MYCOTAXON

Volume LXI, pp. 335-342 January-March 1997

EPULORHIZA INQUILINA SP. NOV. FROM *PLATANTHERA* (ORCHIDACEAE) AND A KEY TO *EPULORHIZA* SPECIES

R. S. Currah
Dept. of Biological Sciences, University of Alberta,
Edmonton, Alberta, T6G 2E9, Canada

L. W. Zettler[1]
Dept. of Biology, Plyler Hall, Furman University
Greenville, South Carolina, 29613, USA

and

T. M. McInnis
Dept. of Biological Sciences, 132 Long Hall, Clemson University
Clemson, South Carolina, 29634-1903, USA

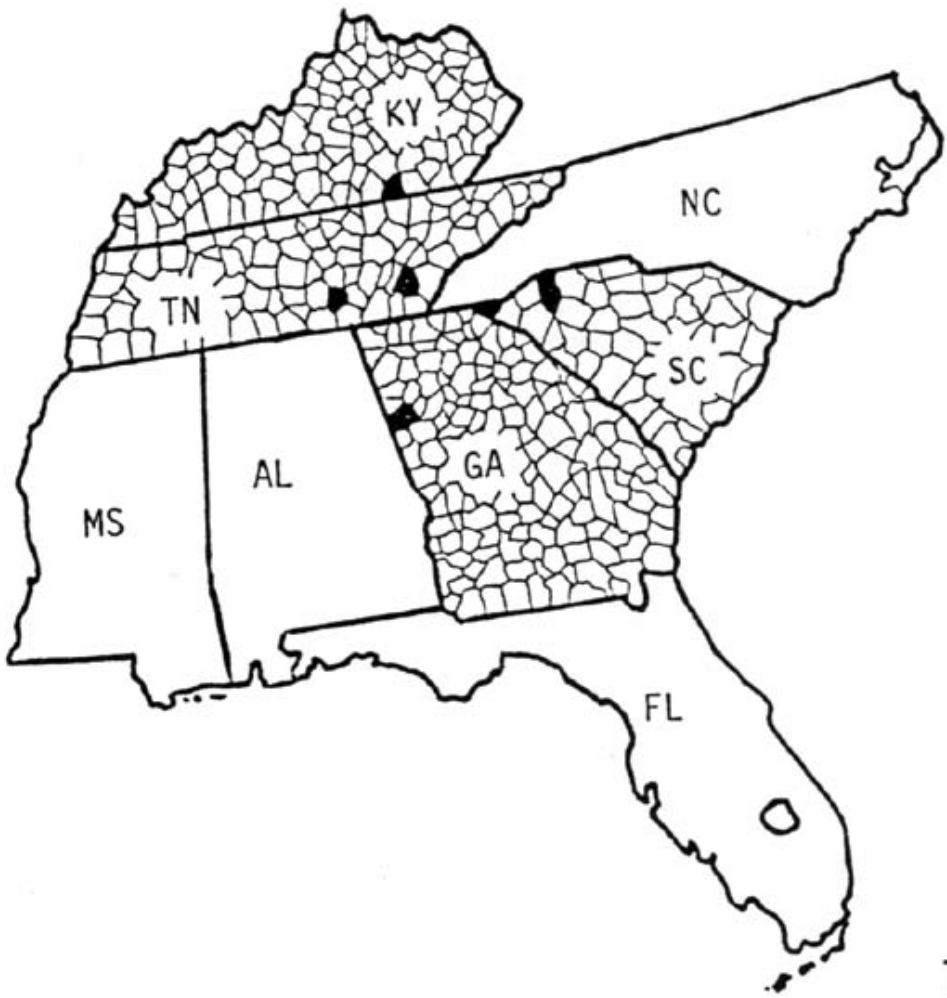
Abstract

Epulorhiza inquilina sp. nov. is described from the mycorrhizas of mature plants of *Platanthera clavellata, P. cristata* and *P. integrilabia*, coexisting terrestrial orchids native to bogs in the southern Appalachians. The new taxon was isolated consistently and exclusively from these orchid taxa over a seven-year period. Protocorms from seeds of *P. integrilabia* planted in their native habitat were also colonized by *E. inquilina* and consequently some specificity in the symbiosis is suspected. *E. inquilina* is binucleate and has imperforate parenthesomes but differs from other species in the genus because of its woolly colonial morphology, tan colour on artificial media, and its elongate monilioid cells that develop in relatively short chains. The five species of *Epulorhiza* are distinguished in a key.

Introduction

Epulorhiza Moore (1987) is one of the most common and distinctive form-genera of Basidiomycotina that form mycorrhizas with terrestrial orchids (Currah and Zelmer, 1992). It appears to be less common, if it occurs at all, in the mycorrhizas of tropical epiphytic representatives of the Orchidaceae (Richardson and Currah 1995, Richardson et al., 1993). Recently, one of us (LWZ) recovered a number of strains of an undescribed species of *Epulorhiza* from the mycorrhizas of three species of *Platanthera* (*P. clavellata* (Michaux) Luer, *P. cristata* (Michaux) Lindley and *P. integrilabia* (Correll)

[1] Dept. of Biology, Illinois College, 1101 West College Avenue, Jacksonville, Illinois, 62650-2299, USA



1

Fig. 1. Collecting sites among four states in the southern Appalachians where orchid mycorrhizas containing *Epulorhiza inquilina* were collected.

Luer) native to shaded bogs of the southeastern United States (Fig. 1). The fungus was not isolated from thirteen other orchid species in the same region. Thus, some degree of specificity between the new fungal species and the three species of *Platanthera* that share similar habitats is suspected. The new species is effective at stimulating germination of the seeds of *P. integrilabia*, both *in situ* and *in vitro* and may have potential for conservation and horticulture (Zettler and McInnis 1992). In this paper we provide a name and diagnosis for the new species, provide some notes concerning its occurrence in the southeastern United States, and provide a key to the five species of *Epulorhiza* known from terrestrial orchids.

Materials and Methods

Healthy roots of mature plants of *P. clavellata, P. cristata* and *P. integrilabia* were obtained between 1989 and 1995 from orchids growing in Georgia, Kentucky, South Carolina and Tennessee. Vouchers representing the orchid taxa were deposited in Clemson University Herbarium (CLEMS). Mycorrhizal endophytic fungi were isolated following the methods of Currah et al. (1987) except root segments were surface sterilized 1 min in an aqueous solution (deionized water) containing 5% ethanol and 5% sodium hypochlorite and rinsed twice in dionized water. The epidermal layer was removed and clumps of cortical cells containing pelotons were macerated and plated on MMN (Marx 1969). Isolation plates were incubated in the dark at 22 C. Pure cultures and growth rates were obtained using the methods in Currah et al. (1987). Colony colours were determined using the Methuen colour codes (Kornerup and Wanscher 1983) after 40 days incubation in the dark at 22 C on Potato Dextrose Agar (PDA, Difco).

Isolation of mycorrhizal fungi from protocorms germinated in the natural habitat was carried out following the methods of Rasmussen and Whigham (1993). Seeds from mature green capsules of *Platanthera integrilabia* were collected October 1992 in McMinn Co., TN, and stored six months as outlined in Zettler and McInnis (1994). Between 50 and 500 seeds were placed in nylon packets (sifting material, 95 μm pore size, Carolina Biological Supply Co., #65-2222M) bound in plastic 35 mm (Polaroid) slide mounting frames. Each packet was stapled on all four sides, attached to a nylon fishing line, and buried in soil at a depth of ca. 5 cm, within 10-20 cm of the root systems of a mature stand of *P. integrilabia, P. clavellata* and *P. cristata* in late February. After 8 months the packets were retrieved, sealed in plastic bags, and stored in the dark at 6-8 C. Protocorms were removed from packets, surface sterilized 1 min in a solution containing 5 ml ethanol, 5 ml sodium hypochlorite and 90 ml of sterile deionized water and rinsed twice with sterile deionized water. Protocorms were plated on MMN agar and incubated in the dark at 22 C for two weeks. Pure cultures were obtained by subculturing hyphal tips. Living cultures of the type and other representative strains are deposited at the University of Alberta Microfungus Collection and Herbarium (UAMH), Edmonton, Canada.

Tests for the presence of cellulase and polyphenol oxidase follow the methods of Smith (1977) and Davidson et al. (1938), respectively. Reactions for the polyphenol oxidase assays were recorded as outlined in Zelmer and Currah (1995). Nuclear numbers of young hyphal cells were determined using DAPI fluorescent stain (Sneh et al. 1991). Septal ultrastructure was examined

following the protocol given in Currah and Sherburne (1992). Morphology of monilioid cells in culture was noted on colonies grown on corn meal agar (CMA, Difco).

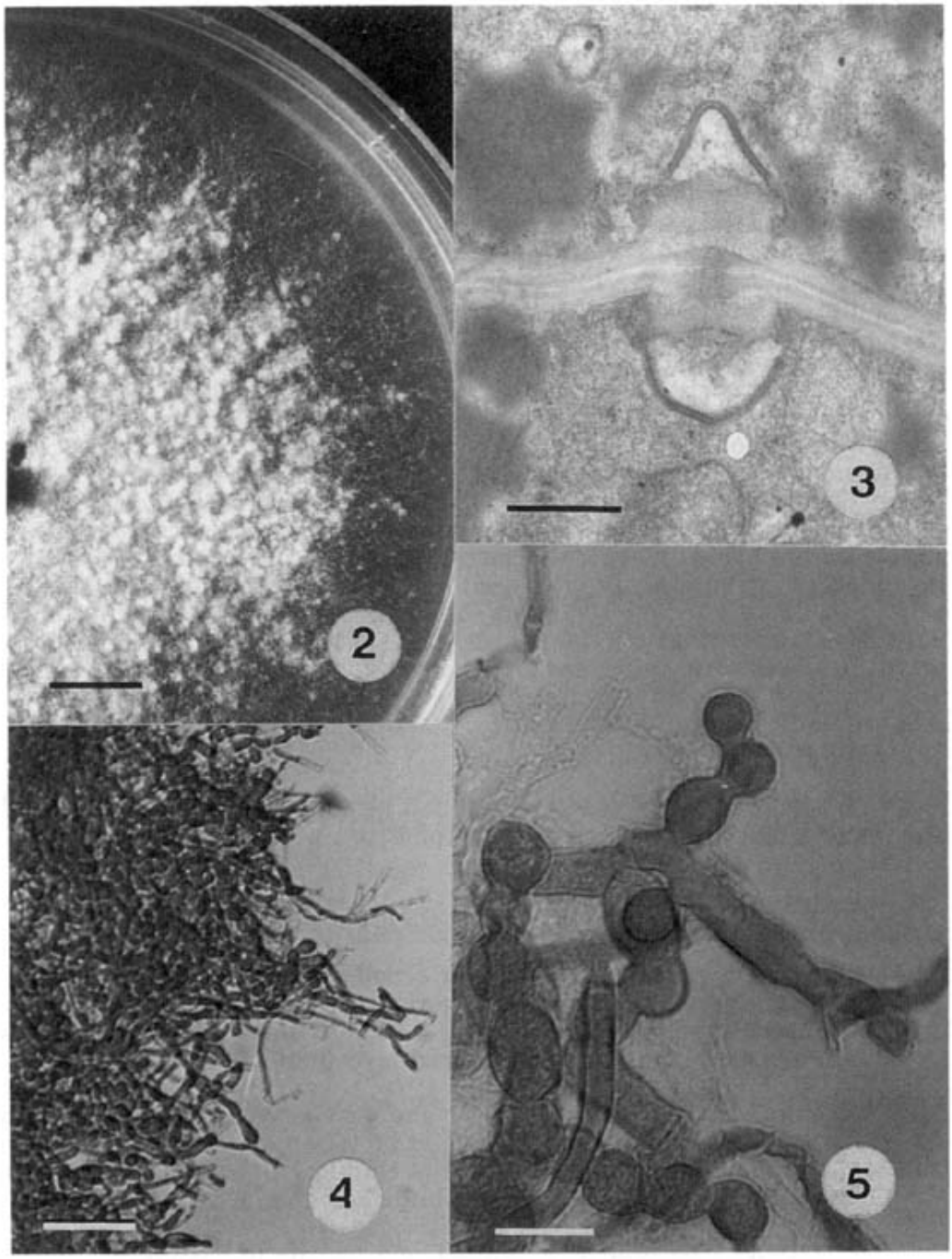
Taxonomic Part

Epulorhiza inquilina sp. nov.

In agaro dextroso solani tuberosi primum glabra, deinde alba vel griseo-brunnea, cristata per auctum mycelii aerii et glomerum cellularum monilioidarum, margo intacta. Cellulae binucleatae, regulariter septatae, parenthesomata imperforata, hyphae currentes 5-6 µm diam, hyalinae et plerumque tenuiter tunicatae. Elongatae ad ellipsoideae vel globosae, 11.1-25.8 x 9.1 - 12.7 µm, catenas simplices cellularum decem vel pauciorum formantes. Cellulae monilioidae glomera magna, deinde cristas vel sclerotia laxa formantes. Holotypus: colonia exsiccata ex UAMH 7632 de Platanthera integrilabia collecta in Pinnacle Mountain, Greenville Co., South Carolina, 1989.

Cultural morphology: On PDA, glabrous at first becoming white to greyish tan (4B2-5B2) and tufted as aerial mycelium (Fig. 2) and clusters of monilioid cells develop, margin entire.
Hyphae: Cells binucleate, regularly septate with imperforate parenthesomes (Fig. 3); runner hyphae 5-6 µm in diameter, hyaline and mostly thin-walled.
Monilioid cells: Elongate to elliptical or spherical, 11.1-25.8 x 9.1-12.7 µm in simple chains of 10 or fewer cells. Monilioid cells developing in loose sclerotial clusters that form tufts (Figs. 4 and 5).
Growth rate: 0.03 - 0.10 mm/hr.
Enzyme assays: polyphenol oxidase negative, weakly cellulolytic but not able to deteriorate cellophane membrane after 55 days.
Holotype: dried colony of UAMH 7632 from *P. integrilabia* collected at Pinnacle Mountain, Greenville Co., South Carolina, 1989, deposited at UAMH.
Additional Isolates: 115 (=UAMH 7811) from *P. clavellata*, Turkey Creek, Carroll Co., Georgia, 1989. 134 and 135 from *P. clavellata*, Tallulah Gorge, Rabun, Co., Georgia, 1989. 89 (=UAMH 7807) and 93 (=UAMH 7808) from *P. integrilabia*, Savage Gulf, Grundy Co., Tennessee, 1989. 136, 155 from *P. integrilabia*, Starr Mountain, McMinn Co., Tennessee, 1989. 235 (=UAMH 7809), 236, 237, 238, 239 from protocorms of *P. integrilabia* germinated *in situ*, Starr Mountain, McMinn Co., Tennesse, 1993. 163 (=UAMH 7810) from *P. cristata*, Starr Mountain, McMinn Co., Tennessee, 1989. 246 from *P. clavellata* growing in type locality of *P. integrilabia*, McCreary Co., Kentucky, 1995.
Etymology: "*inquilina*" refers to an inhabitant or lodger of foreign origin.

Species in the genus *Epulorhiza* usually have narrow vegetative hyphae, (2.5-3.5 µm broad) and produce relatively little aerial mycelium. *E. inquilina* differs from most species in *Epulorhiza* and bears a superficial resemblance to species in the genus *Ceratorhiza*. In most species of *Ceratorhiza* the vegetative hyphae are relatively broad (4-7 µm), and colonies are tufted and have relatively large amounts of aerial mycelia. However, placement of the new species in *Epulorhiza* is supported by the imperforate parenthesomes and the inability of the species to give a positive reaction for polyphenol oxidases on tannic acid media (Zelmer 1994). The species differs from others described in the genus as shown in the key (below).



Figs. 2-5. Characteristics of *Epulorhiza inquilina*. Fig. 2. Colony, 84 days on PDA, of *Epulorhiza inquilina* (ex type strain, UAMH 7632) from *Platanthera integrilabia*. Bar = 12.5 cm. Fig. 3. Section through septum of UAMH 7632 showing imperforate parenthesome. Bar = 1 μm. Fig. 4. Perimeter of a sclerotial aggregation of monilioid cells. Bar = 30 μm. Fig. 5. Short chain of ellipsoid to spherical monilioid cells after 33 days on PDA. Bar = 16 μm.

Peloton morphology in protocorms grown symbiotically lacks distinctive features. Hyphae tend to be narrow and uniform in diameter and form the typical interwoven masses of fungal cells characteristic of the symbiosis (Zettler and McInnis 1992). Monilioid cells have not been observed in the colonized cells of the host orchids.

The presence of this new species of *Epulorhiza* in a rare terrestrial orchid is noteworthy. *P. integrilabia*, currently listed as a C2 candidate for U.S. protection as an endangered species, is indigenous to the wet, shaded boggy areas of the Cumberland Plateau where it often grows in close association with *P. clavellata* and *P. cristata* (Zettler and Fairey 1990). *E. inquilina* was consistently and almost exclusively isolated from *P. clavellata, P. cristata* and *P. integrilabia* growing in shaded bogs in four states (Fig. 1). In all cases, the fungus was located in dull yellow-orange roots and was absent in the tuber. Twelve additional orchid taxa from the southern Appalachians, including two other species of *Platanthera*, which were examined for their mycorrhizal endophytes, did not yield *E. inquilina* . Thus, there may be some specificity between these three orchid species and their mycorrhizal partner. The isolation of *E. inquilina* from *P. integrilabia* seedlings germinated *in situ* suggests that this endangered orchid may rely on this single fungus species to complete its life cycle. If true, this might explain *P. integrilabia*'s limited geographical distribution. *In vitro* seed germination tests have also shown *E. inquilina* to be an effective symbiont with *P. integrilabia* (Zettler and McInnis 1992; 1994) but not with unrelated taxa, e.g., *Spiranthes cernua* (Linnaeus) L. C. Richard and *Goodyera pubescens* (Willdenow) R. Brown (Zettler and McInnis 1993), *Corallorhiza odontorhiza* (Willdenow) Nuttall, *Isotria medeoloides* (Pursh) Rafinesque and *Tipularia discolor* (Pursh) Nuttall (Zettler and McInnis, unpublished).

There have been five species included in *Epulorhiza*. Their principal distinguishing features in culture are emphasized in the following key.

KEY TO THE SPECIES OF *EPULORHIZA*

1. On PDA, runner hyphae usually 5 µm or broader in diam; colonies white to pale cream, grey or tan in colour; aerial mycelium abundant.....2
1. On PDA, runner hyphae usually narrow, < 5 µm in diam; colonies white, pale cream or pale orange in colour, aerial mycelium usually scant.....3

2. On CMA, monilioid cells mostly globose and varying in size in a single chain, 15-24 x 10-12 µm.....*E. albertaensis* Currah and Zelmer
2. On CMA, monilioid cells mostly elongate to elliptical, mostly of the same size, 11-26 x 9-13 µm, forming in short, branched chains.....*E. inquilina*

3. On PDA, colonies pale orange, monilioid cells clavate to irregular, 12-21 x 8-9 µm.....*E. calendulina* Zelmer and Currah
3. On PDA, colonies white, pale cream, tan or grey, monilioid cells in long or short chains and variously shaped.....4

4. On CMA, monilioid cells ellipsoidal, 14-18 x 7-10 µm, in long chains, adjacent cells in a chain connected by a narrow tube-like constriction..... .....*E. anaticula* (Currah) Currah

4. On CMA, monilioid cells more or less globose, 13-18 x 8-17 µm, in chains, the junction between adjacent cells in a chain narrow or broad but not tube-like.....*E. repens* (Bernard) Moore

Acknowledgments

Part of this work was completed with funding provided to LWZ from the American Orchid Society Conservation Committee. We express gratitude to Marta R. Bowen, Irene Kokkala, J. A. Snyder and F. William Zettler for their helpful suggestions. The authors thank Carla Zelmer for technical assistance and advice, Michael Hertwig for the latin translation, and Richard Sherburne for the septum thin section. A review by Dr. Jim Ginns of a previous version of this manuscript was most appreciated.

References cited

Currah, R. S. and Sherburne, R. 1992. Septal ultrastructure of some fungal endophytes from boreal orchid mycorrhizas. Mycol. Res. 96:583-587.

Currah, R. S. and Zelmer, C. D. 1992. A key and notes for genera of fungi mycorrhizal with orchids and a new species in the genus *Epulorhiza*. Rept. Tottori Mycol. Inst. 30:43-59.

Currah, R. S., Sigler, L. and Hambleton, S. 1987. New records and new taxa of fungi from the mycorrhizae of terrestrial orchids of Alberta. Can. J. Bot. 65:2473-2482.

Davidson, R. W., Campbell, W. A. and Blaisdell, D. J. 1938. Differentiation of wood-decay fungi by their reactions on gallic or tannic acid medium. J. Agric. Res. 57:683-695.

Kornerup, A. and Wanscher, J. H. 1983. Methuen handbook of colour. 3rd. ed. Methuen, London.

Marx, D. H. 1969. The influence of ectotrophic mycorrhizal fungi on the resistance of pine roots to pathogenic infections. I. Antagonishm of mycorrhizal fungi to root pathogenic fungi and soil bacteria. Phytopathology 59:153-163.

Moore, R. T. 1987. The genera of *Rhizoctonia*-like fungi: *Ascorhizoctonia, Ceratorhiza* gen. nov., *Epulorhiza* gen. nov., *Moniliopsis* and *Rhizoctonia*. Mycotaxon 29:91-99.

Rasmussen, H. N. and Whigham, D. F. 1993. Seed ecology of dust seeds *in situ*: a new study technique and its application in terrestrial orchids. Am. J. Bot. 80:1374-1378.

Richarson, K. A. and Currah, R. S. 1995. The fungal community associated with the roots of some rainforest epiphytes of Costa Rica. Selbyana 16:49-73.

Richardson, K. A., Currah, R. S. and Hambleton, S. 1993. Basidiomycetous endophytes from the roots of neotropical epiphytic Orchidaceae. Lindleyana 8:127-137.

Smith, R. E. 1977. Rapid tube test for detecting fungal cellulase production. Appl. Environ. Microbiol. 33:980-981.

Sneh, B., Burpee, L. and Ogoshi, A. 1991. Identification of *Rhizoctonia* species. APS press, St. Paul, Minn.

Zelmer, C. D. 1994. Interactions between northern terrestrial orchids and fungi in nature. M.Sc. thesis, Department of Botany, University of Alberta, Edmonton, Alta.

Zelmer, C. D. and Currah, R. S. 1995. *Ceratorhiza pernacatena* and *Epulorhiza calendulina* spp. nov. : mycorrhizal fungi of terrestrial orchids. Can. J. Bot. 73:1981-1985.

Zettler, L. W. and Fairey III, J. E. 1990. The status of *Platanthera integrilabia*, an endangered terrestrial orchid. Lindleyana 5:212-217.

Zettler, L. W. and McInnis, Jr., T. M. 1992. Propagation of *Platanthera integrilabia* (Correll)Luer, an endangered terrestrial orchid, through symbiotic seed germination. Lindleyana 7:154-161.

Zettler L. W. and McInnis, Jr., T. M. 1993. Symbiotic seed germination and development of *Spiranthes cernua* and *Goodyera pubescens* (Orchidaceae: Spiranthoideae). Lindleyana 8:155-162.

Zettler, L. W. and McInnis, Jr. T. M. 1994. Light enhancement of symbiotic seed germination and development of an endangered terrestrial orchid (*Platanthera integrilabia*). Plant Sci. 102:133-138.