Journal of Economic Entomology, 109(3), 2016, 1424–1430
doi: 10.1093/jee/tow060
Advance Access Publication Date: 26 April 2016
Research article

Molecular Entomology

Identification of *Sirex noctilio* (Hymenoptera: Siricidae) Using a Species-Specific Cytochrome C Oxidase Subunit I PCR Assay

Xueting Sun, Jing Tao,[1] Lili Ren, Juan Shi, and Youqing Luo

The Key Laboratory of Beijing for the Control of Forest Pests, Beijing Forestry University, Beijing 100083, China (879795832@qq.com; taojing1029@hotmail.com; 24171397@qq.com; shi_juan@263.net; youqingluo@126.com), and [1]Corresponding author, e-mail: taojing1029@hotmail.com

Received 20 December 2015; Accepted 2 March 2016

Abstract

Sirex noctilio F. (Hymenoptera: Siricidae: Siricinae), a new invasive species in China, is a significant international forestry quarantine pest. Transportation of *Sirex* in logs, and related wood packing materials, has led to environmental damage and substantial economic loss in many countries around the world. Traditional morphological characteristics are not reliable for identification of the Siricidae family, particularly the larvae. Furthermore, specimens are frequently not in a suitable condition to permit morphological identification. The majority of damage is caused by the larval stage, which excavates galleries that can penetrate to the center of tree boles. Thus, development of a rapid, accurate, and effective molecular identification technique for *S. noctilio*, which does not require expert morphological knowledge, is necessary. Here, we describe a molecular identification tool based on the mitochondrial DNA gene, cytochrome C oxidase subunit I (COI). We designed a species-specific COI (SS-COI) PCR assay, which allows direct identification of *S. noctilio*, regardless of developmental stage. Six woodwasp species commonly found in China, *Sirex noctilio*, *Sirex nitobei*, *Sirex* sp., *Tremex fuscicornis* Fabr., *Tremex apicalis* Matsumura, and *Xeris spectrum*, were included in our analyses. Moreover, specimens of *S. noctilio* from 16 different areas were analyzed. The results demonstrate that our molecular assay is effective and accurate, regardless of developmental stage or type of specimen, consistent with use for quarantine purposes, to prevent the harmful consequences of *S. noctilio* spread.

Key words: *Sirex noctilio*, woodwasp, molecular tool, rapid, mitochondrial DNA

Over the past 50 yr, with the rapid expansion of global economic integration, biological invasion of forestry has become an increasingly serious issue (Hulme 2009). The problem warrants increased attention, as the invasion of alien species often leads to destruction of native biodiversity (Simberloff 2000, Clavero and García-Berthou 2005), and can also cause huge economic loss (Everett 2000, Pimentel et al. 2001). Some major invasive species have already caused destruction of forestry resources in China, negatively influencing species diversity and ecological security, and resulting in major economic loss. Avoiding alien species invasions is fundamental to preventing these detrimental effects; therefore, speedy detection and identification of invasive species is vitally important.

Sirex noctilio F. (Hymenoptera: Siricidae: Siricinae), a new invasive species in China, is a significant forestry quarantine pest of worldwide significance. The main harm caused by *S. noctilio* is mediated by the larvae, which feed by boring within the trunks of *Pinus* species trees. The host range of *S. noctilio* is wide, and both natural forests and pine plantations can be host to the pest, especially in overstocked and stressed conditions. *S. noctilio* is easily spread by the transportation of wood and has been found in many countries and regions, where it leads to the destruction of large areas of pine forest. According to the literature, *S. noctilio* spread through *P. radiata* plantations across New Zealand after its first discovery in 1900. Thirty-three percent of trees were killed in the central north region of the island, an area of ~120,000 hm^2, causing huge economic and ecological loss. In 1952, the species was found on the island of Tasmania, Australia (Neumann and Minko 1981), and an outbreak in 1987–1989 killed more than 5 million *P. radiata* trees (Haugen et al. 1990). The pest has also spread widely in South America, with reports of outbreaks in Uruguay in 1980, Argentina in 1985, Brazil in 1988, and Chile in 2000. The mortality rate of pine trees was 60% in the invaded areas of Argentina, with up to 80% mortality reported in Uruguay. In Brazil, a 350,000 hm^2 pine plantation area was destroyed. *S. noctilio* was reported in South Africa in 1994, and in North America and Canada in 2006. Thus, *S. noctilio* is one of the most significant forestry pests in the world, with the North American Plant Protection Organization (NAPPO) and the United States regarding it as a "high risk" invading

organism. The State Forestry Administration management department of China has a particular interest in the pest, and is supporting study of the organism with the aim of controlling population growth and density.

In China, the Eurasian woodwasp *Sirex noctilio* F. was first detected in 2013 in Daqing (N 46.629777°, E 124.430925°), Heilongjiang Province, in the northeast region of the country (Li et al. 2015). Although it is known that the pest is harmful to various kinds of conifers, especially *Pinus*, *Picea*, *Abies*, *Larix*, and *Pseudotsuga*, fieldwork indicated that the only infested pine species in China to date is Scots pine; however, preliminary trap experiments indicate that the wasps also have a preference for *Pinus tabulaeformis* Carr (X.B. Liu, unpublished data). In 2007, the National Forestry Bureau put the insect on the plant importing quarantine pest list of the People's Republic of China. Domestic and foreign experts analyzed the potential distribution area of this insect in China, and their results indicated that a band stretching from Yunnan to Heilongjiang represents a highly suitable area for distribution of the wasp (Dai 2010). The host tree species of *S. noctilio* are widely distributed in China; hence, the potential risk of distribution of this alien species is very high. Given the widespread distribution of *S. noctilio* worldwide and its history of causing huge economic loss, the State Forestry Administration is taking a close interest in its spread and invasion in China, and requires all potentially contaminated material to be inspected and quarantined at entry and exit of the country to ensure strong supervision and management of the threat represented by this pest.

The morphological identification of *S. noctilio* is based on the external characteristics of adults. For adult female specimens, antenna, body color, size of ovipositor pits (near the middle or aligned with the base of the apical section of the sheath), and the proportion of the tarsal pad to the ventral length of the tarsomere, are the main identification characteristics, whereas for male wasps, antenna, body color, and the proportion of the tarsal pad to the ventral length of the tarsomere are key (Schiff et al. 2012). During port inspection and quarantine processes, forestry bureaus and other related management institutions often obtain pupae and larvae of *S. noctilio*, rather than adult specimens. In these cases, it is not possible to identify specimens rapidly and accurately by traditional morphological identification, especially when the intercepted samples are larvae or broken and mutilated specimens. Hence, new molecular identification technology needs to be developed, especially when only immature specimens are available.

The aim of this study was, therefore, to develop a molecular assay to identify *S. noctilio* specimens rapidly and accurately during wood transportation or forestry investigation. Species-specific primer design can facilitate species identification after gel electrophoresis of amplified PCR products of a known size (Chua et al. 2010, Liu et al. 2011, Arif et al. 2012). This technology is based on the design of a specific primer pair from a DNA region shared among other similar species, which is only able to amplify a product from the target species of interest, whereas all of the species could be amplified by generic primer pair. (Rugman-Jones et al. 2009). According to the literature of Wilson and Schiff (Wilson and Schiff 2010, Slippers et al. 2011), mitochondrial cytochrome C oxidase I (COI) gene has recently been used as a molecular tool to identify siricid species. We developed a species-specific PCR assay to amplify the mitochondrial COI gene, by targeting a sequence which varies among woodwasp species. To assess the specificity of the assay, we examined five other woodwasp species common in China, or sharing the distribution of *S. noctilio*: *Sirex nitobei*, *Sirex* sp. (a newly recorded species in China), *Tremex fuscicornis* Fabr., *Tremex apicalis* Matsumura, and *Xeris spectrum*. A variety of developmental stages (larva, pupa, and adult) were analyzed, along with *S. noctilio* samples from 16 different geographical locations. The technology we developed has the potential to tackle *S. noctilio* effectively in China, by aiding prevention of the harmful consequences of further spread of the species through facilitating effective inspection and quarantine processes.

Materials and Methods

Insects

Specimens used in this study were either donated by various local forestry bureaus or obtained from the standard quarantine facility of Beijing Forestry University, where a large number of infested stems were collected locally. Specimens were identified using taxonomic literature on *Sirex* woodwasps (Schiff et al. 2012) (Figs. 1–9) and other related species (Xiao et al. 1991) before DNA extraction. Details of all specimens used in this study are listed in Table 1. All samples were stored in anhydrous ethanol (purity $\geq$ 99.7%).

DNA Extraction and Preparation

DNA was extracted from specimens using the EZgene Insect gDNA Kit (BIOMIGA), following the manufacturer's protocol. For all specimens, DNA was eluted using 70 µl of Elution Buffer and stored at −20°C until use.

Amplification of a Generic COI PCR Product From Six Woodwasp Species

Generic primers to amplify the COI gene sequence from six woodwasp species were synthesized by a commercial company (SinoGenoMax; Beijing). The sequences were forward primer (LCO1490), 5'- GGT CAA CAA ATC ATA AAG ATA TTG G - 3' and reserve primer (HC02198), 5 '- TAA ACT TCA GGG TGA CCA AAA AAT CA - 3' (Vrijenhoek 1994). These primers were used to PCR amplify template DNA from six woodwasp species: *S. noctilio*, *Sirex nitobei*, *Sirex* sp., *Tremex fuscicornis*, *Tremex apicalis*, and *Xeris spectrum*. Reactions were performed in a total volume of 25 µl, containing 12.5 µl of 2× GoTaq Green Master Mix (GoTaq Green Master Mix kit; Promega), 1 µl of each primer, 1.5 µl of DNA template, and 9 µl of ultrapure water. Reaction conditions were as follows: an initial denaturation for 2 min at 94°C, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 45°C for 30 s, and extension at 68°C for 2 min, with a final extension at 68°C for 10 min. For each reaction, a negative control, where sterile distilled water was used in place of the template, was performed.

PCR products (3 µl) were analyzed by electrophoresis on a 1.5% (w/v) agarose gel (1× TAE), alongside a DNA marker (D2000; Takara). After electrophoresis at 130 V for 20 min, the PCR products were visualized using ethidium bromide and ultraviolet light. Finally, products clearly visible after electrophoresis were sent to a commercial company (SinoGenoMax; Beijing) for sequencing in both directions.

Design of an *S. noctilio* SS-COI PCR Assay

Using the results of sequencing of the generic COI PCR products from the six species in this experiment, in addition to comparative analysis using sequences from 11 species of *Sirex* woodwasps available in the GenBank database (Table 2), we designed *S. noctilio* SS-COI primers, SNSSF1 and SNSSR1, using the software Primer Premier 5.0. Primers were synthesized by SinoGenoMax (Beijing). After amplification with these primers, the products were sequenced

Table 1. Chinese woodwasp specimens used in this study

Species	Specimen ID	Developmental stage	Locality	Host tree
Sirex noctilio	XL	Adult	Harbin, Heilongjiang	*Pinus sylvestris*
Sirex noctilio	DJ	Adult	Ningan, Heilongjiang	*Pinus sylvestris*
Sirex noctilio	CH	Adult	Hailin, Heilongjiang	*Pinus sylvestris*
Sirex noctilio	SGLK	Adult	Mudanjiang, Heilongjiang	*Pinus sylvestris*
Sirex noctilio	AC	Adult	Acheng, Heilongjiang	*Pinus sylvestris*
Sirex noctilio	BMT	Adult	Muleng, Heilongjiang	*Pinus sylvestris*
Sirex noctilio	SY	Adult	Suiyang, Heilongjiang	*Pinus sylvestris*
Sirex noctilio	DM	Larva	Dumeng, Heilongjiang	*Pinus sylvestris*
		Pupa Adult	Dumeng, Heilongjiang	*Pinus sylvestris*
Sirex noctilio	HG	Adult	Hegang, Heilongjiang	*Pinus sylvestris*
Sirex noctilio	HNC	Adult	Jiamusi, Heilongjiang	*Pinus sylvestris*
Sirex noctilio	FJC	Adult	Fujin, Heilongjiang	*Pinus sylvestris*
Sirex noctilio	YS	Adult	Yushu, Jilin	*Pinus sylvestris*
Sirex noctilio	CC	Adult	Changchun, Jilin	*Pinus sylvestris*
Sirex noctilio	MZL	Adult	Manzhouli, Inner Mongolia	*Pinus sylvestris*
Sirex noctilio	HDQ	Adult	Keyihe, Inner Mongolia	*Pinus sylvestris*
Sirex noctilio	HHEJ	Adult	Hulunbeir, Inner Mongolia	*Pinus sylvestris*
Sirex sp.	XJ	Adult	Xinjiang urumchi	*Picea schrenkiana*
Sirex nitobei	DQG	Adult	Inner Mongolia	*Pinus sylvestris*
Tremex fuscicornis	YJ	Adult	Xinjiang	*Acer negundo* Linn.
Tremex apicalis	BJ	Adult	Beijing	*Salix babylonica* L.
Xeris spectrum	41	Adult	Changchun, Jilin province	*Pinus sylvestris*

Table 2. *Sirex* species sequences used for comparative analysis

Species	Accession no.
Sirex sp. '*xerophilus*'	JQ619799
Sirex juvencus	KF936647
Sirex cyaneus	JQ619790
Sirex nigricornis	JQ619794
Sirex sp.*near nitidus*	JQ619793
Sirex sp.*near californicus*	JQ619792
Sirex longicauda	JQ619791
Sirex juvencus californicus	JQ619797
Sirex behrensii	JQ619788
Sirex areolatus	JQ619787
Sirex sp. '*abietinus*'	JQ619786

by a commercial company (SinoGenoMax; Beijing) to ensure they were derived from *S. noctilio*.

Specificity and Sensitivity of the SS-COI PCR Assay

In order to test the specificity of the SS-COI primers (SNSSF1 and SNSSR1), we used DNA extracted from five species common in China (Table 1) as the template, with *S. noctilio* as the positive control. PCR amplification reaction components were the same as those for the generic primer pair. Reaction conditions were an initial denaturation of 2 min at 94°C, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 53°C for 30 s, and extension at 72°C for 1.5 min, with a final extension at 72°C for 10 min. A negative control reaction, using sterile distilled water as the template, was performed.

PCR products were electrophoresed on 1.5% (w/v) agarose gels (1× TAE) alongside a DNA marker (D2000; Takara) for 20 min, followed by visualization using ethidium bromide and ultraviolet light. The SS-COI assay was tested using DNA extracted from samples at different developmental stages (larva, pupa, and adult) of *S. noctilio* as the template.

To assess the sensitivity of the SS-COI assay, we performed PCR using serially diluted *S. noctilio* template DNA at concentrations of 40 ng, 4 ng, 400 pg, 40 pg, 4 pg, and 400 fg.

Results

Amplification of Woodwasp COI Gene Sequences

Using DNA from six common woodwasp species (*Sirex noctilio*, *Sirex nitobei*, *Sirex* sp., *Tremex fuscicornis*, *Tremex apicalis*, and *Xeris spectrum*) as the template, we performed PCR amplification using the generic COI primer pair LCO1490/HC02198. Gel electrophoresis demonstrated that the generic primer pair amplified clear products from each of the six species (Fig. 10). Products (~600 bp) detected by electrophoresis were sequenced in both directions by a commercial company (SinoGenoMax; Beijing).

Specificity and Sensitivity of SS-COI Primers

We designed a SS-COI primer pair, SNSSF1/SNSSR1, to amplify a 400 bp product only from *S. noctilio*, but not other woodwasp species. Using template DNA from six woodwasp species common in China, we performed PCR amplification using the SS-COI primer pair. The sequence of the forward primer, SNSSF1, was 5'-ATT CTG ACT CCT TCC TCC TGC TTT-3' and that of the reverse primer, SNSSR1, was 5'-AGT GAT TGC TCC AGC AAG AAC A-3'. Gel electrophoresis demonstrated a clearly visible band amplified from *S. noctilio* DNA, with no amplification from DNA of the five other species, indicating that the primers are specific for *S. noctilio* (Fig. 11). In order to verify the reliability of the assay, we performed reactions using template DNA extracted from different developmental stages of *S. noctilio*: larva, pupa, and adult. Gel electrophoresis confirmed that the SS-COI primers can be used for identification of these life stages (Fig. 12A). Subsequently, PCR products were sequenced to verify that they were indeed from *S. noctilio* samples.

To examine the sensitivity of the SS-COI primers, we performed the assay using serial diluted *S. noctilio* DNA as the template (Fig. 12B). Reactions containing 40 ng, 4 ng, and 400 pg of the

Fig. 1. Back of the female *S. noctilio* (Online figure in color).

Fig. 2. Back of the male *S. noctilio* (Online figure in color).

template resulted in vivid bands after electrophoresis, and the minimum detection limit of the method was 200 pg DNA template (data not shown). These results indicate that this assay is sufficiently sensitive and meets the requirements for quarantine testing.

Investigation of *S. noctilio* Samples From Different Geographical Locations

To investigate the specificity of the SS-COI assay, we tested DNA derived from *S. noctilio* samples collected from 16 different cities in Heilongjiang, Jilin, and Inner Mongolia. The assay was able to amplify the target fragment from all samples (Fig. 13). Lane 10 shows weak amplification because the size of the specimen is small, which is the only one obtained.

Discussion

In insect taxonomy, some special characteristics are generally sufficient for identification of well-preserved specimens, such as the size of the ovipositor pits in females and the proportion of the tarsal pad to the

Fig. 3. Side of the female *S. noctilio*, showing antenna and top of tarsal black (Online figure in color).

Fig. 4. Side of the male *S. noctilio*, showing antenna black, the color from segment 3–7 of abdomen orange, top of tarsal black, and postpedes black (Online figure in color).

ventral length of the tarsomere in males. Such traditional methods of identification require specialized knowledge and are slow. Additional complicating factors, including the requirement to identify specimens of immature developmental stages, cryptic species, and those which have been damaged, can further increase the difficulty of identification.

DNA methods have the potential to be much more reliable tools than traditional morphology for species identification. Molecular tools enable faster and more widely applicable identification of species and are becoming increasingly popular in so many fields, such as the development of sequence-characterized amplified region (SCAR) markers for crop species identification like wheat, barley, rice, tomato, and grape (Paran and Michelmore 1993, Liu et al. 1999, Wu et al. 2005). For fungi, the internal transcribed spacer (ITS) sequence analysis is used because there are frequently obvious interspecies differences discernible using this method in these organisms. Moreover, the fragments produced using the ITS method are relatively small and easy to analyze (Zheng et al. 2003, Chen and Zheng 2007). Not only for fungi, but also for insects, ITS with COI and Cytb genes are genetic sequence markers for species identification, especially COI (Chen et al. 2004, Rugman-Jones et al. 2006, Timm et al. 2008). Such use of DNA barcoding technology simplifies and standardizes species identification, facilitating international data exchange. Compared with morphological identification methods, the molecular assay we describe is more reliable and efficient. Moreover, it can identify immature stages and discern species which are hard to identify by morphological methods. The use of DNA

Fig. 5. Side of the female *S. noctilio,* showing antenna black (Online figure in color).

Fig. 6. Side of the male *S. noctilio,* showing antenna black (Online figure in color).

barcoding has the potential to achieve a 100% success rate for insect species identification, given the availability of sufficient reference sequences, and is certainly an invaluable tool for taxonomy.

Sirex woodwasps are particularly difficult to identify using traditional morphological methods, even by expert entomologists, due to subtle interspecies differences. *Sirex* woodwasps have been extensively studied by investigators in other countries, but relatively little related research has been conducted in China. Gang-rou Xiao and his research group (Xiao et al. 1991) have researched these insects in China, and they focused on systematic traditional studies of *Sirex* woodwasps species. Since relatively little research has been carried out on these insects in China recently, there have been no reports about their species identification using new techniques. According to the identification guide commonly used internationally (Schiff et al. 2012), there is no key which is 100% sufficient to identify Siricid species, due to the subjectivity of classification by color. Nevertheless, color is a useful characteristic contributing to traditional morphological identification.

In this study, to rapidly accomplish species identification, we used the mitochondrial COI gene as a specific genetic marker, which is already used commonly in siricid species (mentioned in introduction part) compared with ITS and Cytb gene. Moreover, the development of the species-specific primer pair is time-saving especially for import and export quarantine process.

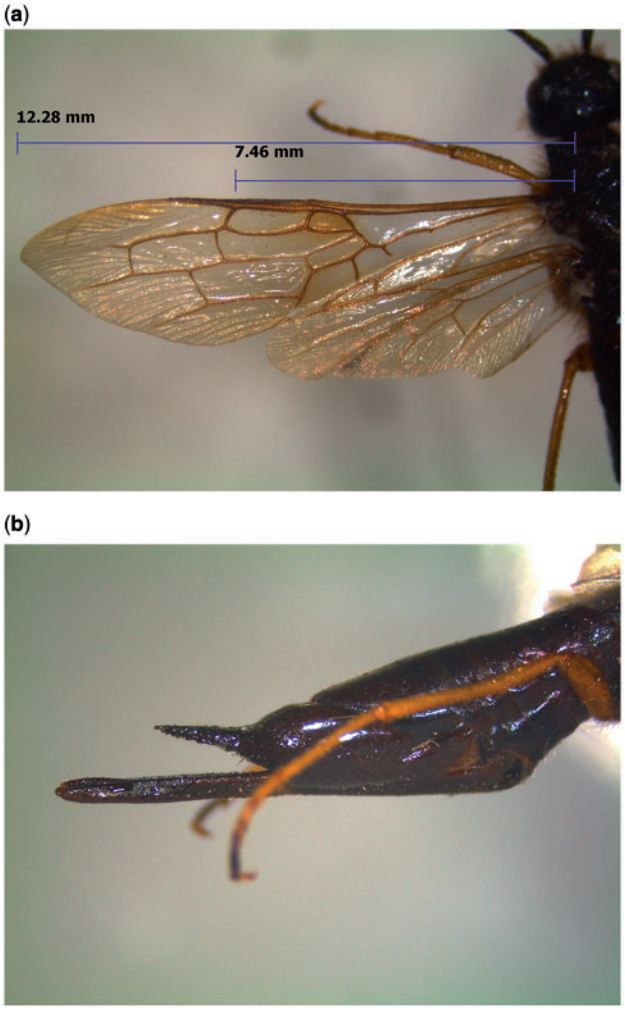

Fig. 7. (**A**) The length of the female forewing. (**B**) The length of the female ovipositor sheath (Online figure in color).

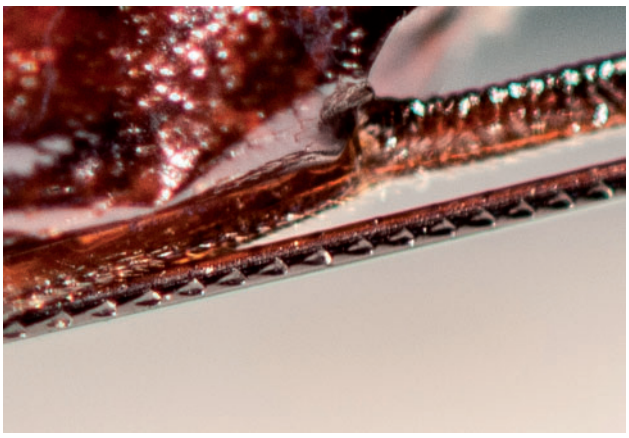

Fig. 8. Ovipositor pits near middle or pits aligned with base of apical section of sheath are at least 0.5 as long as annulus length (Online figure in color).

Acknowledgments

We thank the forestry departments of Heilongjiang and Jilin provinces, Inner Mongolia Autonomous Region, Xinjiang Uygur Autonomous Region, which provided specimens of Sirex woodwasp and other species woodwasp. This

Fig. 9. Tarsal pad is 0.3–0.4 times as long as ventral length of tarsomere (Online figure in color).

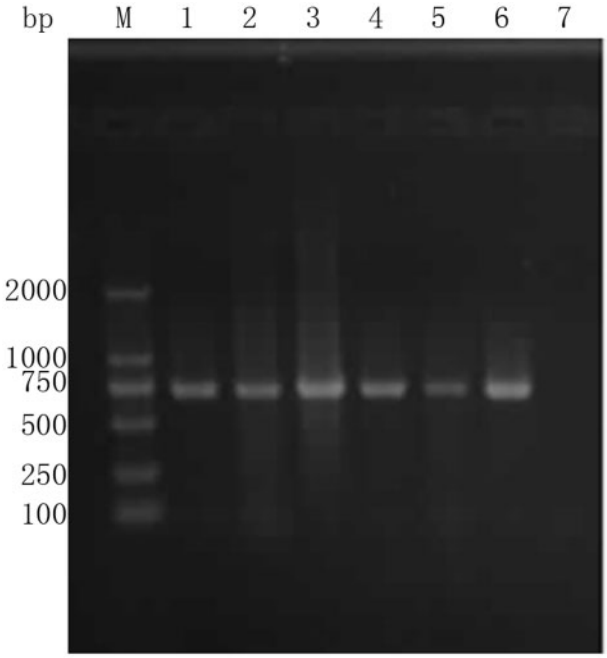

Fig.10. Agarose gel electrophoresis of PCR products amplified from DNA extracted from six species. Species in lane 1–6 are listed in Table 1. Lane 7, negative control.

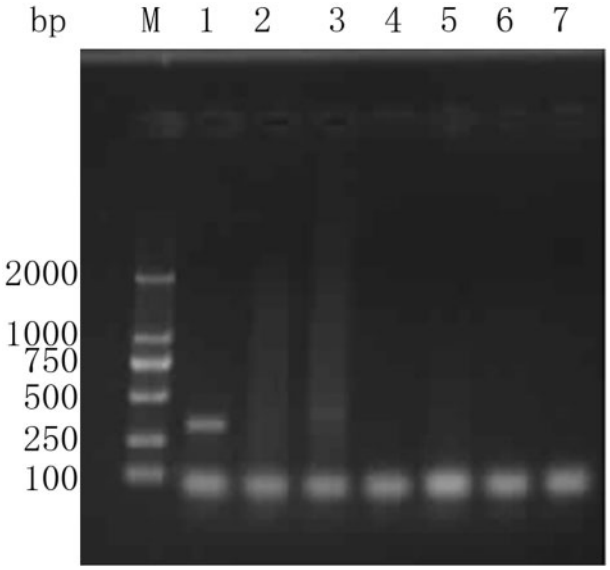

Fig. 11. Agarose gel electrophoresis of PCR products amplified from DNA extracted from six wasp species (lane 1–6) using the SS-COI primer pair. Lane 7: negative control.

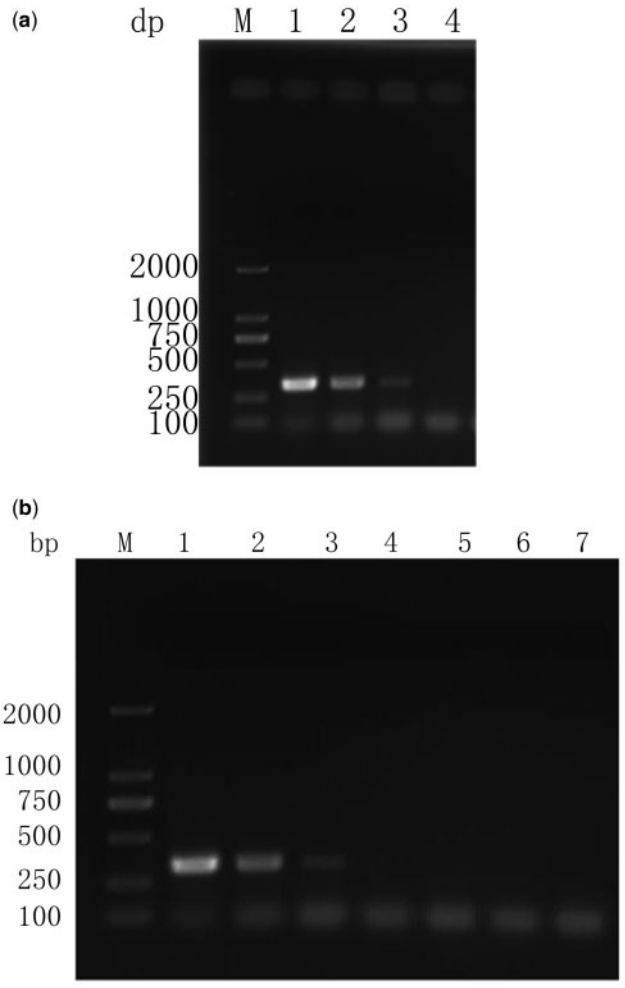

Fig. 12. (**A**) Agarose gel electrophoresis of PCR products amplified from DNA derived from *S. noctilio* larva, pupa, and adult (lanes 1–3, respectively), which are listed in Table 1 from DM using the SS-COI primer pair. Lane 4, negative control. (**B**) Agarose gel electrophoresis of PCR products amplified from serially diluted *S. noctilio* template DNA. Lanes 1–6: 40 ng, 4 ng, 400 pg, 40 pg, 4 pg, and 400 fg of *S. noctilio* DNA, respectively. Lane 7: negative control.

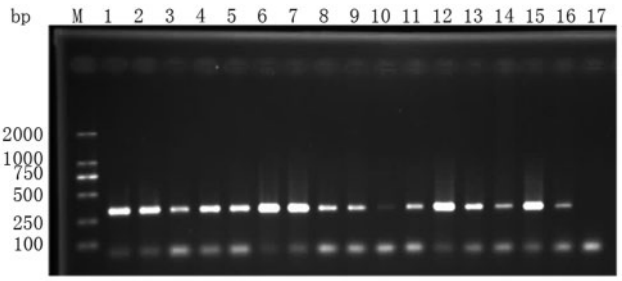

Fig. 13. Agarose gel electrophoresis of PCR products amplified from DNA extracted from *S. noctilio* samples collected from 16 different cities. Specimens in lanes 1–16 are listed in Table 1. Lane 17, negative control. Number 1–16 represent: XL, DJ, CH, SGLK, AC, BMT, SY, DM, HG, HNC, FJC, YS, CC, MZL, HDQ, HHEJ (which are shown in Table 1), respectively.

study was supported by a Special Fund for Forest Scientific Research in the Public Welfare of State Forestry Administration (201504304).

References Cited

Arif, M., F. M. Ochoa-Corona, G. P. Opit, Z.-H. Li, Z. Kučerová, V. Stejskal, and Q.-Q. Yang. 2012. PCR and isothermal-based molecular identification of the stored-product psocid pest *Lepinotus reticulatus* (Psocoptera: Trogiidae). J. Stored Prod. Res. 49: 184–188.

Chen, J., and F. Zheng. 2007. Application of ITS sequences in fungi classification and identification. J. Anhui Agric. Sci. 35: 3785–3786.

Chen, W.-Y., T.-H. Hung, and S.-F. Shiao. 2004. Molecular identification of forensically important blow fly species (Diptera: Calliphoridae) in Taiwan. J. Med. Entomol. 41: 47–57.

Chua, T. H., B. K. Song, and Y.-V. Chong. 2010. Development of allele-specific single-nucleotide polymorphism-based polymerase chain reaction markers in cytochrome oxidase I for the differentiation of *Bactrocera papayae* and *Bactrocera carambolae* (Diptera: Tephritidae). J. Econ. Entomol. 103: 1994–1999.

Clavero, M., and E. García-Berthou. 2005. Invasive species are a leading cause of animal extinctions. Trends Ecol. Evol. 20: 110.

Dai, Q. 2010. Pest risk analysis in China and the economic impacts assessment of the trade measures for *Sirex noctilio*. Master thesis. Beijing Forestry University.

Everett, R. A. 2000. Patterns and pathways of biological invasions. Trends Ecol. Evol. 15: 177–178.

Haugen, D., R. Bedding, M. Underdown, and F. Neumann. 1990. National strategy for control of *Sirex noctilio* in Australia. Australian For. Grower 13: 8.

Hulme, P. E. 2009. Trade, transport and trouble: Managing invasive species pathways in an era of globalization. J. Appl. Ecol. 46: 10–18.

Li, D., J. Shi, M. Lu, L. Ren, C. Zhen, and Y. Luo. 2015. Detection and identification of the invasive *Sirex noctilio* (Hymenoptera: Siricidae) fungal symbiont, *Amylostereum areolatum* (Russulales: Amylostereacea), in China and the stimulating effect of insect venom on laccase production by *A. areolatum* YQL03. J. Econ. Entomol. 108: 1136–1147.

Liu, G.-K., C. Juan, X. Shun, S.-S. Zhang, and D.-M. Pan. 2011. Development of species-specific PCR primers and sensitive detection of the *Tylenchulus semipenetrans* in China. Agric. Sci. China 10: 252–258.

Liu, Z., Q. Sun, Z. Ni, T. Yang, and R. McIntosh. 1999. Development of SCAR markers linked to the Pm21 gene conferring resistance to powdery mildew in common wheat. Plant Breed. 118: 215–219.

Neumann, F., and G. Minko. 1981. The sirex wood wasp in Australian radiata pine plantations. Aust. For. 44: 46–63.

Paran, I., and R. Michelmore. 1993. Development of reliable PCR-based markers linked to downy mildew resistance genes in lettuce. Theor. Appl. Genet. 85: 985–993.

Pimentel, D., S. McNair, J. Janecka, J. Wightman, C. Simmonds, C. O'connell, E. Wong, L. Russel, J. Zern, and T. Aquino. 2001. Economic and environmental threats of alien plant, animal, and microbe invasions. Agric. Ecosyst. Environ. 84: 1–20.

Rugman-Jones, P. F., J. G. Morse, and R. Stouthamer. 2009. Rapid molecular identification of armored scale insects (Hemiptera: Diaspididae) on Mexican 'Hass' avocado. J. Econ. Entomol. 102: 1948–1953.

Rugman-Jones, P. F., M. S. Hoddle, L. A. Mound, and R. Stouthamer. 2006. Molecular identification key for pest species of Scirtothrips (Thysanoptera: Thripidae). J. Econ. Entomol. 99: 1813–1819.

Schiff, N. M., H. Goulet, D. R. Smith, C. Boudreault, A. D. Wilson, and B. E. Scheffler. 2012. Siricidae (Hymenoptera: Symphyta: Siricoidea) of the Western Hemisphere. Can. J. Arthropod Identification 21: 1–305.

Simberloff, D. 2000. Nonindigenous species: A global threat to biodiversity and stability. Nature and human society: the quest for a sustainable world, pp. 325–334. National Academy Press, Washington, DC.

Slippers, B., P. De Groot, and M. J. Wingfield. 2011. The Sirex woodwasp and its fungal symbiont: Research and management of a worldwide invasive pest. pp.4–13 Springer Science & Business Media.

Timm, A., M. Stiller, and J. Frey. 2008. A molecular identification key for economically important thrips species (Thysanoptera: Thripidae) in southern Africa. Afr. Entomol. 16: 68–75.

Vrijenhoek, R. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Mol. Mar. Biol. Biotechnol. 3: 294–299.

Wilson, A., and N. Schiff. 2010. Identification of *Sirex noctilio* and native North American woodwasp larvae using DNA barcodes. J. Entomol. 7: 60–79.

Wu, X., H. Li, H. Wei, L. Fu, Q. Wu, H. Peng, and M. Zhu. 2005. Application of SCAR molecular marker technology in identification of *Lentinula edodes*. Mycosystema 24: 259–266.

Xiao, G., X. Huang, S. Zhou, and J. Wu. 1991. Economic sawfly fauna of China Hymenoptera: Symphyta. pp.37–43 Tian Ze Publishing House.

Zheng, X., H. Yang, D. Li, and W. Han. 2003. Use of 16S-23S intergenic transcribed spacer in identification and community analysis of bacteria. Chin. J. Appl. Eviron. Biol. 9: 678–684.