eDNA and species-specific primer for early detection. A case study on the bivalve *Rangia cuneata*, currently spreading in Europe.

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DNA-based methods for monitoring biological invasions in aquatic environments: eDNA

EARLY DETECTION:
Rangia cuneata

▫ **Native range**: Gulf of Mexico and introduced to the NW Atlantic, where it is predominantly found in estuaries.

▫ **Known introduced range, 2006**: lower portion of the Hudson River, New York and harbor of Antwerp, Belgium, Europe (Verween et al, 2006).

▫ Extremely rapid spread of the species in Europe (Rudinskaya and Gusev, 2012).

- **2010**: *R. cuneata* in Vistula Lagoon of the Baltic Sea
- **2011** : 4,040 ind/m^2 in Kaliningrad Sea channel.
- **2014**: ¿?

EARLY DETECTION: *Rangia cuneata*

- Is recognized as highly invasive species by Invasive Species Specialist Group (www.issg.org/).

- Guilty of the dramatic transformations of the local benthic communities (Rudinskaya and Gusev, 2012).

- Traditional sampling tools → insufficient to detect new invasions, especially in aquatic environments, where organisms are not apparent and are hidden underwater.

- Several studies demonstrate the efficacy of eDNA as a tool for species detection in aquatic environments (Ficetola et al., 2008; Dejean et al., 2011; Jerde et al., 2011; Taberlet et al., 2012; Thomsen et al., 2012).

Detection threshold

- .... If we have many other bivalve species with high density????

  - *Macoma balthica*
  - *Mya arenaria*
  - *Mytilus trossulus*
  - *Cerastoderma glaucum*
  - *Dreissena polymorpha*

¿Rangia cuneata?

- **SOLUTION**: Species-specific primers to detect the presence of a target organism.

EARLY DETECTION:
Rangia cuneata

- **OBJECTIVE**: to develop and validate species-specific molecular markers for early detection and distribution assessment of *R.cuneata*, using eDNA.

- **APPLICATIONS**: biosecurity and monitoring.

- 6 different mollusks were sampled from the Lithuanian coast of the Baltic Sea and the Curonian Lagoon.
  - *Cerastoderma glaucum* (N)
  - *Macoma balthica* (N)
  - *Mytilus trossulus* (N)
  - *Dreissena polymorpha* (EI)
  - *Mya arenaria* (C)

# EARLY DETECTION: *Rangia cuneata*

Water samples from:

- 2 locations within the Baltic Sea coastal zone
- 5 within the Vistula Lagoon

Location	*R. cuneata* larvae density, ind/m^3
Juodkrante	Not detected
Nida	Not detected
1	6791
3	3058
7	1168
9	1946
4	20262

Material and Methods

1. ## DNA extraction.
   - QIAamp® DNA Mini Kit, Quiagen,

   based on silica gel gel columns.

2. ## PCR amplification.
   - 16S rDNA (Palumbi et al., 1996)

3. ## Designing new primer.
   - Alignment with BioEdit (Hall, 1999)
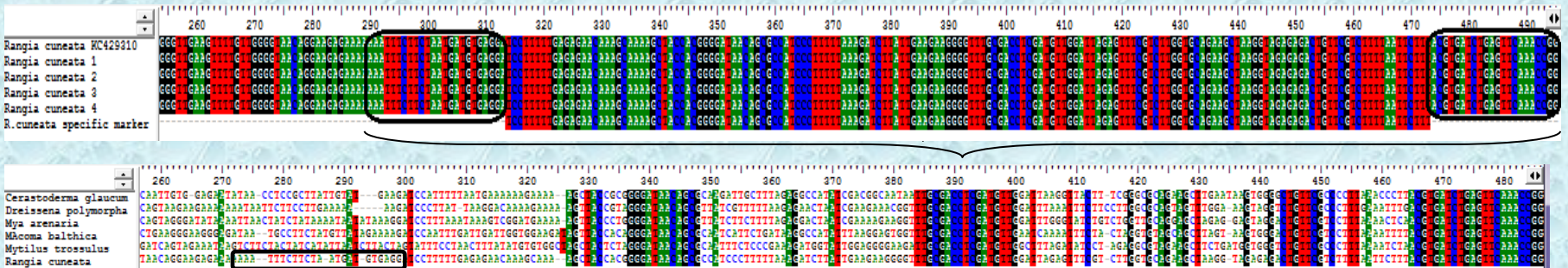
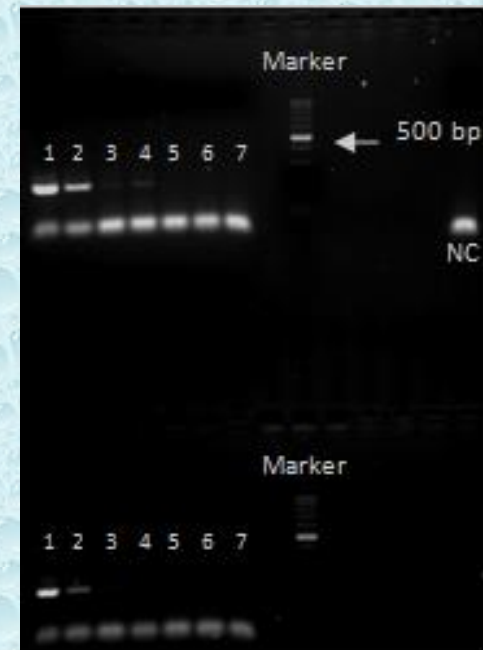# Specificity and sensitivity of the designed primer

RC-16Sar: 5´- AAATTTCTTCTAATGATGTGAGG -3´

16Sbr (Palumbi et al., 1996)

Experimental DNA mixtures
to test the marker's sensitivity.

Cross-amplification?
Testing the new marker specificity

Marker validation

In vitro experiment.
Simulated communities

Validating the designed primers
with the **field samples**

In conclusion, we recommend the application of species-specific markers for screening environmental samples as complimentary routine monitoring tool.