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Gymnomyces xerophilus sp. nov. (sequestrate Russulaceae), an ectomycorrhizal associate of *Quercus* in California

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ABSTRACT

Gymnomyces xerophilus sp. nov., a sequestrate species in the *Russulaceae*, is characterized and described morphologically as a new species from *Quercus*-dominated woodlands in California. ITS sequences recovered from healthy, ectomycorrhizal roots of *Quercus douglasii* and *Q. wislizeni* matched those of *G. xerophilus* basidiomata, confirming the ectomycorrhizal status of this fungus. Phylogenetic analysis of the ITS region places *G. xerophilus* in a clade with both agaricoid (*Russula* in the section Polychromae) and sequestrate (*Gymnomyces*, *Cystangium*) relatives. We include a dichotomous key to the species of *Gymnomyces* associated with *Quercus*.

Introduction

Quercus-dominated ecosystems cover about one third of California's 404,000 km^2 (Pavlik *et al.* 1991). *Quercus* spp. are well adapted to the state's extensive areas of dry, Mediterranean climate, with at least 7 species considered endemic (Nixon 2002). *Quercus*, like other members of the *Fagaceae*, form ectomycorrhizae (EM) with diverse *Ascomycota*, *Basidiomycota*, and *Zygomycota*, including members of the *Russulaceae* (Trappe 1962; Gerdemann & Trappe 1974; Froidevaux & Schwärzel 1977; North 2002; Avis *et al.* 2003; Walker *et al.* 2005). *Quercus* spp. depend on formation of EM for normal function and survival (Frank 1885; Smith & Read 1997). EM fungi are thus vital symbionts for host plants in ideal and harsh environments alike (Allen 1991). In xeric habitats the EM symbiosis provides the host plant access to water reserves via the fungal mycelium, potentially reducing drought stress (Duddridge *et al.* 1980; Parke *et al.* 1983).

Although the EM fungi associated with *Quercus* in California have not been studied extensively, Thiers (1984) and Trappe and Claridge (2005) suggest that seasonally dry climates exert a selection pressure towards a sequestrate fruiting habit in EM fungi. Evidence from seasonally dry locations that have been extensively studied (e.g., coniferous forests in California and Oregon, eucalypt communities in Australia) indicates that sequestrate species make up a significant portion of the EM taxa (Johnson 1994; Waters *et al.* 1997; North 2002). Sequestrate fungi have been shown to produce large amounts of biomass (Luoma *et al.* 1991; Smith *et al.* 2002) and provide important food resources for animals in several temperate forests (Maser *et al.* 1978; Johnson 1994; Trappe & Claridge 2005). Though relatively few groups have been studied in detail,

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most sequestrate fungi form EM with multiple plant hosts (Miller 1983; Trappe & Castellano 1986; Molina *et al.* 1999).

The *Russulaceae*, including sequestrate species, are dominant and diverse EM root symbionts in many forest ecosystems (Horton & Bruns 2001). For example, in a Minnesota oak savanna, *Russula* spp. and other unidentified *Russulaceae* were among the most abundant EM symbionts encountered on roots of mature *Quercus* (Avis *et al.* 2003). Similarly, a study of EM roots of *Quercus* seedlings in southern Appalachia revealed 17 *Russulaceae* species among the 75 taxa encountered (Walker *et al.* 2005). Currently, scant data is available regarding the EM fungi on roots of *Quercus* in California. However, EM roots of another California angiosperm, *Arctostaphylos glandulosa*, were heavily colonized by *Russulaceae* (Horton *et al.* 1999).

Despite the apparent dominance of *Russulaceae* in many EM fungal communites, only three sequestrate species have been conclusively shown to form EM. *Arcangeliella borziana* with *Picea abies* (Peter *et al.* 2001), *Gymnomyces medlockii* (syn. *Martellia medlockii*) with *Pinus contorta* and *Tsuga heterophylla* (Trappe & Castellano 1986), and an unidentified *Gymnomyces* sp. with *Pinus ponderosa* (Stendel *et al.* 1999).

During a study of the EM fungal community associated with *Quercus* in a xeric woodland savanna in northern California, we encountered basidiomata of a previously undescribed *Gymnomyces* sp. (*Russulaceae*). In this paper we describe it as *Gymnomyces xerophilus* sp. nov. and use phylogenetic analysis of the ITS region to place it in a clade of the genus *Russula* containing both agaricoid and sequestrate taxa. In addition we compare *G. xerophilus* with other known *Gymnomyces* spp. associated with *Quercus* and provide a dichotomous key to these taxa.

Materials and methods

Sampling of basidiomata and ectomycorrhizas

Basidiomata and EM roots were sampled at the Koch Natural Area of the University of California Sierra Foothill Research and Extension Center, in Yuba County, California. The terrain consists of low hills 50–650 m above sea level. Overstory vegetation is dominated by three species; *Quercus douglasii*, *Q. wislizeni*, and *Pinus sabiniana* Douglas. The Mediterranean climate is characterized by cool, wet winters and hot, dry summers. Precipitation generally occurs between October and May (annual mean 71 cm, range 23–132 cm) and temperature varies seasonally (mean 17.8 °C, range 10 °C–43 °C) (UCSFREC online: http://danrrec.ucdavis.edu).

Basidiomata of EM fungi were collected under *Q. douglasii* and *Q. wislizeni* between December 2000 and January 2005. A garden cultivator was used to carefully remove litter and soil at random locations beneath mature host canopies. Basidiomata were described, photographed, and taken to the laboratory for tissue sampling and drying on the same day.

For EM, litter was removed and soil cores of ca 900 cm^3 were collected under canopies of *Q. douglasii* and *Q. wislizeni*, stored at 4 °C and processed within 15 d of sampling. Soil was sieved and washed by hand. After cleaning, 100 healthy EM roots per core were randomly selected, pooled, and lyophilized for later DNA extraction and molecular analysis.

Morphological examination of basidiomata

Macroscopic characters were described from fresh specimens. Basidiomata colors are designated by ISCC-NBS terminology (Kelly & Judd 1955). Microscopic characters were determined from two types of mounts: (1) temporary mounts hand-sectioned with a razor blade on the vertical axis of the basidiomata and mounted in 3 % KOH and Melzer's reagent, respectively; and (2) paraffin-embedded specimens sectioned with a sliding microtome to a thickness of 5–10 μm, stained in safranin-fast green, and made into permanent mounts. The basic fuchsin reaction (Romagnesi 1967) was used to test for the presence of dermatocystidia and sulfovanillin was used to test for the presence of encrusted hyphae on the peridial surface (Singer 1986).

All measurements were with the oil-immersion microscope objective at ×1000 magnification. Spore measurements included the largest and smallest spores and at least 20 additional, randomly selected spores from each specimen; spore dimensions excluding ornamentation are reported in this paper. Because spores of sequestrate Basidiomycota are usually statismosporic, spore prints cannot normally be obtained from their basidiomata. Consequently, to best insure that mature spores and their ornamentation are being observed, basidiomata with spore masses so abundant as to obscure the hymenia should be selected. Because spores in the *Russulaceae* are smooth in youth and the ornamentation builds on the surface of the spores as they mature, spores with the tallest and most strongly developed ornamentation should be measured.

Molecular techniques

DNA sequences for basidiomata were generated as in Miller and Buyck (2002). For EM, lyophilized root tips were ground with a micropestle and DNA extracted by a modified CTAB method (Gardes & Bruns 1993) followed by purification with a MO-BIO Soil DNA kit (MO-BIO Laboratories, Solana Beach, CA, USA). PCR was then performed with primers ITS1f (Gardes & Bruns 1993) and LR3 (Hopple & Vilgalys 1994). The reaction protocol began with initial denaturation of 94 °C for 5 min followed by 20 cycles of 1 min. (94 °C); 1 min. (55 °C); 4 min. (72 °C) followed by a final extension of 72 °C for 7 min. Fresh PCR products were cloned with a TOPO-TA kit (Invitrogen, Carlsbad, CA, USA). At least 48 successful clones per reaction were grown overnight in LB media amended with 100 μg/ml of ampicillin. Cloned fragments were re-amplified in a PCR reaction using approximately 0.5 μl of the bacterial suspension as template.

Amplicons were digested with the restriction enzymes ALU1 and Hinf1 and electrophoresed through a 1.5 % agarose gel and stained with SYBR Green I (Molecular Probes, Eugene, OR, USA). One to four representative clones of each restriction fragment length polymorphism (RFLP) type were sequenced with ITS1F and LR3 and/or ITS4 with the ABI Big Dye Terminator Sequencing Kit (v3.1). Sequences were read using an ABI13730xl capillary sequencer (Applied Biosystems, Foster City, CA, USA) at the College of Agricultural and Environmental Sciences Genomics Facility, University of California at Davis and edited with Sequencher v.4.1 (Gene Codes Inc., Ann Arbor, MI, USA).

Sequences were selected for the analysis to highlight the relationship of *G. xerophilus* to specific members of the

Russulaceae and to show the position of the *G. xerophilus* clade in relation to a previously published skeleton of the *Russula* phylogeny (Miller & Buyck 2002). Sequences were initially compiled in AlignIR (vers. 1.2, LI-COR) and final alignment was performed manually. Sequences can be found in GenBank under the numbers AY061651–AY061739, AY603102, DQ028473–DQ028477, (*G. redolens*: DQ403803), (*C. theodoroui*: DQ403804), DQ028474, DQ028475, DQ028477 and the alignment is available in TreeBASE (SN2746).

Phylogenetic analysis

Maximum parsimony analyses were performed with PAUP* 4.0b10 (Swofford 2001). Gaps in alignment were treated as missing data and ambiguously alignable sequence regions were excluded from phylogenetic analysis. Heuristic analysis strategies designed by Maddison *et al.* (1992) and Olmstead and Palmer (1994) were used to find islands of parsimony. The relative robustness of individual branches and clade stability were estimated by bootstrap analysis (Felsenstein 1985; Hillis & Bull 1993). Bootstrap values were generated with the settings 100 replicate searches on all parsimony-informative characters with ten random sequence addition replications and TBR (tree-bisection-reconnection) branch swapping algorithms in PAUP*. Likelihood values for the most parsimonious trees were calculated using the Hasegawa-Kishino-Yano (HKY) DNA substitution model (Hasegawa *et al.* 1985). Decay indices were calculated with AutoDecay version 4.0 (Eriksson 1998) and decay values output as a NEXUS tree file displayed with TreeView (Page 1996).

Taxonomic description

Gymnomyces xerophilus M. E. Sm. & Trappe, **sp. nov.** (Figs 1–3)

Etym.*: Latin *xerophilus ("loving dry places") in reference to the habitat of the species.

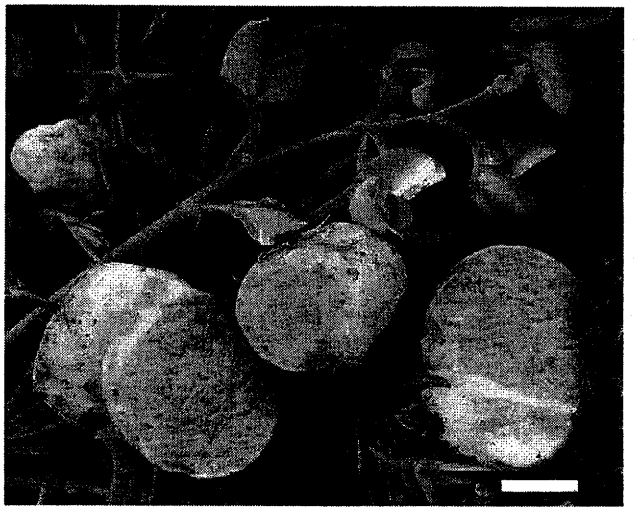

Fig 1 – *Gymnomyces xerophilus*. Fresh basidiomata (SRC–433). Scale = 10 mm.

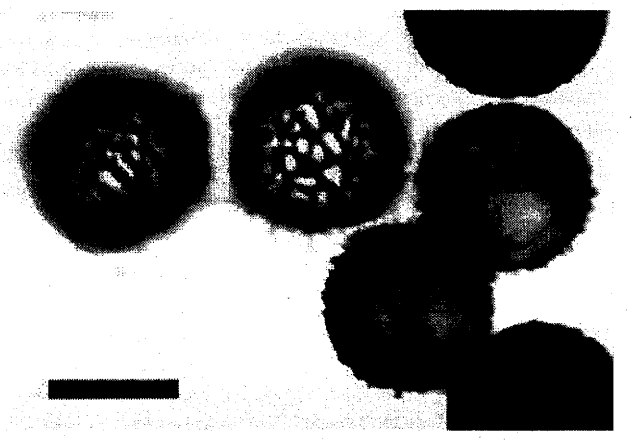

Fig 2 – *Gymnomyces xerophilus*. Basidiospores with a partial to complete reticulum of amyloid ornaments (SRC–433). Scale = 10 μm.

Basidiomata hypogaea, 12–20 × 4–30 mm, globosa, subglobosa vel irregularia. Peridium laeve, juventute album, maturitate cremeum. Gleba juventute alba, maturitate pallide aurantiolutea, loculis labyrinthformis 0.2–3 × 0.1–3 mm. Peridiopellis hyphis adpressis 1–4 μm latis. Subhymenium cellulis isodiametris, 5–20 μm latis. Cystidia, fibulae et sphaerocystae absentes. Basidia clavata, 35–40 × 9–11 (–12) μm. Sporae globosae vel subglobosae, 11–13(–15) × (10–)11–13 μm, juventute verrucis virgisque amyloideis 0.5–1 × 0.3–1 μm, maturitate virgis junctis reticulum partiale completum formantibus.

Typus: **USA**: *California*: Yuba County, UC Sierra Foothill Research and Extension Center, Koch Natural Area, 21 Mar. 2003, *M. E. Smith* SRC-672 (OSC82818 – holotypus; SFSU-isotypus). GenBank accession no. DQ028473.

Basidiomata hypogeous, 12–20 × 4–30 mm, globose to subglobose or irregular. Peridium not readily separable from gleba, smooth, white in youth, becoming yellowish white (ISCC-NBS 92) to pale yellow (ISCC-NBS 89) with some yellow to brown tinged spots in age, not staining when cut or bruised. Gleba dry, white to cream color in youth, soon pale

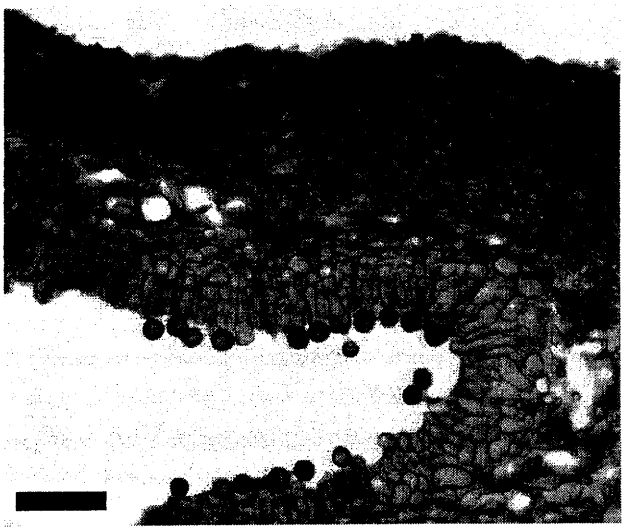

Fig 3 – *Gymnomyces xerophilus*. Overview of peridial cross-section (*Trappe* 3913). Scale = 50 μm.

orange-yellow (ISCC-NBS 73) to light orange-yellow (ISCC-NBS 70) from massed spores lining the labyrinthiform locules 0.2–3 × 0.1–3 mm; white sterile tissue sometimes visible near the peridium and in sterile veins 1–3 mm thick running through the center of some specimens but no true columella present.

Peridium 150–200 µm thick. *Peridiopellis* a prosenchyma of tightly packed, hyaline, thin-walled hyphae (1.5–) 3–5 µm broad with occasional, short, emergent hyphal tips too scattered to form a trichodermial suprapellis; occasional cells inflated up to 10 µm. No encrusted hyphae could be detected with the basic fuschin test and the sulfovanillin test did not reveal dermatocystidia. *Subpellis* of subparallel, hyaline, thin-walled hyphae 1.5–4 µm broad; occasional cells inflated up to 10 µm. Refractive (oleiferous) hyphae present but rare, 2–3 µm broad, staining blue in the presence of sulfovanillin. *Trama* 15–20 µm broad, or subparallel hyphae 2–5 µm broad with occasional cells inflated up to 10 µm. *Subhymenium* of 2 to 3 tiers of ± isodiametric cells 5–12 (–20) µm broad. *Basidia* clavate, 35–40 (–42) × 9–11 (–12) µm, with 2–4 sterigmata 5–8 × 1 µm. *Cystidia, clamp connections* and *sphaerocysts* absent.

Spores globose to subglobose, 11–13(–15) × (10–)11–13 µm excluding the ornamentation, in KOH pale golden yellow singly and golden yellow in mass, the walls ± 1.5 µm thick, ornamented in youth with warts and rods 0.5–1 × 0.3–1 µm, as spores mature many rods gradually joined by ridges ± 0.3 µm tall to form a partial to complete reticulum but some rods and warts remaining isolated; in Melzer's reagent the spore walls smoky brown, the reticular ridges strongly amyloid, the warts and rods erratically beset with strongly amyloid spots at tips or on sides; sterigmal appendage weakly amyloid; plage lacking. *Odour* not distinctive. *Taste* unknown.

Distribution, habitat, and season. Hypogeous in low-elevation oak woodlands of California's Sierra Nevada in association with *Quercus douglasii*, *Q. wislizeni*, and *Q. kelloggii* Newb. at ca 50–600 m elev.; March through May. All but one collection known from the University of California Sierra Foothill Research and Extension Center in Yuba County, California.

Additional collections examined: **USA California:** *Yuba County*: UC Sierra Foothill Research and Extension Center, Koch Natural Area, 1 March 2003, *M. E. Smith SRC–648*, (OSC 82219), GenBank accession #DQ028476; 6 April 2002, *M. E. Smith SRC–433* (OSC 82220), GenBank accession no. AY603102 *El Dorado County*: 5 km north of Cool, 18 May 1974, *J. M. Trappe 3913* (OSC 82217).

Remarks: The scattered inflated cells in the peridium, trama and subhymenium of *G. xerophilus* are not clustered and hence not formed in the manner described as typical for the *Russulaceae* (Watling & Nicoll 1980). Moreover, they are much smaller than is usual for sphaerocysts. *Gymnomyces xerophilus* differs from other oak-associated members of the genus by its reticulate spore ornamentation ≤ 1 µm tall. It closely resembles *G. abietis* Trappe and Castellano macroscopically, but that species has a well developed, trichodermial suprapellis and smaller spores, and is associated with *Abies* spp. in moist, montaine to subalpine forests (Trappe & Castellano 2000).

Provisional key to *Gymnomyces* associated with *Quercus*

This provisional key is based to a large degree on published descriptions, which often do not include complete morphological data for comparison between species. In some cases it was not possible to distinguish between two species on the basis of the descriptions. We now know that several sequestrate *Russulaceae* are white or near white in early developmental stages but develop brown patches as they mature and become brown overall at full maturity. The browning character, once used to distinguish species such as *G. cinnamomeus* Singer and A.H. Sm. from others in the genus, is thus not a useful character. Characters such as height of spore ornamentation or inflation of subhymenial cells can also change as basidiomata mature. As detailed in Materials and Methods, spores should be measured from basidiomata replete with spores to best insure they represent mature character states. Our key is thus presented as an interim aid to identification of the oak-associated *Gymnomyces* species with recognition that most sequestrate *Russulaceae* described in the past need restudy and molecular characterization. Nomenclature follows that of Trappe *et al.* (2002).

1 Spore ornaments up to 3.5–5 µm tall and often forked at the apex; basidia 1–spored ..... **gilkeyae**

Spore ornaments shorter than 2.5 µm, never forked at the apex; basidia 2– or 4–spored 2

2 (1) Spores 14–17 × 12–16 µm excluding ornamentation; hymenial cystidia 43–62 × 12–18 µm ..... **roseomaculatus**

All but exceptional spores 10–14 × 10–12 µm or smaller 3

3 (2) Spores ornamented with cones 2–2.5 µm tall and connected by low, amyloid lines and particles ..... **parksii**

Spore ornamentation ≤ 1 µm tall and spiny or reticulate or, if taller, the ornamentation spiny but not connected by lines or ridges 4

4 (3) Spores partially to completely reticulate at maturity ..... **xerophilus**

Spores with isolated spines, warts, half crescents, or short lines 5

5 (4) Peridial suprapellis with dermatocystidia or a turf of clavate to fusoid cells 6

Peridial suprapellis of appressed hyphae or small end cells 9

6 (5) Suprapellis with abundant clavate to versiform cystidia 45–60 × 10–12 µm; southern Europe ..... **illicis**

Suprapellis cystidia and end cells < 40 µm long; western North America 7

7 (6) Spores ornamented with warts 0.25–0.8 µm tall and broad ..... **californicus** and **fallax** (probably synonyms)
Spore ornamentation 1–1.5 µm tall 8

8 (7) Spores 9–12 × 8–11 µm excluding ornamentation of rods and spines 0.7–1.5 × 0.3–0.7 µm and more strongly amyloid at the apex than at the base ..... **subfulvus**
Spores 10–14 × 9–12 µm excluding ornamentation of strongly amyloid warts ± 1 µm tall and arranged in lines or fused into groups ..... **compactus**

9 (6) Basidia with two sterigmata; peridial suprapellis of minute, granulated cells ..... **rolfalexii**
Some or all basidia with four sterigmata; peridial suprapellis of appressed hyphae 10

10 (9) Spore ornamentation up to 2 µm tall and 2 µm broad at the base; hymenial cystidia exceeding the basidia, mucronate, 35–46 × 12–17 µm ..... **cremeus**
Spore ornamentation not exceeding 1.5 µm tall or broad; hymenial cystidia rare or lacking 11

11 (10) Spores globose, 9–12 µm broad excluding the ornamentation of spines with scattered short lines or fused in crescents or half circles; western North America ..... **cinnamomeus**
Spores subglobose to broadly ellipsoid, 11–13.5 × 9.5–12.5 µm excluding the ornamention of isolated spines; southern Europe ..... **mistiformis**

Results

Molecular identification of ectomycorrhizas

In all, 104 root cores were extracted under *Quercus douglasii* (n = 72) and *Q. wislizeni* (n = 32). Although more than 2000 clones were sequenced from EM near *G. xerophilus* basidiomata, the ITS sequence of *G. xerophilus* was recovered from only 18 clones in 3 root cores (LO–7A, LO–7D, BO–3D). Two cores came from beneath one individual of *Q. wislizeni*, the other from *Q. douglasii*.

Phylogenetic analysis

ITS rDNA amplification products were ca 660–1000 base pairs in length, included two hypervariable regions, one in ITS1 and one in ITS2, and contained approximately 300 phylogenetically informative positions after alignment. An island of 8 most parsimonious trees of 2030 steps was found. Fig 4 shows the best most parsimonious tree (likelihood score of −lnL = 11778.12478) based on equally weighted parsimony with a consistency index (CI) of 0.293 and retention index (RI) of 0.527. Other equally parsimonious trees in this island differed only in minor rearrangements within terminal clades other than the clade containing *G. xerophilus*.

Appropriate divergence occurred among ITS sequences for testing hypotheses on the relationship between *G. xerophilus* and species in the genus *Russula*. Topology of the phylogram (Fig 4) shows that *G. xerophilus* was placed in a terminal clade along with *Russula aurata* (With.) Fr. and *R. romellii* Maire, *Gymnomyces redolens* (Cunn.) Pfist., and *Cystangium theodoroui* Lebel.

Discussion

Gymnomyces xerophilus is easily distinguished from other known *Gymnomyces* spp. by a combination of characters including ectomycorrhizal association with the host genus *Quercus*, prominent orange-yellow gleba, lack of cystidia and trichodermium, and globose to subglobose spores 11–13 µm in diameter with a partial to complete, amyloid reticulum. The spore ornamentation of *G. xerophilus* is notable: few sequestrate species related to *Russula* in the Northern Hemisphere have reticulate spores, although that character is more common in the Southern Hemisphere (Lebel 2002, 2003). Of the *Gymnomyces* spp. associated with the *Fagaceae*, only *G. xerophilus* and *G. redolens* have spores commonly with a complete reticulum; *G. parksii* Singer and A. H. Sm. may sometimes have "almost a broken reticulum" (Singer & Smith 1960).

The phylogeny presented here reiterates an evolutionary pattern common in fungi: sequestrate species have arisen multiple times from agaricoid ancestors (Binder & Bresinsky 2002; Miller *et al.* 2001; Desjardin 2003; Peintner *et al.* 2001; Hansen *et al.* 2001). Our molecular analysis reveals that *G. xerophilus* clusters with both agaricoid and sequestrate members of the *Russulaceae*, including *R. romellii*, *R. aurata*, *Cystangium theodoroui* and *G. redolens*. While the species nested within this clade do not share the same overall fruiting body form, they share other important morphological features.

Russula romellii and *R. aurata* are distinctive because of the vivid yellow to yellow-orange color of their lamellae. Regardless of this notable character, various workers have placed them in different infrageneric groups [including subsection Firmiores by Singer (1986) and section Polychromae (although in separate subsections) by Sarnari (1998)]. *R. romellii* is often used as a comparator for spore print color in the genus *Russula* because it has one of the darkest spore prints known. While less is made of the gleba color (and therefore spore deposit color) in sequestrate forms, the three hypogeous species all share the yellow-orange color of the spores. The gleba color of *G. xerophilus* is striking in fresh fruiting bodies and retains vivid yellow-orange colors when dried. Lebel (2002) described the gleba of *G. redolens* as "white to cream becoming pallid ochraceous" while the gleba of *C. theodoroui* was "cream color in youth, soon pale yellow."(Lebel 2003)

The cuticular structure of *G. xerophilus* with short, emergent hyphal tips is similar to that of *R. romellii* with its attenuate hyphal end cells and that of *R. aurata* in which the cuticular hyphal end cells are short and often conical or papillate. Lebel (2002) describes the peridiopellis of *G. redolens* as "a dense turf of upright to repent, hyaline hyphal tips", and that of *C. theodoroui* as "an intermittent trichodermium of upright to repent, septate, rarely branched hyaline

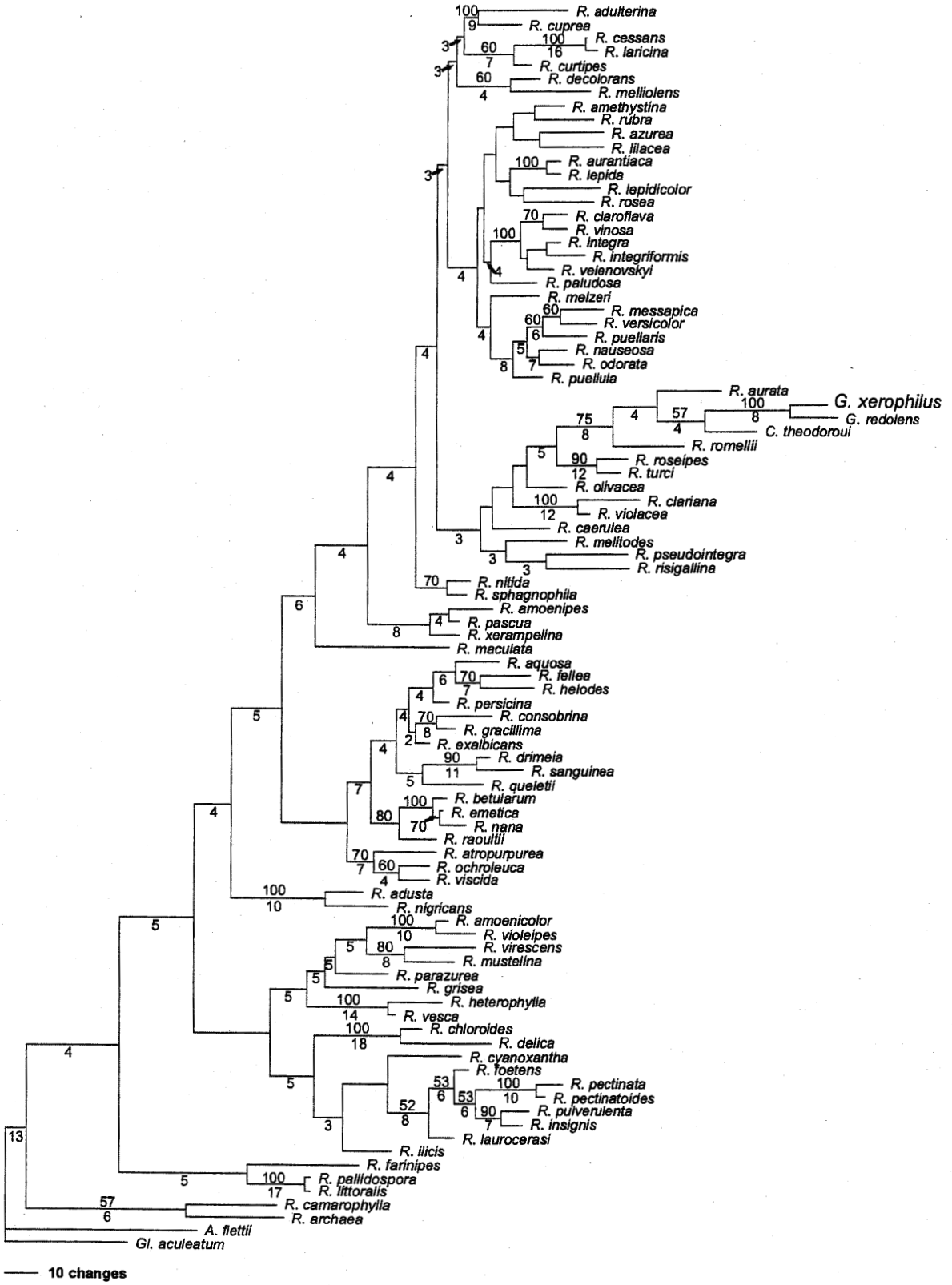

Fig 4 – The best of eight most parsimonious trees of 2030 steps (likelihood score of −lnL = 11778.12478) inferred from equally weighted parsimony analysis of ITS1, 5.8S, and ITS2 nrDNA sequences depicted as a phylogram. The consistency index (CI) was 0.293, and the retention index (RI) was 0.527. Numbers above the branches refer to bootstrap support while numbers below the branches refer to Decay Indices.

hyphae...the terminal elements sometimes inflated, obtuse or fusoid..."(Lebel 2003). The multi-septate nature of the *C. theodoroui* cuticular elements depicted in Lebel (2003) are highly reminiscent of those found in *R. aurata*. Other closely related species of *Russula* frequently have acid resistant incrustations in the cuticular hyphae; *R. romellii* and *R. aurata* are exceptional because they lack of these incrustations (Miller & Buyck 2002).

The basidiospore ornamentation is also similar in all species found in this clade. Most are described as subreticulate to cristate. In *R. romellii* the spores also tend toward subspinose, similar to but less exagerated than the "dense spiny reticulum of warts and spines" found in *G. redolens* (Lebel 2002).

The five species clustered together in the present molecular analysis are all ectomycorrhizal associates of hardwood trees. *Gymnomyces xerophilus* forms EM with *Quercus* in California, *G. redolens* is associated with *Nothofagus* in New Zealand, and *C. theodoroui* is associated with *Eucalyptus* in Queensland, while the European *R. romellii* and *R. aurata* are common associates of *Fagus*.

Based on molecular phylogenies, some authors have recently renamed sequestrate species to reflect the epigeous genera from which they were derived (e.g., Kretzer & Bruns 1997; Peintner *et al.* 2002; Desjardin 2003). While placement of sequestrate fungi within traditionally epigeous genera is a viable nomenclatural option, it detracts from the ecological and morphological information that a sequestrate generic name conveys. In addition, the unsettled taxonomic/phylogenetic status of taxa within the *Russulaceae* (Miller *et al.* 2001; Desjardin 2003; Shimono *et al.* 2004), particularly the genus *Russula*, makes the placement of our new species within that genus untimely. Until a more complete phylogeny of the *Russulaceae* is resolved, including linkages among the various lineages, we prefer to place *G. xerophilus* in the genus *Gymnomyces*. Use of the name *Gymnomyces* does not interfere with the understanding that *G. xerophilus* is derived from within *Russula*.

In this study we used molecular techniques to show that *G. xerophilus* forms ectomycorrhizae with *Quercus* spp. Between December 2002 and May 2004 we sampled 104 large soil cores under *Quercus* spp. but only encountered *G. xerophilus* from three cores under two individual trees. The apparent rarity of *G. xerophilus* on roots is mirrored by its infrequent occurrence as basidiomata; it is known only from four collections. The sporadic detection of *G. xerophilus* may seem unusual, given the dominance of *Russulaceae* in many EM community studies (Horton & Bruns 2001). However, communities of EM fungi on roots are often dominated by one or a few species of *Russulaceae*, with many taxa found only sporadically or not at all (eg Avis *et al.* 2003; Walker *et al.* 2005). Contrary to the results from many community studies of EM fungi on roots, preliminary results indicate that the *Russulaceae* is not a prominent EM family in xeric, low elevation *Quercus* woodlands of California (M. Smith and M. Morris, unpublished data).

In xeric *Quercus* woodlands, basidiomata of *G. xerophilus* were found during March to May, whereas species of *Lactarius* and *Russula* were generally encountered only in the wettest months (January–February) and were restricted to mesic microsites with deep leaf litter (M. Smith, personal observation). The temporal difference in fruiting season between *G. xerophilus* and its epigeous relatives highlights the adaptation of sequestrate fungi to relatively dry conditions in comparison with their epigeous counterparts.

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