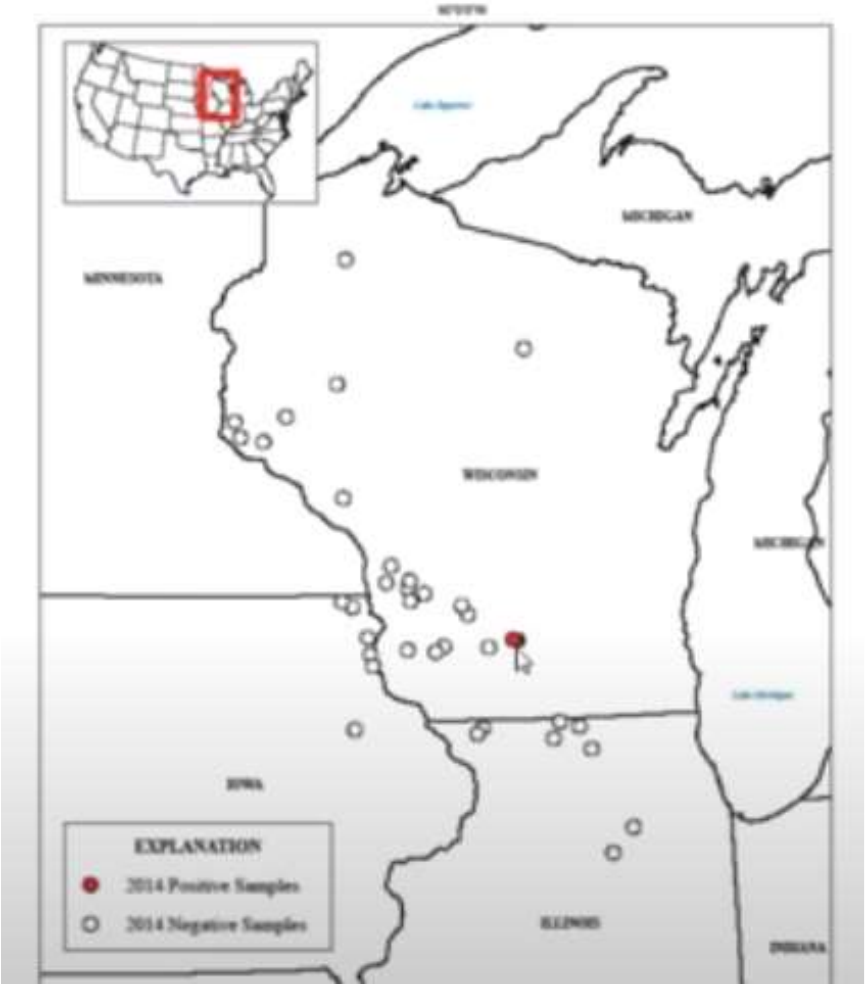
Zoogeography

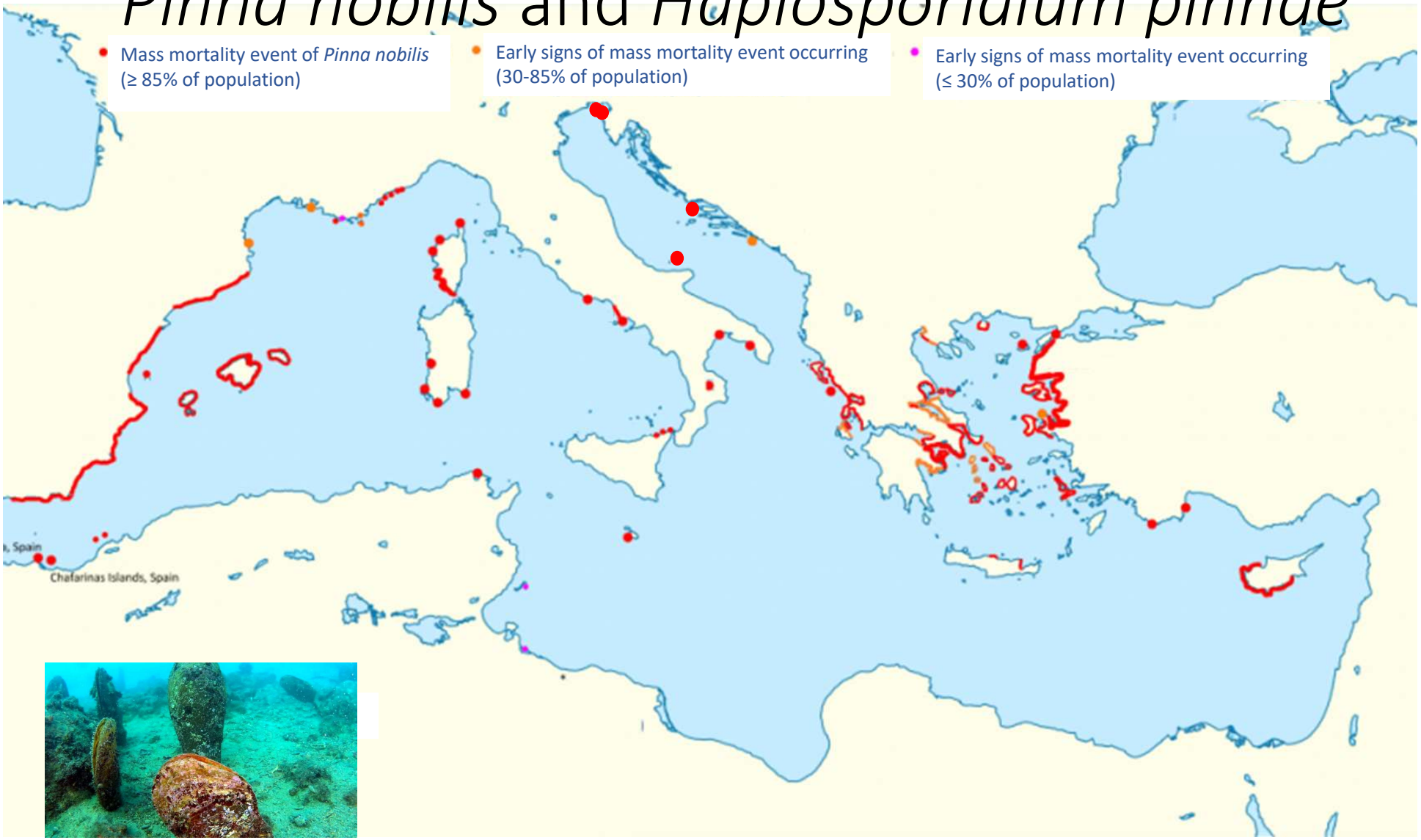
Lesson 12

- New Zealand mud snail (*Potamopyrgus antipodarum*) in Wisconsin

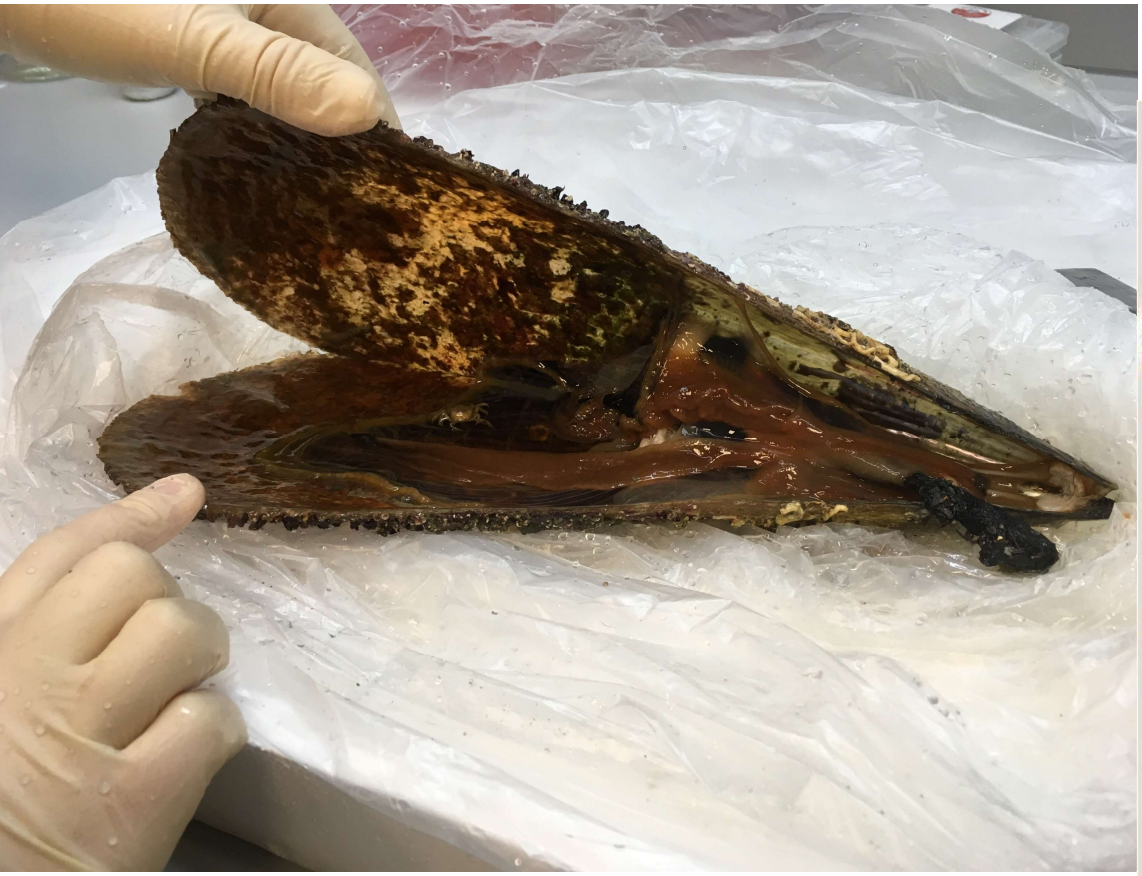


- They test 46 other stations across the states of Wisconsin, Minnesota and Illinois
- They find no other sites positive for the presence of New Zealand mud snails 3 years after the study (2017)
- they identify new positive stations

Pinna nobilis and *Haplosporidium pinnae*

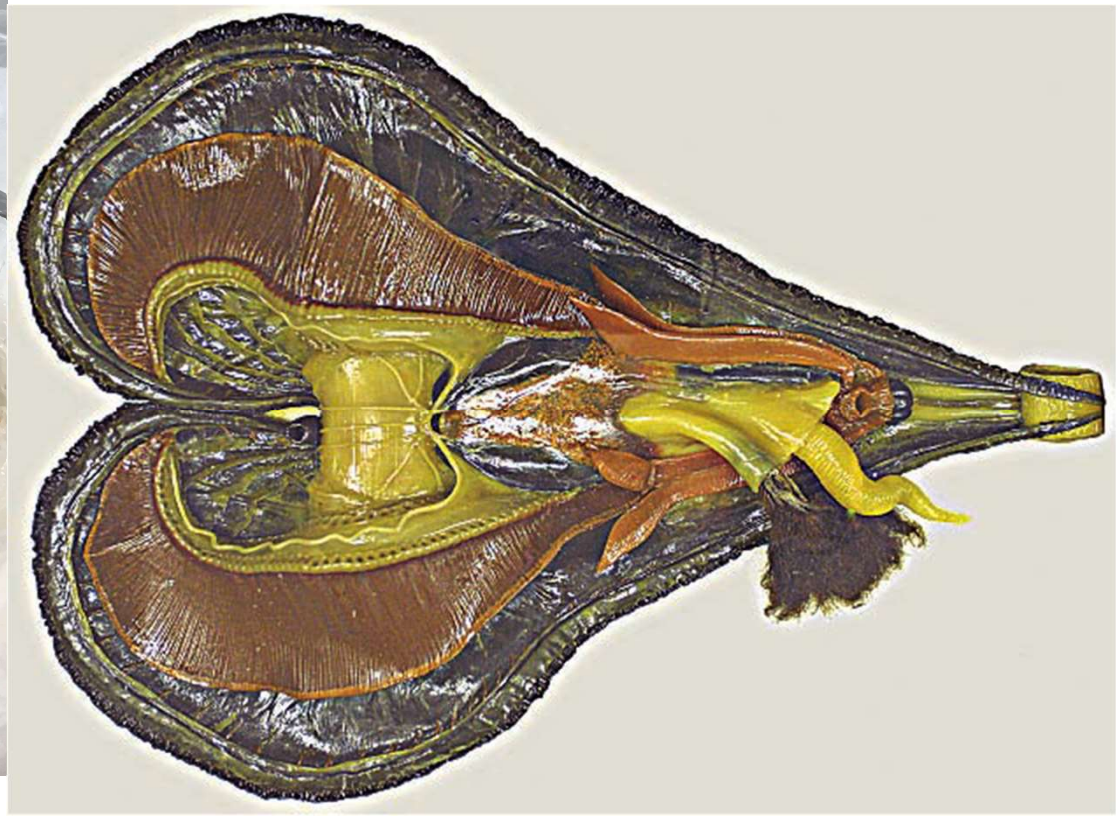


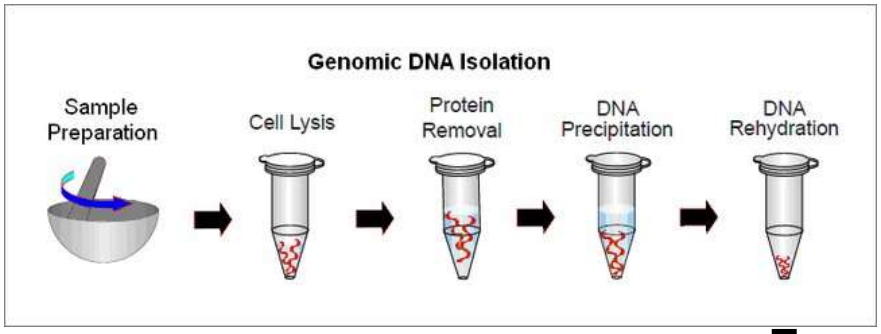
Positive sample

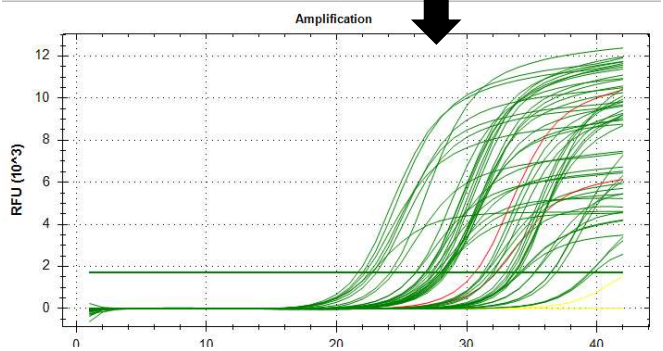


18 specimens analysed

A wax model of *Pinna nobilis* from the Poli's collection of anatomical wax models of molluscs, currently housed in the Muséum National d'Histoire Naturelle in Paris.







PLOS ONE

RESEARCH ARTICLE

Real-Time PCR based test for the early diagnosis of *Haplosporidium pinnae* affecting fan mussel *Pinna nobilis*

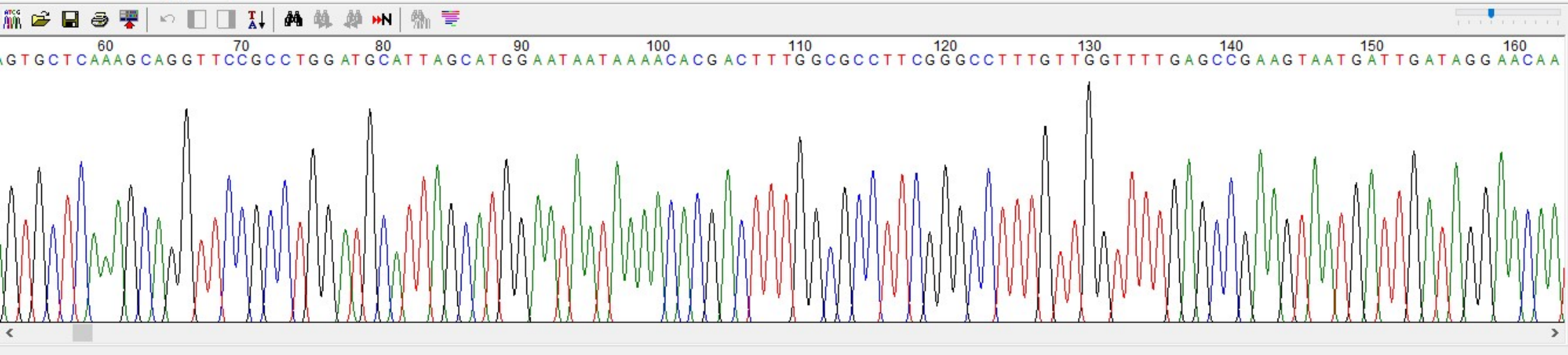
Monserrat López-Sanmartín[1]*, Gaetano Catanese[2,3], Amalia Grau[2,3], José María Valencia[2,3], Jose Rafa García-March[4], José Ignacio Navas[1]

Table 2. Characteristics of the primers used for PCRs.

Primer	Sequence (5′- 3′)	Fragment size (bp)	Temperature annealing (°C)	Reference
18SFr	CGAGCAATAACAGGTCTGTG	200	50°C	Mauri et al 2011
18SRw	GGCAGGGACTTAATCAA			
HPNF3	CATTAGCATGGAATAATAAAACACGAC	600	55°C	Catanese et al 2018
HPNR3	GCGACGGCTATTTAGATGGCTGA			
HpF	GGTACGGAGAATCCGGGGTT	1409	55°C	This study
HpR	ACTTGTCCTTCCTCTAATAATAAGG			
HpF3	GCGGGCTTAGTTCAGGGG	165	60°C	This study
HpR3	ACTTGTCCTTCCTCTAATAATAAGG			

https://doi.org/10.1371/journal.pone.0212028.t002

Results for *Haplosporidium pinnae*

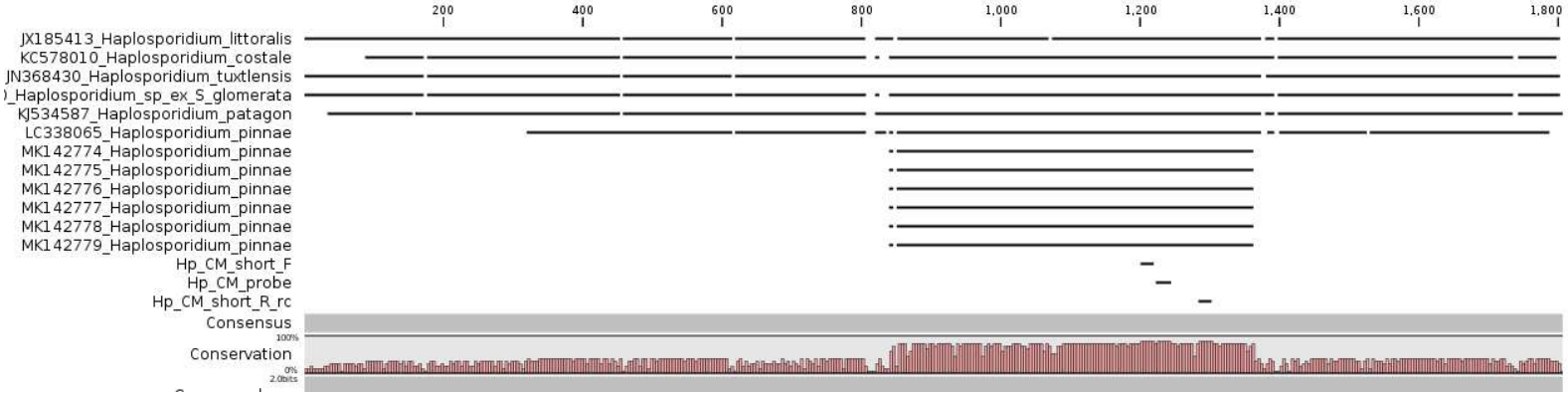


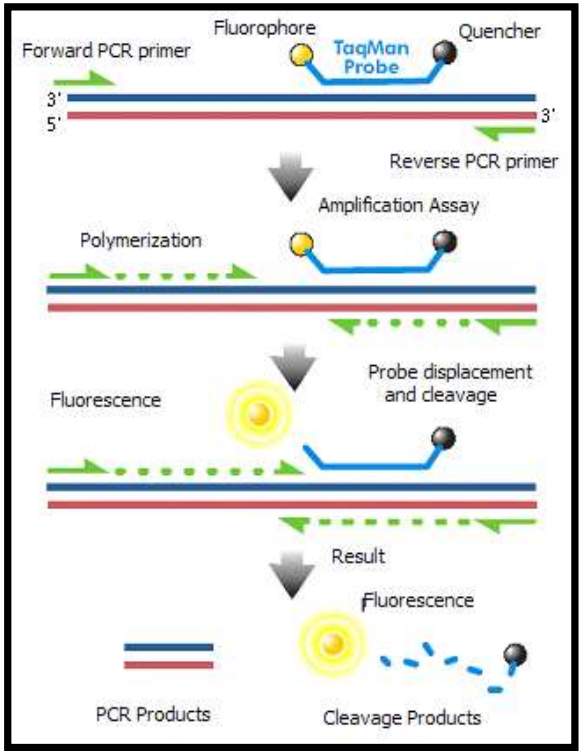
Sequences producing significant alignments Download Manage columns Show 100

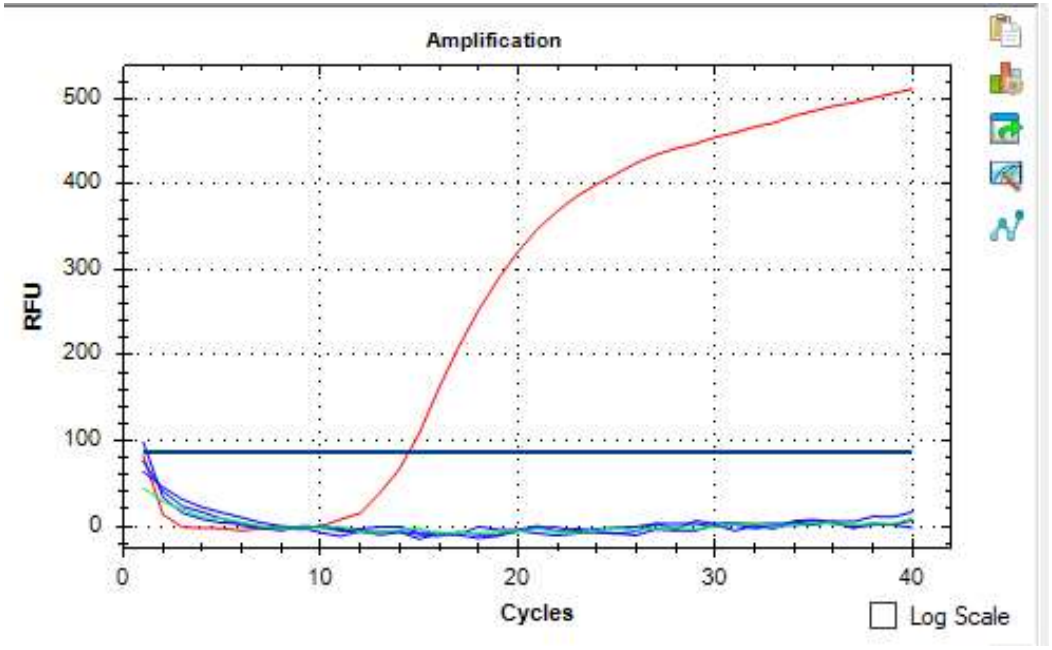
select all *100 sequences selected* GenBank Graphics Distance tree of results

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Haplosporidium pinnae isolate PN1 small subunit ribosomal RNA gene, partial sequence	1842	1842	96%	0.0	99.80%	MN104247.1
Haplosporidium pinnae gene for small subunit ribosomal RNA, partial sequence	1838	1838	97%	0.0	99.41%	LC338065.1

eDNA approach



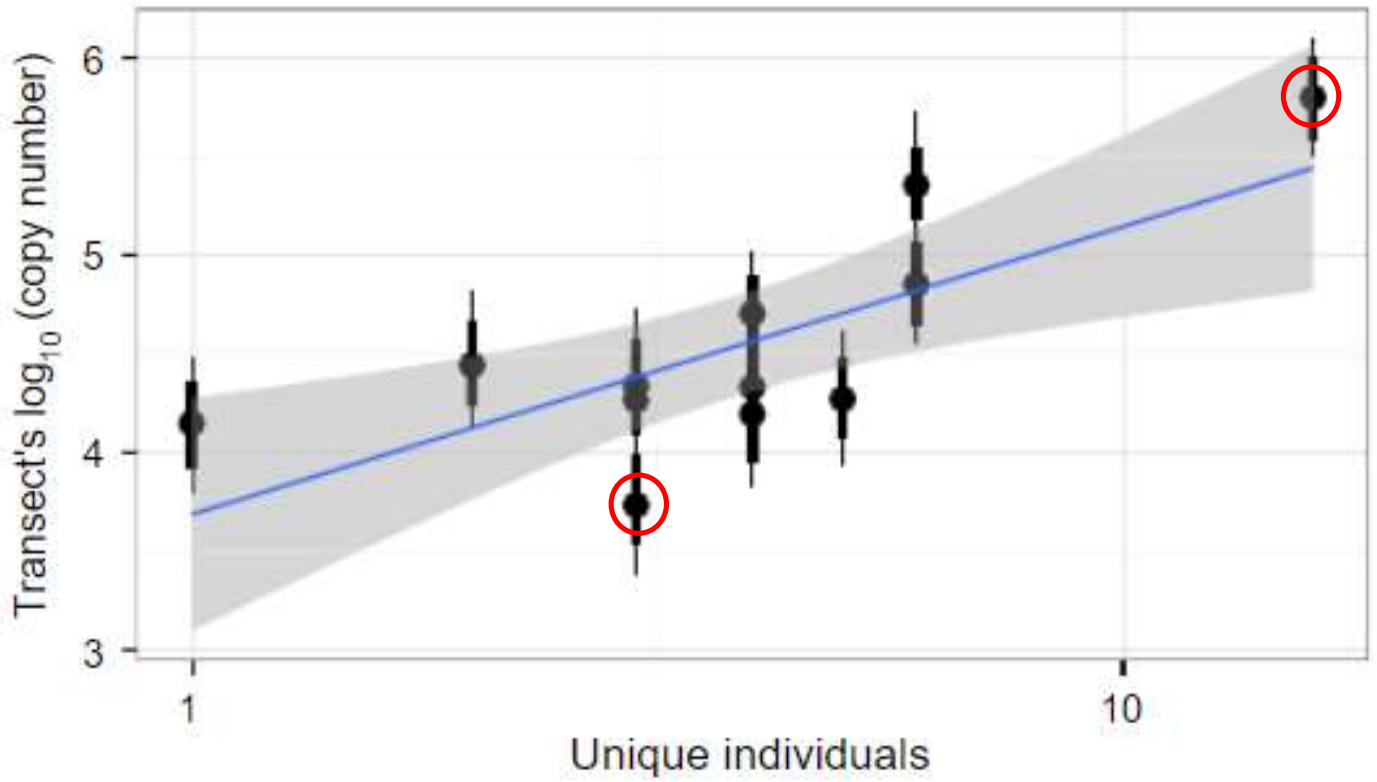




Detecting the movement and spawning activity of bigheaded carps with environmental DNA

RICHARD A. ERICKSON,*[a] Christopher B. Rees,*[1a] ALISON A. COULTER,†[2a]
CHRISTOPHER M. MERKES,* SUNNIE G. MCCALLA,*[a] Katherine F. Touzinsky,† Liza Walleser,*[3]
Reuben R. Goforth†[b] and JON J. AMBERG*[b]

U.S. Geological Survey Upper Midwest Environmental Sciences Center, La Crosse, WI, USA, †Department of Forestry and Natural Resources, Purdue University, West Lafayette, IN, USA



Detecting spawning/ behavioural activity

- Planning the opening and/or closure of some barriers
- Interfere with reproduction of invasive species
- Limiting the collection of target species in particular period of the year or encourage it if they are exotic species
- Activating monitoring actions on target species

Detecting the abundancies changes of species

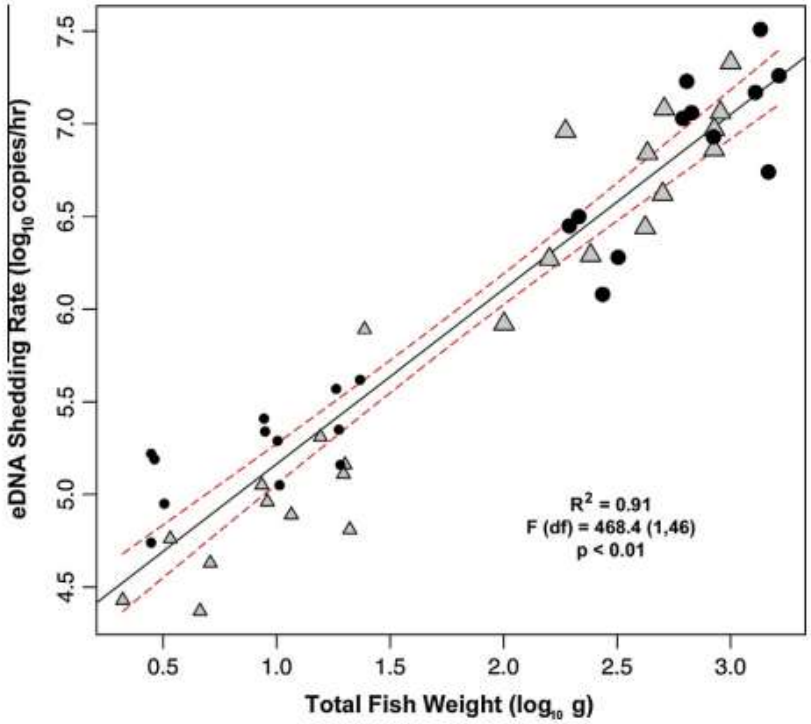


Fig. 2. Combined scatter plots of eDNA shedding rate against biomass of fish in tanks. (Bighead carp sub-adults – large, black circles; bighead carp juveniles –small, black circles; silver carp sub-adults – large, grey triangles; silver carp juveniles – small, grey triangles). Dashed line indicates 95% CI.

Special Issue Article: Environmental DNA

Quantification of eDNA shedding rates from invasive bighead carp *Hypophthalmichthys nobilis* and silver carp *Hypophthalmichthys molitrix* ☆

Katy E. Klymus [a,*], Catherine A. Richter [b], Duane C. Chapman [b], Craig Paukert [c]

[a] Missouri Cooperative Fish and Wildlife Research Unit, Department of Fisheries and Wildlife, University of Missouri, Columbia, MO, USA
[b] U.S. Geological Survey, Columbia Environmental Research Center, Columbia, MO, USA
[c] U.S. Geological Survey, Missouri Cooperative Fish and Wildlife Research Unit, Department of Fisheries and Wildlife, University of Missouri, Columbia, MO, USA

More biomass, more eDNA

Caution with this statement

Detecting the abundancies changes of species

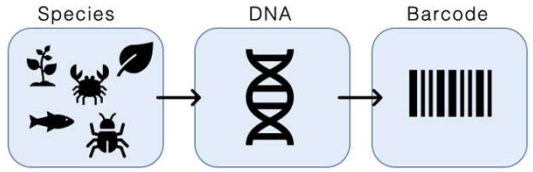
[eDNA] = released DNA – degraded DNA

<u>There are factors that influence the release and degradation rates of the DNA:</u>

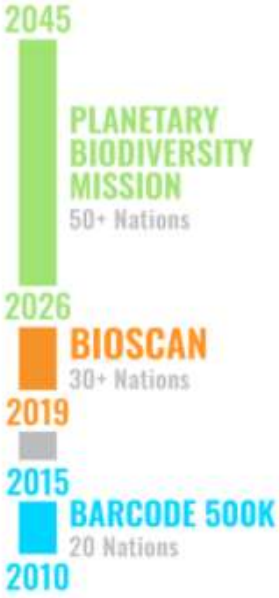
- pH
- Temperature
- Food availability
- Seasonality
- Turbidity
- UVs exposition

Determining species assemblies

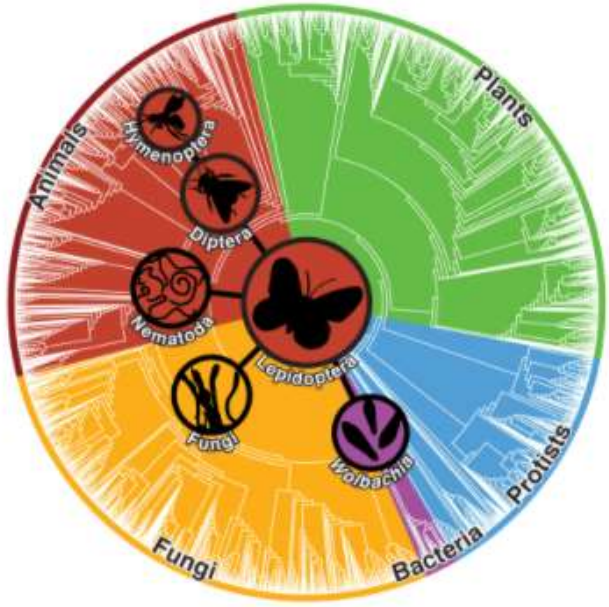
DNA barcoding: species identification from short DNA fragments



TRACKING ECOSYSTEMS



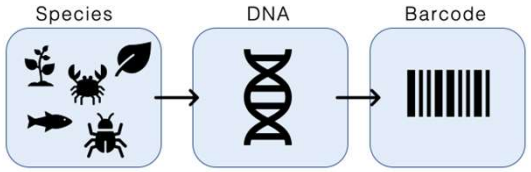
TIMELINE

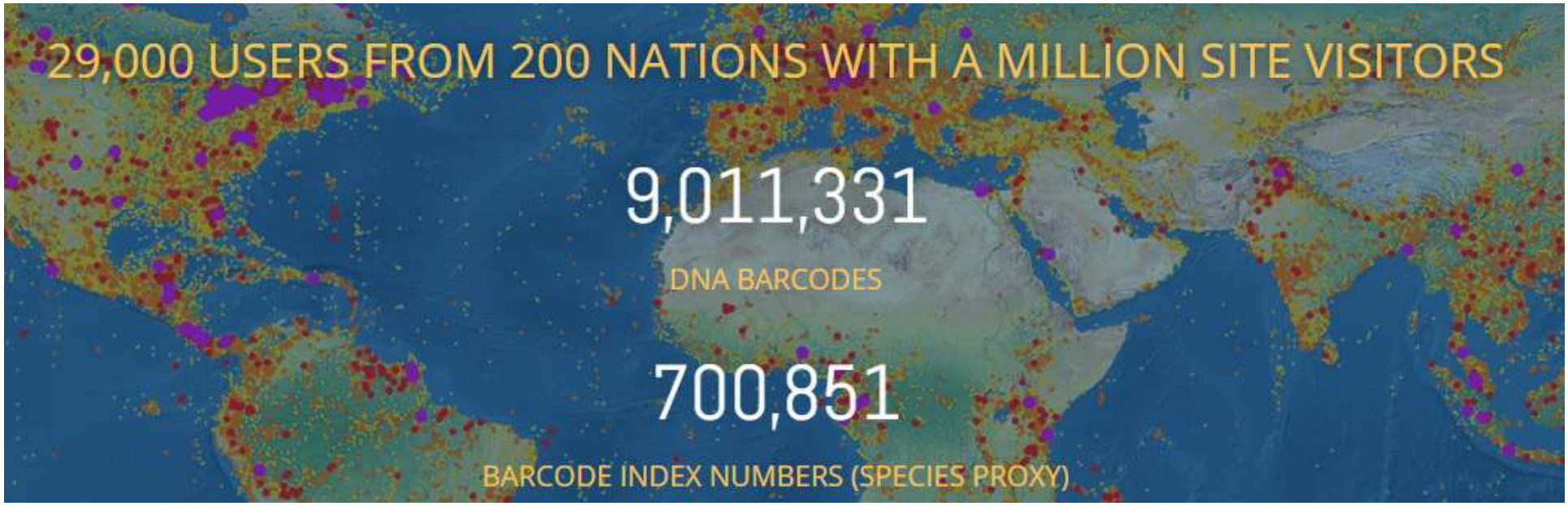


REVEALING SYMBIOMES

Determining species assemblies

DNA barcoding: species identification from short DNA fragments





DNA barcoding

Collection and preservation of the water sample

DNA extraction

DNA amplification using a specific primer pair (qPCR)

Analysis of the qPCR results

✔ Presence of the target species and number of positive replicates

✘ Target species not detected

DNA metabarcoding

Collection and preservation of the sample

DNA extraction

DNA amplification using a universal primer pair (PCR)

Verification of the amplification using capillary electrophoresis

DNA purification

Next generation sequencing of the purified products

Bioinformatic analysis

Result: List of taxa identified and number of associated DNA sequences

Not only recent DNA….Paleoecology

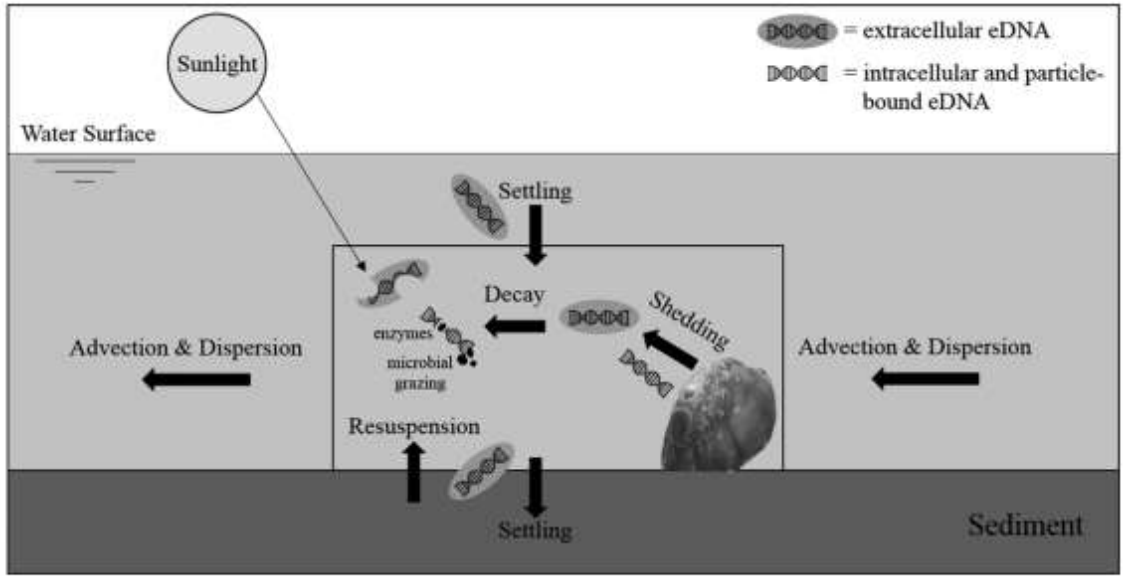


Figure 1. Conceptual model of the source, transport, and fate of eDNA from a freshwater mussel in a stream environment. Processes

Evaluating monitoring actions

Invasive species control/eradication

Restore natural habitats

In both cases eDNA can be used to assess the actions undertaken

Create Databases

- eDNA samples can be used to trace different species
- Multiple testes can be done on the same sample
- Species to monitor today, may not need to be monitored tomorrow
- Samples can be stored frozen or freeze-dried
- Archival samples can be reanalysed years later
- Different laboratories can analyse the same samples

Svalbard Global Seed Vault (Norway)

eDNA: not only research, but chance to develop new employment realities

Monitoring of target taxonomic groups in aquatic ecosystems

This approach has been developed since 2011 by SPYGEN and its partners. It is based on the use of universal primer pairs* and Next Generation Sequencing technologies. This **non-invasive** method enables **blind detection** of all species of a target group present on the study site and thus represents a powerful **environmental monitoring tool**. It enables improved detection of rare species (in comparison with conventional methods), a **reduction in survey costs** and the **avoidance of any risk of introduction of pathogens or invasive species** during sampling.

For more information, you can download: Valentini *et al.* 2016.pdf

**Patented technology (CNRS – Université Grenoble 1)*

Diet analysis from faecal samples

This **non invasive** method was developed in 2007 by the Laboratoire d'Ecologie Alpine in order to improve our knowledge on the **biology** of threatened animal species and their **interactions with the ecosystem**. It is based on the extraction of DNA from faecal samples and its amplification using an universal primer pair*. The amplified DNA is then sequenced (Next Generation Sequencing) and the obtained sequences are compared to the GenBank international reference database using bioinformatics tools.

For more information, you can download: Soininen *et al.* 2009.pdf

**Patented technology (CNRS – Université Grenoble 1)*

eDNA: not only research, but chance to develop new employment realities

Plant composition from a honey sample

Developed in 2010 by the Laboratoire d'Ecologie Alpine, this method is based on the use of an universal primer pair for plants* and Next Generation Sequencing technologies. Using bees as "**environmental samplers**", it is possible to assess the **plant biodiversity** in a study site and to follow its evolution through time, by regularly analysing honey samples. It also represent a powerful tool for the assessment of honey **geographical origin**.

For more information, you can download: Valentini *et al.* 2010.pdf

**Patented technology (CNRS – Université Grenoble 1)*

Next Steps

Instead of spending endless hours doing this…

Develop molecular and bioinformatics methods to analyse massive sequencing

Next Steps

[eDNA] = released DNA – degraded DNA

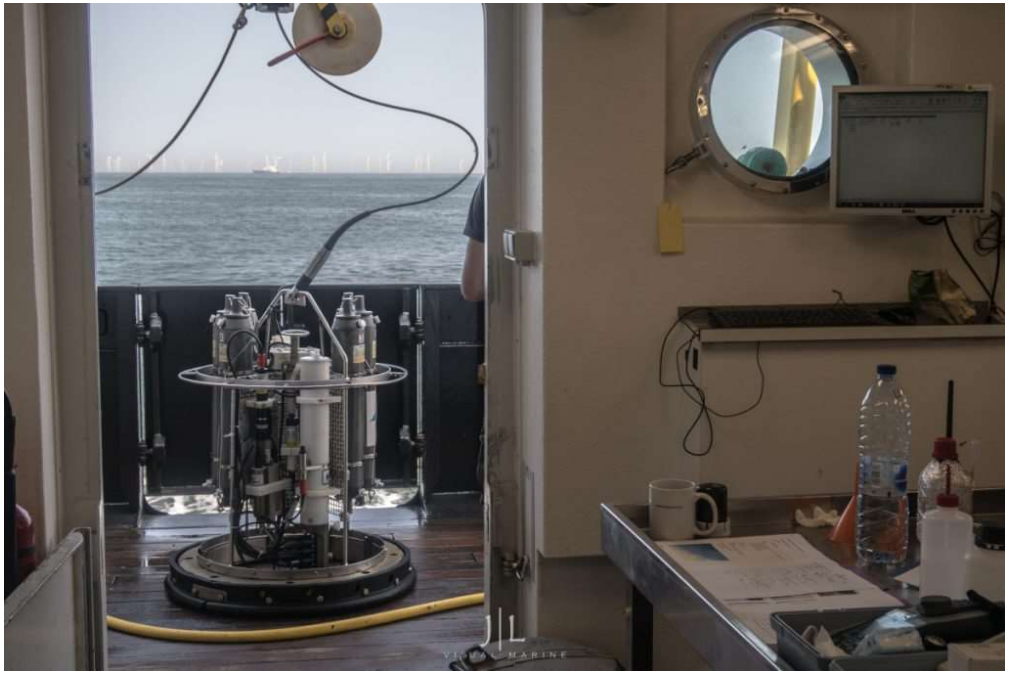
<u>There are factors that influence DNA release and degradation rates:</u>

- pH
- Temperature
- Food availability
- Seasonality
- Turbidity
- Uvs exposition

We have to improve the formula!

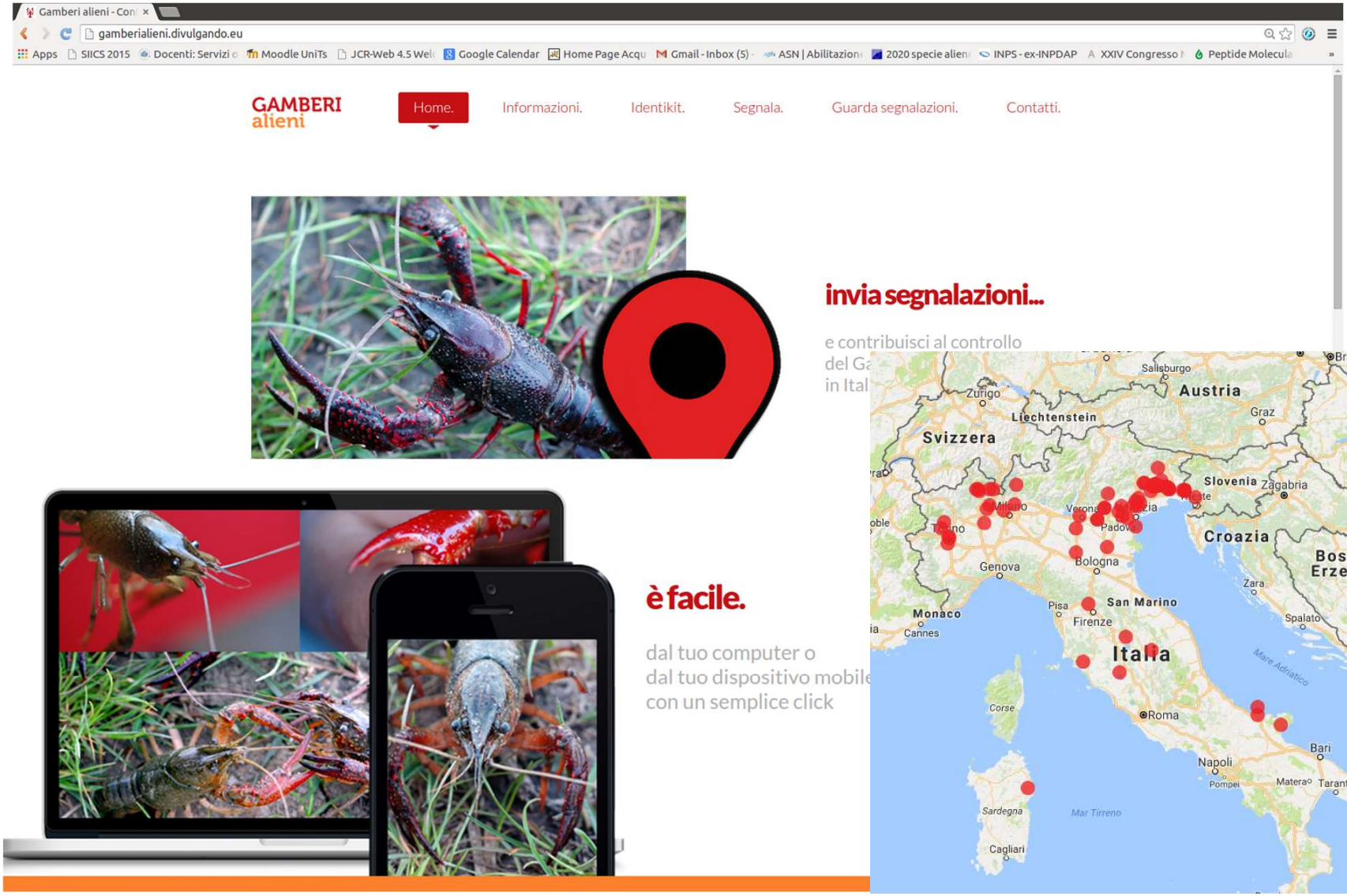
Next Steps

Greater automation for continuous monitoring and long-term data collection



Next Steps

http://gamberialieni.divulgando.eu/



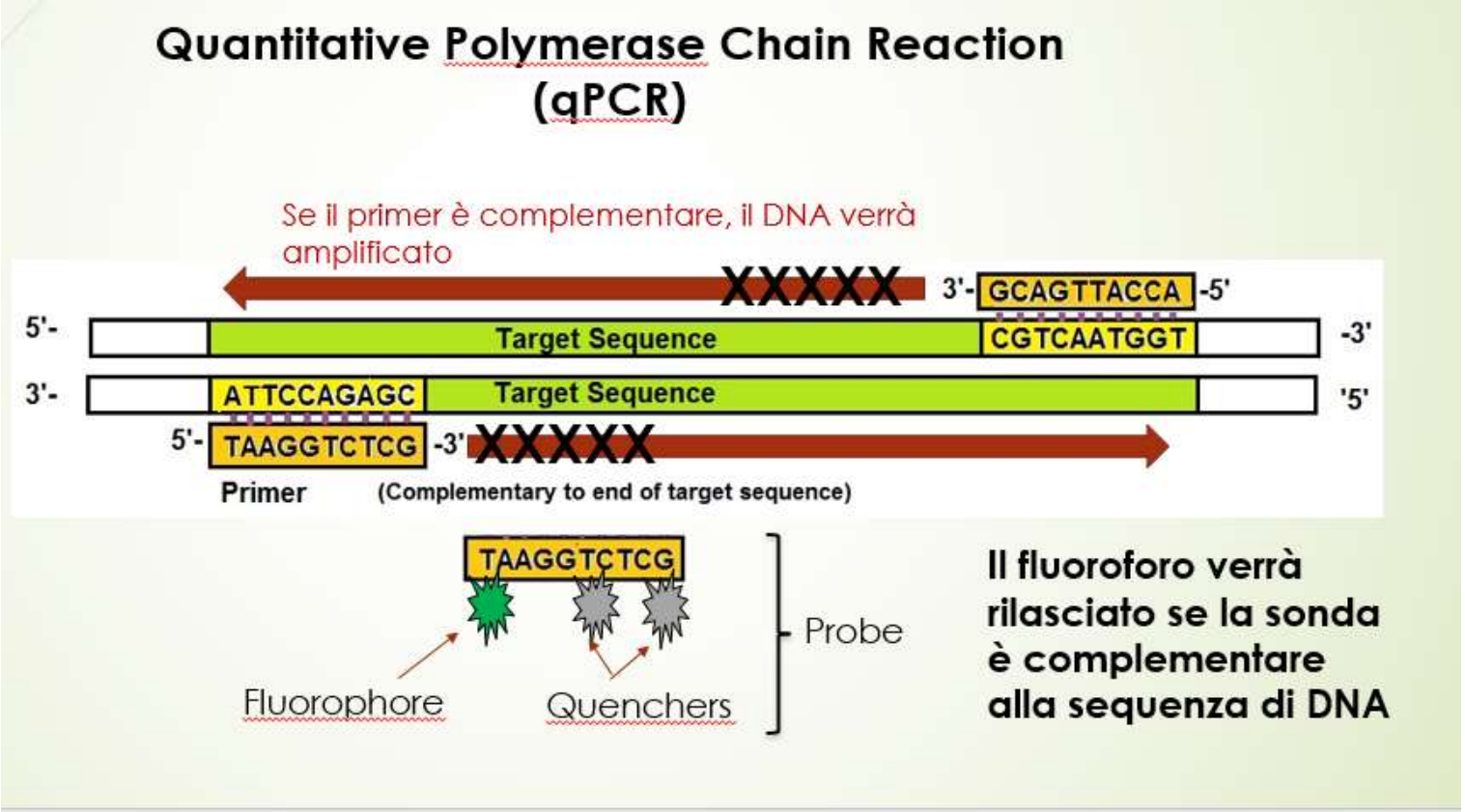
Real demonstration of eDNA sampling

https://vimeo.com/278562904

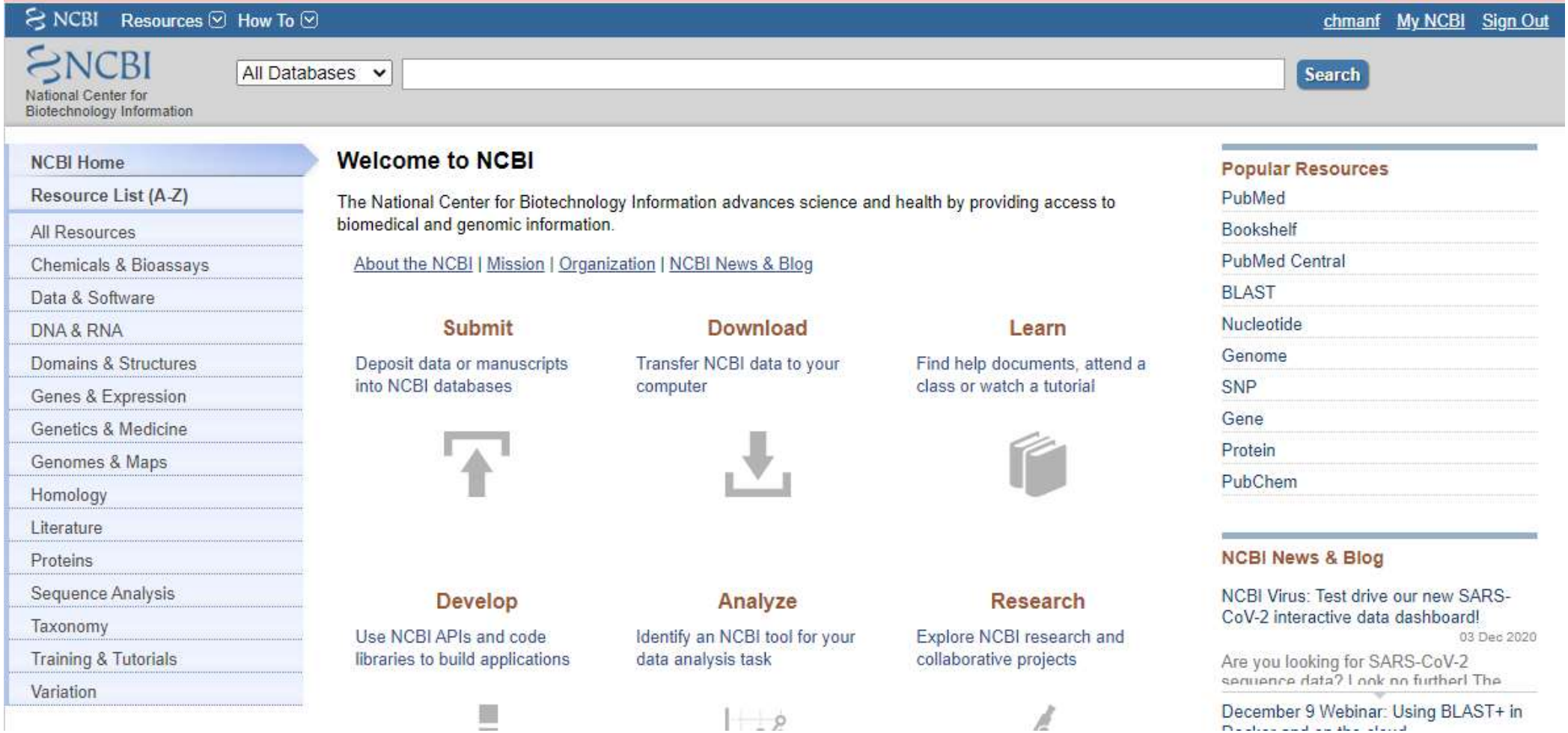
https://vimeo.com/352017863

https://www.youtube.com/watch?v=VQ8zZDPR7OY

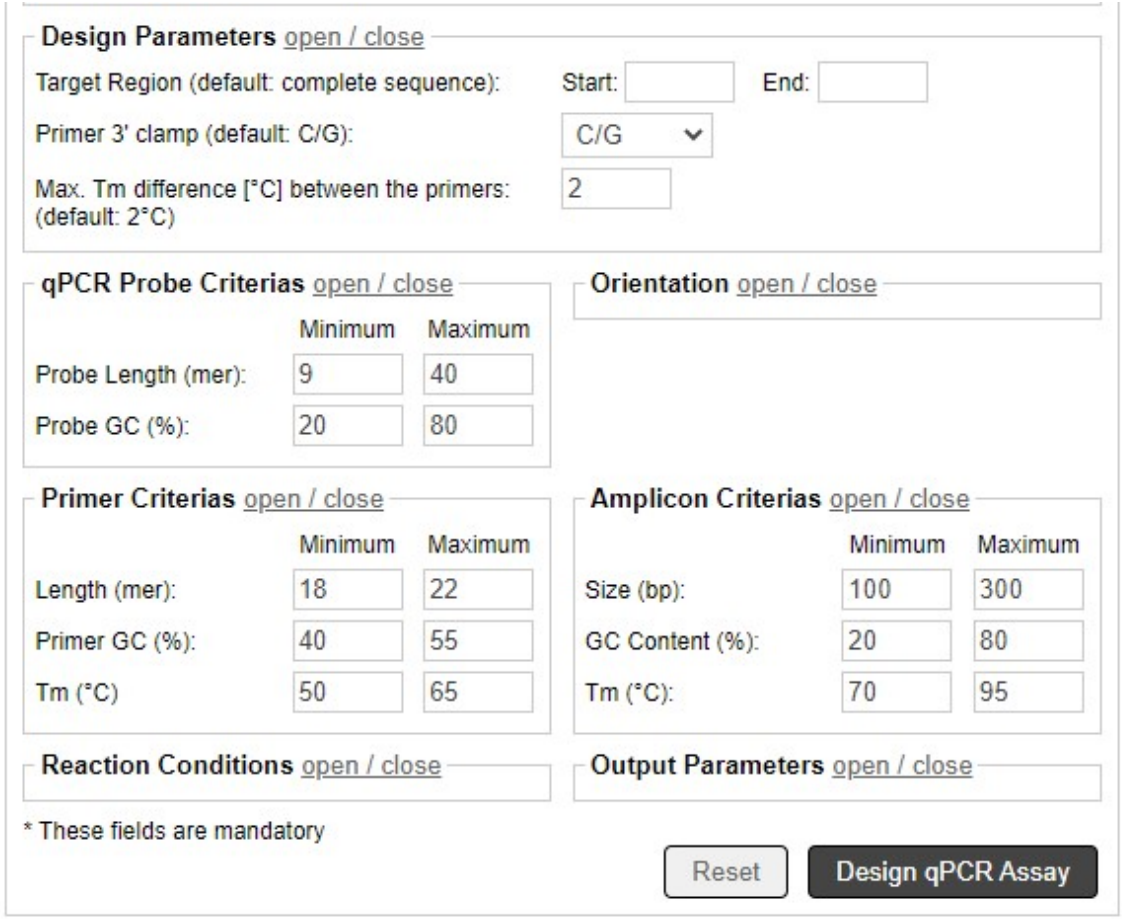
Primer and probe design for qPCR



Databases and target species



- https://www.eurofinsgenomics.eu/en/ecom/tools/qpcr-assay-design/



https://bioinfo.ut.ee/primer3/

Primer3web version 4.1.0 - Pick primers from a DNA sequence.

disclaimer | cautions | code

Select the Task for primer selection: generic

Template masking before primer design (available species)

Select species: Example: Mus musculus
Primer failure rate cutoff: < 0.1
Nucleotides to mask in 5' direction: 1
Nucleotides to mask in 3' direction: 0

Paste source sequence below (5'->3', string of ACGTNacgtn -- other letters treated as N -- numbers and blanks ignored). FASTA format ok. Please N-out undesirable sequence (vector, ALUs, LINEs, etc.) or use a Mispriming Library (repeat library)
NONE

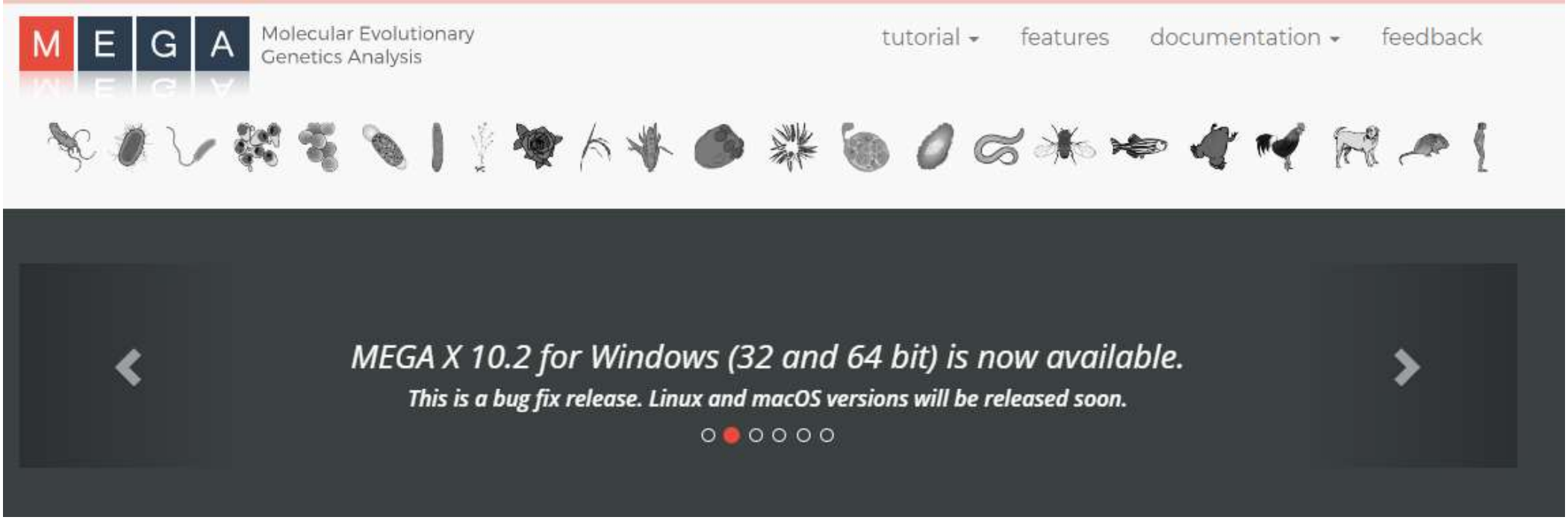
☑ Pick left primer, or use left primer below
☐ Pick hybridization probe (internal oligo), or use oligo below
☑ Pick right primer, or use right primer below (5' to 3' on opposite strand)

Pick Primers | Download Settings | Reset Form

Sequence Id		A string to identify your output.
Targets		E.g. 50,2 requires primers to surround the 2 bases at positions 50 and 51. Or mark the source sequence with [and]: e.g. ...ATCT[CCCC]TCAT.. means that primers must flank the central CCCC.
Overlap Junction List		E.g. 27 requires one primer to overlap the junction between positions 27 and 28. Or mark the source sequence with -: e.g. ...ATCTAC-TGTCAT.. means that primers must overlap the junction between the C and T.
Excluded Regions		E.g. 401,7 68,3 forbids selection of primers in the 7 bases starting at 401 and the 3 bases at 68. Or mark the source sequence with < and >: e.g. ...ATCT<CCCC>TCAT.. forbids primers in the central CCCC.
Pair OK Region List		See manual for help.
Included Region		E.g. 20,400: only pick primers in the 400 base region starting at position 20. Or use { and } in the source sequence to mark the beginning and end of the included region: e.g. in ATC{TTC...TCT}AT the included region is TTC...TCT.
Start Codon Position		
Internal Oligo Excluded Region		

Force Left Primer Start: -1000000 | Force Right Primer Start: -1000000
Force Left Primer End: -1000000 | Force Right Primer End: -1000000

Sequence Quality


MEGA
Molecular Evolutionary
Genetics Analysis
tutorial
features
documentation
feedback
MEGA X 10.2 for Windows (32 and 64 bit) is now available.
This is a bug fix release. Linux and macOS versions will be released soon.