

Project report to DEFRA 1: 3 December 2010

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

Collaborations

An important component of the project is to bring together professional scientists and specialist volunteers to contribute specimens and ecological data, and later on to explore ways of improving the existing recording and monitoring schemes. The project will provide valuable data to aid decision-making for conservation management, for example designation of SSSIs and establishment of red data lists.

The first action was therefore to publicize the project to potential collaborators, and emails and verbal communications took place both directly with known collectors/recording groups and through the British Mycological Society. Excluding those named in the project application, 33 individuals/recording groups have provided specimens and/or identification data. Our initial request was for all species of *Hygrocybe* and *Geoglossaceae*. While a number of these are common and widespread, there are concerns that existing species concepts are too broad and are masking cryptic taxa. Along with specimens that we have collected ourselves, we have received a total of 213 collections belonging to 34 species/varieties of *Hygrocybe* and four species of *Geoglossaceae*. The collections have come from 27 different vice-counties in England, Wales and Scotland. Much of the field season was poor for waxcap species, although a number of species fruited abnormally late in the year. With this in mind, we are very happy with the response we have received from field workers, and there has been a widespread welcome for our project. We are confident that our field collaborators will continue to support our work throughout the project period and beyond.

We have also contacted research groups outside of Britain to make them aware of our work, share results and avoid unnecessary duplication of effort. A group based in Puerto Rico led by Dr Jean Lodge is currently carrying out a phylogenetic survey of the *Hygrophoraceae* (that includes the genus *Hygrocybe*) on a worldwide basis, focusing especially on generic concepts within the family. Unpublished data from that project indicates that *Hygrocybe* is a paraphyletic genus with *Hygrophorus* nested within, and this is likely to lead to the splitting of *Hygrocybe* into several genera. This may have important implications for recording in the UK due to name changes, and we will undertake as part of this project to ensure that the reasons for any changes are made clear to non-specialists. Our own work is complementary to that of Dr Lodge's group as we are focusing on species concepts for taxa within Great Britain and Ireland, and we will benefit from reference sequences etc. that are being gathered by her research group.

We have also contacted Dr David Boertmann from the National Environmental Research Institute at Aarhus University, Denmark. David is an avian ecologist by profession but is a world expert on the genus *Hygrocybe* as a spare-time occupation, and the second edition of his waxcap identification guide was published in September – just after our project commenced. David has expressed a willingness to work with us, and hopefully will be included as a special guest for at least one of the workshops planned for 2011.

Collections

In addition to the fresh material gathered by ourselves and our collaborators, we have also investigated sources of specimens suitable for DNA extraction and sequencing in the major fungaria (dried fungus collections) of Great Britain. Our experience is that specimens older than 10-20 years frequently have DNA that is too poor in quality for efficient amplification, either through

gradual degradation over time, or because of unwittingly destructive preservation methods. The major collection is of course that at Kew, and Appendix 1 contains details of the number of recent specimens for all the potential target species of *Hygrocybe* and *Geoglossaceae*. In summary, there are 2169 specimens of *Hygrocybe* and *Geoglossaceae* in the Kew fungarium, of which 1049 have been collected recently enough to expect that good-quality DNA can be extracted. We also have access to around 1000 more recently collected (between 2002 and 2010) specimens at the University of Aberystwyth, and the collection data are being databased as part of the current project so that they can be included within the NBN. Further collections will be sourced as needed on a more targeted basis, primarily within the UK but from Europe if necessary.

The table in Appendix 1 includes all named species within the Kew collection, which include some that are of uncertain application or are superseded by others. Most of the species with few or no recent collections fall into this category, and will be redetermined or set aside as time allows. Prioritization of species for investigation is still in progress, but those that we are currently planning to study are emboldened in Appendix 1. The first tranche of molecular analysis may uncover anomalies that need further investigation, leading to changes in priorities. If possible we will include sequences of all species currently recognized as occurring in Great Britain and Ireland, and all currently recognized BAP species will receive full investigations.

Initial sequence analysis

The molecular sequencing programme is due to commence shortly, now that the specimens have been sourced. As an initial investigation, we have examined the ITS sequences of all European *Hygrocybe* species that are currently available in public databanks. These include a substantial number of ITS sequences from Kew specimens that were obtained from an earlier project (Brock et al., *New Phytologist* **181**: 719-724, 2009), which will be added to the sequences that we obtain to extend impact of the project. The preserved DNA samples will also be sequenced for other genes.

Alignment of the ITS region of many basidiomycetes is problematic (and *Hygrocybe* is no exception), so as specified in the project proposal we will sequence several other genes to allow a robust phylogenetic analysis. Nevertheless, the ITS sequences may well prove to be of significant utility as unique barcodes for the species concerned, paving the way for molecular diagnostics and projects using next-generation sequencing tools. The ITS sequences may also be of value in identifying cryptic species and misidentified samples. Figure 1 (As Appendix 2 below) shows a preliminary phylogenetic analysis of the sequences so far available. It must be stressed that this is an initial analysis based on an incomplete data set, but there are instances where (a) sequences are either misidentified or come from species that are not monophyletic, (b) significant ITS variation seems to occur within a monophyletic unit, and (c) ITS sequence may either indicate synonymy or cannot distinguish between species.

Ecology

Much of the work on the ecological component of the project is scheduled to take place over the winter of 2010/2011, but several visits to fieldsites have taken place and 15cm³ soil cores centred on *H. conica*/*Geoglossum* fruit bodies are now in pots outside the experimental greenhouse. These will be used as positive controls to test the primers. 10 fresh samples of *H. conica* and *Geoglossum* species (*G. fallax* and *G. cookeanum*) have been gathered from various places in Wales to permit specific primer design, and these are being designed at the moment.

Outreach

In addition to the email and verbal contact outlined above, information relevant to the project has been promulgated through print and internet media. A short announcement about the project is currently in press in *Kew Scientist*, and a rather longer message will be included in the first 2011 edition of *Field Mycologist*. This is the one single publication that reaches the largest proportion of the serious field mycological community. The project will very shortly feature on the Kew website, and a separate project website is already in existence (<http://fungi.myspecies.info/category/projects/waxcap-grassland-fungi>) that includes information about the projects, images of species collected etc. The information here will be added to regularly throughout the project life cycle. There is also an intention to include project information on the University of Aberystwyth waxcap research website, but currently technical problems are preventing this happening.

A major activity in the autumn of 2011 will be a project workshop for scientists, collectors, conservation workers etc. We have reached agreement with the British Mycological Society to hold this event at the Plas Tan y Bwlch centre in Snowdonia during the second week of October, in conjunction with a workshop that was already planned on sand dune fungi. Many of the mycological community will be present, and in addition to the project staff, two guest lecturers that are recognized experts in waxcap research will be there, Dr Eef Arnolds (funded by the BMS) and Dr David Boertmann (funded through the project).

Scotland

Work on the SNH-funded component of the project has not yet formally commenced, but we have identified substantial numbers of Scottish collections for sequencing (see Appendix 1) and we will ensure that species of particular conservation concern in Scotland will be prioritized. A field programme (including searches for Scottish BAP species) will be put together for autumn 2011 and a separate workshop will be held for Scottish participants, hopefully following on directly from the Welsh workshop and with one of its guest speakers in attendance also. Negotiations about the field work and workshop will take place during December 2010/January 2011. Specimens from the Royal Botanic Garden, Edinburgh will be accessed (probably during May 2011) and permission sought to remove small portions of relevant specimens for DNA analysis. Many of these have already been databased and made available to the NBN via an earlier Scottish waxcap project, and details of any remaining specimens will be included in our commitment to make their data accessible on the Internet.

Report submitted by:

A handwritten signature in black ink, appearing to read 'Paul Cannon', with a long horizontal line extending from the end of the signature.

Dr Paul Cannon

CABI and Royal Botanic Gardens, Kew
2 December 2010

Appendix 1. Hygrocybe and Geoglossaceae species from Great Britain: summary data and prioritization for analysis

¹ 41 = included in Section 41, 42 = included in Section 42, SBL = Scottish Biodiversity List, RDL = Provisional Red Data List (Evans et al., 2006), WRDL = Provisional Welsh Red Data List (Rotheroe 2003)

² FRDBI = Foray Records Database of Great Britain and Ireland; ABFG = Association of British Fungus Groups CATE database. There is likely to be some overlap between these datasets.

³ Most recent year recorded in FRDBI.

⁴ E = England, S = Scotland, W = Wales, N = N Ireland.

⁵ ITS sequences present on GENBANK – many not derived from GBI material.

⁶ Name of uncertain affinity – at present the list of uncertain species is incomplete.

⁷ **Priority species for molecular analysis**

Species	Status ¹	P ⁷	FRDBI/ABFG records ²	Most recent ³	Distrib-ution ⁴	ITS ⁵	Dried specimens at Kew					
							England	Wales	Scotland	NI	<10yr old	>10, <20 yr old
Geoglossum arenarium		P	22	2008	ES	0	8	0	2	0	2	1
Geoglossum atropurpureum	41, 42	P	198	2007	ESWN	2	18	15	15	5	15	7
Geoglossum barlae			8	2000	ESN	0	0	0	0	0	0	0
Geoglossum cookeanum			775	2006	ESWN	2	83	13	1	8	37	6
Geoglossum difforme			24	1993	EW	0	0	0	0	0	0	0
Geoglossum elongatum	SBL, RDL	P	25	2005	ESWN	0	4	0	1	1	2	0
Geoglossum fallax			1004	2008	ESWN	2	83	10	7	11	47	15
Geoglossum glabrum			51	1975	ESWN	1	7	0	0	0	0	1
Geoglossum glutinosum	WRDL		474	2006	ESWN	2	41	4	3	6	19	4
Geoglossum nigrum			169	2004	ESWN	1	43	0	9	0	0	8
Geoglossum peckianum	RDL		11	2000	ES	0	0	0	0	0	0	0
Geoglossum simile			4	2006	ES	0	1	0	0	0	0	1
Geoglossum sphagnophilum			1	2004	W	0	0	1	0	0	1	0
Geoglossum starbaeckii	SBL, RDL, WRDL	P	53	2005	ESW	0	5	1	10	0	0	0
Geoglossum uliginosum			9	2006	SWN	0	0	0	1	0	2	0
Geoglossum umbratile	WRDL		385	2006	ESWN	3	30	5		9	38	7
Geoglossum viscosum			12	2005	EW	0	0	0	0	0	0	0
Geoglossum vleugelium			8	2000	ES	0						
Hygrocybe acutoconica (syn. <i>H. persistens</i>)			1796	2008	ESWN	3	37	10	28	2	20	17
Hygrocybe acutoconica var. konradii			258	2008	ESW	0	25	1	0	0	5	5
Hygrocybe aurantia	WRDL	P	9	1987	EW	0	2	2	0	0	0	0
Hygrocybe aurantiosplendens			426	2008	ESWN	3	17	6	3	1	15	4
Hygrocybe calciphila	SBL, RDL, WRDL	P	103	2008	ESWN	1	8	3	1	0	9	3
Hygrocybe calyptriformis	SBL	P	2040	2008	ESWN	5	56	7	7	4	30	19
Hygrocybe canescens			13	2007	SW	0	0	0	1	0	1	0
Hygrocybe cantharellus			1001	2008	ESWN	4	28	3	13	0	13	11
Hygrocybe ceracea			2681	2009	ESWN	4	47	3	14	4	15	5
Hygrocybe chlorophana			4953	2009	ESWN	5	61	10	32	3	21	16

Species	Status ¹	P ⁷	FRDBI/ABFG records ²	Most recent ³	Distrib- ution ⁴	ITS ⁵	Dried specimens at Kew					
							England	Wales	Scotland	NI	<10yr old	>10, <20 yr old
Hygrocybe citrinopallida			15	2008	ESWN	1	0	0	0	0	0	0
Hygrocybe citrinovirens		P	432	2008	ESWN	5	13	8	1	0	9	9
Hygrocybe coccinea			4310	2008	ESWN	7	36	4	17	4	16	9
Hygrocybe coccineocrenata	WRDL	P	132	2008	ESWN	0	4	0	1	0	2	1
Hygrocybe colemanniana			518	2006	ESWN	2	12	3	0	0	8	3
Hygrocybe conica			7027	2009	ESWN	4	54	4	43	2	16	26
Hygrocybe conica var. conicoides		P	413	2008	ESWN	5	12	5	4	1	5	3
Hygrocybe constrictospora			4	2008	S	0	0	0	0	0	0	0
Hygrocybe flavipes		P	587	2006	ESWN	5	18	8	4	3	24	11
Hygrocybe fornicata			787	2006	ESWN	3	32	7	12	4	23	12
Hygrocybe fornicata var. lepidopus			18	2004	ESW	0	1	0	0	0	0	0
Hygrocybe glutinipes			1041	2008	ESWN	4	34	1	6	2	24	4
Hygrocybe glutinipes var. rubra			19	2008	ESW	0	2	0	0	0	0	2
Hygrocybe helobia		P	288	2008	ESWN	3	11	0	3	0	7	6
Hygrocybe ingrata		P	97	2008	ESW	2	3	2	2	0	7	0
Hygrocybe insipida			2567	2008	ESWN	2	54	6	6	5	26	13
Hygrocybe intermedia		P	653	2008	ESWN	2	14	8	11	0	13	9
Hygrocybe irrigata			2103	2009	ESWN	1	49	5	11	4	21	13
Hygrocybe lacmus	WRDL	P	295	2007	ESWN	2	13	5	10	1	6	4
Hygrocybe laeta			2121	2008	ESWN	3	40	6	19	5	19	10
Hygrocybe laeta var. flava			9	2005	EWN	0	0	1	0	0	2	0
Hygrocybe lilacina	SBL, RDL	P	34	2006	ES	0	0	0	0	0	0	0
Hygrocybe marchii		P	483	2007	ESWN	2	8	1	2	0	3	3
Hygrocybe miniata			1540	2009	ESWN	9	83	8	23	0	13	4
Hygrocybe mollis			48	2006	ESWN	0	3	3	6	0	0	0
Hygrocybe mucronella		P	571	2006	ESWN	3	16	4	1	3	18	3
Hygrocybe nitrata		P	459	2008	ESWN	2	13	7	4	0	6	1
Hygrocybe obrussea ⁶			89	2004	ESWN		9	0	1	0	0	0
Hygrocybe ovina			288	2008	ESWN	0	14	6	4	0	6	2
Hygrocybe phaeococcinea	WRDL	P	51	2007	ESWN	3	5	5	0	1	11	0
Hygrocybe pratensis			5749	2009	ESWN	1	53	4	18	2	19	11
Hygrocybe pratensis var. pallida		P	684	2009	ESWN		11	5	12	1	7	7
Hygrocybe psittacina			5951	2008	ESWN	6	41	5	10	2	20	10
Hygrocybe psittacina var. perplexa		P	157	2009	ESWN		3	2	2	0	3	1
Hygrocybe punicea			2392	2008	ESWN	4	25	9	11	7	26	6
Hygrocybe quieta			2399	2008	ESWN	4	40	1	20	5	21	14
Hygrocybe radiata	WRDL	P	42	2006	ESW	0	7	3	0	0	9	1
Hygrocybe reidii			2169	2008	ESWN	2	37	8	15	4	18	14

Species	Status ¹	P'	FRDBI/ABFG records ²	Most recent ³	Distrib- ution ⁴	ITS ⁵	Dried specimens at Kew					
							England	Wales	Scotland	NI	<10yr old	>10, <20 yr old
Hygrocybe russocoriacea			1618	2008	ESWN	1	33	2	10	7	20	6
Hygrocybe salicis-herbaceae		P	17	2005	S	0	0	0	1	0	0	1
Hygrocybe spadicea	41, 42, SBL	P	98	2008	ESW	1	6	1	1	0	2	3
Hygrocybe splendidissima			620	2008	ESWN	2	13	11	4	2	14	3
Hygrocybe subpapillata			17	1999	ES	1	2	0	0	0	0	1
Hygrocybe substrangulata			88	2008	ESN	1	6	0	5	0	1	2
Hygrocybe turunda			141	2008	ESNW	1	7	3	1	0	1	0
Hygrocybe viola	RDL	P	5	2008	E	0	2	0	0	0	1	1
Hygrocybe virginea			7643	2009	ESWN	1	55	3	18	6	14	18
Hygrocybe virginea var. fuscescens			266	2007	ESWN		2	1	0	0	5	0
Hygrocybe virginea var. ochraceopallida			549	2009	ESWN		3	0	0	1	3	2
Hygrocybe vitellina			333	2008	ESWN	2	14	4	4	2	10	2
Hygrocybe xanthochroa	SBL, RDL	P	53	2002	ESWN	2	0	0	2	1	1	1
Microglossum olivaceum	41, 42, SBL	P	391	2008	ESWN		61	32	1	7	45	9
Microglossum viride		P	221	2008	ESWN		31	3	5	2	2	6
Trichoglossum hirsutum			784	2009	ESWN		67	9	1	5	29	6
Trichoglossum rasum	RDL, WRDL		11	1985	EW		4	1	0	0	0	0
Trichoglossum tetrasporum	RDL		5	2000	E		1	0	0	0	1	0
Trichoglossum variabile			12	2003	ESW		1	1	0	0	1	1
Trichoglossum walteri	RDL	P	81	2008	ESWN		10	4	1	1	1	7

Appendix 2.
Preliminary
phylogenetic
arrangement based on
existing ITS sequences

