

<p>Title: Arial text, 12p. bold, upper case for 1st word of title</p>	<p>Molecular identification of fungal endophytes in australian myco-heterotrophic orchids</p>
<p>Abstract: (Your abstract <u>must</u> use Ariel 10 size font and <u>must</u> fit in this space) 400 words</p>	<p>All orchids rely on fungal endophytes for seed germination and growth. Conservation of rare orchid species requires identification of these associated fungi, both for <i>ex situ</i> growth and reintroduction to the wild. The fungal endophytes of Australian myco-heterotrophic orchids have remained unidentified to date as they have been difficult to isolate into pure culture. In this study, direct PCR of colonised orchid roots, cloning and sequencing of fungal ITS regions have been used to identify the fungal endophytes of three species of Australian myco-heterotrophic orchid, namely <i>Dipodium variegatum</i>, <i>Dipodium hamiltonianum</i> and <i>Erythrorchis cassythoides</i>.</p> <p>Genomic DNA was extracted from colonized roots of the three orchids from sites in Queensland and New South Wales, Australia. Fungal DNA was amplified with ITS1F and ITS4 primers and transformed into <i>E. coli</i>. Recombinant plasmids were isolated and fungal ITS inserts sequenced using the T7 primer. Sequences were compared with fungal ITS sequences in Genbank using BLAST searches.</p> <p>The fungal community of <i>Dipodium variegatum</i> included <i>Russula</i>, <i>Verticillium</i> and <i>Trichoderma</i> spp. The fungal community of <i>Dipodium hamiltonianum</i> consisted of <i>Russula</i>, <i>Gymnomyces</i> and <i>Penicillium</i> spp. Analysis of the fungal endophytes of <i>Erythrorchis cassythoides</i> suggests that the orchid is colonized by both ectomycorrhizal fungi such as <i>Russula</i>, <i>Coltricia</i> and <i>Sebacina</i> as well as the saprotrophic <i>Gymnopus</i>.</p> <p>These results suggest that like North American myco-heterotrophic orchids, Australian myco-heterotrophic orchids are commonly colonised by members of the Russulaceae. Conservation strategies for these orchids will require growth of these fungi under laboratory conditions and, as there are such techniques now available, this is a realistic proposition. As these fungi are ectomycorrhizal and the three orchid species typically grow at the base of <i>Eucalyptus</i>, the orchids may be indirect parasites on the trees but this remains to be proven. The involvement of <i>Gymnomyces</i> spp as endophytes in <i>D. hamiltonianum</i> may be one reason why this orchid is becoming rare as the fruit bodies of this species are much sort after food of fungivorous marsupials which may act as dispersal agents for fungal spores. The occurrence of both ectomycorrhizal and saprotrophic fungi as endophytes in <i>Erythrorchis</i> suggests that the orchid may be able to survive the death of its host tree by switching from a parasitic mode of nutrition to a saprophytic one.</p>