First reports of *Phytophthora ramorum* clonal lineages NA1 and EU1 causing Sudden Oak Death on tanoaks in Del Norte County, California

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A year of forest health surveys has led to the first detection of *Phytophthora ramorum* in Del Norte County followed by the first wildland detection of the EU1 clonal lineage (Grunwald et al. 2009) of this pathogen in California. In July 2019, leaves were sampled from two tanoaks (*Notholithocarpus densiflorus*) and 16 California bay laurels (*Umbellularia californica*) in Jedediah Smith State Park in Del Norte County, the northernmost coastal County of California. Leaves displayed lesions normally associated with Sudden Oak Death (SOD) caused by *P. ramorum* and were discovered during the citizen science-based survey known as SOD Blitz (Meentemeyer et al. 2015). Samples were surface sterilized using 75% Ethanol and plated on PARPH-V8 agar (Jeffers and Martin 1986). After plating, DNA was extracted and amplified using two *P. ramorum*-specific assays (Hayden et al. 2006, Kroon et al. 2004). Leaves from two tanoaks exhibiting twig die-back had typical SOD lesions along the midvein, gave positive PCR results and yielded cultures with colony morphology, sporangia and chlamydospores typical of the NA1 lineage of *P. ramorum* originally isolated in California from tanoaks and coast live oaks (*Quercus agrifolia*) (Rizzo et al. 2002). The ITS locus and a portion of the *Cox-1* locus were sequenced from DNA extracts of each culture using primers DC6-ITS4 (Bonants et al. 2004) and COXF4N-COXR4N (Kroon et al. 2004), respectively. ITS sequences (GB MN540639-40) were typical of *P. ramorum* and *Cox-1* sequences (GB MN540142-3) perfectly matched the *Cox-1* sequence of the NA1 lineage (GB DQ832718) (Kroon et al. 2004). Microsatellite alleles were generated as described in Croucher et al. (2013) for the two Del Norte cultures and for eight *P. ramorum* cultures, representative of the four main multilocus genotypes (MLGs) present in California, namely c1 (Santa Cruz/Commercial Nurseries), c3 (San Francisco Bay Area), c2 (Monterey County), and c4 (Humboldt County) (Croucher et al. 2013). The two Del Norte MLGs were identical to one another and most similar to MLG c1, with a single repeat difference at a single locus. SSR results suggest the inoculum source may not be from Humboldt County, neighboring to the South, but from a yet unidentified outbreak, possibly associated with ornamental plants. Jedediah Smith State Park was surveyed for 12 months following the initial detection, however the pathogen has yet to be re-isolated in that location. In July 2020, SOD symptomatic leaves from two tanoak trees exhibiting twig cankers were collected 8 Km north of Jedediah Smith State Park, where three additional tanoak trees displayed rapidly browned

dead canopies consistent with late stage SOD. Leaves were processed as above. Colonies from these samples produced chlamydospores and sporangia typical of *P. ramorum* on PARPH-V8 agar, but displayed a growth rate faster than that of NA1 genotypes and were characterized by aerial hyphae, overall resembling the morphology of EU1 lineage colonies (Brasier 2003). The EU1 lineage was confirmed by the perfect match of the sequence of a portion of the *Cox-1* gene (GB MW349116-7) with the *Cox-1* sequence of EU1 genotypes (GB EU124926). The EU1 clonal lineage has been previously isolated from tanoaks in Oregon forests, approximately 55 Km to the North (Grünwald et al. 2016), but this is the first report for California wildlands and will require containment and government regulations. It is unknown whether the EU1 strains in Del Norte County originated from Oregon forests or elsewhere.

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