Lycopodiella inundata: insights into plant-fungal associations in early vascular plants

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Background: Recent studies have revealed that extant basal vascular plants associate with a wide range of Mucoromycotina and/or Glomeromycotina fungi, paralleling the same in non-vascular liverworts and hornworts. This dispels the long-held paradigm that these early diverging lineages harbour Glomeromycota exclusively. Endophytes belonging to both fungal lineages have also been reported, for the first time, in a Devonian plant (*Horneophyton ligneri*). **Together these discoveries point to much more diverse plant-fungus interactions in early vascular plants than previously assumed**, however our understanding of these remains limited. To gain further insights into the associations between the early diverging Mucoromycotina and basal vascular plants, we are developing the lycophyte *Lycopodiella inundata* as an experimental system because molecular tests and cytological examination of this species (at least at one site in the UK) point to exclusive colonisation by Mucoromycotina.

Findings to date: Through confocal, scanning electron (SEM) microscopy, and molecular analyses, we confirm that *L. inundata* sporophytes can be extensively colonized by Mucoromycotina as seen both in protocorms and roots (Figure 1a,c,f) and fungal sequences extracted from *L. inundata*. Our confocal images also show similar widespread colonization in *L. inundata*'s gametophyte phase, but here the identity of the fungal endophyte has not yet been confirmed. In protocorms, we also observed the 'signature' Mucuromycotina intercellular colonization pattern (Figure 1d,e) which closely resembles that in Haplomitriopsida liverwort (i.e. *Haplomitrium* and *Treubia)*-Mucoromycotina partnerships and the corm of *H. ligneri* (Figure 1g). However while these endopyhtes are known/assumed to enter the host via epidermal cells in Haplomitriopsida liverworts and *H. ligneri*, in *L. inundata* the protocorm hairs function as a conduit for fungal colonization (Figure 1b). Our preliminary phylogram (Figure 3) based on five samples of *L. inundata* from Thursley Common (Figure 2) places the identification of *L. inundata'*s Mucuromycotina fungal strains within an envelope of previously determined fungal sequences extracted from Haplomitriopsida liverworts and closely aligned with two hornworts and the fern *Anogramma*, but separate from reference Mucuromycotina sequences extracted from later-derived angiosperms e.g. *Quercus* and *Endogone* fruiting bodies.

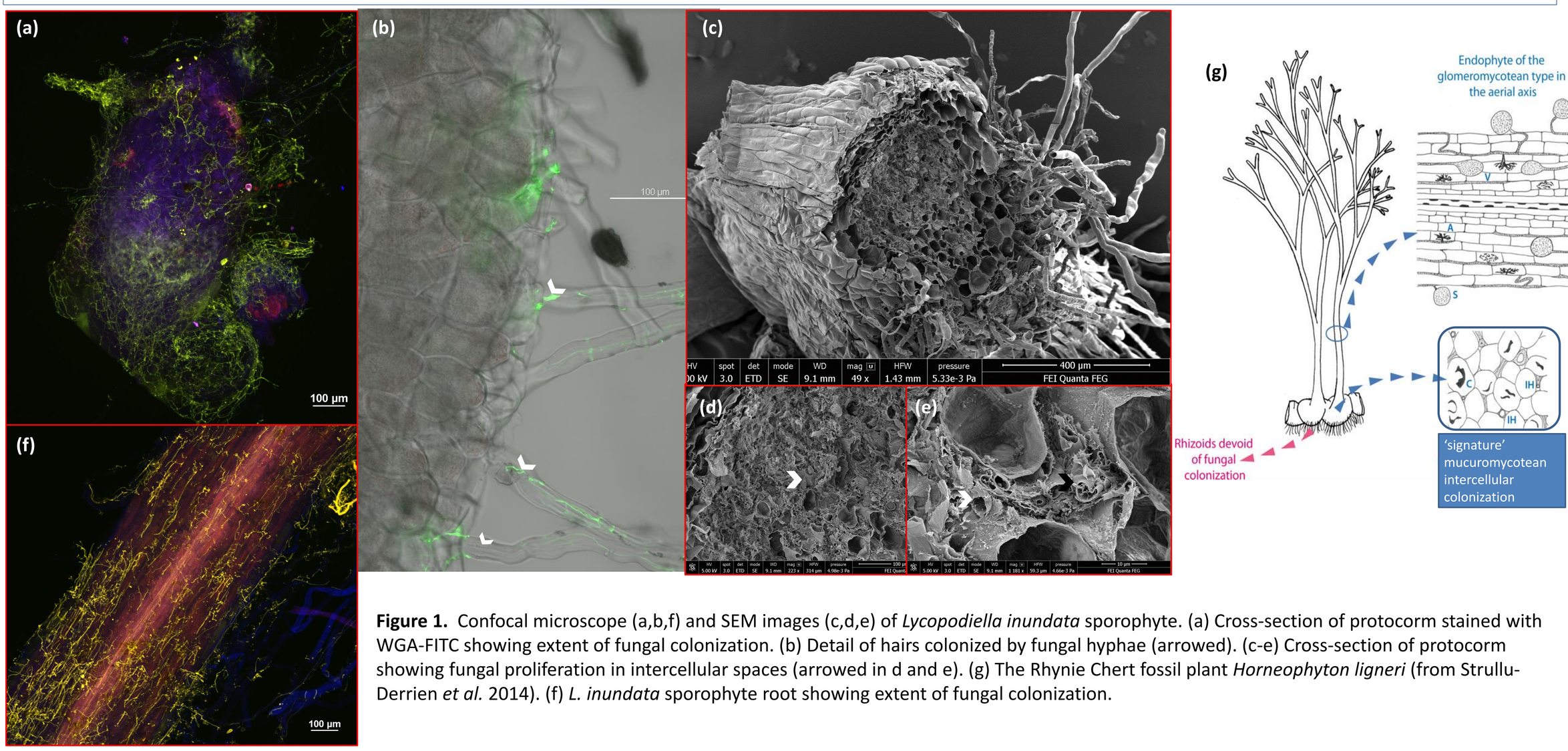

Figure 1. Confocal microscope (a,b,f) and SEM images (c,d,e) of *Lycopodiella inundata* sporophyte. (a) Cross-section of protocorm stained with WGA-FITC showing extent of fungal colonization. (b) Detail of hairs colonized by fungal hyphae (arrowed). (c-e) Cross-section of protocorm showing fungal proliferation in intercellular spaces (arrowed in d and e). (g) The Rhynie Chert fossil plant *Horneophyton ligneri* (from Strullu-Derrien *et al.* 2014). (f) *L. inundata* sporophyte root showing extent of fungal colonization.

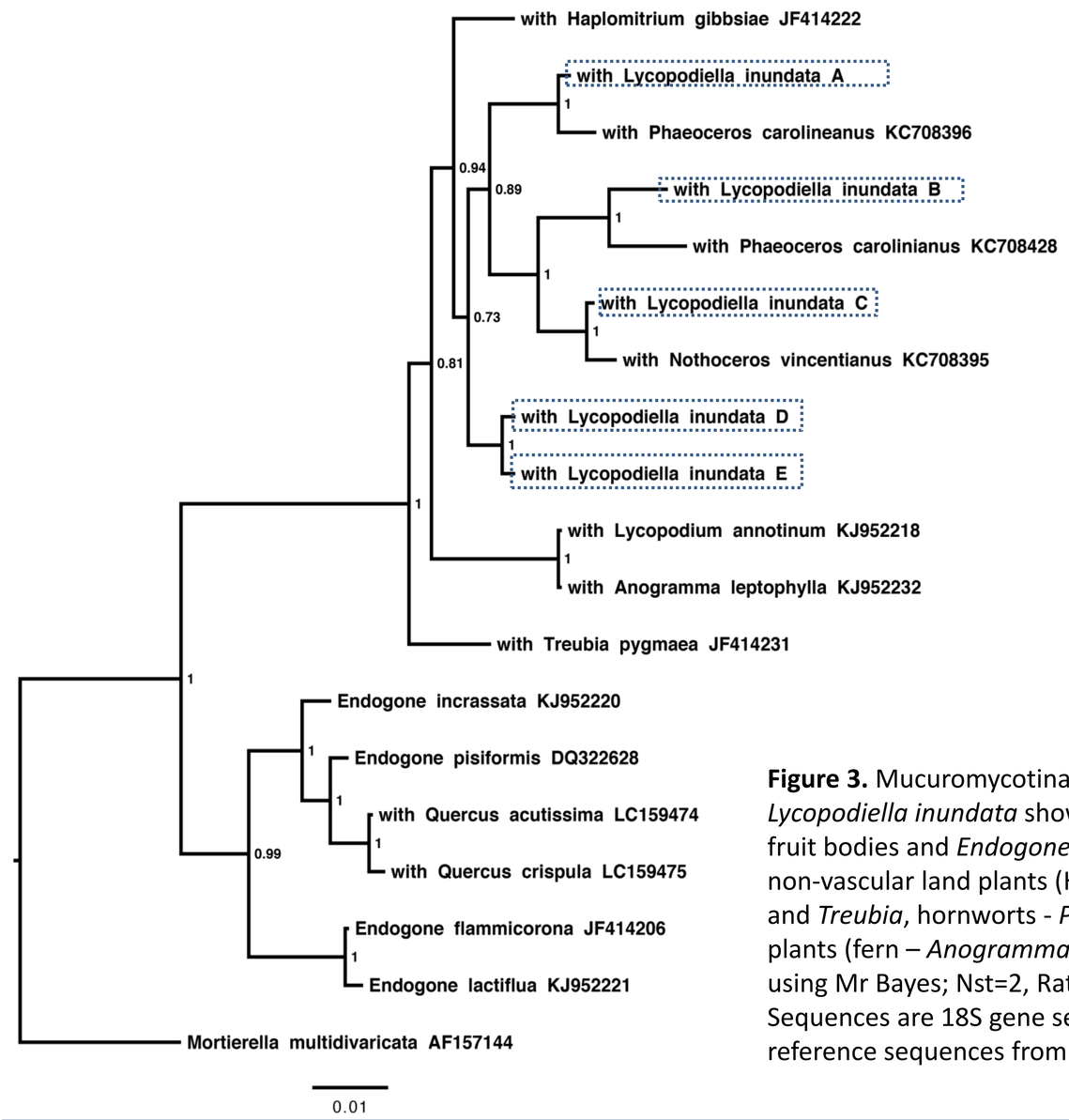

Figure 2. (a) Thursley Common, Surrey wet heathland habitat where *Lycopiella inundata* samples were collected and (b) a close-up colony of *L. inundata*. (c) Picture of young sporophyte, bar = 2mm.

Figure 3. Mucuromycotina fungal sequences extracted from *Lycopodiella inundata* showing their relative position to *Endogone* fruit bodies and *Endogone*-like sequences sourced from wild extant non-vascular land plants (Haplomitriopsida liverworts – *Haplomitrium* and *Treubia*, hornworts - *Phaeoceros* and *Nothoceros)* and vascular plants (fern – *Anogramma*; angiosperm - *Quercus*). Bayesian inference using Mr Bayes; Nst=2, Rates=invgamma; Run 1,000,000 generations; Sequences are 18S gene sequenced using molecular cloning with reference sequences from GenBank.

Future directions: Our current isolation, resynthesis and molecular studies will provide further insights into both host and fungi specificity as well as colonization and mutualism strategies. We also aim to determine the identity of the gametophyte fungal endophyte which remains unknown. In *Lycopodiella inundata*, we see another example of a taxon which appears to associate exclusively with Mucuromycotina fungi while other Lycopods host both Mucoromycotina and Glomeromycotina fungi similarly to the dual colonization observed in thalloid liverworts e.g. *Lunularia.* Whether these differences in symbiotic partners are influenced by external edaphic and ecological conditions remains a key question to better understanding belowground communities.

References: Field *et al.* 2015. New Phytologist 205: 743-756; Field *et al.* Trends in Ecology & Evolution 30: 477-486; Field *et al.* ISME J 10: 1514-1526; Pressel *et al.* 2010. Journal of Systematics and Evolution 54: 666-678; Rimington *et al.* 2015. New Phytologist 205: 1394-1398; Strullu-Derrien *et al*. 2014. New Phytologist 203: 964-979.
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