

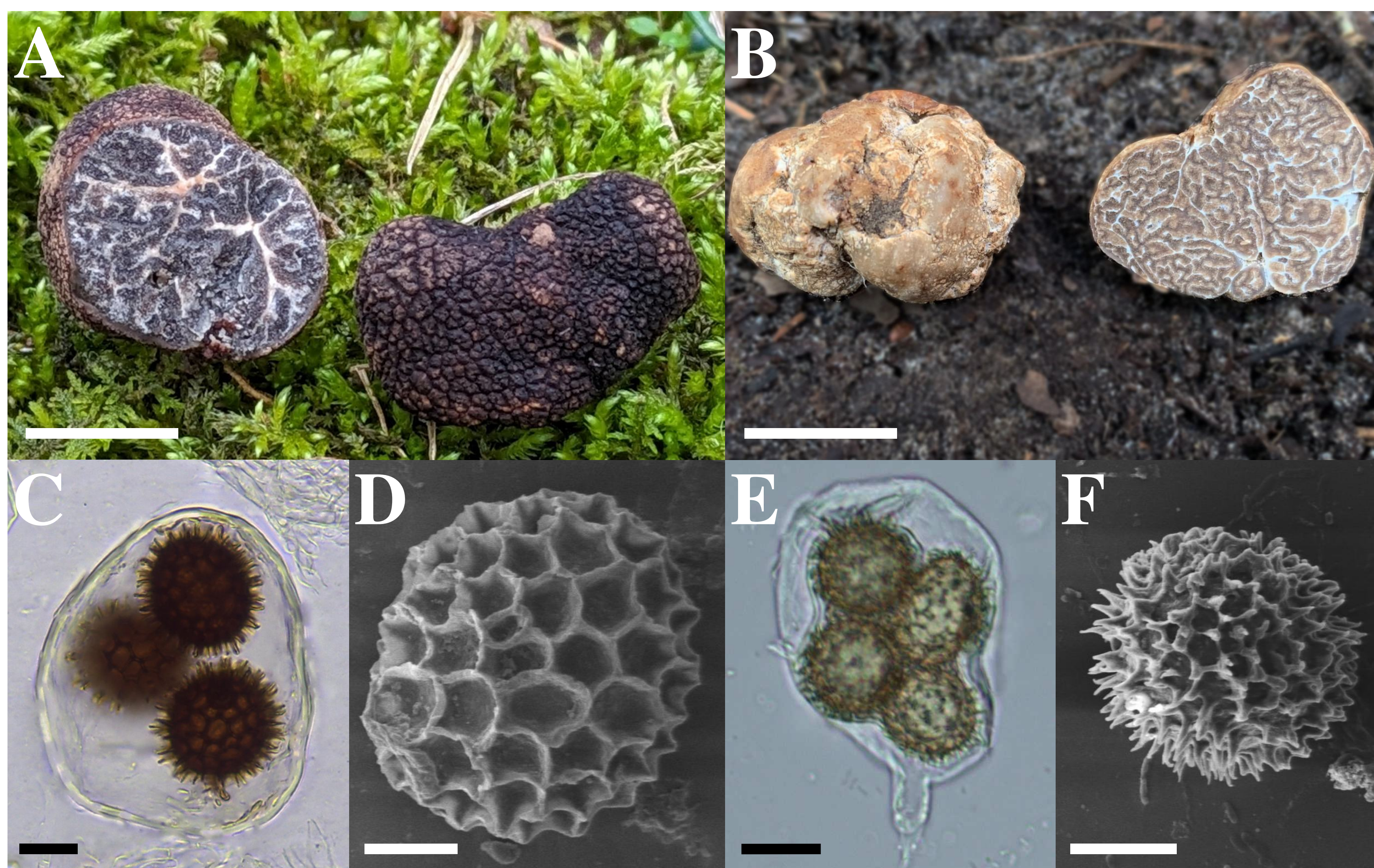
Tuber canirevelatum and *T. cumberlandense*, two new edible *Tuber* species from eastern North America discovered by truffle-hunting dogs

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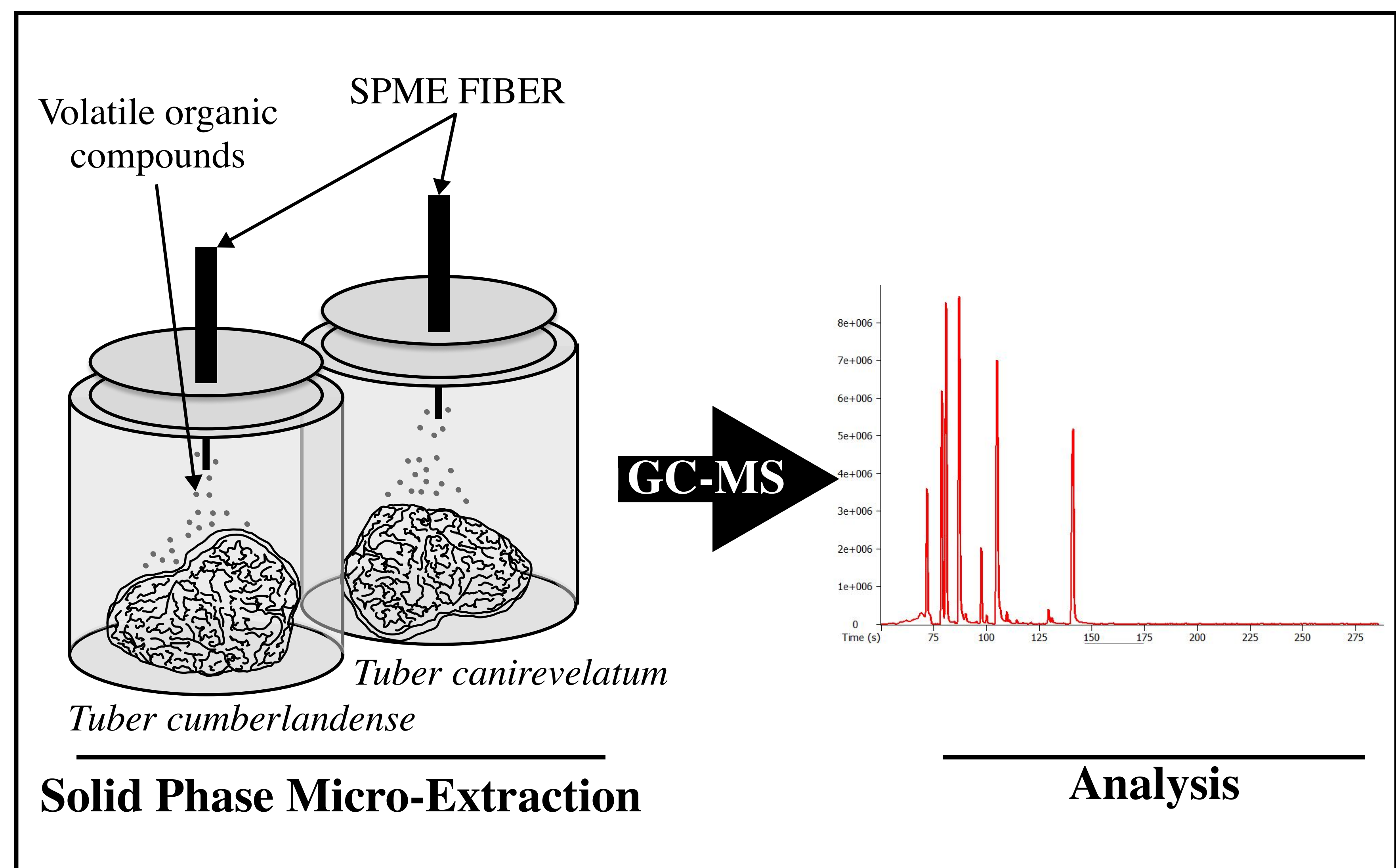
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Background

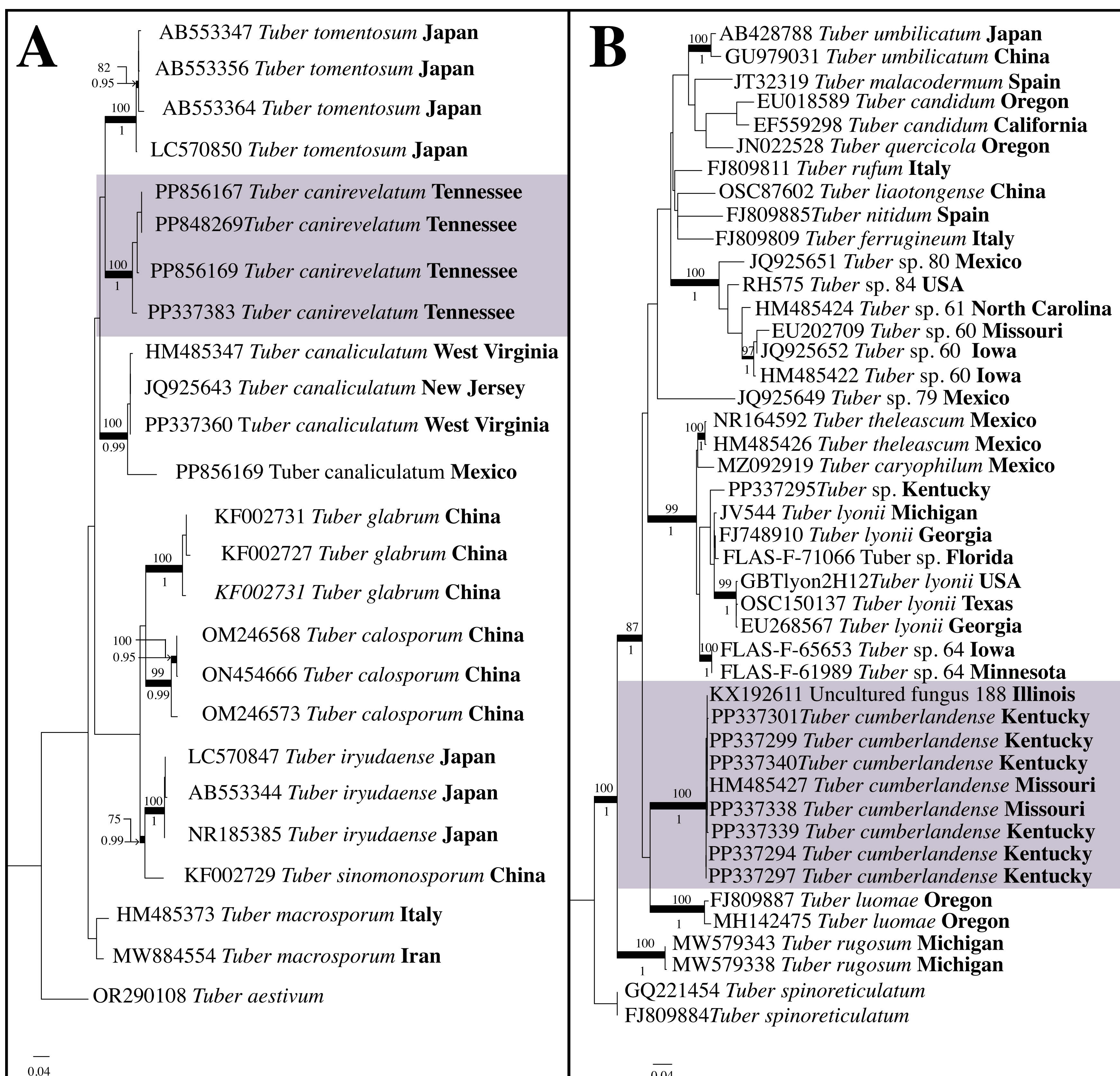
Tuber are ectomycorrhizal fungi whose species form hypogeous fruiting bodies called truffles. Many species are prized due to their edible and aromatic ascomata. Historically, there has been attention on cultivating European truffle species, but there is growing interest in cultivating, wild-harvesting, and selling species of truffles endemic to North America. **North America has many endemic *Tuber* species that remain undescribed, including some with favorable culinary qualities. Here we describe two such *Tuber* species from eastern North America.** We used phylogenetic, morphological, and aromatic analyses to describe and place these species within a phylogenetic context. *Tuber cumberlandense* sp. nov. (previously identified as *Tuber* sp. 66), has been opportunistically harvested from *T. melanosporum* orchards across eastern North America and is sold commercially. *Tuber canirevelatum* sp. nov. belongs in the Macrosporium clade, and thus far is only known from eastern Tennessee, USA. Both new species were discovered with the assistance of trained truffle dogs. In this work, we celebrate and encourage the use of trained truffle-hunting dogs for fungal biodiversity discovery and research.



***Tuber canirevelatum* sp. nov.** ascoma (A), and ascospores (C-D). ***Tuber cumberlandensis* sp. nov.** ascoma (B) and ascospores (E-F). A= 4 mm, B= 5 mm, C= 25 μ m, D= 10 μ m, E= 20 μ m, F= 10 μ m.



Example workflow for extraction and identification of volatile organic compounds released by *Tuber canirevelatum* sp. nov. and *Tuber cumberlandensis* sp. nov. ascoma.



ITS rDNA phylogenies of the Macrosporium clade (A) and Rufum clade (B). Branches with bootstrap support $\geq 70\%$ and Bayesian probabilities ≥ 0.95 are bold.

Results

Tuber canirevelatum

Etymology: In honor of the truffle dogs who have been essential in the discovery and harvesting of truffles.

Distribution: So far only Tennessee under *Quercus* spp.

Diagnosis:

- ascoma hypogeous, ovoid, dark, warty
- amber reticulated ascospores
- spores appear spiny
- gleba light to dark grey
- odor pungent similar to garlic and cilantro

Volatile organic compounds:

- dimethyl disulfide
- dimethyl sulfide
- acetone
- 1-methylthio-propane
- 2-butanol

Tuber cumberlandense

Etymology: Referring to the Cumberland Plateau where *T. cumberlandense* is mostly found.

Distribution: North Eastern US; Kentucky, Tennessee, Missouri

Diagnosis:

- ascoma hypogeous, ovoid, irregularly lobed, rugose, no warts
- yellow-ochre, spiny, ascospores
 - spines connect at the base
- gleba light brown to beige
- odor strong, similar to brassicaceous vegetables, radishes, or green onions

Volatile organic compounds:

- amyl nitrite
- dimethyl sulfide
- dimethyl trisulfide
- 1-methylthio-propane