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DNA Barcodes of the animal species occurring in Italy under the European "Habitats Directive" (92/43/EEC): a reference library for the Italian National Biodiversity Network

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SUMMARY

The present paper reports the development of a public project addressed to build up and publish a DNA barcode reference library for the animal species occurring in Italy listed in the II, IV and V Annexes of the "Habitats Directive" 92/43/EEC. DNA barcoding is a global standard, namely a procedure based on a gene sequence located in a standardized genome region as a diagnostic biomarker for species. DNA barcodes data have been either produced in our laboratories or collected from the literature and international gene databases. They were subsequently used to assemble a database containing both genetic data and information related to the origin of the data. This project represents the first pilot store of DNA sequence data built-in interoperability within the portal of the National Network of Biodiversity of the Italian Ministry of the Environment. The archive, called "DNA Barcode Database of Italian Nature 2000 animal species" (owned by the Zoology and Evolutionary Biology group at Tor Vergata University), was implemented in a relational DBMS with a free license program (PostgreSQL v9.3.4), mapped using the schema ABCD and the extension DNA, and then made interoperable using the software BioCASE (v3.6.0).

INTRODUCTION

The increasing availability of "big data" systems on biodiversity is becoming a powerful tool to understand and mitigate changes occurring in ecosystems at regional and global scale (Kelling et al. 2009; Aronova et al. 2010; Hampton et al. 2013). In Italy, the idea of a national network for the exchange of biodiversity data was outlined in the late 70's in the framework of the "Commissione Fauna" of the Ministry for the Environment, Land and Sea (MATTM). The project was aimed at encouraging taxonomists, ecologists and biogeographers to digitize their data and share them in a national recording system. At that time the project was set aside for the lack of resources. After several decades, the Italian National Network on Biodiversity (NNB) was realized in the framework of the project ''Sistema Ambiente 2010'' promoted by the Ministry of Environment (MATTM, 2013). Its mission is to organize and manage biodiversity data hosted by museums, universities and research centers in Italy, in order to make them widely available on the Web for basic and applied research (Martellos et al. 2011). To date, NNB data are not indexed by the global data aggregators such as Global Biodiversity Information Facility (GBIF) because Italy is not a GBIF participant, however the NNB portal allows an easy access to retrieve information. Institutions, research centers and association participating to NNB are invited to provide data and scientific support in their area of competence in order to enhance sharing and spreading biodiversity information in Italy (MATTM, 2013). Since its formal institution in May 2012, the Italian Biodiversity Network aggregates approximately 1.5 million records from 60 data sources located in several research centers.

In recent years, both basic and applied research on biodiversity combines traditional studies and monitoring approaches with the genetic characterization of populations and species to reveal diversity of taxa and properly identify biological and conservation units (Taylor et al. 2010; Gratton et al. 2016).

DNA barcoding is now a widespread molecular routine using DNA nucleotide sequences as "bar code labels" to support the identification and classification of living organisms (Hebert et al. 2003a). The DNA barcode is the same in any stage of the life cycle of a specific organism, in both sexes and in all its parts, also transformed for commercial or any other purposes. Different species are expected to have different DNA barcodes so that any biological specimens could be identified, and new species discovered (Bergsten et al. 2012). DNA barcoding can serve a dual-purpose, as a new tool in the taxonomist's toolbox supplementing their knowledge as well as a standard device for non-experts who need to achieve a quick identification. These features imply several notably benefits and an increasing global participation in DNA barcoding is occurring in several fields, including conservation biology, public health, environmental monitoring (Adamowicz and Steinke, 2015).

DNA barcoding is a global standard, namely a regulated technique using a gene located in a standardized genome region as a diagnostic biomarker for species. DNA barcoding based on the mitochondrial gene coding for cytochrome c oxidase subunit 1 (COI) has become a widely accepted molecular marker for animal species identification. Other genes have been employed as DNA barcode, but in most metazoans COI sequences, approximately 650 bp from this mtDNA gene, offer an adequate resolving power at the species level and above (Hebert et al. 2003b).

Research projects aimed at genetic characterization of population and species, by means of DNA barcode sequences, meet the requirements of the Convention on Biological Diversity (CBD) on gathering of information about local biodiversity of each signatory CBD Member State (Vernooy et al. 2010). In this perspective, the aim of this paper is to illustrate the results of a pilot project realized within the framework project "Sistema Ambiente 2010" (MATTM, 2013), which promotes the planning and establishing of the Italian National Network of Biodiversity (NNB). This specific activity points toward the genetic characterization, through DNA barcoding, of the animal species occurring in Italy and listed in the II, IV and V Annexes of the "Habitats Directive" 92/43/EEC. The DNA barcode sequences were used to create an archive of enriched primary biodiversity data for animal species protected under the Habitats Directive.

METHODS

The 3rd National Report for the Habitats Directive (2007-2012) by ISPRA (2014) and the Eionet web site listed 225 animal taxa occurring in Italy. Based

on the knowledge obtained over the years, the ISPRA 2014 Report suggested numerous taxonomic and nomenclatural updates over the original Habitat Directive names. As a consequence, our database was structured to collect data for 250 target taxa. We considered the 225 taxa in the ISPRA report with the addition of 25 taxa recently derived from the splitting of some old taxa or doubtfully occurring in Italy. All these taxa were cited in the Codelist for species in Eionet web site (eionet.europa.eu - Reference Portal for Natura 2000).

Since the COI gene has long been employed extensively in molecular phylogeny and phylogeography, a large number of sequences are already available in the international gene databases GenBank (Clark et al. 2016) and BOLD System (Ratnasingham and Hebert, 2007, 2013).

As a first step, we performed the screening for the 250 taxa to retrieve sequences for the COI gene. We favored the DNA barcode sequences derived from Italy or from Italian coastal marine waters for marine wildlife. When different sequences from Italian samples were available, some replicas (up to 5) for each species were included in the database, according to the standard protocols of DNA barcoding. When sequences from Italian specimens were unavailable, we considered DNA barcode sequences of individuals from sites close to national borders. The employment of barcode data from specimens from the nearest countries was already used in Hausmann et al. (2011). In any case we favored sequences from specimen geographically referred as carefully as possible in order to georeference their collecting localities.

To perform the screening, we used the "seqinr" R package (Charif et al. 2014) to retrieve all COI sequences hosted in GenBank for the target taxa. The screening was repeated on BOLD system for the same species. The accession numbers of the sequence were stored in our database together with their references. All the mined sequences were aligned with reference barcode COI fragments of phylogenetically close taxa to verify their overlap by using CodonCode Aligner program (CodonCode Corporation, Dedham, MA, USA). In order to avoid misidentification, most downloaded sequences derived from published papers, assuming a reliable identification at the species level. When not possible, e.g. unpublished paper, we checked the specimen by the "best close match" analysis approach (Meier et al. 2006).

For other target taxa we recovered Italian specimens available in museums or private collections, stored dry, in alcohol or frozen. The search for suitable tissue samples for DNA extraction in some cases required the involvement of taxonomic specialists able to provide properly identified material. All laboratory phases were carried out at the Zoology and Evolutionary Biology (ZEB) Lab , Department of Biology, University of Rome Tor Vergata. DNA extractions were performed according to standard protocols using the GenElute Mammalian Genomic DNA Miniprep Kit (Sigma). The DNA amplification was performed using standard protocols (94°C for 30 sec, 54°C for 30 sec and 72°C for 1 min) and conventional primers for the standard barcode fragment of the COI gene LCO1490/HCO2198 (Folmer et al. 1994). For some specimens, the protocols were slightly modified to obtain a successful amplification: the annealing was set at lower temperature (45ºC) for 5 or 10 initial cycles to allow the primers to bind to the template, then the temperature was raised to 51ºC for the rest of the PCR (35 cycles) to avoid excessive non-specific binding of primers. The DNA sequencing of both strands was performed by using the DNA Analysis Facility on Science Hill at Yale University; all the sequences were submitted to GenBank.

RESULTS

Data mined from international gene databases or directly typified in ZEB lab allowed us to gather DNA barcode data (COI gene) for 202 out of 250 target animal taxa and to assemble 470 DNA nucleotide sequences in our database (Appendix 1).

For each examined taxon we selected one to five sequences, with a median number of three sequences per taxon. When the exact collecting locality for a studied specimen was available, geographic coordinates were calculated and added to the specific dataset record. In literature or public databases, we found 412 sequences for 176 target taxa; in our lab we produced 58 sequences for 34 target taxa; in literature we could not find any sequence for 7 of these 34 target taxa.

For 121 target taxa, at least one sequence resulted from specimens collected in Italy, which

provided 371 out of 470 sequences (79%); for 81 target taxa, we did not find any sequence from specimens collected in Italy; for the remaining 48 target taxa we did not find neither COI sequences nor suitable specimen for DNA extraction.

Table 1 illustrates the Metadata describing the assembled dataset. The store, called "DNA Barcode Database of Italian Nature 2000 animal species" is owned by ZEB group (University of Rome Tor Vergata). The archive is available at the NNB portal and interoperable with other NNB databases through the BioCASE Access Protocol (Holetschek et al. 2012) using ABCD (Access to Biological Collection Data) as the concept schema. The database runs on PostgreSQL (ver.9.3.4; www.postgresql.org) and its accessibility to users through the NNB portal allows sharing information with citizens and stakeholders.

Table 1. Metadata describing the assembled dataset. The archive is available at the Italian National Network on Biodiversity (NNB) portal (http://www.nnb.isprambiente.it/portalino/home_it/dati.php)

Data set	
Authors:	Donatella Cesaroni, Valerio Sbordoni
Authors' contacts:	donatella.cesaroni@uniroma2.it
Object name:	DNA Barcode Database of Italian Nature 2000 animal species
Data set citation:	Zoology and Evolutionary Biology (ZEB) group, Department of Biology, Tor Vergata University, Rome
Summary statistics:	250 species, 470 sequences, ~650bp length
Character encoding:	UTF-8
Format name:	ABCD DNA extension
Format version:	ABCD v2.06
Distribution:	Network Nazionale Biodiversità (Ministero dell'Ambiente e della Tutela del Territorio e del Mare)
	http://www.nnb.isprambiente.it/portalino/home_it/il-network.php
	http://www.nnb.isprambiente.it/portalino/home_it/dati.php
Publication date:	2013; last update February 2016
Language:	English
License of use:	The use of these data is limited to research, educational, non-commercial use. Institution(s) and data producer(s) must be cited as the source of the informations
Metadata language:	English
Management details	
Project title:	DNA Barcode Database of Italian Nature 2000 animal species
Database manager(s):	Stefano De Felici (stefano.de.felici@uniroma2.it)
Record basis:	mtDNA sequences: fragments from cytochrome c oxidase subunit 1 (COI) gene
IT specialist(s):	Stefano De Felici (stefano.de.felici@uniroma2.it)
Funding grants:	This project was funded by the Italian Ministry of the Environment (MATTM) in the framework of the project ‘‘Sistema Ambiente 2010’’ for the development of the Italian National Biodiversity Network (NNB)
Geographic coverage	
Study area:	Italy
Taxonomic coverage	
General description:	All of the species included in this dataset are Metazoan species. The dataset contains 250 target taxa: 225 taxa listed in the 3rd National Report for the Habitats Directive (2007-2012) by ISPRA (2014) with the addition of 25 taxa recently derived from the splitting of some old taxa or doubtfully occurring in Italy. All these taxa were cited in the Codelist for species in Eionet web site (eionet.europa.eu - Reference Portal for Natura 2000)

DISCUSSION

Italy plays a leading role regarding the protection and conservation of the fauna of Community interest. In fact Italy houses more than a third of all species in the Habitats Directive and it is the European country with the highest animal species richness (ISPRA, 2014).

Due to geographic position of the Italian peninsula jutting into the Mediterranean sea and his biogeographical key role, Italy today represents an important biodiversity hotspot with much endemisms. In fact, most temperate species have taken refuge in the southern free-ice part of Italy, and then expanded northward at the end of the last ice age. At present we know about 58000 animal species occurring in Italy. In particular, the terrestrial fauna consists of about 42,000 identified species so far, with about 10% of endemic species, which are of particular interest for biodiversity conservation.

The genetic characterization through the DNA barcoding of Italian animal species protected under the Habitats Directive started with the development of a public project realized within the framework “Sistema Ambiente 2010” (MATTM, 2013) and described in this paper.

Although the work we have carried out has to be intended as a pilot project, it allowed us to collect DNA barcode data for more than 80% of the Italian protected animal species under the Habitats Directive.

The success rate (% of data coverage) of our reference library is quite satisfactory when compared to those obtained in most important DNA barcoding projects restricted to particular taxa or countries. For example, the DNA barcode reference library for Canadian spiders included about two-thirds of the Canadian spider fauna (Blagoev, et al. 2016); the study focusing on the 180 species of butterflies in Romania analyzed 99% of the species (Dincă et al. 2011); the globally largest DNA barcode reference library for well-identified species of Coleoptera of the German fauna included 53% of species (Hendrich, et al. 2015); a study in progress in the State of Bavaria, involved in a research program to build a DNA barcode library for all animal species within its territorial boundaries, provided a comprehensive library for the Geometridae (Lepidoptera) with DNA barcodes for 98% of the known fauna (Hausmann et al. 2011).

The database is open to anyone in order to stimulate interest in the application of DNA barcoding on the entire Italian fauna. DNA barcode reference libraries will play an important role in monitoring programs, biodiversity assessments, and subsequent biological researches (Hausmann et al. 2011). Moreover, the importance of barcoding projects focused on endangered species is well known (Rubinoff, 2006) and the database implemented in this work could be useful to assist future conservation projects.

This work represents a milestone, since it is the first wide-ranging DNA barcode project realized for Italian fauna. Our purpose would be to refine the current results completing the "DNA Barcode Database of Italian Nature 2000 animal species". The future steps will be the implementation of the DNA barcode reference libraries with data for endemic taxa, vulnerable species, and alien species. Despite high levels of Biodiversity in Italy, most species could be considered in decline due to consequences of climate change, extensive land uses changes and alien species introduction. However, protection measures for them are not yet evaluated.

In this context, a future framework program on a national scale for a DNA barcode library for all animal and plant species should be considered necessary; it would propel molecular characterization to support the traditional taxonomy in order to build a cross-referenced database, and this would constitute a definitive advantage and progress for the study of Italian Biodiversity.

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AUTHORS CONTRIBUTION

VS conceived the study. DC supervised the project and prepared the manuscript. SDF designed the archive structure. GR and SDF analysed the data, GR, MC and AV acquired and processed the sequence data. EB handled relationships between Tor Vergata University and MATTM. All authors contributed to revising the manuscript.

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Appendix 1. List of the 250 target animal taxa used to assemble the 470 DNA barcode sequences currently stored in the "DNA Barcode Database of Italian Nature 2000 animal species". For each taxon, this table reports the number of included DNA barcode sequences (records), their accession numbers in the international gene databases and the state of origin of the sampled taxon. Asterisks on the accession numbers indicate that DNA sequences was produced and published for the first time in the present paper. In the Source (Accession number) column, the numbers refer to GenBank, unless BOLD is specified. Details for each specimen (i.e. DNA sequence; collecting locality; geographic coordinates, when available) are accessible at http://www.nnb.isprambiente.it/portalino/home_it/dati.php

Phylum	Class	Order	ScientificName	taxonomic authority	HD Name	Records in DB	Country	Source (Accession number)
Annellida	Hirudinea	Arhynchobdellida	*Hirudo verbana*	Carena, 1820		1	Serbia	KP663465
Annellida	Hirudinea	Arhynchobdellida	*Hirudo medicinalis*	Linnaeus, 1758		1	France	EU100093
Arthropoda	Insecta	Coleoptera	*Bolbelasmus unicornis*	Schrank, 1789		-		
Arthropoda	Insecta	Coleoptera	*Buprestis splendens*	Fabricius, 1775		-		
Arthropoda	Insecta	Coleoptera	*Carabus olympiae*	Sella, 1855		-		
Arthropoda	Insecta	Coleoptera	*Carabus variolosus nodulosus*	Creutzer, 1799	*Carabus variolosus*	1	France	JQ646615
Arthropoda	Insecta	Coleoptera	*Cerambyx cerdo*	Linnaeus, 1758		2	France	KM285874, KM285966
Arthropoda	Insecta	Coleoptera	*Cucujus cinnaberinus*	(Scopoli, 1763)		1	Germany	KM447566
Arthropoda	Insecta	Coleoptera	*Dytiscus latissimus*	Linnaeus, 1758		1	-unknown-	EF670053
Arthropoda	Insecta	Coleoptera	*Graphoderus bilineatus*	(De Geer, 1774)		1	Germany	KM448638
Arthropoda	Insecta	Coleoptera	*Leptodirus hochenwarti*	Schmidt, 1832		2	Italy	**KX241487*, KX241488***
Arthropoda	Insecta	Coleoptera	*Lucanus cervus*	(Linnaeus, 1758)		1	Italy	**KX241489***
Arthropoda	Insecta	Coleoptera	*Morimus asper*	(Sulzer, 1776)	*Morimus funereus*	2	France, Italy	JX969629, KM286055
Arthropoda	Insecta	Coleoptera	*Osmoderma cristinae*	Sparacio, 1994		3	Italy	AJ880679, AM412382, AM412383
Arthropoda	Insecta	Coleoptera	*Osmoderma eremita*	(Scopoli, 1763)		3	Italy	J880680, J880683, M423158
Arthropoda	Insecta	Coleoptera	*Osmoderma italicum*	Sparacio, 2000		1	Italy	AJ880686
Arthropoda	Insecta	Coleoptera	*Rhysodes sulcatus*	(Fabricius, 1787)		-		
Arthropoda	Insecta	Coleoptera	*Rosalia alpina*	(Linnaeus, 1758)		1	Italy	**KX241486***
Arthropoda	Insecta	Coleoptera	*Stephanopachys linearis*	(Kugelann, 1792)		1	Finland	KJ962193
Arthropoda	Insecta	Coleoptera	*Stephanopachys substriatus*	(Paykull, 1800)		1	Finland	KJ963477
Arthropoda	Insecta	Lepidoptera	*Argynnis elisa*	Godart, 1823	*Fabriciana elisa*	1	Italy	**KX241494***
Arthropoda	Insecta	Lepidoptera	*Arytrura musculus*	(Ménétriés, 1859)		-		
Arthropoda	Insecta	Lepidoptera	*Coenonympha oedippus*	(Fabricius, 1787)		1	Germany	GU707147
Arthropoda	Insecta	Lepidoptera	*Erannis ankeraria*	(Staudinger, 1861)		1	-unknown-	GU580770
Arthropoda	Insecta	Lepidoptera	*Erebia calcaria*	Lorkovic, 1953		5	Slovenia	**KX241495*, KX241496*, KX241497*, KX241498*,**

								KX241499*
Arthropoda	Insecta	Lepidoptera	*Erebia christi*	Rätzer, 1890		-		
Arthropoda	Insecta	Lepidoptera	*Eriogaster catax*	(Linnaeus, 1758)		1	Germany	GBLAB286-13 (BOLD)
Arthropoda	Insecta	Lepidoptera	*Euphydryas aurinia*	(Rottemburg, 1775)	*Euphydryas aurinia*	3	France, Italy, Slovenia	**KX241500*,** KT896975, KT897206
Arthropoda	Insecta	Lepidoptera	*Euphydryas glaciegenita*	(Verity, 1928)	*Euphydryas aurinia*	2	Austria, Switzerland	KT896753, KT897061
Arthropoda	Insecta	Lepidoptera	*Euphydryas maturna*	(Linnaeus, 1758)		1	Germany	KP870955
Arthropoda	Insecta	Lepidoptera	*Euphydryas provincialis*	(Boisduval, 1828)	*Euphydryas aurinia*	3	Italy	**KX241501*, KX241502*, KX241503***
Arthropoda	Insecta	Lepidoptera	*Euplagia quadripunctaria*	(Poda, 1761)		3	Italy	**KX241490*, KX241491*, KX241492***
Arthropoda	Insecta	Lepidoptera	*Graellsia isabellae*	(Graells, 1849)		1	Spain	SATWB001-07 (BOLD)
Arthropoda	Insecta	Lepidoptera	*Hyles hippophaes*	(Esper, 1789)		1	Slovakia	JN678057
Arthropoda	Insecta	Lepidoptera	*Lopinga achine*	(Scopoli, 1763)		1	Austria	KP253300
Arthropoda	Insecta	Lepidoptera	*Lycaena dispar*	(Haworth, 1802)		1	Italy	**KX241493***
Arthropoda	Insecta	Lepidoptera	*Maculinea arion*	(Linnaeus, 1758)		1	Austria	KP253531
Arthropoda	Insecta	Lepidoptera	*Maculinea teleius*	(Bergsträsser, 1779)		2	Austria, Slovenia	KM517293, KP253699
Arthropoda	Insecta	Lepidoptera	*Melanargia arge*	(Sulzer, 1776)		5	Italy	**KX241504*, KX241505*, KX241506*, KX241507*, KX241508***
Arthropoda	Insecta	Lepidoptera	*Papilio alexanor*	Esper, 1800		2	Italy	**KX241509*, KX241510***
Arthropoda	Insecta	Lepidoptera	*Papilio hospiton*	Guenée, 1839		2	Italy	F514438, F514443
Arthropoda	Insecta	Lepidoptera	*Parnassius apollo*	(Linnaeus, 1758)		5	Italy	GU947487, GU947498, GU947499, GU947550, GU947563
Arthropoda	Insecta	Lepidoptera	*Parnassius mnemosyne*	(Linnaeus, 1758)		5	Italy	EU836663, EU836671, EU836672, EU836674, EU836675
Arthropoda	Insecta	Lepidoptera	*Proserpinus proserpina*	(Pallas, 1772)		1	France	JN678428
Arthropoda	Insecta	Lepidoptera	*Zerynthia cassandra*	(Geyer, 1828)		5	Italy	KC119712, KC119713, KC119714, KC119715, KC119716

Arthropoda	Insecta	Lepidoptera	*Zerynthia polyxena*	(Denis & Schiffermüller, 1775)		3	Italy	KC119718, KC119723, KC119724
Arthropoda	Insecta	Odonata	*Coenagrion mercuriale*	(Charpentier, 1840)		4	Italy	**KX241511*, KX241512*, KX241513*, KX241514***
Arthropoda	Insecta	Odonata	*Cordulegaster heros*	Theischinger, 1979		-		
Arthropoda	Insecta	Odonata	*Cordulegaster trinacriae*	Waterston, 1976		2	Italy	KF584945, KF584946
Arthropoda	Insecta	Odonata	*Gomphus flavipes*	(Charpentier, 1825)		-		
Arthropoda	Insecta	Odonata	*Leucorrhinia pectoralis*	(Charpentier, 1825)		1	Sweden	JN991193
Arthropoda	Insecta	Odonata	*Lindenia tetraphylla*	(Vander Linden, 1825)		1	Italy	**KX241516***
Arthropoda	Insecta	Odonata	*Ophiogomphus cecilia*	(Fourcroy, 1785)		-		
Arthropoda	Insecta	Odonata	*Oxygastra curtisii*	(Dale, 1834)		1	Italy	**KX241515***
Arthropoda	Insecta	Odonata	*Sympecma paedisca*	(Brauer, 1882)		1	Japan	AB708320
Arthropoda	Insecta	Orthoptera	*Brachytrupes megacephalus*	(Lefevre, 1827)		-		
Arthropoda	Insecta	Orthoptera	*Myrmecophilus baronii*	Baccetti, 1966		-		
Arthropoda	Insecta	Orthoptera	*Saga pedo*	(Pallas, 1771)		1	Hungary	GU206265
Arthropoda	Malacostraca	Decapoda	*Astacus astacus*	(Linnaeus, 1758)		2	Austria, Croatia	AY667146, GU727619
Arthropoda	Malacostraca	Decapoda	*Austropotamobius italicus*	(Faxon, 1984)		4	Italy	HM622599, HM622600, HM622616, HM622617
Arthropoda	Malacostraca	Decapoda	*Austropotamobius pallipes*	(Lereboullet, 1858)		5	Italy	J160743, J160744, J160745, J160747, J160748
Arthropoda	Malacostraca	Decapoda	*Austropotamobius torrentium*	(Schrank, 1803)		2	Croatia, Montenegro	JF293436, JN683352
Arthropoda	Malacostraca	Decapoda	*Scyllarides latus*	(Latreille, 1803)		1	Turkey	KC311421
Chordata	Actinopterygii	Acipenseriformes	*Acipenser naccarii*	Bonaparte, 1836		3	Italy	KJ552424, KJ552671, KJ552707
Chordata	Actinopterygii	Clupeiformes	*Alosa agone*	(Scopoli, 1786)		5	Italy	KJ552379, KJ552455, KJ552649, KJ552682, KJ552733
Chordata	Actinopterygii	Clupeiformes	*Alosa fallax*	(Lacepède, 1803)		1	France	KJ552569
Chordata	Actinopterygii	Cypriniformes	*Alburnus albidus*	(Costa, 1838)		5	Italy	**KX241517***, KJ552477, KJ552552, KJ552619, KJ552716
Chordata	Actinopterygii	Cypriniformes	*Barbus caninus*	Bonaparte, 1839	*Barbus meridionalis*	5	Italy	KJ552786, KJ552837, KJ552916, KJ553046,

								KJ553062
Chordata	Actinopterygii	Cypriniformes	*Barbus plebejus*	Bonaparte, 1839		5	Italy	KJ552791, KJ552884, KJ553115, KJ553128, KJ553292
Chordata	Actinopterygii	Cypriniformes	*Barbus tyberinus*	Bonaparte, 1839		5	Italy	KJ552864, KJ552879 , KJ552901, KJ553061, KJ553184
Chordata	Actinopterygii	Cypriniformes	*Chondrostoma soetta*	Bonaparte, 1840		4	Italy	HM989724, KJ552873, KJ553071, KJ553077
Chordata	Actinopterygii	Cypriniformes	*Cobitis bilineata*	Canestrini, 1865	*Cobitis taenia*	3	Italy	KJ552424, KJ552671, KJ552707
Chordata	Actinopterygii	Cypriniformes	*Cobitis zanandreai*	Cavicchioli, 1965		5	Italy	KJ552927, KJ553001, KJ553015, KJ553193, KJ553270
Chordata	Actinopterygii	Cypriniformes	*Protochondrostoma genei*	(Bonaparte, 1839)	*Chondrostoma genei*	5	Italy	HM560291, HM560292, KJ554091, KJ554121, KJ554251
Chordata	Actinopterygii	Cypriniformes	*Rutilus pigus*	(lacepede, 1803)		2	Italy	HM560326, HM560327
Chordata	Actinopterygii	Cypriniformes	*Rutilus rubilio*	(Bonaparte, 1837)		5	Italy	KJ554129, KJ554200, KJ554212, KJ554290, KJ554352
Chordata	Actinopterygii	Cypriniformes	*Sabanejewia larvata*	(De Filippi, 1859)	*Cobitis larvata*	3	Italy	KJ554664, KJ554774, KJ554832
Chordata	Actinopterygii	Cypriniformes	*Squalius lucumonis*	(Bianco, 1983)	*Leuciscus lucumonis*	5	Italy	HM560346, HM560347, JF317936, KJ552113, KJ552137
Chordata	Actinopterygii	Cypriniformes	*Telestes muticellus*	(Bonaparte, 1837)	*Leuciscus souffia*	5	Italy	HM560367, HM560368, KJ554658, KJ554837, KJ554912

Chordata	Actinopterygii	Cyprinodontiformes	*Aphanius fasciatus*	(Valenciennes, 1821)		5	Italy	KJ552449, KJ552618, KJ552667, KJ552751, KJ709474
Chordata	Actinopterygii	Perciformes	*Knipowitschia panizzae*	(Verga, 1841)	*Knipowitschia punctatissima*	4	Italy	KJ553342, KJ553355, KJ553377, KJ553511
Chordata	Actinopterygii	Perciformes	*Padogobius nigricans*	(Canestrini, 1867)	*Padogobius nigricans*	5	Italy	KJ553763, KJ553788, KJ553962, KJ553991, KJ554001
Chordata	Actinopterygii	Perciformes	*Pomatoschistus canestrinii*	(Ninni, 1883)		-		
Chordata	Actinopterygii	Salmoniformes	*Salmo cettii*	Rafinesque, 1810	*Salmo macrostigma*	2	Italy	**KX241518*, KX241519***
Chordata	Actinopterygii	Salmoniformes	*Salmo fibreni*	Zerunian & Gandolfi, 1990		4	Italy	**KX241520***, KJ554810, KJ554847, KJ554892
Chordata	Actinopterygii	Salmoniformes	*Salmo marmoratus*	Cuvier, 1829		4	Italy	KJ554620, KJ554688, KJ554725, KJ554891
Chordata	Actinopterygii	Salmoniformes	*Thymallus thymallus*	(Linnaeus, 1758)		1	Austria	JX960975
Chordata	Actinopterygii	Scorpaeniformes	*Cottus gobio*	Linnaeus, 1758		4	Italy	KJ552945, KJ552953, KJ553084, KJ553206
Chordata	Amphibia	Anura	*Bombina pachypus*	(Bonaparte, 1839)	*Bombina variegata*	5	Italy	**KX241521*, KX241522***, EU531200, EU531201, EU531204
Chordata	Amphibia	Anura	*Bombina variegata*	(Linnaeus, 1758)	*Bombina variegata*	1	Italy	**KX241523***
Chordata	Amphibia	Anura	*Bufotes viridis*	Laurenti, 1768	*Bufo viridis*	2	Italy	**KX241524*, KX241525***
Chordata	Amphibia	Anura	*Discoglossus pictus*	Otth, 1837		2	France, Malta	JQ626697, JQ626698
Chordata	Amphibia	Anura	*Discoglossus sardus*	Tschudi, 1837		4	Italy	JQ626693, JQ626694, JQ626695, JQ626696
Chordata	Amphibia	Anura	*Hyla arborea*	(Linnaeus, 1758)		-		
Chordata	Amphibia	Anura	*Hyla intermedia*	Boulenger, 1882	*Hyla arborea*	5	Italy	FJ226788, FJ226789, FJ226790, FJ226791, FJ226792

Chordata	Amphibia	Anura	*Hyla meridionalis*	Boettger, 1874		4	Italy	FJ226795, FJ226796, FJ226797, FJ226840
Chordata	Amphibia	Anura	*Hyla sarda*	De Betta, 1857		3	Italy	FJ226815, FJ226816, FJ226843
Chordata	Amphibia	Anura	*Pelobates fuscus insubricus*	(Cornalia, 1873)		-		
Chordata	Amphibia	Anura	*Pelophylax esculenta*	(Linnaeus, 1758)	*Rana esculenta*	1	Poland	JN627424
Chordata	Amphibia	Anura	*Pelophylax lessonae*	(Camerano, 1982)	*Rana lessonae*	1	Poland	JN627426
Chordata	Amphibia	Anura	*Pelophylax ridibundus*	(Pallas, 1771)	*Rana ridibunda*	1	Italy	**KX241526***
Chordata	Amphibia	Anura	*Rana dalmatina*	Fitzinger, 1838		1	Germany	KP697920
Chordata	Amphibia	Anura	*Rana italica*	Dubois, 1987		2	Italy	**KX241527*, KX241528***
Chordata	Amphibia	Anura	*Rana latastei*	Boulenger, 1879				
Chordata	Amphibia	Anura	*Rana temporaria*	Linnaeus, 1758		2	Italy	FN813784, FN813786
Chordata	Amphibia	Urodela	*Euproctus platycephalus*	(Gravenhorst, 1829)		2	Italy	**KX241530***, EU880317
Chordata	Amphibia	Urodela	*Hydromantes ambrosii*	Lanza, 1955		-		
Chordata	Amphibia	Urodela	*Hydromantes flavus*	Stefani, 1969		-		
Chordata	Amphibia	Urodela	*Hydromantes genei*	(Temminck & Schlegel, 1838)		-		
Chordata	Amphibia	Urodela	*Hydromantes imperialis*	Stefani, 1969		-		
Chordata	Amphibia	Urodela	*Hydromantes italicus*	Dunn, 1923	*Hydromantes (Speleomantes) italicus*	1	Italy	AY728215
Chordata	Amphibia	Urodela	*Hydromantes sarrabusensis*	(Lanza, Leo, Forti, Cimmaruta, Caputo & Nascetti, 2001)		-		
Chordata	Amphibia	Urodela	*Hydromantes strinatii*	Aellen, 1958		-		
Chordata	Amphibia	Urodela	*Hydromantes supramontis*	Lanza, Nascetti & Bullini, 1986		-		
Chordata	Amphibia	Urodela	*Lissotriton italicus*	(Peracca, 1898)	*Triturus italicus*	1	Italy	**KX241531***
Chordata	Amphibia	Urodela	*Proteus anguinus*	Laurenti, 1768		1	Italy	**KX241529***
Chordata	Amphibia	Urodela	*Salamandra atra atra*	Laurenti, 1768	*Salamandra atra*	1	Italy	KF645966
Chordata	Amphibia	Urodela	*Salamandra atra aurorae*	Trevisan, 1982		-		
Chordata	Amphibia	Urodela	*Salamandra atra pasubiensis*	Bonato & Steinfartz, 2005		-		
Chordata	Amphibia	Urodela	*Salamandra lanzai*	Nascetti, Andreone, Capula & Bullini, 1988		1	Italy	KF645974
Chordata	Amphibia	Urodela	*Salamandrina perspicillata*	(Savi, 1821)	*Salamandrina terdigitata*	1	Italy	**KX241532***
Chordata	Amphibia	Urodela	*Salamandrina terdigitata*	(Bonnaterre, 1789)	*Salamandrina terdigitata*	2	Italy	**KX241533***, EU880332

Chordata	Amphibia	Urodela	*Triturus carnifex*	(Laurenti, 1768)		5	Italy	EF525958, EF525959, EF525960, EF525961, EF525964
Chordata	Cephalaspidomorphi	Petromyzontiformes	*Lampetra fluviatilis*	(Linnaeus, 1758)		1	France	Y18683
Chordata	Cephalaspidomorphi	Petromyzontiformes	*Lampetra planeri*	(Bloch, 1784)		4	Italy	FJ834326, KJ553784, KJ553838, KJ553859
Chordata	Cephalaspidomorphi	Petromyzontiformes	*Lethenteron zanandreai*	(Vladykov, 1955)		3	Italy	FJ832129, JN027079, JN027080
Chordata	Cephalaspidomorphi	Petromyzontiformes	*Petromyzon marinus*	Linnaeus, 1758		1	Germany	JN028189
Chordata	Mammalia	Artiodactyla	*Capra hircus*	Linnaeus, 1758		-		
Chordata	Mammalia	Artiodactyla	*Capra ibex*	Linnaeus, 1758		1	-unknown-	FJ207526
Chordata	Mammalia	Artiodactyla	*Cervus elaphus corsicanus*	Erxleben, 1777		-		
Chordata	Mammalia	Artiodactyla	*Ovis aries musimon*	(Pallas, 1811)	*Ovis gmelini musimon*	1	-unknown-	EU623452
Chordata	Mammalia	Artiodactyla	*Rupicapra pyrenaica ornata*	Neumann, 1899		1	Italy	KJ184173
Chordata	Mammalia	Artiodactyla	*Rupicapra rupicapra*	(Linnaeus, 1758)		1	Italy	FJ207539
Chordata	Mammalia	Carnivora	*Canis aureus*	(Linnaeus, 1758)		1	-unknown-	AF028186
Chordata	Mammalia	Carnivora	*Canis lupus lupus*	(Linnaeus, 1758)		1	Sweden	AM711902
Chordata	Mammalia	Carnivora	*Felis silvestris silvestris*	Schreber, 1775		1	Poland	FJ958341
Chordata	Mammalia	Carnivora	*Lutra lutra*	(Linnaeus, 1758)		1	Corea	EF672696
Chordata	Mammalia	Carnivora	*Lynx lynx*	(Linnaeus, 1758)		1	Poland	FJ402880
Chordata	Mammalia	Carnivora	*Martes martes*	(Linnaeus, 1758)		1	Italy	**KX241534***
Chordata	Mammalia	Carnivora	*Monachus monachus*	(Hermann, 1779)		1	-unknown-	AY377142
Chordata	Mammalia	Carnivora	*Mustela putorius*	Linnaeus, 1758		1	Italy	**KX241535***
Chordata	Mammalia	Carnivora	*Ursus arctos*	Linnaeus, 1758		1	Poland	GQ901983
Chordata	Mammalia	Cetacea	*Balaenoptera acutorostrata*	Lacépède, 1804		1	France	KF281608
Chordata	Mammalia	Cetacea	*Balaenoptera physalus*	(Linnaeus, 1758)		1	-unknown-	X61145
Chordata	Mammalia	Cetacea	*Delphinus delphis*	Linnaeus, 1758		1	France	KF281612
Chordata	Mammalia	Cetacea	*Globicephala melas*	(Traill, 1809)		1	-unknown-	JF339972
Chordata	Mammalia	Cetacea	*Grampus griseus*	(G. Cuvier, 1812)		1	France	KF281613
Chordata	Mammalia	Cetacea	*Orcinus orca*	(Linnaeus, 1758)		1	Strait of Gibraltar	GU187176
Chordata	Mammalia	Cetacea	*Physeter catodon*	Linnaeus, 1758		1	Island	AJ277029
Chordata	Mammalia	Cetacea	*Pseudorca crassidens*	(Owen, 1846)		1	-unknown-	HM060332
Chordata	Mammalia	Cetacea	*Stenella coeruleoalba*	(Meyen, 1833)		1	France	KF281695
Chordata	Mammalia	Cetacea	*Steno bredanensis*	(G. Cuvier in Lesson, 1828)		1	-unknown-	DQ922633
Chordata	Mammalia	Cetacea	*Tursiops truncatus*	(Montagu, 1821)		1	China	EU557093
Chordata	Mammalia	Cetacea	*Ziphius cavirostris*	Cuvier, 1823		1	USA	EU139297
Chordata	Mammalia	Cetartiodactyla	*Kogia simus*	(Owen, 1866)		1	-unknown-	EU496308

Chordata	Mammalia	Cetartiodactyla	*Megaptera novaeangliae*	(Borowski, 1781)		1	USA	EU139286
Chordata	Mammalia	Chiroptera	*Barbastella barbastellus*	(Schreber, 1774)		3	Italy	FR856638, FR856639, FR856640
Chordata	Mammalia	Chiroptera	*Eptesicus nilssonii*	(Keyserling & Bläsius, 1839)		2	Italy	FR856641, FR856642
Chordata	Mammalia	Chiroptera	*Eptesicus serotinus*	(Schreber, 1774)		5	Italy	FR856643, FR856644, FR856645, FR856646, FR856647
Chordata	Mammalia	Chiroptera	*Hypsugo savii*	(Bonaparte, 1837)		5	Italy	FR856649, FR856650, FR856651, FR856653, FR856654
Chordata	Mammalia	Chiroptera	*Miniopterus schreibersii*	(Kuhl, 1817)	*Miniopterus schreibersi*	5	Italy	FR856655, FR856656, FR856657, FR856658, FR856659
Chordata	Mammalia	Chiroptera	*Myotis alcathoe*	Helversen & Heller, 2001		4	Italy	FR856660, HG325822, HG325823, HG325824
Chordata	Mammalia	Chiroptera	*Myotis bechsteinii*	(Kuhl, 1817)		5	Italy	FR856662, FR856663, FR856664, FR856665, FR856666
Chordata	Mammalia	Chiroptera	*Myotis blythii*	(Tomes, 1857)		5	Italy	FR856667, FR856669, FR856670, FR856673, FR856674
Chordata	Mammalia	Chiroptera	*Myotis brandtii*	(Eversmann, 1845)		1	Italy	FR856675
Chordata	Mammalia	Chiroptera	*Myotis capaccinii*	(Bonaparte, 1837)		3	Italy	FR856676, FR856677, FR856678
Chordata	Mammalia	Chiroptera	*Myotis daubentonii*	(Kuhl, 1817)		5	Italy	FR856679, FR856680, FR856681, FR856682, FR856683
Chordata	Mammalia	Chiroptera	*Myotis emarginatus*	(E. Geoffroy, 1806)		5	Italy	FR856685, FR856687, FR856688, FR856689, FR856690
Chordata	Mammalia	Chiroptera	*Myotis myotis*	(Borkhausen, 1797)		5	Italy	FR856691, FR856699, FR856702, FR856703,

								FR856704
Chordata	Mammalia	Chiroptera	*Myotis mystacinus*	(Kuhl, 1817)		5	Italy	FR856706, FR856707, FR856708, FR856709, FR856710
Chordata	Mammalia	Chiroptera	*Myotis nattereri*	(Kuhl, 1817)		5	Italy	FR856711, FR856718, FR856720, FR856725, FR856730
Chordata	Mammalia	Chiroptera	*Myotis punicus*	Felten, 1977		3	Italy	FR856732, FR856733, FR856734
Chordata	Mammalia	Chiroptera	*Nyctalus lasiopterus*	(Schreber, 1780)		1	Spain	ABBWP001-06 (BOLD)
Chordata	Mammalia	Chiroptera	*Nyctalus leisleri*	(Kuhl, 1817)		5	Italy	FR856754, FR856755, FR856756, FR856757, FR856758
Chordata	Mammalia	Chiroptera	*Nyctalus noctula*	(Schreber, 1774)		1	Italy	FR856759
Chordata	Mammalia	Chiroptera	*Pipistrellus kuhlii*	(Kuhl, 1817)		5	Italy	FR856762, FR856763, FR856764, FR856765, FR856766
Chordata	Mammalia	Chiroptera	*Pipistrellus nathusii*	(Keyserling & Bläsius, 1839)		4	Italy	FR856767, FR856768, FR856769, FR856770
Chordata	Mammalia	Chiroptera	*Pipistrellus pipistrellus*	(Schreber, 1774)		5	Italy	FR856774, FR856775, FR856776, FR856777, FR856778
Chordata	Mammalia	Chiroptera	*Pipistrellus pygmaeus*	(Leach, 1825)		2	Italy	FR856783, FR856784
Chordata	Mammalia	Chiroptera	*Plecotus auritus*	(Linnaeus, 1758)		5	Italy	FR856803, FR856804, FR856806, FR856807, FR856809
Chordata	Mammalia	Chiroptera	*Plecotus austriacus*	(Fischer, 1829)		2	Italy	FR856811, FR856812
Chordata	Mammalia	Chiroptera	*Plecotus macrobullaris*	Kuzjakin, 1965		2	Italy	FR856813, FR856814
Chordata	Mammalia	Chiroptera	*Plecotus sardus*	Mucedda, Kiefer, Pidinchedda & Veith, 2002		3	Italy	FR856815, FR856816, FR856817

Chordata	Mammalia	Chiroptera	*Rhinolophus euryale*	Blasius, 1853		5	Italy	FR856825, FR856826, FR856827, FR856828, FR856829
Chordata	Mammalia	Chiroptera	*Rhinolophus ferrumequinum*	(Schreber, 1774)		5	Italy	FR856831, FR856833, FR856834, FR856835, FR856836
Chordata	Mammalia	Chiroptera	*Rhinolophus hipposideros*	(Bechstein, 1800)		2	Italy	FR856837, FR856838
Chordata	Mammalia	Chiroptera	*Rhinolophus mehelyi*	Matschie, 1901		5	Italy	FR856839, FR856840, FR856841, FR856842, FR856843
Chordata	Mammalia	Chiroptera	*Tadarida teniotis*	(Rafinesque, 1814)		3	Italy	FR856844, FR856845, FR856846
Chordata	Mammalia	Chiroptera	*Vespertilio murinus*	Linnaeus, 1758		1	Switzerland	ABBWP090-06 (BOLD)
Chordata	Mammalia	Lagomorpha	*Lepus timidus*	Linnaeus, 1758		1	China	HM233122
Chordata	Mammalia	Rodentia	*Dryomys nitedula*	(Pallas, 1778)				
Chordata	Mammalia	Rodentia	*Hystrix cristata*	Linnaeus, 1758		1	Italy	**KX241536***
Chordata	Mammalia	Rodentia	*Muscardinus avellanarius*	(Linnaeus, 1758)		1	Russia	JF499319
Chordata	Mammalia	Soricomorpha	*Crocidura sicula*	Miller, 1900		-		
Chordata	Reptilia	Squamata	*Algyroides fitzingeri*	(Wiegmann, 1834)		1	Italy	AF206557
Chordata	Reptilia	Squamata	*Algyroides nigropunctatus*	(Duméril & Bibron, 1839)		-		
Chordata	Reptilia	Squamata	*Archaeolacerta bedriagae*	(Camerano, 1885)	*Lacerta bedriagae*	1	Italy	AF206553
Chordata	Reptilia	Squamata	*Chalcides ocellatus*	(Forskål, 1775)		-		
Chordata	Reptilia	Squamata	*Chamaeleo chamaeleon*	(Linnaeus, 1758)		1	Portugal	EF222198
Chordata	Reptilia	Squamata	*Coluber hippocrepis*	Linnaeus, 1758		1	Morocco	AY039177
Chordata	Reptilia	Squamata	*Coronella austriaca*	Laurenti, 1768		2	France, Switzerland	AY122752, KC997586
Chordata	Reptilia	Squamata	*Cyrtopodion kotschyi*	(Steindachner, 1870)		1	Greece	AY677691
Chordata	Reptilia	Squamata	*Elaphe quatuorlineata*	(Lacepede, 1789)		4	Italy	AY122712, KF728036, KF728058, KF728065
Chordata	Reptilia	Squamata	*Elaphe situla*	(Linnaeus, 1758)		-		
Chordata	Reptilia	Squamata	*Euleptes europaea*	(Gene, 1838)		-		
Chordata	Reptilia	Squamata	*Hemorrhois gemonensis*	(Laurenti, 1768)	*Coluber laurenti*	1	Greece	AY039183
Chordata	Reptilia	Squamata	*Hierophis viridiflavus*	(Lacépède, 1789)	*Coluber viridiflavus*	2	Italy	AY039175, AY039180
Chordata	Reptilia	Squamata	*Iberolacerta horvathi*	(Méhelÿ, 1904)		-		
Chordata	Reptilia	Squamata	*Lacerta agilis*	Linnaeus, 1758		1	-unknown-	KC990830

Chordata	Reptilia	Squamata	*Lacerta viridis*	(Laurenti, 1768)		3	Austria, Italy	AM176577, **KX241537*, KX241538***
Chordata	Reptilia	Squamata	*Natrix natrix cetti*	Gené, 1839		-		
Chordata	Reptilia	Squamata	*Natrix tessellata*	(Laurenti, 1768)		1	Italy	JN871605
Chordata	Reptilia	Squamata	*Podarcis filfolensis*	(Bedriaga, 1876)		-		
Chordata	Reptilia	Squamata	*Podarcis melisellensis*	(Braun, 1877)		-		
Chordata	Reptilia	Squamata	*Podarcis muralis*	(Laurenti, 1768)		1	Austria	KP697817
Chordata	Reptilia	Squamata	*Podarcis raffoneae*	(Mertens, 1952)		-		
Chordata	Reptilia	Squamata	*Podarcis sicula*	(Rafinesque, 1810)		2	Italy	AF206555, FJ460598
Chordata	Reptilia	Squamata	*Podarcis tiliguerta*	(Gmelin, 1789)		-		
Chordata	Reptilia	Squamata	*Podarcis wagleriana*	Gistel, 1868		-		
Chordata	Reptilia	Squamata	*Telescopus fallax*	(Fleischmann, 1831)		-		
Chordata	Reptilia	Squamata	*Vipera ammodytes*	(Linnaeus, 1758)		1	-unknown-	EANAH456-12 (BOLD)
Chordata	Reptilia	Squamata	*Vipera ursinii*	(Bonaparte, 1835)		1	Italy	KC122749
Chordata	Reptilia	Squamata	*Zamenis lineatus*	(Camerano, 1891)	*Elaphe longissima*	1	Italy	HQ392546
Chordata	Reptilia	Squamata	*Zamenis longissimus*	(Laurenti, 1768)	*Elaphe longissima*	4	Italy	AY122696, HQ392540, HQ392543
Chordata	Reptilia	Testudines	*Caretta caretta*	(Linnaeus, 1758)		2	Italy, Mediterranean Sea	**KX241539***, FR694649
Chordata	Reptilia	Testudines	*Chelonia mydas*	(Linnaeus, 1758)		1	China	EU600158
Chordata	Reptilia	Testudines	*Dermochelys coriacea*	(Vandelli, 1761)		1	Australia	GQ152876
Chordata	Reptilia	Testudines	*Emys orbicularis*	(Linnaeus, 1758)		1	Italy	KP697832
Chordata	Reptilia	Testudines	*Emys trinacris*	Fritz et al. 2005		2	Italy	FBHER263-14 (BOLD) FBHER264-14 (BOLD)
Chordata	Reptilia	Testudines	*Eretmochelys imbricata*	(Linnaeus, 1766)		2	Colombia, -unknown-	GQ152887, JN991344
Chordata	Reptilia	Testudines	*Lepidochelys kempii*	(Garman, 1880)		1	USA	GQ152891
Chordata	Reptilia	Testudines	*Testudo graeca*	Linnaeus, 1758		2	Tunisia, Turkey	DQ080049, DQ080050
Chordata	Reptilia	Testudines	*Testudo hermanni*	Gmelin, 1789		1	Italy	**KX241540***
Chordata	Reptilia	Testudines	*Testudo marginata*	Schoepff, 1793		1	Greece	DQ080047
Cnidaria	Anthozoa	Alcyonacea	*Corallium rubrum*	(Linnaeus, 1758)		1	France	AY827536
Echinodermata	Echinoidea	Diadematoida	*Centrostephanus longispinus*	(Philippi, 1845)		-		
Mollusca	Bivalvia	Mytiloida	*Lithophaga lithophaga*	(Linnaeus, 1758)		1	-unknown-	AF120644
Mollusca	Bivalvia	Pterioida	*Pinna nobilis*	Linnaeus, 1758		5	Italy	JX854839, JX854884, JX854968, JX854987, JX855000

Mollusca	Bivalvia	Unionoida	*Margaritifera auricularia*	(Spengler, 1793)		-		
Mollusca	Bivalvia	Unionoida	*Microcondylaea bonellii*	(Ferussac, 1827)		-		
Mollusca	Bivalvia	Unionoida	*Unio glaucinum*			-		
Mollusca	Bivalvia	Unionoida	*Unio mancus*	Lamarck, 1819	*Unio elongatulus*	1	Spain	AY522857
Mollusca	Bivalvia	Unionoida	*Unio pictorum*	(Linnaeus, 1758)	*Unio elongatulus*	1	Austria	AF156499
Mollusca	Gastropoda	Hygrophila	*Anisus vorticulus*	(Troschel, 1834)				
Mollusca	Gastropoda	Patellogastropoda	*Patella ferruginea*	Gmelin, 1791		5	Italy	GQ469872, GQ469873, GQ469874, GQ469875, GQ469876
Mollusca	Gastropoda	Pulmonata	*Helix pomatia*	Linnaeus, 1758		3	Italy	**KX241541*, KX241542*, KX241543***
Mollusca	Gastropoda	Pulmonata	*Vertigo angustior*	Jeffreys, 1830		-		
Mollusca	Gastropoda	Pulmonata	*Vertigo genesii*	(Gredler, 1856)		1	Austria	JN941065
Mollusca	Gastropoda	Pulmonata	*Vertigo geyeri*	Lindholm, 1925		-		
Mollusca	Gastropoda	Pulmonata	*Vertigo moulinsiana*	(Dupuy, 1849)		-		