Phylogeography of the rare bryozoan, *Lophopus crystallinus*
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Lophopus crystallinus (Pallas 1768) is a freshwater bryozoan which belongs to the Class Phylactolaemata and forms small gelatinous colonies in both running and standing water (see Figure). Although it was the first bryozoan to be described, virtually nothing is known about its ecology. The older literature refers to obtaining bucketfuls of *L. crystallinus* colonies from single hauls in the Norfolk Broads (Hurrell 1910), but it is clear from recent surveys that the species has probably entirely vanished from the Broads (O'Dea 2002). *L. crystallinus* has also generally declined throughout Europe (Rieradevall & Busquets 1990). As a consequence of this decline, *L. crystallinus* is listed as a priority species within the UK Biodiversity Action Plan (UK Biodiversity Group 1999) and is classified as 'RARE' in the *British Red Data Book of Invertebrates Other than Insects* (Bratton 1991).

L. crystallinus colonies release resistant, seedlike, asexually-produced propagules (statoblasts) in low numbers continuously throughout the year which are able to float and will therefore enable dispersal within a water body. Recent investigations have demonstrated that migratory waterfowl appear to be important in mediating both historical and ongoing long distance dispersal between populations of the closely related freshwater bryozoan *Cristatella mucedo* (Freeland et al. 2000a,b); however, this has not been shown for populations of *L. crystallinus*. The extent of dispersal of *L. crystallinus* will have important implications to its population biology and conservation management. In particular, low levels of dispersal and reduced gene flow may lead to cryptic speciation, which may explain its apparently wide tolerance of environmental conditions (Rieradevall & Busquets 1990). An understanding of the genetic diversity of *L. crystallinus* is crucial for the production of effective conservation strategies. The Hugh Cary Gilson Award has allowed us to undertake a phylogeographic study to investigate the genetic diversity within and between populations of *L. crystallinus* in order to assess the relatedness of populations over broad spatial scales. Below we outline our approaches and findings. We intend to present our results and analyses in detail in a future publication.

Colonies of *L. crystallinus* for genetic investigations were collected from three sites within the UK (in Oxfordshire, Lincolnshire and Sussex) and from two continental European sites (in Switzerland and Italy). We initially investigated the phylogenetic relationships within and between the continental European and UK populations through the comparison of sequences from the 16S rDNA region of mitochondrial DNA. For this work we used primers that amplify a short fragment of DNA (via the polymerase chain reaction) within the 16S rDNA region. The polymerase chain reaction (PCR) is a basic approach used in molecular biology to 'amplify' or generate multiple copies of regions of DNA of interest. Such amplification is necessary in order to obtain sequence information for these regions. Primers used in PCR are designed to adhere to a particular region of DNA and

amplification of the neighbouring region begins. The more specific primers are to the organism of interest the more specific the amplification procedure will be. Our results revealed few differences amongst the 16S rDNA sequences of *L. crystallinus* from the UK and Europe, but there was a weak indication that the Italian population is genetically isolated from the other populations.

As the mitochondrial sequences proved to be relatively uninformative to the phylogeography of *L. crystallinus,* we subsequently have focused our efforts on the sequencing of nuclear DNA choosing two sites within the region which codes for ribosomes: the internal transcribed spacer region 1 (ITS-1) and the 28S rDNA region. At present we are having encountering problems with contamination of samples with this work. This is because freshwater bryozoans are hosts to many endo- and exosymbionts, and the primers that we are employing are amplifying non-bryozoan DNA sequences. We are hoping to resolve these problems in the near future.

The work described thus far has focused on the sequencing of relatively slowly evolving areas of DNA to gain information on historical gene flow. To try to gain information on more recent gene flow patterns, we needed to use more rapidly evolving markers. Microsatellite primers target non-coding areas of DNA that contain short tandem repeats of DNA motifs (for instance, AAAGAAAGAAAG….) and are ideal to assess ongoing gene flow and diversity within and between populations. As these non-coding areas tend to lose or gain repeats rapidly, microsatellites may vary in size even between individuals within the same population. We tested microsatellite primers originally developed for *C. mucedo.* Unfortunately, none were able to consistently amplify the microsatellite regions of *L. crystallinus.*

Finally, we turned to the use of randomly amplified polymorphic DNA (RAPD) to study patterns of relatedness amongst *L. crystallinus* populations. This approach uses randomly designed primers to amplify unknown regions of the genome. While this has the danger of amplifying non-target DNA of other organisms, controls can help to reduce the misinterpretation of results and the approach is working well. Our results confirm that the Italian population is clearly differentiated from the other populations. This evidence, along with the weak evidence provided by the 16S rDNA sequence analysis, indicates that this population has indeed been genetically isolated from the other populations. The similar patterns revealed by the two independent approaches provide us with confidence in our results. The higher degree of relatedness amongst the non-Italian populations may be due to a higher level of gene flow amongst these populations, or it may be that they have been more recently founded from the same ancestral population. It also appears from our preliminary results that the population from Sussex is more closely related to the Swiss population than to the other UK populations. Clonal diversity within all populations was high as no identical clonal genotypes were discovered, however, sample sizes were small and this work is ongoing.

An understanding of the genetic diversity within and between *L. crystallinus* populations is crucial to producing an effective conservation strategy since genetic diversity is expected to promote long term persistence and could provide an indication of cryptic speciation. This knowledge in combination with a better understanding of dispersal and gene flow will certainly be relevant to the Species Action Plan for *L. crystallinus* (as listed within the UK Biodiversity Action Plan).

Lophopus crystallinus forms small colonies comprised of zooids. Each zooid bears a ciliated tentacular crown (known as a lophophore) used in suspension feeding. Bar indicates 100 μm.

References

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