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Molecular Identification Key for Pest Species of *Scirtothrips* (Thysanoptera: Thripidae)

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ABSTRACT Effective plant quarantine and biological control initiatives require rapid and accurate identification of exotic and potentially invasive taxa that may cause high economic losses or environmental damage. The genus *Scirtothrips* Shull includes several species that are serious agricultural pests, and, because of their minute size and cryptic behavior, prone to undetected transport through international trade of plant material. Although assigning specimens to the genus *Scirtothrips* is straightforward using traditional taxonomic methods, identification of species is much more difficult and requires expert knowledge of the genus. Furthermore, the validity of some *Scirtothrips* species is questionable. Therefore, an easy, accurate, and highly reliable technique is desirable for *Scirtothrips* identification. Here, we provide a simple molecular key based on the internal transcribed spacer regions 1 and 2 (ITS1 and ITS2) of nuclear ribosomal DNA. Individual specimens can be identified by amplification of their ITS1 and ITS2 regions with general primers and determining the size of the products by using standard agarose gel electrophoresis, followed in some instances by DNA digestion with the restriction enzymes SacII or PspOM I. The advantage of this identification system is that nonspecialists are able to quickly and cheaply identify individual specimens. Material analyzed for this work was collected in the United States (California), India, South Africa, Kenya, Mexico, Guatemala, Honduras, Nicaragua, Costa Rica, Panama, Australia, New Zealand and Raiatea (Society Islands French Polynesia). We have identified seven pest species with the molecular-based methods described here. It is hoped that this system can be extended to other members of the genus as their ITS1 and ITS2 sequences become available. We also provide molecular confirmation for two new *Scirtothrips* species, one species from Honduras and one species from New Zealand.

KEY WORDS *Scirtothrips*, *Neohydatothrips*, molecular identification key, exotic pests, nondestructive DNA extraction

The genus *Scirtothrips* Shull currently includes 100 species, according to a world catalog maintained by one of us (L.A.M.). Approximately 10 of these species are recognized as serious pests of agriculture, attacking crops such as avocado, *Persea americana* Miller; citrus (*Citrus* L. spp.); mango, *Mangifera indica* L.; and tea, *Camellia sinensis* L (Mound and Palmer 1981).

Scirtothrips species typically feed on and damage young, growing leaves and immature fruit. Their minute size (often <2 mm in length) and thigmotactic behavior make these insects difficult to detect in fresh vegetation and increase the chances of them being transported through international trade of plant material. In particular, unfumigated plants, especially those with young leaves, may readily carry these insects as eggs, larvae, or adults when transported around the world. Consequently, several species of *Scirtothrips* have (or have the potential to) spread from their natural habitats and hosts to favorable new environments, including valuable agricultural crops (Mound and Palmer 1981, Nakahara 1997) and weeds (Morris and Mound 2004). After translocation to new areas outside of the home range, adventive *Scirtothrips* species may reproduce rapidly and cause significant economic losses. For example, economic losses to the California avocado industry attributable to *Scirtothrips perseae* Nakahara were estimated at $7.6–13.4 million in 1998 (Hoddle et al. 1999). Additionally, unregulated high-density pest populations are a source of invaders that can continue to spread into uninfested areas.

A fundamental requirement of any plant quarantine or biological control program is the rapid and accurate identification of the taxa involved. Traditionally, taxa have been identified using morphological characters, and the generic diagnosis of *Scirtothrips* is fairly straightforward (Mound and Palmer 1981, Johansen and Mojica-Guzman 1998, Moritz et al. 2004). However, identification to species level is much more difficult and requires expert knowledge of the genus

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Table 1. Collection records of thrips specimens

Species	Locality	Host plant	Date	Collector	GenBank no.
S. perseae	California, USA	*Persea americana*	31 March 1997	M. Hand	DQ075004–DQ075033
S. perseae	Oaxaca, Mexico	*Persea americana*	22 Nov. 2003	M. Hoddle	DQ075034–DQ075042
S. aceri	California, USA	*Quercus* sp.	30 April 1997	J. Davidson	DQ075043–DQ075047
S. aurantii	Limpopo Prov., S. Africa	*Citrus* sp.	8 March 2004	M. Gilbert	DQ075048–DQ075051
S. aurantii	Queensland, Australia	*Bryophyllum delagoense*	9 Nov. 2004	D. Morris	DQ075052–DQ075053
S. citri	California, USA	*Citrus* sp.	1 Oct. 2003	L. Robinson	DQ075054–DQ075058
S. dorsalis	Limpopo Prov., South Africa	*Ricinus communis*	25 Feb. 2004	M. Gilbert	DQ075059–DQ075063
S. dorsalis	Bangalore, India	*Mangifera indica*	13 April 2004	G. Mikunthan	DQ075064–DQ075067
S. kenyensis	Gatundu, Kenya	*Camellia sinensis*	13 April 2004	S. Ekesi	DQ075068–DQ075073
S. astrictus	Costa Rica	*Persea americana*	17 March 2004	M. Hoddle	DQ075074–DQ075083
S. oligochaetus	Bangalore, India	*Mangifera indica*	13 April 2004	G. Mikunthan	DQ075084–DQ075085
S. bounites	Oaxaca, Mexico	*Mangifera indica*	21 Nov. 2003	M. Hoddle	DQ075086–DQ075087
S. n. sp.	Uyuca, Honduras	*Persea americana*	10 March 2004	M. Hoddle	DQ075088–DQ075092
S. frondis	Victoria, Australia	*Dicksonia* sp.	10 Jan. 2005	M. Hoddle	DQ075093–DQ075096
S. inermis	Kangaroo Island, Australia	*Prunus persica*	16 Jan. 2005	M. Hoddle	DQ075097–DQ075100
S. drepanofortis	Kangaroo Island, Australia	*Hakea* sp.	16 Jan. 2005	M. Hoddle	DQ075101–DQ075104
S. pan	Hahei, New Zealand	*Knightia excelsa*	8 Dec. 2004	M. Hoddle	DQ075105–DQ075107
S. n. sp.	Coromandel Peninsula, New Zealand	*Melicytus* sp.	9 Dec. 2004	C. Stosic	DQ075108–DQ075110
S. sp. nr. *dobroskyi*	Raiatea, French Polynesia	*Persea americana*	21 Sept. 2004	M. Hoddle	DQ075111–DQ075120
N. geminus	Santiago, Panama	*Manihot esculenta*	22 March 2004	M. Hoddle	DQ075121–DQ075124
N. burungae	Bouquette, Panama	*Persea americana*	22 March 2004	M. Hoddle	DQ075125–DQ075128

(Mound and Palmer 1981, Mound and zur Strassen 2001, Hoddle and Mound 2003). Without fully cleared and expertly slide-mounted specimens, the minute structural details used to diagnose *Scirtothrips* species cannot be studied with accuracy (Mound and zur Strassen 2001, Hoddle and Mound 2003). Furthermore, identification to species is almost impossible without the presence of adults (Brunner et al. 2002). To further complicate matters, the validity of new *Scirtothrips* species collected from mango and avocado in Mexico and described in a recent taxonomic revision (Johansen and Mojica-Guzman 1998) has been questioned because species designations were made according to morphological characters that exhibit high intraspecific variation (Mound and zur Strassen 2001).

The use of genetic markers represents a valuable addition to traditional morphological methods of species diagnosis, an addition that has been deployed in a recent interactive trilingual identification system to 99 species of pest Thripidae of the world (Moritz et al. 2004). The polymerase chain reaction (PCR) (Saiki et al. 1988) and PCR-based typing methods have previously provided ideal markers for identification of difficult to recognize species (Stouthamer et al. 1999, Toda and Komazaki 2002, Borghuis et al. 2004). By its nature, PCR is an ideal tool for use in species diagnosis. It is technically simple, requiring only basic laboratory skills, minimal amounts of DNA, and once established, it is rapid, sensitive, and specific. The objective of this study was to determine whether current PCR-based techniques are suitable to establish a simple molecular identification key for seven important pest species of *Scirtothrips.* Our interest in this area stems from ongoing work on the geographical origin of *S. perseae*, which was discovered attacking avocados in California in 1996 (Nakahara 1997, Hoddle et al. 2002). Confusion currently exists over the taxonomic status of *S. perseae* and the number of *Scirtothrips* species inhabiting avocado in Mexico and Central America (Johansen and Mojica-Guzman 1998, Mound and zur Strassen 2001, Hoddle et al. 2002). Exact knowledge of the systematic identity of this (and other) *Scirtothrips* species is a vital step in the search for the correct natural enemies for potential classical biological control of pestiferous exotic *Scirtothrips* spp. and for enforcement of fair scientifically based plant quarantine and free trade protocols.

Materials and Methods

Collection. The collection records for all the specimens used in this study are listed in Table 1. Specimens were collected into 95% ethanol (see Hoddle et al. 2002 for field collection methods), shipped to our laboratory in California, and stored at −20°C until analyzed. Several nonpestiferous *Scirtothrips* species that were encountered during our collecting efforts were included (Hoddle et al. 2002). Furthermore, two species of *Neohydatothrips* collected from avocado in Central America were included in analyses (Table 1).

DNA Extraction. Individual thrips stored in 95% ethanol were removed from vials and allowed to air dry on filter paper for 2 min. Total DNA was isolated using a "salting-out" protocol adapted from (Sunnucks and Hales 1996). Each individual thrips was placed in a 0.5-ml low-binding microfuge tube containing 100 μl of TNES (50 mM Tris, pH 7.5, 400 mM NaCl, 20 mM EDTA, and 0.5% SDS) and 1.7 μl of proteinase K (10 mg/ml). Rather than grinding the specimen, we pierced one side of the specimen's abdomen by using a sterilized minuten pin. The advantage of this method is that after DNA extraction, a cleared specimen remains for slide mounting (see below). Tubes were incubated at 37°C for 18 h, after which, proteins were precipitated with the addition of 28 μl of 5 M NaCl and 15 s of vigorous shaking. Proteins (and the specimen) were pelleted in a microfuge at 13,000 rpm for 5 min, and the supernatant was transferred to a new microfuge tube. DNA was precipitated from the super-

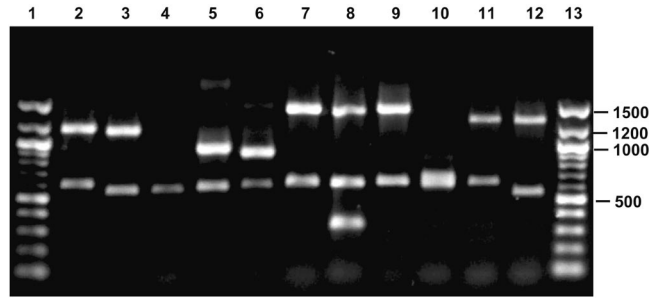

Fig. 1. Gel showing the multiplex PCR products of the ITS1 and ITS2 plus flanking regions of several *Scirtothrips* species. Lane 1, 100-bp ladder size standard (New England Biolabs). Lane 2, *S. aceri*. Lane 3, *S. dorsalis* (ZA). Lane 4, *N. geminus*. Lane 5, *S. citri*. Lane 6, *N. burungae*. Lane 7, *S. perseae* from California. Lane 8, *S. perseae* from Mexico. Lane 9, *S. astrictus*. Lane 10, *S. aurantii*. Lane 11, *S. kenyensis*. Lane 12, *S. dorsalis* (IN). Lane 13, 100-bp ladder size standard (New England Biolabs).

natant by the addition of 1 volume of ice-cold 100% ethanol and incubation for 1 h at −20°C. DNA was then pelleted in a cold (4°C) microfuge at 13,000 rpm for 5 min, washed in ice-cold 70% ethanol, air-dried, and finally dissolved in 30 μl of sterile distilled water. The thrips specimen was retrieved from the original microfuge tube by dissolving the pelleted proteins in 300 μl of 50% ethanol for at least 1 h. The 50% ethanol was then replaced with 70% ethanol, and the specimen was stored at 4°C for slide mounting. Specimens were 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) and identified to species by using morphological characters (Mound and Palmer 1981, Hoddle and Mound 2003, Moritz et al. 2004). All slide-mounted specimens for this project have been deposited at the University of California Riverside Entomology Research Museum, Riverside, CA.

PCR Protocol and Sequencing. The internal transcribed spacer regions 1 and 2 (ITS1 and ITS2) of ribosomal RNA were amplified separately in a single multiplex PCR. The following primer pairs were used to amplify ITS1 and ITS2, respectively: CS249 (5′-TCGTAACAAGGTTTCCG-3′) located in the 18S rRNA coupled with ITS2 (5′-GCTGCGTTCTTCATCGATGC-3′) located in the 5.8S rRNA and ITS2-forward (5′-TGTGAACTGCAGGACACATG-3′) also located in the 5.8S rRNA coupled with CS250 (5′-GTTRGTTTCTTTTCCTC-3′) located in the 28S rRNA (White et al. 1990, Campbell et al. 1993, Moritz et al. 2000). Multiplex-PCR was performed in 25-μl reactions containing 2 μl of DNA template (concentration not determined), 2.5 μl of QIAGEN 10× PCR buffer (containing 15 mM $MgCl_2$) (QIAGEN, Valencia, CA), 20 μM each dNTP, 0.2 μM each primer, 2 μl of QIAGEN HotStarTaq DNA polymerase, and 13.3 μl of sterile distilled water. The PCR was performed in an Eppendorf Mastercycler 5331 (Eppendorf North America Inc., New York, NY) programmed for 15 min at 95°C, followed by 35 cycles of 1 min at 94°C, 1 min at 54°C, and 1 min 45 s at 72°C, with a further 30 min at 72°C after the last cycle. PCR products were electrophoresed on a 0.7% agarose gel and stained with ethidium bromide, allowing us to visualize two bands (Fig. 1).

Where possible, the ITS1 and ITS2 regions were sequenced for at least two individuals of each species. Amplified DNA was cleaned using the Wizard PCR Preps DNA purification system (Promega, Madison, WI) and cloned into plasmids using the pGEM-T Easy Vector kit (Promega, Madison, WI). Plasmids were transformed in JM109 competent cells and grown on LB media petri plates. Insert-positive bacterial colonies were amplified from each individual using M13 primers, and the PCR products were again cleaned using the Wizard PCR Preps DNA purification system. Cleaned products were 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). Sequences were aligned manually in BioEdit version 6.0.7 (Hall 1999) and submitted to GenBank (accession numbers are listed in Table 2).

Restriction. Restriction digestion was performed on 6 μl of the multiplex-PCR product, to which was added 4 U of the restriction enzyme SacII, or PspOM I (New England Biolabs, Beverly, MA), 1 μl of its accompanying buffer, and 2.8 μl of sterile distilled water. Reactions were incubated for 2 h at 37°C and then inactivated with 20 min at 65°C. Restriction products were electrophoresed on a 2% agarose gel and stained with ethidium bromide.

Results

Our DNA extraction method retrieved sufficient DNA for amplification via PCR. In all but one species, gel electrophoresis of the multiplex PCR products yielded two bright bands of a unique size for each species, the larger band including ITS1 and flanking regions and the smaller band including ITS2 and flanking regions (Fig. 1). In the remaining species, *Neohydatothrips geminus* Priesner, the ITS1 region did not amplify as well but was usually still visible as a faint band (Fig. 1). Fragment sizes determined by sequence

Table 2. Size (in base pairs) of the ITS1 and ITS2 multiplex-PCR products and restriction fragments generated by the restriction enzyme SacII

Species	ITS1*	ITS2	Sac II	Comments
S. astrictus	1455	634	134*, 178, 303, 634, 839	ITS2 uncut
S. n. sp. (HN)	1440	619	135, 179, 299, 484, 962*	
S. perseae	1430	623	149, 176, 293, 474, 962*	
S. kenyensis	1343	646	337, 646, 1006*	ITS2 uncut
S. dorsalis (IN)[a]	1333	559	211, 348, 503, 830*	
S. n. sp. (NZ)[b]	1279	615	188, 270, 615, 891*	ITS2 uncut
S. pan	1201	604	72, 131, 184, 604, 814*	ITS2 uncut
S. aceri	1192	615	285, 615, 907*	ITS2 uncut
S. oligochaetus[b]	1180	565	565, 1180*	ITS1 and ITS2 uncut
S. dorsalis (ZA)[a]	1163	570	315, 355,1163*	ITS1 uncut
N. geminus	1148	581	294, 355, 499*, 581	ITS2 uncut
S. frondis	1120	617	617, 1120*	ITS1 and ITS2 uncut
S. inermis	1116	594	594, 1116*	ITS1 and ITS2 uncut
S. drepanofortis	1089	651	651, 1089*	ITS1 and ITS2 uncut
S. sp. nr. *dobroskyi*	998	624	317, 624, 681*	ITS2 uncut
S. citri	941	583	131, 262, 452, 679*	
N. burungae	894	604	604, 894*	ITS1 and ITS2 uncut
S. bounites[b]	890	588	216, 239, 372, 651*	
S. aurantii (AU and ZA)	724	638	220*, 504, 638	ITS2 uncut

Where possible, the ITS1 and ITS2 regions were sequenced for at least two individuals of each species. Sizes correspond to the longest clone sequenced for each product. Species that may be confused with each other based solely on a comparison of the size of their ITS1 and ITS2 products are grouped together. The final species, *S. aurantii*, is distinguishable by the size of its ITS1 and ITS2 products alone. AU, Australia; HN, Honduras; IN, India; ZA, South Africa.

* 35 bp shorter if ITS1 region is amplified alone.

[a] Morphologically identified as the same species.

[b] Only one specimen sequenced.

analysis are shown in Table 2. For some unknown reason, the fragment containing the ITS1 region consistently amplified beyond its reverse primer (ITS2) to include the whole of the ITS2-forward primer plus the short "interprimer" region of 5.8s, a total of 35 extra base pairs. Intraspecific sequence polymorphism was low and largely restricted to microsatellite repeat regions. Indeed, similar levels of sequence polymorphism existed between different clones from a single specimen (for an example of the intraspecific variation in the ITS2 region of *S. perseae* specimens, see Table 3). Although size differences are apparent when all species are electrophoresed on a single agarose gel, species diagnosis may be more ambiguous if specimens are electrophoresed alone (e.g., distinguishing between *S. aceri*, *S. dorsalis*, and *N. geminus;* Fig. 1). However, diagnostic differences also were found by digesting the ITS1 and ITS2 products with an appropriate restriction enzyme (Tables 2 and 4). Together, differences in size of the multiplex PCR products and restriction fragment lengths allow the construction of a simple key to the species studied (Table 5).

Table 3. Intra- and interindividual conformity in the ITS2 region of six *S. perseae* specimens from three distinct localities

Specimen-clone	125–145	236–237	271–278	285–296
PR104-A[a]	T GTCGCCCC-TCGGGG-CGGC	GC	TATAATATCTTTTTT--GCG	
PR104-C[a]	. .....-.....TG....	..	....-.....--...	
PR104-D[a]	. .....C.....-..T.	..	...G-.....--...	
PR101-A[a]	. .....C.....-....	..	....-.....--...	
PR105-A[a]	. .....C.....-....	..	....-.....TT..A	
PR210-6[b]	. .....-.....-....	..	....-.....--...	
PR213-5[b]	. .....C.....-..T.	CT	....-.....T-...	
PR259-1[c]	. .C.....-.....-T...	..	A...-.....--...	
	336 378 382 490–513			
PR104-A	C A C ATTTTTTTTTACTCTTTTTT-CCT			
PR104-C	T . . ..-G.....-...			
PR104-D	T G . .....-...			
PR101-A	T . . ..-.....-...			
PR105-A	T . . ..T...			
PR210-6	T . . ..--.....-...			
PR213-5	T . . ..-.....-...			
PR259-1	T . T .....-.....-...			

Variation in length of the ITS2 region is the result of length polymorphism in microsatellite sequences.

[a] Orange County, California.

[b] Huayapam, Mexico

[c] Chimaltenango, Guatemala

Table 4. Size (in base pairs) of the restriction fragments generated by the restriction enzyme PspOM I

Species	PspOM I	Comments
S. perseae[a]	264, 536, 623, 630*	ITS2 uncut
S. n. sp. (HN)	619, 1440*	ITS1 uncut
S. oligochaetus	354, 565, 826*	ITS2 uncut
S. frondis	143*, 282, 335, 977	
S. inermis[b]	505, 594*, 611	ITS2 uncut
S. drepanofortis	651, 1089*	ITS1 and ITS2 uncut

Restriction fragments created from the ITS1 and ITS2 multiplex PCR products.

* 35 bp shorter if ITS1 region is amplified alone.

[a] Appears as only three bands following gel electrophoresis

[b] Appears as only two bands following gel electrophoresis.

Table 5. Molecular key to pestiferous *Scirtothrips* species based on ITS1 and ITS2 multiplex PCR products

1. Size of the ITS1 band >1,000 bp	15
Size of the ITS1 band <1,000 bp	3
2. Size of the ITS1 band >1,250 bp	8
Size of the ITS1 band <1,250 bp	8
3. Size of the ITS2 band <600 bp	*S. dorsalis* (IN)
Size of the ITS2 band >600 bp	4
4. PCR products restricted with SacII gives 3 bands	*S. kenyensis*
PCR products restricted with SacII gives >3 bands	5
5. PCR products restricted with SacII gives 4 bands	*S.* n. sp. (NZ)
PCR products restricted with SacII gives 5 bands	6
6. Largest SAC II restriction band <900 bp	*S. astrictus*
Largest SAC II restriction band >900 bp	7
7. PCR products restricted with PspOM I gives 3 bands	*S. perseae*
PCR products restricted with PspOM I gives 2 bands	*S.* n. sp. (HN)
8. PCR products restricted with SacII gives 2 bands	9
PCR products restricted with SacII gives >2 bands	12
9. PCR products restricted with PspOM I gives 2 bands	10
PCR products restricted with PspOM I gives >2 bands	11
10. Largest PspOM I restriction band 611 bp	*S. inermis*
Largest PspOM I restriction band 1,089 bp	*S. drepanofortis*
11. PCR products restricted with PspOM I gives 3 bands	*S. oligochaetus*
PCR products restricted with PspOM I gives 4 bands	*S. frondis*
12. PCR products restricted with SacII gives 3 bands	13
PCR products restricted with SacII gives >3 bands	14
13. Largest SacII restriction band 907 bp	*S. aceri*
Largest SacII restriction band 1,163 bp	*S. dorsalis* (ZA)
14. PCR products restricted with SacII gives 4 bands	*N. geminus*
PCR products restricted with SacII gives 5 bands	*S. pan*
15. Size of the ITS1 band <800 bp	*S. aurantii*
Size of the ITS1 band >800 bp	16
16. Size of the ITS1 band <900 bp	17
Size of the ITS1 band >900 bp	18
17. PCR products restricted with SacII gives 2 bands	*N. burungae*
PCR products restricted with SacII gives 4 bands	*S. bounites*
18. PCR products restricted with SacII gives 3 bands	*S.* sp. nr. *dobroskyi*
PCR products restricted with SacII gives 4 bands	*S .citri*

Note that ITS1 is the larger product.

Discussion

DNA sequences generated by PCR have the potential to be extremely useful tools in the identification of species. In the current study, multiplex PCR of the ITS1 and ITS2 regions of rRNA and restriction of the subsequent products provided a set of simple diagnostic characters for several economically important pest species of the genus *Scirtothrips.* The advantage of such an identification system over morphology-based taxonomic methods and other molecular methods (e.g., allozyme variation) is that it is quick, specific, requires only basic laboratory skills, and can be performed with DNA extracted from a single individual, which can be preserved, slide mounted, and used for future reference. Furthermore, we have successfully tested our method on instars that normally would not be identifiable using traditional methods (P.R.-J., unpublished data), and similar systems have been used to identify single eggs (Moritz et al. 2004). Such techniques could greatly improve the efficacy of plant quarantine diagnoses.

One weakness of our identification system is that it still requires identification of specimens to genus via morphological methods, which may be difficult for workers not familiar with thrips identification keys and slide preparation techniques. The design of a set of primers that would result in a genus-specific PCR product would complete our key. However, such a goal is unrealistic because it would mean sequencing thousands of species from other genera to ensure that the primer set used was truly unique to the genus *Scirtothrips.* One further problem that may be encountered stems from the nature of multiplex PCR. In the majority of cases, the QIAGEN HotStarTaq DNA polymerase used in our system (in conjunction with its PCR buffer) results in efficient amplification of both products with minimal production of misprimed products and primer-dimer. Occasionally, a third product may be produced corresponding to a fragment of DNA that approximates to the size of both the ITS1 and ITS2 together (i.e., one primed by CS249 and CS250). However, it is possible that using *Taq* polymerases (and perhaps more importantly their associated buffers) from other manufacturers may result in the preferential amplification of one product (probably the smaller ITS2) at the expense of the other, or excessive mispriming (Henegariu et al. 1997). The option remains to amplify the two regions separately. However, this would result in a reduction in size of the ITS1 region, and subsequently one of the restriction bands, by 35 bp (Table 2). We can find no explanation for why the ITS1 region amplifies beyond its reverse primer in our multiplex PCR.

A recent revision of the genus *Scirtothrips* in Mexico (Johansen and Mojica-Guzman 1998) proposed five new species from avocado. However, the validity of

these species has been questioned because species designations were made according to morphological characters that exhibit high intraspecific variation (Mound and zur Strassen 2001). Despite extensive collecting efforts throughout the avocado growing regions of Mexico and Central America, we failed to detect any of the species proposed by Johansen and Mojica-Guzman (1998), although our system does provide molecular confirmation for a novel *Scirtothrips* species collected from avocado in Honduras. Furthermore, the 1998 revision (Johansen and Mojica-Guzman 1998) proposed several new *Scirtothrips* species from mango. Although we have not extensively surveyed mango as part of this study, the validity of these species must now also be seriously doubted. Indeed, the only *Scirtothrips* species we collected from mango in Mexico (*S. bounties*) is not listed in the revision. This highlights the need for the inclusion of molecular data in taxonomic studies, particularly in groups where "good" morphological characters are sparse.

In *Scirtothrips* inhabiting avocado in Mexico, our molecular data suggest that only one species, *S. perseae*, should be recognized, i.e., the species complex from avocados proposed by Johansen and Mojica-Guzman (1998) is actually a single species, *S. perseae.* In contrast, our molecular data suggest that Indian and South African specimens of *S. dorsalis* (or specimens that "key out" to *S. dorsalis* on the basis of morphological characters) are indeed not the same species. The level of variation, in the sizes and sequences of the ITS1 and ITS2 regions between "*S. dorsalis*" specimens from India and South Africa, is too great for these even to be recognized as different host races (Table 2). Again, this highlights the importance of molecular data in modern taxonomic identifications, this time to separate morphologically identical species. Indeed, if coupled with the type of nondestructive DNA extraction technique we have used, such molecular differences also can be used as a basis to search (postextraction) for subtle morphological differences that previously may have been missed, ignored, or not considered robust enough for species separation.

The main goal of this study was to find a PCR-based method to identify pestiferous species of *Scirtothrips.* Using two regions of rRNA, we have produced a simple and accurate means of identifying seven such pestiferous *Scirtothrips*, along with several nonpestiferous members of the genus and two species of *Neohydatothrips*, at least one of which, *N. burungae*, has been highlighted as a potential future pest of avocado in North America (Hoddle et al. 2002). We also have identified two new *Scirtothrips* species, one species from Honduras and one species from New Zealand, and these species will be described elsewhere. Our molecular key facilitates the speedy and accurate identification of these taxa that should increase the efficiency with which pest species of *Scirtothrips* are identified by plant quarantine facilities and decrease response times for possible eradication programs or the initiation of classical biological control programs. It is envisioned that as their sequences become available, the remaining pestiferous members of the genus (*S. bispinosus, S. longipennis, S. mangifera*e, and *S. manihoti;* Mound and Palmer 1981) also can be incorporated into our key.

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