eration of big patches was noted in January 2007 followed by fertile material and then extensive fruiting in April but the capsules appeared to be aborted, possibly because of lack of rain in the previous four weeks.

Since there is a lack of records of *Tortula freibergii* from natural sandstone in the north-west of England, the Cheshire sandstone ridges and outcrops should be searched, preferably during December to April. Within the present study area, the long-term prospects for *T. freibergii* depend on weather and canal use. The widening of the M60 motorway and the construction of a new marina, both at Stretford, do not seem to have caused any detrimental effect on the species. While access sites cause turf wear which appears to be an advantage, the increased use of long boats is adding to water pollution and oil in particular may have a detrimental effect on the moss. In summary, *T. freibergii* is sporadic along the areas of the Bridgewater Canal that were searched, but seems to be stable.

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Barcoding Britain's liverworts and hornworts: a new project and request for material

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Background

DNA barcoding is an exciting and actively developing field of natural history science. The long-term goal is to develop a universal genetic system to help with the identification of samples of known species, and also to contribute towards the discovery of new species. The principle of the approach is to choose a standard short region of DNA and produce an open-access centralised database of sequences ('barcodes') which can be used as a reference library against which unknown samples can be compared.

In animals, DNA barcoding has already proved very useful. It aims to provide an identification tool in many groups, and has already contributed towards the discovery of new species. The key

breakthrough was finding a region of DNA which showed little or no variation within species, but discontinuity between species. The mitochondrial gene CO1 proved ideal.

In plants, however, finding a 'standard DNA barcoding region' has been more difficult. An ongoing project (coordinated by RBG Kew, in collaboration with the RBG Edinburgh, the NHM London, and eight other labs around the world) will provide guidelines on standard regions of DNA for land plant barcoding. Because levels of genetic variation differ greatly in different plant groups, it seems likely that for the process to work in plants, more than one region of DNA will be needed.

Now that the barcode regions are being defined for plants, the next step is to begin to populate the reference database with these barcode sequences and to thoroughly road-test and refine the approach. As part of this process, and also to produce a resource for the bryological community, the RBGE has undertaken to obtain barcode sequences for >90% of the British liverworts and hornworts.

Figure 1. *Conocephalum salebrosum*, a named recent taxonomic segregate of *C. conicum*, but are there only two genetic species in this group? Photo: David Long.

Relevance to British Bryology

What practical uses could this study have for bryologists? Firstly, it could lead to the discovery of new species. This approach has so far only been applied to a small number of liverwort species and has already led to the discovery of a species new to Britain (Long *et al*. 2006) - the plant formerly known as *Anastrophyllum joergensenii* was shown to consist of two distinct species: *A. alpinum* and *A. joergensenii*. Recent work on *Conocephalum conicum sensu lato* (Figure 1) has revealed multiple genetically isolated races, suggesting hitherto unrecognized biodiversity exists within the lineage (Szweykowski *et al.* 2005). British *Aneura* (Figure 2) also seems to comprise several distinct lineages that bear little resemblance to the current taxonomy of the genus. In some ways this should not be surprising – these are small and relatively simple organisms, with only a limited number of morphological characters available to the naked eye, or with a hand lens or even microscope. However, species boundaries are not constrained by our resolving power. Barcoding could also serve as a pointer to protected 'pseudospecies' that are simply odd phenotypes of common species, thus allowing conservation time and funding to be better focused on genuinely rare taxa.

Secondly, in the mid-term (*c.* 5 years) the database should be sufficiently established to allow routine checking of specimen identities by DNA barcodes. This could provide a useful 'positive feedback mechanism' for bryologists/botanists learning a new group and wanting their identifications checking, and also offers the potential for routine identification (and hence surveying) of very small species in which distinguishing characters are limited, or for which sub-optimal material (*e.g.* sterile or immature material, protonema or fragments) is all that is available. It will also allow reliable identification of both sexes in dioicous species, or morphologically plastic taxa.

Thirdly, there is the real prospect of 'hand-held field DNA identification devices' within the next 10-15 years. These would enable direct 'in-the-field barcoding', which would produce a useful learning tool, again providing positive feedback for the field biologist 'getting their eye in' on a particular group, and also have applications for ecological surveys.

Will DNA barcoding make field bryologists redundant? Absolutely not – the skills of the field bryologist, finding the interesting habitat and working out what to put in the machine, are not automatable! This approach will merely make it easier for identifications to be made or cryptic species to be found once the field sampling has been done.

Will it put professional taxonomists out of a job? No – it should just make their jobs easier and be a tool to support their work by freeing up time previously spent on routine identifications, while pointing them towards hitherto undetected species.

Will it just be a genetic answer that pays no attention to all the past efforts of bryologists and everything we have learnt about morphology? No – building the database requires combining taxonomic/morphological identifications with the genetic data so that it all makes sense in line with liverwort and hornwort taxonomy and biology. Its success depends on its integration with knowledge on morphology and ecology, rather than being independent from them.

Will it lead to the creation of multiple new taxa that can only be recognized through molecular means? This is a harder question to answer. Responsible taxonomists will certainly hesitate before naming lineages that cannot be identified by morphological means. It is to be hoped that after identification of genetically distinct lineages

Figure 2. A large member of the genus *Aneura*, provisionally identified as the Japanese *A. pellioides*, in the Scottish Borders, for which DNA-barcoding is needed to back-up the apparent morphological differences. Photo: David Long.

by barcoding, thorough morphological revision of the material will then find matching traits, allowing taxonomic recognition, as occurred in the *Anastrophyllum* study.

How much will it all cost? If the database was fully populated now, it would cost around ten pounds to identify a sample, assuming batch processing. In the future, these costs are expected to drop rapidly, such that 50p–£1.00 is a realistic future estimate.

Lastly, why here? As an island, Britain is a small, well-defined geographical unit and thus provides an ideal microcosm for our barcoding prototype. It also has what is probably the world's best known flora. Within this, the liverworts and hornworts are an ideal group in many ways – often neglected compared to their more recent relations the angiosperms, and many field botanists feel unhappy identifying them. However, due to a long history of field and herbarium bryology within the UK, our morphological species and their distributions are now well-documented. This greatly simplifies the generation of the reference database of DNA

barcodes.

Request for material

Can you help? There are just over 300 recognised liverwort taxa, and four hornworts, in Britain. Our reference database ideally needs to contain several accessions of each of these, in order to cover the diversity within these taxa as well as the discontinuities between taxa. In practice, this means that we need to have collections for each species from different geographical locations – for example, we might want to get material for *Metzgeria furcata* from south west England, Wales, central Scotland and the far north. We may also want to sample material of a species that has been collected in an atypical habitat. Clearly, even in a well mapped region like Britain this is a big task.

We hope to have convinced you that this is not simply an academic exercise, but is a worthwhile project with a strong practical application. However, the success of the enterprise rests almost solely on the completeness of our taxonomic sampling. As such, this article is a plea for help from active field bryologists. For this to work, we need multiple (about 5) geographically spread collections of every liverwort and hornwort in Britain.

If you would like to contribute material, we will gratefully receive samples of any liverwort or hornwort you collect from England, Wales and Scotland. Postal expenses can be reimbursed. Even if something is common, we may not yet have it from your area (we can add your name to a spreadsheet distribution list if you wish to follow our sampling progress). We usually work with silica-dried material (which keeps indefinitely), but can also use recently air-dried material, and would be very happy to receive fresh material (*e.g.* in small ziplock bags, which we can send out on request). Our DNA extraction method requires only a small amount of plant material – a few stems of a large leafy species, 15 to 20 stems of a tiny species or around a 5 pence coin's worth of thallus. However, because these collections are going to be the standards against which future liverwort and hornwort identifications will be made, we also need enough material to make a good herbarium voucher for RBGE, with the usual details (collector name and collection number if given, date, location, 6-figure grid reference or 8-figure GPS reference and habitat). Rare taxa should be collected only from large populations and with appropriate permits; for red data listed species we will preferentially attempt to use herbarium collections.

For more information on DNA barcoding see barcoding.si.edu/DNABarCoding.htm

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