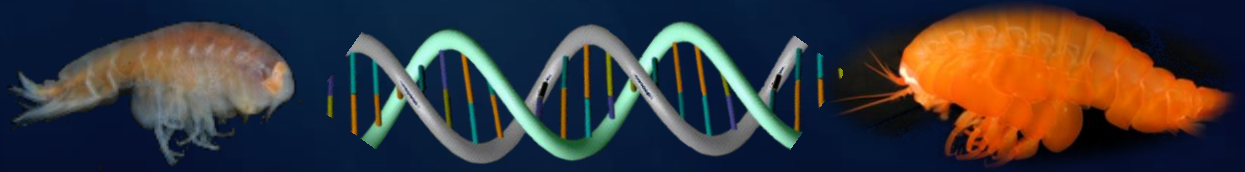
The taxonomy and biogeography of macrofaunal **amphipod crustaceans**, with a focus on the abyssal Pacific fauna relevant to the CCFZ:

Part II: What can DNA tell us?



Charlotte Havermans

museum AWI

Introduction: *Why and how*

Deep-sea macrofaunal taxonomy: challenges

An introduction to DNA barcoding

Molecular deep-sea studies

Molecular work on deep-sea amphipods *(own results & literature)*

Integrative approach (genes & morphology; reverse taxonomy)

Case study 1: DNA barcoding *Orchomene sensu lato*

Case study 2: Phylogeography *Eurythenes gryllus*

« Blind » taxonomic approach (MOTUs)

DNA barcoding for amphipod diversity studies

Outlook: goals beyond species identification

Deep-sea macrofaunal taxonomy

- ✓ Incomplete species descriptions
- ✓ Many species (genera, families) new to science
- ✓ Cryptic species?
- ✓ How to evaluate intraspecific variability, sexual dimorphism?

Deep-sea macrofaunal taxonomy

- ✓ Incomplete species descriptions
- ✓ Many species (genera, families) new to science
- ✓ Cryptic species?
- ✓ How to evaluate intraspecific variability, sexual dimorphism?

A variety of deep-sea molecular studies

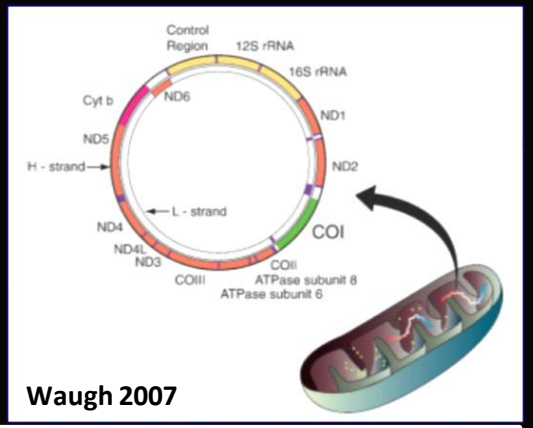
- ✓ **past studies using allozymes: amphipods** (Bucklin et al. 1987, France et al. 1992, France 1994), **polychaetes** (Jollivet et al. 1995), **vent shrimps** (Creasey et al. 1996), **brachiopods** (Valentine & Ayala 1975), **spider crabs** (Creasey et al. 1997)
- ✓ **mt DNA sequence analysis:**
  - 16S rDNA (amphipods: France & Kocher 1996, Escobar-Briones et al. 2010; mollusks: Etter et al. 1999, 2005, Chase et al. 1998, Quattro et al. 2001, Zardus et al. 2006)
  - D-loop, cyt *b* regions (fish: Aboim et al 2005)
  - COI & 16S rDNA (corals: Smith et al. 2004)
- ✓ **combined (e.g. nuclear & mtDNA):**
  - COI + 28S rDNA + calmodulin intron (bivalves: Glazier & Etter 2014)
  - (COI), 16S & 18S rDNA (isopods: Raupach et al. 2007, Brix et al. 2014)
  - COI, 16S, 18S & 28S rDNA, Histone 3 (amphipods: Corrigan et al. 2014)
  - COI and allozyme data (vent mussels: Won et al. 2003, Miyazaki et al. 2004)
  - COI and ribosomal ITS-1 (vent clams: Goffredi et al. 2003)
  - ITS, COI, mitochondrial control region MtC (corals: Miller et al. 2011)
- ✓ **fast-evolving markers:**
  - microsatellites + ITS (corals: Le Goff-Vitry et al. 2004)
  - Amplified fragment length polymorphisms (AFLPs) (vent tubeworms: Shank & Halanych 2007)

DNA barcoding

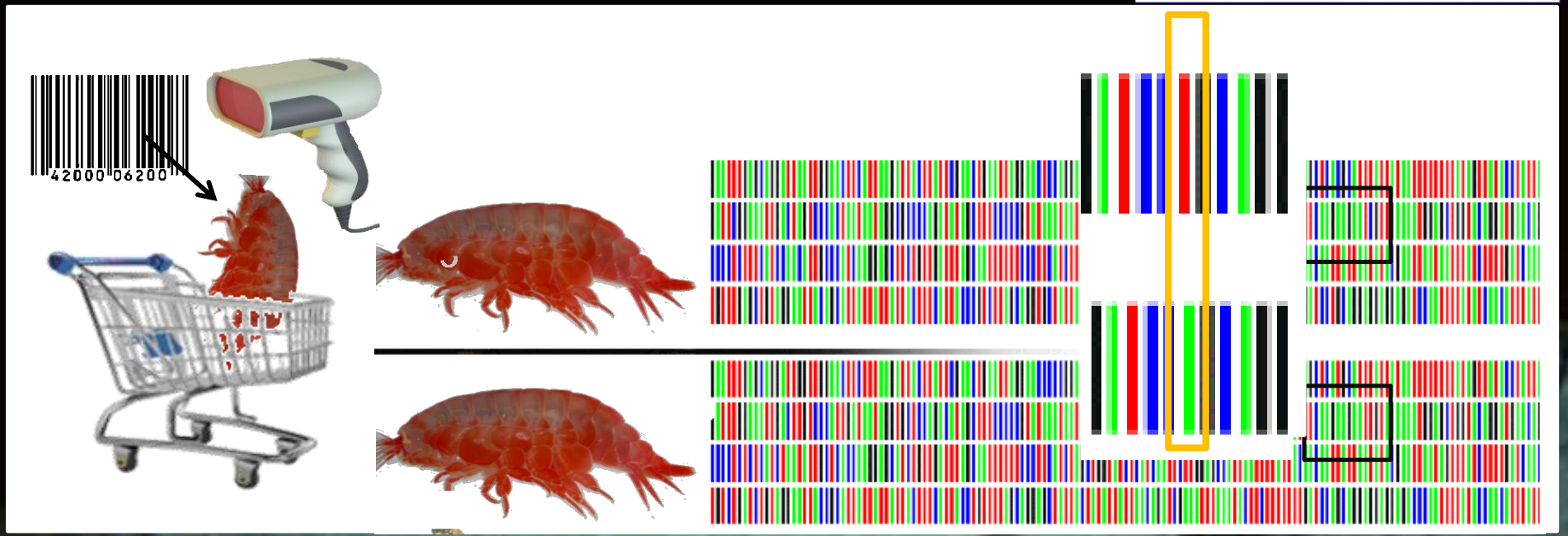
✓ Recent initiative (2003): the use of a standardized short DNA sequence for species identification

→ part of the genome evolving quickly enough for assessing recent speciation events :

cytochrome c oxidase I gene (COI)

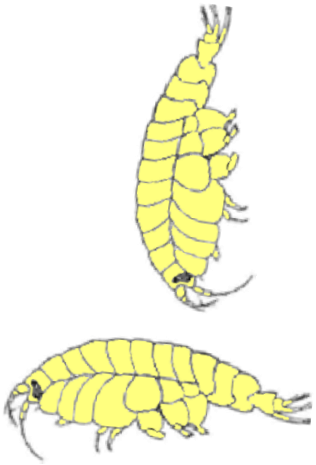


Waugh 2007

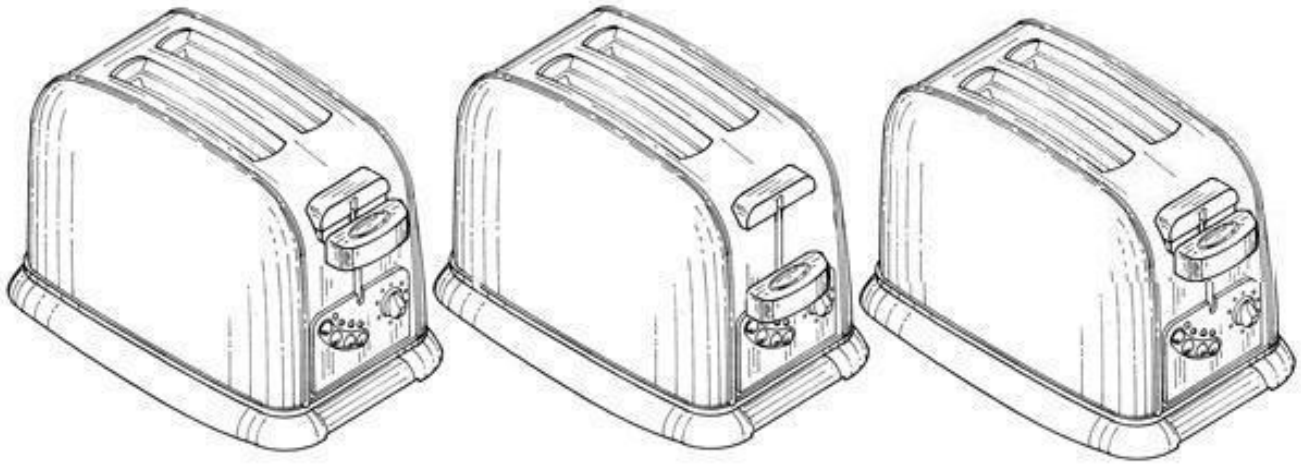


Modified after: http://e360.yale.edu/feature/dna_technology_discovering_new_species/2019/ (Suz Bateson Uni Guelph)

DNA barcoding



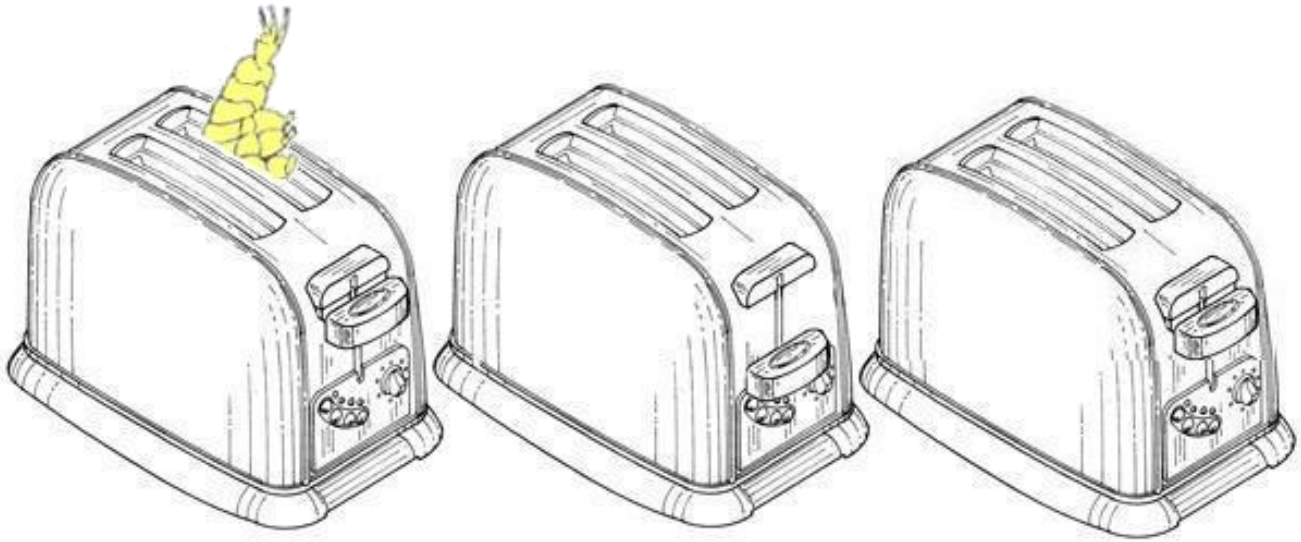
specimen



1

DNA barcoding

specimen

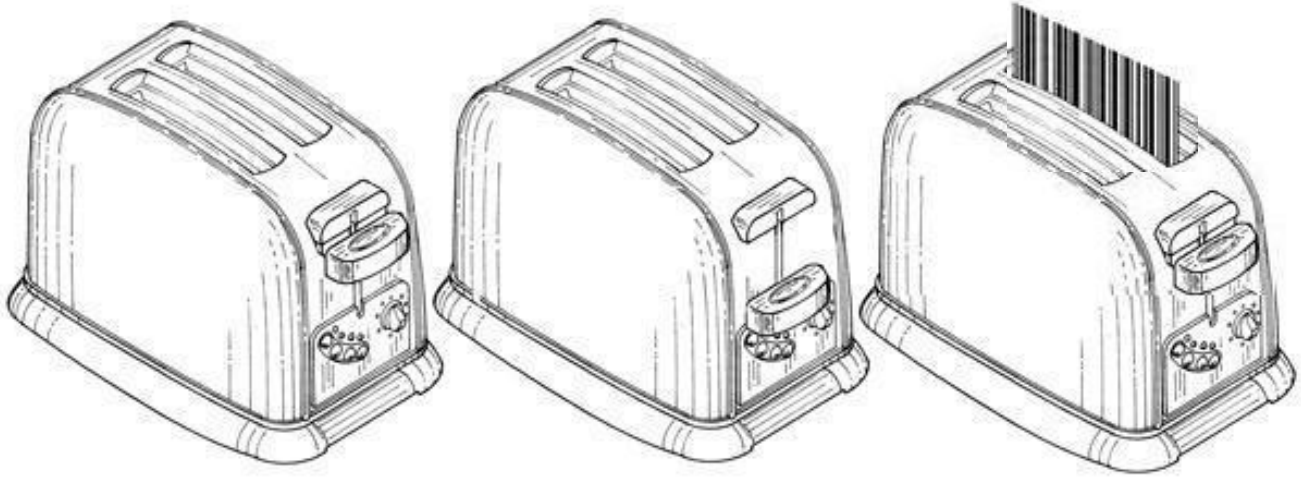


1 2

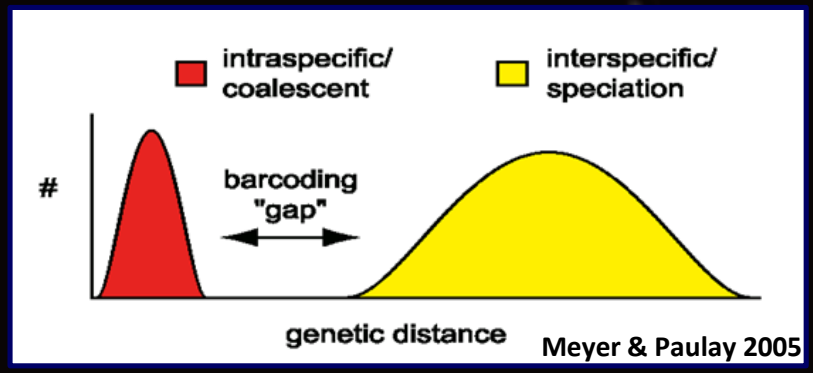
DNA barcoding

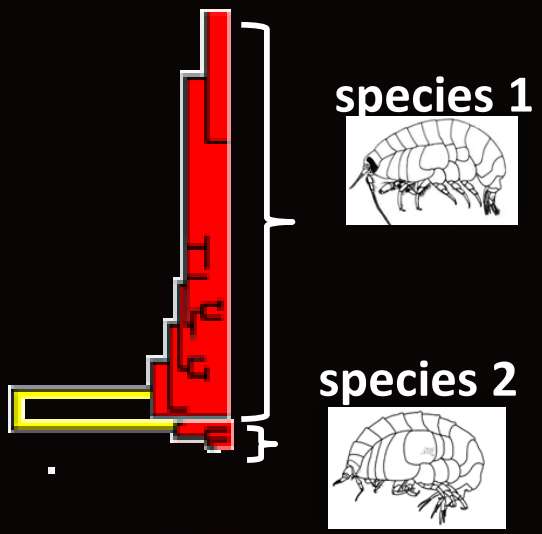
specimen → barcode → match in barcode library → species

specimen



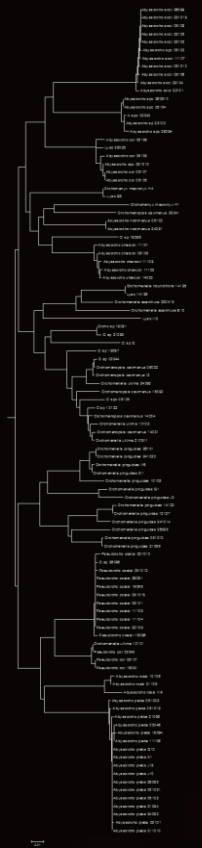
DNA barcoding





intraspecific variation < interspecific divergences

→ differentiating species



Neighbour-Joining tree based on genetic distances between specimens

✓ Some examples of deep-sea studies using COI:

- Octocorals: divergences too low (France & Hoover 2002)
- Vent-endemic gastropods (Kojima et al. 2000), annelids (Black et al. 1997, Hurtado et al. 2004), clams (Peck et al. 1997), mussels (Maas et al. 1999), shrimps (Shank et al. 1999)
- Amphipods (Knox et al. 2012)

Integrative taxonomic studies on lysianassoids

- ✓ thrive in cold waters: shallow waters of high latitudes & deep sea
- ✓ abundant & diverse
- ✓ mostly scavengers; benthic, bentho-pelagic, pelagic species

Case study 1: *Orchomene sensu lato*

DNA barcoding
COI, (28S rDNA)
Reverse taxonomy (*sensu* Kanzaki et al. 2012)
descriptions

Case study 2: *Eurythenes gryllus*

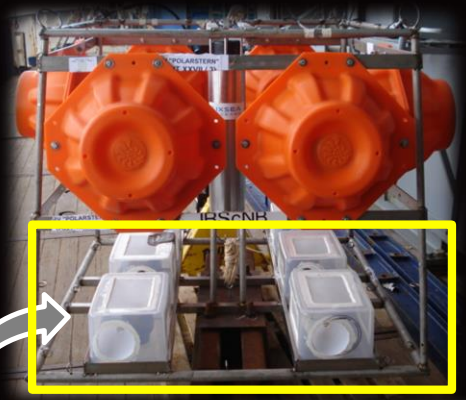
Phylogeography
COI, 16S, 28S rDNA
Reverse taxonomy: descriptions

General methodology

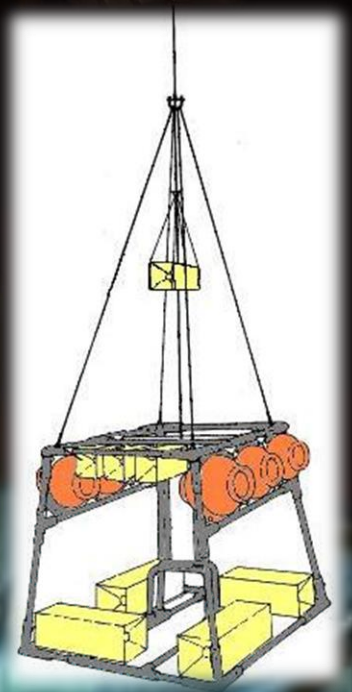
✓ sampling of lysianassoids

Agassiz Trawl

Epibenthic sledge



bait

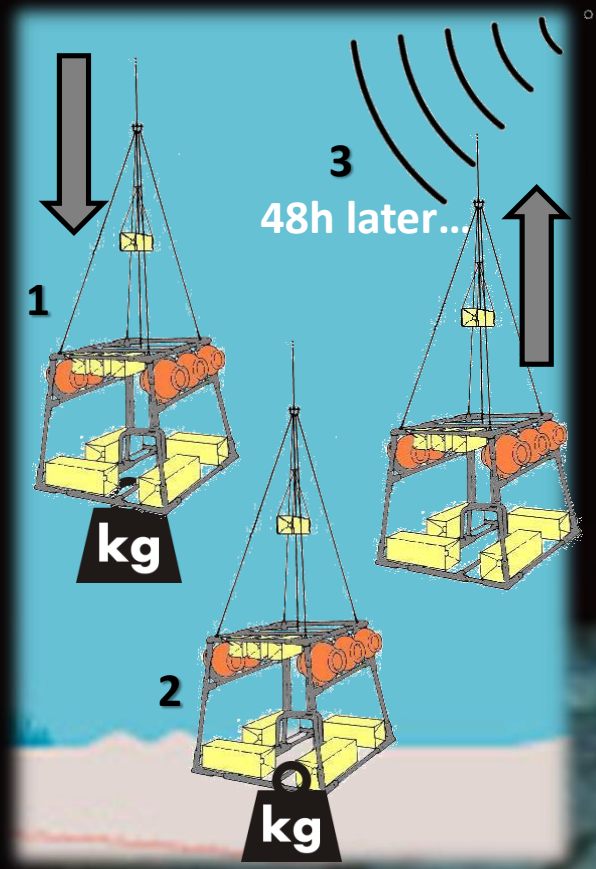


Rauschert dredge

Lander with amphipod traps

General methodology

✓ sampling of lysianassoids



Baited trap samples:

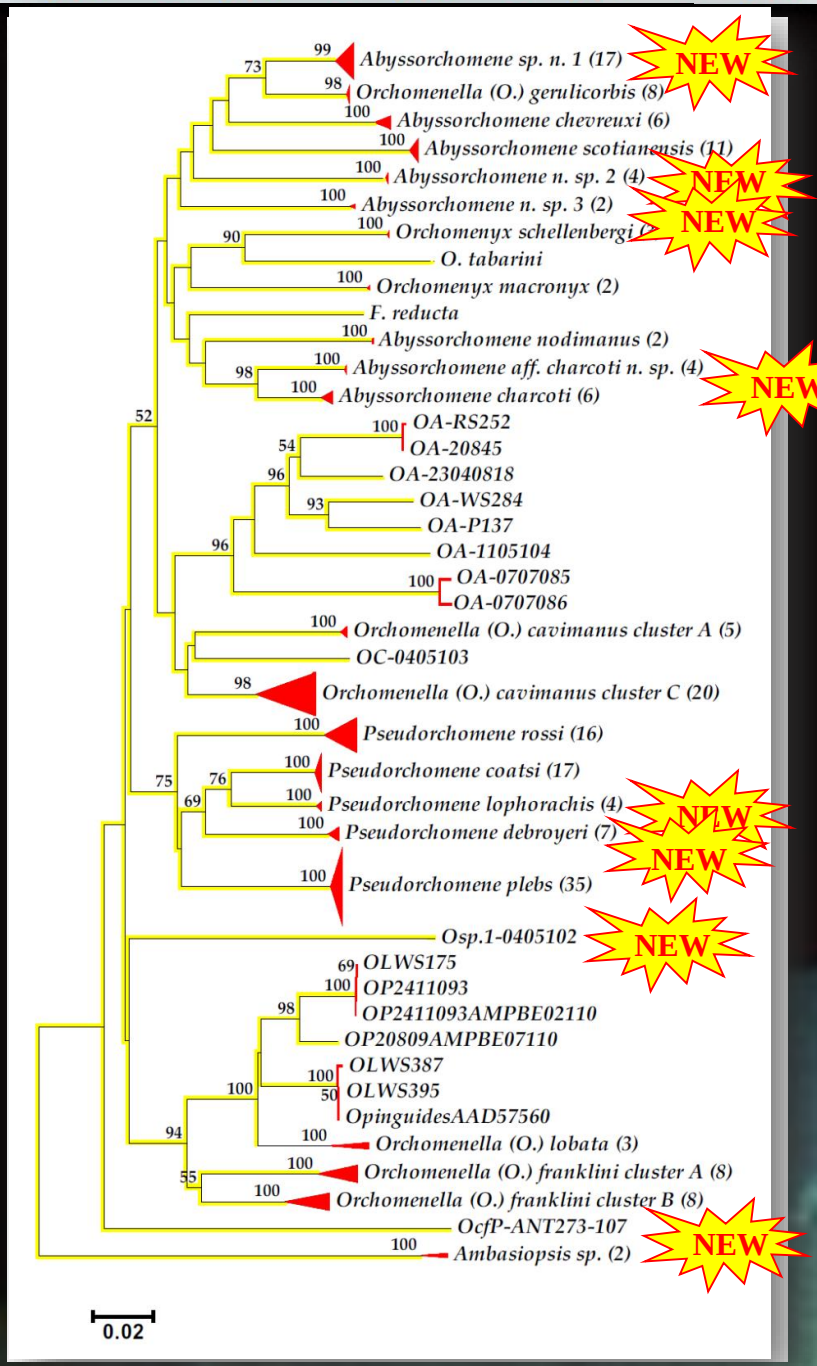
High number of specimens/species

→ Phylogeographic & population genetic studies

General methodology

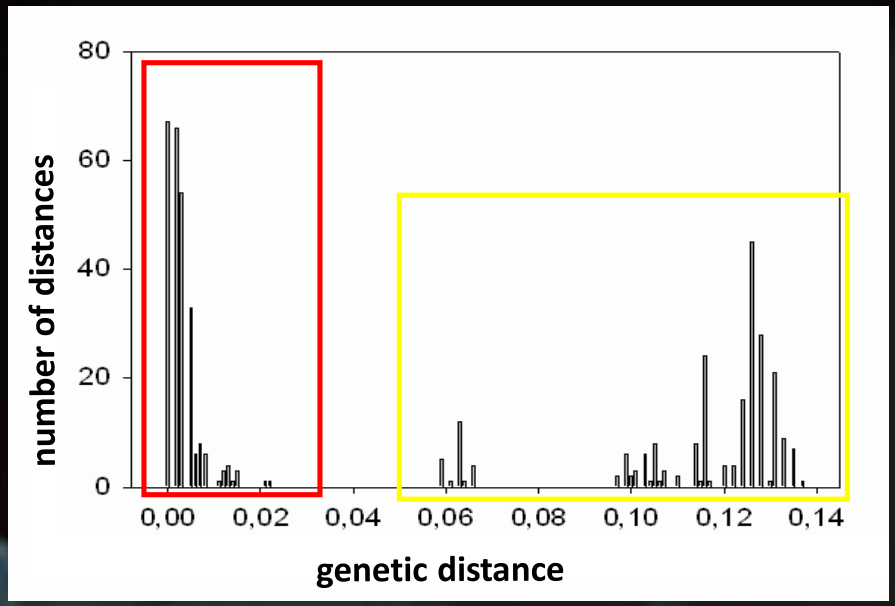
- ✓ preservation of samples for genetics: 96% ethanol or absolute, pre-cooled
- ✓ storage at -20°C
- ✓ DNA extractions using standard kits (Qiagen, Macherey-Nagel)
- ✓ PCR:
  - COI using universal primers (Folmer et al. 1994)
  - 16S rDNA using universal primers (Palumbi et al. 1991)
  - 28S rDNA using amphipod specific primers 28F, 28R (Hou et al. 2007)
- ✓ Sanger sequencing: ABI 3130xl capillary DNA sequencer

Havermans et al. 2010 MPE, 2011 DSRII, 2013 PLoS ONE



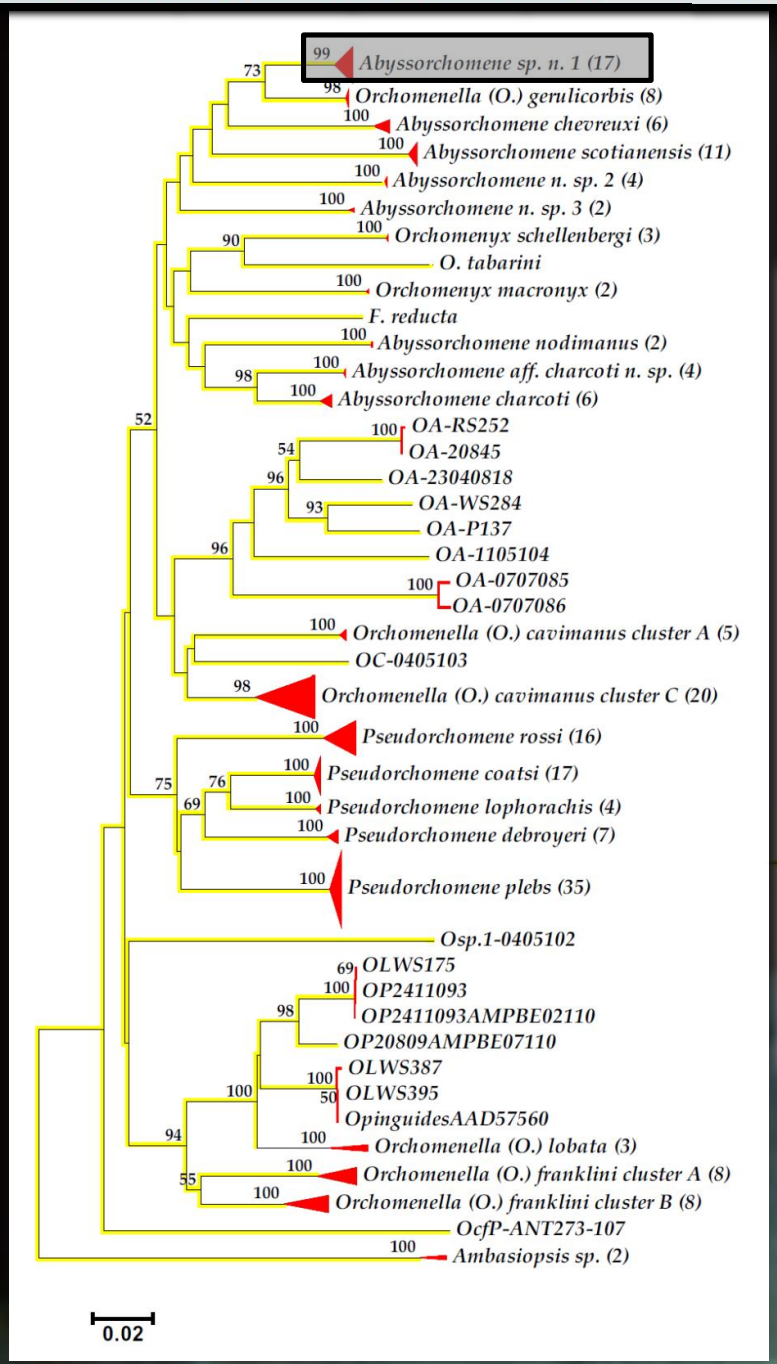
DNA barcoding *Orchomene sensu lato*

- ✓ +/- 250 specimens
- ✓ 25 different species



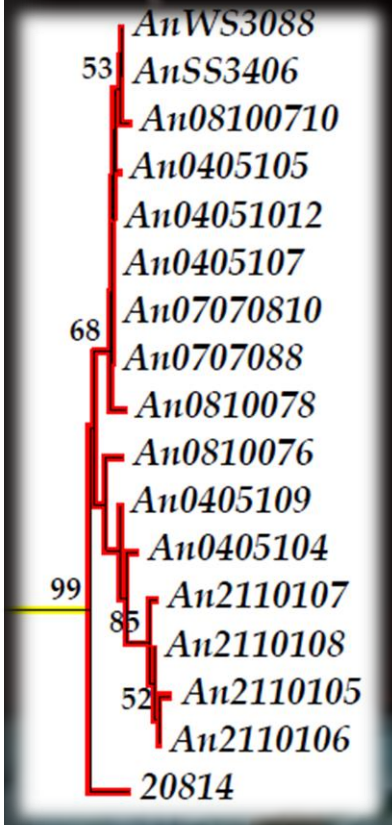
- ✓ 8 new species, 2 described
- ✓ topologies confirmed by 28S rDNA

Havermans et al. 2010 MPE, Havermans et al. 2011 DSRII



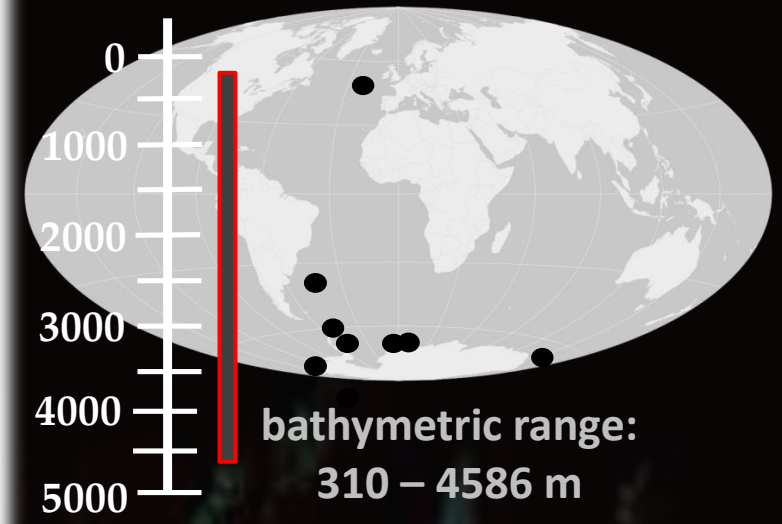
Genetically homogeneous species

Abyssorchomene abyssorum (Stebbing, 1888)



genetic divergence:

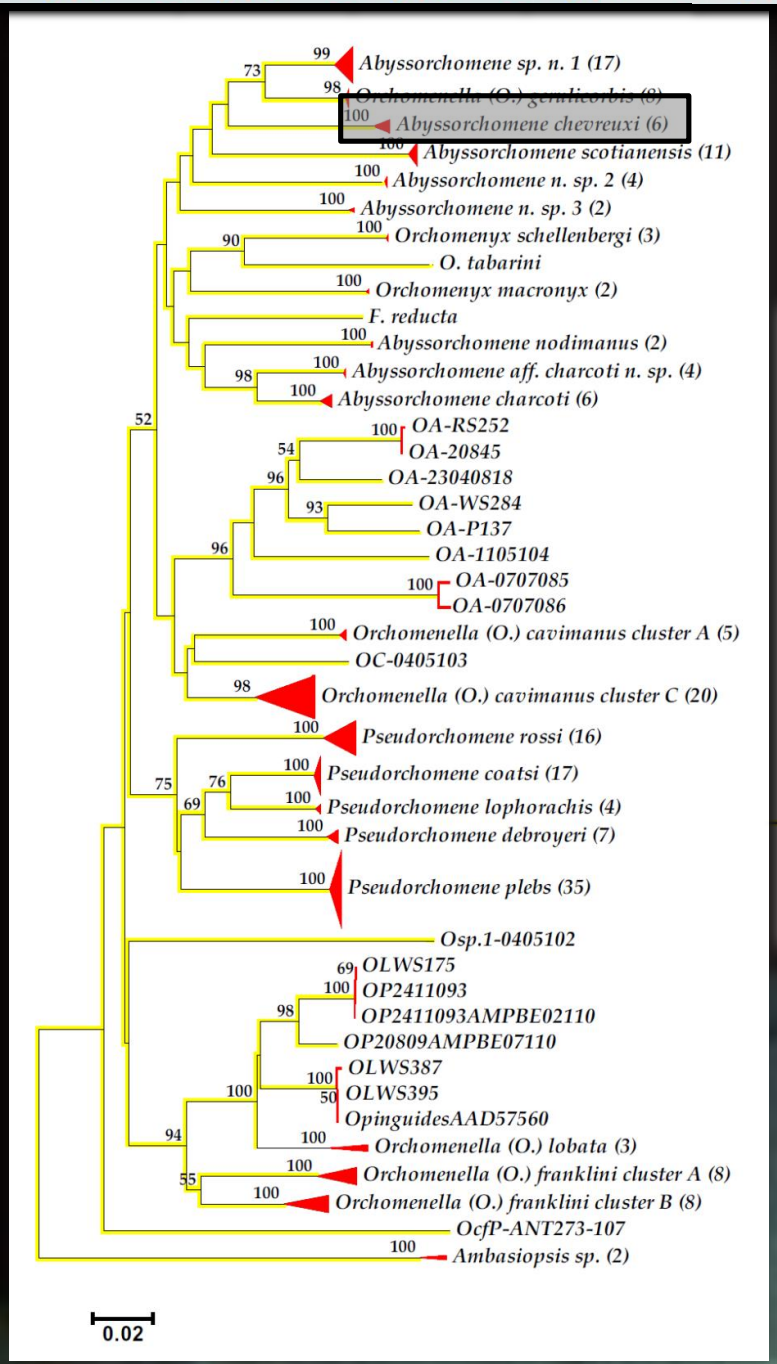
μ = 0.7 % (0.0 – 1.5 %)



bathymetric range:
310 – 4586 m

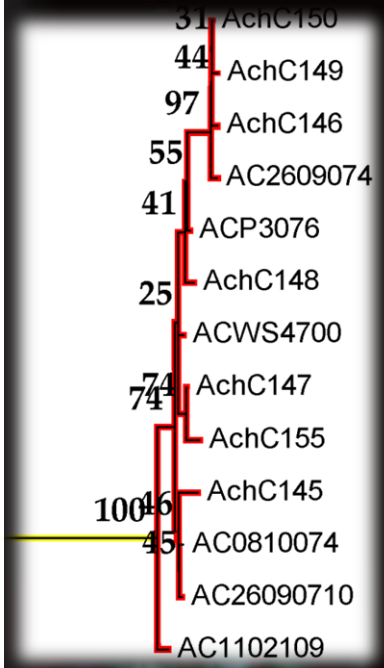
widespread & eurybathic

Havermans et al. 2011 DSRII, Havermans 2014 Biogeogr Atlas of the SO



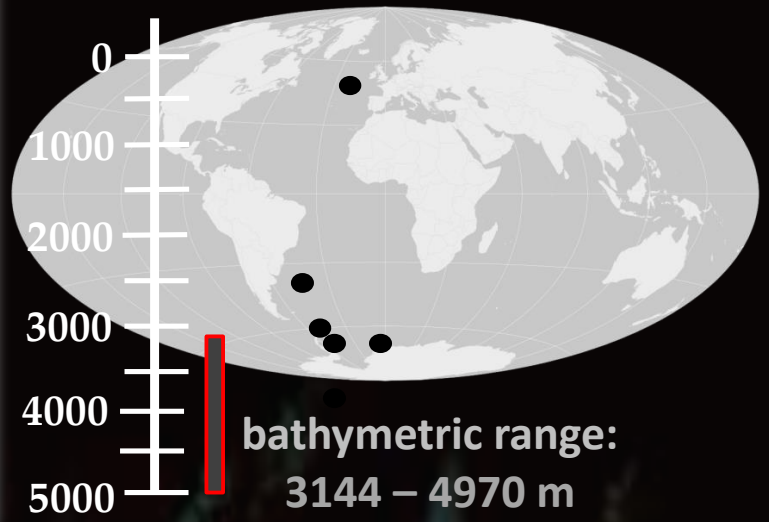
Genetically homogeneous species (2)

Abyssorchomene chevreuxi (Stebbing, 1906)



genetic divergence:

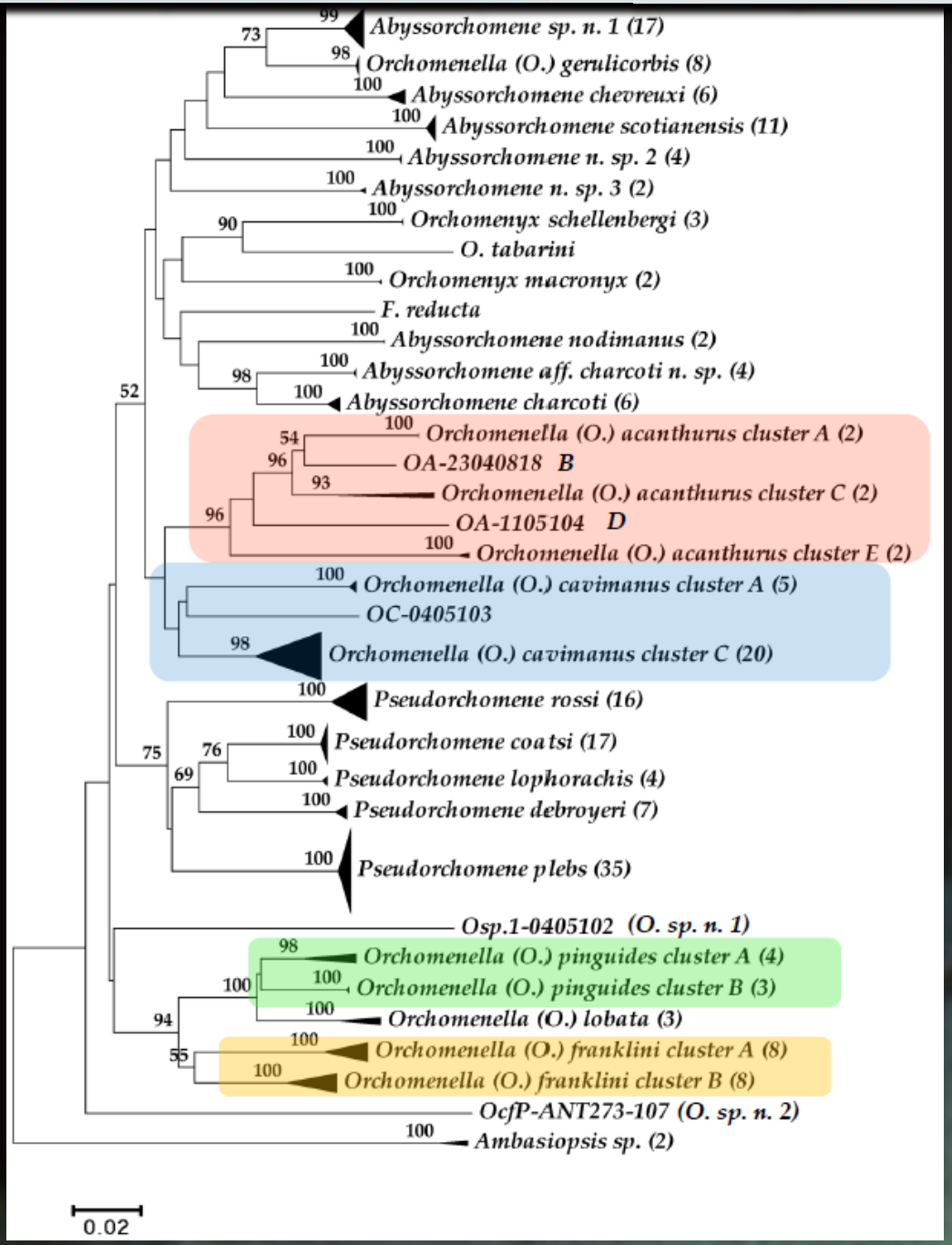
μ = 0.5 % (0.2 – 1.2 %)



bathymetric range:
3144 – 4970 m

widespread

Havermans et al. 2011 DSRII, Havermans 2014 Biogeogr Atlas of the SO



Species complexes

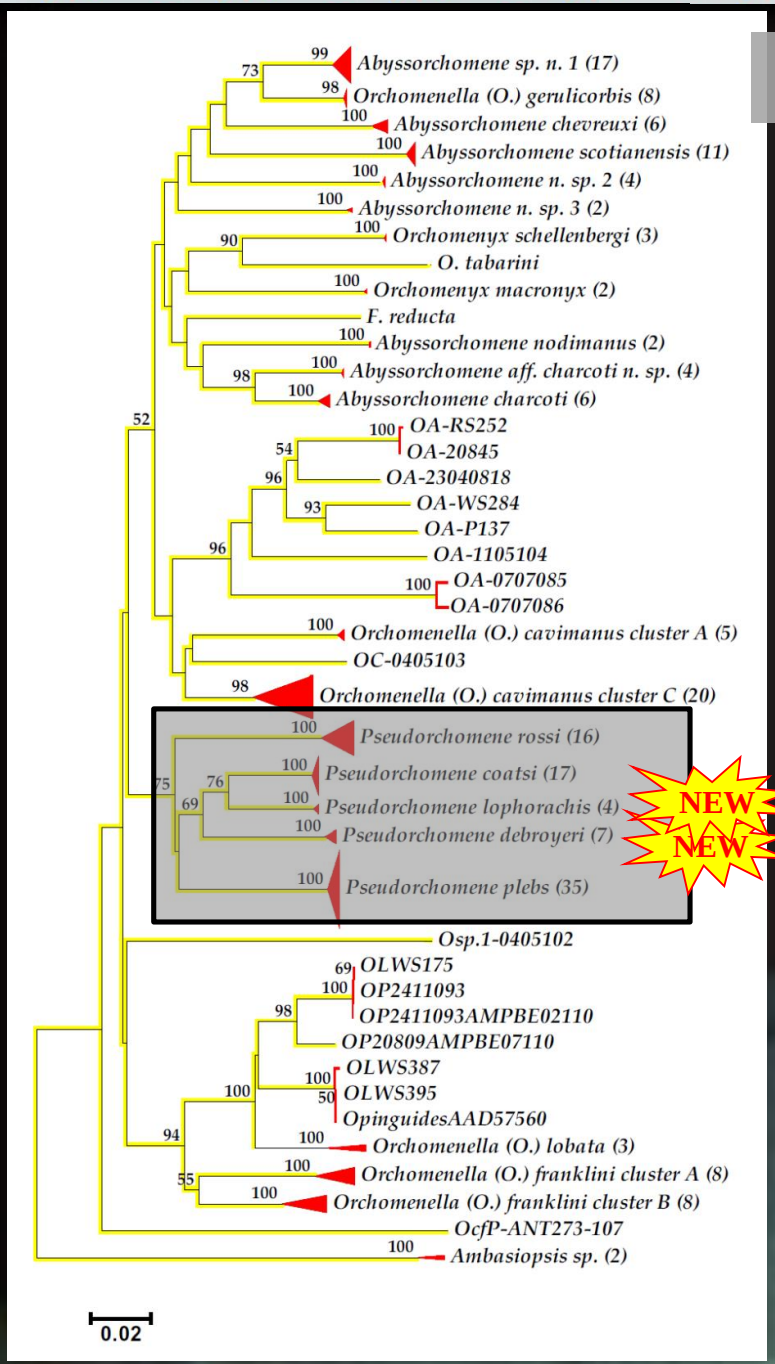
genetic divergences in the range of interspecific distances (μ = **10 - 15 %**)

species complexes:

Orchomenella (Orchomenopsis) acanthurus
Orchomenella (Orchomenopsis) cavimanus
Orchomenella (O.) pinguides
Orchomenella (O.) franklini

restricted & widespread clades

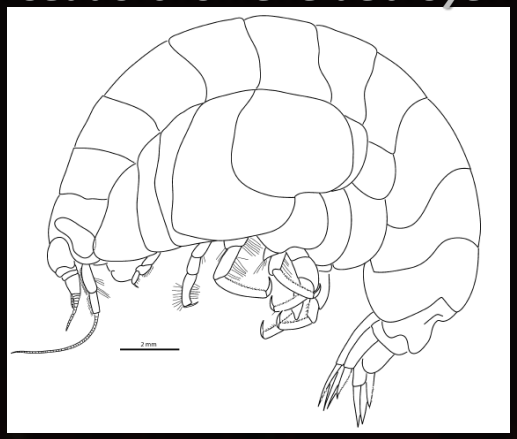
Havermans et al. 2011 DSRII, Havermans 2014 BASO

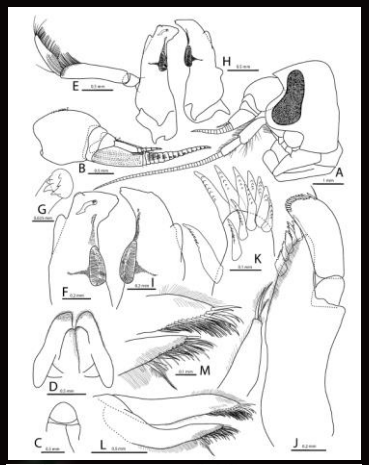


Reverse taxonomy approach for orchomenids

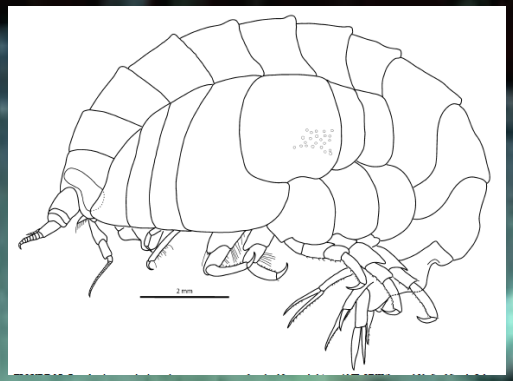
- ✓ **Revision of *Pseudorchomene* systematics:**
  Abyssorchomene → *Pseudorchomene*
- ✓ **Description of two new species:**

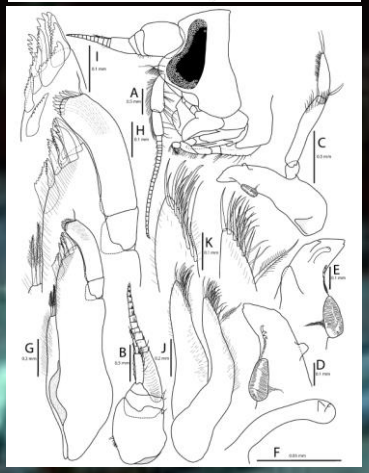
Pseudorchomene debroyeri



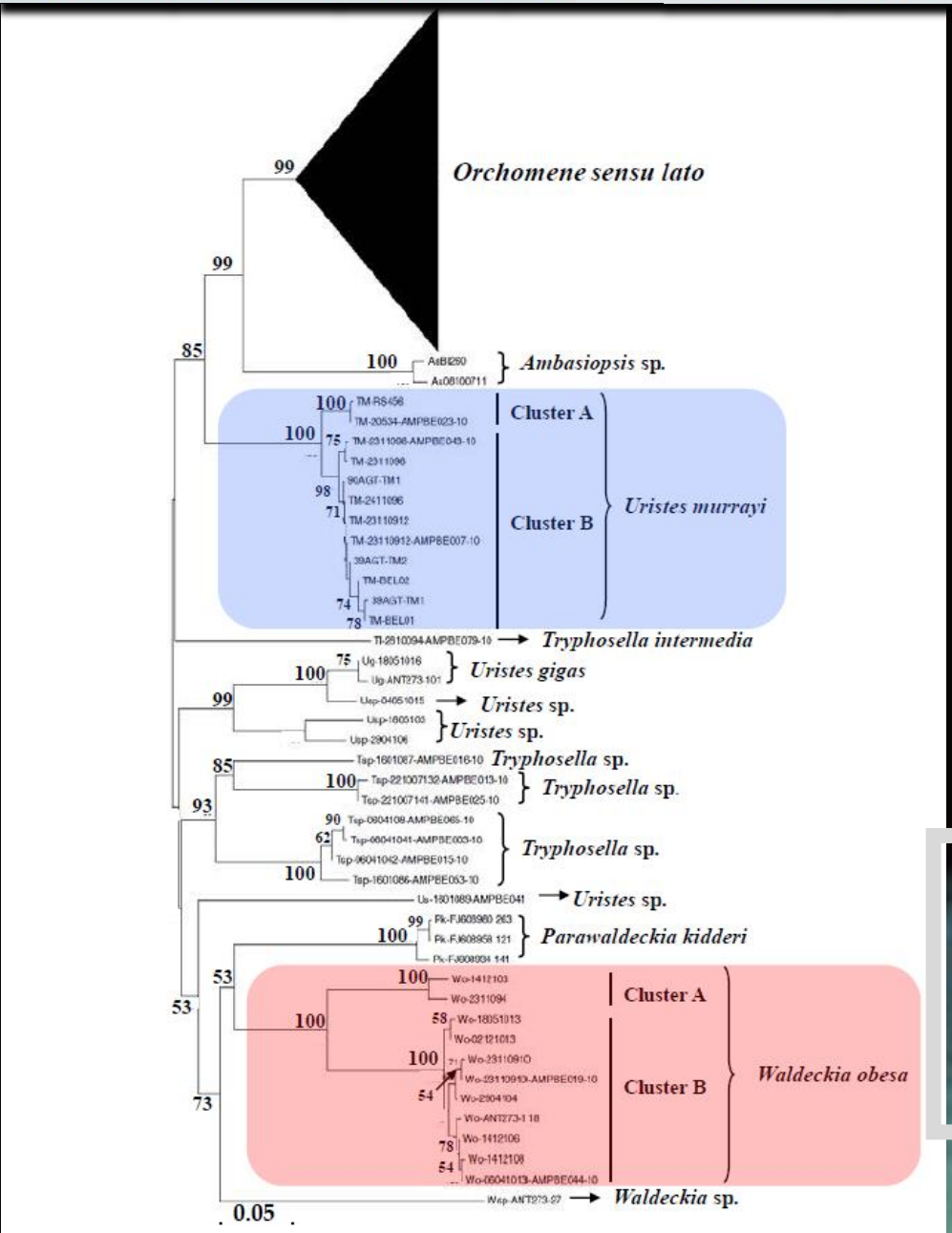


Pseudorchomene lophorachis





d'Udekem d'Acoz & Havermans 2012 Zootaxa



Neighbour-Joining Tree of COI sequences of lysianassoid species (K2P model, bootstrap nreps 2000). Coloured rectangles indicate species complexes.

Extending the DNA barcoding approach to other lysianassoids

Presence of barcoding gap:
Bimodal distribution of intra- (0 – 6.5 %) & interspecific (15.2 – 35.7 %) divergences

species complexes:

Uristes murrayi

Waldeckia obesa

restricted & widespread clades

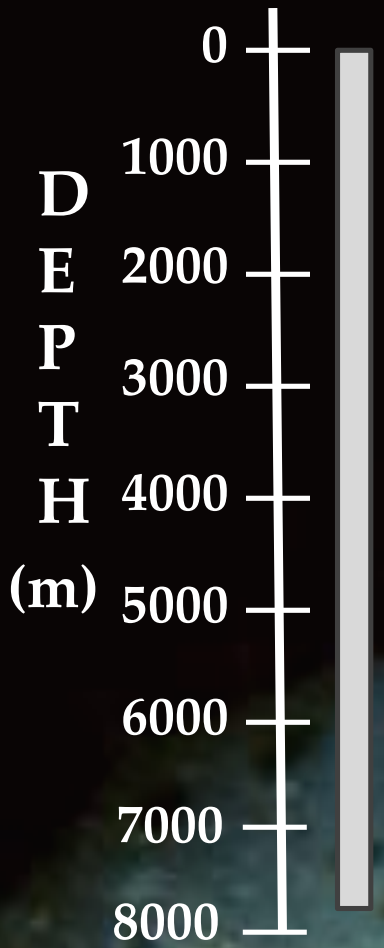
COI also efficient in delimiting species in other amphipod genera:

- Antarctic *Epimeria* (Lörz et al. 2009)
- Deep-sea *Rachothropis* (Lörz et al. 2012)
- Deep-sea Phoxocephalidae (Knox et al. 2012)

Havermans 2012 PhD, Havermans 2014 BASO

Phylogeography of *Eurythenes gryllus*

- ✓ giant, benthopelagic scavenger
- ✓ most widespread amphipod species:

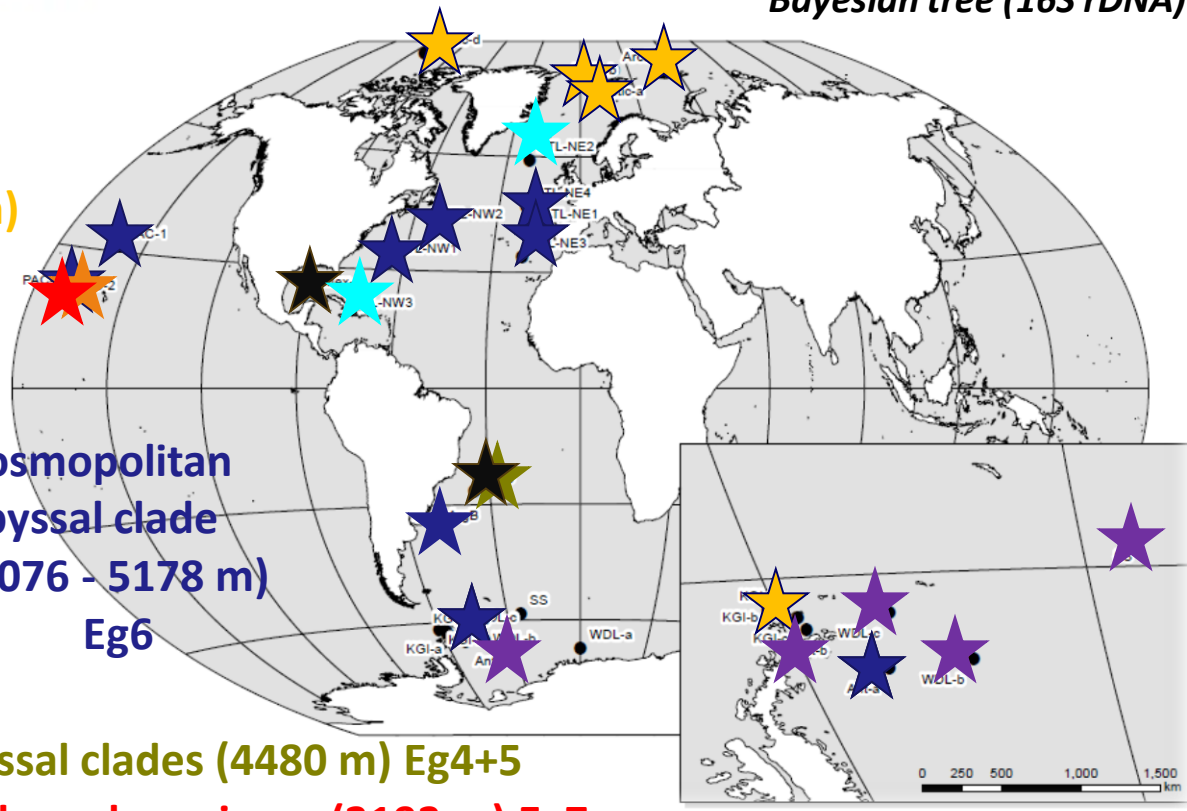


Eurythenes thurstoni

Abyssa sp.

Bayesian tree (16S rDNA)

Bipolar bathyal clade (839 - 2743 m) Eg1



Cosmopolitan abyssal clade (3076 - 5178 m) Eg6

Brazil abyssal clades (4480 m) Eg4+5

Pacific abyssal specimen (3193 m) Eg7

Atlantic abyssal clade (3732 - 4480 m) Eg6

Atlantic bathyal clade (1309 - 2900 m) Eg

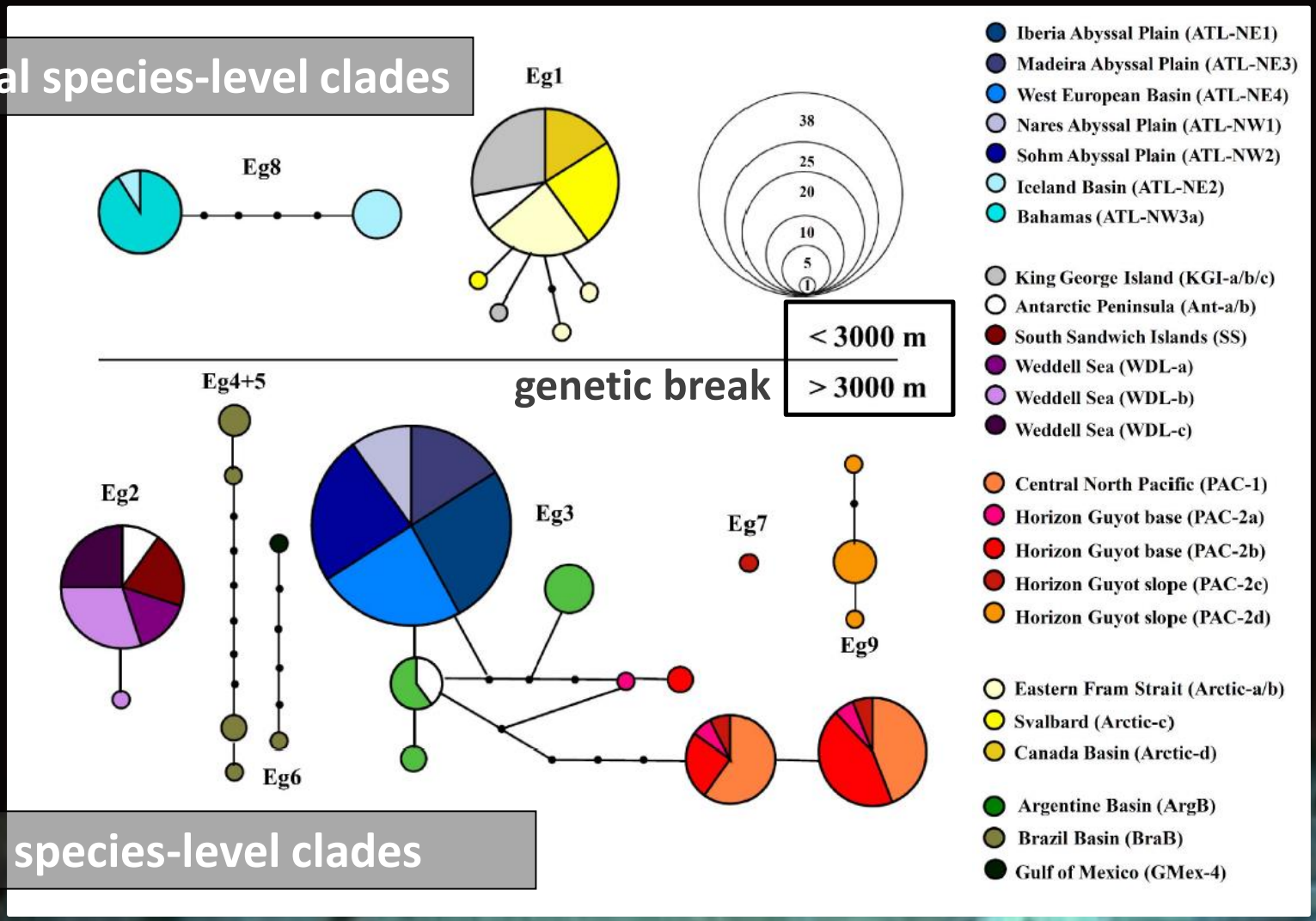
Antarctic abyssal clade (3076 - 4700 m)

Pacific abyssal clade (3193 m)

Havermans et al. 2013 PLoS ONE

16S rRNA gene haplotype

2 bathyal species-level clades



6 abysal species-level clades

Havermans et al. 2013 PLoS ONE

- Species richness compared: <> depth differentiation hypothesis (Etter et al. 2005)

Bathyal

2 species-level clades,

one bipolar and one widespread

⇔

Abyssal

6 species-level clades,

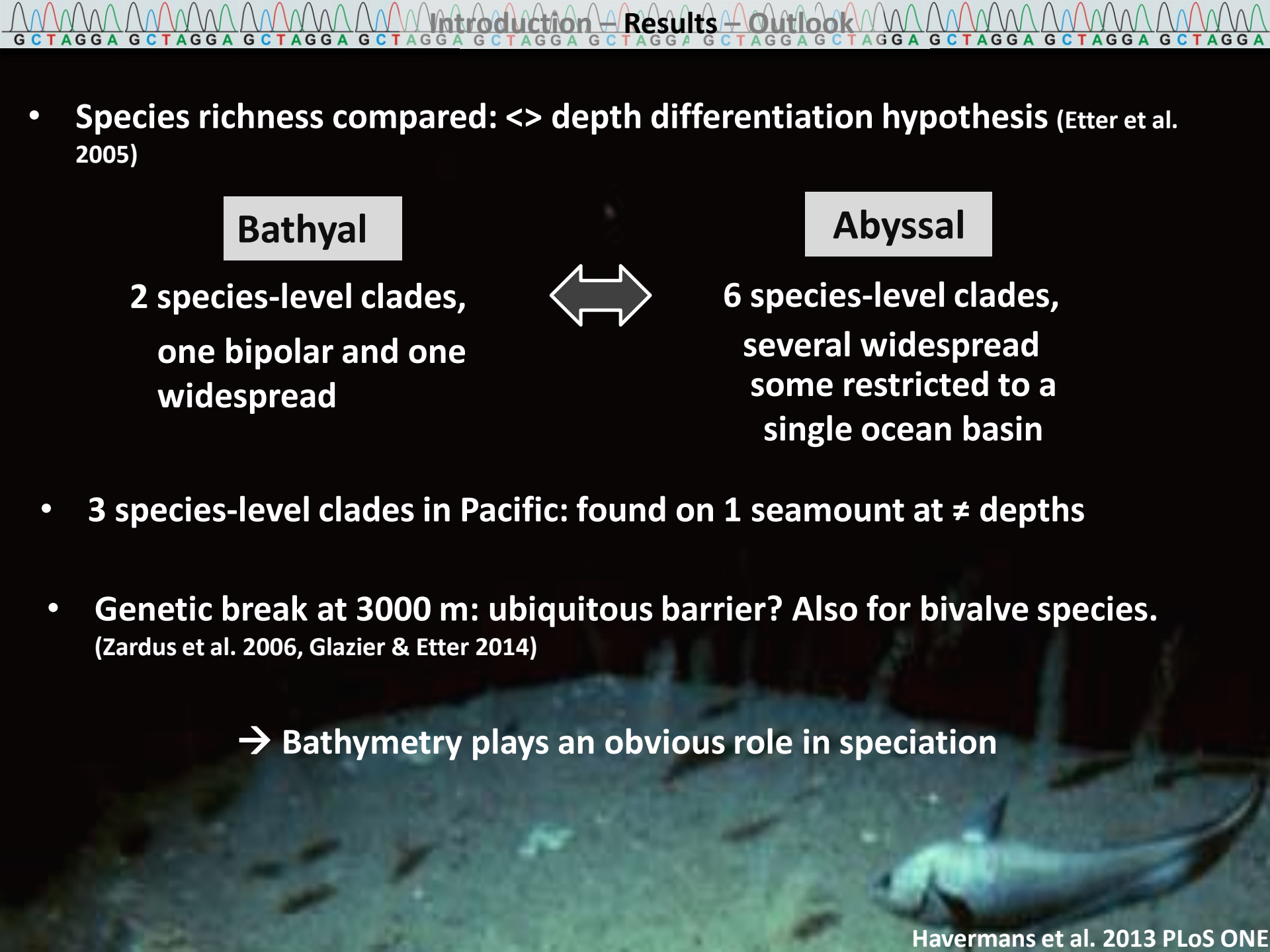
several widespread

some restricted to a single ocean basin

- 3 species-level clades in Pacific: found on 1 seamount at ≠ depths

- Genetic break at 3000 m: ubiquitous barrier? Also for bivalve species. (Zardus et al. 2006, Glazier & Etter 2014)

→ Bathymetry plays an obvious role in speciation



Havermans et al. 2013 PLoS ONE

Table 2. Range and mean of pairwise K2P intraclade and interclade distances for COI, 28S rDNA and 16S rDNA for each clade identified within *Eurythenes gryllus* (sequence data from this study, France and Kocher [12], Escobar-Briones *et al.* [36]).

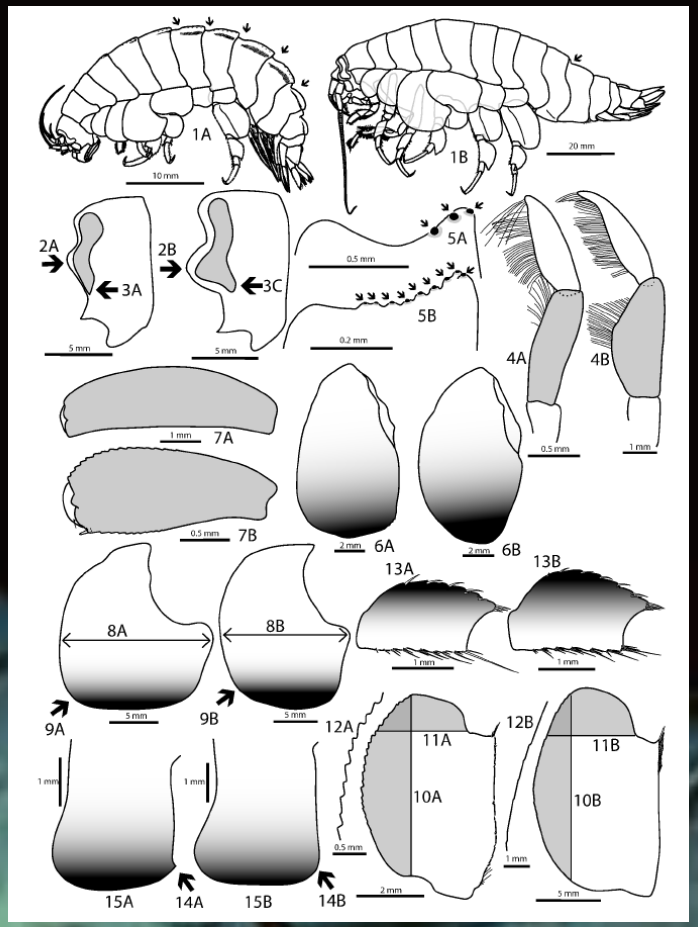
	Intraclade (K2P) divergences		Interclade (K2P) divergences	
	Min. – Max.	Mean	Min. – Max.	Mean
COI				
Antarctic - Arctic bathyal clade (Eg1)	0.0–0.02	0.007	0.085–0.132	0.101
Antarctic abyssal clade (Eg2)	0.0–0.004	0.001	0.085–0.129	0.097
Antarctic - Atlantic abyssal clade (Eg3)	0.0–0.013	0.006	0.090–0.119	0.104
Brazil abyssal clade 1 (Eg4)	0.0–0.0	0.0	0.045–0.115	0.102
Brazil abyssal clade 2 (Eg5)	0.0–0.003	0.002	0.045–0.132	0.110
28S rDNA				
Antarctic - Arctic bathyal clade (Eg1)	0.0–0.001	0.0	0.003–0.007	0.005
Antarctic abyssal clade (Eg2)	0.0–0.0	0.0	0.002–0.006	0.004
Antarctic - Atlantic abyssal clade (Eg3)	0.0–0.001	0.001	0.004–0.007	0.006
Brazil abyssal clade 1 (Eg4)	0.0–0.0	0.0	0.001–0.004	0.003
Brazil abyssal clade 2 (Eg5)	0.0–0.0	0.0	0.001–0.004	0.003
16S rDNA				
Antarctic - Arctic bathyal clade (Eg1)	0.0–0.006	0.001	0.022–0.097	0.035
Antarctic abyssal clade (Eg2)	0.0–0.002	0.0	0.022–0.092	0.038
Antarctic - Atlantic - Pacific abyssal clade (Eg3)	0.0–0.016	0.008	0.024–0.102	0.040
Brazil abyssal clade 1 (Eg4)	0.0–0.002	0.001	0.014–0.090	0.038
Brazil abyssal clade 2 (Eg5)	0.0–0.002	0.001	0.014–0.089	0.048
Atlantic abyssal clade (Eg6)	0.002	/	0.036–0.107	0.044
Pacific abyssal singleton (Eg7)	/	/	0.022–0.092	0.042
Atlantic bathyal clade (Eg8)	0.0–0.009	0.004	0.029–0.092	0.041
Pacific abyssal clade (Eg9)	0.0–0.007	0.002	0.078–0.107	0.090

barcoding gap

Havermans et al. 2013 PLoS ONE

Reverse taxonomy approach for *E. gryllus*

- ✓ « True » *E.gryllus* = bipolar clade Eg1
- ✓ Unique combination of morphological characters for specimens from clades Eg1, 2, 3, 4+5, 6
- ✓ Species Eg1, 2, 3, 4+5 and 6 currently being (re)described



Havermans et al. 2013 PLoS ONE, d'Udekem d'Acoz & Havermans *submitted*

"Blind" taxonomic studies on amphipods

- ✓ MOTUs: Molecular Operational Taxonomic Units
- ✓ Comparative diversity studies on abundant but understudied taxa
- ✓ Thresholds for species assignment (see other amphipod barcoding studies e.g. Witt et al. 2006, Costa et al. 2009, Radulovici et al. 2009, Bradford et al. 2010, Havermans et al. 2011)
- ✓ Knox et al. (2012) – abundance & diversity of deep-sea phoxocephalids

Station	Supranet abundance (ind./1000m^2)	Combined nets raw abundance	Number of COI sequences	Number of MOTUs		
				Total	Singletons	%
CR024	389	207	32	8	1	13
CR041	103	216	27	8	0	0
CR049	190	126	10	4	1	25
CR065	130	88	12	6	2	33
CR083	170	375	29	10	1	10
CR099	310	189	11	5	0	0
CR127	22	24	3	3	1	33
CR136	72	82	12	4	0	0
CR141	19	26	6	3	1	33
CR160	90	99	11	2	0	0
CR178	160	230	18	10	2	20
CR251	780	966	17	8	0	0
CR255	470	544	23	8	1	13
CR276	238	245	12	5	0	0
CR285	100	168	14	8	0	0
CP029	164	231	22	6	3	50
CP051	1	11	5	5	5	100
CP105	28	73	14	6	5	83
CP119	153	138	9	4	2	50
CP139	59	61	10	5	3	60

Singleton molecular operational taxonomic units (MOTUs) are those which occur at a single station only.
COI, cytochrome oxidase *c* subunit I.

Knox et al. 2012 PLoS ONE

Conclusions

✓ DNA barcoding efficient:

- Bimodal distribution of divergences & presence barcoding gap
- Morphospecies retrieved in monophyletic clusters

→ Testing hypotheses on faunal distributions:

endemism vs. eurybathy, cosmopolitism

→ Large-scale comparative biodiversity studies

✓ Lysianassoid species richness clearly underestimated !!

Beyond species identifications…

✓ **genetic connectivity, species' distributions:**

- F-statistics, AMOVA, network analyses, mismatch distribution analysis
  → population expansions, bottlenecks, founder events
  → vulnerability of populations: possibility of recolonization?

✓ **phylogenetic analyses: evolution of deep-sea taxa:**

- **Lysianassoids:** diversification scavengers set at Eocene/Oligocene cooling (Corrigan et al. 2013)

✓ **testing hypotheses in deep-sea biogeography & ecology:**

- Depth-differentiation analysis (Etter et al. 2005): bathyal vs. abyssal
- Source-sink hypothesis (Rex et al. 2005)
- Genetic break around 3000 m ? (bivalves Zardus et al. 2006, Glazier & Etter 2014)

~~« *Abyssal endemism appears to be low* »~~
Etter *et al.* (2005), Rex *et al.* (2005)

~~« *Abyssal populations exhibit only modest genetic structure* »~~
Etter *et al.* (2011)

Thank you!

Amphipod photos from Cédric d'Udekem d'Acoz, Bruno Danis, Martin Rauschert.