Early steps in the molecular biomonitoring of freshwaters in Portugal

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CIBIO-InBIO
Freshwater biodiversity and water quality

New molecular approaches

Ongoing research

1. DNA reference collection
2. Metabarcoding with ethanol of macroinvertebrates
3. Metabarcoding of fish community in the Douro basin

Remarks and next steps
Freshwater biodiversity and water quality

Water of good quality is a worldwide concern. Freshwater habitats and species are highly threatened.

Vörösmarty et al 2010
Freshwater biodiversity and water quality

Water Uses
- drinking
- recreation
- irrigation
- livestock watering
- aquatic life
- wildlife

Water Stressors
- industrial plants
- logging
- mining
- dam
- urban development
- agriculture
Water Framework Directive (WFD)

- AIM: To achieve a **good ecological status** in all EU water bodies
- By 2015: 53% water bodies reached good status
- Reasons for the poor progress:
  - Water biotic indices are diverse
  - Very costly -> often few funds remain to promote the following restoration
  - Complex role of multiple stressors
  - Accumulated impacts in watershed
  - ...

The WFD implementation has been slower than expected
Habitats Directive (HD)

AIM: Establish a network of protected sites to protect a wide range of rare, threatened or endemic species and habitats occurring in EU

EG low overlap of Natura 2000 sites and freshwaters (Hermoso et al. 2014)

EG Additional protected areas need to be defined (Hermoso et al. 2015)

The HD fails to protect freshwater biodiversity
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Environmental DNA (eDNA)

DNA traces from water, feces, bulk, sediment samples to detect species (or OTUS) present in the surveyed ecosystem
New molecular approaches

Metabarcoding

Involves the identification in the same run of a large number of organisms of different species from multiple samples
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Ongoing research: reference collection

IBI – InBIO Barcoding Initiative
Ongoing research: reference collection

Invertebrates and fish

COLLECTIONS

FIELD SAMPLING

Specimen

Sample (leg, fin)

Taxonomists

96% ethanol

Morphological ID

DNA sequencing

DNA BARCODE
Ongoing research: reference collection

DNA barcoding of insects

(23 Orders, 247 Families, 2411 Species)

> 6200 Specimens

> 4900 Morphological ID

> 5000 DNA Barcodes (COI – 658bp)

Folmer, 1994
Ongoing research: DNA reference collection

DNA Barcoding of WFD taxa

EPTO

> 1000 specimens
> 300 species

Ephemeroptera: 10 out of 11 families in Portugal

Plecoptera: all families and over 50% species in Portugal

62% Trichoptera species in Portugal

100% Odonata species in Portugal

Megaloptera: additions to the Portuguese fauna

*Sialis lutaria* Linnaeus, 1758

*Sialis nigripes* Pictet, 1865
Ongoing research: reference collection

DNA barcoding of fish (Douro basin)

COI, 12S, Cytb

35 freshwater fish species (21 native, 14 exotic)
3 specimens per species from geographically distant sites

![Graph showing number of barcoded and not barcoded species by family]

Legend:
- Barcoded areas
- Not Barcoded areas
- Streams
- Main rivers
- River basins
Ongoing research: reference collection

**Cobitis sp.**

![Cobitis sp. diagram](image1.png)

Claudia Baeta

**Phoxinus sp.**

![Phoxinus sp. diagram](image2.png)

Márton Zsoldos

(* CIBIO’s barcoding)
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Remarks and next steps
Ongoing research: from ethanol

AIM: To develop a cost-effective metabarcoding approach for macroinvertebrate community assessment based on the preservative ethanol to meet the criteria of the WFD

Advantages: less time consuming, higher taxonomical resolution (including distinct larvae stages, detection of cryptic species)
Ongoing research: from ethanol

- subsampling time (2 ml ethanol; 1, 2, 3, 5, 7, 14 days after sampling)
- 3 different extraction methods (Column-based & Enzymatic lysis, Column-based & Mechanical lysis, Magnetic Bead-based & Enzymatic lysis)

Tua subbasin (Douro)

- Tradicional kick-net sampling
- Uncleaned, unsorted samples (stored in 96% ethanol; ~3:1)
Ongoing research: from ethanol

Filipa MS Martins et al. in review *Mol Ecol Res*
Ongoing research: from ethanol

- 1-7 days after field sampling: Magnetic Bead-based & Enzymatic lysis
- 7-14 days after field sampling: Magnetic Bead-based/Column-based & Enzymatic lysis extraction methods

Missing taxa in ethanol approach (3 species):
  - Low-frequent species: e.g. Heptageniidae
  - Poor DNA reference database: e.g. Ephemereellidae
  - Primer bias
  - Bioinformatic filtering

Extra taxa from ethanol approach (8 species)

Martins et al. in review *Mol Ecol Res*