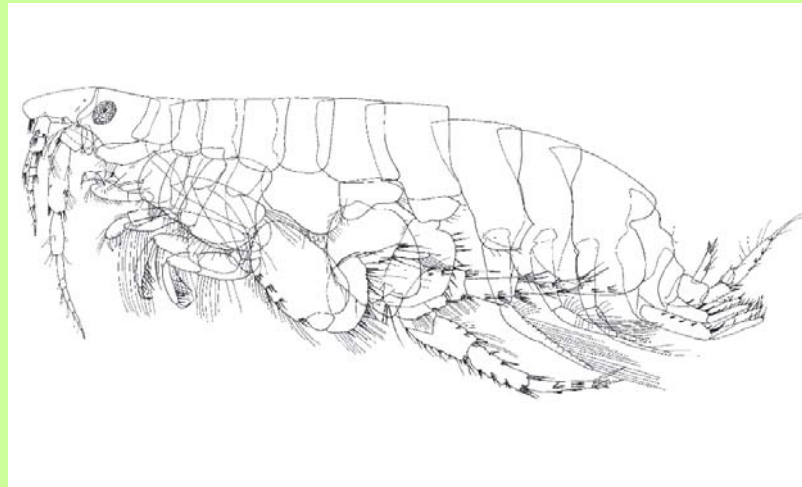


Research into the distribution of the genus *Bathyporeia* (Crustacea, Amphipoda) by means of molecular markers.

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Ghent University, Marine Biology Section, Krijgslaan 281-S8, B-9000 Gent, Belgium



From d'Udekem d'Acoz, 2004



Bathyporeia: classification

Ordo Amphipoda

Subordo Gammaridae

Superfamily Gammaroidea (16 families)

Family Bathyporeiidae BOUSFIELD & SHIH 1994

Genus Bathyporeia LINDSTRÖM 1855

B. borgi d'Udekem d'Acoz & Vader, 2005

B. chevreuxi d'Udekem d'Acoz & Vader, 2005

B. cunctator d'Udekem d'Acoz & Vader, 2005

***B. elegans* Watkin, 1938**

B. elkaimi d'Udekem d'Acoz & Menioui, 2004

B. gladiura d'Udekem d'Acoz & Vader, 2005

B. gracilis G. O. Sars, 1891

B. griffithsi d'Udekem d'Acoz & Vader, 2005

***B. guilliamsoniana* Bate, 1857**

B. ledoyeri d'Udekem d'Acoz & Menioui, 2004

B. lindstromi Stebbing, 1906

B. microceras d'Udekem d'Acoz & Menioui, 2004

B. nana Toulmond, 1966

B. parkeri Bousfield, 1973

***B. pelagica* Bate, 1857**

B. phaiophthalma Bellan-Santini, 1973

***B. pilosa* Lindström, 1855**

B. quoddyensis Shoemaker, 1949

***B. sarsi* Watkin, 1938**

B. tenuipes Meinert, 1877

B. watkini d'Udekem d'Acoz, Echchaoui & Menioui, 2005

Genus Amphiporeia SHOEMAKER 1929

Family Pontoporeiidae DANA 1853

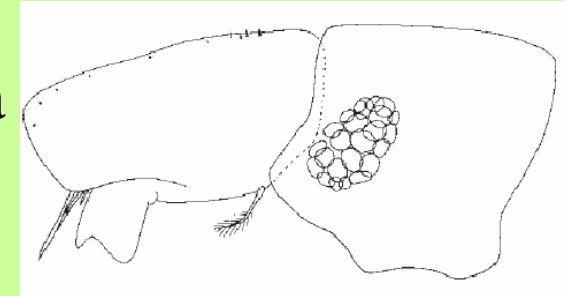
Genus Diporeia BOUSFIELD 1979

Monoporeia BOUSFIELD 1979

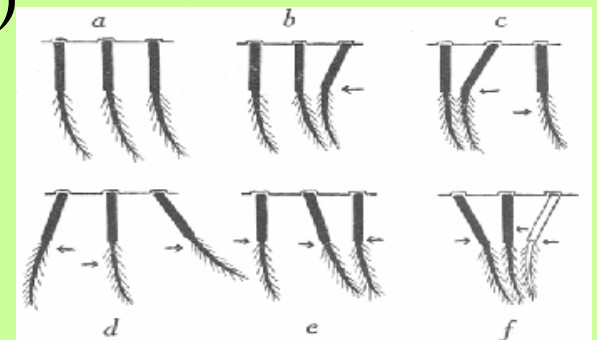
Pontoporeia KROYER 1842

Introduction (1)

- Macrobenthic species
- Pseudorostrum
 - basal part of the peduncle of the first antenna
 - extended along vertical axis
 - geniculate articulation with second article
- Digging species, restricted to sandy bottoms
 - Very fine well-sorted sands
 - < 5-10% silt (except for *B. pilosa*)
 - 90% in first 5 cm, not deeper than 12 cm
- Swimming and digging (Watkin, 1939)
 - pleopods (metachronal rithm)



From d'Udekem d'Acoz, 2004



Introduction (1)

- Atlantic species, not in tropical and south part of West Atlantic
- Selective depositfeeders: micro-organisms, detritus
- Zonation (Watkin, 1939)
 - Not all species present on every shore



- breeders: limited dispersal capacity?



http://en.wikipedia.org/wiki/Brood_pouch_%28Peracarida%29

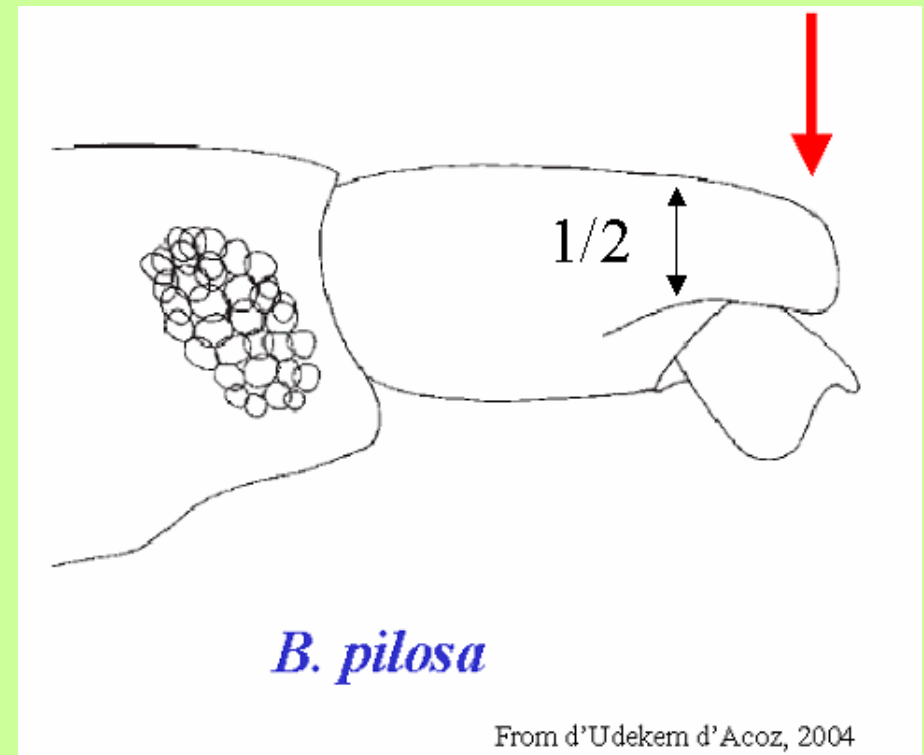
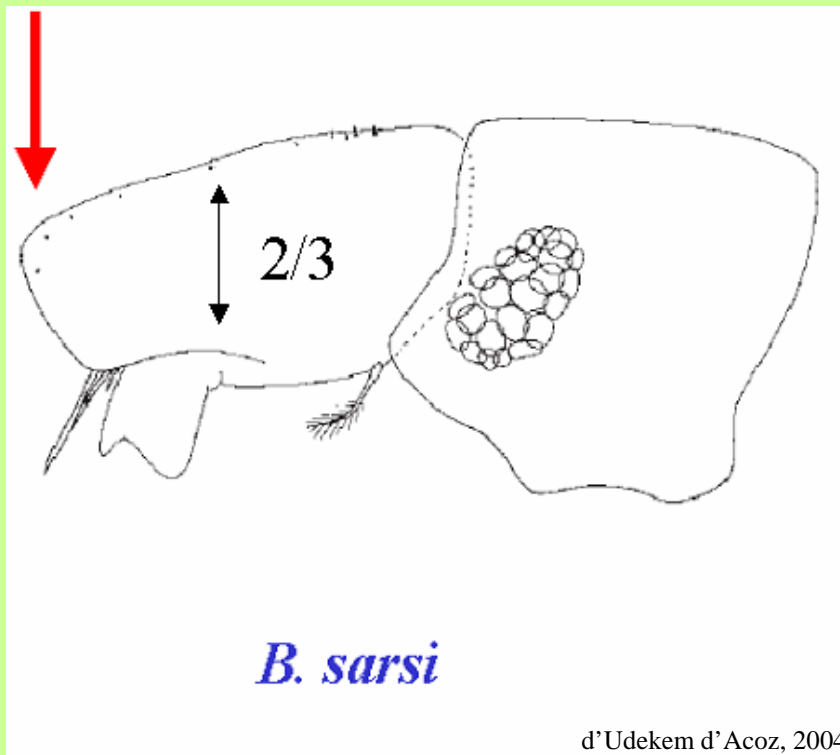
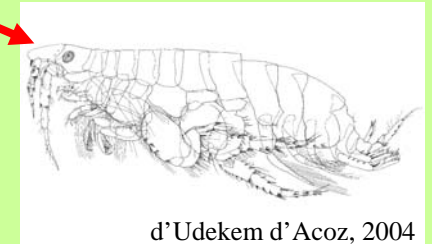
Introduction (2)

- problems concerning **correct taxonomic identification**
 - taxonomic identification of amphipods is complex, great expertise is needed for a correct species identification (Costa *et al.*, 2004)
 - **genus *Bathyporeia***: one of the most problematic taxa concerning species identification (d'Udekem d'Acoz, 2004)

Introduction (2)

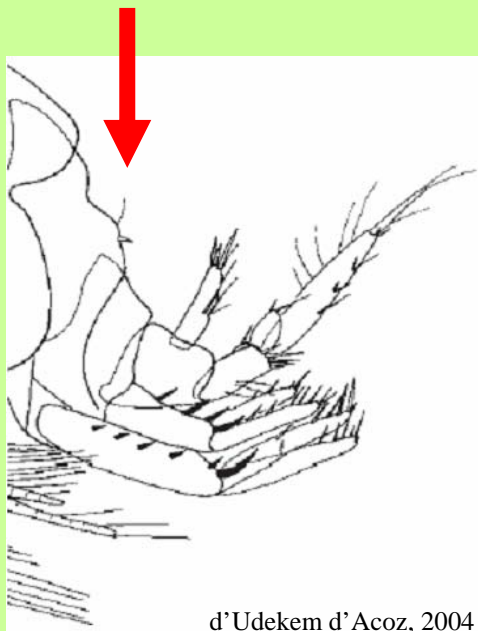
Distinction between intertidal species:

- shape of pseudorostrum
- implantation first antenna

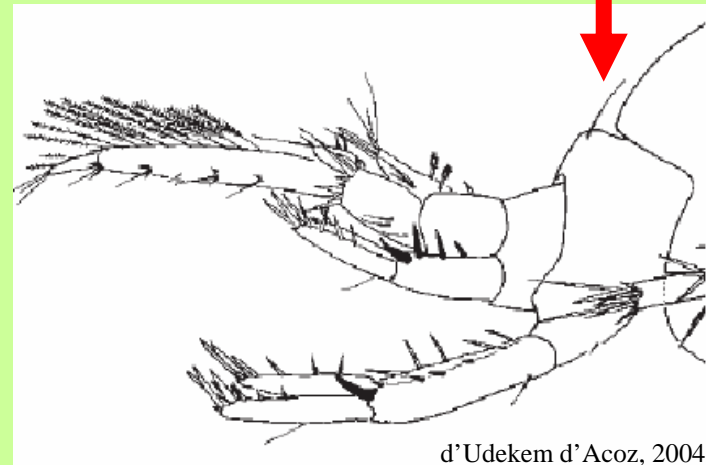
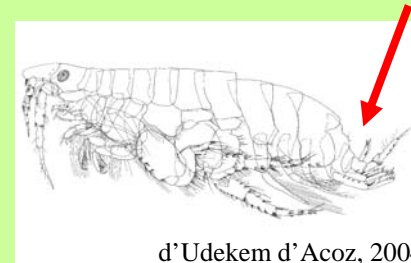


Introduction (2)

Distinction subtidal – intertidal species:
posteriorly directed spines on first urosomite



B. elegans



B. sarsi

From d'Udekem d'Acoz, 2004

Introduction (3)

- problems concerning **correct taxonomic identification**
 - presence of **cryptic species**? (d'Udekem d'Acoz & Vader, 2005)
 - *B. tenuipes* complex: *B. tenuipes*, *B. chevreuxi*, *B. cunctator* & *B. lindstromi*

Introduction (3)

CRYPTIC SPECIES

= “*two or more distinct species that are erroneously classified (and hidden) under one species name*”
(Bickford *et al.*, 2007)

- several reasons:
 - non-visual mating signals: differences in mating pheromones or mating calls
 - morphological stasis: stabilizing selection on morphology due to extreme environmental conditions
- Can be identified using **molecular tools** because they are genetically different

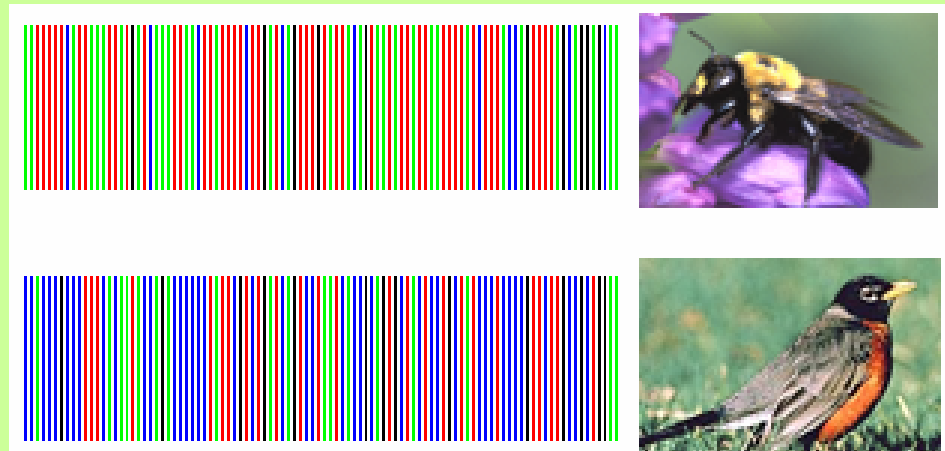
Introduction (3)

Use of molecular data to:

- identify species within the genus using DNA: **DNA-barcoding**
- distinguish morphologically identical species or cryptic species

Introduction (3)

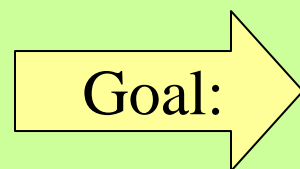
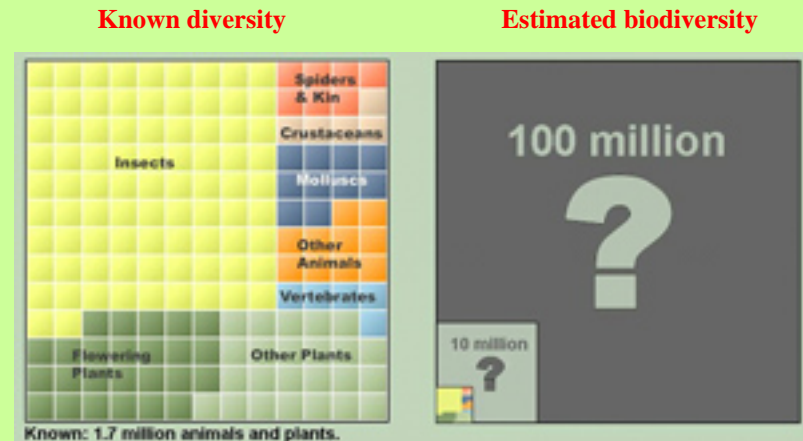
- **DNA-barcoding:** standardized approach to identify plants and animals using minimal DNA sequences, called DNA-barcodes
- **DNA-barcode:** short DNA-sequence, from a uniform locality on the genome, used for identifying species



Introduction (3)

Why use DNA-barcoding?

- quickly and cheaply recognize known species
- discovery of not yet known species
- new tools for appreciating and managing Earth's biodiversity

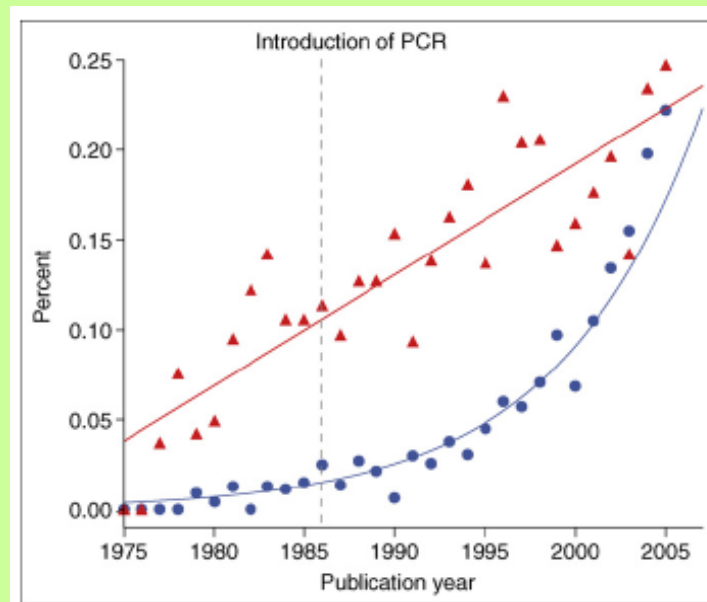


identify quickly and accurately the species of a specimen whatever its condition (egg, larva..)

<http://phe.rockefeller.edu/barcode/>

Introduction (3)

- Molecular techniques highlighted that biodiversity can be strongly underestimated based only on morphological identification
- Increased recognition of cryptic species since mid 1980s (Bickford *et al.*, 2007; 'Trends in Ecology & Evolution')



General Objectives

- (1) Can molecular tools be used to identify the different species and eventually discover cryptic species within this genus?
- (2) Get insight in the degree of genetic variability within the genus
- (3) Get insight in the degree of genetic structuring between and within the intertidal species of the *Bathyporeia* genus.
 - Differences in population genetic structure?
 - Exchange of genetic material limited by absence of a pelagic larva?
 - Cap Griz Nez, harbour of Zeebrugge: barriers?

Materials & Methods (1)

- Collecting intertidal samples along the French-Belgian-Dutch coast and subtidal samples on three sandbanks: Westdiep, Thornton bank, Goote bank
 - Intertidal: digging and sieving
 - Subtidal: Belgica – van Veen grab



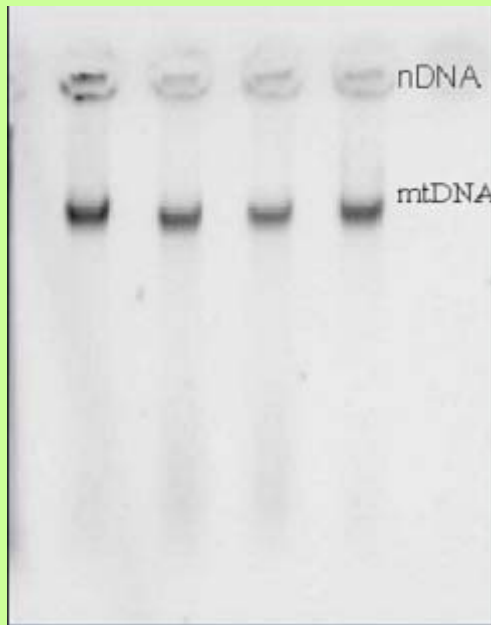
Materials & Methods (2)

Morphological identification of the collected samples: *B. pilosa*, *B. sarsi*, *B. pelagica*, *B. elegans* & *B. guilliamsoniana*

Molecular analysis:

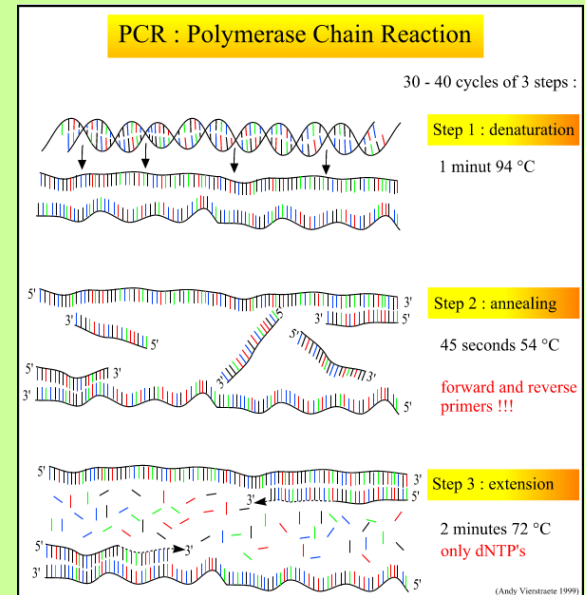
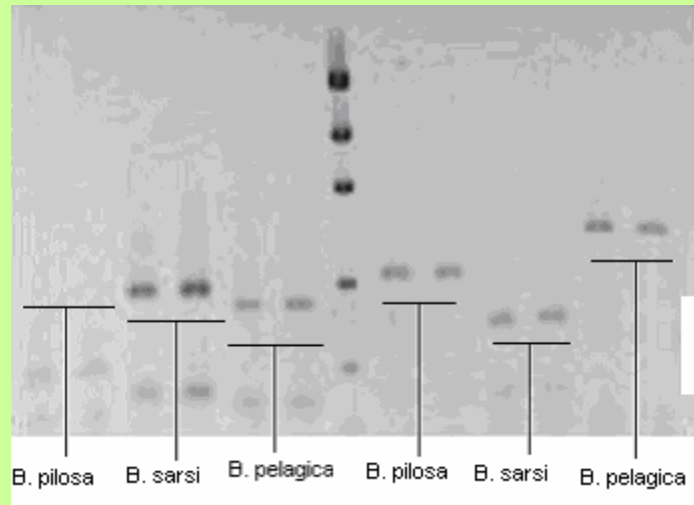
-DNA extraction

- *CTAB extraction using phenol
- *Qiagen Dneasy kit
- * Bionobile QuickPick SML gDNA



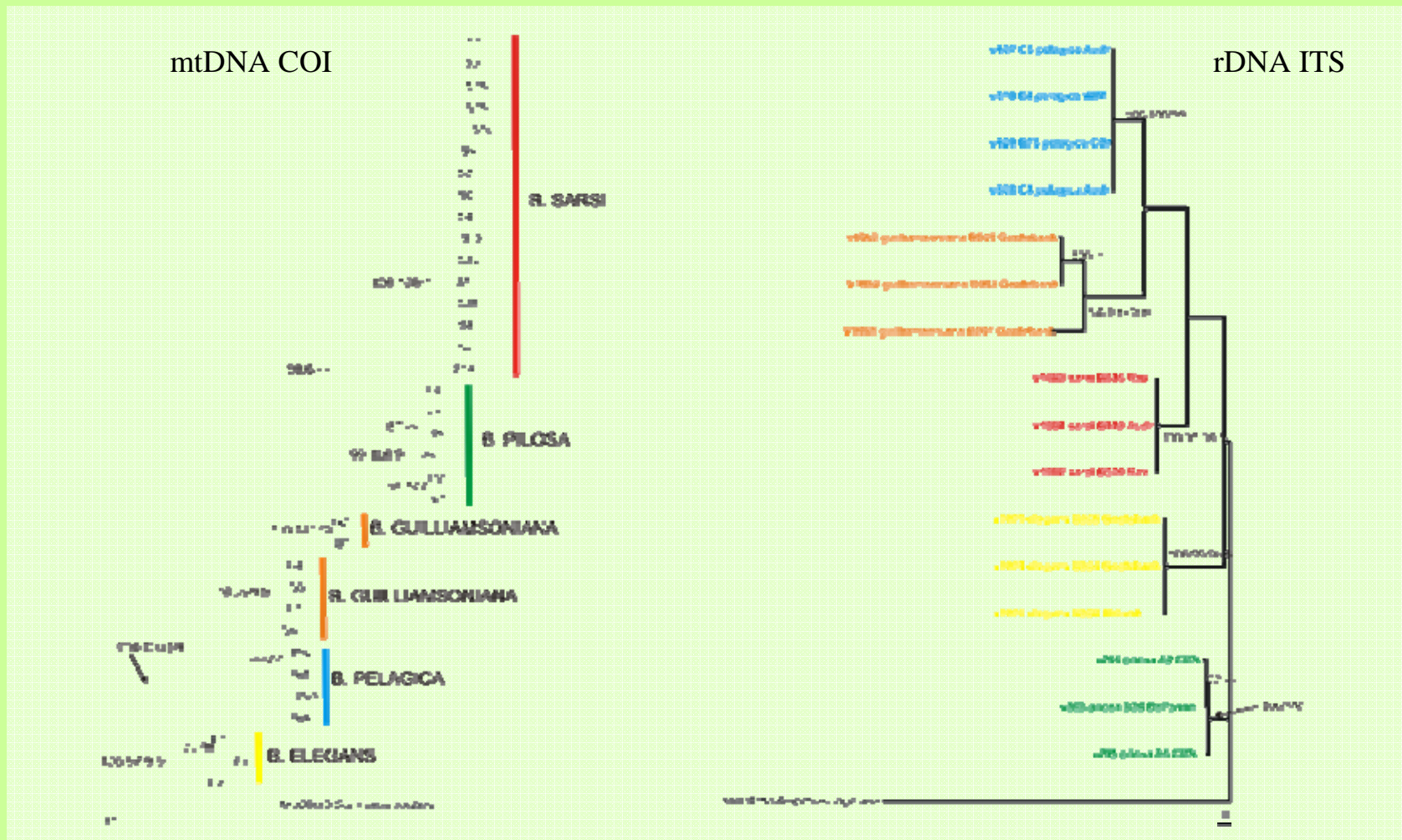
Materials & Methods

- Molecular analysis:
 - DNA amplification: mitochondrial COI gene (“barcoding marker”) and nuclear ITS
 - DNA sequencing
 - RFLP analysis
 - Restriction enzymes MboI and AluI
 - Distinction between intertidal species



Results & Discussion (1)

- Phylogenetic patterns within the genus



Results & Discussion (1)

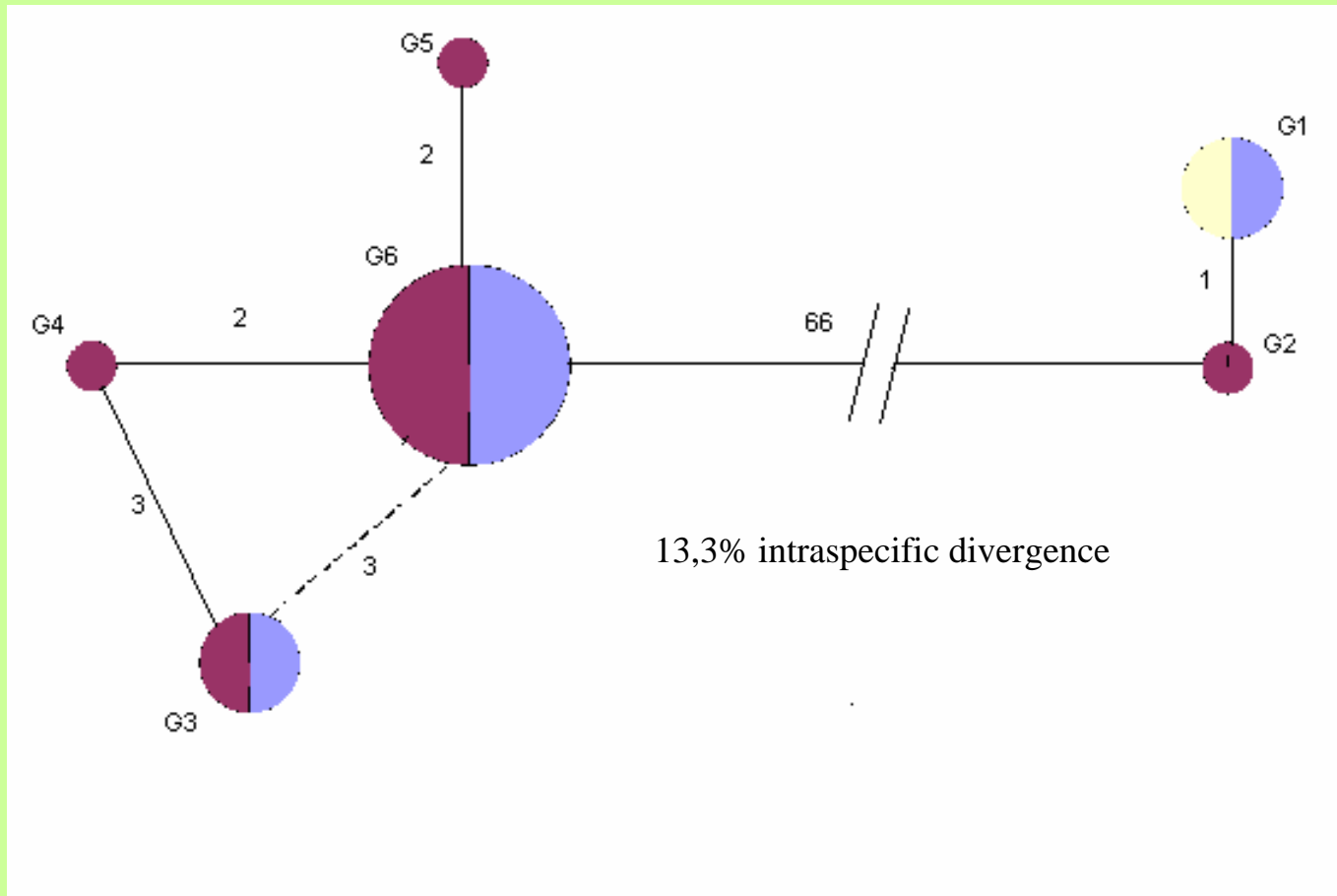
- Phylogenetic patterns within the genus
 - COI and ITS: at least 5 divergent clades → COI useful for DNA barcoding
 - Genetic divergence between different species varying between 12,4 and 20%

	<i>B. pelagica</i>	<i>B. sarsi</i>	<i>B. pilosa</i>	<i>B. guilliamsoniana</i>	<i>B. elegans</i>
<i>B. pelagica</i>	0,7	19,3	17,3	14,3	20
<i>B. sarsi</i>	20	0,8	13,1	13,6	12,4
<i>B. pilosa</i>	18,6	14,5	2	12,4	18,3
<i>B. guilliamsoniana</i>	18,9	18,2	17,6	8,4	15
<i>B. elegans</i>	21,1	19,2	20	20	1,5

Results & Discussion (1)

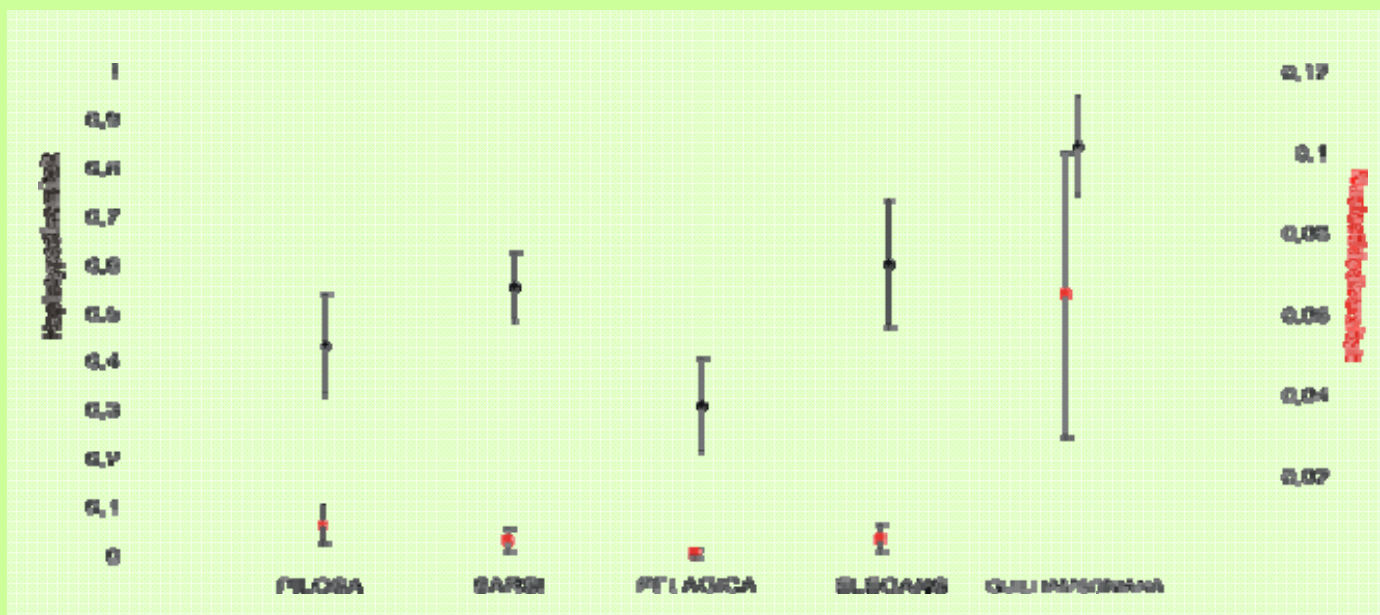
- Phylogenetic patterns within the genus
 - COI and ITS: at least 5 divergent clades → COI useful for DNA barcoding
 - **Large diversity within *B. guilliamsoniana***
 - *Morphologically: one species
 - *Two distinct groups supported by high bootstrap values
 - *High sequence divergence within the ‘species’

	<i>B. pelagica</i>	<i>B. sarsi</i>	<i>B. pilosa</i>	<i>B. guilliamsoniana</i>	<i>B. elegans</i>
<i>B. pelagica</i>	0,7	19,3	17,3	14,3	20
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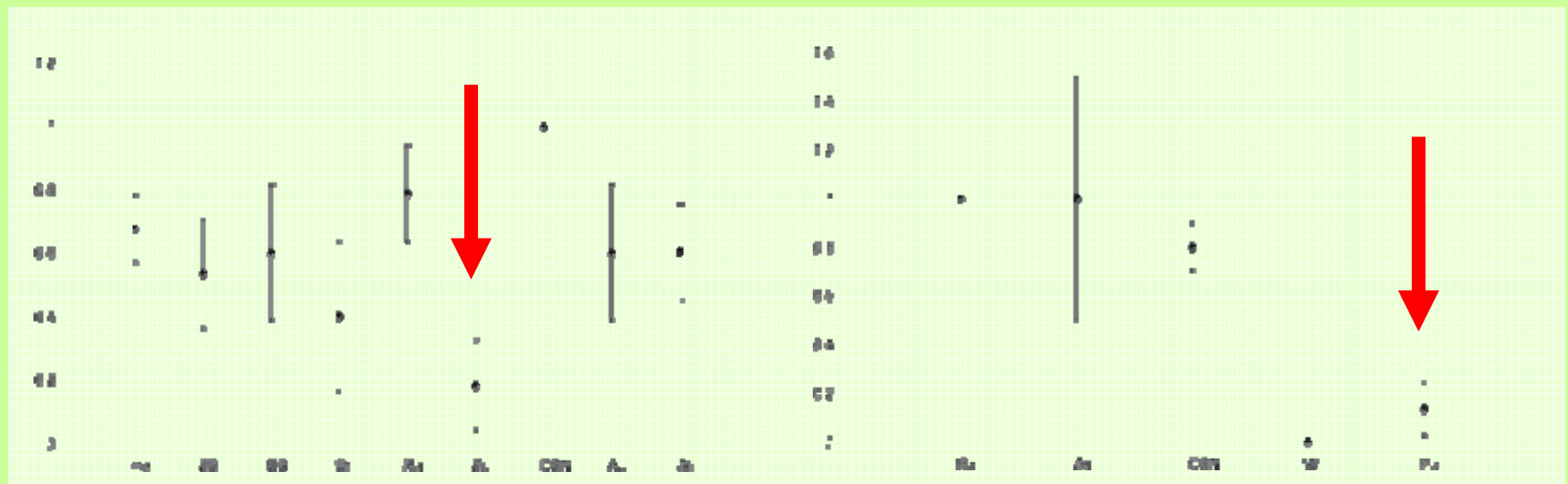
Results & Discussion (2)

- Patterns of genetic diversity:
 - Intertidal species: higher diversity for *B. sarsi* than for *B. pilosa* → zonation on the beach?
 - Higher diversity within subtidal species than within intertidal species → habitat stability?



Results & Discussion (3)

- Population-genetic structure *B. pilosa* – *B. sarsi*
 - Lower haplotype diversity in De Panne & Zuydcoote
→ bottleneck or selective sweep?



Results & Discussion (3)

–**Analysis of Molecular Variance (AMOVA)**: variation mainly explained through differences **within** populations

– ***B. pilosa***: 18,7% variation observed between populations

→ subtle spatial structure present

– ***B. sarsi***: <7% of variation between populations, **no significant** spatial structuring.

- **Link between zonation on the beach and dispersal capacity?**

Conclusions

- Using molecular markers, the different species can be distinguished → COI can be used as a DNA-barcode within this genus
- Genetic diversity within *B. guilliamsoniana* → cryptic species?
- Link between distribution and genetic diversity.
- Link between zonation on the beach and dispersal capacity.