N O T E

A DNA Marker to Track *Conotrachelus nenuphar* (Coleoptera: Curculionidae) Dispersal[1]

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Plum curculio, *Conotrachelus nenuphar* (Herbst), is indigenous east of the Rocky Mountains and north of latitude 50°N in the United States (Amis and Snow. 1985, Handbook of Insect Rearing, Vol. 1) with a population in Utah (Hallman 1998, USDA-ARS Report, Weslaco, TX, USA). It is a phytophagous, true weevil and a pest of pome and stone fruit, i.e., apples, plums, cherries, and peaches (Shapiro-Ilan et al. 2002, J. Nematology 34: 246-349). In Georgia and South Carolina, the peach (*Prunus persica* [L.]) is a major stone fruit commodity. Plum curculio is the most destructive pest of the commodity (Brannon 1927, J. Elisha Mitchell Scientific Soc. 43: 79-83; Leskey and Wright. 2004, Environ. Entomol. 33: 389-396) on which they feed and into which they oviposit. Commercial fruit orchards also have historically been unable to rely on trap monitoring systems to accurately time insecticide applications (Prokopy et al. 2000, J. Entomol. Sci. 35: 411-420; Leskey et al. 2012, http://digitalcommons.unl.edu/usdaars-facpub/1108). Thus, in 1994 Mexico halted importation of peaches from Georgia and South Carolina due to concerns of importing plum curculio with the peaches. But, in 2008 the U.S. Department of Agriculture Animal and Plant Health Inspection Service (APHIS) revisited the plum curculio issue on behalf of the peach growers in Georgia and South Carolina. An agreement was reached in 2011 between the U. S. and Mexico which required that specific procedures be in place to insure that peaches shipped to Mexico from Georgia and South Carolina are free of plum curculio. These procedures included field surveys, trapping, packinghouse inspections, fruit-cutting, and post-entry inspections.

A DNA verification system designed specifically to track dispersal of plum curculio from Georgia is also needed to track and/or verify infestations, particularly if post-entry

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insects are found. The mitochondrial cytochrome oxidase I (COI) gene sequence was used to successfully separate northern from southern strains (Zhang et al. 2008, Ann. Entomol. Soc. Am. 101: 824-832), but, this marker is not sufficiently variable for regional intraspecific identification. Our purpose herein, therefore, is to present preliminary DNA data which suggest that sequence of the PCR-amplified ribosomal DNA (rDNA) intergenic transcribed spacer (ITS) array can be used to track and verify dispersal of plum curculio from Georgia.

Voucher specimens were sent to the Museum of Natural History, University of Georgia, Athens, GA 30602-2603. The intraspecific variability of the ITS region (Jenkins et al. 2007, Mol. Phylogenet. Evol. 42: 612-621) was tested by sampling 6 individuals: 3 from inbred lab colonies from Byron, GA (CN1, CN2, CN3) with the same COI-COII mitochondrial haplotype (GenBank No. KF150698); and, 2 from populations in Utah (CN4, CN5) with the same COI-COII mitochondrial haplotype (GenBank No. KF150699). A 1566 bp fragment of the ITS array (Jenkins et al. 2007) was then amplified from each of these 6 randomly sampled individuals from Georgia and Utah using polymerase chain reaction (PCR) according to an established protocol (Jenkins et al. 2007). Primers successfully used in previous studies (Jenkins et al. 2009, Ann. Entomol. Soc. Am. 102: 386-395) – CS249 (5'-TCGTAACAAGGTTTCCG-3') and CS250 (GTT(A/G)GTTTCTTTTCCTC-3') (Schlötterer et al. 1994, Mol. Biol. Evol. 11: 513-522) – also were used in this study to amplify as well as sequence fragments. Collections were identified as CN for *Conotrachelus nenuphar* plus a number which identified the sample population and a second number which identified the individual, i.e., CN1-1 = *Conotrachelus nenuphar* population 1, individual 1 (GenBank Nos. KF15069-KF150699). Samples in this study included: CN1-1, CN1-3, CN2-1 collected 11-X-2011 by Ann Amis in Byron, GA (32° 39'N; 83° 44' W); CN3-1 collected 2-XI-2011 by Ann Amis in Byron, GA (32° 39'N; 83° 44'W); CN4-1 and CN5-1 collected by Diane Alston on 12-V-2012 in Box Elder Co., Utah (41° 30'N; -113° 5'W). Phylip 3.65 software package (Felsenstein 1993, PHYLIP (Phylogeny Inference Package)

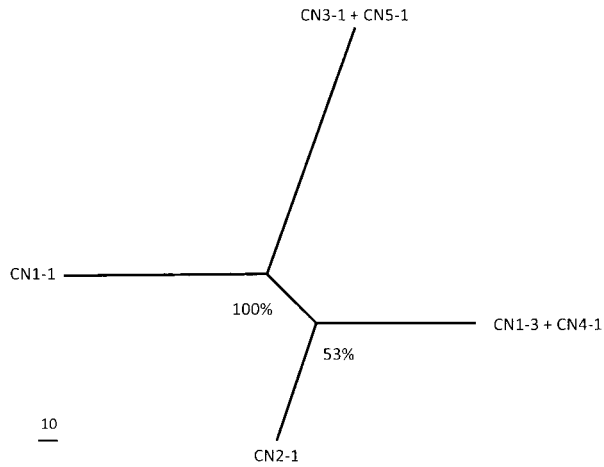

Fig. 1. Unrooted neighbor-joining (NJ) consensus tree of *C. nenuphar* specimens from 3 populations in Byron, GA (CN1, CN2, DN3), and 2 populations in Box Elder Co, UT (CN4, CN5).

Manual, version 3.5. Distributed by the author, University of Washington, Seattle, WA) was used to generate an unrooted neighbor-joining (NJ) consensus tree (Fig. 1). SEQBOOT generated 100 pseudoreplicates, DNADIST with the Kimura-2-parameter model generated the distance matrix, NEIGHBOR generated 100 NJ trees and CONSENSE, majority rule extended, computed a consensus tree with assigned support values at the nodes (Fig. 1). The consensus NJ tree illuminated the discriminating power of this DNA marker as nodes are supported 53% to 100%. Byron (CN1-3) plus Utah (CN4-1) collections formed a consensus sequence showing their extant relationship as did Byron (CN3-1) plus Utah (CN5-1). CN1-1 and CN2-1, although collected on the same day from the same inbred colony with the same maternal lineage, had different sequences. Additional samples are being analyzed to establish a genetic baseline. But, the preliminary data show a level of discrimination not observed with mitochondrial COI sequence (Zhang et al. 2008, Ann. Entomol. Soc. Am. 101: 824-832) or COI-COII sequence. In summary, it appears that the ITS region of the rDNA cluster amplified and sequenced with primers CS249 and CS250 has the discriminating power to provide an efficient, PCR-based, DNA marker with which to track plum curculio dispersal as well as possible source populations.