Mycologia, 102(2), 2010, pp. 438–446. DOI: 10.3852/09-149

Suillus quiescens, a new species commonly found in the spore bank in California and Oregon

Thomas D. Bruns!
Department of Plant and Microbial Biology, University of California, Berkeley, California 94720-3102

Lisa C. Grubisha
USDA-ARS, Department of Plant Sciences, University of Arizona, P.O. Box 210036, Tucson, Arizona 85721

James M. Trappe
Department of Forest Science, Oregon State University, Corvallis, Oregon 97331-7501

Jennifer F. Kerekes
Else C. Vellinga
Department of Plant and Microbial Biology, University of California, Berkeley, California 94720-3120

Abstract: *Suillus quiescens* sp. nov. is common under *Pinus muricata* on Santa Cruz and Santa Rosa Islands in the northern Charmel Islands of California, and we subsequently found it fruiting at Point Reyes National Seashore on the central coast of California. Sequences from the internal transcribed spacer region show that it is distinct from all 44 species of *Suillus* tested, and features of its morphology separate it from all other unsequenced species. *Suillus quiescens* has a broader distribution than coastal California because it also was encountered as ectomycorrhizae on roots of pine seedlings from the eastern Sierra Nevada, coastal Oregon and the southern Cascade Mountains. The reason it had not been identified from these areas might be due to its resemblance to S. *brevipes* at maturity or it might be a rare fruiter that persists in the spore bank.

Key words: bioassay, suilloid-specific primers, *Suillus brevipes*, S. *occidentalis*, S. *volcanalis*

INTRODUCTION

We describe a new species, *Suillus quiescens*, that we encountered in 2002 while collecting on Santa Cruz Island where it and S. *pungens* Thiers & Smith were the only two *Suillus* species that were abundan tly fruiting in association with native *Pinus muricata* (Grubisha et al. 2005).

We determined the nucleotide sequence from the internal transcribed spacer region (hereafter ITS) of several specimens of *Suillus quiescens* to compare it to other similar *Suillus* species. We turned to the **ITS** region because it had been sequenced from a broad sample of genus *Suillus* (Kretzer and Bruns 1997, Kretzer et al. 1996, Manian et al. 2001, Wu et al. 2000). In addition we acquired **ITS** sequence from collections of S. *uolcanalis* Thiel'S and S. *occidentalis* Thiers, two species that are morphologically similar to S. *quiescens* but previously were unsequenced.

Submitted 23 Jun 2009; accepted for publication 9 Sep 2009.
!Corresponding author. E-mail:pogon@berkeley.edu

MATERIALS AND METHODS

Standard methods for describing the basidiocarps were applied with the terminology of Smith and Thiel'S (1971). Color annotations in the macroscopic descriptions are from Kelly and Judd (1976). The notation [72, 7, 7] indicates that measurements were made on 72 spores in seven samples in seven collections. These abbreviations are used: avl for average length, avw for average width, Q for quotient of length and width and avQ for average quotient.

Amplification and sequencing.-DNA was extracted with a modified protocol of the REDExtract-N-Amp^{TM} Plant PCR Kit (Sigma-Aldrich Co., St Louis, Missouri) as follows: 10 uL extraction solution, incubated at 65 C for 10 min followed by 95 C for 30 min, 30 uL dilution solution was added and left at room temperature 3 h. The **ITS** region was generally amplified with the ITS-lf/ITS-4b primer set with an Eppendorf Mastercycler Gradient thermocycler under conditions described by Gardes and Bruns (1993). **In** the case of older specimens or slightly moldy specimens, such as holotypes of S. v*olcanalis* and S. *occidentalis*, we anticipated problems with degraded target DNA or DNA from molds. To circumvent these problems we designed Suilloid specific primers ITS-2S 5'-AAGATTCGATGATTCACTGTAG-3'and ITS-3S 5'GTAAATTCTCAACCCCTCTCGA-3 that are respectively 31 and 108 bp upstream from the ITS 2/ITS3 primer site. We designed these new primers by eye from an alignment with nine *Suillus* species and seven more distantly related basidiomycetes *(Tapinella, Sebacina, Thelephom, Cortinarius, Tricholoma, Coltricia* and *Chaiciporusv,* These primers were designed to be perfect matches with all *Suillus,* while mismatching nonsuilloid taxa at the 3' end. BLAST analyses revealed that the sequences selected were perfect matches only to *Suillus* and the related genera, *Rhizopogon, Truncocolumella, Chroogomphus* and *Gomphidius.* These two primers were used to amplify the **ITSI** and ITS2 regions (White et al. 1990) with primer sets ITS-HI ITS-2S and ITS-3S/ITS-4b under conditions described by Gardes and Bruns (1993). PCR products were cleaned with 0.5 uL ExoSAP IT (USB Corp., Cleveland, Ohio) and cycled at 37 C for 45 min, followed by 80 C for 15 min. Sequenc-

ing was performed with an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, Foster City, California). Sequences were edited and contigs assembled with Sequencher 4.2.2 (Gene Codes Corp., Ann Arbor, Michigan).

Phylogenetic anaivses- Suillus ITS sequences were retrieved from GenBank with the Emerencia interface Website (http://andromeda.botany.gu.se/genussearch.html).Sequences that did not contain both ITSI and ITS 2 spacers were removed, and when large numbers of nearly identical species were encountered a subset of these were selected. Preference was given to published sequences and those in the UNITE database (Koljalg et al. 2005).

These identified *Suillus* sequences from GenBank were used in the analysis: S. *amaranthii:* U74615.1; S. *americamus:* L54103.1; S. *asiaticus.* AF166504, L54090.1; S. *bellinii:* AJ419216.1; S. *bovinus:* AJ272401.1, AJ272402.1, AJ272403.l, AJ419215.1, AJ419934.1, AJ419935.1, AJ493679.], L54077.l; S. *bresadolae:* L540S4.1; S. *breoipes.* AY8S0941.1, L5411 1.1; U74620.1; S. *cauipes:* L540S5.1, L54105.1, L54119.1; S. *collinitus:* AJ410S57.1, L540S9.1; S. *cothurnatus:* AJ419217.1, AJ41921S.1, L54092.1; S. *decipiens:* AF16650S, AF166510, L54079.1; S. *flaoidus:* AY641461.1,UDB00I214,UDB00k54S, *UDBOOI649;Sjluryi:* AJ272407.1; S. *glandulosipes:* L540S7.1; S. *granulatus:* AB2S4447.1, AJ27240S.1, I\J272409.1, AJ272410.1, L54076.1, L54113.1, L54121.1, UDB000650, UDB000666; S. *grevillei:* M91612.1, M91614.1, M91616.1; S *intermedius:* L54074.1; S. *lahei* L540S6.1; S. *laricinus:* AJ272400.1, L54099.1, L54102.1; S. c.f *laricinus* L54120.1; S. *luteus* AFI66511, AJ272411.1, AJ272413.1, AJ272414.1, AJ272415.1, AJ272416.1, AJ419219.1, L540S3.1, L54100.1, L54110.1, UDBOOI650; S. *mediterraneensis:* AJ410S60.1; S. *neoalbidipes:* AYSS0940.1, L54112.1; S. *ochraceoroseu.s:* L54093.1; S. *poluster:* L540S0.1; S. *placidus:* AB2S4441.1, AB2S4443.1, DQ407265.1, L5411S.1, L5410S.1; S. cf. *placilitis:* L5411S.1; S. *plorans.* AJ272417.1; S. *pseudobreoipes:* AY8S093S.1, L54107.1; S. *pu.nctipes:* L5409S.1; S. *pungens* L54095.1; S *serotinus:* L54116.1; S. *spraguei:* AF166519, AF166520, AF166522, AF166525, AY854069.1, M91617.1; S *spect.abilis:* L54104.1; S. *sibiricus.* AF166512, AFI66513, L54117.1; S. *sinrcspaulian.u.s:* L5407S.1; S. *subau.reu.s:* L54109.1; S. *subluteus:* L54075.1, L540SS.1; 5,'. *suilloides:* U74616.1, U74617.1, U7461S.1; S. *tomentosus:* AY8S0937.1, L54106.1, U74614.1; S. *tridentinus:* AJ419220.1, L540S2.1; S. *umbonatus:* AF166526, AY8S0939.1, L54115.1; S. v*ariegatus* AF231914, AJ272419.1, AJ272420.1, AJ27242 1.1, L540Sl.l; S. weaverae L54091.1.

These environmental *Suillus* sequences were retrieved from GenBank: AF476994.1, AJ272405.1, AJ272406.1, AJ410S57.1, AY097052.1, AY5S7754.1, AYSS0932.1, EF45S012.1, EF61976S.1, DQ351501.1, DQ351502, DQ351503.1, DQ351504.

These newly derived sequences were included: S *breoipes* (UC1S06032S) GQ2493SS, (UC1S060327) GQ2493S9; S. *quieseens* (Holotype UC1S603(6) GQ249402, (UC1S0310) GQ249390, (UC1S60312) GQ249391, (UC1S6030S) GQ249393, (UC1S59739) GQ249401, (bioassay-no specimen) GQ249392; S *occidentalis* (HDT2S 175) GQ249394, (STBS6) GQ249395, (STBS2) GQ249396, (STBI41S) GQ249397; S. v*olcanalis* (HDT39S73) GQ24939S, (HDTI2S00 holotype) GQ249399; S. *megaporinus* (UC1S060326) GQ249400.

The initial analysis used 134 ITS sequences representing all *Suillus* species and *Suillus* environmental sequences available (see METHODS and MATERIAS for list). These were aligned with Clustal X 2.09 (Larkin et al. 2007) and trimmed to800 characters (including gaps). The sequences were analyzed by neighbor joining and by parsimony with PAUP 4.0d8I (Swofford 2002) running in classic mode on a Mac (1.9 GHz PowerPC). Neighbor joining trees used likelihood distances with a transition/transversion rate set to 2 and gaps treated as missing data. Parsimony analysis employed a heuristic searches from a random starting tree, TBR branch swapping and MAXTREES set to 10000. The consensus parsimony tree and the neighbor joining tree (not shown) grouped all *Suillus quiescens* sequences with a small set of unidentified environmental sequences in a unique clade nested among other species in the granulatus group *(s. granulatus* [L.] Roussel, S. *granulatus sensu* Thiel's & A.H. Sm. 1971, S. *breuipcs,* S. *glandulosipes* Thiel's & A.H. Sm., S. *neoalbidipes* M.E. Palm & E.L. Stewart, S. *luteus* [L.] Roussel, S. *pseudobreoipes* A.H. Sm. & Thiers, S. *flavidus* [Fr.] J. Presl, S. *mediterraneensis* /Jacquet. & J. Blum] Redeuilh, S. *bellinii* [Inzenga] Watling, S. *collinitus* [Fr.] Kuntze, S *ooicanaliss,*

All latter granulatus-like sequences inclusive of S. *quiescens* sequences, plus additional sequences of S. *collinitus, Sjluryi* Huijsman, S. *occidentalis* and S. *oolcanalis* (63 in total), where realigned with Clustal X and visually adjusted. This alignment, which had many fewer gaps, was trimmed to 695 positions. The dataset was analyzed with neighbor joining and parsimony as indicated above. In addition branch strengths were assessed by 10000 bootstrap replicates with the fast step-wise addition option and with 10000 jackknife with 33% deletion and the fast step-wise addition. Both data matrices and the tree shown were submitted to TreeBASE (study S2452; matrices M4663, M4664)

RESULTS AND DISCUSSION

The four ITS sequences of S. *quiescens* fruiting bodies, combined with five environmental sequences derived from mycorrhizal root tips, form a distinct clade among the granulatus-like species (FIG. 1). Branch lengths, bootstrap and jackknife values that support the S. *quiescens* clade are similar to those associated with other well recognized species in the group. These results show that S. *quiescens* is clearly distinct from S. *brevipes,* S. *valcanalis* and S. *occidentalis,* the three species that it macroscopically resembles.

We initially thought that S. *quiescens* could be a variant of S. *brevipes* because S. *quiescens* differs morphologically from S. *brevipes* primarily by a much paler colored immature pileus and by minute, colored glands on the stipe that develop with age. Although initial BLAST and phylogenetic analyses showed the two to be different all the available S.

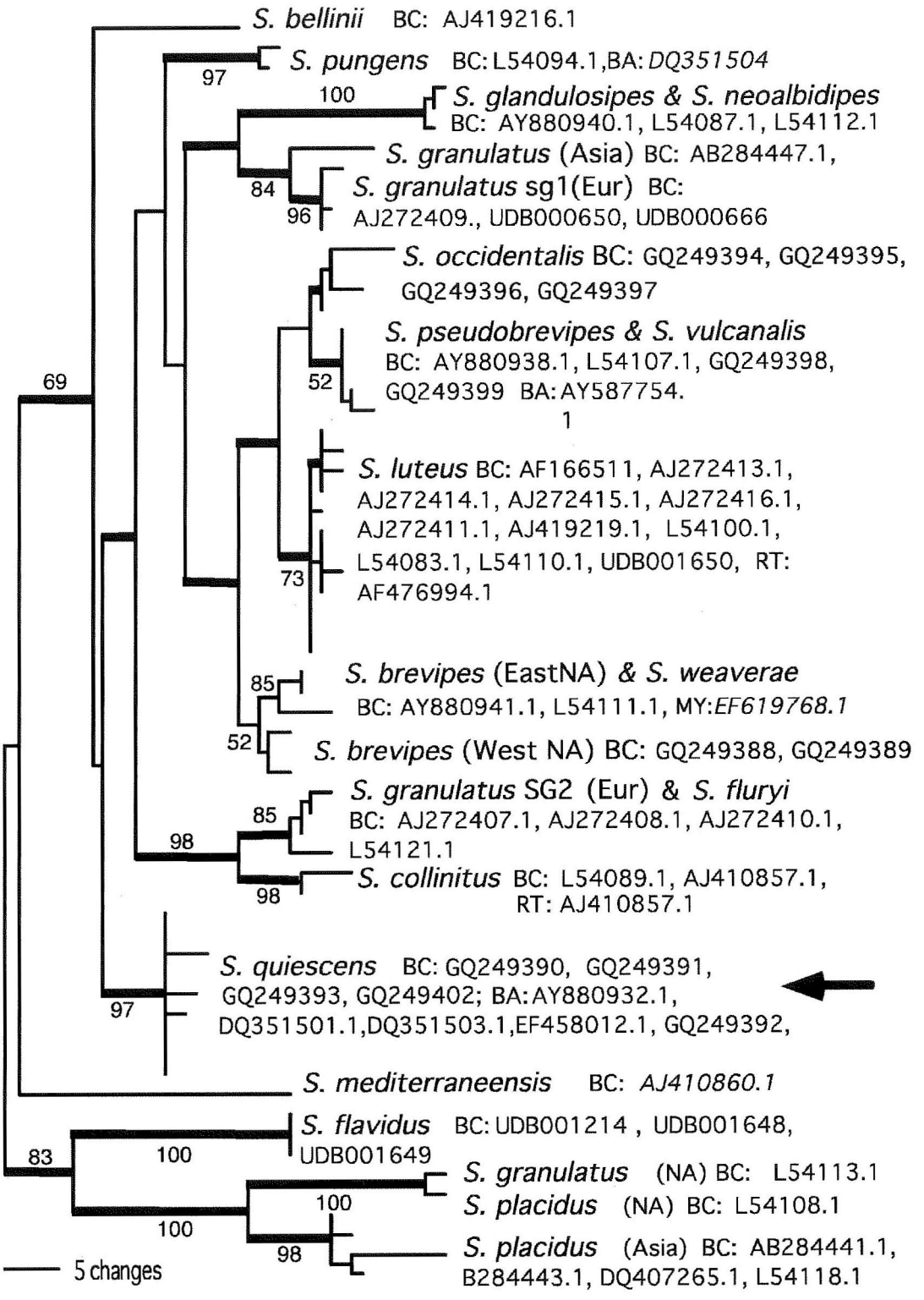

FIG. 1. Parsimony analysis of the ITS sequences for the granulatus group of *Suillus*. Arrow indicates *Suillus quiescens* clade. A random tree from the 10 000 trees of 305 length is shown. Bold branches indicated those present in the strict consensus of all 10 000 trees. Numbers indicate bootstrap support; jackknife values (not shown) were almost identical. GenBank accession numbers for the ITS sequences analyzed are derived from basidiocarps (BC), root tips (RT) of forest trees, soil mycelium (MY) and seedling bioassays of spores (BA).

brevipes sequences were from eastem North America and it seemed possible that the western material might be different and perhaps synonymous with our collections. For that reason we added two sequences of S. *breoipes* from western North America. We found that the sequences derived from western S. *brevipes* were slightly different from those derived from the eastern material, but none were closely related to S. *quiescens* (FIG. 1).

Suillus volcanalis presented a problem for comparison because the key characters of the species seemed like our material (Thiers 1979), but the name S. *volcanalis* is not often applied and is represented by only a few collections. The first ITS sequence that we acquired for it was from a collection *by* Harry Thiers in 1979 at a site near the holotype location. The near perfect match of this sequence to S. *pseudobrevipes* brought up me possibility that the species identification for the S. *volcanalis* collection was wrong. However we were able to acquire partial ITS sequence from the holotype (from 1965) and this too was found to be nearly identical to both S. *pseudobreoipes* and to the later collection of S. v*olcanalis,* but all these sequences were different from S. *quiescens.*

Although S. *occidentalis* is not known from California, it does appear to be reasonably common in Arizona, the area from which it originally was described (Thiers 1976b). Recent photos posted on the Web by Dr Scott Bates of Arizona State University looked very similar to what we were calling S. *quiescens.* However the sequences we obtained from a Thiers collection of S. *occidentalis* and three others derived from material sen t to us from Dr Bates proved to be quite similar to one another but distinct from S. *quiescens* (FIG. 1).

ITS sequences are not yet available for several other species of granulatus-like *Suillus* in western North America, but the morphological characters for these species are distinctly different from S. *quiescens.* Among those species with sparsely glandular, non-annulate stipes and glutinous non fibrillose pilei the following species were considered and rejected as possible identifications for our material. *Suillus brunnescens* A.H. Sm. & Thiers is different by its initially white pileus that stains brown, *by* slightly broader spores (3.8-4.2 vs. 2.4-3.7 11 um)and by its association with five-needle pines (Smith and Thiers 1964). Similarly S. *pallidiceps* A.H. Sm. & Thiers has an initially white pileus and a stipe that lacks dark glands even at maturity (Smith and Thiers 1964). *Suillus wasatchicus* Thiers, although possibly similar in color, has more conspicuous glands on the stipe. S. *kaibabensis* Thiers has pinkish pileus and tubes and a strongly glandular stipe (Thiers 1976a).

Sequences of S. *quiescens* were found to be close matches to some unidentified *Suillus* sequences that were retrieved from mycorrhizae of pine seedlings from multiple locations and studies in Oregon and California (FIG. 1, TABLE). All these reports were from pine seedlings and most were from bioassays in which pines were planted in test soils and grown under laboratory or greenhouse conditions to assay for spores or other resistant propagules. Although *Suillus* species are not uncommon in such assays (TABLE) their frequency of occurrence generally reaches double digits only when the soil is assayed during the fruiting season, without drying or dilution, as was done in the Oregon study by Ashkannejhad and Horton (2006). In California, which tends to be drier than the Oregon site sampled by Ashkannejhad and Horton (2006), such assays usually are dominated by *Rhizopogon* and retrieve only a low percentage of *Suillus* (Kjoller and Bruns 2003, Rusca et al. 2006). Furthermore spatial frequency of *Suillus* spores within a site appears to be much lower than that of *Rhizopogon* (Izzo et al. 2006) and there is some evidence that *Suillus* spores do not retain their viability as long as *Rhizopogon* (Ashkannejhad and Horton 2006). However spores of some *Suillus* species, including S. *quiescens,* do appear to be fairly resistant to soil heating (Peay et al. 2009), and so it is interesting that sequences of S. *quiescens* also were retrieved from pine seedlings planted in steam-pasteurized soil that then were planted in the field near Bend, Oregon (Warren et al. 2008).

The above shows that S. *quiescens* is distributed from at least the northern Channel Islands (near Santa Barbara) to coastal and central Oregon, and the eastern Sierra Nevada (near Mammoth, California), and in most of these areas it is known only from the spore bank. The lack of observed frniting could be because the species has been confused with S. *breoipes* and simply overlooked. However in Point Reyes National Seashore, where the senior author has collected for two decades, it does not appear to be a common frniter, even though it has been retrieved from the spore bank there in two independent studies. Furthermore on Santa Crnz Island, where it is a common fruiter, 18% of the seedling bioassays yielded S. *quiescens* instead of the intended *Rhizopogon* species. In contrast S. *pungens,* which was also a common frniter on the island, colonized only 2% of the seedlings (Grubisha et al. 2007). These results suggest that S. *quiescens* is over-represented in the spore bank studies relative to other *Suillus* species, and we hypothesize that S. *quiescens* is an early successional species mat frnits primarily in young forests and resides in the spore bank for extended periods. Some species in the sister genus *Rhizopogon* are known to have long lived spores and thought to

TABLE I. Reports of *Suillus* species identified from bioassay studies in western USA

Species	%	Region	Reference
S. brevipes	13[a]	Oregon central coast	Ashkannejhad and Horton 2006
	2	California central coast (Point Reyes)	Peay et al. 2009
S. pungens	<1	Southeastern Sierra Nevada	Rusca et al. 2006
	2	California central coast (Point Reyes)	Peay et al. 2009
	8[b]	California central coast (Point Reyes)	Kjøller and Bruns 2003
	2	Santa Cruz Island, California	Unpubl from Grubisha et al. 2007
S. pseudobrevipes	<1	Southwestern Sierra Nevada, California	Izzo et al. 2006
		California central coast	Peay et al. 2009
S. quiescens	8[a]	Oregon central coast	Ashkannejhad and Horton 2006
	<1	Southeastern Sierra Nevada	Rusca et al. 2006
	3	California central coast (Point Reyes)	unpubl from Bruns et al. 2009
	1	California central coast (Point Reyes)	Peay et al. 2009
	18	Santa Cruz Island, California	unpubl from Grubisha et al. 2007
S. tomentosus	20[a]	Oregon central coast	Ashkannejhad and Horton 2006
	3	California central coast (Point Reyes)	Peay et al. 2009
	17[b]	Northern California coast (Salt Point)	Kjøller and Bruns 2003
S. umbonatus	15[a]	Oregon central coast	Ashkannejhad and Horton 2006

[a] Averaged from their three site types.
[b] Based on culture; numbers not fully comparable to others.

exhibit such strategies (Bruns et al. 2009), but so far the limited data on *Suillus* suggest that they have short lived spores (Ashkannejhad and Horton 2006).

Suillus is among the best known genera in North America and Europe, but as shown by S. *quiescens* species remain undescribed even in regions that have been fairly well collected. Some apparently undescribed *Suillus* species are known and common but currently are lumped in morphological concepts that are too broad. S. *granulatus* and *S.placidus* are stellar examples of such species complexes that are clearly illustrated by ITS data shown here (FIG. 1) and reported elsewhere (Kretzer et al. 1996, Manian et al. 2001). *Suillus* also contains species that have been described but are not often collected or perhaps are not recognized when they are collected; ITS can be very useful in these cases as well. In the current study ITS data retrieved from historical collections of S. *vulcanalis* and S. *occidentalis* were essen rial to establish that S. *quiescens* had not already been described. Although some of the specimens involved were more than 40 y old amplifying the ITS region as two pieces and using specific primers let us work around the problems of degraded DNA and contributions from secondary molds. The limitations of ITS for species-level determinations also are illustrated here because some pairs of species are not distinguished by this locus (s. *glandulosipes* and S. *neoalbidipes,* S. *pseudobrevipes* and S. *vulcanalis,* S. *brevipes* and S. *weaverae).* These pairs might be due to over-description (i.e. synonymy) or to the lack of ITS divergence among sibling species, but until other useful loci are developed for the genus this will remain an unresolved issue.

TAXONOMY

Suillus quiescens T.D. Bruns, and E.G Vellinga sp. nov. FIGs. 2-3

MycoBank MB 515081.

A *Suillo* brevipede juventute pileo pallide lutcibrurmeola sed rnaturitate fuscicinnamomescens, punctis glandulosis tenuissimis in stipite producentibus, apice luteolo stipitis, et ordinatione DNA differt. Typus hie designatus: L. Grubisha 747.

Holotype: LG747 (UCI860306) University of California Herbarium (UC).

Etymology. Quiescens refers to the species' ability to lay quiescent in the spore bank until it encounters pine roots.

Pileus broad, hemispheric to broadly convex, moderate to large, 6-12 cm diam; color approaching strong brown (s. Br 55) or deep brown (deep Br 56) on mature pilei, sometimes with olivaceous patches or tones, much paler when young but still some shade of light Brown (IBr 57) or between light brown (57) and light orange (l.0. 52), or grayish yellow; covered in glutinous layer, but glutin often drying in strands to give a slightly fibrillose look to older specimens; margin in-rolled when young with a limited sterile zone of a millimeter or less. *Pileus context* white, generally unchanging, sometimes with brownish stains just under cuticle, and pale yellow tones just above tubes. *Tubes* when young pale yellow (near 89, p.Y.) and with light brown or yellowish brown

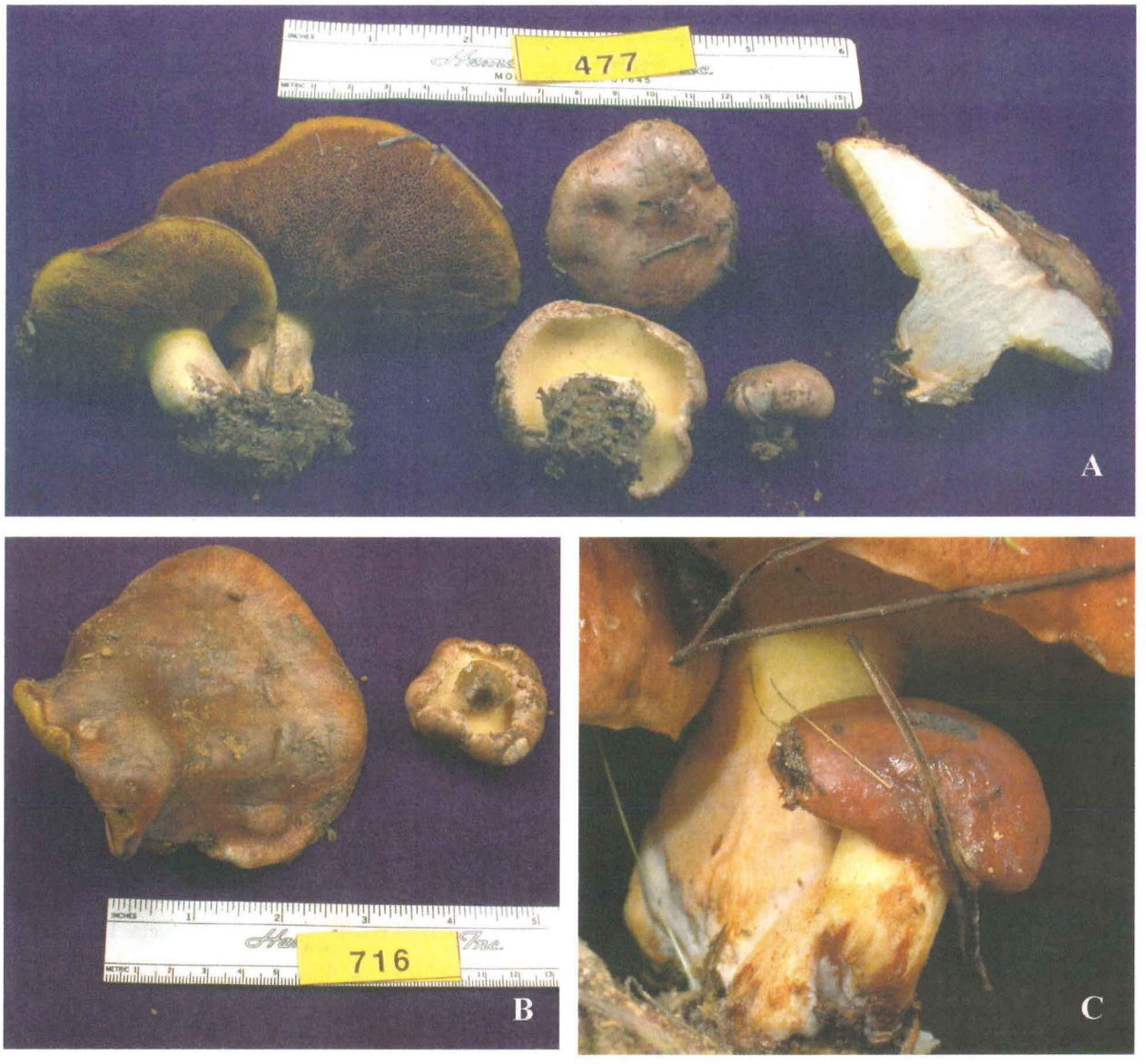

FIG. 2. *Suillus quiescens* basidiomes. A. Holotype UC1860306 collection from Santa Cruz Island. B. Collection UC1860307 from Santa Cruz Island showing in-rolled margin on young specimen. C. Collection UC1859739 from Point Reyes National Seashore showing brown overlay on lower stipe.

glandular secretions; older tubes slightly lighter than brilliant orange yellow (67 Brill Oy), becoming olivaceous yellow (near 84, s.Y) *Mature tube mouths* radially elongate but generally less than 1 mm wide. *Stipe* usually short, typically 2-4 cm long, but sometimes longer (6-8 cm), even, or very slightly bulbous, or tapered to the base, pale yellow (p. Y 89) to light yellow (1. Y. 86) on apical fifth; lower part of stipe same color at apex or white or overlaid with a light brown (l.Br. 57) layer or streaks of glutin as on pileus, occasionally this overlay gives the impression of a annular zone (FIG. 2C); stipe surface finely glandular; glands initially only slightly darker than stipe surface, but sometimes light brown, becoming nearly black after drying. *Stipe context* white and unchanging, but sometimes with orangish stains in older or insect-damaged parts. *Spore deposit* not determined, but probably between strong yellow brown (s.y Br 74) and strong brown (s Br 55) as seen on pileus surface of several specimens.

Spores [72, 7, 7] in side view 6.1-14.7 x 2.4-3.7 u*m*, avl X avw = 7.7-8.7 x 2.8-3.0 u*m*, *Q* = 2.4-3.3, avQ = 2.7-2.9, elongate, with a slight suprahilar depression in side view, and oblong in face view, most with a

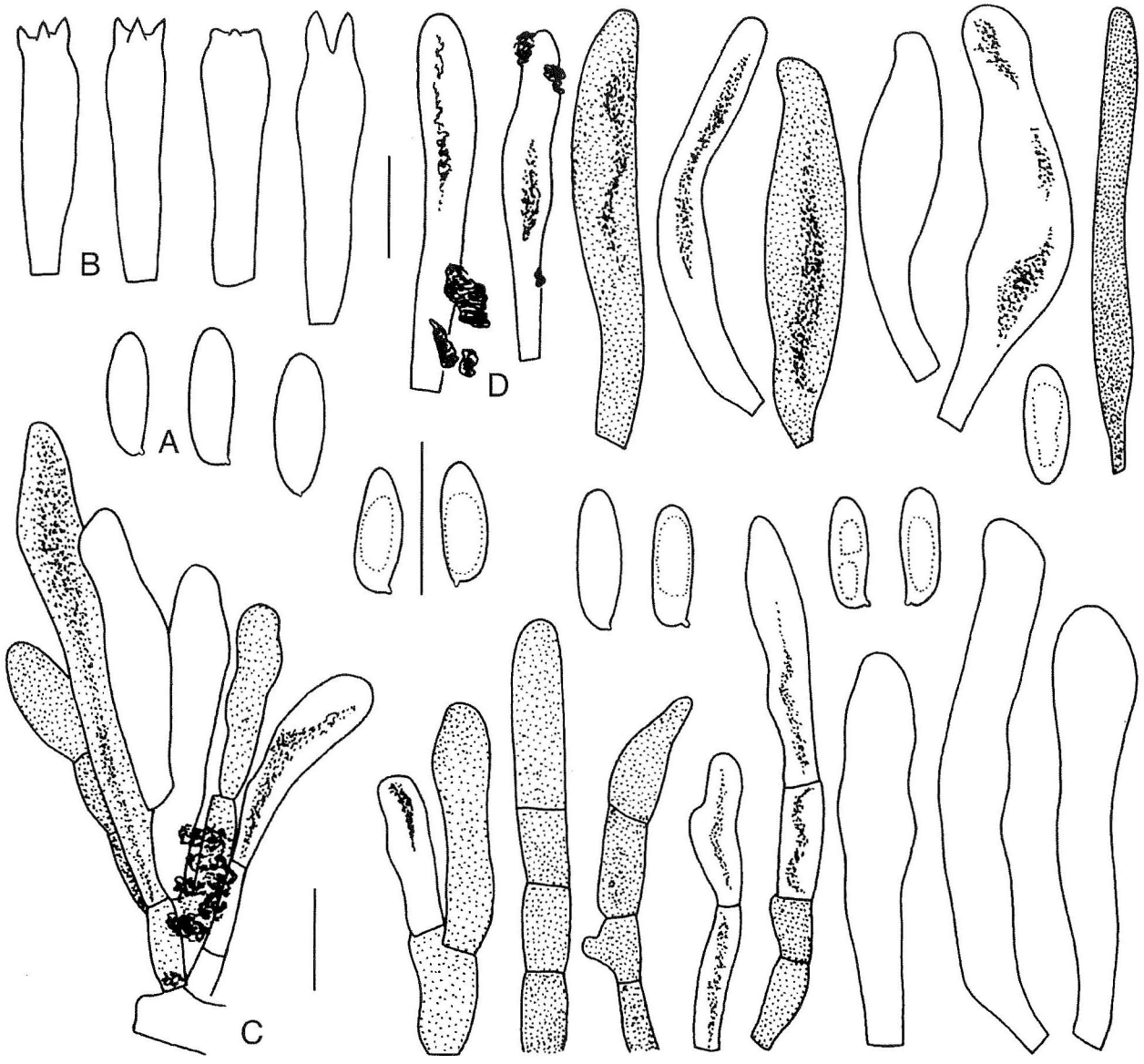

FIG. 3. *Suillus quiescens* microscopic features. A. Spores. B. Basidia. C. Cheilocystidia. D. caulocystidia. Bars = 10 µm.

single large lipid drop, with inconspicuous hilar appendage. *Basidia* 20.2-26.2 X 5.2-6.7 um, avl X avw = 24 x 6.1 um, club-shaped, with 2-4 sterigmata, without clamp connection at base; sterigmata very thick when young; 2- and 4-spored basidia present in most collections. *Tube trama* divergent, composed of hyaline, gelatinizing hyphae diverging from a central strand. Individual hyphae 4.7-8.2 um wide, simple-septate, but with clamp-like swellings at branch points. No clear clamp connections were seen.

Cheilocystidia similar to those in many other granulatus-like *Suillus* species: 21-30 X 3.1-6.2 u.m, either in dense clusters (14-125 um wide) embedded in brown incrusting material, or solitary, primarily narrowly clavate, or cylindrical and then often septate, with yellow-brown inclusions that appear to be shrunken away from the wall, thin-walled; solitary cystidia hyaline, without incrustations, sometimes with mucronate or capitate apices.

Pleurocystidia similar to cheilocystidia, but size and frequency of clusters decreases distal to the tube mouth. *Caulocystidia* in dense clusters with similar sizes and shapes to cheilocystidia, but often more irregular in shape. Fertile basidia frequent on upper stipe surface. *Pileipellis* 100-180 um thick with mostly long, repent, wavy,gelatinized hyphae 3-S J.Imdiam (avw = 4.7 um);hyphae hyaline in KOH; subpellis, similar in thickness to the pileipellis, composed of

hyphae with dark yellow-brown contents and often with granular brownish incrustations in KOH. *Pileus context* composed of inflated (10-24 um wide), branched hyphae; some branches inflated near the septa and in some views giving the impression of clamps, but no clear clamp connections were seen.

Habitat and distribution. Fruiting in small groups especially with young Bishop pine on Santa Cruz Island, where it is the most commonly encountered *Suillus* species. It is also encountered when one uses pine seedlings to bioassay soil from pine forests of the islands, coastal and mon lane areas of California and Oregon. In the Channel Islands it was collected fruiting Jan-Mar, and on the central coast at Point Reyes National Seashore it has been collected fruiting with young Bishop pine mid-November to early December. Given its distribution in Oregon and eastern California other hosts such as lodgepole, jeffrey and ponderosa pines are expected.

Collections studied. USA, CALIFORNIA, Santa Barbara County, Santa Cruz Island, junction of Ridge Road and Sauces Canyon Road, 119°48.692'W, 34°00.49'N, 388 m, under young to moderate aged *Pinus muricata,* 13 Jan 2002, *Lisa Grubisha LG747* HOLOTYPE UC (1860306); USA, CALIFORI'JIA, Santa Barbara County, Santa Cruz Island, junction of Ridge Road and Sauces Canyon Road, 119°48.920'W, 34°00.08'N, 399 m, under young *Pinus muricata,* 1 Mar 2001, *Lisa Grubisha LG359,* UC (1860310); USA, CALIFORNIA, Santa Barbara County, Santa Cruz Island, junction of Ridge Road and Sauces Canyon Road. Eastern most pines along Ridge Road, 119°47.650'W, 34°00.77'N, 388 m, under young to moderate aged *Pinus muricata,* 1 Mar 2001, *Lisa Grubisha LG360,* UC (1860312); USA, CALIFORNIA, Santa Barbara County, Santa Cruz Island, along Sauces Canyon Road. Eastern most pines along Ridge Road, 119°48.990'W, 34°00.11 'N, 399 m, under young *Pinus muricata,* 1 Mar 2001, *Lisa Grubisha LG370,* UC (1860311); USA, CALIFORNIA, Santa Barbara County, Santa Cruz Island, along Sauces Canyon Road. Eastern most pines along Ridge Road, 119°48.990'W, 34°00.11 'N, 388 m, under young to moderate aged *Pinus muricata* with a well developed organic layer, 1 Mar 2001, *Lisa Grubisha LG373,* UC (1860309); USA, CALIFORNIA, Santa Barbara County, Santa Cruz Island, along Sauces Canyon Road. Eastern most pines along Sauces Canyon Road, 119°48.998'W, 34°00.11 'N, 388 m, under young to moderate aged *Pinus muricata* with a well developed organic layer, 1 Mar 2001, *Lisa Grubisha LG372,* UC (1860308); USA, CALIFORNIA, Santa Barbara County, Santa Cruz Island, intersection of Ridge Road and Lagunitas Secus Road. 119° 47.750'W, 34°00.797'N, 423 m, Open forest shrub community of young bishop pine and oak, 13 Jan 2002, *Lisa Grubisha LG830,* UC (1860307).

Summary comparison. Suillus quiescens is most distinctive when young and fresh and then only by its macroscopic characteristics. It gives one the impression of a pale version of the eastern North American S. *breoipes* because of the short stipe and the glutinous pileus. However the light brown pileus when young, the fine glandular dots at the top of the stipe at maturity and the yellowish stipe apex separate S. *quiescens* from S. *breuipes,* which has an almost chocolate (59 d Br.) pileus and a pure white, glandless stipe when young. *Suillus pungens,* which occurs in the same habitats is white when immature and has strong olivaceous tones before becoming similar in color to older S. *quiescens.* The stipe of S. *pungens* is much more prominently glandular. The in-rolled margin of young S. *quiescens* is similar to S. *glandulosipes* or S. *neoalbidipes,* but in contrast to the latter two species it has only a small sterile zone that one might need a hand lens to see. *Suillus occidentalis* appears to be similar in appearance, but at maturity the pileus is lighter colored than that of S. *quiescens.*

ACKNOWLEDGMENTS

We thank the Harry Thiers Herbarium (SFSU) for loans of S. *occidentalis* and S. *oolcanalis* specimens, Peter Kennedy for detennining the ITS sequences of S. *quiescens,* TOB3205, the University of California Santa Cruz Island Natural Reserve and Channel Islands National Park for permission to collect fungal specimens, Lyndal Laughrin and Sarah Chaney for arranging logistical support on Santa Cruz Island and Santa Rosa Island respectively, and *Roy* Halling, Manfred Binder and an anonomous reviewer for many useful suggestions on the manuscript Financial support was supplied in part by NSF DEB-0618293 to TOB and ECV. We dedicate this work to Harry Thiel'S, whose command of the western North American *Suillus* species remains unsurpassed.

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