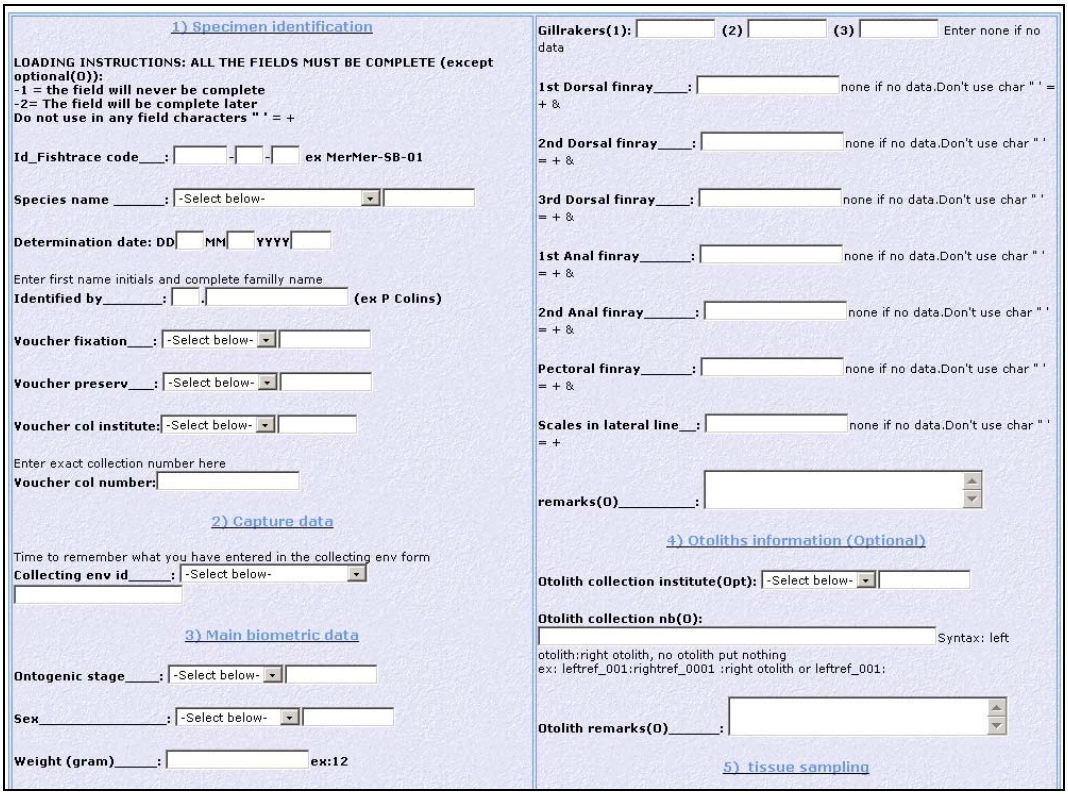


Figure 4.32.- Load Specimen Table.

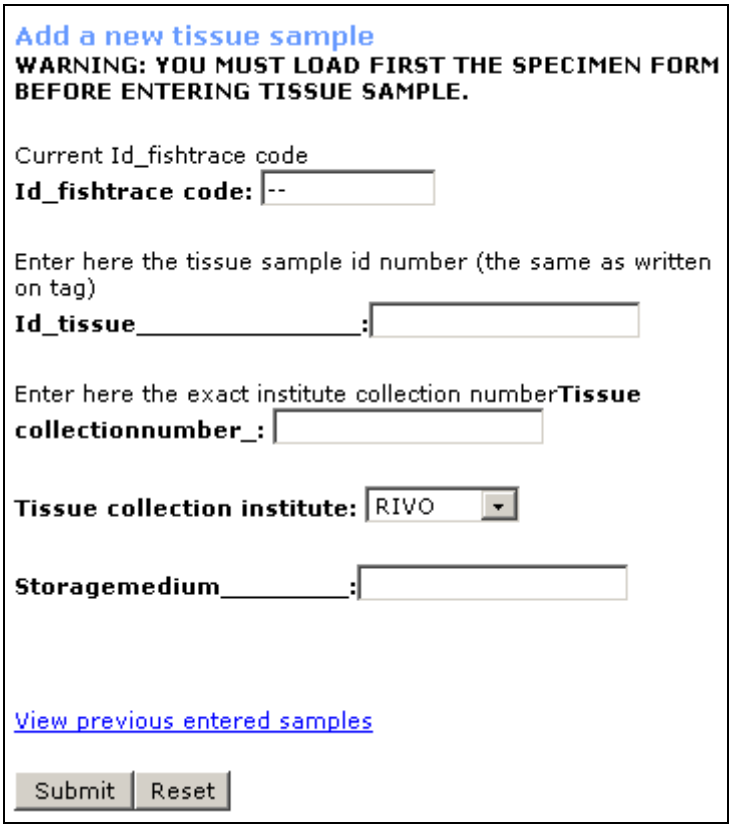


Figure 4.33.- Load Specimen Table: Add a new tissue sample.

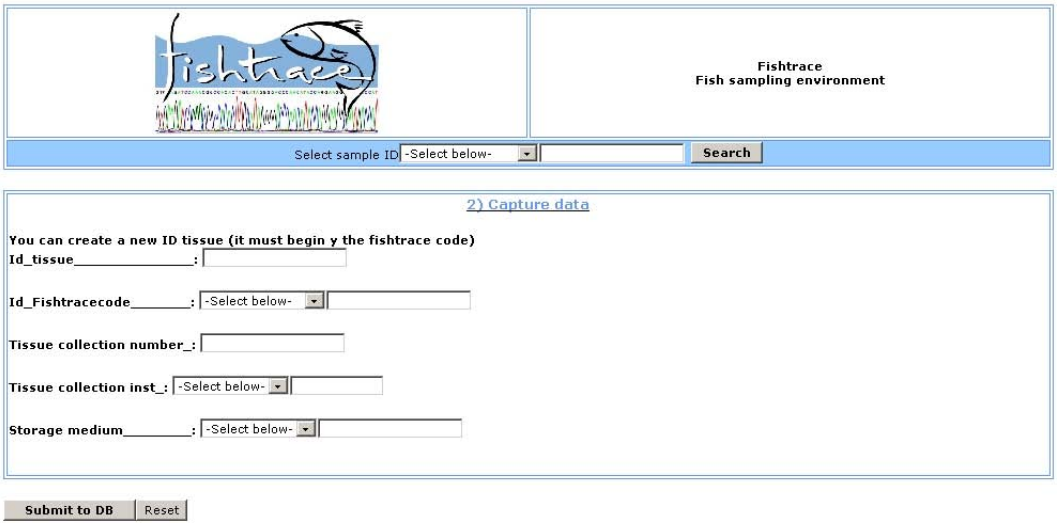


Figure 4.34.- Load Sample Table.

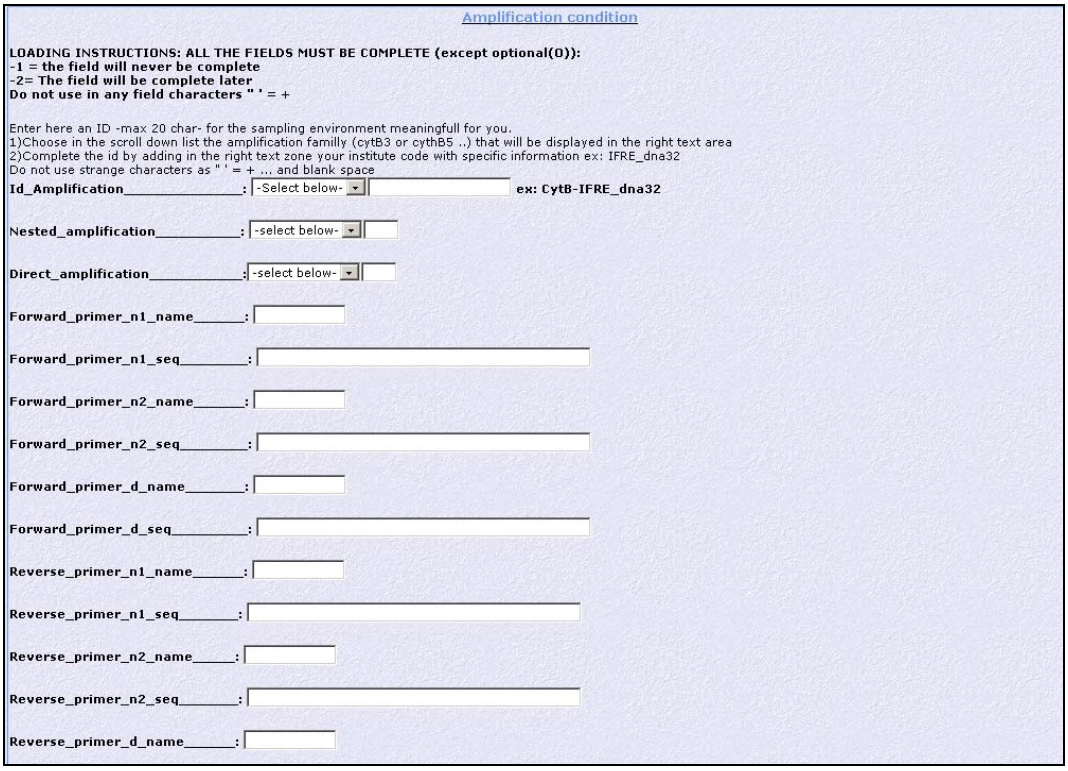


Figure 4.35.- Load Amplification Condition Table (for specimens).

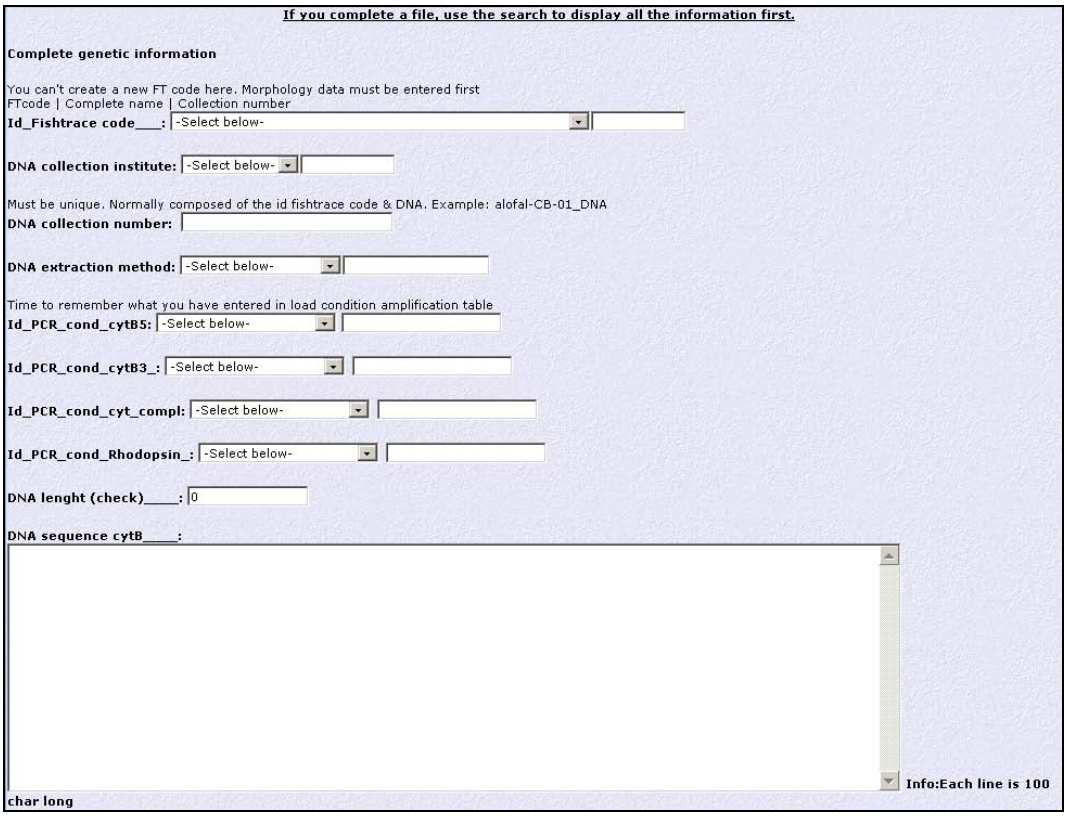


Figure 4.36.- Load Genetic Information (for specimens).

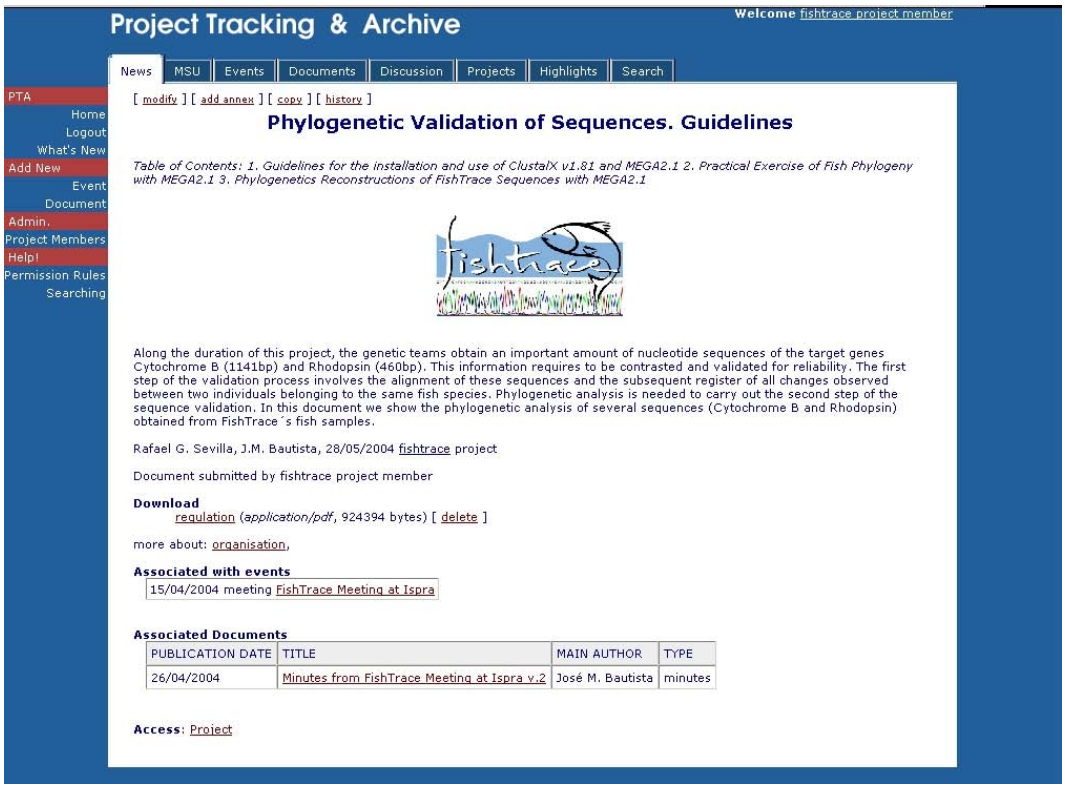


Figure 4.37.- Guidelines for Phylogenetic Validation of Sequences. Link at the PTA web page.

Figure 4.38.- Load Species Table.

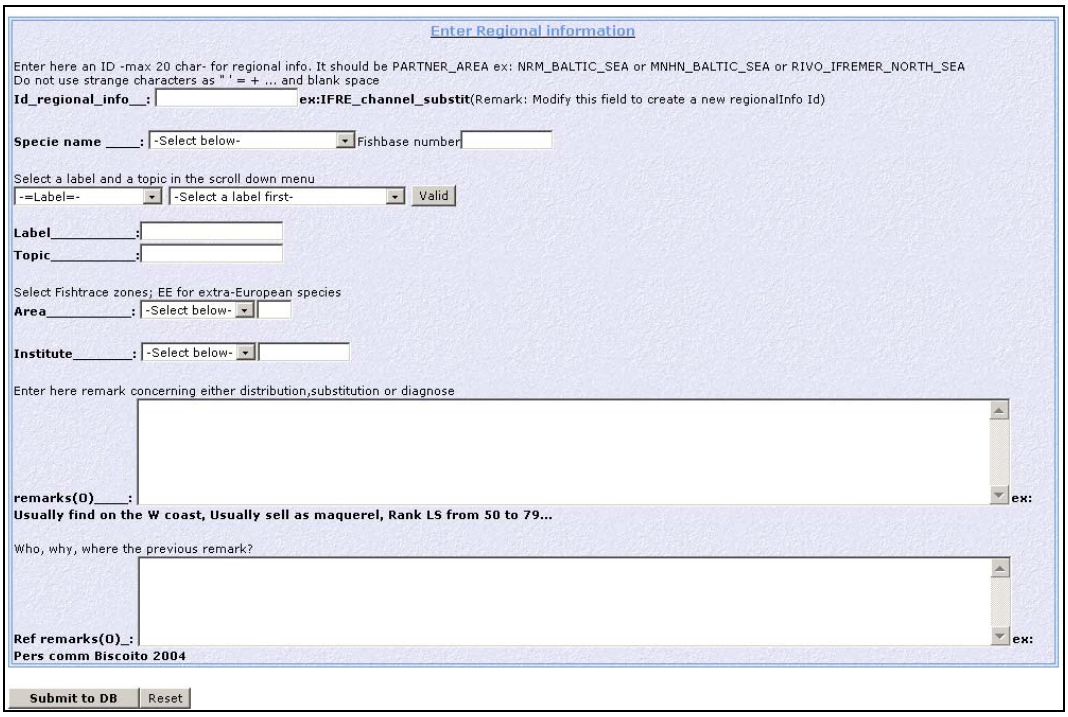


Figure 4.39.- Load Regional Information Table.

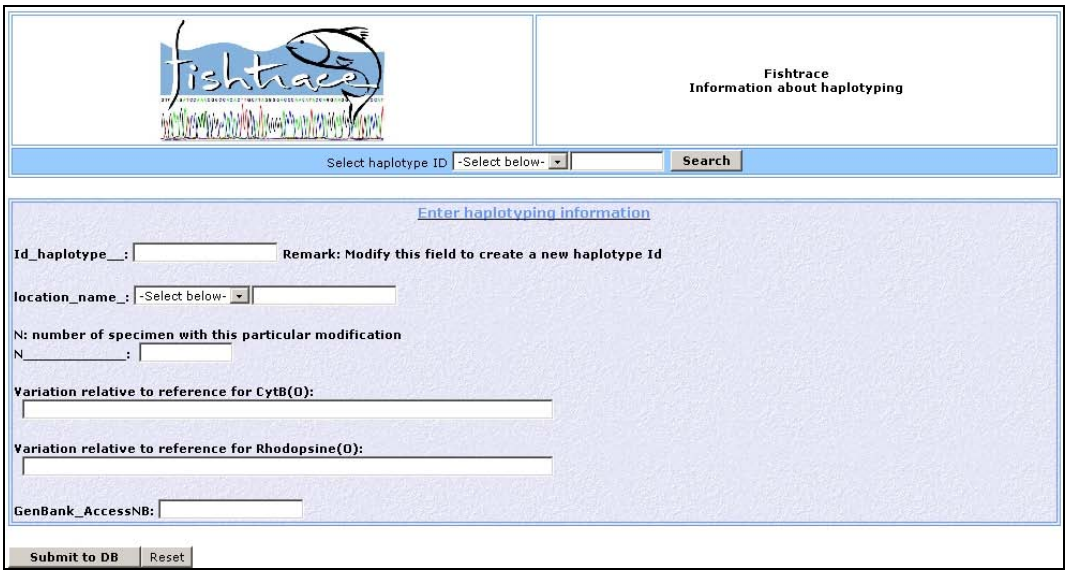


Figure 4.40.- Load Haplotyping Table.

Figure 4.41.-Load Bibliography Table.

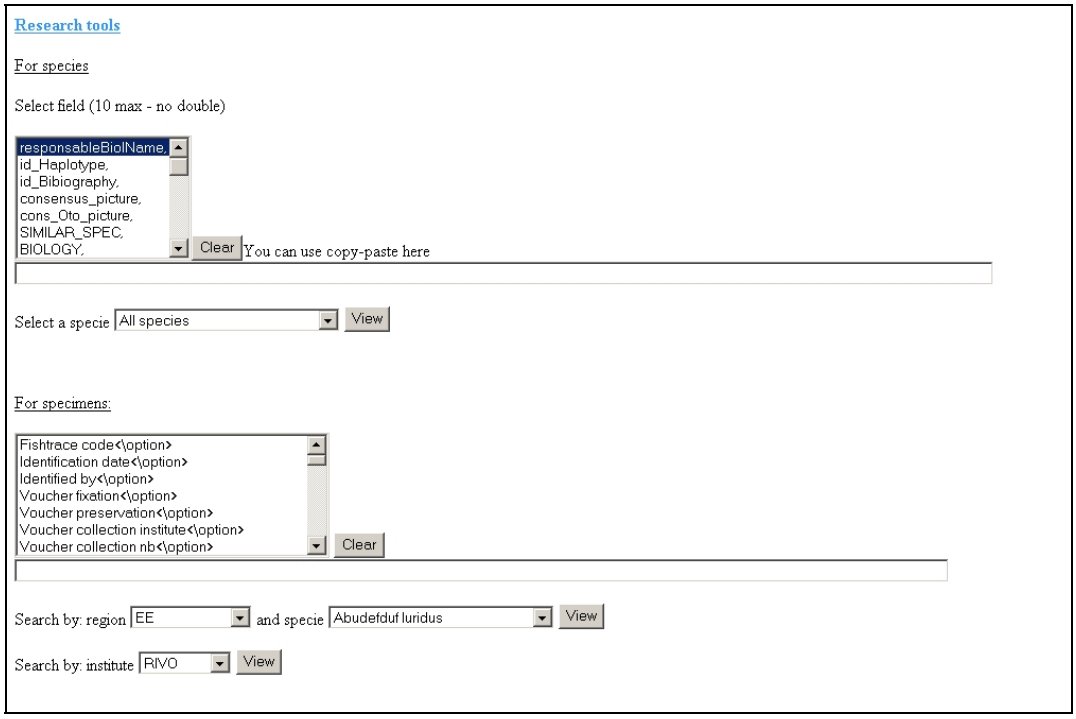


Figure 4.42.- View Data Tool.

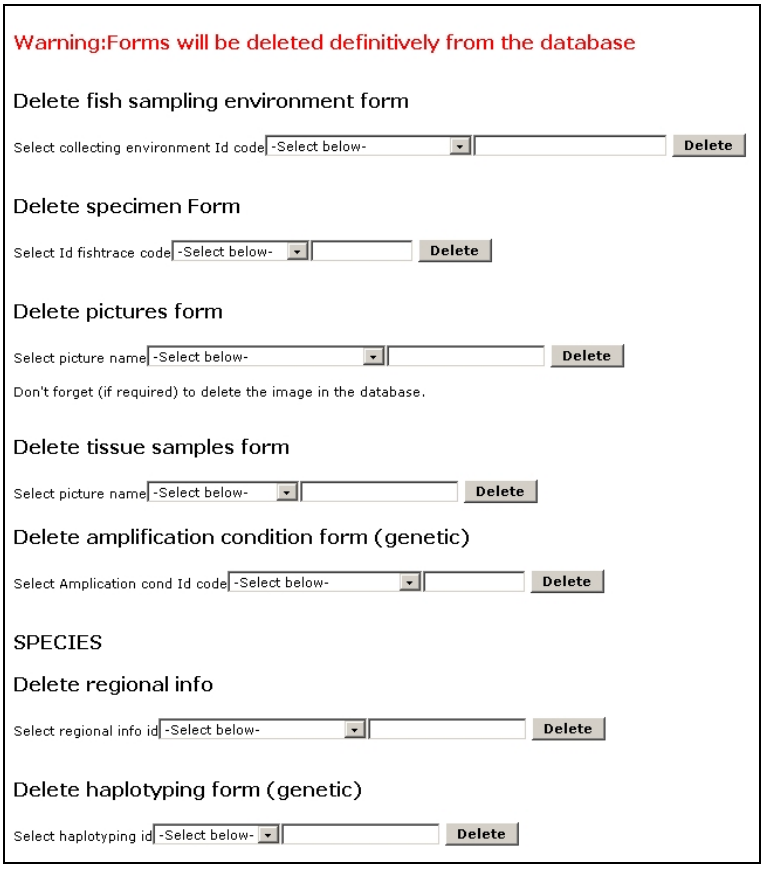
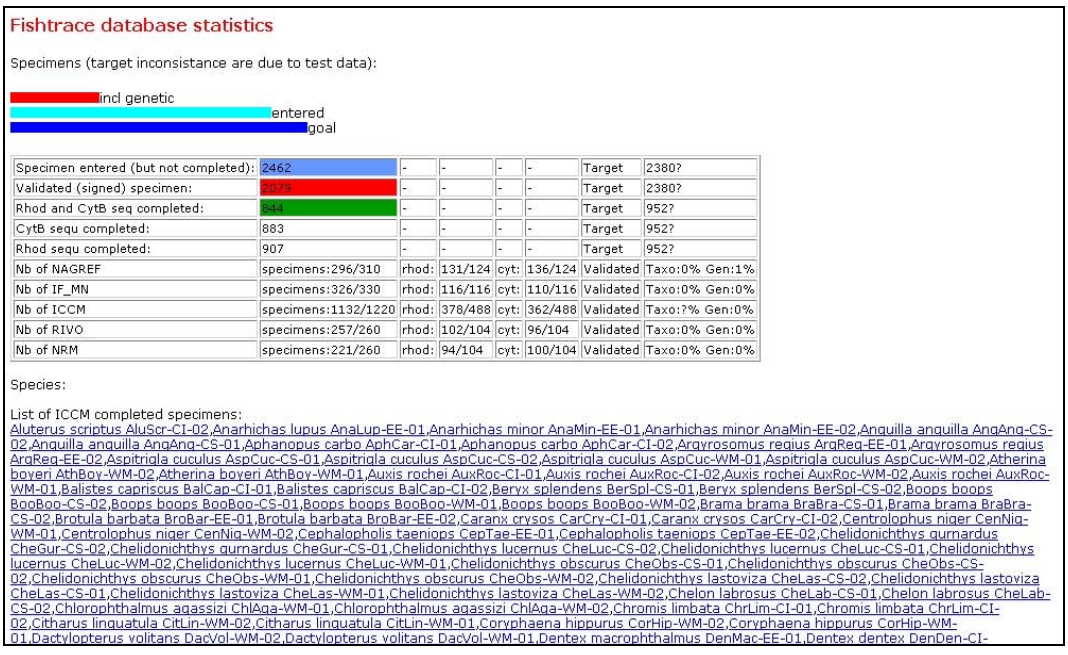


Figure 4.43.- Delete Data Tool.



Specimen entered (but not completed):	2462	-	-	-	-	Target	2380?
Validated (signed) specimen:	2079	-	-	-	-	Target	2380?
Rhod and CytB seq completed:	844	-	-	-	-	Target	952?
CytB sequ completed:	883	-	-	-	-	Target	952?
Rhod sequ completed:	907	-	-	-	-	Target	952?
Nb of NAGREF	specimens:296/310	rhod:	131/124	cyt:	136/124	Validated	Taxo:0% Gen:1%
Nb of IF_MN	specimens:326/330	rhod:	116/116	cyt:	110/116	Validated	Taxo:0% Gen:0%
Nb of ICCM	specimens:1132/1220	rhod:	378/488	cyt:	362/488	Validated	Taxo:?% Gen:0%
Nb of RIVO	specimens:257/260	rhod:	102/104	cyt:	96/104	Validated	Taxo:0% Gen:0%
Nb of NRM	specimens:221/260	rhod:	94/104	cyt:	100/104	Validated	Taxo:0% Gen:0%

Aluterus scriptus AluScr-CI-02,Anarhichas lupus AnaLup-EE-01,Anarhichas minor AnaMin-EE-01,Anarhichas minor AnaMin-EE-02,Anguilla anguilla AngAng-CS-02,Anguilla anguilla AngAng-CS-01,Aphanopus carbo AphCar-CI-01,Aphanopus carbo AphCar-CI-02,Argyrosomus regius ArgReg-EE-01,Argyrosomus regius ArgReg-EE-02,Aspitrigla cuculus AspCuc-CS-01,Aspitrigla cuculus AspCuc-CS-02,Aspitrigla cuculus AspCuc-WM-01,Aspitrigla cuculus AspCuc-WM-02,Atherina boyeri AthBoy-WM-02,Atherina boyeri AthBoy-WM-01,Auxis rochei AuxRoc-CI-01,Auxis rochei AuxRoc-CI-02,Auxis rochei AuxRoc-WM-02,Auxis rochei AuxRoc-WM-01,Balistes capriscus BalCap-CI-01,Balistes capriscus BalCap-CI-02,Beryx splendens BerSpl-CS-01,Beryx splendens BerSpl-CS-02,Boops boops BooBoo-CS-02,Boops boops BooBoo-CS-01,Boops boops BooBoo-WM-01,Boops boops BooBoo-WM-02,Brama brama BraBra-CS-01,Brama brama BraBra-CS-02,Brotula barbata BroBar-EE-01,Brotula barbata BroBar-EE-02,Caranx crysos CarCry-CI-01,Caranx crysos CarCry-CI-02,Centrolophus niger CenNig-WM-01,Centrolophus niger CenNig-WM-02,Cephalopholis taeniops CepTae-EE-01,Cephalopholis taeniops CepTae-EE-02,Chelidonichthys gurnardus CheGur-CS-02,Chelidonichthys gurnardus CheGur-CS-01,Chelidonichthys lucernus CheLuc-CS-02,Chelidonichthys lucernus CheLuc-CS-01,Chelidonichthys lucernus CheLuc-WM-02,Chelidonichthys lucernus CheLuc-WM-01,Chelidonichthys obscurus CheObs-CS-01,Chelidonichthys obscurus CheObs-CS-02,Chelidonichthys obscurus CheObs-WM-01,Chelidonichthys obscurus CheObs-WM-02,Chelidonichthys lastoviza CheLas-CS-02,Chelidonichthys lastoviza CheLas-CS-01,Chelidonichthys lastoviza CheLas-WM-01,Chelidonichthys lastoviza CheLas-WM-02,Chelon labrosus CheLab-CS-01,Chelon labrosus CheLab-CS-02,Chlorophthalmus agassizi ChlAga-WM-01,Chlorophthalmus agassizi ChlAga-WM-02,Chromis limbata ChrLim-CI-01,Chromis limbata ChrLim-CI-02,Citharus linguatula CitLin-WM-02,Citharus linguatula CitLin-WM-01,Coryphaena hippurus CorHip-WM-02,Coryphaena hippurus CorHip-WM-01,Dactylopterus volitans DacVol-WM-02,Dactylopterus volitans DacVol-WM-01,Dentex macrophthalmus DenMac-EE-01,Dentex dentex DenDen-CI-

Figure 4.44.- View Statistics Tool.

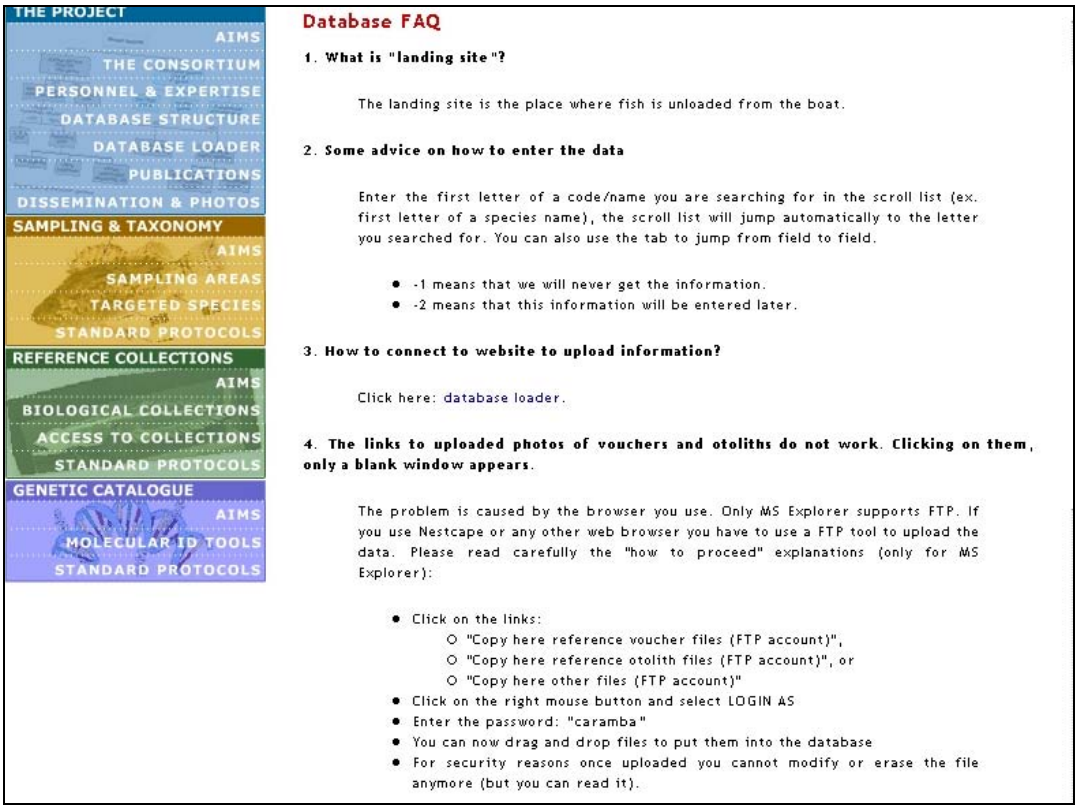
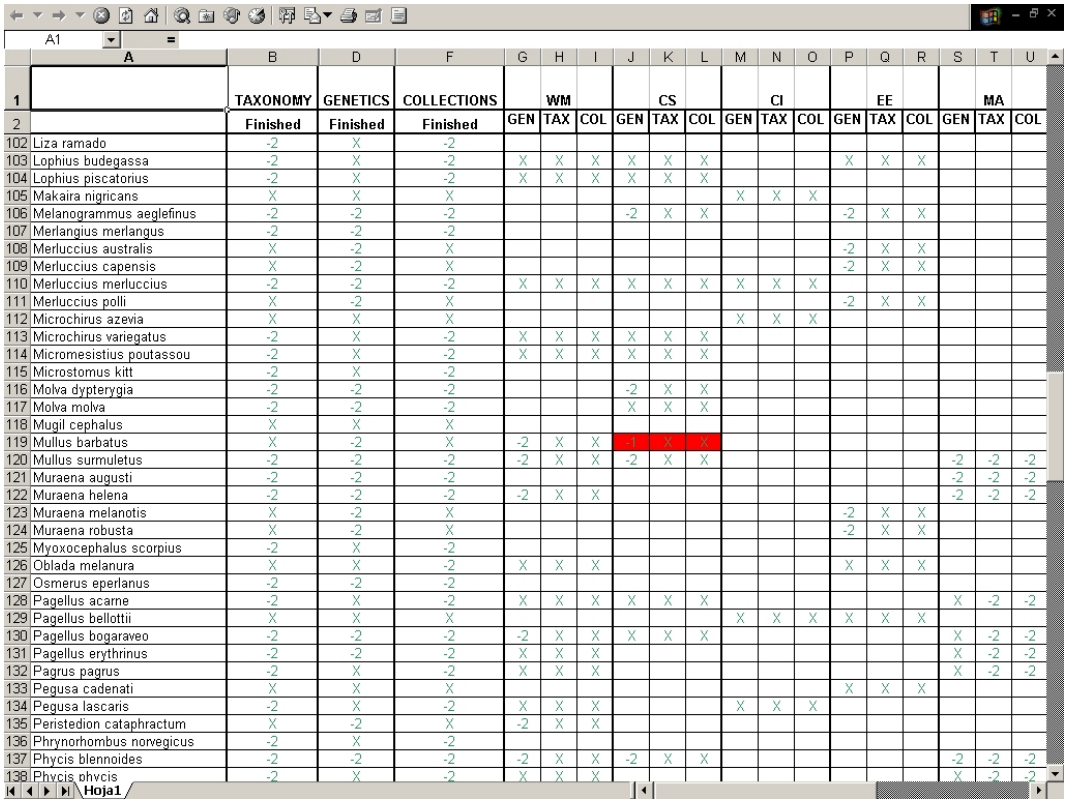


Figure 4.45.- FAQs.



	A	B	D	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U
1		TAXONOMY	GENETICS	COLLECTIONS		WM			CS			CI			EE			MA	
2		Finished	Finished	Finished	GEN	TAX	COL	GEN	TAX	COL	GEN	TAX	COL	GEN	TAX	COL	GEN	TAX	COL
102	Liza ramado	-2	X	-2															
103	Lophius budegassa	-2	X	-2	X	X	X	X	X	X				X	X	X			
104	Lophius piscatorius	-2	X	-2	X	X	X	X	X	X									
105	Makaira nigricans	X	X	X							X	X	X						
106	Melanogrammus aeglefinus	-2	-2	-2				-2	X	X				-2	X	X			
107	Merlangius merlangus	-2	-2	-2															
108	Merluccius australis	X	-2	X										-2	X	X			
109	Merluccius capensis	X	-2	X										-2	X	X			
110	Merluccius merluccius	-2	-2	-2	X	X	X	X	X	X	X	X	X						
111	Merluccius polli	X	-2	X										-2	X	X			
112	Microchirus azevia	X	X	X							X	X	X						
113	Microchirus variegatus	-2	X	-2	X	X	X	X	X	X									
114	Micromesistius poutassou	-2	X	-2	X	X	X	X	X	X									
115	Microstomus kitt	-2	X	-2															
116	Molva dypterygia	-2	-2	-2				-2	X	X									
117	Molva molva	-2	-2	-2				X	X	X									
118	Mugil cephalus	X	X	X															
119	Mullus barbatus	X	-2	X	-2	X	X	-1	X	X									
120	Mullus surmuletus	-2	-2	-2	-2	X	X	-2	X	X							-2	-2	-2
121	Muraena augusti	-2	-2	-2													-2	-2	-2
122	Muraena helena	-2	-2	-2	-2	X	X										-2	-2	-2
123	Muraena melanotis	X	-2	X										-2	X	X			
124	Muraena robusta	X	-2	X										-2	X	X			
125	Myoxocephalus scorpius	-2	X	-2															
126	Oblada melanura	X	X	-2	X	X	X							X	X	X			
127	Osmerus eperlanus	-2	-2	-2															
128	Pagellus acarne	-2	X	-2	X	X	X	X	X	X							X	-2	-2
129	Pagellus bellottii	X	X	X							X	X	X	X	X	X			
130	Pagellus bogaraveo	-2	-2	-2	-2	X	X	X	X	X							X	-2	-2
131	Pagellus erythrinus	-2	-2	-2	X	X	X										X	-2	-2
132	Pagrus pagrus	-2	X	-2	X	X	X										X	-2	-2
133	Pegusa cadenati	X	X	X										X	X	X			
134	Pegusa lascaris	-2	X	-2	X	X	X				X	X	X						
135	Peristedion cataphractum	X	-2	X	-2	X	X												
136	Phrynorhombus norvegicus	-2	X	-2															
137	Phycis blennoides	-2	-2	-2	-2	X	X	-2	X	X							-2	-2	-2
138	Phycis phycis	-2	X	-2	X	X	X										X	-2	-2

Hoja1

Figure 4.46.- Data Validation Flow Document.

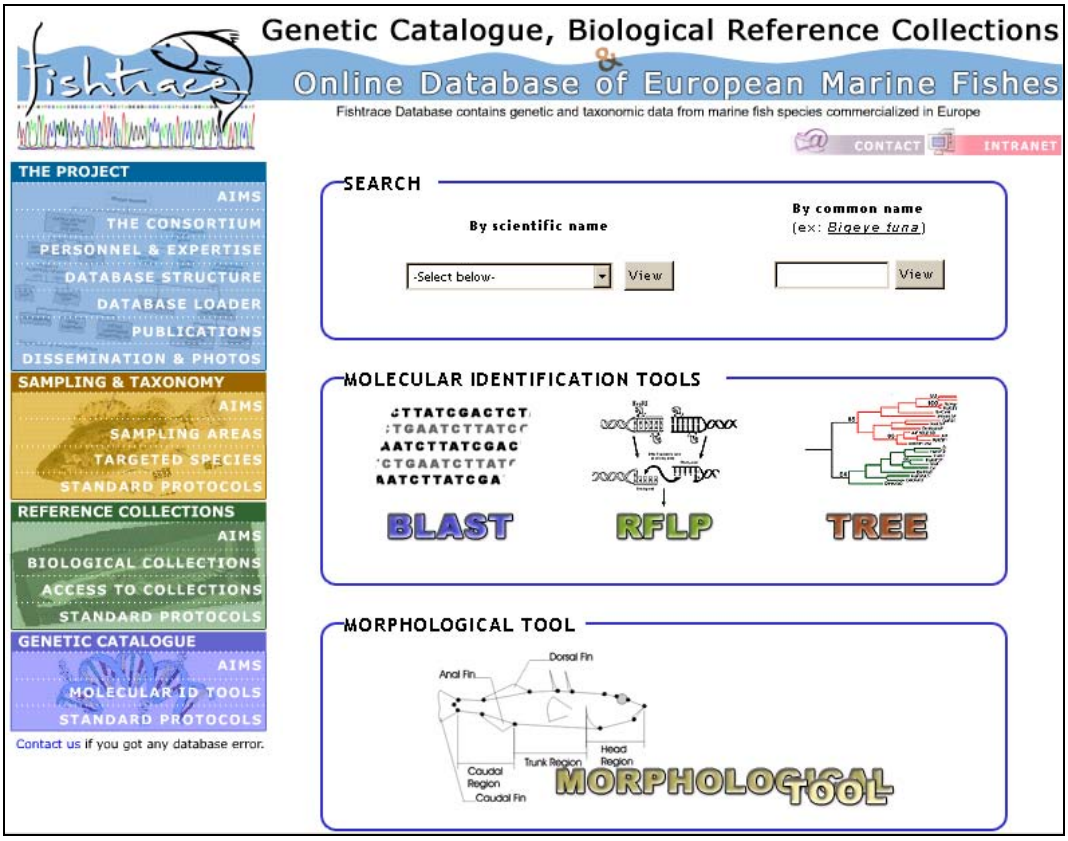


Figure 4.47.- FishTrace web interface Cover Page. Search and Identification tools are shown. The left bar menu corresponds to the Main Menu.

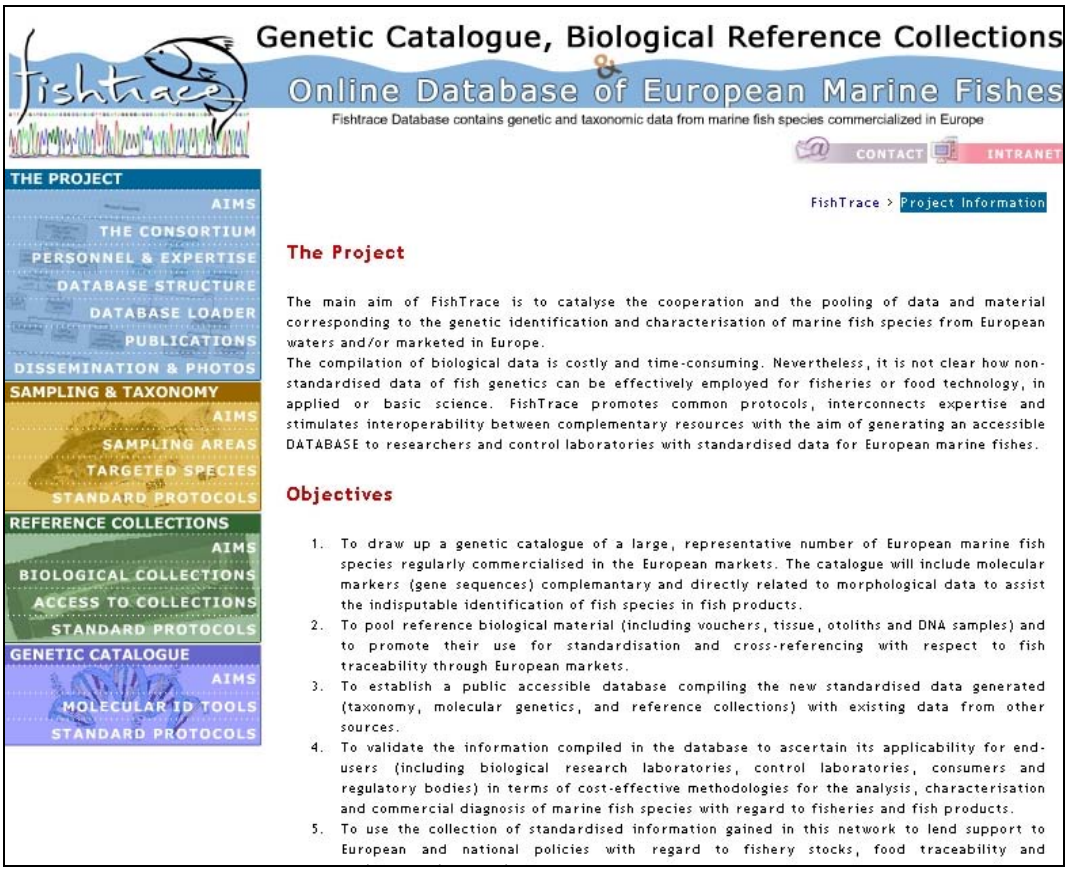


Figure 4.48.- The Project: Aims.

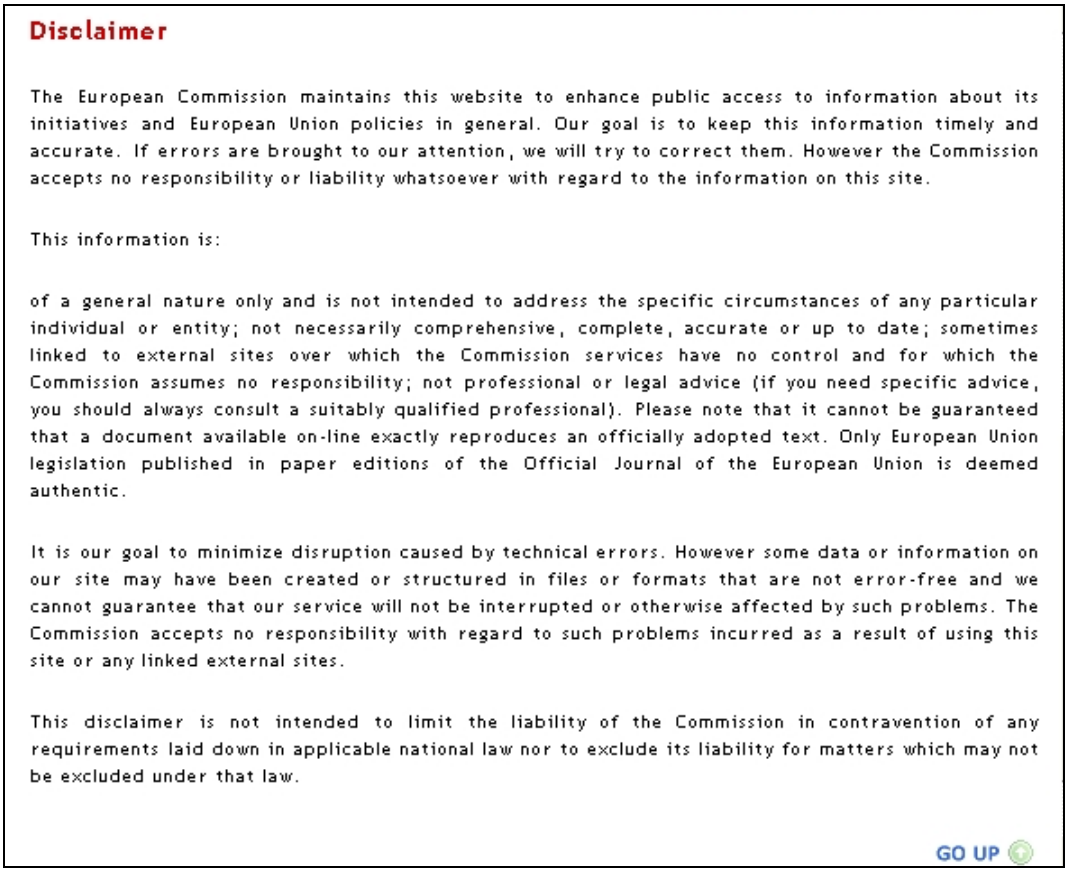


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Figure 4.49.- The Project: Aims.

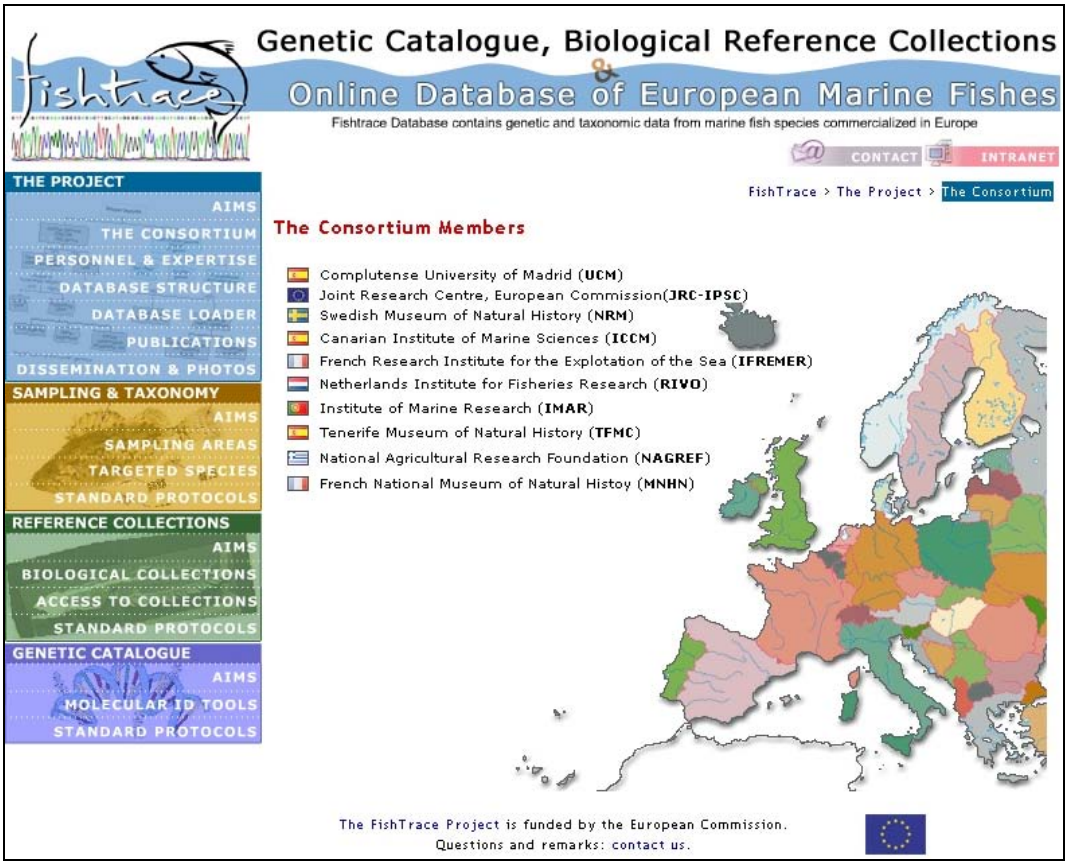
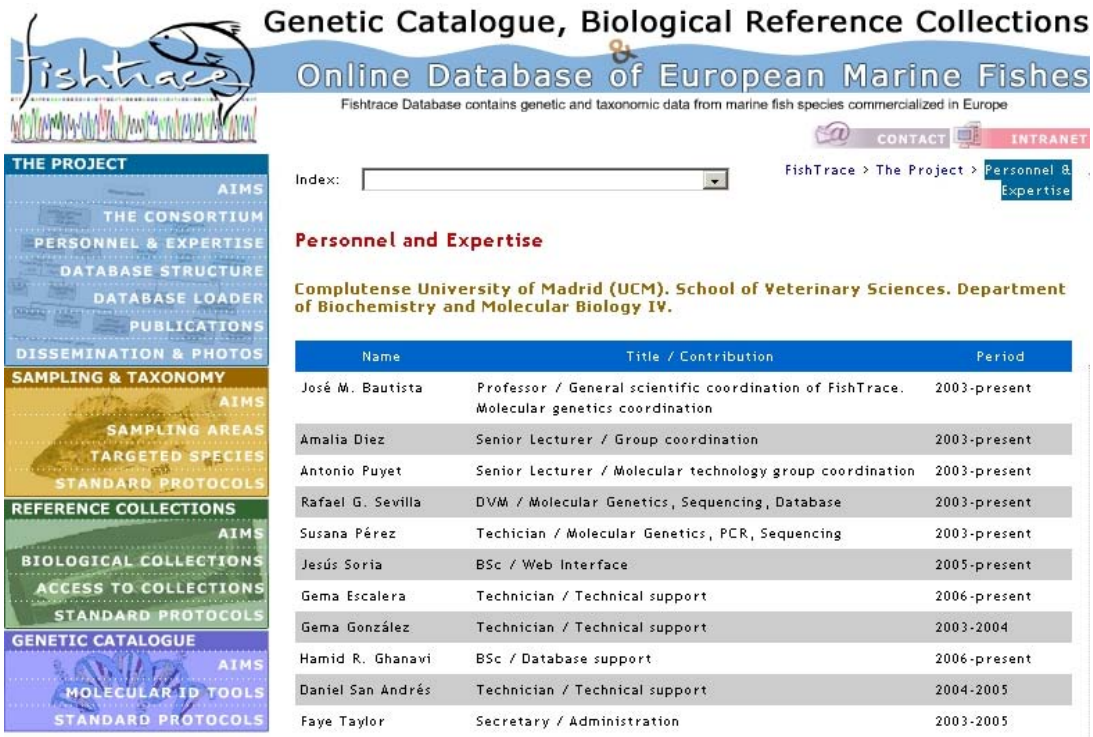


Figure 4.50.- The Project: The Consortium.



Genetic Catalogue, Biological Reference Collections & Online Database of European Marine Fishes

Fishtrace Database contains genetic and taxonomic data from marine fish species commercialized in Europe

CONTACT INTRANET

THE PROJECT
AIMS
THE CONSORTIUM
PERSONNEL & EXPERTISE
DATABASE STRUCTURE
DATABASE LOADER
PUBLICATIONS
DISSEMINATION & PHOTOS
SAMPLING & TAXONOMY
AIMS
SAMPLING AREAS
TARGETED SPECIES
STANDARD PROTOCOLS
REFERENCE COLLECTIONS
AIMS
BIOLOGICAL COLLECTIONS
ACCESS TO COLLECTIONS
STANDARD PROTOCOLS
GENETIC CATALOGUE
AIMS
MOLECULAR ID TOOLS
STANDARD PROTOCOLS

Index:

FishTrace > The Project > Personnel & Expertise

Personnel and Expertise

Complutense University of Madrid (UCM). School of Veterinary Sciences. Department of Biochemistry and Molecular Biology IV.

Name	Title / Contribution	Period
José M. Bautista	Professor / General scientific coordination of FishTrace. Molecular genetics coordination	2003-present
Amalia Diez	Senior Lecturer / Group coordination	2003-present
Antonio Puyet	Senior Lecturer / Molecular technology group coordination	2003-present
Rafael G. Sevilla	DVM / Molecular Genetics, Sequencing, Database	2003-present
Susana Pérez	Techician / Molecular Genetics, PCR, Sequencing	2003-present
Jesús Soria	BSc / Web Interface	2005-present
Gema Escalera	Technician / Technical support	2006-present
Gema González	Technician / Technical support	2003-2004
Hamid R. Ghanavi	BSc / Database support	2006-present
Daniel San Andrés	Technician / Technical support	2004-2005
Faye Taylor	Secretary / Administration	2003-2005

Joint Research Centre (JRC-IPSC). Institute for the Protection and Security of the Citizen.

Name	Title / Contribution	Period
Naouma Kourti	MSc/ General scientific coordination of JRC.	2003-present
Philippe Carreau	MSc / Database coordination	2003-present
Delphine Ortega	MSc / Database management	2003-present
Romas Statkus	PhD/ Fisheries support	2003-2005

Swedish Museum of Natural History (NRM). Vertebrate Zoology and Molecular Systematics.

Name	Title / Contribution	Period
Sven O. Kullander	Professor / General scientific group coordination. Fish taxonomy coordination.	2003-present
MichaelNorén	PhD / Sampling, taxonomy and molecular genetics	2003-present
ErikAlander	Taxonomy and reference collections	
Georg Fridriksson	Taxonomy and reference collections	
Anders Silfvergrip	Reference collections support	

Canarian Institute of Marine Sciences (ICCM). Department of Fisheries Biology.

Name	Title / Contribution	Period
José A. González	PhD / General scientific group coordination. Fish taxonomy. Data validation coordination.	2003-present
José I. Santana	BSc / Specimens collecting and sampling management	2003-present
Ignacio J. Lozano	PhD, Senior Lecturer / Taxonomy management	2003-present
José A. Pérez	BSc / Sampling and reference collection management, and database	2003-present
Antonio M. García	BSc, Pre-doctoral position / Sampling and reference collection management, and database	2003-present
RosaDomínguez-Seoane	BSc / Scientific and technical support for sampling	2004-present
Montserrat Gimeno	BSc / Scientific and technical support for sampling and database structure	2003-2004
Fernanda Marrero	BSc / Scientific and technical support for sampling	2003
Rocío González	BSc / Scientific and technical support for sampling	2003-2004
Miguel Rabassó	BSc / Scientific and technical support for sampling	2003-2004
Víctor M. Tuset	PhD / Scientific support for sampling and taxonomy	2003-present
Prudencio Calderín	Technician / Laboratory support	2003-present

French Research Institute for the Exploitation of the Sea (IFREMER). Department of Marine Product Upgrading.

Name	Title / Contribution	Period
Véronique Verrez	PhD / General scientific group coordination. Molecular genetics coordination	2003-present
Monique Etienne	Scientist / Dissemination management.	2003-2005
Marc Jérôme	Scientist / Molecular Genetics	2003-present
Olivier Mouchel	Technician / Molecular Genetics	2003-present

Netherlands Institute for Fisheries Research (RIVO). Department of Fishtechnology and Fishculture.

Name	Title / Contribution	Period
Hilde Van Pelt	PhD / General scientific group coordination. Molecular genetics coordination.	2003-present
Afne Stein	Tecnician / Molecular genetics, PCR, sequencing.	2003-present
Kees Groeneveld	Taxonomy management	

Institute of Marine Research (IMAR). Natural History Museum of Funchal.

Name	Title / Contribution	Period
Manuel Biscoito	PhD / General scientific group coordination. Reference collections coordination and management.	2003-present
Mafalda Freitas	PhD / Taxonomy management	2003-present
João Delgado	PhD / Sampling and Taxonomy management	2003-present
Rosa Pestana	Sampling and Taxonomy management, Database	

Tenerife Museum of Natural History (TFMC).

Name	Title / Contribution	Period
Sebastián Jiménez	BSc, PhD / General scientific group coordination. Reference collection management. Fish taxonomy.	2003-present
Fátima Hernández	Associate Researcher / Reference Collection management	2003-present
Alejandro De Vera	Associate Researcher / Reference Collection management	2003-present

National Agricultural Research Foundation (NAGREF). Fisheries Research Institute.

Name	Title / Contribution	Period
Grigorios Krey	Associate Researcher / General scientific group coordination. Population and haplotyping data coordination.	2003-present
Panos Leontarakis	MSc / Sampling and Reference Collection management, Taxonomy, Database.	2003-present
Laurence Favre-Krey	MSc / Molecular Genetics, PCR, Sequencing, Haplotyping analysis, Database	2003-present
Angeliki Adamidou	BSc / Taxonomy	2003-2004
Alexis Tsangridis	Associate Researcher / Taxonomy management.	2003-present

French National Museum of Natural History (MNHN). Laboratory of General and Applied Ichthyology.

Name	Title / Contribution	Period
Guy Duhamel	Professor / Curator of ichthyology collection	2003-present
Patrice Pruvost	Collection manager / General scientific group coordination.	2003-present
Samuel Iglésias	PhD / Reference Collection management	2003-2006
Mélyne Hautecoeur	Collection support	2006-present
Romain Causse	Collection support	2003-present
Laurent Nandrin	Collection support	2003-present
Corinne Guchereau	Administrative support	2003-present

GO UP

The FishTrace Project is funded by the European Commission.
Questions and remarks: contact us.

Figure 4.51.- The Project: Personnel and Expertise.

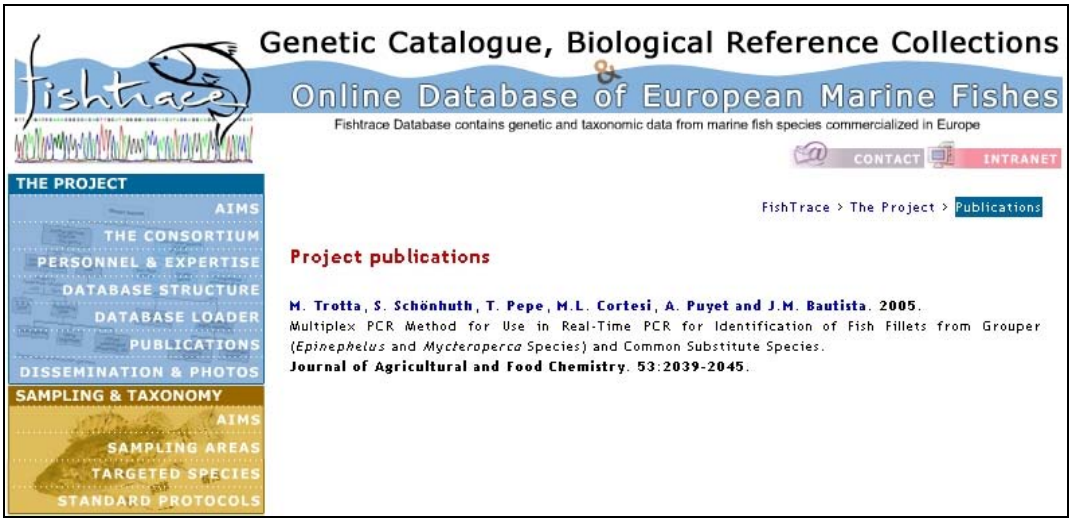


Figure 4.52.- The Project: Publications.

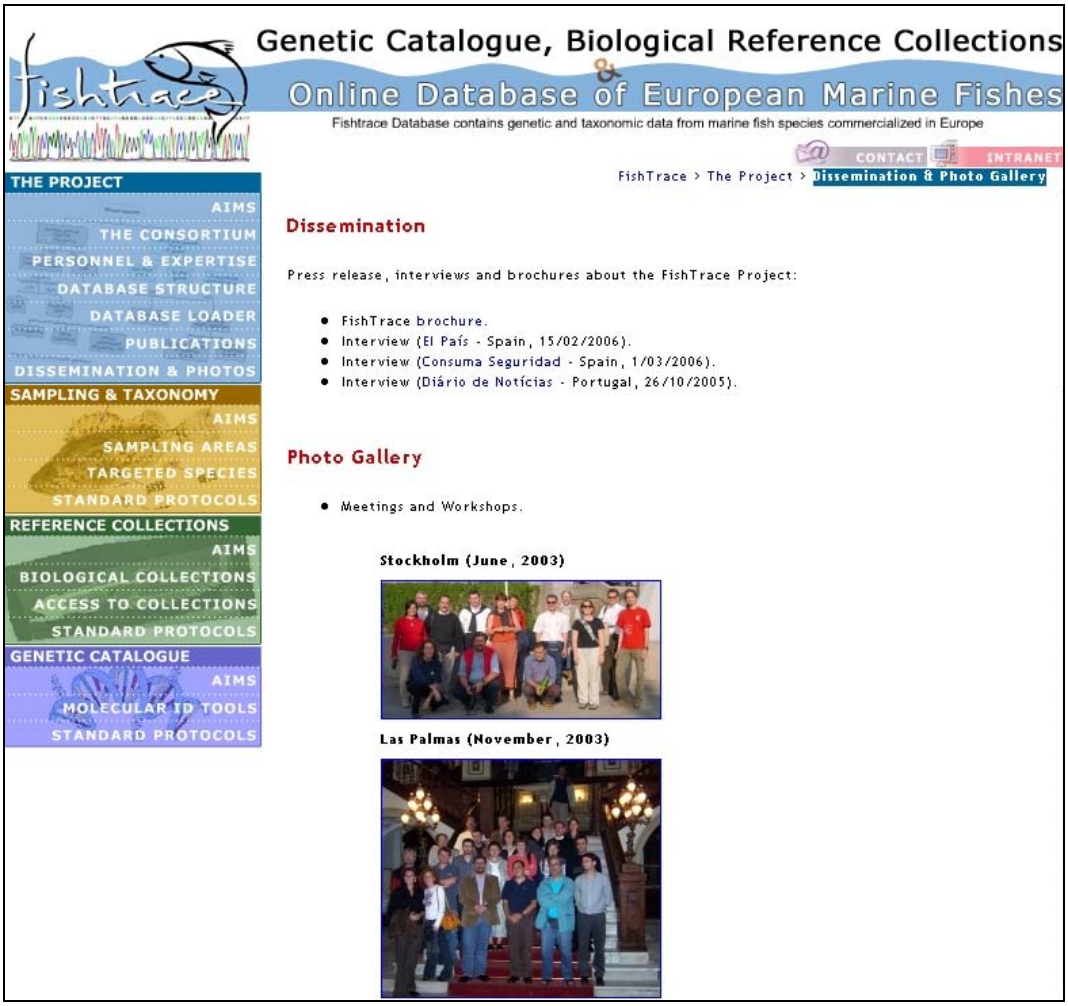


Figure 4.53.- The Project: Disseminations and Photos.

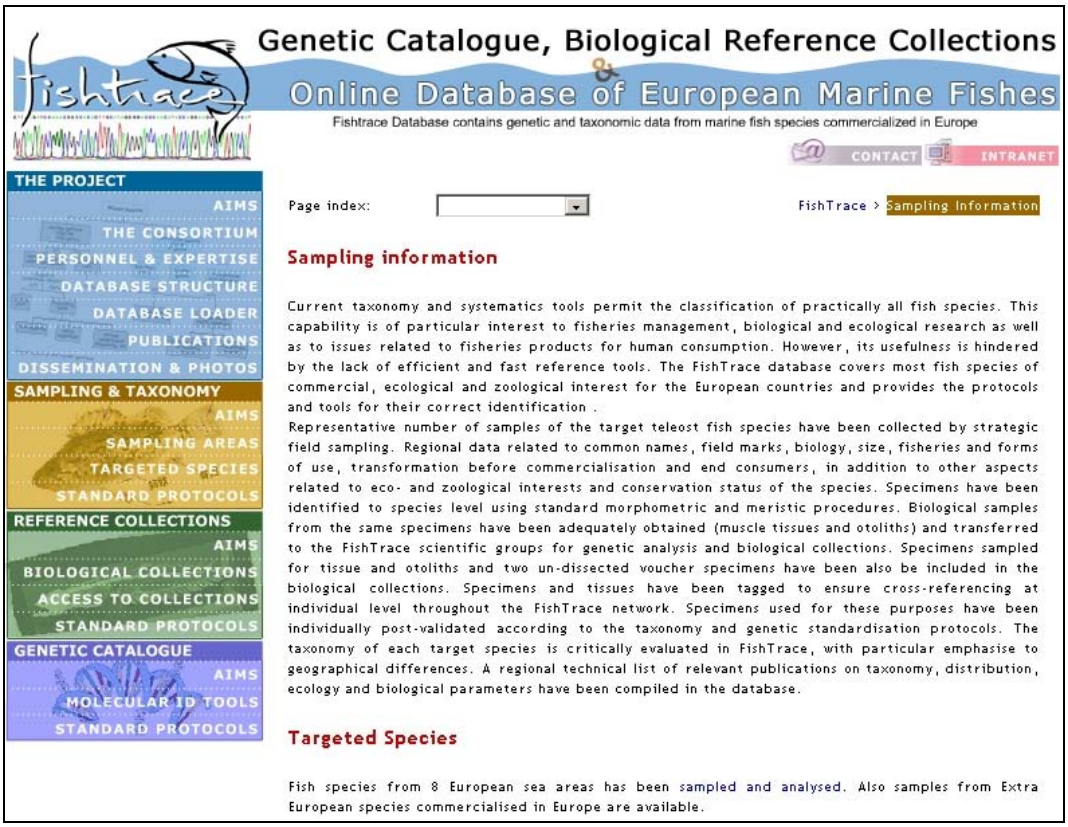


Figure 4.54.- Sampling and Taxonomy: Aims.

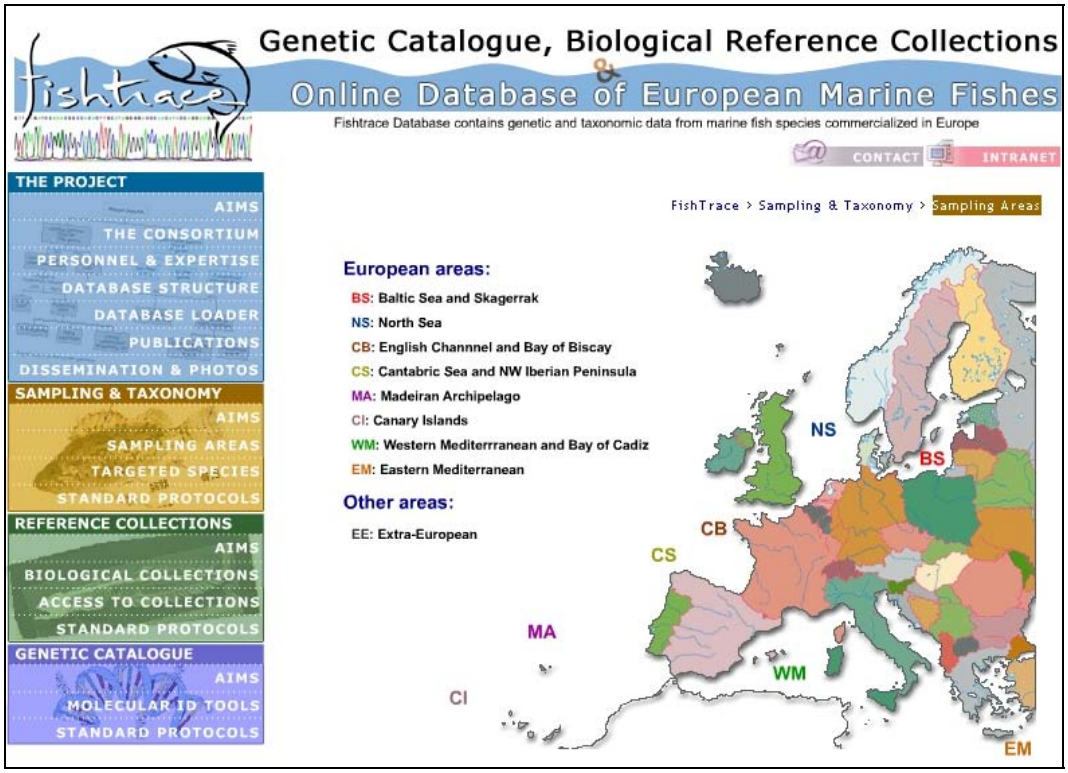


Figure 4.55.- Sampling and Taxonomy: Sampling Areas.

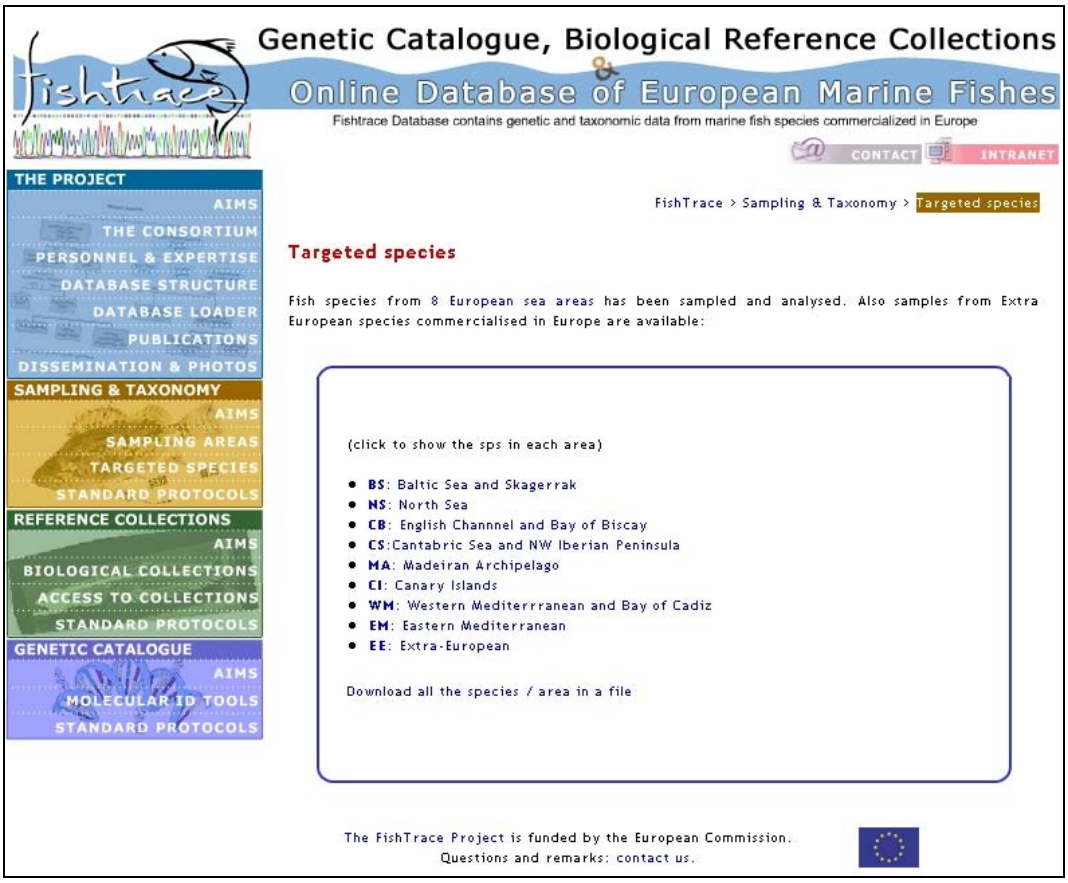


Figure 4.56.- Sampling and Taxonomy: Targeted Species.

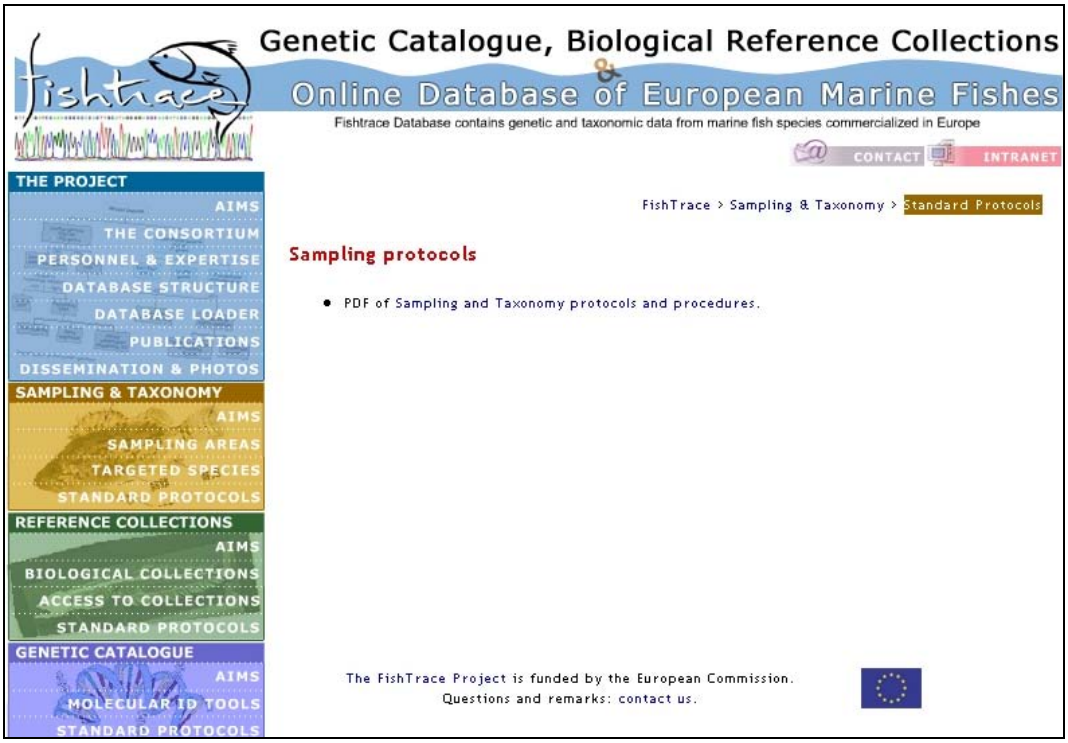


Figure 4.57.- Sampling and Taxonomy: Standard Protocols.

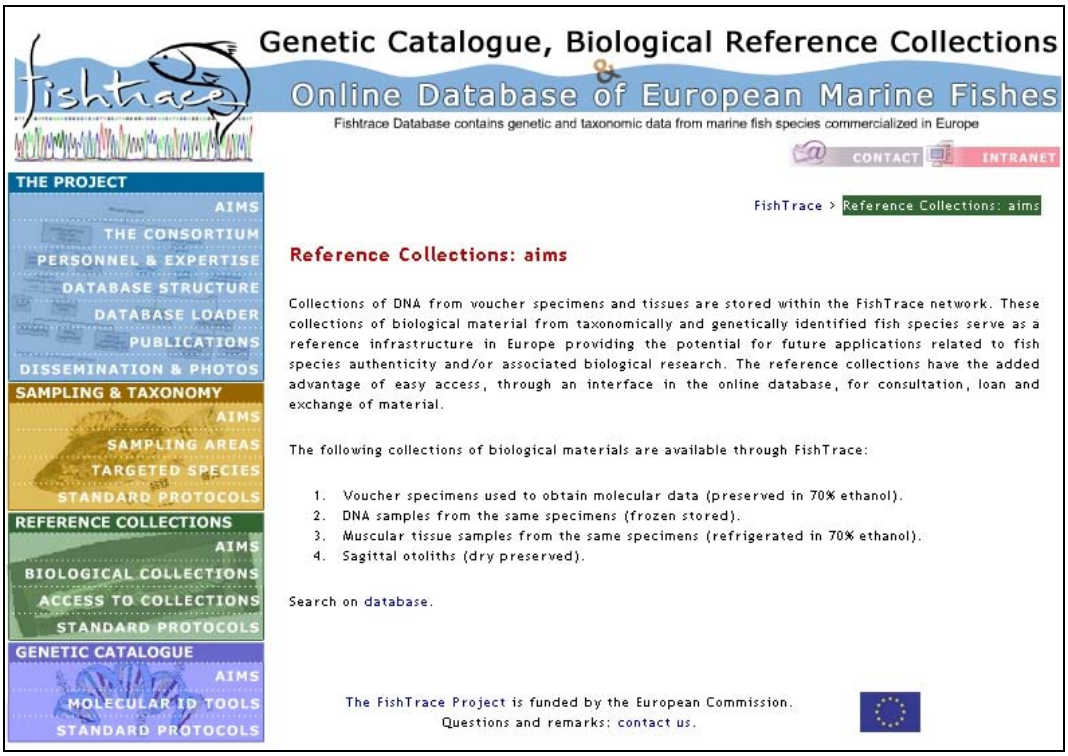


Figure 4.58.- Reference Collections: Aims.

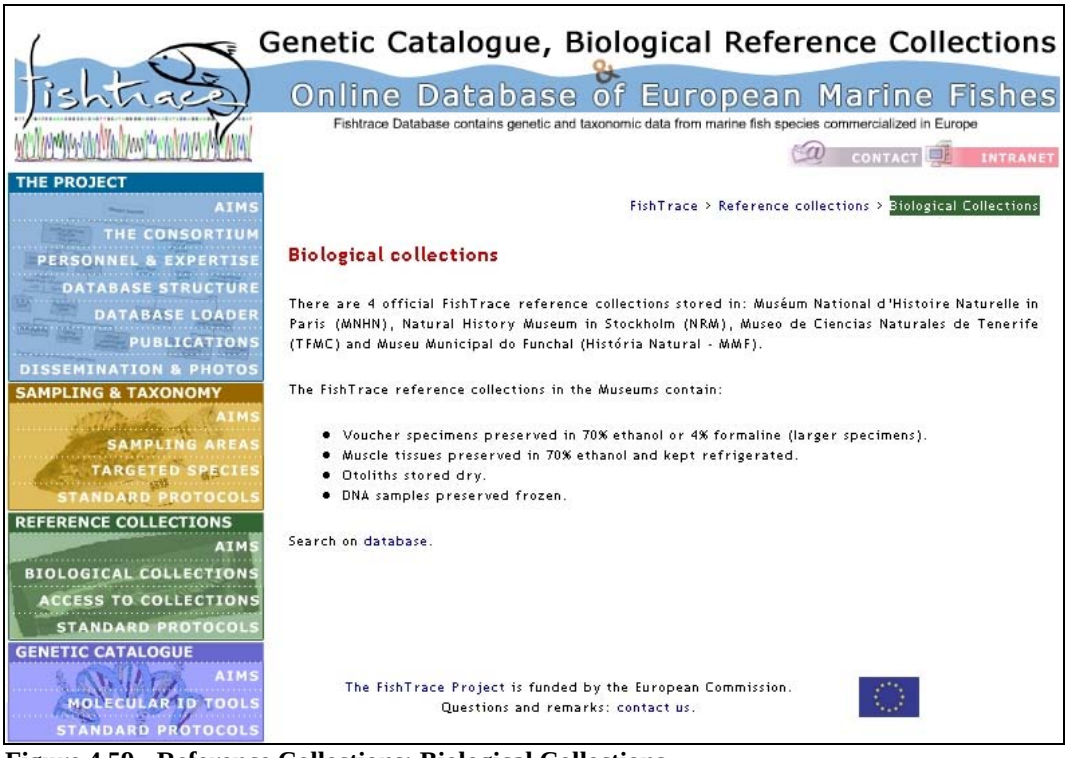


Figure 4.59.- Reference Collections: Biological Collections.

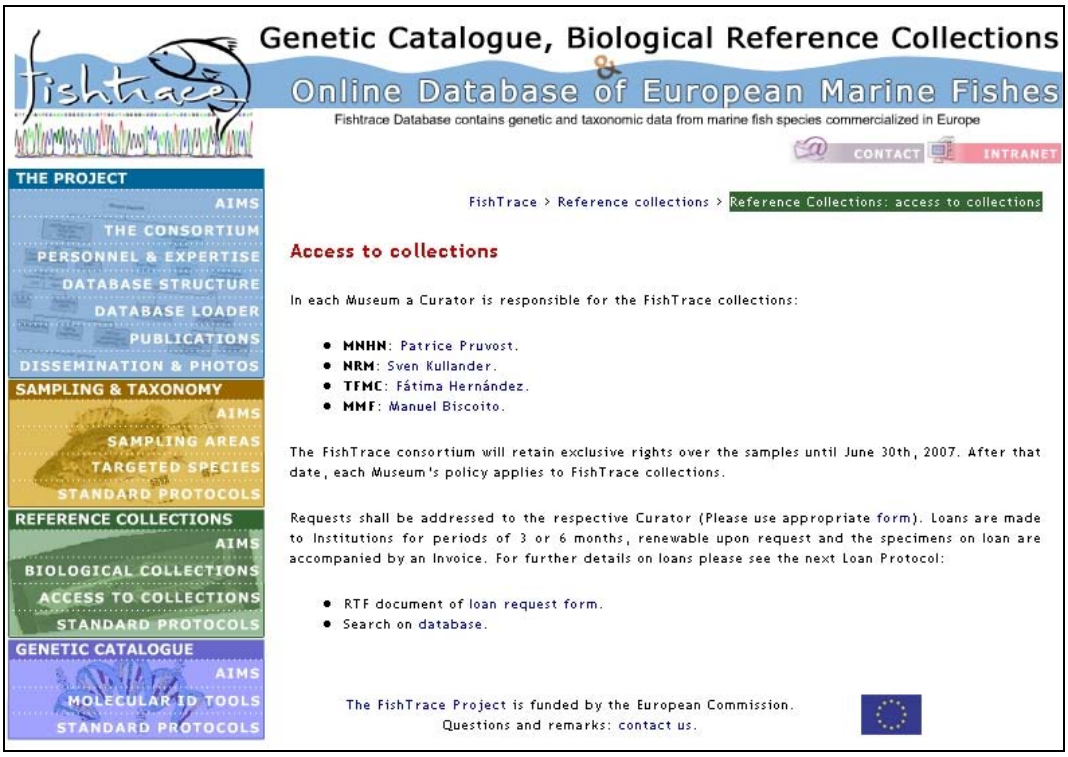


Figure 4.60.- Reference Collections: Access to Biological Collections.

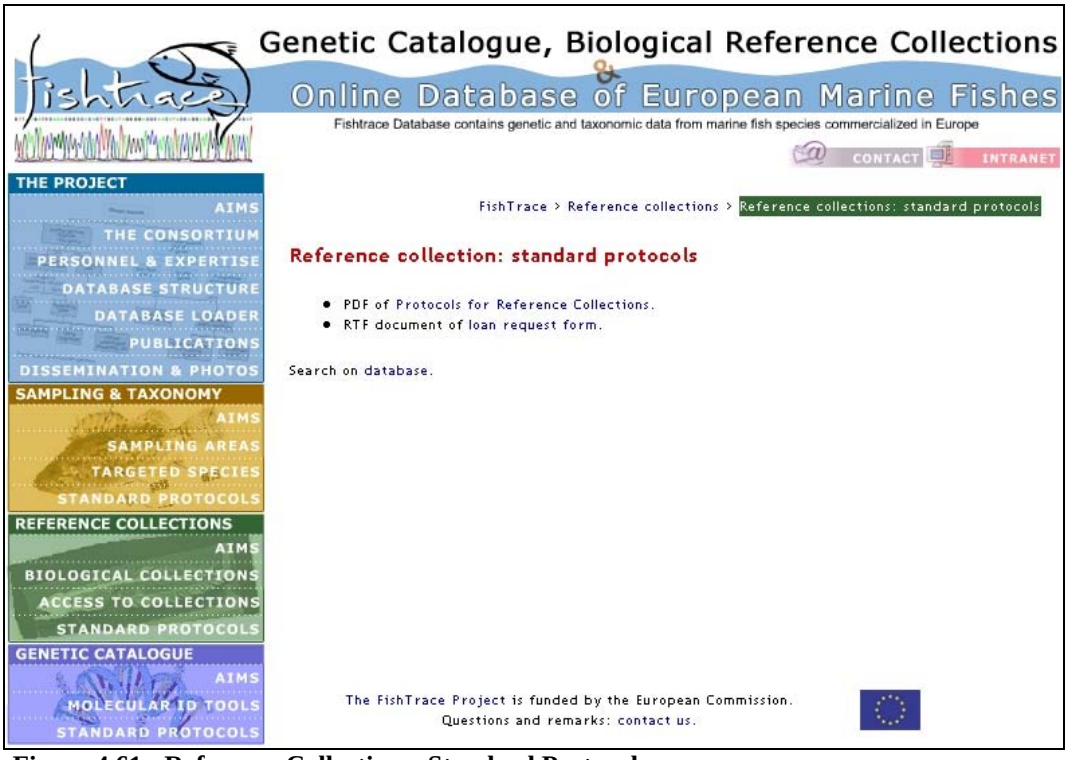


Figure 4.61.- Reference Collections: Standard Protocols.

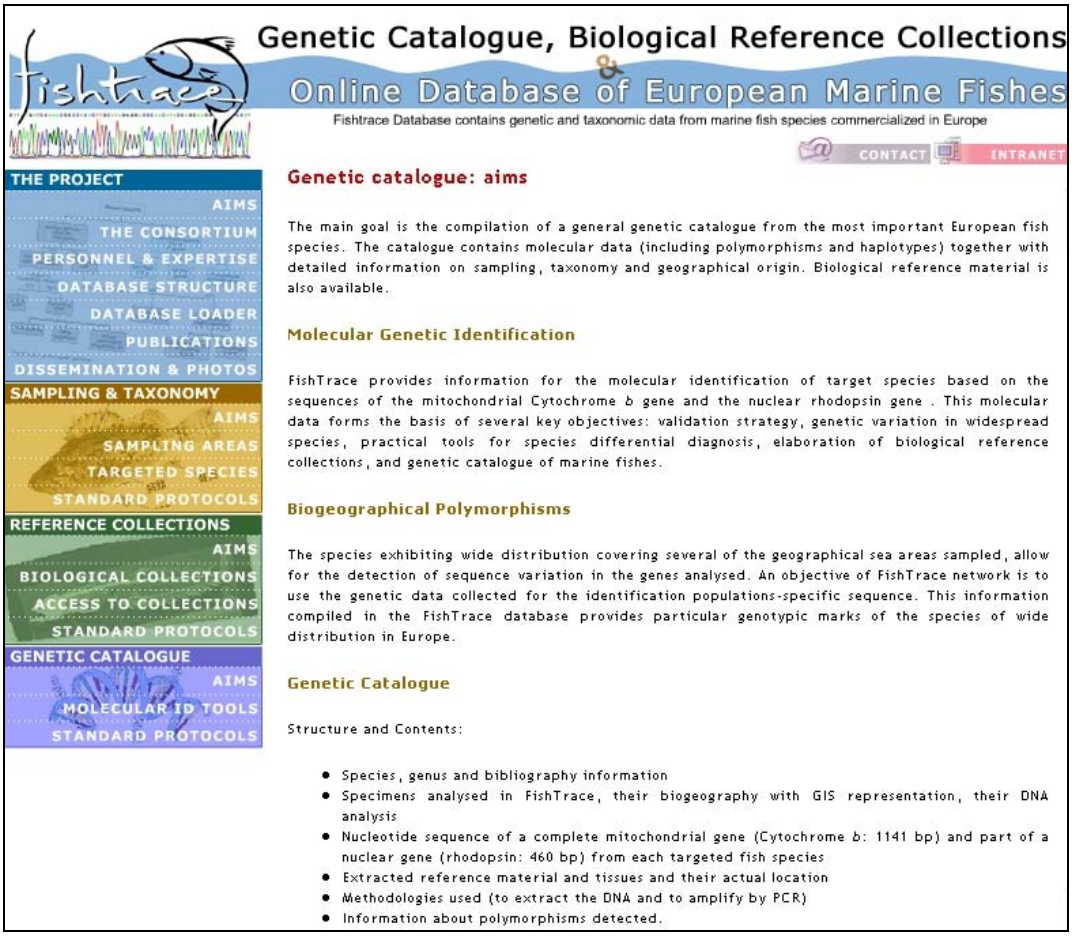


Figure 4.62.- Genetic Catalogue: Aims.

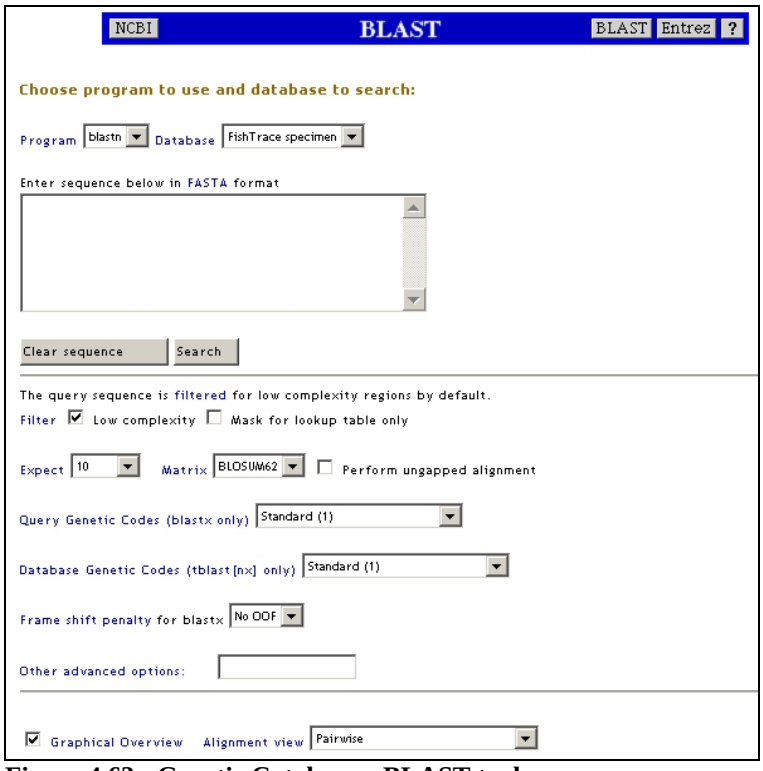


Figure 4.63.- Genetic Catalogue: BLAST tool.

Fishtrace Blast results

C:\jakarta-tomcat\bin>C:\fishtrace\blast\bin\blastall -p blastn -d C:\fishtrace\blast\data\FT_specimen.nt -i C:\fishtrace\blast\tmp\7655289.txt -F L -e 10 -M BLOSUM62 -g T -D 1 -w 0 -m 0 -v 100 -b 50 -T T

BLASTN 2.2.12 [Aug-07-2005]

Reference:
Altschul, Stephen F., Thomas L. Madden, Alejandro A. Schäffer,
Jinghui Zhang, Zheng Zhang, Webb Miller, and David J. Lipman (1997),
"Gapped BLAST and PSI-BLAST: a new generation of protein database search
programs", Nucleic Acids Res. 25:3389-3402.

Query=
(1141 letters)

Database: C:\fishtrace\blast\data\FT_specimen.nt
1677 sequences; 1,340,621 total letters

Sequences producing significant alignments:	Score (bits)	E Value
Mullus-surmuletus(CytB)	2254	0.0
Mullus-surmuletus(CytB)	2254	0.0
Mullus-barbatus(CytB)	2254	0.0
Mullus-surmuletus(CytB)	2222	0.0
Mullus-surmuletus(CytB)	2222	0.0
Mullus-surmuletus(CytB)	2222	0.0
Mullus-surmuletus(CytB)	2214	0.0
Mullus-surmuletus(CytB)	2214	0.0
Mullus-surmuletus(CytB)	2206	0.0
Mullus-surmuletus(CytB)	2183	0.0
Mullus-barbatus(CytB)	1001	0.0
Mullus-barbatus(CytB)	993	0.0
Makaira-nigricans(CytB)	422	e-119
Labrus-bergylta(CytB)	402	e-113
Labrus-bergylta(CytB)	402	e-113
Makaira-nigricans(CytB)	400	e-112
Labrus-bergylta(CytB)	387	e-108
Labrus-bergylta(CytB)	387	e-108
Glyptocephalus-cynoglossus(CytB)	385	e-107

Figure 4.64.- Genetic Catalogue: BLAST results.

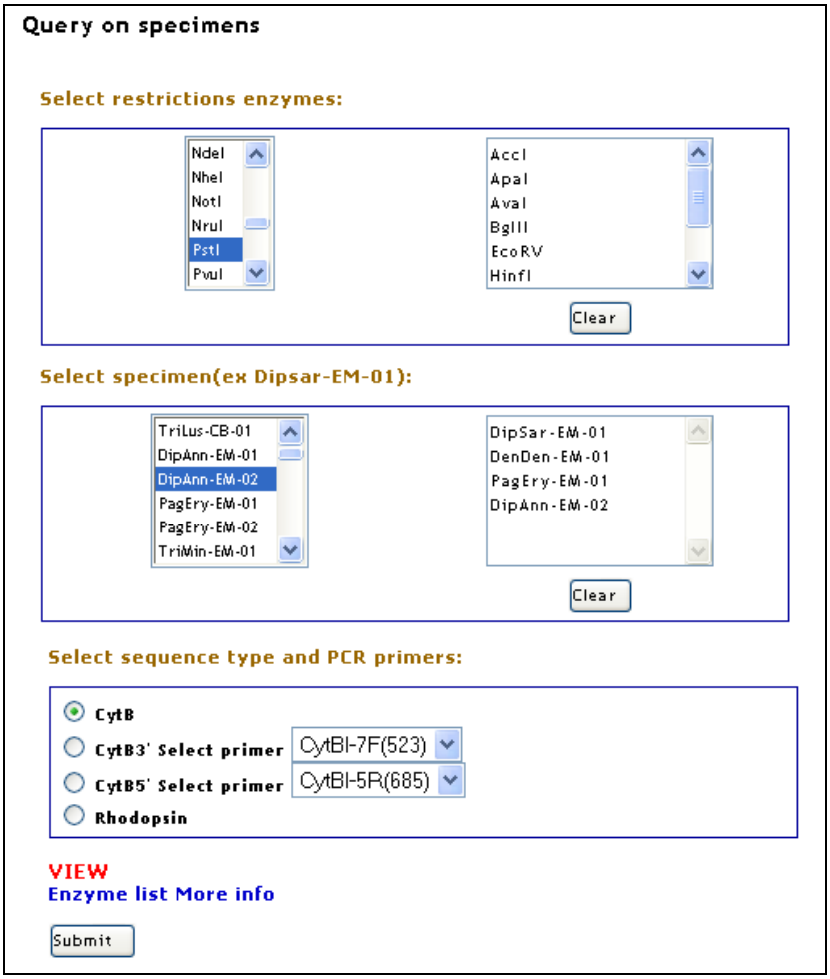
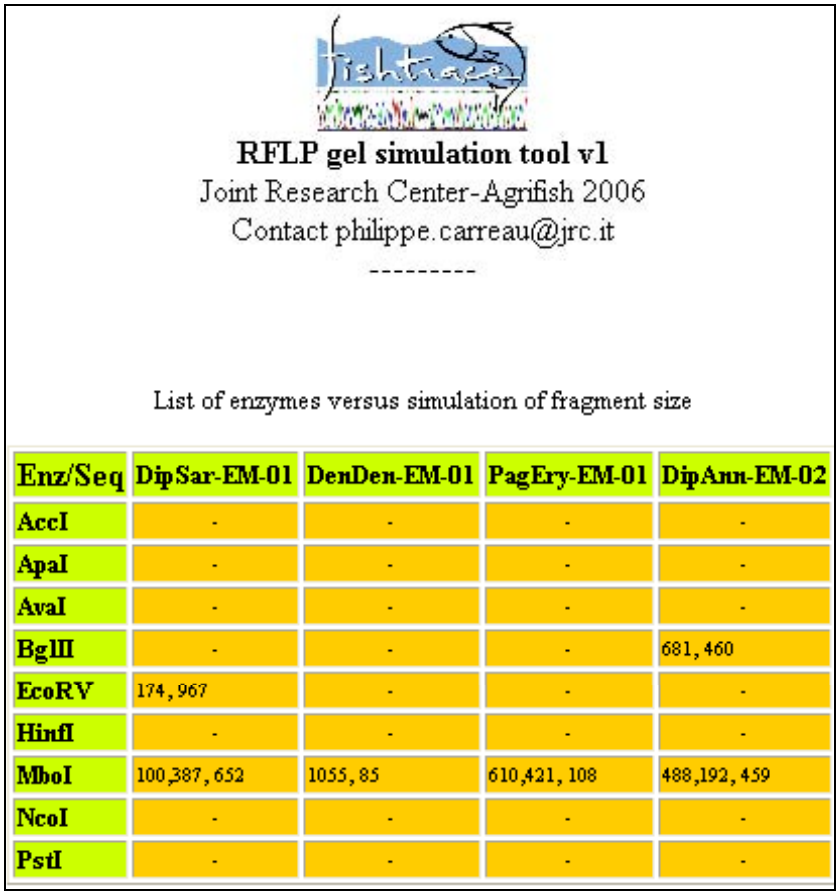


Figure 4.65.- Genetic Catalogue: RFLPs simulator tool.



RFLP gel simulation tool v1

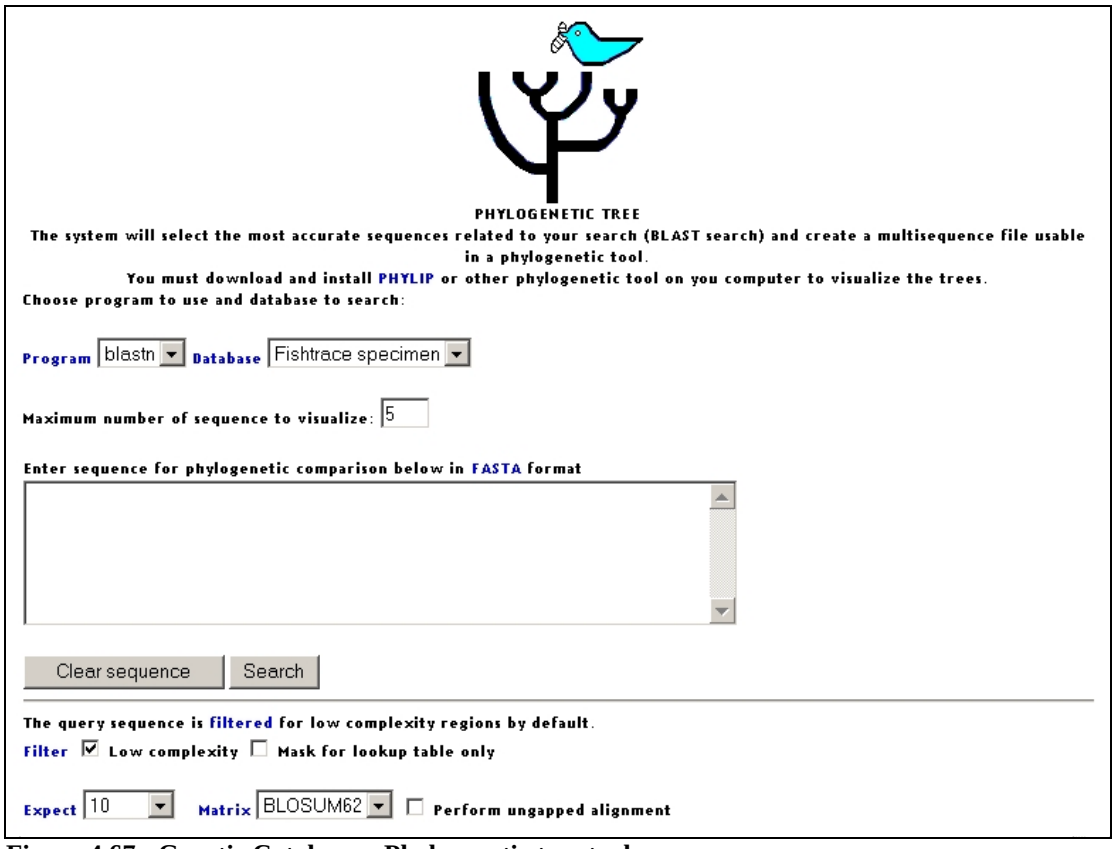
Joint Research Center-Agrifish 2006

Contact philippe.carreau@jrc.it

List of enzymes versus simulation of fragment size

Enz/Seq	DipSar-EM-01	DenDen-EM-01	PagEry-EM-01	DipAnn-EM-02
AccI	-	-	-	-
ApaI	-	-	-	-
AvaI	-	-	-	-
BglII	-	-	-	681, 460
EcoRV	174, 967	-	-	-
HinfI	-	-	-	-
MboI	100,387, 652	1055, 85	610,421, 108	488,192, 459
NcoI	-	-	-	-
PstI	-	-	-	-

Figure 4.66.- Genetic Catalogue: RFLPs simulator results.



PHYLOGENETIC TREE

The system will select the most accurate sequences related to your search (BLAST search) and create a multisequence file usable in a phylogenetic tool.

You must download and install PHYLIP or other phylogenetic tool on you computer to visualize the trees.

Choose program to use and database to search:

Program blastn Database Fishtrace specimen

Maximum number of sequence to visualize: 5

Enter sequence for phylogenetic comparison below in FASTA format

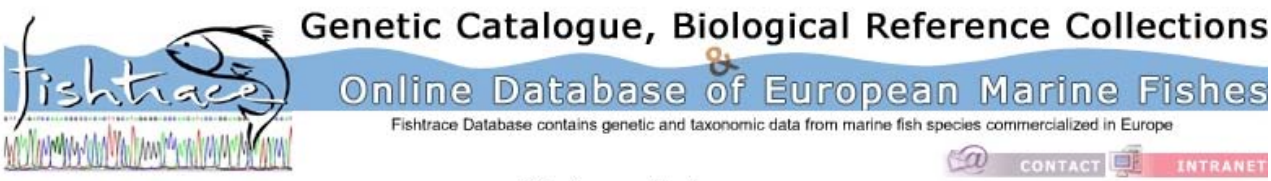
Clear sequence Search

The query sequence is filtered for low complexity regions by default.

Filter Low complexity Mask for lookup table only

Expect 10 Matrix BLOSUM62 Perform ungapped alignment

Figure 4.67.- Genetic Catalogue: Phylogenetic tree tool.



Phylogenetic tree

5 most accurate results (BLAST search) corresponding to your sequence in the FishTrace database

```
>specimen:MulSur-EM-02==Mullus-surmuletus(CytB)
ATGGCCAGCCTACGCAAAACCCACCCACTGATTAAGATTGCAAATGATGCTTTAGTAGACCTCCCCGCTCCCTCCAACAT
CTCGGTATGATGAAACTTCGGCTCTCTGCTAGGCCTCTGCTTAGCCACTCAAATTGTAACAGGACTCTTCCTGGCAATAC
ACTACACCTCTGATATCGCCACAGCTTTCTCCTCCGTTGCCCACATCTGCCGCGACGTTAACTATGGATGATTTATCCGT
AACATACATGCAAACGGAGCATCCTTCTTCTTCATCTGCATCTACATGCACATCGGACGAGGCCTCTACTACGGCTCATA
TCTATACAAAGAGACATGAAACGTCGGCGTTATTCTCCTCCTCCTAGTTATGATGACTGCCTTCGTGGGCTACGTCCTTC
CCTGAGGCCAAATGTCATTCTGAGGTGCTACCGTTATTACAAACTTGATATCTGCCGTCCCTTATGTGGGCAATACACTC
GTTCAATGAATTTGAGGTGGTTTCTCAGTCGACAATGCAACCCTGACCCGCTTCTTTGCATTCCACTTCCTATTCCCCTT
CGTCATTGCCGCAATGACAGTGATTCACCTAATTTTCTTACACGAGACAGGTTCTAACAATCCGACGGGACTAAACTCTG
ACGCCGACAAAATCTCGTTCCACCCCTATTTCTCTTACAAAGACCTCCTCGGATTCGCGGTACTACTCATTGCCCTGTCC
TCCATCGCACTCTTCTCGCCCAACTTACTAGGAGACCCGGACAACTTTACGCCTGCCAACCCGCTTGTAACACCTCCACA
TATTAAGCCTGAGTGGTACTTCCTATTTGCCTACGCCATCCTTCGATCCATCCCTAATAAGCTGGGGGGTGTCCTGGCCC
TTCTATTCTCAATCCTAGTCCTCATGCTCGTACCAATTCTCCACACCTCTAAGCAACGAGGCCTTACATTCCGCCCCCTC
ACACAACTCCTCTTCTGAACCCTTGTGGCTGACGTTATGATTCTAACCTGGATCGGAGGCATGCCAGTCGAGCATCCCTA
CATCATTATTGGTCAAGTCGCCTCTTTCCTCTACTTCTTCCTGTTCCTCTTCCTCATCCCTCTTGCAGGCTGAATGGAGA
ATAAGGCCCTGCAATGAACAT
>specimen:MulSur-EM-01==Mullus-surmuletus(CytB)
ATGGCCAGCCTACGCAAAACCCACCCACTGATTAAGATTGCAAATGATGCTTTAGTAGACCTCCCCGCTCCCTCCAACAT
CTCGGTATGATGAAACTTCGGCTCTCTGCTAGGCCTCTGCTTAGCCACTCAAATTGTAACAGGACTCTTCCTGGCAATAC
ACTACACCTCTGATATCGCCACAGCTTTCTCCTCCGTTGCCCACATCTGCCGCGACGTTAACTATGGATGATTTATCCGT
AACATACATGCAAACGGAGCATCCTTCTTCTTCATCTGCATCTACATGCACATCGGACGAGGCCTCTACTACGGCTCATA
TCTATACAAAGAGACATGAAACGTCGGCGTTATTCTCCTCCTCCTAGTTATGATGACTGCCTTCGTGGGCTACGTCCTTC
CCTGAGGCCAAATGTCATTCTGAGGTGCTACCGTTATTACAAACTTGATATCTGCCGTCCCTTATGTGGGCAATACACTC
GTTCAATGAATTTGAGGTGGTTTCTCAGTCGACAATGCAACCCTGACCCGCTTCTTTGCATTCCACTTCCTATTCCCCTT
CGTCATTGCCGCAATGACAGTGATTCACCTAATTTTCTTACACGAGACAGGTTCTAACAATCCGACGGGACTAAACTCTG
ACGCCGACAAAATCTCGTTCCACCCCTATTTCTCTTACAAAGACCTCCTCGGATTCGCGGTACTACTCATTGCCCTGTCC
TCCATCGCACTCTTCTCGCCCAACTTACTAGGAGACCCGGACAACTTTACGCCTGCCAACCCGCTTGTAACACCTCCACA
TATTAAGCCTGAGTGGTACTTCCTATTTGCCTACGCCATCCTTCGATCCATCCCTAATAAGCTGGGGGGTGTCCTGGCCC
TTCTATTCTCAATCCTAGTCCTCATGCTCGTACCAATTCTCCACACCTCTAAGCAACGAGGCCTTACATTCCGCCCCCTC
ACACAACTCCTCTTCTGAACCCTTGTGGCTGACGTTATGATTCTAACCTGGATCGGAGGCATGCCAGTCGAGCATCCCTA
```

To use the results you must install a phylogetic tree software on your computer as PHILYP, and copy-paste the result below to visualize the phylogenetic tree

Figure 4.68.- Genetic Catalogue: Phylogenetic tree tool results.

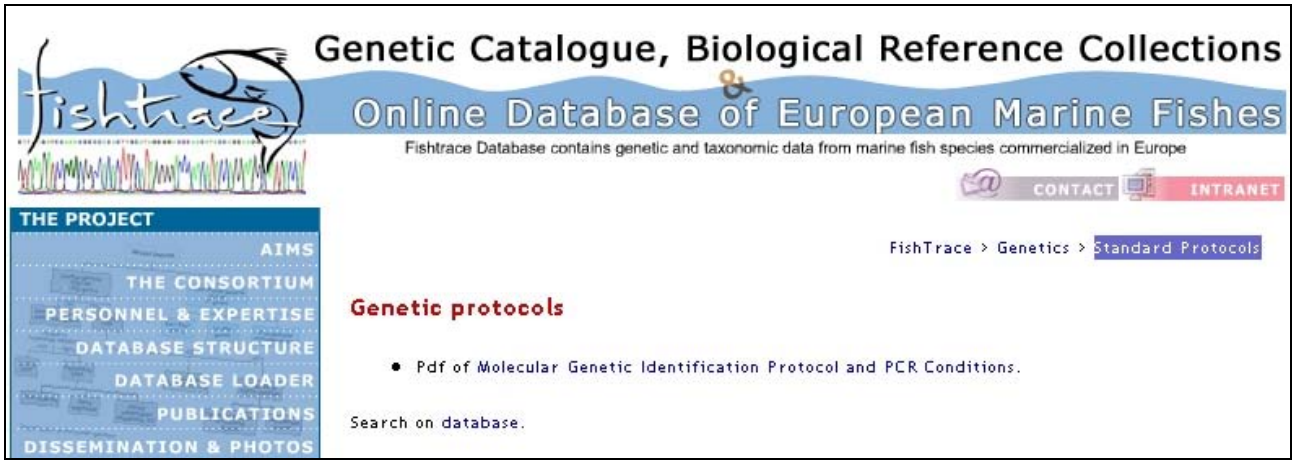


Figure 4.69.- Genetic Catalogue: Standard Protocols for Molecular Genetics procedures.

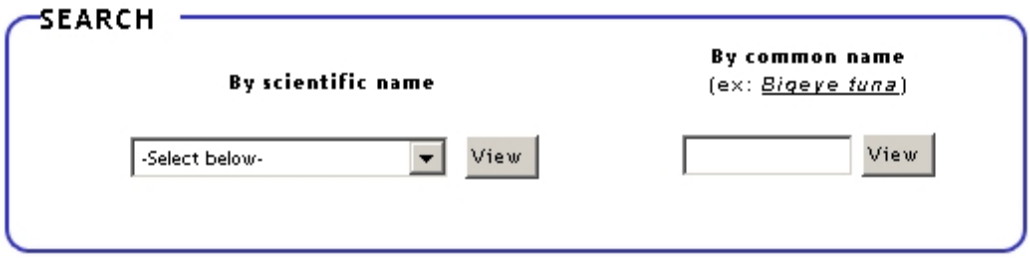


Figure 4.70.- Searching species tool.

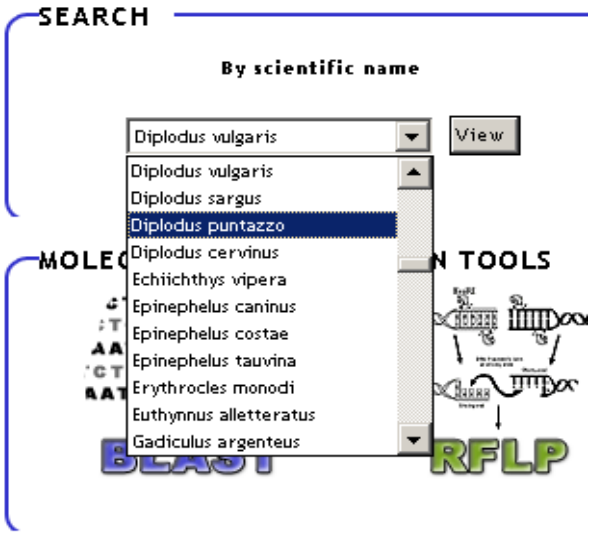


Figure 4.71.- Searching species tool: Search by scientific name.

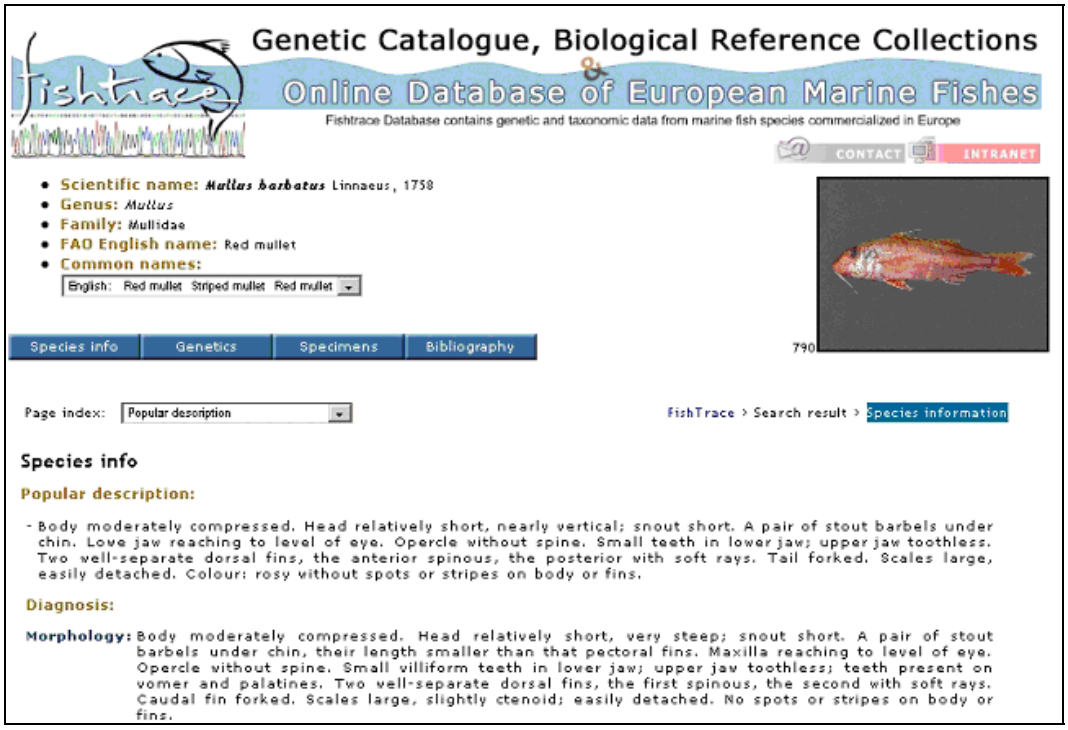


Figure 4.72.- Searching species tool: Result from the search.

Figure 4.73.- Searching species tool: Search by common name: Result from the search.

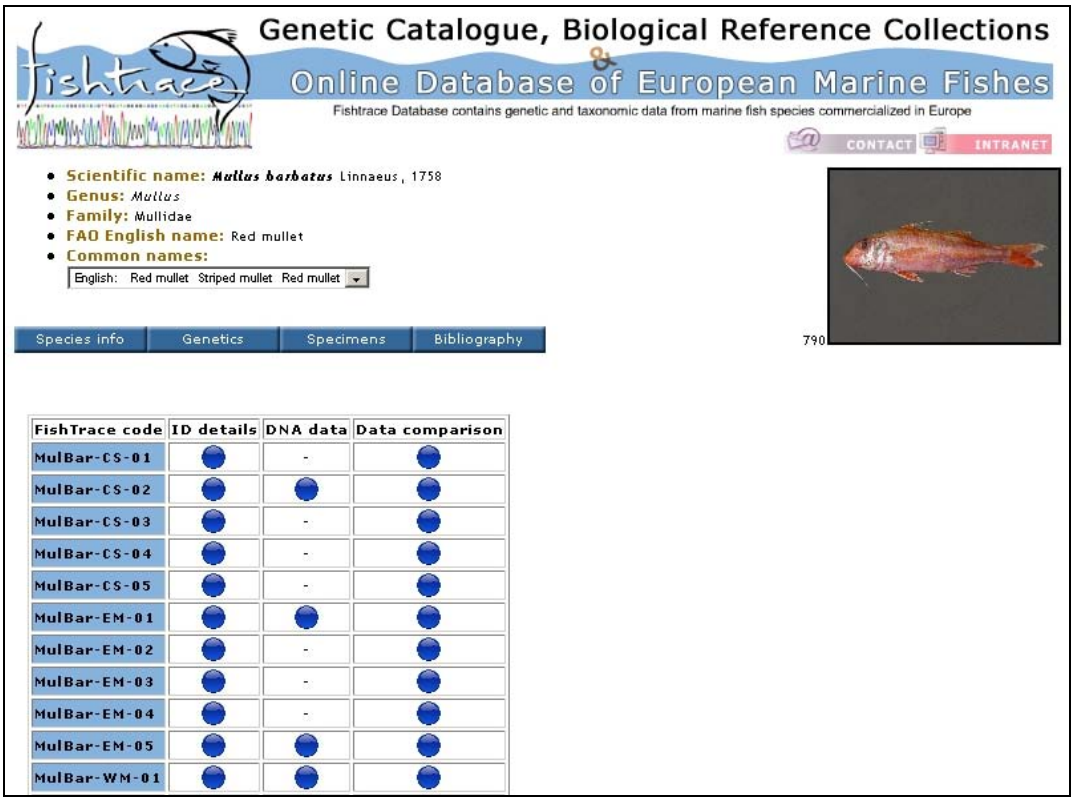


Figure 4.74.- Species Information: Specimens data table.

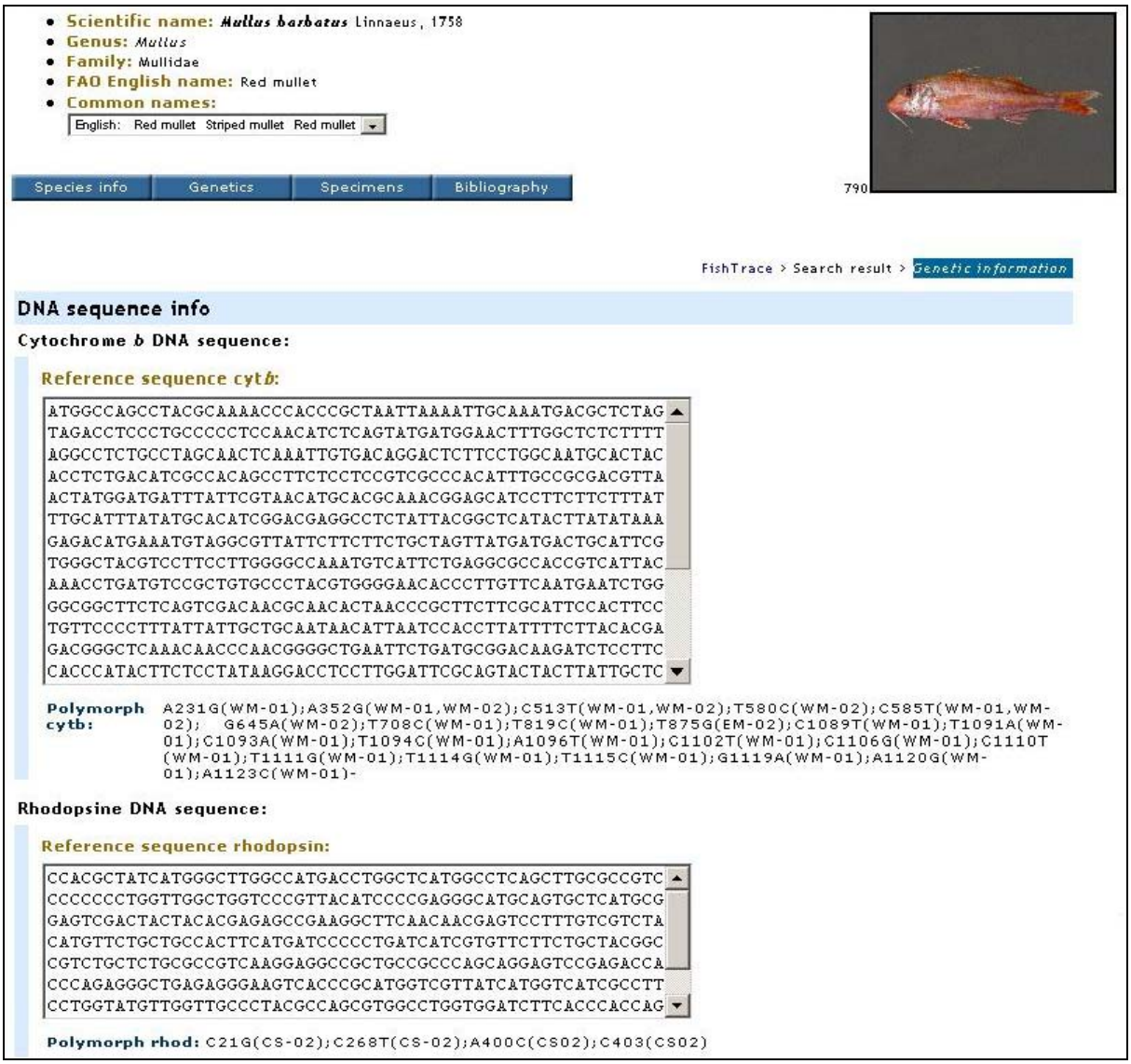


Figure 4.75.- Species Information: Genetics.

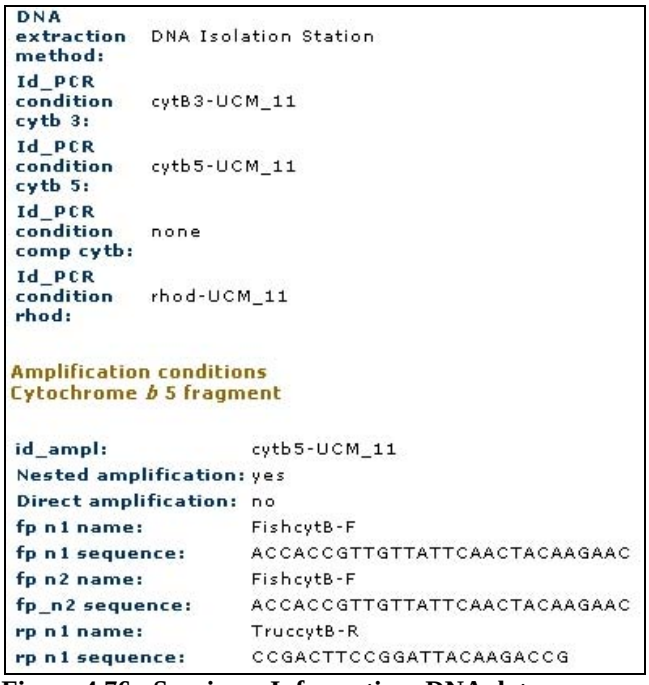


Figure 4.76.- Specimen Information: DNA data.

Location				
locality	ICES VIIIb	ICES VIIIb	ICES VIIIb	ICES VIIIb
depth	-1	-1	-1	-1
fishing_method	bottom trawl	bottom trawl	bottom trawl	bottom trawl
fishmarket	na	na	na	na
lon	2.08333333	2.08333333	2.08333333	2.08333333
lat	44.5	44.5	44.5	44.5
coord_source	other	other	other	other
samp_purch_date	2/23/2004 0:0:0	2/23/2004 0:0:0	2/23/2004 0:0:0	2/23/2004 0:0:0
samp_collector	Gonzalez.JA	Gonzalez.JA	Gonzalez.JA	Gonzalez.JA
cruise_name	Biriatu	Biriatu	Biriatu	Biriatu
landing_site	Port of Pasaia	Port of Pasaia	Port of Pasaia	Port of Pasaia
collect_remarks	na	na	na	na
id_tissue	MulBar-CS-01-a#MulBar-CS-01-b	MulBar-CS-02-a#MulBar-CS-02-b	MulBar-CS-03-b	MulBar-CS-04-b
tissue_col_nb	-2#BMTEJ-VP/1332	-2#BMTEJ-VP/1333	BMTEJ-VP/1334	BMTEJ-VP/1335
tissue_col_inst	UCM#TFMC	UCM#TFMC	TFMC	TFMC
storage_medium	alcohol#alcohol	alcohol#alcohol	alcohol	alcohol
Ampl cond				
id_ampl	na	cytB3-UCM_11#cytb5-UCM_11#rhod-UCM_11	na	na
nested_ampl	na	yes#yes#yes	na	na
direct_ampl	na	no#no#no	na	na
fp_n1_name	na	FishcytB-F#FishcytB-F#Rod-F2B	na	na
fp_n1_seq	na	ACCACCGTTGTTATTCAACTACAAGAAC#ACCACCGTTGTTATTCAACTACAAGAAC#GTCTGCAAGCCCATCAGCAACTTCCG	na	na
fp_n2_name	na	CytBI-7F#FishcytB-F#Rod-F2w	na	na
fp_n2_seq	na	CTAACCCGATTCTTTGCCTTCCACTTCCT#ACCACCGTTGTTATTCAACTACAAGAAC#AGCAACTTCCGCTTCGGTGAGAA	na	na
fp_d_name	na	na	na	na
fp_d_seq	na	na	na	na
rp_n1_name	na	TruccytB-R#TruccytB-R#Rod-5R	na	na
rp_n1_seq	na	CCGACTTCCGGATTACAAGACCG#CCGACTTCCGGATTACAAGACCG#GGTGGTGATCATGCAGTGGCGGAA	na	na
rp_n2_name	na	THR-Fish-R#CytBI-5R#Rod-R4n	na	na
rp_n2_seq	na	ACCTCCGATCTTCGGATTACAAGACC #GGTCTTTGTAGGAGAAGTATGGGTGGAA#GGAACTGCTTGTTCATGCAGATGTAGAT	na	na
rp_d_name	na	na	na	na
rp_d_seq	na	na	na	na
PCR_cycle_n1	na	95-420 / (94-30/55-35/72-120)x35 / 72-420#95-420 / (94-30/55-35/72/120) x35 / 72-420#95-420 / (94-30/62-30/72-30)x40 / 72-420	na	na
PCR_cycle_n2	na	95-420 / (94-30/55-35/72-45)x38 / 72-420#95-420 / (94-30/55-35/72/45) x38 / 72-420#95-420 / (94-30/56-30/72-30)x40 / 72-420	na	na
PCR_cycle_d	na	na	na	na
ampl_remark	na	na	na	na
speccode	na	na	na	na

Figure 4.77.- Specimen Information: Specimen data comparison tool.

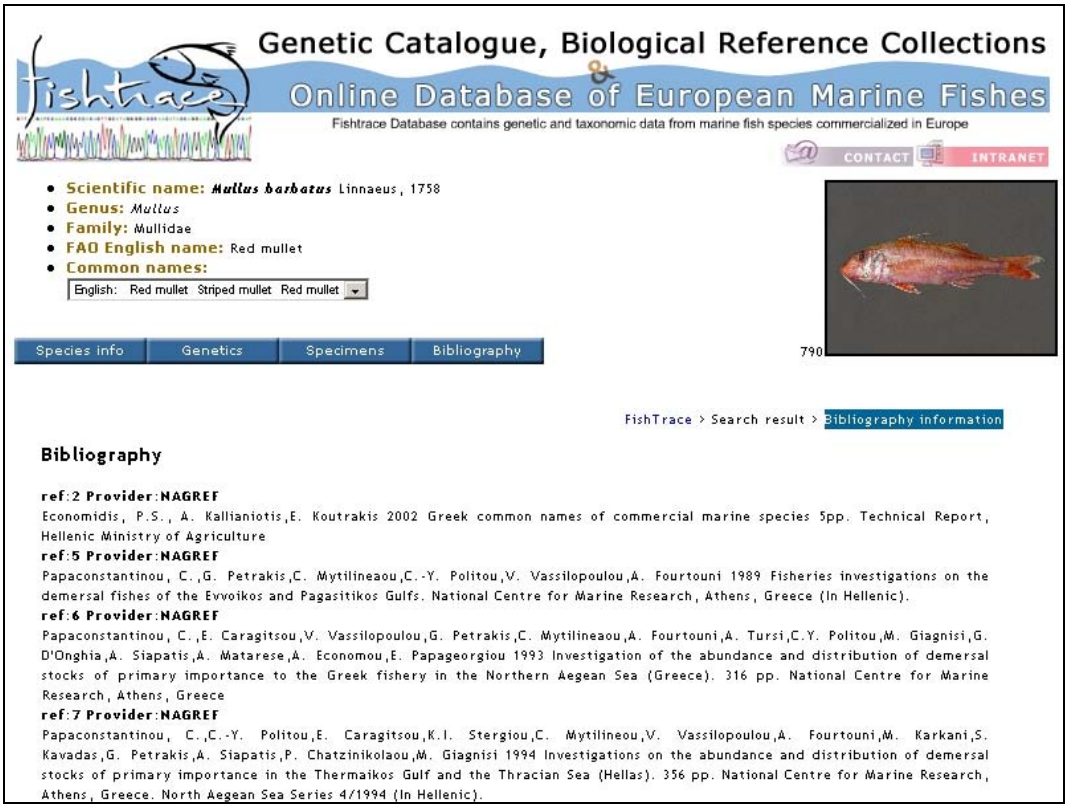


Figure 4.78.- Species Information: Bibliography.

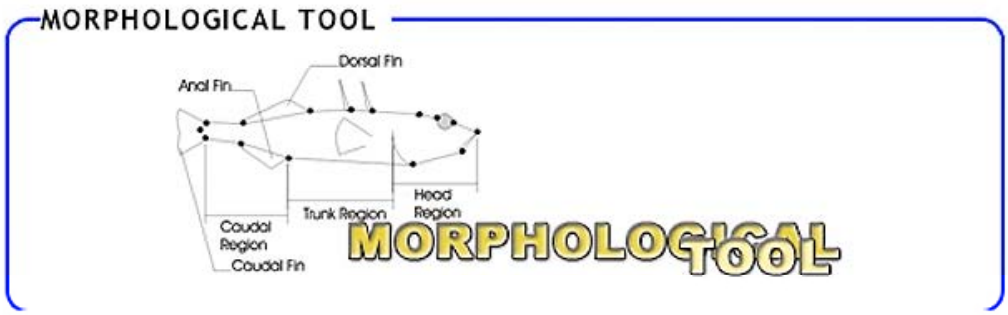


Figure 4.79.- Species Identification Tools: Morphological tool.

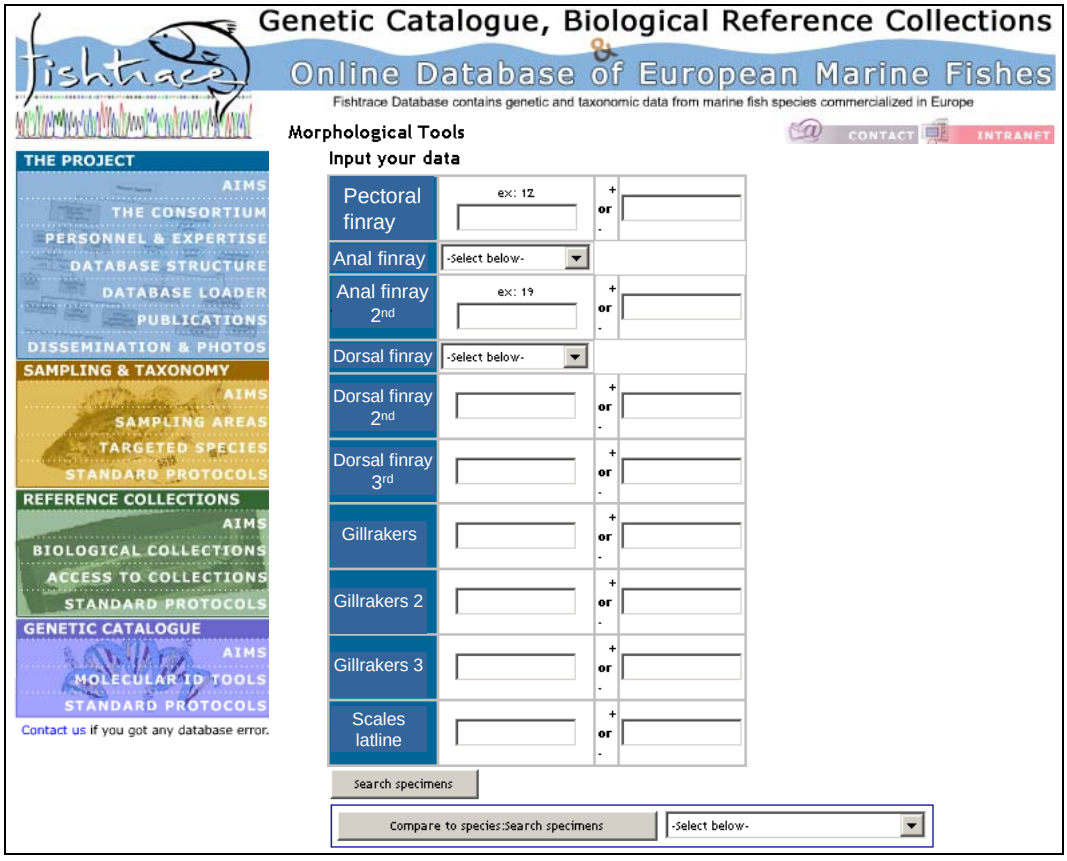


Figure 4.80.- Species Identification Tools: Morphological tool.

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13.- World Wide Web Links

LINK	DESCRIPTION	URL
BoLD	The Barcode of Life Data Systems.	www.barcodinglife.org
CBOL	Consortium for the Bar Code of Life.	http://barcoding.si.edu
COML	The Census of Marine Life.	www.coml.org
FAO	Food and Agriculture Organization of the United Nations.	www.fao.org
FAO-SIDP	FAO Species Identification and Data Programme.	www.fao.org/figis/servlet/static?dom=org&xml=sidp.xml&xp_lang=en&xp_banner=fi
Fish and Chips	EC Fish and Chips Project.	www.fish-and-chips.uni-bremen.de
FishBase	A Global Information System on Fishes.	www.fishbase.org
Fish-BOL	The Fish Barcode of Life Initiative.	www.fishbol.org
GBIF	Global Biodiversity Information System.	www.gbif.org
GenBank	NCBI sequence database.	www.ncbi.nlm.nih.gov/ Genbank/index.html
ICZN	International Code of Zoological Nomenclature.	www.iczn.org
IUCN	The World Conservation Union	www.iucn.org
PescaBase	Estandarización de procedimientos para la identificación y trazabilidad de materias primas de origen pesquero destinadas al consumo.	www.pescabase.org
PTA	European Commission Project Tracking & Archive	http://pta.jrc.cec.eu.int/
Arlequin software	Arlequin: A software for population genetics data analysis	http://anthro.unige.ch/software/arlequin/
MEGA 3.1	MEGA is an integrated tool for automatic and manual sequence alignment, inferring phylogenetic trees, mining web-based databases,estimating rates of molecular evolution, and testing evolutionary hypotheses.	www.megasoftware.net

14.- Abbreviations

16S RNA: Ribosomal subunit (S represents Svedberg units)
BoLD: Barcode of Life Database
BS: Skagerrak and Baltic Sea
CB: English Channel and Bay of Biscay
CI: Canary Islands
COI: Cytochrome *c* oxidase subunit I
CS: Cantabric Sea and NW Iberian Peninsula
cyt*b*: Cytochrome *b*
DNA: Deoxyribonucleic Acid
EC: European Commission
EE: Extra-European
EM: Eastern Mediterranean
ETI: Expert Center for Taxonomic Identification
EU: European Union
FAO: Food and Agriculture Organization
GBIF: Global Biodiversity Information System
HTML: Hypertext Markup Language
ICCM: Instituto Canario de Ciencias Marinas
ICZN: International Code of Zoological Nomenclature
Ifremer: Institut français de recherche pour l'exploitation de la mer
IMAR: Instituto do Mar
IUCN: The World Conservation Union
JRC: EC Joint Research Centre
JSP: Java Server Pages
MA: Madeira Archipelago
MMF: Museu Municipal do Funchal
MNHN: Muséum national d'Histoire Naturelle
mtDNA: Mitochondrial Deoxyribonucleic Acid
NAGREF: National Agricultural Research Foundation
NCBI: U.S. National Center for Biotechnology Information
NRM: Naturhistoriska riksmuseet
NS: North Sea
PCR: Polymerase Chain Reaction
RAPD: Random Amplification of Polymorphic DNA
RFLP: Restriction Fragment Length Polymorphism
rhod: Rhodopsin
RIVO: Netherlands Institute for Fisheries Research
RNA: Ribonucleic Acid
SIDP: Species Identification and Data Programme
SSCP: Single-strand Conformation Polymorphism
TFMC: Museo de Ciencias Naturales de Tenerife
UCM: Universidad Complutense de Madrid
URL: Uniform Resource Locator
WM: Western Mediterranean and Bay of Cadiz
WWW: World Wide Web