

AN ABSTRACT OF THE THESIS OF

Lisa C. Grubisha for the degree of Master of Science in Botany and Plant Pathology presented on June 22, 1998. Title: Systematics of the Genus *Rhizopogon* Inferred from Nuclear Ribosomal DNA Large Subunit and Internal Transcribed Spacer Sequences.

Abstract approved:

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Joseph W. Spatafora

Rhizopogon is a hypogeous fungal genus that forms ectomycorrhizae with genera of the Pinaceae. The greatest number and species of *Rhizopogon* are found in coniferous forests of the Pacific Northwestern United States, where members of the Pinaceae are also concentrated. *Rhizopogon* spp. are host-specific primarily with *Pinus* spp. and *Pseudotsuga* spp. and thus are an important component of these forest ecosystems. *Rhizopogon* includes over 100 species; however, the systematics of *Rhizopogon* have not been well understood. Currently the genus is placed in the Boletales, an order of ectomycorrhizal fungi that are primarily epigeous and have a tubular hymenium. *Suillus* is a stipitate genus closely related to *Rhizopogon* that is also in the Boletales and host-specific with Pinaceae. I examined the relationship of *Rhizopogon* to *Suillus* and other genera in the Boletales. Infrageneric relationships in *Rhizopogon* were also investigated to test current taxonomic hypotheses and species concepts. Through phylogenetic analyses of large subunit and

internal transcribed spacer nuclear ribosomal DNA sequences, I found that *Rhizopogon* and *Suillus* formed distinct monophyletic groups. *Rhizopogon* was composed of four distinct groups; sections *Amylopogon* and *Villosuli* were strongly supported monophyletic groups. Section *Rhizopogon* was not monophyletic, and formed two distinct clades. Section *Fulviglebae* formed a strongly supported group within section *Villosuli*. Taxonomic revisions were proposed. *Suillus*, *Truncocolumella*, and the Gomphidiaceae were transferred to the Rhizopogonaceae. In *Rhizopogon*, sections *Amylopogon*, *Rhizopogon*, and *Villosuli* were elevated to subgenera. Subgenus *Roseoli* was erected to accommodate the second section *Rhizopogon* clade. In section *Fulviglebae*, Stirps *Vinicolor*, *Rhizopogon ochraceisporus*, *R. subclavitisporus*, and *R. clavitisporus* were transferred to subgenus *Villosuli* while the remaining species in section *Fulviglebae* were transferred to subgenus *Rhizopogon*.

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Systematics of the Genus *Rhizopogon* Inferred from Nuclear Ribosomal
DNA Large Subunit and Internal Transcribed Spacer Sequences

by

Lisa C. Grubisha

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APPROVED:

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Major Professor, representing Botany and Plant Pathology

Redacted for Privacy

Chair of Department of Botany and Plant Pathology

Redacted for Privacy

Dean of Graduate School

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CONTRIBUTION OF AUTHORS

Dr. Joseph W. Spatafora participated at all stages of the study including design, data collection and analysis, molecular phylogenetics expert, and editing. Dr. James Trappe was not only the taxonomy consultant and guru, but he participated in the study design, two trips to Idaho to collect specimens, and editing. Dr. Randy Molina also contributed a great deal to these chapters including initiation, design, intellectual development, editing, and advisor on concepts of host specificity and *Rhizopogon* mycorrhizae. All authors passed on knowledge and experience from their various fields of expertise to me and without such assistance this thesis would not have been completed.

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DEDICATION

I dedicate this thesis to my parents, Miriam and Drago Grubisha. Their support always came when I needed it most, and they had the insight to fly me home to Wisconsin for a break from work.

PREFACE

"These dingy, unattractive, potato-like fungi are the Russulas of the underworld--unappreciated except by squirrels. Whereas the Russulas' brittle flesh is irresistible to those who like to trounce things, the Rhizopogons' rubbery texture is a blessing to those who like to *bounce* things."

--David Arora in *Mushrooms Demystified*.

SYSTEMATICS OF THE GENUS *RHIZOPOGON* INFERRED FROM NUCLEAR RIBOSOMAL DNA LARGE SUBUNIT AND INTERNAL TRANSCRIBED SPACER SEQUENCES

CHAPTER 1

INTRODUCTION

Rhizopogon Fries (Basidiomycota, Boletales) is a genus of sequestrate fungi ectomycorrhizal mostly with conifers. The sporocarps are globose to pyriform usually 1-6 cm in diameter, in some cases up to 15 cm in size (Smith and Zeller, 1966). *Rhizopogon* spp. lack a columella. When fresh and mature they have a rubbery texture. The peridium structure may be simplex (composed of single layer of hyphae), or duplex (two distinct hyphal layers) (Zeller and Dodge, 1918). Martin (1996) described five types of peridial structure, four simplex and a single duplex, based on number of hyphal layers and arrangement, and presence of globose cells: *Roseolus*-type, *Abietis*-type, *Luteolus*-type, *Corsicus*-type, and *Villosulus*-type (duplex). The peridium encloses the gleba, which consists of lacunose chambers lined with hymenia. When dried, the gleba varies from very brittle, literally crumbling when sliced with a razor blade, to bone hard. Statismospores are fusoid to oblong or ellipsoid or sometimes versiform, 5-15(-25) × 1.5-8 µm, smooth, with a single, wall, that is thin in most species but may be up to 2 µm thick (Smith and Zeller, 1966), and the spores of some species are amyloid (*Rhizopogon* section *Amylopogon*, Smith, 1964). According to Castellano et al. (1989) *Rhizopogon*

is the only genus of hypogeous fungi that has species with the combination of both smooth and amyloid spores.

CLASSIFICATION

Based on overall gross morphology, 19th century mycologists lumped false-truffles, puffballs, bird's nest fungi, earth stars, and stinkhorns in an artificial grouping of Gasteromycetes (Miller and Miller, 1988; Hibbett et al., 1997). Until relatively recently, these fungi were classified by taxonomists in various orders in the class Gasteromycetes in the Phylum Basidiomycota. A more natural classification system was achieved when ecological data were combined with microanatomical data gathered through scanning and transmission electron microscopy (Smith, 1973; Miller and Miller, 1988). As early as 1933, Morse speculated that the then-called gasteromycete *Podaxis* and the agaric *Coprinus* were related taxa based on morphological similarities. Current molecular data indicate that the hypogeous fungi have arisen independently several times in both the Ascomycota and Basidiomycota (Bruns et al., 1989; Vilgalys et al., 1993; Hibbett et al., 1997; O'Donnell et al., 1997). If taxonomy is to reflect evolutionary history, then genera need to be arranged in monophyletic groups (Vilgalys et al., 1993). True and false-truffles now have been removed from the Gasteromycetes and placed in orders and families that reflect molecular phylogenetic hypotheses in recognition that evolutionary morphological reduction has produced truffle-like morphologies along many independent phylogenetic lines. The four

groups still recognized as “Gasteromycetes” include puffballs, bird’s nest fungi, earth stars, and stinkhorns (Alexopoulos et al., 1996). The continued use of this artificial grouping results from uncertainty about phylogeny of these groups with respect to other fungal lineages. However, Hibbett et al. (1997) have demonstrated that the puffballs have arisen within the Agaricales several times.

Rhizopogon has been placed in two orders (three if we include the Agaricales of Singer which includes the boletes) and three families. Until recently, *Rhizopogon* was placed in the class Gasteromycetes, order Hymenogastrales. Gäumann and Dodge erected the family Rhizopogonaceae in 1928. Dodge (1931) then described the genus *Alpova* Dodge and placed it in the Rhizopogonaceae. Fischer (1933) erected the Melanogastraceae, and included *Alpova* in this family. Hawksworth et al. (1995) list *Alpova* in the Melanogastraceae and placed *Truncocolumella* Zeller, *Amogaster* Castellano, and *Rhizopogon* in Rhizopogonaceae. In 20th century studies, *Rhizopogon* was placed in the Hymenogastraceae (Smith and Zeller, 1966; Smith, 1971; Bruns and Szaro, 1992), Rhizopogonaceae (Zeller, 1939, 1941; 1948, 1949; Lange, 1954; Smith, 1973; Miller and Miller, 1988; and Martin, 1996), and Boletaceae (Miller, 1983; Castellano et al., 1989; Molina et al., 1992; Allen et al., 1998). Although *Rhizopogon* differs strikingly from *Suillus* and other boletes in gross morphology, evidence from scanning electron microscopy of *Rhizopogon* spores support placement of *Rhizopogon* in the Boletales and Boletaceae (Hawker, 1975). Current evidence based on phylogenetic studies of

mitochondrial (Bruns et al., 1989; 1990; 1992; 1998) and nuclear ribosomal DNA sequences (Baura et al., 1992; Kretzer et al., 1996) further demonstrate the close relationship between *Rhizopogon* and *Suillus*.

EVOLUTION OF THE HYPOGEOUS HABIT IN *RHIZOPOGON*

Presently mycologists agree that epigeous and hypogeous fungi are phylogenetically related, although historically a consensus on the direction of evolution was lacking (Heim, 1971; Thiers, 1971, 1984; Trappe and Maser, 1977). The possible phylogenetic relationship between *Rhizopogon* and *Suillus* was hypothesized by Malençon in 1931. This hypothesis was reiterated several times (Smith 1971; Thiers, 1971, 1975, 1984) but agreement on what characters were primitive and derived was not reached. Smith and Singer (1959) proposed the direction of evolution of the "Gastroboletus Series" is from a hypogeous *Rhizopogon* ancestor through *Truncocolumella* and *Chamonixia* to the secotioid *Gastroboletus* to *Boletus*. Smith (1966, 1971, 1973) was a firm advocate that *Rhizopogon* possessed primitive characters from which *Suillus* and finally *Gomphidius* arose. Others disagreed and hypothesized just the opposite, that *Rhizopogon* is derived from a bolete ancestor (Thiers, 1971, 1975, 1984; Bruns et al. 1989). Smith argued that *Rhizopogon* is ancestral because it possesses primitive characters and lacks complex morphology (Smith and Zeller, 1966; Smith, 1971). Thiers (1971, 1975) suggested that *Rhizopogon* evolved from a bolete ancestor. Later Thiers (1984) outlined probable ecological and climatic pressures that would favor

selection of hypogeous growth forms. His ideas centered around his observations from years of experience in the xeric mountains of Idaho and the Sierra Nevadas of California. He hypothesized that in response to a xeric climate with extended periods of drought, selection favored fruitifications in which the pileus did not pull away from the stipe. This resulted in the loss of ballistospory and stipe. The epicutis developed into a peridium and surrounds the hymenium. A chambered gleba resulted from constraints imposed on the hymenium during development from the surrounding peridium. This lacunose gleba thus retained moisture from humidity. This change in morphology subsequently resulted in a change from air dispersal of ballistospores to animal dispersal of statismospores.

Considerable molecular evidence supports the hypothesis that *Rhizopogon* and *Suillus* are indeed close relatives and that *Suillus* is more closely related to *Rhizopogon* than to other boletes (Bruns et al., 1989, 1990, 1992, 1998; Baura et al., 1992). Bruns et al. (1989) hypothesize that an accelerated rate of morphological evolution can best be explained by rapid morphological divergence resulting from selective pressures which may have acted on a small number of developmental genes. Furthermore, the dearth of intermediate forms provides indirect evidence of intense selective pressures.

As for the issue of the direction of evolution between epigeous and hypogeous fungi, currently there is support for both hypotheses in different lineages of fungi: molecular evidence supports hypotheses of the evolution of *Rhizopogon* from a gilled boletoid ancestor (Bruns et al., 1989), and there is

also molecular evidence suggesting that stinkhorns are derived from a *Hysterangium*-like ancestor (Colgan et al., 1997).

TAXONOMY

The first *Rhizopogon* descriptions came from European mycologists (Molina et al., in press). A thorough historical review on the taxonomy of *Rhizopogon* is presented by Martin (1996) and additional insights are provided by Molina et al. (in press). Important early contributions toward North American taxonomic studies of *Rhizopogon* are by Zeller and Dodge (1918), Coker and Couch (1923), and Zeller (1939, 1941, 1948). By far the most comprehensive taxonomic study of the genus was undertaken by Alexander Smith (Smith and Zeller, 1966). In this work, *Rhizopogon* was divided into two subgenera, *Rhizopogonella* and *Rhizopogon*, that were further broken into two and four sections, respectively. He placed 137 species into the four sections, *Amylopogon*, *Fulviglebae*, *Villosuli*, and *Rhizopogon*, of *Rhizopogon* subgenus *Rhizopogon* based on macroscopic and microscopic characters and chemical reactions and bruising of the sporocarp. Later Trappe (1975) transferred the four species in subgenus *Rhizopogonella* to *Alpova*. Our current understanding of the genus *Rhizopogon* is that of Smith and Zeller (1966) without subgenus *Rhizopogonella*.

Subsequently, 25 new North American species have been described, the known distribution of some species was expanded, and some species described in Smith and Zeller (1966) have been reduced to synonymization (Smith,

1966, 1968; Harrison and Smith, 1968; Trappe and Guzmán, 1971; Hosford, 1972, 1975; Hosford and Trappe, 1980; Miller, 1986; Cázares et al., 1992; Allen et al., 1998).

Despite this rich history, the taxonomy of *Rhizopogon* remains poorly understood. Smith and Zeller (1966) point out that, because the sporocarp is so reduced morphologically, few taxonomically informative characters are found on dried specimens. He maintained that characteristics of the fresh specimens were extremely important in species identification. He placed importance on peridial structure, color when fresh, and color changes due to bruising and reactions to KOH and FeSO_4 in his classification scheme (Appendix 1). Smith and Zeller (1966) also recognized that these characters are often missing from descriptions of early taxonomic work, including type descriptions. Many species that stain red often have been misidentified as *R. rubescens* (Smith and Zeller, 1966). Complicating matters further missing or undesignated type specimens in early European collections (e.g. *R. luteolus* Fries and *R. roseolus* Corda) and the use of European keys for identification of North American species have lead to confusion in species identification.

A gradient of developmental stages adds to the difficulties in species identification. Because hypogeous fungi develop more slowly than epigeous fungi, several developmental stages may be found in a single collection (Molina and Trappe, 1994). Ontogenetic stages of a single species may have been described as different species by Smith in Smith and Zeller (1966) (J. Trappe, personal communication). In addition, Smith and Zeller (1966)

placed 11 species morphological affinities to section *Villosuli* in other sections. Additionally, some species with truncate spores were placed in section *Rhizopogon*, not section *Fulviglebae*. Smith's contribution to the understanding of the taxonomy of the genus *Rhizopogon* cannot be overstated; however, even he noted that certain areas of his work on *Rhizopogon* would need to be re-examined.

HOST SPECIFICITY

Ectomycorrhizal fungi may be host-specific for a particular genus or have a broad range of hosts. Many *Rhizopogon* species show strong host-specificity for either Douglas-fir (*Pseudotsuga* spp.) or *Pinus* spp. (Molina et al., in press). Species in *Rhizopogon* section *Villosuli* are found exclusively in Douglas-fir forests (Molina and Trappe, 1994; Molina et al., in press). These species also demonstrate the same host-specificity with Douglas-fir in pure culture synthesis (Molina and Trappe, 1994) and when co-cultured with Douglas-fir and other conifers (Massicotte et al., 1994). Molina and Trappe (1994) found that in pure culture synthesis studies, species within a given *Rhizopogon* section tended to form mycorrhizae with the same hosts giving further support to Smith's (Smith, 1964; Smith and Zeller, 1966) sectional hypotheses.

The other three sections also show a degree of host-specificity or host-preference with certain conifer genera: species in *Rhizopogon* section *Fulviglebae* stirps *Vinicolor* are found with Douglas-fir, *Rhizopogon* section

Rhizopogon is usually found with pines or mixed pine forests, and *Rhizopogon* section *Amylopogon* tend to be found with pines and true firs (*Abies* spp.) or mixtures of Pinaceae. Molina and Trappe (1994) and Molina et al. (in press) suggest that, because of the diversity and abundance of Pinaceae in Pacific Northwestern North America, this region has been a major area for the evolution and speciation of *Rhizopogon*.

Rhizopogon spp. form ectomycorrhizae with five genera of Pinaceae: *Pinus*, *Abies*, *Tsuga*, *Picea*, and *Pseudotsuga* (Molina and Trappe, 1992). Rarely do they form ectomycorrhizae with non-Pinaceae hosts. *Rhizopogon menzei* form ectomycorrhizae with *Adenostoma fasciculatum*, Rosaceae (Allen et al., 1998). A few *Rhizopogon* spp. form ectomycorrhizae with chlorophyllous and achlorophyllous members of the Ericaceae: Pacific madrone (*Arbutus menziesii* Pursh) and common bearberry (*Arctostaphylos uva-ursi* (L.) Spreng) form arbutoid mycorrhizae with *Rhizopogon* spp. in pure culture synthesis (Molina and Trappe, 1982a) and in spore inoculation studies when co-cultured with *Pinus ponderosa* (Molina et al., 1997). Molecular evidence and field observations of sporocarp formation demonstrate that *R. ellena* form ectomycorrhizae with the snow plant, *Sarcodes sanguinea* (Bidartondo et al., 1998).

ECOLOGICAL ROLE

In addition to being an important ectomycorrhizal genus in the Pinaceae forests of the Pacific Northwest, it is also an important ecosystem

component in forest food webs. Hypogeous fungi make up a substantial part of the diet of mycophagous rodents (Maser et al., 1978; Maser et al., 1985). Colgan (1997) demonstrated that diets of the Townsend's chipmunk (*Tamias townsendii*) and the northern flying squirrel (*Glaucomys sabrinus*) were dominated by hypogeous fungi and that *Rhizopogon* was the dominant fungus found in their diet. The northern spotted owl (*Strix occidentalis caurina*) preys on the northern flying squirrel (Forsman et al., 1984; Carey, 1992). Thus, *Rhizopogon* forms important linkages in the forest community through mycorrhizal symbioses with forest trees and its role in the food web of flying squirrels and spotted owls.

Because the taxonomy of *Rhizopogon* is confused, the status of rare and endangered *Rhizopogon* species is unclear. If indeed there are rare species of *Rhizopogon* in the Pacific Northwest, it is important to correctly document their occurrence. Castellano (1997) includes 22 putative rare or endangered species of *Rhizopogon* found in Oregon on a RED list for Oregon macrofungi. Eight species of *Rhizopogon* are also listed as Strategy 3 fungi in the Forest Ecosystem Management Assessment Team (FEMAT) Record of Decision (USDA, 1994). Geographical areas where strategy 3 fungi are found are considered high priority for management by the USDA Forest Service and Bureau of Land Management. Because species concepts in the genus *Rhizopogon* remain confused, species rarity may be under or over

estimated which has important implications for the preservation of the species, for the organisms linked to *Rhizopogon* through mycorrhizal symbioses or forest food webs, and for forest management.

RIBOSOMAL DNA

Ribosomal DNA (rDNA) is commonly used in fungal systematic studies. It is a tandem repeat of three rRNA genes (18S, 5.8S, and 25S in fungi) and internal and external transcribed spacer regions (ITS and ETS, respectively) separating these genes (Bruns et al., 1991; Hamby and Zimmer, 1992; Hibbett, 1992). The tandem repeats are separated by the intergenic spacer region (IGS) that is not transcribed (Hamby and Zimmer, 1992). The IGS is also referred to as the nontranscribed spacer (NTS) region (Bruns et al., 1991; Hibbett, 1992). Through concerted evolution the tandem repeats homogenize rapidly (Arnheim et al., 1980; Zimmer et al., 1980; Dover and Falvell, 1984) and, in general, act as a single copy gene (Bruns et al., 1991).

Some of the attractive features of rDNA are that: 1) it is ubiquitous in living organisms (Hamby and Zimmer, 1992); 2) it is tandemly repeated and easily amplified through polymerase chain reaction (PCR) (Mullis and Falona, 1987; Saiki et al., 1988) (Baldwin et al., 1995); 3) it is a multigene copy in which the motif of alternating gene and spacer regions provide varying levels of nucleotide substitution for addressing questions at higher and lower taxonomic levels (Bruns et al., 1991; Hamby and Zimmer, 1992; Hibbett, 1992); and 4) the more conserved genes provide sites for universal primer

attachment for all eukaryotes from which the less conserved regions may be amplified through PCR (White et al., 1990; Bruns et al., 1991).

Large subunit (25S) rRNA

The large subunit rRNA gene has provided resolution at various taxonomic levels. It is generally useful for analyzing relationships at the familial or generic level (Hibbett and Vilgalys, 1993; Moncalvo et al., 1995; Feibelman et al., 1997; Lutzoni, 1997). Within the large subunit DNA, there are conserved and variable regions. The variable regions are commonly referred to as divergent domains in eukaryotes (Hassouna et al., 1984). They can form stem-loop structures and are a reason for the increase in size of the large subunit rRNA from prokaryotes to eukaryotes (Michot and Bachellerie, 1987).

Internal transcribed spacer region

In molecular systematics, the internal transcribed spacer (ITS) region generally refers to the ITS-1 region, the 5.8S subunit, and the ITS-2 region. The ITS-1 and ITS-2 are flanked by the 18S and 25S, respectively. Although the ITS-1 and ITS-2 regions are part of the repeat unit and are transcribed, they are not part of the actual ribosome (Baldwin, et al., 1995). As a result, a higher degree of nucleotide substitutions and insertion/deletion events (indels) occur within these two spacer regions. ITS-1 and ITS-2 regions typically

provide sufficient resolution for addressing phylogenetic questions at the intergeneric and intrageneric level in fungi (Lee and Taylor, 1991; Baura, 1992; O'Donnell, 1992; Henrion et al., 1994; Hibbett et al., 1995; Monclavo et al., 1995; Kretzer et al., 1996; Harrington and Potter, 1997; Holst-Jensen et al., 1997; Liu, et al., 1997). Although the insertion of gaps into sequence alignment is necessary to maximize positional homology of characters, the placement of indels can be problematic and ambiguous (Baldwin et al., 1995). The problem is that multiple possibilities exist for equally good indel alignments. Indels may contain phylogenetic information, or the indel itself may reflect a phylogenetic history, even if the indel is not considered informative in phylogenetic analyses.

Recoding gaps for phylogenetic analysis is found in phylogenetic studies more often, especially in the plant literature. Gaps may be recoded as a fifth (Swofford, 1993) or binary (presence/absence) character (Wojciechowski et al., 1993), scored as missing data, or excluded entirely from the analysis. Multiple position gaps would be overinflated if every position is recoded as a 5th character (Hibbett et al., 1995). A way to score multiple-base gaps is by adding an additional character to the gap to score the presence of the gap as informative (Bruns et al., 1992).

In this thesis, through phylogenetic analyses of nucleotide sequence data from the nuclear ribosomal large subunit rRNA gene and the internal transcribed spacer region (ITS1, 2 and 5.8S subunit), I examine the phylogenetic relationship of *Rhizopogon* and *Suillus* to determine the

monophyly of these closely related genera. This is accomplished in the studies presented in both Chapters 2 and 3. In Chapter 2, I also investigate the placement of *Rhizopogon* and *Alpova* in the Boletales. I re-examine infrageneric relationships in *Rhizopogon* based on the classification of A. H. Smith (Smith and Zeller, 1966) and then determine the nature of the relationship with *Suillus* in Chapter 3.

CHAPTER 2

PHYLOGENETICS OF *RHIZOPOGON* AND RELATED
GENERA WITHIN THE BOLETALES: EVIDENCE FROM
NUCLEAR LARGE SUBUNIT rDNA SEQUENCES

Lisa Grubisha, James M. Trappe, Randy Molina, and Joseph W. Spatafora.

ABSTRACT

The phylogenetic relationship between the closely related fungal genera *Suillus* and *Rhizopogon* and their placement in the Boletales was tested through maximum parsimony analyses of large subunit (25S) nuclear ribosomal DNA sequences. Genera included in the analyses were *Boletus*, *Tylopilus*, *Xerocomus*, *Phylloporus*, *Boletellus*, *Suillus*, *Rhizopogon*, *Alpova*, *Truncocolumella*, and *Melanogaster*. Species from the Agaricales, Russulales, Ganodermataceae, and Polyporaceae were also included. The average 25S sequence length was 874 base pairs. Conserved regions of sequence alignment interspersed with variable regions were observed for all species. Analyses were conducted with and without these variable regions. In both analyses, the Boletales is strongly supported. Within the Boletales, distinct suilloid and boletoid radiations are also strongly supported. The Boletaceae, as currently conceived, is not monophyletic, and the Melanogastraceae is included in the boletoid radiation. *Alpova* is polyphyletic and *Boletus* is not monophyletic. The suilloid radiation consists of *Suillus*, *Rhizopogon*, and *Truncocolumella*; these genera are incorporated into the family Rhizopogonaceae. *Suillus* and *Rhizopogon* are both monophyletic genera.

Key words: Boletales, Rhizopogonaceae, *Suillus*, *Rhizopogon*, *Alpova*, large subunit rDNA, phylogeny

INTRODUCTION

The Boletales is a large order of Basidiomycetes. Kreisel (1969) placed 11 families in the Boletales that include pored, gilled, resupinate, and hypogeous fungi; however, the Boletales was not always recognized as an order. Smith and Thiers (1971) placed 11 genera, 10 epigeous and one secotioid, in the Boletaceae (Agaricales). Pegler and Young (1981) recognized six families in the Boletales. Singer (1986) classified the boletoid pored and gilled fungi in the Agaricales; the pored boletes were placed in either the Boletaceae or Strobilomycetaceae, while the related gilled fungi were placed in the Paxillaceae or Gomphidiaceae, and the hypogeous fungi were not included in the Agaricales.

Bruns and Szaro (1992) recognized two distinct groups in the boletes as “suilloid” and “boletoid” radiations of the Boletaceae. *Suillus* was the only genus of the Boletaceae in the suilloid radiation. *Gomphidius* and *Chroogomphus* were in the Gomphidiaceae, and they considered *Rhizopogon* in the Hymenogastraceae. Thus, the Boletaceae was not monophyletic in their independent and combined analyses of DNA sequences of mitochondrial and nuclear small subunit rRNA genes. Extensive sampling of rRNA genes from both mitochondrial (Bruns et al., 1989; Bruns and Szaro, 1992; Bruns et al., 1998) and nuclear (Baura et al., 1992; Bruns and Szaro, 1992; Kretzer et al., 1996; Grubisha et al., 1998) loci demonstrate *Suillus*, *Rhizopogon*, *Gomphidius*, and *Truncocolumella* to be more closely related to each other than to other boletes.

Boletales are ectomycorrhizal with woody angiosperms and gymnosperms. *Suillus*, *Rhizopogon*, *Truncocolumella*, and the Gomphidiaceae are obligately ectomycorrhizal and strongly host-specific with genera of the Pinaceae (Molina et al., 1992). Some sections and species in *Suillus* and *Rhizopogon* are host specific with either *Pinus* spp. or Douglas-fir (*Pseudotsuga* spp.) (Molina et al., in press).

Gäumann and Dodge (1928) erected the Rhizopogonaceae to accommodate *Rhizopogon*. Dodge (1931) placed *Alpova* in the Rhizopogonaceae. Later Fischer (1933) included *Alpova* in the Melanogastraceae. Because the phylogenetic placement of the Melanogastraceae has been uncertain, it has been typically placed in the Melanogastrales in the Gasteromycetes. Trappe (1975) discussed the possible evolutionary relationship of *Alpova*, *Melanogaster*, and *Rhizopogon*. As the sporocarp matures in these genera, the glebal cell walls gelatinize, and, in *Alpova* and *Melanogaster*, the basidia autolyse "...leaving the spores suspended in slime" (Trappe, 1975). The most valuable characters for taxonomic separation of these genera include: presence or absence of clamp connections and a hymenial pallisade, spore wall characteristics including color, thickness, laminations, and nature of the basal pore (Trappe, 1975). He concluded that the evolutionary line of *Rhizopogon*-*Alpova*-*Melanogaster* is a continuum and, because *Rhizopogon* and *Melanogaster* differ distinctly but *Alpova* shares characteristics with both, the latter is the obvious intermediate genus.

Nuclear large subunit (25S) ribosomal DNA (rDNA) analyses have been useful for addressing questions at both lower and higher taxonomic levels (Vilgalys and Hester, 1992; Hibbett and Vilgalys, 1993; Vilgalys and Sun, 1994; Chapela et al., 1994; Moncalvo et al., 1995; O'Donnell et al., 1997;

Fiebleman et al., 1997; Platt et al., in prep). The 25S gene is characterized by possessing alternating “core” and “variable” regions (Hassouna et al., 1984; Michot and Bachellerie, 1987). Twelve variable domains have been identified in eukaryotes, including the yeast, *Saccharomyces carlsbergensis* (Hassouna et al., 1984). Large subunit sequences may vary in length as a result of these variable domains.

In this study, we conduct phylogenetic analyses of DNA sequences from the nuclear ribosomal large subunit gene of several genera of the Boletales. Our specific objectives were to: 1) further clarify relationships between the suilloid and boletoid radiation of the Boletales, 2) determine if *Rhizopogon* and *Suillus* are monophyletic groups, and 3) examine the phylogenetic placement of the genus *Alpova*.

MATERIALS AND METHODS

Fungal specimens

Fifteen collections from species of the Boletales were selected for DNA extraction (Table 2.1) and an additional 24 large subunit rDNA sequences from Basidiomycota taxa were obtained from GenBank (Table 2.1).

Nucleic acid extraction, polymerase chain reaction, and DNA sequencing

The protocol for nucleic acid extraction was an SDS-lysis buffer or 2X CTAB method, modified from Bruns et al. (1990) and Doyle and Doyle (1987), respectively. The large subunit (25S) region of the nrDNA was amplified via

Table 2.1. Genbank number, voucher, and collection location of species examined for DNA analysis. Species listed only by Genbank number were not sequenced in this study.

Species	GenBank accession number	Voucher number ^a	Herbarium ^b
<i>Agaricus bisporus</i> (Lange) Imbach	U11911		
<i>Alpova diplophloeus</i> (Zeller & Dodge) Trappe and A.H. Smith	AF071454	JMT 17685	OSC
<i>Alpova trappei</i> Fogel	AF071456	JMT 16394	OSC
<i>Bolbitius vitellinus</i> (Pers. ex Fr.) Fr.	U11913		
<i>Boletellus mirabilis</i> (Murrill) Singer	AF050652		
<i>Boletus edulus</i> Bull. ex Fr.	AF050643		
<i>Boletus edulus</i> Bull. ex Fr.	AF071457	LCG 184	OSC
<i>Boletus retipes</i> Berk. & Curt.	U11914		
<i>Boletus satanas</i> Lenz	AF042015		
<i>Cortinarius stuntzii</i>	U11917		
<i>Crinipellis campanella</i> (Peck) Sing.	U11916		
<i>Filobasidiella neoformans</i> Kwon-Chung	L14068		
<i>Ganoderma lucidum</i> (Curt. ex Fr.) Karsten	X78776		
<i>Ganoderma microsporum</i> Hseu	X78779		
<i>Hebeloma crustuliniforma</i> (Bull. ex St. Amans)	U11918		
<i>Lactarius corrugis</i>	U11919		
<i>Leucoagaricus naucinus</i> (Fr.) Sing.	U11921		
<i>Macrolepiota rachodes</i> (Vitt.) Sing.	U11923		
<i>Marasmius delectans</i> Morgan	U11922		
<i>Melanogaster tuberiformis</i> Corda in Sturm	AF074919	JMT 9666	OSC
<i>Phylloporus rhodoxanthus</i> (Schwein.) Bres.	U11925		

Table 2.1. (continued).

Species	GenBank accession number	Voucher number ¹	Herbarium ²
<i>Pleurotus populinus</i>	U04159		
<i>Pleurotus ostreatus</i> (Jacq. ex Fr.) Kummer	U04147		
<i>Polyporus</i> sp. Fr.			
<i>Rhizopogon hawkeriae</i> Smith	AF071458	JMT15299	OSC
<i>Rhizopogon occidentalis</i> Zeller & Dodge	AF071453	JMT 17564	OSC
<i>Rhizopogon olivaceotinctus</i> A. H. Smith	AF071455	HT 53027	SFSU
<i>Rhizopogon parksii</i> Smith	AF071459	JMT 19446	OSC
<i>Rhizopogon smithii</i> Hosford	AF071460	JMT 12321	OSC
<i>Rhizopogon subpurpurascens</i> Smith	AF071461	JMT 19168	OSC
<i>Rhizopogon truncatus</i> Linder	AF071462	JMT 17993	OSC
<i>Rhizopogon truncatus</i> Linder	AF071463	LCG 212	OSC
<i>Rhizopogon villosulus</i> Zeller	AF071464	JMT 19466	OSC
<i>Russula mairei</i> Sing.	U11926		
<i>Suillus capives</i> (Opat.) Smith & Thiers	AF071535		
<i>Suillus sinuspaulianus</i> (Pomerleau & Smith) Dick & Snell	AF071536		
<i>Truncocolumella citrina</i> Zeller	AF071465	JMT 19184	OSC
<i>Tylopilus fellus</i> (Bull. ex Fries) Karst.	AF071466	JMT 6375	OSC
<i>Xerocomus parasiticus</i> (Bull. ex Fr.) Quél.	AF050646		

¹LCG, Lisa C. Grubisha, HT, Harry Thiers, JMT, James M. Trappe

²OSC, Mycological Collection of the Oregon State University Herbarium, SFSU, San Francisco State University

polymerase chain reaction (PCR) (Mullis and Fallona, 1987; Saiki et al., 1988), using primer pairs LR0R (Monclavo et. al., 1995) and LR5 (Vilgalys and Hester, 1990). Reaction mixtures were made to a total volume of 50 μ L containing double-distilled H₂O, 2 μ L diluted (1:100) DNA template, 10 mM Tris-HCl (pH 8.3), 50 mM potassium chloride, 0.005% Tween 20, 0.005% NP-40, 62.5 μ M dNTPs for each of the four deoxyribonucleotide triphosphates, 2.5 mM MgCl, 0.5 μ M of each primer, and 25 U/ml *Taq* or *Tfl* polymerase. When *Tfl* was used magnesium sulfide was substituted for the magnesium chloride. Reaction mixtures were topped off with 25 μ L of mineral oil and amplified with a MJ Research Programmable Thermal Controller (PTC)-100 thermal cycler (Watertown, Massachusetts). Thermal cycling parameters for amplification consisted of one initial cycle with denaturation at 95 C for 3 min. The following conditions were performed for 35 cycles: denaturation at 95 C for 1 min, annealing at 50 C for 30 s, and extension at 72 C for 45 s (extension temperature of 74 C when *Tfl* polymerase was used in the reaction mixture). The final cycle was set with an extension at 72 C for 2 min. This was followed by a cycle at 4 C for 15 minutes. PCR products, in 5 μ l aliquots, were electrophoresed on 1% agarose gels (Gibco-BRL ultraPURE, Life Technologies) stained with ethidium bromide. Bands were visualized using a transilluminater and sizes were estimated using a 100 bp low mass ladder (Gibco-BRL, Life Technologies).

The PCR products were purified for DNA sequencing either by 3M ammonium acetate and isopropanol precipitation or by using a QIAquick Gel Extraction Kit (QIAGEN, Inc., Valencia, CA) and following manufacturer's instructions. The PCR products were sequenced with primers LR3 (Vilgalys and Hester, 1990), LR0R, and LR 5 on an ABI 377 automated sequencer in the Central Services Laboratory at the Center for Gene Research and

Biotechnology at Oregon State University. Sequences were aligned on a Power Macintosh 7600/132 by direct examination using *SeqApp* version 0.6 (Carmean, 1994) and a color font. Alignment gaps were added to maximize positional homology.

Phylogenetic analysis

Maximum parsimony analyses were performed using PAUP* version 4.0 (Swofford, 1998). One hundred heuristic searches were conducted with random sequence addition and tree bisection-reconnection (TBR) branch-swapping algorithms, collapsing zero-length branches and saving all minimal-length trees (MUL-PARS). *Filobasidiella*, the telomorph of the basidiomycetous yeast, *Cryptococcus*, was chosen as the outgroup for phylogenetic analyses. Four regions of ambiguous alignment were observed in all species. Two maximum parsimony analyses were run: 1) all positions and alignment gaps were included and gaps treated as missing data, and 2) a culled data set when all areas of ambiguous alignment were excluded and remaining gaps treated as missing data. To measure relative support for the resulting clades, 500 bootstrap replications (Felsenstein, 1985) were performed only on phylogenetically informative characters with the following parameters: 5 random sequence additions, TBR, and MUL-PARS off.

RESULTS

25S rRNA sequence variation

The 25S sequence averaged 874 bp in length across all species and ranged from 824 bp in *Crinipellus campanella* to 904 bp in *Rhizopogon occidentalis*. The average sequence length for the major taxonomic groupings are as follows: boletoid radiation of the Boletales is 867 bp, suilloid radiation of the Boletales is 889 bp, Russulales is 880 bp, Agaricales is 861, Ganodermataceae is 876 bp. Four regions of ambiguous alignment were observed and accounted for the length variation between taxa.

Parsimony analyses

An alignment of 935 nucleotide bases was analyzed by maximum parsimony. In the culled data set, 70 most parsimonious trees of 943 steps were recovered (Figure 2.1). From the alignment of 935 nucleotide base pairs, 89 ambiguously aligned positions and uninformative positions were excluded, while 250 characters were considered parsimony informative. The consistency index (CI) was 0.417, the retention index (RI) was 0.661, and the rescaled consistency index was 0.276. The Boletales clade is strongly supported as shown with a bootstrap value of 99. The suilloid radiation comprised the genera *Rhizopogon*, *Suillus*, and *Truncocolumella* and formed a distinct, strongly supported clade with a bootstrap value of 98. *Boletus*, *Tylopilus*, *Xerocomus*, *Boletellus*, *Phylloporus*, *Alpova*, and *Melanogaster* formed the boletoid radiation. Although this clade is also well-supported by a bootstrap

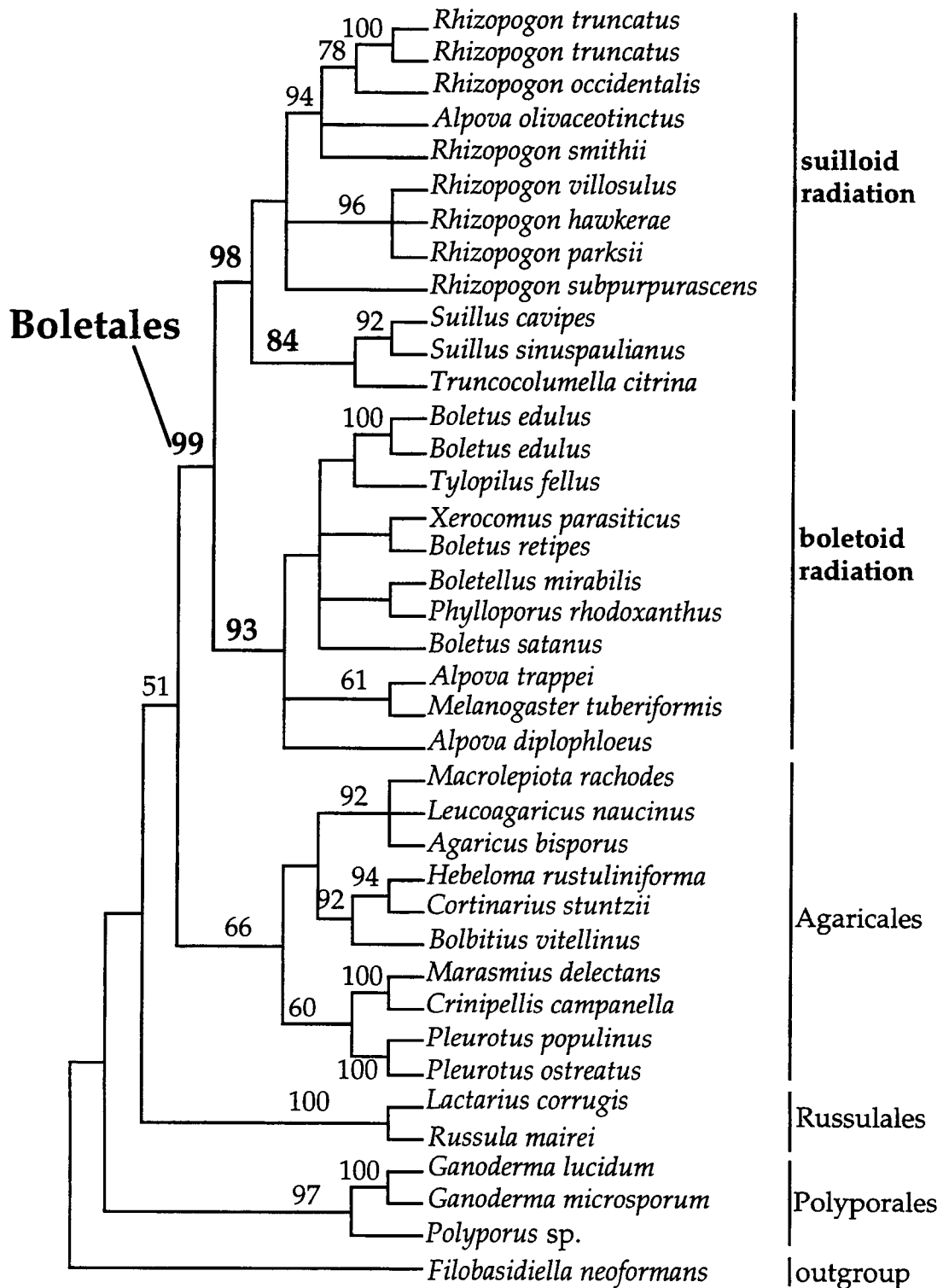


Figure 2.1. Strict consensus cladogram of 70 equally parsimonious trees of 943 steps recovered from maximum parsimony analyses of 25S nrDNA sequences when all ambiguous areas of alignment were excluded. Bootstrap values are noted above the respective internode. CI = 0.417, RI = 0.661.

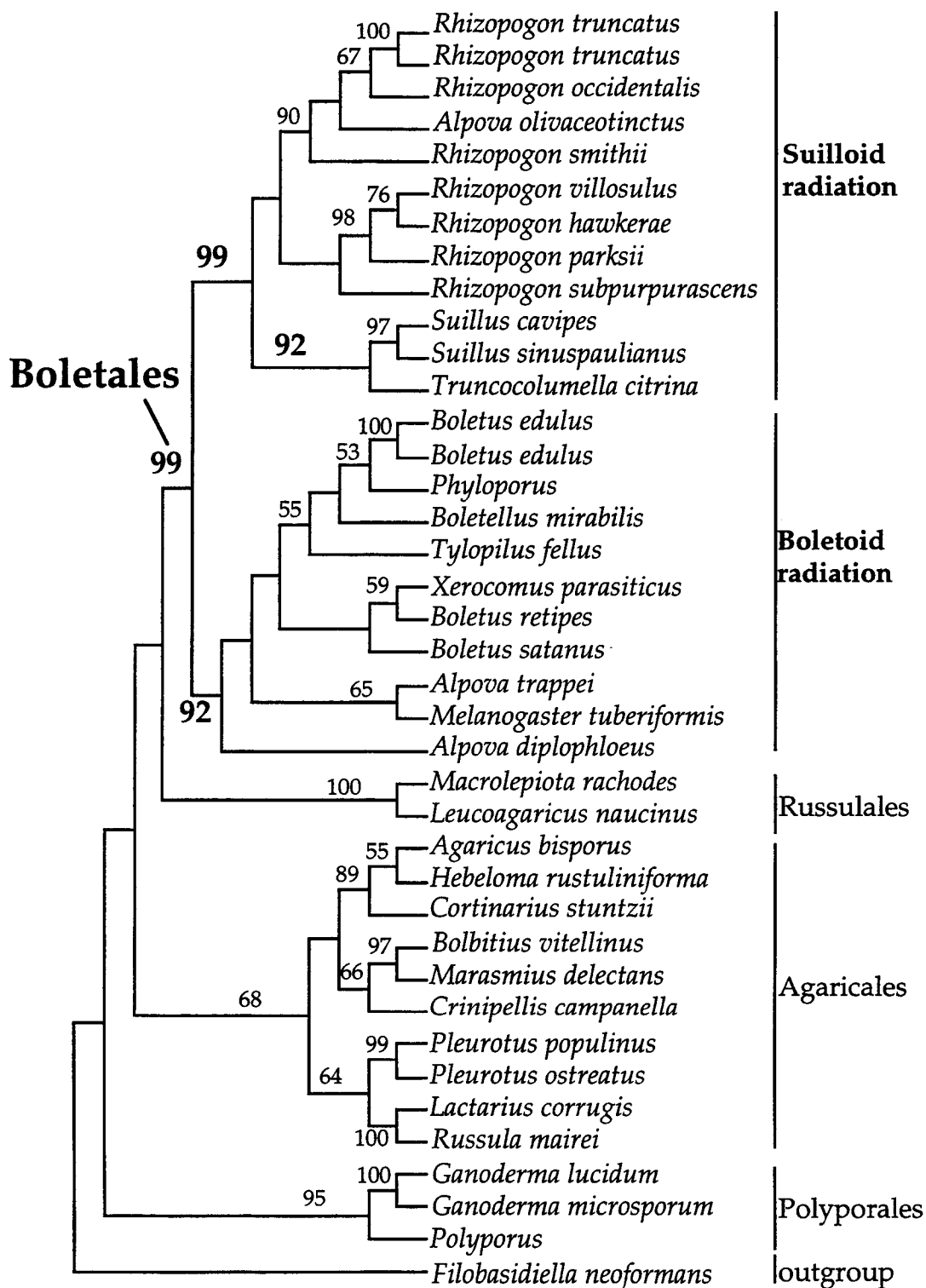


Figure 2.2. One of two equally parsimonious trees of 1031 steps recovered from maximum parsimony analysis of 25S nrDNA sequences when all nucleotide positions were included. Bootstrap values are noted above the respective internode. CI = 0.422, RI = 0.665.

value of 93, generic concepts within the boletoid radiation are not resolved. The Boletales form a sister group to the Agaricales in these analyses, and, although this relationship is only weakly supported, it agrees with results from other studies (Hibbett et al., 1997; Begerow et al., 1997). When all positions were included in the analyses, two equally parsimonious trees were recovered, each of 1031 steps (Figure 2.2). The CI was 0.422, the RI was 0.665, and the RC was 0.281. In this analysis, 276 characters were parsimony informative. Here, the Russulales formed the sister-group to the Boletales, but the bootstrap value is below 50, thus indicating a lack of support for this topology. As in the first analysis, the Boletales was strongly supported with a bootstrap value of 99. The suilloid and boletoid radiations were composed of the same genera as before, and strongly supported by bootstrap values of 99 and 92, respectively. All relationships were resolved within the Boletales clade in this analysis. In both analyses, the Boletaceae and *Boletus* are not monophyletic and *Alpova* is polyphyletic.

DISCUSSION

25S variability and utility

There are four regions of variable alignment in this sequence data. The inclusion or exclusion of these regions affected only the resolution within the Boletales; the remaining clades recovered from the separate analyses containing the same resolution and topology. One variable region varies much less in the Boletales than the other orders. The 25S rRNA gene provided sufficient resolution in this study to address our objectives.

Taxonomic relationships in the Boletales

Boletoid radiation

Suilloid and boletoid radiations of the Boletales as detected by Bruns and Szaro (1992) were based on mitochondria and nuclear small subunit rDNA sequences from *Suillus*, *Rhizopogon*, *Chroogomphus*, *Gomphidius*, *Paxillus*, *Paragyrodon*, *Phylloporus*, *Boletus*, and *Xerocomus*. In a recent phylogenetic study of sequences from the mitochondrial large subunit rRNA gene of 32 genera Bruns et al. (1998) recovered distinct suilloid and boletoid radiations, along with four small clades outside of these main two clades. They found *Boletus*, *Boletellus*, *Xerocomus*, *Phylloporus*, and *Tylopilus* in the boletoid radiation. In our study, genera in the boletoid radiation included *Boletus*, *Xerocomus*, *Boletellus*, *Phylloporus*, *Tylopilus*, *Alpova*, and *Melanogaster*. This group is strongly supported by bootstrap values, but *Boletus* is not monophyletic. Only weak intergeneric resolution is provided when ambiguous areas of the alignment were excluded. Further taxon sampling is needed to refine generic concepts within the bolete radiation.

Although *Alpova* was originally placed in the Rhizopogonaceae by Dodge (1931), phylogenetic placement of *Alpova* and *Melanogaster* is unclear (Thiers, 1984). Occasionally *Alpova* has been placed in the Boletaceae (Molina et al., 1992), but more commonly in the Melanogastraceae (Zeller, 1949; Miller and Miller, 1988, Hawksworth et al., 1995). Besl et al. (1996) suggested that *Melanogaster* was related to the Boletales, and to *Paxillus* in particular.

Results from this study support placement of *Alpova* and *Melanogaster* in the Boletales. Both genera are ectomycorrhizal (Miller and Miller, 1988; Molina et al., 1992) and associate with diverse hosts, e. g., *Alpova diplophloeus* is associated only with alder. Host association with both gymnosperms and woody angiosperms is observed with other members of the Boletales.

Suilloid radiation

Suillus, *Rhizopogon*, and *Truncocolumella* form a distinct, well-supported clade in these analyses. Evolutionary connections between *Suillus*, *Gomphidius*, *Truncocolumella*, and *Rhizopogon*, have been previously hypothesized based on taxonomic and ecological data (Smith and Singer, 1959; Singer, 1962; Smith and Thiers, 1964; Heim, 1971; Smith, 1971; Thiers, 1971, 1975, 1984). Molecular evidence supports the suilloid radiation as a cohesive group distinct from other genera of the Boletaceae (Bruns and Szaro, 1992; Bruns et al., 1998, Grubisha et al., 1998). *Rhizopogon*, *Suillus*, *Gomphidius*, *Chroogomphus*, *Brauniellula*, *Truncocolumella*, and *Alpova olivaceotinctus* were all genera or species found in the suilloid radiation by Bruns et al. (1998). They also found that closely related species and genera had identical or almost identical sequences in their analyses; sequences for some species of *Suillus*, *Rhizopogon*, and the Gomphidiaceae were identical. Based on our results and others (Bruns and Szaro, 1992; Bruns et al., 1998) the Boletaceae is not monophyletic, and *Suillus* is within this separate suilloid radiation. Singer

(1945) segregated *Suillus*, *Psiloboletinus*, and *Boletinus* in the subfamily Suilloideae within the Boletaceae because he believed they were closer to each other than to other boletes. Thiers (1971) suggested that *Suillus* and *Fuscoboletinus* may represent a family distinct from the Boletaceae. He based his reasoning primarily on morphological characteristics; compared to other boletes, *Suillus* fruiting bodies are rather small in size, its spores are usually smaller and often differently shaped than other Boletaceae, and the often decurrent nature of the hymenophore is uncharacteristic of other Boletaceae. In phylogenetic analyses of nucleotide sequences, *Suillus* forms a sister group to *Rhizopogon*. Along with *Truncocolumella*, these three genera form a well supported group distinct from the Boletaceae. Further evidence for the close relationship between *Rhizopogon* and *Suillus* has been documented in other studies (Bruns et al., 1989; Baura et al., 1992; Kretzer et al., 1996; Grubisha et al., 1998). This relationship warrants placement of these three genera in a family separate from the Boletaceae.

Taxonomic revisions

Affinities between *Gomphidius*, *Suillus*, *Rhizopogon*, and *Truncocolumella* are more evident in microscopic characters than gross morphological characters. Similarities of the Gomphidiaceae to *Suillus* is seen in size, shape, and color of the spores (Smith and Thiers, 1964; Thiers, 1971; Pegler and Young, 1981), the conspicuous cystidia that stain dark brown in KOH (Smith and Thiers, 1964; Thiers, 1975), and the divergent gill trama

(Thiers, 1971; Pegler and Young, 1981). Macroscopically the decurrent hymenophore, veil, and ixotrichodermal pileipellis in the Gomphidiaceae resemble those of *Suillus* (Pegler and Young, 1981). *Truncocolumella* and *Rhizopogon* show affinities to this group in spore characters, lacunose to poroid gleba, and trama of the glebal plates (Thiers, 1971).

Suillus, *Rhizopogon*, and Gomphidiaceae display similar mycorrhizal associations. All are host-specific with genera in the Pinaceae with only rare exception (Miller, 1971; Thiers, 1975; Singer, 1986; Molina et al., 1992). Several species are further restricted to specific hosts such as *Pinus* spp. (*Suillus*, *Rhizopogon*, Gomphidiaceae), *Larix* Adans., (*Suillus*, Gomphidiaceae), or *Pseudotsuga* (*Suillus*, *Rhizopogon* section *Villosuli*, *Truncocolumella citrina*, Gomphidiaceae) (Thiers, 1975; Molina et al., 1992). Some species of *Gomphidius* and *Suillus* often fruit together, e.g., *Gomphidius subroseus* and *Suillus lakei* are often found together in association with *Pseudotsuga menziesii* (Agerer, 1990). Singer (1986) discussed how the pairs *Gomphidius roseus*/*Suillus bovinus* and *Gomphidius maculatus*/*Suillus grevillei* not only are typically found together, but often have concrescent hyphae and basal portion of their stipes.

Gomphidius was put in its own family, the Gomphidiaceae, by Maire in 1933 (Miller, 1971; Pegler and Young, 1981) and Singer (1945) erected the subfamily Suilloideae. Gäumann and Dodge recognized the *Rhizopogonaceae* in 1928. Following priority of the family and subfamily rank of these groups, we propose that *Suillus* and the Gomphidiaceae be

transferred to the Rhizopogonaceae, the oldest familial name of the three. *Truncocolumella* has been previously placed in the Rhizopogonaceae (Zeller, 1949; Smith, 1973). Based on evidence from phylogenetic analyses of molecular data of these genera, mycorrhizal host associations, and microscopic and morphological affinities we believe these genera form a distinct group from the rest of the boletes. We also acknowledge placement of the Melanogastraceae in the boletoid radiation of the Boletales. *Rhizopogon olivaceotinctus*, transferred to *Alpova* by Trappe (1975), is returned to the genus *Rhizopogon* where it had been originally described as *R. olivaceotinctus* A. H. Smith (Smith and Zeller, 1966).

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CHAPTER 3

RE-EXAMINATION OF INFRAGENERIC RELATIONSHIPS
WITHIN *RHIZOPOGON* BASED ON nrDNA
INTERNAL TRANSCRIBED SPACER SEQUENCES

Lisa C. Grubisha, James M. Trappe, Randy Molina, and Joseph W. Spatafora.

ABSTRACT

Rhizopogon (Basidiomycota, Boletales) is a genus of hypogeous fungi ectomycorrhizal mostly with members of the Pinaceae. This large genus comprises an estimated 100⁺ species, with the greatest diversity found in coniferous forests of the Pacific Northwestern United States. In this study, maximum parsimony analyses of nuclear ribosomal DNA internal transcribed spacer sequences of 41 *Rhizopogon* and 10 *Suillus* species were conducted to test infrageneric sectional relationships in *Rhizopogon* and examine phylogenetic relationships with the closely related epigeous genus, *Suillus*. Sequences from 10 *Rhizopogon* type collections were included in these analyses. Insertion/deletion events (indels) were problematic yet reflected taxonomic divisions. Separate analyses that addressed differential indel coding revealed no significant differences in tree topology. The results strongly supported the sister relationship of *Rhizopogon* to *Suillus*. The average length of the ITS region varied between 468-584 bases for the four sections of *Rhizopogon* and was 459 bases long for *Suillus*. *Rhizopogon* section *Rhizopogon* is not monophyletic and comprised two clades; one characterized by possessing several long indels. *Rhizopogon* sections *Amylopogon* and *Villosuli* formed well-supported clades, but species concepts within these sections were unresolved with respect to certain species. Four species from section *Fulviglebae* formed a strongly supported clade nested within section *Villosuli*. Infrageneric taxonomic revisions are proposed.

Key words: Boletales, Rhizopogonaceae, *Rhizopogon*, *Suillus*, ITS, indel coding, phylogeny

INTRODUCTION

Rhizopogon Fries (Rhizopogonaceae) is a basidiomycete genus containing more than 100 species (Martin, 1996). *Rhizopogon* is ectomycorrhizal mostly with Pinaceae and its worldwide distribution correlates with natural and exotic Pinaceae forests (Molina et al., in press). Despite this cosmopolitan range, a large diversity of species is found in pine (*Pinus* L.) and Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco] forests of the Pacific Northwestern United States (Smith and Zeller, 1966). *Rhizopogon* is a common ectomycorrhizal fungus in these coniferous forests and thus an important component of the forest ecosystem. *Rhizopogon* spp. are primary dietary components of many forest mammals such as the northern flying squirrel (*Glaucomys sabrinus*) (Maser et al., 1985; Colgan III, W., 1997); this mammal is the primary prey for the northern spotted owl (*Strix occidentalis caurina*) (Forsman et al., 1984; Carey et al., 1992), a threatened and endangered species in the Pacific Northwest.

The systematics of *Rhizopogon* remains in a state of flux that dates back to the early 19th century before type collections were designated, notes on fresh characters were scanty, and only gross morphological characters were used to describe species (Lange, 1954; Smith and Zeller, 1966; Smith, 1971). Our current understanding of *Rhizopogon* taxonomy is based primarily on a

landmark publication by A. H. Smith (Smith and Zeller, 1966) who significantly increased the number of described North American species to 137 including redescribed “European” species found in North America. Smith and Zeller (1966) divided the genus into two subgenera, *Rhizopogonella* and *Rhizopogon*. Species in subgenus *Rhizopogonella* were subsequently moved to *Alpova* (Trappe, 1975). Subgenus *Rhizopogon* was divided into four sections, *Amylopogon*, *Fulviglebae*, *Rhizopogon*, and *Villosuli*, based on macroscopic and microscopic sporocarp characters and color changes on the peridium from chemical reactions and bruising of the sporocarp (Smith, 1964; Smith and Zeller, 1966) (Appendix 1).

Although Smith’s important contribution towards understanding the systematics of this important fungal genus cannot be overstated, several unanswered questions remain. For instance, he placed several species with morphological affinities with section *Villosuli* in other sections, e.g., *R. vinicolor*, *R. clavitisporus*, etc.. He also may have described ontogenetic variations as separate species. Smith (Smith and Zeller, 1966) emphasized that this major taxonomic work was based on techniques available at the time and future revision was expected.

Hypotheses regarding the evolutionary relationship between *Suillus* and *Rhizopogon* are not new (Malençon, 1931; Heim, 1971; Thiers, 1971, 1984), and molecular evidence supports the hypothesis that *Suillus* and *Rhizopogon* are closely related (Bruns et al., 1989, 1990, Bruns and Szaro, 1992, Kretzer et al., 1996; Bruns et al., 1998). Judging from gross morphology, they

do not appear to share a recent common ancestor, but both genera possess boletoid spores and are ectomycorrhizal with conifers. Bruns et al. (1989) suggested that an accelerated rate of morphological change compared to molecular change occurred and may be explained by selective pressures acting on certain developmental genes. Questions remain concerning the nature of this relationship. Has *Rhizopogon* been derived several times within *Suillus*, or is it a monophyletic, but closely related genus?

Phylogenetic analyses of the internal transcribed spacer region (ITS) of the nuclear ribosomal DNA (nrDNA) are commonly used for addressing questions of systematics at the intergeneric and intrageneric level in fungi (Lee and Taylor, 1991; Baura et al., 1992; O'Donnell, 1992; Henrion et al., 1994; Hibbett et al., 1995; Moncalvo et al., 1995; Kretzer et al., 1996; Kretzer and Bruns, 1997; Harrington and Potter, 1997; Holst-Jensen et al., 1997; Liu, et al., 1997) and plants (reviewed in Baldwin et al., 1995). Through phylogenetic analyses of nrDNA ITS sequences, the objectives of this study are: 1) to further qualify the phylogenetic relationship between *Rhizopogon* and *Suillus*; 2) to categorize infrageneric sectional relationships in *Rhizopogon*, and 3) to develop hypotheses for evolution of host-specificity for the genus.

MATERIALS AND METHODS

Fungal specimens

Species representing the sections *Amylopogon*, *Fulviglebae*, *Rhizopogon*, and *Villosuli* of the genus *Rhizopogon* were selected for phylogenetic analysis of nucleotide data (Table 3.1). Forty collections used for DNA extraction were from the University of Michigan Herbarium (MICH) and the Mycological Collection of the Oregon State University Herbarium (OSC). Pieces of ten of these were donated from type collections by MICH. Specimens of *Boletus edulus*, *B. piperatus*, and *Alpova trappei* were also included (Table 3.1). GenBank numbers are given in Table 3.1 for sequences from 10 *Suillus* spp., *Rhizopogon subcaerulescens*, *Truncocolumella citrina*, *Chroogomphus vinicolor*, and *Gomphidius glutinosus*.

Nucleic acid extraction, polymerase chain reaction, and DNA sequencing

The protocol for nucleic acid extraction was modified from either a SDS-lysis buffer (Bruns et al., 1990) or a 2X CTAB method (Doyle and Doyle, 1987). The ITS region of the nrDNA was amplified via polymerase chain reaction (PCR) (Mullis and Fallona, 1987; Saiki et al., 1988). Primer pairs ITS-5 and ITS-4, ITS-5 and ITS-2, ITS-4 and ITS-3 (White et al., 1990) and ITS-1F and ITS-4B (Grades and Bruns, 1993) were used.

It was difficult to obtain DNA sequences from most of the type specimens from MICH. A further modification of the DNA extraction

Table 3.1. Genbank number, voucher, and collection location of species from which the internal transcribed spacer regions and 5.8S subunit were sequenced. Species listed only by Genbank number were not sequenced in this study.

Species	Voucher number ¹	Geographic location	Herbarium ²	GenBank accession number ³
<i>Alpova trappei</i> Fogel	JMT 16394	California, USA	OSC	AF074920
<i>Boletus edulus</i> Bull.:Fr.	LCG 184	Oregon, USA	OSC	AF074921
<i>Boletus piperatus</i> Fr.	LCG 185	Oregon, USA	OSC	AF074921
<i>Choogomphus vinicolor</i> (Peck) Miller				L54095
<i>Gomphidius glutinosus</i> (Schaeff.:Fr.) Fr.				L54114
<i>R. burlinghamii</i> A. H. Smith	JMT 17882	California, USA	OSC	AF058303
<i>R. colossus</i> A. H. Smith	AHS 49480 (Holotype)	Oregon, USA	MICH	AF071441 AF071442
<i>R. diabolicus</i> A. H. Smith	AHS 68424 (Paratype)	Washington, USA	MICH	AF071444 AF071443
<i>R. ellenae</i> A. H. Smith	AHS 66137 (Holotype)	Idaho, USA	MICH	AF071445 AF071446
<i>R. ellenae</i> A. H. Smith	JMT 17476	Oregon, USA	OSC	AF058311
<i>R. evadens</i> A. H. Smith	AHS 65484 (Holotype)	Oregon, USA	MICH	AF062927
<i>R. evadens</i> A. H. Smith	JMT 16402	California, USA	OSC	AF058312
<i>R. fusciorubens</i> A. H. Smith	JMT 17446	South Carolina, USA	OSC	AF058313

Table 3.1. (Continued).

Species	Voucher number ¹	Geographic location	Herbarium ²	GenBank accession number ³
<i>R. gilkeyae</i> A. H. Smith	JMT 19383	Oregon, USA	OSC	AF058304
<i>R. hawkeriae</i> A. H. Smith	AHS 68417 (Paratype)	Washington, USA	MICH	AF071447 AF071448
<i>R. luteolus</i> Fr.	JMT 22516	Uppsala, Sweden	OSC	AF062936
<i>R. occidentalis</i> Zeller & Dodge	JMT 17564	Oregon, USA	OSC	AF058305
<i>R. occidentalis</i> Zeller & Dodge	LCG 211	California, USA	OSC	AF062939
<i>R. ochraceisporus</i> A. H. Smith	AHS 65963 (Paratype)	Idaho, USA	MICH	AF071439
<i>R. ochraceisporus</i> A. H. Smith	JMT 17944	Oregon, USA	OSC	AF058306
<i>R. ochraceisporus</i> A. H. Smith	JMT 17916	Oregon, USA	OSC	AF062935
<i>R. ochraceorubens</i> A. H. Smith	AHS 59643 (Holotype)	Idaho, USA	MICH	AF062928
<i>R. ochraceorubens</i> A. H. Smith	JMT 19192 (Topotype)	Idaho, USA	OSC	AF071440
<i>R. parksii</i> A. H. Smith	JMT 17679	Oregon, USA	OSC	AF062930
<i>R. parksii</i> A. H. Smith	JMT 19446	Oregon, USA	OSC	AF058314
<i>R. parvulus</i> A. H. Smith	AHS 68364 (Paratype)	Idaho, USA	MICH	AF071449 AF071450
<i>R. rogersii</i> vSmith	JMT 17228	Oregon, USA	OSC	AF071437
<i>R. roseolus</i> Corda	JMT 17998	California, USA	OSC	AF062931
<i>R. rubescens</i> (Tul. & Tul.)Tul. & Tul.	JMT 8227	California, USA	OSC	AF058315

Table 3.1. (Continued).

Species	Voucher number ¹	Geographic location	Herbarium ²	GenBank accession number ³
<i>R. semireticulatus</i> A. H. Smith	JMT 7899	Oregon, USA	OSC	AF058307
<i>R. semireticulatus</i> A. H. Smith	JMT 17562	Oregon, USA	OSC	AF062940
<i>R. smithii</i> Hosford	JMT 12321	California, USA	OSC	AF062932
<i>R. sp. nov.</i>	JMT 17466	Oregon, USA	OSC	AF071438
<i>R. subcaerulescens</i> A. H. Smith				M91613
<i>R. subgelatinosus</i> A. H. Smith	JMT 7624	Oregon, USA	OSC	AF062937
<i>R. subpurpurascens</i> A. H. Smith	AHS 65669 (Paratype)	Idaho, USA	MICH	AF062929
<i>R. subpurpurascens</i> A. H. Smith	JMT 19168	Idaho, USA	OSC	AF058308
<i>R. subsalmonius</i> A. H. Smith	JMT 17218	Oregon, USA	OSC	AF062938
<i>R. succosus</i> A. H. Smith	JMT 19321	West Virginia, USA	OSC	AF062933
<i>R. villescens</i> A. H. Smith	JMT 17681	Oregon, USA	OSC	AF058309
<i>R. villosulus</i> Zeller	AHS 59143	Idaho, USA	MICH	AF071451 AF071452
<i>R. villosulus</i> Zeller	JMT 19466	Washington, USA	OSC	AF058310
<i>R. vinicolor</i> A. H. Smith	JMT 17899	Oregon, USA	OSC	AF058316
<i>R. vinicolor</i> A. H. Smith	JMT 20787	Idaho, USA (check)	OSC	AF062941
<i>R. vulgaris</i> (Vitt.) M. Lange	JMT 19154	Oregon, USA	OSC	AF062934
<i>R. zelleri</i> A. H. Smith	JMT 12974	New Mexico, USA	OSC	AF062942
<i>Suillus americanus</i> (Peck) Snell				L54103
<i>S. brevipes</i> (Peck) Kuntze				L54111

Table 3.1. (Continued).

Species	Voucher number ¹	Geographic location	Herbarium ²	GenBank accession number ³
<i>S. caerulescens</i> Smith & Thiers				L54096
<i>S. cavipes</i> (Opat.) Smith & Thiers				L54085
<i>S. grevillei</i> (Klotzsch) Singer				M91614
<i>S. granulatus</i> (Fries) Kuntze				L54113
<i>S. luteus</i> (Fries) Gray				L54100
<i>S. lakei</i> (Murrill) Smith & Thiers				L54086
<i>S. sinuspaulianus</i> (Pomerleau & Smith) Dick & Snell				L54078
<i>S. tomentosus</i> (Kauffmann) Singer, Snell & Dick				L54106
<i>Truncocolumella citrina</i> Zeller				L54097

¹LCG, Lisa C. Grubisha, AHS, Alexander H. Smith; JMT, James M. Trappe

²MICH, Herbarium of the University of Michigan; OSC, Mycological Collection of the Oregon State University Herbarium

³When one GenBank number is given it is for the sequence of the entire ITS region, ITS 1, ITS 2 and 5.8S subunit. When two GenBank numbers are given one is for the ITS 1 and partial 5.8S subunit sequence, and the second is for the sequence for the ITS 2 region and partial 5.8S subunit.

succeeded. After the tissue was ground in liquid nitrogen and SDS or 2X CTAB buffer was added, the tubes were incubated alternately between the 60 C waterbath and an ethanol/dry ice bath for five minute intervals several times. Furthermore, most successfully sequenced type specimens did not amplify or sequence well unless amplified as the separate and smaller ITS 1 and ITS 2 spacer regions.

PCR amplification, quantification, purification, sequencing, and alignment of sequences were previously described (Grubisha et al., 1998). In this study the annealing temperature was changed to 53 C. The ITS-1 and ITS-2 spacer regions and 5.8S subunit were sequenced with combinations of the primers ITS-5, ITS-2, ITS-4, ITS-3, ITS-1F, and ITS-4B.

Phylogenetic analysis

An alignment of 892 nucleotide bases representing the ITS-1, ITS-2, and 5.8S subunit was analyzed for four different insertion/deletion (indel) treatments. The PAUP NEXUS alignment file is included as Appendix 2. Alignment gaps were treated as follows: 1) All set--all characters were included and gaps treated as missing data; 2) Culled set--multiple-base indels and areas of ambiguous alignment were excluded, remaining gaps treated as missing data; 3) Indel "I" coded set-- a new character "I" was inserted to indels, ambiguous areas deleted, and remaining gaps treated as missing data; and 4) Binary coded set--indels were excluded and re-coded as presence/absence (0,1)

in the data matrix at the end of the alignment, remaining single-base gaps treated as missing, and ambiguous areas of the alignment were deleted.

Maximum parsimony analyses were performed using PAUP* version 4.0 (Swofford, 1998). One hundred heuristic searches were conducted with random sequence addition and tree bisection-reconnection (TBR) branch-swapping algorithms, collapsing zero-length branches and saving all minimal-length trees (MUL-PARS). To measure relative support for the resulting clades, 100 bootstrap replications (Felsenstein, 1985) were performed only on phylogenetically informative characters with the following parameters: 5 random sequence additions, TBR, and MUL-PARS off.

Choice of outgroup

Entire ITS sequences were obtained from *Boletus edulus*, *B. piperatus*, and *Alpova trappei*, with the intention of using species from the boletoid radiation of the Boletaceae as a more distantly related outgroup; however, sequences from these species were highly divergent and simply too difficult to align with *Suillus* and *Rhizopogon* sequences. Introduction of excessive and ambiguous alignment gaps was necessary and resulted in the loss of phylogenetic information. These outgroup species were removed from the alignment and not included in the phylogenetic analyses. Thus, the outgroup chosen for these analyses was selected from within the Rhizopogonaceae of the Boletales: *Truncocolumella citrina*, *Chroogomphus vinicolor*, and

Gomphidius glutinosus and were easily aligned with *Suillus* and *Rhizopogon* sequences.

RESULTS

Parsimony analyses

No major differences in tree topology could be inferred from the four indel treatments. Bootstrap values varied slightly, but remained essentially unchanged, except for the Culled set (treatment 2), when all ambiguous areas and large indels were removed. In this case somewhat lower bootstrap values were recovered. Results from the four analyses are summarized in Table 3.2. The number of most parsimonious trees ranged from 16 for the All (treatment 1) and Culled sets (treatment 2) to 32 for the Binary coded set (treatment 4) and 80 for the Indel "I" set (treatment 3). Tree length ranged from 630 for the Culled set to 931 steps for the All set.

The strict consensus tree for the Indel "I" coded treatment is presented in Fig. 3.1. Maximum parsimony analysis yielded 80 trees of 739 steps. For these trees, the consistency index (CI) was 0.512, retention index (RI) was 0.801, and the rescaled consistency index (RC) was 0.411. There were 234 parsimony informative characters. Bootstrap values greater than 50% are indicated above the respective internode.

Table 3.2. Results from maximum parsimony analysis of four insertion/deletion (indel) coding strategies.

Analysis Treatment ¹	Indel coding			Most parsimonious trees					
	Gaps as missing	"I" inserted to indel	Presence/absence (0,1)	Number of Characters ²	Number	Length	CI	RI	RC
1	yes	no	no	291	16	931	0.522	0.767	0.400
2	yes	no	no	199	16	630	0.515	0.792	0.408
3	yes	yes	no	234	80	739	0.512	0.801	0.411
4	yes	no	yes	221	32	687	0.516	0.801	0.414

¹Different treatments of indels (see text for further discussion):

1 = All set: all characters states were included, even ambiguous areas of the alignment, all gaps scored as missing data;

2 = Culled set: ambiguous areas of alignment and large inserts were excluded, gaps treated as missing data;

3 = Indel "I" set: ambiguous areas of alignment excluded, character "I" inserted into gaps;

4 = Binary coded set: ambiguous areas of alignment excluded; large gaps excluded and coded as presence/absence.

²Number of parsimony informative characters included in the analysis.

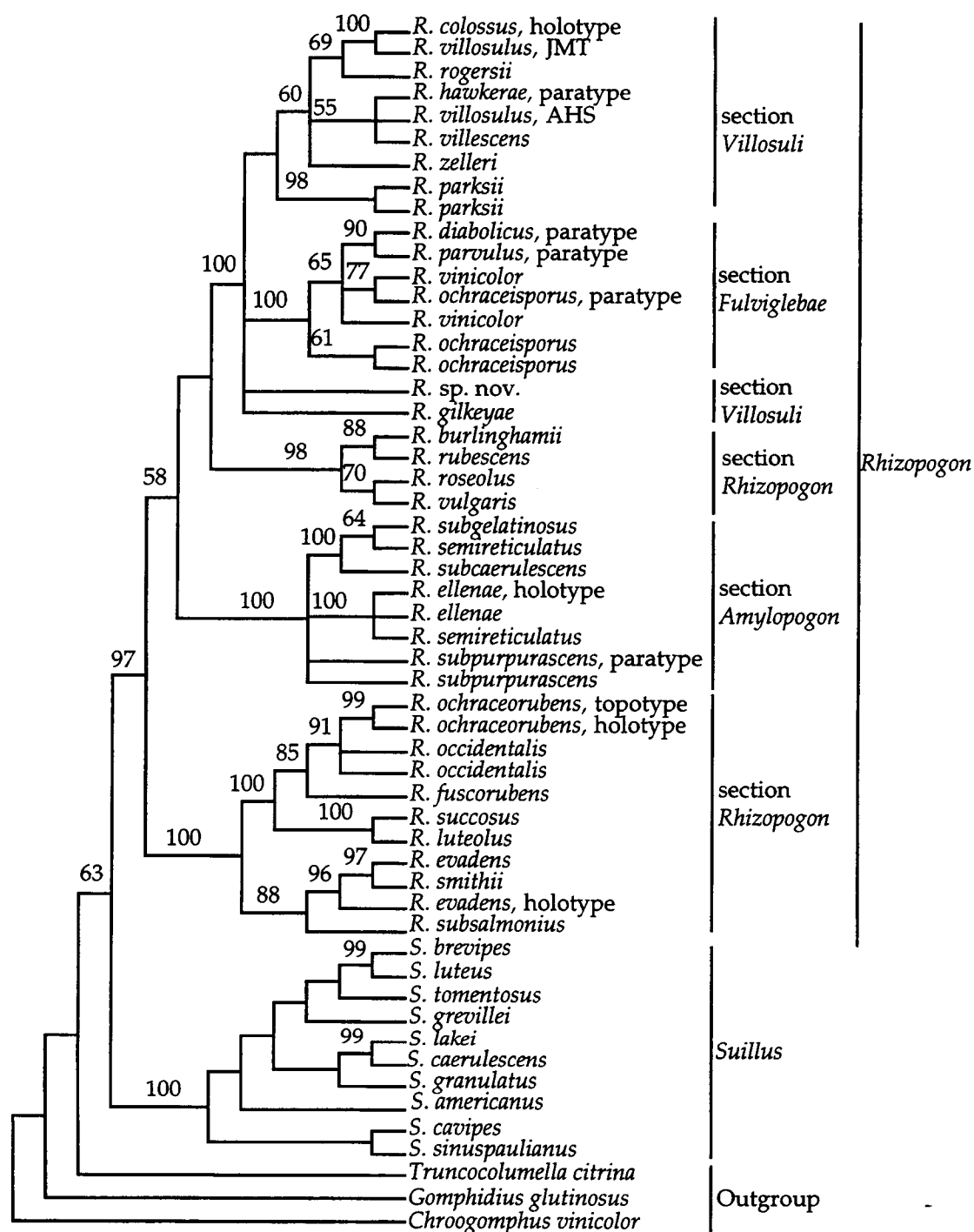


Figure 3.1. Strict consensus cladogram of 80 equally parsimonious trees of 739 steps based on complete ITS nrDNA sequences when indels were coded with "I" and ambiguous areas of the alignment were excluded. Bootstrap values are indicated at the respective internode. CI = 0.512, RI = 0.801. Placement of species in sections of genus *Rhizopogon* is according to Smith and Zeller (1966).

ITS sequence analysis

Length variation of the ITS 1 and ITS 2 between *Suillus* and *Rhizopogon* and within *Rhizopogon* sections is recorded in Table 3.3. The 5.8S region was 159 bases long for all taxa, with four exceptions that differed

Table 3.3. ITS 1 and ITS 2 size and length variation for *Suillus* and *Rhizopogon*.

Taxa	Average size and length variation ¹		
	ITS 1	ITS2	ITS + ITS 2
<i>Suillus</i>	209 205-218	249 247-251	459 456-465
<i>Rhizopogon</i>	247 218-344	256 232-284	510 461-621
section <i>Amylopogon</i>	224 220-228	262 248-264	468 466-468
section <i>Fulviglebae</i>	220	248 246-248	468 466-468
Section <i>Rhizopogon</i> A ²	318 282-344	267 254-284	584 544-621
Section <i>Rhizopogon</i> B	223	254 242-267	477 465-490
Section <i>Villosuli</i>	219 218-220	247 232-254	471 461-493

¹Alignment length of the ITS 1 = 420 and ITS 2 = 313 bases.

²Section *Rhizopogon* A refers to the group of species that have long indels in the ITS. These also form the basal section *Rhizopogon* clade in Fig. 3.2. Section *Rhizopogon* B includes species from section *Rhizopogon* lacking the long inserts in ITS and forming the second, more derived clade in Fig. 3.2.

only by 1 base. Determination of the length of the ITS 1, ITS 2, and 5.8S subunit was based on the 5.8S length of *Rhizopogon subcaerulescens* and *Suillus sinuspaulianus* as identified by Cullings and Vogler (in press). The average range between *Rhizopogon* sections was 219-318 bp for the ITS 1 and 247-267 bp for the ITS 2. The alignment length of the ITS 1 was 420 bp and ITS 2 was 313 bp. The majority of the long indels was restricted to the ITS 1. The ITS 2 was easily aligned, except for two areas which were excluded from all analyses.

Most species sampled from section *Rhizopogon* possess 2 long inserts of 24 and 33 bp. Within this group, *R. succosus* and *R. luteolus* contain two additional inserts of 8 and 11 (*R. luteolus* has a two base-pair deletion in this insert) bases long. *R. smithii* and *R. evadens* (JMT 16402) share two long inserts of 9 and 29 bases. Three species, *R. rubescens* (*R. roseolus*), *R. vulgaris*, and *R. burlinghamii*, sampled from section *Rhizopogon*, lack these inserts. Multiple-base indels are mapped onto a phylogram in Figure 3.2. Indel 14 and 27 represents an AG rich region in the beginning of the ITS 1 in the basal section *Rhizopogon* clade and *Suillus*, respectively. A TC rich region that ranges in length between 4 -11 bases also in the basal section *Rhizopogon* clade, indel 16, and *Suillus*, indel 28, are also included on Fig. 3.2. These two areas were excluded as ambiguous alignment areas in three of the four maximum parsimony analyses. They are included on Fig. 3.2 although the alignment is not certain, because the indel itself is believed to show

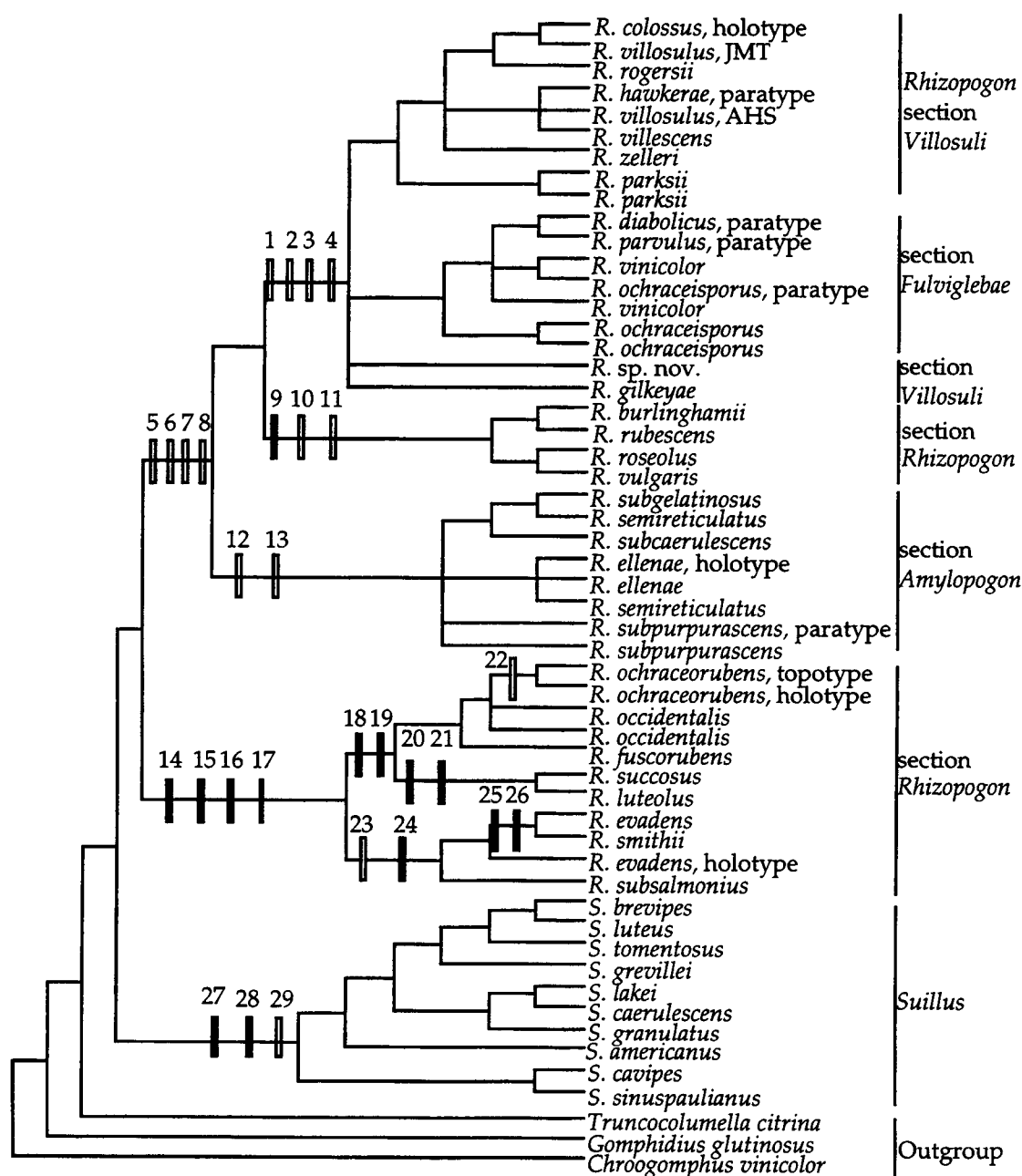


Figure 3.2. The cladogram presented in Fig. 3.1 with multiple-base insertion/deletions (indels) mapped to respective branches. Solid bar indicates insertions, open bar indicates deletions. Position in alignment is as follows: 1=193-195, 2=160-163, 3=624-630, 4=638-639, 5=152-155, 6=58-82, 7=290-318, 8=141-151, 9=120-124, 10=156-159, 11=190-196, 12=156-159, 13=190-194, 14=58-82, 15=109-112, 16=141-151, 17=233-264, 18=190-196, 19=55-58, 20=47-54, 21=67-77, 22=267-288, 23=190-195, 24=830-834, 25=221-229, 26=290-318, 27=58-82, 28=141-151, 29=185-288. Placement of species in sections of the genus is according to Smith and Zeller (1966)

phylogenetically important information; both of these indels are not present in the more derived clades of genus *Rhizopogon*. The indels followed sectional concepts as defined A. H. Smith (Smith and Zeller, 1966).

DISCUSSION

Sequence analyses

Multiple-base indels, especially in ITS analyses, are commonly treated as missing data or are excluded entirely from phylogenetic analyses. A universal method to code indels resulting from length variable sequences in phylogenetic analyses is presently unclear (Liston, et al., in press). Excluding indels or treating them as missing data may result in the loss of phylogenetic information (Baum et al., 1994). Single-base gaps in a conserved region may be treated as a fifth character (Swofford, 1993); however, coding all nucleotide positions of a single event multiple-base gap as fifth characters will result in overweighing the gap (Baum et al., 1994; Hibbett et al., 1995). Another approach is the insertion of an additional character to a multiple-base indel so as to retain any phylogenetic information and record the presence of the indel itself as informative but not overweigh the gap as a series of independent characters (Bruns et al., 1992; Hibbett et al., 1995; Kretzer et al., 1996, Kretzer and Bruns, 1997). Alternatively, a long indel may be downweighed by recoding the parsimony informative characters as a single character (Holst-Jensen et al., 1997). Recoding gaps as binary, presence/absence, characters is

not uncommon (Wojciechowski et al., 1993; Hibbett et al., 1995; Manos, 1997; Moller and Cronk, 1997; Vargas et al., 1998; Downie et al., 1998). Another method is to use test statistics to determine the level of noise versus phylogenetic signal in indels. Lutzoni (1997) determined indel-rich and indel-poor regions of an ITS alignment and tested these regions for presence of phylogenetic signal from g_1 values and the PC test. Indel rich regions were rejected if the phylogenetic signal as determined by the g_1 values and the PC test was considered artifactual. Hibbett et al. (1995) discuss gap coding strategies.

The degree of multiple-base indels present in this alignment of *Rhizopogon* ITS sequences seems to be uncommon and has not previously appeared in published alignments. In addition, most of the indels strongly related to taxonomic groupings. Several approaches of indel coding were attempted to determine their importance in adding support for these groups. Results indicate the indels offer additional support but are not crucial to resulting tree topology; data strongly support the resulting topology when indels are excluded.

Tree topology did not significantly differ between the four analyses run with the different treatment of indels. Variations in tree topology from these analyses were restricted to 1) the placement *R. gilkeyae* between basal to section *Villosuli* or basal to the section *Fulviglebae* clade, and 2) the branching order of *R. subcaerulescens*, *R. subgelatinosus*, and *R. semireticulatus* (JMT 7899). Bootstrap values were slightly higher in the indel

coded analyses, an observation consistent with other studies that compared coding indels.

Phylogenetic relationship between Rhizopogon and Suillus

Sister-group relationship between *Suillus* and *Rhizopogon* is strongly supported by the results presented here. *Suillus* and *Rhizopogon* both form well-supported monophyletic groups with bootstrap values of 100 and 97, respectfully. *Suillus* species in this study associate with a variety of conifer hosts as indicated by Kretzer et al. (1996). Although previous studies have shown that *Suillus* and *Rhizopogon* are closely related, the monophyly of these two respective genera was uncertain due to limited species sampling and because the choice of loci was less variable than the ITS region. We attempted to include enough species from both genera to represent the range of conifer associates. Similar results were observed in a recent phylogenetic study of the large subunit nrDNA sequences of *Suillus*, *Rhizopogon*, *Truncocolumella*, *Alpova*, and other genera from the boletoid radiation of the Boletaceae (Grubisha et al., 1998).

Examination of infrageneric relationships in Rhizopogon

Most sectional relationships, as defined by Smith (1964; Smith and Zeller, 1966), are well-supported. Sections *Villosuli* and *Amylopogon* are strongly supported groups with bootstrap values of 100. Section *Rhizopogon*

is not monophyletic and forms two well-supported non-sister clades with high bootstrap values of 100 and 98. The other section that deviates from Smith's classification is that some species of section *Fulviglebae*, are nested within section *Villosuli* and form a strongly supported group by a bootstrap value of 100.

Section *Rhizopogon*

Smith and Zeller (1966) divide *Rhizopogon* section *Rhizopogon* into two subsections, two series, and 11 stirps. In this study, we sampled 15 sequences from 12 species representing both subsections and series and six stirps. The subsections are separated by spore width: species in subsection *Rhizopogon* have spores 3-5 μm wide, whereas species in subsection *Angustispori* have spores 1.6-3 μm wide. In both subsections the next division depends on whether the peridium (1) develops yellow colors during development and bruises red, (2) lacks yellow at all developmental stages and bruises red, or (3) lacks yellow coloration and does not bruise red (Table 3.4).

In *Rhizopogon* section *Rhizopogon* species that stain red and have most spores 3 μm or more wide are placed in subsection *Rhizopogon* stirps *Rubescens*. Three species sampled from stirps *Rubescens* are *R. rubescens*, *R. succosus*, and *R. roseolus*. Only *R. luteolus* was sampled from subsection *Rhizopogon* stirps *Luteolus*. *Rhizopogon succosus* and *R. luteolus* share several morphological characters but are distinct species (Miller, 1986; Hosford and Trappe, 1988). Based on peridium coloration, microscopic characters, and

Table 3.4. Taxonomic divisions in *Rhizopogon* section *Rhizopogon* based on spore width and peridium coloration as defined by A. H. Smith (Smith and Zeller, 1966).

<i>Rhizopogon</i> section <i>Rhizopogon</i>	Spore width (μm)	Peridium coloration		
		Yellow ¹	Bruises red	Other
Subsection <i>Rhizopogon</i>	3.5-5			
Stirps <i>Rubescens</i>		yes ²	yes	
Stirps <i>Luteolus</i>		yes	no	
Subsection <i>Angustispori</i>	1.6-3			
Series <i>Lutei</i>		yes		
Stirps <i>Vulgaris</i>		yes	yes	
Stirps <i>Ochraceorubens</i>		yes	no	
Series <i>Versicolores</i> ³		no		yes
Stirps <i>Subsalmonius</i>			no	peach-pink to salmon pink
Stirps <i>Evadens</i>			yes	

¹Yellow color refers to whether the peridium develops yellow colors during some time of its development, and should not be confused with bruising yellow.

²A. H. Smith identifies three species in Stirps *Rubescens* that do not have a yellow stage, including *R. roseolus* Corda sensu Smith.

³Only two of the seven stirps in Series *Versicolores* are mentioned here.

the glass-hard consistency of the dried gleba, Miller (1986) suggested that a better placement of *R. succosus* is in stirps *Luteolus*. These observations are supported by the data presented here. The relationship between these two species is supported by a bootstrap of 100. In addition to being morphologically similar, they share similar long insertions in the ITS 1 sequences.

Smith (Smith and Zeller, 1966) placed *R. vulgaris* in subsection *Angustispori*, stirps *Vulgaris* because it has narrow spores, stains red, and is yellow at some point of its development. Smith did recognize the similarity of species in stirps *Vulgaris* with those in stirps *Rubescens* and mentions that stirps *Vulgaris* is a continuation of stirps *Rubescens* into the narrow spored species. *Rhizopogon rubescens* sensu Smith and Zeller has yellow coloration on the peridium whereas *R. roseolus* sensu Smith and Zeller does not. Smith's descriptions of *R. rubescens*, *R. vulgaris*, and *R. roseolus*, included in Smith and Zeller (1966) are based on examinations of North American collections. These three species were originally described from Europe in the nineteenth century (Molina et al., in press). This study supports the close relationship of these species, sensu A. H. Smith. *Rhizopogon rubescens*, *R. roseolus*, *R. vulgaris*, and *R. burlinghamii*, form a distinct clade separate from the other species sampled from section *Rhizopogon*. These species also lack several large indels present in species found in the other section *Rhizopogon* clade. These results and the morphological similarities of these species support their separation from section *Rhizopogon*.

The two holotypes from MICH that were sampled from section *Rhizopogon*, *R. ochraceorubens* and *R. evadens*, are from subsection *Angustispori*, series *Lutei* and *Versicolores* respectively. Species in series *Versicolores* do not have a yellow ontogenetic stage. *Rhizopogon ochraceorubens* and *R. fuscorubens* are closely related and placed in strips *Ochraceorubens*. Smith indicates that the major difference between these two

is the rhizomorphs on the peridium of *R. fuscorubens* dry black and the peridium dries yellow. The rhizomorphs on the peridium of *R. ochraceorubens* do not dry black and the peridium dries red. When revived in KOH, the sectioned peridium is bright red for both species and very prominent in the holotype specimen. *Rhizopogon occidentalis*, originally placed in stirps *Rubescens*, appears to be closely related to both *R. ochraceorubens* and *R. fuscorubens*, although the sectioned peridium lacks the bright red reaction to KOH. All three species fruit in association with pines and generally form ectomycorrhizae only with pines in pure culture syntheses (Molina and Trappe, 1982, 1994). *Rhizopogon occidentalis* will form mycorrhizae with *Arctostaphylos* and *Arbutus* spp. if pines are present as the primary host (Molina et al., 1997).

Two species were sampled from Series *Versicolores*, *R. subsalmonius* and *R. evadens* and belong to stirps *Subsalmonius* and *Evadens* respectively. *Rhizopogon subsalmonius* does not stain red when cut. *Rhizopogon evadens* stains red, but the peridium is white and lacks yellow coloration. The peridium does not stain bright red when sections are treated with KOH. Hosford (1972, 1975) placed *R. smithii* in *Rhizopogon* section *Rhizopogon*. He indicates that *R. smithii* shares several microscopic and peridial characteristics with *R. evadens* var. *evadens*, but *R. evadens* var. *evadens* differs in lacking a yellow stage and intense olive green reaction to FESO_4 on the fresh peridium, as seen in *R. smithii*. The results of this study show that *R. evadens* var. *evadens* and *R. smithii* are closely related.

Section *Fulviglebae*

The four species sampled from section *Fulviglebae* were selected because they shared some peridial characters with section *Villosuli* and, as with the *Villosuli*, are associated with Douglas-fir. They form a tight clade with a bootstrap value of 100 within section *Villosuli*. Because these species are such a distinct clade, this placement may be considered a sister-group to section *Villosuli*. *Rhizopogon gilkeyae* does share some morphological affinities with these species. Smith and Zeller (1966) placed it in stirps *Viridis* (section *Villosuli*) because the peridium lacks the green to olive reaction to KOH, as do *R. vinicolor*, *R. diabolicus*, *R. parvulus*, and *R. ochraceisporus*. Smith and Zeller (1966) note that the base of the spores is “obscurely” truncate. *Rhizopogon gilkeyae* does have flagellate hyphae as do many other members of section *Villosuli*. This species may represent the transition from stirps *Vinicolor* to the remaining species in section *Villosuli*. *Rhizopogon parvulus* and *R. diabolicus* are closely related species, both morphologically (Smith and Zeller, 1966) and based on our data. Their relationship to *R. vinicolor* and *R. ochraceisporus* remains to be determined.

Species in stirps *Vinicolor* and *R. ochraceisporus* (stirps *Thaxteri*) in section *Fulviglebae* are morphologically similar. Although Smith and Zeller (1966) mention that within stirps *Vinicolor* there is a trend towards brown-walled hyphae in the peridium, a characteristic of species in section *Villosuli*, descriptions of brown-walled hyphae are not included in species descriptions

for stirps *Vinicolor*. The species in stirps *Vinicolor* and *R. ochraceisporus* also associate with Douglas-fir.

R. vinicolor and *R. ochraceisporus* may be ontogenetic stages of a single species. However, except for glebal color, these two species are very similar morphologically. The two sequences from *R. ochraeisporus* (JMT 17944 and JMT 17916) are from the same field collection, but were separated because of glebal color. The gleba of 17916 is rusty to olive brown whereas the gleba of JMT 17944 is dark greenish olive. As described by Smith and Zeller (1966) *R. vinicolor* typically has an olive gleba whereas *R. ochraceisporus* is rusty. That one *R. vinicolor* sequence is closer to the paratype of *R. ochraceisporus* than it is to the other *R. vinicolor* implies conspecificity of the two species.

Unfortunately the paratype of *R. vinicolor* did not sequence well. Further sampling is needed to address this question, especially sequencing the holotype, paratype, or topotype of *R. vinicolor*. *Rhizopogon vinicolor* is strongly host-specific with Douglas-fir. Several studies have demonstrated that *R. vinicolor* only forms ectomycorrhizae with Douglas-fir, even when other conifers are present (Molina, 1980; Molina and Trappe, 1982, 1994; Massicote et al., 1994; Molina et al., 1997).

Section *Villosuli*

Smith (1964) recognized twenty-one species of *Rhizopogon* in section *Villosuli*. These are separated from the other three sections by having brown-walled hyphae that form a distinct epicutis in the peridium and nontruncate,

nonamyloid spores. Internal transcribed spacer sequences from the holotype of *R. colossus* and paratypes of *R. villosulus* and *R. hawkeriae* are similar in these analyses.

Based on the findings presented here, *R. colossus*, *R. villosulus*, *R. rogersii*, *R. hawkeriae* and *R. villescens* could be a single species that shows variation or several very closely related species. Some years after publication of Smith and Zeller (1966), Smith concluded from additional collecting that *R. colossus* was a developmental stage of *R. villosulus* (personal communication to J. M. Trappe), and we agree based on morphological and molecular evidence. Further sampling of types from other species in the *Villosuli* is needed to clarify the relationships among these species. Perhaps sampling a gene which showed more interspecific variation would be appropriate for these close relatives.

Some species concepts in section *Villosuli* remain unresolved because of the gradient of ontogenic stages that may currently be identified as individual species and the placement of species with similar morphological characteristics in other sections of *Rhizopogon*. In this study, four species in section *Fulviglebae*, *R. vinicolor*, *R. ochraceisporus* (paratype), *R. diabolicus* (paratype), and *R. parvulus* (paratype) are nested within section *Villosuli*. Based on morphological similarities and our molecular phylogenetic results, either *R. gilkeyae* should be moved to section *Fulviglebae* and this clade considered a sister-group to section *Villosuli*, or these four species could be transferred to section *Villosuli*. Because *R. gilkeyae* is clearly a member of the

Villosuli from a morphological standpoint and the four species in section *Fulviglebae* differ from the *Villosuli* primarily in having truncate spores, we suggest the second option. Spore truncation simply reflects the breadth of attachment of the spore to the basidium, a character of doubtful phylogenetic significance. Furthermore, these fungi are all host-specific to Douglas-fir, a character not seen within other *Rhizopogon* sections (Molina and Trappe, 1994; Massicotte et al., 1994)

Section *Amylopogon*

Section *Amylopogon* is monophyletic and forms a well-supported clade with a bootstrap value of 100. The holotype of *R. ellенаe* and a paratype of *R. subpurpurascens* were sampled. Martin (1996) moved *R. ellенаe* to section *Rhizopogon* because it does not have amyloid spores. In our results, the holotype of *R. ellенаe* is found in the strongly supported section *Amylopogon* clade. Smith and Zeller (1966) stated that although not all species in section *Amylopogon* have amyloid spores, all *Rhizopogon* species with amyloid spores are placed in this section. Section *Amylopogon* is supported by anatomy, the olive to green, blue, pink or red reaction of the peridium to KOH, and, when present, amyloid spores. Species in section *Amylopogon* are the most broad-ranging in the genus in terms of mycorrhizal hosts, but they typically occur in conifer forests with pines and true firs (*Abies* Mill). *Rhizopogon subcaerulescens* forms ectomycorrhizae

with Douglas-fir in laboratory conditions (Massicote, et al., 1994), although they are not known to be associated with Douglas-fir forests.

Host specificity and evolution

Rhizopogon spp. show a great deal of host specificity with members of the Pinaceae (Molina et al., 1992). Smith and Zeller (1966) noted that the greatest species diversity occurs in the coniferous forests of the Pacific Northwest of the United States. In general, sections of *Rhizopogon* show a certain degree of specificity for particular genera of Pinaceae and some species show specificity with either *Pinus* spp. or Douglas-fir (Molina et al., in press). For several *Rhizopogon* species host specificity was supported by pure culture synthesis (Molina and Trappe, 1982b, 1994) and spore inoculation studies (Massicote et al., 1994; Molina et al., 1997). These data offer further support to Smith's (1964; Smith and Zeller, 1966) sectional hypotheses (Figure 3.3). Molina and Trappe (1994) and Molina et al. (in press) suggest because of its diversity and quantity of Pinaceae hosts the Pacific Northwestern United States, has been a major area for the evolution and speciation of *Rhizopogon* and their conifer hosts.

Evolutionary relationships at the generic level of the Pinaceae are not strongly supported in phylogenetic studies (Prager et al, 1976; Price et al., 1987; Chaw et al., 1997; Stefanovic et al., 1998). Hart's (1987) cladistic analysis of morphological characters includes the genera *Larix*, *Pseudotsuga*, *Pinus*,

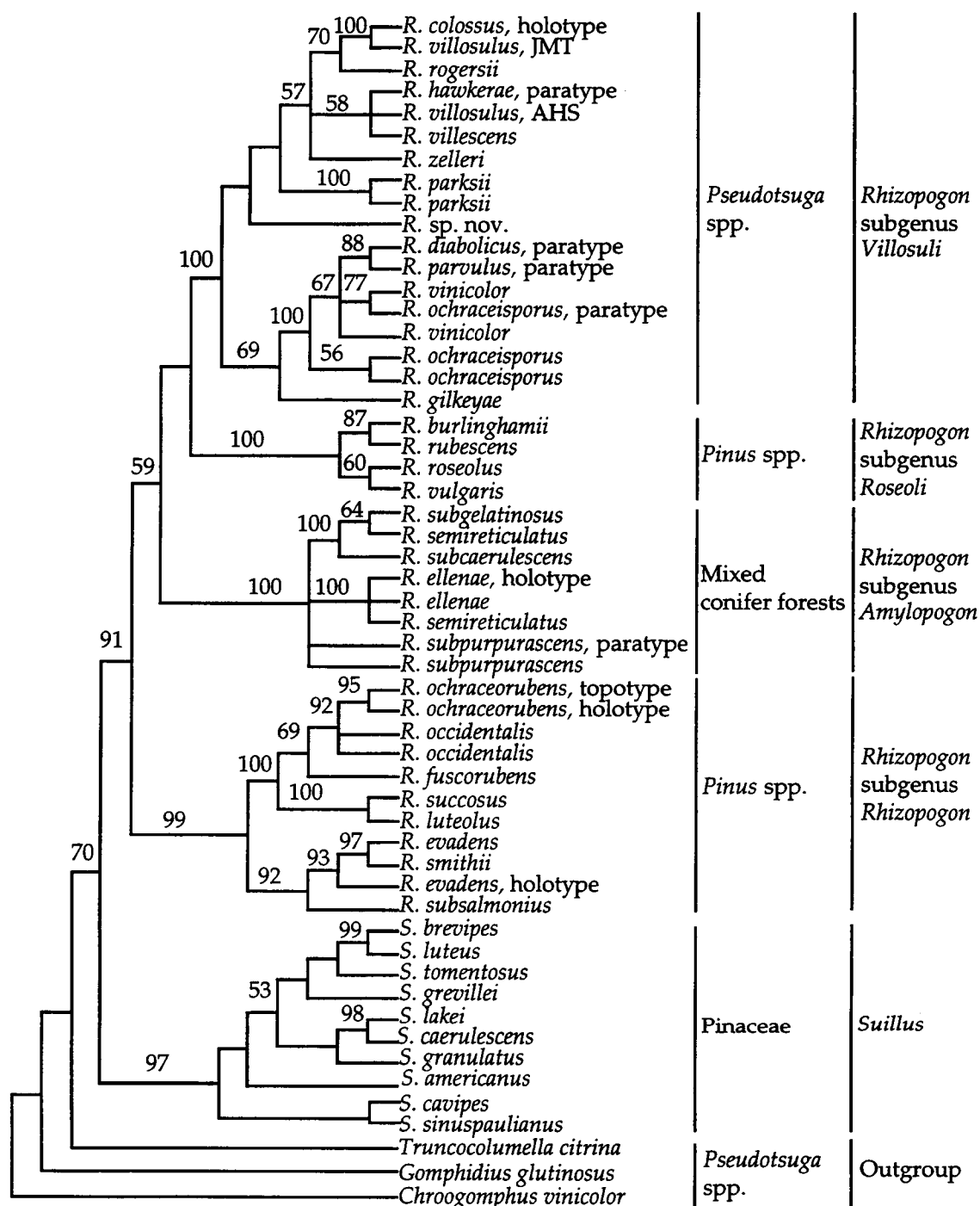


Figure 3.3. Strict consensus cladogram of 32 equally parsimonious trees using binary (presence/absence) gap coding. Bootstrap values are noted above the respective internode. Primary ectomycorrhizal hosts are listed. Sections are elevated to subgenera and appear to be associated with certain Pinaceae genera, with the exception of subgenus *Amylopogon* that is associated with a broad range of hosts. Host information is from collection data and pure culture synthesis studies (Molina et al., 1992).

Abies, *Picea*, and *Tsuga*, but provides no measure of support for the resulting clades. In that study, *Pinus* appeared to be the ancestral genus, while the pairs *Pseudotsuga/Larix* and *Abies/Tsuga* formed a sister group. *Rhizopogon* section *Rhizopogon* appear to be the ancestral *Rhizopogon* section and is strongly associated with *Pinus* spp. The degree of host specificity with all species in *Rhizopogon* section *Villosuli* to *Pseudotsuga* to the complete exclusion of other Pinaceae, implies that they have been co-speciating for a long time and may have developed unique recognition factors.

Taxonomic revisions

As suggested by Molina (1980), we propose the elevation of the following sections to subgenera with appropriate emendations:

section *Amylopogon* to subgenus *Amylopogon*

section *Villosuli* to subgenus *Villosuli*

section *Rhizopogon* to subgenus *Rhizopogon*

We propose the erection of subgenus *Roseoli* with *R. roseolus* as the type, to accommodate the species in stirpes *Rubescens* and *Vulgaris*. We include all species in these two stirps with the realization that further research is needed to further test the proposed classification. The transfer of *R. succosus* to stirps *Luteolus* and *R. occidentalis* to stirps *Ochraceorubens*.

We propose transfer of the seven species in stirpes *Vinicolor* to subgenus *Villosulus* and the erection of section *Vinicolor* to complement section *Villosuli*. We include all species in stirps *Vinicolor* because they are

closely related to *R. vinicolor* morphologically (Smith and Zeller, 1966) and ecologically (Molina and Trappe, 1994; Massicote et al., 1994), but further morphological and molecular analyses are needed to fully resolve their standing. *Rhizopogon ochraceisporus* is transferred to subgenus *Villosuli*, section *Vinicolor*, but further research is needed to determine its relationship to *R. vinicolor*. *R. clavitisporus* and *R. subclavitisporus* (section *Fulviglebae*, stirps *Clavitisporus*) possess brown-walled and flagellate hyphae (Smith and Zeller, 1966). These two species are transferred to subgenus *Villosuli*, section *Villosuli*. The remaining species in section *Fulviglebae*, *R. exiguus*, *R. hysterangioides*, *R. variabilisporus*, *R. griseogleba*, *R. pannosus*, *R. fragmentatus*, *R. truncatus*, *R. tsugae*, *R. thaxteri*, *R. atlanticus*, and *R. lutescens* are transferred as section *Fulviglebae* to subgenus *Rhizopogon* with *R. exiguus* as the type of the section. Smith and Zeller (1966) also recognized the similarities between section *Fulviglebae* and section *Rhizopogon*. We anticipate, as did Smith, that further research on individual species in the *Fulviglebae* will lead to refinement and probably additional reclassifications of some species.

While the ITS provided a great deal of resolution to determine subgeneric relationships in *Rhizopogon*, distinctions between closely related or potentially conspecific species were unclear. A gene that possesses more interspecific variation between closely related species, perhaps the IGS or β -tubulin gene, would be desirable for future studies of species concepts.

The inclusion of sequences from type collections reinforced and clarified relationships within *Rhizopogon* and was invaluable in this study. This database of *Rhizopogon* sequences may also be used as a database for sequences from *Rhizopogon* ectomycorrhizal root tips. Bidartondo et al. (1998) demonstrated that *R. ellенае* form ectomycorrhizae with the snow plant, *Sarcodes sanguinea*, based on the ITS sequence of the holotype of *R. ellенае* provided by this study combined with sporocarp collection data.

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CHAPTER 4

CONCLUSIONS

SUMMARY

Rhizopogon systematics

In this thesis, I have tested hypotheses of the systematics of the ectomycorrhizal genus *Rhizopogon* through phylogenetic analyses of nucleotide sequence data from ribosomal DNA. First, the relationship of the genus *Rhizopogon* with other genera of the Boletales was examined, with special emphasis on the genus *Suillus* (Chapter 2). Secondly, Smith's (Smith and Zeller, 1966) hypotheses on infrageneric relationships in *Rhizopogon* were tested (Chapter 3).

The Boletales is a large and complex order. Relationships in the boletoid radiation were not clarified, but *Boletus*, *Boletellus*, *Xerocomus*, *Phylloporus*, *Tylopilus*, *Alpova*, and *Melanogaster* were well supported as a group. *Boletus* was not monophyletic and *Alpova* was polyphyletic in these analyses. Although placement of *Melanogaster* in the Boletales has recently been suggested (Besl et al., 1996), ours may be the first report of *Melanogaster* as part of the boletoid radiation within the Boletales. *Rhizopogon*, *Suillus*, and *Truncocolumella* formed a distinct and strongly supported group. The suilloid radiation is a cohesive group of closely related genera. In order to

show the natural classification of these closely related genera, I placed *Suillus*, and *Truncocolumella* and the Gomphidiaceae (*Gomphidius*, *Chroogomphus*, *Cystogomphus*, *Brauniellula*, and *Gomphogaster*) in the Rhizopogonaceae.

The Boletales are obligately ectomycorrhizal. Genera in the boletoid radiation associate with either gymnosperm or angiosperm hosts. Within and between genera there is a wide range of host associations, although certain genera or species may show a narrow range of host specificity, e. g., *Alpova diplophloeus* is host specific with alders. This contrasts with the suilliod radiation. A striking feature of the Rhizopogonaceae is the host specificity to Pinaceae with only rare exception. Within *Rhizopogon* (Chapter 3) and *Suillus* (Thiers, 1975; Kretzer et al., 1996) certain subgenera or species are host specific with *Pinus* spp., *Pseudotsuga* spp., or *Larix* spp.. *Chroogomphus*, and *Truncocolumella* are host specific with Douglas-fir.

In general, infrageneric relationships in *Rhizopogon* as defined by A. H. Smith (Smith and Zeller, 1966) were well supported. Section *Amylopogon* and *Villosuli* were both well supported and monophyletic. Many species of section *Fulviglebae* were nested within section *Villosuli*. Section *Rhizopogon* was not monophyletic. These three sections were elevated to subgenera. A new subgenus, *Roseoli*, was erected for the *Rhizopogon roseolus*/*R. vulgaris* group. A summary of the taxonomic revisions proposed in this thesis is outlined below. Subgenera in *Rhizopogon* also reflect host associations, e. g., species in subgenus *Villosulus* are host specific with *Pseudotsuga*. Groupings within *Rhizopogon* subgenera *Amylopogon* and

Rhizopogon remain the same as outlined by Smith and Zeller (1966), but stirps are elevated to sections. Transfer of species between sections and subgenera was discussed in Chapter 3.

Summary of taxonomic revisions:

Rhizopogonaceae

Subfamily *Rhizopogonoideae*

Rhizopogon

Subgenus *Amylopogon*

Subgenus *Roseoli*

Section *Roseoli*

Section *Vulgares*

Subgenus *Villosuli*

Section *Villosuli*

Section *Vinicolores* (formerly stirps
Vinicolor)

R. ochraceisporus

R. clavitisporus

R. subclavitisporus

Subgenus *Rhizopogon*

Section *Rhizopogon*

Section *Fulviglebae*

Subfamily *Suilloideae*

Suillus

Truncocolumella

Subfamily *Gomphidioideae* (formerly Gomphidiaceae)

Gomphidius

Chroogomphus

Cystogomphus

Brauniellula

Gomphogaster

Utility of nuclear ribosomal DNA

Approximately the first 900 bases of the nuclear large subunit rDNA were sequenced to test phylogenetic relationships at the generic level within the Boletales. The internal transcribed spacer regions 1 and 2, and the 5.8S rRNA subunit gene were sequenced to address infrageneric questions in *Rhizopogon*. These areas of rDNA provided sufficient resolution of intergeneric and infrageneric relationships addressed in each study. The ITS sequences were only alignable for genera in the suilloid radiation of the Boletales, the genera I have placed in the Rhizopogonaceae. It appears that within the Boletales, the ITS is useful for examining intergeneric and infrageneric questions from closely related genera, e. g., the suilloid radiation. Relationships between closely related species could not be clarified in this study.

Areas of ambiguous alignment and multiple-base insertion/deletion (indel) regions were present in both data sets. Indels in the ITS alignment strongly followed taxonomic divisions; however, this was not observed in the large subunit alignment. In the ITS alignment it was not difficult to code most of the indel regions. Coding the indels in the large subunit alignment was not possible because these areas were also considered to be areas of ambiguous alignment.

RECOMMENDATIONS FOR FUTURE RESEARCH

Generic relationships in the Boletales

The new members of the Rhizopogonaceae, *Gomphidius*, *Chroogomphus*, and *Brauniellula*, need to be tested and included in subsequent analyses. I anticipate that within the suilloid radiation they will form a basal group to the sister group of *Suillus* and *Rhizopogon*. More genera, e. g., *Strobiliomyces*, *Gyrodon*, *Leccinum*, within the boletoid radiation should be sampled in order to clarify relationships in this group. Sampling of additional *Melanogaster* and *Alpova* is needed to further explore the polyphyly of *Alpova* and the relationship of the Melanogastraceae in the Boletales.

Species concepts in Rhizopogon

Although this thesis has provided clarification on subgeneric concepts in *Rhizopogon*, infrasubgeneric species concepts remain confused. Further study is needed in all four subgenera. Future molecular phylogenetic studies should employ another gene, perhaps the β -tubulin gene or the intergenic spacer region, not only to substantiate the results presented here, but to locate a gene which offers more reliability in distinguishing closely related species. Special emphasis should be placed on subgenus *Villosulus*. In subgenus *Villosulus* species concepts in both section *Villosuli* and section *Vinicolor*

need further refinement. Studies of these sections should involve the combination of a detailed morphological study and a molecular phylogenetic analyses.

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APPENDICES

APPENDIX 1. Primary morphological characters used to distinguish the four sections of *Rhizopogon* based on A. H. Smith's classification (Smith, 1964; Smith and Zeller, 1966).

<i>Rhizopogon</i> section	Peridium	Hyphae	Gleba	Spores
<i>Amylopogon</i>	layer of interwoven rhizomorphs present; olive to blue or pink in KOH; form orange to brown pigment balls in Melzer's solution	not well-differentiated and not forming an epicuticular layer in the peridium		amyloid or dextrinoid
<i>Fulviglebae</i>		in some species brown-walled and forming a distinct epicuticular layer in the peridium	fulvous to cinnamon to dark yellow-brown; often yellow when immature	truncate; inamyloid but sometimes dextrinoid
<i>Rhizopogon</i> ¹			white to olive buff or olive to olive-brown	
<i>Villosuli</i>	reaction to 2% KOH produces a red or an olive to green or both red to green reaction	brown-walled and forming a distinct epicuticular layer in the peridium		

¹Smith states that these species lack characters used to define other sections.

APPENDIX 2. PAUP NEXUS alignment file of 53 sequences of the internal transcribed spacer (ITS) regions 1 and 2 and the 5.8S subunit from *Rhizopogon*, *Suillus*, *Gomphidius*, *Truncocolumella*, and *Chroogomphus* used in the maximum parsimony analyses in Chapter 3.

#NEXUS

[LISA.ITS.4.20.98-1318870123 -- data title]

begin data;

dimensions ntax=54 nchar=927;

FORMAT symbols = "A G C T N X Y W R S K M I 0 1 2 3" MISSING=.

EQUATE="-=." INTERLEAVE;

OPTIONS MSTAXA=UNCERTAIN [GAPMODE=NEWSTATE];

matrix

	10	20	*start of ITS-1	40	50
Suillus brevipes	GTGAACCTGC	GGAAGGATCA	TTAATGAANN	NTATAATCANN	GGCGAGI---
S. luteus	GTGAACCTGC	GGAAGGATCA	TTAATGAANN	NTATAATCANN	GGCGAGI---
S. tomentosus	GTGAACCTGC	GGAAGGATCA	TTAAAGAA--	I-ATAATCANN	--CGAGI---
S. americanus	GTGAACCTGC	GGAAGGATCA	TTAATGAANN	NTATAATCANN	GGCGAGI---
S. granulatus	GTGAACCTGC	GGAAGGATCA	TTAACGAATT	CACG-ATTNN	GGCGAGI---
S. grevillei	-----	--AAGGATCA	TTATCGAANN	NTATAATCANN	GGCGAGI---
S. cavipes	GTGAACCTGC	GGAAGGATCA	TTAAAGAANN	NTATAATCANN	GGCGAAI---
S. caerulescens	GTGAACCTGC	GGAAGGATCA	TTAACGAANN	NTATAATCANN	GGCGAGI---
S. lakei	GTGAACCTGC	GGAAGGATCA	TTAATGAANN	NTATAATCANN	GGCGAGI---
S. sinuspaulinus	GTGAACCTGC	GGAAGGATCA	TTATCGAANN	NTATAATCANN	GACGAAI---
Chroogomphus	GTGAACCTGC	GGAAGGATCA	TTAATGAA--	ITATAATTNN	GGTCGGI---
Gomphidius	GTGAACCNGC	GGAAGGATCA	TTAACGAA--	ICGTAAATTNN	GGCGT-I---
Truncocolumella	GTGAACCTGC	GGAAGGATCA	TTAACGAA--	ITCTAATCANN	GCCGA-I---
Rhizopogon evadens	GTGAAC-TGC	GGAAGGA-CA	TTAATGAA--	ITATAACANN	GGAGG-I---
R. evadens (T)	GTGAACCTGC	GGAAGGATCA	TTAATGAA-T	GT--AACANN	GGAGG-I---
R. smithii	GTGAACCTGC	GGAAGGATCA	TTAATGAA-T	GT--AACANN	GGAGG-I---
R. subsalmonius	GTGAAC-TGC	GGAAGGA-CA	TTAATGAA-T	GT--AACANN	GGAGG-I---
R. fuscorubens	GTGAACCTGC	GGAAGGATCA	TTAATGAA-T	GTATAAATNN	GGCGA-I---
R. ochraceorubens(TT)	NGNGCGCGGG	GNGGNGCNCA	TTTCNGNC-T	NCANAAATNN	GGCGA-I---
R. ochraceorubens(HT)	GTGAACCTGC	GGAAGGATCA	TTAATGAA-T	GTATAAATNN	GGCGA-I---
R. occidentalis	GTGAACCTGM	GGAAGGATCA	TTAATGAA-T	GTATAAATNN	GGCGA-I---
R. occidentalis	GTGAACCTGC	GGAAGGATCA	TTAATGAA-T	GTATAAATNN	GGCGA-I---
R. succosus	GTGAACCTGC	GGAAGGATCA	TTAATGAAAT	GTGTAAATGA	TGCACCTCTT
R. luteolus	GTGAACCTGC	GGAAGGATCA	TTAATGAAAT	GTGTAAATGA	TGCACCTTTT
R. burlinghamii	GTGAACCTGC	GGAAGGATCA	TTAACGAA--	ITATAAATTNN	GGAGGGI---
R. rubescens	GTGAAC-TGC	AGAAATGATCA	TTAACGAA--	ITATAAATTNN	GGAGGGI---
R. roseolus	GTGAAC-TGC	GGAAGGATCA	TTAACGAA--	ITATAAATTNN	AGAGGGI---
R. vulgaris	GTGAACATGM	GGAA-GATCA	TTAAYGAA--	ITATAAATTNN	AGAGGGI---
R. colossus (HT)	GTGAACCTGC	GGAAGGATCA	TTAACGAA--	ITATAAATNN	GGAAAGI---
R. hawkeriae (PT)	GTGAACCTGC	GGAAGGATCA	TTAACGAA--	ITATAAATNN	GGAAAGI---
R. parksii (T17679)	GTGAAC-TGC	GGAAGGATCA	TTAACGAA--	ITATAAATNN	GGAAAGI---
R. parksii (T19446)	GTGAACCTGC	GGAAGGATCA	TTAACGAA--	ITATAAATNN	GGAAAGI---
R. villosulus (AHS)	GGGACC-TGC	CGGAGGGTTM	ATWAMGGAA-	IWWTAATTNN	GGAAASI---
R. villosulus (JMT)	GTGAACCTGC	GGAAGGATCA	TTAACGAA--	ITATAAATNN	GGAAAGI---
R. rogersii	GTGAACATGM	GGAAGGATCA	TTAACGAA--	ITATAAATNN	GGAAAGI---
R. sp. nov	GTGAAC-TGC	GGAAGGATCA	TTAACGAA--	ITATAAATNN	GGAAAGI---
R. gilkeyae	GTGAACCTGC	GGAAGGATCA	TTAACGAA--	ITATAAATNN	GGTCGGI---
R. zelleri	GTGAACCWGC	GGAAGGATCA	TTAACGAA--	ITATAAATNN	GGAAAGI---
R. villescens	GCGAAC-TGC	GG-AGG-CCA	TTAACGGA--	ITATAAATNN	GGAAAGI---
R. diabolicus	-----	GGARGGTTCA	TTAA-GGANN	ITTTAATTNN	GGAAAGI---
R. parvulus	-----	GGARGGTTCW	TTAA-GGANN	ITWTAATTNN	GGAAAGI---
R. vinicolor (T17899)	GTGAACCTGC	GGAAGGATCA	TTAACGAA--	ITATAAATNN	GGAAAGI---
R. vinicolor (T20787)	GTGAACCTGC	GGAAGGATCA	TTAACGAA--	ITATAAATNN	GGAAAGI---
R. ochraceisorus (PT)	GTGAACCTGC	GGAAGGATCA	TTAACGAA--	ITATAAATNN	GGAAAGI---
R. ochraceisorus (17916)	GTGAACCTGC	GGAAGGATCA	TTAACGAA--	ITATAAATNN	GGAAAGI---
R. ochraceisorus (17944)	GTGAAC-TGC	GGAAGGATCA	TTAACGAA--	ITATAAATNN	GGAAAGI---
R. subcaerulescens	-----	--AAGGATCA	TTAACGAA--	ITATAAATTNN	GAGGGGI---
R. ellenae (HT)	GTGAACCTGC	GGAAGGATCA	TTAACGAA--	ITATAAATTNN	GAGGGGI---
R. ellenae	GTGAACCTGC	GGAAGGATCA	TTAACGAA--	ITATAAATTNN	GAGGGGI---
R. subpurpurascens (PT)	GTGAAC-TGC	GGAAGGA-CA	TTAACGAA--	ITATAAATTNN	GAGGGGI---
R. subpurpurascens	GTGAACATGC	GGAAGGA-CA	TTAACGAA--	ITATAAATTNN	GAGGGGI---
R. subgelatinosus	GTGAAC-TGC	GGAAGGATCA	TTAACGAA--	ITATAAATTNN	GAGGGGI---
R. semireticulatus (17562)	GTGAAC-TGM	G-AAGGA-CA	TTAACGAA--	ITATAAATTNN	GAGGGGI---
R. semireticulatus (7899)	GTGAAC-TGC	GGAAGGATCA	TTAACGAA--	ITATAAATTNN	GAGGGGI---

	60	70	80	90	100
<i>Suillus brevipes</i>	----I-----	GGAA--AGG	-CGGAGAG--	--TTGTAGCT	GGCCCC----
<i>S. luteus</i>	----I-----G	GGAA--AGG	-CGGAGAG--	--TTGTAGCT	GGCCTCCA--
<i>S. tomentosus</i>	----I---GG	CCGAT--GGA	AAGGAGAGAG	GGTTGTAGCT	GGCGT-----
<i>S. americanus</i>	----I-----	-ATGAA--GGA	-CGAGGA----	--CTGTCGCT	GGCCTTTC--
<i>S. granulatus</i>	----I---GGG	GAAGGCCGAG	GG-----	--TTGTAGCT	GGCCTTTTTC
<i>S. grevillei</i>	----I---GGG	AAAGG--GGG	GAG-----	--TTGTCGCT	GGCCTTTTAC
<i>S. cavipes</i>	----I---AGG	GGGAT--GGG	GGAAG-----	--CTGTCGCT	GGCCTTTTGC
<i>S. caerulescens</i>	----I-----G	GGAA--GGA	-CGAGAG----	--TTGTCGCT	GGCCTTTT--C
<i>S. lakei</i>	----I-----G	GGAA--GGA	-CGAGAG----	--TTGTCGCT	GGCCTTTT--C
<i>S. sinuspaulinus</i>	----I-----G	GGAA--CGAG	TCGG-----	--CTGTCGCT	GGCCTTTT--C
<i>Chroogomphus</i>	----I---CG	GGAA--GGA	GGGAG-----	--CTGTCGCT	GGCCTTTT---
<i>Gomphidius</i>	----I---GG	GGGAT--GGA	GGGAG-----	--CTGTCGCT	GGCCTTTTTC
<i>Truncocolumella</i>	----I---GR	GGGATTCGGG	GGACAAA----	--GCTGTCGCT	GGCCTTTT---
<i>Rhizopogon evadens</i> (HT)	----I---GA	GGAAAAACGGG	GGATAAAGCA	AGCTGTGGCT	GGCCTTTG---
<i>R. smithii</i>	----I---GA	GGAAAAACAGG	GGTTAAAGCA	AGCTGTGGCT	GGTCTTTG---
<i>R. subsalmonius</i>	----I---GA	GGAAATGCAGG	GGGGAAAAAA	AGCTGTGCT	GGCCTTTT---
<i>R. fusciorubens</i>	----AGTCGA	GGAAATAC--AG	GGGTAAAAACA	-GCTGGCTGT	CGCTGGTCTT
<i>R. ochraceorubens</i> (TT)	----AGTCAA	GGAAATTT--GG	GGGAAAAACA	-GCTGTGCT	GGCCTTTG---
<i>R. ochraceorubens</i> (HT)	----AGTCGA	GGAAATTT--GG	GGGAAAAACA	-GCTGTGCT	GGCCTTTG---
<i>R. occidentalis</i>	----AGTCGA	GGAAATTT--GG	GGGAAAAACA	-GCTGTGCT	GGCCTTTG---
<i>R. occidentalis</i>	----AGTCGA	GGAAATTT--GG	GGGAAAAACA	-GCTGTGCT	GGCCTTTG---
<i>R. succosus</i>	AAGTAGTTGA	GGAAATGCAGG	GGGTAAAAAT	AGTTGTTGCT	GGCCTTTG--TT
<i>R. luteolus</i>	AAGTAGTTGA	GGAAATACAGG	GGGTAAAAAT	AGTTGTTGCT	GGCCTTTG---
<i>R. burlinghamii</i>	-----	-----	-----	-GCTGTAGCT	GGCCTTTG---
<i>R. rubescens</i>	-----	-----	-----	-GCTGTAGCT	GGCCTTTG---
<i>R. roseolus</i>	-----	-----	-----	-GCTGTAGCT	GGCCTTCG---
<i>R. vulgaris</i>	-----	-----	-----	-GCTGTAGCT	GGCCTTTG---
<i>R. colossus</i> (HT)	-----	-----	-----	--CTGTCGCT	GGCCTTTC---
<i>R. hawkeriae</i> (PT)	-----	-----	-----	--CTGTCGCT	GGCCCTCT--
<i>R. parksii</i> (T17679)	-----	-----	-----	--CTGTCGCT	GGCCCTCGC-
<i>R. parksii</i> (T19446)	-----	-----	-----	--CTGTCGCT	GGCCCTCGC-
<i>R. villosulus</i> (AHS)	-----	-----	-----	--YGTTCGYK	G--CCCTCT--
<i>R. villosulus</i> (JMT)	-----	-----	-----	--CTGTCGCT	GGCCTTTC---
<i>R. rogersii</i>	-----	-----	-----	--CTGTCGCT	GGCCTTCT--
<i>R. sp. nov</i>	-----	-----	-----	--CTGTCGCT	GGCCCTCGC-
<i>R. gilkeyae</i>	-----	-----	-----	--TTGTCGCT	GGCCTTTC---
<i>R. zelleri</i>	-----	-----	-----	--CTGTCGCT	GGCCCTCT--
<i>R. villescens</i>	-----	-----	-----	--CTGTCGCT	GGCCCTCT--
<i>R. diabolicus</i>	-----	-----	-----	--CTGTCGCT	GGCCCTCT--
<i>R. parvulus</i>	-----	-----	-----	--CTGTCGCT	GGCCCTCT--
<i>R. vinicolor</i> (T17899)	-----	-----	-----	--CTGTCGCT	GGCCCTCT--
<i>R. vinicolor</i> (T20787)	-----	-----	-----	--TTGTCGCT	GGCCCTCT--
<i>R. ochraceisporus</i> (PT)	-----	-----	-----	--TTGTCGCT	GGCCCTCT--
<i>R. ochraceisporus</i> (17916)	-----	-----	-----	--CTGTCGCT	GGCCCTCT--
<i>R. ochraceisporus</i> (17944)	-----	-----	-----	--CTGTCGCT	GGCCCTCT--
<i>R. subcaerulescens</i>	-----	-----	-----	--TTGTCGCT	GGCCTCGCTC
<i>R. ellenae</i> (HT)	-----	-----	-----	--TTGTCGCT	GGCCTCGCTC
<i>R. ellenae</i>	-----	-----	-----	--TTGTCGCT	GGCCTCGCTC
<i>R. subpurpurascens</i> (PT)	-----	-----	-----	--TTGTCGCT	GGCCTCGCTC
<i>R. subpurpurascens</i>	-----	-----	-----	--TTGTCGCT	GGCCTCGCTC
<i>R. subgelatinosus</i>	-----	-----	-----	--TTGTCGCT	GGCCTCGCTC
<i>R. semireticulatus</i> (17562)	-----	-----	-----	--NTGTCGCT	GGCCTCGCTC
<i>R. semireticulatus</i> (7899)	-----	-----	-----	--TTGTCGCT	GGCCTCGCTC

	110	120	130	140	150
Suillus brevipes	-----	-----	---IAGGGCA	T-GTGCACGC	TCTCTTCC--
S. luteus	-----	-----	---IGGGGCA	T-GTGCACGC	TCTCTTCC--
S. tomentosus	-----	-----	---I-AAGCA	C-GTGCACGC	CCTCTTTCTC
S. americanus	-----A TC	-----	---IGAGGCA	T-GTGCACGC	TCTCTTCT--
S. granulatus	-----A AA	-----	---IGAGGCA	C-GTGCACGC	TCTCTTCCCT
S. grevillei	-----	-----	---IGAGGCA	T-GTGCACGC	TCTCCTTT--
S. cavipes	G-----	-----	---I-AGGCA	T-GTGCACGC	TTTCTTCT--
S. caerulescens	GC-----	-----	---IGAGGCA	T-GTGCACGC	TTTCTTCT--
S. lakei	GC-----	-----	---IGAGGCA	T-GTGCACGC	TTTCTTCT--
S. sinuspaulinus	GC-----	-----	---IGAGGCA	T-GTGCACGC	TTTCTTTTCT
Chroogomphus	-----	-----	---I--GGCA	T-GTGCACGC	TCTCTTTT--
Gomphidius	CG-----	-----	---IAGGGCA	T-GTGCACGC	TTTCTTTC--
Truncocolumella	C-----	-----	---IGAGGCA	T-GTGCACGC	TTTTCTTTTC
Rhizopogon evadens	-----TA AT	-----	---IGAGGCA	T-GTGCACGC	TCCCC-----
R. evadens (HT)	-----TA AT	-----	---IGAGGCA	T-GTGCACGC	TGCGTCCC--
R. smithii	-----TA AT	-----	---IGAGGCA	T-GTGCACGC	TGCGTCCC--
R. subsalmonius	-----A AC	-----	---IGAGGTA	T-GTGCACGC	TTTTTTCCCC
R. fuscorubens	G-----TA AT	-----	---IGAGACA	T-GTGCACGC	TGCGTCCC--
R. ochraceorubens(TT)	-----TA ATT	-----	---IGAGGCA	T-GTGCACGC	T----CCC--
R. ochraceorubens(HT)	-----TA ATT	-----	---IGAGGCA	T-GTGCACGC	T----CCC--
R. occidentalis	-----TA ATTAAATTAT	-----	---IGAGGCA	T-GTGCACGC	T----CCC--
R. occidentalis	-----TA ATTAAATTAT	-----	---IGAGGCA	T-GTGCACGC	T----CCC--
R. succosus	G-----TTAT	-----	---IGAGGCA	TTGTGCACAC	TACCTC----
R. luteolus	-----TCAT	-----	---IGAGGCA	TTGTGCACAC	TACCTC----
R. burlinghamii	-----	-----G AAACGAGGCA	T-GTGCACGC	-----	-----
R. rubescens	-----	-----G AAACGAGGCA	T-GTGCACGC	-----	-----
R. roseolus	-----	-----G AAACGAGGCA	T-GTGCACGC	-----	-----
R. vulgaris	-----	-----G AAACGAGGCA	T-GTGCACGC	-----	-----
R. colossus (HT)	-----	-----	---I-GGGCA	T-GTGCACGC	C-----
R. hawkeriae (PT)	-----	-----	---I-GGGCA	T-GTGCACGC	C-----
R. parksii (T17679)	-----	-----	---I-GGGCA	T-GTGCACGC	T-----
R. parksii (T19446)	-----	-----	---I-GGGCA	T-GTGCACGC	T-----
R. villosulus (AHS)	-----	-----	---I-GGGCA	T-GTGCACGC	Y-----
R. villosulus (JMT)	-----	-----	---I-GGGCA	T-GTGCACGC	C-----
R. rogersii	-----	-----	---I-GGGCA	T-GTGCACGC	C-----
R. sp. nov	-----	-----	---I-GGGCA	T-GTGCACGC	T-----
R. gilkeyae	-----	-----	---I-GGGCA	T-GTGCACGC	T-----
R. zelleri	-----	-----	---I-GGGCA	T-GTGCACGC	T-----
R. villescens	-----	-----	---I-GGGCA	T-GTGCACGC	C-----
R. diabolicus	-----	-----	---IGGGGCA	T-GTGCACGC	T-----
R. parvulus	-----	-----	---IGGGGCA	T-GTGCACGC	T-----
R. vinicolor(T17899)	-----	-----	---IGGGGCA	T-GTGCACGC	T-----
R. vinicolor(T20787)	-----	-----	---IGGGGCA	T-GTGCACGC	T-----
R. ochraceisporus (PT)	-----	-----	---IGGGGCA	T-GTGCACGC	T-----
R. ochraceisporus (17916)	-----	-----	---IGGGGCA	T-GTGCACGC	T-----
R. ochraceisporus (17944)	-----	-----	---IGGGGCA	T-GTGCACGC	T-----
R. subcaerulescens	GCGC-----	-----	---IGAGGCA	T-GTGCACGC	T-----
R. ellenae (HT)	GCTCGCGC--	-----	---IGAGGCA	T-GTGCACGC	T-----
R. ellenae	GCTCGCGC--	-----	---IGAGGCA	T-GTGCACGC	T-----
R. subpurpurascens (PT)	GC-----	-----	---IGAGGCA	T-GTGCACGC	T-----
R. subpurpurasc(T)	GC-----	-----	---IGAGGCA	T-GTGCACGC	T-----
R. subgelatinosus	GCGC-----	-----	---IGAGGCA	T-GTGCACGC	T-----
R. semireticulatus (17562)	GCTCGCGC--	-----	---IGAGGCA	T-GTGCACGC	T-----
R. semireticulatus (7899)	GCGC-----	-----	---IGAGGCA	T-GTGCACGC	T-----

	160	170	180	190	200
<i>Suillus brevipes</i>	-I--GGAC-T	-TTCGCI---	-----CGI	-TATI-----	-----
<i>S. luteus</i>	-I--GGACCT	-TTCGCI---	-----CGI	-TATI-----	-----
<i>S. tomentosus</i>	-I--GACCT	AG-GTCI---	-----CTI	-TATI-----	-----
<i>S. americanus</i>	-I--GGAC-T	-TTCGCI---	-----CGI	-TATI-----	-----
<i>S. granulatus</i>	-I--AAC-T	-CTCGCI---	-----CGI	-TATI-----	-----
<i>S. grevillei</i>	-I-GGAACCT	-TT-GCI---	-----CTI	-TATI-----	-----
<i>S. cavipes</i>	-I--GAACCC	-TCAGCI---	-----CGI	-TATI-----	-----
<i>S. caerulescens</i>	-I--GGAC-T	-TTCGCI---	-----CGI	-TATI-----	-----
<i>S. lakei</i>	-I--GGAC-T	-TTCGCI---	-----CGI	-TATI-----	-----
<i>S. sinuspaulinus</i>	-I----ACCI	-TTCGTI---	-----CGI	-TATI-----	-----
<i>Chroogomphus</i>	-I-GGAACGI	-TTGGTI---	-----CGT	CTTTCATATT	TTI-----CA
<i>Gomphidius</i>	-I-GGAGCCC	-TCGTI---	-----CGT	TTTTCATATC	TTI-----CA
<i>Truncocolumella</i>	-I--GGACCC	-TTCGTI---	-----CGT	CTTTCAAACT	AI-----A
<i>Rhizopogon evadens</i>	-IGAGGAAC	C-TCGAI---	-----CGT	CTTTCATCTI	----TCTCA
<i>R. evadens</i> (HT)	-TGAGGACCT	C-TCGAI---	-----CGT	CTTTCATCTI	----TCTCA
<i>R. smithii</i>	-TGAGGACCT	C-TCGAI---	-----CGT	CTTTCATCTI	----TCTCA
<i>R. subsalmonius</i>	TGAGGACCT	C-TCGAI---	-----CGT	CTTTCATCTI	----TCTCA
<i>R. fuscorubens</i>	-TGAGGACCT	CTTCGAI---	-----CGT	CTATCATCTC	ATCTCTCTCA
<i>R. ochraceorubens</i> (TT)	-TGAGGACCT	CTTCGAI---	-----CGT	CTATCATCTC	ATCTCTCTCA
<i>R. ochraceorubens</i> (HT)	-TGAGGACCT	CTTCGAI---	-----CGT	CTATCATCTC	ATCTCTCTCA
<i>R. occidentalis</i>	-TGAGGACCT	CTTCGAI---	-----CGT	CTATCATATC	ATCTCTCTCA
<i>R. occidentalis</i>	-TGAGGACCT	CTTCGAI---	-----CGT	CTATCATATC	ATCTCTCTCA
<i>R. succosus</i>	-TGAGGACCT	CTTCGAAATA	GGGGGTGTG	CTATCATCTC	ATI-CTCTCA
<i>R. luteolus</i>	-TGAGGACCT	CTTCGAAACTT	--GGGTGTG	CTATCATCTC	ATI-CTCTCA
<i>R. burlinghamii</i>	-I---I---T	CTTCTI---	-----IGT	TTTTCACAAI	-----CTCA
<i>R. rubescens</i>	-I---I---T	CTTCTI---	-----IGT	TTTTCACAAI	-----CTCA
<i>R. roseolus</i>	-I---I---T	CTTCTI---	-----IGT	TTTTCATAAI	-----CTCA
<i>R. vulgaris</i>	-I---I---T	CTTCTI---	-----IGT	TTTTCATAAI	-----CTCA
<i>R. colossus</i> (HT)	-I---GACCI	-----	-----IGT	GTTTCATAAA	TTI--TCTCA
<i>R. hawkeriae</i> (PT)	-I---GACCI	-----	-----IGT	GTTTCATAAA	TTI--TCTCA
<i>R. parksii</i> (T17679)	-I---GACCI	-----	-----IGT	GTTTCATAAA	TTI--TCTCA
<i>R. parksii</i> (T19446)	-I---GACCI	-----	-----IGT	GTTTCATAAA	TTI--TCTCA
<i>R. villosulus</i> (AHS)	-I---GACCI	-----	-----IGT	GTTTCATAAA	TTI--TCTCA
<i>R. villosulus</i> (JMT)	-I---GACCI	-----	-----IGT	GTTTCATAAA	TTI--TCTCA
<i>R. rogersii</i>	-I---GACCI	-----	-----IGT	GTTTCATAAA	TTI--TCTCA
<i>R. gilkeyae</i>	-I---GACCI	-----	-----IGT	GTTTCAACAA	TTI--TCTCA
<i>R. zelleri</i>	-I---GACCI	-----	-----IGT	GTTTCATAAA	TTI--TCTCA
<i>R. villescens</i>	-I---GACCI	-----	-----IGT	GTTTCATAAA	TTI--TCTCA
<i>R. diabolicus</i>	-I---GACCI	-----	-----IGT	GTTTCAAAAA	TTI--TCTCA
<i>R. parvulus</i>	-I---GACCI	-----	-----IGT	GTTTCAAAAA	TTI--TCTCA
<i>R. vinicolor</i> (T17899)	-I---GACCI	-----	-----IGT	GTTTCAAAAA	TTI--TCTCA
<i>R. vinicolor</i> (T20787)	-I---GACCI	-----	-----IGT	GTTTCAAAAA	TTI--TCTCA
<i>R. ochraceisporus</i> (PT)	-I---GACCI	-----	-----IGT	GTTTCAAAAA	TTI--TCTCA
<i>R. ochraceisporus</i> (17916)	-I---GACCI	-----	-----IGT	GTTTCAAAAA	TTI--TCTCA
<i>R. ochraceisporus</i> (17944)	-I---GACCI	-----	-----IGT	GTTTCAAAAA	TTI--TCTCA
<i>R. subcaerulescens</i>	-I---I---T	CTCI-----	-----IGT	TTTTCTCTCA	AI----CTCA
<i>R. ellenae</i> (HT)	-I---I---T	CTTI-----	-----IGT	TTTTCATAAI	----CTCTCA
<i>R. ellenae</i>	-I---I---T	CTTI-----	-----IGT	TTTTCATAAI	----CTCTCA
<i>R. subpurpurascens</i> (PT)	-I---I---T	CTTI-----	-----IGT	TTTTCATAAI	----CTCA
<i>R. subpurpurascens</i>	-I---I---T	CTTI-----	-----IGT	TTTTCATAAI	----CTCA
<i>R. subgelatinosus</i>	-I---I---T	CTCI-----	-----IGT	TTTTCTCAAI	----CTCA
<i>R. semireticulatus</i> (17562)	-I---I---T	CTTI-----	-----IGT	TTTTCATAAI	----CTCTCA
<i>R. semireticulatus</i> (7899)	-I---I---T	CTCI-----	-----IGT	TTTTCTCAAI	----CTCA

	210	220	230	240	250
Suillus brevipes	-----	-----	-----	-----	I-----
S. luteus	-----	-----	-----	-----	I-----
S. tomentosus	-----	-----	-----	-----	I-----
S. americanus	-----	-----	-----	-----	I-----
S. granulatus	-----	-----	-----	-----	I-----
S. grevillei	-----	-----	-----	-----	I-----
S. cavipes	-----	-----	-----	-----	I-----
S. caerulescens	-----	-----	-----	-----	I-----
S. lakei	-----	-----	-----	-----	I-----
S. sinuspaulinus	-----	-----	-----	-----	I-----
Chroogomphus	CCAGTGCACC	T-AATGTAGG	-----	-----	I-----
Gomphidius	CCCGTGCACA	C-AATGTAGG	-----	-----	I-----
Truncocolumella	CTTGTGCACC	T-AATGTAGG	-----	-----	I-----
Rhizopogon evadens	CCTGTGCACC	TCGATGTAGG	-----IT	CIGGCATGGT	TTTGGTAAAA
R. evadens (HT)	CTTGTGCACC	T-AATGTAGG	A-TTCAGAGT	CAGGCATGGT	TTTGGTAAAA
R. smithii	CCTGTGCACC	T-AATGTAGG	AGTTCAGAGT	CGGGCATGGT	TTTGGTAAAA
R. subsalmonius	CCTGTGCAC T	T-AATGTAGG	-----IT	CIGGCATGGT	TTTGGTAAAA
R. fuscorubens	CCTGTGCACC	T-AATGTAGG	-----IT	CITGCATGGT	TTTGGTAAAA
R. ochraceorubens(TT)	CCTGTGCACC	T-AATGTAGG	-----IT	CITGCATGGT	TTTGGTAAAA
R. ochraceorubens(HT)	CCTGTGCACC	T-AATGTAGG	-----IT	CITGCATGGT	TTTGGTAAAA
R. occidentalis	CCTGTGCACC	T-AATGTAGG	-----IT	CITGCATGGT	TTTGGTAAAA
R. occidentalis	CCTGTGCACC	T-AATGTAGG	-----IT	CITGCATGGT	TTTGGTAAAA
R. succosus	CCTGTGCACC	T-AATGTAGG	-----IT	CITGCATGGT	TTTGGTAAAA
R. luteolus	CCTGTGCAC T	T-AATGTAGG	-----IT	CITGCATGGT	TTTGGTAAAA
R. burlinghamii	CCTGTGCACC	T-AATGTAGG	-----	-----	I-----
R. rubescens	CCTGTGCACC	T-AATGTAGG	-----	-----	I-----
R. roseolus	CCTGTGCACC	T-AATGTAGG	-----	-----	I-----
R. vulgaris	CCTGTGCACC	T-AATGTAGG	-----	-----	I-----
R. colossus (HT)	CCTGTGAACC	T-AATGTAGG	-----	-----	I-----
R. hawkerae (PT)	CCTGTGAACC	T-AATGTAGG	-----	-----	I-----
R. parksii (T17679)	CCTGTGAACC	T-AATGTAGG	-----	-----	I-----
R. parksii (T19446)	CCTGTGAACC	T-AATGTAGG	-----	-----	I-----
R. villosulus (AHS)	CCTGTGAACC	T-AATGTAGG	-----	-----	I-----
R. villosulus (JMT)	CCTGTGAACC	T-AATGTAGG	-----	-----	I-----
R. rogersii	CCTGTGAACC	T-AATGTAGG	-----	-----	I-----
R. sp. nov	CCTGTGAACC	T-AATGTAGG	-----	-----	I-----
R. gilkeyae	CCTGTGAACC	T-AATGTAGG	-----	-----	I-----
R. zelleri	CCTGTGAACC	T-AATGTAGG	-----	-----	I-----
R. villescens	CCTGTGAACC	T-AATGTAGG	-----	-----	I-----
R. diabolicus	CCTGTGAACC	T-AATGTAGG	-----	-----	I-----
R. parvulus	CCTGTGAACC	T-AATGTAGG	-----	-----	I-----
R. vinicolor(T17899)	CCTGTGAACC	T-AATGTAGG	-----	-----	I-----
R. vinicolor(T20787)	CCTGTGAACC	T-AATGTAGG	-----	-----	I-----
R. ochraceisporus (PT)	CCTGTGAACC	T-AATGTAGG	-----	-----	I-----
R. ochraceisporus (17916)	CCTGTGAACC	T-AATGTAGG	-----	-----	I-----
R. ochraceisporus (17944)	CCTGTGAACC	T-AATGTAGG	-----	-----	I-----
R. subcaerulescens	CCTGTGCACC	T-AATGTAGG	-----	-----	I-----
R. ellenae (HT)	CCTGTGCACC	T-AATGTAGG	-----	-----	I-----
R. ellenae	CCTGTGCACC	T-AATGTAGG	-----	-----	I-----
R. subpurpurascens (PT)	CCTGTGCACC	T-AATGTAGG	-----	-----	I-----
R. subpurpurascens	CCTGTGCACC	T-AATGTAGG	-----	-----	I-----
R. subgelatinosus	CCTGTGCACC	T-AATGTAGG	-----	-----	I-----
R. semireticulatus (17562)	CCTGTGCACC	T-AATGTAGG	-----	-----	I-----
R. semireticulatus (7899)	CCTGTGCACC	T-AATGTAGG	-----	-----	I-----

	260	270	280	290	300
Suillus brevipes	-----	-----	-----GG	GCGCGGGGCG	-----
S. luteus	-----	-----	-----GG	GCGCGGGG	-----
S. tomentosus	-----	-----	-----GG	GCGCGGGGCG	-----
S. americanus	-----	-----	-----GG	GCGCGGGGCC	-----
S. granulatus	-----	-----	-----GG	GCGCGGGGCG	-----
S. grevillei	-----	-----	-----GG	GCGCGGGGCG	-----
S. cavipes	-----	-----	-----GG	GCGCGGGGCG	-----
S. caerulescens	-----	-----	-----GG	GCGCGGGGCC	-----
S. lakei	-----	-----	-----GG	GCGCGGGGCG	-----
S. sinuspaulinus	-----	-----	-----GG	GCGCGGGG	-----
Chroogomphus	-----	---ATGCCCT	CTCCTTT-GG	GAGGGGGG	-----
Gomphidius	-----	---ATGCCC	CTCCTTC-GG	GAGGGGG	-----
Truncocolumella	-----	---ATGCCC	TTTCTTTTCGG	GAGAGGGGG	-----
Rhizopogon evadens	CGAAATCATG	CGAG-TGCCCT	TT-CCCT-GC	GGGGGGAGG-	-----
R. evadens (HT)	CGAAATCGTG	TGAGNNGCCT	TTTCCCTGTG	GGGGGGAGGT	AAATCCCCCTT
R. smithii	CGAAACTGTG	CGAGNNGCCT	TTTCCCT-GC	GGGGGGAGGT	GAGCGTCTTT
R. subsalmonius	CAGAACTGTG	CGAGTTACCT	CTCTCT--GC	GGGGGGAGGT	-----
R. fuscorubens	CAGAAACCGTG	CTAGA-GCCT	CTCTCTC-TG	CGGGGGGA--	-----
R. ochraceorubens(TT)	CAGAACTGTG	CTAGNN-I--	-----	-----	-----
R. ochraceorubens(HT)	CAGAACTGTG	CTAGNN-I--	-----	-----	-----
R. occidentalis	CAGAACTGTG	CTAAA-GCCT	CTCTCTC-TG	SGGGGGGA--	-----
R. occidentalis	CAGAACTGTG	CTAGA-GCCT	CTCTCTC-TG	CGGGGGGA--	-----
R. succosus	CAGAACTGTG	CTAGA-GCCT	CTCTCTCTGC	GGGGGGAA--	-----
R. luteolus	CAGAACTGTG	CTAGA-GCCT	CTCTCTCTGC	GGGGGGAA--	-----
R. burlinghamii	-----	---ATGCCCT	CTCTTTC-GG	GAGGGGGG	-----
R. rubescens	-----	---ATGCCCT	CTCTTTC-GG	GAGGGGGG	-----
R. roseolus	-----	---ATGCTC	CTCCTTC-GG	GAGGGGGG	-----
R. vulgaris	-----	---ATGCTC	CTCCTTC-GG	GAGGGGGG	-----
R. colossus (HT)	-----	---ATGCCCT	TTCTTTC-GG	GAGAGGGG	-----
R. hawkeriae (PT)	-----	---ATGCCCT	CTCTTTC-GG	GAGGGGGG	-----
R. parksii (T17679)	-----	---ATGCCC	CTCCTCC-GG	GAGGGGGG	-----
R. parksii (T19446)	-----	---ATGCCC	CTCCTCC-GG	GAGGGGGG	-----
R. villosulus (AHS)	-----	---ATGCCCT	CTCTTTC-GG	GAGGGGGG	-----
R. villosulus (JMT)	-----	---ATGCCCT	TTCTTTC-GG	GAGAGGGG	-----
R. rogersii	-----	---ATGCCCT	TTCTTTTCGG	GAGGGGGG	-----
R. sp. nov	-----	---ATGCCCT	CTCCTCC-GG	GAGGGGGG	-----
R. gilkeyae	-----	---ATGCCCT	CTCCTCC-GG	GAGGGGGG	-----
R. zelleri	-----	---ATGCCCT	CTCCTCC-GG	GAGGGGGG	-----
R. villescens	-----	---ATGCCCT	CTCTTTC-GG	GAGGGGG	-----
R. diabolicus	-----	---ATGCCCT	CTCCTCT-GG	GAGAGGGG	-----
R. parvulus	-----	---ATGCCCT	CTCCTCT-GG	GAGAGGGG	-----
R. vinicolor(T17899)	-----	---ATGCCCT	CTCCTCT-GG	GAGAGGGG	-----
R. vinicolor(T20787)	-----	---ATGCCCT	CTCCTCT-RG	GAGAGGGG	-----
R. ochraceisporus (PT)	-----	---ATGCCCT	CTCCTCT-AG	GAGAGGGG	-----
R. ochraceisporus (17916)	-----	---ATGCCCT	CTCCTCT-GG	GAGGGGGG	-----
R. ochraceisporus (17944)	-----	---ATGCCCT	CTCCTCT-GG	GAGGGGGG	-----
R. subcaerulescens	-----	---ATGCCCT	CTCCTCC-GG	GAGGGGGG	-----
R. ellenae (HT)	-----	---ATGCCCT	CTCCTCC-GG	GAGGGGGG	-----
R. ellenae	-----	---ATGCCCT	CTCCTCC-GG	GAGGGGGG	-----
R. subpurpurascens (HT)	-----	---ATGCCCT	CTCCTCC-GG	GAGGGGGG	-----
R. subpurpurascens	-----	---ATGCCCT	CTCCTCC-GG	GAGGGGGG	-----
R. subgelatinosus	-----	---ATGCCCT	CTCTTCC-GG	GAGGGGG	-----
R. semireticulatus (17562)	-----	---ATGCCCT	CTCCTCC-GG	GAGGGGGG	-----
R. semireticulatus (7899)	-----	---ATGCCCT	CTCCTCC-GG	GAGGGGGG	-----

	310	320	330	340	350
<i>Suillus brevipes</i>	-----	-----	-----ACC	CGCGTCTTCA	TA---TACCI
<i>S. luteus</i>	-----	-----	-----ACC	CGCGTCTTCA	TA---TACCI
<i>S. tomentosus</i>	-----	-----	-----ACC	CGCGTCTTCA	TA---AGCC
<i>S. americanus</i>	-----	-----	-----ACC	CGCGTCTTCA	TA---TACCI
<i>S. granulatus</i>	-----	-----	-----GACC	CGCGTCTTTC	ATM--TCACCI
<i>S. grevillei</i>	-----	-----	-----ACC	CGCGTCTTCA	TA---TACCI
<i>S. cavipes</i>	-----	-----	-----ACC	CGCGTCTTCA	TA---TACCC
<i>S. caerulescens</i>	-----	-----	-----ACC	CGCGTCTTCA	TG---CACCI
<i>S. lakei</i>	-----	-----	-----ACC	CGCGTCTTCA	TG---CACCI
<i>S. sinuspaulinus</i>	-----	-----	-----ACC	CGCGTCTTCA	TA---TACCI
<i>Chroogomphus</i>	-----	-----	-----ACC	TATGTCTTCT	TCAGACACCI
<i>Gomphidius</i>	-----	-----	-----TACC	TATGTCTTCA	TA---TACAI
<i>Truncocolumella</i>	-----	-----	-----ACC	TATGTATTCA	TA---CCAI
<i>Rhizopogon evadens</i>	-----	-----T	CGAGT--ACC	TATGTATTAA	AAA--TACAI
<i>R. evadens</i> (HT)	CCCCGGGGGG	GGGAGGTTGG	CGAGAATCC	TATGTATTGA	AAAA--TACAI
<i>R. smithii</i>	CCCTGCGGGG	G--AGGTTGA	CTAGAATCC	TATGTATTGA	AAGA--TACAI
<i>R. subsalmonius</i>	-----	-----	CGAGA--ACC	TATGTATTTA	TAAAAATACGI
<i>R. fuscorubens</i>	-----	-----AAGGA	CGAAAGTACC	TATGTCTTCA	AA---TACAI
<i>R. ochraceorubens</i> (TT)	-----	-----	-----ACC	TATGTCTTCA	AAA--TACAI
<i>R. ochraceorubens</i> (HT)	-----	-----	-----ACC	TATGTCTTCA	AAA--TACAI
<i>R. occidentalis</i>	-----	-----AAGGA	C-AAAGTACC	TATGTCTTCA	AAA--TACAI
<i>R. occidentalis</i>	-----	-----AAGGA	C-AAAGTACC	TATGTCTTCA	AAA--TACAI
<i>R. succosus</i>	-----	-----AGGA	CAAAAGTACC	TATGTATTCA	AA---TACAI
<i>R. luteolus</i>	-----	-----AGGA	CGAAAGTACC	TATGTCTTCA	TA---TACAI
<i>R. burlinghamii</i>	-----	-----	-----ACC	TATGTCTTCA	TA---CGCCI
<i>R. rubescens</i>	-----	-----	-----ACC	TATGTCTTCA	TA---TGCCI
<i>R. roseolus</i>	-----	-----	-----ACC	TATGTCTTCA	TA---TACCI
<i>R. vulgaris</i>	-----	-----	-----ACC	TATGTCTTCA	TA---CGCCI
<i>R. colossus</i> (HT)	-----	-----	-----ACC	TATGTCTTCA	TA---TACAI
<i>R. hawkeriae</i> (PT)	-----	-----	-----ACC	TATGTCTTCA	TA---TACAI
<i>R. parksii</i> (T17679)	-----	-----	-----ACC	TATGTCTTCA	TA---TACAI
<i>R. parksii</i> (T19446)	-----	-----	-----ACC	TATGTCTTCA	TA---TACAI
<i>R. villosulus</i> (AHS)	-----	-----	-----ACC	TATGTCTTCA	TA---TACAI
<i>R. villosulus</i> (JMT)	-----	-----	-----ACC	TATGTCTTCA	TA---TACAI
<i>R. rogersii</i>	-----	-----	-----ACC	TATGTCTTCA	TA---TACAI
<i>R. sp. nov</i>	-----	-----	-----ACC	TATGTCTTCA	TA---TACAI
<i>R. gilkeyae</i>	-----	-----	-----ACC	TATGTCTTCA	TA---TACAI
<i>R. zelleri</i>	-----	-----	-----ACC	TATGTCTTCA	TA---TACAI
<i>R. villescens</i>	-----	-----	-----ACC	TATGTCTTCA	TA---TACAI
<i>R. diabolicus</i>	-----	-----	-----ACC	TATGTCTTCA	TA---TACAI
<i>R. parvulus</i>	-----	-----	-----ACC	TATGTCTTCA	TA---TACAI
<i>R. vinicolor</i> (T17899)	-----	-----	-----ACC	TATGTCAATCA	TA---TACAI
<i>R. vinicolor</i> (T20787)	-----	-----	-----ACC	TATGTCTTCA	TA---TACAI
<i>R. ochraceisporus</i> (PT)	-----	-----	-----ACC	TATGTCTTCA	TA---TACAI
<i>R. ochraceisporus</i> (17916)	-----	-----	-----ACC	TATGTCTTCA	TA---TACAI
<i>R. ochraceisporus</i> (17944)	-----	-----	-----ACC	TATGTCTTCA	TA---TACAI
<i>R. subcaerulescens</i>	-----	-----	-----ACC	TATGTCTTCG	TA---ACAI
<i>R. ellenae</i> (HT)	-----	-----	-----ACC	TATGTCTTCA	TA---TACAI
<i>R. ellenae</i>	-----	-----	-----ACC	TATGTCTTCA	TA---TACAI
<i>R. subpurpurascens</i> (PT)	-----	-----	-----ACC	TATGTCTTCG	TA---TACAI
<i>R. subpurpurascens</i>	-----	-----	-----ACC	TATGTCTTCA	TA---TACAI
<i>R. subgelatinosus</i>	-----	-----	-----ACC	TATGTCTTCG	TA---ACAI
<i>R. semireticulatus</i> (17562)	-----	-----	-----ACC	TATGTCTTCA	TA---TACAI
<i>R. semireticulatus</i> (7899)	-----	-----	-----ACC	TATGTCTTCG	TA---ACAI

	360	370	380	390	400
<i>Suillus brevipes</i>	TCTTCGTGTA	GAAAGTCTTT	GAATGTTATT	----ACCATC	ATCGAGTCGC
<i>S. luteus</i>	TCTTCGTGTA	GAAAGTCTTT	GAATGTTT--	----ACCATC	ATCGAGTCGC
<i>S. tomentosus</i>	CCTTCGTGTA	GAAAGTCWAT	GAATGTTTTT	----ACCATC	ATCGACTCGC
<i>S. americanus</i>	TCTTCGTGTA	GAAAGTCTTT	GAATGTTTTT	----ACAATC	ATCGAGTCGT
<i>S. granulatus</i>	TCTTCGTGTA	GAAAGTCTTT	GAATGTTTTT	----ACCATC	ATCGAGTCGC
<i>S. grevillei</i>	TCTTCGTGTA	GAAAGTCTTC	GAATGTTTAT	----ATTATC	ATCGAGCCGC
<i>S. cavipes</i>	CCTTCGTGTA	GAAAGTCTTT	GAACGTTAT-	----AAAAATC	ATCGAGTCGC
<i>S. caerulescens</i>	TCTTCGTGTA	GAAAGTCTTT	GAATGTTAT-	----ACCATC	ATCGAGTCGC
<i>S. lakei</i>	TCTTCGTGTA	GAAAGTCTTC	GAATGTTAT-	----ACTATC	ATCGAGYCGC
<i>S. sinuspaulinus</i>	TCTTCGTGTA	GAAAGTCTTT	GAATGTTTTT	TTTTACAATC	GTCGAGTCGC
<i>Chroogomphus</i>	CCACAGTTAA	GAAAGTCTCA	GAATGTTT--	----ACTATC	GTCGAGCCAC
<i>Gomphidius</i>	--ACAGTTTA	GAAAGTCTCA	GAACGTTT--	----ACTATC	GTCGAGCCGC
<i>Truncocolumella</i>	TCTTCGTGTA	GAAAGTCTCA	GAATGTTTTT	T---ACTACC	GTCGAGTCGC
<i>Rhizopogon evadens</i>	TCTTCGTGTA	GAAAGTCTTT	GAATGTTT--	----ACGATC	ATAGAGCCGC
<i>R. evadens</i> (TT)	TCTTCGTGTA	GAAAGTCTTT	GA-TGTTT--	----ACGATC	ATAGAGCCGC
<i>R. smithii</i>	TCTTCGTGTA	GAAAGTCTTT	GAATGTTT--	----ACGATC	ATAGAGCTGC
<i>R. subsalmonius</i>	TCTTCGTGTA	GAAAGTCTTT	GAATGTTT--	----ACGATC	AGAGAGCCGC
<i>R. fuscorubens</i>	TCTTCGTGTA	GAAAGTCTTT	GAATGTTTT-	----ACTATC	ATAGAGTCGC
<i>R. ochraceorubens</i> (TT)	TCTTCGTGTA	GAAAGTCTTA	GAATGTTT--	----ACTATC	ATTGAGTCGC
<i>R. ochraceorubens</i> (HT)	TCTTCGTGTA	GAAAGTCTTA	GAATGTTT--	----ACTATC	ATTGAGNCGC
<i>R. occidentalis</i>	TCTTCGTGTA	AAAAGTCTTA	GAATGTTT--	----ACTATC	ATTGAGTCGC
<i>R. occidentalis</i>	TCTTCGTGTA	GAAAGTCTTA	GAATGTTT--	----ACTATC	ATTGAGTCGC
<i>R. succosus</i>	TCTTTGTGTA	GAAAGTCATT	GAATGTTT--	----ACTATC	ATTGAGTCAC
<i>R. luteolus</i>	TCTTCGTGTA	GAAAGTCTTT	GAATGTTT--	----ACTATC	ATTGAGTCAC
<i>R. burlinghamii</i>	TCTTCGTGTA	GAAAGTCTTA	GAATGTTT--	----ACTATC	AGAGAGTCGC
<i>R. rubescens</i>	TCTTCGTGTA	GAAAGTCTTA	GAATGTTT--	----ACTATC	AGAGAGTCGC
<i>R. roseolus</i>	TCTTCGTGTA	GAAAGTCTTA	GAATGTTT--	----ACTATC	AGAGAGTCGC
<i>R. vulgaris</i>	TCTTCGTGTA	GAAAGTCTTA	GAATGTTT--	----ACTATC	AGAGAGTCGC
<i>R. colossus</i> (HT)	TCTCCGTGTA	GAAAGTCTTA	GAATGTTT--	----ACTATC	ATAGAGTCGC
<i>R. hawkeriae</i> (PT)	TCTCCGTGTA	CAAAGTCTWA	GAATGTTT--	----ACTATC	ATAGAGTTGC
<i>R. parksii</i> (T17679)	TCTTCGTGTA	GAAAGTCTTA	GAATGTTT--	----ACTATC	AGACAGTCGC
<i>R. parksii</i> (T19446)	TCTTCGTGTA	GAAAGTCTTA	GAATGTTT--	----ACTATC	AGAGAGTCGC
<i>R. villosulus</i> (AHS)	TCTCCGTGTA	GAAAGTCTTA	GAATGTTT--	----ACTATC	ATAGAGTTGC
<i>R. villosulus</i> (JMT)	TCTCCGTGTA	GAAAGTCTTA	GAATGTTT--	----ACTATC	ATAGAGTCGC
<i>R. rogersii</i>	TCTCCGTGTA	GAAAGTCTTA	GAATGTTT--	----ACTATC	ATAGAGTCGC
<i>R. sp. nov</i>	TCTCCGTGTA	GAAAGTCTTA	GAATGTTT--	----ACTATC	AGAAAGTCGC
<i>R. gilkeyae</i>	TCTTCGTGTA	GAAAGTCTTA	GAATGTTT--	----ACTATC	AGAGAGTCGC
<i>R. zelleri</i>	TCTCCGKGT	GAAAGTCTTA	GAATGTTT--	----ACTATC	ATAGAGTCGC
<i>R. villescens</i>	TCTCCGTGTA	GAAAGTCTTA	GAATGTTT--	----ACTATC	ATAGAGTCGC
<i>R. diabolicus</i>	TCTTCGTGTA	GAAAGTCTTA	GAATGTTT--	----ACTATC	AGAGAGTCGC
<i>R. parvulus</i>	TCTTCGTGTA	GAAAGTCTTA	GAATGTTT--	----ACTATC	AGAGAGTCGC
<i>R. vinicolor</i> (T17899)	TCTTCGTGTA	GAAAGTCTTA	GAATGTTT--	----ACTATC	AGAGAGTCGC
<i>R. vinicolor</i> (T20787)	TCTTCGTGTA	GAAAGTCTTA	GAATGTTT--	----ACTATC	AGAGAGTCGC
<i>R. ochraceisorus</i> (PT)	TCTTSGTGTA	GAAAGTCTAA	GAATGTTT--	----ACTATC	AGAGAGTCGC
<i>R. ochraceisorus</i> (17916)	TCTTCGTGTA	GAAAGTCTTA	GAATGTTT--	----ACTATC	AGAGAGTCGC
<i>R. ochraceisorus</i> (17944)	TCTTCGTGTA	GAAAGTCTTA	GAATGTTT--	----ACTATC	AGAGAGTCGC
<i>R. subcaerulescens</i>	TCTTCGTGTA	GAAAGTCTTT	GAATGTTT--	----ACTATC	ATCGAGTCGC
<i>R. ellenae</i> (HT)	TCTTCGTGTA	GAAAGTCTTT	GAATGTTT--	----ACTATC	ATCGAGTCGC
<i>R. ellenae</i>	TCTTCGTGTA	GAAAGTCTTT	GAATGTTT--	----ACTATC	ATCGAGTCGC
<i>R. subpurpurascens</i> (PT)	TCTTCGTGTA	GAAAGTCTTT	GAATGTTT--	----ACTATC	ATCGAGTCGC
<i>R. subpurpurascens</i>	TCTTCGTGTA	GAAAGTCTTT	GAATGTTT--	----ACTATC	ATCGAGTCGC
<i>R. subgelatinosus</i>	TCTTCGTGTA	GAAAGTCTTT	GAATGTTT--	----ACTATC	ATCGAGTCGC
<i>R. semireticulatus</i> (17562)	TCTTCGTGTA	GAAAGTCTTT	GAATGTTT--	----ACTATC	ATCGAGTCGC
<i>R. semireticulatus</i> (7899)	TCTTCGTGTA	AAAAGTCTTT	GAATGTTT--	----ACTATC	ATCGAGTCGC

	410	420	430	440	*5.8S start
Suillus brevipes	GACTTCTAGG	AGACGCGA-T	TCCTTTGAGAC	AAAAGTTIAT	TACAACTTTC
S. luteus	GACTTCTAGG	AGACGCGA-T	TCCTTTGAGAA	AAAAGTTIAT	TACAACTTTC
S. tomentosus	GACTTCTAGG	AGACGCGA-T	TCCTTTGAGAC	AAAAGTTIAT	TACAACTTTC
S. americanus	GACTTCTAGG	AGACGCGA-T	TCCTTTGAGAC	AAAAGTTIAT	CACAACTTTC
S. granulatus	GACTTCTAGG	AGACGCGA-T	TCCTTTGAGAC	AAAAGTTIAT	TACAACTTTC
S. grevillei	GACTTCTAGG	AGACGCGG-T	TCCTTTGAGAC	AAAAGTTIAT	TACAACTTTC
S. cavipes	GACTTCTAGG	AGACGCGA-T	TCCTTTGAGAC	AAAAGTTAAT	TACAACTTTC
S. caerulescens	GACTTCTAGG	AGACGCGA-T	TCCTTTGAGAC	AAAAGTTIAT	TACAACTTTC
S. lakei	GACTTCTAGG	AGACGCGG-T	TCCTTTGAGAC	AAAAGTTIAT	TACAACTTTC
S. sinuspaulinus	GACTTCTAGG	AGACGCGA-T	TCCTTCGAGAC	AAAAGTTIAT	TACAACTTTC
Chroogomphus	GACTTCCAGG	AGACGTGGG-	TCGGCGAGAT	AAAAGTTIAT	TACAACTTTC
Gomphidius	GACTTCCAGG	AGACGCGGG-	TCGGCGAGAC	AAAAGTTIAT	CACAACTTTC
Truncocolumella	GACTTCTAGG	AGACGCGAA-	TCCTTCGGGAC	AAAAGTTIAT	TACAACTTTC
Rhizopogon evadens	GACTTCTAGG	AGACGCGGG-	TCCTTTGAGAT	AAAAGTTIAT	TACAACTTTC
R. evadens (HT)	GACTTCTAGG	AGACGCGGG-	TCCTTTGAGAT	AAAAGTTIAT	TACAACTTTC
R. smithii	GACTTCTAGG	AGACGCGGG-	TCCTTTGAGTT	AAAAGCTIAT	TACAACTTTC
R. subsalmonius	GACTTCTAGG	AGACGCGG--	TCCTTTGAGAT	AAAAGRTIAT	TACAACTTTC
R. fuscorubens	GACTTCTAGG	AGACGCGGG-	TCCTTTGAGTT	AAAAGTTTAT	TACAACTTTC
R. ochraceorubens(TT)	GACTTCTAGG	AGACGCGGG-	TCCTTTGAGTT	AAAAGTTTAT	TACAACTTTC
R. ochraceorubens(HT)	GACTTCTAGG	AGACGCGGG-	TCCTTTGAGTT	AAAAGTTTAT	TACAACTTTC
R. occidentalis	GACTTCTAGG	AGACGCGGG-	TCCTTTGAGTT	AAAAGTTTAT	TACAACTTTC
R. occidentalis	GACTTCTAGG	AGACGCGGG-	TCCTTTGAGTT	AAAAGTTTAT	TACAACTTTC
R. succosus	GACTTCTAGG	AGATGTGGG-	TCCTTTGAGAT	AAAAGTTTAT	TACAACTTTC
R. luteolus	GACTTCTAGG	AGATGTGGG-	TCCTTTGAGAT	AAAAGTTTAT	TACAACTTTC
R. burlinghamii	GACTTCTAGG	AAACGCGAA-	TCCTTGAGAT	AAAAGTTAAT	TACAACTTTC
R. rubescens	GACTTCTAGG	AGACGCGAA-	TCCTTGAGAT	AAAAGTTAAT	TACAACTTTC
R. roseolus	GACTTCTAGG	AGACGCGAA-	TCCTTGAGAT	AAAAGTTAAT	TACAACTTTC
R. vulgaris	GACTTCTAGG	AGACGCGAA-	TCCTTGAGAT	AAAAGTTAAT	TACAACTTTC
R. colossus (HT)	GACTTCTAGG	AGACGCGAA-	TCGTGAGAT	AAAAGTTIAT	TACAACTTTC
R. hawkeriae (PT)	GACTTCTAGG	AGACGCGAA-	TCGTGAGAT	AAAAGTTIAT	TACARCTTTC
R. parksii (T17679)	GACTTCTAGG	AGACGCGAA-	TCCTTTGAGAT	AAAAGTTIAT	TACAACTTTC
R. parksii (T19446)	GACTTCTAGG	AGACGCGAA-	TCCTTTGAGAT	AAAAGTTIAT	TACAACTTTC
R. villosulus (AHS)	GACTTCTAGG	AGACGCGAA-	TCGTGAGAT	AAAAGTTIAT	TACAACTTTC
R. villosulus (JMT)	GACTTCTAGG	AGACGCGAA-	TCGTGAGAT	AAAAGTTIAT	TACAACTTTC
R. rogersii	GACTTCTAGG	AGACGCGAA-	TCGTGAGAT	AAAAGTTIAT	TACAACTTTC
R. sp. nov	GACTTCTAGG	AGACGCGAA-	TCCTTTGAGAT	AAAAGTTIAT	TACAACTTTC
R. gilkeyae	GACTTCTAGG	AGACGCGAAA	CTCTTGAGAT	AAAAGTTIAT	TACAACTTTC
R. zelleri	GACTTCTAGG	AGACGCGAAT	TCGTGAGAT	AAAAGTTIAT	AACAACTTTC
R. villescens	GACTTCTAGG	AGACGCGAA-	TCGTGAGAT	AAAAGTTIAT	TACAACTTTC
R. diabolicus	GTCTTCTAGG	AGTCGCGAAA	-CTCTGAGAT	AAAAGTTIAT	TACANCTTTC
R. parvulus	GACTTCTAGG	AGACGCGAAA	-CTCTGAGAT	AAAAGTTIAT	TACAACTTTC
R. vinicolor(T17899)	GACTTCTAGG	AGACGCGAAA	-CTCTGAGAT	AAAAGTTIAT	TACAACTTTC
R. vinicolor(T20787)	GACTTCTAGG	AGACGYGAAA	-CTCTGAGAT	AAAAGTTIAT	TACAACTTTC
R. ochraceisporus (PT)	GACTTCTAGG	AGACGCGAAA	-CTCTGAGAT	AAAAGTTIAT	TACAACTTTC
R. ochraceisporus (17916)	GACTTCTAGG	AGACGTGAAA	-CTCTGAGAT	AAAAGTTIAT	TACAACTTTC
R. ochraceisporus (17944)	GACTTCTAGG	AGACGTGAAA	-CTCTGAGAT	AAAAGTTIAT	TACAACTTTC
R. subcaerulescens	GACTTCTAGG	AGACGCGA-T	TCCTTTGAGAT	AAAAGTTIAT	TACAACTTTC
R. ellenae (HT)	GACTTCTAGG	AGACGCGA-T	TCCTTTGAGAT	AAAAGTTIAT	TACAACTTTC
R. ellenae	GACTTCTAGG	AGACGCGA-T	TCCTTTGAGAT	AAAAGTTIAT	TACAACTTTC
R. subpurpurascens (PT)	GACTTCTAGG	AGACGCGA-T	TCCTTTGAGAT	AAAAGTTIAT	TACAACTTTC
R. subpurpurascens	GACTTCTAGG	AGACGCGA-T	TCCTTTGAGAT	AAAAGTTIAT	TACAACTTTC
R. subgelatinosus	GACTTCTAGG	AGACGCGA-T	TCCTTTGAGAT	AAAAGTTIAT	TACAACTTTC
R. semireticulatus (17562)	GACTTCTAGG	AGACGCGA-T	TCCTTTGAGAT	AAAAGTTIAT	TACAACTTTC
R. semireticulatus (7899)	GACTTCTAGG	AGACGCGA-T	TCCTTTGAGAT	AAAAGTTIAT	TACAACTTTC

	460	470	480	490	500
<i>Suillus brevipes</i>	AGCAACGGAT	CTCTTGGCTC	TCGCATCGAT	GAAGAACGCA	GCGAAATCGCG
<i>S. luteus</i>	AGCAACGGAT	CTCTTGGCTC	TCGCATCGAT	GAAGAACGCA	GCGAAATCGCG
<i>S. tomentosus</i>	AGCAATGGAT	CTCTTGGCTC	TCGCATCGAT	GAAGAACGCA	GCGAAATCGCG
<i>S. americanus</i>	AGCAATGGAT	CTCTTGGCTC	TCGCATCGAT	GAAGAACGCA	GCGAAATCGCG
<i>S. granulatus</i>	AGCAATGGAT	CTCTTGGCTC	TCGCATCGAT	GAAGAACGCA	GCGAAATCGCG
<i>S. grevillei</i>	AGCAATGGAT	CTCTTGGCTC	TCGCATCGAT	GAAGAACGCA	GCGAAATCGCG
<i>S. cavipes</i>	AGCAATGGAT	CTCTTGGCTC	TCGCATCGAT	GAAGAACGCA	GCGAAATCGCG
<i>S. caerulescens</i>	AGCAACGGAT	CTCTTGGCTC	TCGCATCGAT	GAAGAACGCA	GCGAAATCGCG
<i>S. lakei</i>	AGCAACGGAT	CTCTTGGCTC	TCGCATCGAT	GAAGAACGCA	GCGAAATCGCG
<i>S. sinuspaulinus</i>	AGCAATGGAT	CTCTTGGCTC	TCGCATCGAT	GAAGAACGCA	GCGAAATCGCG
<i>Chroogomphus</i>	AGCAATGGAT	CTCTTGGCTC	TCGCATCGAT	GAAGAACGCA	GCGAAATTGCG
<i>Gomphidius</i>	AGCAATGGAT	CTCTTGGCTC	TCGCATCGAT	GAAGAACGCA	GCGAAATTGCG
<i>Truncocolumella</i>	AGCAATGGAT	CTCTTGGCTC	TCGCATCGAT	GAAGAACGCA	GCGAAATCGCG
<i>Rhizopogon evadens</i>	AGCAATGGAT	CTCTTGGCTC	TCGCATCGAT	GAAGAACGCA	GCGAAAAGCG
<i>R. evadens</i> (HT)	AGCAATGGAT	CTCTTGGCTC	TCGCATCGAT	GAAGAACGCA	GCGAAAAGCG
<i>R. smithii</i>	AGCAATGGAT	CTCTTGGCTC	TCGCATCGAT	GAAGAACGCA	GCGAAAAGCG
<i>R. subsalmonius</i>	AGCAATGGAT	CTCTTGGCTC	TYGCATYGRN	GAAGAACGCA	GCGAAAAGCG
<i>R. fuscorubens</i>	AGCAATGGAT	CTCTTGGCTC	TCGCATCGAT	GAAAA-CGCA	GCGAAACGCG
<i>R. ochraceorubens</i> (TT)	AGCAATGGAT	CTCTTGGCTC	TCGCATCGAT	GAAGAACGCA	GCGAAACGCG
<i>R. ochraceorubens</i> (HT)	AGCAATGGNT	-TCTTGGTCN	CNNNATCGAT	GAAGAACGCA	GCGAAACGCG
<i>R. occidentalis</i>	AGCAATGGAT	CTCTTGGCTC	TCGCATCGAT	GAAGAACGCA	GCGAAACGCG
<i>R. occidentalis</i>	AGCAATGGAT	CTCTTGGCTC	TCGCATCGAT	GAAGAACGCA	GCGAAACGCG
<i>R. succosus</i>	AGCAATGGAT	CTCTTGGCTC	TCGCATCGAT	GAAGAACGCA	GCGAAACGCG
<i>R. luteolus</i>	AGCAATGGAT	CTCTTGGCTC	TCGCATCGAT	GAAGAACGCA	GCGAAACGCG
<i>R. burlinghamii</i>	AGCAATGGAT	CTCTTGGCTC	TCGCATCGAT	GAAGAACGCA	GCGAAAAGCG
<i>R. rubescens</i>	AGCAATGGAT	CTCTTGGCTC	TCGCATCGAT	GAAGAACGCA	GCGAAAAGCG
<i>R. roseolus</i>	AGCAATGGAT	CTCTTGGCTC	TCGCATCGAT	GAAGAACGCA	GCGAAAAGCG
<i>R. vulgaris</i>	AGCAATGGAT	CTCTTGGCTC	TCGCATCGAT	GAAGAACGCA	GCGAAAAGCG
<i>R. colossus</i> (HT)	AGCAATGGNN	NNCTTGGCNC	NNNNATCGAT	GAAGAACGCA	GCGAAAAGCG
<i>R. hawkeriae</i> (PT)	AGCAATGGAT	CTCTTGGCTC	TCNNNTCGAT	GAAGAACGCA	GCGAAAAGCG
<i>R. parksii</i> (T17679)	AGCAATGGAT	CTCTTGGCTC	TCGCATCGAT	GAAGAACGCA	GCGAAAAGCG
<i>R. parksii</i> (T19446)	AGCAATGGAT	CTCTTGGCTC	TCGCATCGAT	GAAGAACGCA	GCGAAAAGCG
<i>R. villosulus</i> (AHS)	AGCAATGGAT	CTCTTGGCTC	TCNNNTCGAT	GAAGAACGCA	GCGAAAAGCG
<i>R. villosulus</i> (JMT)	AGCAATGGAT	CTCTTGGCTC	TCGCATCGAT	GAAGAACGCA	GCGAAAAGCG
<i>R. rogersii</i>	AGCAATGGAT	CTCTTGGCTC	TCGCATCGAT	GAAGAACGCA	GCGAAAAGCG
<i>R. sp. nov</i>	AGCAATGGAT	CTCTTGGCTC	TCGCATCGAT	GAAGAACGCA	GCGAAAAGCG
<i>R. gilkeyae</i>	AGCAATGGAT	CTCTTGGCTC	TCGCATCGAT	GAAGAACGCA	GCGAAAAGCG
<i>R. zelleri</i>	AGCAATGGAT	CTCTTGGCTC	TCGCATCGAT	GAAGAACGCA	GCGAAAAGCG
<i>R. villescens</i>	AGCAATGGAT	CTCTTGGCTC	TCGCATCGAT	GAAGAACGCA	GCGAAAAGCG
<i>R. diabolicus</i>	AGCAATGGNN	NNCTTGGNNN	NNNNATCGAT	GAAGAACGCA	GCGAAAAGCG
<i>R. parvulus</i>	AGCAATGGNN	NNCTTGGNNN	NNNNATCGAT	GAAGAACGCA	GCGAAAAGCG
<i>R. vinicolor</i> (T17899)	AGCAATGGAT	CTCTTGGCTC	TCGCATCGAT	GAAGAACGCA	GCGAAAAGCG
<i>R. vinicolor</i> (T20787)	AGCAATGGAT	CTCTTGGCTC	TCGCATCGAT	GAAGAACGCA	GCGAAAAGCG
<i>R. ochraceisporus</i> (PT)	AGCATTTGGAT	CTCTTGGCTC	TCGNATCGAT	GAAGAACGCA	GCGAAAAGCG
<i>R. ochraceisporus</i> (17916)	AGCAATGGAT	CTCTTGGCTC	TCGCATCGAT	GAAGAACGCA	GCGAAAAGCG
<i>R. ochraceisporus</i> (17944)	AGCAATGGAT	CTCTTGGCTC	TCGCATCGAT	GAAGAACGCA	GCGAAAAGCG
<i>R. subcaerulescens</i>	AGCAACGGAT	CTCTTGGCTC	TCGCATCGAT	GAAGAACGCA	GCGAAAAGCG
<i>R. ellenae</i> (HT)	AGCAATGGNN	CCCTTGGNNN	NNNNATCGAT	GAAGAACGCA	GCGAAAAGCG
<i>R. ellenae</i>	AGCAATGGAT	CTCTTGGCTC	TCGCATCGAT	GAAGAACGCA	GCGAAAAGCG
<i>R. subpurpurascens</i> (PT)	AGCAATGGAT	CTCTTGGCTC	TCGCATCGAT	GAAGAACGCA	GCGAAAAGCG
<i>R. subpurpurascens</i>	AGCAATGGAT	CTCTTGGCTC	TCGCATCGAT	GAAGAACGCA	GCGAAAAGCG
<i>R. subgelatinosus</i>	AGCAACGGAT	CTCTTGGCTC	TCGCATCGAT	GAAGAACGCA	GCGAAAAGCG
<i>R. semireticulatus</i> (17562)	AGCAATGGAT	CTCTTGGCTC	TCGCATCGAT	GAAGAACGCA	GCGAAAAGCG
<i>R. semireticulatus</i> (7899)	AGCAACGGAT	CTCTTGGCTC	TCGCATCGAT	GAAGAACGCA	GCGAAAAGCG

	510	520	530	540	550
<i>Suillus brevipes</i>	ATATGTAATG	TGAATTGCAG	ATCTACAGTG	AATCATCGAA	TCTTTGAACG
<i>S. luteus</i>	ATATGTAATG	TGAATTGCAG	ATCTACAGTG	AATCATCGAA	TCTTTGAACG
<i>S. tomentosus</i>	ATATGTAATG	TGAATTGCAG	ATCTACAGTG	AATCATCGAA	TCTTTGAACG
<i>S. americanus</i>	ATATGTAATG	TGAATTGCAG	ATCTACAGTG	AATCATCGAA	TCTTTGAACG
<i>S. granulatus</i>	ATATGTAATG	TGAATTGCAG	ATCTACAGTG	AATCATCGAA	TCTTTGAACG
<i>S. grevillei</i>	ATATGTAATG	TGAATTGCAG	ATCTACAGTG	AATCATCGAA	TCTTTGAACG
<i>S. cavipes</i>	ATATGTAATG	TGAATTGCAG	ATCTACAGTG	AATCATCGAA	TCTTTGAACG
<i>S. caerulescens</i>	ATATGTAATG	TGAATTGCAG	ATCTACAGTG	AATCATCGAA	TCTTTGAACG
<i>S. lakei</i>	ATATGTAATG	TGAATTGCAG	ATCTACAGTG	AATCATCGAA	TCTTTGAACG
<i>S. sinuspaulinus</i>	ATATGTAATG	TGAATTGCAG	ATCTACAGTG	AATCATCGAA	TCTTTGAACG
<i>Chroogomphus</i>	ATATGTAATG	TGAATTGCAG	ATCTACAGTG	AATCATCGAA	TCTTTGAACG
<i>Gomphidius</i>	ATATGTAATG	TGAATTGCAG	ATCTACAGTG	AATCATCGAA	TCTTTGAACG
<i>Truncocolumella</i>	ATATGTAATG	TGAATTGCAG	ATCTACAGTG	AATCATCGAA	TCTTTGAACG
<i>Rhizopogon evadens</i>	ATATGTAATG	TGAATTGCAG	ATCTACAGTG	AATCATCGAA	TCTTTGAACG
<i>R. evadens</i> (HT)	ATATGTAATG	TGAATTGCAG	ATCTACAGTG	AATCATCGAA	TCTTTGAACG
<i>R. smithii</i>	ATATGTAATG	TGAATTGCAG	ATCTACAGTG	AATCATCGAA	TCTTTGAACG
<i>R. subsalmonius</i>	ATATGTAATG	TGAATTGCAG	ATCTACAGTG	AATCATCGAA	TCTTTGAACG
<i>R. fusciorubens</i>	ATATGTTATG	TGAATTGCAG	ATCTACAGTG	AATCATCGAA	TCTTTGAACG
<i>R. ochraceorubens</i> (TT)	ATATGTAATG	TGAATTGCAG	ATCTACAGTG	AATCATCGAA	TCTTTGAACG
<i>R. ochraceorubens</i> (HT)	ATATGTAATG	TGAATTGCAG	ATCTACAGTG	AATCATCGAA	TCTTTGAACG
<i>R. occidentalis</i>	ATATGTAATG	TGAATTGCAG	ATCTACAGTG	AATCATCGAA	TCTTTGAACG
<i>R. occidentalis</i>	ATATGTAATG	TGAATTGCAG	ATCTACAGTG	AATCATCGAA	TCTTTGAACG
<i>R. succosus</i>	ATATGTAATG	TGAATTGCAG	ATCTACAGTG	AATCATCGAA	TCTTTGAACG
<i>R. luteolus</i>	ATATGTTATG	TGAATTGCAG	ATCTACAGTG	AATCATCGAA	TCTTTGAACG
<i>R. burlinghamii</i>	ATATGTTATG	TGAATTGCAG	ATCTACAGTG	AATCATCGAA	TCTTTGAACG
<i>R. rubescens</i>	ATATGTAATG	TGAATTGCAG	ATCTACAGTG	AATCATCGAA	TCTTTGAACG
<i>R. roseolus</i>	ATATGTAATG	TGAATTGCAG	ATCTACAGTG	AATCATCGAA	TCTTTGAACG
<i>R. vulgaris</i>	ATATGTAATG	TGAATTGCAG	ATCTACAGTG	AATCATCGAA	TCTTTGAACG
<i>R. colossus</i> (HT)	ATATGTAATG	TGAATTGCAG	ATCTACAGTG	AATCATCGAA	TCTTTGAACG
<i>R. hawkeriae</i> (PT)	ATATGTAATG	TGMATTGCAG	ATCTACAGTG	AATCATCGAA	TCTTYGAACG
<i>R. parksii</i> (T17679)	ATATGTAATG	TGAATTGCAG	ATCTACAGTG	AATCATCGAA	TCTTTGAACG
<i>R. parksii</i> (T19446)	ATATGTAATG	TGAATTGCAG	ATCTACAGTG	AATCATCGAA	TCTTTGAACG
<i>R. villosulus</i> (AHS)	ATATGTAATG	TGAATTGCAG	ATCTACAGTG	AATCATCGAA	TCTTTGAACG
<i>R. villosulus</i> (JMT)	ATATGTAATG	TGAATTGCAG	ATCTACAGTG	AATCATCGAA	TCTTTGAACG
<i>R. rogersii</i>	ATATGTAATG	TGAATTGCAG	ATCTACAGTG	AATCATCGAA	TCTTTGAACG
<i>R. sp. nov</i>	ATATGTAATG	TGAATTGCAG	ATCTACAGTG	AATCATCGAA	TCTTTGAACG
<i>R. gilkeyae</i>	ATATGTAATG	TGAATTGCAG	ATCTACAGTG	AATCATCGAA	TCTTTGAACG
<i>R. zelleri</i>	ATATGTAATG	TGAATTGCAG	ATCTACAGTG	AATCATCGAA	TCTTTGAACG
<i>R. villescens</i>	ATATGTAATG	TGAATTGCAG	ATCTACAGTG	AATCATCGAA	TCTTTGAACG
<i>R. diabolicus</i>	ATATGTAATG	TGAATTGCAG	ATCTACAGTG	ATTCAATCGAA	TCTTAGAACG
<i>R. parvulus</i>	ATATGTAATG	TGAATTGCAG	ATCTACAGTG	AATCATCGAA	TCTTTGAACG
<i>R. vinicolor</i> (T17899)	ATATGTAATG	TGAATTGCAG	ATCTACAGTG	AATCATCGAA	TCTTTGAACG
<i>R. vinicolor</i> (T20787)	ATATGTAATG	TGAATTGCAG	ATCTACAGTG	AATCATCGAA	TCTTTGAACG
<i>R. ochraceisporus</i> (PT)	ATATGTAATG	TGAATTGCAG	ATCTACAGTG	AATCATCGAA	TCTTTGAACG
<i>R. ochraceisporus</i> (17916)	ATATGTAATG	TGAATTGCAG	ATCTACAGTG	AATCATCGAA	TCTTTGAACG
<i>R. ochraceisporus</i> (17944)	ATATGTAATG	TGAATTGCAG	ATCTACAGTG	AATCATCGAA	TCTTTGAACG
<i>R. subcaerulescens</i>	ATATGTAATG	TGAATTGCAG	ATCTACAGTG	AATCATCGAA	TCTTTGAACG
<i>R. ellenae</i> (HT)	ATATGTAATG	TGAATTGCAG	ATCTACAGTG	AATCATCGAA	TCTTTGAACG
<i>R. ellenae</i>	ATATGTAATG	TGAATTGCAG	ATCTACAGTG	AATCATCGAA	TCTTTGAACG
<i>R. subpurpurascens</i> (PT)	ATATGTAATG	TGAATTGCAG	ATCTACAGTG	AATCATCGAA	TCTTTGAACG
<i>R. subpurpurascens</i>	ATATGTAATG	TGAATTGCAG	ATCTACAGTG	AATCATCGAA	TCTTTGAACG
<i>R. subgelatinosus</i>	ATATGTAATG	TGAATTGCAG	ATCTACAGTG	AATCATCGAA	TCTTTGAACG
<i>R. semireticulatus</i> (17562)	ATATGTAATG	TGAATTGCAG	ATCTACAGTG	AATCATCGAA	TCTTTGAACG
<i>R. semireticulatus</i> (7899)	ATATGTAATG	TGAATTGCAG	ATCTACAGTG	AATCATCGAA	TCTTTGAACG

	560	570	580	590	600
Suillus brevipes	CACCTTGCGC	TCCTCGGTGT	TCCGAGGAGC	ATGCCGTGTTT	GAGTGTCACT
S. luteus	CACCTTGCGC	TCCTCGGTGT	TCCGAGGAGC	ATGCCGTGTTT	GAGTGTCACT
S. tomentosus	CACCTTGCGC	TATCGGTGT	TCCGATGAGC	ATGCCGTGTTT	GAGCGTCATT
S. americanus	CACCTTGCGC	TCCTCGGTGT	TCCGAGGAGC	ATGCCGTGTTT	GAGCGTCAGT
S. granulatus	CACCTTGCGC	TCCTCGGTGT	TCCGAGGAGC	ATGCCGTGTTT	GAGCGTCAGT
S. grevillei	CACCTTGCGC	TCCTCGGTGT	TCCGAGGAGC	ATGCCGTGTTT	GAGCGTCAGT
S. cavipes	CACCTTGCGC	TCCTCGGTGT	TCCGAGGAGC	ATGCCGTGTTT	GAGTGTCACT
S. caerulescens	CACCTTGCGC	TCCTCGGTGT	TCCGAGGAGC	ATGCCGTGTTT	GAGTGTCACT
S. lakei	CACCTTGCGC	TCCTCGGTGT	TCCGAGGAGC	ATGCCGTGTTT	GAGTGTCACT
S. sinuspaulinus	CACCTTGCGC	TCCTCGGTGT	TCCGAGGAGC	ATGCCGTGTTT	GAGTGTCACT
Chroogomphus	CACATTGCGC	TCCTCGGTGT	TCCGAGGAGC	ATGCCGTGTTT	GAGTGTCACT
Gomphidius	CACATTGCGC	TCCTCGGTGT	TCCGAGGAGC	ATGCCGTGTTT	GAGTGTCACT
Truncocolumella	CACCTTGCGC	TCCTCGGTGT	TCCGAGGAGC	ATGCCGTGTTT	GAGCGTCAGT
Rhizopogon evadens	CACCTTGCGC	TCCTTGGTAT	TCCGAGGAGC	ATGCCGTGTTT	GAGTGTCACT
R. evadens (HT)	CACCTTGCGC	TCCTTGGTAT	TCCGAGGAGC	ATGCCGTGTTT	GAGTGTCACT
R. smithii	CACCTTGCGC	TCCTTGGTAT	TCCGAGGAGC	ATGCCGTGTTT	GAGTGTCACT
R. subsalmonius	CACCTTGCGC	TCCTTGGTAT	TCCGAGGAGC	ATGCCGTGTTT	GAGTGTCACT
R. fuscorubens	CACCTTGCGC	TCCTTGGTAT	TCCGAGGAGC	ATGCCGTGTTT	GAGTGTCACT
R. ochraceorubens(TT)	CACCTTGCGC	TCCTTGGTAT	TCCGAGGAGC	ATGCCGTGTTT	GAGTGTCACT
R. ochraceorubens(HT)	CACCTTGCGC	TCCTTGGTAT	TCCGAGGAGC	ATGCCGTGTTT	GAGTGTCACT
R. occidentalis	CACCTTGCGC	TCCTTGGTAT	TCCGAGGAGC	ATGCCGTGTTT	GAGTGTCACT
R. occidentalis	CACCTTGCGC	TCCTTGGTAT	TCCGAGGAGC	ATGCCGTGTTT	GAGTGTCACT
R. succosus	CACCTTGCGC	TCCTTGGTAT	TCCGAGGAGC	ATGCCGTGTTT	GAGTGTCACT
R. luteolus	CACCTTGCGC	TCCTTGGTAT	TCCGAGGAGC	ATGCCGTGTTT	GAGTGTCACT
R. burlinghamii	CACCTTGCGC	TCCTCGGTGT	TCCGAGGAAC	ATGCCGTGTTT	GAGTGTCACT
R. rubescens	CACCTTGCGC	TCCTCGGTGT	TCCGAGGAGC	ATGCCGTGTTT	GAGTGTCACT
R. roseolus	CACCTTGCGC	TCCTCGGTGT	TCCGAGGAGC	ATGCCGTGTTT	GAGTGTCACT
R. vulgaris	CACCTTGCGC	TCCTCGGTGT	TCCGAGGAGC	ATGCCGTGTTT	GAGTGTCACT
R. colossus (HT)	CACCTTGCGC	TCCTCGGTGT	TCCGAGGAGC	ATGCCGTGTTT	GAGTGTCACT
R. hawkeriae (PT)	CACCTTGCGC	TCCTCGGTGT	TCCGAGGAGC	ATGCCGTGTTT	GAGTGTCACT
R. parksii (T17679)	CACCTTGCGC	TCCTCGGTGT	TCCGAGGAGC	ATGCCGTGTTT	GAGTGTCACT
R. parksii (T19446)	CACCTTGCGC	TCCTCGGTGT	TCCGAGGAGC	ATGCCGTGTTT	GAGTGTCACT
R. villosulus (AHS)	CACCTTGCGC	TCCTCGGTGT	TCCGAGGAGC	ATGCCGTGTTT	GAGTGTCACT
R. villosulus (JMT)	CACCTTGCGC	TCCTCGGTGT	TCCGAGGAGC	ATGCCGTGTTT	GAGTGTCACT
R. rogersii	CACCTTGCGC	TCCTCGGTGT	TCCGAGGAGC	ATGCCGTGTTT	GAGTGTCACT
R. sp. nov	CACCTTGCGC	TCCTCGGTGT	TCCGAGGAGC	ATGCCGTGTTT	GAGTGTCACT
R. gilkeyae	CACCTTGCGC	TCCTCGGTGT	TCCGAGGAGC	ATGCCGTGTTT	GAGTGTCACT
R. zelleri	CACCTTGCGC	TCCTCGGTGT	TCCGAGGAGC	ATGCCGTGTTT	GAGTGTCACT
R. villescens	CACCTTGCGC	TCCTCGGTGT	TCCGAGGAGC	ATGCCGTGTTT	GAGTGTCACT
R. diabolicus	CACCTTGCGC	TCCTCGGTGT	TCCGAGGAGC	AWGCCGTGGTC	GAGTGTCAKT
R. parvulus	CACCTTGCGC	TCCTCGGTGT	TCCGAGGAGC	ATGCCGTGTTT	GAGTGTCACT
R. vinicolor(T17899)	CACCTTGCGC	TCCTCGGTGT	TCCGAGGAGC	ATGCCGTGTTT	GAGTGTCACT
R. vinicolor(T20787)	CACCTTGCGC	TCCTCGGTGT	TCCGAGGAGC	ATGCCGTGTTT	GAGTGTCACT
R. ochraceisorus (PT)	CACCTTGCGC	TCCTCGGTGT	TCCGAGGAGC	ATGCCGTGTTY	GAGTSTCACT
R. ochraceisorus (17916)	CACCTTGCGC	TCCTCGGTGT	TCCGAGGAGC	ATGCCGTGTTT	GAGTGTCACT
R. ochraceisorus (17944)	CACCTTGCGC	TCCTCGGTGT	TCCGAGGAGC	ATGCCGTGTTT	GAGTGTCACT
R. subcaerulescens	CACCTTGCGC	TCCTCGGTGT	TCCGAGGAGC	ATGCCGTGTTT	GAGTGTCACT
R. ellenae (HT)	CACCTTGCGC	TCCTCGGTGT	TCCGAGGAGC	ATGCCGTGTTT	GAGTGTCACT
R. ellenae	CACCTTGCGC	TCCTCGGTGT	TCCGAGGAGC	ATGCCGTGTTT	GAGTGTCACT
R. subpurpurascens (PT)	CACCTTGCGC	TCCTCGGTGT	TCCGAGGAGC	ATGCCGTGTTT	GAGTGTCACT
R. subpurpurascens	CACCTTGCGC	TCCTCGGTGT	TCCGAGGAGC	ATGCCGTGTTT	GAGTGTCACT
R. subgelatinosus	CACCTTGCGC	TCCTCGGTGT	TCCGAGGAGC	ATGCCGTGTTT	GAGTGTCACT
R. semireticulatus (17562)	CACCTTGCGC	TCCTCGGTGT	TCCGAGGAGC	ATGCCGTGTTT	GAGTGTCACT
R. semireticulatus (7899)	CACCTTGCGC	TCCTCGGTGT	TCCGAGGAGC	ATGCCGTGTTT	GAGTGTCACT

	*5.8S end; start ITS 2	620	630	640	650
<i>Suillus brevipes</i>	AAATTCTCA	ACCCCTCTCG	ATT-TGCTTC	GAAAGGGCGC	TTGGATG-TT
<i>S. luteus</i>	AAATTCTCA	ACCCCTCTCG	ATT-TGCTTC	GAGCGGGTGC	TTGGATG-TT
<i>S. tomentosus</i>	AAATTCTCA	ACCCCTCTCG	ATT-NNCTTC	GAGAGGGTGC	TTGGATA-GT
<i>S. americanus</i>	AAATTCTCA	ACCCCTCTCG	ATT-TGCTTC	GAGTGGGG-C	TTGGATA-GT
<i>S. granulatus</i>	AAATTCTCA	ACCCCTCTCG	ATT-TGCTTC	GAGAGGGCGC	TTGGATG-CT
<i>S. grevillei</i>	AAATTCTCA	ACCCCTCTCG	ATT-AGCTTC	GAAAGGGCGC	TTGGATA-GT
<i>S. cavipes</i>	AAATTCTCA	ACCCCTCTCG	ATT-TGCTTC	GAGCGGGTGC	TTGGATG-TT
<i>S. caerulescens</i>	AAATTCTCA	ACTCCTCTCG	ATT-TGTTTT	GAGCGGACGT	TTGGATA-GT
<i>S. lakei</i>	AAATTCTCA	ACCCCTCTCG	ATT-TGTTTT	GAGCGGGCGT	TTGGATA-GT
<i>S. sinuspaulinus</i>	AAATTCTCA	ACCCCTCTCG	ATT-TTCTTC	GACTGGGAGT	TTGGATA-GT
<i>Chroogomphus</i>	AAATTCTCA	ACCCCTCTTG	ATT-TGCTTC	AAGGGGGAGC	TTGGATA-GT
<i>Gomphidius</i>	AAATTCTCA	ACCCCTCTCG	ATT-TACTTC	GAGGGGGAGC	TTGGATGGGT
<i>Truncocolumella</i>	AAATTCTCA	ACCCCTCTCG	ATT-TGCTTC	GAGAGGGTGC	TTGGATA-GT
<i>Rhizopogon evadens</i>	TAATTCA	ACCCCTCTCG	ATT-AGCTTC	GAGGGGGAGC	TTGGATA-GT
<i>R. evadens</i> (HT)	TAATTCA	ACCCCTCTCG	ATT-AGCTTC	GAGAGGGAGC	TTGGATA-GT
<i>R. smithii</i>	TAATTCA	ACCCCTCTCG	ATT-AGGTTT	GAGGGGGAGC	TTGGATA-GT
<i>R. subsalmonius</i>	-AATTCA	ACCCCTCTCG	ATT-AGCTTC	GAGAGGGAGT	TTGGATA-GT
<i>R. fuscorubens</i>	AATCTCA	ACCCCTCTCG	ATT-AGCTTC	GAGGGGGAGC	TTGGATA-GT
<i>R. ochraceorubens</i> (TT)	AAATTCTCA	ACCCCTCTTG	ATT-AGCTTC	GAGGGGGAGT	TTGGATA-GT
<i>R. ochraceorubens</i> (HT)	AAATTCTCA	ACCCCTCTTG	ATT-AGCTTC	GAGGGGGAGT	TTGGATA-GT
<i>R. occidentalis</i>	AAATTCTCA	ACCCCTCTTG	ATT-AGCTTC	GAGGGGGAGT	TTGGATA-GT
<i>R. occidentalis</i>	AAATTCTCA	ACCCCTCTCG	ATT-AGCTTC	GAGGGGGAGT	TTGGATA-GT
<i>R. succosus</i>	AAATTCTCA	ACCCCTTTCA	ATT-AACTTT	GAAATGGGAGC	TTGGATA-GT
<i>R. luteolus</i>	AAATTCTCA	ACCCCTTTCA	ATT-AACTTT	GAAATGGGAGC	TTGGATA-GT
<i>R. burlinghamii</i>	AAATTCTCA	ACCCCTCTCG	ATT-CGTTTT	GAGGGGGAGC	TTGGATG-GT
<i>R. rubescens</i>	AAATTCTCA	ACCCCTCTCG	ATT-TGTTTT	GAGGGGGAGC	TTGGATA-GT
<i>R. roseolus</i>	AAATTCTCA	ACCCCTCTTG	ATTTTTTTTT	GAGGGGGAGC	TTGGATG-GT
<i>R. vulgaris</i>	AAATTCTCA	ACCCCTCTCG	ATT-TTTTTT	GAGGGGGAGT	TTGGATG-GT
<i>R. colossus</i> (HT)	AAATTCTCA	ACCCCTCTTG	ATT-----I-	GAGGGAG-IT	TTGGATA-GT
<i>R. hawkeriae</i> (PT)	AAATTCTCA	ACCCCTCTTG	ATT-----I-	GAGGGAG-IT	TTGGATA-GT
<i>R. parksii</i> (T17679)	AAATTCTCA	ACCCCTCTTG	ATT-----I-	GAGGGAG-IT	TTGGATA-GT
<i>R. parksii</i> (T19446)	AAATTCTCA	ACCCCTCTTG	ATT-----I-	GAGGGAG-IT	TTGGATA-GT
<i>R. villosulus</i> (AHS)	AAATTCTCA	ACCCCTCTTG	ATT-----I-	GAGGGAG-IT	TTGGATA-GT
<i>R. villosulus</i> (JMT)	AAATTCTCA	ACCCCTCTTG	ATT-----I-	GAGGGAG-IT	TTGGATA-GT
<i>R. rogersii</i>	AAATTCTCA	ACCCCTCTTG	ATT-----I-	GAGGGAG-IC	TTGGATA-GT
<i>R. sp. nov</i>	AAATTCTCA	ACCCCTCTTG	ATT-----I-	GAGGGAG-IT	TTGGATA-GT
<i>R. gilkeyae</i>	AAATTCTCA	ACCCCTCTTG	ATTATATTTT	GAGGGAG-IT	TTGGATA-GT
<i>R. zelleri</i>	AAATTCTCA	ACCCCTCTTG	ATT-----I-	GAGGGAG-IT	TTGGATA-GT
<i>R. villescens</i>	AAATTCTCA	ACCCCTCTTG	ATT-----I-	GAGGGAG-IT	TTGGATA-GT
<i>R. diabolicus</i>	AAAKTCTCA	ACCCCTCTTG	AGT-----I-	GAGGGAG-IT	TTGGATA-GT
<i>R. parvulus</i>	AAATTCTCA	ACCCCTCTTG	ATT-----I-	GAGGGAG-IT	TTGGATA-GT
<i>R. vinicolor</i> (T17899)	AAATTCTCA	ACCCCTCTTG	ATT-----I-	GAGGGAG-IT	TTGGATA-GT
<i>R. vinicolor</i> (T20787)	AAATTCTCA	ACCCCTCTTG	ATT-----I-	GAGGGAG-IT	TTGGATA-GT
<i>R. ochraceisporus</i> (PT)	AAATTCTCA	ACCCCTCTTG	ATT-----I-	GAGGGAG-IT	TTGGATA-GT
<i>R. ochraceisporus</i> (17916)	AAATTCTCA	ACCCCTCTTG	ATT-----I-	GAGGGAG-IT	TTGGATA-GT
<i>R. ochraceisporus</i> (17944)	AAATTCTCA	ACCCCTCTTG	ATT-----I-	GAGGGAG-IT	TTGGATA-GT
<i>R. subcaerulescens</i>	AAATTCTCA	ACCCCTCTCG	ATT-AGCTTC	GAGAGGGCGC	TTGGATA-GT
<i>R. ellenae</i> (HT)	AAATTCTCA	ACCCCTCTCG	ATT-AGCTTC	GAGAGGGTGC	TTGGATA-GT
<i>R. ellenae</i>	AAATTCTCA	ACCCCTCTCG	ATT-AGCTTC	GAGAGGGTGC	TTGGATA-GT
<i>R. subpurpurascens</i> (PT)	AAATTCTCA	ACCCCTCTCG	ATT-AGCTTC	GAGAGGGCGC	TTGGATA-GT
<i>R. subpurpurascens</i>	AAATTCTCA	ACCCCTCTCG	ATT-AGCTTC	GAGAGGGCGC	TTGGATA-GT
<i>R. subgelatinosus</i>	AAATTCTCA	ACCCCTCTCG	ATT-AGCTTC	GAGAGGGCGC	TTGGATA-GT
<i>R. semireticulatus</i> (17562)	AAATTCTCA	GCCCCCTCTCG	ATT-AGCTTC	GAGAGGGTGC	TTGGATA-GT
<i>R. semireticulatus</i> (7899)	AAATTCTCA	ACCCCTCTCG	ATT-AGCTTC	GAGAGGGCGC	TTGGATA-GT

	660	670	680	690	700
<i>Suillus brevipes</i>	GGGGGC T -GC	CGGAGACAC T	GGAC TC ----	-----	---- GTCCA
<i>S. luteus</i>	GGGGGC T -GC	CGGAGACAC T	GGAT TC ----	-----	---- GTCCA
<i>S. tomentosus</i>	GGAGGC T -GC	CGGAGAC CT -	G TTTTT ----	-----	----- TCA
<i>S. americanus</i>	GGGGGC T -GC	CGGAGAC CT -	GGA ATT ----	-----	--- CATTC TG
<i>S. granulatus</i>	GGGGGC T -GC	CGGAGAC CT -	GG TTTC ----	-----	---- GTCCA
<i>S. grevillei</i>	GGGGGC T -GC	CGGAGAT CT -	GGAC TT ----	-----	-- TTCGT CTG
<i>S. cavipes</i>	GGGGGC T -GC	CGGAGAC TT -	GGAT TC ----	-----	---- GTCCA
<i>S. caerulescens</i>	GGGGGC T -GC	CGGAGAC CT -	GGAT TT ----	-----	--- ATTTCA
<i>S. lakei</i>	GGGGGC T -GC	CGGAGAC CT -	GRAT TT ----	-----	--- ATTT CG
<i>S. sinuspaulinus</i>	GGGGGC T -GC	CGGAGAC TC -	GAAT TC ----	-----	--- ATT - CG
<i>Chroogomphus</i>	GGGGGC T -GC	CAGAGAC TT -	GGAT TT ----	-----	-- GTG TCCA
<i>Gomphidius</i>	GGGGG TT -GC	CGGAG-----	-----	-----	----- TCCA
<i>Truncocolumella</i>	GGGGGC T -GC	CGGAG-----	-----	-----	----- TC TCA
<i>Rhizopogon evadens</i>	GGGGG TT -GC	CGGAGAC TTG	GGAC-----	-----	-- TAG TTCCA
<i>R. evadens</i> (HT)	GGGGG TT -GC	CGGAGAC TTG	GGAC-----	-----	-- TAG TTCCA
<i>R. smithii</i>	GGGGG TT -GC	CGGAGAC TTG	GGAC T -----	--- ACTAG -	-- TAG TTCCA
<i>R. subsalmonius</i>	GGGGG TT -GC	CGGAG TCTT -	-----	-----	----- A
<i>R. fuscorubens</i>	GGGGG TTTGC	AGGAAAC TT -	GGAC TTTT --	--- CATTAG -	----- TCTA
<i>R. ochraceorubens</i> (TT)	GGGGG TTTGC	AGGAAAC TTG	GG-C-----	TTTC - TAG -	TTAAAGTCTA
<i>R. ochraceorubens</i> (HT)	GGGGG TTTGC	AGGAAAC TTG	GG-C-----	TTTC - TAG -	TTAAAGTCTA
<i>R. occidentalis</i>	GGGGG TTTGC	AGGAAAC TTG	GG-C TTTGGC	TTTC - TAG -	TTAAAGTCTA
<i>R. occidentalis</i>	GGGGG TTTGC	AGGAAAC TTG	GG-C TTTGGC	TTTC - TAG -	TTCAAGTCTA
<i>R. succosus</i>	GGGGG TTTGC	AGGAAAC TTG	GATC TTTTTC	TTTCATTAGA	AAAAAGTCTA
<i>R. luteolus</i>	GGGGG TTTGC	AGGAAAC TTG	GATAT TTT -C	TTTAAC TAG -	AAAAAGTCTA
<i>R. burlinghamii</i>	GGGGGC T -GC	CGGAGAA T -A	GGAT TC ----	-----	---- GTCC T
<i>R. rubescens</i>	GGGGGC T -GC	CGGAGAC T -A	GGAT TC ----	-----	---- GTCC T
<i>R. roseolus</i>	GGGGGC T -GC	CGGAGAC T -G	GGAT TT ----	-----	---- GTCC T
<i>R. vulgaris</i>	GGGGGC T -GC	CGGAGAC T -A	GGAC TC ----	-----	---- GTCC T
<i>R. colossus</i> (HT)	GGGG S TTTGC	CGGAGAC TT -	--- TTT ----	-----	-----
<i>R. hawkeriae</i> (PT)	GGGG S TT -GC	CGGAGAC TT -	-----	-----	----- A
<i>R. parksii</i> (T17679)	GGGGGC T -GC	CGGAGAC TT -	GGAT TC ----	-----	---- GTCTA
<i>R. parksii</i> (T19446)	GGGGGC T -GC	CGGAGAC TT -	GGAT TC ----	-----	---- GTCTA
<i>R. villosulus</i> (AHS)	GGGGG TT -GC	CGGAGAC TT -	-----	-----	----- A
<i>R. villosulus</i> (JMT)	GGGGG TTTGC	CGGAGAC TT -	--- TTT ----	-----	-----
<i>R. rogersii</i>	GGGGG TT -GC	CGGAGAC TT -	-----	-----	----- A
<i>R. sp. nov</i>	GGGGG TT -GC	CGGAGAC TT -	GGAT TC ----	-----	---- GTCTA
<i>R. gilkeyae</i>	GGGGG TT -GC	CGGAGAC TAA	-----	-----	----- TA
<i>R. zelleri</i>	GGGGG TT -GC	CGGAGAC TT -	-----	-----	----- A
<i>R. villescens</i>	GGGAG TT -GC	TGGAGAC TT -	-----	-----	----- A
<i>R. diabolicus</i>	GGGGG TT -GC	TGGAGAC TT -	--- TTA ----	-----	----- TT
<i>R. parvulus</i>	GGGGG TT -GC	TGGAGAC TT -	--- TTA ----	-----	----- TT
<i>R. vinicolor</i> (T17899)	GGGGG TT -GC	TGGAGAC TT -	--- TTA ----	-----	----- TT
<i>R. vinicolor</i> (T20787)	GGGGG TT -GC	TGGAGAC TT -	--- TTA ----	-----	----- TT
<i>R. ochraceisporus</i> (PT)	GGGGG TT -GC	TGGAGAC TT -	--- TTA ----	-----	----- TT
<i>R. ochraceisporus</i> (17916)	GGGGG TT -GC	TGGAGAC TT -	--- TTA ----	-----	----- TT
<i>R. ochraceisporus</i> (17944)	GGGGG TT -GC	TGGAGAC TT -	--- TTA ----	-----	----- TT
<i>R. subcaerulescens</i>	GGAGG TT -GC	CGGAGAC TT -	GGAT TC ----	-----	---- GTCCG
<i>R. ellenae</i> (HT)	GGAGG TT -GC	CGGAGAC TC -	GGAT TC ----	-----	---- GTCCA
<i>R. ellenae</i>	GGAGG TT -GC	CGGAGAC TC -	GGAT TC ----	-----	---- GTCCA
<i>R. subpurpurascens</i> (PT)	GGAGG TT -GC	CGGAGAC TT -	GGAT TC ----	-----	---- GTCCA
<i>R. subpurpurascens</i>	GGAGG TT -GC	CGGAGAC TT -	GGAT TC ----	-----	---- GTCCA
<i>R. subgelatinosus</i>	GGAGG YT -GC	CGGAGAC TT -	GGAT TC ----	-----	---- GTCCG
<i>R. semireticulatus</i> (17562)	GGAGG TT -GC	CGGAGAC TC -	GGAT TC ----	-----	---- GTCCA
<i>R. semireticulatus</i> (7899)	GGAGG YT -GC	CGGAGAC TT -	GGAT TC ----	-----	---- GTCCG

	710	720	730	740	750
<i>Suillus brevipes</i>	GGACTCGGGC	TCCTCTTAAA	TGAATCGGCT	TGCGGITCGA	CTTTCGACTT
<i>S. luteus</i>	GGACTCGGGC	TCCTCTTAAA	TGAATCGGCT	CGCGGITCGA	CTTTCGACTT
<i>S. tomentosus</i>	GGACTCGGGC	TCCTCTGAAA	TGTATTGGCT	TGCGGITCGA	CTTTCGACTG
<i>S. americanus</i>	GGACTCGGGC	TCCTCTGAAA	TGCATCGGCT	TGCGGITCGA	CTTTCGACTT
<i>S. granulatus</i>	GGACTCGGGC	TCCTCTGAAA	TGTATCGGCT	TGCGGITCGA	CCTTCGACTT
<i>S. grevillei</i>	GGACTCGGGC	TCCTCTGAAA	TGAATGGGCT	TGCGGITCGG	CTTTCGACTA
<i>S. cavipes</i>	GGACTCGGGC	TCCTCTTAAA	TGAATCGGCT	TGCGGITCGA	CTTTCGACTT
<i>S. caerulescens</i>	GGACTCGGGC	TCCTCTGAAA	TGCATCGGCT	TGCGGITCGA	CTTTCGACTT
<i>S. lakei</i>	GGACTCGGGC	TCCTCTGAAA	TGCATTGGCT	TGCRGITTTGA	CTTTCGACTT
<i>S. sinuspaulinus</i>	AGACTCGGGC	TCCTCTGAAA	TGCATCGGCT	TGCGGITCGA	CTTTCGACTT
<i>Chroogomphus</i>	GGACTTGGGC	TCCTCTGAAA	TGCATCGGCT	TGCGAITTTGA	CTTTCGACTT
<i>Gomphidius</i>	GGACTCGGGC	TCCTCTGAAA	TGCATTGGCT	TGCGGITCGA	CTTTCTCCTT
<i>Truncocolumella</i>	GGACTCGGGC	TCCTCTGAAA	TGCATTGGCT	TGCGGITCGA	TTTTTCGACTT
<i>Rhizopogon evadens</i>	GGACTCGGGC	TCCTCTGAAA	TGCATCGGCT	TGCGGITCGA	CTTTCGACTT
<i>R. evadens</i> (HT)	GGACTCGGGC	TCCTCTGAAA	TGCATTGGCT	TGCGGITAGA	CTTTCGAGTT
<i>R. smithii</i>	GGACTCGGGC	TCCTCTTAAA	TGCATTGGCT	TGCGGITAGA	CTTTCGAGTT
<i>R. subsalmonius</i>	GGACTCGGGC	TCCTCTGAAA	TGCATTGGCT	TGCAGITCGA	CTTTCGACTT
<i>R. fuscorubens</i>	GGATTTCGAGC	TCCTCTGAAA	TACATTGGCT	TGCGGITCGA	CTTTCGACTT
<i>R. ochraceorubens</i> (TT)	GGATTTCGAGC	TCCTCTGAAA	TACATTGGCT	TTTCGGITCGA	CTTTCGACTT
<i>R. ochraceorubens</i> (HT)	GGATTTCGAGC	TCCTCTGAAA	TACATTGGCT	TTTCGGITCGA	CTTTCGACTT
<i>R. occidentalis</i>	GGATTTCGAGC	TCCTCTGAAA	TGCATTGGCT	TTTCGGITCGA	CTTTCGACTT
<i>R. occidentalis</i>	GGATTTCGAGC	TCCTCTGAAA	TACATTGGCT	TTTCGGITCGA	CTTTCGACTT
<i>R. succosus</i>	GGATTTCGAGC	TCCTCTGAAA	TACATTGGCT	TGCGGGTCTA	CTTTCGACTT
<i>R. luteolus</i>	GGATTTCGAGC	TCCTCTGAAA	TACATTGGCT	TGCGGGTCTA	CTTTCGACTT
<i>R. burlinghamii</i>	TGACTTCGGGC	TCCTCTTAAA	TGCATCGGCT	TGCGGITCGA	CTTTCGACTT
<i>R. rubescens</i>	TGACTTCGGGC	TCCTCTTAAA	TGCATCGGCT	TGCGGITCGA	CTTTCGACTT
<i>R. roseolus</i>	TGACTTCGGGC	TCCTCTTAAA	TGCATTGGCT	TGCGGITCGA	CTTTCGACTT
<i>R. vulgaris</i>	TGACTTCGGGC	TCCTCTTAAA	TGCATTGGCT	TGCGGITCGA	CTTTCGACTT
<i>R. colossus</i> (HT)	-GACTTCGGGC	TCCTCTGAAA	TGCATCGGCT	TGCGGITCGA	CTTTCGACTA
<i>R. hawkeriae</i> (PT)	GGACTTCGGGC	TCCTCTGAAA	TGCATCGGCT	TGCGGITCGA	CTTTTGACTA
<i>R. parksii</i> (T17679)	GGACTTCGGGC	TCCTCTGAAA	TGCATCGGCT	TGCGGITCGA	CTTTCGACTA
<i>R. parksii</i> (T19446)	GGACTTCGGGC	TCCTCTGAAA	TGCATCGGCT	TGCGGITCGA	CTTTCGACTA
<i>R. villosulus</i> (AHS)	GGACTTCGGGC	TCCTCTGAAA	TGCATCGGCT	TGCGGITCGA	CTTTYGACTA
<i>R. villosulus</i> (JMT)	-GACTTCGGGC	TCCTCTGAAA	TGCATCGGCT	TGCGGITCGA	CTTTCGACTA
<i>R. rogersii</i>	GGACTTCGGGC	TCCTCTGAAA	TGCATCGGCT	TGCGGITCGA	CTTTCGACTA
<i>R. sp. nov</i>	GGACTTCGGGC	TCCTCTGAAA	TGCATCGGCT	TGCGGITCGA	CTTTCGACTT
<i>R. gilkeyae</i>	AGACTTCGGGC	TCCTCTGAAA	TGCATTGGCT	TGCGAITAGA	CTTTCGACTT
<i>R. zelleri</i>	GGACTTCGGGC	TCCTCTGAAA	TGCATCGGCT	TGCGGITCGA	CTTTCGACTA
<i>R. villescens</i>	GGACTTCGGGC	TCCTCTGAAA	TGCATCGGCT	TGCGGITCGA	CTTTCGACTA
<i>R. diabolicus</i>	AGACTTCGAGC	TCCTCTGAAA	TGCATCGGCT	TGCGGITCGA	CTTTCGACTT
<i>R. parvulus</i>	AGACTTCGAGC	TCCTCTGAAA	TGCATCGGCT	TGCGGITCGA	CTTTCGACTT
<i>R. vinicolor</i> (T17899)	AGACTTCGAGC	TCCTCTGAAA	TGCATCGGCT	TGCGGITCGA	CTTTCGACTT
<i>R. vinicolor</i> (T20787)	AGACTTCGAGC	TCCTCTGAAA	TGCATCGGCT	TGCGGITCGA	CTTTCGACTT
<i>R. ochraceisporus</i> (PT)	AGACTTCGAGC	TCCTCTGAAA	TGCATCGGCT	TGCGGITCGA	CTTTCGACTT
<i>R. ochraceisporus</i> (17916)	AGACTTCGAGC	TCCTCTGAAA	TGCATCGGCT	TGCGGITCGA	CTTTCGACTT
<i>R. ochraceisporus</i> (17944)	AGACTTCGAGC	TCCTCTGAAA	TGCATCGGCT	TGCGGITCGA	CTTTCGACTT
<i>R. subcaerulescens</i>	AGACTTCGGGC	CTTCTGAAA	TGCATCGGCT	TGCGGITCGA	CTTTCGACTA
<i>R. ellenae</i> (HT)	GGACTTCGGGC	CTTCTGAAA	TGCATTGGCT	TGCGGITCGA	CTTTCGACTA
<i>R. ellenae</i>	GGACTTCGGGC	CTTCTGAAA	TGCATTGGCT	TGCGGITCGA	CTTTCGACTA
<i>R. subpurpurascens</i> (PT)	GGACTTCGGGC	CTTCTGAAA	TGCATCGGCT	TGCGGITCGA	CTTTCGACTA
<i>R. subpurpurascens</i>	GGACTTCGGGC	CTTCTGAAA	TGCATCGGCT	TGCGGITCGA	CTTTCGACTA
<i>R. subgelatinosus</i>	AGACTTCGGGC	CTTCTGAAA	TGCATCGGCT	TGCGGITCGA	CTTTCGACTA
<i>R. semireticulatus</i> (17562)	GGACTTCGGGC	CTTCTGAAA	TGCATTGGCT	TGCGGITCGR	CTTTCGACTA
<i>R. semireticulatus</i> (7899)	AGACTTCGGGC	CTTCTGAAA	TGCATCGGCT	TGCGGITCGA	CTTTCGACTA

	760	770	780	790	800
<i>Suillus brevipes</i>	TGCATGACAA	GGCCTTTGGC	GTGATAATGA	TCGCCGTTCTG	CCGAAGTGCA
<i>S. luteus</i>	TGCATGACAA	GGCCTTTGGC	GTGATAATGA	TCGCCGTTCTG	CCGAAGTGCA
<i>S. tomentosus</i>	TGCATGACAA	GGCCTTTGGC	GTGATAATGA	TCGCCGTTCTG	CCGAAGTGCA
<i>S. americanus</i>	TGCGCGACAA	AGCTTTTCGGC	GTGATAATGA	TCGCCGTTCTG	CTGAAGCGCA
<i>S. granulatus</i>	TGCGCGACAA	GGCCTTTGGC	GTGATAATGA	TCGCCGTTCTG	CCGAAGCGCA
<i>S. grevillei</i>	TGCATGACAA	GGCTTTTGGC	GTGATAATGA	TCGCCGTTCTG	CCGAAGTGCA
<i>S. cavipes</i>	TGCGCGACAA	GGCTTTTCGGC	GTGATAATGA	TCGCCGTTCTG	CTGAAGCGCA
<i>S. caerulescens</i>	TGCATGACAA	GGCCTTTCGGC	GTGATAATGA	TCGTCGTCGG	CCGAAGTGCA
<i>S. lakei</i>	TGCATGACAA	GGCCTTTCGGC	GTGATAATGA	TCGTCGTTAG	CTGAAGTGCA
<i>S. sinuspaulinus</i>	TGCACGACAA	GGCCTTTCGGC	GTGATAATGA	TCGCCGTTCTG	CCGAAGTGCG
<i>Chroogomphus</i>	TGCACGACAA	GGCTTTTCGGT	GTGATAATGA	TCGCCGTTCTC	CCGAAGCGCA
<i>Gomphidius</i>	TGCGCGACAA	GGCTTTTCGGT	GTGATGATGA	TCGCCGTTCTG	CCGAAGCGCA
<i>Truncocolumella</i>	TGCGCGACAA	GGCTTTTCGGC	GTGATAATGA	TCGCCGTTCTG	CCGAAGCGCA
<i>Rhizopogon evadens</i>	TGCGCGAAAA	GGCTTTTCGGC	GTGATAATGA	TCGCCGTTGTG	CTGAAGTGCA
<i>R. evadens</i> (HT)	TGCGGGAAAA	GGCTTTTCGGC	GTGATAATGA	TCGCCGTTGTG	CTGAAGCGCA
<i>R. smithii</i>	TGCGGGAAAA	GGCTTTTCGGC	GTGATAATGA	TCGCCGTTGTG	CTGAAGCGCA
<i>R. subsalmonius</i>	TGCGCGAAAA	GGCTTTTCGGC	GTGATAATGA	TCGCCGTTGTG	CTGAAGTGCA
<i>R. fuscorubens</i>	TGCGCGATAA	GGCTTTTCGGC	GTGATAATGA	TCGCCGTTGTG	CTGAAGCGCA
<i>R. ochraceorubens</i> (TT)	TGCGTGACAA	GGCTTTTCGGC	GTGATAATGA	TCGCCGTTGTG	CTGAAGCGCA
<i>R. ochraceorubens</i> (HT)	TGCGTGACAA	GGCTTTTCGGC	GTGATAATGA	TCGCCGTTGTG	CTGAAGCGCA
<i>R. occidentalis</i>	TGCGCGACAA	GGCTTTTCGGC	GTGATAATGA	TCGCCGTTGTG	CTGAAGCGCA
<i>R. occidentalis</i>	TGCGCGACAA	GGCTTTTCGGC	GTGATAATGA	TCGCCGTTGTG	CTGAAGCGCA
<i>R. succosus</i>	TGTGTGATAA	GGCTTTTCGGT	GTGATAATGA	TCACCGTTGTG	CTGAAGTGCA
<i>R. luteolus</i>	TGTGCGATAA	GGCTTTTCGGT	GTGATAATGA	TCACCGTTGTG	CTGAAGTGCA
<i>R. burlinghamii</i>	TGCGCGACAA	GGCTTTTCGGC	GTGATAATGA	TCGCCGTTCTG	CTGAAGCGCA
<i>R. rubescens</i>	TGCGCGACAA	GGCTTTTCGGC	GTGATAATGA	TCGCCGTTCTG	CTGAAGCGCA
<i>R. roseolus</i>	TGCGCGACAA	GGCTTTTCGGC	GTGATAATGA	TCGCCGTTCTG	CTGAAGCGCA
<i>R. vulgaris</i>	TGCGCGACAA	GGCTTTTCGGC	GTGATAATGA	TCGCCGTTCTG	CTGAAGCGCA
<i>R. colossus</i> (HT)	TGCGCGACAA	GGCTTTTCGGC	GTGATAATGA	TCGCCGTTCTG	CTGAAGTGTA
<i>R. hawkeriae</i> (PT)	TGCGCGACAA	GGCTTTTCGGC	GTGATAATGA	TCGCCGTTCTG	CTGAAGTGCA
<i>R. parksii</i> (T17679)	TGCGCGACAA	GGCTTTTCGGC	GTGATAATGA	TCGCCGTTCTG	CTGAAGTGCA
<i>R. parksii</i> (T19446)	TGCGCGACAA	GGCTTTTCGGC	GTGATAATGA	TCGCCGTTCTG	CTGAAGTGCA
<i>R. villosulus</i> (AHS)	TGCGCGACAA	GGCTTTTCGGC	GTGATAATGA	TCGCCGTTCTG	CTGAAGYGCA
<i>R. villosulus</i> (JMT)	TGCGCGACAA	GGCTTTTCGGC	GTGATAATGA	TCGCCGTTCTG	CTGAAGTGTA
<i>R. rogersii</i>	TGCGCGACAA	GGCTTTTCGGC	GTGATAATGA	TCGCCGTTCTG	CTGAAGCGCA
<i>R. sp. nov</i>	TGCGCGACAA	GGCTTTTCGGC	GTGATAATGA	TCGCCGTTCTG	CTGAAGCGCA
<i>R. gilkeyae</i>	TGCGCGACAA	GGCTTTTCGGC	GTGATAATGA	TCGCCGTTCTG	CTGAAGCGCA
<i>R. zelleri</i>	TGCGCGACAA	GGCTTTTCGGC	GTGATAATGA	TCGCCGTTCTG	CTGAAGTGCA
<i>R. villescens</i>	TGCGCGACAA	GGCTTTTCGGC	GTGATAATGA	TCGCCGTTCTG	CTGAAGCGCA
<i>R. diabolicus</i>	TGCGCGACAA	GGCTTTTCGGC	GTGATAATGA	TCGCCGTTCTG	CTGAAGCGCA
<i>R. parvulus</i>	TGCGCGACAA	GGCTTTTCGGC	GTGATAATGA	TCGCCGTTCTG	CTGAAGCGCA
<i>R. vinicolor</i> (T17899)	TGCGCGACAA	GGCTTTTCGGC	GTGATAATGA	TCGCCGTTCTG	CTGAAGCGCA
<i>R. vinicolor</i> (T20787)	TGCGCGACAA	GGCTTTTCGGC	GTGATAATGA	TCGCCGTTCTG	CTGAAGCGCA
<i>R. ochraceisporus</i> (PT)	TGCGCGACAA	GGCTTTTCGGC	GTGATAATGA	TCGCCGTTCTG	CTGAAGCGCA
<i>R. ochraceisporus</i> (17916)	TGCGCGACAA	GGCTTTTCGGC	GTGATAATGA	TCGCCGTTCTG	CTGAAGCGCA
<i>R. ochraceisporus</i> (17944)	TGCGCGACAA	GGCTTTTCGGC	GTGATAATGA	TCGCCGTTCTG	CTGAAGCGCA
<i>R. subcaerulescens</i>	TGTGCGACAA	GGCTTTTCGGC	GTGATAATGA	TCGCCGTTCTG	CTGAAGCGCA
<i>R. ellenae</i> (HT)	TGCTCGACAA	GGCTTTTCGGC	GTGATAATGA	TCGCCGTTCTG	CCGAAGCGCA
<i>R. ellenae</i>	TGCTCGACAA	GGCTTTTCGGC	GTGATAATGA	TCGCCGTTCTG	CCGAAGCGCA
<i>R. subpurpurascens</i> (PT)	TGCTCGACAA	GGCTTTTCGGC	GTGATAATGA	TCGCCGTTCTG	CTGAAGCGCA
<i>R. subpurpurascens</i>	TGCGCGACAA	GGCTTTTCGGC	GTGATAATGA	TCGCCGTTCTG	CTGAAGCGCA
<i>R. subgelatinosus</i>	TGTGCGACAA	GGCTTTTCGGC	GTGATAATGA	TCGCCGTTCTG	CTGAAGCGCA
<i>R. semireticulatus</i> (17562)	TGCTCGACAA	GGCTTTTCGGC	GTGATAATGA	TCGCCGTTCTG	CCGAAGCGCA
<i>R. semireticulatus</i> (7899)	TGTGCGACAA	GGCTTTTCGGC	GTGATAATGA	TCGCCGTTCTG	CTGAAGCGCA

	810	820	830	840	850
<i>Suillus brevipes</i>	AGACAGAACG	GTCCCGTGCC	TCTAAYGC--	-I----GTCG	ACGC-----
<i>S. luteus</i>	AGACAGAATG	GTCCCGTGCC	TCTAATGC--	-I----GTCG	ACGC-----
<i>S. tomentosus</i>	CGAACGAATG	GTCTCGTGCC	TCTAATCA--	-I----GTCG	ATGC-----
<i>S. americanus</i>	TCAATGAATG	GTCCCGTGCC	TCTAATCA--	-I----GTCG	ACGC-----
<i>S. granulatus</i>	TGAATGAACC	GTCCCGCGCC	TCCAATCC--	-I----GTCG	ACGC-----
<i>S. grevillei</i>	TGAATGAACG	GTCCCGTGCC	TCTAATGC--	-I----GTCG	ATGC-----
<i>S. cavipes</i>	TGAATGAA-G	GTCTCGCGCC	TCTAANAC--	-I----GTCG	ACCC-----
<i>S. caerulescens</i>	CGAATGAACC	GTCCCGCGCC	TCTAATAC--	-I----GTCG	ACGC-----
<i>S. lakei</i>	TGAATGAACC	GTCTCGCGCC	TCTAATAC--	-I----GTCG	ACGC-----
<i>S. sinuspaulinus</i>	CGAATGAAAC	GTCCCGCGCC	TCTAATGC--	-I----GTCG	ACCC-----
<i>Chroogomphus</i>	TGA-TAGAAG	GTCTCGTGCC	TCCAACAT--	-I----GTCG	ACGATG----
<i>Gomphidius</i>	TGAACG-AAG	GTCTCGTGCC	TCCAATAC--	-I----GTCG	ACCC-----
<i>Truncocolumella</i>	TGAATG-AAC	GTCCCGTGCC	TCTAATAA--	-I----GTCG	ACTGGTACCT
<i>Rhizopogon evadens</i>	TGAATGA-TG	GTTCCGTGCC	TYTAATA--C	GCAT--ICG	ACC-----
<i>R. evadens</i> (HT)	TGAATGAAAG	GATCTGTGCC	TCTAATA--C	ACAT--ING	ACTCG-----
<i>R. smithii</i>	TGAATGAAAG	GTTCCGTGCC	TCTAATA--C	GCAT--ICG	ACTTGT-----
<i>R. subsalmonius</i>	TGAATGAA-G	GTCTGTGCC	TCTAATATAC	GCATGTGTCG	ACTGAT-----
<i>R. fuscorubens</i>	TGACTGTGAA	GTTCCGTGCC	TCTAATAC--	-I----GTCG	ACC-----
<i>R. ochraceorubens</i> (TT)	TGACTGTAAA	GTTCCGTGCC	TCTAATAT--	-I----GTCG	ACTGAC-----
<i>R. ochraceorubens</i> (HT)	TGACTGTAAA	GTTCCGTGCC	TCTAATAT--	-I----GTCG	ACTGAC-----
<i>R. occidentalis</i>	TGACTGTAAA	GTTCCGTGCC	TCTAATAT--	-I----GTCG	ACCGACGAC--
<i>R. occidentalis</i>	TGACTGTAAA	GTTCCGTGCC	TCTAATAT--	-I----GTCG	ACCGACGAC--
<i>R. succosus</i>	TAACTGTAAA	GTTCCGTGCC	TCTAATATT--	-I----GTCG	A-----
<i>R. luteolus</i>	TAACTGTAAA	GTTCCGTGCC	TCTAATATT--	-I----GTCG	A-----
<i>R. burlinghamii</i>	TGAATGAAAT	GTTCCGTGCC	TCTAATAC--	-I----GTCG	AC-----
<i>R. rubescens</i>	CGAATGAAAT	GTTCCGTGCC	TCTAATAC--	-I----GTCG	AC-----
<i>R. roseolus</i>	TGAATG-AAG	GTTCCGTGCC	TCTAATACT--	-I----GTCG	ACAG-----T
<i>R. vulgaris</i>	TGAACG-AAG	GTTCCGTGCC	TCTAATACC--	-I----GTCG	ACGCG-----T
<i>R. colossus</i> (HT)	TGAATG-AAG	GTTACGCGCC	TCTAATAT--	-I----GTCG	ACT-----CT
<i>R. hawkeriae</i> (PT)	TGAATG-AAA	GTTTCGCGCC	TCTAATAC--	-I----GTCG	ACT-----CT
<i>R. parksii</i> (T17679)	TGAATG-AAA	GTTTCGCGCC	TCTAATAC--	-I----GTCG	ACT-----CT
<i>R. parksii</i> (T19446)	TGAATG-AAA	GTTTCGCGCC	TCTAATAC--	-I----GTCG	ACT-----CT
<i>R. villosulus</i> (AHS)	TGAATG-AAR	GTTTCGCGCC	TCTAATAC--	-I----GTCG	ACT-----CT
<i>R. villosulus</i> (JMT)	TGAATG-AAG	GTTACGCGCC	TCTAATAT--	-I----GTCG	ACT-----CT
<i>R. rogersii</i>	TGAATG-AAG	GTTTCGCGCC	TCTAATAT--	-I----GTCG	ACT-----CT
<i>R. sp. nov</i>	TGAATG-AAG	GTTTCGCGCC	TCTAATAC--	-I----GTCG	ACT-----CT
<i>R. gilkeyae</i>	TGAATG-AAG	GTTTCGCGCC	TCTAATAT--	-I----GTCG	ACT-----CT
<i>R. zelleri</i>	TGAATG-AAG	GTTTCGCGCC	TCTAATAT--	-I----GTCG	ACA-----CT
<i>R. villescens</i>	TGAATG-AAG	GTTTCGCGCC	TCTAATAY--	-I----GTCG	ACN-----CT
<i>R. diabolicus</i>	TGACTG-AAG	GTTTCGCGCC	TCTAATAC--	-I----GTCG	ACT-----CT
<i>R. parvulus</i>	TGACTG-AAG	GTTTCGCGCC	TCTAATAC--	-I----GTCG	ACT-----CT
<i>R. vinicolor</i> (T17899)	TGACTG-AAG	GTTTCGCGCC	TCTAATAC--	-I----GTCG	ACT-----CT
<i>R. vinicolor</i> (T20787)	TGACCG-AAG	GTTTCGCGCC	TCTAATAC--	-I----GTCG	ACT-----CT
<i>R. ochraceisporus</i> (PT)	TGACTG-AAG	GTTTCGCGCC	TCTAATAC--	-I----GTCG	ACT-----CT
<i>R. ochraceisporus</i> (17916)	TGACTG-AAG	GTTTCGCGCC	TCTAATAC--	-I----GTCG	ACT-----CT
<i>R. ochraceisporus</i> (17944)	TGACTG-AAG	GTTTCGCGCC	TCTAATAC--	-I----GTCG	ACT-----CT
<i>R. subcaerulescens</i>	TGACTG-AAC	GTCCCGTGCC	TCTAATGT--	-I----GTCG	ACCG-----CT
<i>R. ellenae</i> (HT)	TGACCG-AAG	GTTCCGTGCC	TCTAATAC--	-I----GTCG	ACCA-----CT
<i>R. ellenae</i>	TGACCG-AAG	GTTCCGTGCC	TCTAATAC--	-I----GTCG	ACCA-----CT
<i>R. subpurpurascens</i> (PT)	TGACYG-AGG	GTTCCGTGCC	TCTAATAC--	-I----GTCG	ACCA-----CT
<i>R. subpurpurascens</i>	TGACTG-AGG	GTTCCGTGCC	TCTAATAC--	-I----GTCG	ACCA-----CT
<i>R. subgelatinosus</i>	TGACTG-AAC	GTCCCGTGCC	TCTAATAC--	-I----GTCG	ACCG-----CT
<i>R. semireticulatus</i> (17562)	TGACCG-AA-	GTTCCGTGCC	TCTAATAC--	-I----GTCG	ACCG-----CT
<i>R. semireticulatus</i> (7899)	TGACTG-AAC	GTCCCGTGCC	TCTAATAC--	-I----GTCG	ACCG-----CT

	860	870	880	890	900
<i>Suillus brevipes</i>	-----CT CTC-----	-----GGGGC	CGTCTTCCTC	A---TTGACG	
<i>S. luteus</i>	-----CT CT-----	-----AGGGG	CGTCTTCCTC	A---TTGACG	
<i>S. tomentosus</i>	-----CTTT TCGAA-----	-----GG	CGTCTTCCTC	A---TTGACG	
<i>S. americanus</i>	-----CTT CT-----	-----GGG	CGTCTTCCTT	A---TAGACG	
<i>S. granulatus</i>	-----TTT CGA-----	-----G	CGTCTTCCTC	A---TTGACG	
<i>S. grevillei</i>	-----CTT CT-----	-----GG	CGTCTTCCTT	A---TTGACT	
<i>S. cavipes</i>	-----CCT AAC-----	-----GGCC	CGTCTTCCTT	C---TTGACG	
<i>S. caerulescens</i>	-----TTT CT-----	-----GG	CGTCTTCCTT	A---TTGACG	
<i>S. lakei</i>	-----TTT CCA-----	-----G	CGTCTTCCTT	A---TTGACG	
<i>S. sinuspaulinus</i>	-----CTT CC-----	-----GGG	CGTCTTCCTT	A---TTGACG	
<i>Chroogomphus</i>	-----CCT CTCAAAGGGC	GAT-----	-GTCCTTCCTT	T---TTTTATT	
<i>Gomphidius</i>	-----TTC TCT-----	GAAGG-----	-GTCCTTCCTT	--CCTTATG	
<i>Truncocolumella</i>	-----CTC TTAGGAGAGC	GAA-----	GGTCTTCCTT	--CCTGACG	
<i>Rhizopogon evadens</i>	-----TTT TACTAG-----	-----	-GTCCTTGAA	A-TTTTGACA	
<i>R. evadens</i> (HT)	-----TCT TC-----	-----GGACT	CGTCTTTC--	--TTTGACA	
<i>R. smithii</i>	-----	C TGTCGGGACT	CGTCTTTC--	--TTTGACA	
<i>R. subsalmonius</i>	--CTATCTTA ACTGAGAGGA	AT-----G	GTCCTTCACTT	-CATTTGACA	
<i>R. fuscorubens</i>	-TTTCTCTCT CCAGGGAGGG	GA-----G	TGTCCTTCCTT	A-ATT-GAC-	
<i>R. ochraceorubens</i> (TT)	-----TCT CAGTT-----	-----G	G-TCTTCCTT	A-ATT-GACA	
<i>R. ochraceorubens</i> (HT)	-----TCT CAGTT-----	-----G	G-TCTTCCTT	A-ATT-GACA	
<i>R. occidentalis</i>	-----TAT CAGTAGTC--	-----G	G-TCTTCCTT	A-ATT-GACA	
<i>R. occidentalis</i>	-----TAT CAGTAGTC--	-----G	G-TCTTCCTT	A-ATT-GACA	
<i>R. succosus</i>	-----TTC TACTAGTA-A	TA-----G	GATCTTCCTT	ATAT--GACA	
<i>R. luteolus</i>	-----TCCC TA-TAGTATA	TACTATATAG	GATCTTCCTT	ATAAT-GACA	
<i>R. burlinghamii</i>	-TTTTATT--	-----	-GTCCTTCCT-	-CATT-GA-A	
<i>R. rubescens</i>	--TTTTT--	-----	-GTCCTTCCT-	-CATT-GAC-	
<i>R. roseolus</i>	TACTATCTCT TCGGAGAGAA	AGC-----	-GTCCTTCCTT	-CATT-GAC-	
<i>R. vulgaris</i>	CTCTATCTCT TCGGAGAGAA	AGCAC-----	-GTCCTTCCTT	-CATT-GAC-	
<i>R. colossus</i> (HT)	TATTATCTCT TAGGAGAGAA	AA-----	GGTCTTCCTT	--ATT-GAC-	
<i>R. hawkeriae</i> (PT)	TATYATCTCT TCGGAGAGAA	AA-----	GGTCTTCCTT	--ATT-GAC-	
<i>R. parksii</i> (T17679)	TATTATCTCT TCGGAGAGAA	AA-----	-GTCCTTCCTT	-CATT-GAC-	
<i>R. parksii</i> (T19446)	TATTATCTCT TCGGAGAGAA	AA-----	GGTCTTCCTT	-CATT-GAC-	
<i>R. villosulus</i> (AHS)	TATTATCTCT TCGGAGAGAA	AA-----	GGTCTTCCTT	--ATT-GAC-	
<i>R. villosulus</i> (JMT)	TATTATCTCT TAGGAGAGAA	AA-----	GGTCTTCCTT	--ATT-GAC-	
<i>R. rogersii</i>	TATTAT-ACT	AA-----	GGTCTTCCTT	--ATT-GAC-	
<i>R. sp. nov</i>	TATTAT-TCT TCGGAGAGAA	AA-----	-GTCCTTCCTT	-CATT-GAC-	
<i>R. gilkeyae</i>	TATTATCTCT TCGGAGAGAA	AA-----	GGTCTTCCTT	--ATT-GAC-	
<i>R. zelleri</i>	TATTATCTCT TCGGAGAGAA	AA-----	GGTCTTCCTT	--ATT-GAC-	
<i>R. villescens</i>	TATTANCTCT TCGGAGAGAA	AA-----	-GCCYCTTT	--ATT-GAC-	
<i>R. diabolicus</i>	TATTATCTCT TCGGAGAGAA	AA-----	GGTCTTCCTT	--ATT-GAC-	
<i>R. parvulus</i>	TATTATCTCT TCGGAGAGAA	AA-----	GGTCTTCCTT	--ATT-GAC-	
<i>R. vinicolor</i> (T17899)	TATTATCTCT TCGGAGAGAA	AA-----	GGTCTTCCTT	--ATT-GAC-	
<i>R. vinicolor</i> (T20787)	TATTATCTCT TCGGAGAGAA	AA-----	GGTCTTCCTT	--ATT-GAC-	
<i>R. ochraceisporus</i> (PT)	TATTATCTCT TCGGAGAGAA	AA-----	GGTCTTCCTT	--ATT-GAC-	
<i>R. ochraceisporus</i> (17916)	TATTATCTCT TCGGAGAGAA	AA-----	GGTCTTCCTT	--ATT-GAC-	
<i>R. ochraceisporus</i> (17944)	TATTATC-CT TCGGAGAGAA	AA-----	-GNCTTCCTT	--ATT-GAC-	
<i>R. subcaerulescens</i>	TATTATCTCT CCGGAGAGAA	CA-----G	GGTCTTCCTT	--ATT-GAC-	
<i>R. ellenae</i> (HT)	TATTATCTCT CCGGAGAGAA	CA-----G	GGTCTTCCTT	--ATT-GAC-	
<i>R. ellenae</i>	TATTATCTCT CCGGAGAGAA	CA-----G	GGTCTTCCTT	--ATT-GAC-	
<i>R. subpurpurascens</i> (PT)	TACTATCTCT CCGGAGAGAA	CA-----G	GGTCTTCCTT	--ATT-GAC-	
<i>R. subpurpurascens</i>	TACTATCTCT CCGGAGAGAA	CA-----G	GGTCTTCCTT	--ATT-GAC-	
<i>R. subgelatinosus</i>	TATTATCTCT CCGGAGAGAA	CA-----G	G-TCTTCCTT	--ATT-GAC-	
<i>R. semireticulatus</i> (17562)	TATTATCTCT CCGGAGAGAA	CA-----G	GGYCTTCCTT	--ATT-GGC-	
<i>R. semireticulatus</i> (7899)	TATTATCTCT CCGGAGAGAA	CA-----G	GGTCTTCCTT	--ATT-GAC-	

	910	915[1234567890123]
<i>Suillus brevipes</i>	ITTTGACC-T	CAAAAT00000000000000
<i>S. luteus</i>	ITTTGACC-T	CAAAAT00000000000000
<i>S. tomentosus</i>	ITTTGACC-T	CAAAAT00000000000000
<i>S. americanus</i>	ITTTGACC-T	CAAAAT00000000000000
<i>S. granulatus</i>	ITTTGACC-T	CGAAAT00000000000000
<i>S. grevillei</i>	ITTTGACCC-T	CAAAAT00000000000000
<i>S. cavipes</i>	ITTTGACC-T	CAAAAT00000000000000
<i>S. caerulescens</i>	ITTTGACC-T	CGAAAT00000000000000
<i>S. lakei</i>	ITTTGACC-T	CGAAAT00000000000000
<i>S. sinuspaulinus</i>	ITTTGACC-T	CAAAAT00000000000000
<i>Chroogomphus</i>	ITTTGACC-T	CAAAAT00000000000010
<i>Gomphidius</i>	ITTTGACC-T	CAAAAT00000000000010
<i>Truncocolumella</i>	ITTTGACC-T	CAAAAT00000000000010
<i>Rhizopogon evadens</i>	TTTTGACC-T	CAAAAT00001000001111
<i>R. evadens</i> (HT)	TTTTGACC-T	CAAAAT00001000011111
<i>R. smithii</i>	TATTGACC-T	CAAAAT00001000011111
<i>R. subsalmonius</i>	TTTTGACC-T	CAAAAT01001000001111
<i>R. fuscorubens</i>	TTTTGACC-T	CAAAAT01001000001110
<i>R. ochraceorubens</i> (TT)	TTTTGACC-T	CAAAAT01001000001110
<i>R. ochraceorubens</i> (HT)	TTTTGACC-T	CAAAAT01001000001110
<i>R. occidentalis</i>	ATTTGACC-T	CAAAAT01001000001110
<i>R. occidentalis</i>	TTTTGACC-T	CAAAAT01001000001110
<i>R. succosus</i>	TTATGACC-T	CAAAAT11001000101110
<i>R. luteolus</i>	TTTTGACC-T	CAAAAT11001000101110
<i>R. burlinghamii</i>	TTTTGAAC-T	CCAAT0011210100010
<i>R. rubescens</i>	TTTTGACC-T	CAAAAT0011210100010
<i>R. roseolus</i>	TTTTGACC-T	CAAAAT0011210100010
<i>R. vulgaris</i>	TTTTGACC-T	CAAAAT0011210100010
<i>R. colossus</i> (HT)	TTTTGACC-T	CAAAAT0010201200010
<i>R. hawkeriae</i> (PT)	TTTTGACC-T	CAAAAT0010201200010
<i>R. parksii</i> (T17679)	TTTTGACC-T	CAAAAT0010201200010
<i>R. parksii</i> (T19446)	TTTTGACC-T	CAAAAT0010201200010
<i>R. villosulus</i> (AHS)	TTTTGACC-T	CAAAAT0010201200010
<i>R. villosulus</i> (JMT)	TTTTGACC-T	CAAAAT0010201200010
<i>R. rogersii</i>	-TTTGACC-T	CAAAAT0010201200010
<i>R. sp. nov</i>	TTTTGACC-T	CAAAAT0010201200010
<i>R. gilkeyae</i>	TTTTGACC-T	CAAAAT0010201200010
<i>R. zelleri</i>	TTTTGACC-T	CAAAAT0010201200010
<i>R. villescens</i>	TTTTGACC-T	CAAAAT0010201200010
<i>R. diabolicus</i>	TTTTGACC-T	CAAAAT0010201200010
<i>R. parvulus</i>	TTTTGACC-T	CAAAAT0010201200010
<i>R. vinicolor</i> (T17899)	TTTTGACC-T	CAAAAT0010201200010
<i>R. vinicolor</i> (T20787)	TTTTGACC-T	CAAAAT0010201200010
<i>R. ochraceisporus</i> (PT)	TTTTGACC-T	CAAAAT0010201200010
<i>R. ochraceisporus</i> (17916)	TTTTGACC-T	CAAAAT0010201200010
<i>R. ochraceisporus</i> (17944)	TTTTGACC-T	CAAAAT0010201200010
<i>R. subcaerulescens</i>	TTTTGACC-T	CAAAAT0010210200010
<i>R. ellenae</i> (HT)	TTTTGACC-T	CAAAAT0010210200010
<i>R. ellenae</i>	TTTTGACC-T	CAAAAT0010210200010
<i>R. subpurpurascens</i> (PT)	TTTTGACC-T	CAAAAT0010210200010
<i>R. subpurpurascens</i>	TTTTGACC-T	CAAAAT0010210200010
<i>R. subgelatinosus</i>	TTTTGACC-T	CAAAAT0010210200010
<i>R. semireticulatus</i> (17562)	TTTTGGCC-T	CAAAAT0010210200010
<i>R. semireticulatus</i> (7899)	TTTTGACC-T	CAAAAT0010210200010


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end;
begin PAUP;

[exclude ambig and large inserts, Xlgam.gap3]
[exclude 1-22 47-81 98-124 141-152 167-177 221-264 289-327 343-345 379-384 459-
462 468-475 668-699 828-837 842-881;]

[Igap3]
[exclude 1-22 56-81 98-119 120-123 141-151 [152-155] [156-159] [160-164] [166-177]
[196-198] [221-229] 289-327]
[343-345 379-384 459-462 468-475 670-695 [829-836] 844-881;]

[allincl.gap3]
[exclude 1-22 459-462 468-475;]

[binary coding, ambig ex]
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exclude 1-22 47-54 55-58 59-81 98-119 120-123 141-151 152-155 156-159 160-163

           [8]  [9]  [10]  [11]  [12]
164-165 166-177 221-229 230-264 265-278 289-327 343-345 379-384 459-462 468-475

           [13]
670-695 829-836 844-881;
end;

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