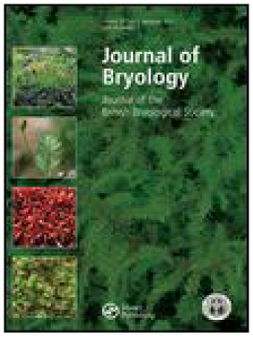

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Population status and ecology of the episodic moss *Physcomitrium eurystomum* Sendtn. in Britain

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ABSTRACT

Introduction. *Physcomitrium eurystomum* is rare in Europe and threatened with extinction. This study investigates its status and ecology in Britain.

Methods. A detailed search was made for *P. eurystomum* at all sites where it has been reported in Britain, plus four other locations nearby. Geographic coordinates of colonies were recorded with GPS units and used to derive counts of occupied Ordnance Survey (OS) grid cells at resolutions of 1, 10 and 100 m. DNA barcoding was used to help identify non-fruiting plants. Habitat and community composition were recorded by relevés.

Results. In this study *Physcomitrium eurystomum* was found at five locations, one in Hertfordshire and four in West Norfolk, and occupied 109 OS 1 m grid cells. A small pond in West Norfolk (Wicken Pond) supported the majority (61%) of the population. DNA barcoding helped confirm its occurrence at Wilstone Reservoir (Hertfordshire), at which it was thought extinct. The moss occured exclusively within the drawdown zone of seasonally fluctuating freshwater bodies, both natural and artificial. Vegetation was dominated by vascular plants, most frequently *Agrostis stolonifera, Persicaria lapathifolia, Ranunculus sceleratus, Rorippa palustris* and *Stellaria aquatica*. Soil pH varied from strongly acidic to slightly alkaline.

Conclusions. Unsuccessful searches of five sites with historic records raises concern for the species. The significant importance of Wicken Pond has not been recognised previously and suggests statutory protection of the site is warranted. The non-native and invasive *Crassula helmsii* is a major risk to *P. eurystomum* at sites in Britain and elsewhere in Europe.

KEYWORDS
Crassula helmsii; DNA barcoding; Funariaceae

Introduction

Physcomitrium Mitt. (Funariales: Funariaceae) is a genus of ephemeral acrocarpous mosses, including 66 species accepted by MBG (2019). *Physcomitrium eurystomum* Sendtn. (Norfolk Bladder-moss), a species that appears to have arisen from a hybridisation event between two lineages in the *Physcomitrium–Physcomitrella* complex (McDaniel et al. 2010; Beike et al. 2014), is a Temperate Eurasian species (Hill and Preston 1998), reported from Africa (Kenya, Malawi, Tanzania and Uganda), Asia (China, Hong Kong, India, Japan and Turkey), Australasia (New Guinea) and Europe (Porley 2013). It is categorised as 'Vulnerable' on the IUCN Red List of bryophytes in Europe (Hodgetts et al. 2019) and is widespread but rare across the continent, occurring in Austria, Belarus, Belgium ('threatened'), Britain (Endangered), Bulgaria, Croatia, Czech Republic (Vulnerable), Denmark, Estonia (Vulnerable), France, Germany (Vulnerable), Hungary (Endangered), Italy (mainland and Sicily), Lithuania, Montenegro (Stešević et al. 2020). The Netherlands ('susceptible'), Poland, Romania, Russian Federation (Central European Russia), Serbia, Slovakia (Vulnerable), Slovenia (Data Deficient), Switzerland (Vulnerable) and Ukraine (Papp et al. 2013; Hodgetts 2015; Puglisi and Privitera 2018; Rimac et al. 2019). In Britain, the species was first reported from Langmere (West Norfolk, v.-c. 28) by Ducker and Warburg (1961) and has so far been found from a small number of locations in Hertfordshire (v.-c. 20) and elsewhere in West Norfolk (Blockeel et al. 2014). The aim of this study was to investigate the population status and ecology of the species in Britain to help inform conservation decisions and research priorities. Taxonomy follows Hill et al. (2008) for bryophytes and Stace (2019) for vascular plants.

Methods

Distribution and abundance

Geographic coordinates in this paper follow the Ordnance Survey (OS) National Grid reference system. An inventory of all locations from which *P. eurystomum* has been reported in Britain was prepared from: (1) a review of biological records within the national recording database of the British Bryological Society (BBS), held by the Biological Records Centre (Wallingford, UK); (2) details on vouchers held in herbaria (BBSUK, BM, E and NMW); (3) published literature (Ducker and Warburg 1961; Warburg 1962; Bloom 1982, 1983; Swann 1982; Smith 1996; Beckett et al. 1999; Smith 2004; Tipper 2009, 2010; Porley 2013; Blockeel et al.

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2014) and (4) correspondence with local experts. All sites within the subsequent inventory ($n = 9$), plus four other locations nearby, were surveyed during 7–10 October 2019. A brief return to some sites was made during 27–28 December 2019. At each site, a detailed search was made for *P. eurystomum* and locations of fruiting colonies indicated by a temporary marker flag, ignoring further locations if <1 m from an existing flag. Non-fruiting plants were ignored, since sporophyte morphology provides the only reliable diagnostic feature in the field. Coordinates of all flagged locations were recorded with a hand-held GPS (Garmin GPSMAP 64s, Garmin Ltd, Olathe, USA), which consistently reported accuracy of ≤4 m. At one site, Wicken Pond, a Trimble Geo 7XT (Trimble, Sunnyvale, USA) was used, which reported mean accuracy of 0.42 m (range = 0.35–0.58 m). Subsequent mapping was undertaken using Quantum GIS (Quantum GIS Development Team, 2019), including counts of OS grid cells occupied by flagged colonies at resolutions of 1, 10 and 100 m.

DNA barcoding

Non-fruiting patches of Funariaceae were frequent at some sites. Samples of such ($n = 4$) were collected during October 2019 from three sites (Fowl Mere, Ringmere and Wilstone Reservoir). These were subject to DNA barcoding and compared with a voucher specimen of *P. eurystomum* from another site (Wicken Pond), plus vouchers of other Funariaceae previously sequenced. DNA was extracted with the commercial kit NucleoSpin Plant II Mini (Macherey-Nagel, Düren, Germany) following the instructions provided by the manufacturer (PL1 as lysis buffer). Considering the hybrid origin of the species, different *loci* were targeted in both the plastid and nuclear genomes by PCR. Within the plastid genome, two *loci* were targeted: *psb*A-*trn*H and *trn*L-F, while within the nuclear compartment, we sequenced two flanking regions associated to the genes 4780 and 7379 used in the Targeted Enrichment capture approach described in Medina et al. (2019). The primers used for the nuclear *loci* were newly designed by Matt Johnson and Nikisha Patel: 4780F = ATGGACGGCGCACTTGTTA; 4780R = CTTGTAACGTCGCTTCAGATTTT; 7379F = TCACGTTGGACCATGTGACG; and 7379R = CGTTCAAACGCCTCTCATTG.

Polymerase Chain Reactions (PCRs) were conducted in final volumes of 50 µL, with 0.15 µL of GoTaq DNA polymerase (Promega, Madison WI, USA), 1 µL of 10 µM dNTP mix, 1 µL of each primer (10 µM) and 1 µL of DNA extract. The thermal cycler was programmed with a hot start denaturation step of 5 min at 94°C, followed by 30 cycles of denaturation (1 min, 94°C), annealing (1 min, 50°C) and extension (1 min, 70°C), ended by a final extension step of 10 min. Amplification products were visualised in 1% agarose gels, cleaned used the ExoSAP-IT protocol (USB-Affymetrix, Cleveland OH, USA), and sent for sequencing to Macrogen through the EZ-sequencing service. Contigs were generated using Geneious (Kearse et al. 2012; http://www.geneious.com), keeping track of all double peaks shown in both reads of each sequence of the nuclear *loci* using the standard IUPAC ambiguous nucleotide letters in the consensus sequence, and then compared visually on PhyDe (Müller et al. 2006; http://www.phyde.de).

Habitat and community composition

Relevés were recorded to describe habitat conditions and community composition of locations occupied by *P. eurystomum*, generally following the method of Bates (2011). Sample locations were selected to represent the full range of conditions occupied by the moss. Relevés measured 50 × 25 cm, and within each, percentage cover of each species of bryophyte, vascular plants, lichen and macroalgae present was estimated. Percentage cover of dead plant material ('litter') and bare ground was also recorded in each relevé. Shade was recorded according to the following index: 1 = fully exposed to sunlight at all times; 2 = shaded from direct sunlight for up to half the day; 3 = receiving significant direct sunlight but for less than half the day; 4 = moderately shaded from direct sunlight, e.g. by a light-medium deciduous tree canopy; 5 = permanently shaded from direct sunlight but otherwise open to the sky (i.e. with north-facing aspect); 6 = in deep woodland (e.g. coniferous) shade with no sunflecks; 7 = in perpetual, very deep shade. Slope was measured with a digital clinometer, recorded as the average angle (°) from horizontal in the direction of greatest slope. Aspect was recorded as the bearing (°) of the relevé using the above GPS unit. Soil pH was measured by mixing a 1:1 ratio of soil with distilled water, leaving to stand for 30 mins and taking a pH reading of the slurry with a calibrated Hanna HI-98130 unit (Hanna Instruments, Woonsocket, USA).

Results

Distribution and abundance

Physcomitrium eurystomum was found at five locations, one in Hertfordshire and four in West Norfolk (Table 1).

Table 1. Number of Ordnance Survey grid cells occupied by *Physcomitrium eurystomum* during present survey.

		N° occupied OS grid cells		
Site	OS grid reference	1 m	10 m	100 m
Hertfordshire				
Wilstone Reservoir	SP905131	2	1	1
West Norfolk				
Fenmere	TL909881	21	4	1
Langmere	TL906884	4	3	1
Ringmere	TL909879	15	9	3
Wicken Pond	TF836314	67	5	2
TOTAL:		**109**	**22**	**8**

Wicken Pond (West Norfolk) supported the majority of the national population, including 67 (61%) occupied OS 1 m grid cells (Figure 1). Figure 2 shows occupancy of OS 10 km grid cells in Britain. Three locations from which the moss has not previously been recorded, but are close to historic sites, were searched without success, including College Lake (Buckinghamshire; SP932142), Marsworth Reservoir (Hertfordshire; SP921137) and Tringford Reservoir (Hertfordshire; SP918133). Five locations with historic records were also searched without success, including Devil's Punchbowl (West Norfolk; TL878891), Fowl Mere (West Norfolk; TL879895), Home Mere (West Norfolk; TL893896), Startop's End Reservoir (Hertfordshire; SP918138), and an unnamed small mere south of Mouse Hall (TL855908).

DNA barcoding

Four non-fruiting Funariaceae specimens (87708, 87709, 87710 and 87735) were barcoded and compared with sequences of the reference voucher (87743) for *P. eurystomum* (Table 2). The sequences of specimen 87735, from Wilstone Reservoir, matched the latter identically in all nucleotides of sequences of pastid loci *psb*A-*trn*H and *trn*L-F. Regarding the nuclear loci, this sample also matched in all nucleotides the reference sequences of loci 4780 and 7379, including a number of sites that showed double peaks in the forward and reverse reads of the chromatograms (six unique double peak singletons in 4780 and three in 7379). The remaining samples (87708, 87709 and 87710) were identical but did not match the reference *P. eurystomum*, differing in both plastid (six substitutions in *psb*A-*trn*H, and seven substitutions and four indels in *trn*L-F) and nuclear *loci* (partially compatible with the reference, but showing a different pattern of double peaks not present in *P. eurystomum*). Based on available data on GenBank from the plastid loci, the non-*P. eurystomum* samples can be ascribed to a clade of *P. pyriforme*.

Habitat and community composition

Physcomitrium eurystomum was found exclusively within the drawdown zone of seasonally fluctuating freshwater bodies (Figure 3). Results from relevés ($n = 20$; Table 3) show the moss occupied vegetation dominated by vascular plants, most frequently *Agrostis stolonifera, Persicaria lapathifolia, Ranunculus sceleratus*, *Rorippa palustris* and *Stellaria aquatica*, with affinities to the phytosociological communities OV28 *Agrostis stolonifera—Ranunculus repens*, OV31 *Rorippa palustris—Filaginella uliginosa* and OV32 *Myosotis scorpioides—Ranunculus sceleratus* (Rodwell 2000). Macroalgae and lichens were absent from relevés, and liverworts rare. Bare ground occurred in all relevés, with up to 70% cover (mean = 28%; range = 2–70%). Plant litter was frequent, though usually in small quantity (mean = 5.7%; range = 0–50%). Significant shade was mostly absent. Soils were either sandy loams (Fenmere, Langmere and Ringmere) or silty clays (Wicken Pond and Wilstone Reservoir), with pH averaging neutral (mean = 6.6), though ranging from strongly acidic (4.7) to slightly alkaline (7.6). All relevés were on very gently sloping ground (mean slope = 3.5°; range = 0.3–7°), with no aspect preference.

Site accounts

The account below provides details of locations where *P. eurystomum* has been recorded historically and/or during the present survey. Figure 4 shows locations of the species recorded during the present survey.

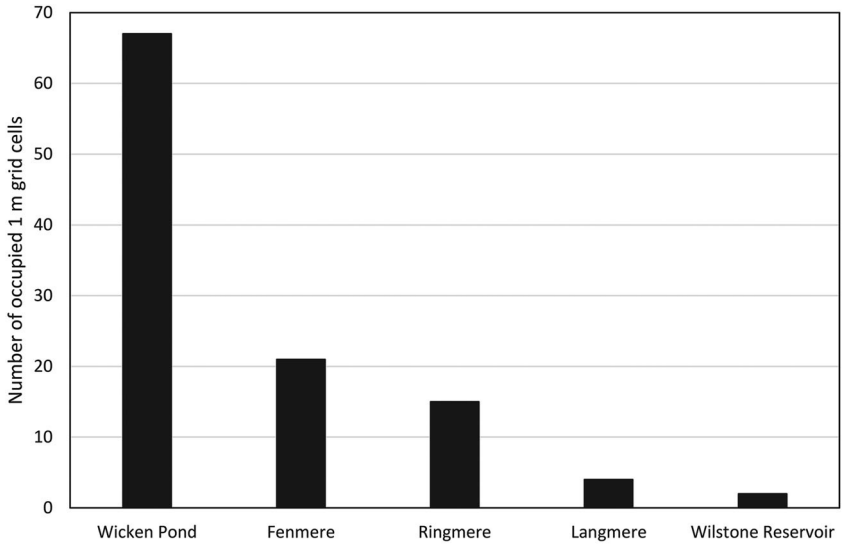

Figure 1. Number of Ordnance Survey 1 m grid cells occupied by *Physcomitrium eurystomum* during present survey.

Figure 2. Occupancy of Ordnance Survey 10 km grid cell by *Physcomitrium eurystomum* in Britain.

Table 2. Details of samples investigated by DNA barcoding.

Sample	Site	OS grid reference	Provisional identification	Confirmed identification	GenBank accessions *trn*L-F; *psb*A-*trn*H; reg4780; reg7379
87743	Wicken Pond	TF8361331444	n/a	*Physcomitrium eurystomum*	MT158326; MT158331; MT158336; MT158340
87708	Fowl Mere	TL8795989347	Funariaceae (no sporophytes)	*Physcomitrium pyriforme*	MT158327; MT158332; MT158337; ————
87709	Fowl Mere	TL8796489482	Funariaceae (no sporophytes)	*Physcomitrium pyriforme*	MT158328; MT158333; ————–; MT158341
87710	Ringmere	TL9099687947	Funariaceae (no sporophytes)	*Physcomitrium pyriforme*	MT158329; MT158334; MT158338; MT158342
87735	Wilstone Reservoir	SP9008913059	Funariaceae (immature sporophytes)	*Physcomitrium eurystomum*	MT158330; MT158335; MT158339; MT158343

Figure 3. Habitat and locations (red dots) of *Physcomitrium eurystomum* at (A) Fenmere (8 October 2019), (B) Langmere (9 October 2019), (C) Ringmere (8 October 2019), (D) Wicken Pond (7 October 2019) and (E) Wilstone Reservoir (27 December 2019). Photographs: D. A. Callaghan.

Table 3. Percentage cover of plant species and environmental data from relevés occupied by *Physcomitrium eurystomum* within the study sites.

	Relevés (% cover)[a]																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Liverworts																				
Riccia cavernosa																	0.5	0.2		
Mosses																				
Aphanorrhegma patens	0.5														2	2	1	1	1	
Bryum klinggraeffii																				0.1
Drepanocladus aduncus						0.1	3	2		0.1	0.1		0.1	0.1		3	0.5	0.1	0.1	
Leptobryum pyriforme		0.1	0.1	0.1						0.1	0.1	0.1	0.1	0.1	0.1	1				5
Physcomitrium eurystomum	1	1	2	7	5	2	0.5	0.5	2	8	10	20	7	5	8	80	15	17	8	5
Vascular plants																				
Agrostis stolonifera	50	2	5	1	5	50	55	20	35	80	90	50	25	65		1	0.5			
Chenopodium rubrum															15	5			4	
Cirsium arvense										1										
Conyza canadensis																1	1			
Crassula helmsii																				5
Epilobium hirsutum																		5		
Epilobium sp.		0.5		1											1	0.5	2			
Juncus bufonius	7	5	15	10	20															
Lycopus europaeus	1																			
Lythrum portula			0.5																	
Medicago lupulina										1										
Mentha aquatica	1									0.5			4	4	1					
Persicaria lapathifolia	5		4	2	5										10	3	30	10	8	
Phalaris arundinacea													5	2						
Potentilla anserina						6	15	10	5	0.5			10	2						
Ranunculus repens						15	15	8	5											
Ranunculus sceleratus	6	15	15	35	30											0.5	1	0.5	4	
Ranunculus sp. (subgen. *Batrachium*)			0.1												0.5		0.5	0.2	1	0.1
Rorippa palustris	2		3		2	2		6	4						10		10	10	8	
Rumex crispus	2	5		10	6															
Rumex maritimus															3					
Sagina procumbens							1													
Sonchus asper															2	4		5	1	
Stellaria aquatica	2					18	5	35	30	5	1	0.5	5	1						5
Urtica dioica									5						0.1	1			0.5	
Veronica catenulata																				1
Bare ground (%)	20	70	55	35	25	7	5	10	10	5	2	10	40	20	50	4	40	55	70	30
Litter (%)	3	3	3	5	2	1	1	10	4	3	0	20	4	1	0	1	0	0	3	50
Shade Index	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	1	2
Aspect (°)	60	76	63	40	36	358	35	40	232	198	222	246	39	271	352	309	8	56	98	51
Slope (°)	3	5	3.5	5	4.5	2.5	7	4.3	5.6	4.4	4.5	2.4	6	5	0.3	1.3	1	0.4	0.8	4
Soil pH	6	5.8	6.1	5.8	6.2	5.3	5.1	4.7	5.3	7.4	7.4	7.5	7.6	7.5	7.1	7.3	7.3	7.4	7.2	7.4
Soil texture[b]	SL	SL	SL	SL	SL	SL	SL	SL	SL	SL	SL	SL	SL	SL	SC	SC	SC	SC	SC	SC

[a]Relevés 1–5 = Fenmere, 6–9 = Langmere (south-west basin), 10–14 = Ringmere, 15–19 = Wicken Pond, 20 = Wilstone Reservoir.
[b]SL: Sandy Loam; SC: Silty Clay.

Figure 4. Locations of *Physcomitrium eurystomum* recorded during the present survey, at (A) Fenmere (northern waterbody) and Ringmere (southern), (B) Langmere, (C) Wicken Pond, and (D) Wilstone Reservoir. Satellite image captured 27 September 2018. Satellite image © Google, DigitalGlobe.

West Norfolk (vice-county 28)

Devil's Punchbowl (TL878891). Owned and managed by the Ministry of Defence (MOD) and within Stanford Training Area Site of Special Scientific Interest (SSSI). The only report of *P. eurystomum* is an unconfirmed record listed by Swann (1982), without details. The waterbody was dry during the present survey and the bed covered in a dense mat of vegetation, largely comprising *Phalaris arundinacea*. No *P. eurystomum* was found and no suitable habitat patches were present.

Fenmere (TL909881). Owned and managed by Norfolk Wildlife Trust and within East Wretham Heath SSSI. There are no previous records of *P. eurystomum*, but a good population was discovered on 8 October 2019 (Figures 3A and 4A), including 21 occupied OS 1 m grid cells (Table 1). During a return visit on 28 December 2019, all suitable habitat was under water.

Fowl Mere (TL879895). Owned and managed by the Ministry of Defence (MOD) and within Stanford Training Area SSSI. There is a single record of *P. eurystomum* from this waterbody, on the west side of the mere by R. P. Libbey and E. L. Swann in 1973. The waterbody was dry during the present survey and the bed covered in a dense mat of vegetation, especially *Agrostis stolonifera* and a dense central stand of *Persicaria lapathifolia*. Mammal activity maintains some small patches of bare ground and non-fruiting tufts of Funariaceae occurred occasionally. DNA sequencing of two samples confirmed the identity of both as *Physcomitrium pyriforme* (Table 2).

Home Mere (TL893896). Owned and managed by the Ministry of Defence (MOD) and within Stanford Training Area SSSI. *Physcomitrium eurystomum* has been found once at this location, by A. L. Bull (det. C. R. Stevenson) on 29 March 1990. The waterbody was dry during the present survey and the bed covered in a

dense mat of vegetation, comprising *Agrostis stolonifera* and *Potentilla anserina*. Disturbance from vehicle activity had created some bare ground, but it was dry and no *P. eurystomum* was found.

Langmere (TL906884). Owned and managed by Norfolk Wildlife Trust and within East Wretham Heath SSSI. A waterbody comprising three adjacent basins, Langmere is the best-known location for *P. eurystomum* in Britain. It was found here first by B. F. T. Ducker on 8 January 1961, from which it was described new for the British Isles (Ducker and Warburg 1961). A large population has sometimes been reported, for example in 1973, 2005 and 2016. During the present survey, all three basins were dry, and the drawdown zone of the banks largely comprised a dense turf of *Agrostis stolonifera*, often with abundant *Drepanocladus aduncus* and *Potentilla anserina*. The beds of the basins were dominated by *Stellaria aquatica* and *Urtica dioica*. A small amount of *P. eurystomum* was present on the bed of the south-west basin (four occupied OS 1 m grid cells; Figures 3B and 4B). Small patches of non-fruiting Funariaceae were scattered around all three basins.

Mouse Hall, unnamed mere south of (TL855908). Owned and managed by the Ministry of Defence (MOD) and within Stanford Training Area SSSI. The only report of *P. eurystomum* was by J. A. Paton, of uncertain date but likely 1961 (J. A. Paton pers. comm.). No specimen has been traced and no further information is thought to exist. The small waterbody, mostly dry during the present survey, now offers no suitable habitat for the species, with vegetation dominated by a dense turf of grasses and rushes, plus *Salix* scrub.

Ringmere (TL909879). Owned and managed by Norfolk Wildlife Trust and within East Wretham Heath SSSI. The first record of *P. eurystomum* from Ringmere was by P. W. Richards on 18 June 1972, with subsequent reports in 1990, 2005 and 2011. The waterbody was dry during the present survey and the bed covered in a dense mat of vegetation, comprising *Agrostis stolonifera*, *Stellaria aquatica* and a central stand of *Persicaria lapathifolia*. *Physcomitrium eurystomum* was found scattered widely around the periphery in small quantity (15 occupied OS 1 m grid cells; Figures 3C and 4A). Non-fruiting tufts of Funariaceae occurred more frequently, including higher in the drawdown zone than *P. eurystomum*. DNA sequencing of a single non-fruiting sample from the upper drawdown zone confirmed its identity as *P. pyriforme* (Table 2).

Wicken Pond (TF836314). Owned and managed by Norfolk Wildlife Trust. No statutory protection, but adjacent to Syderstone Common SSSI. The first record of *P. eurystomum* from this small (0.09 ha) waterbody is from 17 December 2006 by C. Dunster (det. C. R. Stevenson). There are no subsequent records until the present survey, when a strong population was found

Figure 5. Mature and immature sporophytes of *Physcomitrium eurystomum*, amongst non-fruiting *Leptobryum pyriforme*, at Wilstone Reservoir, 27 December 2019. Photograph: D. A. Callaghan.

(67 occupied OS 1 m grid cells; Figures 3D and 4C). Wicken Pond was significantly deepened some time before 1983, to increase water storage capacity for use in farmland irrigation. It was subsequently donated to The Norfolk Wildlife Trust and for conservation reasons, in January 1997 the bed was raised by infilling with chalk overburden from a local quarry. Vegetation is cut annually, and arisings removed to adjacent ground. DNA barcoding of a reference sample was undertaken (Table 2).

Hertfordshire (vice-county 20)

Startop's End Reservoir (SP918138). Owned by the Canals and Rivers Trust, managed by Herts and Middlesex Wildlife Trust, and within Tring Reservoirs SSSI. The only records of *P. eurystomum* are from October and November 1969, represented by several herbarium vouchers (BM and NMW). During the present survey, the only potential habitat for the moss was a small area of exposed mud at the southern end of the reservoir, bordering *Salix* scrub. The non-native and invasive *Crassula helmsii* blankets large parts of the upper drawdown zone. No *P. eurystomum* was present.

Wilstone Reservoir (SP905131). Owned by the Canals and Rivers Trust, managed by Herts and Middlesex Wildlife Trust, and within Tring Reservoirs SSSI. *Physcomitrium eurystomum* was first found here by E. F. Warburg in December 1961, shortly after its discovery in Britain, 125 km north-east at Langmere. It was

seen again in 1962 and 1969. An unsuccessful search was made in 1995 (Smith 1996). A very small population was found during the present survey (two occupied OS 1 m grid cells; Figures 3E, 4D and 5), towards the upper margin of the drawdown zone in a shallow depression dominated by *Stellaria aquatica*, amidst extensive mats of *Crassula helmsii*. DNA barcoding of a sample was undertaken (Table 2).

Discussion

This is the first detailed assessment of the status of *P. eurystomum* in Britain, a species threatened with extinction in Europe. Results confirm its presence at five waterbodies, including one newly discovered during the present survey. Rediscovery of a small population within Tring Reservoirs, specifically Wilstone Reservoir, is significant, since the last record from this reservoir complex was in 1969 and the species was considered locally extinct. The significant importance of Wicken Pond for this species, illustrated by the present study, is especially noteworthy given the habitat restoration measures undertaken, and suggests statutory protection of the site is warranted. The moss is known to have responded positively to habitat restoration measures elsewhere, including excavation of shallow pools in an area of the Moravian Karst, Czech Republic (Nováková et al. 2015). Counts of grid cells occupied by *P. eurystomum* are not available from surveys at other sites, either within Britain or elsewhere in Europe. Although hopefully the method will be adopted for future work, especially since occupancy of 1 m grid cells corresponds with the concept of 'individual equivalents', developed recently for assessment of bryophyte species against the IUCN Red List criteria (Bergamini et al. 2019). Counts of sporophytes in The Netherlands, with no more than 40 reported from any site (Nieuwkoop 2016), suggests Dutch locations tend to support much smaller populations than some of the British sites.

The present study failed to locate *P. eurystomum* at five sites from where it has been recorded historically, which raises concern for the species. Such sites may have only been occupied briefly, by sink populations of short longevity due to low habitat quality, or they may have been more permanent populations that have undergone long-term decline, for example due to habitat deterioration. The moss is known to form a persistent diaspore bank (Poschlod 1993; Eckstein 2006; Malkowsky et al. 2018), and likely survives underground as dormant spores for prolonged periods, similar to the related *Aphanorrhegma patens* and *Physcomitrium sphaericum* (Furness and Hall 1981; Malkowsky et al. 2018). Its continued presence at historic sites that lack recent records therefore deserves further investigation via analysis of diaspore banks. Research into the longevity of its spores under natural conditions would also be valuable.

Physcomitrium eurystomum is the result of a historic hybridisation event (McDaniel et al. 2010; Beike et al. 2014); consistent with the double peaks observed clearly during the present study in reads of chromatograms of the nuclear *loci*, likely the signature of a heterozygous admixture of alleles resulting from allopolyploidy. DNA barcoding confirmed the presence of *P. eurystomum* at Wilstone Reservoir based on a sample with immature sporophytes collected on 10 October 2019. The complete match in the four tested *loci* between this specimen and the reference voucher of *P. eurystomum*, including all nuclear heterozygous sites, indicates both share exactly the same ancestry lineages. During a return visit to Wilstone Reservoir on 27 December 2019, mature sporophytes were present and *P. eurystomum* was confirmed morphologically.

Results of this study confirm that *P. eurystomum* is confined in Britain to the drawdown zone of fluctuating freshwater ponds, lakes and reservoirs. Elsewhere in Europe it is known from similar habitat, but also seasonal pools in river floodplains and clay banks of rivers (Touw and Rubers 1989; Nieuwkoop 2016), rarely in wet meadows and ditches (Hodgetts et al. 2019). Curiously, Zechmeister et al. (2002) lists its occurrence in various habitats of Austrian agricultural landscapes, including arable fields with root crops, fallow lands, field margins, mining areas, periodic streams and low intensity meadows. Some of these habitats are common in the range of the species in Britain, but despite locally intensive bryophyte recording the plant has never been found beyond the drawdown zone of freshwater bodies. Whilst the moss attains peak abundance on open ground with limited competition, results of the present study show it can survive in small quantity within closed vegetation dominated by mats of *Agrostis stolonifera*, referable to the OV28 *Agrostis stolonifera-Ranunculus repens* community (Rodwell 2000). However, its survival in such conditions is critically dependent on disturbance events, providing competitive release on patches of bare soil. At Langmere and Ringmere, this occurred along trails of grazing animals, including domestic sheep and *Muntiacus reevesi* Ogilby (Reeves' Muntjac), and in areas subject to digging activity by *Oryctolagus cuniculus* (L.) (European Rabbit).

The non-native and invasive *Crassula helmsii* is a major risk to survival of *P. eurystomum* within Tring Reservoirs, and a potential threat at all locations for the moss in Britain. The plant has also become established within the range of *P. eurystomum* in mainland Europe and is spreading rapidly (Denys et al. 2014). Since it is a high profile threat to many freshwater wetland species, various methods of control have been trialled, including application of hot foam,

various herbicides, liquid nitrogen and hydrogen peroxide, plus burning with flame-throwers, burial beneath synthetic materials and mechanical excavation of plants and top soil (Leach and Dawson 2000; Bridge 2005; Sims and Sims 2016; Hussner et al. 2017; van Kleef et al. 2017; van der Loop et al. 2018). Several types of biological control offer potential, though none has yet been applied (Gassmann et al. 2006). Spraying with the broad-spectrum, post-emergence herbicide glyphosate (*N*-phosphonomethyl glycine) is a common strategy in Britain, and a method used at Tring Reservoirs (J. Wells pers. comm.). Glyphosate works by inhibiting the enzyme 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase and disrupting the shikimate pathway, the functionality of which is required for the survival of plants (Funke et al. 2006). Different species of bryophyte exhibit varying degrees of sensitivity to glyphosate (Pihakaski and Pihakaski 1980; Stjernquist 1981; Newmaster et al. 1999; Newmaster and Bell 2002; Oesau 2008), though no experimental research has been undertaken regarding effects on *P. eurystomum*, or indeed any other member of Funariaceae.

Observations accompanying records in Britain indicate large variation in abundance of *P. eurystomum* at sites between years. For example, at Langmere it was noted by D. T. Holyoak as 'locally plentiful' in the north basin in October 2005 and by F. R. Rumsey as 'locally abundant' in the south-east basin in February 2016, but was not found in either of these basins during the present survey, with just small amounts detected in the south-west basin. A major reason for this variation appears related to hydrological conditions during the previous annual cycle. It would be valuable to investigate the hydroecology of *P. eurystomum* so that favourable water level regimes may be defined. This is especially important for waterbodies where water levels can be managed and where stabilisation of water levels for leisure activities, such as fishing and boating, poses a threat. It is also of relevance regarding Fenmere, Langmere and Ringmere, all fed by a groundwater aquifer projected to undergo a long-term decline in average annual recharge during the present century due to climate change (Herrera-Pantoja et al. 2012).

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