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DNA Barcoding survey of dune slack *Bryum* in Wales



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Evidence Report No 669

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1. Crynodeb Gweithredol

Mae'r genws o fwsoglau *Bryum* yn cynnwys rhai rhywogaethau sy'n anodd iawn i'w hadnabod oherwydd yr angen am gapsiwlau sborau aeddfed i'w hadnabod. O'r herwydd, ni ellir adnabod planhigion *Bryum* ifanc trwy ddulliau confensiynol, gan ei gwneud hi'n anodd penderfynu pa rywogaethau *Bryum* sy'n cytrefu pantiau sydd newydd eu crafu yn y twyni. Mae defnyddio codau bar DNA yn ddull dibynadwy o adnabod *Bryum*, hyd yn oed o samplau bach o blanhigion ifanc.

Cafodd 100 sampl o fwsogl *Bryum* eu casglu o bantiau twyni mewn chwe system o dwyni yng Nghymru. Cafodd y rhan fwyaf o'r samplau (82) eu hadnabod gan ddefnyddio codau bar DNA fel y rhywogaeth *B. pseudotriquetrum*, sy'n gyffredin mewn twyni/ffeniau/llyfolchdir, ond roedd 12 yn cynrychioli *B. algovicum*, sy'n rhywogaeth arbenigol mewn pantiau twyni, roedd dau ohonynt yn *B. pallens*, sy'n weddol gyffredin, roedd un yn un o arbenigwyr prin y twyni *B. marratii*, ac roedd un yn fwsogl prin arbenigol arall mewn twyni, *B. warneum*. Nodwyd mai genera eraill oedd dau sampl, o ganlyniad i is-samplu casgliadau cymysg. Roedd yn hysbys bod y ddau safle lle nodwyd y rhywogaethau prin yn cefnogi'r rhywogaethau hynny, ond roedd y cytrefi oedd newydd eu darganfod o leiaf 400m o unrhyw gytrefi a oedd yn hysbys cyn hynny.

Mae defnyddio codau bar DNA yn ddull defnyddiol o fonitro sut mae bryoffytau'n cytrefu pantiau a grafwyd yn y twyni, er ei bod yn annhebygol y byddai casgliadau ar hap gan rywun nad yw'n arbenigwr yn dod o hyd i rywogaethau prinnach neu'n esgor ar lwyddiant. Ymddengys mai'r ffordd orau o fonitro poblogaethau o fryoffytau'r twyni yw cyfuno arolygon maes traddodiadol gyda gwaith i gasglu samplau er mwyn defnyddio codau bar DNA.

2. Executive summary

The moss genus *Bryum* includes some species that are very difficult to identify because of the need for ripe spore capsules for identification. Young plants of *Bryum* cannot therefore be identified by conventional methods, making it difficult to determine which *Bryum* species are colonising newly scraped dune slacks. DNA Barcoding provides a reliable method for identifying *Bryum*, even from small samples of young plants.

100 samples of *Bryum* moss were collected from dune slacks at six dune systems in Wales. The majority of the samples (82) were identified by DNA Barcoding as the common dune/fen/flush species *B. pseudotriquetrum*, but 12 represented the common dune slack specialist *B. algovicum*, two were the moderately common species *B. pallens*, one was the rare dune specialist *B. marratii* and one was the rare dune specialist *B. warneum*. Two samples were determined as other genera resulting from sub-sampling of mixed collections. Both sites where the rare species were identified were known to support those species, but the newly discovered colonies were at least 400m from any previously known colonies.

DNA Barcoding is a useful approach for monitoring the colonisation of scraped dune slacks by bryophytes, although random collection by a non-specialist is considered unlikely to locate rarer species or demonstrate success. Combining a traditional field bryophyte survey with the collection of samples for DNA Barcoding appears to be the best way of monitoring dune bryophyte populations.

3. Introduction

Dune slacks are host to a suite of specialist mosses and liverworts (Blockeel *et al.*, 2014), with the moss genus *Bryum* (now split by some taxonomists into several genera, including *Ptychostomum*) particularly well represented. Studies by David Holyoak (2002 & 2015) have shown significant declines among Welsh dune *Bryum* species, with *B. calophyllum* lost entirely, *B. knowltonii* lost from two of its three sites, and *B. warneum* lost from four of its seven sites. Dune systems are naturally dynamic, and the low-lying, winter-wet dune slacks where pioneer *Bryum* species grow tend to fill with sand over time and become overgrown with taller vegetation. New slacks will develop and dune parabola slacks will move if the dune system is dynamic, so there is typically a supply of early successional slacks that *Bryum* species can colonise. However, decades of environmental change, including N-deposition and altered rainfall patterns, grazing loss, myxomatosis, and recovery from historic periods of more intense (often) military disturbance have led to substantial stabilisation on almost every dune system (Litt *et al.*, 2021), resulting in significant reductions in mobile and early successional dune habitats. This loss of mobility is thought to be the cause of the observed declines in dune *Bryum* mosses, along with similar declines in specialist dune invertebrates and flowering plants.

Natural Resources Wales have responded to the declines in many dune species by initiating a widespread programme of dune rejuvenation: initially on a small number of National Nature Reserves and subsequently more widely under the Sands of LIFE Programme (Natural Resources Wales, 2019) and Dynamic Dunescape Project. Interventions include scrub removal, scraping of over-mature dune slacks to remove the humic upper soil horizons, destabilising dune faces to encourage sand movement, and cutting notches in the frontal dunes to allow sand ingress from the beach and focus wind to move sand from the front of the system. Baseline bryophyte monitoring took place before the interventions and will continue after the project ends (Callaghan, 2020).

Observations of some slacks excavated prior to Sands of LIFE suggested that the edges of scrapes sometimes supported notable species which had grown from exposed subterranean propagules – most notably *Amblyodon dealbatus* at Pembrey Burrows (Callaghan, 2017a). Subsequent work by Callaghan *et al.* (2020) demonstrated the importance of the propagule bank for bryophyte colonisation of scraped dune slacks, identifying using DNA Barcoding 34 species that grew in cultivation from shallow sand/soil cores, including five Red List bryophytes. However, pioneer *Bryum* spp. (rather than tuber- or bulbil-producing species) were notably absent among the 34 which grew from cultivation, suggesting that scraping might not revitalise populations of pioneer *Bryum* such as *B. calophyllum* and *B. warneum*; these would need to colonise as spores from elsewhere.

The bare sand of some scraped slacks at Kenfig NNR was seen to support small patches of *Bryum* during a visit in 2016 (Sam Bosanquet pers. obs.), but identification was impossible because of the need to examine spore capsules to identify many *Bryum* species. This raised the possibility that some of the rarer pioneer *Bryum* were colonising scraped slacks. Full assessment of the success of scrape interventions, including those of Sands of LIFE, in reversing the declines of specialist dune species is not possible without determining whether or not they are working for *Bryum*. The current project aims to establish whether DNA Barcoding of small samples of *Bryum*, too small or juvenile to identify, might provide a way of evaluating which bryophytes are colonising dune scrapes.

4. Methods

Six dune systems across Wales were selected for survey: three in south Wales and three in north Wales (Fig. 1). Five of these sites had experienced dune rejuvenation works over recent years, whilst the sixth, Morfa Dyffryn, has the best-developed dune slack bryophyte assemblage in Wales and is effectively acting as a control site because of the lack of rejuvenation works there. Half a day was spent on each site, involving the collection of up to 10 *Bryum* samples from up to five slacks per site. Samples were collected by Sam Bosanquet – an experienced bryologist capable of identifying *Bryum* to genus – from locations separated by at least 50 cm within a slack, in an attempt to avoid collecting from the same individual moss patch (see Figs. 10-41). Sampling aimed to represent the range of morphology among the *Bryum* within a particular slack, and included shoots considered likely to be *B. pseudotriquetrum* as well as those believed to be potentially less common species. Samples were kept deliberately small in order to avoid damage to any populations: 3–5 shoots collected into a folded paper packet (Fig. 37), on to which the sample number was written. A 10-figure (1m accuracy) GPS reading was recorded using a Garmin GPSMap 66 for each sample, and habitat notes were recorded for each sampled slack. The moss samples were slowly air dried and then sent to the Royal Botanic Garden Edinburgh (RBGE) for DNA Barcoding.



Figure 1. Map showing the six Welsh dune systems from which *Bryum* samples were collected for DNA Barcoding. © Crown Copyright and database right 2022. Ordnance Survey licence number 100019741. © Hawlfraint a hawliau cronfa ddata'r Goron 2022. Rhif Trwydded yr Arolwg Ordnans 100019741

Up to three moss stems were selected from each packet using standard RBGE protocols for bryophyte material (Forrest *et al.* 2023a). The DNA extraction followed the methodology in Forrest *et al.* 2023b. The PCR amplification of the plastid loci *rbcl* and *psbA-trnH* and the nuclear ribosomal repeat ITS2 followed the methodology in Forrest *et al.* 2023c, as did the Sanger sequencing. Sequencing products were sent to Dundee University for running on an ABI 3730.

Sequence traces were assembled into contigs and manually edited using Sequencer version 5.4.6. The cleaned edited sequences were exported in FASTA format and uploaded to the open access Barcode of Life database, BOLD, following the methodology in Forrest *et al.* 2023d. They were also compared to the NCBI public database using a BLASTn search.

The DNA barcode identifications of the plant specimens were made by comparing the DNA barcode sequences to a reference database of DNA sequences from over 270 verified specimens that represent c. 70 species of *Bryum s.l.*, using a combination of tree-based methods and direct visual comparison of aligned sequences.

5. Results

5.1. Ecology

Samples were collected from 21 slacks on the six dune systems, with multiple samples from the majority of slacks (Table 1). Additional slacks were photographed on most sites, particularly when no *Bryum* was seen. Ecological notes were made for a total of 36 slacks (Table 2, p. 10), including some which had been scraped and held almost no plants at all, and others which had not been scraped and supported diverse assemblages of dune plants including *Anagallis (Lysimachia) tenella*, *Dactylorhiza incarnata*, *Epipactis palustris*, *Equisetum variegatum*, *Salix repens* and *Trifolium fragiferum*. A full set of 32 photographs is included at the end of this report.

Table 1. Summary information on sites surveyed for dune *Bryum* in 2022.

Site	Date	Slacks visited	Slacks sampled	Samples	Slacks photographed	Photos
Merthyr Mawr Warren SSSI	31/05/2022	10	3	20	6	7
Kenfig SSSI	31/05/2022	8	3	8	7	7
Morfa Dyffryn SSSI	29/06/2022	5	5	25	5	5
Tywyn Aberffraw SSSI	29/06/2022	7	5	16	7	7
Newborough Warren SSSI	30/06/2022	4	3	25	3	5
Whiteford Burrows SSSI	26/07/2022	2	2	6	1	1

5.2. DNA Barcoding

All but two of the 100 *Bryum* samples yielded DNA that matched to a *Bryum* species; the two that matched to other families were probably the result of subsampling from a mixed collection.

- 82 match to *Bryum (Ptychostomum) pseudotriquetrum*, although this disguises quite significant variation in the DNA among these samples because *B. pseudotriquetrum* as currently defined is genetically rather variable;
- 12 from Merthyr Mawr, Morfa Dyffryn and Newborough Warren match to *Bryum algovicum/Ptychostomum compactum* var. *rutheanum*, a species that has been renamed following its transfer from *Bryum* to *Ptychostomum*;
- 2 from Tywyn Aberffraw match to *Bryum/Ptychostomum pallens*;
- 1 from Morfa Dyffryn (SH5651123826) matches to *Bryum/Ptychostomum warneum*;
- 1 from Whiteford Burrows (SS4319294146) matches to *Bryum marratii*.

6. Discussion

Most *Bryum* samples were matched to *B. pseudotriquetrum*. This is a large, distinctive species by the standards of the genus, and the majority of the samples were expected to be *B. pseudotriquetrum* when they were collected. Many of the samples that were considered

likely to be species other than *B. pseudotriquetrum* in the field were indeed confirmed to be other taxa, although some were just very young *B. pseudotriquetrum*. An experienced field bryologist could probably collect only samples that do not resemble *B. pseudotriquetrum* and significantly reduce the expense of the DNA analysis by reducing the sample size.

Two rare dune *Bryum* species were identified from the samples, which suggests that the protocol of collecting small samples and using DNA Barcoding to identify them works in principal. Its effectiveness is further evidenced by the ability of the technique to identify the *Bryum marratii* sample from Whiteford Burrows, because this was extremely small and would probably have been passed over as an unidentifiable juvenile *Bryum* despite its relatively distinctive leaf shape. *Bryum marratii* was already known from Whiteford Burrows (Bosanquet, 2012), but only from the dune-saltmarsh transition on the east side of the dune ridge, about 1.4 km from the colony identified during this survey (Fig. 2). The only *Bryum warneum* identified during the survey did have the sporophytes necessary for identification without DNA, so a bryologist would probably have collected a specimen for microscope checking if it had been encountered. However, it was from an area of Morfa Dyffryn from which *B. warneum* was not previously recorded (Fig. 3) despite expert survey (Callaghan 2017b), again demonstrating the potential of DNA Barcoding.



Figure 2: Colony of *Bryum marratii* (red square) identified by DNA Barcoding in the ‘new slack’ at Whiteford Burrows compared with the previous known distribution of this species on the site (blue circles). © Crown Copyright and database right 2022. Ordnance Survey licence number 100019741. © Hawlfraint a hawliau cronfa ddata'r Goron 2022. Rhif Trwydded yr Arolwg Ordans 100019741.

The scraping is working to a certain extent, although only three of the 34 samples from scrapes were the common dune slack specialist *Bryum algovicum*, with the remainder being the more robust slack/fen/flush species *B. pseudotriquetrum*. The three samples of *B. algovicum* all came from a scrape at Newborough Warren that was scraped in winter 2013-

2014. There is an area of unscraped dune slack with *B. algovicum* close to the scrape, and it is unclear whether the *B. algovicum* colonised the scrape as spores or via fragments. Nevertheless, it is encouraging that *B. pseudotriquetrum* is not the only *Bryum* which benefits from dune destabilisation work.

Some small scrapes at Merthyr Mawr Warren had been colonised by *B. pseudotriquetrum* and the uncommon liverwort *Riccia cavernosa* (e.g. Figs. 34 & 35). In contrast the large scrapes there (e.g. Figs. 29 & 32) still have very mobile sand and completely lack bryophyte colonisation. Small scrapes scattered through a slack appear more likely to be colonised by bryophytes in the short term, but larger excavated slacks might be colonised after several years once vascular plants have established and stabilised the sand.



Figure 3: Colony of *Bryum warneum* (red square) identified by DNA Barcoding at Morfa Dyffryn compared with the previous known distribution of this species on the site (blue circles). © Crown Copyright and database right 2022. Ordnance Survey licence number 100019741. © Hawlfraint a hawliau cronfa ddata'r Goron 2022. Rhif Trwydded yr Arolwg Ordnans 100019741.

The current work suggests that DNA Barcoding could be a very useful tool for monitoring the condition of dune slack bryophyte communities, especially for identifying pioneer *Bryum*

mosses in recently scraped slacks. This might allow rarer colonists to be safeguarded during follow-up interventions, or perhaps ‘gardened’ to aid their establishment. Waiting several years for sporophyte production as colonies mature could lead to delays in these potential benefits, or to the unwitting loss of pioneer colonies. The technique would also allow the identification of *Bryum* species even at times of year when sporophytes are absent.

Random sampling of mosses by an inexperienced non-specialist might work, but would be unlikely to include any rare species; random samples of *Bryum* by an experienced non-specialist would increase the chances of collecting rarer *Bryum*, but *B. pseudotriquetrum* would likely dominate the samples; targeted collect by a specialist of *Bryum* that appears different to *B. pseudotriquetrum* would maximise results and minimise the costs of DNA analysis. This kind of specialist sampling could also be combined with conventional bryophyte survey to give the best possible monitoring results.

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8. Acknowledgements

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9. Data tables, maps and photographs

Table 2: Ecological notes on dune slacks, indicating how many *Bryum* samples were taken from each slack and how many photographs were taken.

Site	Date	OSGR	Samples	Sample Code	Photo	HabitatNotes
Tywyn Aberffraw SSSI	29/06/2022	SH36776923	0	0	A1	Elongate scrape in former Meesia slack, F Anagallis tenella but no Bryum or Meesia
Tywyn Aberffraw SSSI	29/06/2022	SH36776924	2	AA	A2	Small hand-dug scrapes in former Meesia slack, F Cx serotina and small Sx repens
Tywyn Aberffraw SSSI	29/06/2022	SH36776936	3	AB	A3	Scrape in former Bryum calophyllum site A Equisetum variegatum, F Calliergonella, R Bryum
Tywyn Aberffraw SSSI	29/06/2022	SH36726947	5	AC	A4	Flushed dune grassland with Pinguicula & Selaginella, O Bryum LF Preissia
Tywyn Aberffraw SSSI	29/06/2022	SH36446930	0	0	A5	Scrape from 2020 very dry with no Bryum
Tywyn Aberffraw SSSI	29/06/2022	SH35746843	1	AD	A6	Scrape from 2020 with F Anagallis tenella & short Sx repens, R Bryum
Tywyn Aberffraw SSSI	29/06/2022	SH35856836	5	AE	A7	Unscraped slack with A Equisetum & F Preissia
Morfa Dyffryn SSSI	29/06/2022	SH56512380	10	DA	D1	Open slack with A Epipactis, O Bryum in hollows with Eq variegatum & Aneura
Morfa Dyffryn SSSI	29/06/2022	SH56452403	3	DB	D2	Linear hollow at landward side of large slack, with A Campylium
Morfa Dyffryn SSSI	29/06/2022	SH56342415	3	DC	D3	Wide open slack with A short Salix repens and A black algal biocrust
Morfa Dyffryn SSSI	29/06/2022	SH56252437	4	DD	D4	Hollow at landward edge of large slack, A short Sx repens & Cx serotina, A Bryum cf pseud
Morfa Dyffryn SSSI	29/06/2022	SH56002446	5	DC	D5	Large slack with A Epipactis & short Sx repens, F Aneura, A Bryum among blown sand
Kenfig SSSI	31/05/2022	SS79518239	0	0	K1	Large slack scraped in 2021, no bryophytes just A Chara
Kenfig SSSI	31/05/2022	SS78978262	2	KA	K2	Elongate slack scraped in 2018-19, widespread vascular plant growth, occasional Bryum
Kenfig SSSI	31/05/2022	SS78968284	0	0	K3	Slack scraped in 2013, now well vegetated, occasional Calliergonella, no Bryum
Kenfig SSSI	31/05/2022	SS78908274	0	0	K4	Slack scraped in 2012, frequent Baldellia & Calliergonella, occasional Campylium, no Bryum
Kenfig SSSI	31/05/2022	SS78358224	0	0	K5	Excavations scraped in 2011-12, Agrostis colonising but no Bryum
Kenfig SSSI	31/05/2022	SS78388235	0	0	0	Excavations scraped in 2012-13, Agrostis colonising but no Bryum
Kenfig SSSI	31/05/2022	SS78508237	3	KB	K6	Inland end of excavations, unscraped vegetation with Eleocharis & Anagallis, F Bryum
Kenfig SSSI	31/05/2022	SS78678231	3	KC	K7	Slack scraped in 2013 Locally frequent Calliergonella & Campylium, O Bryum
Merthyr Mawr Warren SSSI	31/05/2022	SS86267635	0	0	MM1	Damp sand but no plants or bryophytes, scraped in 2013 but probably too large and mobile
Merthyr Mawr Warren SSSI	31/05/2022	SS86257633	10	MMA	MM2, MM3	Adjacent to 2013 scrape, A Equisetum variegatum, A Dactylorhiza spp etc
Merthyr Mawr Warren SSSI	31/05/2022	SS86207632	0	0	0	Seaward end of this large scrape, too mobile for colonisation
Merthyr Mawr Warren SSSI	31/05/2022	SS86127641	0	0	MM4	Excavated in 2021, no bryophyte colonisation, scraps of Rubus caesius
Merthyr Mawr Warren SSSI	31/05/2022	SS86327661	0	0	MM5	Small scrape excavated December 2020, edges have Rubus & Agrostis but no bryophytes
Merthyr Mawr Warren SSSI	31/05/2022	SS86257663	0	0	0	Small scrape excavated December 2020, looks ideal but no bryophytes
Merthyr Mawr Warren SSSI	31/05/2022	SS86237663	5	MMB	MM6	Small scrape excavated December 2020, F Riccia cavernosa
Merthyr Mawr Warren SSSI	31/05/2022	SS86207661	5	MMC	MM7	Small scrape excavated December 2020, F Riccia cavernosa
Merthyr Mawr Warren SSSI	31/05/2022	SS86287661	0	0	0	Small scrape excavated December 2020, looks good but no bryophytes
Merthyr Mawr Warren SSSI	31/05/2022	SS86197661	0	0	0	Small scrape excavated December 2020, looks good but no bryophytes
Newborough Warren SSSI	30/06/2022	SH41796324	10	NA	N1, N2	Parabola slack in excellent condition, A Equisetum, Anagallis, short Sx repens, A Bryum
Newborough Warren SSSI	30/06/2022	SH42076340	5	NB	N3	Scrape in parabola extensively colonised by Equisetum, Cx flacca & short Sx repens, O Aneura
Newborough Warren SSSI	30/06/2022	SH42246350	0	0	0	Deep scrape with Juncus articulatus & Samolus, no bryophytes
Newborough Warren SSSI	30/06/2022	SH42256336	10	NC	N4, N5	Scrape in parabola with A Equisetum, Eleo quinque etc, O Bryum but LA Bryum in SW corner
Whiteford Burrows SSSI	26/07/2022	SS43199414	2	WA	0	25 year old slack formed on beach, with A Calliergonella and LA Phragmites
Whiteford Burrows SSSI	26/07/2022	SS43439430	4	WB	W1	Sandy track with LF Bryum, F Trifolium fragiferum, O Sagina nodosa



Figure 4. Map of Tywyn Aberffraw showing locations of slacks where ecological notes were taken and a number indicating how many samples were collected in that slack. Purple is SSSI boundary. © Crown Copyright and database right 2022. Ordnance Survey licence number 100019741. © Hawlfraint a hawliau cronfa ddata'r Goron 2022. Rhif Trwydded yr Arolwg Ordnans 100019741.

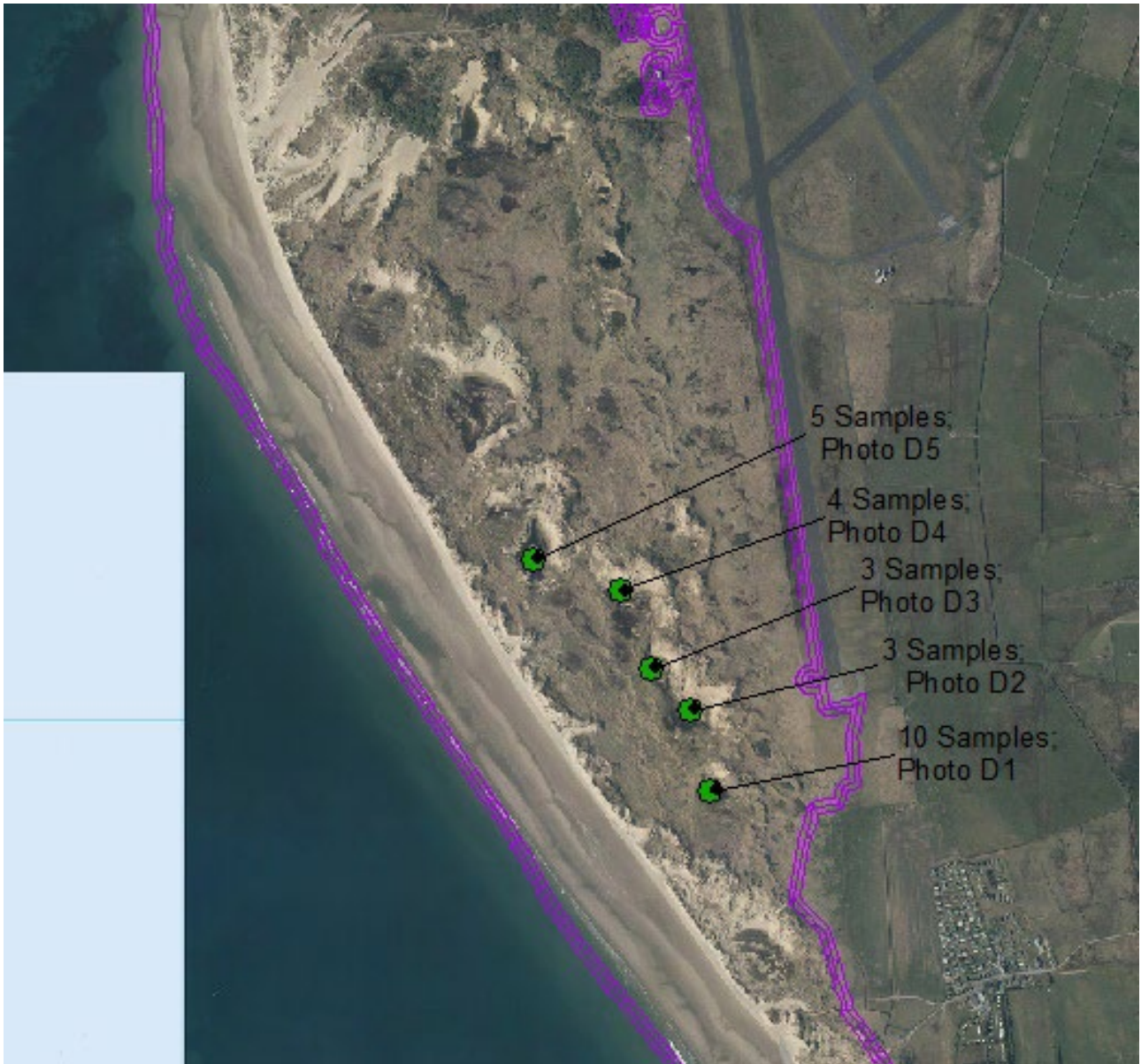


Figure 5. Map of Morfa Dyffryn showing locations of slacks where ecological notes were taken and a number indicating how many samples were collected in that slack. Purple is SSSI boundary. © Crown Copyright and database right 2022. Ordnance Survey licence number 100019741. © Hawlfraint a hawliau cronfa ddata'r Goron 2022. Rhif Trwydded yr Arolwg Ordnans 100019741.

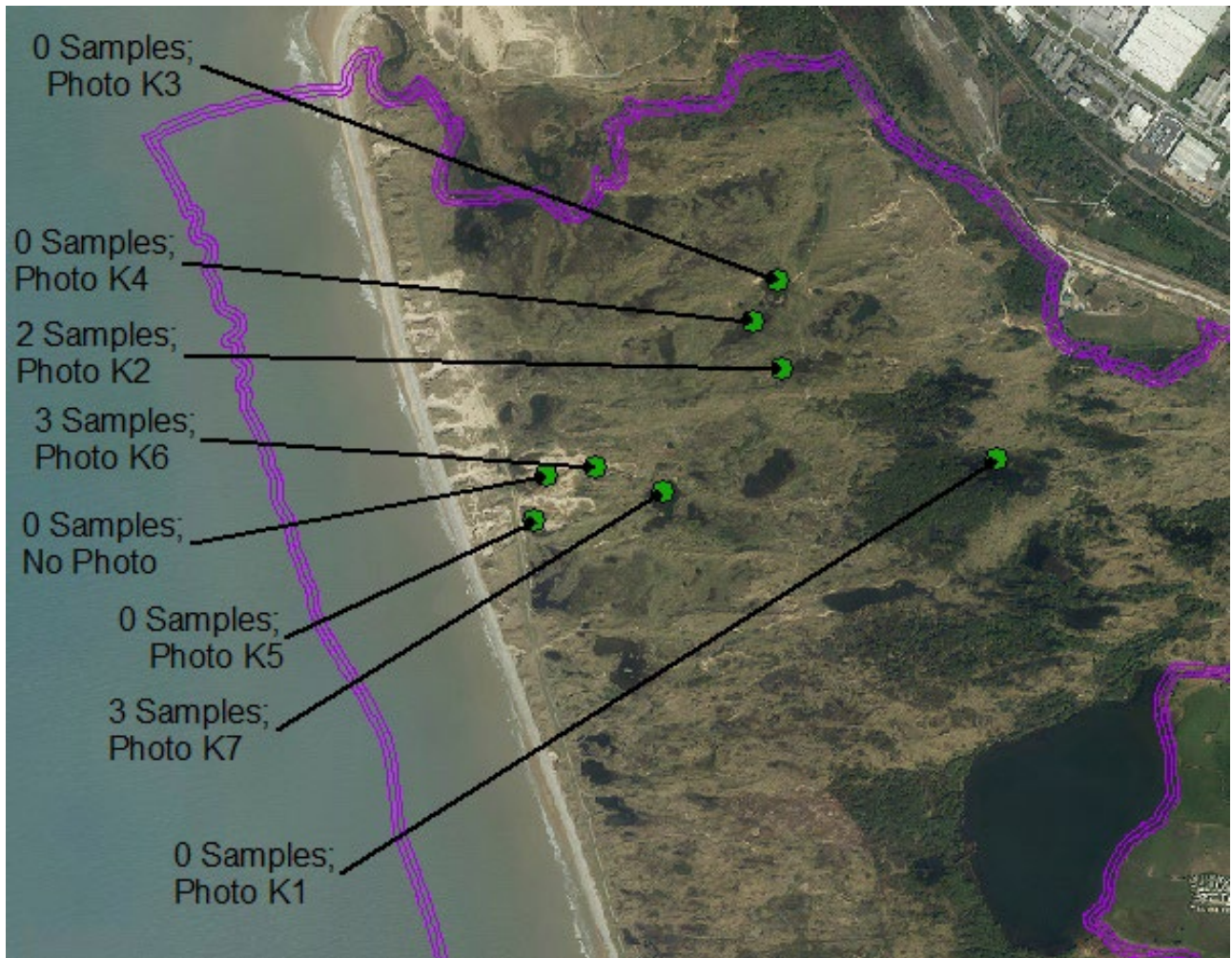


Figure 6. Map of Kenfig Burrows showing locations of slacks where ecological notes were taken and a number indicating how many samples were collected in that slack. Purple is SSSI boundary. © Crown Copyright and database right 2022. Ordnance Survey licence number 100019741. © Hawlfraint a hawliau cronfa ddata'r Goron 2022. Rhif Trwydded yr Arolwg Ordnans 100019741.

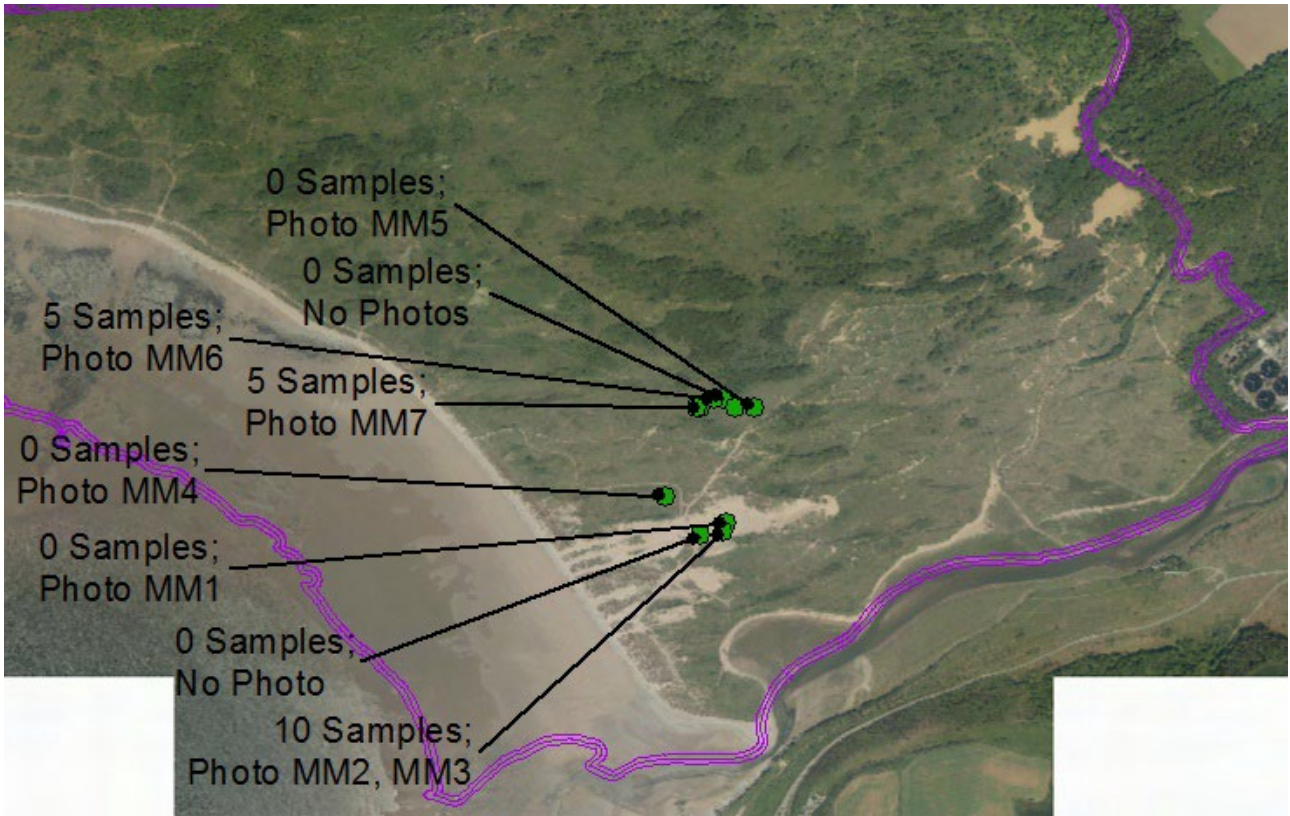


Figure 7. Map of Merthyr Mawr Warren showing locations of slacks where ecological notes were taken and a number indicating how many samples were collected in that slack. Purple is SSSI boundary. © Crown Copyright and database right 2022. Ordnance Survey licence number 100019741. © Hawlfraint a hawliau cronfa ddata'r Goron 2022. Rhif Trwydded yr Arolwg Ordnans 100019741.



Figure 8. Map of Newborough Warren showing locations of slacks where ecological notes were taken and a number indicating how many samples were collected in that slack. Purple is SSSI boundary. © Crown Copyright and database right 2022. Ordnance Survey licence number 100019741. © Hawlfraint a hawliau cronfa ddata'r Goron 2022. Rhif Trwydded yr Arolwg Ordnans 100019741.



Figure 9. Map of Whiteford Burrows showing locations of slacks where ecological notes were taken and a number indicating how many samples were collected in that slack. Purple is SSSI boundary. © Crown Copyright and database right 2022. Ordnance Survey licence number 100019741. © Hawlfraint a hawliau cronfa ddata'r Goron 2022. Rhif Trwydded yr Arolwg Ordnans 100019741.



Figure 10. Photo A1. Elongate scrape in former *Meesia uliginosa* slack at Aberffraw, F *Anagallis tenella* but no *Bryum* or *Meesia*.

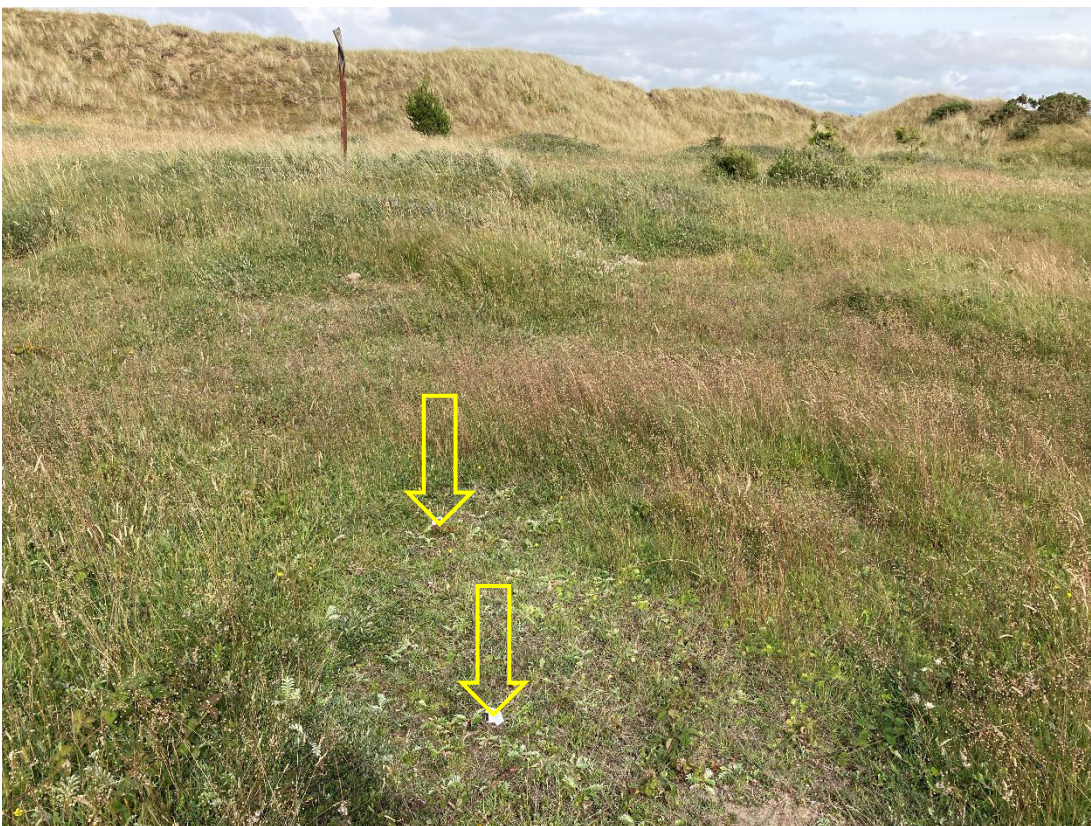


Figure 11. Photo A2. Small hand-dug scrapes in former *Meesia* slack, F *Cx serotina* and small *Sx repens*. The locations of two samples (DNA *Bryum pseudotriquetrum*) are indicated with arrows.



Figure 12. Photo A3. Scrape in former *Bryum calophyllum* site with A *Equisetum variegatum*, F *Calliergonella*, R *Bryum* (DNA *B. pseudotriquetrum*). Locations of two of the three samples are marked.



Figure 13. Photo A4. Flushed dune grassland with *Pinguicula* & *Selaginella*, O *Bryum* LF *Preissia quadrata*. Locations of three samples are indicated; DNA identified *B. pallens* and *B. pseudotriquetrum*.



Figure 14. Photo A5. Scrape from 2020 at Tywyn Aberffraw, very dry with no *Bryum*.



Figure 15. Photo A6. Scrape from 2020 at Tywyn Aberffraw with F *Anagallis tenella* & short *Sx repens*, R *Bryum* (DNA *B. pseudotriquetrum*). Sampling location is indicated.

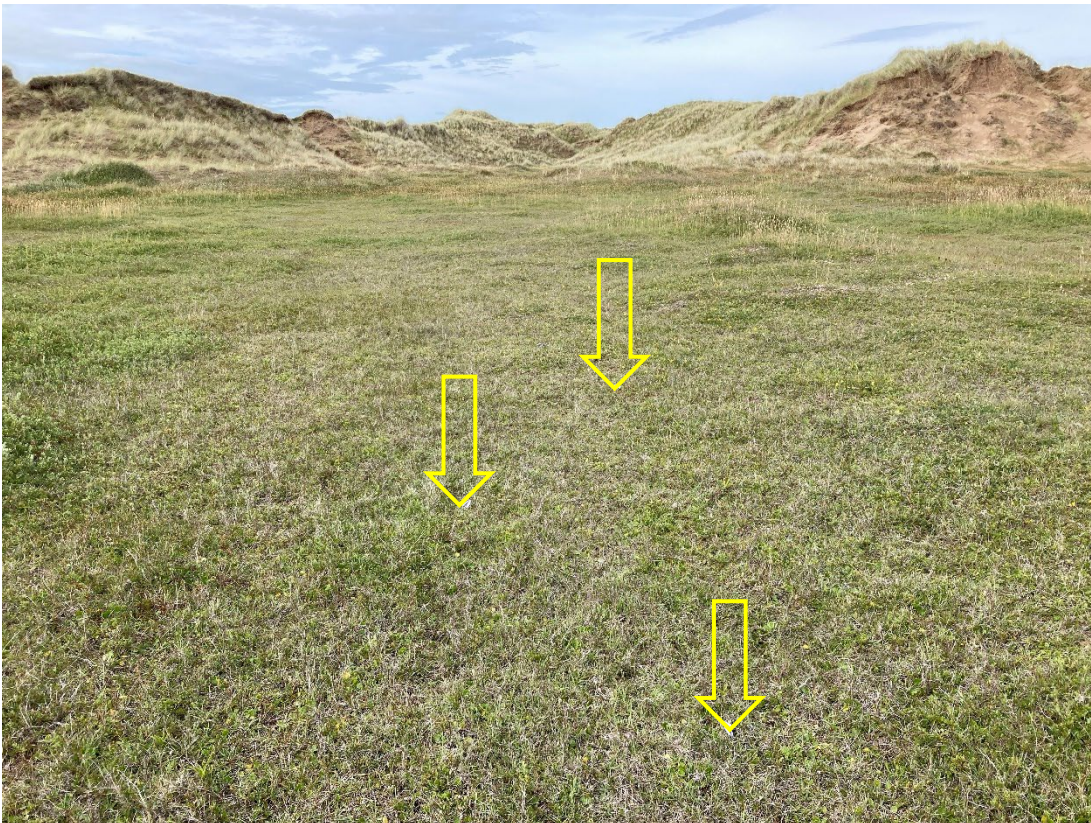


Figure 16. Photo A7. Unscrapped slack at Aberffraw with *A Equisetum variegatum* & *F Preissia quadrata*. Locations of three of five samples (DNA *B. pseudotriquetrum*) are indicated.

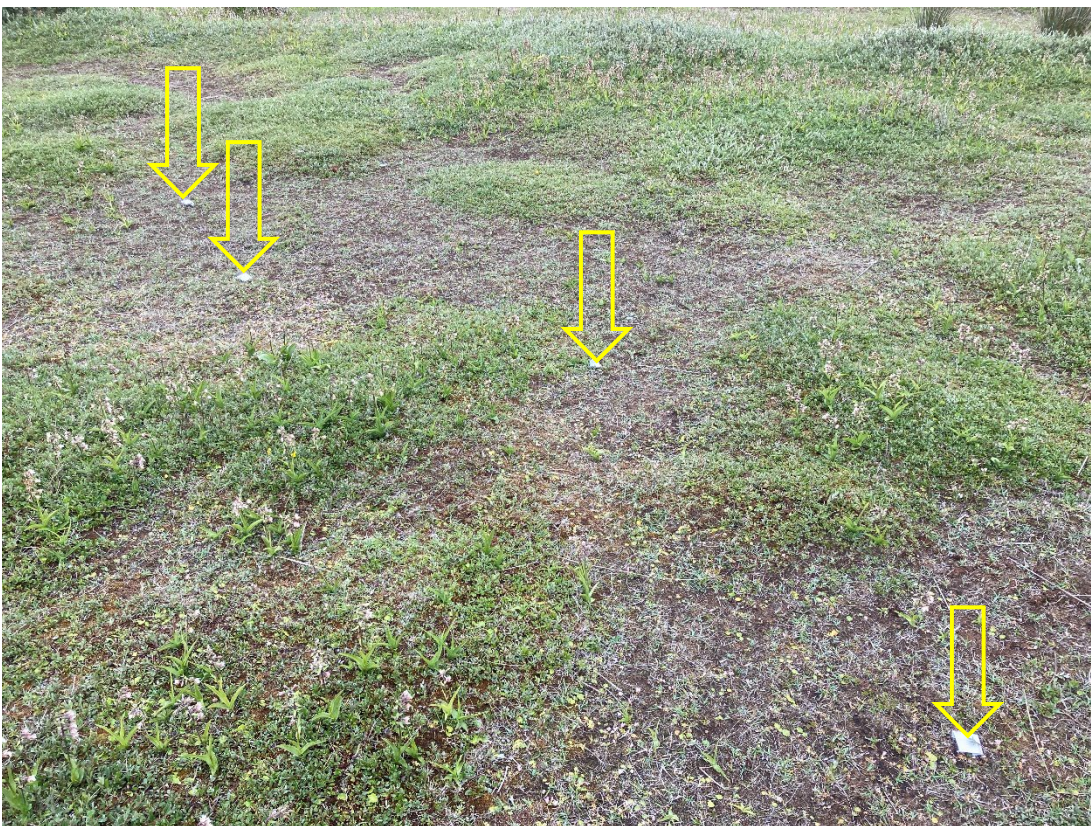


Figure 17. Photo D1. Open slack at Morfa Dyffryn with *A Epipactis palustris*, *O Bryum* in hollows with *Eq variegatum* & *Aneura*. Samples in low-lying areas: 9 were *B. pseudotriquetrum*; 1 was *B. warneum*.



Figure 18. Photo D2. Linear hollow at landward side of large slack at Morfa Dyffryn, with A *Campylium*. Locations of all 3 samples (DNA *B. pseudotriquetrum*) marked.

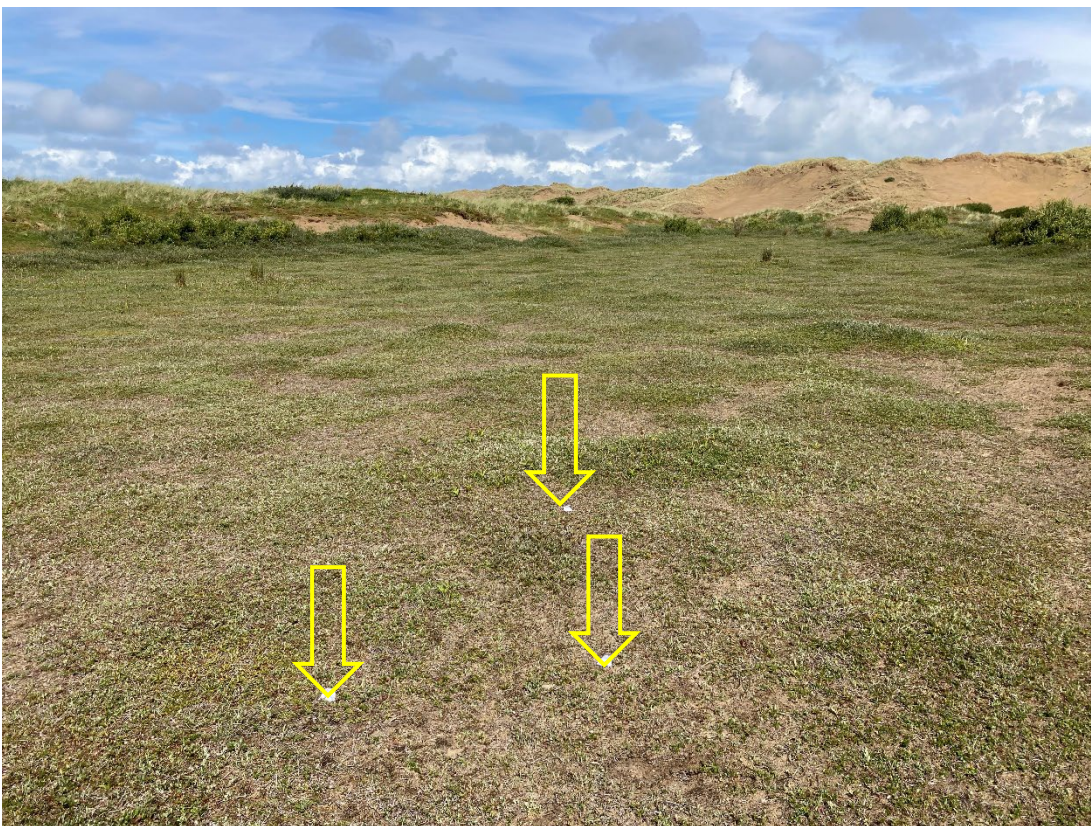


Figure 19. Photo D3. Wide open slack at Morfa Dyffryn, with A short *Salix repens* and A black algal biocrust. Locations of all 3 samples (DNA 2x *B. pseudotriquetrum*, 1x *B. algovicum*) are marked.



Figure 20. Photo D4. Hollow at landward edge of large slack at Morfa Dyffryn, A short *Salix repens* & *Carex serotina*, A *Bryum pseudotriquetrum* (DNA confirmed). All 4 sample locations are marked.



Figure 21. Photo D5. Large slack at Morfa Dyffryn with A *Epipactis palustris* & short *Salix repens*, F *Aneura*, A *Bryum* (DNA *B. pseudotriquetrum*) among blown sand. 3 of the 5 samples are indicated.



Figure 22. Photo K1. Large slack at Kenfig scraped in 2021, no bryophytes just A *Chara*.



Figure 23. Photo K2. Elongate 'Runway Scrape' slack at Kenfig scraped in winter 2018/19, widespread vascular plant growth, occasional *Bryum* (DNA *B. pseudotriquetrum*).



Figure 24. Photo K3. 'Bush Grass Two' Slack at Kenfig scraped in February 2013, now well vegetated, occasional *Calliergonella*, no *Bryum*.



Figure 25. Photo K4. Slack at Kenfig scraped in February 2012, frequent *Baldellia* & *Calliergonella*, occasional *Campylium*, no *Bryum*.



Figure 26. Photo K5. Excavations at the seaward end of Kenfig Burrows scraped in winter 2011-2012, known as 'Rejuvenation 1'. *Agrostis* is colonising but no *Bryum*.



Figure 27. Photo K6. Inland end of winter 2012-2013 excavations ('Rejuvenation 2.2') at Kenfig, unscraped vegetation with *Eleocharis* sp. & *Anagallis tenella*, F *Bryum* (DNA *B. pseudotriquetrum*).



Figure 28. Photo K7. Slack east of 'Rollercoaster' at Kenfig scraped in October 2013 Locally frequent *Calliergonella* & *Campylium*, *O Bryum* (DNA *B. pseudotriquetrum*).



Figure 29. Photo MM1. Slack at Merthyr Mawr with damp sand but no plants or bryophytes, scraped in 2013 but probably too large and mobile.



Figure 30. Photo MM2. Area of unscraped dune slack at Merthyr Mawr adjacent to 2013 scrape, A *Equisetum variegatum*, A *Dactylorhiza* spp etc. 8 of 10 samples indicated (DNA 8x *B. algovicum*, 2x *B. pseudotriquetrum*).



Figure 31. Photo MM3. *Bryum algovicum* (DNA confirmed) with sporophytes at Methyr Mawr.



Figure 32. Photo MM4. Slack at Merthyr Mawr Warren excavated in 2021, no bryophyte colonisation, scraps of *Rubus caesius*.



Figure 33. Photo MM5. Small scrape at Merthyr Mawr excavated December 2020, edges have *Rubus* & *Agrostis* but no bryophytes.



Figure 34. Photo MM6. Small scrape excavated at Merthyr Mawr in December 2020, *F Riccia cavernosa* and *O Bryum* (DNA *B. pseudotriquetrum*). Locations of 5 samples are shown.



Figure 35. Photo MM7. Small scrape excavated at Merthyr Mawr in December 2020, *F Riccia cavernosa* and *O Bryum* (DNA *B. pseudotriquetrum*). Locations of 5 samples are shown.

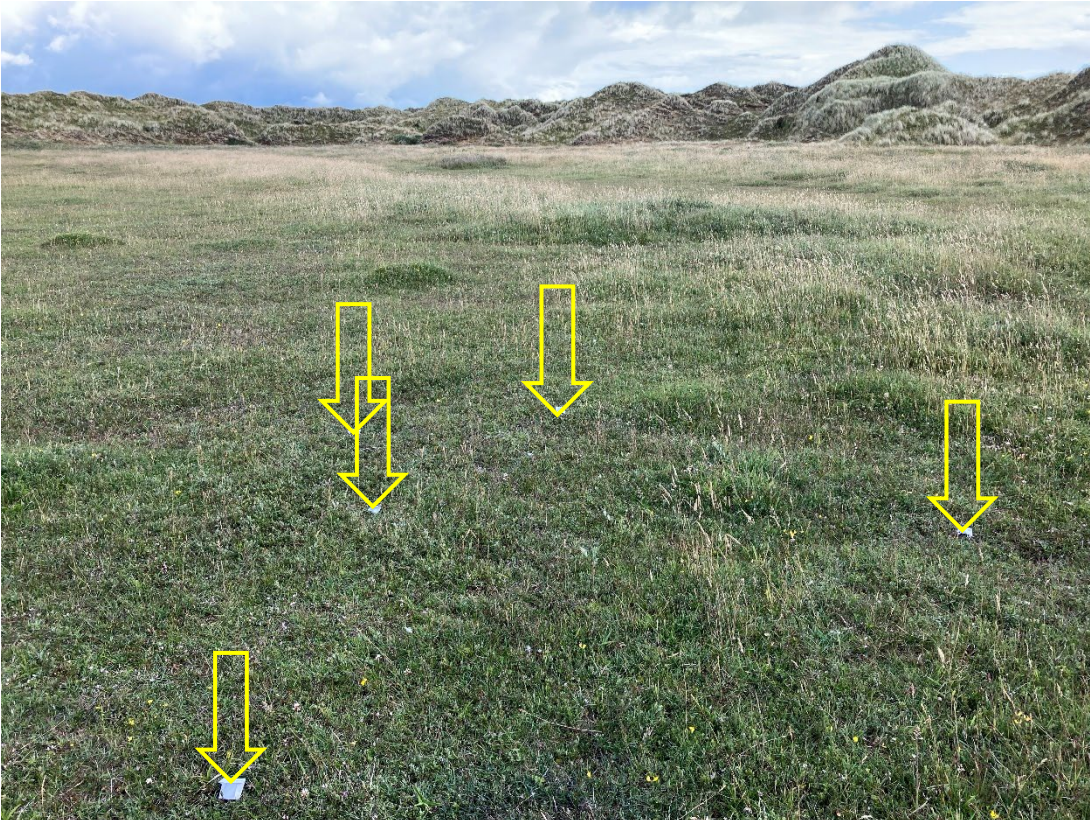


Figure 36. Photo N1. Parabola slack at Newborough Warren in excellent condition, A *Equisetum*, *Anagallis*, short *Sx repens*, A *Bryum*. 5 of 10 samples (DNA *B. pseudotriquetrum*) are indicated.



Figure 37. Photo N2. Collection of *Bryum* (DNA *B. pseudotriquetrum*) from unscrapped slack at Newborough Warren, with *Anagallis tenella*, *Equisetum variegatum* and *Salix repens*.



Figure 38. Photo N3. Scrape in parabola at Newborough Warren, extensively colonised by *Equisetum*, *Cx flacca* & short *Sx repens*, *O Aneura*. One of 5 samples (DNA *B. pseudotriquetrum*) is indicated.



Figure 39. Photo N4. Scrape in parabola at Newborough with *A Equisetum*, *Eleocharis quinqueflora* etc, *O Bryum* but LA *Bryum* in SW corner where *B. algovicum* was collected. 3 samples indicated.



Figure 40. Photo N5. Sample of fruiting *Bryum algovicum* from scrape in dune parabola slack at Newborough Warren. This sample returned DNA of *Didymodon tophaceus* due to mixed sampling.



Figure 41. Photo W1. Sandy track at Whiteford Burrows with LF *Bryum pseudotriquetrum* (DNA confirmed), F *Trifolium fragiferum*, O *Sagina nodosa*.

Full data table: excluded from the Accessible version of the report

Data Archive Appendix

Data outputs associated with this project are archived on server-based storage at Natural Resources Wales.

The data archive contains:

- [A] The final report in Microsoft Word and Adobe PDF formats.
- [B] Two spreadsheets of data in Microsoft Excel format.
- [C] Photographs in JPEG format.

The metadata for this project is held as record no 125500.

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