SEPTAL PORE CAP ULTRASTRUCTURE OF FUNGI IDENTIFIED AS *EPULORHIZA* SP. (*SENSU SEBACINA*) ISOLATED FROM AUSTRALIAN ORCHIDS

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Abstract

The ultrastructure of the septal pore cap of six non-sporulating orchid mycorrhizal fungi tentatively identified as *Epulorhiza* sp. (*Sebacina*) was examined with the transmission electron microscope in an attempt to elucidate their taxonomic identity. Each fungus has morphological chartacteristics visible in the light microscope which are consistent with their tentative identity. Five of the fungi were found to have imperforate septal pore caps. One fungus was found to have perforate septal pore caps indicating that it is not a species of *Epulorhiza*.

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Introduction

Determining the identity of fungi that do not sporulate is particularly difficult in the absence of molecular information. Fungi isolated from peletons within roots of orchids usually have broad, smooth hyphae in culture and resemble *Rhizoctonia* (*sensu lato*). Analysis of the nuclear number and hyphal morphology of these fungi with the light microscope usually enables the fungi to be placed in either *Ceratorhiza* or *Rhizoctonia* (Moore 1987). However, fungi with fine hyphae cannot be identified as easily. They are usually placed in *Epulorhiza* (Moore 1987). This group, formerly called *Sebacina*, appears to be a complex of related fungi with various functional and morphological differences (Warcup 1988). Warcup & Talbot (1966) reported the diameter of hyphae of species of *Sebacina* to range from 2 to 4 micrometers. The fungi in the genera *Ceratorhiza* and *Rhizoctonia* are placed in the Order Ceratobasidiales by Wells (1994) or the Euholobasidiomycetes (Class 8) by Walker (1996). The fungi in the genus *Sebacina* are placed in the Order Auriculariales by Wells (1996) or the Holobasibial jelly fungi (Class 7) by Walker (1996).

Species of *Epulorhiza* (*sensu Sebacina*) have been reported in the roots of wild collected terrestrial orchids (Currah & Sherburne 1992, Perkins *et al.* 1995, Warcup 1988), but appear to be rare in epiphytes in nature, even though a fungus identified as *Epulorhiza* sp. (SS1) has been isolated from the epiphyte *Stanhopea tigrina* Bateman ex Lindl. being maintained in a glasshouse (Table 1; Esnault *et al.* 1994). However, this latter plant may have been colonized by *Epulorhiza* sp. in the absence of appropriate fungi with *in situ* specificity in its vicinity (Masuhara & Katsuya 1994, Perkins, Masuhara & McGee 1995). Thus the isolation of a fungus (SSO) with cultural and morphological characteristics that placed it in the genus *Epulorhiza* from the temperate epiphytic orchid *Sarcochilus olivaceous* Lindl. was unexpected (Markovina & McGee 2000). Subsequently, a similar fungus (SDF) has been isolated from a second epiphyte, *Dendrobium falcorostrum* Fitzg. (Perkins unpublished) indicating that *Epulorhiza* sp. may be more common among epiphytes from the field than we originally thought.

Because so many of our fungi have been identified as *Epulorhiza* sp. entirely from their cultural characteristics and hyphal morphology visible in the light microscope, confirmation of their taxonomic placement using other techniques is necessary. Members of the *Epulorhiza* group have flattened, imperforate septal pore caps over the dolipore septum (Currah & Sherburne 1992, Moore 1987, Williams & Thilo 1989). These contrast with the dome-shaped, perforate septal pore caps of *Ceratobasidium*, *Thanatephorus* and *Moniliopsis* (Currah & Sherburne 1992, Wells 1994). Variation in the ultrastructure of the dolipore septum is also discussed by Moore (1996). Since molecular approaches were unavailable to us, we examined the ultrastructure of a range of isolates from orchids putatively identified as *Epulorhiza* spp. to determine if the type of septal pore structure supported our identifications.

Materials and Methods

Eleven isolates of fungi from our culture collection (Table 1) were grown in NDY/6 broth medium in 250 ml Erlenmeyer flasks at room temperature for ten to 12 days. The composition of the medium (g/l) was as follows: Sucrose 5.0, Yeast Extract 0.08, $NaNO_3$ 0.34, KH_2PO_4 0.17, KCl 0.08, $MgSO_4$.7H2O 0.08, and $FeSO_4$ 0.002.

After growth in the medium, hyphal fragments were removed from the flasks, washed with distilled water on a nylon mesh filter and then fixed with 2.5% glutaraldehyde in 0.2M cacodylate buffer at pH 7.2 for three hours. After washing again with 0.2M cacodylate buffer, the hyphae were postfixed in 1% OsO_4 in 0.1M cacodylate buffer for two hours. The samples were then washed with distilled water, dehydrated in a series of graded ethanol and finally acetone solutions, and infiltrated and embedded in Spurr's resin. The resin was polymerized at 60% for two days. The blocks were sectioned with a diamond knife (Diatome). Ultrathin sections were examined with a Philips EM400 or a Philips CM12 transmission electron microscope.

Results

All isolates of *Ceratorhiza* (CL1, CSO and CSF) and *Rhizoctonia* (RsSo and AG6) that we examined have dome-shaped, perforated septal caps (Fig. 1). In contrast the five isolates of putative *Epulorhiza* (SS1, SDF, SM1P, SC1 and SM2) have flattened, imperforate septal caps (Figs 2 and 3). One isolate (SSO) with narrow hyphae, initially identified as *Epulorhiza* sp., was found to have dome-shaped, perforate septal caps (Fig. 4) similar to those found in the isolates of *Ceratorhiza* and *Rhizoctonia.*

Discussion

The structure of the septal pore cap has taxonomic significance. For example, Currah & Sherburne (1992) and Muller *et al.* (2000) used the ultrastructure of the septal pore cap as a characteristic to distinguish between genera of Basidiomycota. Currah & Sherburne (1992) reported flattened, imperforate septal pore caps in three isolates of *Epulorhiza* (including *Sebacina*). Also, imperforate septal pore caps were observed in *Epulorhiza* and *Sebacina* by Moore (1987) and by Williams & Thilo (1989). However, the Sebacinaceae is not the only group of Basidiomycota known to have imperforate septal pore caps. Wells (1994) listed groups of fungi in the Basidiomycota with imperforate septal pore caps including Auriculariales, Dacrymycetales and Tulasnellales. In contrast all members of the Ceratobasidiales have perforate caps according to Wells (1994). Walker (1996) placed fungi with imperforate septal pore caps over dolipore septa within either the Phragmobasidial jelly fungi or the Holobasidial jelly fungi. Recently, Muller *et al.* (2000) found imperforate septal pore caps in *Asterodon* and *Coltricia* (Hymenochaetaceae). Weiss & Oberwinkler (2001) consider that the Hymenochaetaceae are distantly related to the Sebacinaceae based on ribosomal DNA sequences. It is therefore possible that the flattened imperforate septal cap evolved more than once. Thus without molecular information, the fungi we consider to be *Epulorhiza* sp. (*Sebacina*) may be in either of two classes in the Basidiomycota. The taxonomic placement of the groups of fungi in the Basidiomycota, including orchid mycorrhizal fungi, is still under active consideration (Weiss & Oberwinkler 2001).

We believe that, based upon our investigation, we have five isolates of *Epulorhiza* sp. in culture, three of which came from terrestrial orchids, *Caladenia* and *Microtis*, and two from epiphytic orchids, *Stanhopea* and *Dendrobium*. However, it is clear that two different distantly related groups of fungi with two different types of septal pore structure can colonise a single orchid, and that these groups of fungi are found in both terrestrial and epiphytic orchids in the field (Table 1). We have no evidence that *Epulorhiza* sp. (SSO) colonises *S. olivaceous* in the field, although we have germinated seed with SS1 in the laboratory (McGee & Markovina unpublished) indicating possible nonspecific fungal associations (Masuhara & Katsuya 1994).

SSO was initially identified as *Epulorhiza* sp. because the hyphal diameter fell within the range given by Warcup & Talbot (1966) for this genus. Based on septal pore ultrastructure, the initial identification of SSO as *Epulorhiza* sp. is incorrect. This fungus should be placed within the Euholobasidiomycetes. These conclusions are justified as long as the present interpretation of taxonomic relationships is correct. The placement of SSO remains unknown, but it is a functional orchid mycorrhizal fungus and probably distantly related to *Epulorhiza* sp.

Table 1: Isolates of fungi with their tentative names and origins from orchid fungi collection.

ISOLATE	NAME AND ORIGIN
CL1	*Ceratobasidium* isolated from leaf litter around *Pterostylus ophioglossa* R. Br. at Bligh Park
CSO	*Ceratobasidium* isolated from *Sarcochilus olivaceus* Lindl. on 4/6/97 at Waterfall, Royal National Park
AG6	*Rhizoctonia solani* (OHT-1-1) isolated from soil in Hokkaido, Japan
CSF	*Ceratobasidium* sp. isolated from *Sarcochilus falcatus* R. Br. on 29/5/97 at Platypus pool, Wangat Lodge, Dungog
RsSo	*Rhizoctonia solani* isolated from *Sarcochilus olivaceus* Lindl. on 4/6/97 at Waterfall, Royal National Park
SSO	*Epulorhiza* sp. isolated from *Sarcochilus olivaceus* Lindl. on 4/6/97 at Waterfall, Royal National Park
SS1	*Epulorhiza* sp. isolated from *Stanhopea tigrina* Bateman ex Lindley on 1/4/93 in Sydney University glasshouse
SDF	*Epulorhiza* sp. isolated from *Dendrobium falcorostrum* Fitzg. on 22/4/97 in the Chichester forest, Mountaineer. Orchid collected from a dead beech tree growing at 1000 m altitude
SM1P	*Epulorhiza* sp. from *Microtis parviflora* R. Br. on 21/7/93 at Pitt Town then reinoculated and reisolated from protocorm
SC1	*Epulorhiza* sp. isolated from *Caladenia catenata* (Smith) Druce on 27/6/94 at South Windsor
SM2	*Epulorhiza* sp. isolated from *Microtis parviflora* R. Br. on 21/7/93 at Pitt town

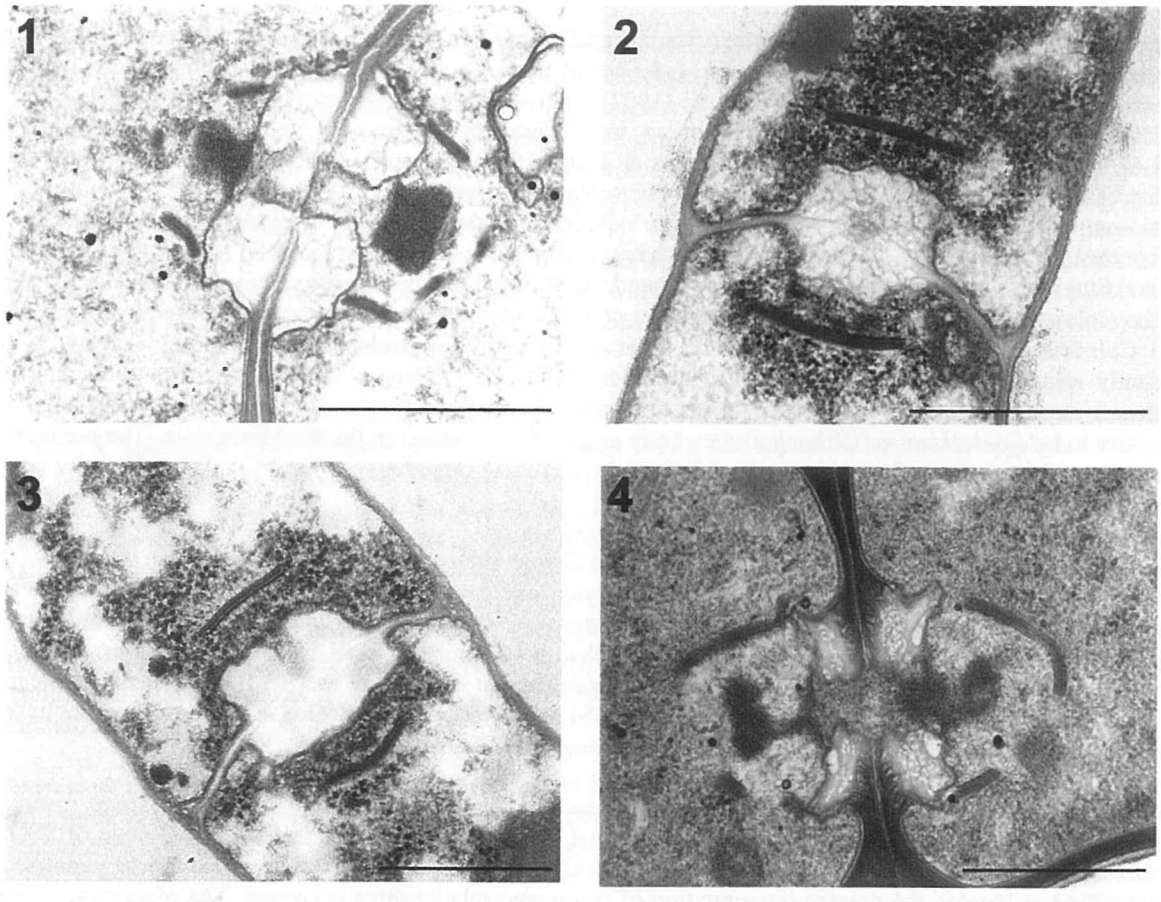

Figure 1. Dome-shaped, perforate septal pore caps in CSF. Scale bar equals 0.5 micrometer; **Figure 2.** Flattened, imperforate septal pore caps in SM2. Scale bar equals 0.5 micrometer; **Figure 3.** Flattened, imperforate septal pore caps in SSI. Scale bar equals 0.5 micrometer; **Figure 4.** Dome-shaped, perforate septal pore caps in SSO. Scale bar equals 0.5 micrometer.

Care should be taken with identifications based solely on morphological features as seen in the light microscope. Final placement of each of these fungi probably will rely on the use of molecular data. It is possible that the taxonomy of orchid mycorrhizal fungi requires substantial clarification.

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