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Amanita augusta, a new species from California and the Pacific Northwest

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ABSTRACT: *Amanita augusta*, formerly known under the misapplied European name *A. franchetii*, is described as a new species from California and the Pacific Northwest. Here we discuss the phylogeny of *A. augusta* in the context of genus *Amanita*, subsection *Validae*, and also provide taxonomic and phylogenetic notes on *A. franchetii* s.s. and its varieties.

Key words: *Amanita*, *Amanitaceae*, fungal taxonomy, nrITS data.

Introduction: Europe was the point of origin for the study and taxonomy of mushrooms and other fungi; and there was a long standing tradition for some North American mycologists to apply European names to taxa perceived to be the same species based solely on morphology. Recently, the refinement of species concepts and the utilization of molecular genetics have helped gain insight into the degree of relatedness of many North American and European species sharing the same name. Often, similar species are clearly related but not identical; in other cases, the European names were clearly misapplied and referred to species that were not closely related (in part due to the fact that North American researchers had relatively little field experience with European taxa); and thirdly, in some cases, the same species indeed occurs in both continents.

The species described here has been known for several decades as *Amanita aspera* (Pers.) Pers.

sensu Thiers (Thiers 1982), and then *Amanita franchetii* (Boud.) Fayod *sensu* Thiers as the

former name became an illegitimate synonym. The first reference to *A. aspera* in Western North America appears to be in Smith (1949), while Jenkins (1986) refers to the taxon as one with a broad distribution in North America. It has long been noted by mycologists that western North American *A. franchetii* is clearly not the same as the European species (Tulloss 2012, Lindgren 1998). Herein we described and contrast *A. augusta* from California and western North America with *A. franchetii* from Europe using morphological and phylogenetic data. Future research will address the related species from the other parts of North America.

Materials and Methods: Methods for morphological studies and DNA extraction, PCR conditions and primers, PCR product clean-up and sequencing were employed as outlined in Bojantchev & Davis (2011). For the purposes of this study, the nrITS gene region was sequenced from seven of our collections and the sequences were deposited in GenBank. Collections are stored in the private herbarium of the senior author or at the University of California herbarium in Berkeley (UC) where noted. All examined collections are by D. Bojantchev except where noted. The senior author examined several collections from the “Thiers Herbarium” at San Francisco State University, collected by Harry D. Thiers and his students. Extensive iconography of the species described here is available at <http://www.mushroomhobby.com>. The following genus abbreviations are used for the host trees in this manuscript: *P* for *Pinus*, *Pi* for *Picea*, *Ps* for *Pseudotsuga*, *T* for *Tsuga*, *N* for *Notholithocarpus*, *Q* for *Quercus*.

Phylogenetic Analysis: All *Amanita* nrITS sequences from the public databases GenBank (<http://www.ncbi.nlm.nih.gov>) and UNITE (<http://unite.ut.ee/>) were downloaded, reviewed, and selected for quality and identification congruity. Sequence alignments were generated with MAFFT v6.821b (Kato et al. 2002) with the G-INS-i global alignment

iterative refinement strategy. No gap opening and extension penalties were set for better resolution of the variable sectors within nrITS. The alignments were visually inspected and corrected where needed.

Thirty-five nrITS sequences of *Amanita* subsect. *Validae* (Quél.) Drehmel, Vilgalys & Moncalvo were selected for higher resolution phylogenetic analysis. These included our collections of *A. augusta* and *A. franchetii* as well as the most closely related sequences (98% or above BLAST similarity index) from the public databases. Three older collections of *A. franchetii* f. *queletii* from France, provided by Serge Poumarat failed to yield useable nrITS after repeated attempts. The evolutionary history was inferred by using the Maximum Likelihood method as implemented by MEGA5 (Tamura et al. 2007) with the bootstrap consensus tree inferred from 1000 replicates (FIG. 1).

***Amanita augusta* Bojantchev & R.M. Davis sp. nov. FIGS. 2–7
MycoBank number: MB 801321**

Pileo 30–150 mm lato, hemispherico, dein planoconvexo, subglutinoso, brunneo dein flavo, margine non striato, cum velo generali floccis flavo-luteis. Lamellis confertis, liberis, albis. Stipite 40–150 mm longo, cylindrico, velo basali subfloccoso, interdum flavido, basi bulbosis. Annulo supero, reflexo lacero, flavo. Carne albido. Sapore miti. Sporis 8.5–9.3 × 6–6.8 μm, ellipsoideae, hyalinis, amyloideae. Basidiis 35–62 × 8–12 μm, tetrasporigeris, fibulis absentibus, cellulis marginalis spheropedonculatibus interdum subglobosis.

TYPE: USA. CALIFORNIA: SONOMA COUNTY, Salt Point State Park, 0.25 mi. South of Hwy 1 (38°33'36"N 123°18'50"W), under *Notholithocarpus densiflorus*, *Pinus muricata*, *Pseudotsuga menziesii*, 11 Dec 2011, Bojantchev DBB49390 (**Holotype** UC 1851352; Genbank nrITS JQ9372

ETYMOLOGY: In Latin Augustus means majestic or venerable, a name befitting this distinctive *Amanita* species.

PILEUS 30–150 mm diam., hemispherical to convex when young, plano-convex to plano-concave with age; margin incurved to straight, occasionally ribbed striate near the margin; color dark brown to brown to yellow brown, sometimes yellow to grayish yellow at age, typically paler towards the margin; the universal veil forming rows of concentric warts (finer towards the edge), yellow, but fading to grayish-white with age, easily removable, often partially or completely washed out or rubbed away, leaving the pileus partially or fully glabrous. LAMELLAE crowded, 9–20 mm broad, white to yellowish near the cap margin, even, narrowly attached to free; lamellulae common. STIPE 40–150 mm long, 10–30 mm wide, context stuffed, cylindrical to tapering towards the apex, often with a distinct bulb; color yellow when young, often fading to whitish at age, typically yellowish above the annulus; surface above the bulb forming scaly girdles (finer towards the annulus) with yellow apices, often fading to glabrous at age; surface above the annulus distinctly longitudinally striate. ANNULUS superior, membranous, pendant, upper surface distinctly striate, thinning, but rarely collapsing with age, typically fimbriate, pale yellow to yellow on both sides, developing darker yellow brown zones at age. STIPE BASE prominent bulb in most basidiomata, girdled with veil remnants, distinctly rufescent in age. UNIVERSAL VEIL yellow. CONTEXT white to pale yellow. ODOR mild. TASTE mild. SPORE DEPOSIT white.

BASIDIOSPORES (7.8–)8.5–9.3(–11.2) × (5.2–)6.0–6.8 (–7.8) μm (mean 8.9 × 6.4 μm), Q = 1.27–1.57, Q_{av} = 1.41 (N = 201, 6 basidiomata, 3 collections), broadly ellipsoid to ellipsoid, slightly to distinctly inequilateral in 30–40% of the cases, with a prominent lateral apiculus, hyaline, amyloid. BASIDIA 35–62 × 8–12 μm, 4-spored, clavate; sterigmata 4–6 μm long; no clamps observed. SUBHYMENIAL LAYER composed of

several layers of irregular to pyriform cells 9–31 × 7–21 μm, not clamped. LAMELLAR TRAMA divergent, composed of filamentous to swollen hyphae 6–22 μm wide, no clamps observed. LAMELLAR MARGINAL CELLS subglobose to sphaeropedunculate 10–33 × 7–21 μm (Fig. 4a). PILEIPELLIS an ixocutis of densely interwoven hyphae 2–7 μm wide with the upper layer slightly gelatinized. ANNULUS composed of filamentous cells, 2–6 μm wide, on the lower surface with clusters of inflated subglobose to pyriform cells, 10–30 × 8–24 μm (Fig. 4b), with a few inflated cells on the upper surface; clamp-connections not observed. UNIVERSAL VEIL formed of dense filamentous hyphae 2–8 μm wide, interspersed with broadly ellipsoid to subglobose vesiculose cells, 30–80 × 14–60 μm; no clamps observed. STIPE TRAMA acrophysalidic, composed of filamentous hyphae 2–6 μm wide and inflated hyphae 62–176 × 16–44 μm; no clamps observed. OLEIFEROUS HYPHAE abundant, 7–20 μm wide with refractive content (Fig. 4c).

HABITAT AND DISTRIBUTION — *Amanita augusta* is common in the mixed coastal woods of California, the Pacific Northwest, and Alaska (Fig. 8). Reports of collections from the Sierra-Cascade mountain ranges are scarce, particularly in California. The primary fruiting period is autumnal but basidiomata may be collected in the late summer and fall in the Pacific Northwest and in the winter and spring in California. It tends to fruit in small numbers, typically 1-3 basidiomata, rarely more. *Amanita augusta* is an ectomycorrhizal species and along the range of its distribution it is found in mixed conifer and broadleaved habitats, amongst a number of potential hosts. The exact associations are not fully mapped out yet, but amongst the established ectomycorrhizal hosts are *P. muricata* (Bruns et al. 2002) and *Ps. menziesii* (Smith et al. 2002), while *Tsuga heterophylla* is listed as probable (Trappe 1960). In addition, we have observed *A. augusta* in solid stands of live oak (*Quercus agrifolia*) in California and Sitka spruce (*Picea*

sitchensis) in Alaska, as well as in mixed conifer spruce (*Pi. engelmannii*) in Oregon. Tanoak (*N. densiflorus*) is a common tree in much of its distribution in California. Future research will undoubtedly shed more light on the exact associations from the number of other potential conifer and broadleaved hosts.

ADDITIONAL COLLECTIONS EXAMINED: USA.

CALIFORNIA: MARIN COUNTY, Point Reyes N.S., off Mt. Vision Rd., (38°05'03"N 122°52'18"W), elev. 1220 ft., under *P. muricata*, 10 Dec 2005, DBB00014; SAN MATEO COUNTY, Long Ridge Open Space Preserve, off Skyline Blvd (37°16'21"N 122°09'13"W), elev. 2520 ft., under *Q. agrifolia*, 1 Apr 2012, DBB50192; San Francisco Watershed, 6 Jan 1967, coll. by H. D. Thiers, Breckon 673 (SFSU), 12 Jan 1967, coll. by R. Keller, Keller 338 (SFSU), 16 Dec 1969, coll. by H. D. Thiers, Keller 604 (SFSU); MENDOCINO COUNTY, Jackson State Demonstration Forest, off Little Lake Rd (39°18'27"N 123°42'47"W), under *N. densiflorus*, *Ps. menziesii*, *T. heterophylla*, *Sequoia sempervirens*, 14 Nov 2009, DBB26180; 3 Nov 1962, coll. by H. D. Thiers, HDT 9376 (SFSU); 19 Dec 1967, coll. by G. Breckon, Breckon 297 (SFSU); SONOMA COUNTY, Salt Point State Park, 0.8 mi. North of Hwy 1 (38°36'25"N 123°21'40"W), under *P. muricata*, 23 Dec 2007, DBB03543; 23 Dec 2007, DBB03547; 0.5 mi. South of Hwy 1 (38°33'40"N 123°18'49"W), mixed woods with *P. muricata*, *Ps. menziesii* and *N. densiflorus*, 24 Nov 2011 DBB48532 (Genbank nrITS JQ937286), 23 Nov 2006, DBB01312; 11 Nov 2006, DBB00982; **OREGON:** CURRY COUNTY, Samuel Boardman State Park, (42°08'52"N 124°21'28"W), elev. 100 ft., mixed woods composed of *Pi. sitchensis*, *P. contorta* var. *contorta* and *N. densiflorus*, 11 Nov 2009, Bojantchev DBB25368 (Genbank nrITS JX515565); **WASHINGTON:** CLALLAM COUNTY, Olympic Peninsula, off Hot Spring Rd. (48°04'29"N 123°57'18"W), elev. 1200 ft., under *Pi. sitchensis*, *Pi. engelmannii*, *Ps. menziesii*, 21 Oct 2009, DBB21873 (Genbank nrITS JX515564). **ALASKA:** KENAI PENINSULA COUNTY,

forests that include various pines and Engelmann off Seward Hwy. (60°19'33"N 149°21'42"W), elev. 580 ft., under *Pi. sitchensis*, 22 Aug 2007, DBB00873.

DISCUSSION — Several SFSU herbarium collections of *A. aspera* made by Hary D. Thiers and his students were examined and on account of the macroscopic features of the desiccata (Fig. 7), microscopic detail, and collection notes, there is no doubt that taxonomically they represent the concept of *A. augusta*. Based on the phylogenetic analysis (Fig. 1), *A. augusta* forms a distinct clade within subsect. *Validae* with several closely related members from Asia and North America. *Amanita franchetii* s.s. is not a sister species, but belongs to a separate clade, which shares a common ancestry with *A. spissa* (Fr.) P. Kumm. *Amanita franchetii* is fairly variable in appearance and there are three morphological infraspecific taxa accepted currently (Neville & Poumarat 2004) – the classic yellowish capped *A. franchetii* f. *franchetii* (Boud.) Fayod (Fig. 9a), the pale whitish *A. franchetii* f. *lactella* Neville & Poumarat (Fig. 9a,b), and a forma with dark pilei, *A. franchetii* f. *queletii* (Bon & Dennis) Neville & Poumarat. *Amanita franchetii* f. *queletii* most closely resembles *A. augusta*, but the latter has more yellow hues on the stipe and pronounced reddening on the bulb at age. In our collections the yellow and pale varieties (Fig. 9) had identical nrITS, but within other collections from Europe there are differences of 1-2 base pairs, which suggests that *A. franchetii* may be a species complex in need of further study.

The current literature does not recommend *A. augusta* as an edible species. *Amanita* subsect. *Validae* is not known to contain strongly toxic species, and at least one of them, *A. rubescens*, is a well-known and accepted edible in Europe. Still, caution should be advised as in the raw state *A. rubescens* is known to contain the haemolytic protein rubescenslysin (Odenthal et al. 1982) and should never be consumed raw. Other members of subg. *Lepidella* are also known to contain

haemolytic lectins (Antonyuk & Stoika 2012), thus the presence of these substances in *A. augusta* is likely. These toxins are known also from macromycetes of other genera and require heat treatment prior to consumption. The notable mycologist and naturalist David Arora has been serving *A. augusta* to test groups since the mid 90's after a carefully developed initial test program (personal communication). He also reports that a similar species, also named *A. franchetii*, is sold on the markets in Mexico. The first author sampled *A. augusta* on two occasions without ill effects. Additional caution should be urged because *A. augusta* can easily be confused with *A. pantherina* (DC.) Krombh. and other species known for severe toxic reactions.

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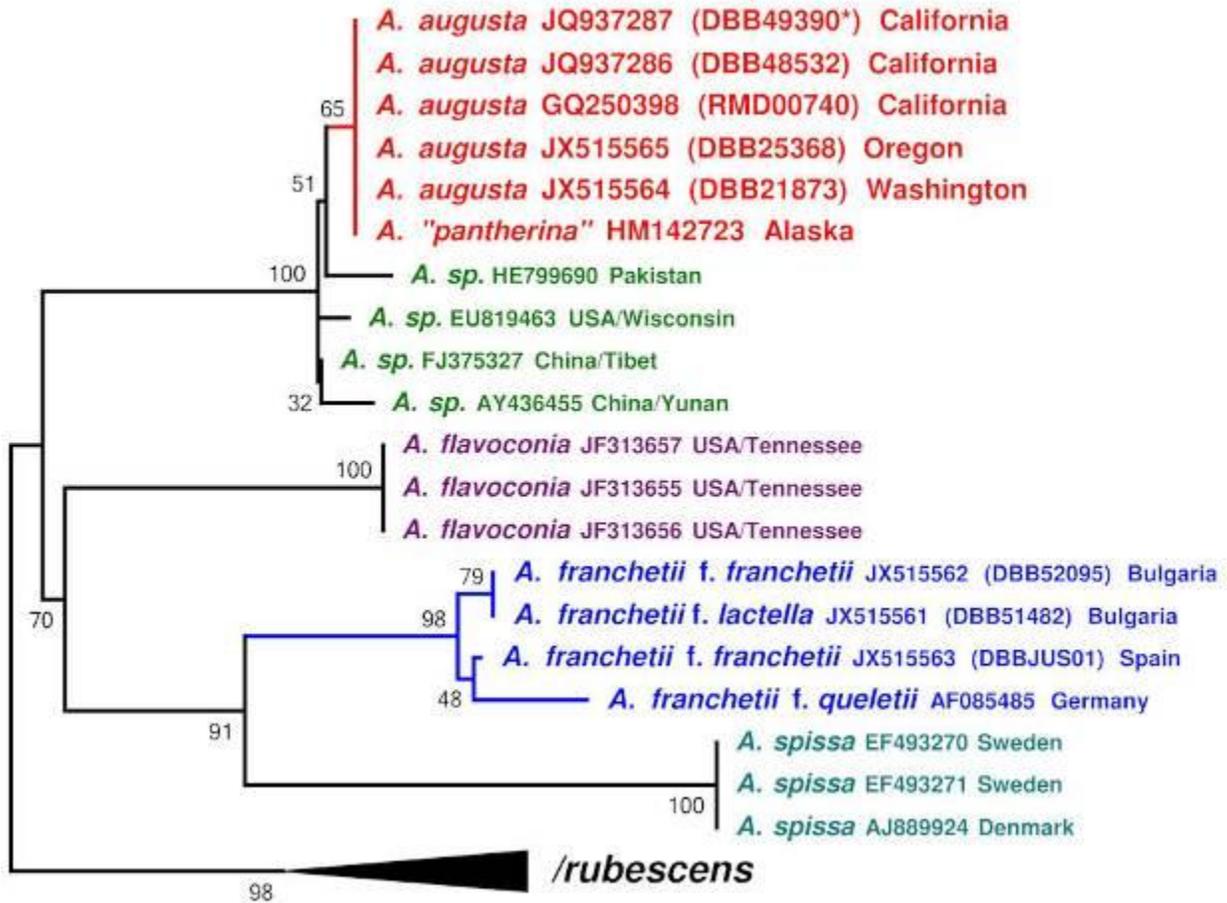


Fig. 1. Phylogenetic tree inferred by maximum likelihood analysis of 35 *Amanita* nrITS sequences (20 shown, the rest are collapsed under the /rubescens clade). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. The tree shows the position of *A. augusta* relative to its closest neighbors in the subsect. *Validae*. The /rubescens clade has been collapsed for clarity. The GenBank accession numbers are presented after the species names and the author's collection numbers are enclosed in brackets. The holotype collection is marked with *.



Fig. 2. *Amanita augusta*. DBB00014



Fig. 3. *Amanita augusta* (UC 1851352, holotype) a) Basidiospores b) Basidiomata

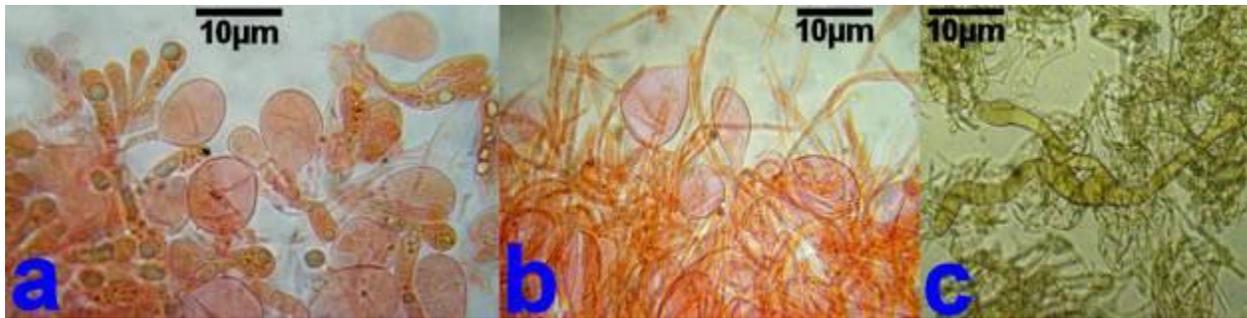


Fig. 4. *Amanita augusta* (UC 1851352, holotype) a) Marginal cells on the lamellar edge (mounted in Congo Red) b) Filamentous and vesiculose cells from the lower surface of the annulus c) Oleiferous hyphae



Fig. 5. *Amanita augusta* a) DBB03543 b) DBB03547 c) DBB21873 d) DBB50192



Fig. 6. *Amanita augusta* a) DBB48532 b) DBB00982 c) DBB01312



Fig. 7. SFSU herbarium collections of *Amanita augusta* a) Breckon 297 b) Keller 604

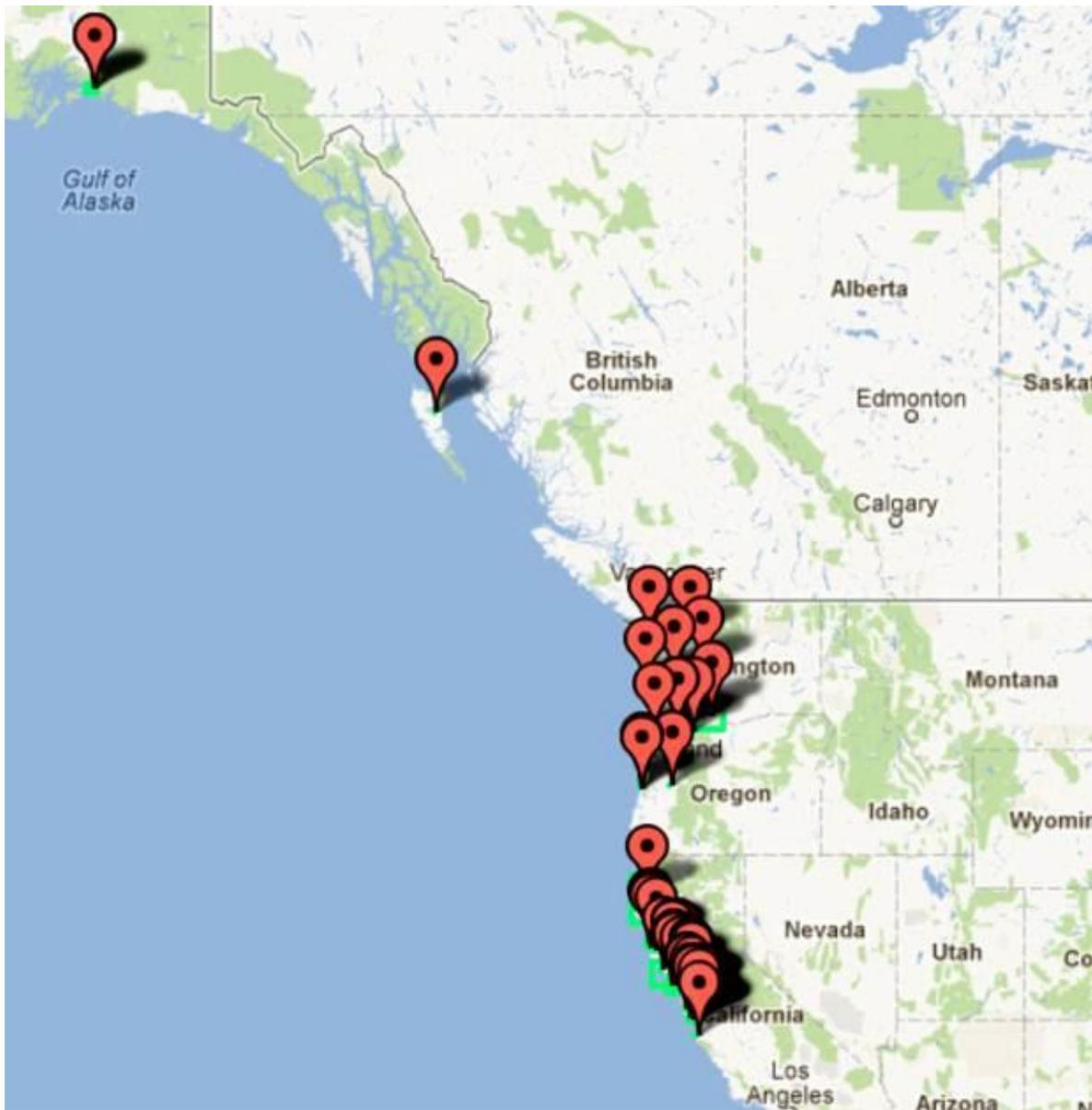


Fig. 8. Distribution pattern of *Amanita augusta*, based on mostly non-vouchered, but well-illustrated amateur reports in MushroomObserver.org (Wilson & Hollinger, 2012). The predominantly coastal distribution pattern matches the observations of the authors.



Fig. 9. a) DBB52095 *Amanita franchetii* f. *franchetii* b) c) DBB51482 *Amanita franchetii* f. *lactella* – the two collections have identical nrITS.



Fig. 10. *Amanita franchetii* f. *queletii* — a) b) c) the darker form of *A. franchetii*, which more closely resembles *A. augusta*, but is distinguished by the whitish stipe and pale bulb. This collection was not sequenced, but used as an illustration. Photos by Patrice Tanchaud, France.