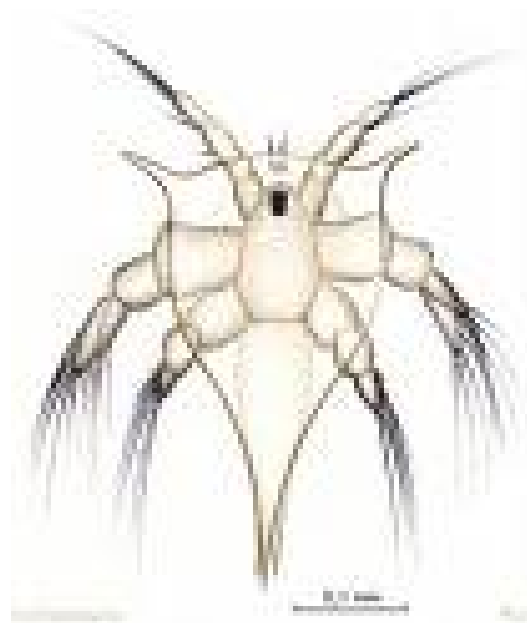


# DNA Barcoding



**Millport 2010**

# DNA barcodes

a DNA sequence that can be used

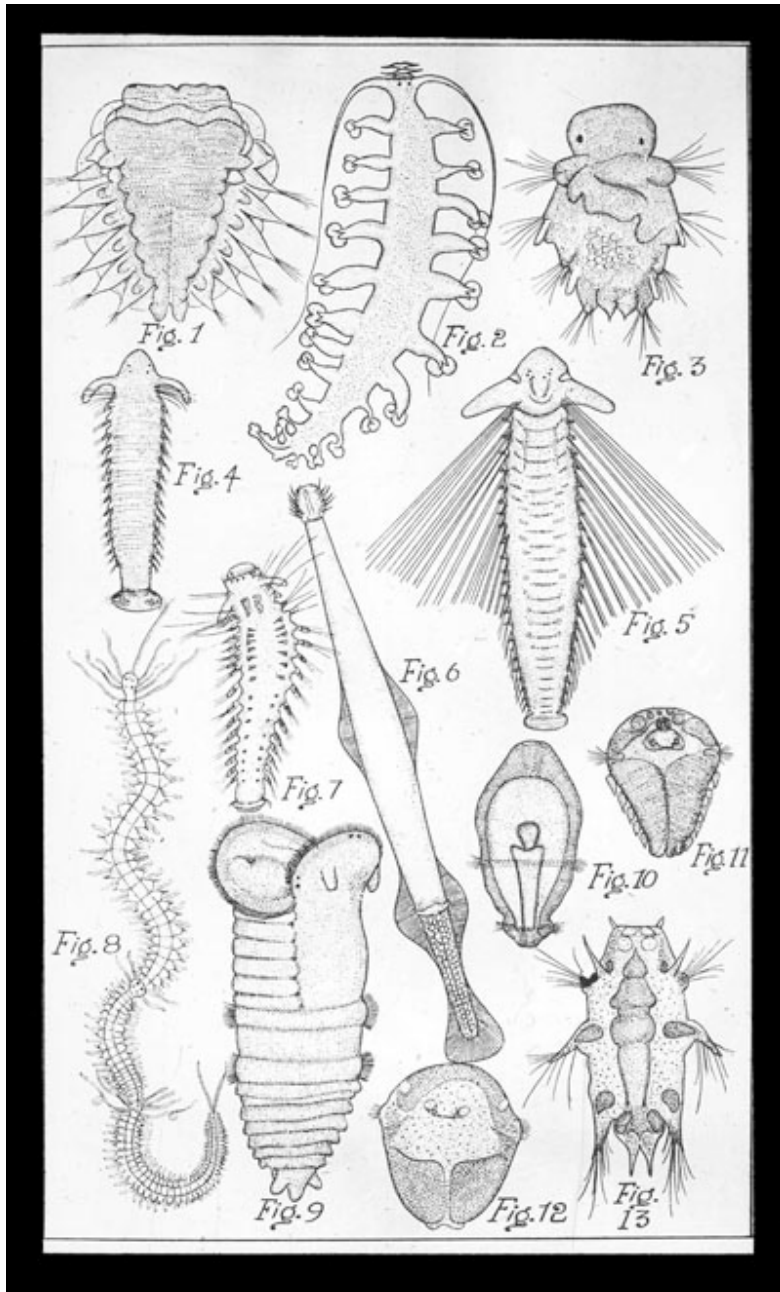
to identify a specimen to species

or to classify specimens in to  
taxa (putative species)

# DNA Barcoding at Millport 2009

## AIM:

- To generate DNA barcodes from adult and planktonic (larval) crustaceans of the Millport area
  - To link planktonic larvae to their littoral / sessile adults



The zooplankton

home to  
'permanent  
residents'

and those just  
passing through.

# **DNA Barcoding at Millport 2009**

**FOCUS:**  
**Barnacles**



Nauplius larvae....



Nauplius larvae... of crustaceans,  
but which crustaceans?



*Cthamalus montagui*



# We will

- \* select adult and larval crustaceans
- \* extract their DNA
- \* use PCR to amplify a fragment of the mitochondrial cytochrome oxidase I (COX1) gene
- \* check that the PCR has worked using gel electrophoresis
- \* send the successful ones off for sequencing

# First.

- Take a sample of the plankton, and look at it under the binocular microscopes.
- Select FOUR zooplankter, preferably nauplii. Place them in the first four wells of your sample strip tube.

# First...

- Take as much sea water out of the strip tubes as possible, and add 20  $\mu\text{l}$  LYSis buffer.
- Keep the strip tube on ice.

***LABEL YOUR TUBES***

## Second.

- Take four adult crustaceans, including barnacles of at least two and preferably all three common species), and remove a *SMALL* portion of an appendage.
- Put 40  $\mu\text{l}$  of LYSis buffer in wells 5-8 of your strip tube, and add the fragment of adult.

*no, really*

***LABEL YOUR TUBES***

***LABEL YOUR TUBES***

***LABEL YOUR TUBES***

# Third.

- Once everyone has their eight tubes with LYSis buffer ready...
- The lab head honchos will put them in the PCR machine and run the LYSIS protocol...

This takes 40 mins or so...

# Fourth.

- Set up the PCRs in a new strip tube using

  - 17  $\mu\text{l}$  of PCR Master Mix

  - 3  $\mu\text{l}$  of lysate

*mix the lysate by pipetting up and down first*

***LABEL YOUR TUBES***

# Fifth

- Once everyone has their PCR reactions ready, the lab head honchos will put them in the PCR machine and run the COX1 PCR protocol...

*This takes 2.5 hrs...*

***LABEL YOUR TUBES***

## Sixth.

- set up another **LABELLED** strip tube with 4  $\mu\text{l}$  of Load buffer in each well.
- transfer 4 $\mu\text{l}$  of your PCR to the corresponding wells of the strip tube
- load the samples in the agarose gels, in the order

**Ladder (marker), sample 1-8, Ladder,**  
filling in the gel loading sheet so you know which samples are which...

# Seventh.

- electrophorese at ~100 mA for ~1 hr
- once electrophoresis is done, we will go to the dark room to look and see how successful we have been

# **Eighth.**

- The lab head honchos will collect each successful PCR reaction into a master plate to be sent to Edinburgh for sequencing



Nauplius larvae....