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A new species of *Russula,* subgenus *Compactae* from California

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Abstract: A new species of *Russula,* subgenus *Compactae*, is described growing in association with coast live oak (*Quercus agrifolia*) in California. This species has previously been referred to locally as *Russula subnigricans* Hongo and *Russula eccentrica* Peck, but morphological and DNA evidence show that it is a distinct species which we call *R. cantharellicola*. Our analysis shows that there are multiple clades of what is called *R. subnigricans,* and that the California species clearly falls within one of those clades. As *R. subnigricans* has caused deaths in Japan, China, and Taiwan, the California species should be cautioned as potentially dangerous to those who consume it.

Key words: *Russula,* California, fungal diversity, toxicity

Introduction: *Russula* Pers. (Russulales, Russulaceae) is a large, prominent, and important genus of ectomycorrhizal woodland mushrooms. *Russula* subg. *Compactae* (Fr.) Bon stands apart from the rest of the genus in having large, persistent, dull-colored, hard-bodied basidiocarps, numerous lamellulae alternating with the lamellae, and a poorly differentiated pileipellis that does not separate easily from the context. Ongoing molecular studies reveal a number of clades within subgenus *Compactae* (Bart Buyck, pers. comm.), each embracing well-known species with blackening basidiocarps such as *R. nigricans* Fr., *R. densifolia* Secr. ex Gillet, and *R. albonigra* (Krombh.) Fr., plus several additional clades with sequences called *R. subnigricans* Hongo – an Asian species that stains dingy reddish in age but does not blacken.

While several of the blackening species are widely eaten, from Mexico to Russia, China and Thailand (Boa 2004), *R. subnigricans* is notable for being deadly poisonous, having caused numerous fatalities in Japan, southern China and Taiwan due to rhabdomyolysis (Lee et al. 2001; Matsuura et al. 2009; Chen et al. 2014).

Shaffer (1962) included *R. subnigricans* in his treatment of "*Russula* subsection *Compactae*" but did not cite any collections from North America. Since the 1970s the senior author has repeatedly collected a species closely resembling *R. subnigricans* in the oak woodlands of central coastal California, always in association with the California oak chanterelle, *Cantharellus californicus* Arora & Dunham (2008). Arora (1986) referred this species to the *"Russula subnigricans* group" pending further study. Weber & Smith (1985) depicted *R. subnigricans* from the southern USA, but Bills (1985) showed that the southern USA taxon was already named *R. eccentrica* Peck (Peck 1911) based on specimens collected in Missouri, USA, and that this name had priority over *R. subnigricans* should the two species prove to be identical. Thiers (1998), in his treatise on California *Russula*, applied the name *R. eccentrica* to the California species. His description fits that of the species described by Arora (1986) as "*Russula subnigricans* group," but Thiers cited only one collection and we were unable to locate it in San Francisco State University (SFSU) for sequencing. In this study we determined that this California taxon with a noticeable affinity for *Cantharellus* differs from both *R. eccentrica* and *R. subnigricans*, and we describe it as a new species.

Materials and Methods

Morphological examinations.—A description was drawn up from fresh material with special emphasis on the staining reactions, and the collections were photographed in the field. Spores and other micro characteristics were examined at magnifications of 400× and 1000×. *DNA extraction and sequencing*.—DNA was extracted from powdered tissue of dry basidiocarps, hydrated with 2% CTAB (cetyltrimethylammonium bromide) buffer, with 2% PVP (polyvinylpyrrolidone), followed by standard methods for chloroform extraction and ethanol precipitation. DNA was extracted from oak root-tips using the Sigma Extract-N-Amp kit (Sigma Aldrich, St. Louis, MO). The ITS region was amplified using the standard fungal specific primers ITS1F (Gardes & Bruns 1993) and ITS4 (White et al. 1990) using dilute DNA template. Sequences were produced using the standard BigDye Terminator v3.1 Kit and were run on an ABI Prism 3700 Genetic Analyzer (Life Technologies) using the above PCR primers. Each automated sequence was examined and manually interpreted, and the ambiguous bases were corrected using Sequencher 4.7 (Gene Codes Corporation). All sequences were deposited in GenBank as KF306036–KF306039.

Phylogenetic methods.— ITS sequences were taken from GenBank that had a similarity score of greater than 90% to the taxon of interest using BLAST. The sequences were from both vouchered and environmental samples such as ectomycorrhizal root-tips. *Russula cerolens* Shaffer was chosen as the outgroup. Sequences were aligned using MAFFT (Katoh et al. 2002) and the alignment was manually optimized. The final dataset was restricted to members of subgenus *Compactae* and analyzed using maximum likelihood with the GTRGAMMA model of nucleotide substitution in RAxML (Stamatakis 2006). Maximum likelihood bootstrapping was performed locally in RAxML using stringent bootstrapping criteria with 1000 replications (Stamatakis 2006).

Results

Morphological examination of specimens showed that the new taxon differed from other *Russula* species described from California and the eastern USA, including *R. eccentrica*. Phylogenetic reconstruction of the available data for subgenus *Compactae* (FIG. 1) showed that there are many clades, with one or multiple names, and some without names and only from ectomycorrhizal root-tips. The name *R. subnigricans* has been broadly applied and can be found in several clades. The new California taxon described here is in a well-supported clade (93%), with specimens of "*Russula subnigricans*" from Japan and Korea, *R. eccentrica* from Tennessee, and *R. polyphylla* Peck from an unknown locality. The new taxon is sister to a specimen of "*Russula subnigricans*" from Japan, and the available phylogenetic information indicates that these are two different taxa. Using phylogenetic and morphological data the new species is described below.

Russula cantharellicola D. Arora & N.H. Nguyen, sp. nov. FIGS. 2 & 3

Medium-sized to large basidiocarps with white pileus becoming brownish, sordid reddish or grayish-brown, white gills that stain sordid reddish and eventually become entirely sordid red, sturdy stalk, unpleasant odor when mature, and subglobose to ovoid, weakly ornamented spores.

Holotype: USA, California, Santa Cruz County, near Aptos, with *Quercus agrifolia* and *Cantharellus californicus*. 36.983069°N 121.860901°W. 1 November 2011, Arora 11404 (SFSU); Isotype: UC1999420.

Mycobank : MB804625; GenBank KF306036 (ITS sequence)
Etymology: c*antharelli*- (Latinized Greek), referring to the chanterelle genus *Cantharellus*; *cola* (from L. *colere,* to dwell among).

Pileus 8–30 cm broad when mature, broadly convex with a depressed center becoming plano-depressed or vase-shaped; pellicle not easily separable from the context; the surface slightly viscid when moist, glabrous, whitish when young but soon developing pale smoky-brown or dingy reddish-brown shades, sometimes also with slight ochre stains or a mixture of all the above colors but *not* blackening; margin at first inrolled, smooth, not becoming striate. Flesh thick, crisp, white, typically bruising slowly dingy reddish (this process may take one to 20 minutes or more) and sometimes hardly at all, then usually slightly browner or grayer within an hour but not blackening; odor strong and unpleasant at maturity or upon drying; taste mild to slightly bitter.

Lamellae thick, brittle, fairly close, usually alternating long and short, adnate to slightly decurrent, at first pallid but soon developing sordid reddish or brick-red stains; in weathered specimens often entirely dark, dull reddish. Stipe 5–13 × 3–7 cm thick, hard, firm, rigid, solid, equal or with a tapered base, the surface glabrous; white at first, staining sordid reddish and/or smoky-brown with age or handling but not blackening.

Spores deposit white (Codice Romagnesi Ia), spores 7–10 × 6–8 µm (avQ=1.2, *n*=30), subglobose, broadly ellipsoid to ovoid, weakly ornamented with low (<0.5 µm) amyloid warts connected in some places by very fine lines (the amyloid warts are so small and close together that the whole spore appears weakly amyloid). Pleurocystidia and cheilocystidia 50–90 × 6–12 µm, elongate-fusoid. Basidia 48–63 µm long, 5–

7.5 µm thick; sterigmata 5–6.3 µm long. Pileocystidia not observed. Pileipellis 200–334 µm thick, embedded in a clear layer of gluten up to 250 µm thick; Epicuticular hyphae with free tips, 1.6–4.7 µm thick, interwoven and interspersed with thick-walled hyphae 3.1–4.7 µm.

Additional Collections Examined: USA, CALIFORNIA: Contra Costa County: Tilden Regional Park, under *Quercus agrifolia*, 18 October 2009, O. Gannot (GenBank KF306037); Santa Cruz County, near Aptos, under *Q. agrifolia*, 7 October 2012, UC1999436.

Occurrence: Single or in groups on ground in association with oak, especially *Q. agrifolia,* usually fruiting in relatively warm weather; common in the summer and fall, occasionally also in the spring but usually absent during the coolest months. Basidiocarps of *Cantharellus californicus* are typically seen fruiting simultaneously with *R. cantharellicola* (FIG. 2) or after *R. cantharellicola* has finished fruiting. During simultaneous fruiting, basidiocarps of the two species can even be found attached to each other at the bases of their stipes. We have confirmed that *R. cantharellicola* is ectomycorrhizal with *Q. agrifolia* from sequencing of the root-tips of the host tree (GenBank KF306038).

Distribution: Presently known only from the central California coastal region; specimens sequenced came from Santa Cruz and Contra Costa counties but the senior author has observed it in the central California coastal region from Monterey County north to Sonoma County.

Comments: This species is easily distinguished from other California members of *Russula* subgenus *Compactae* by the tendency of the basidiocarps to develop sordid dark red stains as they age, at least on the gills, and by the failure to subsequently blacken with age or after handling. The name *R. adusta* Pers. (Fr.) has been applied to one or more conifer-associated species in western North America that may exhibit a slight reddening of bruised areas but also typically discolor grayish and do not exhibit the strong reddening of the gills at maturity characteristic of *R. cantharellicola* (see FIG. 2). The surface of the stipe in *R. cantharellicola* may stain dingy reddish when bruised but never exhibits the bright red-orange to red to black phases characteristic of *R. nigricans*, *R. dissimulans* Shaffer or *R. densifolia* (a process that normally takes 20 minutes or less in those species). *Russula cantharellicola* is also separated from these latter species based on molecular evidence (FIG. 1). *Russula nigricans*, *R. dissimulans*, and *R. densifolia* belong to one well-supported clade (99% bootstrap) while *R. cantharellicola* belongs to another well-supported clade (93% bootstrap). Though the name *Russula eccentrica* has been historically applied to *R. cantharellicola* (Thiers 1998), evidence at hand suggests that *R. eccentrica* is a southeastern USA species that does not occur in California.

Molecular evidence based on ITS does not help to identify the edibility of *R. cantharellicola*. It does not belong to the *R. subnigricans* clade identified as causing 45 deaths in southern China (Chen et al. 2014), but it may still be poisonous. Though a number of mushroom collectors gather russulas for the table without being sure of the species, the unpleasant odor of the mature basidiocarps of *R. cantharellicola* may act as a deterrent to consumption.

It is interesting to note the affinity of this species for the common edible mushroom, *Cantharellus californicus*. In over 30 years of field observation, the senior author has never seen *R. cantharellicola* without *C. californicus* fruiting in the immediate vicinity either simultaneously or subsequently, whereas the latter fruits in many localities where the russula is not present. It is unknown at this time whether there is a direct relationship between the two fungi, as has been inferred for species of *Gomphidius* and *Suillus* (Olsson 2000) and *Chalciporus* and *Amanita muscaria* (Spooner & Roberts 2005), or whether their occurrence in association with each other is coincidental. A similar relationship with chanterelles has not been noted in the literature for either *R. subnigricans* or *R. eccentrica* or any other member of subgenus *Compactae*.

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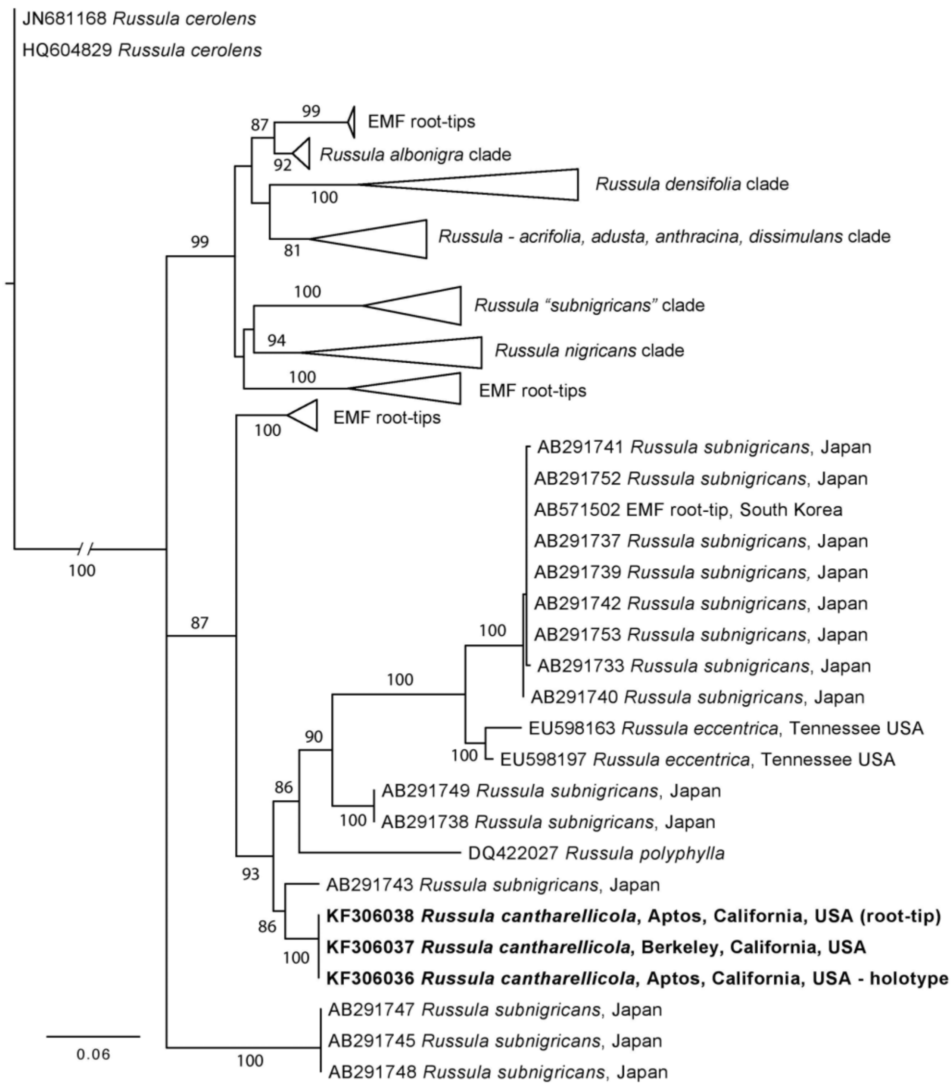

FIGURE 1. Maximum likelihood ITS tree of *Russula* sequences in subgenus *Compactae* with *Russula cerolens* (subgenus *Ingratula*) as the outgroup. Clades of less related species to *R. cantharellicola* have been truncated for clarity. The alphanumeric code preceding species names are GenBank accession numbers. Values on the branches are maximum likelihood bootstrap that are > 50%. The bold face highlights *R. cantharellicola*.

FIGURE 2. *Russula cantharellicola* (holotype, Arora 11404) fruiting with *Cantharellus californicus* in association with coast live oak (*Quercus agrifolia*). (Photo by David Arora)

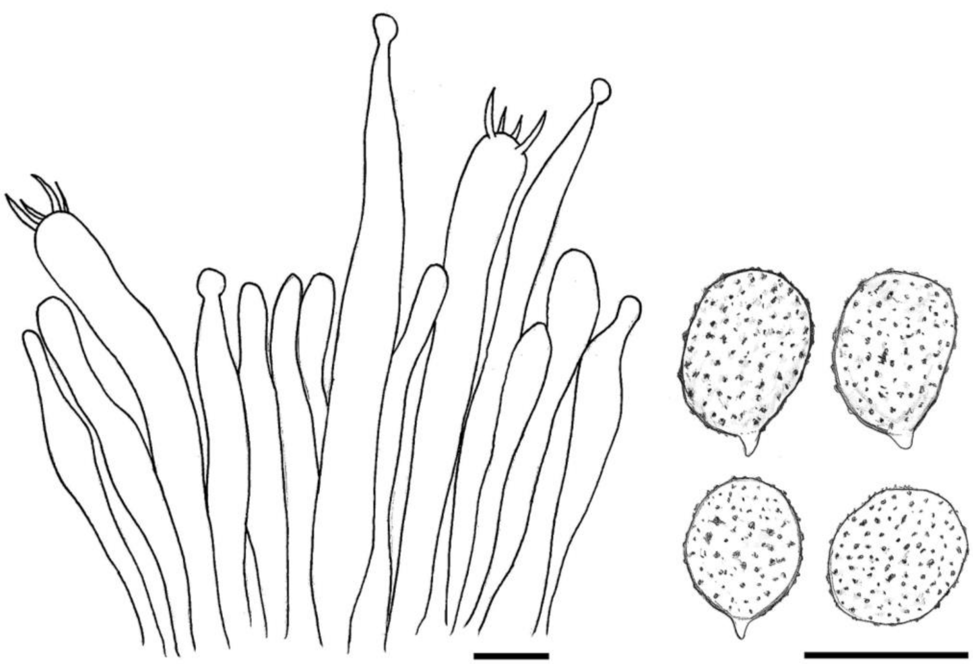

FIGURE 3. Line drawing of *Russula cantharellicola* (holotype, Arora 11404) showing cystidia, basidia, sterile cells, and basidiospores. Line markers = 10 μm.