Relationships Among Species of *Scirtothrips* (Thysanoptera: Thripidae, Thripinae) Using Molecular and Morphological Data

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Ann. Entomol. Soc. Am. 101(3): 491–500 (2008)

ABSTRACT Results of analyses of molecular sequence (COI, 28S-D2) and morphology (21 character states) data, both alone and combined, were used to determine relationships between 18 species of *Scirtothrips* (Thysanoptera: Thripidae, Thripinae). Two species of *Neohydatothrips* from Panama were used as the outgroup. Five major pest *Scirtothrips* were included in these analyses: *S. aurantii*, *S. citri*, *S. dorsalis*, *S. kenyensis*, and *S. perseae*. Trees generated from a parsimony analysis of molecular data and Bayesian analysis of molecular and morphology data combined showed high levels of congruence. The morphology-only tree had several differences in species groupings when compared with trees derived using molecular data. Both parsimony and Bayesian analyses using molecular data indicated that *Scirtothrips* species associated with avocados were monophyletic, a result not observed with morphological analyses. No support was found for a monophyletic *S. dorsalis* clade when sampled from six different countries. The *S. dorsalis* complex may be comprised of at least three separable groups identifiable at the molecular level, but indistinguishable morphologically. The implications of this finding for *S. dorsalis*, a thrips pest of global significance with high invasion potential, are discussed.

KEY WORDS COI, 28S-D2, identification, *Neohydatothrips*, phylogeny

The genus *Scirtothrips* Shull is characterized by small, pale, active thrips (≈1.5 mm in length) that feed and breed primarily on young foliage and that may occasionally be collected from flowers. The majority of species seem to be mono- or stenophagous (e.g., *S. perseae* Nakahara on avocados [*Persea americana* Miller] and *S. frondis* Hoddle & Mound on *Dicksonia* sp. ferns), whereas others are highly polyphagous, and they can be serious pests of a variety of unrelated agricultural crops and ornamental plants (e.g., *S. dorsalis* Hood; Hoddle and Mound 2003). The genus is comprised of ≈100 described species with endemic representation in temperate and tropical areas of Africa, Asia, Australia, North, Central, and South America, New Zealand, and perhaps some island groups of the South Pacific (Bailey 1964, Mound and Palmer 1981, Johansen and Mojica-Guzmán 1998, Hoddle and Mound 2003, Hoddle et al. 2007a). Of this approximate world total, 32 new species have been described from Mexico attacking avocados and mangos (*Mangifera indica* L.) (Johansen and Mojica-Guzmán 1998). However, there is considerable doubt over the validity of many of these Mexican species because of highly variable morphological characters used to describe new species (Mound and zur Strassen 2001). Five of these new *Scirtothrips* species infesting avocados in Mexico were recently synonomized with the well recognized *S. perseae* (Hoddle et al. 2007b), a serious pest of avocados in California (USA), Mexico, and Guatemala (Nakahara 1997; Hoddle et al. 2002, 2003).

Scirtothrips are economically important because several species are serious agricultural pests of unrelated perennial and annual crops, have demonstrated high invasion potential, and they are a serious quarantine concern for many countries (Mound and Palmer 1981, Nakahara 1997; Morse and Hoddle 2006). Nonpestiferous native species are of interest from the standpoints of biodiversity and evolutionary relationships with endemic host plants. Given the global distribution of *Scirtothrips* species, the varying levels of polyphagy, the diverse host plant affinities, the biodiversity interests, the invasion potential and associated biosecurity threats, there has been high recent interest in understanding the validity of currently ascribed taxonomic identities and developing an accurate tool for separating species (Mound and zur Strassen 2001, Rugman-Jones et al. 2006, Hoddle and Mound 2007).

Species of *Scirtothrips* are difficult to identify because of high intraspecific morphological variation, even among members of populations collected at the same time from the same host plant (Hoddle and

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Mound 2003). Difficulties in species identification relying solely on slide-mounted specimens have been reduced by the development of a simple molecular key by using internal transcribed spacer regions 1 and 2 (ITS1 and ITS2) of nuclear ribosomal DNA for 16 species of *Scirtothrips*, of which several are major pest species (Rugman-Jones et al. 2006).

In this study, sufficient molecular data have been acquired to investigate the phylogenetic relationships between 18 unambiguously recognized species of *Scirtothrips* from Africa, Asia, Australia, North and Central America, New Zealand, and French Polynesia that were collected from at least 19 species of host plant in 17 families. This article presents the findings of molecular and morphology-based phylogenetic analyses for these 18 *Scirtothrips* species and is the first attempt to provide some understanding of the relationships between species of this genus.

Materials and Methods

Collection of *Scirtothrips* Specimens for Analysis. Collection records for specimens used in this study are listed in Table 1. Specimens were collected into 95% ethanol (see Hoddle et al. 2002, 2006; Rugman-Jones et al. 2006 for field collection methods) and stored at −20°C until analyzed. Two species of *Neohydatothrips* (Thysanoptera: Thripidae, Sericothripinae) collected from avocados in Panama were included in analyses as an outgroup.

DNA Extraction, Amplification, and Sequencing. Total DNA was isolated from individual thrips using a "salting-out" protocol adapted from Sunnucks and Hales (1996) and detailed in Rugman-Jones et al. (2006). The advantage of this method is that the specimen remains intact, preserving the integrity of morphological characters. After DNA extraction, all specimens were recovered, cleared in 5% NaOH for 12 h, taken through an alcohol dehydration series, placed in clove oil, and then slide mounted in balsam (Mound and Marullo 1996). They were identified to species by using morphological characters (Mound and Palmer 1981, Hoddle and Mound 2003, Moritz et al. 2004). Specimens from which DNA was extracted and subsequently slide mounted for this project have been deposited at the University of California-Riverside Entomology Research Museum, and their museum and GenBank accession numbers are included in Table 1.

After DNA extraction, two separate gene regions were amplified using polymerase chain reaction (PCR): the relatively conserved 28S-D2 domain of the large subunit rRNA and the cytochrome *c* subunit I (COI) of mitochondrial DNA (mtDNA) (Rugman-Jones et al. 2007). An ≈553-bp section of the 28S-D2 domain was amplified in 25-μl reactions containing 2 μl of DNA template (concentration not determined), 1× PCR buffer (containing 2 mM $MgSO_4$), 20 μM each dNTP, 0.2 μM each of the primers CF and CR (Campbell et al. 1993; 2000), 1 μl of bovine serum albumin (BSA) (New England Biolabs, Ipswich, MA), and 1 μl of *Taq* polymerase (New England Biolabs). Amplification was performed in a Mastercycler 5331 (Eppendorf, Hamburh, Germany) programmed for an initial denaturing step of 3 min at 94°C, followed by 30 cycles of 45 s at 94°C, 30 s at 50°C, and 1 min 30 s at 72°C; and a final extension of 30 min at 72°C. An ≈663-bp segment of the COI gene also was amplified in 25-μl reactions containing 2 μl of DNA template (concentration undetermined), 1× PCR buffer (containing 2 mM $MgSO_4$), 20 μM each dNTP, 2 mM $MgCl_2$, 0.2 μM each of the primers LCO1490 and HCO2198 (Folmer et al. 1994), 1.25 μl of BSA, and 1 U of *Taq* polymerase. The thermocycler was programmed for an initial denaturing step of 1 min at 94°C; followed by five cycles of 30 s at 94°C, 1 min 30 s at 45°C, and 1 min at 72°C; followed by a further 35 cycles of 30 s at 94°C, 1 min 30 s at 51°C, and 1 min at 72°C; and a final extension of 5 min at 72°C. PCR products were visualized on a 1% agarose gel stained with ethidium bromide.

Amplified DNA was cleaned using the Wizard PCR Preps DNA Purification System (Promega, Madison, WI) and direct sequenced in both directions at the University of California-Riverside Genomics Institute Core Instrumentation Facility by using an Applied Biosystems 3730 DNA Analyzer with a Big-Dye version 3.1 kit (Applied Biosystems, Foster City, CA). Raw sequences were trimmed in BioEdit (version 7.0.5.3) (Hall 1999) by removing the primers, and they were deposited in GenBank (Table 1).

Morphological Data. Twenty-one morphological characters were scored for all 18 *Scirtothrips* species together with *N. geminus* and *N. burungae* (Table 3). Selected morphological states have been described previously and used to define diagnostic characters in *Scirtothrips* species (Mound and Palmer 1981). The morphological characters listed in Table 3 and used in analyses are useful in recognizing and defining species because they exhibit high levels of stability, are unambiguous in interpretation, and combinations of these characters have been used in the construction of dichotomous keys that use morphology for separation of *Scirtothrips* species (Mound and Palmer 1981, Hoddle and Mound 2003).

Alignment and Phylogenetic Analyses. Sequences were initially aligned in Clustal X (version 1.83.1) (Thompson et al. 1997) and optimized by eye. The COI sequences were all of equal length (663 bases), and they had no stop codons, with the first position equal to codon position 1, making alignment easy. In contrast, 28S-D2 (553 bases aligned) included six ambiguous regions that were relatively conserved but still variable and difficult to align (Table 2). Additional character state coding was implemented for these six regions to recognize morphologically similar blocks of data (same length and sequence) (Wheeler 1999, Lutzoni et al. 2000, Geiger 2002, Rozas et al. 2003, Gillespie 2004; Table 2). This mixed alphabetical and numeric state coding (maximum of K = 21 states) was treated as unordered and combined with the other data, including the ambiguous regions. INNASE step coding of the states allocated to the ambiguous regions (Lut-

Table 1. Collection records of *Scirtothrips* species used in molecular and morphological phylogenetic analyses

Species[a] (no. sequenced [28S-D2:COI])	Locality collected (native range)	Host plant	Date (mo/d/yr)	Collector	GenBank and (UCR Museum) accession nos.
Scirtothrips aceri (CA) (1:3)	California, USA (native to North America)	*Quercus* sp.	04/30/1997	J. Davidson	EU100969/EU100998 (UCRC ENT 023759)
S. astrictus (CR) (2:2)	Costa Rica (Native to Costa Rica)	*Persea americana*	03/17/2004	M. Hoddle	EU100967/EU100996 (UCRC ENT 023760)
S. aurantii (ZA) (2:3)	Limpopo Prov., S. Africa (native South Africa)	*Citrus* sp.	03/08/2004	M. Gilbert	EU100965/EU100994 (UCRC ENT 023761)
S. aurantii (AU) (1:1)	Brisbane, Australia (invasive)	*Bryophyllum delagoense*	1/15/2003	D. Morris	EU100966/EU100995 (UCRC ENT 023762)
S. bounties (MX) (1:1)	Oaxaca, Mexico (native to Mexico)	*Mangifera indica*	11/21/2003	M. Hoddle	EU100977/EU101006 (UCRC ENT 023763)
S. citri (CA) (1:3)	California, USA (native to North America)	*Citrus* sp.	10/01/2003	L. Robinson	EU100975/EU101004 (UCRC ENT 023764)
S. citri (MX) (1:1)	Oaxaca, Mexico	*Mangifera indica*	11/21/2003	M. Hoddle	EU100976/EU101005 (UCRC ENT 023765)
S. dobroskyi (FP) (2:2)	Raiatea, French Polynesia (native to the South Pacific)	*Persea americana*	09/21/2004	M. Hoddle	EU100968/EU100997 (UCRC ENT 023766)
S. dorsalis (ZA) (1:1)	Limpopo Prov., S. Africa (native to South Africa)	*Ricinus communis*	02/25/2004	M. Gilbert	EU100956/EU100985 (UCRC ENT 023767)
S. dorsalis (IN) (1:1)	Bangalore, India (native to Australasia)	*Mangifera indica*	04/13/2004	G. Mikunthan	EU100955/EU100984 (UCRC ENT 023768)
S. dorsalis (AU1) (1:1)	Kunurra, Western Australia	*Sesbania* sp.	02/22/2005	L. Mound	EU100957/EU100986 (UCRC ENT 023769)
S. dorsalis (AU2) (1:1)	Kunurra, Western Australia	*Khaya* sp.	02/24/2005	L. Mound	EU100958/EU100987 (UCRC ENT 023770)
S. dorsalis (JP) (2:2)	Okinawa, Japan	*Erythrina* sp.	12/14/2004	K. Minoura	EU100978/EU101007 (UCRC ENT 023771)
S. dorsalis (TH1) (2:2)	Chiang-Mai, Thailand	Unidentified herb	01/28/2005	C. Stosic	EU100960/EU100989 (UCRC ENT 023772)
S. dorsalis (TH2) (2:2)	Chiang-Mai, Thailand	*Mangifera indica*	01/28/2005	C. Stosic	EU100961/EU100990 (UCRC ENT 023773)
S. dorsalis (TH3) (2:2)	Chiang-Mai, Thailand	*Citrus* sp.	01/28/2005	C. Stosic	EU100962/EU100991 (UCRC ENT 023774)
S. dorsalis (TW) (2:2)	Tai Chung, Taiwan	*Vitis* sp.	01:6:2003	Liang-Jong Wang	EU100959/EU100988 (UCRC ENT 023775)
S. drepanofortis (AU) (1:1)	Kangaroo Island, Australia (native to Australia)	*Hakea* sp.	01/16/2005	M. Hoddle	EU100972/EU101001 (UCRC ENT 023776)
S. frondis (AU) (1:1)	Victoria, Australia (native to Australia)	*Dicksonia* sp.	01/10/2005	M. Hoddle	EU100974/EU101003 (UCRC ENT 023777)
S. helenae (AU) (1:1)	Wyndham, Australia (native to Australia)	*Acacia* sp.	02/26/2005	L. Mound	EU100979/- (UCRC ENT 023778)
S. inermis (AU) (2:2)	Kangaroo Island, Australia (Uncertain, South Pacific?)	*Prunus persica*	01/16/2005	M. Hoddle	EU100973/EU101002 (UCRC ENT 023779)
S. kenyensis (KE) (1:1)	Gatundu, Kenya (native to Africa)	*Camellia sinensis*	04/13/2004	S. Ekesi	EU100964/EU100993 (UCRC ENT 023780)
S. moneres (AU) (1:1)	Fitzroy Crossing, Australia (native to Australia)	*Acacia ancistrocarpa*	02/27/2005	L. Mound	EU100980/- (UCRC ENT 023781)
S. oligochaetus (IN) (1:1)	Bangalore, India (native to Asia)	*Mangifera indica*	04/13/2004	G. Mikunthan	EU100963/EU100992 (UCRC ENT 023782)
S. pan (NZ) (2:2)	Hahei, New Zealand (native to New Zealand)	*Knightia excelsa*	12/08/2004	M. Hoddle	EU100981/- (UCRC ENT 023783)
S. perseae (CA) (2:12)	California, USA (Native to Mexico & Guatemala)	*Persea americana*	03/31/1997	M. Hand	DQ075176/DQ075132 (UCRC ENT 023784)
S. perseae (UR) (1:4)	Uruapan, Mexico	*Persea americana*	11/26/2003	M. Hoddle	DQ075184/DQ075162 (UCRC ENT 023785)
S. perseae (CD) (2:3)	Chilapa de Diaz, Mexico	*Persea americana*	02/19/2003	M. Hoddle	DQ075186/DQ075148 (UCRC ENT 023786)
S. perseae (SC) (3:3)	San Cristobal de las Casas, Mexico	*Persea americana*	02/11/2003	M. Hoddle	DQ075178/DQ075167 (UCRC ENT 023787)
S. perseae (GT) (2:3)	Chimaltenango, Guatemala	*Persea americana*	03/08/2004	M. Hoddle	DQ075188/DQ075170 (UCRC ENT 023788)
S. n. sp. 1 (HN) (2:2)	Uyuca, Honduras (native to Honduras)	*Persea americana*	03/10/2004	M. Hoddle	EU100954/EU100983 (UCRC ENT 023789)
S. n. sp. 2 (NZ) (1:1)	Coromandel Peninsula, New Zealand (native to New Zealand)	*Melicytus* sp.	12/09/2004	C. Stosic	EU100982/- (UCRC ENT 023790)
Neohydatothrips geminus (PA) (1:1)	Santiago, Panama (native to Central America)	*Manihot esculenta*	03/22/2004	M. Hoddle	EU100970/EU100999 (UCRC ENT 023791)
N. burungae (PA) (1:2)	Boquette, Panama (Native to Central and South America)	*Persea americana*	03/22/2004	M. Hoddle	EU100971/EU101000 (UCRC ENT 023792)

[a] Country code is provided in parenthesis for cross referencing to Table 2 and Figs. 1A and B and 2.

zoni et al. 2000) was deemed unnecessary given the results using unordered data.

Parsimony analyses using 500 random addition sequences and random starting trees were done on 1) the complete molecular data set (28S-D2 and COI), 2) molecular data with all 28S-D2 ambiguous region data excluded, 3) molecular data and ambiguous state coding, 4) molecular data including ambiguous state cod-

Table 2. Ambiguous region coding for 28S-D2 region in *Neohydatothrips* and *Scirtothrips*

Species and country code	Ambig-1	Ambig-2	Ambig-3	Ambig-4	Ambig-5	Ambig-6
N. geminus (PA)	[CTT-TT] 0	[T–G-GTC] 0	[TGAA] 0	[-T–CGCTTCGGT–GGC] 0	[A-TT] 0	[TGTAG—-CAGGCTA-CA] 0
N. burungae (PA)	[-TT-TT] 1	[G–G-TTC] 1	[TTAT] 1	[-T–CGTTTCGGC–GGC] 1	[–TT] 1	[CGTAA—-CGGGCTA-CA] 1
S. perseae (CA)	[CGG–C] 2	[G—-G-TTC] 1	[TTAT] 1	[GT-AGGCTCGTCTT-GCC] 2	[–TT] 1	[CGCAC—-CGGGCTA-CA] 2
S. perseae (SC)	[CGG–C] 2	[G—-G-TTC] 1	[TTAT] 1	[GT-AGGCTCGTCTT-GCC] 2	[–TT] 1	[CGCAC—-CGGGCTA-CA] 2
S. perseae (UR)	[CGG–C] 2	[G—-G-TTC] 1	[TTAT] 1	[GT-AGGCTCGTCTT-GCC] 2	[–TT] 1	[CGCAC—-CGGGCTA-CA] 2
S. perseae (CD)	[CGG–C] 2	[G—-G-TTC] 1	[TTAT] 1	[GT-AGGCTCGTCTT-GCC] 2	[–TT] 1	[CGCAC—-CGGGCTA-CA] 2
S. perseae (GT)	[CGG–C] 2	[G—-G-TTC] 1	[TTAT] 1	[GT-AGGCTCGTCTT-GCC] 2	[–TT] 1	[CGCAC—-CGGGCTA-CA] 2
S. n. sp. *1* (HN)	[CGG–C] 2	[G—-G-TTC] 1	[TTAT] 1	[GT-AGGCTTGTCTT-GCC] 3	[–TT] 1	[CGCAC—-CGGGCTA-CA] 2
S. dorsalis (IN)	[CGG–C] 2	[G—-G-TTC] 1	[G–T] 2	[GT-GGGCTTG-CTC-GCT] 4	[A-TT] 0	[CGTTT—-CGGGCTG-TT] 3
S. dorsalis (ZA)	[CGG–C] 2	[G—-G-TTC] 1	[G–T] 2	[GT-GGGCTTG-CCC-GCC] 5	[A-TT] 0	[CGTTT—-CGGGCTG-CT] 3
S. dorsalis (AU1)	[CGG–C] 2	[G—-G-TTC] 1	[G–T] 2	[GT-GGGCTTG-CCC-GCT] 6	[A-TT] 0	[CGTTT—-CGGGCTG-TT] 3
S. dorsalis (AU2)	[CGG–C] 2	[G—-G-TTC] 1	[G–T] 2	[GT-GGGCTTG-CTC-GCC] 7	[A-TT] 0	[CGTTT—-CGGGCTG-TT] 3
S. dorsalis (TW)	[CGG–C] 2	[T-TG-TTC] 2	[G–T] 2	[GT-GGGCTTG-CTC-GCT] 4	[A-TT] 0	[CGTTT—-CGGGCTG-TT] 3
S. dorsalis (TH1)	[CGG–C] 2	[T-CG-TTC] 3	[G–T] 2	[GT-GGGCTTG-CTC-GCT] 4	[A-TT] 0	[CGTTT—-CGGGCTG-TT] 3
S. dorsalis (TH2)	[CGG–C] 2	[T-TG-TTC] 2	[G–T] 2	[GT-GGGCTTG-CTC-GCT] 4	[A-TT] 0	[CGTTT—-CGGGCTG-TT] 3
S. dorsalis (TH3)	[CGG–C] 2	[T-TG-TTC] 2	[G–T] 2	[GT-GGGCTTG-CTC-GCT] 4	[A-TT] 0	[CGTTT—-CGGGCTG-TT] 3
S. dorsalis (JP)	[CGG–C] 2	[G–G-TTC] 1	[G–T] 2	[GT-GGGCTTG-CYC-GCT] 5,7	[CAT-] 4	[CGTTT—-CGGGCTG-TT] 3
S. oligochaetus (IN)	[CGG–C] 2	[G—-G-TTC] 1	[G–T] 2	[GT-GGGCTTG-CCC-GCC] 5	[A-TT] 0	[CGTTT—-CGGGCTG-TT] 3
S. kenyensis (KE)	[CGA–C] 3	[G—-G-TTC] 1	[-TGT] 3	[GT-GGCTTTCGGGCCGCC] 8	[–TT] 1	[TGATTTCATCGGGC TA-CA] 4
S. aurantii (ZA)	[TGT–T] 4	[G—-G-TTC] 1	[ATAT] 4	[CA-GGCTT-GTCTT-GC-] 9	[–TT] 1	[CGCAT—-CGGGCTA-CG] 5
S. aurantii (AU)	[TGT–T] 4	[G—-G-TTC] 1	[ATAT] 4	[CA-GGCTT-GTCTT-GC-] 9	[–TT] 1	[CGCAT—-CGGGCTA-CG] 5
S. astrictus (CR)	[CGG–C] 2	[G—-G-TTC] 1	[TCAT] 5	[GT-AGGCTTGTCTT-GCC] 3	[–TT] 1	[CGTAC—-CGGGCTA-CA] 6
S. dobroskyi (FP)	[CGG–C] 2	[T-CG-TTC] 3	[G-A-] 6	[GT-GGGCTTG-CCT-GCC] A	[–TT] 1	[CGTAT—-CGGGCTA-CA] 7
S. aceri (CA)	[CGG–G] 5	[G—-G-TTC] 2	[TTAT] 1	[GT-AGGCTTG-CTT-GCC] B	[–TT] 1	[CGTAT—-CGGGCTA-TA] 8
S. drepanofortis (AU)	[CAGTTC] 6	[G—-G-TTC] 2	[TCAT] 5	[GC-AGGCTTG–CTTGCC] C	[ATTT] 2	[CTCAA–TT-GGGCTA-CA] 9
S. inermis (AU)	[CGG–C] 2	[T–CG-AC] 4	[G–T] 2	[GT-GGGCT-CGTCTCGCC] D	[–TT] 1	[CGCAA—-CGGGCTG-TA] A
S. frondis (AU)	[CGT–T] 7	[GTTG-TTC] 5	[–AA] 7	[GATTGGTTTCGGCCTTCC] E	[ATTT] 2	[CGCCCG—CGGGCTA-CG] B
S. citri (CA)	[CGT–T] 7	[G–GTTTC] 6	[G-AT] 8	[GT–GGTT-CGCC–GCC] F	[-TCA] 3	[CGTAC—-CGGGCTA-CA] C
S. citri (MX)	[CGG–C] 2	[G–GTTTC] 6	[G-AT] 8	[GT–GGTT-CGCC–GCC] F	[-TCA] 3	[CGTAC—-CGGGCTA-CA] C
S. bounites (MX)	[CGG–C] 2	[G–GTTTC] 6	[G-AT] 8	[GT–GGTT-CGCC–GCC] F	[-TCA] 3	[CGTAC—-CGGGCTA-CA] 5
S. helenae (AU)	[CGC–C] 8	[TATTAGGC] 7	[ATAT] 4	[C–AGATTTATTTTTGC-] G	[A-TT] 0	[CTCAA—-CGGGCTAT CA] D
S. moneres (AU)	[GGC–G] 9	[G–G-TTC] 1	[G-AT] 8	[GTTGGGCTT-GCTCATCC] H	[–TT] 1	[CGCAT—-CGGGCTG-TA] E
S. pan (NZ)	[CGA–C] 3	[T–G-TTT] 8	[C-AT] 9	[GT–GGTTTCGGCC-GCC] I	[–TT] 1	[-TCAC—-GGCTA-CA] F
S. n. sp. *2* (NZ)	[CGA–C] 3	[T–G-TTC] 9	[T-AT] A	[GT-GGGCTCGTTCTCGCC] K	[–TT] 1	[CCTTT—GGGGGCTA-GA] G

Ambiguous regions, in brackets, correspond to the following 28S-D2 site positions: Ambig-1 (190–195), Ambig-2 (327–334), Ambig-3 (340–343), Ambig-4 (372–389), Ambig-5 (443–446), and Ambig-6 (481–499), which are each followed by the state codes. Country codes in parentheses (see Table 1).

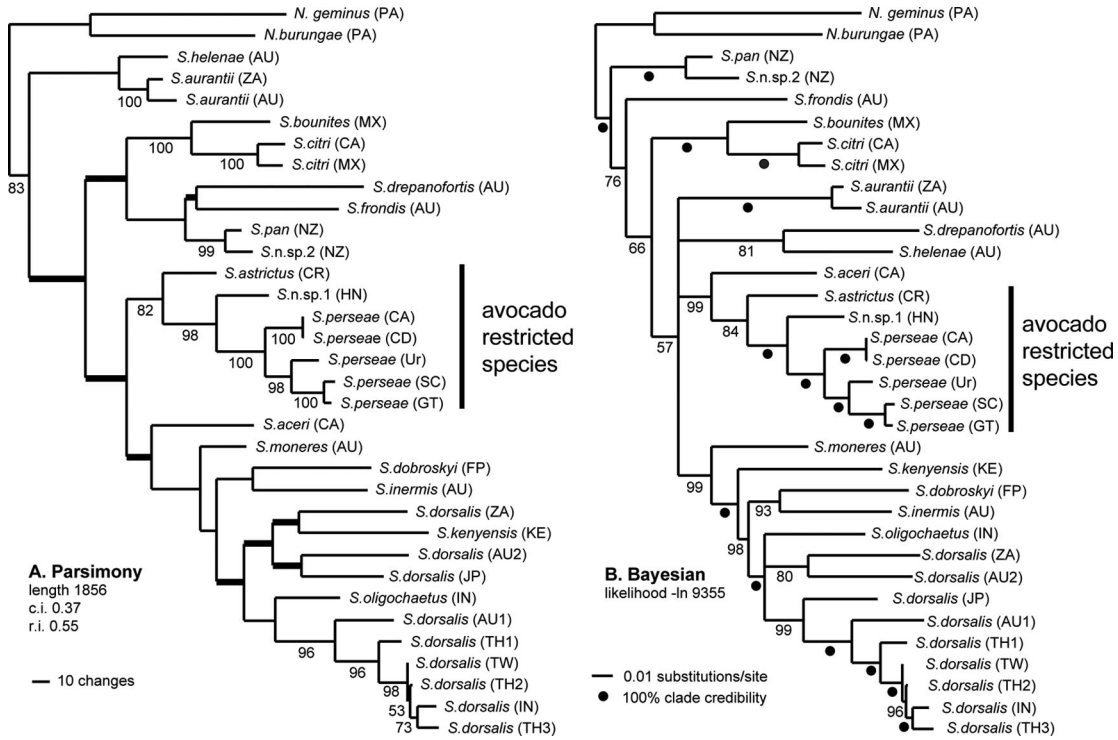

Fig. 1. (A) Single most parsimonious tree from combined 28S-D2 and COI with ambiguous state coding; thinner branches collapse in consensus of nine trees if state coding is not used. (B) The 50% compatible tree from Bayesian analysis of 500,000 generations after 150,000 burnin generations area discarded; relationships are the same for the 28S-D2 and COI only (−ln 0966) or for 28S-D2, COI, and morphology (−ln 9355) as shown; ambiguous state coding excluded.

ing and morphological data, and 5) the morphological data set alone using PAUP 4.0* (Swofford 2002). Bootstrap (BS) partitions were run using 1,000 BS repetitions using two random heuristic searches at each step. Ambiguous region codings were treated as unordered. Bayesian mcmc analyses were done on molecular data alone and also combined with morphological data (see below) by using MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003). The ambiguous state coding, which has more than nine states, could not be handled in the current version of MrBayes so was excluded from these analyses. Molecular data in the Bayesian analyses were treated as five partitions (28S-D2 unambiguous, 28S-D2 ambiguous [Table 2], and codon positions 1, 2, and 3 for COI) by using fully variable (GTR+I+Γ) parameters (Nylander et al. 2004). Morphological data were treated as a single parameter with equal rate variation. Four starting chains from random trees with uniform priors were used in each of three separate analyses of 500,000 generations, sampling every 100 generations. Results are based on a burnin of 1,500 trees (150,000 generations). Tree distributions were visualized and likelihood scores obtained using Tracer version 1.3 (Rambout and Drummond 2005). Unambiguous morphological state changes were identified and plotted on both the molecular, combined, and morphology only trees by using MacClade 4.0.

Results

Parsimony analysis of the morphology data alone produced two trees (length 60, c.i. 407, r.i. 0.75; Fig. 1) that differed only in the monophyly of *S. pan* and *S.* n. sp. two (NZ). The morphology tree was weakly supported, with bootstrap support obtained only for *Scirtothrips* (98%), and *S. kenyensis* + *S. moneres* (54%) (Fig. 2). *S. aurantii* was placed as the sister group of *S. dorsalis* based on two morphological characters: 1) the abdominal tergites having a dark median area and 2) sternites IV–VI with microtrichial fields present medially; and, in a clade with *S. oligochaetus* based on the presence of sternites IV–VI having microtrichial fields present medially. The results were not stable to successive approximation weighting (Carpenter 1988), and they yielded trees that were two steps longer, and which did not support the monophyly of *S. dorsalis* + *S. aurantii.* In the unweighted analyses, the avocado-restricted species (*S. astrictus*, *S.* n. sp. 1 [HN], and *S. perseae*) were paraphyletic with respect to *S. drepanofortis* (Fig. 1).

Parsimony analyses of the complete molecular data set, including ambiguous region coding, resulted in a single tree (length 1856, c.i. 0.35, r.i. 0.55; Fig. 1A). Using only the molecular data and excluding the state codes, searches found nine trees in three islands (sensu Maddison 1991), with one of the trees having

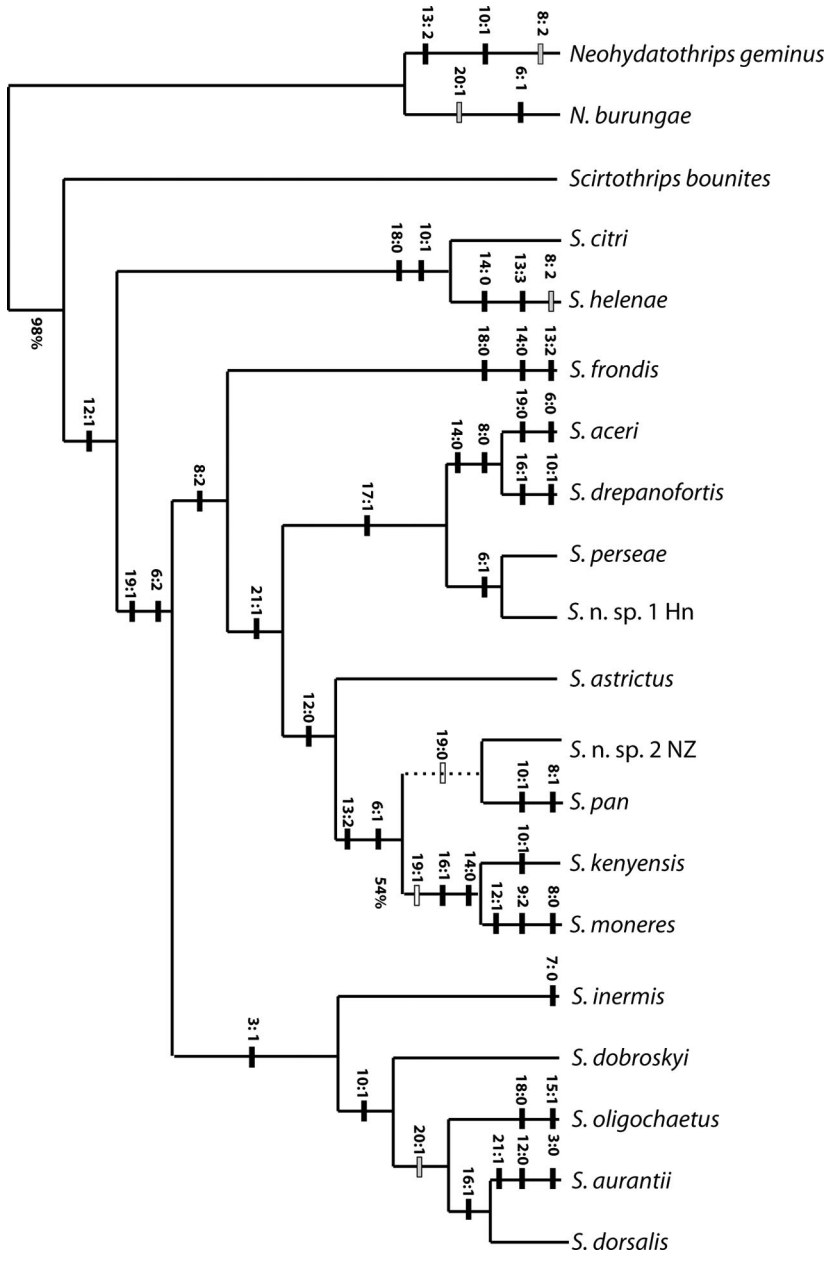

Fig. 2. One of two most parsimonious trees for *Scirtothrips* and *Neohydatothrips* species based on morphology (see Table 3). Numbered bars indicate unambiguous character state changes. Dotted line indicates the collapse in a strict consensus of the two trees, with the different unambiguous optimizations of character 19 (white bar). Homoplasy characters are indicated with gray bars.

the same topology as in Fig. 1A. Overall the consensus solution of the nine trees had several areas of conflict and collapse (thin lines, Fig. 1A). Furthermore, exclusion of the 28S-D2 ambiguous regions and ambiguous state codes produced five trees (length 1645, c.i. 0.34, r.i. 0.53), with a shift in some of the basal relationships (*S. helenae* and *S. aurantii* became paraphyletic), but maintaining the same relationships within the *S. astrictus–S. perseae* ([SAP], which are avocado-restricted species) and the *S. moneres–S. dorsalis* (SMD) clades. Furthermore, analyses with only 28S-D2 had less resolution (48 trees) but again supported the SAP and SMD clades but COI alone supported only the SAP clade. Similarly, the inclusion of all data, including morphology, produced two trees (length 1933, c.i. 0.36, r.i. 0.56) that also maintained the SAP and SMD clades, but with *S. aceri* and *S. drepanofortis* being monophyletic and sister to SMD. *Sciro-*

Table 3. Character state and scoring for *Scirtothrips* and *Neohydatothrips* species used in morphology analyses (see Fig. 2)

Species	Character state																				
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Neohydatothrips geminus	0	2	0	1	1	0	1	2	2	1	1	0	2	1	1	0	1	1	0	0	0
N. burungae	0	2	0	1	1	1	1	1	2	0	1	0	1	1	1	0	1	1	0	1	0
Scirtothrips aceri	1	1	0	0	0	0	1	0	1	0	0	1	1	0	0	0	1	1	0	0	1
S. astrictus	1	1	0	0	0	2	1	2	1	0	0	0	1	1	0	0	0	1	1	0	1
S. aurantii	1	1	0	0	0	2	1	1	1	1	0	0	1	1	0	1	0	1	1	1	1
S. bounites	1	1	0	0	0	0	1	1	0	0	0	0	1	1	0	0	0	1	0	0	?
S. citri	1	1	0	0	0	0	1	1	1	1	0	1	1	1	0	0	0	0	0	0	0
S. dobroskyi	1	1	1	0	0	2	1	1	1	1	0	1	1	1	0	0	0	1	1	0	0
S. dorsalis	1	1	1	0	0	2	1	1	1	1	0	1	1	1	0	1	0	1	1	1	0
S. drepanofortis	1	1	0	0	0	2	1	0	1	1	0	1	1	0	0	1	1	1	1	0	1
S. frondis	1	1	0	0	0	2	1	2	1	0	0	1	2	0	0	0	0	0	1	0	0
S. helenae	1	1	0	0	0	0	1	2	0	1	0	1	3	0	0	0	0	0	0	0	0
S. inermis	1	1	1	0	0	2	0	1	1	0	0	1	1	1	0	0	0	1	1	0	0
S. kenyensis	1	1	1	0	0	1	1	2	1	1	0	0	2	0	0	1	0	1	1	0	1
S. moneres	1	1	1	0	0	1	1	0	2	0	0	1	2	0	0	1	0	1	1	0	1
S. oligochaetus	1	1	1	0	0	2	1	1	1	1	0	1	1	1	1	0	0	0	1	1	0
S. pan	1	1	1	0	0	1	1	1	1	1	0	0	2	1	0	0	0	1	0	0	1
S. perseae	1	1	0	0	0	1	1	2	1	0	0	1	1	1	0	0	1	1	1	0	1
S. n. sp. HN1	1	1	0	0	0	1	1	2	1	0	0	1	1	1	0	0	1	1	1	0	?
S. n. sp. NZ1	1	1	0	0	0	1	1	2	1	0	0	0	2	1	0	0	0	1	0	0	?

See Mound and Palmer (1981) for more details on character state descriptions and scoring. 1, forewing first vein setal row: 0 complete; 1 incomplete; 2, forewing second vein setal row: 0 complete; 1 incomplete; 2 absent; 3, forewing posteriomarginal cilia: 0 wavy; 1 straight; 4, antennal segment VIII sensorium base: 0 round or oval; 1 elongate; 5, female tergite IX marginal setae: 0 four setae; 1 eight setae; 6, ocellar setae pair III position: 0 anteromarginal; 1 within triangle; 2 between hind ocelli; 7, ocellar setae III length: 0 longer than distance between hind ocelli; 1 shorter than distance between hind ocelli; 8, sculpture within ocellar triangle: 0 absent; 1 transversely striate; 2 irregularly reticulate; 9, number of pairs of prominent postocular setae: 0 three pairs; 1 two pairs; 2 one pair; 10, pronotal striae separation: 0 greater than diam of setal pore; 1 less than setal pore; 11, pronotal blotch: 0 absent; 1 present; 12, metanotal median setae origin: 0 at anterior margin; 1 behind anterior margin; 13, metanotal sculpture: 0 lines; 1 linear reticulation; 2 broadly reticulate; 3 transverse; 14, tergites IV and V median setae: 0 shorter than distance between bases; 1 longer than distance between bases; 15, tergite VII posteromarginal comb: 0 incomplete medially; 1 complete medially; 16, abdominal tergites with dark median area: 0 absent; 1 present; 17, abdominal tergites III-V with dark lateral area: 0 absent; 1 present; 18, abdominal tergites III–VI antecostal ridge: 0 pale; 1 dark; 19) sternites III–V antecostal ridge: 0 pale; 1 dark; 20, sternites IV–VI microtrichial fields: 0 absent medially; 1 present medially; and 21, male tergite IX drepanae: 0 absent; 1 present.

thrips pan and *S.* n. sp. N.Z. shifted to the sister group of *S. frondis* (Fig. 1B), and *S. aurantii* (AU and SA) grouped with *S. drepanofortis* and *S. helenae.* In none of the trees that included molecular data, even with COI data excluded from analyses, did *S. aurantii* group with *S. dorsalis* or *S. oligochaetus,* even though all three taxa share at least one distinct morphological synapomorphy (sternites IV–VI with microtrichial fields present medially; character 20: state 1 [Table 3; Fig. 2]). Additionally, in all of the parsimony analyses, *S. oligochaetus* rendered *S. dorsalis* polyphyletic, and the parsimony analysis of the molecular data proposes, albeit weakly, that *S. oligochaetus* is the sister group of the *S. dorsalis* population from South Africa (Fig. 1A).

The 50% compatibility tree resulting from the Bayesian analyses, which is topologically identical for the molecular only and combined molecular and morphology data, is presented in Fig. 1B, along with the clade credibility values, which were generally very high. Regions of collapse correspond to the same uncertain relationships resulting from the molecular-only parsimony results. In the parsimony (Fig. 1A), Bayesian (Fig. 1B), and morphology (Fig. 2) consensus trees (that included all compatible groupings [<50%] for molecular analyses), *S. oligochaetus* (Karny) always rendered *S. dorsalis* paraphyletic.

The lack of molecular-only (Fig. 1A) and molecular and morphology (Fig. 1B) support for monophyly of the *S. dorsalis* complex suggests that this "species" may actually be comprised of several morphologically indistinguishable species which can only be separated using molecular analyses, a possibility that has been suggested previously (Rugman-Jones et al. 2006). Molecular data presented here, and the high level of observed variation in the sizes and sequences of ITS1 and ITS2 regions between "*S. dorsalis*" specimens reported by Rugman-Jones et al. 2006, is suggestive that molecular-based differences between specimens is probably too great for representatives of these populations to be considered as different host races, even though they are readily identified morphologically as being one species, *S. dorsalis.* In both of the molecular-based analyses presented here, *S. dorsalis* is separated into two (Bayes) or three clades (parsimony). In comparison, the different *S. dorsalis* molecular types could not be distinguished from each other by morphology.

Discussion

Regardless of which analyses were used that included molecular data, the avocado-restricted species of *Scirtothrips* formed a monophyletic group with clade credibility values ranging from 82 to 100% (Fig. 1A and B). This relationship was not as robust in the morphological analysis (Fig. 2). By tracking character state changes in the morphology matrix, *S. astrictus* was separated from the other avocado associated *Scirtothrips* species based primarily on the position of the

metanotal seta arising at the anterior margin (character 12:state 0) as opposed to arising behind the margin as seen in *S. perseae* and *S.* n. sp. HN (12:1) (Fig. 2). Overall, there is strong support from molecular and combined molecular and morphological analyses for an avocado-associated group of *Scirtothrips.* This result suggests that the evolutionary relationship among species in this group could be affected by their close host association (perhaps mono- or stenophagous) with *P. americana,* a plant native to Mexico and Central America that has potent secondary chemical defenses to deter generalist herbivores (Rodriguez-Soana et al. 1999).

Scirtothrips aurantii Faure, native to South Africa, is a serious economic pest of citrus and mangoes. This thrips is invasive in Queensland Australia and curiously is currently restricted to feeding and breeding on just one host plant, the invasive weed *Bryophyllum delagoense* (Ecklon and Zeyher) Schinz. The polyphagous and monophagous *S. aurantii* grouped together with 100% support in molecular analyses (Fig. 1A and B). *S. aurantii* was paraphyletic to *S. dorsalis* in the morphology-only analyses based on the presence of a dark median area on the abdominal tergites (16:1) (Fig. 2). The results of the molecular analyses sustains the contention of Morris and Mound (2004), that the polyphagous native South African population of *S. aurantii* and the monophagous population invasive to Australia are closely related, and they are probably the same species.

S. aurantii typically has a broad host range in its proposed area of origin and adults are characterized, in part, morphologically by strong microtrichial fields extending fully across abdominal sternites IV–VI. This defining morphological character is shared with *S. dorsalis, S. oligochaetus,* and the outgroup species, *N. burungae* (20:1). *S. aurantii* does not group closely to either of these two *Scirtothrips* species in any analyses including molecular data (Fig. 1A and B). These three *Scirtothrips* species group together only in the morphology-only analyses (Fig. 2), although this grouping is not supported in the successive weighting analyses that focused on overweighting those characters with the best fit to the tree topology. The presence of microtrichia medially on sternites (20:1) is an important feature defining the relationship between these three species, although this character has demonstrated homoplasy within the outgroup. Molecular analyses suggest that *S. aurantii* is not closely related to *S. dorsalis* and *S. oligochaetus* as morphology, biogeography, and feeding habits would suggest. Another native African species, *S. kenyensis* Mound (possibly a polyphagous species as it is a pest on tea, a plant species not represented naturally in Africa) groups closely to *S. dorsalis* and *S. oligochaetus* in the two molecular analyses but not the morphological analysis. Character state analyses indicated that the position of ocellar setae III (6:1), sculpturing within the ocellar triangle (8:2), and patterning of metanotal sculpture (13:2) were significant in separating *S. kenyensis* from *S. dorsalis* and *S. oligochaetus* in the morphology tree.

The two New Zealand species of *Scirtothrips* were monophyletic in both molecular-based analyses, and the morphological analysis. In the parsimony analysis, *S. pan* Mound and Walker and *S.* n. sp. N.Z. were the sister group of the native Australian species *S. frondis* Hoddle & Mound, and *S. drepanofortis* Hoddle & Mound. These latter two Australian species were more closely related to each other than to their New Zealand congenerics, a result not seen in the morphological analysis where separation occurred based on the position of ocellar setae III and color of the antecostal ridge of the sternites. A similar relationship was not observed in the Bayesian analysis, but *S. frondis* did consistently group closer to the New Zealand *Scirtothrips* than the other three native Australian species. Furthermore, in both molecular-based analyses, *S. drepanofortis* formed a single clade with one other native Australian species, either *S. frondis* (Parsimony) or *S. helenae* Palmer & Mound (Bayesian). In the morphological analysis, *S. drepanofortis* formed a single clade with *S. aceri,* an oak feeding thrips native to the Americas. Character state analyses indicated that the support for the *S. drepanofortis* and *S. aceri* clade was unambiguously supported by the absence of sculpturing within the ocellar triangle and the short distance between the bases of median setae on the tergites. *S. moneres* Hoddle & Mound, a specialist on native Australian *Acacia* species, did not group with any other native Australian *Scirtothrips,* and it was consistently paraphyletic to *S. dorsalis* and two other species that may possibly be native to the South Pacific, *S. inermis* Priesner and *S. dobroskyi* Moulton. In the morphological analysis, *S. moneres* formed a single clade with the African *S. kenyensis* which was unambiguously supported by the short distance between the bases of median setae on the tergites and the presence of a dark median area on the abdominal tergites.

Given the pest status of *S. dorsalis* and its propensity for invasion, detailed work to resolve the species identities of members in the *S. dorsalis* group is needed. To better quantify genetic and morphological variation, which could help resolve species identity and status for members of this group, future studies should include investigation of more gene regions, of a greater number of specimens, collected over a wider geographic area through the assumed native range. Ecologically oriented field studies looking at host plant preferences and population phenology, combined with cross mating studies of isofemale lines established from different populations within the *S. dorsalis* group would be needed to complement the results of future molecular-oriented studies. Molecular characterization of microbial endosymbionts may also be useful in understanding geographic groupings of *S. dorsalis.* This high level of molecular, biological, and ecological resolution will be needed to mitigate quarantine disputes arising from detection of morphologically indistinguishable members of the *S. dorsalis* group.

The possibility of new species of *Scirtothrips* unexpectedly emerging as economic pests is an increasingly serious threat because of expanding global com-

merce that emphasizes "free trade." The rapid irruption of an unknown thrips pest was realized in California, when a previously unknown species of *Scirtothrips* established on avocados in 1996 (Nakahara 1997). The native range of this pest was determined to be Mexico and Guatemala (Hoddle et al. 2002), and the likely area of origin within this home range was identified as Coatepec-Harinas in Mexico (Rugman-Jones et al. 2007). This pest has subsequently caused millions of dollars (US$) in crop losses annually in California (Hoddle et al. 2003). Even though *S. perseae* seems to be closely associated with avocados, nothing was known about this pest until it established in California. This is remarkable given that Mexico has the largest commercial avocado growing areas in the world and highlights the fact that very little is known about most *Scirtothrips* species even on commercial crops.

The invasion threat posed by *Scirtothrips* species could be mitigated by better understanding of the biology, ecology, taxonomy (morphological and molecular), and phylogenetic relationships between species. This may improve our ability to detect and identify potential pests, determine areas of origin, assist with the identification of invasion pathways, and help with the design of control strategies, especially biological control. This ecologically oriented control technology is becoming increasingly sophisticated with its use of molecular tools to identify invasive pest genotypes, so host specific natural enemies adapted to that specific pest subpopulation can be located within the invader's home range (Rugman-Jones et al. 2007, Ryan et al. 2007). Finally, improved knowledge of taxonomic (i.e., molecular and morphological) identities; biogeographic, host plant, and endosymbiont associations; and biological compatibility would help with providing science-based answers for resolving quarantine disputes that arise when produce and plants contaminated with *Scirtothrips* species are detected at ports of entry.

Acknowledgments

We are indebted to Phil A. Phillips for assistance with collecting thrips in Mexico and Central America. The staff of the Core Instrumentation Facility of the University of California-Riverside Genomics Institute provided assistance with sequencing work. We also thank our colleagues listed in Table 1 whose collecting efforts contributed greatly to this study. Financial support for this work came, in part, from the University of California Exotic and Invasive Pests and Diseases Research Program.

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Received 19 October 2007; accepted 14 January 2008.