Mycologia, 88(5), 1996, pp. 776–785.

Internal transcribed spacer sequences from 38 recognized species of *Suillus* sensu lato: Phylogenetic and taxonomic implications

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Abstract: Internal transcribed spacer regions of the nuclear ribosomal repeat have been sequenced from 47 isolates belonging to 38 recognized species of *Suillus* sensu lato. The sequences have been analyzed for phylogenetic and taxonomic implications using parsimony as well as distance methods. Based on these data, the genera *Boletinus* and *Fuscoboletinus,* that are often recognized within *Suillus* sensu lato, are not monophyletic. Isolates of *Suillus granulatus* derived from either North America or Europe and Asia are polyphyletic and seem to represent at least two different species. Our data also suggest that within *Suillus* sensu lato, mycorrhizal associations with *Larix* are primitive and host changes to *Pinus* and *Pseudotsuga* seem to have occurred only once. Changes among host species of the same genus appear to be more frequent. Finally, the most primitive clade within *Suillus* sensu lato seems to be formed by organisms with a strongly boletinoid hymenophore.

Key Words: Basidiomycota, Boletales, ITS sequences, phylogeny, taxonomy

INTRODUCTION

The genus *Suillus* sensu lato (Basidiomycota, Boletales) comprises approximately 70–80 species that produce epigeous mushrooms with tubular hymenophores. Its closest relatives are *Rhizopogon* (and other hypogeous fungi closely related to *Rhizopogon*; Bruns et al., 1989), and the Gomphidiaceae (Bruns and Szaro, 1992); collectively, these organisms have been referred to as the suilloid radiation in the Boletales (Bruns and Szaro, 1992). With few exceptions, *Suillus* and other members of the suilloid group are ectomycorrhizal associates of the Pinaceae. Due to the fairly high degree of host specificity especially within *Suillus* sensu lato, detailed knowledge of the phylogeny provides a unique opportunity to examine the pattern of ectomycorrhizal host-symbiont evolution and potential cospeciation.

Suillus sensu lato includes organisms sometimes also classified as *Boletinus* or *Fuscoboletinus*. Delineation of *Suillus* sensu stricto from the related genera *Boletinus* and *Fuscoboletinus* has remained controversial (Pomerleau and Smith, 1962; Smith and Thiers, 1971; Pegler and Young, 1981; Singer, 1986; Sutara, 1987). *Boletinus* is a rather old name and the genus has traditionally been recognized by its radially elongated pores (boletinoid hymenophore) (Kalchbrenner, 1867). Other characters that have been used to differentiate *Boletinus* from *Suillus* are the presence of clamp connections, a sterile stipe surface without glandular dots and the absence of fasciculate encrusted pleurocystidia. Delineations based on these characters are, however, inconsistent (Singer, 1962). Finally, Singer (1986) restricted the genus to three species (*B. cavipes, B. paluster* and *B. asiaticus*) which are characterized by the presence of clamp connections in their fruitbodies. It is well known, however, that species of *Suillus* sensu stricto are also able to develop clamp connections in mycelium cultures (Hübsch, 1961; Pantidou and Groves, 1966). *Fuscoboletinus* on the other hand is a comparably recent segregate in which Pomerleau and Smith (1962) have grouped together all *Suillus* or *Boletinus* species that give a vinacious to purple brown spore print.

In the present study, we have determined internal transcribed spacer (ITS) sequences from 47 isolates belonging to 38 different species of *Suillus* sensu lato. We have used those data to perform phylogenetic analyses and to clarify the confused state of taxonomy.

MATERIALS AND METHODS

Fungal specimens and DNA extraction.—All fungal specimens used in this study are listed in TABLE I. Crude DNA extracts were prepared by either of two methods, a CHELEX method (Taylor and Swann, 1994) or a modified CTAB method (Gardes and Bruns, 1993). The former was preferably used for extractions from fresh mycelia, and the latter for extractions from dried fruitbody collections. Either method yielded crude extracts with unquantified DNA contents. From these extracts, a range of dilu-

tions was made in water (usually around 10^{-2} to 10^{-3}) and used for PCR amplification.

Polymerase chain reaction.—Reaction mixtures were made up from equal volumes of DNA solution and a master mix containing all the other necessary components. Final concentrations were: 10 mM Tris/HCl pH 8.3, 50 mM KCl, 2.5 mM $MgCl_2$, 0.1 mg/mL gelatin, 200 μM of each of the four deoxyribonucleotide triphosphates, 0.5 μM of each of two different primers, 25 U/mL *Taq* polymerase, and empirically determined amounts of template DNA (see paragraph on DNA extractions). Varying sets of primers were used in the reactions. In most cases, use of the primer pairs ITS5/ITS4 or ITS5/ITS4-S (that amplify both ITS regions including the 5.8S rDNA) was found to be convenient. From older specimens, however, yields could be significantly increased by individual amplification of the two ITS regions with the primer pairs ITS5/ITS2 and ITS3/ITS4-S. Primer sequences and maps have been published before (White et al., 1990; Gardes and Bruns, 1993) except for ITS4-S the sequence of which is: 5′-CCTCCGCTTATTGATATGCTTAAG-3′. PCR reactions were overlaid with a drop of mineral oil (Sigma) and subjected to 35 amplification cycles on a Techne Thermal Cycler (model PHC-2). Conditions for the thermal cycling are described in Bruns and Gardes (1993). If the first amplifications yielded some but not enough DNA, aliquots of the amplified fragment were run on a 1.5% low melt agarose gel, bands were cut out, melted into TE buffer (10 mM Tris/HCl pH 8.0, 1 mM EDTA) at 65–70 C, diluted (10^{-2} in water) and subjected to another round of 35 amplification cycles.

Sequencing.—For use in sequencing, PCR products were cleaned from the reaction mixture either by successive dilution with H_2O and reconcentration using centrifugal filters (Millipore Ultrafree-MC filters) or with the BIO 101 Geneclean II kit. In the latter case, DNAs were first run on an agarose gel (1% normal agarose, 2% NuSieve agarose (FMC BioProducts), bands were cut out and subsequently cleaned according to the kit instructions. Sequencing was done by the cyclic reaction termination method using fluorescence labeled dideoxyribonucleotide triphosphates. The sequence reaction and the processing of the reaction products for electrophoresis were performed following the instructions for the sequencing kit (PRISM™ Ready Reaction DyeDeoxy™ Terminator Cycle Sequencing Kit, Perkin-Elmer Corporation). Electrophoresis and data collection were done on an ABI Model 373A DNA Sequencer (Perkin-Elmer Corporation) to which a Macintosh Quadra 650 computer was connected. DNA Sequencing Analysis (version 2.01) and SeqEd (version 1.03) were used for processing the raw data. A few sequences were determined by conventional dideoxy termination reaction with ^{35}S labeled ATP as described by Bruns et al. (1990).

Phylogenetic analysis.—Sequences were aligned by visual estimation using a matrix created in PAUP 3.1.1 (Swofford, 1993) and a color font. Computer alignment was not found to be useful due to short hypervariable areas in the ITS-sequences. Obvious insertions/deletions (indels) were considered to be informative and were weighted equal to one or two transition(s) (except for a very large insertion present in the outgroups that was weighted as four transitions). Technically, this was done as follows: Alignment gaps were treated as missing data, but a new character state "I" was introduced at deletion sites. The weight attributed to the proper deletion was taken into account by the number of "I"s being introduced. As far as possible, only gaps with exactly the same length and position were coded for with "I"s introduced at precisely the same position. Phylogenetic analysis was done using both parsimony (Fitch, 1971) and distance methods (neighbor joining, Saitou and Nei, 1987). Searches for most parsimonious trees were done in PAUP 3.1.1, and neighbor joining was performed as follows: First a distance matrix was created in PAUP 3.1.1 that was subsequently adjusted manually to precisely fit the output format of DNADIST, a program of the PHYLIP 3.5 package that computes distance matrixes from sequence data. The advantage of using PAUP 3.1.1 over DNADIST for computing distances was that PAUP 3.1.1 recognized the introduced character state "I", when the data type was not defined to be "DNA". Finally, the edited distance matrix was fed into NEIGHBOR, another program of the PHYLIP 3.5 package, for neighbor joining. Bootstrap analyses were done under the parsimony criterion in PAUP 3.1.1, and values are based on 500 replicates. *Gomphidius glutinosus* and *Chroogomphus vinicolor* have been chosen as outgroups for the phylogenetic analyses, because both are closely related to *Suillus* (Bruns and Szaro, 1992). *Rhizopogon subcaerulescens, Truncocolumella citrina* and *Gastrosuillus laricinus* were also included in the study. *Rhizopogon subcaerulescens* and *Truncocolumella citrina* have been shown before to be closely related to *Suillus* (Bruns et al., 1989; Bruns et al., 1990). *Gastrosuillus laricinus* is a recent derivative of *Suillus grevillei* (Baura et al., 1992).

RESULTS

Complete, two directional sequences of both ITS regions (1 and 2) were determined from the speci-

TABLE I. Specimens used in this study: *B.* = *Boletinus*, *C.* = *Chroogomphus*, *F.* = *Fuscoboletinus*, *G.* = *Gomphidius*, *Ga.* = *Gastrosuillus*, *R.* = *Rhizopogon*, *S.* = *Suillus*, *T.* = *Truncocolumella*. Only selected synonyms are given

Specimen	Collection number	Geographic origin	Location[a]
C. vinicolor (Peck) Miller	TDB-1010	USA, California	TDB
Ga. laricinus (Singer & Both) Thiers	EB-2031[b]	USA, New York	BM
G. glutinosus (Schaeff. ex Fr.) Fr.	TDB-935B	USA, California	TDB
R. subcaerulescens Smith	F-2882[b]	USA, Colorado	MICH
S. americanus (Peck) Snell ex Slipp & Snell	TDB-581	USA, Michigan	MICH
S. asiaticus (Sing.) Kretzer & Bruns	JV-4850F	Finland	MICH
= *B. asiaticus* Singer			
S. bovinus (L. ex Fr.) Kuntze	AD-5	Sweden	TDB
S. bresadolae (Quél.) Gerhold	HB-399	Germany	HB
= *S. aeruginascens* var. *bresadolae* (Quél. in Bres.) Mos.			
S. brevipes (Peck) Kuntze	TDB-834	USA, Michigan	TDB
S. caerulescens Smith & Thiers[c]	TDB-1028	USA, California	TDB
S. cavipes (Opat.) Smith & Thiers	WJS-618	USA, Idaho	SFSU
= *B. cavipes* (Opat.) Kalchbr.	TDB-646	USA, Michigan	TDB
	HDT-31407	Switzerland	SFSU
S. collinitus (Fr.) Kuntze	J3.15.2	France	MNHN
S. cothurnatus Singer	NSW-4662	USA, Louisiana	MICH
S. decipiens (Berk. & Curt.) Kuntze	DG-1451	USA, Texas	MICH
= *B. decipiens* (Berk. & Curt.) Peck			
S. glandulosipes Smith & Thiers	HDT-8394	USA, California	MICH
S. granulatus (Fries) Kuntze	AD-11	Sweden	TDB
S. granulatus (Fries) Kuntze	TDB-878	USA, Michigan	TDB
S. c. f. granulatus (Fries) Kuntze	VC-1042	Nepal	VC
S. grevillei (Klotzsch) Singer	EB-2040A[b]	USA, New York	BM
S. grisellus (Peck) Kretzer & Bruns	TDB-574	USA, Michigan	TDB
= *F. grisellus* (Peck) Pomerleau & Smith			
= *B. grisellus* Peck			
S. intermedius (Smith & Thiers) Smith & Thiers	ACAD-15271	Canada, Nova Scotia	TDB[d]
= *S. acidus* var. *intermedius* Smith & Thiers			
S. lakei (Murrill) Smith & Thiers	HDT-44112	USA, California	SFSU
= *B. lakei* (Murill) Singer			
S. laricinus (Berkeley ex Hooker) Kuntze[e]	VC-1231	Nepal	VC
= *S. viscidus* (Fr. & Hök) Rauschert in Dörfelt	TDB-561	USA, Michigan	TDB
= *S. aeruginascens* (Secr.) Snell in Slipp & Snell	TDB-576	USA, Michigan	TDB
= *F. aeruginascens* (Secr.) Pomerleau & Smith			
S. luteus (Fries) Gray	TDB-571	USA, Michigan	MICH
S. luteus (Fries) Gray	TDB-824	USA, Michigan	TDB
S. luteus (Fries) Gray	HB-348	Germany	HB
S. neoalbidipes Palm & Stewart	TDB-835	USA, Michigan	TDB
S. ochraceoroseus (Snell) Singer	SAR-84-137	USA, Washington	MICH
= *F. ochraceoroseus* (Snell) Pomerleau & Smith			
= *B. ochraceoroseus* Snell			
S. paluster (Peck) Kretzer & Bruns	GT-831015/15	Canada, Ontario	DAOM-190075
= *B. paluster* (Peck) Peck			
= *F. paluster* (Peck) Pomerleau			
S. placidus (Bonorden) Singer	TDB-725	USA, Michigan	MICH
S. c. f. placidus (Bonorden) Singer	VC-1022	Nepal	VC
S. pseudobrevipes Smith & Thiers	TDB-663	USA, Colorado	TDB
S. punctipes (Peck) Singer	TDB-265	USA, Minnesota	TDB
S. pungens Smith & Thiers	TDB-1001	USA, California	TDB
S. serotinus (Frost) Kretzer & Bruns	TJB-6597	USA, New York	CORT
= *F. serotinus* (Frost) Smith & Thiers			
S. sibiricus (Singer) Singer	VC-1040	Nepal	VC
S. sinuspaulianus (Pomerleau & Smith) Dick & Snell	DAOM-66995	Canada, Quebec	DAOM-66995
= *F. sinuspaulianus* Pomerleau & Smith			

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Specimen	Collection number	Geographic origin	Location[a]
S. spectabilis (Peck) Kuntze = *F. spectabilis* (Peck) Pomerleau & Smith = *B. spectabilis* (Peck) Murrill	TDB-641	USA, Michigan	MICH
S. spraguei (Berk. & Curt.) Kuntze = *S. pictus* (Peck) Smith & Thiers = *B. pictus* (Peck) Peck	TDB-638[b]	USA, Michigan	MICH
S. subalutaceus (Smith & Thiers) Smith & Thiers = *S. acidus* var. *subalutaceus* Smith & Thiers	ACAD-15288	Canada, Nova Scotia	TDB[d]
S. subaureus (Peck) Snell in Slipp & Snell	TDB-780	USA, Massachusetts	TDB
S. subluteus (Peck) Snell ex Slipp & Snell	IB-13-8/19/72	USA, Michigan	MICH
S. tomentosus (Kauffmann) Singer, Snell & Dick	TDB-661	USA, Colorado	MICH
S. tridentinus (Bres.) Singer	HB-347	Germany	HB
S. umbonatus Dick & Snell	TDB-978	USA, California	TDB
S. variegatus (Swartz ex Fr.) Kuntze	HB-325	Germany	HB
S. weaverae (Smith & Shaffe) Kretzer & Bruns = *F. weaverae* Smith & Shaffer	MGW-1992	USA, Minnesota	MIN-794257
T. citrina Zeller	TDB-2001	USA, California	TDB

[a] BM, Buffalo Museum, New York; CORT, Herbarium of the State University of New York—College at Cortland; DAOM, National Mycological Herbarium of Canda; HB, H. Besl, University of Regensburg, Germany; MIN, University of Minnesota Herbarium; MICH, Herbarium of the University of Michigan; MNHN, Museum National d'Histoire Naturelle, Paris, France; SFSU, San Francisco State University Herbarium; TDB, T. D. Bruns, UC Berkeley; VC, Van Cotter, Cyanamid Forschung GmbH, Schwabenheim, Germany.

[b] ITS sequences of these specimens have been published previously (Baura et al., 1992).

[c] The specimen TDB-1028 was identified as *S. caerulescens* without differentiating between *S. caerulescens* Smith & Thiers and *S. ponderosus* Smith & Thiers which are distinguished ony by a dry versus gelatinous annulus.

[d] Cultures were obtained from K. A. Harrison, Acadia University, Nova Scotia.

[e] The specimens TDB-561 and TDB-576 have been identified as *S. laricinus* without differentiating between *S. laricinus* (Berkeley ex Hooker) Kuntze and *S. serotinus* (Frost) Kretzer & Bruns, two varieties that have been raised to the level of separate species by Smith and Thiers (1971). They differ in a bluish-green versus bluish to dark bluish-gray to fuscous color reaction.

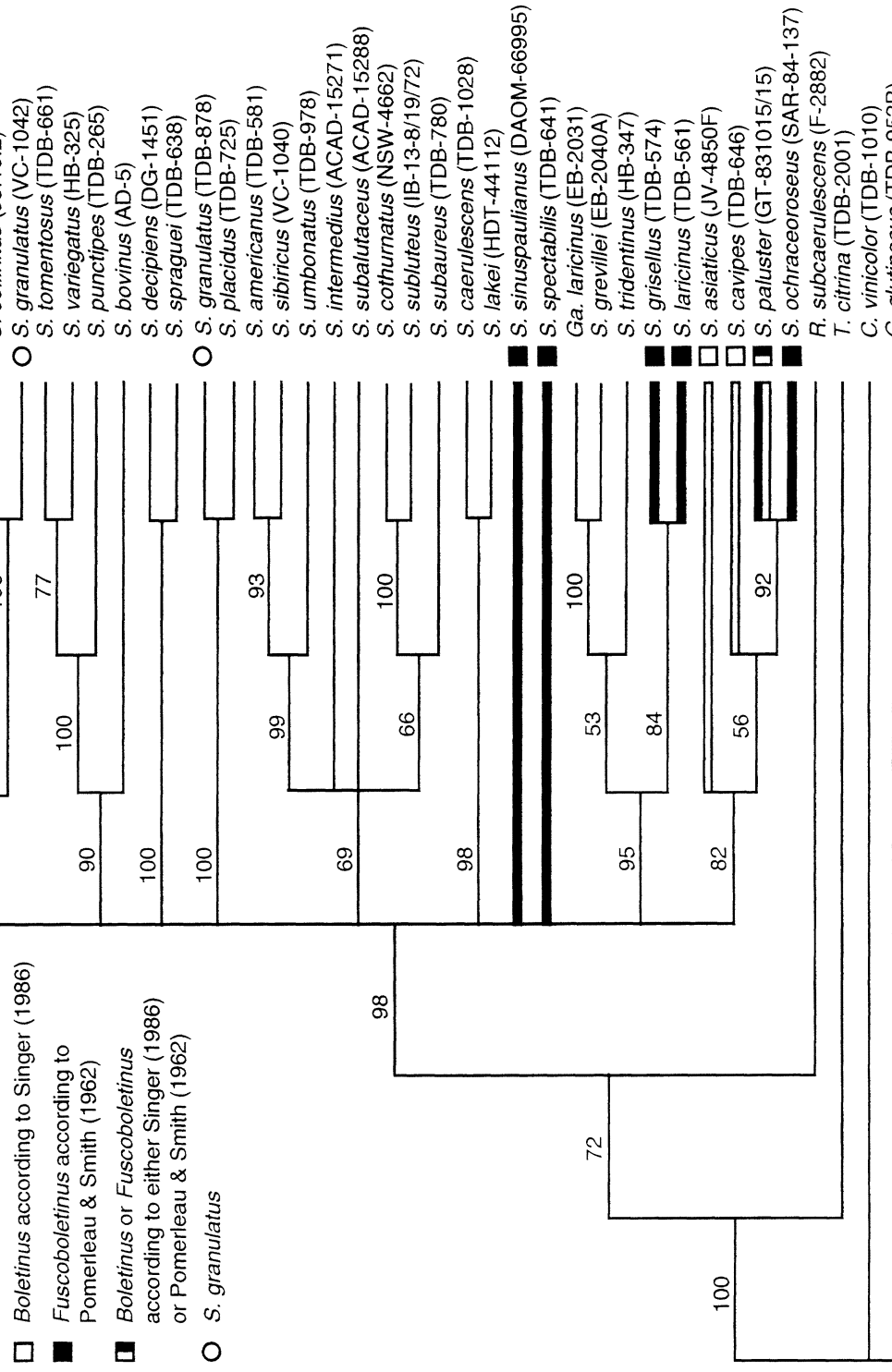
mens listed in TABLE I. In most cases, the sequences include the complete 5.8S rRNA gene. They have been submitted to the Genome Sequence Data Base under the accession numbers L54074 to L54121.

Alignment of the full set of sequences was in some areas ambiguous. Inclusion or exclusion of these areas did not, however, seem to influence the main conclusions drawn from the study, nor did differential weighting of transitions and transversions (either equal or transversions twice over transitions). Long branches essentially proved to be well supported independent of the treatment, whereas short branches were unstable. Results presented in FIGS. 1 and 2 are based on unweighted analyses from which areas of ambiguous alignment have been excluded. Under these conditions, the data matrix comprised 676 characters out of which 168 were cladistically informative. FIG. 1 summarizes results from parsimony analysis; only branches with more than 50% support by bootstrap analysis are shown. FIG. 2 presents a neighbor joining tree derived from the same dataset. Although a number of branches on the neighbor joining tree are weakly supported (as can be seen by comparison to FIG. 1), it currently represents our best phylogenetic estimate.

Finally, we have assessed intraspecific sequence variation and relationships between closely related taxa in a number of separate analyses, and the results are shown in FIG. 3. Trees were constructed from the corresponding subset of taxa by neighbor joining. As only closely related species were included in each dataset, sequences could be aligned unambiguously, and no areas of ambiguous alignment needed to be excluded. Transitions and transversions were, again, weighted equally.

DISCUSSION

Molecular phylogenetic analyses presented in this paper strongly support the concept of a genus *Suillus* sensu lato as introduced by Bruns and Palmer (1989) which comprises both the genera *Boletinus* and *Fus-*



coboletinus. Based on ITS data, neither *Boletinus* nor *Fuscoboletinus* as defined by Singer (1986) and Pomerleau and Smith (1962), respectively, represents a monophyletic group. Several well supported branches render *Fuscoboletinus* polyphyletic, and the shortest trees showing *Fuscoboletinus* as a monophyletic genus were 33 steps longer than the shortest trees found overall (731 versus 698 steps). *Boletinus,* on the other hand, was found to be paraphyletic, because a strongly supported branch placed *Suillus ochraceoroseus* in the middle of the *Boletinus* clade. *S. ochraceoroseus* had originally been described as *Boletinus ochraceoroseus* (Snell and Dick, 1941) but was moved into *Suillus* by Singer, when he narrowed the concept of *Boletinus* to those species that had clamp connections in their fruitbodies (= *B./S. asiaticus, B./S. cavipes, B./S. paluster*) (Singer, 1962, 1986). According to Singer's older and broader genus concept however, *Boletinus* as a whole is again not monophyletic but polyphyletic, because it includes *S. ochraceoroseus* with *S. spraguei, S. spectabilis, S. grisellus, S. lakei, S. decipiens* and some additional taxa that were not treated in this study (Singer, 1962). Altogether, the only natural clade that seems to correlate fairly well with any concept for the genus *Boletinus* comprises the species *asiaticus, cavipes, paluster,* and *ochraceoroseus*. Whether these species are to be kept in a separate genus, seems to be a matter of taste. However, we do not support it for several reasons: (i) Beside molecular evidence, there are no morphologic or physiologic synapomorphies known that differentiate this clade from *Suillus*. (ii) Based on our molecular data, the clade is not strikingly distant from *Suillus*. (iii) In fact, there is no strong evidence for the "*Boletinus*" clade being basal within *Suillus* sensu lato, but a basal position is a necessary requirement for both *Suillus* and "*Boletinus*" to be monophyletic.

Other than *Boletinus* and *Fuscoboletinus, Suillus* sensu lato appears to be monophyletic with the exception of *Gastrosuillus laricinus* which is a recent derivative of *Suillus grevillei* (Baura et al., 1992) and probably other Gastrosuilli (unpublished results). As we are planning more detailed studies on the genus *Gastrosuillus,* we will discuss this problem at a later point. For now, we propose to collapse both the genera *Boletinus* and *Fuscoboletinus* into *Suillus*. As neither *Boletinus* nor *Fuscoboletinus* has been consistently recognized by authors in the past, most of the species have at some point already been included in *Suillus*. Those species that haven't and that are treated or mentioned in this study are transferred below.

Isolates of *Suillus granulatus* derived from either North America (*S. granulatus* TDB-878) or Europe and Asia (*S. granulatus* AD-11 and *S. c. f. granulatus* VC-1042) were widely separated into different clades by strongly supported branches. These findings are in agreement with earlier studies based on mating behavior and the physiology of mycorrhiza formation which indicated that isolates from America and Europe (Fries and Neumann, 1990) or America and Asia (Jacobson and Miller, 1992) might represent two different species. To our knowledge, no comparison has so far been made between isolates from Europe and Asia. Although isolates from Sweden and Nepal were more closely related to each other in this study than either of them was to North American isolates, they were still separated by a strongly supported branch which puts *S. collinitus* next to *S. granulatus* from Nepal and makes these two organisms paraphyletic to the Swedish *S. granulatus* and others. In addition, *S. granulatus* associates with a very different pine in Sweden than in Nepal (*Pinus sylvestris* L. section *Pinus* versus *Pinus wallichiana* Jackson section *Strobus*). Finally, *S. c. f. granulatus* from Nepal also seems to be morphologically distinct from other organisms being classified as *S. granulatus* in that it has a less mottled cap and sometimes stains slightly blue green to blue at the stipe base (Cotter, 1987). Overall, current evidence suggests that there might be three different taxa to be recognized within the *Suillus granulatus* complex, but more isolates need to be included in future studies to fully resolve the genetic heterogeneity within this complex. There are at least two more members of the *S. granulatus* complex that need to be examined: (i) another Asian *S. granulatus* from Korea which grows with *Pinus densiflora* Sieb. and Zucc. from the section *Pinus* (in contrast to the Nepalian isolate included in this study that grows with *Pinus wallichiana* from the section *Strobus*); (ii) *S. granulatus* found in the southwestern United States growing with *Pinus ponderosa* Laws. (section *Pinus*), while other North American isolates (includ-

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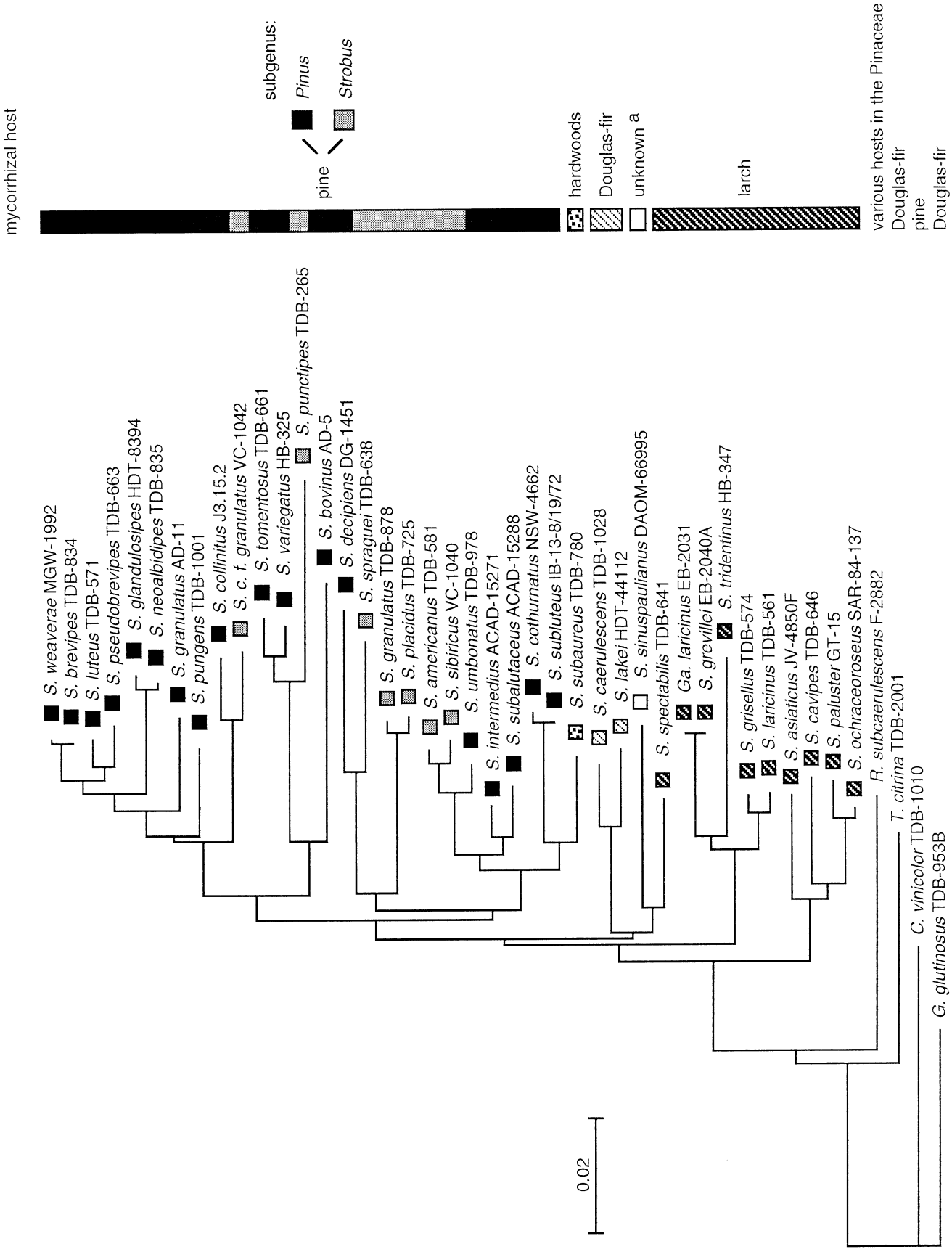
FIG. 1. Phylogenetic analysis of the ITS sequence data: Bootstrap analysis under parsimony criterion showed that most of the major lineages are recognized with reasonable confidence, while relationships among them are not. From the major lineages it is evident that neither *Boletinus* nor *Fuscoboletinus* as defined by Singer (1986) and Pomerleau and Smith (1962), respectively, is monophyletic. Furthermore, isolates of *S. granulatus* derived from either North America (*S. granulatus* TDB-878) or Europe (*S. granulatus* AD-11) and Asia (*S. c. f. granulatus* VC-1042) are polyphyletic and do not seem to represent the same species. Branch lengths are arbitrary.



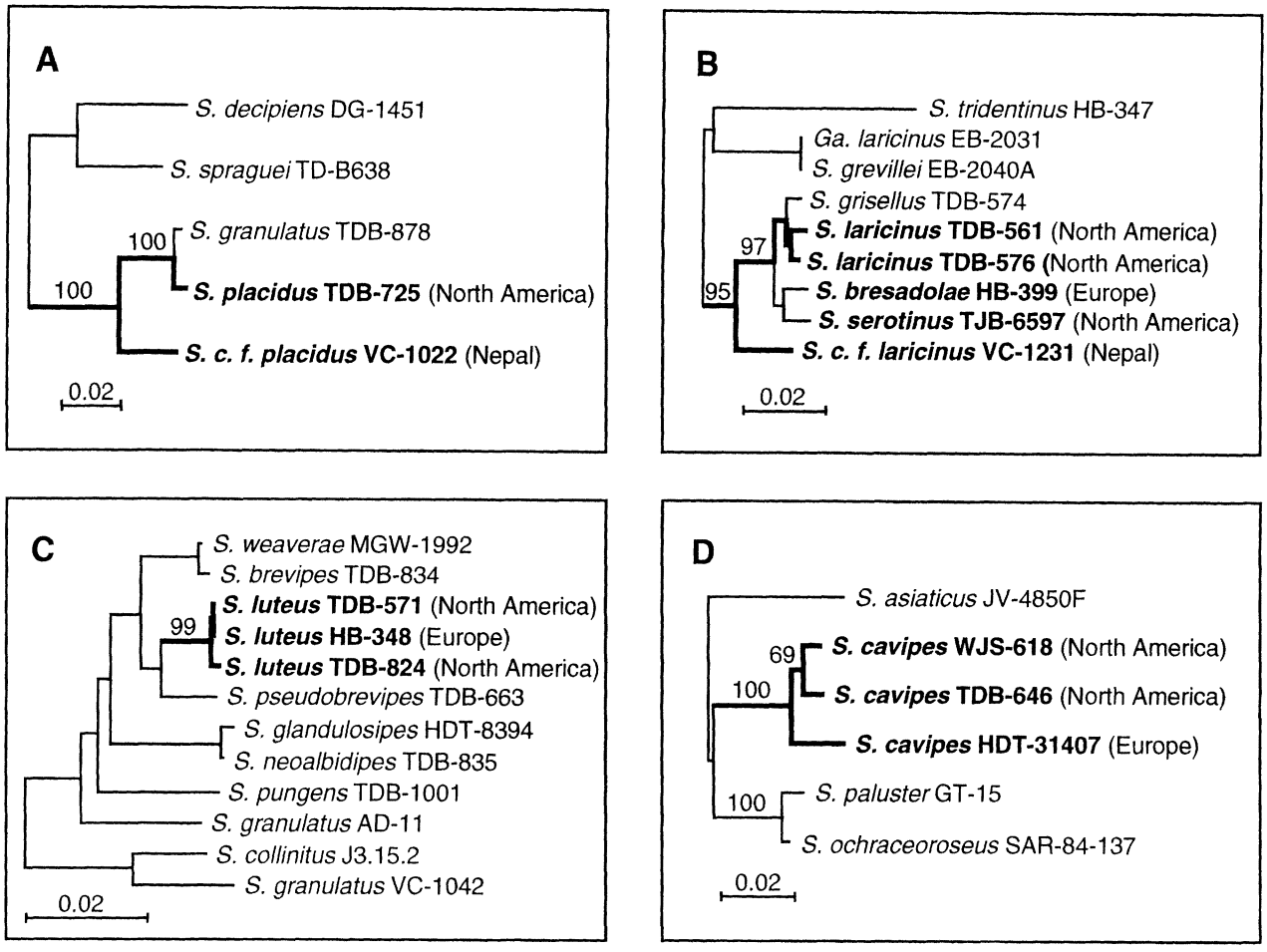


FIG. 3. Assessing intraspecific sequence divergence: Bootstrap values are given for selected branches of interest only when above 60%. Horizontal branch lengths represent mean distances (Swofford, 1993).

ing TDB-878) grow with *Pinus strobus* L. (section *Strobus*).

Besides the *S. granulatus* problem, minor conflicts with current species concepts have been observed in two more cases both of which involve specimens from Nepal: (i) *Suillus c. f. placidus* from Nepal has been described as fitting current species descriptions well but not completely, although observed differences were not explicitly stated (Cotter, 1987). We found *S. c. f. placidus* VC-1022 (Nepal) and *S. placidus* TDB-725 (North America) to be fairly closely related, but separated by a well supported branch which puts American *S. placidus* TDB-725 and American *S. granulatus* TDB-878 tightly together and makes the two *S. placidus* isolates from either North America or Nepal paraphyletic instead of monophyletic (see FIG. 3A). (ii) Finally, the isolate VC-1231 (Nepal) of *Suillus laricinus* fits into the clade of *S. laricinus* and relatives but is paraphyletic to *S. laricinus* sensu stricto (FIG. 3B). The species concept for *S. laricinus* has been considerably narrowed in the last decades by elevating the former variants *S. bresadolae* and *S. serotinus* to the level of independent species (Gerhold, 1985, and Smith and Thiers, 1971, respectively). Fu-

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FIG. 2. Neighbor joining tree representing a best tree estimate: Within the phylogeny of *Suillus* sensu lato, mycorrhizal associations with larches seem to be primitive and switches to different host genera such as pines, Douglas-fir, or hardwoods seem to have occurred only once. On the other hand, host switches between pines of the different subgenera *Pinus* or *Strobus* seem to be rather frequent. The shown host specificities are based on observations of fruiting habits both by the authors and other authorities (Smith and Thiers, 1964; Smith and Thiers, 1971; Singer, 1986; Cotter, 1987). Horizontal branch lengths represent the mean distances (Swofford, 1993). [a] Fruitbodies have been found in mixed conifer forests under *Pinus, Abies* and *Picea*.

ture work including more isolates should show whether this refined species concept is supported by molecular data. If that is the case, a new species will be needed to accommodate "*S. laricinus*" from Nepal which might also be supported by the unusual staining pattern observed on these specimens (Cotter, 1987).

Intraspecific sequence divergence was less pronounced in two more cases examined and did not conflict with current species concepts (FIG. 3C and D). *Suillus luteus* was the least divergent species looked at with only one base difference even between isolates from Europe and North America. This finding is in agreement with the idea of a recent introduction of *S. luteus* into North America. *Suillus cavipes* formed a divergent but monophyletic group, with the European isolate HDT-31407 being more distantly related to the two American isolates WJS-618 and TDB-646.

Our best tree estimate shown in FIG. 2 suggests some evolutionary aspects that are interesting to note though they are not strongly supported by the data. First, the pattern of host specificity within *Suillus* sensu lato does not seem to have evolved through cospeciation with the ectomycorrhizal hosts. In conflict with the idea of cospeciation is primarily the finding that, while *Larix* is considered to be more recently derived than *Pinus* (Hart, 1987), larch association seems to be primitive in *Suillus,* and associations with pines, Douglas-fir, and hardwoods seem to be derived. Rather than cospeciation, multiple jumps to new host species and genera seem to have occurred during the evolution of *Suillus* sensu lato. While changes in host specificity to closely related hosts seem to be fairly frequent events (at least five changes between the pine subsections *Pinus* and *Strobus* are implied in FIG. 2), all changes to new host genera seem to be unique events in the evolution of *Suillus* sensu lato. Changes in host specificity have also occurred in the outgroups, *Rhizopogon, Truncocolumella* and the Gomphidiaceae, but are not discussed in this paper. Finally, the most primitive clade within *Suillus* sensu lato seems to be formed by organisms with a very pronounced boletinoid hymenophore, that is with radially strongly elongated pores. It seems that the boletinoid hymenophore represents an intermediate evolutionary state between the gills found in related genera such as *Gomphidius* and *Chroogomphus* and the pores found in *Suillus*. This is basically an old idea; Smith and Thiers (1964) stated: "We find a tendency toward a lamellate type of hymenophore in *Fuscoboletinus paluster,* and it is only a step from this group to the *Gomphidiaceae,* whose species are truly lamellate." The lines and directions of evolution, however, have started to become clear only recently: Within the suilloid group, tubes seem to be derived from gills (Bruns and Szaro, 1992).

TRANSFER OF SPECIES

Because the molecular evidence presented in this paper suggests that both genera *Boletinus* and *Fuscoboletinus* should be collapsed into *Suillus,* we formally propose to transfer a number of species included in this analysis to *Suillus*:

Suillus asiaticus (Singer) Kretzer & Bruns, comb. nov.
Basionym: *Boletinus asiaticus* Singer. *Rev. Mycol.* 3. 164. 1938.

Suillus grisellus (Peck) Kretzer & Bruns, comb. nov.
Basionym: *Boletinus grisellus* Peck. *Mem. N. Y. State Museum* 4: 169. 1900.
Synonym: *Fuscoboletinus grisellus* (Peck) Pomerleau and Smith. *Brittonia* 14: 168. 1962.

Suillus paluster (Peck) Kretzer & Bruns, comb. nov.
Basionym: *Boletus paluster* Peck. *Ann. Rept. New York State Cab.* 23: 132. 1872.
Synonyms: *Boletinus paluster* (Peck) Peck. *Bull. N.Y. State Museum* 2: 78. 1889.
Boletinellus paluster (Peck) Murrill. *Mycologia* 1: 8. 1909.
Fuscoboletinus paluster (Peck) Pomerleau and Smith. *Mycologia* 56: 708. 1964.

Suillus serotinus (Frost) Kretzer & Bruns, comb. nov.
Basionym: *Boletus serotinus* (Frost) *Bull. Buffalo Soc. Nat. Sci.* 2: 100. 1874.
Synonym: *Fuscoboletinus serotinus* (Frost) Smith and Thiers. *Boletes of Michigan,* p. 85. 1971.

Suillus weaverae (Smith and Schaffer) Kretzer & Bruns, comb. nov.
Basionym: *Fuscoboletinus weaverae* Smith and Schaffer, *Mich. Bot.* 4: 27. 1965.

ACKNOWLEDGMENTS

The authors want to thank Dr. Van Cotter, Dr. Anders Dahlberg, Dr. Helmut Besl, and Dr. Daniel Mousain for providing mycelium cultures, fruitbody collections, or fungal DNA, Dr. Dennis Desjardin for proofreading the manuscript, and the DFG (Deutsche Forschungsgemeinschaft) for financial support.

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