Developing Molecular Mayfly Markers

Developing accurate markers for environmental DNA (eDNA) surveys of UK river invertebrates of ecological importance

Summer Browne *, John Bythell
Cellular and Molecular Biology, s.browne@ncl.ac.uk, 160169825
School of Natural and Environmental Sciences

Aims:

- Identifying which haplotypes (set of genes inherited from single parent) and cryptic species (are morphologically identical but different species) occur in a region and establish their distribution.
- Relationship between *Baetis rhodani* species complex haplogroups and morphological characteristics – are the cryptic species morphologically identifiable?

Introduction:

- *Baetis rhodani* is a species of mayfly commonly found in freshwater habitats, used by the Environment Agency as bio-indicators of river water quality. Morphological (physical) characteristics do not allow differentiation of suspected cryptic species (morphologically identical but different species). Molecular phylogenetics can identify this 'hidden' diversity. Due to reduction in cost of sequencing, environmental DNA (eDNA) approaches will become routine to detect such cryptic diversity.
- Several studies - Williams et al. (2006); Lucientini et al. (2011) and Múrria et al. (2007) - have established a total of 16 haplogroups proposed as cryptic species of *B. rhodani.*
- This study aimed to establish which haplogroups occur in the northeast. Further objectives were to determine whether *Baetis atlanticus,* a recently identified species in the region, represents a valid (distinct) species using molecular indicators.
- Understanding cryptic diversity in these mayflies will allow more accurate use of these bio indicators in assessing water quality.

Methods & Analysis:

Samples were collected from the northeast. A QIAGEN kit was used for DNA extraction following PCR methods to isolate and amplify the COI gene: a highly conservative protein coding gene in the mitochondria (Folmer et al. 1994). PCR products were sequenced and analysed using finch TV (figure 1) and MEGA7 (figure 2).

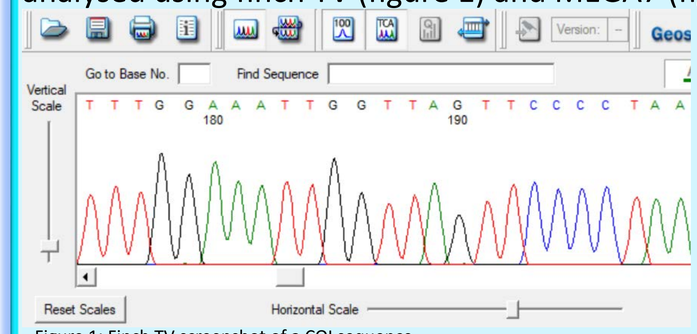

Figure 1: Finch TV screenshot of a COI sequence

Finch TV

Mega7

Figure 2: MEGA 7 screenshot of COI sequences

Results:

- 25 unique, high-quality sequences were obtained from *B. rhodani* morphospecies from various locations- shown next to coloured dots in figure 3.
- Most (21) were identified as haplogroup 3, 2 as haplogroup 4 and 2 samples did not fall into any of the 16 haplogroups of the *B. rhodani* complex .
- Samples identified from morphology alone as *B. atlanticus* fell into group 3 (pink dot).
- The samples that did not fall into any of the 16 *B. rhodani* haplogroups were identified as *B. scambus/fuscatus* (red dot).

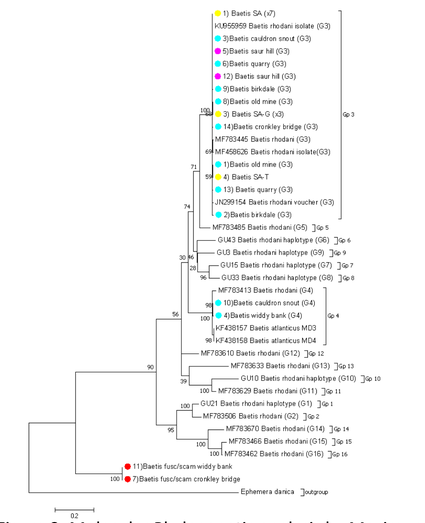

Figure 3. Molecular Phylogenetic analysis by Maximum Likelihood method

Conclusions:

- Two samples that were identified via morphology as *B. rhodani* were identified using molecular analysis as a distinct species *B. scambus/fuscatus,* indicating that there is more cryptic diversity than first thought.
- Further analysis is needed to determine whether *B. atlanticus* is a valid species, but this study suggests that at least 3 cryptic species occur in Northumberland that are routinely mis-identified as a single bioindicator species in water quality monitoring programmes.
- There are at least two of the known haplogroups of the *B. rhodani* complex co-occurring in the northeast.

References:

- Folmer O, Black MB, Wr H, Vrrjenhoek RC (1994) DNA primers for amplification of mitochondrial Cytochrome C oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology 3:294-9
- Williams HC, Ormerod SJ, Bruford MW (2006) Molecular systematic and phylogeography of the cryptic species complex Baetis rhodani (Ephemeroptera, Baetidae. Molecular Phylogenetics and Evolution 40:370-382
- Lucentini L, Rebora M, Puletti E, Gigliarelli L, Fontaneto D, Gaino E, Panara F (2011) Geographical and seasonal evidence of cryptic diversity in *the Baetis rhodani* complex (Ephemeroptera, Baetidae) revealed by means of DNA taxonomy. Hydrobiologia 673:215-228
- Múrria et al. (2007) Local environment rather than past climate determines community composition of mountain stream macroinvertebrates across Europe. Wiley Molecular Ecology 26:6085– 6099

Acknowledgments: Thanks to Newcastle University for funding the summer research project and to John Bythell, Ros Brown and Miriam