

Systematics, barcoding and ecology of fungi from waxcap grasslands in Britain

1. Milestones

This report describes activities since April 2011, and covers Milestones 4 (initial phylogeny available, potential cryptic species identified; end July 2011 – **see section 4**) and 5 (project workshop takes place; end September 2011 – **see section 5**). SNH additionally requested a second project workshop in Scotland, and survey work to establish whether BAP species were still present in historical locations – **see section 6**.

2. Collaborations

Collaborations between the project team and volunteer surveyors/collectors have continued to progress well, with over 70 volunteers (as individuals or members of recording groups) now actively involved in the project, primarily through collection of material for phylogenetic analysis. There has been very active interest in our project, as evidenced by the numbers of samples received and the high level of participation in the two project workshops.

We have also developed further collaborations with two US-based research groups carrying out complementary work. These are a group headed by Dr Jean Lodge (USDA Forest Service) studying the phylogeny of the *Hygrophoraceae* with special reference to generic limits and US/European differences in species name application, and one headed by Dr Andy Taylor (Illinois Natural History Survey) which focuses on phylogeny of the *Geoglossomycetes*. We have agreed to share data with both groups and will be included in relevant publications (with appropriate acknowledgement to DEFRA as funding source). These collaborations will provide a robust phylogenetic backbone for our work, and enable us to focus more strongly on the UK species-level work that is the priority for this project.

3. Collections

The 2011 field season is not yet complete, but we have already acquired over 200 further waxcap samples and around 80 collections of earthtongues. The unexpectedly high molecular phylogenetic diversity in *Hygrocybe* that was referred to in Project Report 2 meant that we needed to focus our collection efforts on problem areas within the genus, illuminated by further phylogeny research carried out in late spring and summer 2011. We have therefore requested our collaborators to send us material only from the following categories:

1. *Hygrocybe spadicea* (as a priority BAP species)
2. Taxa required for further investigation of potentially cryptic species: (*Hygrocybe acutoconica (persistens)* s.l., *H. conica* s.l., *H. insipida*, especially specimens which seem to lie between this and *H. ceracea*, *H. laeta* s.l., *H. psittacina* colour variants, *H. punicea* and *H. virginea* s.l., especially off-white specimens.)
3. Taxa required because no specimens sequenced yet by the project team (including *Hygrocybe H. calyptriformis* var. *domingensis*, *H. citrinopallida*, *H. colemanniana*, *H. subpapillata* and *H. turunda*)
4. Specimens from a list of about 20 species for which sequence data is incomplete.

A substantial number of new collections were made this autumn, in conjunction with the two workshops (for details see below) and in a survey for earthtongues requested by SNH.

4. Phylogenetic analysis

The initial results described in Project Report 2 have now been expanded upon and re-analyzed, and it is now clear that the morphological species concepts adopted in the most recent *Hygrocybe* monograph (Boertmann, 2010¹) are too broad, with many of the taxa recognized in that work consisting of multiple phylogenetic species.

Results are presented as a global ITS tree (embedded here), and summarized in Table 1 (see



HYGROCYPBE_BIG_TR
EE.pdf

Appendix).

The global tree contains sequences from other studies as well as our own, including some generated from environmental sequencing; our own can be recognized as those with references beginning with WX, KM, CAH or E00.

Further work on the waxcap samples has uncovered a somewhat higher degree of what appear to be misidentifications than was indicated in Project Report 2, in material already accessed into the Kew and Edinburgh collections as well as from our volunteer collectors. We are currently investigating these discrepancies as a high priority, via morphological examination of the material concerned and re-sequencing where appropriate.

The work on *Geoglossaceae* has lagged behind that on the waxcaps, partly due to problems in accessing fresh material but also because of prioritizing our understanding of the waxcap species aggregates. A tree containing the results to date is embedded here



Geoglossaceae_Phy
ML.pdf

and recent collecting activities have substantially increased the number of fresh samples available. Our results to date indicate that in the *Geoglossaceae* in their current accepted sense, the number of cryptic species is less than in *Hygrocybe*. However, there does seem to be a substantial degree of genetic variation within the *Microglossum* group, which contains the BAP species "*Thuemenidium*" *atropurpureum* and *Microglossum olivaceum*. There is also substantial morphological variation within these taxa and it is likely that cryptic species will be confirmed. We were able to make a number of new collections of these species last month, and will be working on them over the winter.

5. Project workshops

Two project workshops have taken place this autumn, one in Braemar (Scotland) and the other in Plas Tan y Bwlch, the Snowdonia National Park field centre.

The Braemar workshop took place between 23 and 25 September 2011, with 17 participants from a wide range of Scottish groups and institutions and three tutors – Gareth Griffith (University of Aberystwyth), Martyn Ainsworth (Kew/Natural England) and Liz Holden (Mar Lodge). Gareth and Martyn belong to the project consortium, while Liz is an expert fungal survey consultant. The workshop included presentations on waxcap grassland fungi and the sites to be visited, ecological aspects of waxcap fungi, a project progress report detailing the phylogenetic work to date, and priorities for survey work in the 2011 season. Practical sessions included survey work on five local Deeside grassland sites, where the target species proved to be abundant, with the BAP species *Thuemenidium atropurpureum* being recorded from one location. Best practice for recording was emphasized, including photography of the specimens in situ, GPS data, notes of smell, texture etc., and the limitations of field identification were discussed. Samples were brought back to the lab and further analyzed using microscopic techniques, and use of the recommended key was demonstrated. Feedback from the workshop has been very positive.

The Snowdonia workshop was held between 7 and 14 October 2011, in partnership with the British Mycological Society. The workshop focused on fungi of sand dunes and unimproved grasslands (both key habitats for our target species), with presentations and practical sessions in the evenings following survey work during the day. Field sites included sand dune ecosystems at Ynislas, Morfa Harlech and Newborough Warren, and non-dune grasslands were surveyed on acid rocks in the vicinity of the field centre and on the Lley Peninsula, and limestone at the Great Orme, Llandudno. The BMS provided all the local organization for the meeting, and paid (with funds from a Heritage Lottery grant to support fungal recording) for Dr Eef Arnolds, a well-known expert on waxcap fungi from the Netherlands, to participate as expert tutor. Presentations were also made by three members of the project team, Gareth Griffith (ecology and nutrition of waxcaps), Bryn Dentinger (progress in phylogenetic research and DNA methods) and Martyn Ainsworth (surveying fungi of conservation concern). The workshop had about 30 participants, including many of the knowledgeable volunteer collectors that have been working with us from the outset of the project. There was considerable interest in the heterogeneous nature of waxcap species as evidenced by their ITS sequences. As with the Braemar workshop, the opportunity was taken to discuss priorities for future sampling work. Around 200 collections were made and processed for DNA examination.

6. Survey of historical BAP sites

SNH requested that surveys were carried out of some of the sites in Scotland known historically to harbour BAP species from the taxonomic groups covered by the project. The first of these covered five sites in southern Scotland and the Edinburgh region at which *Hygrocybe spadicea* had been recorded, took place between 15 and 17 October 2011 and was subcontracted to Liz Holden (see section 5 above). We have not yet received a formal report of this study, but *H. spadicea* was not found fruiting at any of the locations surveyed. The work included general surveys of waxcap species (including collection of target species for phylogenetic analysis) and site condition monitoring. Two further Scottish sites were surveyed for *H. spadicea* in 2011, Pinbain Hill by Nev Kilkenny and a site in south Ayrshire by Alan Silverside; again no *H. spadicea* was found. Short-term surveys are not the ideal method for establishing the continuing presence of *Hygrocybe* species, but indications are that *H. spadicea* may either have been lost from the sites concerned, or only rarely produces fruit-bodies. Further site monitoring is to be recommended.

The second survey focused on earthtongue species. It was based on Skye, which has seven sites from which the BAP species *Thuemenidium atropurpureum* was found in the last thirty years, and a further locality for *Microglossum olivaceum*. Four mycologists took part, Paul Cannon (CABI/Kew, project leader), Dave Genney (SNH), Vince Hustad (University of Illinois) and Alan Silverside (University of the West of Scotland), with a local SNH staff member joining the group on one day. Six of the eight historical sites were re-surveyed, with one of the remaining proving impossible to locate due to ambiguous locality information. *T. atropurpureum* was re-found at three of the seven historical sites (Camasunary, Loch na Sguabaidh and Milovaig), and discovered at four further localities (Quiraing, Culnamean and Carbost Burn (both in Glen Brittle) and a further site near Milovaig. *Microglossum olivaceum* was re-found at Quiraing, and also discovered in three separate colour forms at seven new sites (Carbost Burn, Glen Eynort, Holmisdale, Milovaig, Camas Bàn, Camasunary and Talisker Bay). Full locality data, accompanying images etc. will be provided separately to SNH. One 100m square at Milovaig harboured five separate species of *Geoglossaceae* and could well be worthy of SSSI status.

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Table 1. Summary of sequencing activity to date

Species	Status	FRDBI/ABFG records	Most recent	Distribution	ITS sequences generated/available from other sources	Phylogenetic species (provisional)	Priority for further research
<i>Hygrocybe acutoconica</i>		1551	2008	ESWN	30	? 5	High
<i>Hygrocybe aurantiosplendens</i>		426	2008	ESWN	5	1	
<i>Hygrocybe calciphila</i>	RDL, WRDL	103	2008	ESWN	4	1	
<i>Hygrocybe calyptriformis</i>	ex RDL	2040	2008	ESWN	14	1	
<i>Hygrocybe canescens</i>		13	2007	SW	1	1	
<i>Hygrocybe cantharellus</i>		1001	2008	ESWN	7	3	
<i>Hygrocybe ceracea</i>		2681	2009	ESWN	13	2	
<i>Hygrocybe chlorophana</i>		4953	2009	ESWN	16	3	
<i>Hygrocybe citrinopallida</i>		15	2008	ESWN	2	1	
<i>Hygrocybe citrinovirens</i>		432	2008	ESWN	10	? 3	
<i>Hygrocybe coccinea</i>		4310	2008	ESWN	15	1	
<i>Hygrocybe coccineocrenata</i>	WRDL	132	2008	ESWN	4	1	
<i>Hygrocybe colemanniana</i>		518	2006	ESWN	2	1	
<i>Hygrocybe conica</i>		7027	2009	ESWN	21	? 8	High
<i>Hygrocybe constrictospora</i>		4	2008	S	3	1	
<i>Hygrocybe flavipes</i>		587	2006	ESWN	6	2	
<i>Hygrocybe fornicata</i>		787	2006	ESWN	5	4 or 5	
<i>Hygrocybe glutinipes</i>		1041	2008	ESWN	7	4	
<i>Hygrocybe helobia</i>		288	2008	ESWN	7	1	
<i>Hygrocybe ingrata</i>		97	2008	ESW	6	? 2	
<i>Hygrocybe insipida</i>		2567	2008	ESWN	9	2	High
<i>Hygrocybe intermedia</i>		653	2008	ESWN	6	1	
<i>Hygrocybe irrigata</i>		2103	2009	ESWN	7	3	High
<i>Hygrocybe lacmus</i>	WRDL	295	2007	ESWN	4	1	
<i>Hygrocybe laeta</i>		2121	2008	ESWN	10	2 or 3	
<i>Hygrocybe lilacina</i>	RDL	34	2006	ES	2	1	
<i>Hygrocybe marchii</i>		483	2007	ESWN	2	2	
<i>Hygrocybe miniata</i>		1540	2009	ESWN	14	5	
<i>Hygrocybe mucronella</i>		571	2006	ESWN	8	1	
<i>Hygrocybe nitrata</i>		459	2008	ESWN	5	? 3	
<i>Hygrocybe ovina</i>		288	2008	ESWN	2	1	
<i>Hygrocybe phaeococcinea</i>	WRDL	51	2007	ESWN	4	1	
<i>Hygrocybe pratensis</i>		5749	2009	ESWN	11	5	High

Species	Status	FRDBI/ABFG records	Most recent	Distribution	ITSsequences generated/available from other sources	Phylogenetic species (provisional)	Priority for further research
<i>Hygrocybe psittacina</i>		5951	2008	ESWN	8	3 or 4	High
<i>Hygrocybe punicea</i>		2392	2008	ESWN	18	3	High
<i>Hygrocybe quieta</i>		2399	2008	ESWN	13	2	High
<i>Hygrocybe radiata</i>	WRDL	42	2006	ESW	1	1	
<i>Hygrocybe reidii</i>		2169	2008	ESWN	12	1	
<i>Hygrocybe russocoriacea</i>		1618	2008	ESWN	4	1	
<i>Hygrocybe salicis-herbaceae</i>		17	2005	S	7	1	
<i>Hygrocybe spadicea</i>	BAP	98	2008	ESW	4	1	High
<i>Hygrocybe splendidissima</i>		620	2008	ESWN	10	2	
<i>Hygrocybe subpapillata</i>		17	1999	ES	1	1	High
<i>Hygrocybe substrangulata</i>		88	2008	ESN	5	2	
<i>Hygrocybe turunda</i>		141	2008	ESNW	1	1	High
<i>Hygrocybe viola</i>	RDL	5	2008	E	2	1	
<i>Hygrocybe virginea</i>		7643	2009	ESWN	18	7	High
<i>Hygrocybe vitellina</i>		333	2008	ESWN	5	1	
<i>Hygrocybe xanthochroa</i>	RDL	53	2002	ESWN	5	2	