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## Biology of the ectomycorrhizal genus *Rhizopogon*. V. Phylogenetic relationships in the Boletales inferred from LSU rDNA sequences

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**Abstract:** The phylogenetic relationship between *Alpova*, *Chroogomphus*, *Gomphidius*, *Rhizopogon*, *Suillus*, and *Truncocolumella* and their placement in the Boletales was tested through maximum parsimony analyses of large subunit nuclear ribosomal DNA sequences. Taxon sampling included representatives of the genera *Alpova*, *Boletellus*, *Boletus*, *Chroogomphus*, *Gomphidius*, *Melanogaster*, *Paragyrodon*, *Phylloporus*, *Rhizopogon*, *Suillus*, *Truncocolumella*, *Tylopilus*, and *Xerocomus*, as well as species from the Agaricales, Russulales, and Polyporales. The order Boletales was strongly supported as monophyletic. Furthermore, within this order, two major groups emerged: the boletoid radiation and a Melanogastraceae/*Paragyrodon sphaerosporus* paraphyletic assemblage, and the suilloid radiation. The boletoid radiation and a Melanogastraceae/*Paragyrodon sphaerosporus* assemblage was strongly supported. Although the boletoid radiation was not strongly supported by bootstrap analysis, the clade did not collapse in the strict consensus topology. This clade comprised species from the genera *Boletellus*, *Boletus*, *Phylloporus*, *Tylopilus*, and *Xerocomus*. The Boletaceae sensu Singer was not monophyletic. Although species relationships within the boletoid radiation were not well resolved, the genus *Boletus* did not appear to be monophyletic. *Alpova diplophloeus*, *A. trappei*, *Melanogaster tuberiformis*, and

*Paragyrodon sphaerosporus* formed a paraphyletic assemblage basal to the boletoid radiation clade. The suilloid clade was strongly supported and included *Alpova olivaceotinctus*, *Chroogomphus*, *Gomphidius*, *Rhizopogon*, *Suillus* and *Truncocolumella citrina*. Species in the genus *Alpova* therefore were grouped both within and outside the suilloid radiation. Within the suilloid radiation, *Suillus* was inferred to be more closely related to *Truncocolumella citrina* and the Gomphidiaceae than to *Rhizopogon*, suggesting a more distant relationship between *Suillus* and *Rhizopogon* than previously hypothesized. *Rhizopogon* was not supported as monophyletic, although its monophyly could not be rejected by the data. *Alpova olivaceotinctus* grouped within the genus *Rhizopogon*, consistent with previous classifications, and is returned to that genus.

**Key Words:** *Alpova*, *Melanogaster*, phylogeny, Rhizopogonaceae, *Suillus*

### INTRODUCTION

The Boletales is a morphologically diverse and species-rich order of Basidiomycetes that includes poroid, gilled, resupinate, hypogeous, and epigeous gasteroid fungi (Bruns et al 1998, Hibbet et al 1997, Hughey et al 2000, Kreisel 1969). Bruns et al (1998) identified six groups within the Boletales in a study of mitochondrial large subunit rDNA (mt-LrDNA) sequences that included 80 genera of Hymenomycetes from the Agaricales, Aphyllophorales, and Boletales.

Bruns and Szaro (1992) identified two distinct groups as “suilloid” and “boletoid” radiations within the Boletales. Presently our understanding of taxa that comprise the suilloid radiation include *Alpova olivaceotinctus*, *Brauniellula albidipes*, *Truncocolumella citrina* and species from the genera *Chroogomphus*, *Gomphidius*, *Rhizopogon*, and *Suillus* (Bruns et al 1998, Kretzer and Bruns 1999). The boletoid radiation comprises species from eleven genera including *Boletus*, *Boletellus*, *Chamonixia*, *Leccinum*, *Phylloporus*, *Strobilomyces*, *Tylopilus*, and *Xerocomus* (Bruns et al 1998, Kretzer and Bruns 1999).

The relationships between these seven genera identified within the suilloid radiation are not well understood and require further investigation. Hypotheses on the evolution from epigeous to hypoge-

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ous forms were developed from inferred phylogenetic relationships between *Rhizopogon* and *Suillus*. Thiers (1984) proposed the secotioid syndrome hypothesis to explain this relationship based on his observations of *Rhizopogon* and *Suillus* fruiting bodies found in the dry Sierra Nevada. He proposed that morphological change occurred along a gradient from a poroid *Suillus*-like ancestor through a secotioid intermediate to *Rhizopogon*, which represented the hypogeous and most derived form. Molecular studies have supported individual components of this hypothesis by showing that (i) secotioid taxa are derived from within *Suillus* (Kretzer and Bruns 1997) and (ii) that *Suillus* and *Rhizopogon* are genetically extremely closely related (Bruns et al 1989). However, based on nuclear ribosomal large subunit sequences (nuc-LSU-rDNA) Bresinsky et al (1999) found that *Suillus viscidus* and *Boletinus* (*Suillus*) *cavipes* are more closely related to *Chroogomphus rutilus* and *Gomphidius glutinosus* than they are to *Rhizopogon luteolus*.

Although *Alpova olivaceotinctus* and *Truncocolumella citrina* were both found in the suilloid radiation by Bruns et al (1998), it is unclear if they are most closely related to *Rhizopogon*. *Truncocolumella citrina* was placed in the *Rhizopogonaceae* (Zeller 1949) but a recent study based on ATPase subunit 6 sequences suggests it may be more closely related to *Suillus* than to *Rhizopogon* (Kretzer and Bruns 1999). *Alpova olivaceotinctus* was one of four *Rhizopogon* species that were previously transferred to *Alpova* (Trappe 1975). *Alpova olivaceotinctus* lacks clamp connections in the peridium, a character that ties it to *Rhizopogon*. However all four species transferred to *Alpova* lack a hymenium, a feature that ties them to *Melanogaster*. Trappe (1975) considered *Alpova olivaceotinctus* to be an intermediate species between *Rhizopogon* and *Alpova* as illustrated by the presence of a vestigial tangled hymenium and the lack of clamp connections in the peridium. He stated that because *Alpova olivaceotinctus* shares characters with both *Alpova* and *Rhizopogon* it could be placed in either genus.

Two cyclopentenones, chamonixin and involutin, have been isolated from *Melanogaster broomeianus* (Besl et al 1996). These cyclopentenones are found in the Boletales including the Paxillaceae, and the genera *Chamonixia*, *Gyrodon*, *Gyroporus*, and *Leccinum* (Besl and Bresinsky 1997). This suggests that *Melanogaster broomeianus* may be related to members of the Boletales including *Paxillus* (Besl et al 1996). In addition, Bresinsky et al (1999) found that *Melanogaster broomeianus* grouped with *Gyrodon lividus*, *Paxillus filamentosus*, and *P. involutus* based on analysis of nuc-LSU-rDNA sequences. *Gyrodon lividus*, *Paxillus filamentosus*, and *P. involutus* are clearly outside

both the boletoid and suilloid radiations (Kretzer and Bruns 1996). These conclusions raise questions about the placement of *Alpova* in the Boletales. *Alpova olivaceotinctus* appears to be closely related to *Rhizopogon* (Bruns et al 1998, Kretzer et al 1996), but the type species of *Alpova*, *A. diplophloeus*, shares morphological characteristics with the genus *Melanogaster*, not *Rhizopogon*.

A weakness of previous molecular phylogenetic studies is that relationships within the suilloid radiation remain largely unresolved. The largest taxon sampling so far was conducted by Bruns et al (1998). Although 16 suilloid species were sampled, the region of the mt-LrDNA that was sequenced was too conserved to resolve relationships within this clade. Sequences were found to be identical or almost identical between species of *Suillus*, *Rhizopogon*, and the Gomphidiaceae (Bruns et al 1998). Therefore, we chose to investigate the nuclear large subunit rRNA gene (nuc-LSU-rDNA). The nuc-LSU-rDNA is less conserved than the mt-LrDNA and may provide additional information to help clarify relationships within the suilloid radiation. The nuc-LSU-rDNA has been used previously in phylogenetic studies of the Boletales, including examination of relationships within the Paxillaceae, and between *Boletellus* and *Xerocomus* (Binder and Fisher 1997, Bresinsky et al 1999, Jarosch and Bresinsky 1999).

In this study, we conducted phylogenetic analyses of DNA sequences from the nuclear ribosomal large subunit gene of several genera in the Boletales. Our specific objectives were to: (i) further examine the phylogenetic placement of *Alpova* by including *A. diplophloeus*, the type of the genus and (ii) clarify relationships within the suilloid radiation.

#### MATERIALS AND METHODS

*Fungal specimens.*—Herbarium and fresh collections of fifteen species of the Boletales were selected for DNA extraction (TABLE I). Herbarium specimens were obtained from the Mycological Collection of the Oregon State University Herbarium (OSC). All fresh collections were deposited as vouchers in the OSC. *Alpova olivaceotinctus* genomic DNA was donated by Annette Kretzer and obtained from a voucher housed at the San Francisco State University Herbarium (SFSU). The identification was confirmed through Restriction Fragment Length Polymorphism (RFLP) analysis of internal transcribed spacer nuclear ribosomal DNA sequences from three different *Alpova olivaceotinctus* vouchers. The RFLP patterns were identical for all three *A. olivaceotinctus* vouchers and different from other *Alpova* species tested (Kretzer pers comm).

Taxon sampling of the suilloid radiation was intended to approximate that of a previous phylogenetic study of the Boletales (Bruns et al 1998), so as to provide a comparison

TABLE I. Species examined for DNA analysis

Species <sup>a</sup>	GenBank	Voucher number <sup>b</sup>	Herbarium <sup>c</sup>
<i>Agaricus bisporus</i>	U11911		
<i>Alpova diplophloeus</i>	AF071454	JMT 17685	OSC
<i>Alpova trappei</i>	AF071456	JMT 16394	OSC
<i>Bolbitius vitellinus</i>	U11913		
<i>Boletellus mirabilis</i>	AF050652		
<i>Boletus edulis</i>	AF050643		
<i>Boletus edulis</i>	AF071457	LCG 184	OSC
<i>Boletus retipes</i>	U11914		
<i>Boletus satanas</i>	AF042015		
<i>Boletus satanas</i>	AF071528		
<i>Chroogomphus vinicolor</i>	AF071529		
<i>Cortinarius stuntzii</i>	U11917		
<i>Crinipellis campanella</i>	U11916		
<i>Ganoderma lucidum</i>	X78776		
<i>Ganoderma microsporum</i>	X78779		
<i>Gomphidius glutinosus</i>	AF07153		
<i>Hebeloma crustuliniforme</i>	U11918		
<i>Lactarius corrugis</i>	U11919		
<i>Leucoagaricus naucinus</i>	U11921		
<i>Macrolepiota rachodes</i>	U11923		
<i>Marasmius delectans</i>	U11922		
<i>Melanogaster tuberiformis</i>	AF074919	JMT 9666	OSC
<i>Paragyrodon sphaerosporus</i>	AF071531		
<i>Phylloporus rhodoxanthus</i>	U11925		
<i>Pleurotus cornucopiae</i>	U04135		
<i>Pleurotus ostreatus</i>	U04147		
<i>Rhizopogon hawkerae</i>	AF071458	JMT 15299	OSC
<i>Rhizopogon occidentalis</i>	AF071453	JMT 17564	OSC
<i>Rhizopogon olivaceotinctus</i>	AF071455	HT 53027	SFSU
<i>Rhizopogon parksii</i>	AF071459	JMT 19446	OSC
<i>Rhizopogon smithii</i>	AF071460	JMT 12321	OSC
<i>Rhizopogon subcaerulescens</i>	AF071534		
<i>Rhizopogon subpurpurascens</i>	AF071461	JMT 19168	OSC
<i>Rhizopogon truncatus</i>	AF071462	JMT 17993	OSC
<i>Rhizopogon truncatus</i>	AF071463	LCG 212	OSC
<i>Rhizopogon villosulus</i>	AF071464	JMT 19466	OSC
<i>Russula mairei</i>	U11926		
<i>Suillus capives</i>	AF071535		
<i>Suillus sinuspaulianus</i>	AF071536		
<i>Truncocolumella citrina</i>	AF071465	JMT 19184	OSC
<i>Tylopilus felleus</i>	AF071466	JMT 6375	OSC
<i>Xerocomus chrysenteron</i>	AF071537		

<sup>a</sup> Species listed only by GenBank number were not sequenced in this study.

<sup>b</sup> LCG, Lisa C. Grubisha, HT, Harry Thiers, JMT, James M. Trappe.

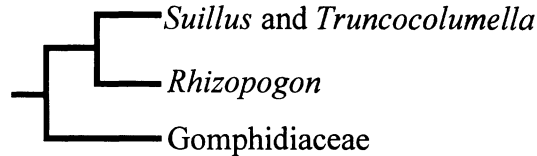
<sup>c</sup> OSC, Mycological Collection of the Oregon State University Herbarium, SFSU, San Francisco State University.

with a more variable gene. To complete the taxon sampling for comparison of the Boletales to members of the Agaricales, an additional 30 nuc-LSU-rDNA sequences from Basidiomycota taxa were retrieved from GenBank (TABLE I).

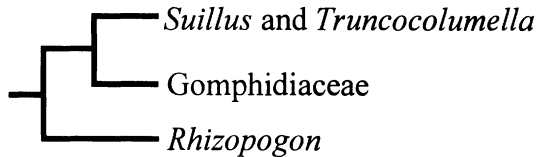
*Nucleic acid extraction, polymerase chain reaction, and DNA sequencing.*—The protocol for nucleic acid extraction was a modified SDS-lysis buffer (Bruns et al 1990) or 2X CTAB (Doyle and Doyle 1987), and is outlined by Platt and Spatafora (2000). The nuc-LSU-rDNA was amplified using the primer

pair LR0R (Moncalvo et al 1995) and LR5 (Vilgalys and Hester 1990). Polymerase chain reaction (PCR) protocol was previously described (Platt and Spatafora 2000), but for this study thermal cycling parameters were changed to: 95 C for 3 min, 35 cycles of 95 C for 1 min, 50 C for 30 s, and 72 C for 45 s then 72 C for 2 min. and finally 4 C for 15 min.

Purification of PCR products and sequencing follow Platt and Spatafora (2000) except that PCR products were sequenced with primers LR3 (Vilgalys and Hester 1990),



A.



B.

FIG. 1. Depiction of constraints II and III used for Kishino-Hasegawa tests. In both A and B *Truncocolumella citrina* is constrained with *Suillus*, *Rhizopogon* includes nine *Rhizopogon* species and *Alpova olivaceotinctus*, and the Gomphidiaceae includes *Gomphidius glutinosus* and *Chroogomphus vinicolor*. A. Constraint II, *Rhizopogon* and *Suillus* are sister groups and the Gomphidiaceae is basal to this clade. B. Constraint III, *Suillus* and the Gomphidiaceae are sister groups and *Rhizopogon* is monophyletic and basal to this clade.

LR0R, and LR5. Sequences were aligned manually in SeqApp version 1.9a169 (D. G. Gilbert, <http://iubio.bio.indiana.edu/soft/molbio/seqapp/>). The sequence alignment has been deposited in TreeBASE as S514.

**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 (MulTrees). The outgroups chosen were the Russulales and Polyporales. Areas of ambiguous alignment and non-informative characters were excluded from the analyses. Gaps were treated as missing data. To measure relative support for the resulting clades, 1000 bootstrap replications (Felsenstein 1985) were performed using parsimony informative characters only with the following parameters: 10 random sequence additions, TBR, and MulTrees.

To test alternative phylogenetic relationships in the suilloid radiation, the Kishino-Hasegawa maximum likelihood ratio test (Kishino and Hasegawa 1989) was performed as implemented in PAUP\* (Swofford 1998). The Hasegawa-Kishino-Yano model of mutation was used with a transition: transversion ratio of 2 (Hasegawa et al 1985). Three constraints (hypotheses) were tested: (I) *Rhizopogon* is monophyletic, (II) *Rhizopogon* and *Suillus* are sister-groups and the Gomphidiaceae is basal to this clade, and (III) *Suillus* and the Gomphidiaceae are sister-groups and *Rhizopogon* is monophyletic and basal to this clade (FIG. 1). In constraint

I and III, *Truncocolumella citrina* was constrained with the *Suillus* clade. Constraints were constructed using MacClade 3.0 (Maddison and Maddison 1992). After each constraint was loaded in PAUP\*, maximum parsimony analyses were conducted with the same parameters as above. The constrained and unconstrained trees were compared using the Hasegawa-Kishino-Yano model for maximum likelihood analyses in PAUP\* (Hasegawa et al 1985, Swofford 1998).

## RESULTS

**Parsimony analyses.**—The data set comprised 931 aligned characters, of which 699 were excluded because they were either ambiguously aligned or parsimony-uninformative, and 232 were parsimony-informative. A total of 107 most parsimonious trees were recovered with 866 steps [consistency index (CI) = 0.396, retention index (RI) = 0.684, rescaled consistency index = 0.271, FIG. 2].

The Boletales clade is supported with a bootstrap value of 100 and forms a sister group to the Agaricales (FIG. 2). Although this relationship is not well-supported by a bootstrap value (61), it agrees with results from other studies (Begerow et al 1997, Hibbett et al 1997). Two major groups are identified within the Boletales clade. The boletoid radiation and a *Alpova/Melanogaster/Paragyrodon* paraphyletic assemblage formed a well-supported clade with a bootstrap value of 97. The boletoid radiation and is composed of species from the genera *Boletellus*, *Boletus*, *Phylloporus*, *Tylopilus*, and *Xerocomus*. Although there was low bootstrap support for this clade, it does not collapse in the strict consensus tree topology. *Alpova* s. s. (minus *A. olivaceotinctus*) and *Boletus* do not appear to be monophyletic, but resolution of species-level relationships is very low (FIG. 2).

The suilloid radiation included *Alpova olivaceotinctus*, *Chroogomphus*, *Gomphidius*, *Rhizopogon*, *Suillus*, and *Truncocolumella* and formed a strongly supported clade with a bootstrap value of 97. *Truncocolumella citrina* grouped with *Suillus* and it appears to be more closely related to *Suillus* than is *Rhizopogon*. *Rhizopogon* appears to be a sister-group to the clade of *Chroogomphus*, *Gomphidius*, *Truncocolumella*, and *Suillus*. Three clades of *Rhizopogon* were inferred and are consistent with sectional classifications of the genus (Smith and Zeller 1966), with the exception of *R. truncatus*, a species that has been classified in section *Fulviglebae* but grouped in this study with species in section *Rhizopogon* (*R. occidentalis* and *R. smithii*).

Although *Rhizopogon* appears to form a paraphyletic grade, the monophyly of *Rhizopogon* and its sister-group relationship to the *Suillus/Gomphidius* clade could not be rejected based on results from Kishino-Hasegawa maximum likelihood ratio tests

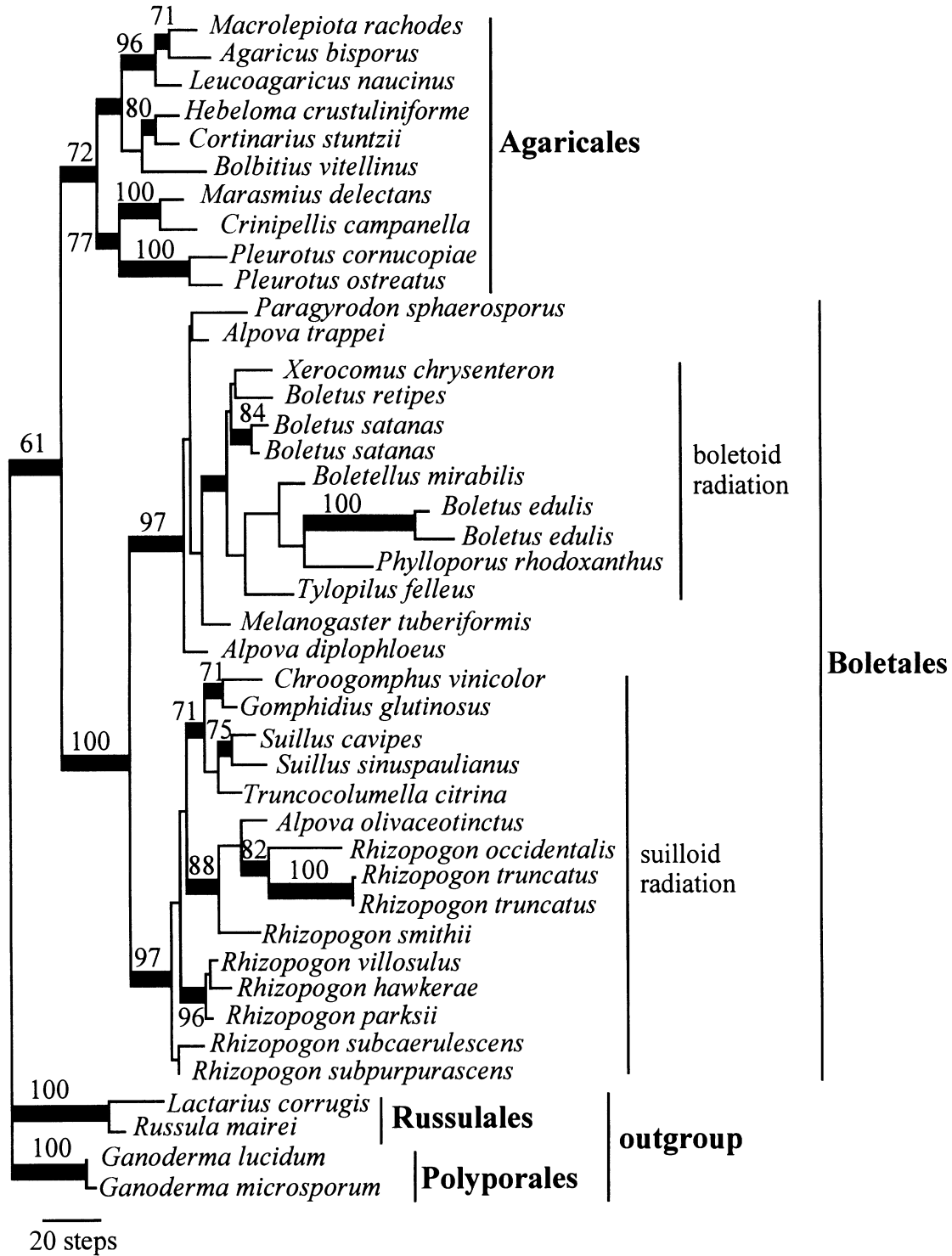


FIG. 2. One of 107 most parsimonious trees each of 866 steps based on nuc-LSU-rDNA sequence analysis (consistency index = 0.396, retention index = 0.684). The tree presented has the highest log likelihood value resulting from maximum likelihood analyses based on the Hasegawa-Kishino-Yano model. Bootstrap values above 60 are located at the appropriate internode. Branches that do not collapse in a strict consensus tree are indicated by thickened lines. Placement of species into boletoid and suilloid radiations follows Bruns et al (1998).

TABLE II. Results from Kishino-Hasegawa tests

Tree	# Trees <sup>a</sup>	Tree length	Best constrained tree		
			ln L	T	P <sup>b</sup>
Best unconstrained tree	107	866	-4064.47	—	—
Constraint I <sup>c</sup>	316	853	-4069.75	1.11	0.27
Constraint II <sup>c</sup>	66	854	-4079.78	1.94	0.05
Constraint III <sup>c</sup>	223	853	-4069.75	1.11	0.27

<sup>a</sup> The number of trees determined from maximum parsimony enforcing topological constraints.

<sup>b</sup> Probability of getting a more extreme T-value under the null hypothesis of no difference between the two trees (two-tailed test).

<sup>c</sup> Constraint I: *Rhizopogon* is monophyletic, Constraint II: *Rhizopogon* and *Suillus* are sister groups with the Gomphidiaceae basal to this clade, Constraint III: *Suillus* and the Gomphidiaceae are sister groups and *Rhizopogon* is monophyletic and basal to this clade.

(TABLE II). While the constraints enforcing *Suillus* and the Gomphidiaceae as a sister-group and *Rhizopogon* as basal (constraint III, FIG. 1) and the monophyly of the genus *Rhizopogon* (constraint I) could not be rejected ( $P \gg 0.05$ ), the constraint enforcing *Rhizopogon* and *Suillus* as sister-groups and the Gomphidiaceae as basal to this group (constraint II, FIG. 1) was rejected ( $P = 0.05$ ).

#### DISCUSSION

The phylogenetic placement of *Alpova* remains disputed in modern classification of fungi and it has been placed in both the Rhizopogonaceae (Dodge 1931), and Boletaceae (Castellano et al 1989, Molina et al 1992). However, the genus is more often considered part of the Melanogastraceae (Fischer 1933, Hawksworth et al 1995, Miller and Miller 1988, Zeller 1949). Because a previous study found *Alpova olivaceotinctus* to be closely related to species in the suilloid radiation (Bruns et al 1998), we wanted to see if other *Alpova* species would also cluster with the suilloid clade. Based on our results *Alpova* is polyphyletic with at least one species (*Alpova olivaceotinctus*) in the suilloid radiation and two species (*Alpova diplophloeus* and *A. trappei*) that are closely related to *Melanogaster tuberiformis* and *Paragyrodon sphaerosporus*.

Trappe (1975) noted the similarities between several *Alpova* species and *Rhizopogon*, including *Alpova olivaceotinctus*. Based upon these data (FIG. 2) *Alpova olivaceotinctus* (Trappe 1975) is returned to *Rhizopogon*, where it had been originally described as *R. olivaceotinctus* A. H. Smith (Smith and Zeller 1966). It would not be surprising if additional *Alpova* species from *Alpova* subg. *Alpova* sect. *Rhizopogonella* were found to be more closely related to *Rhizopogon* than to other *Alpova* species in future studies.

*Alpova diplophloeus* and *A. trappei* do not appear to form a monophyletic *Alpova* clade. However, the

few number of isolates sampled precludes us from making a definitive statement on the monophyly of the non-suilloid *Alpova* species. *Alpova diplophloeus* is also the type species for the genus indicating that the genus *Alpova* is not part of the suilloid radiation. Although our results support the hypothesis that *Alpova* and *Melanogaster* are closely related, they are in conflict with the proposal of a *Rhizopogon-Alpova-Melanogaster* evolutionary continuum (Trappe 1975).

Consistent with previous studies (Bruns et al 1998, Bruns and Szaro 1992) *Paragyrodon sphaerosporus* was found outside the boletoid radiation. In this study it formed a paraphyletic grade with *Alpova diplophloeus*, *A. trappei*, and *Melanogaster tuberiformis*. A better understanding of the relationships amongst these species is emerging. *Paragyrodon sphaerosporus* and *Paxillus involutus* were found to be closely related (Bruns et al 1998), while Bresinsky et al (1999) concluded that *Melanogaster broomeianus* was related to *Gyrodon lividus*, *Paxillus filamentosus*, and *P. involutus*. It is likely that *Alpova diplophloeus*, *A. trappei*, and *Melanogaster tuberiformis* are also related to *Gyrodon lividus*, *Paxillus filamentosus*, and *P. involutus*. Future studies that provide extensive taxon sampling of these genera are needed to elucidate the evolutionary relationships among these fungi.

In a recent phylogenetic study of sequences from the mt-LrRNA gene of 32 genera Bruns et al (1998) recovered six clades in the Boletales, including distinct suilloid and boletoid groups. They found *Boletellus*, *Boletus*, *Chamonixia*, *Gastroboletus*, *Leccinum*, *Phylloporus*, *Tylopilus*, *Strobilomyces*, *Xerocomus* and others in the boletoid radiation. The results of our study support the findings of Bruns et al (1998). The genera in the boletoid radiation were *Boletellus*, *Boletus*, *Phylloporus*, *Tylopilus*, and *Xerocomus*. *Boletus* does not appear to be monophyletic, but because there is not much resolution between species within

this clade, we cannot make strong inferences about relationships within the boletoid radiation.

Evolutionary relationships between *Gomphidius*, *Rhizopogon*, *Suillus*, and *Truncocolumella* have previously been hypothesized based on morphological and ecological data (Heim 1971, Singer 1986, Smith 1971, Smith and Singer 1959, Smith and Thiers 1964, 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, Kretzer and Bruns 1999, Kretzer et al 1996). We chose to further investigate the suilloid radiation with the goal examining relationships within this group with a variable locus. *Alpova olivaceotinctus*, *Chroogomphus*, *Gomphidius*, *Rhizopogon*, *Suillus*, and *Truncocolumella* form a distinct, well-supported clade in these analyses (FIG. 2).

Because topologies from mitochondrial small unit rRNA and nuclear small subunit rRNA gene regions presented in a previous study (Bruns and Szaro 1992) were partially in conflict with the topology found in our analyses, the Kishino-Hasegawa maximum likelihood ratio tests were performed to test alternative phylogenetic hypotheses in the suilloid clade. Constraint I tested the hypothesis that *Rhizopogon* is monophyletic. This hypothesis could not be rejected ( $P \gg 0.05$ , TABLE II). Therefore, although the species of *Rhizopogon* sampled in this study do not form a monophyletic clade with strong bootstrap support, the monophyly of *Rhizopogon* could not be rejected. Constraint II, the hypothesis that *Rhizopogon* and *Suillus* form sister-groups and that the Gomphidiaceae is ancestral was rejected ( $P \leq 0.05$ , FIG. 1, TABLE II). Constraint III, the final hypothesis tested, that *Rhizopogon* morphology is the ancestral state and that *Suillus* and Gomphidiaceae are derived and constrained as sister-groups, could not be rejected ( $P = 0.27$ , FIG. 1, TABLE II). An ancestral morphology for the suilloid radiation cannot be inferred from the results of the parsimony analyses (FIG. 2). However, the results from the Kishino-Hasegawa tests suggest that the *Rhizopogon*-type morphology may reflect the ancestral morphological state for the suilloid radiation.

The data presented in this paper suggest that *Rhizopogon* and *Suillus* are not sister-groups, which conflicts with conclusions from earlier studies (Bruns et al 1989, Bruns and Szaro 1992). In our analysis, *Suillus* is most closely related to *Truncocolumella* and the Gomphidiaceae, not *Rhizopogon*. In a separate study using nuc-LSU-rDNA sequences, Bresinsky et al (1999) found that *Suillus viscidus* and *Boletinus (Suillus) cavipes* were more closely related to *Gomphidius glutinosus* and *Chroogomphus rutilus* than they were to *Rhizopogon luteolus*, and corroborates the results

presented here. *Truncocolumella citrina* has been previously classified in either the Rhizopogonaceae (Hawksworth et al 1995, Smith 1973) or the Boletaceae (Castellano et al 1989), neither of these hypotheses is supported by our data. Based on the conclusions from this study it is important that future studies directed at examining evolutionary relationships in the suilloid radiation include not only species from *Rhizopogon* and *Suillus*, but members of the Gomphidiaceae and *Truncocolumella citrina*.

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