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Evidence Project Final Report

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Project identification

1. Defra Project code: CTE 0933
2. Project title: Systematics, barcoding and ecology of fungi from waxcap grasslands in England and Wales
3. Contractor organisation(s): Royal Botanic Gardens, Kew; CABI; University of Aberystwyth
4. Total Defra project costs (agreed fixed price): £ 77267
5. Project: start date 01/08/2010
   end date 31/01/2013

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Executive Summary

7. The executive summary must not exceed 2 sides in total of A4 and should be understandable to the intelligent non-scientist. It should cover the main objectives, methods and findings of the research, together with any other significant events and options for new work.

Grasslands with relatively low nitrogen levels (i.e. those that are grazed or mown and receiving no or only low levels of nitrogen fertilizers and airborne nitrogen pollution) are important and diminishing wildlife habitats in Britain. They are of particular conservation interest due to their characteristic fungal communities, many of which are highly sensitive to nitrogen ("N") inputs, and disappear (or at least stop producing fruit bodies) following even modest N deposition. The effect is long-term, and recovery of fruiting populations (i.e sexually reproductive) can take decades once the elevated N input has ceased. The fungi in such habitats are diverse, and their communities are important at a European scale. Some, such as the waxcaps (*Hygrocybe* sensu lato), produce conspicuous, brightly coloured fruit bodies.

The fungi of these so-called unimproved or semi-improved grasslands have been the subject of substantial conservation-related activity in recent years, primarily programmes of identification workshops linked to citizen science-led or specially commissioned surveys. The number of species from a set of four groups of fungi from these habitats (*Clavariaceae*, *Hygrocybe*, *Entoloma* and *Geoglossaceae*; the so-called CHEG taxa) has been used as an indicator of fungal interest, ecological continuity and site quality. Sites have been ranked according to their CHEG scores and the "best" examples have been listed as Important Fungal Areas, and, following production of appropriate guidelines, proposed for designation as Sites of Special Scientific Interest. The most significant threshold within the current guidelines is that a grassland site can be considered for SSSI status if 18 waxcap species have been recorded there.

Species delimitation in several of these groups is controversial. Many cannot be reliably identified using field characters alone, and it can be difficult to distinguish between related taxa even when using microscopic analysis. Our project was designed to test existing species concepts using molecular sequencing methods, leading to the designation of diagnostic "barcode" sequences that can be used as an objective method to identify species.

We focused on two groups of fungi from the grassland system, the waxcaps themselves (*Hygrocybe* spp.) and the earthtongues (*Geoglossum* and relatives). Both groups have been the subject of long-term site surveys, they have excited the attention of non-specialists, their presence has led to the designation of a few SSSIs for their non-lichenized fungal interest. Three species were included in the UK's Biodiversity Action Plan signposting exercise (*Hygrocybe spadicea*, *Geoglossum atropurpureum* and *Microglossum olivaceum*), 11 taxa were placed on the unofficial Great Britain and Isle of Man Red Data List for fungi, and two (*G. atropurpureum* and *H. calyptriformis*) were unsuccessfully proposed for inclusion in Appendix 1 of the Bern Convention. All four national statutory conservation

bodies have funded work on naturally occurring populations of these fungi. Despite all of this interest, neither group has been the subject of a modern systematic revision using molecular methods; few authenticated DNA sequences are available and the work to identify diagnostic DNA barcode has not been carried out.

With the help of around 85 individuals and volunteer recording groups throughout the UK, we were able to access nearly 700 collections of *Hygrocybe* and *Geoglossaceae*. Alongside these fresh gatherings, we also made extensive use of dried reference samples in the national fungaria (mycological herbaria) of Kew and Edinburgh. These included several type specimens, the authentic material on which fungal species names are based.

We rapidly discovered that the level of species diversity within *Hygrocybe* is substantially greater than that presented in the current guidebooks and scientific treatments. Further research is needed, but we currently believe that at least 96 species are present in the UK (as defined by DNA sequence-based methods), compared with the 51 species (plus 8 varieties) currently accepted. This newly discovered diversity has profound implications for conservation management, and the relevant SSSI guidelines based on species numbers will need some reassessment. Bearing in mind the diversity we have uncovered and the knowledge that western European waxcap habitats are considered of high conservation value on a continental scale, it would not be surprising if some of the species were found to be endemic to the British Isles.

The diversity of waxcaps was also investigated at family and generic levels using multigene DNA analysis, in partnership with an international consortium of researchers led by an investigator in the USA. The species included in this globally relevant dataset fall into multiple distinct groups at the subfamily and generic ranks, and the genus *Hygrocybe* is confirmed to be an artificial assemblage of distantly related taxa that are being segregated into separate genera. This is part of a major taxonomic monograph of the family in which waxcaps belong (*Hygrophoraceae*), which is in the final stages of preparation.

The existence of such a substantial assemblage of cryptic species also has important implications for fieldworkers and identification guidebooks. In some cases we have been able to correlate DNA-based (phylogenetic) taxa with species that are currently placed in synonymy, thus providing a sound basis for resurrecting some historically recognised species concepts. In others, however, genetic divergence seems to be associated with a series of overlapping morphological characters. We need to do a lot more work to investigate potential diagnostic features (ecological and geographical in addition to morphological) of our newly recognized species, but it remains probable that some will need sequence-based methods to identify them in a reliable and robust fashion. However, we do believe that there is a future for citizen scientist survey and monitoring of waxcap habitats. In a handful of cases where we have been able to scrutinise the sequence-based diagnosis of taxa, we found that the current morphology-defined species can be separated easily into cryptic taxa. Conversely, there were only a small number of instances where morphospecies were found to encompass distantly related phylogenetic species. Future surveys based on newly-defined species aggregates rather than phylogenetic species will be scientifically useful, and it will be possible, at least in part, to integrate future and historic field-based survey results.

We are in the process of formally describing the new species we have detected; the first paper (dealing with two species related to the well-known Parrot Waxcap, *Hygrocybe psittacina*) is nearing completion. One of these has a distinctive purple fruit body, and has been found in central England and western Wales, whereas the other is thus far only known from Wales. A series of scientific papers will follow. Our project was featured on national news programmes (radio and TV) and we shall also make our findings known to the citizen science community via websites, popular articles and at field meetings, etc.

The project has demonstrated a healthy and mutually valuable working relationship between academic scientists, conservation agencies and volunteer surveyors. We are immensely grateful to the extensive list of recording groups and interested individuals who have contributed to the project.

Project Report to Defra

8. As a guide this report should be no longer than 20 sides of A4. This report is to provide Defra with details of the outputs of the research project for internal purposes; to meet the terms of the contract; and to allow Defra to publish details of the outputs to meet Environmental Information Regulation or Freedom of Information obligations. This short report to Defra does not preclude contractors from also seeking to publish a full, formal scientific report/paper in an appropriate scientific or other journal/publication. Indeed, Defra actively encourages such publications as part of the contract terms. The report to Defra should include:
   - the objectives as set out in the contract;
   - the extent to which the objectives set out in the contract have been met;

- details of methods used and the results obtained, including statistical analysis (if appropriate);
- a discussion of the results and their reliability;
- the main implications of the findings;
- possible future work; and
- any action resulting from the research (e.g. IP, Knowledge Exchange).

The objectives as set out in the project proposal are as follows:

1. To establish a multidisciplinary research task force for the programme with representation from a wide range of stakeholders.
2. To establish a species target list for systematic research and conservation management.
3. To acquire fresh or recently collected material of at least 25 English and Welsh species of *Hygrocybe* and *Geoglossaceae*, including all BAP and provisional RDL species but including other taxa to allow the classification of listed species to be placed in context.
4. To elucidate their systematics using multigene sequencing technologies, improving their circumscription and reducing subjectivity of species definition.
5. To identify cryptic species, which may have distinct conservation requirements, using molecular methods and correlating these phylogenetic taxa with morphological traits.
6. To identify suitable barcoding sequences for all of the species considered to fix their application in a modern context and inform molecular detection systems.
7. To research into the ecology of *Hygrocybe* species, specifically their nutrition and mycorrhizal status and initially using a simplified natural system, to inform future management plans
8. To disseminate results from the project in an appropriate manner to allow improvement of survey, monitoring and management methodologies for these fungi.

Substantial progress was made on most of the objectives.

1. **Achieved**. Productive collaborations occurred throughout the life of the project. These can be divided into various categories.

   Academic partnerships to strengthen the scientific backbone of the project were established with, among others: Dr D Jean Lodge, USDA Forest Service, Puerto Rico, Dr Brandon Matheny, University of Tennessee, USA, Dr David Boertmann, Aarhus University, Denmark, Dr Eef Arnolds, Dutch Mycological Society and Vincent Hustad, University of Illinois Urbana-Champaign, USA.

   Collaborations occurred with a wide range of individuals and volunteer fungus recording groups throughout the UK. This resulted in the acquisition of nearly 700 freshly collected specimens for morphological and molecular analysis, along with images and field data. There was considerable interest and enthusiasm shown for the project, and many of the groups responded actively to a series of requests to target particular species at various stages throughout the project.

   Matching funding of £10K was obtained from Scottish Natural Heritage, enabling the expansion of project activities in Scotland including a monitoring programme for key species.

   A good working relationship was maintained with the British Mycological Society. This included running of a dune and grassland field meeting and workshop attended by Dr Eef Arnolds in North Wales, with matching funding from the Heritage Lottery Fund. A further waxcap identification workshop took place in Scotland in collaboration with the Grampian Fungus Group, which was partially supported by the project.
2. **Achieved**. An initial target list of species was established, with selection criteria including appearance on provisional red data lists and number of recent records in the two national fungus recording databases for the UK. This list was used as the basis for requests to collectors, and proved a satisfactory method for identifying taxa potentially of particular conservation concern. For a number of reasons, however, it has not yet been possible to transform the initial list into a focused target list for monitoring purposes. This is primarily due to the low information baseline for the fungi concerned in both taxonomic and distributional terms, and to the shortcomings of recording based exclusively on fruit body observations.
3. **Achieved**. Collections of most taxa from *Hygrocybe* sensu lato and *Geoglossaceae* recorded from the British Isles were acquired as part of the project, and these were augmented with dried reference samples from the national fungus collections, including type specimens. The initial approach was to accept all offers of specimens to ensure a ready supply of experimental material, before making a series of more targeted requests in subsequent years

of the project. The strategy was as successful as could be expected, given that by their nature the rarer and more recalcitrant species complexes targeted towards the end of the project were both more difficult to collect and more difficult to resolve taxonomically. The strategy also allowed us to make an assessment of the accuracy of field identifications, and to identify particular problem areas. An extension to the project allowed us to include some samples from the 2012 field season, but the number was small due to the very poor fruiting of most waxcap species.

4. **Achieved**. Multigene phylogenies have been generated for both the *Hygrophoraceae* and the *Geoglossales*, in partnership with an international consortium of colleagues and led by two researchers in the USA. Both of these studies resulted in substantial changes to the systematic arrangements. *Hygrocybe* as circumscribed by Boertmann (2010), the most recent morphology-based monograph, has been found to be polyphyletic and in need of splitting into seven genera. The red- and yellow-pigmented taxa will remain in *Hygrocybe* sensu stricto, with that genus name retained for the most iconic species. *H. calyptriformis*, a pale pink species once listed as a priority BAP species and red-listed as Vulnerable, but now known to be fairly common in Britain, is assigned to the genus *Porpolomopsis*, and the *psittacina/laeta* complex to the genus *Gliophorus*. *H. ovina* and its relatives will be placed in a further genus, *Neohygrocybe*, and a small cluster of species related to *H. xanthochroa* will be transferred to *Chromosera*. Most species with white or pale brown fruit bodies will go to the genus *Cuphophyllus*, which is only distantly related to *Hygrocybe* s. str. and may not even belong to the *Hygrophoraceae*.

   British species traditionally assigned to the *Geoglossaceae* have also been demonstrated to be highly divergent, supporting a preliminary study by Ohenoja et al. (2010). Within the *Geoglossaceae*, *G. glutinosum* has been assigned to a new genus *Glutinoglossum*, and *G. arenarium* to a further new genus *Sabuloglossum*. *G. atropurpureum*, at one time confused with *G. arenarium* (Cannon et al. 1985) is now known to be only very distantly related to the *Geoglossaceae*. It is now recognized as belonging to the *Helotiales* and related to the genus *Microglossum*, a further group of stipitate grassland species that has been recognized as of conservation concern. Further work is needed to establish whether *G. atropurpureum* should be placed in *Microglossum* (as was suggested historically) or in a genus of its own. A paper based on this work has been accepted for publication in a high impact-factor journal (Hustad et al. in press).

5. **Substantial progress**. The approach taken here was to generate rDNA ITS sequences for as large a number of collections as possible, followed by morphological analysis of the specimens forming phylogenetic clusters and correlation where possible with phenotypic characters. A total of 418 ITS sequences have been generated and validated to date, and these combined with all publically available *Hygrocybe* s.l. sequences into a comprehensive dataset.

   The level of cryptic speciation within *Hygrocybe* was found to be substantially greater than anticipated, with more than 1/4 [~27%] of morphologically defined species being found to contain distinct phylogenetic taxa (Appendix 1). *H. conica*, for example, variously treated as common and widespread or containing several morphospecies, has now been found to contain at least six phylogenetic taxa. Other well-known highly variable taxa such as *H. acutoconica*, *H. psittacina* and *H. virginea* can similarly be split into at least 4, 7, and 7 cryptic species, respectively. More unexpectedly, the group including *H. ceracea*, *H. constrictopora* and *H. insipida* was resolved into at least 8 sequence-based taxa, and *H. pratensis* with its varieties appears to be composed of at least 8 cryptic taxa. The entire list of cryptic taxa within *Hygrocybe* s. l. remains to be fully characterized, but some have been found to correspond to historically recognized taxa placed into synonymy by Boertmann (2010) but accepted by earlier authors including Orton (1960), Arnolds (1990), and Boertmann (1995). There is some prospect therefore that a number of the phylogenetic taxa detected in the current study can be correlated with phenotypic characters and thus be recognizable with care and experience using traditional methods.

   It has been possible to make some generalized conclusions as to the overall distribution patterns of waxcap species in the light of our studies. Some species names have been used for populations on both sides of the Atlantic, but our work indicates strongly that *Hygrocybe* s.l. species do not in general have intercontinental distributions, and that the same name has been often given to different species in Europe and North America. Some of this evidence is to be published in Lodge et al. (in prep.). We have also found in a number of instances that British and central European populations of traditional morphotaxa belong to different cryptic species. For example the well-known parrot waxcap, *H. psittacina* (now to be included in the separate genus *Gliophorus*), may not to be present in the UK, but instead there may be a series of independent but morphologically confusable cryptic taxa. This has important consequences for conservation policy on a European level and further emphasizes the unique

condition of British *Hygrocybe* s.l. populations.

There is a substantial further body of work to be done before the genera that are the subject of this project are fully revised according to modern phylogenetic principles. This process has been commenced, and the first of a series of scientific papers is almost complete. A confidential draft will be included as an appendix to this report (Ainsworth et al., in prep.).

6. **Substantial progress**. All of the sequences generated during this project have the necessary metadata to be used as species barcodes. These cannot be adopted formally in some cases as taxonomic issues are outstanding, especially where names need neo- and/or epitypification, or where type material needs sequencing to establish which of the cryptic species is associated with the original name. Genetic barcodes will be specified in a series of taxonomic papers to be published in the coming months and years.
7. **Some progress**. The ecological status of waxcap fungi has been contentious for many years, in particular the issue of whether *Hygrocybe* s.l. species have mycorrhizal associations, and if so with which plants. We focused on the *H. conica* aggregate, which includes populations in plant species-poor sand-dune environments. The initial stage of the work involved the design of targeted primers for *H. conica* s. l.. The substantial genetic diversity detected within this species complex made this task difficult. Various primer combinations were tested and the most satisfactory of these showed good amplification of DNA from a diverse set of *H. conica* sequences, but also weak amplification from other *Hygrocybe* species. This does not however negate the utility of the primer set and it may be possible to modify the annealing conditions to improve specificity. Amplification tests on root samples from pot cultures containing translocated *H. conica* fruiting patches were positive, demonstrating the utility of the system for ecological research.

   This initial study showed that *H. conica* DNA could be detected in model systems, but did not address the relationship between *H. conica* mycelium and plant roots within the pots – i.e. whether there is a nutritional connection between fungus and plant. A visualisation experiment using the fluorescent in-situ hybridisation (FISH) technique was therefore designed. This would allow us to detect living fungal hyphae within, and in intimate contact with, plant roots, providing strong indications of a symbiotic association. Unfortunately, our efforts have been unsuccessful to date, due to autofluorescence of the plant roots that obscure any signal from hybridization with *Hygrocybe* hyphae.

   We are now involved in a new programme with matching funding to address this research issue. We are using whole-genome analytical techniques to detect genes associated with mycorrhizal function from one of the specimens collected during the DEFRA-funded project.
8. **Substantial progress**. Project results have been disseminated via a number of channels appropriate to different stakeholder groups. One paper is accepted for publication, and two others are at an advanced state of development, one of which is a major taxonomic monograph of the family *Hygrophoraceae*. The project and its findings have been made available to volunteer stakeholders and others by means of workshops and email communications, and the project reports have been made accessible on the Fungi of Great Britain & Ireland website (http://fungi.myspecies.info). This is currently being upgraded and a preview of the new version is available on http://s2.fungi.myspecies.info. Various web communities have also been used periodically to make requests for specimens etc. A non-specialist summary of the project will be submitted for publication in a popular journal such as *Field Mycology*; this remains to be completed as we wanted to wait for final results before drafting the paper.

Methods and results

In general terms, the methods used during this project have been standard in nature, and have been detailed at various stages in previous project reports. The methods used in the molecular analysis are detailed in Dentinger et al. (2010) and Ainsworth et al. (in prep.).

A summary of the sequencing activity for the project is given below in Table 1. The process is still continuing, so final figures for the project will be somewhat larger than given here. All of the sequences will be made publically available in accordance with Kew policies. We have also had access to substantial numbers of further sequences from *Hygrocybe* s.l. species, including published sequences from GenBank and unpublished information from our collaborators.

Source/clade	*Hygrocybeae*	*Humidicutae*	*Cuphophyllus*	*Chromoserae*	Total
Fresh samples	**162**	**62**	**67**	**7**	**298**
Project sequences from RBG Kew	**67**	**6**	**14**	**3**	**90**
Project sequences from RBG Edinburgh	**20**	**4**	**3**	**3**	**30**
Unpublished sequences from collaborators	50	23	11	4	88
Published GenBank sequences	244	22	34	6	305
Grand total	543	117	129	23	811

Table 1. Sequences used in the project. Those generated specifically are shown in **bold**.

Only a small number of sequences from *Geoglossaceae* are currently available. The extreme cryptic diversity found within *Hygrocybe* s.l. means that these fungi were accorded priority within the project, as advised in previous reports.

Figure 1 (below) is a graphic demonstrating the very substantial phylogenetic diversity we have found within *Hygrocybe* as it is currently circumscribed. For information, "tribe Hygrocybeae" (in red) contains the main body of species within *Hygrocybe* as traditionally accepted. "Tribe Humidicutae" (in green) includes the *H. psittacina*, *H. calyptriformis* and *H. ovina* clades (to be treated as *Gliophorus*, *Porpolomopsis*, and *Neohygrocybe,* respectively). The "Cuphophylloid grade" (in blue) contains the white and pale brown species of *Hygrocybe* in its traditional sense (to be treated as species of *Cuphophyllus*), and "tribe *Chromoserae*" (in yellow) contains the relatives of *H. xanthochroa*, which will be placed in a separate genus *Chromosera*. The sequence labels and bootstrap values have been removed to aid appreciation of the overall diversity pattern; this information is contained in the original figures submitted as appendices to this report. We are also providing updated phylogenies for each Tribe that have been interpreted to define only the recognized terminal phylogenetic taxa (i.e. "species").

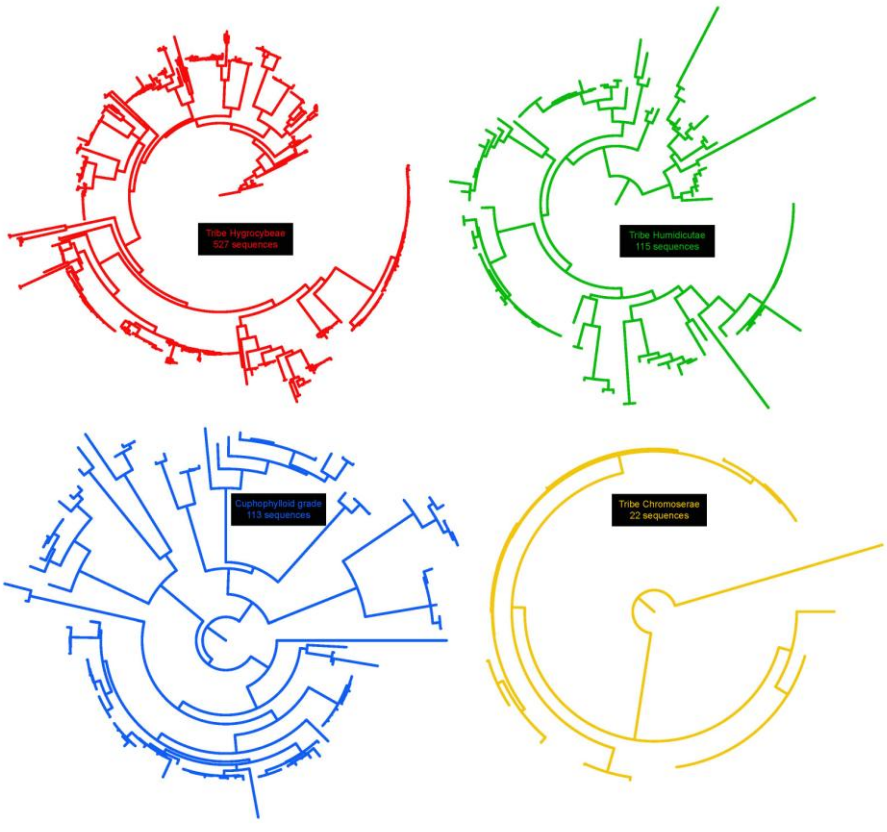

Figure 1. Overall phylogenetic diversity patterns for the four major clades within *Hygrocybe* as

traditionally circumscribed.

Hygrocybe s.l. species, demonstrating the range of pigmentation in the fruit bodies

Discussion and implications

The phylogenetic diversity within *Hygrocybe* as traditionally circumscribed is high, both at species and higher levels. A large proportion (~1/4) of species identified using morphological methods have been found to be polyphyletic, containing up to 8 or more cryptic species. In a number of cases it has been possible to adopt existing names for the newly recognized taxa, principally by sequencing Peter Orton's type collections held at Kew. This suggests that the most recent monograph (Boertmann 2010) uses too broad a species concept in some cases, and that it may be possible to separate segregate taxa using morphometric methods. In others, however, further work is required to investigate whether there are any consistent morphological characters that correlate with the phylogenetic diagnosis of these cryptic species, which could be of practical use to fieldworkers. Ultimately, however, some cryptic taxa may only be detectable by DNA sequence comparisons and an updated identification handbook will be required to summarise our results for the recording community.

If phylogenetic species concepts are adopted, our results will have a profound impact on survey and monitoring methodologies for waxcaps, at least those that rely upon citizen science approaches. In addition, the "CHEG" scoring system for grassland fungal diversity might need to be substantially altered if it is used as a simple measure of biodiversity. Our results are necessarily preliminary, but it seems commonplace for multiple cryptic species to occur within small areas, thereby potentially inflating the CHEG score. Comparison of sites also becomes more complex; sites that share morphospecies may in reality not be so similar when cryptic species are taken into account.

The project has identified the need for a complete revision of *Hygrocybe* s.l. using polyphasic techniques. We have begun this process, and anticipate that a whole series of taxonomic papers will be prepared to update our understanding of different species groups. We can then relate phylogenetic species concepts to traditional morphospecies. Once this has been done, we will be able to make a reassessment of the scientific validity of fungal surveys that rely only on field observations and/or microscopic characters provided that voucher specimens have been deposited in accessible fungaria.

We are optimistic that waxcap surveys carried out by citizen scientists will be largely confirmed as robust activities in scientific terms. Only in rare instances did we find that traditional morphospecies are paraphyletic. In general terms, therefore, it should be possible to use them to measure diversity, but in some cases by identifying species aggregates rather than phylogenetic taxa. Even so, the costs for environment-level sequencing are decreasing rapidly, and it may be more practical in future to assess site quality using such methods and an appropriate sampling methodology.

Our study has enabled us to make some assessment of the accuracy of identifications using field/microscopic methods, by comparing them with those based on sequencing. Misidentifications were common but often redeterminable using microscopic examination, but only after illumination by DNA sequencing. Misidentifications were present in both the new material that was collected for this project and in preserved specimens within the national fungaria, including specimens identified by well-known mycologists. For example, we discovered that more than1/4 of sequences generated from a recent study of fungarium specimens (Brock et al. 2009) have been misidentified, and these are currently used as reference sequences in GenBank This is to be expected bearing in mind the historically inadequate staffing levels in fungal collections; it is only practical to check the identity of a small proportion of the specimens accessed, and these institutions have ever had enough experts to represent all taxonomic groups.

Our project has involved one of the largest surveys for waxcap fungi to date, on a national scale. We have detected surprisingly high levels of phylogenetic diversity within *Hygrocybe* s.l., but the density of survey work is still insufficient for us to gain an accurate picture of the total diversity of this fungus group in the UK. Further surveys, whether carried out by traditional or environmental sequencing techniques, will be likely to result in the recognition of yet further species. The UK is rightly regarded as one of the most important areas in Europe for waxcap diversity, and our studies have further emphasized the quality of our habitats. Protection of species-rich traditionally managed grasslands should continue to be a high priority for conservation managers.

Fruit bodies of the *Hygrocybe conica* aggregate, showing the range of morphological variation. We have discovered at least 7 cryptic phylogenetic species within this aggregate.

Future work

The highest priority must be to complete the taxonomic research stimulated by this project, ensuring that the cryptic species we have detected are robust, and relate effectively to traditionally circumscribed taxa. That may well mean further surveys in partnership with our volunteer groups. There is continuing

enthusiasm for this work amongst the citizen science community, and it will be important to maintain the close relationship between fieldworkers and lab-based professional mycologists.

We are already involved in small-scale work using matching funding to investigate further the ecology and nutritional status of waxcap species. We also think it would be important to investigate environmental sequencing techniques for fungal surveys of grasslands. This may reveal a further layer of species-level diversity, which will need to be related to that detectable using existing methods. Environmental sequencing offers the potential for more objective and more rapid surveys for fungi that do not depend on the appearance of fruit bodies. There would however need to be an extended period of testing to investigate seasonal effects and patchiness of distribution.

References to published material

9. This section should be used to record links (hypertext links where possible) or references to other published material generated by, or relating to this project.

Ainsworth, A.M., Dentinger, B.T.M. & Cannon, P.F. (in preparation). Conservation of grassland fungi: Morphology and ITS barcoding reveal two new species of waxcap mushrooms (Hygrophoraceae) in Britain.

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