



DISCOVERY OF CRYPTIC *ARMILLARIA SOLIDIPIPES* GENOTYPES WITHIN THE COLORADO PLATEAU

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ABSTRACT

Armillaria solidipes (= *A. ostoyae*) is a root-disease pathogen that causes severe losses in growth and productivity of forest trees throughout the Northern Hemisphere. This species is genetically diverse with variable disease activities across different regions of the world. In North America, *A. solidipes* in the Colorado Plateau exists in drier habitats and causes more disease on hardwoods in comparison with *A. solidipes* in the northwestern USA (McDonald 1999). Here, we address 1) the discovery of cryptic genotypes of *A. solidipes* located within or near the Colorado Plateau region, 2) the analytical issues associated with rDNA sequences containing high levels of heterogeneity, and 3) the significance of these cryptic *A. solidipes* genotypes.

Methods Used To Discover Cryptic *A. solidipes* Genotypes

The intergenic spacer 1 (IGS-1) rDNA region of a single *A. solidipes* [pending vote to conserve *A. ostoyae* (Redhead et al. 2011)] isolate (U5) was cloned and sequenced to examine sequence heterogeneity. The resulting IGS-1 rDNA sequences (8 sequence types derived from 100 clones) demonstrated high heterogeneity within the IGS-1 rDNA region (Table 1). The sequence types of the cloned isolate (U5) are representative of other *A. solidipes* isolates from the Colorado Plateau (Figure 1).

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We then compared the IGS-1 rDNA sequence types from this study with sequences of *A. solidipes* and closely related species from Asia, Europe, and North America. All sequences were edited and aligned by hand/eye and analyzed using the neighbor-joining method for reconstructing phylogenetic trees in MEGA 5.05 (Figure 2) (Saitou and Nei 1987, Tamura et al. 2011).

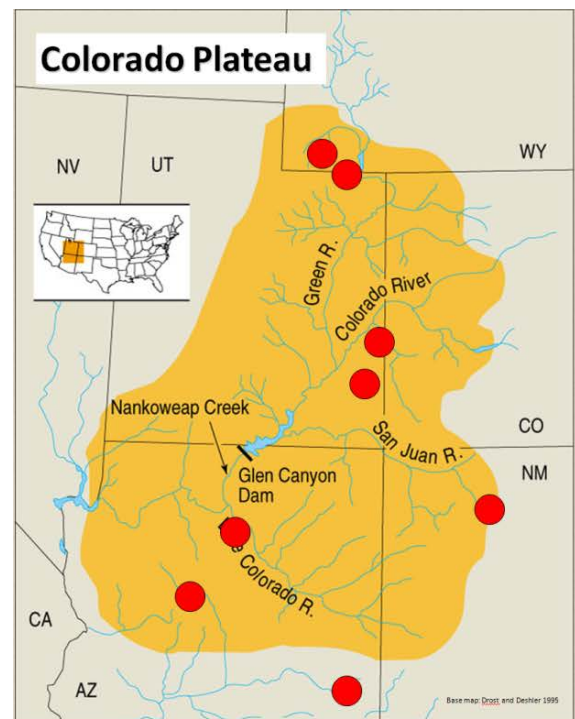


Figure 1: Approximate known locations of isolates putatively regarded as belonging to the “Colorado Plateau” *A. solidipes* group. These isolates contain numerous single nucleotide polymorphisms (SNPs) within the intergenic spacer 1 (IGS-1) rDNA region (Table 1). This indicates that each individual has heterogeneous (hybrid) intraspecific genotypes that include sequence types of both Colorado Plateau and Circumboreal (formerly Rockies group) *A. solidipes* clades (Figure 2).

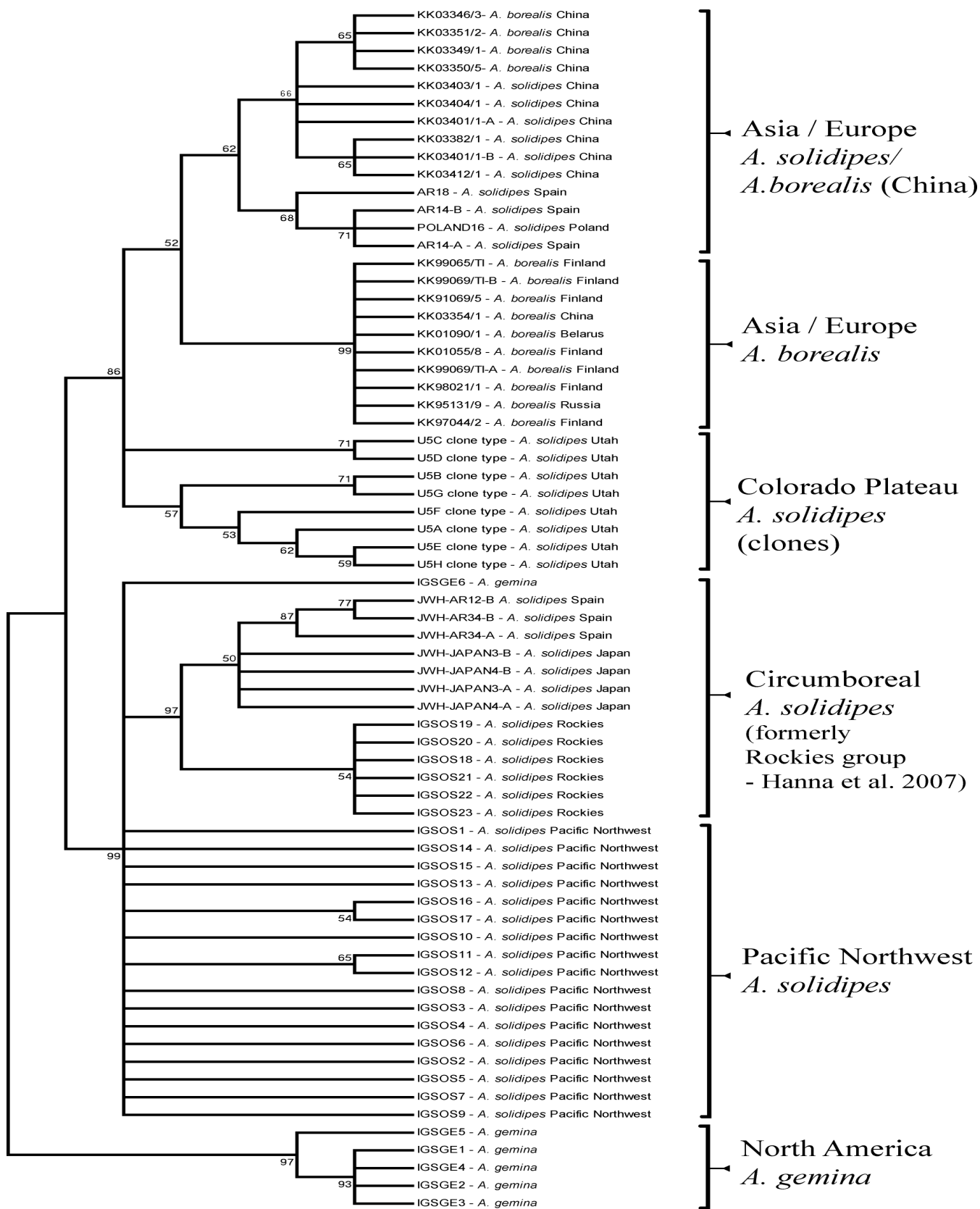


Figure 2: Neighbor-joining 50 percent majority-rule, consensus tree based on 591 basepairs within the IGS-1 rDNA region. The number at each node represents percent bootstrap values out of 500 replicates.

Table 1: SNPs (single nucleotide polymorphisms) within a sequence obtained using direct PCR versus eight IGS-1 sequence types derived from 100 clones of the same isolate (U5).

Base Pair	25	48	195	226	270	300	311	361	366	372	403	411	450	457	483	517	529	542	545
Isolate U5 Direct PCR	Y	Y	Y	Y	Y	R	R	Y	W	Y	R	K	R	Y	S	K	W	Y	R
U5 - Clone Type A	T	C	C	T	T	G	G	T	T	C	A	G	G	T	C	G	T	T	G
U5 - Clone Type B	T	C	C	T	T	G	G	T	T	C	A	G	G	T	C	G	T	C	A
U5 - Clone Type C	C	T	T	C	C	G	G	T	T	C	A	G	G	T	C	G	T	T	G
U5 - Clone Type D	C	T	T	C	C	A	G	T	T	C	A	G	G	T	C	G	T	T	G
U5 - Clone Type E	T	C	C	T	T	G	G	T	T	C	A	G	G	T	C	G	T	T	G
U5 - Clone Type F	C	C	C	T	T	G	G	T	T	C	A	G	G	T	C	G	T	T	G
U5 - Clone Type G	T	C	C	T	T	G	G	T	T	C	A	G	G	T	C	G	A	C	A
U5 - Clone Type H	T	C	C	T	T	G	G	T	T	C	A	G	G	T	C	G	T	T	G

SNP codes:
K = G or T
R = A or G
Y = C or T
S = C or G
W = A or T

Significance Of Cryptic *A. solidipes* Genotypes From The Colorado Plateau

Previous studies of genetic diversity within *A. solidipes* have shown that using heterogeneous sequence types can potentially reduce phylogenetic signal by collapsing clades between that of its ancestral/parental origins (Hanna et al. 2007). For this reason, we examined clones of the IGS-1 region from one of several highly heterogeneous isolates identified from the Colorado Plateau (Figure 1).

Surprisingly, the resulting sequence types derived from the U5 IGS-1 clones are more closely related to *A. borealis* sequence types than with other North American *A. solidipes* groups (Figure 2). Furthermore, a high level of apparent recombination within the clones was revealed (Table 1), and moreover, the eight sequence types from the 100 clones did not account for all the sequence variability observed by direct-PCR sequencing methods (Table 1). The genetic differences between this cryptic genotype and other North American *A. solidipes* isolates may correspond with the differences in disease activity/potential vegetation groups from that of *A. solidipes* in the Northwestern United States (Table 2) (McDonald 1999).

These cryptic *A. solidipes* genotypes from the Colorado Plateau could potentially represent ancient genotypes of *Armillaria* that have occupied the Colorado Plateau for over 200 million years, dating back to an ancient time when present-day geographic regions of North America, Europe, and Asia were contiguous. More studies are needed to determine the ecological significance of these cryptic *A. solidipes* genotypes from the Colorado Plateau.

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Table 2: Table from McDonald et al. 1999 showing that disease activity of *Armillaria solidipes* differs in Utah from that of *A. solidipes* from the Pacific Northwest.

Disease activity of <i>Armillaria solidipes</i> in Utah (Colorado Plateau) is different from Inland Northwest				
G. I. McDonald 1999		Armillaria Present		
Potential Veg Group	Location	Yes	No	%Present
Dry Fir/Dry Shrub	NW	0	8	0
	Utah	0	5	0
Cold Fir/Dry Shrub	NW	0	8	0
	Utah	5*	7	42
Cold Fir/Dry Herb	NW	11*	9	55
	Utah	3*	3	50



**A. solidipes* pathogenic on aspens and conifers.
**A. solidipes* is pathogenic on conifers.

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