

Developing molecular pipelines to provide samples for barcoding and other analyses

Helena Wiklund, Natural History Museum, London, UK

Sampling the deep

- Deep-sea sampling is expensive
 - Most sampling gears cover small area
 - Animal distribution often patchy
 - Animals in bad shape when reaching surface
- > We need to do as much as possible with the very few animals we actually can get!

Some questions

- What animals are down there?
- What / how do they live, eat and reproduce?
Mobile or sessile?
- Distribution ranges – dispersal or endemics?

Both morphology and molecular data are needed for answering these questions, and we need to try to answer them if we're going to assess impacts on the fauna

At sea

- Minimize time on deck for samples
- Sieve carefully, in chilled seawater!
- Preferably live-sorting under stereo microscope
- Photographing every specimen to document e.g. colours and appendages that can fall off during preservation
- Fix in 80-95% ethanol and store chilled/frozen

Photography equipment



Canon camera mounted
on stereo microscope



Canon with macro lens
MP-E 65 mm, 5x



P/14 #	Canon micro	Lumix	Canon HW	Family (or higher)	Species	indiv	Site	Ship sample #	Lat
130	989			Spionidae?		1	PS-2 Cawsand Bay		
131	991			Spionidae		3	PS-2 Cawsand Bay		
132		850		Chaetopterida	Chaetopterus sp.	1	PS-4 Eddystone dredge		
133			1274	Bryozoa			PS-4 Eddystone dredge		

Back on land and in lab

- Photograph and measure specimens
- Photograph details in light microscope
- Tissue sample for DNA extraction



Sequencing

- Barcoding gene, COI
- If possible, also other genes that are informative for the group in question (e.g. 18S, 28S, 16S, cytb, H3)
- Important to keep track of vouchers for each sequence



Plymouth2014_DNA011_COI

Analyses

- Sequences - used for identification (if reference sequences are available)
- Sequences - used for distribution and connectivity analyses
- Sequences - used for phylogenetic analyses and for publishing species descriptions

Analyses

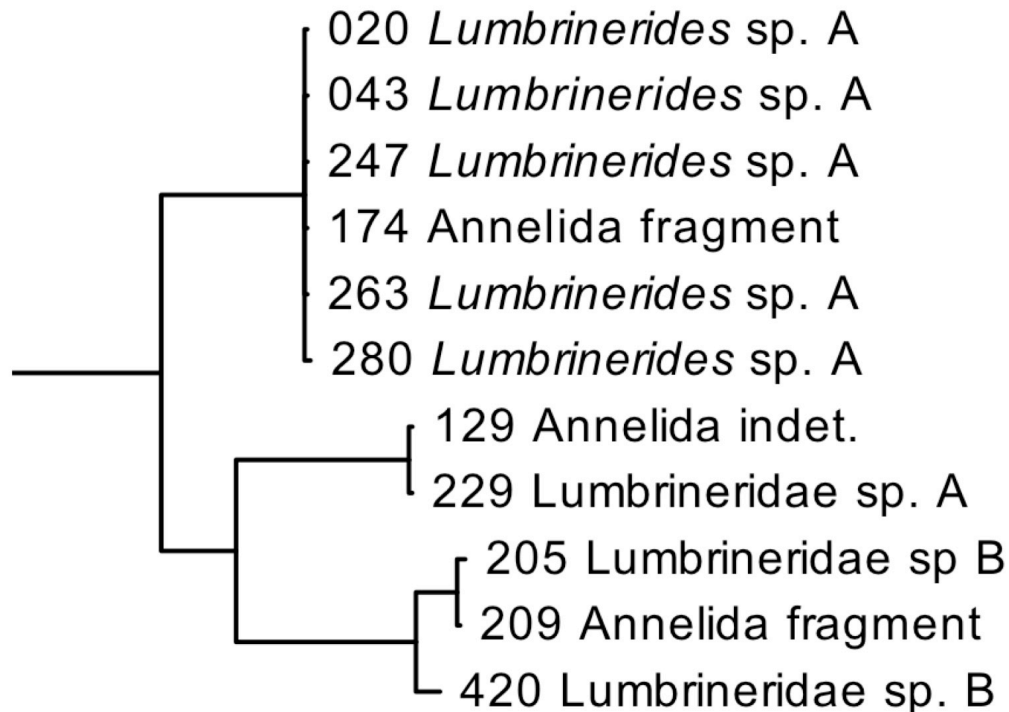
- Sequences used for identification
e.g. using BLAST on GenBank / Barcode of Life

	Description	score	score	cover	value	ident	Accession
<input type="checkbox"/>	Scoloplos normalis voucher Sno_001 18S ribosomal RNA gene, partial sequence	2745	2745	100%	0.0	96%	FJ612494.1
<input type="checkbox"/>	Orbinia swani 18S small subunit ribosomal RNA gene, partial sequence	2734	2734	100%	0.0	96%	DQ790087.1
<input type="checkbox"/>	Orbinia cf. swani 18S ribosomal RNA gene, partial sequence	2734	2734	100%	0.0	96%	AY532363.1

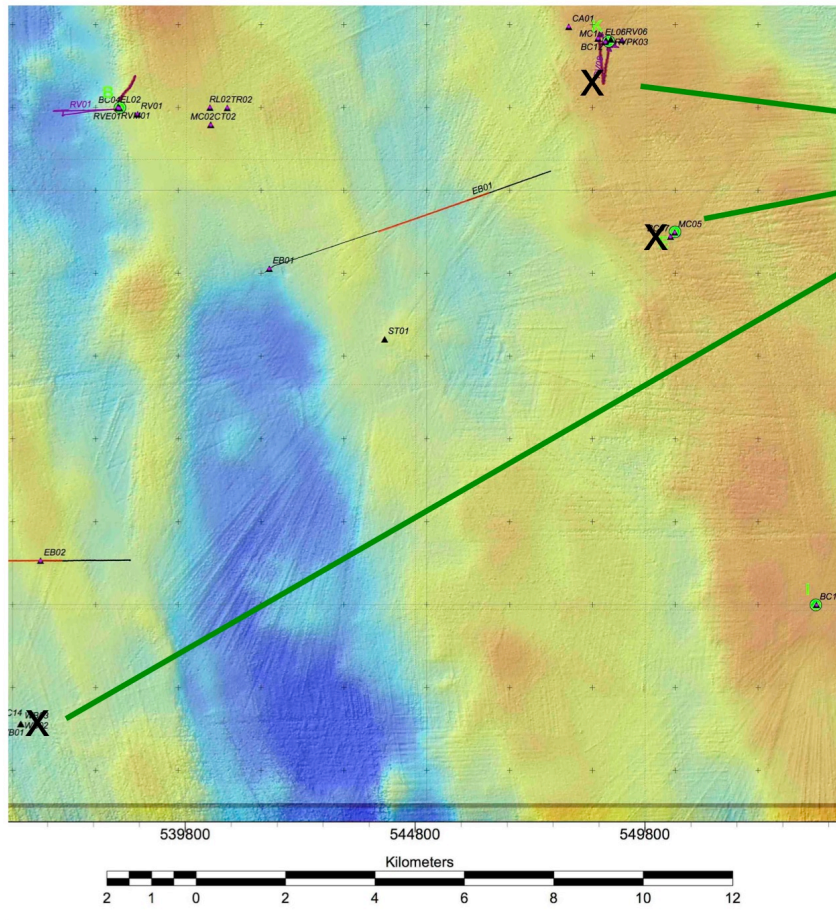
BLAST – Basic Local Alignment Search Tool

Analyses

- Sequences used for identification – matching fragments with complete specimens



Analyses

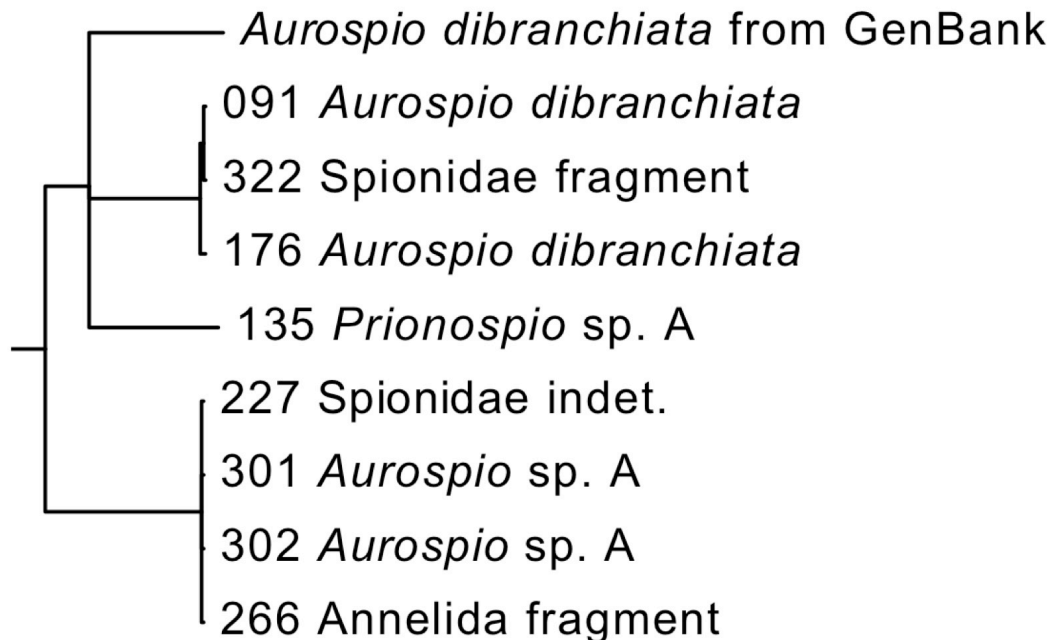


Lumbrinerides sp. A

Sequences used for
distribution and
connectivity analyses




Analyses

- Sequences for phylogenetic analyses and species descriptions



Resulting information

Dorvilleidae Worksheet

Sample Data		Specimen Data		Taxonomy Data	
Locale: Santa Cruz Basin, CA		Specimen: CrsRef_148	juvenile?	CRS Dorvilleidae sp. 6	
Habitat: WF					
Dive TD 504	Coll. Date 30Oct. '02	Photos: IA @ UH-M 'crsRef_148'	Slide	Species	
Sample Notes: WF Bone Slurp		Orig. specimen names: CRS Dorvilleid sp. 6 – Juvenile??	Fixed in Formaldehyde 4%5W Stored in Etoh 80%	ID person /Date	I.Altamira mar'09
<p>Specimen is Entire Number of Chaetigers = [17], plus [1] asetigerous segment adjacent to pygidium, with highly reduced parapodia, suggesting a juvenile stage. Length, overall = 1.1 mm. Length to Ch10 = 0.7 mm. Width at Ch1 = 0.15 mm</p> <p>Ovigerous: No</p> <p>Pigmentation: Some small amorphous globules inside body.</p> <p>Prostomium longer than wide.</p> <p>Paired Antennae simple, digiform w. rounded tips.</p> <p>Paired Palps simple, subequal in size to antennae.</p> <p>Ciliary banding, throughout.</p> <p>Mandibles: Heavy sclerotization.</p> <p>Mandibles: Reduced L-shape w. serrations on anterior margins and bifid on lateral margins.</p> <p>Superior Basal Plates:</p> <p>Inferior Basal Plates: pres. / abs.</p> <p>Superior Free Denticles: pres./ > 8; ≤ 8 / abs.</p> <p>Inferior Free Denticles: pres. / abs.</p> <p>Dorsal Branchiae: absent. Ventral Branchiae absent</p> <p>Dorsal Cirri: Highly reduced digiform knob, inserted sub-distally on parapodia. Cirrostyle: Absent</p> <p>Notoaciaculae absent. Anterior Ch's w/o specialized setae.</p> <p>Furcate Setae absent. Ventral Cirri absent, but inferior parapodial lobe.</p> <p>Parapodia fan out distally.</p> <p>Supra-acicular capillary setae present. Supra-acicular chaetae were not clearly visualized.</p> <p>Sub-acicular, compound falcigers present. Serrations not visualized.</p> <p>Length ratio of smallest to largest blade = 1 : 1.3</p> <p>Ventral Neuropodial Setal lobe: Present. Inferiormost Seta of each does not differ from other sub-acicular setae.</p> <p>Ventral Cirri absent. A ventral nub-like process protrudes distally from each parapodium.</p> <p>Anal cirri: One pair visible on ventral side</p> <p>Cirrus length in relation to pygidium.= 1 : 1</p> <p>Pygidial style: Inserted mid-ventrally</p>					
				<p>Fig. 1. crsRef_148. Entire specimen, dorsal view at 100x. PhotoShop composite - IA.</p>	
				<p>Fig. 2. crsRef_148. Anterior parapodia with simple and compound chaetae @ 400x.</p>	
				<p>Fig. 3. crsRef_148. Mandibles @ 400x.</p>	

```
>Ophryotrocha_langstrumpae Ophryotrocha langstrumpae 16S
ribosomal RNA gene, partial sequence; mitochondrial gene
for mitochondrial product
GCCTGTTTATCAAAAACATAGCTTTGTGAATACAAAATACAAAGTAACTCCTGCCAGT
GAAATCTTAAACGGCCGCTTTAGTGCTAAGGTAGCATAATCACTTGCCCTTTAATTGGG
GGCTGGAATGAATGGAACACATAAATACTTTCTTTTTACGACTCTAAAAATTAAT
CTTTATGTGAAAAATCATAAATTAATAAAAAAGACCAAAGACCCTACCGAGCTTAAAT
TTATAACCATTATTGTCAAATAAATTTTTGTTGGGGTGATACTGGATACCCATCCTATT
TTATTTTTAACTTAACTTATAAATTTTCGCTTAGCTACCCTAGGGATAACAGGCTACT
CTTTTTTAAAAGTCAAATTTACAAAAGACCCAGCACCTCGATGTTGGCTTAAGAATA
AATTAAGTGCAGCAGCTTAATCTAAGGGCTTGTTCGCCATAATTTCTTACGTGATCT
GA
```

Ophryotrocha langstrumpae sp. nov. (Figs 14–19)

Material examined: East Pacific, Santa Cruz Basin, 33°30'N, 119°22'W, wood parcel at 1672 m depth, one specimen, 4.8 mm long, 31 chaetigers, preserved in formaldehyde, HOLOTYPE (NHMUK2012.19); same location, two specimens preserved in ethanol, PARATYPES (NHMUK2012.20-2012.21); same location, 1056 specimens preserved in formaldehyde or in ethanol. San Nicolas slope, 33°20'N, 119°59'W, whale-fall at 960 m depth, 32 specimens preserved in formaldehyde or ethanol. East Pacific, Santa Cruz Basin, 33°30'N, 119°22'W, whale-fall at 1675 m depth, 2 specimens preserved in formaldehyde or in ethanol. East Pacific, Santa Catalina Basin, 33°12'N, 118°30'W, whale-fall at 1240 m depth, 19 specimens preserved in formaldehyde or ethanol. East Pacific, Santa Catalina Basin, 33°12'N, 118°30'W, wood parcel at 1244 m depth, 13 specimens preserved in formaldehyde or ethanol.

Description: Body shape dorso-ventrally compressed, elongated, tapering slightly at posterior end (Fig. 14). Prostomium wider than long, with long digitiform paired antennae inserted dorsally, reaching back to first chaetiger.

Archiving

- Keep tissue for sequence voucher and/or type material for description
 - deposit these samples in an open repository, e.g museum collection, where other people can request them for comparison and future studies
- If not publishing the result – keep reference collection in an open repository as above

Summary

