

Is There a Cryptic Species Within *Aulacorthum solani* (Hemiptera: Aphididae)?

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ABSTRACT Examination of DNA sequences of the 5' end of the mitochondrial cytochrome oxidase I gene of *Aulacorthum solani* (Kaltenbach) (Hemiptera: Aphididae) reveals little variation between samples from broad geographic provenances. The apparent genetic similarity despite *A. solani*'s morphological and biological differences contrasts with the species complexes of other aphid pests.

KEY WORDS foxglove aphid, glasshouse potato aphid, cytochrome oxidase I

Most aphids are host specialists, but of the nearly 4,700 species of aphids (Remaudière and Remaudière 1997), 18–35 species are highly polyphagous (Blackman and Eastop 1994, 2000, 2006). One of these polyphagous species is *Aulacorthum solani* (Kaltenbach) (Hemiptera: Aphididae). Considered native to Europe, *A. solani* is nearly cosmopolitan in its distribution. This aphid was first described on potato (*Solanum tuberosum* L., Solce), but it has since been recorded from an extremely large range of both mono- and dicotyledonous, herbaceous, and woody plant families (Blackman and Eastop 1994, 2000, 2006). The basic life cycle of *A. solani* is also variable, the species exhibiting both holocycly and anholocycly. Along with this range in the life history, there is an equal amount of morphological variability. This combination of variability has led some to propose additional species and subspecies (Blackman and Eastop 2006, Damsteegt and Voegtlin 1990, Müller 1976) or to suggest that *A. solani* be treated as a species complex (Müller 1985). With all of these complications, it is no surprise that *A. solani* ranks third among aphids for most recognized junior synonyms, with 37 (Remaudière and Remaudière 1997).

Clearing the taxonomic difficulty associated with *A. solani* is of great economic importance considering the its combination of extreme polyphagy and its known transmission of 45 plant viruses (Chan et al. 1991). Despite damaging crops in Asia, Australia, and Europe (Southall and Sly 1976, Hwang et al. 1981, Johnstone and Rapley 1981), *A. solani* is generally not considered a serious pest on field crops in North America, and in particular rarely colonizes soybean, *Glycine max* (L.) Merr. However, there have been recent outbreaks of *A. solani* on soybean in Asia (Nagano et al. 2001, Saito et al. 2001). This difference in host use suggests *A.*

solani may represent more than one species and that its global movement should be more carefully monitored and controlled. The present study was conducted to ascertain whether there is molecular evidence for cryptic species within what is currently recognized as *A. solani*.

Materials and Methods

Specimens were collected by hand at various sites in the United States, China, and Japan. Other sites (i.e., Colombia, The Netherlands, New Zealand, and Panama) are represented by specimens intercepted at quarantine facilities in the United States (Table 1). Because The Netherlands is a re-exporter of cut flowers for the worldwide market, this country may or may not represent the actual point of origin for the pertinent samples.

Using the technique described by Favret (2005), we extracted whole genomic aphid DNA nondestructively from a single individual in each collection sample using kits from QIAGEN (Valencia, CA). Specimen body contents are cleared via this system and individuals were subsequently preserved on microscope slides in Canada balsam. Specimens were determined as *A. solani* by using standard identification keys and then deposited in the National Aphidoidea Collection of the U.S. National Museum of Natural History, located at the USDA, Systematic Entomology Laboratory (Beltsville, MD).

A portion of the mitochondrial cytochrome oxidase I (COI) gene was amplified by polymerase chain reaction (PCR) using aphid-specific primers (Favret and Voegtlin 2004). The fragment of DNA was sequenced in both directions using these same primers with BigDye kits, version 3.1 (Applied Biosystems, Foster City, CA) and read on an Applied Biosystems sequencer. Sequences were assembled and aligned with BioEdit version 7.0.8. The number of base pair differences was hand-counted, and sequences were deposited in GenBank (accession nos. FJ009047,

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Table 1. Sample origins, variable sites, and accession numbers

Origin	Selected sequence position							GenBank accession
	9	18	33	68	76	342	348	
United States ^a	A	G	C	C	T	C	C	FJ009047
China ^b	C	A	T	C	C	C	C	FJ009046
Japan ^c	C	A	T	C	C	C	C	FJ009045
New Zealand (1) ^d	A	G	C	C	T	C	C	FJ009044
New Zealand (2) ^d	A	A	C	C	C	T	A	FJ009043
Netherlands (1) ^d	A	G	C	C	T	C	C	FJ009040
Netherlands (2) ^d	A	A	C	C	C	T	A	FJ009042
Netherlands (3) ^d	A	A	C	C	T	C	C	FJ009039
Netherlands (4) ^d	A	A	T	T	T	T	A	FJ009041
Portugal ^d	A	A	T	C	T	T	A	FJ009035
United Kingdom ^d	A	G	C	C	T	C	C	FJ009037
Colombia ^d	A	G	C	C	T	C	C	FJ009038
Panama ^d	A	G	C	C	T	C	C	FJ009036

^a Ten collections from nine host genera in the Great Smoky Mountains National Park in North Carolina and Tennessee, U.S.A.

^b Two collections from soybean in Xiuyan, Liaoning Province, China.

^c Two samples from soybean in Morioka, Iwate Prefecture, and one sample from soybean in Tsukuba, Ibaraki Prefecture, Japan.

^d Intercepted at ports-of-entry in the United States.

FJ009046, FJ009045, FJ009044, FJ009043, FJ009040, FJ009042, FJ009039, FJ009041, FJ009035, FJ009037, FJ009038, and FJ009036).

Results and Discussion

Even with a broad range of host plants (we collected *A. solani* on 23 host species in the Great Smoky Mountains National Park alone) and the great geographic range from which we obtained specimens, we observed very little COI sequence variation, because only five different genotypes were found. In the 551 bp sequenced, only seven were variable (Table 1), with no more than five base pair differences between any two individuals. Sequences from the specimens from the Americas, the United Kingdom, and one of The Netherlands samples and one of the New Zealand specimens were identical, and another pair of samples from New Zealand and The Netherlands was identical to each other. Sequences from the Japanese and Chinese samples were identical. This divergence of 0.7% is far lower than the 2% sequence divergence often cited for species level differences (Hebert et al. 2003). However, a Templeton, Crandall, and Sing (TCS) analysis suggested that the Southeast Asian haplotype was not nested with the other haplotypes.

Numerous other aphids have been considered as part of species groups or complexes (e.g., *A. frangulae*), which has confused their taxonomy and rendered species identifications difficult. Taxonomic resolution has been achieved with some of these complexes by using molecular techniques (e.g., Lozier et al. 2008) or have led to evidence for a single species rather than a complex (e.g., Clements et al. 2000). In the current study, the lack of large genetic divergence seems to support a single species concept for *A. solani*. Our findings corroborate those of Valenzuela et al. (2007) who also found no significant sequence differences

between specimens from The Netherlands, western United States, and Australia.

If *A. solani* does not represent a complex of cryptic species, why is it a pest on soybean in Asia but not in North America? One possibility is that of biotypes that are sometimes recognized and labeled for morphologically indistinguishable insect populations harboring biological differences with usually the presence or absence of virulence on a particular host variety. Of the described biotypes of insect pests of agricultural crops, nearly half are aphids (Saxena and Barrion 1987). Along with biotypes, it is possible that host plant use may be tied to facultative endosymbionts as has been shown with the pea aphid, *Acyrtosiphon pisum* (Harris) (Tsuchida et al. 2004). In this case, the presence of the U-type symbiont provided its aphid host improved fitness on certain plant hosts (Tsuchida et al. 2004). Alternately, different host cultivars may react differently to aphid clones. For example, Shufran et al. (2007) demonstrated the ability of different aphid clones to cause varying degrees of plant damage to the same aphid-resistant crop variety.

Whatever may be the causes of variation in the biology of *A. solani*, our current species concept of *A. solani* remains intact based upon our collected specimens. This is important from a taxonomic and regulatory point of view. However, our sampling was limited and more genetically divergent lineages of *A. solani* may yet be found.

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