



Taxonomic notes on *Weissia* subgenus *Astomum*, including *Weissia wilsonii* D.A.Callaghan, a new species from Europe

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ABSTRACT

Introduction. The present study investigates the taxonomy of European members of *Weissia* Hedw. subgenus *Astomum* Hampe., a group considered taxonomically difficult and including species of high conservation concern.

Methods. A broad set of samples were subject to DNA sequencing and morphological analysis, plus a review of type material was undertaken.

Key Results and Conclusions. Three taxonomic additions and changes are supported. (1) *Weissia longifolia* var. *angustifolia* (Baumgartner) Crundw. & Nyholm is raised to species rank, *W. angustifolia* (Baumgartner) D.A.Callaghan, *comb. et. stat. nov.* (2) The type of *W. multicapsularis* (Sm.) Mitt. comprises an intermix of *Phascum cuspidatum* Hedw. and *W. longifolia* Mitt., and the protologue refers to unique features of *P. cuspidatum* but no such features of *W. longifolia*. *Weissia multicapsularis* is therefore described as a new synonym of *P. cuspidatum*. (3) Plants that have been named as *W. multicapsularis* by modern authors comprise an undescribed species, here named *W. wilsonii* D.A.Callaghan, *sp. nov.* An illustrated key to European species of *Weissia* subgenus *Astomum* is provided.

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Introduction

Weissia Hedw. subgenus *Astomum* Hampe. (Pottiaceae) has been used to place species with, mostly, a short seta, cleistocarpic capsule and well-differentiated perichaetial leaves. Crundwell and Nyholm (1972) recognised six European species within this subgenus, *W. levieri* (Limpr.) Kindb., *W. longifolia* Mitt., *W. mittenii* (Bruch & Schimp.) Mitt., *W. multicapsularis* (Sm.) Mitt., *W. rostellata* (Brid.) Lindb. and *W. sterilis* Nichols., all of which are known from Britain (Smith 2004; Blockeel et al. 2014). In England, Section 41 of The Natural Environment and Rural Communities Act 2006 (as amended) lists species considered national priorities for biodiversity conservation, including, due to their rarity, *W. levieri*, *W. multicapsularis* and *W. sterilis*. *Weissia multicapsularis* is also listed as 'Critically Endangered' on the IUCN World Red List (IUCN 2017) and, in England, is a designated interest feature of two Sites of Special Scientific Interest (SSSI), Carricknath Point to Porthbean Beach SSSI and Talland Barton Farm SSSI. Recent work undertaken as part of efforts to conserve these species, in particular *W. multicapsularis* (Holyoak 1999; Callaghan 2016), revealed uncertainty regarding identification of some species and potential taxonomic problems. This is a concern because a well-resolved taxonomy coupled with clear identification characters is fundamental to species conservation efforts, helping to ensure scarce resources are spent efficiently. The aim of the present study is to use a combination of

DNA sequence and morphological data to resolve taxonomic issues related to British species of *Weissia* subgenus *Astomum* and provide guidance for identification.

Materials and methods

Sampling

Specimens belonging to five species of *Weissia* subgenus *Astomum* present in Britain from BBSUK, BM, CGE, E, NMW and various private herbaria, were inspected as were type specimens of *W. longifolia* var. *longifolia* (NY) and *W. multicapsularis* (LINN). Fresh field collections of *W. levieri*, *W. longifolia*, *W. multicapsularis* and *W. sterilis* were made during 2017, from sites in England and Wales. The putative hybrid *W. mittenii* and other unnamed hybrids known from Britain (Nicholson 1905, 1906, 1910) were excluded from consideration.

Morphology

Following consideration of characters used in published identification keys (Braithwaite 1887; Dixon and Jameson 1924; Crundwell and Nyholm 1972; Smith 2004; Frey et al. 2006), variation in morphological traits across specimens was reviewed, including capsule length, leaf margin incurvature, operculum length, perichaetial leaf length, plant height, presence of an abscission zone at capsule mouths, presence of branching, ratio of operculum length to urn length,

ratio of stem leaf length to perichaetial leaf length, ratio of urn width to urn length, seta length, spore papillosity, spore size, stem leaf length, urn length and urn width. To accelerate rehydration of dried cleistocarpous sporophytes, material was soaked in a warm 2% KOH solution.

DNA extraction, PCR amplification and sequencing

A total of 64 specimens from *Weissia* subgenus *Astomum* were newly sampled for molecular data, four identified as *W. levieri*, 20 as *W. longifolia* var. *angustifolia* (Baumgartner) Crundw. & Nyholm, 17 as *W. longifolia* var. *longifolia*, 12 as *W. multicapsularis*, seven as *W. rostellata*, and four as *W. sterilis* (Appendix), while sequences from a single specimen of *W. jamaicensis* (Mitt.) Grout were obtained from GenBank and used as an outgroup. It was clear from our pilot studies as well as from previously published research (e.g. Werner et al. 2005) that *Weissia* subgenus *Astomum* is unlikely to be monophyletic, and that some widespread species in other subgenera, most notably *W. controversa* Hedw., are polyphyletic. As our objective was to investigate boundaries between morphologically very similar species in subgenus *Astomum* in Britain, and as none of these species are likely to be confused with taxa in other subgenera, we restricted our sampling to species traditionally included in section *Astomum* and selected an outgroup that pilot studies showed to be outside of a clade that includes all our ingroup taxa.

Genomic DNA was isolated using the Invisorb spin plant mini kit (Invitex, Berlin, Germany) and eluted DNA stored in the supplied buffer, or else using DNeasy® Plant mini kits (Qiagen) and eluted in 100 µL of a Tris-EDTA buffer for storage.

Amplification using PCR was attempted for one plastid and one nuclear marker, the *trnL-trnF* (*trnL-F*) and ITS1 and 2 regions respectively. The *trnL-F* locus was amplified and sequenced using the primer pair *trnL.C* and *trnL.F* (Taberlet et al. 1991), while the ITS1 and ITS2 loci were either amplified and sequenced separately, using the primer pairs ITS1_F and ITS1_R (Sawicki and Szczecińska 2011) for ITS1, and seqITS2 (Olsson et al. 2009) and ITS4bryo (Stech 1999; as 25R in Stech et al. 2003) for ITS2, or in a single amplification using primer pair ITS1_F and ITS4bryo. The same primers were used for sequencing.

The *trnL-F* PCR amplifications were performed in 20 µL reactions containing 2 µL of the supplied 10x PCR reaction buffer, 2 µL of dNTPs (each at 2 mM), 0.6 µL of MgCl₂ (at 50 mM), 4 µL of a PCR enhancer additive—either TBT-PAR (Samarakoon et al. 2013) or CES (Ralsler et al. 2006; both at 5x), 2 µL of each primer (at 10 µM), 0.25 µL of Biotaq DNA polymerase (Bioline, London, UK; at 5 units/µL) and 1 µL template DNA, made up to 20 µL with Sigma PCR-grade water.

The PCR protocol consisted of an initial step of 94 °C for 4 min, followed by 31 cycles of 94 °C for 1 min, 50 °C for 1 min, 72 °C for 1 min 45 s, and then a final extension step of 72 °C for 5 min.

The ITS PCR amplifications were performed in 20 µL reactions containing 2 µL of the supplied 10x PCR reaction buffer, 2 µL of dNTPs (each at 2 mM), 1 µL of MgCl₂ (at 50 mM), 0.75 µL of each primer (at 10 µM), 0.15 µL of Biotaq DNA polymerase (Bioline, London, UK) and 1 µL template DNA, made up to 20 µL with Sigma PCR-grade water. The PCR protocol consisted of an initial step of 95 °C for 4 min, followed by 35 cycles of 94 °C for 1 min, 55 °C for 1 min, 72 °C for 1 min 30 s, and then a final extension step of 72 °C for 7 min.

The success of the PCR was assessed using a 1% agarose Tris-Borate-EDTA gel with SYBR®Safe (Invitrogen) gel stain visualised under blue light. Successfully amplified products were cleaned using ExoSap-IT (Affometrix) according to the manufacturer's instructions and sequenced using 1/16th reactions with BigDye® Terminator v. 3.1 chemistry. Sequencing reactions were sent to the Genepool facility at the University of Edinburgh for clean-up and Sanger sequencing on an ABI 3730.

Sequence alignment, phylogenetic analysis and haplotype networking

Where they had been generated as two separate amplicons, sequences of ITS1 and ITS2 were concatenated prior to the combined ITS data and the *trnL-F* data being aligned manually using PhyDE v.0.997 (Müller et al. 2011). There was very little alignment ambiguity in either region, nearly all of which was in the form of indels in short single base-pair repeat regions. Maximum Likelihood (ML) analyses were conducted using Randomized Accelerated Maximum Likelihood (RAxML) v.7.4.2 (Stamatakis 2006) with the raxmlGUI v.1.3 front end (Silvestro and Michalak 2012). We chose ML over Bayesian methods for this study due to the relatively low amount of informative data and the correspondingly potentially significant influence of even supposedly 'flat' priors on posterior distributions (e.g. Wang and Yang 2014). Separate RAxML analyses were conducted for the concatenated ITS1 and 2 data, for the *trnL-F* data, and for a combined dataset including all the terminals for which ITS data was obtained together with *trnL-F* data where it was available for these specimens. For analysis of ITS1 and 2 data alone a General Time Reversible (GTR) model of nucleotide substitution with a proportion of invariable sites (GTR + I) was assumed based on the Akaike Information Criterion (AIC) as implemented in MrModeltest 2.3 (Nylander 2004). This model was also used for the combined ITS and *trnL-F* analysis, while for analysis of the *trnL-F* data alone the basic GTR model was used. The 'ML + thorough bootstrap' option within the raxml GUI (RAxML option '-b' followed by an ML search) was

used for all analyses, with 50 runs and 1000 replications. In all cases the data were treated as a single partition, as total variability was low and thus accurate estimation of model parameters potentially problematic. As ambiguous morphological species boundaries and low levels of divergence in the generally highly variable ITS region suggested that reticulate as well as tree-like processes might be present, PopART v1.7 (<http://www.leigh.net.nz/software.shtml>) was used to generate 95% TCS haplotype networks (statistical parsimony; Templeton et al. 1992) for the concatenated ITS1 and ITS2 matrix and the *trnL-F* matrix. Sites with undefined states for some terminals (gaps) were removed prior to haplotype analysis, while a small number of sites with ambiguous base calls were changed to single bases where there was otherwise no variation in that character (i.e. the character was assumed to be invariant in the sampled terminals and thus uninformative). Terminals with much missing data or many ambiguous base calls were removed.

Results and discussion

Taxonomy

Synonymisation of *Weissia multicapsularis* with *Phascum cuspidatum*

Phascum cuspidatum Hedw., Sp. Musc. Frond.: 22. 1801. = *Phascum multicapsulare* Sm., Fl. Brit. 3: 1152. 1804 ≡ *Weissia multicapsularis* (Sm.) Mitt., Ann. Mag. Nat. Hist., ser. 2, 8: 317. 1851. *syn. nov.*

Type. [UK] Clapham springs near Bedford, February 1805, Rev. Dr Abbot (holotype: LINN!)

Discussion. The type of *Weissia multicapsularis* (LINN!) comprises four small tufts of material glued to a herbarium sheet, each an intermix of *Phascum cuspidatum* and *W. longifolia* (Figure 1). The protologue (Smith 1804) is brief and lacks illustrations but highlights characters that are clearly referable to *P. cuspidatum*, in particular

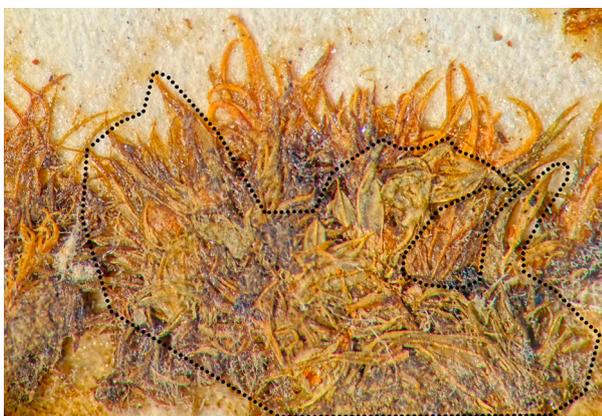


Figure 1. Part of the type material of *Weissia multicapsularis* (LINN), glued to a herbarium sheet, showing a mixture of *Phascum cuspidatum* (within marked zone) and *W. longifolia* (outside marked zone).

wide perichaetial leaves that are never curled when dry; it does not mention characters clearly referable only to the *Weissia*. The material was collected by Rev. Dr Abbot from 'Clapham springs near Bedford, February 1805' and is described in the protologue as occurring 'in cart-ruts in Clapham Park wood, near Clapham Ford end, and other places near Bedford [UK]'. Clapham Park Wood, now a local nature reserve, was searched for *Weissia* on 6 October 2017, but little suitable habitat was present, with the rides generally grass-dominated. Margins of an arable field bordering the north of the wood provided better habitat, supporting frequent *P. cuspidatum* and, at one location (TL 04754 53356), a very small amount of *W. longifolia*.

Elevation of *Weissia longifolia* var. *angustifolia* to species rank

Weissia angustifolia (Baumgartner) D.A.Callaghan, *comb. et stat. nov.*

≡ *Astomum crispum* var. *angustifolium* Baumgartner, Denkschr. Kaiserl. Akad. Wiss., Wien Math.-Naturwiss. Kl. 92: 323. 1915 (basionym) ≡ *Weissia longifolia* var. *angustifolia* (Baumgartner) Crundw. & Nyholm, J. Bryol. 7: 14. 1972.

Type. [Croatia] above Porto Slatina, island of Sant'Andrea (Sveti Andrija), west of Lissa (Vis), 43°01'33"N, 15°44'56"E, macchie, 6 June 1911, A. Ginzberger (holotype: W 1964-0021668).

Discussion. Baumgartner (1915) was reluctant to describe this taxon at the rank of species because he had sparse material available for study, but noted it was clearly different to *Weissia longifolia* and could, ultimately, be considered a species. Two morphological characters, both recognised by Baumgartner (1915), are particularly characteristic. Firstly, margins along the distal half of perichaetial leaves are tightly involute, a feature unique amongst European *Weissia* subgenus *Astomum*, in which margins are normally plane to loosely incurved. Secondly, an abscission zone is present at the junction of the operculum and urn, comprising a ring of small and strongly differentiated cells, allowing clean detachment of the lid from the urn, a feature shared only with *W. levieri*. Note that, however, the lid normally remains attached to the urn in nature, with dehisced capsules rarely seen, whereas in *W. levieri* the lids are commonly shed once capsules are mature.

Weissia wilsonii as a replacement name for the taxon referred to previously as *W. multicapsularis*

Weissia wilsonii D.A.Callaghan, *sp. nov.*

(Figures 2, 3 and 4)

Diagnosis. Similar to *Weissia longifolia* but distinguished by the large operculum, (0.21–)0.23–0.50(–0.59) times as long as urn in *W. wilsonii* compared to 0.11–0.24(–0.26) in *W. longifolia* (Figures 5 and 6).

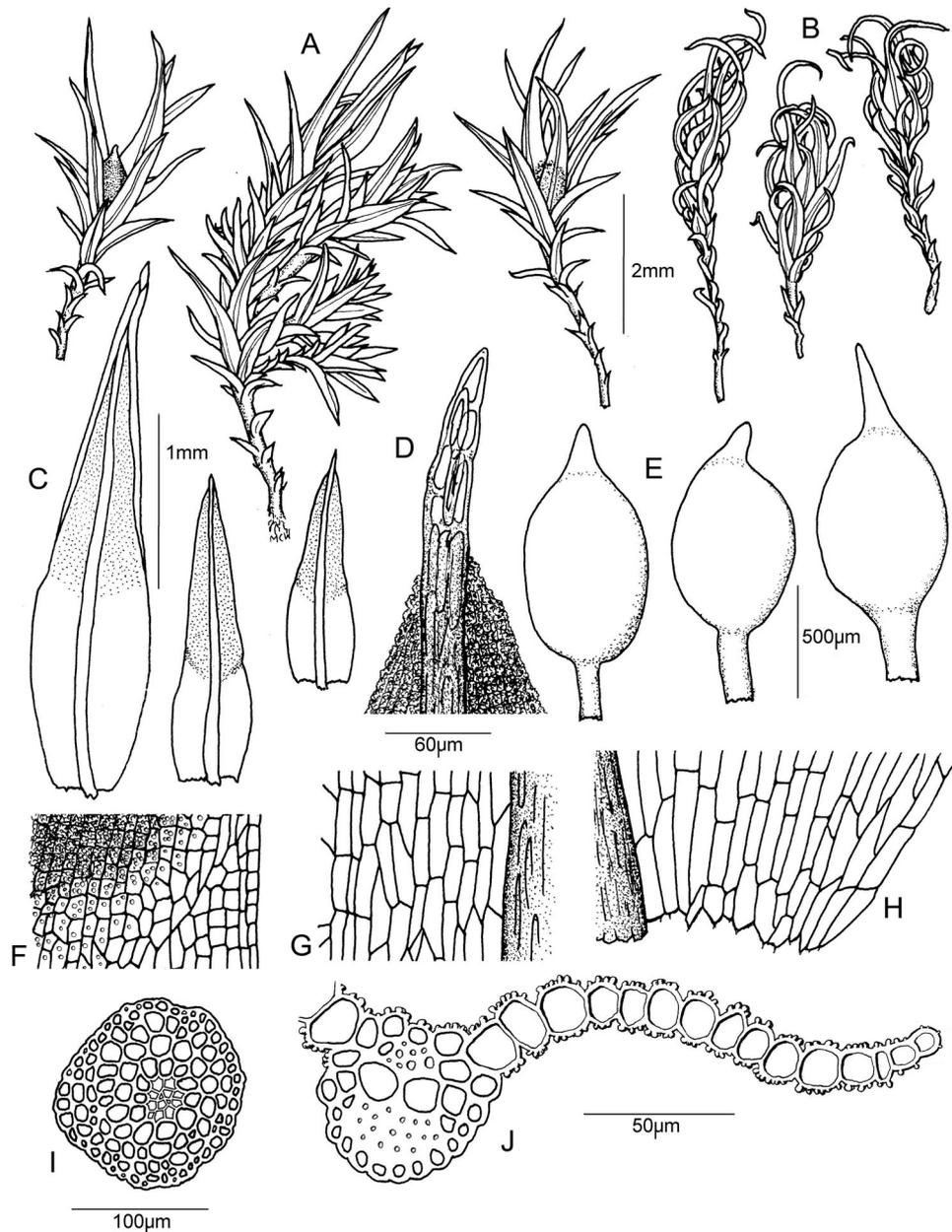


Figure 2. *Weissia wilsonii* D.A.Callaghan. (A) Habit hydrated. (B) Habit dry. (C) Stem leaves. (D) Stem leaf tip. (E) Mature capsules. (F) Perichaetial leaf cells at transition between smooth lower cells and papillose upper cells. (G) Perichaetial leaf lower cells beside costa. (H) Perichaetial leaf basal cells. (I) Stem cross-section. (J) Perichaetial leaf cross-section. Drawn by Malcolm Watling from holotype (BM).

Type. UK: Talland Barton Farm, Cornwall, 50°20'14"N, 4°29'3"W, bare soil beside coastal footpath along edge of horse pasture, 35 m a.s.l., 22 January 2018, D. A. Callaghan, Coll. No. 81462 (holotype: BM).

Description. Plants small, typically about 5 mm tall, usually forming small cushions, green, unbranched or with sympodial branching, branches ca. 1–2.5 mm long. Rhizoids reddish-brown, smooth. Stems ca 130 µm wide, transverse section rounded, central strand narrow, ca. 25 µm wide, hyalodermis absent. Stem leaves patent to recurved, axis of leaf tip 30–90° from axis of stem, opaque, mid-green to dark-green, up to 1.5 mm long, ovate-lanceolate, moderately expanded at base, ca. 3.4 times longer than wide, apex acute; leaf margins plane, untoothed. Lowermost

leaves tiny and scale-like. Leaf lamina unistratose; median leaf cells quadrate to shortly oblong, ca. 8 µm wide, with dense, simple papillae; marginal cells not differentiated; basal cells strongly differentiated, the inner and paracostal cells pale yellow, short to long rectangular, mostly 30–60 µm long and 6–9 µm wide, smooth, with yellowish walls; marginal basal cells shorter and narrower, ca. 25 µm long and 5 µm wide, with the walls not thicker than those of the inner cells. Nerve single, shortly excurrent, by ca. 60 µm, moderately stout, ca. 65 µm wide and ca. 1/8 width of leaf at base.

Autoicous. Male inflorescence terminal on a branch, female inflorescence terminal on the main stem. Perichaetial leaves erect when wet, strongly curled when



Figure 3. Sporophytic shoots of *Weissia wilsonii*, Greeb Point, Cornwall, UK (Callaghan 66365). Photo: D. A. Callaghan.

dry, much longer than vegetative leaves, 2.6–5.0 mm long, margins plane to loosely incurved. Seta 0.3–0.5 mm. Capsules cleistocarpous, ovoid to sub-spherical, urn (0.30–)0.60–0.96(–0.98) mm long (excluding operculum) and (0.27–)0.35–0.71 mm wide, ratio of urn width to urn length 0.60–1.04. Operculum erect to inclined, 0.12–0.43 mm long, (0.21–)0.23–0.50(–0.59) times as long as urn. Calyptra 0.4–0.7 mm long. Spores 15–23 μ m.

Etymology. Named in honour of the eminent bryologist William Wilson (1799–1871), who first collected the plant near to his home town of Warrington, Cheshire, UK.



Figure 4. Cushions of *Weissia wilsonii* (arrowed) with *Entosthodon fascicularis* (Hedw.) Müll.Hal., *Oxyrrhynchium hians* (Hedw.) Loeske and *Tortula truncata* (Hedw.) Mitt., in stubble (wheat) field, Greeb Point, Cornwall, UK, 27 February 2018. Photo: D. A. Callaghan.

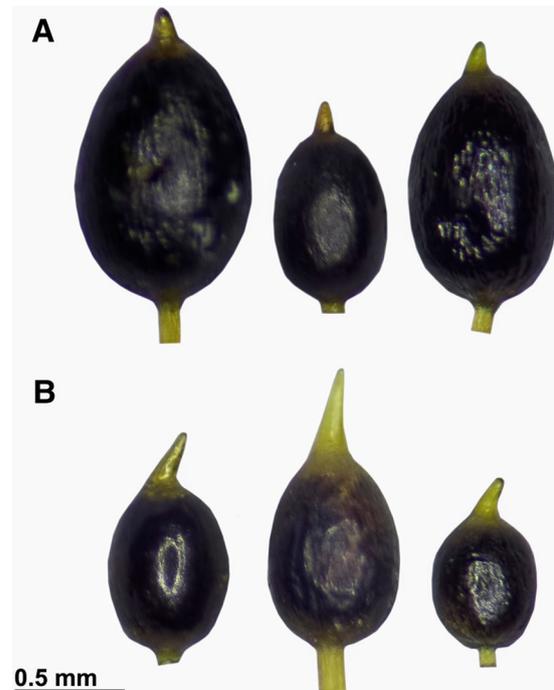


Figure 5. Capsules of (A) *Weissia longifolia* (BBSUK C.2004.007.300 [left]; NMW C.2009.001.308 [middle]; BBSUK C.2001.019.452 [right]) and (B) *W. wilsonii* (Callaghan 81462 [left]; NMW C.2010.034.031 [middle]; Callaghan 66365 [right]), illustrating the characteristically large operculum of the latter. Photo: D. A. Callaghan.

Paratype. UK: Greeb Point, Cornwall, 50°09'55"N, 4°58'30"W, loam soil of stubble (wheat) field, 40 m a.s.l., 27 February 2018, D. A. Callaghan, Coll. No. 81480 (BM, E, NICH).

Distribution. Presently known from a small number of sites in England and Wales, formerly also a single site in France. Appears to have undergone a long-term decline, likely due mainly to the intensification of agricultural land, in particular related to grazing and the widespread loss of winter-stubble fields.

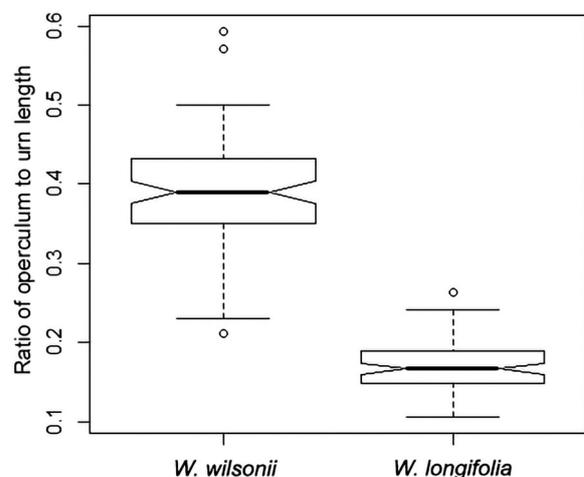


Figure 6. Boxplot of ratio of operculum length to urn length in *Weissia wilsonii* and *W. longifolia*. Measures of *W. wilsonii* are from 87 capsules from 20 populations. Measurements of *W. longifolia* are from 77 capsules from 16 populations.

Ecology and habitat. A pioneer of insolated to partly shaded soil in open habitats, such as quarries, arable fields, woodland rides, track-sides, earth banks and short grassland (Figure 7). Conditions are usually circum-neutral, though acidic to calcareous soils can be occupied. The largest known population inhabits an arable field at Greeb Point, Cornwall, where the associated bryophyte flora conforms with the *Dicranella staphylina*–*Riccia glauca* assemblage described by Preston et al. (2010), a widespread arable community with a westerly bias in Britain. Exposed coasts can also be occupied, sometimes close to high tides, perhaps indicating a degree of salt tolerance.

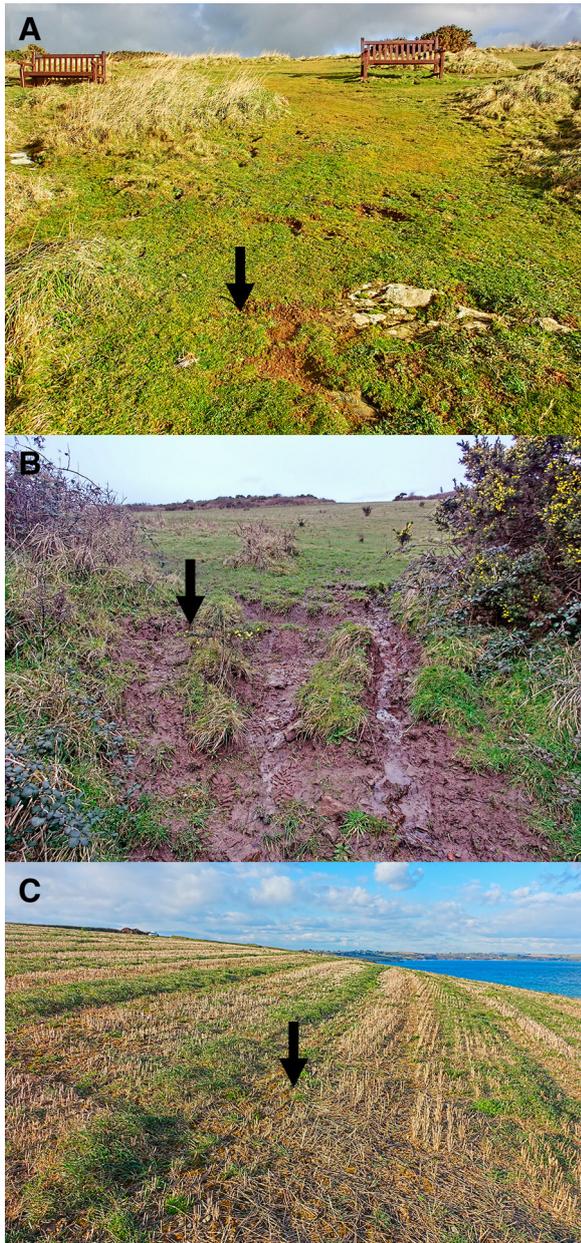


Figure 7. Examples of habitat occupied by *Weissia wilsonii* in Cornwall, UK, including (A) bare patches of soil in coastal grassland, Pentire Point East, 13 February 2014, (B) cattle-poached track beside pasture, Gwendreath, 4 February 2014, and (C) stubble (wheat) field, Greeb Point, 27 February 2018. Photos: D. A. Callaghan.

Discussion. This is the plant commonly referred to *Weissia multicapsularis* (Mitten 1851; Wilson 1855; Braithwaite 1887; Nicholson 1908; Dixon and Jameson 1924; Jones 1952; Paton 1969; Crundwell and Nyholm 1972; Holyoak 1999, 2012; Smith 2004; Frey et al. 2006; Blockeel et al. 2014; Callaghan 2016). This new species was first collected by William Wilson from fields at Appleton, Cheshire, UK, in March 1836 (BM000725388!, BM000725411!, BM000725416!, BM000725420!), and illustrated by him in *Bryologia Britannica* (Wilson 1855; Figure 8). The main diagnostic character, the large operculum, has been recognised by some authors, but often it has been over-looked while other unreliable features have been highlighted.

Molecular sequence variation

Plant DNA barcoding has been used successfully to identify lineages in the liverworts *Herbertus* Gray (Bell et al. 2012) and *Aneura* Dumort. (Bączkiewicz et al. 2017) and the mosses *Dicranum* Hedw. (Lang et al. 2014), *Racomitrium* Brid. (Stech et al. 2013) and *Schistidium* Bruch & Schimp. (Hofbauer et al. 2016). However, preliminary data generated from the plastid supplementary barcode locus *psbA-trnH* using primers 501F (Cox in Forrest and Crandall-Stotler 2004) and *trnHR* (Sang et al. 1997) only had three variable nucleotides between four accessions, *W. multicapsularis* (17-0049062), *W. longifolia* var. *angustifolia* (17-0049055), *W. sterilis* (17-0049059) and *W. levieri* (17-0049061), so we focused our study on ITS and *trnL-F*, in line with previous work on the genus.



Figure 8. Plate from Wilson (1855) showing *Weissia wilsonii*, based on material collected by him from Appleton, Cheshire, UK. NB: shoots bottom-left and bottom-right, each marked by an asterisk, are *W. mittenii*.

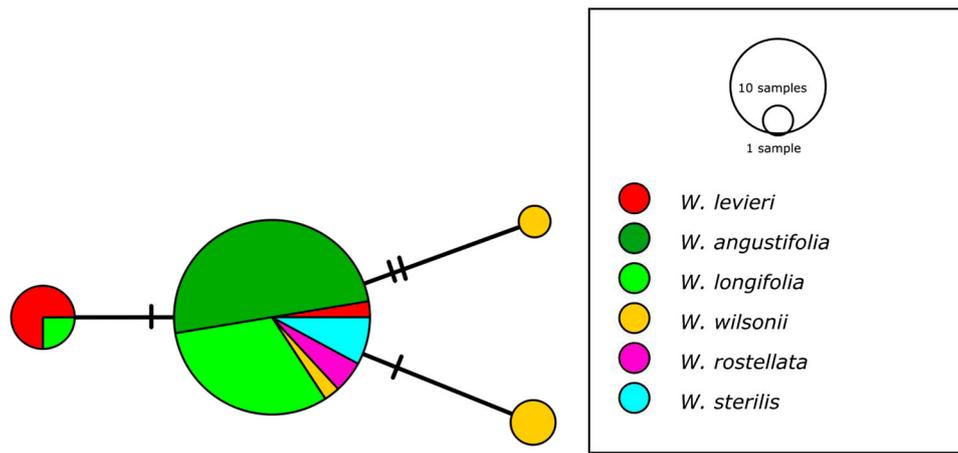


Figure 9. Haplotype network based on the *trnL-F* matrix constructed using statistical parsimony (TCS) in the program PopART 1.7.

Within *Weissia*, Inoue and Tsubota (2017) generated DNA sequence data for some cleistocarpic Japanese species, with two DNA lineages that they recognise as species, *W. kiiensis* (S.Okamura) Y.Inoue & H.Tsubota and *W. parajaponica* Y.Inoue & H.Tsubota. In common with Werner et al. (2005), they show the common, widespread species *W. controversa* (subgen. *Weissia*) to be highly polyphyletic. In fact, in Werner et al.'s study, none of the four species of *Weissia* that were sampled more than once resolved as monophyletic, suggesting that there are serious problems with morphological circumscription within the genus. Both molecular studies also show that, in common with other moss taxa (e.g. Funariaceae, Liu et al. 2012), the cleistocarpous capsule morphotype has probably evolved multiple times, making the current subgeneric classification of *Weissia* highly doubtful, despite its value in facilitating morphological identifications within the genus.

Amplification of at least some of the ITS region was successful for 64 specimens while the *trnL-F* region amplification was successful for 45 specimens. The matrices for the independent analyses of the ITS and *trnL-F* data comprised these sequences together with the outgroup. The combined matrix, including all

terminals with ITS data and with *trnL-F* data added where available, comprised 65 terminals including the outgroup, with 41 of these having both ITS and *trnL-F* data and 24 ITS data alone. The concatenated ITS matrix consisted of 709 characters, of which 40 were variable and the *trnL-F* matrix 366 characters, of which only 12 were variable. The combined matrix thus consisted of 1075 characters, of which 52 were variable.

Phylogeny and haplotype network analysis

Of the 12 variable characters in the aligned *trnL-F* matrix, only six represented substitutions and only four of these were characters for which no terminals had missing data or multiple ambiguous base calls, thus allowing inclusion in the haplotype network analysis. The results of this are shown in Figure 9. Two substitutions distinguish a single sample of *W. wilsonii*, another distinguishes two further samples of *W. wilsonii* and the fourth separates three of the four sampled specimens of *W. levieri* together with one sample of *W. longifolia*. The results of the ML analysis including indels and missing data (not shown) provide resolution supported by bootstrap values >50% for only two nodes, one representing all ingroup taxa other

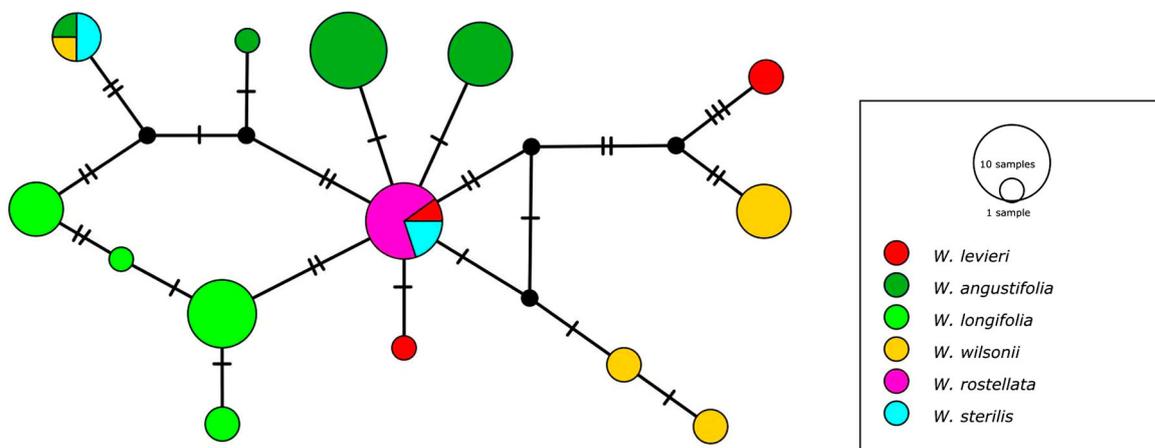


Figure 10. Haplotype network based on the combined ITS1 and ITS2 matrix constructed using statistical parsimony (TCS) in the program PopART 1.7.

than the two samples of *W. wilsonii* that are distinguished by a single substitution (60% bootstrap), and the other corresponding to the three samples of *W. levieri* and the single specimen of *W. longifolia* separated in the haplotype network (62% bootstrap).

Twenty-three of the 40 variable characters in the combined ITS1 and 2 matrix represented substitutions in characters for which no terminals had missing data or multiple ambiguous base calls and were able to be included in the haplotype network analysis, the results of which are shown in Figure 10. Fourteen haplotypes are distinguished in the network, three corresponding to samples of *W. wilsonii*, four to samples of *W. longifolia*, three to samples of *W. angustifolia* and two to samples of *W. levieri*. One taxonomically mixed haplotype corresponds to all seven sampled specimens of *W. rostellata*, two of *W. sterilis* and one of *W. levieri*, while another groups together single specimens of *W. wilsonii* and *W. angustifolia* with two specimens of *W. sterilis*. Maximum likelihood analysis of the matrix

including indels and missing data produced a topology (not shown, but nearly identical to that resulting from analysis of the total combined matrix as discussed below and illustrated in Figure 11) in which 10 nodes are resolved with bootstrap values >50%. These are two clades of seven and 10 terminals of *W. angustifolia*, supported by bootstrap values of 64% and 68% respectively, one clade containing four specimens of *W. wilsonii* (72%), another containing a clade of seven specimens of *W. wilsonii* (50%) and two specimens of *W. levieri* (98%) together supported by a 57% bootstrap value, a clade of five specimens of *W. longifolia* (78%), and finally a clade with three specimens of *W. angustifolia*, two of *W. sterilis* and one of *W. wilsonii* (56%), within which a terminal clade supported by a 94% bootstrap value is distinct from two of the *W. angustifolia* specimens, one of these being sister to the terminal clade (78% bootstrap). Although there is no bootstrap support for the node, all 17 specimens of *W. longifolia* form a clade.



Figure 11. Maximally likely topology from the RAXML analysis of the concatenated *trnL-F*, ITS1 and ITS2 matrix. Numbers on branches are bootstrap support values.

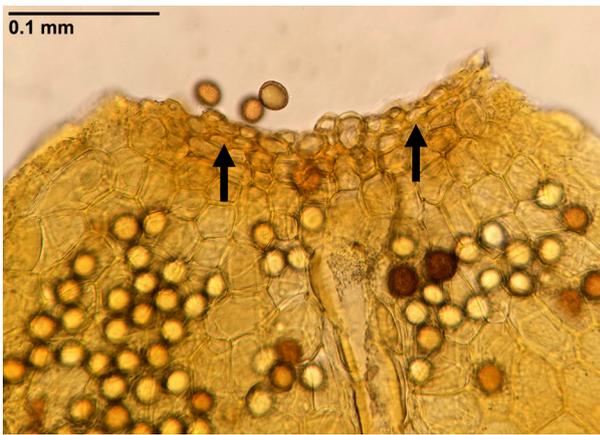


Figure 12. Dissected urn of *W. angustifolia* (Callaghan 80535), with lid detached, showing abscission zone of small, strongly differentiated cells at mouth. Photo: D. A. Callaghan.

As the conflict between the *trnL-F* and combined ITS1 and 2 matrices is based on a very small number of informative characters in the *trnL-F* alignment and has low bootstrap support (60–62%), it was considered appropriate to perform an analysis on the total combined matrix. The results of this are shown in [Figure 11](#). The topology is effectively identical to that described above for the combined ITS1 and 2 analysis. Bootstrap support values are broadly similar for most nodes, although notably they are improved for the clades in which all but one of the *W. wilsonii* specimens occur. The clade containing four specimens of *W. wilsonii* is supported at 83%, the combined clade of *W. wilsonii* and two specimens of *W. levieri* at 67%, and the clades containing seven specimens of *W. wilsonii* and two of *W. levieri* are supported at 77% and 98% respectively. This suggests that the signal in the ITS and *trnL-F* datasets distinguishing the samples of *W. wilsonii* is broadly congruent (compare also the haplotype networks in [Figures 9](#) and [10](#)).

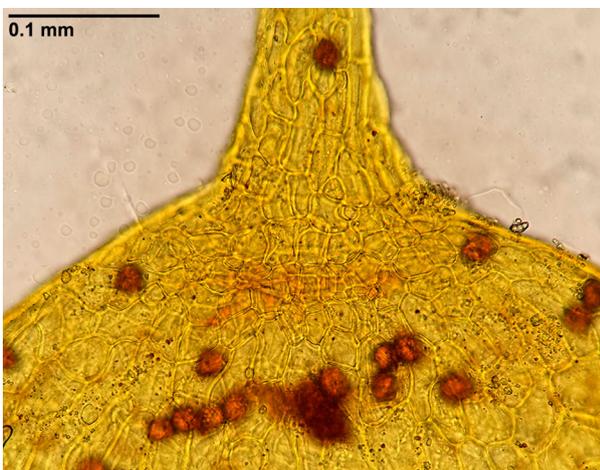


Figure 13. Dissected urn of *W. sterilis* (NMW C90.31.53), showing no abscission zone of small, strongly differentiated cells at mouth. Photo: D. A. Callaghan.

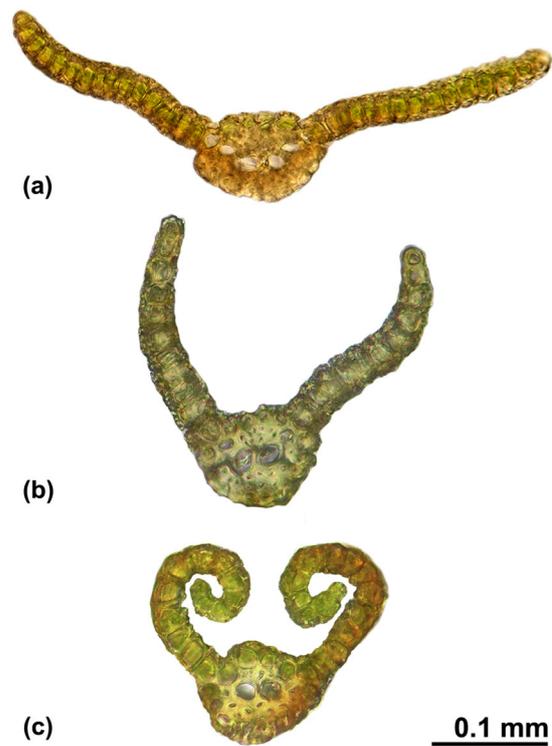


Figure 14. Section from distal half of perichaetial leaf of (A) *W. levieri* (Callaghan 21722), (B) *W. wilsonii* (Callaghan 22600) and (C) *W. angustifolia* (Callaghan 80535). Note, *W. levieri* can exhibit loosely incurved margins, as shown here by *W. wilsonii*. Photo: D. A. Callaghan.

Clearly all six of the UK species of *Weissia* subgenus *Astomum* sampled in this study are closely related, and the markers used here are insufficiently variable to definitively circumscribe them. This is entirely consistent with the difficulty in separating the species morphologically and suggests that species entities, where they exist, are of rather recent origin, that some gene flow is still occurring between them and certainly that lineage sorting is incomplete. Nonetheless most of the haplotypes recovered represent single morphological species, different haplotypes of the same species are generally similar to each other, and phylogenetic analysis tends to recover clades corresponding to species or to subsets of samples from species. In particular, there is fairly convincing evidence for separation of nearly all plants identified as *W. wilsonii* from the morphologically similar *W. longifolia* and *W. angustifolia*, even if two genetically distinct forms seem to be present. However, the anomalous positions of a small number of specimens, such as *W. wilsonii* 17-0049121, and the existence of taxonomically mixed haplotypes strongly hints at ongoing reticulate evolutionary processes, a full understanding of which would require a much greater volume of informative data. The group would provide an excellent subject for a population genetics study or a phylogenetic analysis based on high-throughput sequencing methods, but neither of these approaches were within the scope of the present work.



Figure 15. Shoots of (A) *Weissia longifolia* (BM001146982), (B) *W. wilsonii* (BM000725415) and (C) *W. sterilis* (BM001146975), showing relatively short stem leaves of (A) and (B), and relatively long stem leaves of (C). Note, (A) is a mature but short plant and possesses few stem leaves, mostly very small and scale-like (in area arrowed). Mature plants of *W. wilsonii* can be as short as (A) but such plants of *W. sterilis* have not been detected. *W. longifolia* can be as tall as (B) and (C). Photo: D. A. Callaghan.

Key to *Weissia* subgenus *Astomum* in Europe

Note, the putative hybrid *W. mittenii* and other unnamed hybrids, are excluded from consideration. Also excluded are unreliable characters utilised in previously published keys, such as spore papillosity, the degree to which dry perichaetial leaves become curled, colour of exothelial cells of capsule, amount of branching (all species exhibit varying amounts of sympodial branching), spore colour, plant size and number of sporophytes per perichaetium.

- 1 Seta >1 mm long (longer than capsule)
 *W. rostellata*
- 1 Seta <1 mm long (as long as or shorter than capsule) 2
- 2 Ring of small, strongly differentiated cells at mouth of capsule, lid easily detached from mature sporophytes (Figure 12) 3
- 2 No ring of small, strongly differentiated cells at mouth of capsule, lid not easily detached from mature sporophytes (Figure 13) 4
- 3 Margins along distal half of perichaetial leaves tightly involute (Figure 14C), lids rarely becoming detached from mature sporophytes in nature
 *W. angustifolia*
- 3 Margins along distal half of perichaetial leaves plane to loosely incurved (Figure 14A), lids commonly becoming detached from mature sporophytes in nature. *W. levieri*
- 4 On shoots or branches with sporophytes, stem (non-perichaetial) leaves mostly ≥ 1.6 mm and frequently >1.9 mm long (Figure 15C) *W. sterilis*
- 4 On shoots or branches with sporophytes, stem (non-perichaetial) leaves <1.6 mm long (Figure 15A,B) 5

- 5 Operculum relatively small, 0.11–0.24(–0.26) times as long as urn (Figure 5A). *W. longifolia*
- 5 Operculum relatively large, (0.21–)0.23–0.50(–0.59) times as long as urn (Figure 5B) *W. wilsonii*

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Appendix. Specimens used in molecular study

Taxon	Specimen code	Genbank	Site	Country	Habitat	Date	Leg.	Herbarium
<i>W. levieri</i>	16-0045795	ITS1 MH520771 ITS2 MH520809 <i>trnL</i> MH545632	Middle Hope, North Somerset	UK	Thin, stoney, S-facing limestone grassland	9 March 2012	D.A. Callaghan	Priv. Herb. D.A. Callaghan 21722
<i>W. levieri</i>	17-0049116	ITS MH545644	Lookout Station, Glamorgan	UK	Insolated soil over limestone on coastal cliff	12 July 2017	D.A. Callaghan	Priv. Herb. D.A. Callaghan 80528
<i>W. levieri</i>	17-0049128	ITS MH545664 <i>trnL</i> MH545630	Purn Hill, North Somerset	UK		1 January 2017	P. Bowyer	Priv. Herb. D.A. Callaghan 81464
<i>W. levieri</i>	17-0049135	ITS MH545646 <i>trnL</i> MH545622	Selçuk, İzmir	Turkey	Soil amongst bushes on rocky slope	15 April 1972	E. Nyholm	E <i>EN1519</i>
<i>W. longifolia</i> var. <i>angustifolia</i>	16-0045796	ITS1 MH520773 ITS2 MH520811 <i>trnL</i> MH545595	Southgate Cliffs, Glamorgan	UK	S-facing limestone grassland on sea cliff	19 February 2014	S. Pilkington	Priv. Herb. D.A. Callaghan 52026
<i>W. longifolia</i> var. <i>angustifolia</i>	16-0045797	ITS1 MH520774 ITS2 MH520812 <i>trnL</i> MH545585	West Cliff, Dorset	UK	Compacted soil beside rabbit burrow in limestone grassland	29 March 2012	D.A. Callaghan	Priv. Herb. D.A. Callaghan 16733
<i>W. longifolia</i> var. <i>angustifolia</i>	16-0045798	ITS1 MH520775 ITS2 MH520813	Bratton Camp, South Wiltshire	UK	Thin chalk grassland	21 February 2014	S. Pilkington	Priv. Herb. D.A. Callaghan 52146
<i>W. longifolia</i> var. <i>angustifolia</i>	16-0045799	ITS1 MH520776 ITS2 MH520814 <i>trnL</i> MH545579	Brean Down, North Somerset	UK	Red clay soil of steep S-facing limestone grassland	8 March 2012	D.A. Callaghan	Priv. Herb. D.A. Callaghan 21637
<i>W. longifolia</i> var. <i>angustifolia</i>	16-0045802	ITS1 MH520806 ITS2 MH520844	Leckhampton Quarry, Gloucestershire	UK	Insolated limestone grassland bank of old quarry	30 April 2015	D.A. Callaghan	Priv. Herb. D.A. Callaghan 66363
<i>W. longifolia</i> var. <i>angustifolia</i>	17-0049055	ITS MH545659 <i>trnL</i> MH545606	Tears Point, Glamorgan	UK	Thin soil over limestone on coastal cliff	12 July 2017	D.A. Callaghan	Priv. Herb. D.A. Callaghan 80530
<i>W. longifolia</i> var. <i>angustifolia</i>	17-0049056	ITS MH545649 <i>trnL</i> MH545578	Scottsquar Hill, Gloucestershire	UK	Insolated soil patch on steep limestone grassland	12 September 2017	D.A. Callaghan	Priv. Herb. D.A. Callaghan 80533
<i>W. longifolia</i> var. <i>angustifolia</i>	17-0049057	ITS MH545650 <i>trnL</i> MH545580	Painswick Beacon, Gloucestershire	UK	Insolated soil patch on steep limestone grassland	12 September 2017	D.A. Callaghan	Priv. Herb. D.A. Callaghan 80535
<i>W. longifolia</i> var. <i>angustifolia</i>	17-0049058	ITS MH545651 <i>trnL</i> MH545583	Haresfield Beacon, Gloucestershire	UK	Insolated soil patch on steep limestone grassland	17 September 2017	D.A. Callaghan	Priv. Herb. D.A. Callaghan 80640
<i>W. longifolia</i> var. <i>angustifolia</i>	17-0049060	ITS MH545652 <i>trnL</i> MH545599	Selsley Common, Gloucestershire	UK	Insolated soil patch on steep limestone grassland	12 September 2017	D.A. Callaghan	Priv. Herb. D.A. Callaghan 80534
<i>W. longifolia</i> var. <i>angustifolia</i>	17-0049107	ITS MH545653 <i>trnL</i> MH545582	Uphill, North Somerset	UK	Soil over coastal limestone	1 January 2017	P. Bowyer	Priv. Herb. D.A. Callaghan 81463
<i>W. longifolia</i> var. <i>angustifolia</i>	17-0049108	ITS MH545660 <i>trnL</i> MH545598	Longhole Cliff, Glamorgan	UK	Insolated soil over limestone on coastal cliff	12 July 2017	D.A. Callaghan	Priv. Herb. D.A. Callaghan 80531
<i>W. longifolia</i> var. <i>angustifolia</i>	17-0049112	ITS MH545654 <i>trnL</i> MH545605	Avon Gorge, Gloucestershire	UK	Insolated soil patch over steep limestone slope	8 September 2017	D.A. Callaghan	Priv. Herb. D.A. Callaghan 80532
<i>W. longifolia</i> var. <i>angustifolia</i>	17-0049113	ITS MH545655 <i>trnL</i> MH545600	St Catherine's Point, Isle of Wight	UK	Thin soil over rock on grassy coastal slope	16 March 2002	D.G. Long	E <i>DL31015</i>
<i>W. longifolia</i> var. <i>angustifolia</i>	17-0049117	ITS MH545656 <i>trnL</i> MH545596	Shide Chalk Pit, Isle of Wight	UK	Short turf of old chalk pit	18 March 2002	D.G. Long	E <i>DL31039</i>
<i>W. longifolia</i> var. <i>angustifolia</i>	17-0049126	ITS MH545648 <i>trnL</i> MH545621	Coteau de Mesnil Soleol, Lower Normandy	France	On gravel amongst old aerodrome buildings	19 March 2013	D.G. Long	E <i>DL42350</i>
<i>W. longifolia</i> var. <i>angustifolia</i>	17-0049129	ITS MH545657 <i>trnL</i> MH545620	Salisbury Plain, Wiltshire	UK	Side of bomb hole in chalk grassland	16 January 2009	M.O. Hill	Priv. Herb. M.O. Hill
<i>W. longifolia</i> var. <i>angustifolia</i>	17-0049130	ITS MH545647 <i>trnL</i> MH545615	Kaiserstuhl, Baden- Württemberg	Germany	Bare soil on open grassy slope in disused vulcanite quarry	12 April 2015	D.G. Long	E <i>DL43618</i>
<i>W. longifolia</i> var. <i>angustifolia</i>	17-0049131	ITS MH545661 <i>trnL</i> MH545597	Lookout Station, Glamorgan	UK	Insolated soil over limestone on coastal cliff	12 July 2017	D.A. Callaghan	Priv. Herb. D.A. Callaghan 80529
<i>W. longifolia</i> var. <i>angustifolia</i>	17-0049151	ITS MH545658 <i>trnL</i> MH545584	Brightstone Down, Isle of Wight	UK	Short turf of old chalk pit	15 March 2002	D.G. Long	E <i>DL30990</i>

(Continued)

Appendix. Continued.

Taxon	Specimen code	Genbank	Site	Country	Habitat	Date	Leg.	Herbarium
<i>W. longifolia</i> var. <i>longifolia</i>	16-0045770	ITS1 MH520777 ITS2 MH520815 trnL MH545625	Home Willow Farm, nr., Bedfordshire	UK	Side of ditch beside road to farm	2 January 1984	M. Yeo	NMW C.2001.019.437
<i>W. longifolia</i> var. <i>longifolia</i>	16-0045772	ITS1 MH520779 ITS2 MH520817	Middle Winterslow, South Wiltshire	UK	Acid fallow field	26 March 1992	H.W. Matcham	NMW C.2001.019.452
<i>W. longifolia</i> var. <i>longifolia</i>	16-0045773	ITS1 MH520780 ITS2 MH520818	Lower Smite Farm, Worcestershire	UK	Arable field	1 March 2003	M. Lawley	NMW C.2004.007.235
<i>W. longifolia</i> var. <i>longifolia</i>	16-0045774	ITS1 MH520781 ITS2 MH520819 trnL MH545624	Dickleburgh Moor, East Norfolk	UK	2–3 year old set aside field, soil loam	14 April 2003	S.V. O'Leary	NMW C.2004.007.300
<i>W. longifolia</i> var. <i>longifolia</i>	16-0045775	ITS1 MH520782 ITS2 MH520820	Harmeston, Pembrokeshire	UK	Cereal stubble field	4 January 2003	S.D.S. Bosanquet	NMW C.2004.007.334
<i>W. longifolia</i> var. <i>longifolia</i>	16-0045776	ITS1 MH520783 ITS2 MH520821 trnL MH545612	College Wood, North Lincolnshire	UK	Arable field	5 December 2004	F.R. Lammiman	NMW C.2005.006.282
<i>W. longifolia</i> var. <i>longifolia</i>	16-0045777	ITS1 MH520784 ITS2 MH520822 trnL MH545623	Denton, N. of, South Lincolnshire	UK	Wet soil in set aside field near canal	24 March 2005	N.G. Hodgetts	NMW C.2006.003.234
<i>W. longifolia</i> var. <i>longifolia</i>	16-0045778	ITS1 MH520785 ITS2 MH520823 trnL MH545608	Overley Wood, Gloucestershire	UK	Disturbed ground at base of recently planted tree	30 December 2006	R.V. Lansdown	NMW C.2008.002.109
<i>W. longifolia</i> var. <i>longifolia</i>	16-0045779	ITS1 MH520786 ITS2 MH520824	Brookwood Cemetery, Surrey	UK	Ditch bank	19 April 2008	J.G. Duckett	NMW C.2009.001.308
<i>W. longifolia</i> var. <i>longifolia</i>	16-0045780	ITS1 MH520787 ITS2 MH520825 trnL MH545609	Highgate Cemetery, Middlesex	UK	Clay bank exposed ca. 4 years previously	5 February 2008	J.G. Duckett	NMW C.2009.001.350
<i>W. longifolia</i> var. <i>longifolia</i>	16-0045781	ITS1 MH520788 ITS2 MH520826 trnL MH545611	Bryn Gomer, Pembrokeshire	UK	Among planted shrubs beside road	3 February 2007	S.D.S. Bosanquet	NMW C.2010.034.031
<i>W. longifolia</i> var. <i>longifolia</i>	16-0045782	ITS1 MH520789 ITS2 MH520827 trnL MH545627	Bosherston, Pembrokeshire	UK	Anthill	3 February 2005	S.D.S. Bosanquet	NMW C.2010.034.187
<i>W. longifolia</i> var. <i>longifolia</i>	16-0045812	ITS1 MH520790 ITS2 MH520828	Tibby Fowler's Glen, Berwickshire	UK	Anthill on calcareous grassland slope	1 March 2000	D.G. Long	E
<i>W. longifolia</i> var. <i>longifolia</i>	17-0049103	ITS MH545666 trnL MH545610	Pentyrch, Glamorgan	UK	Bare soil in garden	10 November 2016	B. Stewart	Priv. Herb. D.A. Callaghan 81465
<i>W. longifolia</i> var. <i>longifolia</i>	17-0049106	ITS MH545663 trnL MH545619	Hinchingbrooke Country Park, Huntingdonshire	UK	Bare ground beside woodland track	7 December 2011	M.O. Hill	Priv. Herb. M.O. Hill
<i>W. longifolia</i> var. <i>longifolia</i>	17-0049115	ITS MH545662 trnL MH545618	Dinkelberg, Schopfheim	Germany	Soil patch on hilly calcareous pasture	1 March 2017	M. Lüth	Priv. Herb. D.A. Callaghan 81466
<i>W. longifolia</i> var. <i>longifolia</i>	17-0049150	ITS MH545665 trnL MH545614	Whitadder Water, Berwickshire	UK	Anthill on old grassland slope	15 November 1992	D.G. Long	E DL23134
<i>W. rostellata</i>	16-0045792	ITS1 MH520799 ITS2 MH520837 trnL MH545604	Bignor Park, West Sussex	UK	Calcareous clay bank of ditch across meadow	2 September 2015	T.W. Ottley	Priv. Herb. D.A. Callaghan 71080
<i>W. rostellata</i>	16-0045800	ITS1 MH520800 ITS2 MH520838 trnL MH545587	Sutton Reservoir, Cheshire	UK	Exposed mud of reservoir margin	11 October 2014	D.A. Callaghan	Priv. Herb. D.A. Callaghan 61369
<i>W. rostellata</i>	16-0045801	ITS1 MH520801 ITS2 MH520839	Roadford Reservoir, North Devon	UK	Exposed mud of reservoir margin	22 August 2011	D.A. Callaghan	Priv. Herb. D.A. Callaghan 18512
<i>W. rostellata</i>	16-0045805	ITS1 MH520802 ITS2 MH520840	Lower Quilley, Londonderry	UK	Damp clay-loam soil in stubble field	19 October 2008	D.T. Holyoak	NMW
<i>W. rostellata</i>	16-0045806	ITS1 MH520803 ITS2 MH520841	Wollaston, Shropshire	UK	Arable field	1 February 2006	M. Lawley	NMW

(Continued)

Appendix. Continued.

Taxon	Specimen code	Genbank	Site	Country	Habitat	Date	Leg.	Herbarium
<i>W. rostellata</i>	16-0045807	ITS1 MH520804 ITS2 MH520842	Red Hill, Pembrokeshire	UK	Wheat stubble field on clay loam	22 January 2005	S.D.S. Bosanquet	NMW
<i>W. rostellata</i>	16-0045808	ITS1 MH520805 ITS2 MH520843	Llys-y-fran Reservoir, Pembrokeshire	UK	Inundation zone of reservoir	25 November 2006	S.D.S. Bosanquet	NMW
<i>W. sterilis</i>	16-0045803	ITS1 MH520807 ITS2 MH520845 <i>trnL</i> MH545617	Malling Chalk Pits, East Sussex	UK	S-facing bank in chalk pit	6 December 2015	T.W. Ottley	Priv. Herb. D.A. Callaghan 81467
<i>W. sterilis</i>	16-0045814	ITS1 MH520808 ITS2 MH520846 <i>trnL</i> MH545616	Fort Widley, nr., South Hampshire	UK	N-facing shallow ridge between paths in chalk grassland	13 December 2014	J.A. Norton	Priv. Herb. D.A. Callaghan 81468
<i>W. sterilis</i>	17-0049059	ITS MH545673 <i>trnL</i> MH545626	Selsley Common, Gloucestershire	UK	Insolated soil patch on steep limestone grassland	12 September 2017	D.A. Callaghan	Priv. Herb. D.A. Callaghan 80536
<i>W. sterilis</i>	17-0049144	ITS MH545675	Royston Heath, Hertfordshire	UK	Broken chalk turf	2 March 1968	H.J.B. Birks	E E00427373
<i>W. multcapsularis</i>	16-0045845	ITS1 MH520772 ITS2 MH520810	Petit Tor Point, South Devon	UK	Bare red clay soil at top of sea cliff above limestone quarry	27 February 1999	D.T. Holyoak	NMW C.2005.006.12
<i>W. multcapsularis</i>	16-0045771	ITS1 MH520778 ITS2 MH520816 <i>trnL</i> MH545607	Southwell, E. of, Dorset	UK	On partly bare clayey soil among scattered grasses and herbs on unshaded flat ground near quarries	17 March 2000	D.T. Holyoak	NMW C.2001.019.451
<i>W. multcapsularis</i>	16-0045783	ITS1 MH520791 ITS2 MH520829	Dingestow, Monmouthshire	UK	Damp sandy clay bank of lane-side	1 December 1980	G.W. Garlick	NMW C.2001.019.343
<i>W. multcapsularis</i>	16-0045784	ITS1 MH520792 ITS2 MH520830	Downderry, nr., East Cornwall	UK	Edge of newly seeded field	March 1962	J.A. Paton	NMW C.2001.019.348
<i>W. multcapsularis</i>	16-0045785	ITS1 MH520793 ITS2 MH520831	Cadgewith, West Cornwall	UK	Soil on rocks in old quarry on cliffs	15 March 1962	J.A. Paton	NMW C.2001.019.611
<i>W. multcapsularis</i>	16-0045787	ITS1 MH520794 ITS2 MH520832	Gwendreath, West Cornwall	UK	Wet clay soil at edge of steep track leading onto horse pasture	4 February 2014	D.A. Callaghan	Priv. Herb. D.A. Callaghan 51801
<i>W. multcapsularis</i>	16-0045788	ITS1 MH520795 ITS2 MH520833 <i>trnL</i> MH545581	Pentire Point East, West Cornwall	UK	Soil at edge of turf over rock in coastal, rabbit-grazed grassland	13 February 2014	D.A. Callaghan	Priv. Herb. D.A. Callaghan 51916
<i>W. multcapsularis</i>	16-0045789	ITS1 MH520796 ITS2 MH520834	Talland Barton Farm, East Cornwall	UK	Loam soil on grassland bank through coastal grassland	23 January 2013	D.A. Callaghan	Priv. Herb. D.A. Callaghan 29900
<i>W. multcapsularis</i>	16-0045790	ITS1 MH520797 ITS2 MH520835 <i>trnL</i> MH545576	Greeb Point, East Cornwall	UK	Bare soil on earth bank	29 April 2015	D.A. Callaghan	Priv. Herb. D.A. Callaghan 66365
<i>W. multcapsularis</i>	16-0045791	ITS1 MH520798 ITS2 MH520836	Bryn Gomer, Pembrokeshire	UK	Among planted shrubs beside road	3 February 2007	S.D.S. Bosanquet	NMW C.2010.034.031
<i>W. multcapsularis</i>	17-0049062	ITS MH545667 <i>trnL</i> MH545575	Greeb Point, East Cornwall	UK	Soil in margin of coastal arable field	11 March 2017	D.A. Callaghan	Priv. Herb. D.A. Callaghan 80001
<i>W. multcapsularis</i>	17-0049121	ITS MH545668 <i>trnL</i> MH545613	Hayley Wood, Cambridgeshire	UK	Disturbed ground inside deer fence near woodland edge	1 February 2003	M.O. Hill	Priv. Herb. M.O. Hill