

ATLAS Deliverable 4.3

Report on selected protocols for RAD/genome scan on each species retained for ATLAS

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Introduction

The deliverable includes:

1. Species ID Sheets for Genotyping By Sequencing, GBS, (RAD-seq and microsatellite analyses)

The objective of this deliverable was to pave the road for further analyses on species for which we hope to gather samples allowing population genomics/ connectivity assessment through genome scan analysis (several geographic locations, with 15-50 specimens per location). The present document thus reports tests performed during the first two years of Atlas on selected species and on the performance of restriction site-associated DNA sequencing (RAD-seq) on cold-water coral species and Genotyping by Sequencing (GBS) of microsatellites of three commercial fisheries species. RAD protocols are standard, once the suitability of the genome is tested and the enzyme selected, the ID sheets attached therefore contain the required information using the same RAD protocol as other labs. As for the genome scan using microsatellites, the protocol is described in the first publication (Farrell et al. 2017) and the same protocol was used for the two other fisheries species. While the microsatellite loci deployed on boarfish (*Capros aper*) are available in Farrell et al. 2017 (open access), loci used for horse mackerel (*Trachurus trachurus*) and Dublin bay prawn (*Nephrops norvegicus*) will be released on publication.

This test stage comprised successful RAD-seq efforts for five coral species, including the reef-forming and solitary scleractinian corals and one octocoral. Two enzymes were assessed for performance. The appendix only includes information obtained from the enzyme delivering the best coverage of the genome (depth of coverage generally above 15x; thousands of loci recovered). Future steps include increased sample numbers to allow stricter data filtering, and complete population genomics on a subset of those species (e.g. *Lophelia pertusa*, *Madrepora oculata* and *Dendrophyllia cornigera*). Major constraints include availability of samples (tissue), poor preservation conditions of pre-Atlas collections (room temperature, ethanol evaporation, mold and freeze thawed), causing degraded DNA and the need to further optimise DNA extraction protocols for individual species.

GBS of microsatellites has been targeted for three commercial fisheries species, boarfish, horse mackerel and Dublin Bay prawn. The initial detection of microsatellites has been performed for all three species through shotgun sequencing (MiSeq 300PE or 250PE) and primers for microsatellites have been designed for these species. These primers are subsequently used on a large number of individuals (c.1,000 individuals per species) in a single MiSeq run per species and subsequently genotyped. The study focused on boarfish has been completed and published (Farrell et al. 2017), while the GBS data for horse mackerel and Dublin bay prawn are currently being analysed.

References

Farrell ED, Carlsson JEL & Carlsson J. 2016. Next Gen Pop Gen: implementing a high-throughput approach to population genetics in boarfish (*Capros aper*). Royal Society Open Science. 2016 3 16065. <http://dx.doi.org/10.1098/rsos.160651>

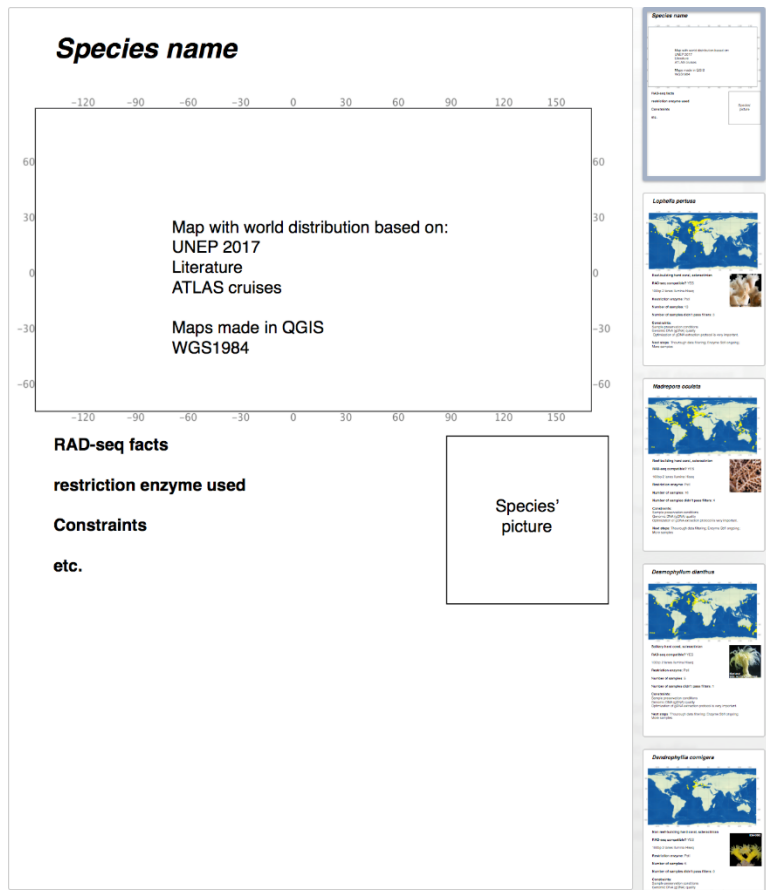
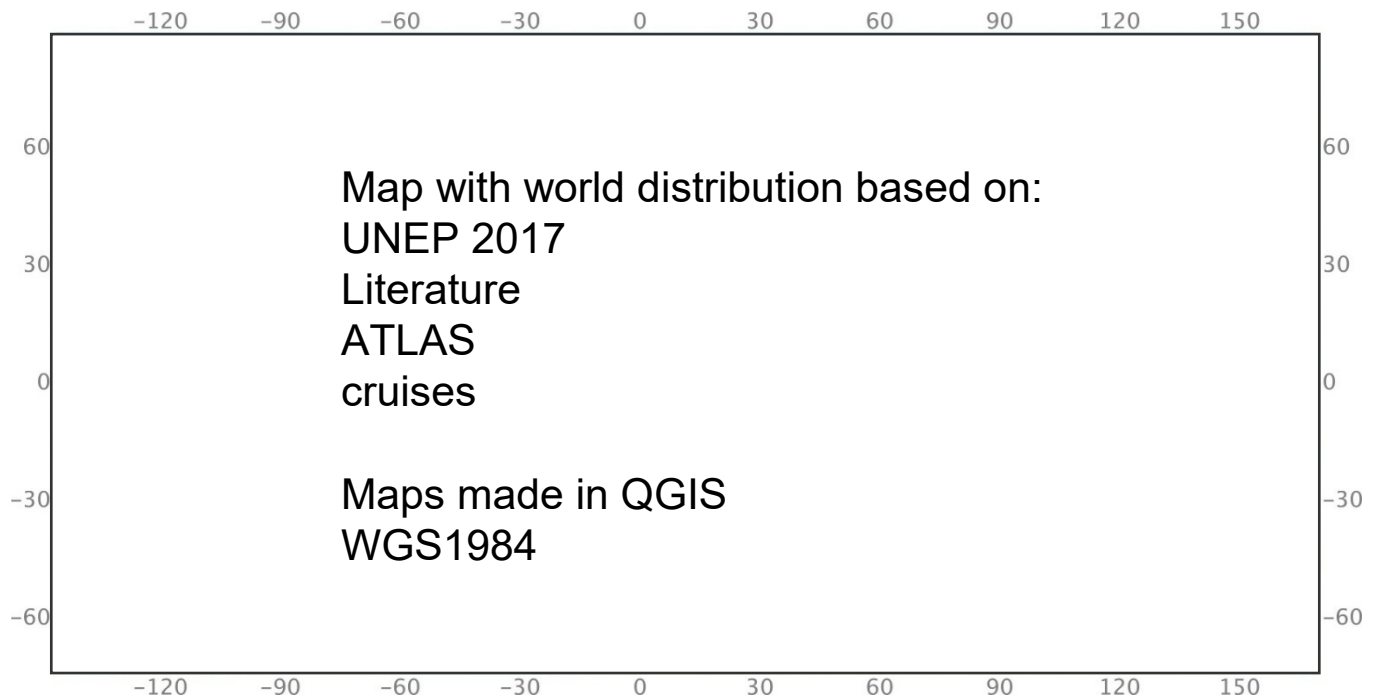


Figure 1 - Overview of the RAD-seq species sheets. The first page shows an outline of the configuration of each species page (species name, map with global distribution, picture, and general species-specific information). Full species sheets are in the appendix.

Appendix

Full species sheets are found in the following pages.

Species name

Type of coral

Restriction enzymes used and number of lanes sequenced

Number of samples sequenced

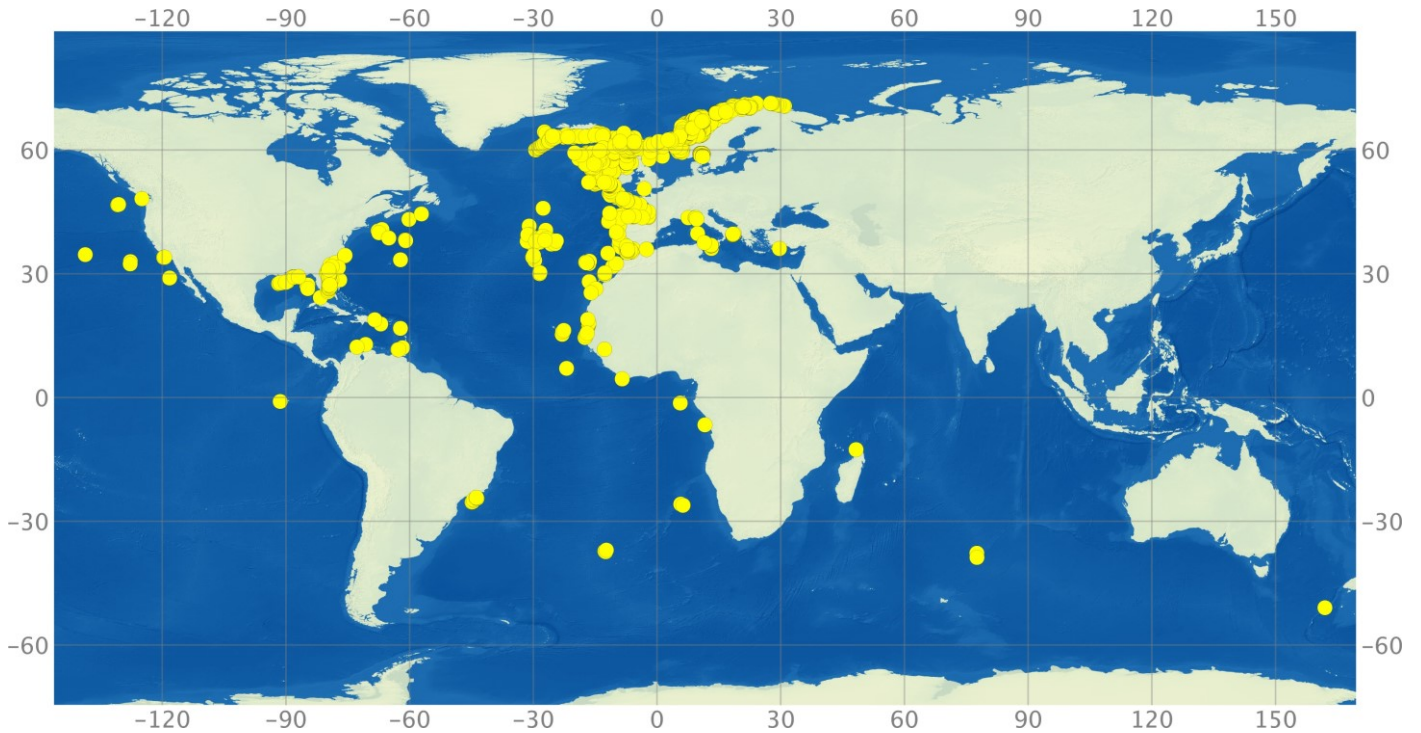
Number of samples retained after data filtering for each enzyme

Geographical location of samples

Number of polymorphic loci for each enzyme

Species
picture

Lophelia pertusa



Reef-building hard coral, scleractinian

RAD-seq compatible? YES

Restriction enzyme:

SbfI 1 lane and *PstI* 2 lanes, 100bp

Number of samples: 13

After filtering:

PstI 10

SbfI 10

Location: Alboran Sea, Bay of Cadiz, Azores archipelago, Iceland, Rockall Bank, Bay of Biscay

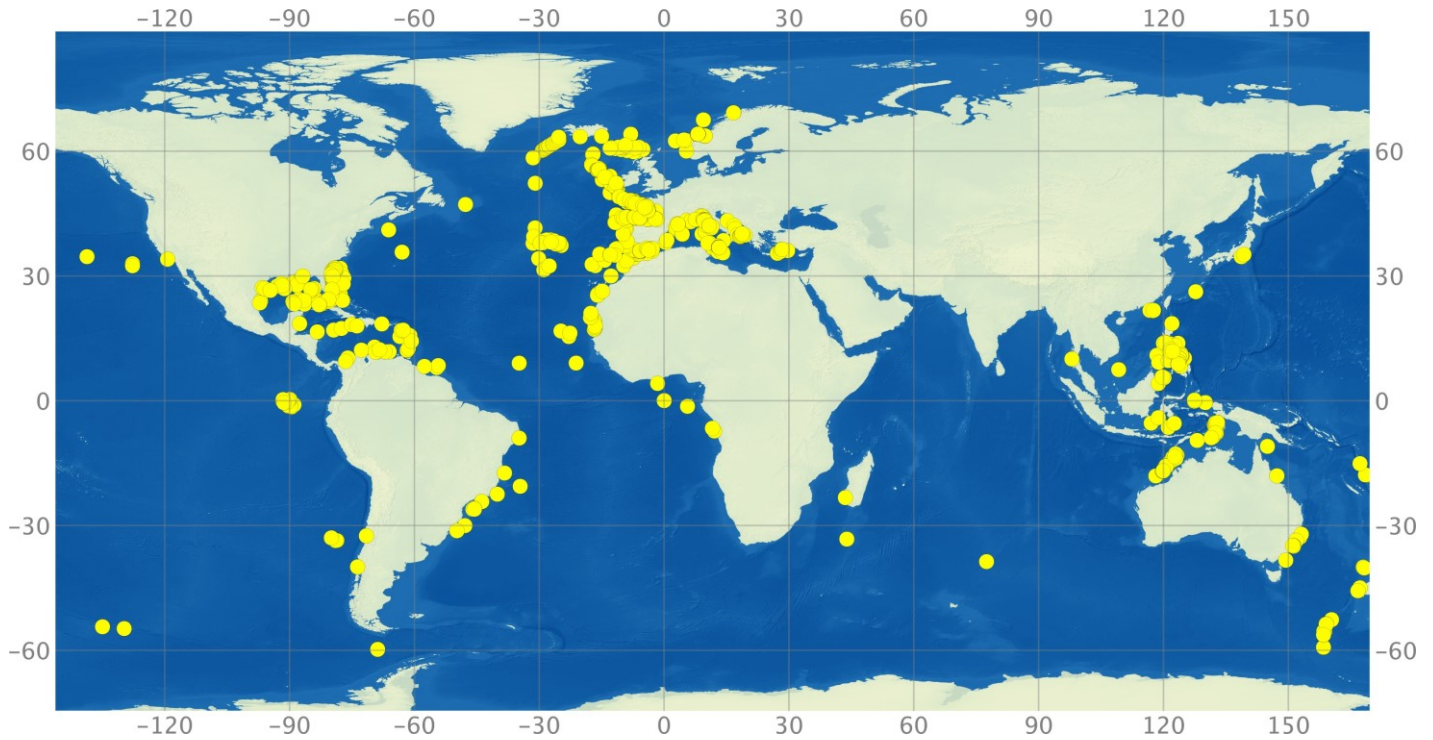
Polymorphic loci:

PstI 116 059

SbfI 9 776



Madrepora oculata



Reef-building hard coral, scleractinian

RAD-seq compatible? YES

Restriction enzyme:

SbfI 1 lane and *PstI* 2 lanes, 100bp

Number of samples: 16 (*PstI*) 13 (*SbfI*)

After filtering:

PstI 10

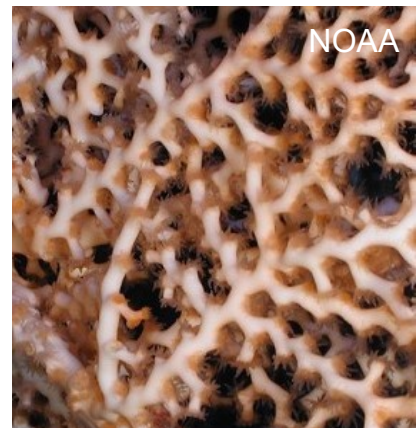
SbfI 10

Location: Alboran Sea, Azores archipelago, Rockall Bank, Bay of Biscay

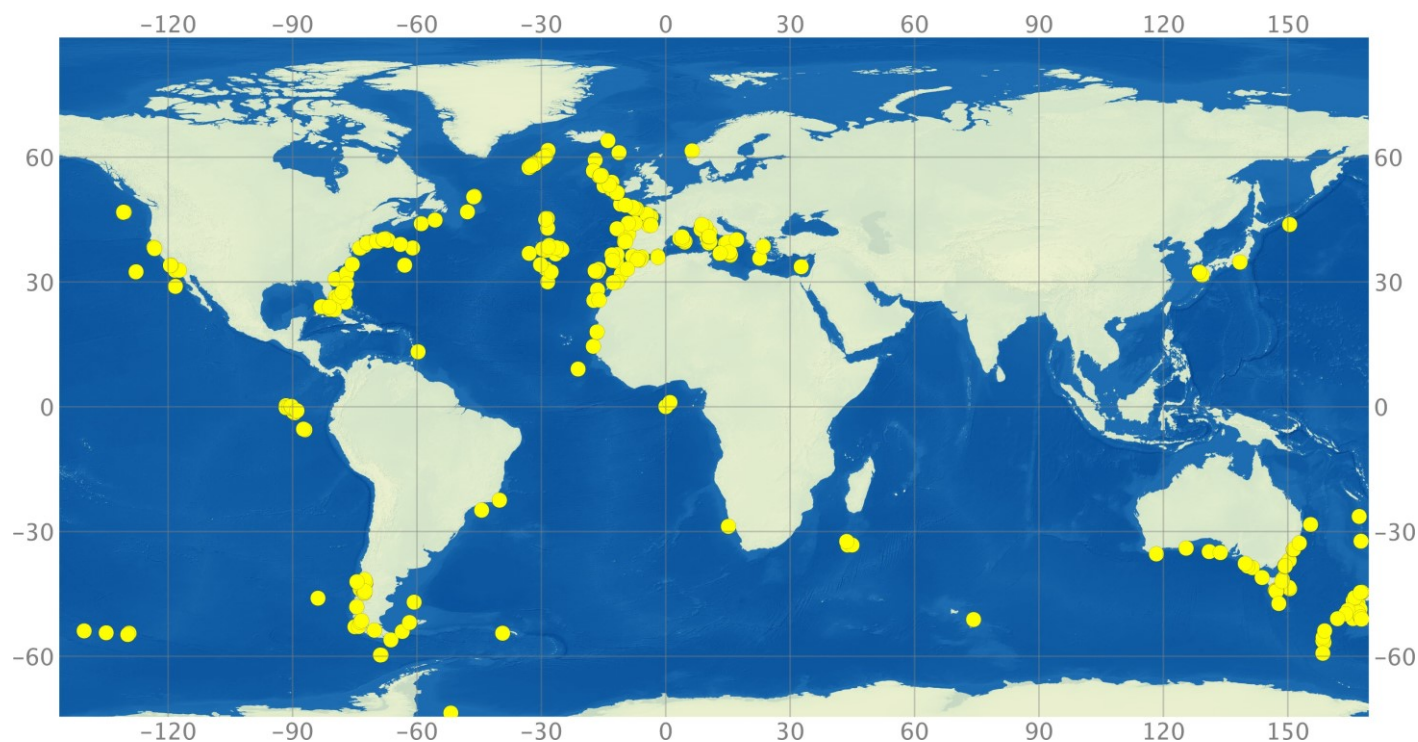
Polymorphic loci:

PstI 110 186

SbfI 5 315



Desmophyllum dianthus



Solitary hard coral, scleractinian

RAD-seq compatible? YES

Restriction enzyme:

Sbf 1 lane and *Pst* 2 lanes, 100bp

Number of samples: 4

After filtering:

Pst 4

Sbf 1

Location: Azores archipelago, Ionian Sea

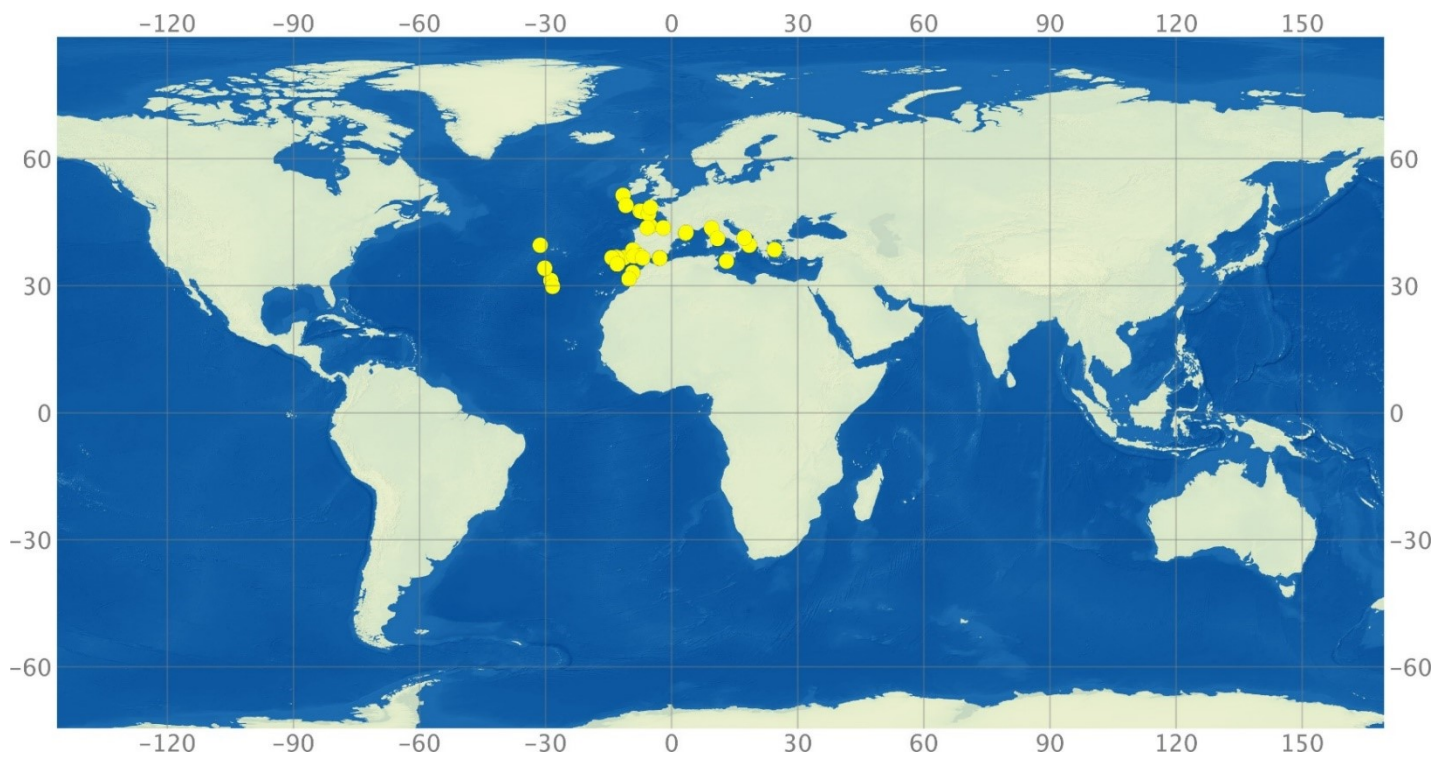
Polymorphic loci:

Pst 61 915

Sbf 5 047



Dendrophyllia cornigera



Non reef-building hard coral, scleractinian

RAD-seq compatible? YES

Restriction enzyme:

SbfI 1 lane and *PstI* 2 lanes, 100bp

Number of samples: 5

After filtering:

PstI 5

SbfI 5

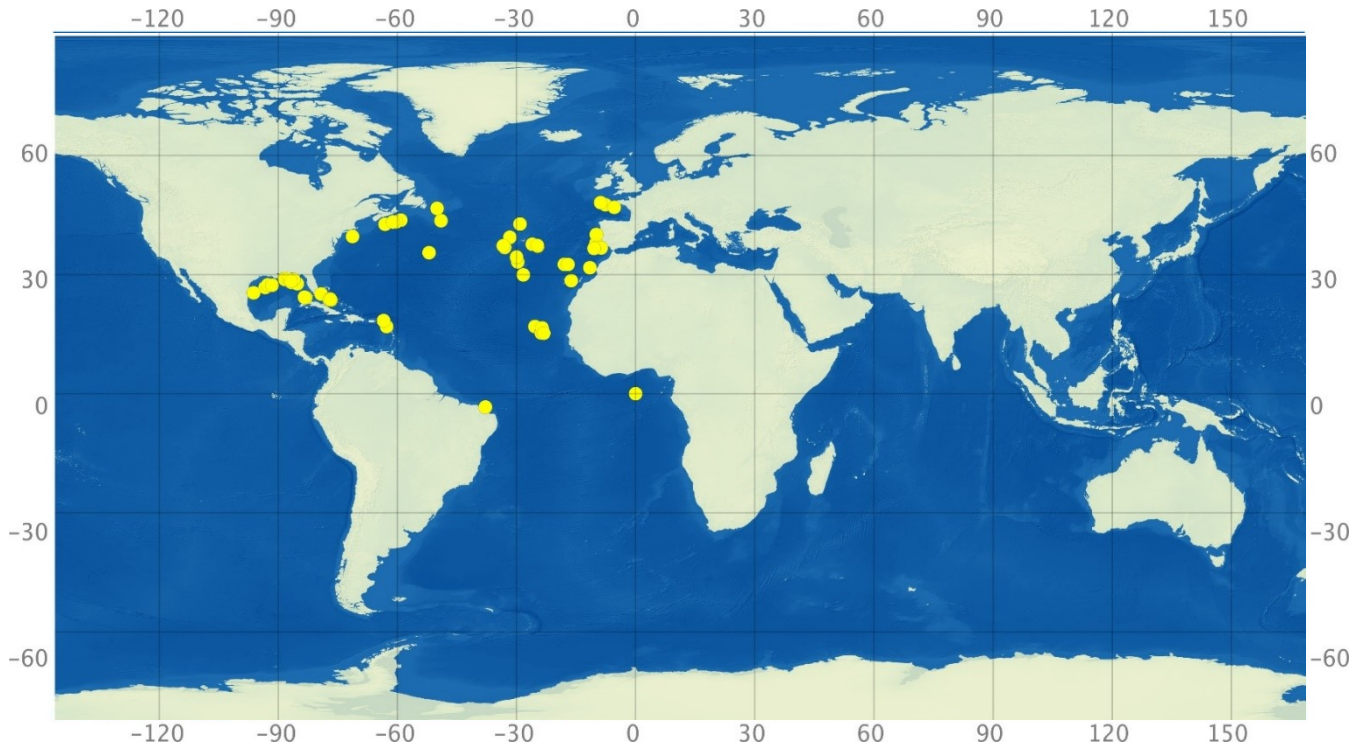
Location: Bay of Cadiz, Alboran Sea

Polymorphic loci:

PstI 166 581

SbfI 9 660



Acanella arbuscula

Bamboo-coral, octocoral

RAD-seq compatible? YES

Restriction enzyme:

SbfI 1 lane and *PstI* 2 lanes, 100bp

Number of samples: 4

After filtering:

PstI 3

SbfI 4

Location: Azores archipelago

Polymorphic loci:

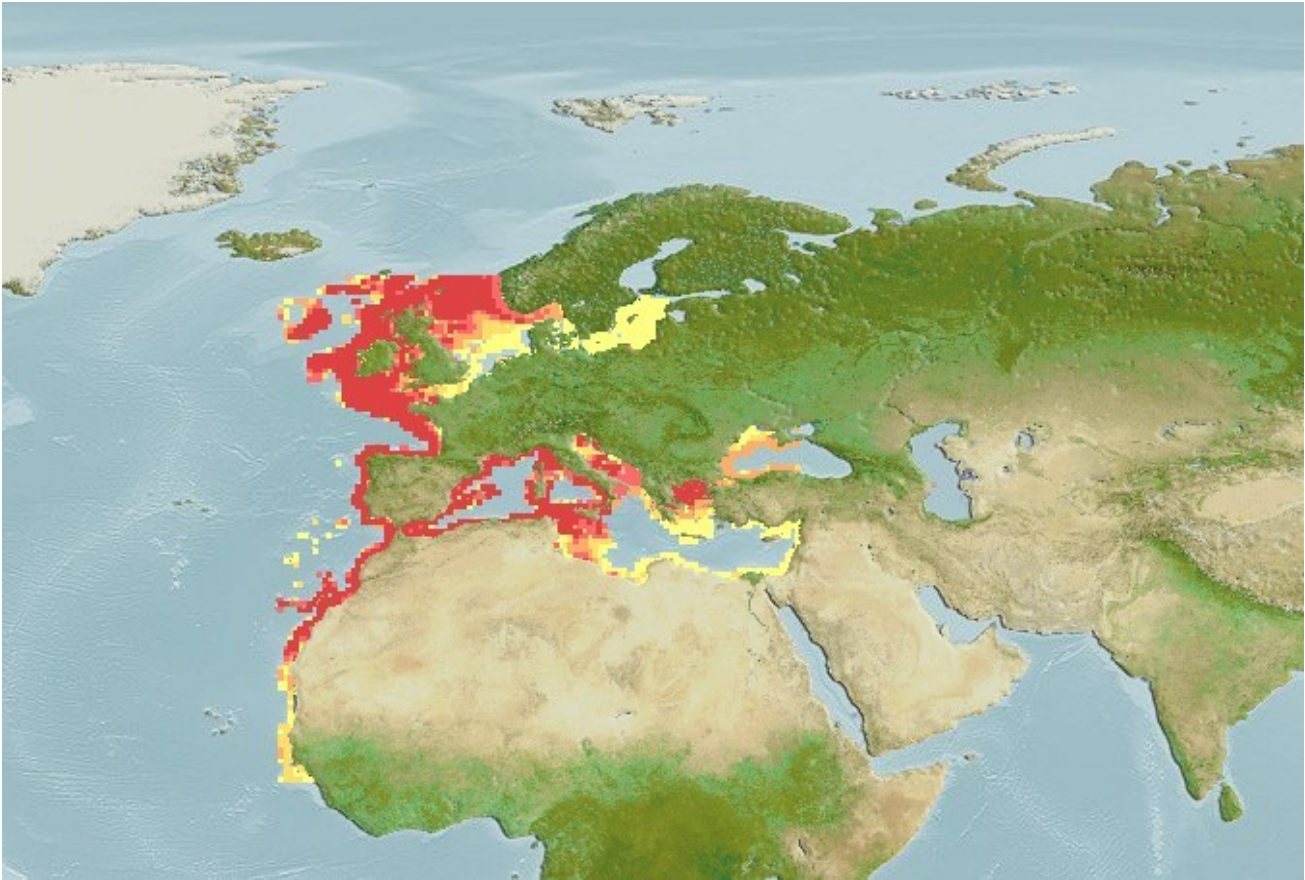
PstI 69 259

SbfI 6 151



Medwaves 2017 EU-ATLAS

Capros aper



Distribution from fishbase.org

Marine pelagic continental shelf fish (40-780m depth)

Shotgun sequence compatible? YES (MiSeq 300PE)

Genome target: Microsatellites (but also SNPs)

Number of samples in shotgun run: 2 (Irish and Mediterranean)

Number of microsatellite loci detected: 34,666

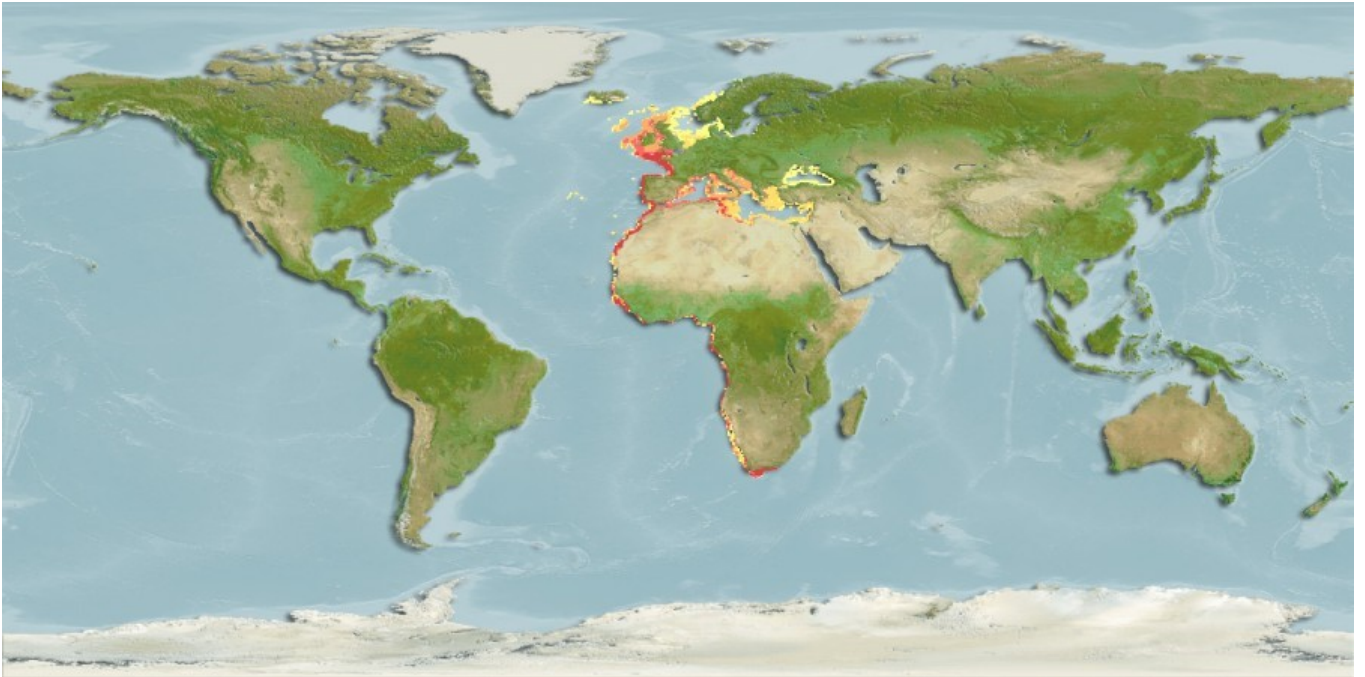
Locations for deployment: Range wide (c.f. Farrel et al. 2017)

Status: Genotyping by sequencing complete (Farrell ED, Carlsson JEL & Carlsson J. 2016. Next Gen Pop Gen: implementing a high-throughput approach to population genetics in boarfish (*Capros aper*). Royal Society Open Science. 2016 3 16065. <http://dx.doi.org/10.1098/rsos.160651>)



Capros aper - Boarfish.
Photographed by Pierluigi Angioi.

Trachurus trachurus



Distribution from fishbase.org



Trachurus trachurus - Horse mackerel.
Photographed by Kare Kare.

Marine benthopelagic fish (0-1000m depth)

Shotgun sequence compatible? YES (MiSeq 300PE)

Genome target: Microsatellites (but also SNPs)

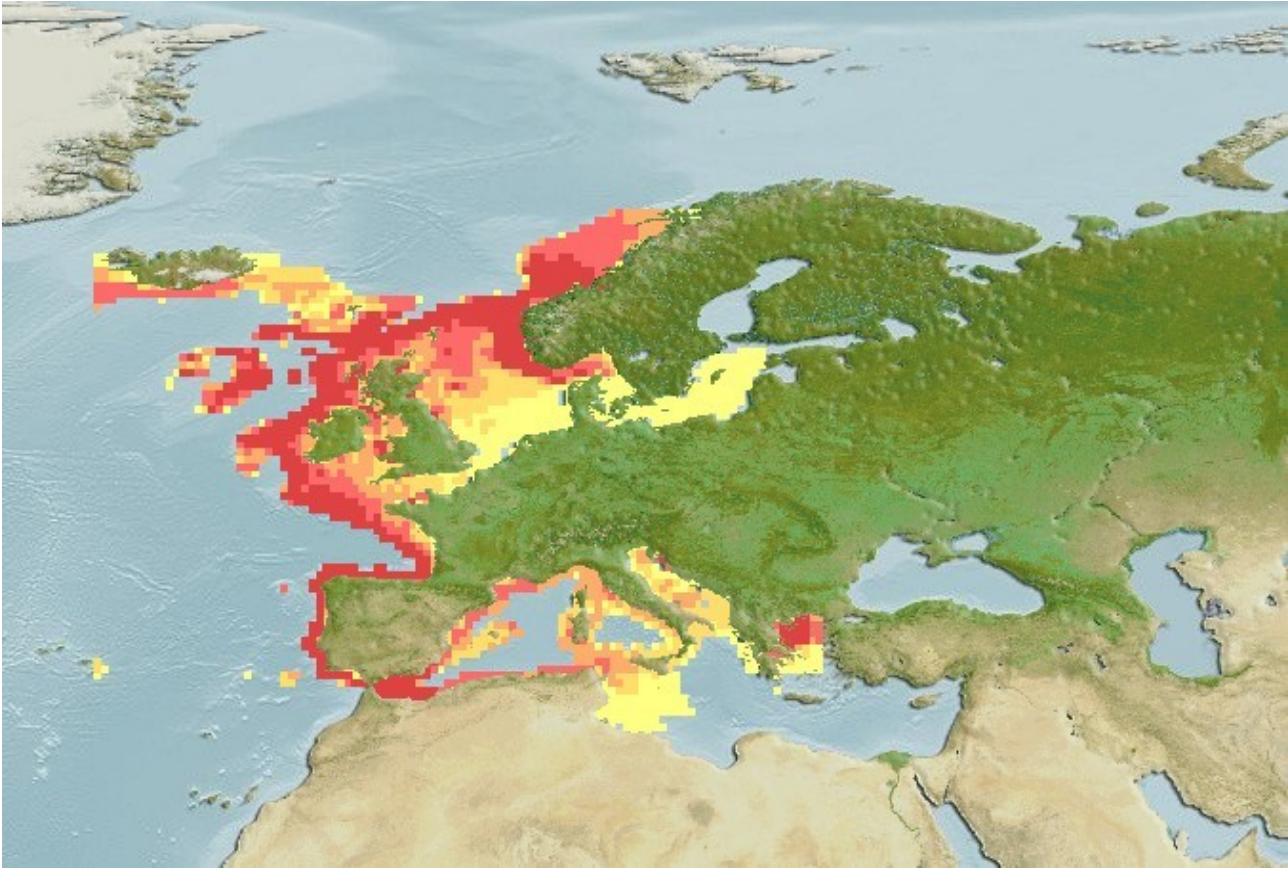
Number of samples in shotgun run: 2 (Western Atlantic and North Sea)

Number of microsatellite loci detected: 5,041

Locations for deployment: Atlantic Range wide (Mediterranean sample pending)

Status: Genotyping by sequencing currently being deployed on range-wide Atlantic samples

Nephrops norvegicus



Distribution from aquamaps.org

Marine crustacean (20-1000m depth)

Shotgun sequence compatible? YES (MiSeq 250PE)

Genome target: Microsatellites (but also SNPs)

Number of samples in shotgun run: 2 (West and East Ireland)

Number of microsatellite loci detected: 2,000+

Locations for deployment: Range wide

Status: Genotyping by sequencing currently being deployed



Nephrops norvegicus - Dublin bay prawn. Photographed by Hans Hillewaert.

