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Ephemerella readeri Müll. Hal. (*Physcomitrella readeri* (Müll. Hal.) I.G. Stone & G.A.M. Scott, Funariidae, Bryophyta): a genus and species new to Europe

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SUMMARY

A morphological and molecular analysis of a *Physcomitrella*, collected from a reservoir margin in the north of England, revealed this to be *P. readeri*, a species new to Europe. The present study clarifies previous confusion over the taxonomy of *P. readeri* showing it to be clearly distinct in both sporophytic and gametophytic characters from *P. patens* and uniform across its world range from England to USA, Japan and Australasia. While phylograms of the ITS1 region from both the *Physcomitrella* species, *Physcomitrium pyriforme* (Hedw.) Bruch & Schimp., *Enthosthodon attenuatus* (Dicks.) Bryhn and *Funaria hygrometrica* Hedw., place the first two in separate clades, in ITS2 phylograms they occur as sister taxa. This, together with previous genealogical studies on the speciation history of the *Physcomitrella–Physcomitrium* species complex, and morphology, suggests that generic rank is appropriate for *P. readeri*. We therefore reinstate the original name *Ephemerella readeri* Müll. Hal. Recent records at several reservoirs in England indicate that *E. readeri* may be native to UK, though remarkable congruence in ITS1 with Australian plants also suggests recent arrival as an alternative possibility.

KEYWORDS: hybridization, ITS sequences, *Physcomitrella patens*, reservoir bryophytes, scanning electron microscopy, stomata

INTRODUCTION

The moss genus *Physcomitrella* Bruch & Schimp. comprises a single European species *Physcomitrella patens* (Hedw.) Bruch & Schimp. (*Aphanorhegma patens* (Hedw.) Lindb. (Corley *et al.*, 1982)). *P. patens* is widely distributed across the northern hemisphere, extending from the USA to Europe (excluding the Mediterranean) and Siberia (Tan, 1979; Smith, 2004). The only other generally recognised taxon in the genus, *P. readeri* (Müll. Hal.) I.G. Stone & G.A.M. Scott, has until now been recorded only from California, Australia, New Zealand and Japan (Dixon, 1926; Ochi, 1968; Scott & Stone, 1976; Goffinet, 2007).

However, the taxonomic status of *P. readeri* based on morphology has been a highly contentious issue with opinions ranging from generic rank to conspecificity with *P. patens*. It was originally called *Ephemerella readeri* Müll. Hal. (Müeller, 1902), but renamed *Physcomitridium readeri* (Müll. Hal.) Roth, by Roth (1911) and subsequently by him (Roth, 1914) as *Physcomitrella austropatens* Broth. ex Roth. Sainsbury (1955) retained Roth's (1911) original name *Physcomitridium readeri* but doubted the generic distinction from *Physcomitrella* stating this to be based solely on slightly immersed stomata with an elongate slit in *Physcomitridium* compared to small, elliptic and superficial counterparts in *Physcomitrella*. Stone & Scott (1973) and Scott & Stone (1976) reverted to using *Physcomitrella readeri* as Australian specimens have superficial stomata and because *readeri* takes precedence over the epithet *austropatens*. Fife (1982) and Catcheside (1980) retained the name *Physcomitrella readeri* but the latter author stated that the South Australian plants are congeneric with *Physcomitrella patens* in the northern hemisphere. The most recent general account of New Zealand mosses by Beever, Allison & Child (1992) and Fife's (1995) New Zealand checklist both followed Tan (1979) who argued for the subdivision of *P. patens* into four subspecies, based mostly on geographical distribution. These are; subsp. *patens* B.C. Tan, comprising most plants from the northern hemisphere, subsp. *readeri* from Australia and New

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Zealand, subsp. *californica* (H. A. Crum & L. E. Anderson) B.C. Tan from the USA and Japan and subsp. *magdalenae* Sloover, B.C. Tan (formerly *P. magdalense* Sloover) from Rwanda. In the latest North American flora, Goffinet (2007) used *P. readeri*, supported by clear cut morphological differences in both sporophytes and gametophytes from *P. patens*, instead of *P. patens* ssp. *californica* or *P. californica* H. A. Crum & L. E. Anderson in earlier works (Crum, Steere & Anderson, 1973).

Recent molecular studies have now done much to clarify the situation. Goffinet *et al.* (2005) reported that a 71-kb inversion in the plastid genome, previously considered diagnostic of *Physcomitrella*, also occurs in two other funarialean genera *Funaria* and *Enthosthodon.* A more recent study by Goffinet *et al.* (2007) confirmed *Physcomitrella* as nesting with all the other genera in the Funariaceae whereas the Ephemeraceae, a family with gymnostomous cleistocarpous capsules, was transferred from the Funariales into a derived clade in the Pottiaceae. Most recently, a genealogical analysis of six loci from several populations of *P. patens* (three collections from France, Germany and England), *P. readeri* and related species in *Physcomitrium* (McDaniel *et al.*, 2010) revealed that the genus arose at least three times from distinct ancestors within *Physcomitrium* and that *P. patens* and *P. readeri* had different origins. In this investigation, *P. patens* subsp. *readeri* from Australia was found to be sister to *P. patens* subsp. *californica* from both California and Japan and both these taxa were strongly supported as sister to isolates of *Physcomitrium sphaericum* from Germany and France.

Physcomitrella patens has been used extensively as a model organism, and there is now a wealth of information available on the molecular genetics of this moss (e.g. Schaefer & Zrÿd 1997; Frank, Ratnadewi & Reski, 2005; Lang *et al.*, 2005; Kamisugi *et al.*, 2008), including sequencing of both nuclear (Rensing *et al.*, 2008) and plastid (Sugiura *et al.*, 2003) genomes. Despite such close attention to the genetics of *P. patens*, there has been little or no attempt to investigate genetic variation within the species, as has been done in several other mosses (Wilson & Provan, 2003; Spagnuolo *et al.*, 2007; Korpelainen *et al.*, 2008; Goffinet & Shaw, 2009). In the course of the first investigation into the population genetics of *P. patens* (Hooper, 2008) that included sampling of several widely separated sites in the UK, EJH, in the autumn of 2006, collected sporophyte-bearing *Physcomitrella* plants from the margin of Lindley Wood Reservoir in the Washburn Valley in West Yorkshire, a long known locality for *P. patens* (Duckett & Duckett, 1980). Further examination and axenic culturing revealed that, in addition to typical *P. patens,* some of the Lindley Wood Reservoir plants were markedly different but matched exactly the descriptions of *P. readeri*: a moss not previously recorded in Europe.

This paper presents the results of a detailed study of the novel Yorkshire *Physcomitrella*, including molecular comparisons with *P. patens* and other members of the Funariaceae. The present and previous (McDaniel *et al.*, 2010) sequence analyses, together with morphological data obtained from both wild and cultured materials, are used to reassess the taxonomic status of *P. readeri*. The possible geographic origins of the Yorkshire *P. readeri* are also discussed.

Materials and Methods

Population details and sample collections

The origins of the plants used in this investigation are listed in Table 1. Although the population of *P. readeri* at Lindley Wood Reservoir was the only population found during 2006, it was a particularly large population, and it occupied the entire extent of bare mud in the reservoir, which covered an area of 65,581 m^2. A similarly extensive population also covered the bare mud at Lindley Wood Reservoir in 2010. Axenic cultures were established from spores following surface sterilization of intact mature capsules. Plants were then grown either on BCD medium containing 1 mM $CaCl_2$ (Knight *et al.*, 2002) or on full strength or 10% Parker nutrient medium (Klekowski, 1969) solidified with 1% Phytogel (Duckett & Ligrone, 1992) maintained at 20–25°C under constant illumination from fluorescent tubes giving an irradiance of approximately 50 W m^{-2}.

Well-established cultures of *P. readeri* with mature gametophores were transferred to a 8 : 16 hour light/dark cycle and maintained at 15°C. This lower temperature and short day length regime, routinely used to induce sporophyte production in *P. patens* (Cove, 2005), proved equally effective with *P. readeri.* To test for the production of possible hybrids, colonies of both *P. patens* and *P. readeri* were grown together under the short day 15°C regime and sporophyte production was monitored along the contact zones.

Nuclear ITS region analysis

In an analysis of genetic diversity among *Physcomitrella* populations collected from eight UK locations, kinship analysis based on AFLP profiles (Hooper, 2008; Hooper *et al.* unpublished data) identified plants collected at Lindley Wood Reservoir, Yorkshire, as differing greatly from plants from the other seven UK populations, as well from accessions from Germany, France, Switzerland and Ukraine.

Upon culturing the Lindley Wood samples for production of gametophytic tissue and sporophytes, further morphological difference between these plants and *P. patens* became evident. To clarify the identity of these plants, analysis of the internal transcribed sequence (ITS) region of ribosomal DNA was conducted.

DNA was extracted from individual plants as described by Knight *et al.* (2002). The ITS1–5.8S rRNA–ITS2 sequence of the nuclear genome was amplified from each sample by PCR using the primer sequences

TCGTAACAAGGTTTCCGTAGGTG (at the 3′ end of the 18S rDNA) gene and ATTTTCAAGCTGGGCTC-TTTCC (at the 5′ end of the 26S rDNA gene). Primer sequences were based on the published sequence of the *Physcomitrella* rDNA repeat (Capesius, 1997) and were designed using Primer3 (Rozen & Skaletsky, 1998). The conservation of the primer sequence in the plant kingdom was determined by a BLAST search of the 'Viridiplantae' subset non-redundant nucleotide database.

PCR reactions (50 μl) contained 10 mM Tris-Cl, pH 9.0, 50 mM KCl, 2.5 mM $MgCl_2$, 0.2 mM dNTP mix, 0.3 μM each of forward and reverse primer and 2 μl DNA (maximum concentration: 20 ng/μl). The PCR comprised 35 cycles; pre-melt for 2 minutes at 94°C, denaturation at 94°C for 20 seconds, annealing at 58°C for 40 seconds and extension at 72°C for 60 seconds, with a final extension at 72°C for 10 minutes. PCR products were purified using a QIAquick PCR purification kit (QIAGEN Ltd, Crawley, UK). The purified products were either directly sequenced or cloned by blunt-end ligation into the EcoRV site of pBluescript II, SK^+. Cycle-sequencing was performed by the Integrated Genomic and Gene Expression Analysis Facility at the University of Leeds, and analysed using an ABI 3130 Genetic Analyser (Applied Biosystems, Foster City, CA, USA). The ITS sequences obtained in this study have been submitted to GenBank (GU590792-GU590800 bankit 1296615).

Sequence alignment and analysis

The sequences were viewed and assembled using the ABI trace viewer in BioEdit, version 7.0.9.0, with the ITS1 and ITS2 regions treated separately. Sequences were first automatically aligned in a matrix using ClustalW version 2 (Chenna *et al*., 2003), before manually editing in the Sequence Alignment Editor in BioEdit. The ITS region sequence from *Funaria hygrometrica* Hedw. was obtained from GenBank (Benson *et al*., 2006) and included in the alignment matrix.

After sequence alignment, most parsimonious phylogenetic trees were produced using the phylogenetic analysis package TNT (Goloboff, Farris & Nixon, 2008), by performing a branch-and-bound search using the implicit numeration option in TNT, which guarantees to find all trees. Support for each clade was tested by bootstrap using 1000 replicates.

Maximum likelihood analysis was also conducted, using the free software package TREEFINDER (Jobb, 2008). Optimal substitution models for each ITS region were automatically selected using the model proposer dialogue in TREEFINDER. Of the 32 models proposed, a HKY+G model of evolution that produces the tree with the highest maximum likelihood score (Hasegawa, Kishino & Yano, 1985) was selected for each ITS region. This was then used to reconstruct phylogenies in TREEFINDER. Bootstrap analysis was performed using 1000 replicates.

Morphological analysis

Living specimens were mounted in water and photographed with a digital camera under a Leica DM RXAZ microscope using differential interference contrast optics. For scanning electron microscopy (SEM), samples were dehydrated over 24 hours in a graded ethanol/acetone series, critical point dried, coated with a 20 nm layer of gold and observed with a Hitachi S570 scanning electron microscope operating at 20 kV (Pressel, Matcham & Duckett, 2007). Wild and cultured specimens of *P. readeri* were compared with herbarium specimens from Japan, Australia and the USA.

RESULTS

ITS sequence analysis

The ITS sequence analyses included two strains discovered in the Villersexel population in France (Table 1). One strain (K4) was found to be very similar to plants from Gransden, while the other (K3) is the most genetically divergent strain of the *P. patens* accessions available (Kamisugi *et al*., 2008).

Table 1. *Provenance of the mosses used in the phylogenetic reconstruction and the morphological analyses.*

Species	Population/location	County/region	Country
Physcomitrella readeri	Lindley Wood Reservoir	West Yorkshire	England
P. readeri†	Melton Reservoir	Victoria	Australia
P. readeri†	Masu pond	Okayama-shi	Japan
*Physcomitrella patens**	Lindley Wood Reservoir	West Yorkshire	England
*P. patens**	Bough Beech Reservoir	East Sussex	England
P. patens	Gransden	Cambridgeshire	England
P. patens†	Villersexel	Franche-Comté	France
Physcomitrium pyriforme	Carbondale	Illinois	USA
Entosthodon obtusus	St Agnes	Cornwall	England
*E. attenuatus**	Lizard Downs	Cornwall	England
Funaria hygrometrica	Leeds	West Yorkshire	England

*Collections used for morphological analysis alone.

†Strains obtained from the University of Freiburg collection (http://www.cosmoss.org/ecomap.content).

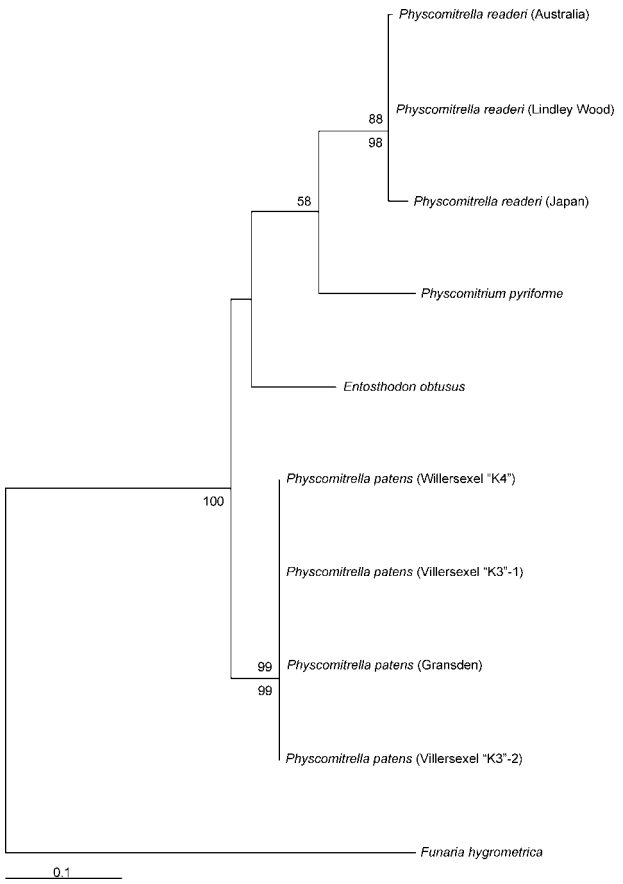

Figure 1. ITS1 maximum likelihood phylogram. Likelihood=−1132. Bootstrap values (log likelihood) from the maximum likelihood analysis are shown above branches, with bootstrap values from the parsimony analysis shown below (1 tree, 159 steps, C.I.=0.86 and R.I.=0.80).

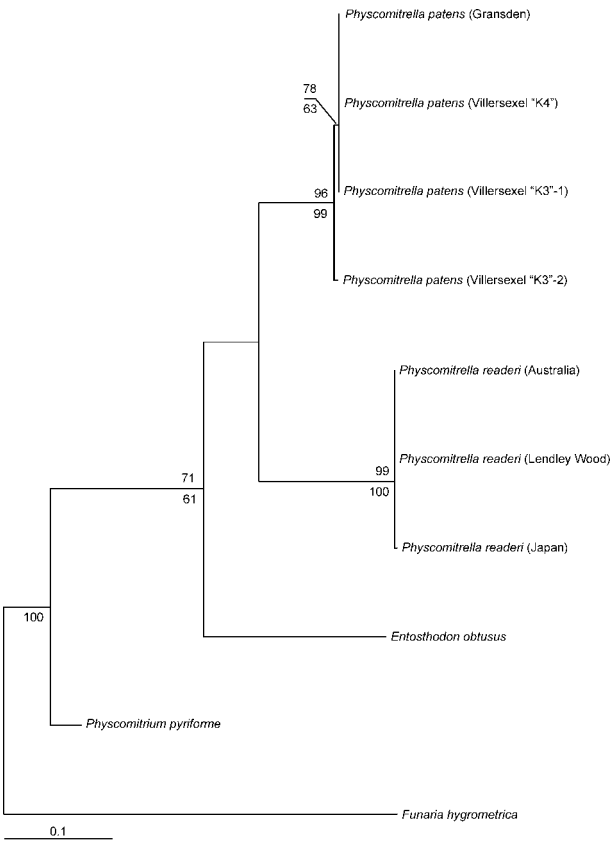

Figure 2. ITS2 maximum likelihood phylogram. Likelihood=−1225. Bootstrap values (log likelihood) from the maximum likelihood analysis are shown above branches, with bootstrap values from the parsimony analysis shown below (1 tree, 167 steps, C.I.=0.89 and R.I.=0.84).

Figures 1 and 2 show the maximum likelihood phylograms produced using the ITS1 and ITS2 sequences from the 10 samples analysed. The most parsimonious trees are not shown as the topologies obtained were identical using both methods. Both trees place the sample collected from Lindley Wood Reservoir with *P. readeri*, from Japan and Australia, and in separate clades from the *P. patens* plants from the UK and France.

The complete sequence alignments of the entire ITS region for all the accessions are given in the Appendix, while Fig. 3 depicts the relevant section of sequence alignment between the known *P. readeri* sample from Australia and the sample from Lindley Wood, produced using BLASTN (Altschul *et al*., 1990). This figure shows that within these two regions there is a single-base mismatch between these samples, in ITS1. Likewise, only a single-base mismatch (in ITS2) differentiates the Lindley Wood ITS sequences from those of a Japanese accession designated as *P. patens* ssp. *californica*. This result is in contrast with the comparison between the Lindley Wood sample and the *P. patens* sample from the UK, where the percentage identity is only 86% (averaged over all characters in BLAST alignment). The ITS1 and ITS2 region sequences from the Gransden and Villersexel K4 strains of *P. patens* were identical, indicating that there is little variation between these accessions. However, the Villersexel K3 strain exhibited a difference: the initial sequence obtained being found to be a mixed trace containing more than one sequence. These were distinguished by cloning the PCR products to derive two separate sequences for this strain. The percentage identity between the Gransden and K4 strains and the two sequences derived from the K3 strain was 99%. The K3 strain has been shown to be the most genetically distinct when compared to all other samples of *P. patens* in laboratory culture, and was used to produce the *P. patens* genetic linkage map (Kamisugi *et al*., 2008).

Morphological analysis

As in *P. patens, P. readeri* has a typical funarialean protonemata with highly branched chloronemal filaments arising from the distal ends of the cells of the main caulonemal axes (Fig. 4a). The chloronemal cells tend to be slightly swollen and are highly vacuolate with abundant peripheral ovoid chloroplasts. Apart from oblique cross-walls most of the cells of the main axes lack longitudinally-aligned endoplasmic arrays of elongate plastids characteristic of

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Score = 852 bits (443),  Expected = 0.0
Identities = 555/556 (99%),   Gaps = 0/556 (0%)
Strand = Plus/Plus

Query  1     CACACACAAAAACTGCAAACAACCCCCTTTTTGAGAACTATCTTATTTGTACAAAACC 60
             ||||||||||||||||||||||||||||||||||| ||||||||||||||||||||
Sbjct  1     CACACACAAAAACTGCAAACAACCCCCTTTTTGAGAATTATCTTATTTGTACAAAACC 60
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Figure 3. Section of the BLAST sequence alignment between *P. readeri* from Lindley Wood (top strand) and *P. readeri* from Australia (bottom strand). The single-base mismatch in the ITS1 region is highlighted.

caulonemal cells in *Funaria* (Pressel, Ligrone & Duckett, 2008). Instead, many of these cells contain between 300 and 400 minute spherical plastids (Fig. 4b). Figure 4c illustrates a typical obovate leaf of *P. readeri* with the costa only extending half the length of the leaf. The apices are serrate (Figs 4d, 5d) and cells are thin-walled throughout the lamina and larger near the costa (Fig. 4e). SEM images best illustrate the rather lax habit of *P. readeri*; it has clearly exserted sporophytes with an apiculate capsule (Fig. 5a–c). Numerous stomata are visible below the calyptra at the base of the capsule (Fig. 5e) and at higher magnification (Fig. 5f), it can be seen that the guard cells are swollen and superficial.

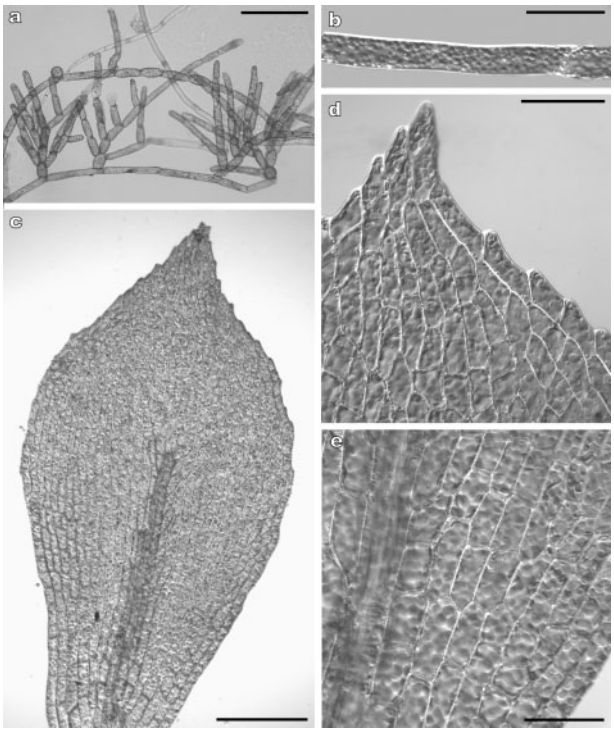

Figure 4. Light micrographs of *Physcomitrella readeri* from Lindley Wood Reservoir. (a) General aspect of protonema showing a main caulonemal axis with highly ramified choronemal side branches. (b) Cell of main axis packed with minute spherical chloroplasts. (c) Typical leaf showing the costa extending half way up the lamina and the serrate apex. (d) Detail of serrate leaf apex. (e) Slightly elongate thin-walled lamina cells adjacent to costa near a leaf base. Scale bars: a, c=200 μm; b, d, e=50 μm.

Sexual incompatibility

Cultures of *P. patens* (Gransden) and *P. readeri* were co-cultured to isolate hybrid sporophytes. Such sporophytes isolated from the contact zones between the parental plants contained spores that were abnormal in size and shape, and non-viable. This is indicative of a meiotic incompatibility between two distinct species.

Discussion

The morphological comparisons conducted in this study together with the molecular data of McDaniel *et al.* (2010) support the view that our accessions identified as *Physcomitrella readeri* and *P. patens* ssp. *californica* are conspecific with *Physcomitrella readeri.* On the basis of our molecular and morphological data, we reinstate the generic status and original name *Ephemerella readeri* Müll. Hal. The key morphological features that immediately distinguish *Ephemerella* from *Physcomitrella* are its lax habit, obovate leaves with the costa extending only half way up, exserted sporophytes and apiculate capsules. Ironically the present study rules out sunken stomata (Fig. 5f), the original criterion (Roth, 1911) for the generic distinction, as these are clearly superficial in both *P. patens* and *P. readeri.*

Description

***Ephemerella readeri* Müll. Hal.** *Ephemeral* green clumps or scattered plants. *Stems* 0.5–5 mm high. *Leaves* 1.5–1.8 mm long, erect when moist, slightly crisped when dry, oblanceolate, spathulate or obovate with a short apiculus, margins plane or slightly reflexed when dry, entire near the base, serrate in distal third, costa ending far short of the apex; cells large and thin-walled, rectangular or rhomboidal to hexagonal, proximal cells near costa 65–140 × 15–45 μm distal cells 35–70 × 10–30 μm. *Paroicous*; antheridia in clusters of 4–5 in the leaf axils just below the archegonia at the apex. *Capsules* cleistocarpous, lacking a line of dehiscence, slightly emergent. Setae very short, usually half the length of the capsules. Capsules very variable in size; globular or oblong to apiculate; dark brown to black at maturity. Peristome absent, exothecial cells very thin-walled. Up to 32 superficial stomata at base of capsule. *Spores* dark rusty brown, 30–45 μm, spinulose with slightly hooked spines.

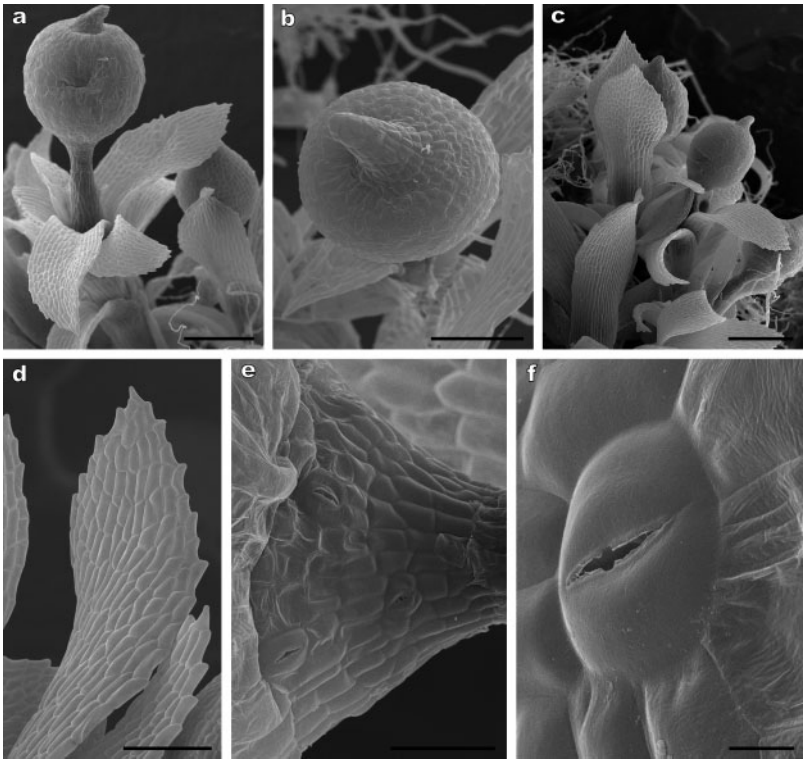

Figure 5. Scanning electron micrographs of *Physcomitrella readeri* from Lindley Wood Reservoir. (a–c) Sporophytes; (d) leaf; (e) stomata around the base of a capsule; (f) detail of a superficial stoma. Scale bars: a, c=500 μm; b, d=200 μm; e=50 μm; f=10 μm.

The Yorkshire *E. readeri* plants differ from *P. patens* in both sporophyte and gametophyte morphology and match exactly herbarium specimens of *P. readeri* from Japan, Australia, New Zealand and the USA and the descriptions of this species in the North American (Goffinet, 2007), Australian (Scott & Stone, 1976; Catcheside, 1980) and New Zealand floras (Sainsbury, 1955). Adding the ITS sequences common to plants from Japan, Australia and UK, and further closely similar loci identified by McDaniel *et al.* (2010), we conclude that, not only is *E. readeri* a rather uniform species throughout its world range, but is also clearly distinct from *P. patens*. Reinforcing the last conclusion is the failure of crosses to produce viable spores. It should be noted that all other interspecific hybrid sporophytes recorded in the Funariales including those from the intergeneric hybrid *Funaria hygrometrica* × *Physcomitrium pyriforme* widely used in studies on apogamy and apospory, all lack viable spores (Bryan, 1957; Tan, 1978; Chopra & Kumar, 1988; Natcheva & Cronberg, 2004). In this context, it is interesting to note that McDaniel *et al.* (2010) found no genealogical evidence of hybridization between species with major differences in sporophyte morphology.

Our ITS sequence data (and the more extensive molecular information obtained by McDaniel *et al.* (2010)), with *P. patens* and *E. readeri* falling into separate clades in both maximum likelihood and most parsimonious trees underlines that the two are highly distinct. Whereas the ITS2 phylogram placing the two as sister taxa would perhaps have been predictable before the study by McDaniel *et al.* (2010), the ITS1 was not. Indeed, the ITS1 phylogram with *E. readeri* in a clade with *Entosthodon obtusus* and *Physcomitrium pyriforme*, and separation of the two in the genealogical trees of McDaniel *et al.* (2010) suggests that *E. readeri* and *P. patens* are correctly placed in separate genera. Certainly the treatment in McDaniel *et al.* (2010), namely, *P. patens* subsp. *patens*, *P. patens* subsp. *readeri* and *P. patens* subsp. *californica*, is now outmoded. Thus, our study confirms Tan's (1979) prediction that sampling of *P. patens* over a wide geographical range would render his subspecific divisions unsustainable.

There are two possible explanations for our conflicting data between the ITS1 and ITS2 regions. On the one hand, the longer ITS1 sequences may be inherently more variable than those from ITS2. On the other hand, they may reflect an ancient natural hybridization event in *Physcomitrium,* a genus with this phenomenon firmly embedded in its evolutionary history (McDaniel *et al.*, 2010).

The development and morphology of the protonemata of *Physcomitrella readeri*, *P. patens* and *Physcomitrium pyriforme* are indistinguishable. All three, together with *Funaria hygrometrica,* produce widely spreading colonies with buds developing all along the main caulonemal axes. In *Funaria* the two kinds of filaments are more clearly distinct (more highly pigmented walls and smaller, more elongate sparser plastids) than in the other three species (Kingham *et al.*, 1995; Pressel *et al.*, 2008). The frequent occurrence of caulonemal cells packed with minute spherical plastids (Fig. 4b) is the one unusual feature of the protonemata of *E. readeri*. On media low in nutrients

the side branches develop into rhizoids with progressively narrower diameters (results not shown), whereas on standard media extensively branched aerial chloronemata are produced as documented previously in *Funaria* and *P. patens* (Goode, Duckett & Stead, 1992). In ageing cultures, the latter differentiate into chains of spherical thin-walled brood cells in all three species (Goode, Duckett & Stead, 1993). *Funaria hygrometrica* is the only moss in the Funariales known to date where chloronematal side branches produce gemmae with elongate abscission cells (Bopp *et al*., 1991). A unique feature of *Funaria*, unlike other mosses where high nutrient status promotes gemma production, is that gemmae formation typically occurs on nutrient-depleted medium. The only other example of specialized propagule production on the filament systems of the Funariales are the unique starch-filled tubers in *Discelium* Brid. (Duckett & Pressel, 2003). Unlike all the aforementioned taxa, the protonemata of the two *Entosthodon* Schwägr. species, plus *Goniomitrium* Hook. f. & Wilson and three of four genera in the Gigaspermaceae (*Chamaebryum* Thér. & Dixon, *Gigaspermum* Lindb. and *Oedipodiella* Dixon), are slow growing, scarcely differentiated into caulo- and chloronemata, do not spread widely across the medium and produce central clumps of gametophores (Pressel & Duckett, unpublished data).

The occurrence of *E. readeri* on otherwise bare reservoir mud at Lindley Wood Reservoir is closely in line with its ecology elsewhere in the world, namely, mudflats along lake banks in the USA (Goffinet, 2007), along river banks and in billabongs in Australia (Catcheside, 1980) and along periodically inundated ditch banks in New Zealand (Duckett, personal observation). As far as can be gleaned from this rather sparse information, ecologically *E. readeri* is closely similar to *P. patens*, but the latter species appears to be more frequent and is found in a greater range of habitats including stream banks, ditches, wet farm tracks, woodland rides and wet meadows (Hill, Preston & Smith, 1994). Both belong to a suite of species that depend on periodic inundation including the mosses *Micromitrium tenerum* (Bruch & Schimp.) Crosby, *Physcomitrium eurystomum* Sendtn., *P. sphaericum* (C. F. Ludw.) Fürnr., *Ephemerum cohaerens* (Hedw.) Hampe, *E. sessile* (Bruch) Müll. Hal., *E. spinulosum* Bruch & Schimp. and *E. hibernicum* D.T. Holyoak & V.S. Bryan (Hill *et al*., 1994; Holyoak, 2001; Holyoak & Bryan, 2005), the liverworts *Riccia canaliculata* Hoffm., *R. cavernosa* Hoffm. *and R. huebeneriana* Lindenb. (Hill, Preston & Smith, 1991) and the gametophytes of *Equisetum* L. (Duckett & Duckett, 1980). Records for these sporadically-growing species depend on their chance sighting by a small number of bryologists in particular years where there have been suitable fluctuations in water levels. Although there is bound to be some under recording of species like these that grow only in widely scattered temporary habitats, many of them do appear to be genuinely rare; in fact all but *Riccia cavernosa* are bryophyte red data book species (Church *et al.*, 2001).

It is unsurprising therefore that the first detailed systematic survey of reservoirs in Ireland (Holyoak, 2001; Holyoak & Bryan, 2005) yielded two new species of *Ephemerum* and numerous new records for these reservoir specialists. More remarkably, in a similar detailed survey of reservoir bryophytes in northern England, Hodgetts (2003) reported that much of the *Physcomitrium*-like material growing at Lindley Wood Reservoir in 2001 and 2003 appeared to be a hybrid between *P. sphaericum* and *Aphanoregma patens*. His description, namely, 'seta intermediate in length, slightly curved, capsules variable in size, spores very spinosely papillose, leaves slightly toothed', exactly matches that of *E. readeri*. It may well be that a North American variety of *Physcomitrella patens* var. *pedicellata*, which has an exerted capsule and has been considered to be a hybrid between *P. patens* and *Physcomitrium sphaericum* (Tan, 1978), is also *E. readeri*.

Hodgetts (2003) also found putative hybrids between *P. sphaericum* and *Aphanoregma patens*, which are almost certainly *E. readeri*, at several other reservoirs including Bosley in Cheshire and Wayoh in Lancashire. Just as native status for all the other reservoir species listed above has never been disputed, despite their almost exclusive occurrence in man-made habitats, it is most likely that *E. readeri* is native to the British Isles but has hitherto been overlooked. Taking into account the Hodgetts (2003) records, it now has more recorded sites in the UK than in the USA (Goffinet, 2007). On the other hand, our failure to find *E. readeri* in any of the numerous herbarium specimens of *P. patens* collected from the Washburn valley and other northern England reservoirs over many decades in the twentieth century still leaves open the possibility of fairly recent introduction. If this is the case, given climatic and particularly the habitat conditions in the UK being not markedly different from those where it occurs elsewhere in the world, new localities for *E. readeri* are to be anticipated.

If *E. readeri* is a new arrival in the UK, one possible source that immediately comes to mind is that its spores were inadvertently introduced along with *Crassula helmsii.* This highly invasive alien from Australasia has spread widely on reservoir margins in the UK since first reported as naturalized in the 1950s. Isoenzyme studies suggest that all the wild *Crassula helmsii* in the UK derive from plants growing along the River Murray (Dawson, 1996), which is also a well-documented locality for *E. readeri* (Catcheside, 1980). Remarkable congruence in the ITS1 sequence between the Australian and Lindley *E. readeri* supports this suggestion.

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TAXONOMIC ADDITIONS AND CHANGES: *Ephemerella readeri* Müll. Hal. Reinstated.

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Appendix

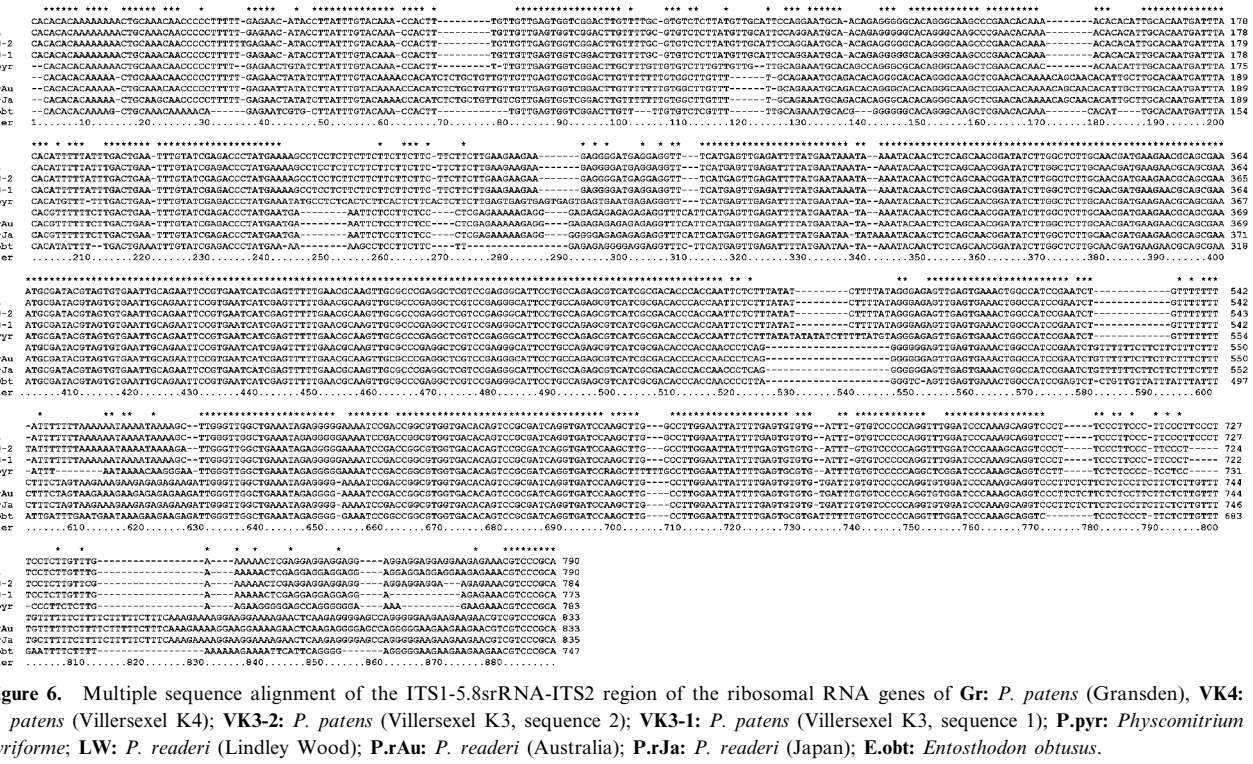

Figure 6. Multiple sequence alignment of the ITS1-5.8srRNA-ITS2 region of the ribosomal RNA genes of **Gr:** *P. patens* (Gransden), **VK4:** *P. patens* (Villersexel K4); **VK3-2:** *P. patens* (Villersexel K3, sequence 2); **VK3-1:** *P. patens* (Villersexel K3, sequence 1); **P.pyr:** *Physcomitrium pyriforme*; **LW:** *P. readeri* (Lindley Wood); **P.rAu:** *P. readeri* (Australia); **P.rJa:** *P. readeri* (Japan); **E.obt:** *Entosthodon obtusus*.

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