

## Hybridisation and introgression in British *Helosciadium* (Apiaceae)

Stuart D. Desjardins<sup>1\*</sup>; Andrew G. Shaw<sup>2</sup>; Judith A. Webb<sup>3</sup>

<sup>1</sup>University of Leicester, UK; <sup>2</sup>Rare British Plants Nursery, Builth Wells, UK;

<sup>3</sup>Kidlington, Oxfordshire, UK

\*Corresponding author: Stuart D. Desjardins: [stuart.desjardins@gmail.com](mailto:stuart.desjardins@gmail.com)

This pdf constitutes the Version of Record published on 26<sup>th</sup> February 2020

### Abstract

Reticulation between *Helosciadium repens* (Jacq.) W.D.J. Koch and *H. nodiflorum* (L.) W.D.J. Koch (Apiaceae) has been the source of much speculation, but until now supporting evidence has remained largely anecdotal. In the current study inter-specific hybridisation and introgression between the two species was confirmed using DNA barcoding. The parentage of three putative hybrids collected from Port Meadow, Oxfordshire (UK) was determined using a maternally-inherited chloroplast marker (*rps16-trnK*) and two biparentally-inherited nuclear markers (*LEAFY2*, *ITS*). Two of the individuals are early-generation hybrids between *H. repens* and *H. nodiflorum*, F1 or otherwise, while the third is most likely a backcross to *H. repens*. These individuals are the first confirmed hybrids/hybrid derivatives between the two parental species, and represent a new addition to the British flora. The hybrids closely resemble *H. nodiflorum* var. *longipedunculatum* F.W. Schultz, and in our view should be treated as *H. × longipedunculatum* (F.W. Schultz) Desjardins.

**Keywords:** *Apium*; DNA barcoding; molecular systematics; reticulate evolution.

### INTRODUCTION

*Helosciadium* W.D.J. Koch (Apiaceae) is a small genus of helophytic, perennial umbellifers that originated in Europe around 6 mya (Spalik & Downie, 2006). Five species are extant worldwide, and they form a strongly-supported monophyletic clade (Hardway *et al.*, 2004; Spalik *et al.*, 2009). The species are: *H. bermejoi* (L. Llorens) Popper & M.F. Watson, *H. crassipes* W.D.J. Koch ex Rchb., *H. inundatum* (L.) W.D.J. Koch, *H. nodiflorum* (L.) W.D.J. Koch and *H. repens* (Jacq.) W.D.J. Koch (Ronse *et al.*, 2010). The taxa were previously placed within *Apium* L. *sensu lato* in tribe Apieae (Wolff, 1927; Tutin, 1968; Pimenov & Leonov, 1993), but recent molecular phylogenetic studies have them as a separate genus in tribe Oenantheae alongside other genera typical of aquatic/paludal habitats (e.g. *Berula* Besser ex W.D.J. Koch, *Cicuta* L., *Oenanthe* L. and *Sium* L.; Hardway *et al.*, 2004; Spalik *et al.*, 2009).

*Helosciadium* is remarkable within the Apiaceae in that it displays an unusual propensity for spontaneous hybridisation (Stace *et al.*, 2015). To date three hybrid taxa have been formally described: (i) *Helosciadium × moorei* (Syme) Warren – a hybrid between *H. nodiflorum* and *H. inundatum* (Desjardins, 2016; O'Mahony, 2016); (ii) *Helosciadium × clandestinum* Rita, Capó & Cursach – a hybrid between *H.*

*nodiflorum* and Menorcan endemic *H. bermejoi* (Rita *et al.*, 2016; 2018); and (iii) × *Beruladium procurrens* A.C. Leslie – an intergeneric hybrid between *H. nodiflorum* and *Berula erecta* (Huds.) Coville (Desjardins *et al.*, 2015; Leslie and Desjardins, 2018). The current study focuses on a potential new hybrid combination involving the closely-related species *H. repens* and *H. nodiflorum*.

*Helosciadium repens* is a small plant that grows in open, wet places; it has a creeping habit and roots freely at every node (Stace, 2019). In horticultural conditions it behaves as a long-lived clonal perennial, but in the wild it usually behaves as an annual, or at best, a short-lived perennial that is killed off annually by prolonged winter flooding, summer droughts and/or heavy grazing by stock. These extreme seasonal conditions being an essential requirement for the maintenance of the open bare ground that is required by *H. repens*. The taxon is predominantly distributed in Western Europe, but is also found in parts of Central and Southern Europe, and there are isolated populations in North Africa (McDonald & Lambrick, 2006). It is in decline all over its European range (Lansdown, 2011) and the remaining wild populations are reported to have a narrow genetic base (Herden *et al.*, 2019). The taxon was formerly scattered around the British Isles, being found in parts of Eastern England and the Scottish Lowlands (Stace, 2019), but it is now restricted to just two sites, one at Port Meadow, Oxfordshire (v.c.23) and another at Walthamstow Marshes, Essex (v.c.18). An introduced population is also present at North Hinksey Meadow, Oxfordshire (v.c.23; JNCC, 2013; Stroh *et al.*, 2016).

*Helosciadium nodiflorum* is much more common than *H. repens*. It is widely distributed in Europe, often being found wherever there is suitable habitat (Tutin, 1980). It is also much more variable, displaying high levels of phenotypic plasticity and presenting in a wide variety of forms, depending on the environmental conditions. The typical upright form, var. *vulgare* F.W. Schultz, is easy to distinguish, but smaller creeping forms, such as var. *pseudo-repens* H.C. Watson and *f. simulans* Ridd., can resemble *H. repens* and be mistaken for it in the field (Riddelsdell & Baker, 1906; Riddelsdell, 1914b). This overlap between the two species has led to ongoing identification problems for field botanists, particularly in the absence of flowering material, and has led some to question whether *H. repens* is even resident to the British Isles (Tutin, 1962). Furthermore, a number of common garden experiments have suggested that British plants appearing to be *H. repens* in the wild, tend to revert to a more typical *H. nodiflorum* state in cultivation (Druce, 1927; Grassly *et al.*, 1996). However, a genetic study using random amplified polymorphic DNA (RAPD) markers was able to confirm the presence of pure *H. repens* at Port Meadow. The genetic profiles of British *H. repens* clustered with reference populations from central Europe and, when cultivated, retained the distinctiveness of their field morphology (Grassly *et al.*, 1996).

*Helosciadium repens* and *H. nodiflorum* therefore appear to be closely-related, but distinct species and can be separated using a combination of characters (*H. nodiflorum* vs. *H. repens*): (i) leaflets longer than wide vs. as long as wide; (ii) leaflets apically acute vs. asymmetrically bifid (i.e. unequally lobed); (iii) peduncles shorter than rays vs. longer than rays; (iv) bracts 0-2 vs. 3-7; and (v) fruits longer than wide vs. wider than long (Riddelsdell & Baker, 1906; Tutin, 1980; Stace, 2019; O'Mahony in Stace *et al.*, 2015).

However, discrimination of the two species is further complicated by accounts of putative interspecific hybrids. Riddelsdell (1917b) collected a number of

individuals from Binsey Common, Oxfordshire (v.c.23), which appeared to combine traits of *H. repens* and *H. nodiflorum* with intermediate leaflets, intermediate peduncles, variable bracts, umbels resembling *H. repens* and coarse reddish stems typical of *H. nodiflorum*. At the time these were treated as *H. repens* × *H. nodiflorum*, and Druce (1928) made the combination *Apium* × *riddelsdellii* Druce *nomen nudum*. However, when Tutin (1975) examined these specimens he regarded them as only variants of *H. nodiflorum*. Walters (1980) presented a collection from Chippenham Fen, Cambridgeshire (v.c.29), which resembled a small, creeping *H. nodiflorum*, but with poor pollen and without ripe fruits. It was initially thought to be a possible example of *H. repens* × *H. nodiflorum*, but was eventually revealed to be × *Beruladium procurrens* – the intergeneric hybrid between *B. erecta* and *H. nodiflorum* (Desjardins *et al.*, 2015). Crackles (1976) reported a number of putative *H. repens* × *H. nodiflorum* specimens from Hornsea Mere, Yorkshire (v.c.61); similar to *H. repens*, but with less than three bracts at the base of most umbels, and apparently sterile fruits.

Previous accounts of *H. repens* × *H. nodiflorum*, while tantalising, are based on morphological determination alone, and have not been verified by alternative methods (e.g. secondary chemistry, cytology, DNA barcoding etc.), neither have they been compared with artificially resynthesised specimens (Tutin, 1975). The current study therefore aimed to unequivocally determine the parentage of three putative hybrid specimens collected from Port Meadow, where the prospective parental species grow together. Sequence data from three gene regions were used: *rps16-trnK*, the ITS and *LEAFYi2*. *rps16-trnK*, an intergenic spacer, is a maternally-inherited chloroplast marker and was used to identify the female parent. The ITS, ribosomal DNA (rDNA), and *LEAFYi2*, an intron of a low-copy nuclear gene (LCNG), are biparentally-inherited nuclear markers and were used to identify the male and female parents.

## Materials and Methods

### *Plant Material*

Six specimens were sampled from Port Meadow (Table 1). Two *H. repens* (HR1 & HR3), one *H. nodiflorum* (HN2), and three putative hybrids (RXN1; RXN2, RXN5). The protected *H. repens* specimens were sampled non-destructively as offshoots, under Natural England License Number: 2017-32055-SCI-SCI. Where possible, voucher specimens were made and deposited in **LTR**.

The putative hybrids were identified in the field and collected as follows. In July 2001, A.G.S collected RXN1 from SP 500 078, which was displaying the leaflet and petiole characters of *H. nodiflorum* with the bract and peduncle characters of *H. repens* (O'Mahony in Stace, 2015). In August 2014 J.A.W collected RXN2 from amongst a population of *H. repens* at SP 500 075 with *H. nodiflorum* growing nearby. This specimen resembled *H. repens* in the field, but was generally bigger with larger-lobed leaflets (Fig. 1). In October 2017, J.A.W collected RXN5 from SP 499 078, observing that it was clearly different from the surrounding *H. repens*.

**Table 1. Accessions collected from Port Meadow, Oxfordshire, and used in the current study.**

<b>Code</b>	<b>Voucher specimen</b>
HN2	<i>Helosciadium nodiflorum</i> (L.) W.D.J. Koch, England, Oxfordshire, Port Meadow, SP 500 078, 29 August 2015, <i>S.D. Desjardins</i> <b>LTR.</b>
HR1	<i>Helosciadium repens</i> (Jacq.) W.D.J. Koch, England, Oxfordshire, Port Meadow, SP 500 076, 17 October 2017, <i>J.A. Webb</i> <b>LTR.</b>
HR3	<i>Helosciadium repens</i> (Jacq.) W.D.J. Koch, England, Oxfordshire, Port Meadow, SP 499 079, 17 October 2017, <i>J.A. Webb</i> (Silica-dried material only).
RXN1	<i>Helosciadium</i> × <i>longipedunculatum</i> (F.W. Schultz) Desjardins (( <i>H. nodiflorum</i> × <i>H. repens</i> ) × <i>H. repens</i> ), England, Oxfordshire, Port Meadow, SP 500 078, 28 July 2001, <i>A.G. Shaw</i> <b>LTR.</b>
RXN2	<i>Helosciadium</i> × <i>longipedunculatum</i> (F.W. Schultz) Desjardins ( <i>H. nodiflorum</i> × <i>H. repens</i> ), England, Oxfordshire, Port Meadow, SP 500 075, 30 August 2014, <i>J.A. Webb</i> <b>LTR.</b>
RXN5	<i>Helosciadium</i> × <i>longipedunculatum</i> (F.W. Schultz) Desjardins ( <i>H. nodiflorum</i> × <i>H. repens</i> ), England, Oxfordshire, Port Meadow, SP 499 078, 17 October 2017, <i>J.A. Webb</i> <b>LTR.</b>



**Figure 1. *Helosciadium* × *longipedunculatum* (RXN2) at Port Meadow, Oxfordshire, with larger-lobed leaflets than typically observed in *H. repens*.**

### *DNA extraction, amplification and sequencing*

Total genomic DNA (gDNA) was isolated from dried leaf material (20 mg) using the DNeasy Plant Mini Kit (Qiagen). *rps16-trnK* and the ITS were amplified by PCR, purified and sequenced as per Desjardins *et al.* (2015). *LEAFY2* was amplified with the primers *LFsx/1-2* (5' CAC CCA CGA CCI TTY ATI GTI ACI GAR CCI GGI GA 3') and *LFtxr* (5' CCT GCC IAC RTA RTG ICK CAT YTT IGG YTT 3'), under the cycling conditions 95 °C/02:00 + 35 × (95 °C/00:30, 55 °C/00:30, 72 °C/01:00) + 72 °C/07:00 (Frohlich & Meyerowitz, 1997). Samples that gave mixed signals were also sequenced from clones. Cloning was conducted using the pGEM<sup>®</sup>-T Easy Vector System (Promega) and α-Select Competent Cells taken from *E. coli* (Bioline). Recombinant plasmids were selected for by blue-white screening and the size of the insert determined by colony PCR with M13 primers. Plasmid DNA was isolated from cell cultures using the E.Z.N.A.<sup>®</sup> Plasmid Mini Kit (Omega Bio-tek). A minimum of five colonies were sequenced per accession. Sanger sequencing reactions were outsourced to GATC Biotech (Konstanz, Germany).

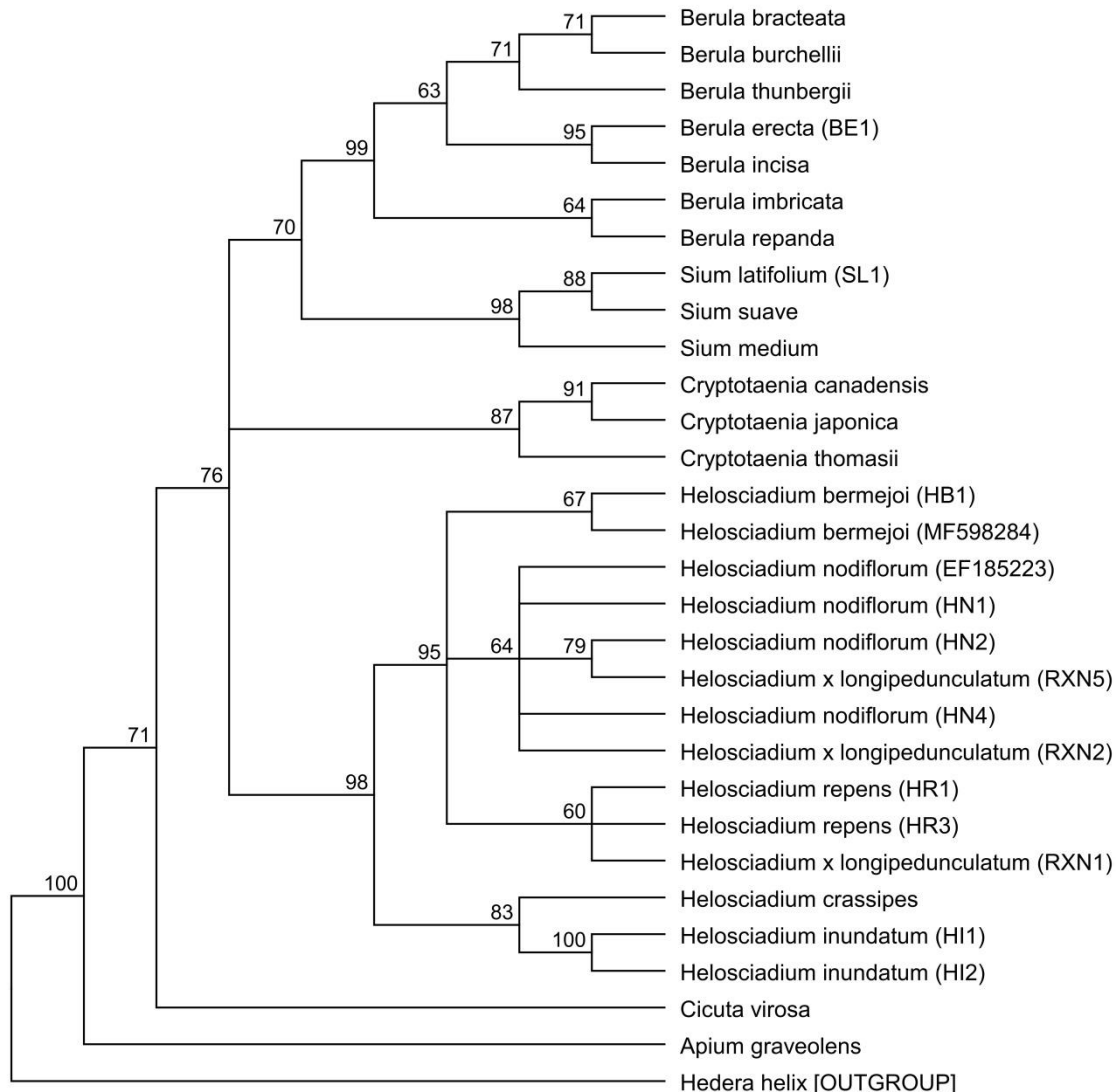
### *Phylogenetic analysis*

Generated sequence reads were viewed, trimmed and blasted with Geneious R7 (created by Biomatters; available from <http://www.geneious.com/>). Additional sequences were downloaded from the GenBank database (Supplementary information 1). Sequences were aligned using the Clustal W algorithm, and adjusted by eye. Length-mutational events (indels) were incorporated for the analysis of *LEAFY2* using a simple gap coding method (Simmons & Ochoterena, 2000). Copies acquired from the putative hybrid specimens were investigated by direct sequence comparison with reference taxa and by phylogenetic analysis. Maximum parsimony (MP) analysis was conducted on sequence data using PAUP\* 4.0 (Swofford, 2002). *Hedera helix* L. (Araliaceae) was used as the OUTGROUP for MP analysis of *rps16-trnK* and the ITS, but no sensible alignment could be made with ingroup taxa for *LEAFY2*, so *Sium latifolium* L. and *B. erecta* were used instead. Topology searches were carried out using a branch and bound algorithm with the addition method FURTHEST. Bootstrapping = 1000 replicates.

## **Results**

### *Chloroplast DNA marker*

None of the *rps16-trnK* sequences from the three putative hybrids was identical (99.02 - 99.76 % identity), indicating three distinct haplotypes. RXN1 clustered with *H. repens* in a weakly-supported clade (60% bootstrap support, BS; Fig. 2), while RXN2 and RXN5 clustered with *H. nodiflorum* in a weakly-supported clade (64% BS). Within the *H. nodiflorum*-clade RXN5 was further sister to HN2 with moderate support (79% BS). The putative hybrids therefore appear to have independent maternal lineages with RXN1 possessing a *H. repens* haplotype, and RXN2 and RXN5 possessing different *H. nodiflorum* haplotypes.

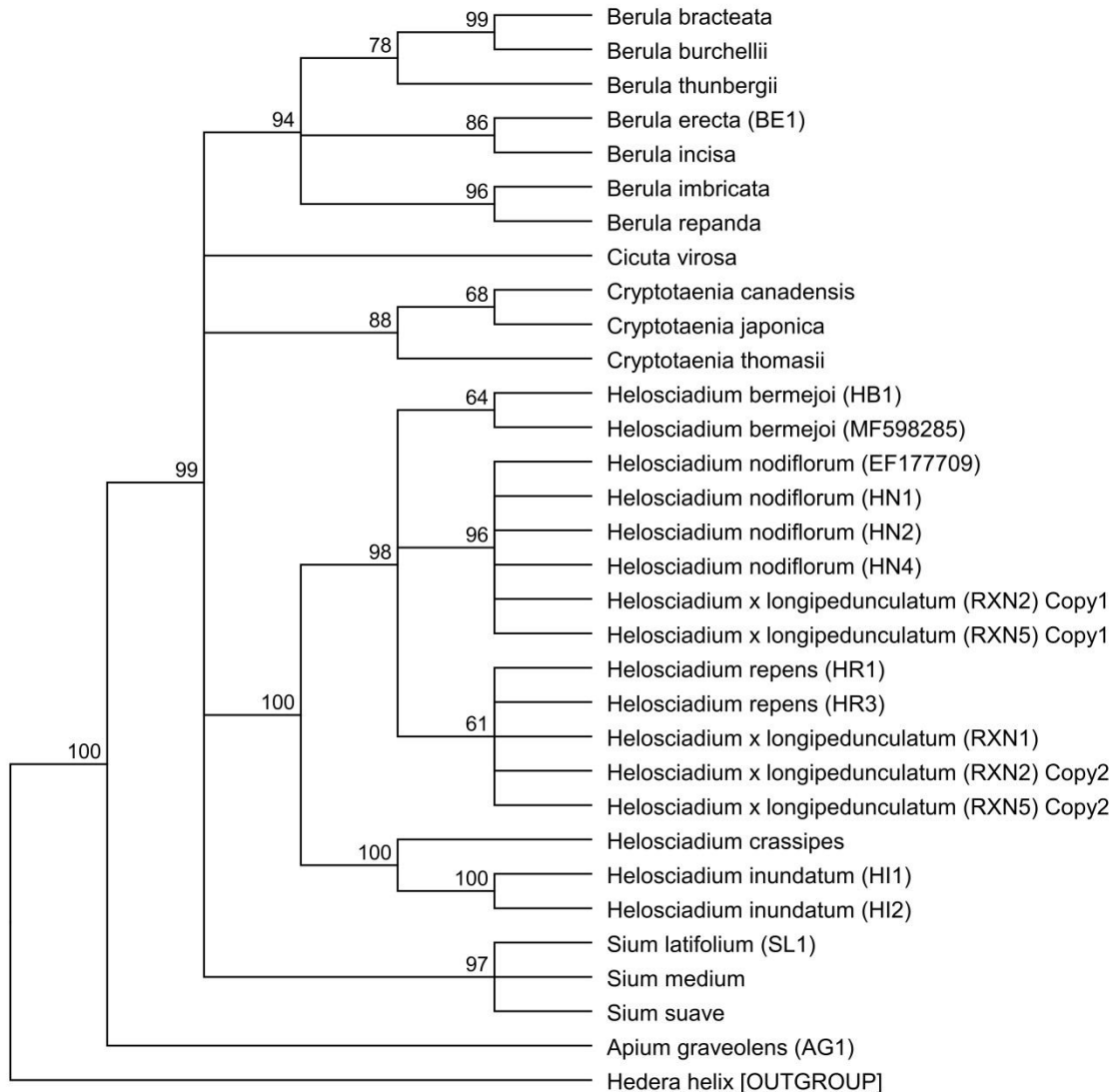


**Figure 2. A 50% majority-rule consensus tree of the 4 shortest trees (289 steps) generated by a maximum parsimony analysis of *rps16-trnK* sequence data. RXN2 and RXN5 were placed in a *H. nodiflorum* clade (64% BS), while RXN1 was placed in a *H. repens* clade (60% BS). BS values are displayed above nodes.**

#### *Internal transcribed spacer*

The ITS ribotypes of *H. repens* and *H. nodiflorum* differ at five polymorphic sites. A direct sequence comparison of the ITS ribotype of putative hybrid RXN1 matched *H. repens* at all five of these sites, and the phylogenetic analysis placed it in a weakly-supported *H. repens* clade (61% BS; Fig. 3). When sequenced directly (5' → 3') the ITS sequences of putative hybrids RXN2 and RXN5 were visibly heterozygous at all five of these polymorphic sites, and subsequent gene cloning detected the presence of two distinct copies in both individuals, which were designated copy 1 and 2. Copy 1 copies possessed the *H. nodiflorum* character state at all five polymorphic sites, and the phylogenetic analysis placed them in a strongly-supported *H. nodiflorum* clade (96% BS). Copy 2 copies possessed the *H. repens* character state at all five polymorphic sites, and the phylogenetic analysis placed them in the *H. repens* clade. Putative hybrids RXN2 and RXN5 therefore possess two divergent forms of the ITS,

one matching *H. nodiflorum* and another matching *H. repens*, while putative hybrid RXN1 is apparently homozygous and possesses only a single form of the ITS, which matches *H. repens*.

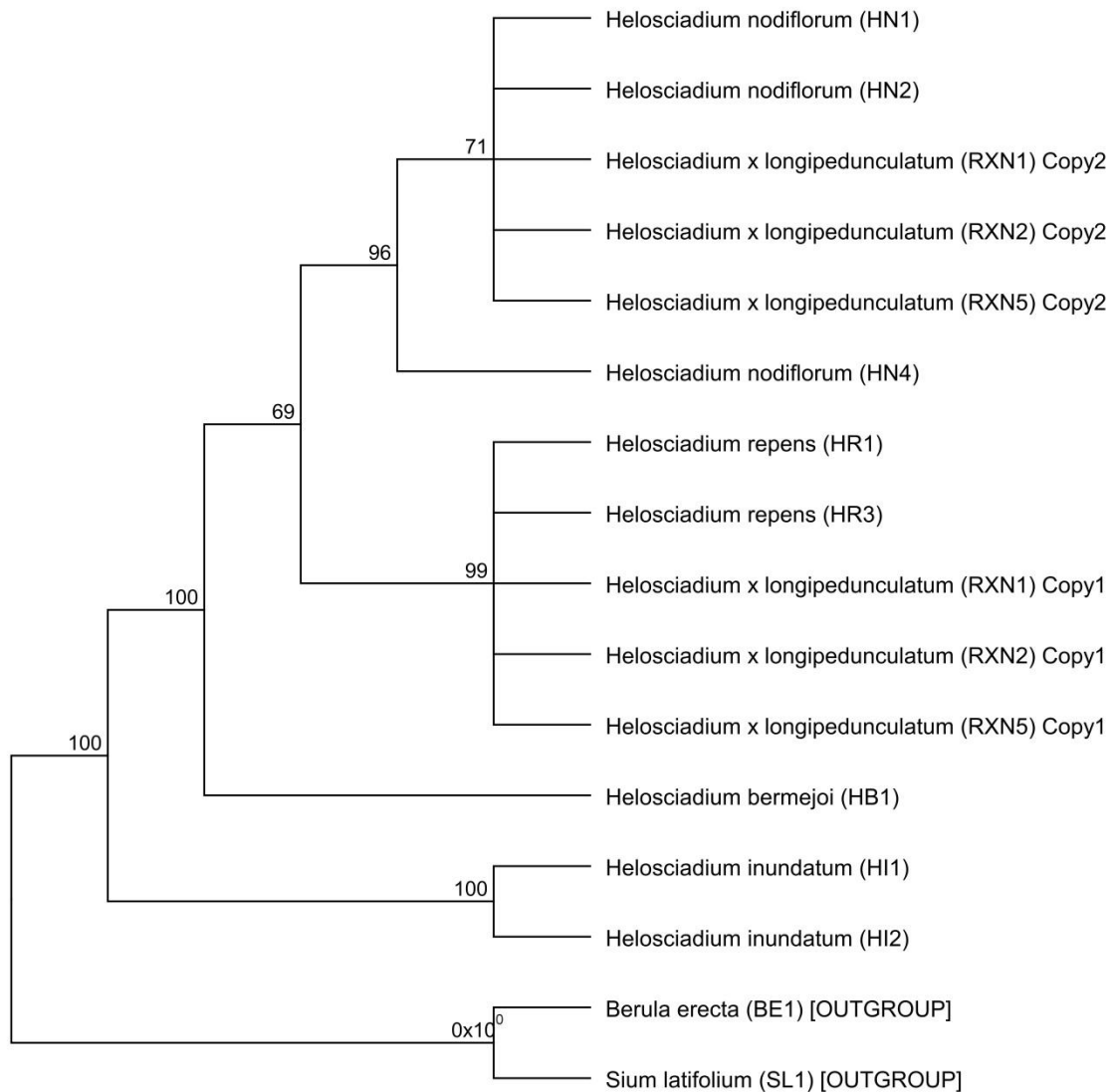


**Figure 3. A 50% majority-rule consensus tree of the 9 shortest trees (447 steps) generated by a maximum parsimony analysis of ITS sequence data. RXN1 is apparently single copy and was placed in a *H. repens* clade (61% BS). Two distinct copies were detected in RXN2 and RXN5, copy 1 copies were placed in a *H. nodiflorum* clade (96% BS) and copy 2 copies were placed in the *H. repens* clade. BS values are displayed above nodes.**

### *LEAFY2*

When sequenced directly (5' → 3') the *LEAFY2* sequences of all three putative hybrids (RXN1, RXN2, RXN5) gave a clean signal for the first 250 bp, with clear double peaks at sites where *H. repens* and *H. nodiflorum* are polymorphic. However, after 250 bp the sequence reads became mixed and unintelligible, presumably due to a frameshift induced by a 4 bp-indel that exists between *H. repens* and *H. nodiflorum*. Gene cloning detected the presence of two distinct copies of *LEAFY2* in

the putative hybrids, designated copy 1 and copy 2. A direct sequence comparison of copy 1 copies matched *H. repens*, and the phylogenetic analysis placed them in a strongly-supported *H. repens*-clade (99% BS; Fig. 4). A direct sequence comparison of copy 2 copies matched *H. nodiflorum*, and the phylogenetic analysis placed them in a strongly-supported *H. nodiflorum*-clade (96% BS). The putative hybrids therefore all appear to possess two divergent copies of the LCN *LEAFY2*, one originating from *H. repens* and another from *H. nodiflorum*.



**Figure 4. A 50% majority-rule consensus tree of the 65 shortest trees (186 steps) generated by a maximum parsimony analysis of *LEAFY2* sequence and coded indel data. Two distinct copies were detected in RXN1, RXN2 and RXN5, copy 1 copies were placed in a *H. repens* clade (99% BS) and copy 2 copies were placed in a *H. nodiflorum* clade (96% BS). BS values are displayed above nodes.**

## Discussion

*Helosciadium repens* and *H. nodiflorum* are closely allied species (Spalik *et al.*, 2009; Ronse *et al.*, 2010), and diverged from their common ancestor relatively recently



(~1 mya; Spalik & Downie, 2006). The taxa are still capable of interbreeding and, upon cross-fertilisation, can give rise to viable offspring. In the current study three individuals collected from Port Meadow, Oxfordshire, were established as being of a hybrid origin, including two early-generation hybrids, most likely F1s, and a backcross to *H. repens*. The hybrids and the backcross all appear to be fertile, and in cultivation developed ripe fruits when growing in close proximity to other *Helosciadium* species.

Reticulation, in the form of hybridisation and introgression, therefore appears to be ongoing between *H. repens* and *H. nodiflorum*. It is tempting to conclude that the species boundary is being maintained by some other means than reproductive isolation. However, it is possible that intermixed *H. repens* and *H. nodiflorum* is a relatively recent occurrence and that hybridisation is now occurring more frequently than in the past. Climate change, atmospheric pollution, surrounding land use and alterations to the site's hydrology are some of the factors that may have caused recent changes in the distribution and abundance of the hybrid, and its parents, at Port Meadow. Hybridisation may even be a potential threat to the long-term survival of pure *H. repens* in the British Isles, particularly if the remaining individuals are subjected to excess gene flow from the more-abundant *H. nodiflorum* (i.e. genetic swamping; Levin *et al.*, 1996; Todesco *et al.*, 2016).

#### *Molecular Confirmation*

In the case of RXN2 and RXN5 both biparentally-inherited nuclear markers (*LEAFYi2* and the ITS) revealed contributions from *H. nodiflorum* and *H. repens*, with the maternally-inherited chloroplast marker (*rps16-trnK*) identifying *H. nodiflorum* as the female parent. RXN2 and RXN5 therefore appear to be early-generation hybrids between *H. nodiflorum* and *H. repens*. Interestingly, while the direction of hybridisation is the same, RXN2 and RXN5 possess distinct chloroplast haplotypes and appear to have arisen independently.

In the case of RXN1 the ITS and *rps16-trnK* sequences matched *H. repens*, with no apparent contribution from *H. nodiflorum*, but its *LEAFYi2* sequence revealed contributions from both species. RXN1 is therefore most likely a backcross to *H. repens*, for while it retains both parental copies of the LCNG, *LEAFYi2*, it has seemingly lost the *H. nodiflorum* copy of the ITS. Discrepancy between nuclear datasets, e.g. rDNA and LCNGs, is not uncommon and can be due to differential patterns of inheritance (Small *et al.*, 2004). In first-generation hybrids nuclear markers are typically inherited in a predictable, biparental and additive fashion but, in the case of repetitive DNA, such as the ITS, additional copies can be lost from generation to generation. This is because repeat-units tend to evolve in unison via mechanisms of concerted evolution, e.g. gene conversion, unequal crossovers etc. (Baldwin *et al.*, 1995). In the wake of a reticulate event, two divergent copies, initially present in the amalgamated genome, can be lost if the sequence becomes homogenised to a single parental type (Álvarez & Wendel, 2003). Homogenisation can be bidirectional (Wendel *et al.*, 1995), but in backcrosses it typically occurs in the direction of the recurrent parent (Aguilar *et al.*, 1999). LCNGs, like *LEAFYi2*, are thought to be less likely to undergo concerted evolution than rDNA, and can maintain the signal of reticulation even when it has been lost from rDNA markers (Small *et al.*, 2004). This appears to be the case here and in RXN1 the signal of hybridisation, while apparently lost from the ITS sequence, is preserved in *LEAFYi2*.

This trace would have been entirely overlooked if only the ITS had been relied upon, as is so often the case in phylogenetic and hybridisation studies (Álvarez & Wendel, 2003), and highlights the importance of analysing additional nuclear datasets.

### *Taxonomy*

The hybrid *H. repens* × *H. nodiflorum* appears to be a permanent fixture of the British flora; having arisen independently in a number of different locations (Crackles, 1976; Stace *et al.*, 2015). A validly published hybrid binomial is therefore warranted, and is in line with other hybrid combinations in this genus (e.g. Riddelsdell, 1914a; Desjardins *et al.*, 2015; Rita *et al.*, 2016). Druce (1928) proposed the name *Apium* × *riddelsdellii*, but this is invalid as it was published without description. A number of described varieties/forms of *H. nodiflorum* are also said to possess intermediate features between *H. repens* and *H. nodiflorum* (e.g. var. *ochreatum* (DC.) DC., var. *pseudo-repens*, var. *longipedunculatum* F.W. Schultz).

The best candidate among these is *H. nodiflorum* var. *longipedunculatum*, which has been linked with the hybrid by a number of eminent botanists (e.g. Rothmaler, 1963; Stace, 1997). This variety was originally recognised by Schultz (1854), and later described in further detail by Riddelsdell & Baker (1906) who examined a number of specimens from Duddingston Loch, Edinburgh (v.c.83) and Gullane Links, East Lothian (v.c.82). While Riddelsdell (1917a) never regarded this variety as the cross between *H. repens* and *H. nodiflorum*, preferring his own candidates from Binsey Common (Riddelsdell, 1917b), it appears to be a strong contender for a number of reasons. (i) Its leaflets are somewhat intermediate between *H. repens* and *H. nodiflorum*, being ovate to broadly ovate, coarsely serrate and occasionally lobed; (ii) its peduncles resemble those of *H. repens*, being typically longer than the rays of the umbel; and (iii) an involucre of 1-3 bracts is always present, a rare thing in pure *H. nodiflorum* (Riddelsdell & Baker, 1906).

A comparison of *H. repens* × *H. nodiflorum* hybrids from Port Meadow with *H. nodiflorum* var. *longipedunculatum* specimens from the Natural History Museum (N.H.M.), London, revealed a close resemblance. Sheets examined included, from Gullane Links: J.R. Scott & W. Jameson, 1819, BM001154369 – G. Lawson, 1845, BM001144095 (x2) – G. Don, date unknown, BM001144094; from Duddingston Loch: J.T. Syme, 1850, BM001154368 – C. Bailey, 1882, BM001187309, BM001187310, BM001154374). In both cases the leaflets are ovate to broadly ovate, with or without lobes, peduncles are always present and are equal to or longer than rays; bracts are also present at the base of most umbels. Ancient DNA analysis of the type specimens would be definitive but, given the age and historical value of the specimens, destructive sampling was not considered. Nevertheless, morphological comparison alone has convinced us that var. *longipedunculatum* does most likely represent the hybrid between *H. repens* and *H. nodiflorum*, in line with Rothmaler (1963) and Stace (1997).

There is a valid hybrid binomial under *Apium* (see Rothmaler, 1963), but no valid combination exists under the genus *Helosciadium*. A number of entries have been submitted to the Tela Botanica online database by B. Bock (see <http://www.tela-botanica.org/bdtfx-nn-82040>), but these do not constitute valid publication (Kanchi Gandhi, pers. comm.; Jean-Marc Tison, pers. comm.; Clive Stace, pers. comm.). Furthermore, the combinations do not appear in TaxRef (accessed 19.2.2020), the French taxonomic index managed by the Museum National d'Histoire

Naturelle, nor are they cited in the International Plant Names Index (IPNI; accessed 19.2.2020). A new combination for *Helosciadium* has therefore been made here (see below).

The original author, Schultz (1854), did not designate a holotype, neither did he give a precise locality nor cite the collector of the specimens he procured. However, he writes that the material was originally collected from the Edinburgh area and that he received it by way of London, presumably on loan from the British Museum. He further notes that the consignment consisted of two specimens, affixed to a single sheet, and that they were catalogued under the name *H. repens* Koch. It was from these two specimens that Schultz (1854) made his original description.

A survey of *H. nodiflorum* var. *longipedunculatum* sheets in the N.H.M. revealed the specimens in question – BM001144095 (Fig. 5.). Two small examples, adjacent to one another, collected by G. Lawson from Gullane Links in July 1845, and archived under the name *H. repens* Koch, which are almost certainly the original syntypes. We here designate the larger of the two (left) as the lectotype. Note that in the current mounting these two specimens occupy only the top right hand corner of a sheet, the rest being taken up by another larger specimen (BM001144094). This third specimen was also collected from Gullane Links, by G. Don (date unknown), and Riddelsdell & Baker (1906) cited it as a further example of var. *longipedunculatum*. However, the label reads 30. *Sium repens* Jacq., and Schultz (1854) makes no mention of such a specimen. The conclusion being that Schultz did not have this additional specimen to hand when making his original description of var. *longipedunculatum*, and that the two collections were combined at a later date. For ease of viewing this additional specimen has been cropped out of Fig. 5., with only a terminal leaflet still visible in the bottom left hand corner.

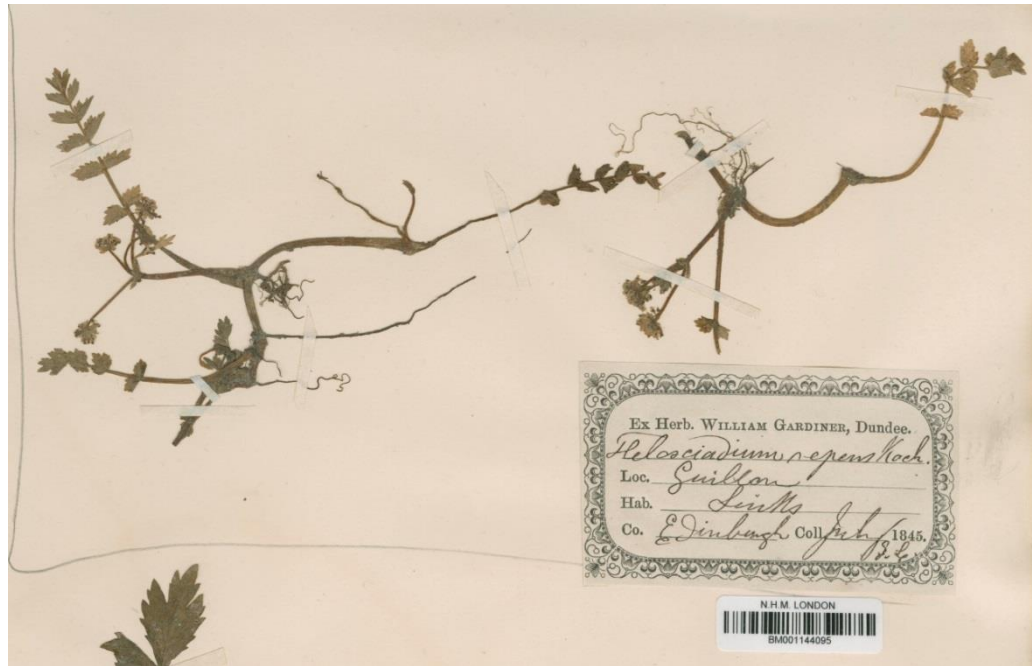
***Helosciadium* × *longipedunculatum*** (F.W. Schultz) Desjardins, *comb. nov.*  
= *Helosciadium nodiflorum* var. *longipedunculatum* F.W. Schultz, *Bonplandia* 2: 237. 1854 [basionym].  
= *Apium* × *longipedunculatum* (F.W. Schultz) Rothm. *Exkursionsflora* 4: 233. 1963 [synonym].

**Lectotype:** Scotland, East Lothian, Gullane (“Guillon”) Links. July 1845. G.L. (“George Lawson”). BM001144095 (**BM**; Fig. 5, left).

### **Acknowledgements**

Tony O’Mahony and Magdalena Vicens (Jardi Botanic de Soller, Mallorca) for providing plant material; Fred Rumsey and John Hunnux (Natural History Museum, London) for providing high-resolution images of herbarium specimens; Clive Stace for his invaluable guidance on plant taxonomy; Doug McKean and Henry Noltie for identifying the collector and location of the original syntypes of var.

*longipedunculatum*. This work was funded by a BSBI Science & Research grant, which was awarded to S.D.D.



**Figure 5. Syntypes of *Helosciadium* × *longipedunculatum* collected by G.L. (“George Lawson”) from Gullane (“Guillon”) Links, Scotland in July 1845 (BM001144095). Specimen to left herein designated as the lectotype.**

Image reproduced under a cc-by licence

(<http://creativecommons.org/licenses/by/4.0/>)

© The Trustees of the Natural History Museum, London.

## References

- Aguilar, J.F, Rosselló, J., & Feliner, G.N. 1999. Nuclear ribosomal DNA (ITS) concerted evolution in natural and artificial hybrids of *Armeria* (Plumbaginaceae). *Molec. Ecol.* 8: 1341-1346.
- Álvarez, I. & Wendel, J.F. 2003. Ribosomal ITS sequences and plant phylogenetic inference. *Molec. Phylogen. Evol.* 29: 417-434.
- Baldwin, B.G., Sanderson, M.J., Porter, J.M., Wojciechowski, M.F., Campbell, C.S. & Donoghue, M.J. 1995. The ITS region of nuclear ribosomal DNA: a valuable source of evidence on angiosperm phylogeny. *Ann. Missouri Bot. Gard.* 82: 247-277.
- Crackles, F.E. 1976. *Apium repens* (Jacq.) Lag. and *A. nodiflorum* (L.) Lag. × *A. repens* (Jacq.) Lag. in the East Riding. *Naturalist (Hull)* 937: 74.
- Desjardins, S.D, Leslie, A.C., Stace, C.A., Schwarzacher, T. & Bailey, J.P. 2015. Intergeneric hybridisation between *Berula erecta* and *Helosciadium nodiflorum* (Apiaceae). *Taxon* 64: 115-125.
- Desjardins, S.D. 2016. Molecular confirmation of *Helosciadium* × *moorei* (*H. inundatum* × *H. nodiflorum*; Apiaceae) from County Cork, Ireland. *New Journal of Botany* 6: 90-97.
- Druce, G.C. 1927. *The Flora of Oxfordshire*. Oxford: Clarendon Press.
- Druce, G.C. 1928. *British Plant List*, 2<sup>nd</sup> ed. Arbroath: T. Buncle & Co.
- Frohlich, M.W. & Meyerowitz, E.M. 1997. The search for flower homeotic gene homologs in basal angiosperms and Gnetales: a potential new source of data on the evolutionary origin of flowers. *Int. J. Pl. Sci.* 158: S131-S142.

- Grassly, N.C., Harris, S.A. & Cronk, Q.C.B. 1996. British *Apium repens* (Jacq.) Lag. (Apiaceae) status assessed using random amplified polymorphic DNA (RAPD). *Watsonia* 21: 103-111.
- Hardway, T.M., Spalik, K., Watson, M.F., Katz-Downie, D.S. & Downie, S.R. 2004. Circumscription of Apiaceae tribe Oenantheae. *S. African J. Bot.* 70: 393-406.
- Herden, T., Bönisch, M. & Friesen, N. 2019. Genetic diversity of *Helosciadium repens* (Jacq.) W.D.J. Koch (Apiaceae) in Germany, a Crop Wild Relative of celery. *Ecology and Evolution*. 00: 1-16.
- JNCC (Joint Nature Conservation Committee). 2013. *Third Report by the United Kingdom under Article 17 on the implementation of the Habitats Directive from January 2007 to December 2012; conservation status assessment for creeping marshwort (Apium repens)*. Peterborough: JNCC.
- Lansdown, R.V. 2011. *Apium repens*. The IUCN Red List of Threatened Species 2011. [online] e.T161949A5518019. Accessed 15 June 2018.
- Leslie, A.C. & Desjardins, S.D. 2018. A new location for  $\times$  *Beruladium procurrans* (Apiaceae) at Carlton Marshes, Suffolk. *BSBI News* 139: 25-26.
- Levin, D.A., Francisco-Ortega, J. & Jansen, R.K. 1996. Hybridization and the extinction of rare plant species. *Conservation Biology* 10: 10-16.
- McDonald, A.W. & Lambrick, C.R. 2006. *Apium repens creeping marshwort; Species Recovery Program 1995-2005*. Peterborough: English Nature.
- O'Mahony, T. 2016. A taxonomic comparison of *Helosciadium inundatum* (L.) W.D.J. Koch, *H. nodiflorum* (L.) W.D.J. Koch and their interspecific hybrid, *H. \times moorei* (Syme) Druce (Apiaceae). *Irish Botanical News* 26: 15-21.
- Pimenov, M.G. & Leonov, M.V. 1993. *The Genera of the Umbelliferae*. London: Kew Publishing.
- Riddelsdell, H.J. 1914a. *Helosciadium moorei*. *Irish Naturalist* 23:1-11.
- Riddelsdell, H.J. 1914b. The British forms of *Helosciadium*. *Irish Naturalist* 23: 95-101.
- Riddelsdell, H.J. 1917a. *Helosciadium* in Britain. *Rep. Bot. Exch. Club Soc. Brit. Isles* 4: 409-412.
- Riddelsdell, H.J. 1917b. *H. repens \times nodiflorum?*. *Rep. Bot. Exch. Club Soc. Brit. Isles* 4: 570.
- Riddelsdell, H.J. & Baker, E.G. 1906. British forms of *Helosciadium nodiflorum* Koch. *Journal of Botany* 44: 185-190.
- Rita, J., Capó, M. & Cursach, J. 2016. *Helosciadium \times clandestinum* un nuevo híbrido aparecido en Menorca (Islas Baleares). *Flora Montiberica* 63: 130-136.
- Rita, J., Capó, M., Moragues, E., Bota, J. & Cursach, J. 2018. Hybridization processes in an introduced subpopulation of an endangered plant: Management strategies to guarantee the conservation of *Helosciadium bermejoi* (Apiaceae). *Journal for Nature Conservation* 41: 26-34.
- Ronse, A.C., Popper, Z.A., Preston, J.C. & Watson, M.F. 2010. Taxonomic revision of European *Apium* L. s.l.: *Helosciadium* W.D.J. Koch restored. *Pl. Syst. Evol.* 287: 1-17.
- Rothmaler, W. 1963. *Excursionsflora von Deutschland IV*, 1<sup>st</sup> ed. Berlin: Volk und Wissen Volkseigener.
- Schultz, F.W. 1854. *Helosciadium nodiflorum* Koch. *Bonplandia* 2: 237-238.
- Simmons, M.P. & Ochoterena, H. 2000. Gaps as characters in sequence-based phylogenetic analyses. *Syst. Biol.* 49: 369-381.

- Small, R.L., Cronn, R.C. & Wendel, J.F. 2004. Use of nuclear genes for phylogeny reconstruction in plants. *Austral. Syst. Bot.* 17: 145-170.
- Spalik, K. & Downie, S. R. 2006. The evolutionary history of *Sium sensu lato* (Apiaceae): dispersal, vicariance, and domestication as inferred from ITS rDNA phylogeny. *Amer. J. Bot.* 93: 747-761.
- Spalik, K., Downie, S.R. & Watson, M.F. 2009. Generic delimitations within the *Sium* alliance (Apiaceae tribe Oenantheae) inferred from cpDNA *rps16-5'trnK<sup>(UUU)</sup>* and nrDNA ITS sequences. *Taxon* 58: 735-748.
- Stace, C.A. 1997. *New Flora of the British Isles*, 2<sup>nd</sup> ed. Cambridge: Cambridge University Press.
- Stace, C.A. 2019. *New Flora of the British Isles*, 4<sup>th</sup> ed. Middlewood Green, Suffolk: C & M Floristics.
- Stace, C.A., Preston, C.D. & Pearman, D.A. 2015. *The Hybrid Flora of the British Isles*. London: Botanical Society of Britain & Ireland.
- Stroh, P.A., Webb, J. & Rumsey, F.J. 2016. *Apium repens* Creeping Marshwort. Species Account. *Botanical Society of Britain and Ireland*. [online] [Accessed 15 June 2018] Available at: [https://bsbi.org/wp-content/uploads/dlm\\_uploads/Apium\\_repens\\_species\\_account.pdf](https://bsbi.org/wp-content/uploads/dlm_uploads/Apium_repens_species_account.pdf)
- Swofford, D.L. 2002. *PAUP\*: phylogenetic analysis using parsimony (and other methods)*. Sunderland, Massachusetts: Sinauer Associates.
- Todesco, M., Pascual, M.A., Owens, G.L., Ostevik, K.L., Moyers, B.T., Hübner, S., Heredia, S.M., Hahn, M.A., Caseys, C., Bock, D.G. & Rieseberg, L.H. 2016. Hybridization and extinction. *Evolutionary Applications* 9(7): 892-908.
- Tutin, T.G. 1962. Umbelliferae. Pp. 495-531 in: Clapham, A.R., Tutin, T.G. & Warburg, E.F. (eds.), *Flora of the British Isles*, 2<sup>nd</sup> ed. Cambridge: Cambridge University Press.
- Tutin, T.G. 1968. *Flora Europaea. Vol. 2. Rosaceae to Umbelliferae*. London: Cambridge University Press.
- Tutin, T.G. 1975. *Apium*. Pp. 268-269. In: Stace, C.A. (ed.), *Hybridization and the Flora of the British Isles*. London: Academic Press.
- Tutin, T.G. 1980. *Umbellifers of the British Isles*. London: Botanical Society of Britain & Ireland.
- Walters, S.M. 1980. *Apium repens* (Jacq.) Reichb. f.. *Watsonia* 13: 170.
- Wendel, J.F., Schnabel, A. & Seelanan, T. 1995. Bidirectional interlocus concerted evolution following allopolyploid speciation in cotton (*Gossypium*). *Proc. Natl. Acad. Sci.* 92: 280-284.
- Wolff, H. 1927. Umbelliferae-Apioideae-Ammineae-Carinae, Ammineae novemjugatae et genuinae. Pp. 1-398. In: Engler, A. (ed.), *Das Pflanzenreich, Heft 90*. Berlin: Engelmann.

Copyright retained by author(s). Published by BSBI under the terms of the [Creative Commons Attribution 4.0 International Public License](https://creativecommons.org/licenses/by/4.0/).

ISSN: 2632-4970

<https://doi.org/10.33928/bib.2020.02.027>

**Supplementary information 1.** Accessions used in the current study and their associated metadata. Sequences generated in the current study are denoted with a double asterisk (\*\*).

**Taxon** – country of origin; voucher specimen; *LEAFYi2* GenBank accession number; ITS GenBank accession number; *rps16-trnK* GenBank accession number.

---

***Apium graveolens*** L. – a) England; *M.W. Chase 2523 (K)*; *LEAFYi2* MT110306\*\*; ITS MT108800\*\*; *rps16-trnK* n/a; b) France; *Downie 258 (ILL)*; *LEAFYi2* n/a; ITS n/a; *rps16-trnK* AF110545. ***Berula bracteata*** (Roxb.) Spalik & S.R. Downie – Saint Helena; *V. Williams 1 (WA)*; *LEAFYi2* n/a; ITS AY353982; *rps16-trnK* EF367712. ***Berula burchellii*** (Hook. f.) Spalik & S.R. Downie – Saint Helena; *V. Williams 2 (WA)*; *LEAFYi2* n/a; ITS AY353983; *rps16-trnK* EF367713. ***Berula erecta*** (Huds.) Coville – England; *A.C. Leslie BE1 (LTR)*; *LEAFYi2* MT110307\*\*; ITS KP871508; *rps16-trnK* KP871504. ***Berula imbricata*** (Schinz) Spalik & S.R. Downie – Tanzania; *Kayombo & Kayombo 217 (MO)*; *LEAFYi2* n/a; ITS AY360228; *rps16-trnK* EF367695. ***Berula incisa*** (Torr.) G.N. Jones – USA; *Holmgren & Holmgren 4577 (ILL)*; *LEAFYi2* n/a; ITS DQ005646; *rps16-trnK* EF367698. ***Berula repanda*** (Hiern) Spalik & S.R. Downie – South Africa; *Rogers 9101 (G)*; *LEAFYi2* n/a; ITS AY353977; *rps16-trnK* EF367715. ***Berula thunbergii*** (DC.) H. Wolff – Yemen; *Heckel & Wood Y1215 (E)*; *LEAFYi2* n/a; ITS DQ005660; *rps16-trnK* EF367702. ***Cicuta virosa*** L. – Finland; *Lee & Downie 75 (ILL)*; *LEAFYi2* n/a; ITS U78372; *rps16-trnK* DQ168974. ***Cryptotaenia canadensis*** (L.) DC. – USA; *Downie 817 (ILL)*; *LEAFYi2* n/a; ITS U79613; *rps16-trnK* EF185213. ***Cryptotaenia japonica*** Hassk. – China; *Downie 402 (ILL)*; *LEAFYi2* n/a; ITS AY360236; *rps16-trnK* EF185217. ***Cryptotaenia thomasii*** (Ten.) DC. – Italy; *Brookes, Haddad & Jury 5710 (E)*; *LEAFYi2* n/a; ITS DQ516348; *rps16-trnK* EF367703. ***Hedera helix*** L. – a) Spain; *R. Vargas 5PV97 (LIV)*; *LEAFYi2* n/a; ITS AJ131227; *rps16-trnK* n/a; b) Unknown; *Chase 2743 (K)*; *LEAFYi2* n/a; ITS n/a; *rps16-trnK* GQ983991. ***Helosciadium bermejoi*** (L. Llorens) Popper & M.F. Watson – a) Balearic Islands, Spain; *J.L. Gradaille 10240 (HJBS) HB1*; *LEAFYi2* MT110308\*\*; ITS MT109374\*\*; *rps16-trnK* MT095022\*\*; b) Balearic Islands, Spain; *16878-CN* (Balearic Islands Univ. Herb.); *LEAFYi2* n/a; ITS MF598285; *rps16-trnK* MF598284. ***Helosciadium crassipes*** W.D.J. Koch ex Rchb. – France; *Reduron s.n. (ILL)*; *LEAFYi2* n/a; ITS AY360239; *rps16-trnK* EF185222. ***Helosciadium inundatum*** (L.) W.D.J. Koch – a) Wales; *A.G. Shaw HI1 (LTR)*; *LEAFYi2* MT110309\*\*; ITS KX513939; *rps16-trnK* KX513935; b) Ireland; *T. O'Mahony HI2* (silica-dried material only); *LEAFYi2* MT110310\*\*; ITS KX513940; *rps16-trnK* KX513936. ***Helosciadium × longipedunculatum*** (F.W. Schultz) Desjardins – a) England; *A.G. Shaw*

*RXN1* (**LTR**); *LEAFYi2* copy1 MT110316\*\*, copy2 MT110317\*\*; ITS MT108804\*\*; *rps16-trnk* MT095026\*\*; b) England; *J.A. Webb RXN2* (**LTR**); *LEAFYi2* copy1 MT110318\*\*, copy2 MT110319\*\*; ITS copy 1 MT108805\*\*, copy 2 MT108806\*\*; *rps16-trnk* MT095027\*\*; c) England; *J.A. Webb RXN5* (**LTR**); *LEAFYi2* copy1 MT110320\*\*, copy2 MT110321\*\*; ITS copy 1 MT108807\*\*, copy 2 MT108808\*\*; *rps16-trnk* MT095028\*\*. ***Helosciadium nodiflorum*** (L.) W.D.J. Koch – a) England; *A.C. Leslie HN1* (**LTR**); *LEAFYi2* MT110311\*\*;  
ITS KP871514; *rps16-trnk* KP871507; b) England; *S.D. Desjardins HN2* (**LTR**) *LEAFYi2* MT110312\*\*;  
ITS MT108801\*\*;  
*rps16-trnk* MT095023\*\*;  
c) Ireland; *T O'Mahony HN4* (silica-dried material only); *LEAFYi2* MT110313\*\*;  
ITS KX513941; *rps16-trnk* KX513937; d) France; *Downie 317* (**ILL**); *LEAFYi2* n/a; ITS EF177709; *rps16-trnk* EF185223.

***Helosciadium repens*** (Jacq.) W.D.J. Koch – a) England; *J.A. Webb HR1* (**LTR**); *LEAFYi2* MT110314\*\*;  
ITS MT108802\*\*;  
*rps16-trnk* MT095024\*\*;  
b) England; *J.A. Webb HR3* (silica-dried material only); *LEAFYi2* MT110315\*\*;  
ITS MT108803\*\*;  
*rps16-trnk* MT095025\*\*. ***Sium latifolium*** L. – England; *A.C. Leslie SL1* (**LTR**); *LEAFYi2* MT110322\*\*;  
ITS MT108809\*\*;  
*rps16-trnk* MT095029\*\*. ***Sium medium*** Fisch. & C.A. Mey. – Kyrgyzstan; *Konnov & Kotshgareva 456* (**LE**); *LEAFYi2* n/a; ITS DQ005674; *rps16-trnk* EF185268. ***Sium suave*** Walter – Canada; *Downie 12* (**ILL**); *LEAFYi2* n/a; ITS AY360263; *rps16-trnk* EF185274.