N O T E

Wolbachia Strains Associated with Univoltine and Multivoltine Plum Curculios (Coleoptera: Curculionidae)[1]

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Plum curculio, *Conotrachelus nenuphar* (Herbst), is a serious fruit pest in eastern North America. Two strains have been identified, one univoltine (northern) and one multivoltine (southern) (Chapman 1938, NY Agric. Exp. Stn. Bull. 684; Racette et al. 1992, Phytoprotection 73: 85-100). Decreased fecundity and reproductive incompatibility have been reported from crosses of univoltine females with multivoltine males (Stevenson and Smith 1961, J. Econ. Entomol. 54: 283-284) and multivoltine females with univoltine males (Padula and Smith 1971, Ann. Entomol. Soc. Am. 64: 665-668). Because similar anomalies have been attributed to *Wolbachia* infection (Werren 1998, Pp. 245-260, *In* D. J. Howard and S. H. Berlocher [eds.], Endless Forms Species and Speciation, Oxford University Press, New York; Bandi et al. 2001, Trends Parasitol. 17: 88-94), we hypothesized that the two strains of *C. nenuphar* are infected with different, perhaps incompatible, *Wolbachia* strains.

Zhou et al. (1998, Proc. Royal Soc. London B 265: 509-515) used *Wolbachia* surface protein (*wsp*) gene sequence to divide the genus into supergroups A and B and to create reference groups based on sequence divergence of <2.5%. To determine if *C. nenuphar* were infected with *Wolbachia* and to classify infecting strain(s), univoltine adults from Massachusetts (R. Prokopy, UMASS) and multivoltine adults from Georgia (D. Horton, UGA) and Florida (R. Mizell, UFL) were screened using a *wsp* polymerase chain reaction (PCR) assay. DNA from individual weevils was prepared following Ashburner (1989, Preparation of DNA from single flies. *In Drosophila:* A laboratory manual, Cold Spring Harbor Laboratory Press, NY). PCR was performed as described by Zhou et al. (1998) with 300 nM 81F and 691R primers and 5-10 ng of DNA. Amplimers were cloned and sequenced in both orientations. Amplimers from three individuals per population were sequenced. From the first individual, three plasmid clones were sequenced. One clone each from the second and third individuals was sequenced.

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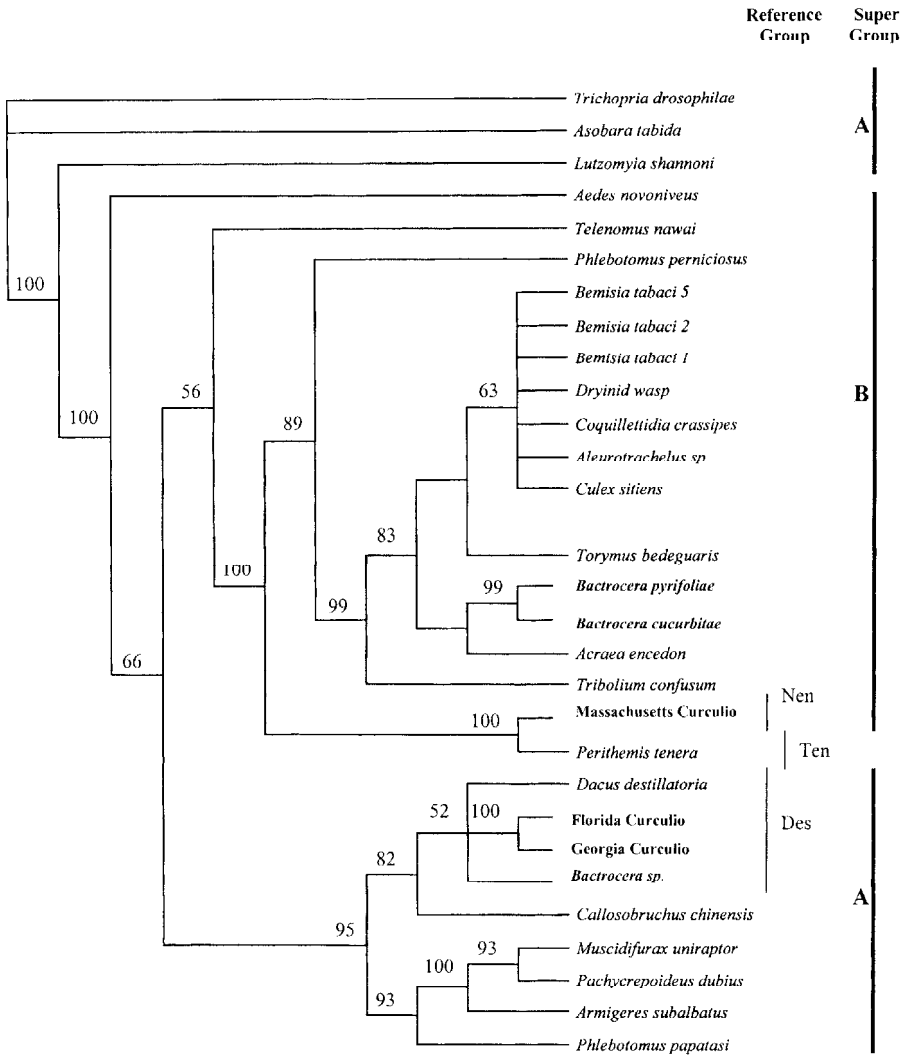

Fig. 1. Parsimonious midpoint rooted tree generated for 29 *Wolbachia* strains (shown by host) using a CLUSTAL W alignment of nucleotide sequences. All bootstrap values detected were above 50% for 100 replicates. Branches without bootstrap values were collapsed for bootstrap analysis. A total of five clones from three individuals were used to generate consensus sequences for Massachusetts, Florida and Georgia curculios. All strains within a reference group are less than 2.5% divergent.

Consensus sequences for MA, GA, and FL amplimers were aligned with 26 partial *wsp* sequences using CLUSTAL W (Thompson et al. 1994, Nuc. Acids Res. 22: 4673-4680). A phylogenetic tree of the 507-nucleotide data set (Fig. 1) was constructed using PAUP* 4 (Sinauer Associates, Sunderland, MA) and analyzed by

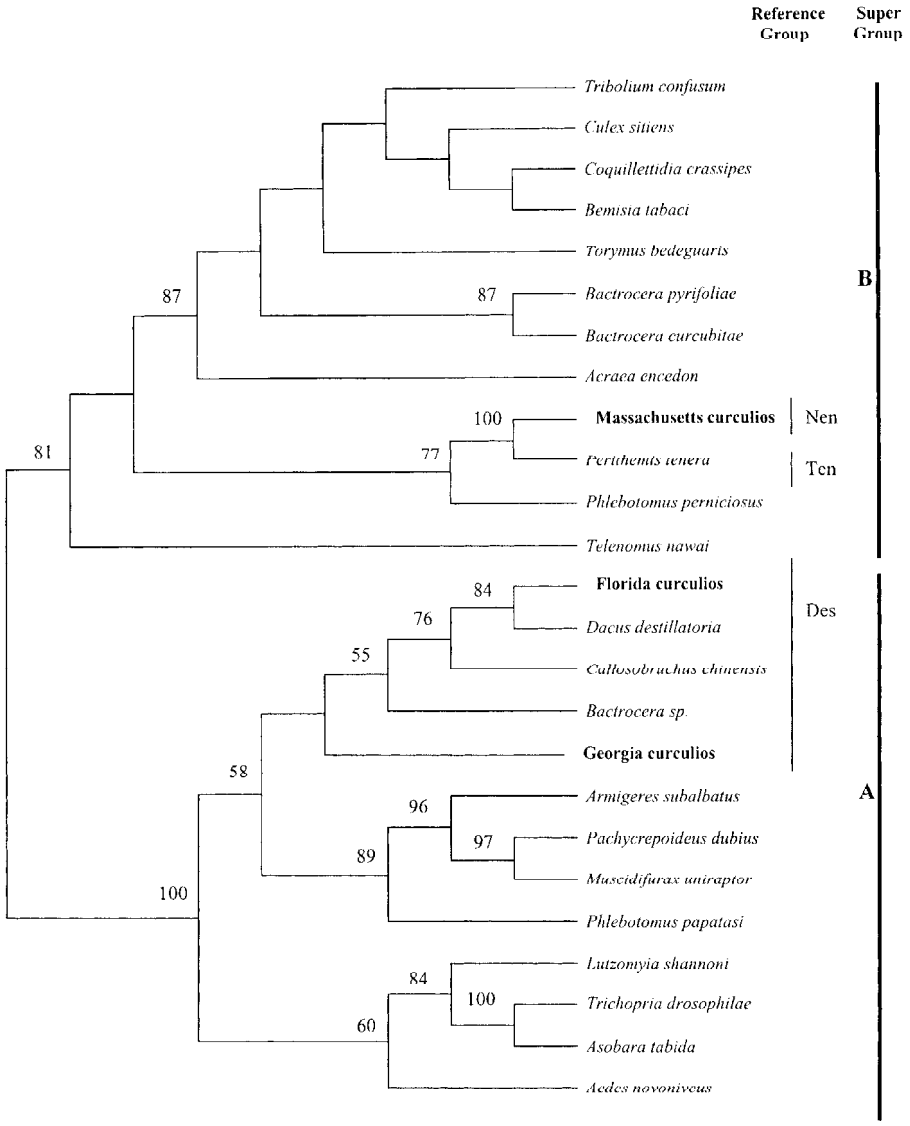

Fig. 2. Neighbor joining mid-point rooted tree generated for 24 *Wolbachia* strains (shown by host) using a CLUSTAL W alignment of predicted protein sequences. All bootstrap values detected were above 50% for 100 replicates. Branches without bootstrap values were collapsed for bootstrap analysis.

maximum parsimony with branch and bound searches. A phylogenetic tree (Fig. 2) of 24 predicted protein sequences (169 characters) was constructed using PAUP* 4 and analyzed by the neighbor-joining method using a heuristic search. Bootstrap analyses for both data sets were performed with 100 replications.

Our results suggest that univoltine and multivoltine weevils carry different strains of *Wolbachia* (Figs. 1, 2). *Wolbachia* detected in MA weevils is in supergroup B and is 91.48% similar to *w*Ten-B1 (host *Perithemis tenera*). Following convention (Stouthamer et al. 1999, Annu. Rev. Microbiol. 53: 71-102, Jeyaprakash and Hoy 2000, Insect Molec. Biol. 9: 393-405), this strain shall be called "*w*Nen" and the reference group "Nen". *Wolbachia* detected in FL and GA weevils are in supergroup A (Figs. 1, 2). While *Wolbachia* from GA curculios is unique (not shown), *Wolbachia* from FL curculios is 99.6% identical to that associated with *D. destillatoria*. The FL and GA strains belong in the reference group "Des", with the GA strain known hereafter as "*w*Cnen".

In addition to studying the impact of *Wolbachia* infection on *C. nenuphar* reproduction, we are exploring the *wsp* PCR assay as a diagnostic tool to monitor strain boundaries in states such as Virginia. Because the multivoltine strain hinders fruit export, use of this technology could delineate affected regions, limiting economic costs of *C. nenuphar*.

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