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

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Abstract: Suillus quiescens sp. nov. is common under Pinus muricata on Santa Cruz and Santa Rosa Islands in the northern Channel 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 abundantly 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. volcanalis Thiers and S. occidentalis Thiers, two species that are morphologically similar to S. quiescens but previously were unsequenced.

MATERIALS AND METHODS

Standard methods for describing the basidiocarps were applied with the terminology of Smith and Thiers (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™ Plant PCR Kit (Sigma-Aldrich Co., St Louis, Missouri) as follows: 10 μL extraction solution, incubated at 65 °C for 10 min followed by 95 °C for 30 min, 30 μL 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. volcanalis 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. These primers were designed by eye from an alignment with nine Suillus species and seven more distantly related basidiomycetes (Tapinella, Sebacina, Thelephom, Cortinarius, Tricholoma, Coltricia and Chaetiporus). 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, Truncocolussella, Ctenogomphus and Gomphidius. These two primers were used to amplify the ITS1 and ITS2 regions (White et al. 1990) with primer sets ITS-H/ITS-2S and ITS-3S/ITS-4b under conditions described by Gardes and Bruns (1993). PCR products were cleaned with 0.5 μL ExoSAP IT (USB Corp., Cleveland, Ohio) and cycled at 37 °C for 45 min, followed by 80 °C for 15 min. Sequenc-
Phylogenetic analysis. Suillus ITS sequences were retrieved from GenBank with the Emerecne interface Website (http://andromeda.botany.gu.se/genussearch.html). Sequences that did not contain both ITS 1 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 environmental Suillus sequences were retrieved from GenBank: AF476699.1, AJ272401.1, AJ272406.1, AJ410587.1, AJ410587.2, AY587754.1, AY850932.1, EF455012.1, EF651976.1, DQ351501.1, DQ351502, DQ351503.1, DQ351504.

These newly derived sequences were included: S. brevis: (UC15060323) GQ249355, (UC15060327) GQ249359; S. quiescens (Holotype UC1506036) GQ249402, (UC15060310) GQ249390, (UC15060312) GQ249391, (UC15060308) GQ249393, (UC15059739) GQ249401, (bioassay-no specimen) GQ249392; S. occidentalis (HDT2S 175) GQ249394, (STB5) GQ249395, (STB2) GQ249396, (STB418) GQ249397; S. volcanalis (HDT39873) GQ249398, (HDT2S00 holotype) GQ249399; $\text{megagorinus}$ (UC15060326) GQ249400.

The initial analysis used 134 ITS sequences representing all Suillus species and Suillus environmental sequences available (see METHODS and MATERIALS for list). These were aligned with Clustal X 2.09 (Larkin et al. 2007) and trimmed to 800 characters (including gaps). The sequences were analyzed by neighbor joining and by parsimony with PAUP 4.0b8i (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 unidentifed environmental sequences in a unique clade nested among other species in the granulatus group (s. granulatus [L.] Roussel, S. granulatus sensu Thieb's & A.H. Sm. 1971, S. brevis, S. glandulosipes Thieb's & A.H. Sm., S. neoalbidipes M.E. Palm & E.L. Stewart, S. luteus [L.] Roussel, S. pseudobrevis A.H. Sm. & Thiers, S. flavipes [Fr.] J. Presl, S. mediterraneensis [Jacquet. & J. Blum] Redeuilh, S. bellini [Inzenza] Watling, S. collinitus [Fr.] Kuntze, S. ocellatus.)

All latter granulatus-like sequences inclusive of S. quiescens sequences, plus additional sequences of S. collinitus, S. fijiensi, S. occidentalis and S. volcanalis (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. brevis, S. volcanalis and S. occidentalis, the three species that it macroscopically resembles.

We initially thought that S. quiescens could be a variant of S. brevis because S. quiescens differs morphologically from S. brevis 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 *Sulites*. Arrow indicates *Sulites 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).
brévipes sequences were from eastern 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. brévipes from western North America. We found that the sequences derived from western S. brévipes 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. pseudobrévipes brought up the 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. pseudobrevipes and to the later collection of S. volcanalis, 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 sent 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 gluttonous 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 11μm) 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 waxatchicus 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. All samples from the Oregon study were collected 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 fruiting could be because the species has been confused with S. brévipes 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 fruiter, even though it has been retrieved from the spore bank there in two independent studies. Furthermore on Santa Cruz 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 fruiter 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 that fruits predominantly 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
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.

MycoBank MB 515081.


Holotype: LG747 (UC1860306) 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 (.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

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

<table>
<thead>
<tr>
<th>Species</th>
<th>%</th>
<th>Region</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. brevipes</em></td>
<td>13''</td>
<td>Oregon central coast</td>
<td>Ashkannejhad and Horton 2006</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>California central coast</td>
<td>Peay et al. 2009</td>
</tr>
<tr>
<td><em>S. fungens</em></td>
<td>&lt;1</td>
<td>Southeastern Sierra Nevada</td>
<td>Ruscus et al. 2006</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>California central coast</td>
<td>Peay et al. 2009</td>
</tr>
<tr>
<td></td>
<td>8''</td>
<td>California central coast</td>
<td>Kjoller and Bruns 2003</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Santa Cruz Island, California</td>
<td>Unpubl from Grubisha et al. 2007</td>
</tr>
<tr>
<td><em>S. pseudobrevipes</em></td>
<td>&lt;1</td>
<td>Southwestern Sierra Nevada, California</td>
<td>Peay et al. 2009</td>
</tr>
<tr>
<td><em>S. quiescens</em></td>
<td>8''</td>
<td>Oregon central coast</td>
<td>Ashkannejhad and Horton 2006</td>
</tr>
<tr>
<td></td>
<td>&lt;1</td>
<td>Southeastern Sierra Nevada</td>
<td>Ruscus et al. 2006</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>California central coast</td>
<td>unpubl from Bruns et al. 2009</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>California central coast</td>
<td>Peay et al. 2009</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>Santa Cruz Island, California</td>
<td>unpubl from Grubisha et al. 2007</td>
</tr>
<tr>
<td><em>S. tomentosus</em></td>
<td>20''</td>
<td>Oregon central coast</td>
<td>Ashkannejhad and Horton 2006</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>California central coast</td>
<td>Peay et al. 2009</td>
</tr>
<tr>
<td></td>
<td>17''</td>
<td>Northern California coast</td>
<td>Kjoller and Bruns 2003</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(Salt Point)</td>
<td></td>
</tr>
<tr>
<td><em>S. umbonatus</em></td>
<td>15''</td>
<td>Oregon central coast</td>
<td>Ashkannejhad and Horton 2006</td>
</tr>
</tbody>
</table>

*averaged from their three site types.

*Based on culture; numbers not fully comparable to others.*
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 (1.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 μm, av1 X avw = 7.7-8.7 x 2.8-3.0 μm 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
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.lmdiam (avw = 4.7 um); hyphae hyaline in KOH; subpellis, similar in thickness to the pileipellis, composed of 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.

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 both California 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*, 1 Mar 2001, Lisa Grubisha LG370, UC (1860311); USA, CALIFORNIA, Santa Barbara County, Santa Cruz Island, junction of Ridge Road and Sauces Canyon Road, 119°48.750°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. brevipes* 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. brevipes*, 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. glandulosispes* 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**

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**LITERATURE CITED**


Grubisha LC, Bergemann SE, Bruns TD. 2007. Host islands within the California northern Channel Islands create


