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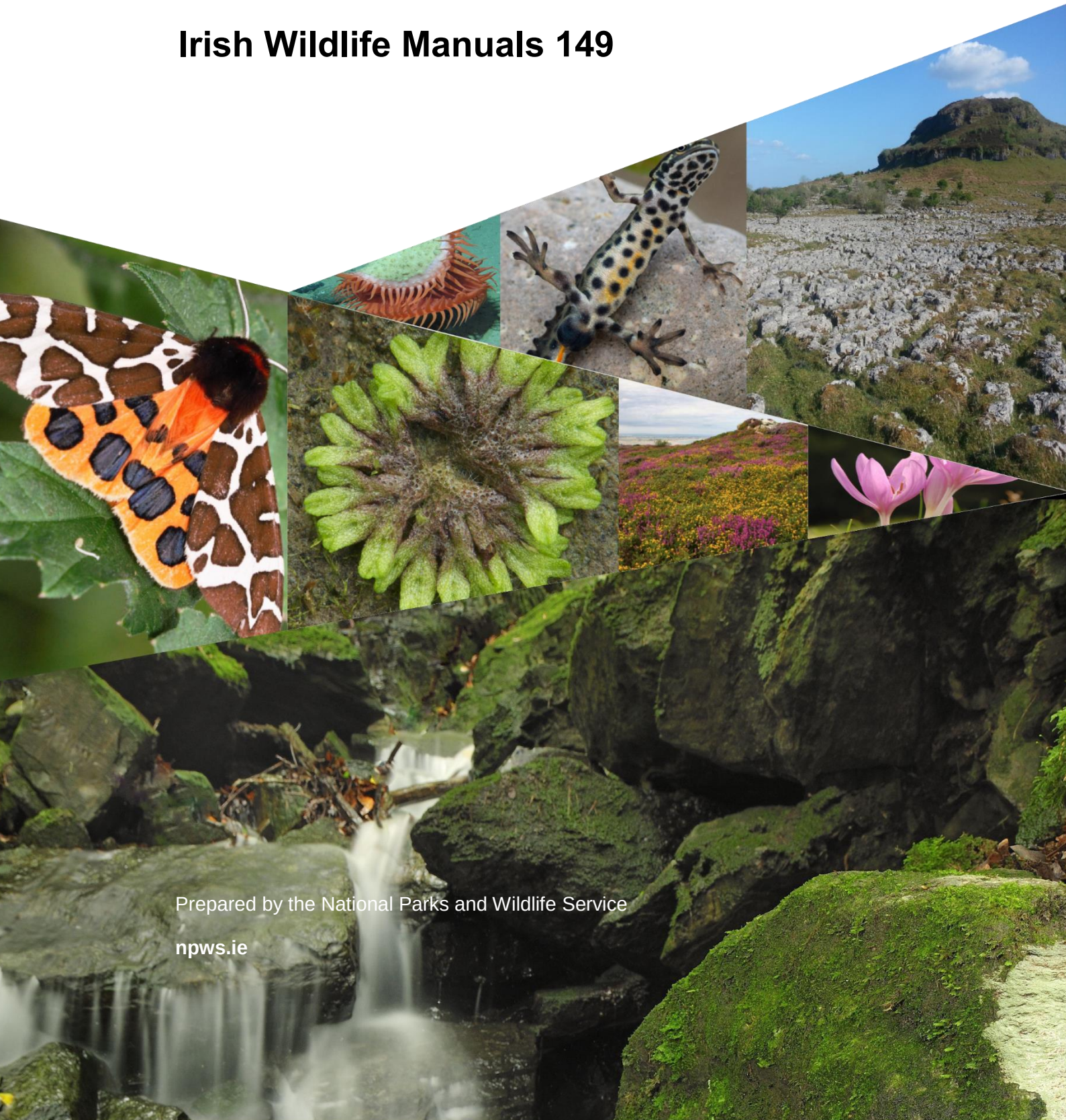
agus Fiadhúlra
National Parks and Wildlife Service

Assessing genetic variation and taxonomic integrity of selected bryophyte taxa in Ireland

Irish Wildlife Manuals 149



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Front cover, from left to right and top to bottom:

A deep water fly trap anemone *Phelliactis* sp., Yvonne Leahy; **Common Newt** *Lissotriton vulgaris,* Brian Nelson; **Limestone pavement**, Bricklieve Mountains, Co. Sligo, Andy Bleasdale; **Garden Tiger** *Arctia caja,* Brian Nelson; **Violet Crystalwort** *Riccia huebeneriana*, Robert Thompson; **Coastal heath**, Howth Head, Co. Dublin, Maurice Eakin; **Meadow Saffron** *Colchicum autumnale*, Lorcan Scott

Bottom photograph: **Irish Pouncewort** *Lejeunea hibernica*, Kylemore, Co. Galway, Robert Thompson

Assessing genetic variation and taxonomic integrity of selected bryophyte taxa in Ireland

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Contents

Executive Summary

The results of a molecular and morphological investigation into some of Ireland's rare and interesting bryophytes (mosses and liverworts) are presented. Fourteen target species were subjected to DNA analysis and microscope studies. There are significant molecular and morphological differences both within Irish populations and between Irish and non-Irish populations of several species, with especially significant results for *Didymodon maximus, Hamatocaulis vernicosus, Hypnum uncinulatum, Lejeunea eckloniana, L. flava, L. mandonii* and *Radula carringtonii*. It is suggested that there are likely to be many other bryophyte taxa in Ireland with hidden genetic diversity.

Acknowledgements

We would like to thank the following people who have helped this project in various ways, including providing specimens and expert advice, and guidance in the field: Neil Bell (Royal Botanic Gardens Edinburgh, Scotland), John Brinda (Missouri Botanical Garden, USA), Richard Caners (Royal Alberta Museum, Canada), Len Ellis (Natural History Museum, London, England), Susana Fontinha (Madeira), Rosalina Gabriel (Universidade dos Açores, Azores), Rory Hodd (Ireland), Martin Hutten (Glacier Bay National Park & Preserve, Alaska, USA), Misha Ignatov (Russian Academy of Sciences, Moscow, Russia), Juan Jiménez (Universidad de Murcia, Spain), Jan Kučera (University of South Bohemia, Czech Republic), Carlos Lobo (Botanic Garden of Madeira), David Long (Royal Botanic Gardens Edinburgh, Scotland), Tamás Pócs (Eszterházy Károly Catholic University, Hungary), Ron Porley (Portugal), Lesley Scott (Royal Botanic Gardens Edinburgh, Scotland), James Walton (National Park Service, Alaska, USA).

1 Introduction

1.1 Project Rationale

A co-ordinated research programme led by the National Parks and Wildlife Service (NPWS) of the Department of Housing, Local Government and Heritage over the last two decades has resulted in an enormous increase in our knowledge of mosses and liverworts (bryophytes) in Ireland. Ten years of fieldwork from 1999 to 2009 was followed by the publication of *Rare & Threatened Bryophytes of Ireland* (Lockhart *et al*., 2012a). Further work resulted in detailed documents on the occurrence of rare and threatened species at specific sites, a scientifically-backed revision of the Flora Protection Order, and detailed conservation assessments for Irish species listed on Annex II of the EU Habitats Directive. Leadership and support by NPWS has enabled the international importance of Ireland's bryophytes to be recognised through the publication of the IUCN European Red List (Hodgetts *et al*., 2019), the Checklist of European Bryophytes (Hodgetts *et al*., 2020), the Distribution and Country Status of European Bryophytes (Hodgetts & Lockhart, 2020) and Important Bryophyte Areas of Europe (in prep.).

The mosses and liverworts of Ireland are now known to be of international significance. In particular, there is a suite of species that are restricted globally to the Atlantic fringes of Europe and a few other places in the world, for which Ireland holds internationally important populations. However, an aspect of the recent research has identified a number of questions about the Irish bryophyte flora that still need answering, in order to place the Irish bryophyte flora in a global context and to further its conservation:

- How unique is the Irish bryophyte flora? How do Irish specimens compare with other taxa and is there any geographical variation within the taxa?
- What are the affinities of some widespread Irish taxa in which there have been shown to be cryptic species?
- What are the true identities and relationships of some species in Ireland?
- How does the Irish Atlantic bryophyte flora relate to other Atlantic bryophyte floras?
- How significant are Irish populations of globally rare species?
- What are the conservation implications and proposed actions?

The traditional route to answering questions on biological identity and uniqueness is the morphological and ecological route, which can be broadly considered together as the phenotype. We can assess the morphological and ecological similarities of taxa and establish species boundaries and ecological preferences. However, the use of molecular evidence has grown hugely in ecological work since the 1990s and is now used on a regular basis. DNA can be used to distinguish species and to discover cryptic variation, which may not be evident at a macroscopic level. This variation is important in terms of conservation, as it is the first level of biodiversity, *i.e.* genetic diversity. Thus, to address the questions above, we used DNA sequence analysis in combination with morphological investigation.

1.2 Target taxa

The target taxa for this project (14 taxa, see Table 1) were chosen by NGH, in discussion with NL. Most are strongly oceanic in their distribution and more or less confined in Ireland to the south-west of the country, with further populations in Macaronesia and in some cases further afield. However, *Didymodon maximus* is confined to the Dartry Mountains, with disjunct occurrences in North America and Siberia, and *Hamatocaulis vernicosus* is a more widespread species of mires and is listed on Annex II of the EU Habitats Directive. All taxa were chosen to test the hypothesis that Irish material might differ significantly from material from elsewhere. Specimens of non-target taxa (*Didymodon giganteus*, *Lejeunea canariensis, L. cavifolia*,

Plagiochila punctata and *Solenostoma obovatum*) were also included in the analyses, in order to compare them with target taxa.

Table 1 Target taxa selected for DNA analysis. IUCN Red List Categories: (EN) Endangered, (VU) Vulnerable, (NT) Near Threatened, (LC) Least Concern, (NA) Not Applicable. Red list status (Ireland) follows Lockhart *et al*., 2012a, 2012b; (Europe) Hodgetts *et al*., 2019.

Taxon	IUCN Status in Ireland/Europe	Distribution
Acrobolbus wilsonii Nees	VU/VU	W Ireland, W Scotland, Macaronesia, Faroe Is.
Cephalozia crassifolia Lindenb. & Gottsche (*Fuscocephaloziopsis crassifolia* (Lindenb. & Gottsche) Váňa & L.Söderstr.)	EN/LC	SW Ireland, Macaronesia, Spain, Central & S America
Didymodon maximus (Syed & Crundw.) M.O.Hill	NT/VU	NW Ireland, Siberia, Arctic Russia, N America
Hamatocaulis vernicosus (Mitt.) Hedenäs	NT/VU	Circumpolar, Turkey, N Africa, Central & S America
Hypnum uncinulatum Jur.	NT/LC	SW Ireland, Macaronesia, Portugal, Spain, Tunisia (?)
Lejeunea eckloniana Lindenb.	NT/LC	SW Ireland, W Scotland, Macaronesia, Portugal, Spain, Africa
Lejeunea flava (Sw.) Nees	VU/NT	SW Ireland, Macaronesia, pantropical
Lejeunea hibernica Bischl., H.A.Mill. & Bonner ex Grolle	NT/NT	SW Ireland, Macaronesia
Lejeunea mandonii (Steph.) Müll.Frib.	EN/VU	SW Ireland, W Britain, Macaronesia, Portugal, Spain
Plagiochila bifaria (Sw.) Lindenb.	LC/LC	Ireland, W Britain, W Europe, Central and S America, Galapagos Is.
Plagiochila heterophylla Lindenb. ex Lehm.	EN/LC	SW Ireland, W Britain, NW France, Central & S America
Radula carringtonii J.B.Jack	NT/NT	SW Ireland, W Scotland, Macaronesia
Radula holtii Spruce	NT/NT	SW Ireland, W Scotland, Macaronesia, Portugal, Spain, France
Solenostoma subellipticum (Lindb. ex Kaal.) Schust.	NT/NA	Circumboreal

2 Materials and Methods

2.1 General

This section gives only the general methodologies used. More detail for each taxon is provided in the Results (Section 3). Bryophyte nomenclature is according to the British and Irish bryophyte checklist (Blockeel *et al*., 2021), with names from the European bryophyte checklist (Hodgetts *et al*., 2020) also included where they differ. Taxa not included in these checklists have their authority included in the text. Species status is listed according to the Irish Red List (Lockhart *et al*., 2012b) and the European Red List (Hodgetts *et al*., 2019).

2.2 Samples

Fresh material was obtained for all the target taxa, supplemented with recent herbarium specimens where necessary. Fresh material from Ireland was collected mainly during targeted fieldwork by NGH in September 2021; material from Madeira was collected by NGH and Ron Porley during March 2022. Further fresh material from the Azores was collected by Rosalina Gabriel in May 2022, and from Monchique (Algarve, mainland Portugal) by Ron Porley and Nick Hodgetts in 2019 and 2020. Herbarium material was sourced from the National Botanic Gardens of Ireland, Glasnevin, Dublin (**DBN**) and the Royal Botanic Garden, Edinburgh (**E**). Additional herbarium material for study was received from Misha Ignatov in Russia.

All material was checked and identified or verified microscopically by NGH. Specimens were also subjected to further morphological study, both before molecular analysis and afterwards, in light of the molecular results.

Most of the molecular analyses were done by CTK and FO at Glasnevin, but analysis of *Hamatocaulis vernicosus* was done in Sweden by LH.

2.3 DNA Extraction and PCR

About one plantlet was taken for the DNA extraction. Samples were either herbarium specimens or silica dried specimens. Samples were homogenised using tungsten-carbide beads and a Qiagen TissueLyser II. Total genomic DNA was isolated from samples using Machery-Nagel NucleoSpin Plant II kits, following the manufacturer's instructions with modifications. The protocol using Buffer PL1 was used and samples were incubated for one hour at 56 °C in a water bath. A double elution was performed to give a total DNA sample of 130 µl. DNA extracts were run by electrophoresis on a 1.5% agarose gel stained with SYBRSafe to assess quality. DNA concentration was assessed using a Nanodrop 2000 Spectrophotometer (ND-2000). An aliquot of DNA was taken for analysis and the remainder is being stored in the DNA bank in the National Botanic Gardens (Appendix 1).

Target regions were amplified by PCR in an Applied Biosystems SimpliAmp Thermal Cycler. Cycling conditions were as follows: initial denaturing at 94 °C for 5 mins, 30 cycles of 94 °C for 1 min, 58 °C for 1 min, 72 °C for 1 min, followed by a final extension at 72 °C for 10 mins.

The 30 µl volume PCR reactions consisted of 15 µl (1 X) Bioline MyTaq HS Mix or PCRBIO Taq Mix, 11 µl of H_2O, 2 µl of template DNA (approx. 10 ng), and 1 µl each of the forward and reverse primers at 0.2 µM concentration. The PCR product was stained and checked after gel electrophoresis on a SYBRSafe stained 1.5% agarose gel. The Bioline EasyLadder 1 was used to estimate bp (base pair) size of the PCR products in order to confirm the correct region was being amplified.

2.4 DNA Sequencing and Analysis

PCR products were cleaned using Bioline SureClean and sent for Sanger sequencing to Macrogen Europe. Electropherograms were processed and aligned using Geneious 2021.0.1. Sequences were BLAST searched (Altschul *et al*., 1990) against NCBI/GenBank (https://www.ncbi.nlm.nih.gov/) data to confirm identification. To compare the samples against known taxa, DNA sequences were downloaded from GenBank and used to create a sequence alignment and a Neighbor-Joining tree using a Tamura-Nei genetic distance model in Geneious. The analysis from the Neighbor-Joining trees can be used to define genetic groups or haplotypes. A haplotype is a combination of genetic markers on a single chromosome or markers linked through inheritance. In this case, the haplotypes are from a single chromosome in the chloroplast or as an inherited set of markers in the ITS nuclear region. To show the relationships of the different genetic groupings, haplotype networks were created using PopArt version 1.7 (Leigh *et al*., 2015). The haplotype networks were created using the Templeton-Crandall-Sing (TCS) method, an agglomerative algorithm that progressively combines clusters sharing one or more connecting edge (Templeton *et al*., 1992). The TCS algorithm is designed to be particularly accurate in analysis of divergence at the population level, where ancestral haplotypes are likely to occur commonly in the population and variation is relatively low (Clement *et al*., 2000). The network diagrams were annotated using InkScape version 1.3.1 (www.inkscape.org).

The two main regions used for the molecular analysis were the *trn*L-F (Taberlet *et al*., 1991) and the ITS (White *et al*., 1990). The *trn*L-F region is located in the chloroplast DNA and tends to be relatively conserved but is useful for detecting variation between species and can also be used for assessing variation between populations. The ITS is a nuclear DNA region and tends to be more variable, so while it can be more informative, it can also be more difficult to work with, as a single individual can contain multiple copies. Additional regions were used for specific taxa to give greater resolution - for *Didymodon* taxa additional chloroplast regions were used; *atp*B-*rbc*L, *rps*4, *trn*G, *trn*V-*trn*M (Jiménez *et al*., 2021; Kučera & Ignatov, 2015).

As this project primarily involved rare species, there were limitations associated with the analysis, in particular the number of samples analysed. Rare species by their nature have limited ranges and limited population numbers and so the resolution of the data can be limited. See Appendix 1 for sample details. Out of 143 samples tested, 116 were successfully analysed for the *trn*L-F region, while only 40 were successfully analysed for the ITS region. The low success rate for the ITS region was due mainly to difficulties in amplification for the liverwort taxa. Despite these limitations, a number of significant results could be determined for each taxon. The results are presented below by taxon.

Molecular analysis of *Hamatocaulis vernicosus* concentrated on the nuclear internal transcribed spacers 1 and 2 (ITS) and the plastid ribosomal protein 16 G2 intron (*rpl*16) and *trn*L intron and *trn*L_{UAA}-*trn*F_{GAA} intergeneric spacer (*trn*L-*trn*F). The molecular laboratory work was performed as by Hedenäs & Eldenäs (2007) and Hedenäs (2018). Sequence editing and analyses included the following, as described in more detail by Hedenäs *et al*. (2022). Nucleotide sequence fragments for each DNA region were edited and assembled using PhyDE® 0.9971 (http://www.phyde.de/index.html; accessed 2 March 2021). After manual alignment and exclusion of partially incomplete data in the beginning and end of the sequences, gaps were coded using the simple indel coding of Simmons & Ochoterena (2000) in SeqState (Müller, 2005) and were included in the analysis.

Because no incongruence between the nuclear and plastid markers was found in earlier studies of *H. vernicosus* (Hedenäs & Eldenäs, 2007, 2008), all molecular data was analysed in combination. Since reticulation occurs in *H. vernicosus s.l.* (Hedenäs & Eldenäs, 2007; Hedenäs, 2018), relationships were evaluated in the context of other European samples (from Hedenäs *et al*., 2022) with the program TCS (Clement *et al*., 2000). Specimens for which only one or two of the molecular markers could be generated were referred to either of the two cryptic species, based on their available sequences, but were not included in the haplotype analyses.

3 Results and Discussion

3.1 *Acrobolbus wilsonii* Nees

Figure 1 *Acrobolbus wilsonii* Nees. Photograph Nick Hodgetts.

Acrobolbus wilsonii (Wilson's Pouchwort) (Figure 1) is a rare oceanic liverwort restricted to the west of Ireland, Scotland, Macaronesia and the Faroe Islands. It is listed on the Flora (Protection) Order, 2022.

There was limited data available for *Acrobolbus wilsonii*. Only three samples were analysed successfully with molecular techniques, two from Madeira and one from Ireland, and there is limited variation in both the *trn*L-F and the ITS regions. Of these three samples, only 172 bp (base pairs) were aligned. Very little inference is possible based on the data generated, although the ITS data suggest that there could be distinct differences between the Irish and the Madeira populations. The *trn*L-F region indicates that the Irish sample (22D084) groups with the UK sample from GenBank, and the other samples from Madeira (22D086 and 22D087) group separately (Figure 2). However, the data is very limited and needs further investigation.

There is also no discernible morphological difference between Irish and Macaronesian material. We cannot infer much with the data generated, except that the Irish and Madeira populations are different.

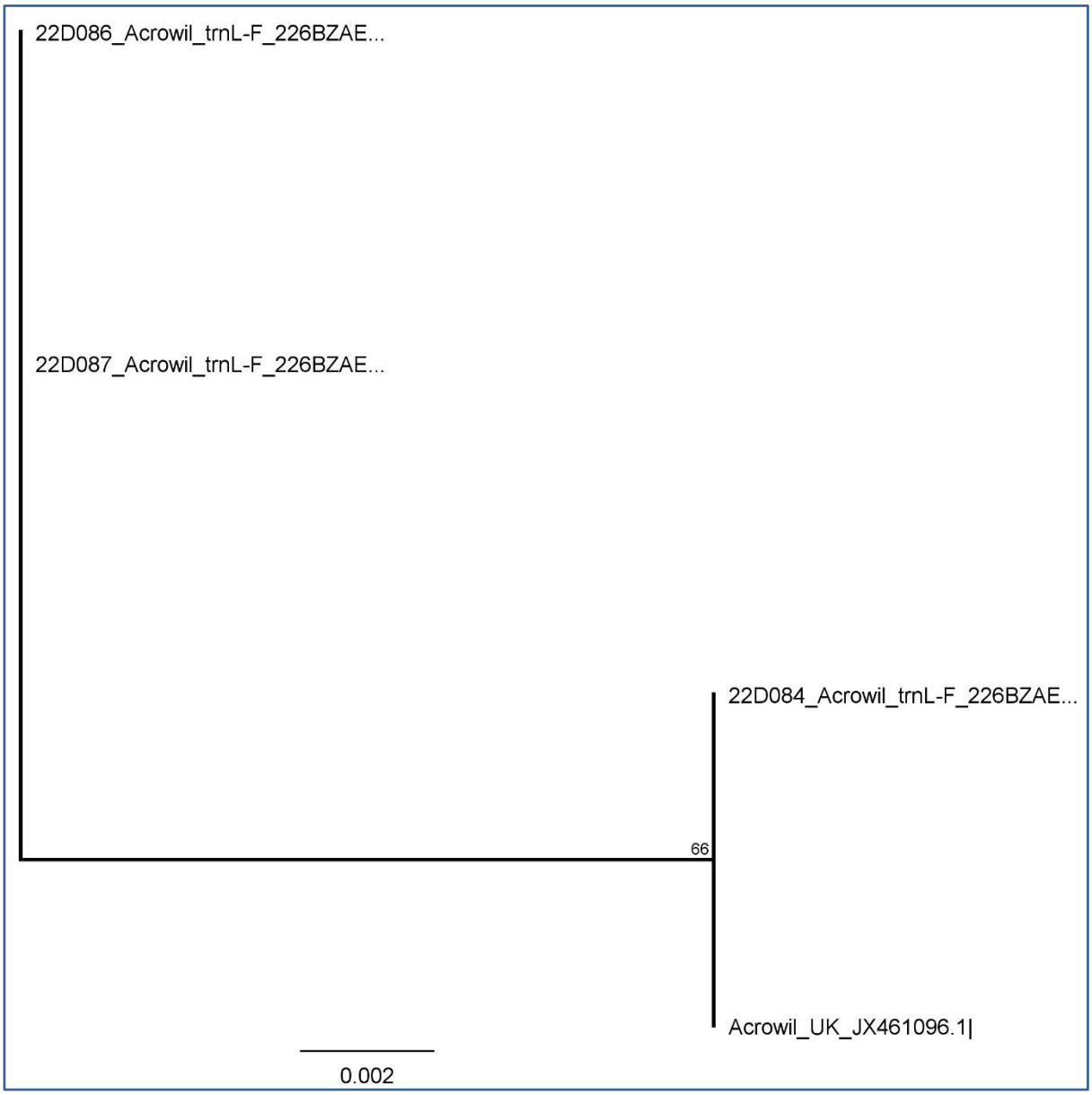

Figure 2 Neighbor-Joining tree of the *trn*L-F region from *Acrobolbus wilsonii* samples analysed here and one from GenBank. The scale represents the number of nucleotide changes and the numbers on the branch nodes indicate the level of confidence of the grouping.

3.2 *Cephalozia crassifolia* Lindenb. & Gottsche

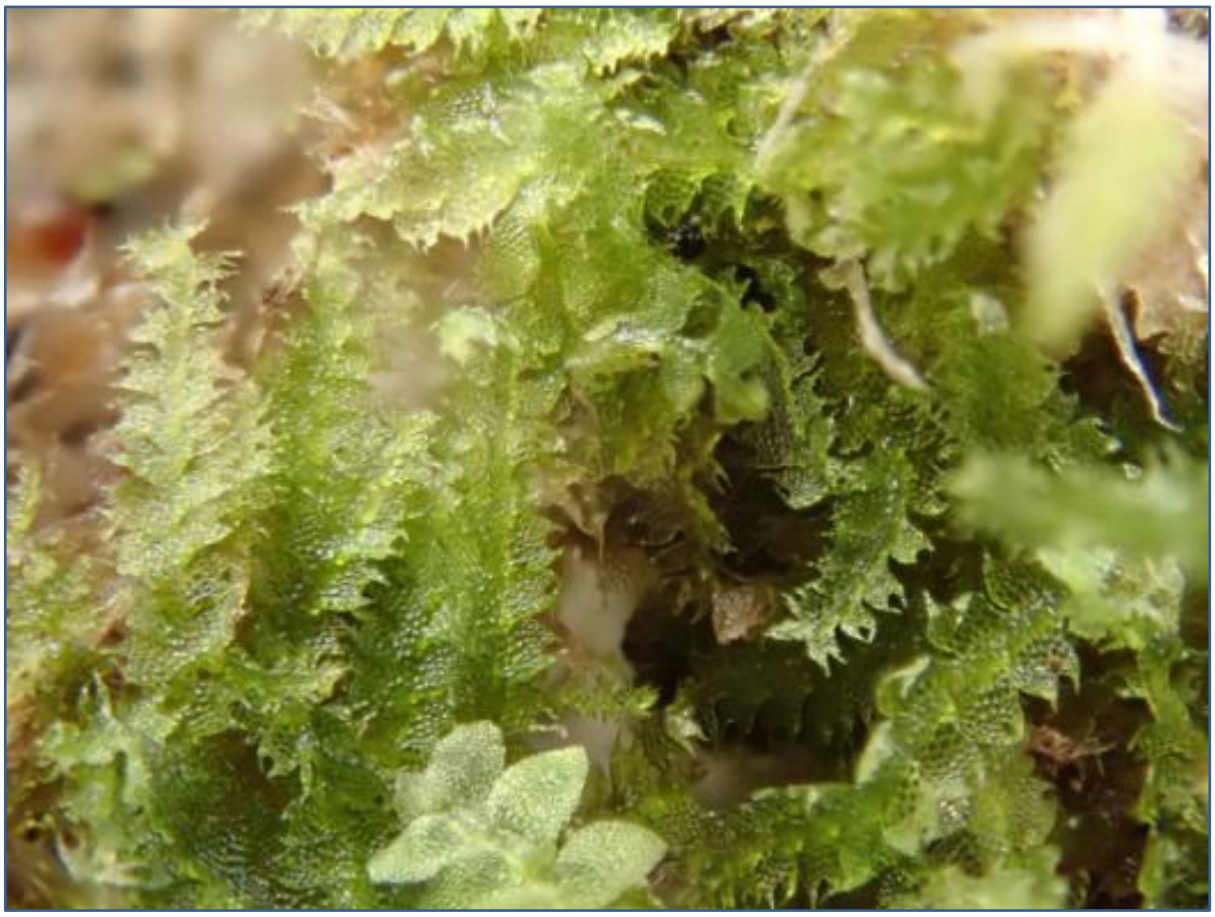

Figure 3 *Cephalozia crassifolia* Lindenb. & Gottsche [*Fuscocephaloziopsis crassifolia* (Lindenb. & Gottsche) Váňa & L.Söderstr.)] Photograph © British Bryological Society.

Cephalozia crassifolia (Irish Pincerwort) (Figure 3) is a rare oceanic liverwort restricted to the south-west of Ireland, Spain, Macaronesia and Central and South America. It is listed on the Flora (Protection) Order, 2022. Until recently it was regarded as *C. hibernica* Spruce ex Pearson, a European endemic, but this was synonymised with the Neotropical *C. crassifolia* by Váňa (1988), a synonymy that was accepted by Grolle & Long (2000) and Hodgetts *et al*. (2020).

There was limited data available for *Cephalozia crassifolia*. A total of five specimens were successfully analysed using molecular techniques. The data show limited variation in both the *trn*L-F and the ITS regions, and the samples from Ireland and the Azores group together with no distinct differentiation. However, they do group separately (using GenBank data) from specimens from Panama, although no morphological differences have so far been identified. There is also no noticeable difference between Irish and Azorean specimens morphologically. Very little inference is possible based on the data generated.

3.3 *Didymodon maximus* (Syed & Crundw.) M.O.Hill

Figure 4 *Didymodon maximus* (Syed & Crundw.) M.O.Hill. Photograph Nick Hodgetts.

Didymodon maximus (Irish Beard-moss) (Figure 4) is a very rare plant restricted in Ireland to the Dartry Mountains of Cos. Sligo and Leitrim, and apparently also occurring disjunctly in North America, Siberia and Arctic Russia. It is listed on the Flora (Protection) Order, 2022.

A total of 14 samples were analysed across five gene regions (*trn*L-F, ITS, *atp*B-*rbc*L, *rps*4, *trn*G, and *trn*V-*trn*M). The samples were from Ireland (10), and from Siberia (two) and Wrangel Island (two) in Russia (Figure 5). The specimens were mostly labelled as *D. maximus*, with one specimen of the closely-related *D. giganteus* from Wrangel Island. A Neighbor-Joining tree of the samples shows the Irish samples grouping onto three different branches on the tree (Figure 6). Most samples group together (haplotype 1, H1), along with other samples from GenBank. Although this shows GenBank samples of *D. giganteus* in the same grouping, nothing should be read into this, as more detailed work (Kučera *et al*., 2018) clearly shows *D. maximus* and *D. giganteus* on different, albeit closely related, branches of the tree. The *trn*L-F data in Figure 6 is based on an alignment of 317 bp and the *trn*G data in Figure 7 is based on a 460 bp alignment. A limited number of outgroups were also added. Additional data and additional samples should increase the resolution of this analysis.

Three specimens group together as a separate group (haplotype 2, H2). One specimen, 22D045 from the DBN herbarium (DBN0001130, Benbulbin, summit area, NGH4494), is anomalous, grouping with *D. spadiceus* in GenBank and using genetic data from *trn*L-F and ITS (Figure 7), but morphologically identical to *D. ferrugineus*. The same pattern of haplotypes is repeated in the other gene regions analysed, *e.g. trn*G (Figure 7).

Morphological studies confirm the differences between haplotypes 1 and 2. Haplotype 1 conforms morphologically to the type specimen of *D. maximus* (Ben Bulben, Sligo, 1871, Moore s.n., **BM**). These specimens are generally large, the shoots more than 3 cm long. The

leaves are ovate-lanceolate, (1.5–)2–2.8 mm long x 0.5–1.1 mm wide, strongly recurved to squarrose when moist, not undulate. The lamina turns dark red with KOH. The costa is *c.* 55–75 µm wide near the base, convex on the abaxial surface, ± flat adaxially, with six well-differentiated guide cells and the stereids in *c.* two rows both abaxially and adaxially. The lamina cells are nearly smooth or only very weakly papillose.

Haplotype 2 is morphologically distinct. The costa is *c.* 120 µm wide near the base, in transverse section ± biconvex. The guide cells are less well-differentiated from the stereids compared with haplotype 1, being only somewhat larger and in a row of 6–10. There are 4–6 rows of stereids abaxially, 2–3 rows adaxially. The lamina cells are strongly papillose. These specimens are molecularly very close to *D. fallax* according to GenBank and Jan Kučera (pers. comm. 2023, Figure 8). The Irish distribution of the two haplotypes is shown in Figure 9.

D. maximus is closely related to *D. ferrugineus*, which is differentiated by its smaller size, the leaves (0.5–)0.7–2 mm long, and the costa in transverse section without adaxial stereids (Jiménez, 2006). According to Syed & Crundwell (1973), “The large size of both stem and leaves is of itself sufficient to distinguish *D. maximus* from all forms of *D. ferrugineus*, a species that is much less variable in size and other characters than the related *D. fallax*. The more incrassate basal cells of the leaves, the larger size of the central strand of the stem and the thinner walls of its cells are also reliable diagnostic characters”.

D. giganteus is close to *D. maximus* but not identical, coming out in a distinct group in GenBank. Indeed, it is a very distinctive species, distinguished by its relatively enormous size, as it can reach as much as 23 cm in length, and by its ovate-lanceolate, keeled leaves (2–2.5)4–5 mm long x 0.7–1.1 mm wide, flexuous-appressed when dry, spreading to recurved moist, with undulate margins in the upper half. The laminal cells are very sinuous and irregularly thick-walled, much more so than in *D. maximus*, and distinctly papillose, the basal cells porose (Jiménez *et al*., 2005; Jiménez, 2006; Noguchi, 1988). The costa is *c.* 75 µm wide near the base, in transverse section: ± concave on the adaxial surface to ± biconvex. There are 4–6 guide cells, which are much larger than, and well-differentiated from, the stereids. The Asian *D. erosodenticulatus* (Müll.Hal.) K.Saito is very similar to *D. giganteus*, but has dentate leaf margins (Noguchi, 1988).

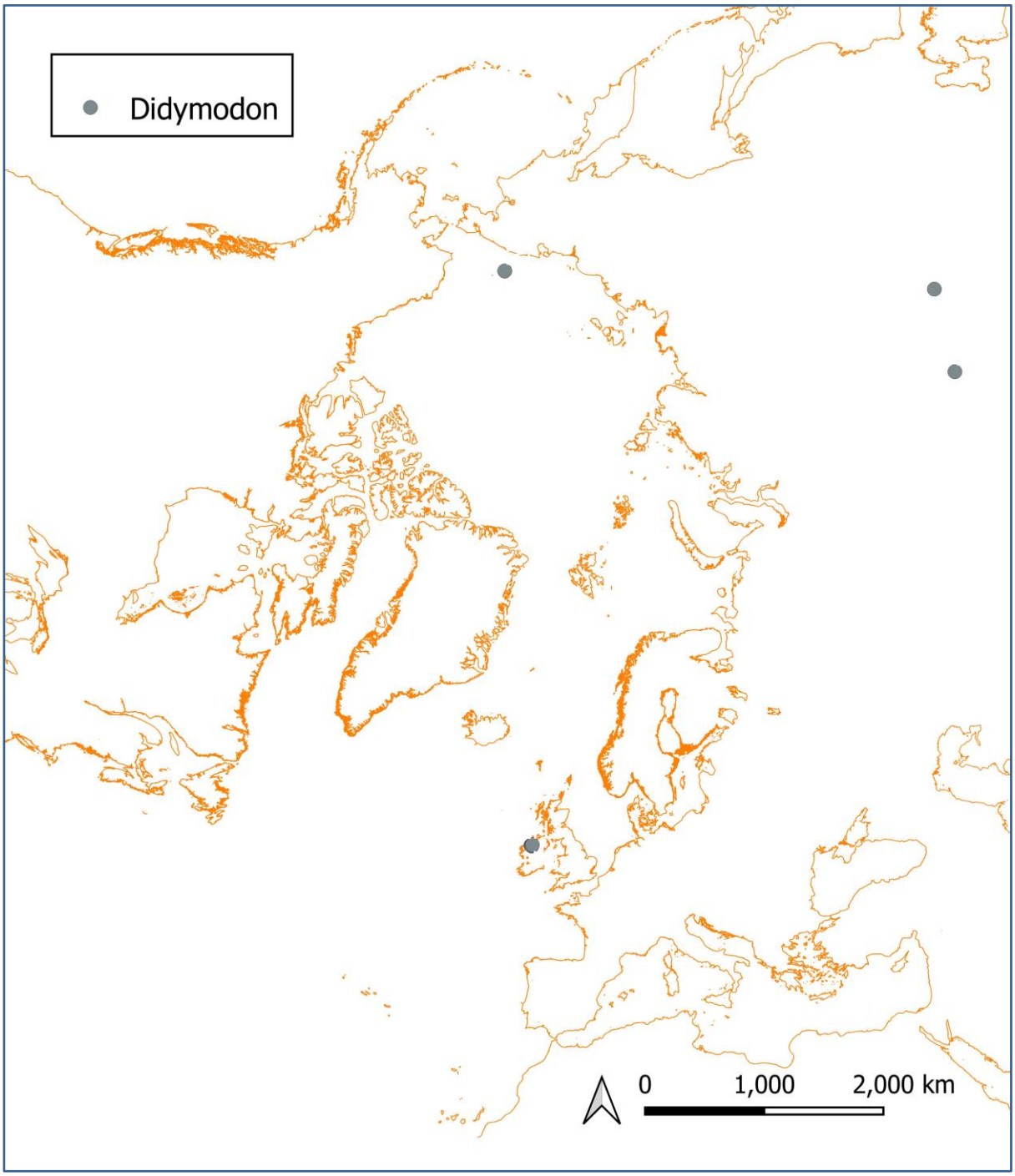

Figure 5 Map showing the locations of the *Didymodon* samples used in the analysis. The samples were from Ireland, Siberia and Wrangel Island, Russia.

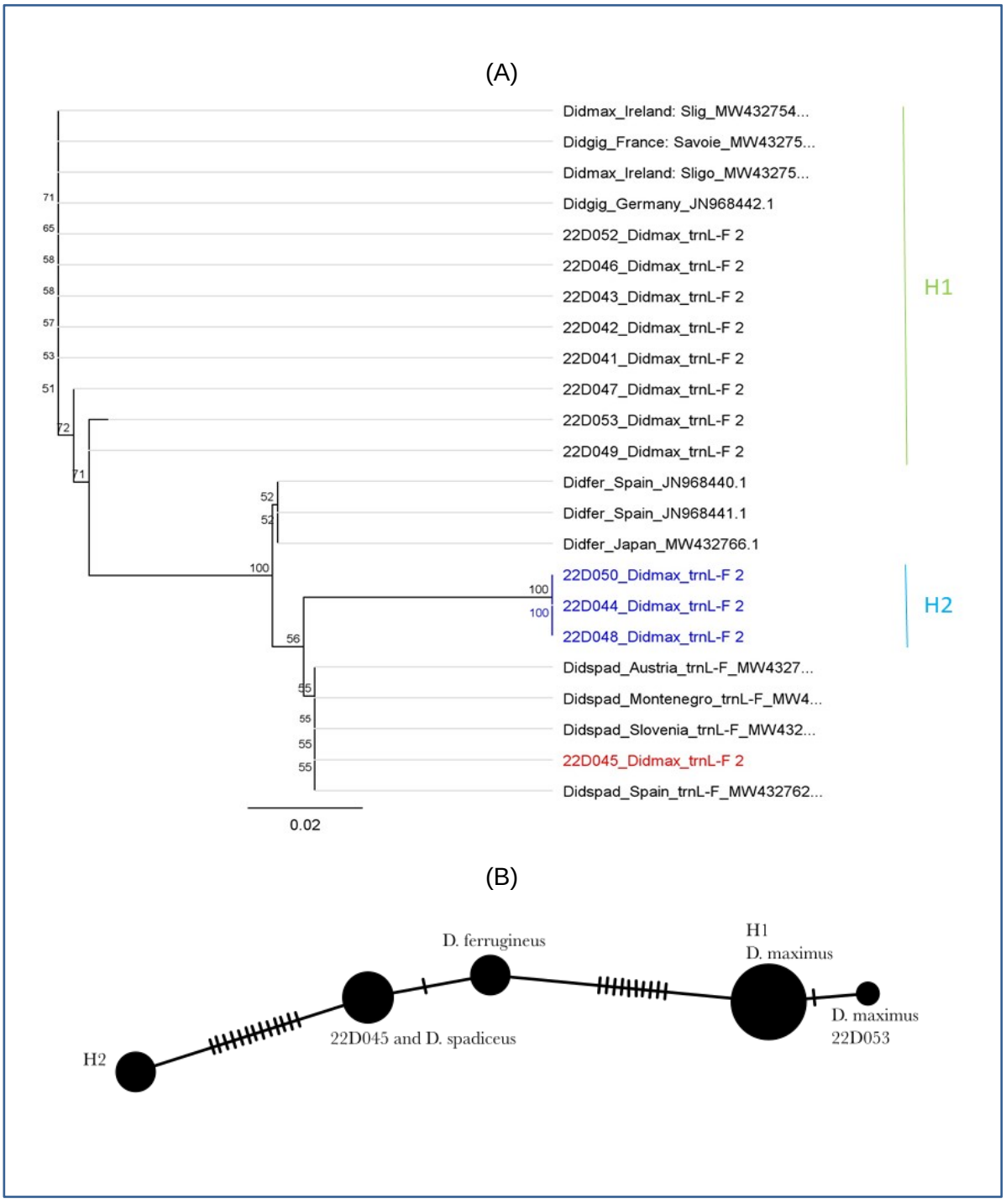

Figure 6 (A) Neighbor-Joining tree of the *trn*L-F region from *Didymodon* samples, including outgroup species *D. ferrungineus* and *D. spadiceus*. Most samples group together into a group that is composed of other *D. maximus* samples from GenBank (H1). Three specimens group together as a separate group (H2). The suspected *D. spadiceus* specimen (22D045) groups with other *D. spadiceus* specimens from GenBank. The scale represents the number of nucleotide changes and the numbers on the branch nodes indicate the level of confidence of the grouping. (B) Haplotype network of the *trn*L-F data from the same specimens. Circles represent haplotype groups and the size of the circles is dependent on the number of individuals in the group. The cross-bars represent the number of differences between haplotypes.

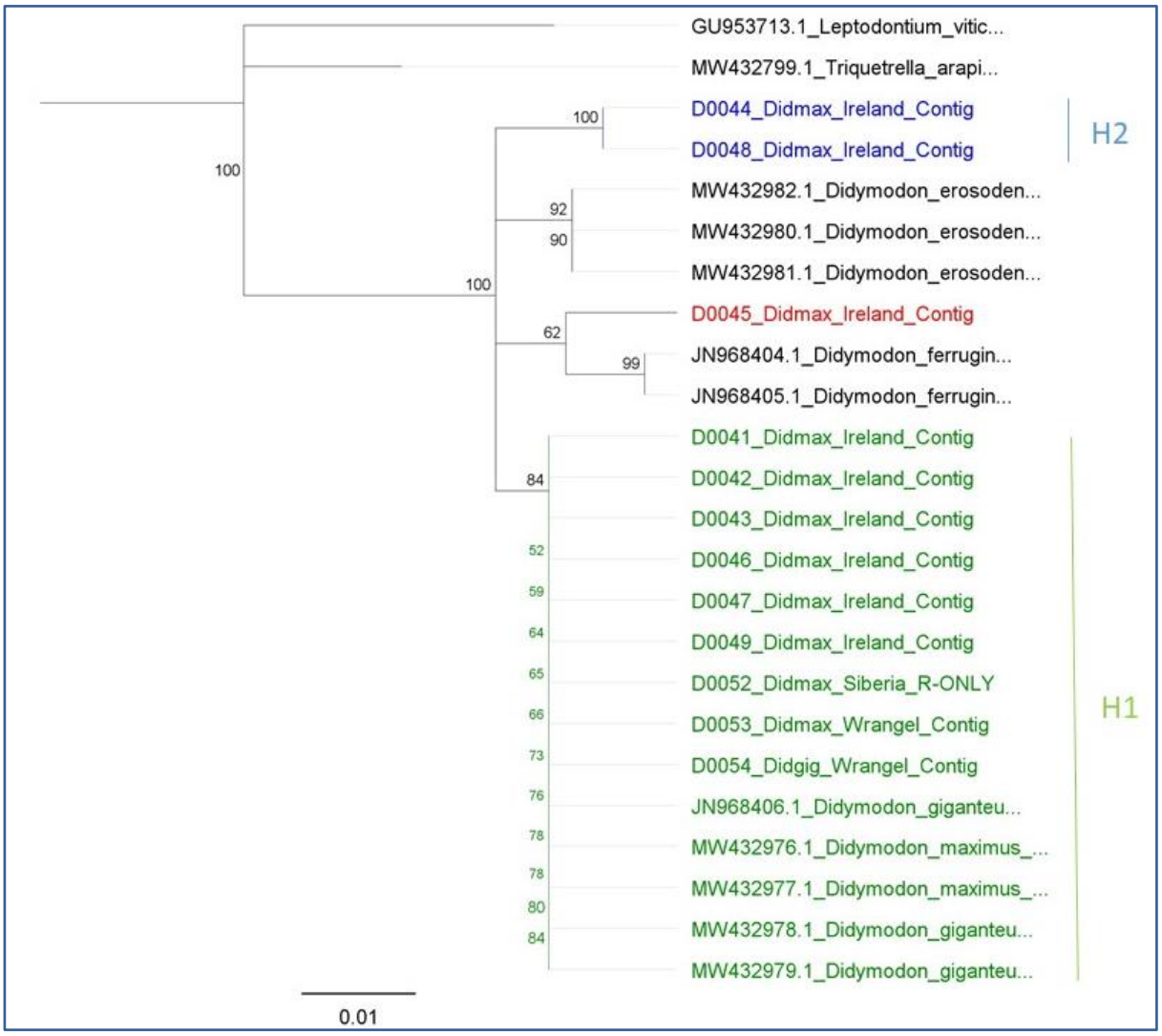

Figure 7 Neighbor-Joining tree of the *trn*G region from *Didymodon* samples. The data from this tree shows a similar pattern to that in Figure 6. Most of the Irish specimens group with other *D. maximus* specimens in H1. Only two of the three from H2 group separately and the suspected *D. spadiceus* sample, coloured red, groups out with other related taxa. The scale represents the number of nucleotide changes and the numbers on the branch nodes indicate the level of confidence of the grouping.

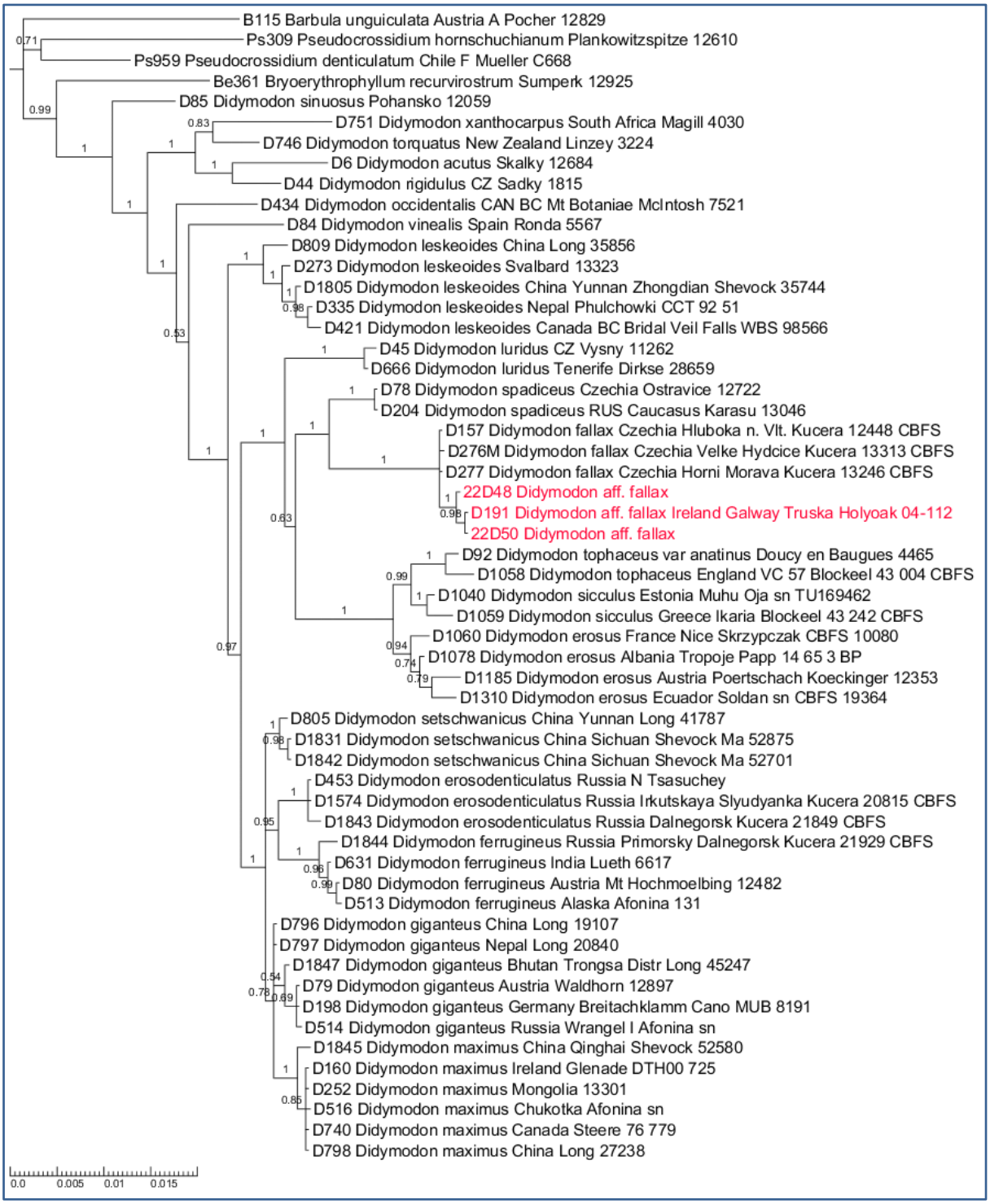

Figure 8 A majority consensus tree from a Bayesian analysis of the *trn*V-*trn*M loci for *Didymodon* species. The Irish H2 samples are highlighted in red. (Figure supplied by J. Kučera.)

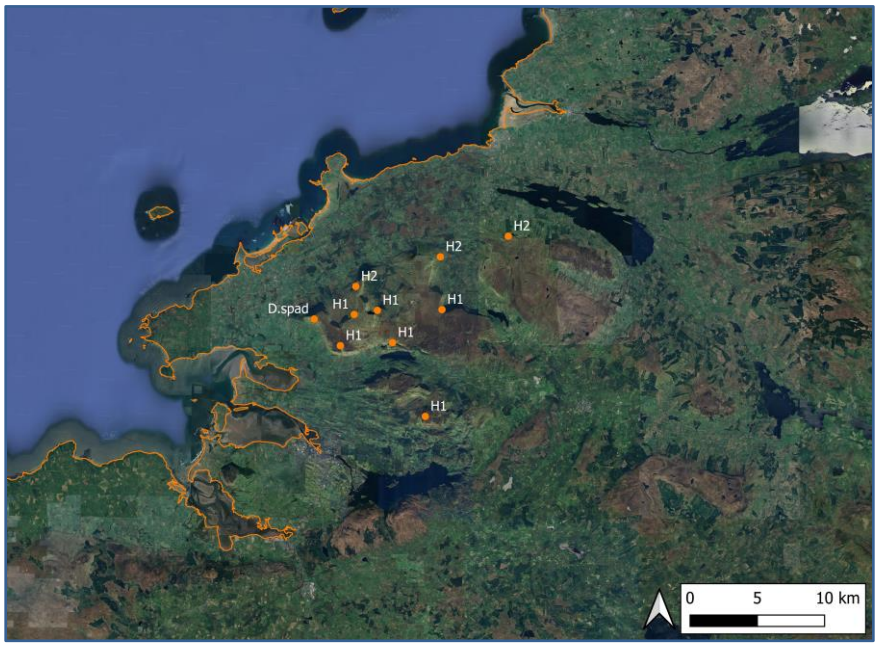

Figure 9 Map showing the locations of the Irish samples in the Dartry Mountains in Sligo and Leitrim, with labels showing the haplotype designation and the specimen identified as *D. spadiceus*.

In summary, three groups were found among the Irish specimens labelled as *Didymodon maximus*. This was found across multiple gene regions as shown here for *trn*L-F (Figure 6 A and B) and *trn*G (Figure 7). Haplotype 1 (H1) corresponds to *D. maximus* and groups with other *D. maximus* specimens from GenBank (Figure 6 A and B). The Neighbor-Joining tree (Figure 6 A) shows the individual samples in the different groups, whereas the haplotype network (Figure 6 B) shows the overall structure of the groups. Although individuals in the haplotype 2 (H2) group were labelled as *D. maximus*, they clearly are not, based on the molecular data (Figure 6 A and B). When analysed against a larger dataset (*trn*V-*trn*M data, Jan Kučera, pers. comm.) using other molecular markers, the specimens from H2 group closer to *D. fallax* than *D. spadiceus* (Figure 8). However, they do not group with *D. fallax* and are suspected to be unique taxa. The third group indicates that one individual collected in Ireland is *D. spadiceus* as it groups with other *D. spadiceus* specimens from GenBank. However, morphologically it is very like *D. ferrugineus*. There is no obvious geographical pattern or clustering of the different haplotypes (Figure 9). The samples are found across the Dartry mountain range. Indeed, a specimen analysed by Jan Kučera, which also groups with other H2 individuals, was collected in Galway.

Historically, there has been considerable confusion surrounding *D. maximus*. Using the results of this study, GenBank and information from Jan Kučera, it is now confirmed that *D. maximus* is a rare disjunct species occurring in Ireland, Siberia and Canada, although some specimens from Siberia and Alaska labelled '*D. maximus*' have been redetermined as *D. giganteus*, including the Alaskan Hutten 16650 (**CAS**) (Juan Jiménez, pers. comm. November 2021). The clear morphological differences between *D. maximus* and *D. giganteus* are confirmed. However, some specimens from Ireland labelled '*D. maximus*' have been misidentified, including the three identified as 'haplotype 2' in the present study, and Long 14626 (**E**), which was (unfortunately) used to illustrate *D. maximus* by Jiménez (2006), and also corresponds morphologically to 'haplotype 2', although it has not been subjected to molecular analysis. There are also specimens from Scotland in the private herbarium of NGH labelled *D. spadiceus* that are morphologically indistinguishable from 'haplotype 2'. Further study is needed to clarify what these plants should be named. It seems likely that a full revision of herbarium specimens of *D. maximus* and *D. spadiceus* is required in the light of these results. The individual in the third group is problematic. It appears to be *D. ferrugineus* morphologically, but groups with *D. spadiceus* using molecular analysis.

3.4 *Hamatocaulis vernicosus* (Mitt.) Hedenäs

Figure 10 *Hamatocaulis vernicosus* (Mitt.) Hedenäs. Photograph Nick Hodgetts.

Hamatocaulis vernicosus (Slender Green Feather-moss) (Figure 10) is listed on Appendix 1 of the Bern Convention and Annex II of the EU Habitats Directive, and is covered by the Flora (Protection) Order, 2022 in Ireland. It is however widespread in mesotrophic fen habitats throughout temperate parts of the Northern Hemisphere. Previous work (Hedenäs & Eldenäs, 2007; Hedenäs, 2018; Hedenäs *et al*., 2022) has shown that *Hamatocaulis vernicosus* occurs in Europe as two genetically distinct but morphologically indistinguishable cryptic species. All the Irish material examined corresponds to one of these ('cryptic species 1' in Figures 11 and 12). Figure 11 shows the haplotype network for Irish specimens. Figure 12 shows the position of the Irish haplotypes within the European haplotype network.

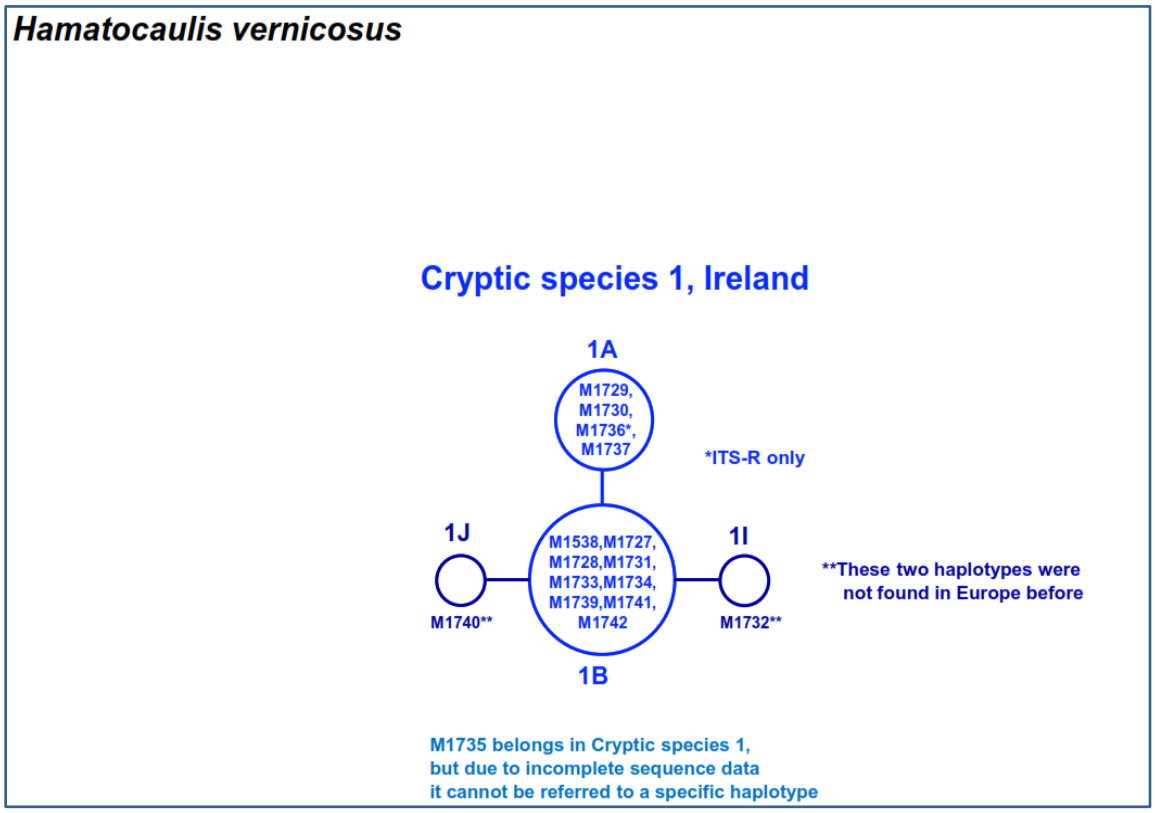

Figure 11 Irish specimens of *Hamatocaulis vernicosus* analysed and assigned to different haplotypes of cryptic species 1.

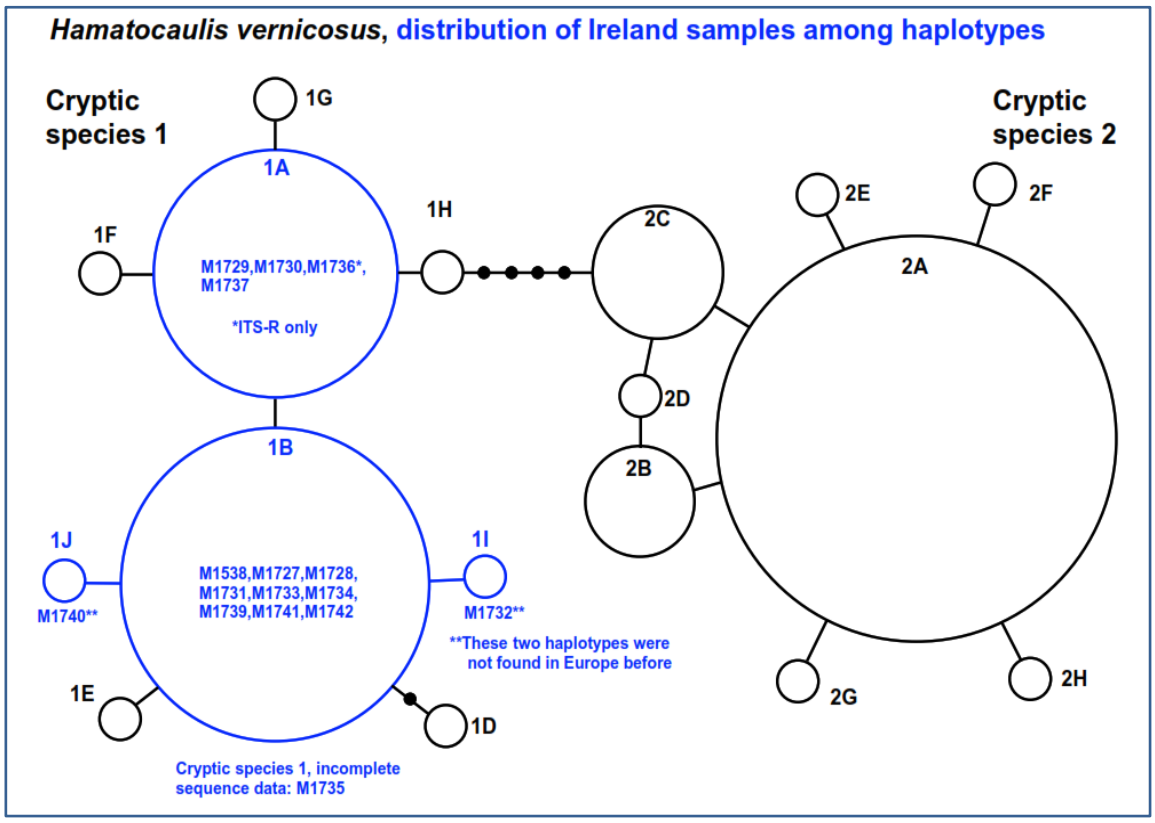

Figure 12 Irish specimens of *Hamatocaulis vernicosus* showing the position of the Irish haplotypes within the European haplotype network.

Although no morphological differences have been found between the cryptic species or the haplotypes, there is clearly considerable genetic diversity within *H. vernicosus* in Ireland. This

includes two unique haplotypes, 1I and 1J. 1I is from Scragh Bog, Westmeath and 1J is from Largan More, Mayo. Scragh Bog therefore has two haplotypes, suggesting that, although sporophytes are very rare in *H. vernicosus*, they are produced occasionally, leading to genetic variation. It is interesting that the newly identified population from Lough Patrick, which is not far from Scragh Bog, is haplotype 1B, not the same as that of Scragh Bog. There does not seem to be any convincing geographical pattern to the distribution of haplotypes (Figure 13).

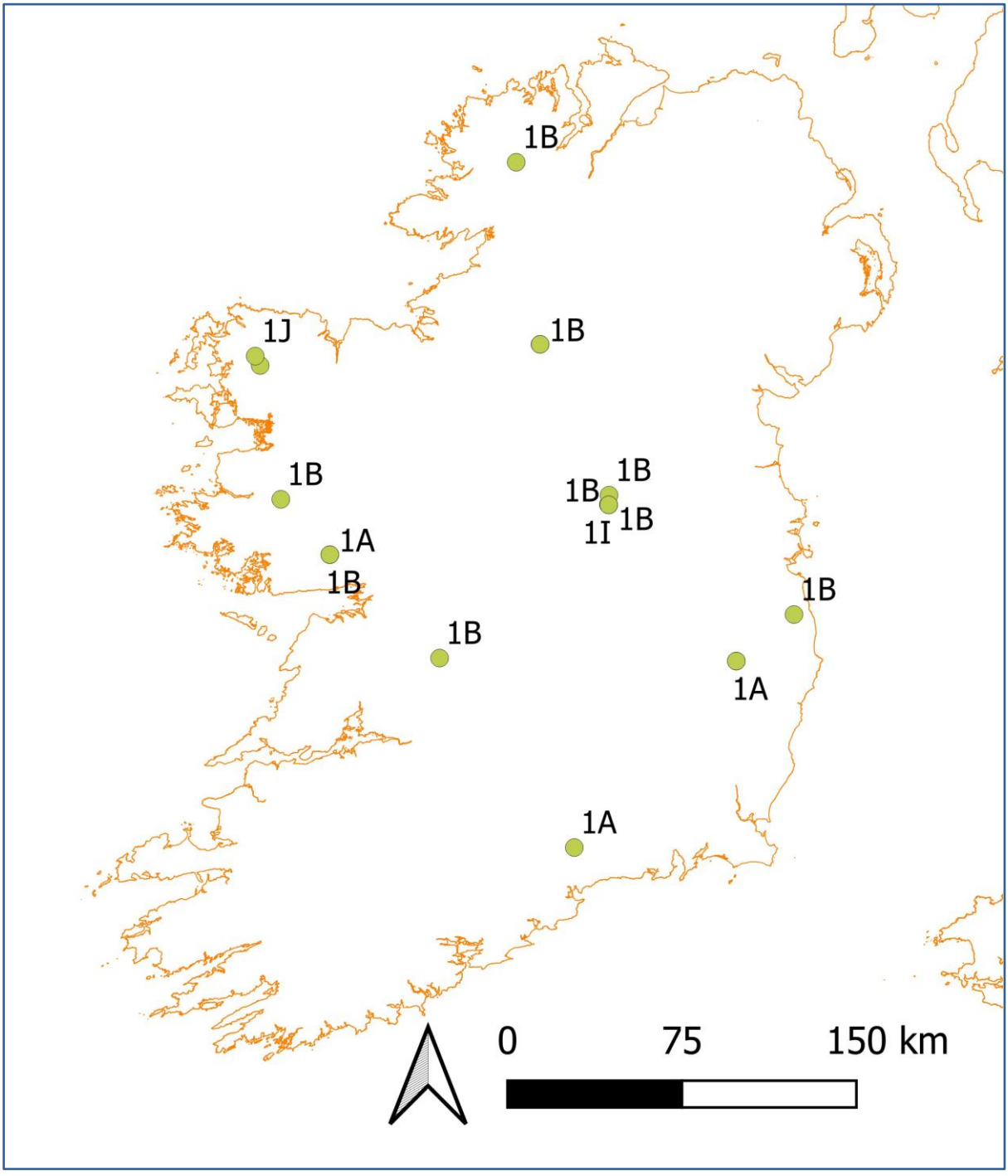

Figure 13 Map showing the locations of the *Hamatocaulis vernicosus* samples and their haplotype designation.

3.5 *Hypnum uncinulatum* Jur.

Figure 14 *Hypnum uncinulatum* Jur. Photograph Robert Thompson.

Hypnum uncinulatum (Hooked Plait-moss) (Figure 14) is a rare moss restricted to the south-west of Ireland, Macaronesia, Portugal and Spain. It is listed on the Flora (Protection) Order, 2022.

A total of 17 *Hypnum uncinulatum* specimens were used in the analysis, consisting of eight samples from Ireland, three from Madeira, three from Monchique (mainland Portugal) and three from the Azores (Figure 15). The main finding from the Neighbor-Joining tree analysis was the emergence of two distinct haplotypes (Figure 16), one from mainland Portugal and South Kerry, the other from Macaronesia and North Kerry. Although not all of the samples gave sufficiently clear ITS data, the samples that were successful show a similar pattern to the *trn*L-F data (Figure 17). Sample 22D066 groups separately. The distribution of the two haplotypes in Ireland is shown in Figure 18.

Microscope study revealed consistent morphological differences between the two main haplotypes:

Group 1: Portuguese mainland and South Kerry. These tend to be smaller plants, with the stem leaves more or less the same size as the branch leaves. The leaves are entire to weakly denticulate to about halfway down, and not cordate at the base. The alar cells form auricles, with the 'notch' between the auricle and the lamina occurring 2–3 or more cells above the 1–2 large hyaline cells in the extreme corner – *i.e.* most of the alar cells are within an auricle. The alar region is not or weakly excavate and consists of many (usually >36) small, thick-walled isodiametric/wider than long cells, which tend to ascend up the margin. The transition from alar cells to lamina cells is rather abrupt.

Group 2: Macaronesia and North Kerry. These tend to be larger plants, with the stem leaves tending to be larger than the branch leaves. The leaves (or at least some of them) are strongly denticulate in the acuminate apex, and often denticulate to about ⅔ the way down, except in really poor material, and tending to be slightly cordate at the base. The alar cells hardly form auricles, the 'notch' occurring almost immediately above the 1–2 large hyaline cells in the

extreme corner – *i.e.* most of the alar cells are not in an auricle. The alar region is distinctly excavate and consists of few (<30, usually <20) small, thick-walled isodiametric/wider than long cells, not or hardly ascending up margin. The transition from alar cells to lamina cells is relatively gradual.

Combining our data with data from GenBank, haplotype 1 groups neatly with *H. andoi*, and haplotype 2 with *H. uncinulatum* (Figures 16 and 17). This also shows that some material in GenBank identified as *H. uncinulatum* is more likely to be *H. andoi*.

One additional specimen from Ireland (22D066) consistently groups outside of the *H. uncinulatum* and the *H. andoi* groups. This specimen groups with *H. uncinulatum*, but is sufficiently different to separate out from the main grouping. It is possibly a form or a different haplotype of *H. uncinulatum*.

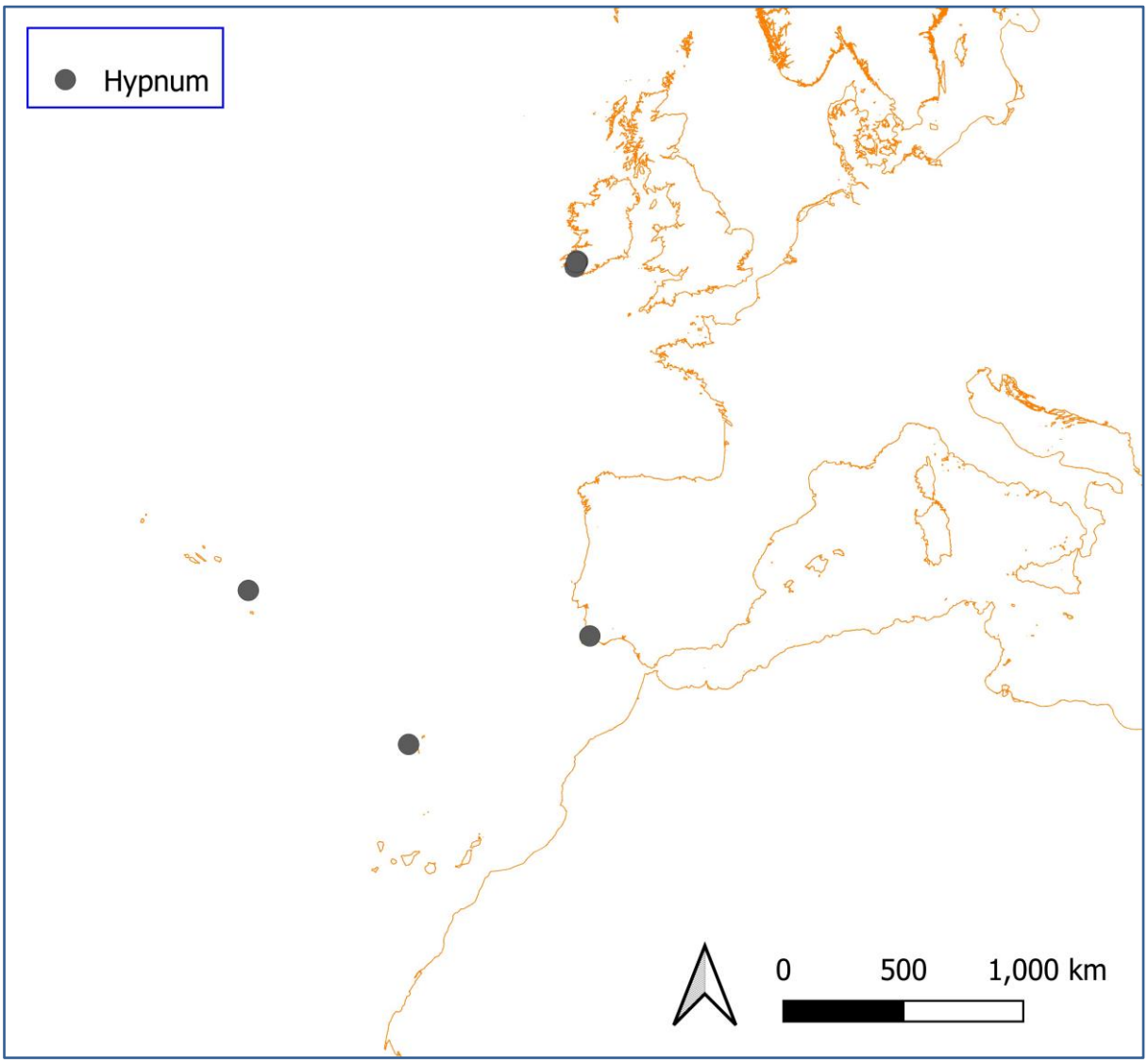

Figure 15 Map of the locations of the *Hypnum cf. uncinulatum* specimens used for the analysis. The specimens consisted of samples from Ireland, Madeira, Portugal and the Azores.

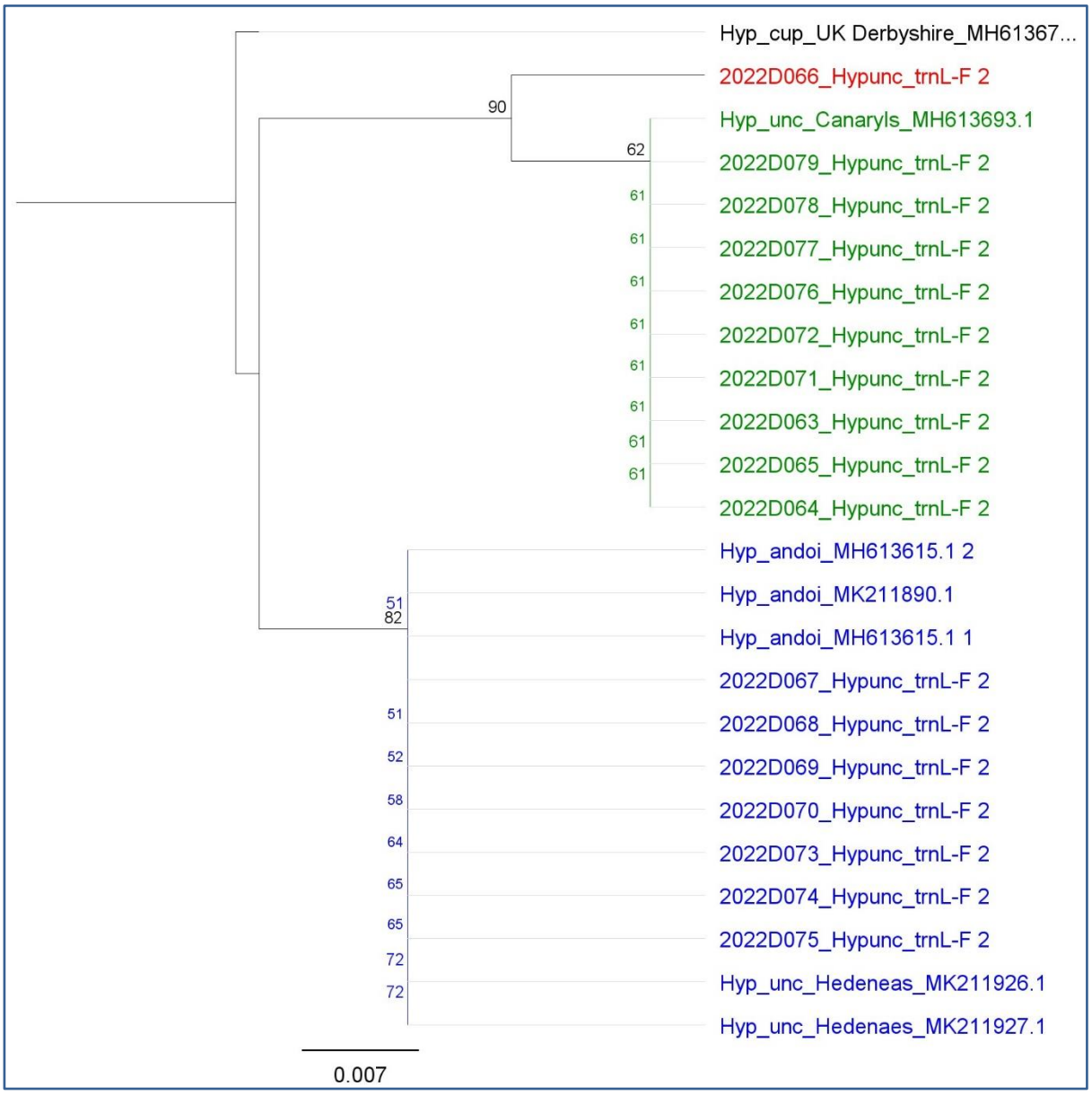

Figure 16 Neighbor-Joining tree of the *trn*L-F data for the *Hypnum* specimens. The samples separate into two distinct groups, one which can be classed as true *H. uncinulatum* (green) and the other as *H. andoi* (blue) – based on comparison with GenBank data. One sample, coloured red, groups with *H. uncinulatum* but contains sufficient variation to isolate it from the main group. The tree is rooted on a specimen of *H. cupressiforme*.

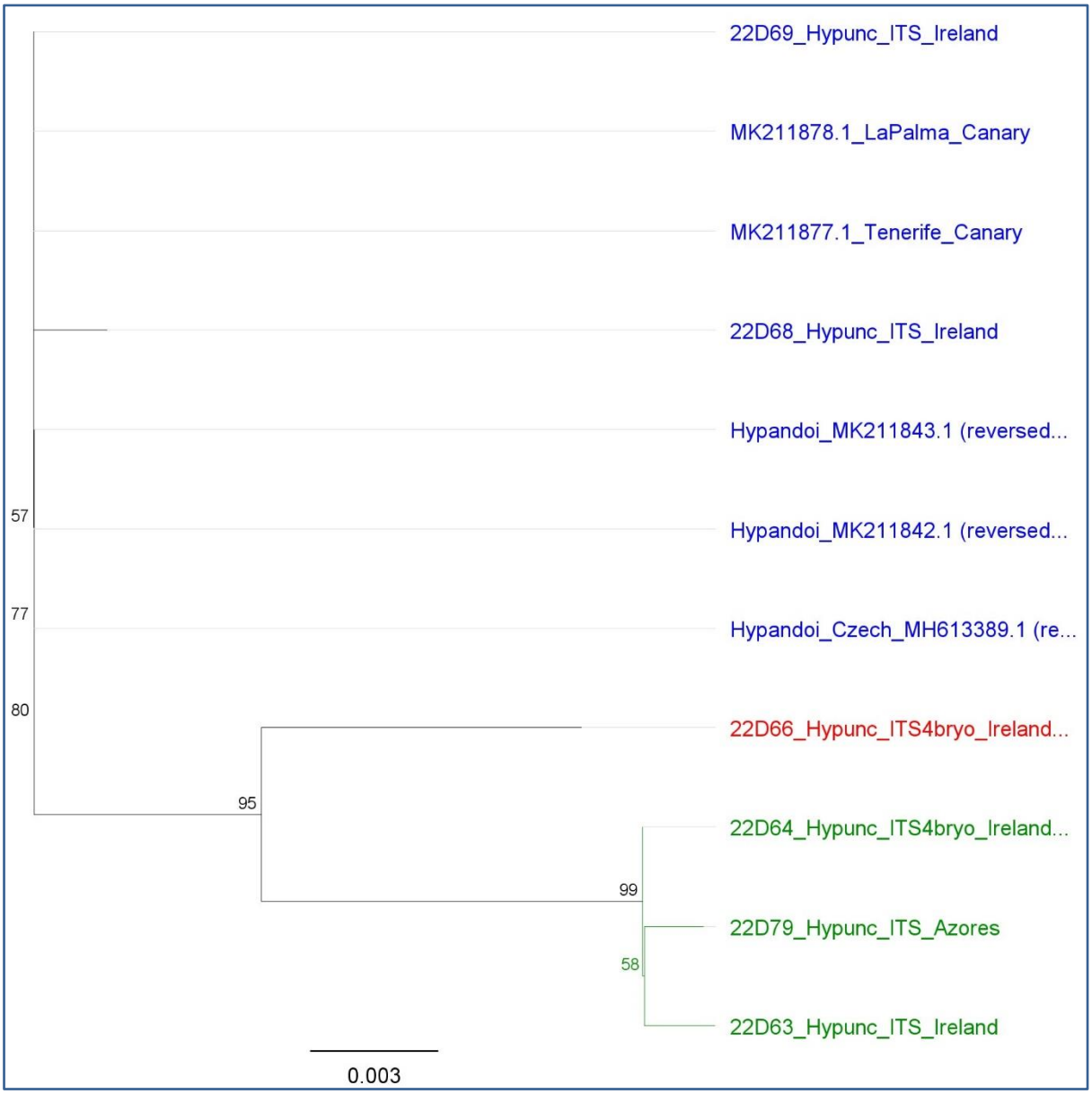

Figure 17 Neighbor-Joining tree of the ITS data for some of the *Hypnum* specimens along with GenBank data. The *H. uncinulatum* are coloured green and *H. andoi* blue. The same sample (22D66) that separated from the *H. uncinulatum* group in the *trn*L-F analysis does similar here for the ITS data.

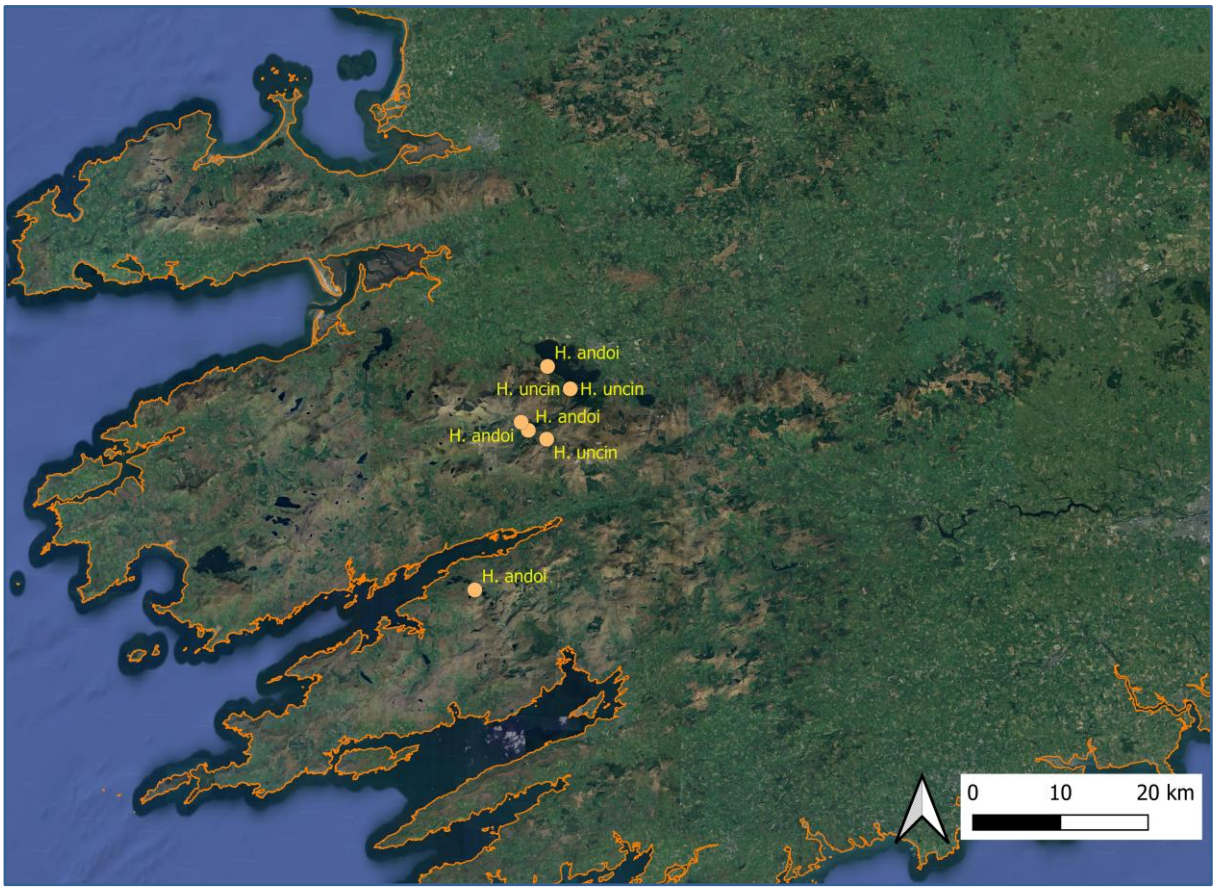

Figure 18 Distribution of the two main groups of specimens initially considered to be '*H. uncinulatum*' in Ireland. Although all samples were initially identified as *H. uncinulatum*, this taxon seems to be less common than *H. andoi*.

The main finding from the analysis was the potential misidentification of many specimens of '*H. uncinulatum*', as these clearly group with *H. andoi* (Figure 16). Indeed, some of the specimens on GenBank used in this analysis also seem to be misidentified.

H. uncinulatum (group 2) and *H. andoi* (group 1) are both extremely variable morphologically, just as *H. cupressiforme* is. However, they can be separated using the characters given above. The widely-recognised differences in the alar cells between *H. andoi* and *H. uncinulatum* are largely supported by this study. According to most of the literature, *H. andoi* has longer, slightly inclined, less curved capsules, not wide-mouthed, and with a mamillate lid. *H. uncinulatum* has shorter, wide-mouthed capsules with whiter peristome teeth and a rostrate lid. Lüth (2019) shows differences in the pseudoparaphyllia: narrow and filamentous in *H. andoi*; wide and foliose in *H. uncinulatum*. No such distinction is made by Smith (2004) or Guerra & Brugués (2018), and the distinction is not supported in examination of the specimens used in this study, with the pseudoparaphyllia variable across both haplotypes.

According to the *Flora Briofítica Ibérica* (Guerra & Brugués, 2018), *H. uncinulatum* has the alar group strongly excavate and delimited by 4–10 basal cells and (3–)4–10 marginal cells. *H. andoi* has the alar group not or weakly excavate and delimited by 4–14 basal cells and 5–23 marginal cells. This distinction is more or less borne out in the specimens used in the present study.

The same source also says: "Ando & Townsend (1980) also highlight other characters for identification. According to these authors, the apex of the stem leaf of *H. uncinulatum* tapers more gradually and is somewhat more falcate, flat and toothed than in *H. andoi*. After the study of numerous samples of *H. uncinulatum*, these differences have been verified, but also the variability they show, for which reason it is considered that they should be treated together with the characteristics of the alar cells. Traditionally, two sporophytic characters have been used

as differentials between *H. uncinulatum* and *H. andoi*: the size of the operculum and the shape of the urn. The operculum (lid) of *H. uncinulatum* is shortly rostellate compared to the mamillate operculum of *H. andoi*. Regarding the shape of the capsule, in *H. uncinulatum* it is ovate and inclined, and more frequently curved, while the capsule of *H. andoi* tends to be cylindrical and less inclined. Both sporophytic characters show great variability, so their taxonomic value is low."

This low taxonomic value placed on the sporophyte is interesting, because sporophytic characters have been used extensively in the past for identifying *H. uncinulatum* (*e.g.* Smith, 2004). However, the results of this study, which link the molecular data with the morphology of the gametophyte, tend to support the views in *Flora Briofítica Ibérica*. Only one collection examined of *Hypnum cf. uncinulatum* from Monchique (Portuguese mainland) had sporophytes, and these had a mamillate lid, so the specimen can be referred to *H. andoi*.

As far as the Irish collections are concerned, these results suggest that *H. uncinulatum* may be very much rarer and more geographically restricted in Ireland than previously thought, with many specimens likely to be the common *H. andoi* (Figure 18). A full revision of herbarium material, using the morphological characters described here, would further clarify its status.

3.6 *Lejeunea eckloniana* Lindenb.

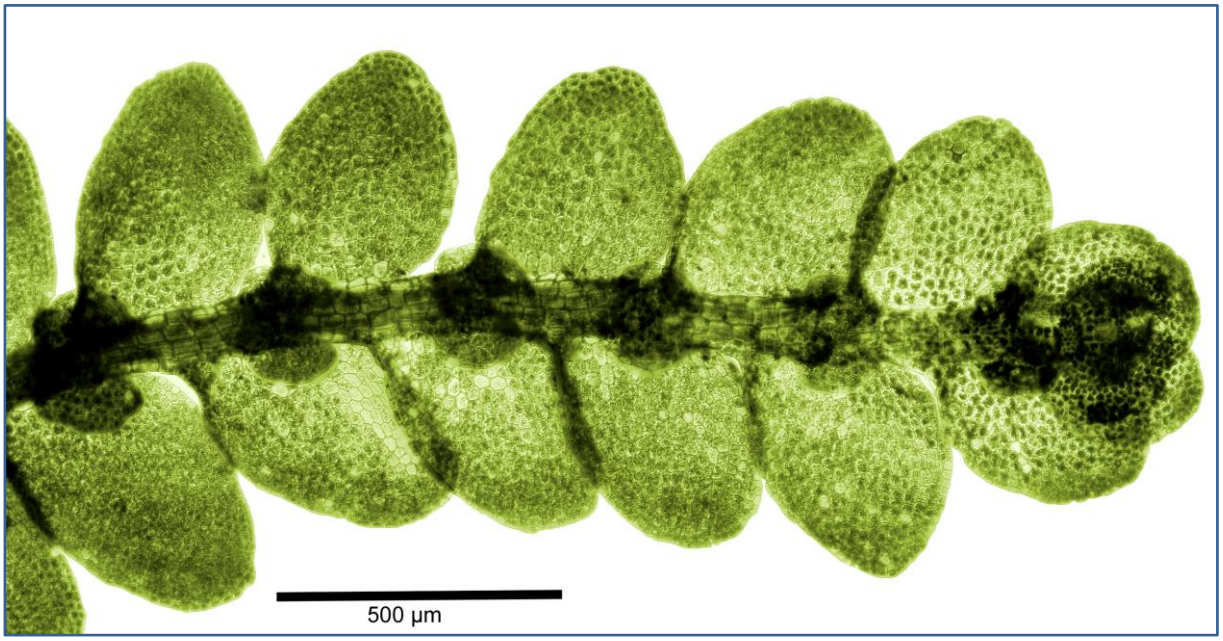

Figure 19 *Lejeunea eckloniana* Lindenb. Photograph Alan Orange.

Lejeunea eckloniana (Holt's Pouncewort) (Figure 19) is restricted to the south-west of Ireland, western Scotland, Macaronesia, Portugal, Spain and tropical and southern Africa. It is not very rare in Ireland, but is restricted in habitat and geography, and is a distinctive member of the oceanic bryophyte community.

Twenty two specimens labelled *Lejeunea eckloniana* were examined during the molecular study, from Africa (seven), Ireland (three), Madeira (eight) and Portugal (four).

Although the *trn*L-F molecular data was limited to a short alignment of 211 bp (base pairs), the analysis still gave good resolution and suggests *L. eckloniana* populations can be split into three groups - an African group (haplotype 1), a Portuguese mainland group (haplotype 2) and a Macaronesia/Ireland group (haplotype 3) (Figure 20). The ITS data was not usable. A specimen from Ghana was equivocal.

There are also significant morphological distinctions between the three haplotypes. Irish and Macaronesian *L. eckloniana* differs in several respects morphologically from African *L. eckloniana*, and was therefore known for many years as a separate species, *L. holtii* Spruce (see Jones, 1974; Paton, 1999); it was, however, synonymised with *L. eckloniana* by Dirkse *et al.* (1993), who had a broad concept of *L. eckloniana*, considering it to be a widespread and variable species. The differences between haplotypes 1 and 3, which largely confirm the differences between *L. eckloniana* and *L. holtii* mentioned in Paton (1999), are summarized below.

Haplotype 1: Africa. Leaves ovate, rather broadly rounded at the apex, with the base of the antical margin nearly always reaching at least halfway across the stem, and frequently crossing it. Underleaves relatively large, 2–4 times wider than the stem, with the sinus usually narrowly to widely acute but very variable even within the same specimen, with some underleaves having an obtuse or even a lunate sinus; the underleaf margin tends to be somewhat crenulate with convex cells. Female bracts with the lobe obovate, rather broadly rounded to widely obtuse at the apex, and the lobule ovate-lanceolate, obtuse to acute at the apex. The perianth is normally clavate, normally widest in the middle, or sometimes pyriform, and ± sharply keeled but hardly winged (see Wigginton, 2004, for illustration).

Haplotype 3: Macaronesia/Ireland. Leaves oval, narrow at the apex, with the base of the antical margin usually not reaching halfway across the stem, and very rarely crossing it. Underleaves

relatively small, 1.5–3 times wider than the stem, with the sinus usually widely acute to lunate but sometimes narrower; the underleaf margin is usually entire, not or hardly crenulate. Female bracts with the lobe ovate-lanceolate, obtuse to acute at the apex, and the lobule lanceolate, acute to acuminate at the apex. The perianth is pyriform to strongly obovoid, widest at the apex, and broadly winged (see Paton, 1999, for illustration).

Supposed differences in shoot size and the size and shape of cortical stem cells could not be confirmed in the specimens examined, these characters being very variable in both haplotypes, with significant overlap. Similarly, underleaf characters were found to be very variable throughout the range of *L. eckloniana sens*. *lat*., and therefore of limited value. On the whole, African specimens tend to have slightly larger underleaves with a more narrowly acute sinus, but the sinus is very variable throughout African and European/Macaronesian material. Both African and European/Macaronesian material may have blunt teeth or poorly defined 'shoulders' on the underleaf margins.

The plants from Monchique (Portuguese mainland, haplotype 2) differ significantly from both African and Macaronesian/Irish material. They are considerably smaller than either of the other haplotypes, the shoots up to *c.* 0.75 mm wide, with smaller leaves (10–18 cells wide *vs.* 18–25 cells wide) that are somewhat concave ventrally when dry (± flat in African *L. eckloniana*) and smaller leaf cells (18–25 µm wide *vs.* 20–35 µm) with no 'ochraceous bodies', which both the other two haplotypes possess. The cortical stem cells and the perianth are similar to those of haplotype 1 rather than haplotype 3. The underleaves are rather variable but are more similar to those of haplotype 3, with most having a ± lunate sinus and often a poorly-defined lateral tooth. There may also be a difference in the oil bodies: typical African *L. eckloniana* has many (20–30) small, simple oil bodies, although it can have as few as 6–8 (Tamás Pócs pers. comm. May 2020). The Monchique specimens have, on average, *c.* 2–25 oil bodies per cell.

It seems clear that the specimens labelled '*L. eckloniana*' analysed contain three separate taxa: haplotype 1 from Africa is 'true' *L. eckloniana* – the type specimen was described (as *Eulejeunea ecklonii*) from South Africa (Stephani, 1890); haplotype 2 appears to be an as yet undescribed species; haplotype 3 is what used to be called *L. holtii*. The latter is certainly very close to *L. eckloniana*, but the morphological differences described above, along with the molecular difference and the geographical isolation suggest that it can legitimately be regarded as a separate taxon. The molecular data also indicate that while many individuals group into the three haplotype groups, some individuals are sufficiently divergent to group outside of these haplotypes. For example, the sample from Ghana (22D114) is quite divergent from H2. Further work to assess this variation is necessary.

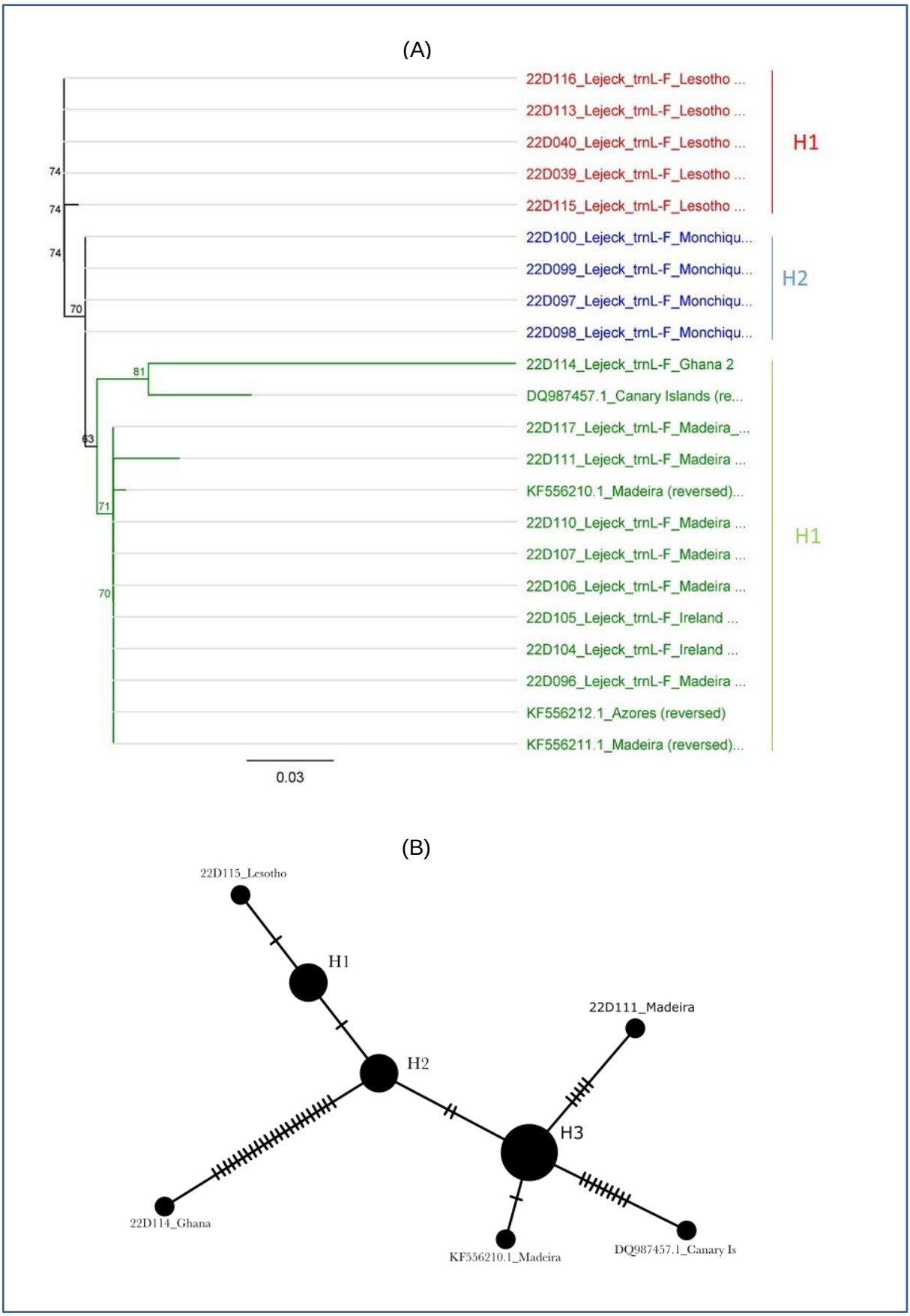

Figure 20 Data analysis using the *trn*L-F region. (A) Neighbor-Joining tree of the *trn*L-F data for *L. eckloniana*. (B) Haplotype network showing the three main haplotypes and the other individuals.

3.7 *Lejeunea flava* (Sw.) Nees

Figure 21 *Lejeunea flava* (Sw.) Nees. Photograph © British Bryological Society.

Lejeunea flava (Yellow Pouncewort) (Figure 21) is primarily a pantropical species, also occurring in south-western Ireland and Macaronesia. Material of *L. flava* from Ireland and Macaronesia is currently considered to be subsp. *moorei*, which is endemic. The species is represented in Africa by subsp. *flava* and subsp. *tabularis* (Spreng.) S.W. Arnell. Several other varieties and subspecies have been described, but it is often simply referred to without a subspecific epithet (*e.g*. in South America). Schuster (1980) considers the common American expression of this species to be subsp. *flava*.

Fourteen specimens labelled *Lejeunea flava* were examined, from Africa (five), the Azores (two), Madeira (two) and Ireland (five).

The data indicate that the African samples (with the exception of the Ghana sample, 22D123) separate out from the Irish and Azores samples (Figure 22). The sample from Ghana was of lower quality than the others and therefore less reliable. However, when GenBank data from multiple origins are added to the analysis, the African samples are mixed across the clusters (Figure 23). There is one main haplotype with some minor haplotypes distributed outside of the main haplotype. There is no strong support for different haplotypes in *L. flava* as the node supports are always under 70 (Figures 22 and 23).

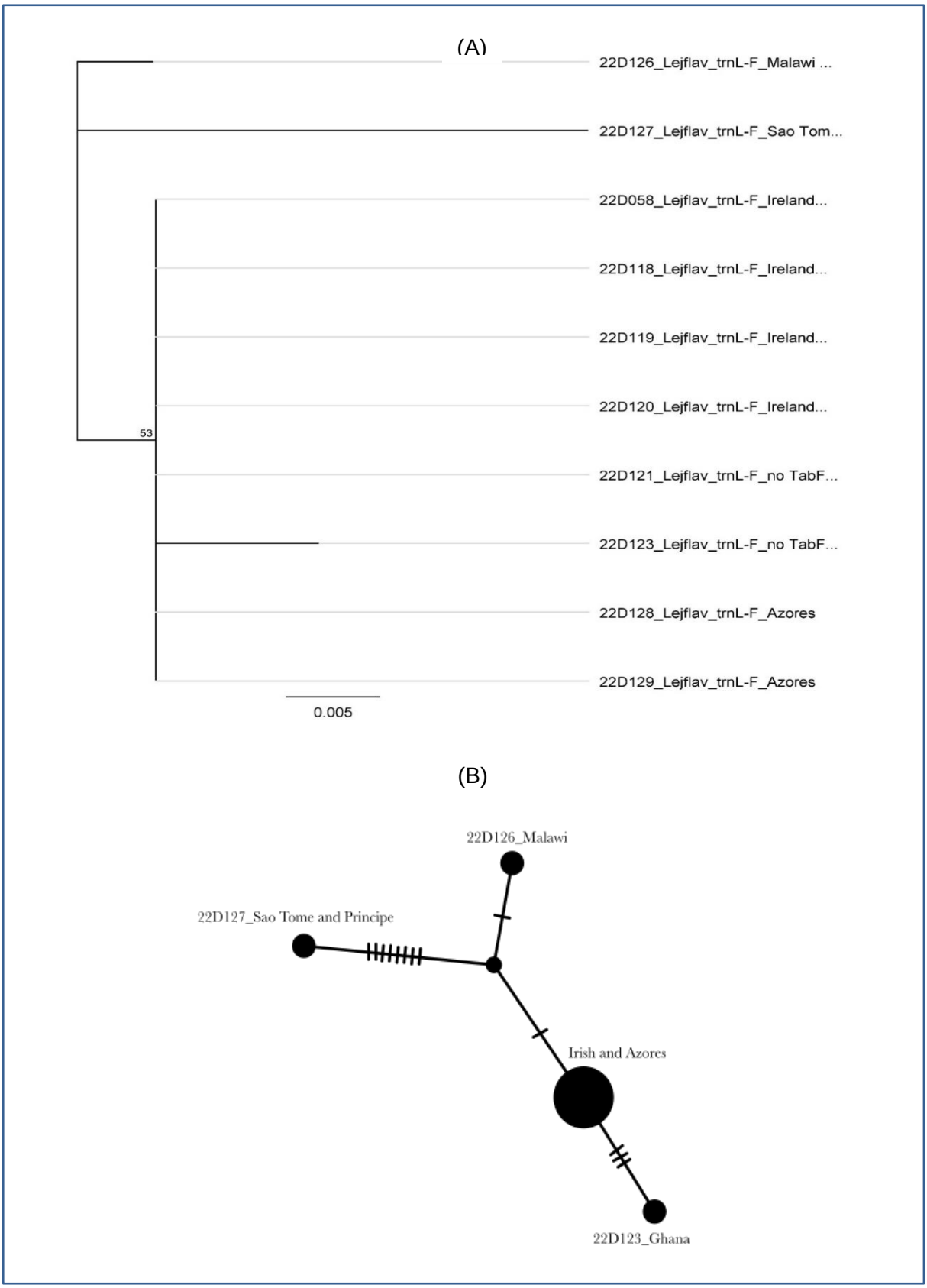

Figure 22 (A) Neighbor-Joining tree of the *trn*L-F data for *L. flava* individuals analysed. The groupings show African specimens separate from the Irish and Azores individuals. The exception is the sample from Ghana (22D123), which groups closer to the Irish and Azores samples. (B) Haplotype network showing one main haplotype, with some minor haplotypes distributed outside the main haplotype.

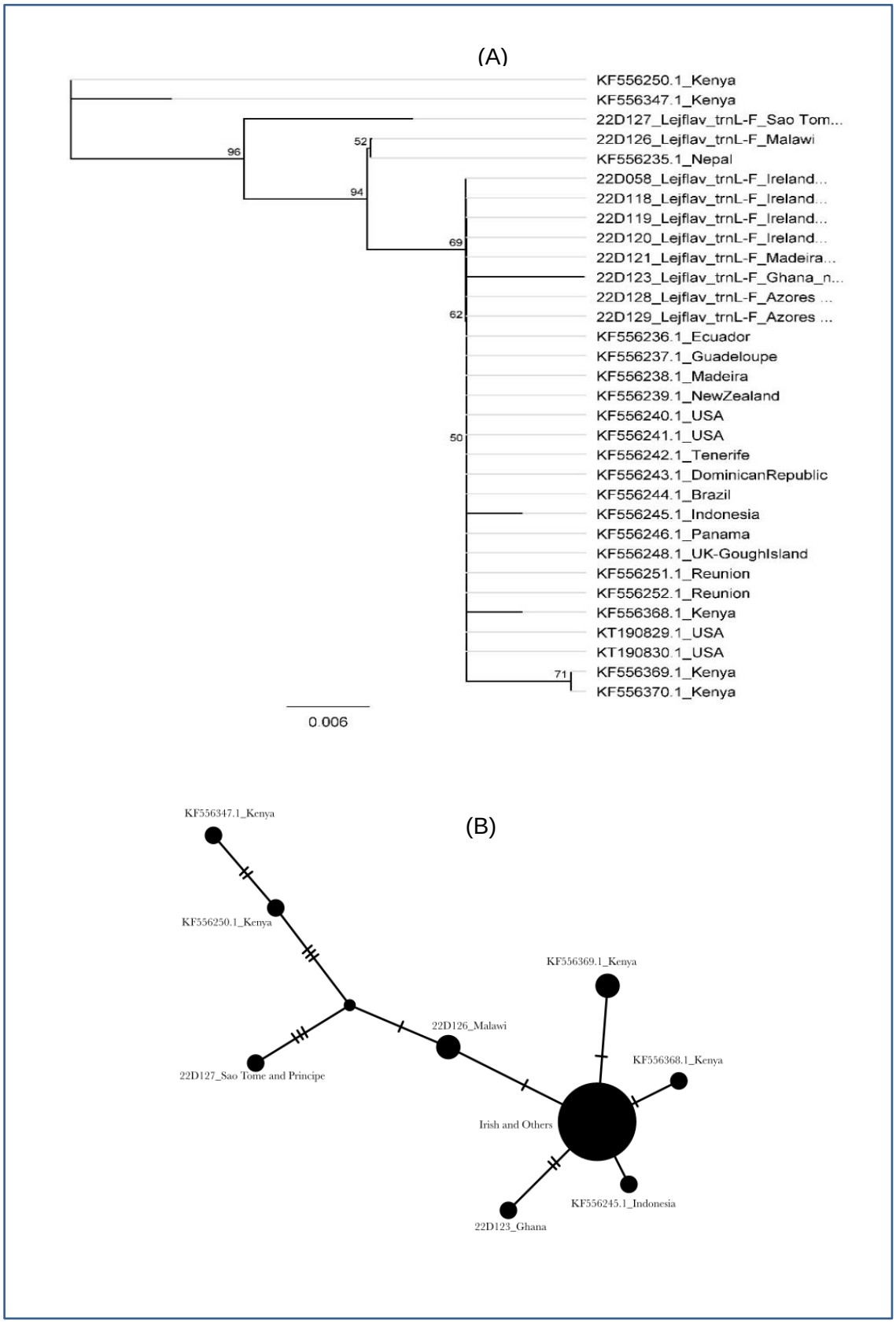

Figure 23 Results of clustering analyses undertaken on the *L. flava trn*L-F data, including GenBank data. (A) Neighbor-Joining tree of the *trn*L-F data for *L. flava* individuals analysed along with GenBank accessions. The African samples are found across multiple groupings in this analysis. (B) Haplotype network showing most of the samples group into one main haplotype with some minor haplotypes distributed outside of the main group.

The morphological differences between Irish/Macaronesian subsp. *moorei* and other subspecies of *L. flava* are rather slight, but possibly significant, and are described by Schuster (1980) and Paton (1999). Irish/Macaronesian plants (subsp. *moorei*) are described as having narrower leaves, very slightly smaller leaf cells, the underleaves less cordate and longer than wide (0.75–0.90 times as broad as long, but subsp. *tabularis* has underleaves of similar dimensions, subsp. *flava* has the underleaves truncate at the base rather than cordate, and subsp. *moorei* often has markedly cordate underleaves), and the perianths less distinctly keeled and with a short wide beak. Paton (1999) describes the perianths of subsp. *moorei* as being keeled in the upper ⅓; Wigginton (2004) states that African material has the perianths keeled in the upper ½. On the other hand, Wigginton (2004) refers to the '*tubata* form' of African *L. flava*, which has perianths with "...the rostrum mouth...flared out like the bell of a trumpet", which is very similar to the Irish/Macaronesian plants. Wigginton (2004) suggests that "...it does not appear to be a character of particular taxonomic value". Unfortunately, perianths are scarce in the material used for the current study. Paton also says that the perianth keels in Irish/Macaronesian plants are "usually strongly crenulate", while illustrations in Wigginton (2004) show African material with ± smoothly keeled perianths.

Microscopic examination of the specimens used in this study, and comparison between the Irish/Macaronesian material and the African material, largely confirms these character states. However, even based on the small amount of fertile material available, the perianth characters seem to be quite variable. African material does mostly have longer perianth keels, but these range from smooth to weakly crenulate. The rostrum tends to be wider than long with a flared apex in Irish/Macaronesian material, but is variable in African material, ranging from very similar to Irish/Macaronesian material to longer than wider and without any flaring. No convincing differences could be detected in the leaf shape, the size of leaf cells, or underleaf shape and size.

The molecular analysis of this species is rather inconclusive. There does seem to be some difference between the Irish/Macaronesian material and the African material, and there are some minor morphological differences, so in the absence of further information it seems appropriate to retain the *status quo* and recommend that the Irish (and Macaronesian) material continues to be known as subsp. *moorei*.

3.8 *Lejeunea hibernica* Bischl., H.A.Mill. & Bonner ex Grolle

Figure 24 *Lejeunea hibernica* Bischl., H.A.Mill. & Bonner ex Grolle. Photograph Robert Thompson.

Lejeunea hibernica (Irish Pouncewort) (Figure 24) is a rare oceanic liverwort restricted to south-western Ireland and Macaronesia. It is listed on the Flora (Protection) Order, 2022.

Eight specimens of *Lejeunea hibernica* were examined molecularly, from Ireland (five) and Madeira (three). The specimens emerge as a distinct group, supporting the identity of *L. hibernica* as a species. There appears to be very little variation throughout its range, so there is no reason to postulate any taxonomic difference between Irish and Macaronesian populations, but the data are very limited.

3.9 *Lejeunea mandonii* (Steph.) Müll.Frib.

Figure 25 *Lejeunea mandonii* (Steph.) Müll.Frib. Photograph Des Callaghan.

Lejeunea mandonii (Atlantic Pouncewort) (Figure 25) is a very rare oceanic liverwort restricted to the west of Ireland, western Britain, Portugal, Spain and Macaronesia. It is listed on the Flora (Protection) Order, 2022.

Only one Irish specimen and one Scottish specimen were sequenced. The Madeiran specimens consist of one that was confidently identified (using morphological characters) as *L. mandonii*, because it looks like Irish/Scottish *L. mandonii*, and three that were collected as '*L. mandonii/canariensis*' because they did not look typical for *L. mandonii* in the field, but *L. canariensis* is also a very rare species with which we were unfamiliar. While the data is therefore limited, the *trn*L-F tree shows at least two closely-related haplotypes (Figure 26), with both represented in Madeira. The Irish and Scottish specimens group with one of the Madeiran samples in one haplotype; the other Madeiran samples group together as the other haplotype. There is good support for these groups as the nodes marking the two groups have a 100% value. There is no *L. mandonii* data on GenBank, so the data generated here will be a useful addition to this database.

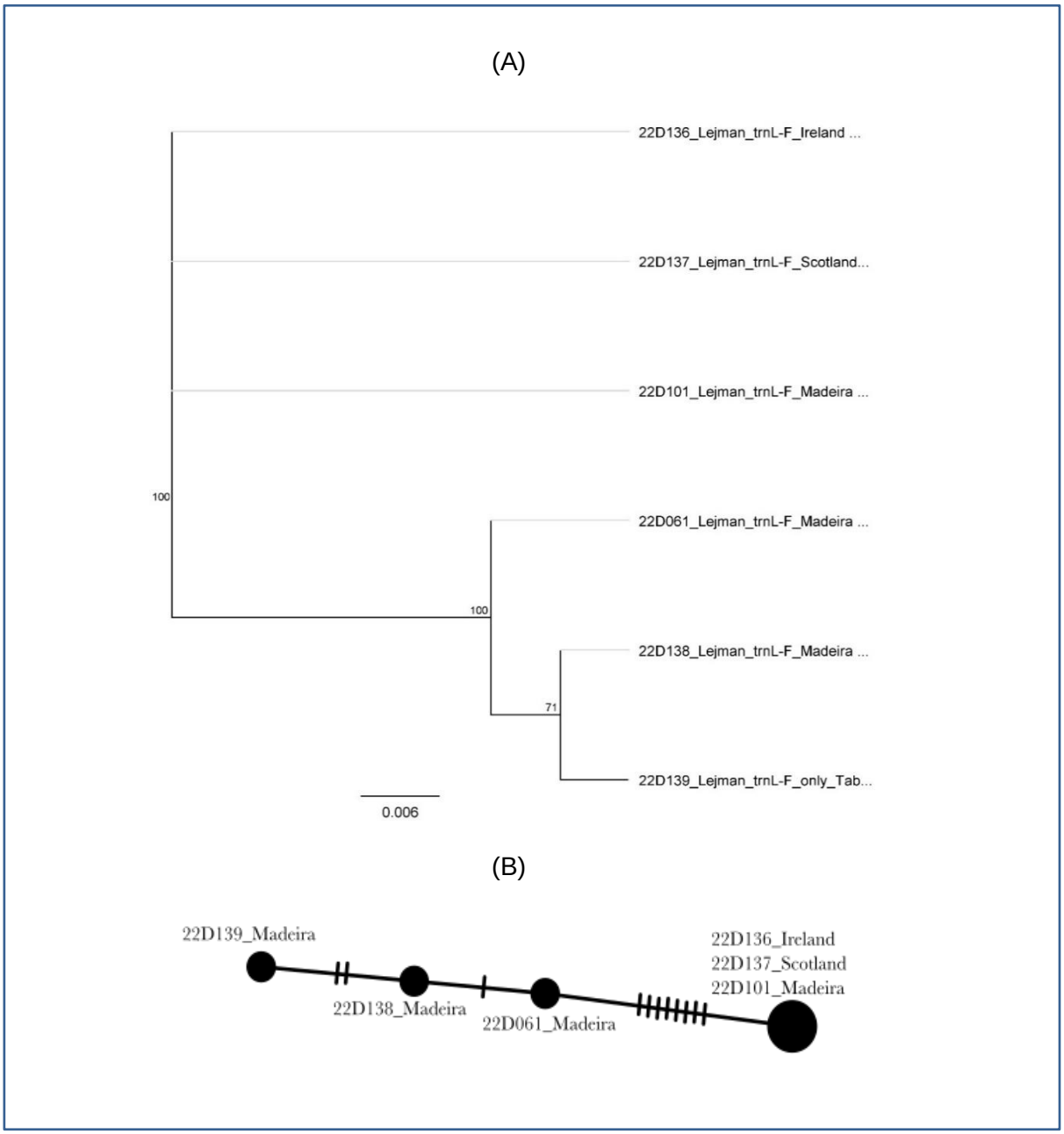

Figure 26 Results of clustering analysis from *trn*L-F data in *L. mandonii*. (A) Neighbor-joining tree of the *trn*L-F data for *L. mandonii*. (B) Haplotype network showing grouping of the Irish, Scottish and a Madeiran sample, with the other Madeiran samples grouping separately.

On examining the specimens microscopically and comparing them with herbarium material, it is clear that the two haplotypes represent two species, *L. mandonii*, present in Ireland, Scotland and Madeira, and *L. canariensis*, present only (in our collections) in Madeira. Although *L. canariensis* is very small, it resembles a small form of *L. flava*, being bright yellow-green in colour and opaque, presumably because of the cell structure and different oil bodies. *L. mandonii* is a darker, duller green, and more translucent. The literature on *L. canariensis* is very sparse, and it is intended to produce a paper with a full description and illustrations. The relationship between *L. canariensis* and the very similar American *L. laetevirens* Nees & Mont. requires further study.

3.10 *Plagiochila bifaria* (Sw.) Lindenb.

Figure 27 *Plagiochila bifaria* (Sw.) Lindenb. © British Bryological Society.

Plagiochila bifaria (Killarney Featherwort) (Figure 27) is an oceanic liverwort occurring in Ireland, western Britain, western Europe, central and southern America and the Galapagos Islands. It is not particularly rare, but was until recently known as *P. killarniensis* Pearson, until synonymised with the Neotropical *P. bifaria* (Heinrichs *et al*., 1998, 2004).

A total of 15 specimens from our collections were analysed, ten being initially identified as *P. bifaria*, five as *P. bifaria/punctata*, as the distinction between these two species is not always straightforward. Data were generated from three gene regions, *trn*L-F, *rbc*L and ITS. Eight specimens were from Ireland, one from Scotland, four from Madeira and two from the Azores (Figure 28). Additional data from GenBank was incorporated to confirm the identity of the newly-collected specimens. As expected, *P. bifaria* and *P. punctata* emerge in two distinct groups, with *P. punctata* confined (among our specimens) to Ireland and those representing *P. bifaria* occurring in Madeira, the Azores and Scotland. The results show that some specimens had initially been misidentified and others that were uncertain could be confirmed using the molecular analysis. The molecular analysis has a high level of confidence, with the main groups having above 70% confidence. The separation of *P. bifaria* and *P. punctata* from *P. heterophylla* has 100% confidence in the Neighbor-Joining tree bootstrapping for *rbc*L and ITS (Figures 28 and 29).

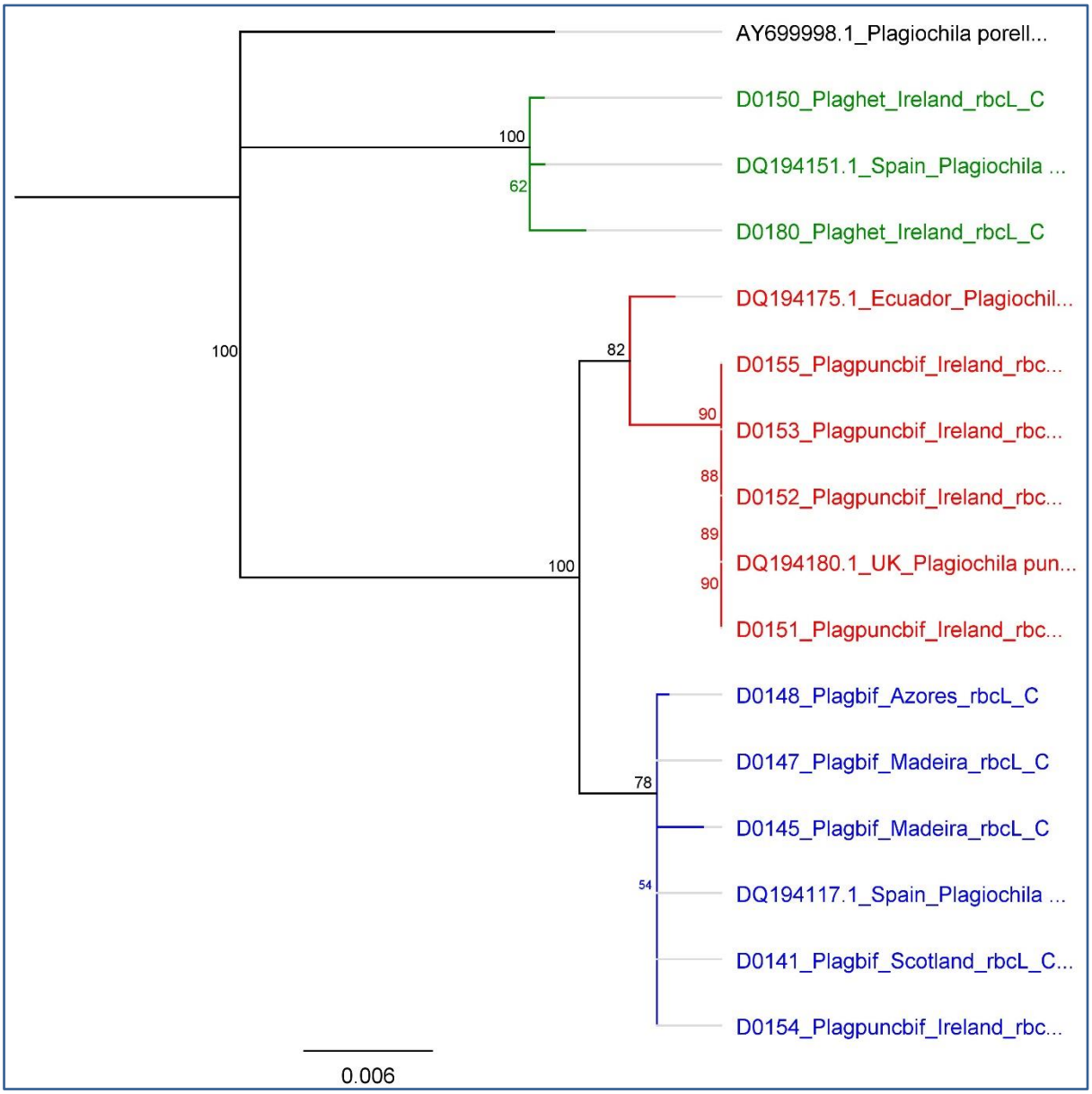

Figure 28 Neighbor-Joining tree of the *rbc*L data for *P. bifaria* (blue), *P. punctata* (red) and *P. heterophylla* (green), including GenBank data. An outgroup of *P. porelloides* was used to root the tree.

The results establish that molecular techniques are very useful in identifying dubious specimens that are difficult to assign to one or the other taxon morphologically. There are no surprises, with *P. bifaria* and *P. punctata* coming out clearly as separate taxa, and there are no significant differences between plants of either species from Ireland and Macaronesia. Furthermore, the synonymy of plants of *P. bifaria* (‘*P. killarniensis*’) from Europe and Macaronesia with those from Central and South America is confirmed, using data from GenBank (Figures 28 and 29). Specimens from different geographic locations are grouped together on the trees according to taxon rather than geography. For example, samples from Brazil, Bolivia and Costa Rica group with *P. bifaria* samples from Ireland, Scotland and Madeira (Figure 29).

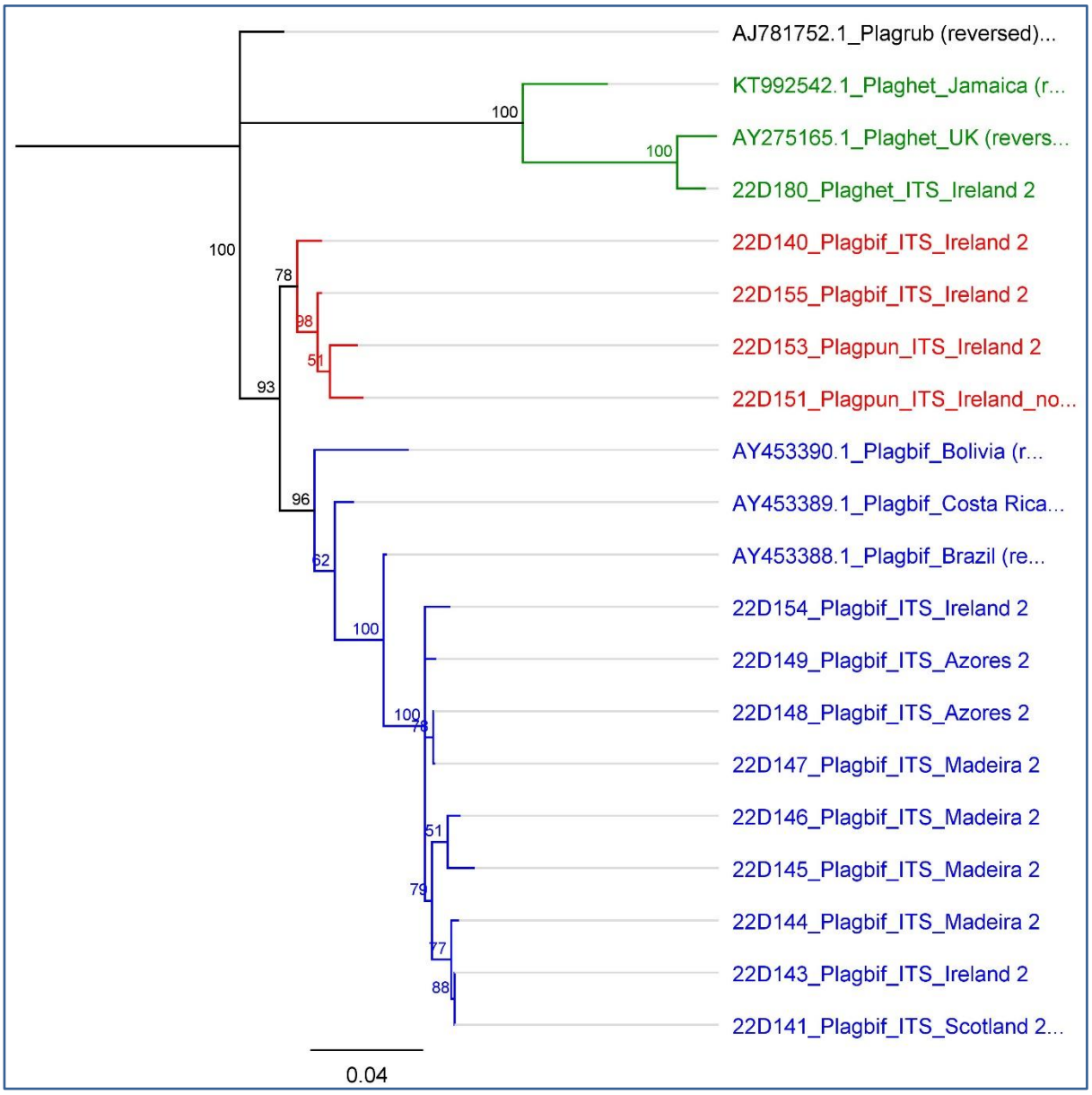

Figure 29 Neighbor-Joining tree of the ITS data for *P. bifaria* (blue), *P. punctata* (red) and *P. heterophylla* (green), including GenBank data. An outgroup of *P. porelloides* was used to root the tree.

3.11 *Plagiochila heterophylla* Lindenb. ex Lehm.

Figure 30 *Plagiochila heterophylla* Lindenb. ex Lehm. Photograph Nick Hodgetts.

Plagiochila heterophylla (Western Featherwort) (Figure 30) is a rare oceanic liverwort restricted to the west of Ireland, western Britain, north-western France and central and southern America. Until recently it was regarded as a rare European endemic, *P. atlantica* F.Rose, but it was synonymised with the Neotropical *P. heterophylla* by Heinrichs (2002). It is listed on the Flora (Protection) Order, 2022.

It is a very rare plant in Ireland, and only a single specimen was sequenced. As expected, the results show that it is clearly distinct from other *Plagiochila* species (Figures 28 and 29), and also, using GenBank data, confirms the synonymy of the European '*P. atlantica*' with *P. heterophylla*. Figure 29 shows a sample from Jamaica grouping with samples from Ireland and the UK.

3.12 *Radula carringtonii* J.B.Jack

Figure 31 *Radula carringtonii* J.B.Jack. Photograph David Holyoak.

Radula carringtonii (Carrington's Scalewort) (Figure 31) is a rare oceanic liverwort restricted to the west of Ireland, western Scotland and Macaronesia.

Six specimens labelled *R. carringtonii* from Ireland and four from Madeira were analysed, and data from GenBank added. All the specimens from Ireland collected as *R. carringtonii* were confirmed as that species. Most of the material collected from Madeira as *R. carringtonii* emerged as *R. aquilegia*, with one specimen (22D164) probably a mixture of the two taxa. Combining our data with GenBank data, samples of *R. carringtonii* from Madeira and the Canary Islands represent one haplotype, whereas the Irish specimens are composed of two haplotypes, one the same as the Macaronesian haplotype, the other distinct (Figure 32), which are geographically separated into North and South Kerry populations (Figure 33).

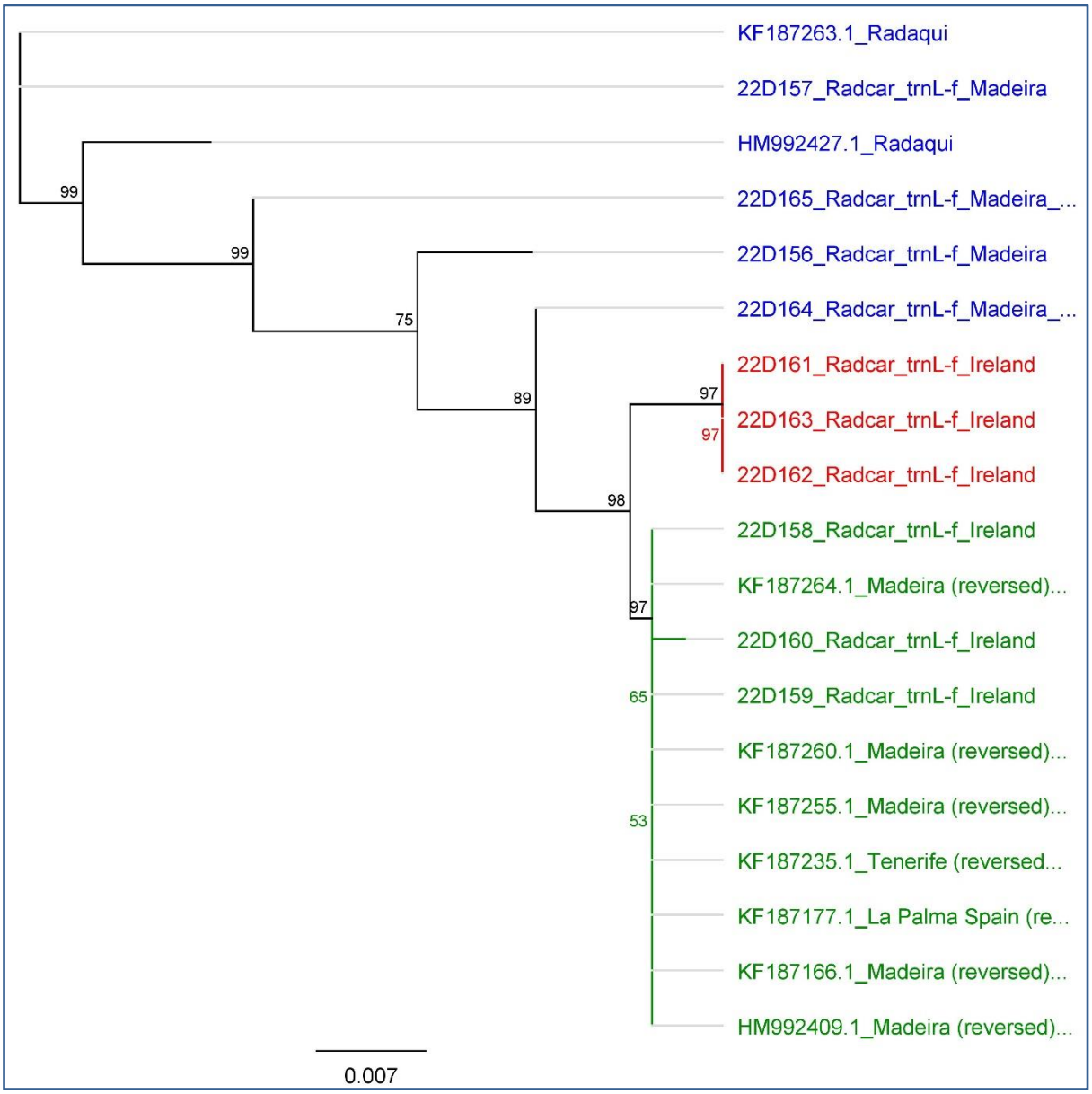

Figure 32 Neighbor-Joining tree of the *trn*L-F data from specimens initially identified as *Radula carringtonii*. The tree shows individuals re-determined as *R. aquilegia* (blue) and two other haplotypes, H1 (red) and H2 (green). The haplotype groupings have good support (97%).

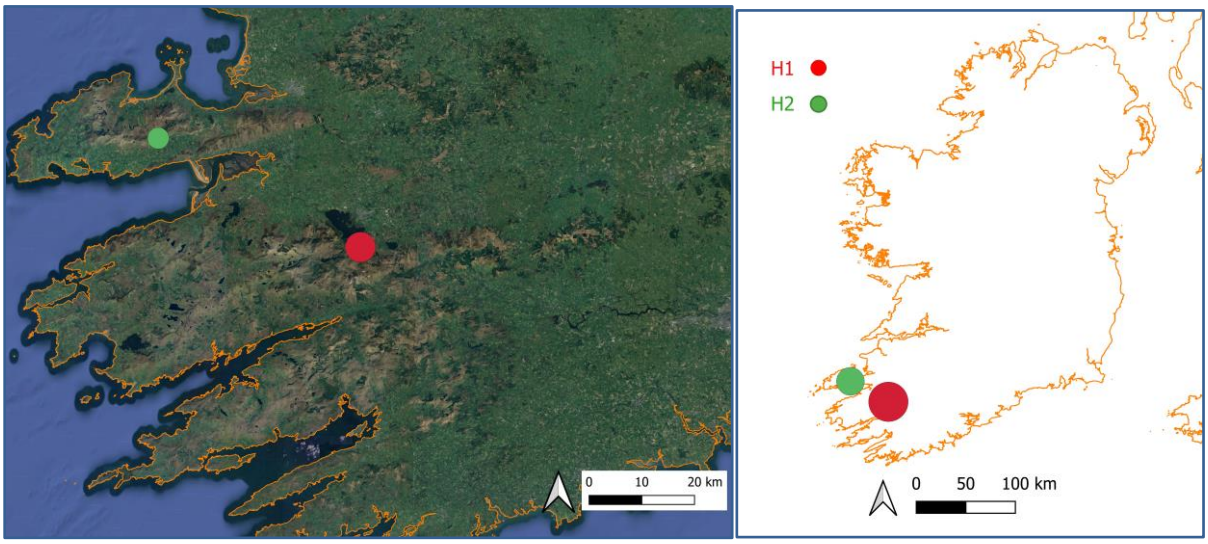

Figure 33 Maps showing the location of H1 and H2 from *Radula carringtonii* populations in Ireland. The detail on the left shows the locations of the populations sampled in Kerry. Background terrain mapping data from Google.

R. aquilegia and *R. carringtonii* can be very difficult to distinguish from each other morphologically, as evidenced by our initial misidentifications from Madeira. While good material of each species is distinct, some material appears to be intermediate using both morphological and molecular data. The emergence of two haplotypes of *R. carringtonii* in Ireland is of interest, as one (in South Kerry) seems to be unique to Ireland. As yet, microscope study has revealed no distinct morphological difference between the two haplotypes. There may be some minor differences in the stem transverse section, but more study is needed to establish whether this is significant. It may be that the two haplotypes are best regarded as cryptic species, in much the same way as in *Hamatocaulis vernicosus*.

3.13 *Radula holtii* Spruce

Figure 34 *Radula holtii* Spruce. Photograph Neil Lockhart.

Radula holtii (Holt's Scalewort) (Figure 34) is a rare oceanic liverwort restricted to south-western Ireland, western Scotland, Macaronesia, Portugal, Spain and France. It is listed on the Flora (Protection) Order, 2022.

Eight specimens of *R. holtii* were sequenced from our collections, five from Ireland and three from Madeira, and data on *R. holtii* from GenBank was added to the analysis (Figure 35). Most of the material from Ireland groups together using both molecular and morphological information, and this corresponds to *R. holtii*. One specimen, 22D171 (NGH11253), came out with *R. carringtonii*, and further microscopic examination showed that this specimen was indeed a sample of *R. carringtonii* that had initially been misidentified as *R. holtii*. Two of the three specimens from Madeira proved, on microscopic examination, to be the Macaronesian endemic *R. jonesii*, which is superficially similar but not closely related to *R. holtii* (Devos *et al*., 2011). The third Madeiran specimen, 22D173 (NGH11284), is a mixture of *R. jonesii* and *R. carringtonii*, the latter being the specimen represented in the molecular work. GenBank data shows that both *R. holtii* and *R. jonesii* occur in Macaronesia, but we did not collect any *R. holtii*. Specimens 22D172 (NGH11253) and 22D174 (Porley Chão da Ribeira-Fanal) did not work for the sequencing, but morphological evidence suggests they are both *R. jonesii*.

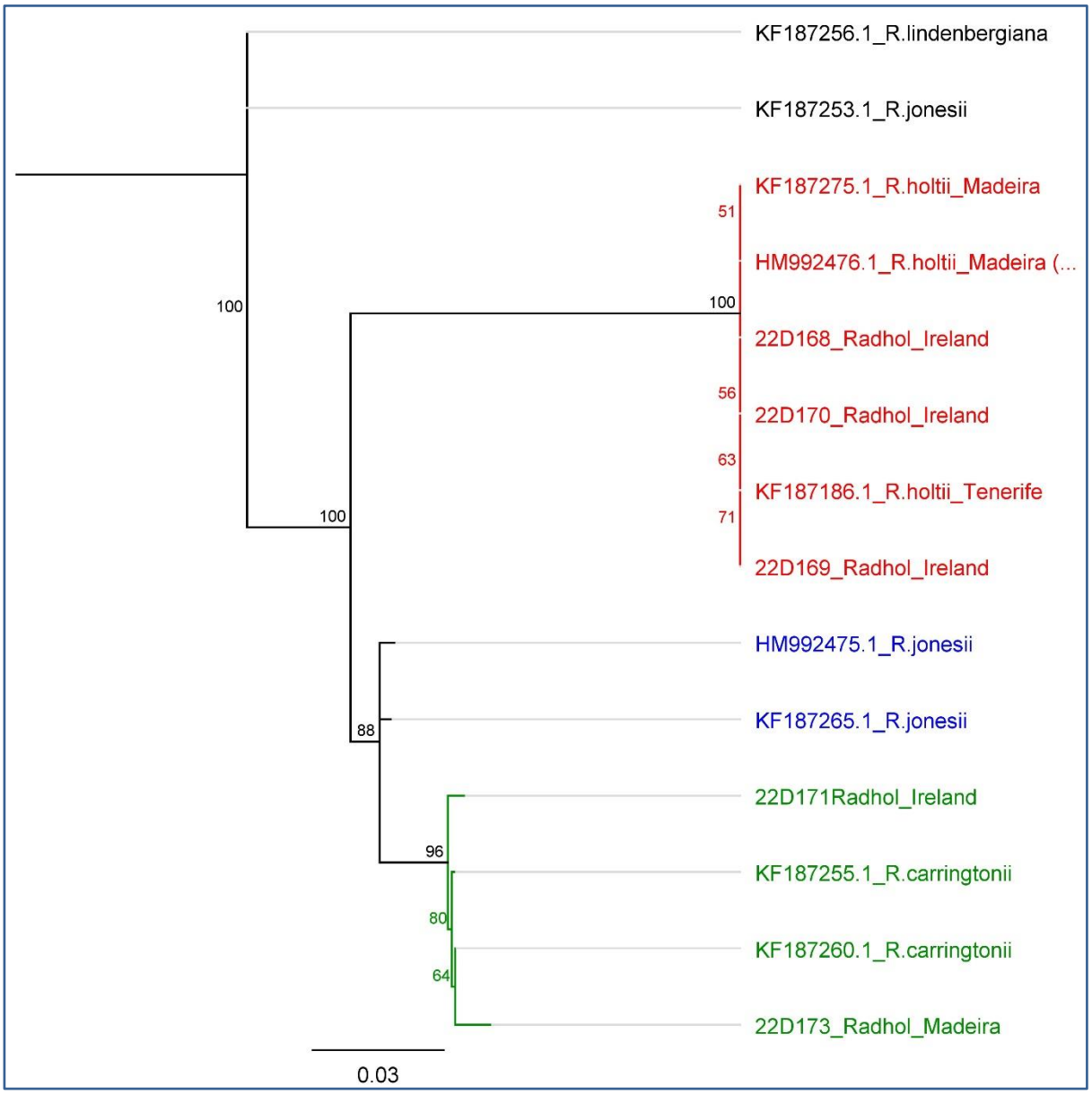

Figure 35 Neighbor-Joining tree of the *trn*L-F data from *Radula holtii*, including GenBank data from related taxa. The tree shows at least two, possibly three distinct groups of individuals. *R. lindenbergiana* is included to root the tree.

According to this limited analysis, populations of *R. holtii* are genetically uniform. They differ slightly from Macaronesian material (GenBank) but essentially group very closely. *R. jonesii* is not known from Ireland.

3.14 *Solenostoma subellipticum* (Lindb. ex Kaal.) Schust.

Figure 36 *Solenostoma subellipticum* (Lindb. ex Kaal.) Schust. Photograph Neil Lockhart.

Solenostoma subellipticum (Two-lipped Flapwort) (Figure 36) is a circumboreal liverwort, and not particularly rare. *S. obovatum* and *S. subellipticum* have long been regarded as separate species in Britain and Ireland. *S. subellipticum* tends to be smaller, with less highly coloured rhizoids and growing in more base-rich habitats, but is otherwise almost indistinguishable from *S. obovatum*. However, it was nested within *Solenostoma obovatum* in a molecular study by Shaw *et al*. (2015) and thus reduced to a synonym, a treatment followed by Söderström *et al*. (2016). It was, however, included in the European checklist (Hodgetts *et al*., 2020), albeit with some hesitation and disagreement. It was included in this project in order to test the synonymy.

Four samples of *Solenostoma* were analysed, all from Ireland, representing two closely related species, *Solenostoma obovatum* and *S. subellipticum* (Figure 37), and this data was combined with data from GenBank. There is very little variation in the sequences, and no geographical patterns are obvious (Figure 37). 22D177 is slightly different from the others, but this is probably not significant. The results are inconclusive, but if anything tend to support the synonymy of *S. subellipticum* with *S. obovatum*.

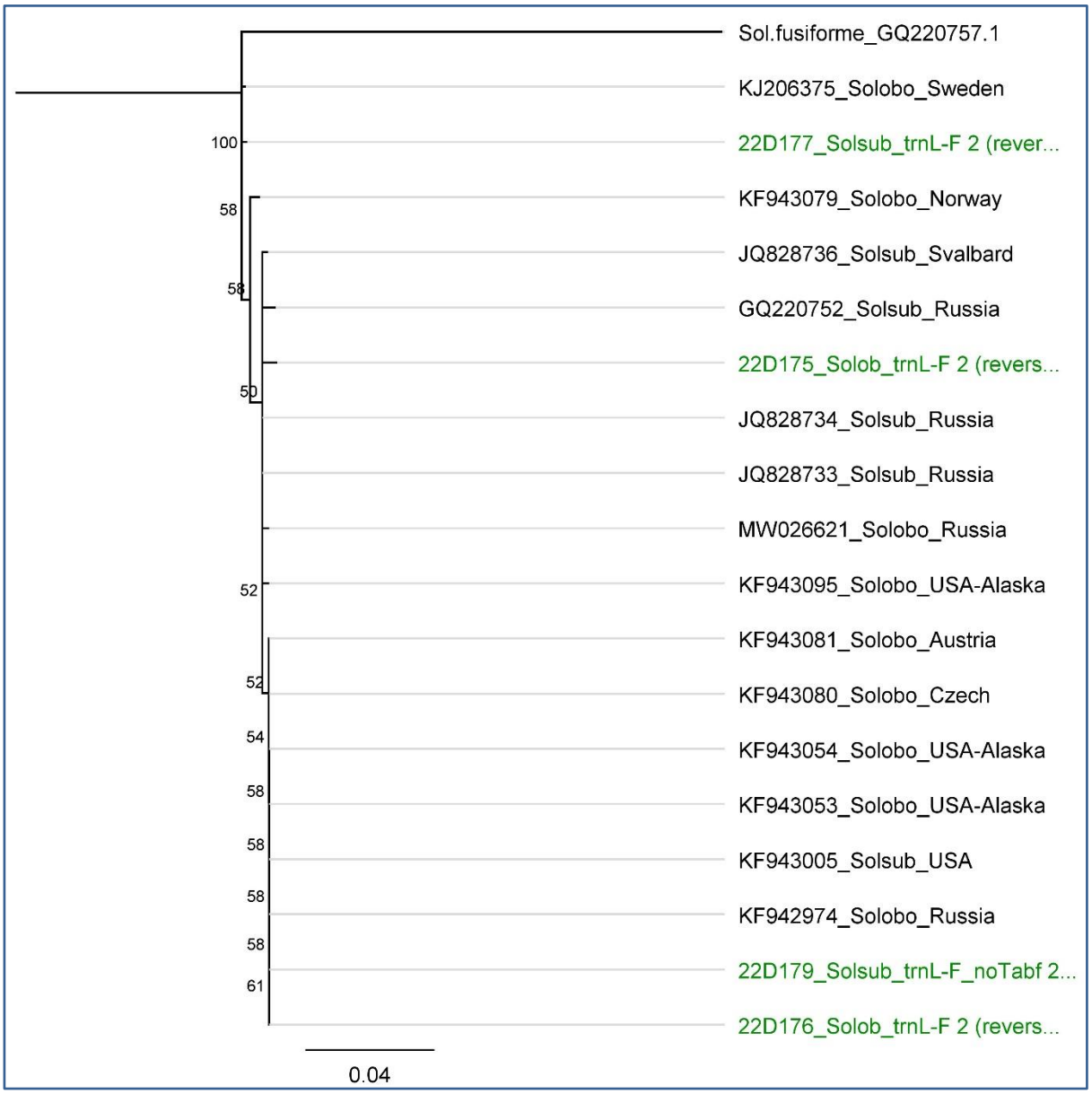

Figure 37 Neighbor-Joining tree of the *trn*L-F data from *Solenostoma obovatum* and *S. subellipticum*. Additional data was added from GenBank and the tree was rooted using *S. fusiforme*. The tree lacks clarity and the level of consensus is low (below 70%). The Irish specimens (coloured green) are scattered across the tree, as are the other specimens.

4 Conclusions

The specific findings about the taxa addressed in this project can be summarised as follows:

- *Acrobolbus wilsonii*: too little data are available for firm conclusions, but there is an indication that Irish and Madeiran material may be different from each other.
- *Cephalozia crassifolia*: too little data are available for firm conclusions, but there are indications that Azores and Irish material group together, and separately from Central and southern American material. Further work is needed to clarify the relationship between the American and the European/Macaronesian plants.
- *Didymodon maximus*: a globally rare disjunct species occurring in Siberia, Arctic Russia, North America and Ireland. Some Irish specimens represent a different taxon closely related to *D. fallax* and *D. spadiceus* that is likely to be more widespread, at least in Britain and Ireland. One specimen remains problematic, being identical with *D. ferrugineus* morphologically but emerging as *D. spadiceus* molecularly. Further work, including a revision of herbarium specimens, is necessary to elucidate further information.
- *Hamatocaulis vernicosus*: one cryptic species occurs in Ireland with four haplotypes represented, including two that are unique to Ireland (Scragh Bog and Largan More). This emphasises the importance of protecting *all* populations of rare species, in order to be sure of conserving genetic diversity.
- *Hypnum uncinulatum*: many specimens of the common *H. andoi* have been misidentified as *H. uncinulatum*. It appears that *H. uncinulatum* is a Macaronesian species considerably rarer in Ireland than previously thought, and possibly restricted to North Kerry; much of the material examined so far from South Kerry and the Portuguese mainland is *H. andoi*. A full herbarium revision of Irish '*H. uncinulatum*' is needed to confirm these findings.
- *Lejeunea eckloniana*: this species is probably confined to Africa. Irish and Macaronesian material, originally named *L. holtii*, is different, and the old name should probably be resurrected. Material from Monchique (mainland Portugal) is different from both and will be described as a new species.
- *Lejeunea flava*: there are slight morphological and molecular differences between African material and Irish/Macaronesian material, but these are not consistent or very marked. It is therefore appropriate to retain the taxonomic *status quo*, with Irish and Macaronesian material retained as a subspecies (subsp. *moorei*).
- *Lejeunea hibernica*: apparently genetically and morphologically uniform throughout its range, but the data are too sparse to arrive at any further conclusions.
- *Lejeunea mandonii*: this is confirmed as a rare endemic restricted to Ireland, Britain and Macaronesia. *L. canariensis* is confirmed as a separate taxon, and will be the subject of a future paper.
- *Plagiochila bifaria*: this is a distinct amphi-Atlantic species with little molecular (but some morphological) variation across its range. Molecular examination can be useful to distinguish problematic specimens from *P. punctata*.
- *Plagiochila heterophylla*: this is a distinct amphi-Atlantic species with little molecular or morphological variation across its range.
- *Radula carringtonii*: this is endemic to Macaronesia, Ireland and Scotland. Two haplotypes were identified, one apparently unique to Ireland (South Kerry), but these appear to be morphologically identical. Molecular techniques can be useful to distinguish *R. carringtonii* from *R. aquilegia*. Further molecular work on Scottish *R. carringtonii* would be desirable.

- *Radula holtii*: Irish material of this species is genetically uniform; it also occurs in Macaronesia, where it is very slightly different genetically. Material collected from Madeira as *R. holtii* all turned out to be the Macaronesian endemic *R. jonesii*.
- *Solenostoma subellipticum*: results were inconclusive but tend to support the synonymy of *S. subellipticum* with *S. obovatum*.

In addition, a number of potentially important general conclusions can be drawn from these studies:

- The Irish bryophyte flora has features which are unique and therefore important to conserve in a global context.
- Molecular analysis of the Irish bryophyte flora can reveal new, interesting and often unexpected results.
- Morphological uniformity can sometimes hide genetic diversity, with implications for the conservation of biodiversity as a whole.
- There are likely to be many other bryophyte taxa with hidden genetic diversity, and much more research is needed in order to investigate this, especially in the case of the globally important oceanic flora.

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Appendix 1

Samples used for DNA extraction and data analysis. Aliquots of DNA are being stored in the DNA bank in the National Botanic Gardens of Ireland. Not all of the samples were successful in the molecular analysis. Samples from which no DNA was successfully extracted are 'greyed out'. For details of the samples used in the analyses, refer to the Results and Discussion sections of the text.

DNA ID	Taxon (specimen label)	Taxon (after analysis)	Location	Accession/Voucher Number
22D083	*Acrobolbus wilsonii*	*Acrobolbus wilsonii*	Ireland	DBN0001271
22D084	*Acrobolbus wilsonii*	*Acrobolbus wilsonii*	Ireland	NGH11139
22D085	*Acrobolbus wilsonii*	*Acrobolbus wilsonii*	Ireland	NGH11198
22D086	*Acrobolbus wilsonii*	*Acrobolbus wilsonii*	Madeira	NGH11312
22D087	*Acrobolbus wilsonii*	*Acrobolbus wilsonii*	Madeira	NGH11336
22D088	*Cephalozia crassifolia*	*Cephalozia crassifolia*	Azores	TER-20220520/07
22D089	*Cephalozia crassifolia*	*Cephalozia crassifolia*	Azores	TER-20220520/08a
22D090	*Cephalozia crassifolia*	*Cephalozia crassifolia*	Ireland	DBN0001276
22D091	*Cephalozia crassifolia*	*Cephalozia crassifolia*	Ireland	DBN0001280
22D092	*Cephalozia crassifolia*	*Cephalozia crassifolia*	Ireland	DBN0001285
22D093	*Cephalozia crassifolia*	*Cephalozia crassifolia*	Ireland	NGH11170
22D094	*Cephalozia crassifolia*	*Cephalozia crassifolia*	Ireland	NGH11171
22D054	*Didymodon giganteus*	*Didymodon maximus* (H1)	Wrangel I.	LE; Afonina 18.8.1985
22D041	*Didymodon maximus*	*Didymodon maximus* (H1)	Ireland	DBN0001133, NGH4404
22D042	*Didymodon maximus*	*Didymodon maximus* (H1)	Ireland	DBN0001134, NGH4504
22D043	*Didymodon maximus*	*Didymodon maximus* (H1)	Ireland	DBN0001128
22D044	*Didymodon maximus*	*Didymodon fallax/spadiceus* (H2)	Ireland	DBN0001129, NGH4483
22D045	*Didymodon maximus*	*Didymodon cf. ferrugineus/spadiceus*	Ireland	DBN0001130, NGH4494
22D046	*Didymodon maximus*	*Didymodon maximus* (H1)	Ireland	DBN0001131, NGH4309
22D047	*Didymodon maximus*	*Didymodon maximus* (H1)	Ireland	DBN0001135
22D048	*Didymodon maximus*	*Didymodon fallax/spadiceus* (H2)	Ireland	DBN0001137, NGH4467
22D049	*Didymodon maximus*	*Didymodon maximus* (H1)	Ireland	DBN0001138
22D050	*Didymodon maximus*	*Didymodon fallax/spadiceus* (H2)	Ireland	DBN0001139
22D051	*Didymodon maximus*	Pottiaceae, unrelated to *D. maximus*	Siberia, Khamar-Daban	MHA; Ignatov 18-4393; MHA 9029070
22D052	*Didymodon maximus*	*Didymodon maximus* (H1)	Siberia, Tyva	Pisarenko, ex NVS
22D053	*Didymodon maximus* (+ *D. asperifolius*)	*Didymodon maximus* (H1)	Wrangel I.	LE; Afonina 26.7.1985
M1538	*Hamatocaulis vernicosus*	*Hamatocaulis vernicosus* (H1B)	Ireland	Beata Papp 185753

DNA ID	Taxon (specimen label)	Taxon (after analysis)	Location	Accession/Voucher Number
M1727	*Hamatocaulis vernicosus*	*Hamatocaulis vernicosus* (H1B)	Ireland	GF Smith 2005015
M1728	*Hamatocaulis vernicosus*	*Hamatocaulis vernicosus* (H1B)	Ireland	NGH7191
M1729	*Hamatocaulis vernicosus*	*Hamatocaulis vernicosus* (H1A)	Ireland	K Duff
M1730	*Hamatocaulis vernicosus*	*Hamatocaulis vernicosus* (H1A)	Ireland	K Duff
M1731	*Hamatocaulis vernicosus*	*Hamatocaulis vernicosus* (H1B)	Ireland	DBN0001209
M1732	*Hamatocaulis vernicosus*	*Hamatocaulis vernicosus* (H1I)	Ireland	DBN0001213
M1733	*Hamatocaulis vernicosus*	*Hamatocaulis vernicosus* (H1B)	Ireland	DBN0001214
M1734	*Hamatocaulis vernicosus*	*Hamatocaulis vernicosus* (H1B)	Ireland	DBN0001215
M1735	*Hamatocaulis vernicosus*	*Hamatocaulis vernicosus*	Ireland	DBN0001217
M1736	*Hamatocaulis vernicosus*	*Hamatocaulis vernicosus* (H1A)	Ireland	DBN0001219
M1737	*Hamatocaulis vernicosus*	*Hamatocaulis vernicosus* (H1A)	Ireland	DBN0001222
M1738	*Hamatocaulis vernicosus*	*Hamatocaulis vernicosus*	Ireland	DBN0001224
M1739	*Hamatocaulis vernicosus*	*Hamatocaulis vernicosus* (H1B)	Ireland	DBN0004748
M1740	*Hamatocaulis vernicosus*	*Hamatocaulis vernicosus* (H1J)	Ireland	DBN0004749
M1741	*Hamatocaulis vernicosus*	*Hamatocaulis vernicosus* (H1B)	Ireland	DBN0004751
M1742	*Hamatocaulis vernicosus*	*Hamatocaulis vernicosus* (H1B)	Ireland	DBN0004752
22D063	*Hypnum uncinulatum*	*Hypnum uncinulatum*	Ireland	DBN0001227
22D064	*Hypnum uncinulatum*	*Hypnum uncinulatum*	Ireland	DBN0001228
22D065	*Hypnum uncinulatum*	*Hypnum uncinulatum*	Ireland	DBN0001229
22D066	*Hypnum uncinulatum*	*Hypnum cf. uncinulatum*	Ireland	DBN0001231
22D067	*Hypnum uncinulatum*	*Hypnum andoi*	Ireland	DBN0001234
22D068	*Hypnum uncinulatum*	*Hypnum andoi*	Ireland	DBN0001235
22D069	*Hypnum uncinulatum*	*Hypnum andoi*	Ireland	DBN0001237
22D070	*Hypnum uncinulatum*	*Hypnum andoi*	Ireland	DBN0001240
22D071	*Hypnum uncinulatum*	*Hypnum uncinulatum*	Madeira	NGH11280
22D072	*Hypnum uncinulatum*	*Hypnum uncinulatum*	Madeira	NGH11362
22D073	*Hypnum uncinulatum*	*Hypnum andoi*	Monchique	Porley Barbelote
22D074	*Hypnum uncinulatum*	*Hypnum andoi*	Monchique	Porley Foia
22D075	*Hypnum uncinulatum*	*Hypnum andoi*	Monchique	Porley Ribeira da Cerca
22D076	*Hypnum uncinulatum*	*Hypnum uncinulatum*	Madeira	Porley Chão dos Louros
22D077	*Hypnum uncinulatum*	*Hypnum uncinulatum*	Azores	TER-20220520/13
22D078	*Hypnum uncinulatum*	*Hypnum uncinulatum*	Azores	TER-07052022/18
22D079	*Hypnum uncinulatum*	*Hypnum uncinulatum*	Azores	TER-07052022/05
22D102	*Lejeunea cavifolia*	*Lejeunea cavifolia*	Scotland	NGH8192
22D103	*Lejeunea cavifolia*	*Lejeunea cavifolia*	Scotland	NGH10288
22D080	*Lejeunea ?cavifolia*	*Lejeunea cavifolia*	Madeira	NGH11247
22D081	*Lejeunea ?cavifolia*	*Lejeunea lamacerina*	Monchique	Porley 20; Penedo do Buraco

DNA ID	Taxon (specimen label)	Taxon (after analysis)	Location	Accession/Voucher Number
22D082	*Lejeunea ?cavifolia*	*Lejeunea cavifolia*	Monchique	Porley 18; Vale de Cova da Serra
22D095	*Lejeunea ?cavifolia*	*Lejeunea cavifolia*	Monchique	Porley 19; Barbelote
22D039	*Lejeunea eckloniana*	*Lejeunea eckloniana* (H1)	Lesotho	E00206750
22D040	*Lejeunea eckloniana*	*Lejeunea eckloniana* (H1)	Lesotho	E00206751
22D104	*Lejeunea eckloniana*	*Lejeunea cf. holtii* (H3)	Ireland	DBN0001439
22D105	*Lejeunea eckloniana*	*Lejeunea cf. holtii* (H3)	Ireland	NGH11151
22D106	*Lejeunea eckloniana*	*Lejeunea cf. holtii* (H3)	Madeira	NGH11223
22D107	*Lejeunea eckloniana*	*Lejeunea cf. holtii* (H3)	Madeira	NGH11242
22D108	*Lejeunea eckloniana*	*Lejeunea eckloniana*	Madeira	NGH11246
22D109	*Lejeunea eckloniana*	*Lejeunea eckloniana*	Madeira	NGH11251
22D110	*Lejeunea eckloniana*	*Lejeunea cf. holtii* (H3)	Madeira	NGH11288
22D111	*Lejeunea eckloniana*	*Lejeunea cf. holtii* (H3)	Madeira	NGH11302
22D112	*Lejeunea eckloniana*	*Lejeunea eckloniana*	Uganda	NGH U4353
22D113	*Lejeunea eckloniana*	*Lejeunea eckloniana* (H1)	Lesotho	NGH 3166b
22D114	*Lejeunea eckloniana*	*Lejeunea cf. eckloniana* (H2/3)	Ghana	E00728676, Adu-Gyamfi et al. 8675
22D115	*Lejeunea eckloniana*	*Lejeunea eckloniana* (H1)	Lesotho	E00206863, Duckett et al. 3121d
22D116	*Lejeunea eckloniana*	*Lejeunea eckloniana* (H1)	Lesotho	E00206864, Duckett et al. 3091b
22D181	*Lejeunea eckloniana*	*Lejeunea eckloniana*	Ireland	NGH11180
22D117	*Lejeunea eckloniana 'pale form'*	*Lejeunea cf. holtii* (H3)	Madeira	NGH11224
22D096	*Lejeunea ?eckloniana*	*Lejeunea cf. holtii* (H3)	Madeira	NGH11363
22D097	*Lejeunea ?eckloniana*	*Lejeunea sp. nov.* (H2)	Monchique	Porley 13; Vale de Cova da Serra
22D098	*Lejeunea ?eckloniana*	*Lejeunea sp. nov.* (H2)	Monchique	Porley 9; Foía
22D099	*Lejeunea ?eckloniana*	*Lejeunea sp. nov.* (H2)	Monchique	Porley 6; Ribeira de Cerca
22D100	*Lejeunea ?eckloniana*	*Lejeunea sp. nov.* (H2)	Monchique	Porley 4; Vale de Cova da Serra
22D057	*Lejeunea flava*	*Lejeunea flava*	Ireland	DBN0001298
22D058	*Lejeunea flava*	*Lejeunea flava* subsp. *moorei*	Ireland	NGH11154
22D118	*Lejeunea flava*	*Lejeunea flava* subsp. *moorei*	Ireland	NGH11158
22D119	*Lejeunea flava*	*Lejeunea flava* subsp. *moorei*	Ireland	NGH11169
22D120	*Lejeunea flava*	*Lejeunea flava* subsp. *moorei*	Ireland	NGH11194
22D121	*Lejeunea flava*	*Lejeunea flava* subsp. *moorei*	Madeira	NGH11278
22D122	*Lejeunea flava*	*Lejeunea flava*	Madeira	NGH11401
22D123	*Lejeunea flava*	*Lejeunea flava*	Ghana	NGH8685
22D124	*Lejeunea flava*	*Lejeunea flava*	Malawi	NGH M2102a
22D125	*Lejeunea flava*	*Lejeunea flava*	Uganda	NGH U4068h
22D126	*Lejeunea flava*	*Lejeunea flava*	Malawi	E00990695, NGH2266d
22D127	*Lejeunea flava*	*Lejeunea flava*	Sao Tome & Principe	E00990698, Shevock 34787
22D128	*Lejeunea flava subsp. moorei*	*Lejeunea flava* subsp. *moorei*	Azores	TER-07052022/01

DNA ID	Taxon (specimen label)	Taxon (after analysis)	Location	Accession/Voucher Number
22D129	*Lejeunea flava subsp. moorei*	*Lejeunea flava* subsp. *moorei*	Azores	TER-20220520/01
22D059	*Lejeunea hibernica*	*Lejeunea hibernica*	Ireland	DBN0001386
22D060	*Lejeunea hibernica*	*Lejeunea hibernica*	Ireland	DBN0001391
22D130	*Lejeunea hibernica*	*Lejeunea hibernica*	Ireland	DBN0001397
22D131	*Lejeunea hibernica*	*Lejeunea hibernica*	Ireland	NGH11157
22D132	*Lejeunea hibernica*	*Lejeunea hibernica*	Ireland	NGH11179
22D133	*Lejeunea hibernica*	*Lejeunea hibernica*	Madeira	NGH11347
22D134	*Lejeunea hibernica*	*Lejeunea hibernica*	Madeira	NGH11368
22D135	*Lejeunea hibernica*	*Lejeunea hibernica*	Madeira	NGH11422
22D136	*Lejeunea mandonii*	*Lejeunea mandonii*	Ireland	NGH11173
22D137	*Lejeunea mandonii*	*Lejeunea mandonii*	Scotland	NGH11457
22D138	*Lejeunea mandonii/canariensis*	*Lejeunea canariensis*	Madeira	NGH11404
22D139	*Lejeunea mandonii/canariensis*	*Lejeunea canariensis*	Madeira	NGH11420
22D061	*Lejeunea ?mandonii*	*Lejeunea canariensis*	Madeira	NGH11243
22D062	*Lejeunea ?mandonii*	*Lejeunea ?mandonii*	Madeira	NGH11254
22D101	*Lejeunea ?mandonii*	*Lejeunea mandonii*	Madeira	NGH11393
22D140	*Plagiochila bifaria*	*Plagiochila bifaria*	Ireland	DBN0001490
22D141	*Plagiochila bifaria*	*Plagiochila bifaria*	Scotland	NGH11129
22D142	*Plagiochila bifaria*	*Plagiochila bifaria*	Ireland	NGH11133
22D143	*Plagiochila bifaria*	*Plagiochila bifaria*	Ireland	NGH11153a
22D144	*Plagiochila bifaria*	*Plagiochila bifaria*	Madeira	NGH11237
22D145	*Plagiochila bifaria*	*Plagiochila bifaria*	Madeira	NGH11274
22D146	*Plagiochila bifaria*	*Plagiochila bifaria*	Madeira	NGH11351
22D147	*Plagiochila bifaria*	*Plagiochila bifaria*	Madeira	NGH11398
22D148	*Plagiochila bifaria*	*Plagiochila bifaria*	Azores	TER-20220520/03
22D149	*Plagiochila bifaria*	*Plagiochila bifaria*	Azores	TER-07052022/03
22D183	*Plagiochila bifaria*	*Plagiochila bifaria*	Ireland	NGH11168
22D151	*Plagiochila bifaria/punctata*	*Plagiochila punctata*	Ireland	NGH11138
22D152	*Plagiochila bifaria/punctata*	*Plagiochila punctata*	Ireland	NGH11155
22D153	*Plagiochila punctata/bifaria*	*Plagiochila punctata*	Ireland	NGH11159
22D154	*Plagiochila bifaria/punctata*	*Plagiochila bifaria*	Ireland	NGH11161
22D155	*Plagiochila bifaria/punctata*	*Plagiochila punctata*	Ireland	NGH11175
22D150	*Plagiochila heterophylla*	*Plagiochila heterophylla*	Ireland	NGH11193
22D180	*Plagiochila heterophylla*	*Plagiochila heterophylla*	Ireland	NGH11192
22D158	*Radula carringtonii*	*Radula carringtonii* (H2)	Ireland	DBN0001484
22D159	*Radula carringtonii*	*Radula carringtonii* (H2)	Ireland	NGH11153
22D160	*Radula carringtonii*	*Radula carringtonii* (H2)	Ireland	NGH11156
22D161	*Radula carringtonii*	*Radula carringtonii* (H1)	Ireland	NGH11174
22D162	*Radula carringtonii*	*Radula carringtonii* (H1)	Ireland	NGH11183
22D163	*Radula carringtonii*	*Radula carringtonii* (H1)	Ireland	NGH11186
22D164	*Radula carringtonii*	*Radula aquilegia* + *R. carringtonii*	Madeira	NGH11304
22D165	*Radula carringtonii*	*Radula aquilegia*	Madeira	NGH11325

DNA ID	Taxon (specimen label)	Taxon (after analysis)	Location	Accession/Voucher Number
22D166	*Radula carringtonii*	*Radula aquilegia*	Madeira	NGH11381
22D156	*Radula ?carringtonii*	*Radula aquilegia*	Madeira	NGH11279
22D157	*Radula ?carringtonii*	*Radula aquilegia*	Madeira	NGH11397
22D167	*Radula holtii*	*Radula holtii*	Ireland	DBN0001469
22D168	*Radula holtii*	*Radula holtii*	Ireland	NGH11134
22D169	*Radula holtii*	*Radula holtii*	Ireland	NGH11136
22D170	*Radula holtii*	*Radula holtii*	Ireland	NGH11164
22D171	*Radula holtii*	*Radula carringtonii* (H1)	Ireland	NGH11184
22D172	*Radula holtii*	*Radula jonesii*	Madeira	NGH11253
22D173	*Radula holtii*	*Radula carringtonii* (H2) + *R. jonesii*	Madeira	NGH11284
22D174	*Radula holtii*	*Radula jonesii*	Madeira	Porley Chão da Ribeira-Fanal
22D175	*Solenostoma obovatum*	*Solenostoma obovatum*	Ireland	NGH11142
22D176	*Solenostoma obovatum*	*Solenostoma obovatum*	Ireland	NGH11147
22D177	*Solenostoma subellipticum*	*Solenostoma cf. obovatum*	Ireland	DBN0001493
22D178	*Solenostoma subellipticum*	*Solenostoma cf. obovatum*	Ireland	DBN0001498
22D179	*Solenostoma subellipticum (?)*	*Solenostoma cf. obovatum*	Ireland	NGH11144
22D182	*Solenostoma subellipticum (?)*	*Solenostoma cf. obovatum*	Ireland	NGH11146

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