

## Isolation and Identification of Mycorrhizal Fungi from Eleven Terrestrial Orchids

Pornpimon Athipunyakom<sup>1,2</sup>, Leka Manoch<sup>2</sup> and Chitrapan Piluek<sup>3</sup>

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### ABSTRACT

Mycorrhizal fungi were isolated from eleven terrestrial orchid species, namely *Calanthe rubens*, *Calanthe rosea*, *Cymbidium sinense*, *Cymbidium tracyanum*, *Goodyera procera*, *Ludisia discolor*, *Paphiopedilum concolor*, *P. exul*, *P. godefroyae*, *P. niveum* and *P. villosum*. Healthy roots of orchid hosts were collected from various parts of the country. Isolation of mycorrhizal fungi from pelotons was carried out using a modification of Masuhara and Katsuya method. Identification was based on morphological characteristics. Nuclei were stained with safranin O using Bandoni's method. Seven genera and fourteen species of mycorrhizal fungi were identified: *Ceratorhiza cerealis*, *C. goodyerae-repentis*, *C. pernacatena*, *C. ramicola*, *Ceratorhiza* sp., *Epulorhiza calendulina*, *E. repens*, *Rhizoctonia globularis*, *Sistotrema* sp., *Trichosporiella multisporum*, *Tulasnella* sp. *Waitea circinata* and two *Rhizoctonia* species. Nuclear staining revealed that all strains were binucleate except for *W. circinata* which was multinucleate. Pure cultures were maintained on PDA slant and liquid paraffin at the Culture Collection, Department of Plant Pathology, Kasetsart University.

**Key words** : *Ceratorhiza*, *Epulorhiza*, *Rhizoctonia*, mycorrhizal fungi, terrestrial orchids

### INTRODUCTION

Thailand is one of few countries in the world that is rich in orchid species. They are found in all different habitats with approximate a total of 170 genera and 1,230 species of which 150 species are considered endemic to the country. Among these 80% are epiphytic; most of the rest are terrestrial, and only few species are saprophytic orchids (Nanakorn, 2001). Terrestrial orchids, in their habitats, require the presence of suitable fungi in the living cells of the plant embryo and development multicellular absorptive structures

in order to develop and mature successfully (Currah *et al.*, 1990).

Mycorrhizal fungi are associated with the root systems of more than 90% of terrestrial plant species in a mutual symbiosis. The fungi obtain sugars from photosynthesis by the host plant in exchange for essential ions, namely phosphate and nitrate (Clements, 1988). In nature, all orchids utilize endomycorrhizal fungi to initiate seed germination, and seedling development; the availability of each fungi, therefore, is an absolute requirement of the orchid life cycle. The orchid-fungus symbiosis is initiated when orchid seeds

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<sup>1</sup> Plant Pathology Group, Plant Protection Research and Development Office, Department of Agriculture, Bangkok 10900, Thailand.

<sup>2</sup> Department of Plant Pathology, Faculty of Agriculture, Kasetsart University, Bangkok 10900, Thailand.

<sup>3</sup> Department of Horticulture, Faculty of Agriculture, Kasetsart University, Bangkok 10900, Thailand.

are infected by a suitable fungus (Arditti, 1982; Rasmussen, 1995). Compared with other angiosperms, orchid seeds are extremely small, almost dust-like, and they contain a very small food reserve, making them light. When an orchid seed lands on a substrate, the germination process is initiated when the fungus penetrates the seed testa and invades to the embryo (Clements, 1988; Rasmussen, 1995). Fungal hyphae infect cortical cells, forming masses of tightly-interwoven coils called pelotons. Pelotons are considered to be the most distinctive characteristic of orchid mycorrhiza (Currah and Zelmer, 1992). The pelotons are digested by the host orchid in a controlled manner, this enables carbohydrates tied up within the hyphal cells to be released and absorbed. Most peloton-forming fungi have been classified as members of the anamorphic form-genus *Rhizoctonia*, on the basis of overall morphological characteristics. Most isolates remain without reproductive structures in pure culture. In rare case when the teleomorphic stage is induced, basidia and basidiospores are borne in the absence of a fruiting body (Zettler, 1997).

Moore (1987) described new anamorphic genera formally placed in *Rhizoctonia*. He based the new treatments on morphological characteristics such as nuclear condition and septal ultrastructure, as well as teleomorphic stage. Three new genera emerged that are currently recognized: *Ceratorhiza*, *Epulorhiza*, and *Moniliopsis*. Binucleate strains of *Rhizoctonia* with perforate parentheses, and *Ceratobasidium* teleomorphs are included in *Ceratorhiza*. Binucleate strains of *Rhizoctonia* with imperforate parentheses and *Tulasnella* or *Sebacina* teleomorphs are placed in *Epulorhiza*. Multinucleate strains with perforate parentheses and *Thanatephorus* or *Waitea* teleomorphs are in *Moniliopsis*.

In Thailand, researchs on orchid mycorrhizal fungi were conducted by Manoch *et al.* (2000), Kummuang *et al.* (2000), Sangthong and Smitamana (2002) and Athipunyakom *et al.*

(2004).

Because of a virtual lack of knowledge of the biodiversity of the mycorrhizal fungi of tropical Orchidaceae, the distribution and identification of fungi from a variety of tropical terrestrial orchids were examined. The objectives were to isolate and identify the naturally occurring mycorrhizal fungi of terrestrial orchids from various habitats.

## MATERIALS AND METHODS

### Collection sites

Healthy roots of eleven terrestrial orchids, namely *Calanthe rubens*, *Calanthe rosea*, *Cymbidium sinense*, *Cymbidium tracyanum*, *Goodyera procera*, *Ludisia discolor*, *Paphiopedilum concolor*, *P. exul*, *P. godefroyae*, *P. niveum* and *P. villosum* were collected from various locations, Mae Hong Son, Chiang Mai, Bangkok, Chanthaburi, Kanchanaburi, Nakhon Ratchasima, Udon Thani, Trang, Krabi and Satun during 1999-2001 (Table 1). They were taken, wrapped in tissue paper, put in plastic bag and kept in an ice box during transportation to the laboratory.

### Fungal isolation

Mycorrhizal fungi were isolated using a modification of Masuhara and Katsuya methods (Athipunyakom *et al.*, 2004). The root segments were treated with 5% sodium hypochlorite (NaOCl) for surface sterilization. After shaking for 5 min, they were rinsed three times with sterile distilled water. The roots were then cut into longitudinal sections and observed for the presence of hyphal coils (pelotons) on a glass slide under a stereo-microscope in sterile condition. The epidermal layer was then removed to avoid contamination. The pelotons were transferred from the inner cortex, macerated with a sterile fine needle, placed in a few drop of sterile water in a watch glass and rinsed 3-5 times in sterile water. A single peloton was placed on Masuhara and Katsuya 1/6 NDY medium containing 100 mg/l

streptomycin and 50 mg/l tetracycline in a petridish and incubated in the dark at 28 °C. After 3 to 7 days incubation, hyphal tips were transferred onto a potato dextrose agar (PDA) slant. Pure cultures were maintained on PDA slant at the Culture Collection, Department of Plant Pathology, Faculty of Agriculture, Kasetsart University for identification.

### Fungal identification

Macroscopic features examined were colony growth pattern, color and sclerotia formation. Fungal growth rate was measured from the colony on PDA. For microscopic examination, fertile hyphae were mounted in sterile water on a microscopic slide, covered with a cover slip, and examined under a light microscope. Hyphal and

**Table 1** Mycorrhizal fungi isolated from healthy roots of eleven terrestrial orchids collected from different locations.

KUFC No.	Mycorrhizal fungi	Orchid hosts	Location	Collection time
5-6	<i>Ceratorhiza cerealis</i>	<i>Goodyera procera</i>	QSB*, Chiang Mai	Dec/ 1999
19-1	<i>C. goodyerea-repentis</i>	<i>Ludisia discolor</i>	KU*, Bangkok	May/2000
36-1	<i>C. goodyerae-repentis</i>	<i>Ludisia discolor</i>	QSB*, Chiang Mai	Oct/2000
5-10	<i>C. goodyerae-repentis</i>	<i>Goodyera procera</i>	QSB*, Chiang Mai	Dec/1999
32-2	<i>C. goodyerae-repentis</i>	<i>Goodyera procera</i>	Mae Hong Son	Oct/2000
13-1	<i>C. pernacatena</i>	<i>Calanthe rubens</i>	KU*, Bangkok	Jan/2000
12-5	<i>C. ramicola</i>	<i>Paphiopedilum exul</i>	KU*, Bangkok	Jan/2000
32-1	<i>Ceratorhiza</i> sp.	<i>Goodyera procera</i>	Mae Hong Son	Oct/2000
4-2	<i>Epulorhiza calendulina</i>	<i>Paphiopedilum concolor</i>	Kanchanaburi	Jan/2000
27-9	<i>E. repens</i>	<i>Calanthe rosea</i>	Chanthaburi	Jul/2000
23-2	<i>E. repens</i>	<i>Cymbidium sinense</i>	QSB*, Chiang Mai	Dec/ 1999
16-4	<i>E. repens</i>	<i>Cymbidium tracyanum</i>	QSB*, Chiang Mai	May/2000
6-2	<i>E. repens</i>	<i>Paphiopedilum concolor</i>	KU*, Bangkok	Dec/1999
18-10	<i>E. repens</i>	<i>Paphiopedilum concolor</i>	Udon Thani	Mar/2000
17-2	<i>E. repens</i>	<i>Paphiopedilum exul</i>	KU*, Bangkok	Jan/2000
37-2	<i>E. repens</i>	<i>Paphiopedilum exul</i>	Krabi	Mar/2000
21-1	<i>E. repens</i>	<i>Paphiopedilum villosum</i>	QSB*, Chiang Mai	May/2000
5-21	<i>Rhizoctonia globularis</i>	<i>Goodyera procera</i>	QSB*, Chiang Mai	Dec/1999
11-2	<i>Rhizoctonia</i> sp. 1	<i>Paphiopedilum niveum</i>	KU*, Bangkok	Dec/1999
37-5	<i>Rhizoctonia</i> sp.2	<i>Paphiopedilum exul</i>	Krabi	Oct/2000
33-1	<i>Sistotrema</i> sp.	<i>Paphiopedilum godefroyae</i>	Trang	Oct/2000
11-13	<i>Trichosporiella multisporum</i>	<i>Paphiopedilum niveum</i>	KU*, Bangkok	Dec/1999
16-2	<i>Tulasnella</i> sp.	<i>Cymbidium tracyanum</i>	QSB*, Chiang Mai	May/2000
39-1	<i>Waitea circinata</i>	<i>Paphiopedilum niveum</i>	Satun	Aug/2001

QSB\* = Queen Sirikit Botanic Garden, Chiang Mai

KU\* = Kasetsart University, Bangkok

monilioid cells were measured. The number of nuclei per cell was determined by examination after staining with safranin O-KOH using Bandoni's method (Bandoni, 1979).

## RESULTS AND DISCUSSION

Mycorrhizal fungi associated with eleven terrestrial orchids including seven genera and fourteen species were found: *Ceratorhiza cerealis*, *C. goodyerae-repentis*, *C. pernacatena*, *C. ramicola*, *Ceratorhiza* sp., *Epulorhiza calendulina*, *E. repens*, *Rhizoctonia globularis*, *Sistotrema* sp., *Trichosporiella multisporum*, *Tulasnella* sp., *Waitea circinata* and two *Rhizoctonia* species (Table 1). Nuclear staining revealed that cells of all strains were binucleate except for *W. circinata* which had multinucleate cells. Of the seven genera, six were orchidaceous rhizoctonias based on the board, clampless hyphae bearing monilioid cells (Burgeff, 1959; Sneh *et al.*, 1991). *Sistotrema* sp. produced clamp connection (Figure 2C) and *Trichosporiella multisporum* formed conidia. In this study, *Rhizoctonia* species failed to produced the teleomorph stage in culture. However, *Waitea circinata* isolated from the peloton of *Paphiopedilum niveum* formed a structure resembled basidium on PDA (Figure 2E) but no basidiospore produced.

The results from the present study showed that a number of mycorrhizal fungi were associated with a single orchid host, for example *Ceratorhiza ramicola*, *Epulorhiza repens* and *Rhizoctonia* sp. 2 associated with *Paphiopedilum exul*; *Epulorhiza calendulina* and *Epulorhiza repens* associated with *Paphiopedilum concolor*; and *Waitea circinata*, *Trichosporiella multisporum* and *Rhizoctonia* sp. 1 associated with *Paphiopedilum niveum*. In contrast with a specific mycorrhizal fungi, *Sistotrema* sp. merely associated with a single orchid host, *Paphiopedilum godefroyae* (Table 1).

*Ceratorhiza cerealis* (Van der Hoeven) Moore

KUFC 5-6

Class Agonomycetes, Order Agonomycetales

Basionym: *Rhizoctonia cerealis* Van der Hoeven

Teleomorph: *Ceratobasidium cornigerum* (Bourdot) D.P. rogers  
(*Ceratobasidium cereal* Murray & Burpee)

On PDA, colony grew rapidly, 9 cm in diam. after 5 days incubation at 28°C. Colony was white to light brown in two weeks. Vegetative hyphae 2.7-6.7 µm wide, septate, binucleate. Monilioid cells barrel-shaped to cylindrical, 9.3 – (19.5) – 27.3 × 8.0 – (10.3) – 13.3 µm in diam. Aerial and submerged hyphae formed on culture media, sometimes aggregated to form loose hyphae and turned to brown sclerotia.

Host : Root of *Goodyera procera*, Queen Sirikit Botanic Garden, Chiang Mai.

This study indicated that *Ceratorhiza cerealis* was isolated from *Goodyera procera*. The teleomorph state of this fungus, *Ceratobasidium cerealis* is a cosmopolitan, saprotrophic, plant parasitic and orchid endomycorrhizal species. Burpee (1980) reported that *Rhizoctonia cerealis* caused chlorosis and blight of turfgrasses. However, this fungus was also used to germinate orchid seeds. Weber and Webster (2001) reported that *Rhizoctonia cerealis* causing sharp eyespot on the stem base of cereals and grasses. This fungus was tested to stimulate the germination of *Dactylorhiza maculata* ssp. *ericetorum* seeds *in vitro*. The result showed that the orchid seeds could germinate within 30 days and the protocorm developed a green shoot tip after expose to daylight within 90 days after sowing. Smreciu and Currah (1989) reported that *Ceratobasidium cerealis*, teleomorphic state of *Ceratorhiza cerealis* stimulated germination of eleven orchid species in Canada.

***Ceratorhiza goodyerae - repentis*** (Costantin and Dufour) Moore (Figure 1A - 1D)

KUFC 19-1, KUFC 36-1, KUFC 5-10, KUFC 32-2

Class Agonomycetes, Order Agonomycetales

Basionym: *Rhizoctonia goodyerae-repentis*  
Costantin & Dufour

Teleomorph: *Ceratobasidium cornigerum*  
(Bourd.) Rogers

On PDA, colony grew rapidly, 9 cm in diam after 4 days incubation at 28°C, white to cream when young turned to orange-brown to dark brown at maturity, with concentric zonation (Figure 1B). After 14 days, aerial hyphae hyaline to tan, fluffy, sometimes aggregated to form loose hyphae and turned to sclerotia. Vegetative hyphae 4.0 – 5.3 µm wide, septate, binucleate (Figure 1D). Monilioid cells barrel-shaped to elliptical 15.4 – (20.6) – 26.4 × 6.9 – (10.6) – 11.3 µm (Figure 1C).

HOST : Root of *Goodyera procera*, QSB (Chiang Mai) and Mae Hong Son, Root of *Ludisia discolor*, QSB (Chiang Mai) and Kasetsart University (Bangkok).

*Ceratorhiza goodyera - repentis* was first reported from mycorrhiza of terrestrial orchid, *Goodyera repens* R. Br. by Costantin and Dufour in 1920. Later on, there were several reports from terrestrial orchids roots in Canada and U.S.A. (Alexander and Hadley, 1985; Currah *et al.*, 1990; Richardson *et al.*, 1993; Zelmer and Currah, 1995).

***Ceratorhiza pernacatena* (Zelmer and Currah)**  
KUFC 13-1 (Figure 1E - 1F)

Class Agonomycetes, Order Agonomycetales

Teleomorph: *Ceratobasidium*

On PDA, colony grew rapidly, 9 cm in diam after 5 days incubation at 28°C, white to light buff, aerial hyphae bearing scattered patches of dense, cottony, with concentric zonation (Figure

1F). Vegetative hyphae 3.5 – 5.8 µm wide, hyaline septate, binucleate. Monilioid cells developing blastically, globose, 18-21 (23) µm wide, forming chain of monilioid cells with narrow, tubular connections and septa between adjacent cells (Figure 1E), aggregated to form loose sclerotia (Figure 1F).

HOST : Root of *Calanthe rubens*, Kasetsart University (Bangkok).

Zelmer and Currah (1995) isolated *Ceratorhiza pernacatena* sp., nov. from terrestrial orchid, *Platanthera praeclara*. The fungus produced nearly globose monilioid cell, 20-23 (25) µm wide, connected by a narrow septate isthmus. The monilioid cells of *C. pernacatena* in this study formed constrictions at the septa between adjacent monilioid cells which was the main characteristic of this species. The size and shape of monilioid cells are similar to our isolate.

***Ceratorhiza ramicola*** (Weber and Roberts) Moore  
KUFC 12-5 (Figure 1G - 1H)

Class Agonomycetes, Order Agonomycetales

Basionym: *Rhizoctonia ramicola* Weber & Roberts

Teleomorph: *Ceratobasidium cornigerum*  
(Bourd.) Rogers

*Ceratobasidium ramicola* Tu,  
Roberts & Kimbrough

On PDA, colony reaching 9 cm in diam after 5 days incubation at 28°C, white, aerial hyphae, cottony, turned to light brown with age (Figure 1H). Vegetative hyphae 3.5-6 µm wide, septate and frequently anastomosed, binucleate, hyaline when young turned to brown, rich in oil globules and granular matters (Figure 1G), branching often upright angles. Monilioid cells and sclerotia absent.

HOST : Root of *Paphiopedilum exul*, Kasetsart

University (Bangkok).

*Ceratorhiza* sp. (KUFC 36-6)

Class Agonomycetes, Order Agonomycetales

On PDA, colony grew rapidly, 9 cm in diam after 5 days incubation at 28°C, white to light brown, turned to dark brown with age, with concentric zonation, and abundant aerial hyphae. Vegetative hyphae septate, binucleate, hyaline to light brown, branching at upright angle, rich in oil globule. Monilioid cells elongate shape, hyaline to light brown.

HOST : Root of *Goodyera procera*, Mae Hong Son.

*Epulorhiza calendulina* Zelmer and Currah (KUFC 4-1)

Class Agonomycetes, Order Agonomycetales

On PDA, colony reaching 3.5 cm in diam. after 10 days incubation at 28°C, salmon buff to light ochraceous buff, submerged with short dense aerial hyphae on the surface. Vegetative hyphae 3.3-4.0 µm. wide, septate, binucleate. Monilioid cells clavate to irregular shaped, developing blastically in chain. The fungus failed to produce teleomorphic stage in culture.

HOST : Root of *Paphiopedilum concolor*, Kanchanaburi

*E. calendulina* is distinguished from *E. repens* by the colony color and irregular shaped of monilioid cells. However, *E. calendulina* is similar to the other *Epulorhiza* species in having slow growth and colony with leathery, plane aerial mycelial mat on PDA, and the formation of flattened imperforate parentheses (Currah and Sherburne, 1992). Zelmer and Currah (1995) isolated this mycorrhizal fungi from native terrestrial orchid, *Amerorchis rotundifolia*, in Alberta, Canada and

identified as a new species, *Epulorhiza calendulina* sp. nov.

*Epulorhiza repens* (Bernard) Moore KUFC 27-9, KUFC 23-2, KUFC 16-4, KUFC 6-2, KUFC 18-10, KUFC 17-2, KUFC 37-2, KUFC 21-1

Class Agonomycetes, Order Agonomycetales

Basionym: *Rhizoctonia repens* (Bernard)

Teleomorph: *Tulasnella calospora* (Boud.) Juel

On PDA, colony reaching 9 cm in diam after 10 days incubation at 28°C, white to cream, submerged with glabrous surface and concentric zonation. On CMA, mycelium submerged, white to cream, sclerotia minute, composing of loosely arranged clusters of monilioid cells. Vegetative hypha 2.5-4.0 µm wide, hyaline, septate, binucleate, constricted at branch points. Monilioid cells, hyaline, ellipsoidal to spherical, 7.5-14.2 × 6.5-10.8 µm, in short branched or unbranched.

HOST : Root of *Calanthe rosea*, Chanthaburi; *Cymbidium sinense* (QSB, Chiang Mai); *Cymbidium tracyanum* (QSB, Chiang Mai); *Paphiopedilum concolor*, KU (Bangkok), Udon Thani; *Paphiopedilum exul*, KU, (Bangkok), Krabi; *Paphiopedilum villosum* (QSB, Chiang Mai)

*Rhizoctonia repens*, a ubiquitous species of mycorrhizal fungus frequently found in orchids, a originally isolated and described by Bernard (1909) as orchid symbiont with *Laelio-Cattleya canhamiana*. The fungus produced monilioid cells and pelotons formed in culture of the fungus. The main characteristics of this taxon were creamy white color of hyphae and the presence of monilioid cells in short chains. After Bernard (1909), there were several reports of this mycorrhizal fungi from various orchid species, such as Burgeff (1959), Curtis (1939). Curtis (1939) isolated orchid mycorrhizal fungi from Wisconsin and identified as *Rhizoctonia repens* based on the lack of aerial mycelium and the size and shape of monilioid cells

as described Bernard. Warcup and Talbot (1967) used a soil agar casing method and succeeded to induce *Tulasnella calospora* (Boudier) Juel teleomorph of *R. repens*. Currah *et al.* (1987) described *Epulorhiza repens* from the root of *platanthera obtusata* in Alberta, Canada. In Thailand, Athipuyakom *et al.* (2004) isolated several strains of *Epulorhiza repens* from terrestrial orchid, *Spathoglottis plicata* in Chiang Mai and Chanthaburi. These mycorrhizal fungi were tested to promote germination and development of *S. plicata* seeds *in vitro*. The result indicated that *E. repens* (KUFC 14-6) was the most effective mycorrhizal fungi to stimulate growth and development of *S. plicata*. Viable seedling became mature enough for planting within 127 days.

***Rhizoctonia globularis*** Saksena and Vaartaja  
KUFC 5-21

Class Agonomycetes, Order Agonomycetales

Basionym: *Opadorhiza globularis* (H.K. saksena & vaartaja) T.F. Andersen & R.T. Moore

Telomorph: *Endoperplexa enodulosa* (Hauerslev) P. Roberts

On PDA, colony white to pale pink, submerged, with thin smooth aerial mycelium in the centre. Vegetative hyphae septate, 2.5-3.5  $\mu\text{m}$  wide, branching often at nearly upright angles but more commonly at 45°. Monilioid cells, hyaline, globose, 8-10.5  $\mu\text{m}$  diam, produced in short branched chain of 3-8 cells.

*R. globularis* is close to *R. repens* as described by Burgeff and Curtis. The conspicuous radial growth of surface mycelium with concentric zonation and the smaller monilioid cells with typical mode of attachment in a chain distinguishing *R. globularis* from *R. repens* (Saksena and Vaartaja, 1960).

HOST : Root of *Goodyera procera*, QSB (Chiang Mai)

***Rhizoctonia sp. 1*** KUFC 11-2

Class Agonomycetes, Order Agonomycetales

On PDA, colony reaching 9 cm in diam after 7 days incubation at 28°C, white to cream, submerged, sometimes zonation occurred. Vegetative hyphae 3.2-4.5  $\mu\text{m}$  wide, septate, binucleate, containing numerous oil globules. Monilioid cells hyaline, spherical to oval or dumbbell shaped, 7.5×14.0–10.0-12.5  $\mu\text{m}$  in diam, containing oil globule. Cluster of monilioid cells loosely arranged, submerged and developed to minute sclerotia.

Host : Root of *Paphiopedilum niveum*, KU, Bangkok

On PDA, colony color of *Rhizoctonia sp. 1* was similar to that of *Epulorhiza repens*, but no oil globule produced in neither vegetative hyphae nor monilioid cells.

***Rhizoctonia sp. 2*** KUFC 37-5

Class Agonomycetes, Order Agonomycetales

On PDA, colony grew moderately, reaching 5.6 cm in diam after 10 days incubation at 28°C, white to cream, submerged with dense aerial hyphae, forming granular aggregations in the centre. Vegetative hyphae septate, branching at upright angles. Monilioid cells absent.

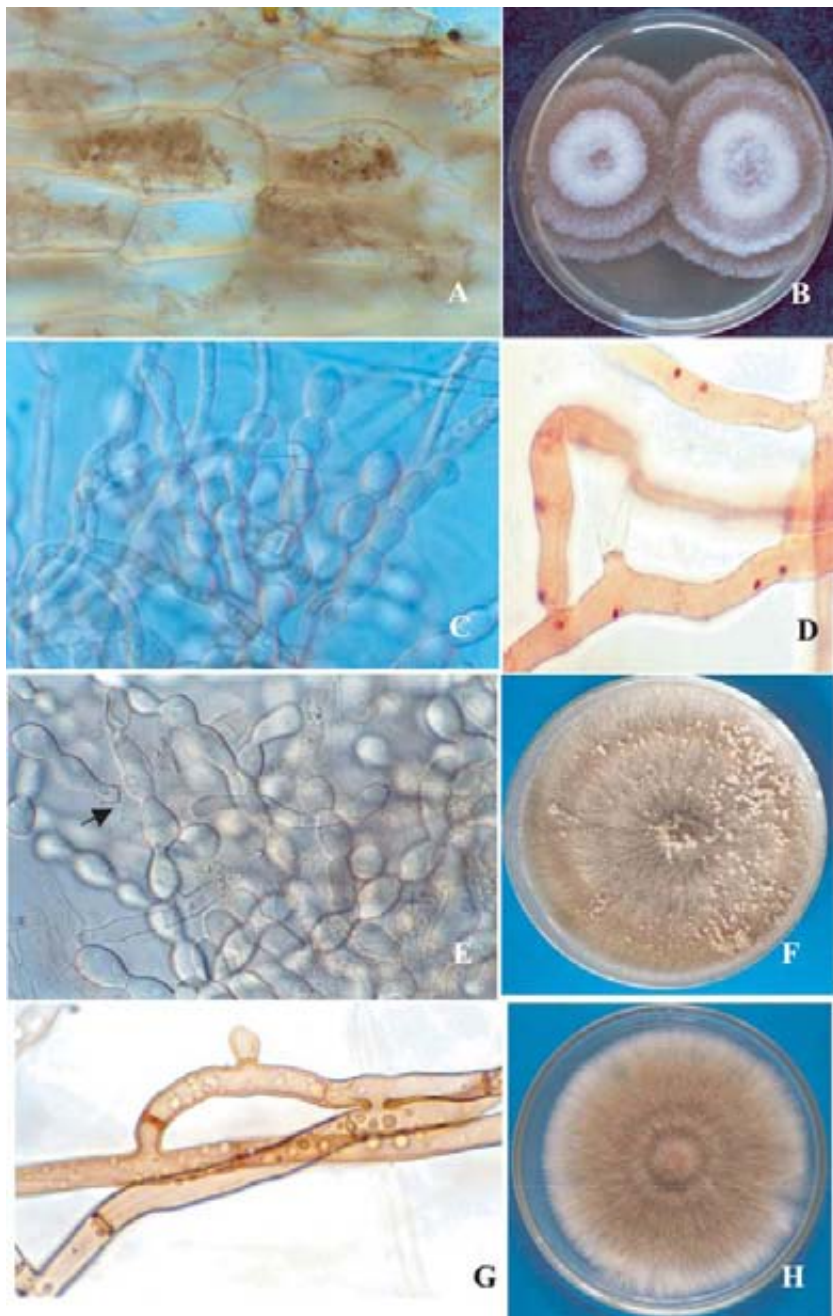
Host ; Root of *Paphiopedilum exul*, Krabi

***Sistrotrema sp.*** KUFC 33-1 (Figure 2A - 2D)

Class Basidiomycetes, Order Stereales, Family Sistotremataceae

On PDA, colony reaching 5 cm in diam after 10 days incubation at 28°C, cream to yellow, with aerial hyphae (Figure 2B).

On PDA, colony reaching 5 cm in diam after 10 days incubation at 28°C, cream to yellow.



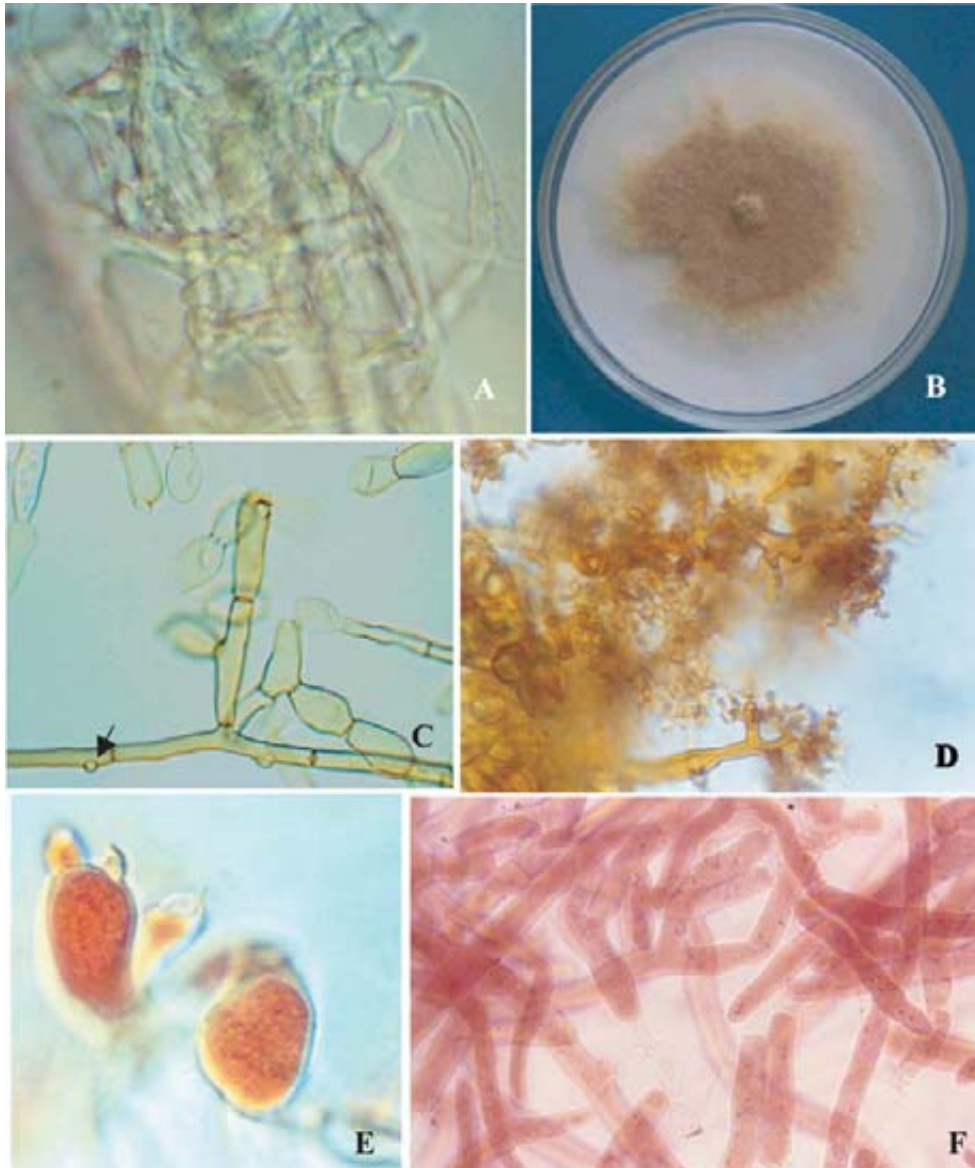
**Figure 1** *Ceratorhiza goodyerae repentis* (KUFC 32-1): pelotons within root cortical cells 200X (A); colony on PDA, 7 days at 28°C, showing concentric zonation (B); barrel shape monilioid cells on PDA, 14 days at 28°C 400X (C); binucleate cells on V-8 agar, 3 days at 28°C 1,000X (D); *Ceratorhiza pernacatena* (KUFC 13-1): chain of monilioid cells with slight tubular constriction (arrowhead) 400X (E); colony on PDA, 14 days at 28°C (F); *Ceratorhiza ramicola* (KUFC 12-5): vegetative hyphae with oil globules 1,000X (G); colony on PDA, 5 days at 28°C (H).



with aerial hyphae (Figure 2B). Vegetative hyphae 2-3  $\mu\text{m}$  wide, septate. Monilioid cells 7.5-11.0  $\mu\text{m}$  wide, hyaline to yellow brown, with clamp connection (Figure 2C).

HOST : Root of *Paphiopedilum godefroyae*, Trang

*Sistrotrema* sp. was the only mycorrhizal fungi with clamp connection. Currah *et al.* (1990)



**Figure 2** *Sistrotrema* sp. (KUFC 33-1): pelotons within root cortical cells 1,000X (A); colony on PDA, 10 days at 28°C (B); clavate monilioid cells and vegetative hypha with clamp connection (arrow head) 400X (C); *Waitea circinata* (KUFC 39-1): branching hyphae 400X (D); swollen cells structure resembled basidia producing basidiospores 500X (E); multinucleate cell 1,000X (H).

reported that this fungus was first described as mycorrhizal fungus associated with terrestrial orchids, *Piperia unalascensis* and *Platanthera obtusata* in Alberta, Canada.

***Waitea circinata*** Warcup and P.H.B. Talbot  
KUFC 39-1 (Figure 2D - 2F)

Class Basidiomycetes, Order Stereales, Family  
Botryobasidiaceae

Anamorph: *Rhizoctonia zeae* Voorhees

On PDA, colony pink to brown, drying pinkish buff. Vegetative hyphae were convoluted branching (Figure 2D). This fungus formed a structure resembling to basidia  $10-18 \times (4.5) 5.5-6.5 (-8) \mu\text{m}$  wide, and basidiospore on PDA (Figure 2E). Vegetative hyphae  $2.5-4.6 \mu\text{m}$  wide, septate, multinucleate cells, convoluted branching hyphae (Figure 2D).

Host : Root of *Paphiopedilum niveum*, Satun.

*Waitea circinata* was the only multinucleate strain of orchid mycorrhizal fungi isolated from the pelotons in the cortical cells of *Paphiopedilum niveum* root. It is the first report of this taxon from the orchid host.

Warcup and Talbot (1967) isolated and described *W. circinata* from soil in Australia and reported that Dr. O. Vaartaja could induce sporulation of *W. circinata* on cornmeal agar. Symbiotic germination tests with non-orchidaceous isolates of *W. circinata* and a species of multinucleate *Rhizoctonia* from buckwheat stems failed to stimulate terrestrial orchid, *Spiranthes sinensis* seeds *in vitro* (Masuhara *et al.*, 1994).

***Trichosporiella multisporum*** Sigler and Currah  
KUFC 11-13

Mitosporic Fungi

On PDA colony reaching 5 cm after 20 days incubation at  $28^{\circ}\text{C}$ , initially flat, yellow, with

white aerial hyphae. Vegetative hyphae  $2-3.5 \mu\text{m}$  wide, septate, bearing abundant lateral conidia. Conidia smooth walled, globose to ellipsoidal,  $2.5-5.5 \times 4-5 \mu\text{m}$ . wide. Colony resemble *Rhizoctonia* in culture features.

Host : Root of *Paphiopedilum niveum*, KU, Bangkok

Currah *et al.* (1987) described mycorrhizal fungi isolated from terrestrial orchid, *Coeloglossum viride* in Alberta, Canada and identified as *Trichosporiella multisporum* Sigler and Currah sp. nov. On PDA, colony reaching 4 cm in diam after 28 days. flat, sulcate, glabrous and yellow, developing sparse tufts of white aerial mycelium toward the center of the colony, margin entire, reverse golden yellow to pale orange. Vegetative hyphae septate,  $2.5-3 \mu\text{m}$  in diam, bearing abundant lateral conidia, rarely sessile, short-pedicellate, conidia smooth-walled, globose to ellipsoidal,  $3-5 \times 3.5-4.5 \mu\text{m}$ .

Most orchid mycorrhizal fungi are in the form genus *Rhizoctonia*. However the classification of *Rhizoctonia* spp. is complicated due to the lack of spore production (Sneh *et al.*, 1991). Attempt was made to identify *Tulasnella*, *Ceratobasidium* and *Thanatephorus* teleomorphs of *Rhizoctonia* by using molecular technique (Pope and Carter, 2001; Otero *et al.*, 2002; Shan *et al.*, 2002). With this regard, the study on orchid mycorrhizae would be most successful in term of the specificity between mycorrhizal fungi and the orchid hosts.

The important plant pathogen species complex *Rhizoctonia solani* (teleomorphs *Thanatephorus*, *Ceratobasidiales*) possess multinucleate cells, binucleate cells have found in the genus *Ceratobasidium* (*Ceratobasidiales*) and *Tulasnella* (*Tulasnelles*). Uninucleate strains occur in anamorphs of *Ceratobasidium*, but have rarely been reported (Otero *et al.*, 2002).

An addition way to classify these fungi is by anastomosis groups (AG). *Rhizoctonia solani* has 13 AGs. The binucleate *Rhizoctonia* spp.

include 21 AGs (Sneh *et al.*, 1991), and the uninucleate *Rhizoctonia* spp. include only one AG to date (Sen *et al.*, 1999). The *Rhizoctonia solani* (multinucleate) AGs known to be associated with orchids are AG-6 and AG-12 (Carling *et al.*, 1999). Nevertheless, the most common group of orchid mycorrhizal fungi is binucleate (Sneh *et al.*, 1991). Uninucleate *Rhizoctonia* have not been previously reported from orchid roots (Otero *et al.*, 2002).

### CONCLUSION

Seven genera and fourteen species of mycorrhizal fungi were isolated from the peloton in the root cortical cells of eleven terrestrial orchids. They were cultivated on PDA and identified as *Ceratorhiza cerealis*, *C. goodyerae-repentis*, *C. pernacatena*, *C. ramicola*, *Ceratorhiza* sp., *Epulorhiza calendulina*, *Epulorhiza repens*, *Rhizoctonia globularis*, *Sistotrema* sp., *Trichosporiella multisporum*, *Tulasnella* sp. *Waitea circinata* and two *Rhizoctonia* species. Nuclear staining revealed that cells of all strains were binucleate except for *W. circinata*, which had multinucleate. *Epulorhiza repens* was the most common mycorrhizal fungus found. It was abundant in association with the root cortex of six different terrestrial orchids, namely *Calanthe rosea*, *Cymbidium sinense*, *Cymbidium tracyanum*, *Paphiopedilum concolor*, *Paphiopedilum exul* and *Paphiopedilum villosum*; *Ceratorhiza goodyerae-repentis* associated with two species of terrestrial orchids, *Goodyera* and *Ludisia* discolor. Mycorrhizal fungi associated with a single orchid host were *Ceratorhiza cerealis* with *Goodyera procera*, *Ceratorhiza pernacatena* with *Calanthe rubens*, *Ceratorhiza ramicola* with *Paphiopedilum exul*, *Ceratorhiza* sp. with *Goodyera procera*, *Epulorhiza calendulina* with *Paphiopedilum concolor*, *Rhizoctonia globularis* with *Goodyera procera*, *Rhizoctonia* sp.1 with *Paphiopedilum niveum*, *Rhizoctonia* sp. 2 with *Paphiopedilum exul*, *Sistotrema* sp. with *Paphiopedilum*

*godefroyae*, *Trichosporiella multisporum* with *Paphiopedilum niveum*, *Tulasnella* sp. with *Cymbidium tracyanum* and *Waitea circinata* with *Paphiopedilum niveum*.

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