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Short Communication

First Record of a Phoretic Astigmatid Mite (*Sancassania* sp.; Acaridae: Astigmata) on *Oryctes agamemnon* (Coleoptera: Scarabaeidae) in UAE

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ABSTRACT

Phoresy is a relationship between two different species of organisms in which the larger, or host, organism transports a smaller organism, the guest. The purpose of this research was to identify phoretic mites associated with *Oryctes agamemnon* Burmeister (Coleoptera: Scarabaeidae) and to provide a genetic profile using randomly amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR). The *Oryctes agamemnon* beetle is one of the major pests of date palm trees in the United Arab Emirates (UAE). Based on traditional taxonomy, the collected phoretic mites belonged to the family Acaridae and the genus *Sancassania*. The molecular technique, RAPD-PCR, was employed to make a genetic profile of this phoretic mite. Nine random primers were used. The reaction products were developed with agarose gel electrophoresis. The total number of bands produced by the nine primers was 57. Each primer produced many bands of which few were intense and distinguished bands. The current study is the first record documenting the presence of *Sancassania* sp. in UAE and adds these mites to the list of species biodiversity. © 2010 Friends Science Publishers

Key Words: RAPD-PCR; Acaridae; *Sancassania*; *Oryctes*; Phoresy; UAE

INTRODUCTION

Phoresy is a symbiotic interaction that results in dispersal, benefiting the relocated organisms without negatively impacting the phoretic host (Holte *et al*., 2001). Scarab beetles are commonly associated with phoretic astigmatid mites (Acaridae: Astigmata) (Houck & Oconnor, 1991). The fruit-stalk borer, *Oryctes agamemnon* (Burm.) (Coleoptera: Scarabaeidae) is an important insect pest on date palm trees (*Phoenix dactylifera* L.) in the United Arab Emirates (UAE). This insect is active from late March until the end of September and both adults and larvae cause damage (Gassouma, 2000). Species recognition of phoretic mites is in some cases difficult, because of the conserved deutonymphal morphology. Species discrimination is often possible only through the use of molecular techniques (Houck, 1989). This fact applies to the genus *Sancassania*, which is morphologically conserved and notorious for its intraspecific variability. Seventy five *Sancassania* species and one subspecies have been described, but most (approximately 79%) are known from the original descriptions only (Klimov *et al*., 2004). The majority of *Sancassania* sp. is not recognizable from the published descriptions therefore a molecular method such as the random amplified polymorphic DNA (RAPD) can be useful for establishing a genetic profile, which can be used in identification. The RAPD-PCR technique has been proven to be a valuable aid to taxonomists and has been used successfully and reliably (Brown *et al*., 1997; Black *et al*., 2001; Barman *et al*., 2003).

The desert ecosystem is characterized by low biodiversity. UAE is located in the desert biome and therefore has a limited number of animal species. In addition, microarthropod species compositions in general and phoretic mites in particular, are relatively un-explored and there is significant potential for discovering new species. The addition of a genus or species to the roster of the known organisms in UAE would be important for biodiversity and species conservation. The phoretic stage of *Sancassania* sp., the deutonymph, disperses in soil until they encounter and attach to *O. agamemnon* beetle larvae or adults. The mites remain with the beetles as phoretic stages. To our knowledge, this study is the first record of a phoretic astigmatid mite of the genus *Sancassania* on *O. agamemnon* in UAE. Also, this is the first research on phoretic mites in UAE. More work is undergoing toward studying the rest of phoretic astigmatid mites in the UAE desert ecosystem, which has not been previously explored. There is a possibility that the species collected in UAE represents a new species in the genus *Sancassania*.

The purpose of this research was to identify phoretic mites associated with *O. agamemnon* and to provide a

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genetic profile using DNA-RAPD-PCR.

MATERIALS AND METHODS

Collecting area: The collection of specimens was carried out in infested date palm plantations at Al-Ain (24º 11´ N, 55º 45´ E), Abu Dhabi, UAE, during the period from June to September in 2007 and 2008.

Insects and mites: *Oryctes agamemnon* beetles were captured at night using light traps, brought to the laboratory and examined under a dissecting microscope. Mites were removed using a fine camel hair brush and were stored in 70% ethanol. Mite specimens were cleared in Nesbitt's solution, mounted in Hoyer's medium on microscope slides (Krantz, 1978; Evans, 1992) and examined under a compound microscope for identification.

DNA extraction: Chromosomal DNA was extracted from collected mites (deutonymphs & tritonymphs) using a Maxwell 16 Tissue DNA purification kit, which is designed for use with the Maxwell 16 instrument (Promega, CA, USA), according to the manufacturer's protocol. All mites collected from each individual *O. agamemnon* beetle were combined and DNA was extracted for later use in PCR amplification. Thus each DNA sample represented the total mite population of one beetle.

RAPD-PCR amplification: This is a relatively easy procedure, which can process a large number of marker loci across the entire genome. This feature is the major advantage of using the RAPD-PCR approach (Lynch & Milligan, 1994). A set of nine decamer primers (Operon Technologies, Inc., USA) were examined for RAPD-PCR analysis. The sequences of these primers are presented in Table I. These primers were selected as they generated consistent and reproducible RAPD banding patterns. DNA amplification was performed with a PCR thermocycler (Genius, Techne, UK). HotStarTaq™Master Mix kit (Qiagen, CA, USA) was used for PCR reactions. For the RAPD-PCR assay, the reaction mixture included 20 ng template DNA, 12.5 µL HotStarTaq™ Master Mix (1 × PCR buffer, 1.25 units HotStarTaq™ polymerase, 200 µM of each dNTP), 1.5 mM $MgCl_2$ and 50 pM primer in a total volume of 25 µL. The program of thermal cycling was as follows: initial activation step at 95°C for 5 min followed by 40 cycles of 1 min at 94°C, 1 min at 36°C and 2 min at 72°C, with a final extension step at 72°C for 5 min. To detect contamination, a negative control, in which all PCR components except DNA template were included, was run in every experiment. In order to insure reproducibility of the individual bands, PCR reactions on the extracted DNA were conducted at least three times.

Electrophoresis of PCR products: RAPD PCR products were analyzed by electrophoresis in 1.8% agarose gel pre-stained with ethidium bromide and run at 10 V/cm for 3.5 h in TBE buffer. Gels were observed under UV illumination (UV Transilluminator, TFX 35, Life Technologies, CA, USA) and images were acquired with a Gel-Doc 1000 (Bio-Rad Laboratories, CA, USA). The molecular size of the amplified products was estimated by comparison with a 100 bp DNA ladder (Promega, CA, USA).

RESULTS

Mite identification: Based on traditional taxonomy using morphological identification characters the phoretic mite belonged to the order Acari, suborder Astigmata, family Acaridae, genus *Sancassania* (= *Caloglyphus*). Two immature stages were found, deutonymphs (Fig. 1) and tritonymphs (Fig. 2). The majority of collected mites were deutonymphs, with only few tritonymphs. Ventral suckers, which are present on the body of deutonymphs, were used to attach to the *O. agamemnon* body surface. The anterior tarsui of tritonymphs have expanded foliate setae (Fig. 3). Most of the deutonymphs and tritonymphs were found in the sub-elytral space and fewer mites were found on the thoracic and abdominal sternal plates or legs of *O. agamemnon* beetles. In some cases, mites were found on the beetle head.

DNA RAPD-PCR: By developing the reaction products with agarose gel electrophoresis, it became evident that DNA fragments were amplified with all the primers used (Fig. 4). The total number of bands produced per individual primer is presented in Table I. Every primer produced at least one intense band together with fainter bands. The number of intense bands produced by each primer was: 3, 2, 2, 1, 3, 4, 2, 4 and 4, respectively. For example, the molecular size of the three bands produced by primer one was 400, 450 and 750 bp and the intense band produced by primer four was 350 bp.

Voucher specimens of collected mites have been deposited to the Entomology and Parasitology Laboratory in the United Arab Emirates University.

DISCUSSION

RAPD PCR is a useful molecular technique that can aid in species taxonomy. Atienzar and Awadhesh (2006) reviewed more than 9000 papers that used RAPD and concluded that with proper optimization, RAPD is a reliable, sensitive and reproducible assay and has the potential to detect a wide range of DNA damage as well as

Table I: Band number of random primers used for PCR amplification of *Sancassania* sp.

Primer	Sequence (5'-3')	G+C %	Total No. of bands
OPA-04	AATCGGGCTG	60	7
OPA-07	GAAACGGGTG	60	5
OPA-09	GGGTAACGCC	70	8
OPA-13	CAGCACCCAC	70	6
OPA-18	AGGTGACCGT	60	6
OPA-20	GTTGCGATCC	60	8
OPC-02	GTGAGGCGTC	70	5
OPD-02	GGACCCAACC	70	6
OPE-02	GGTGCGGGAA	70	6
Total	--	--	57

Fig. 1: *Sancassania* sp. deutonymph ventral view (under compound microscope (x100))

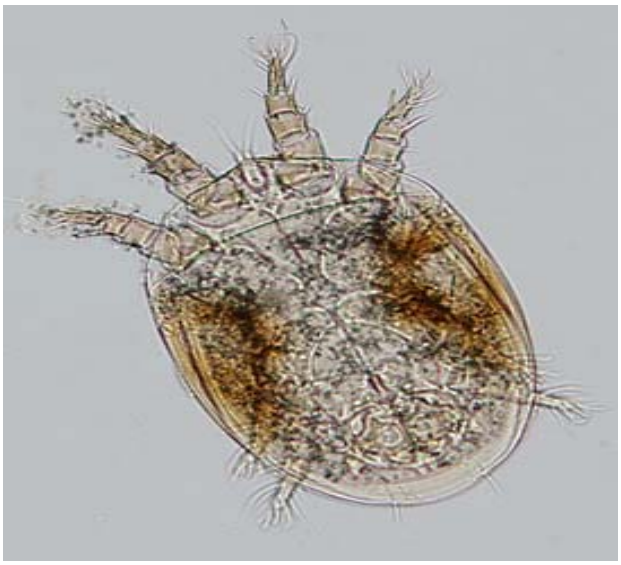


Fig. 2: *Sancassania* sp. tritonymph (under compound microscope (x100))

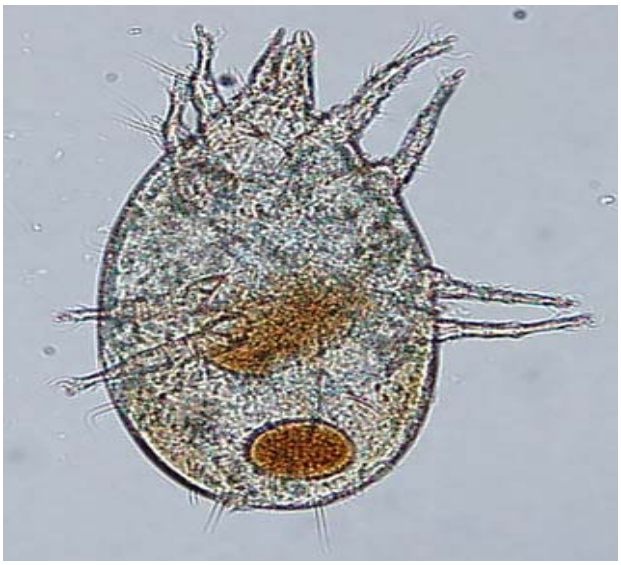


Fig. 3: The expanded foliate setae of the anterior tarsus of *Sancassania* sp. tritonymph (under compound microscope (x400))

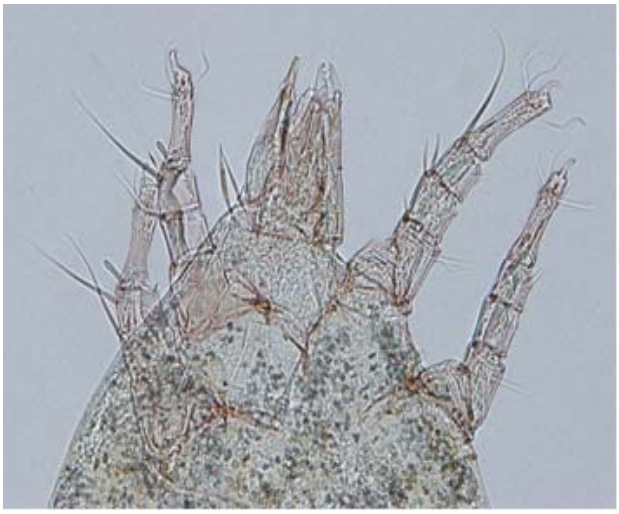
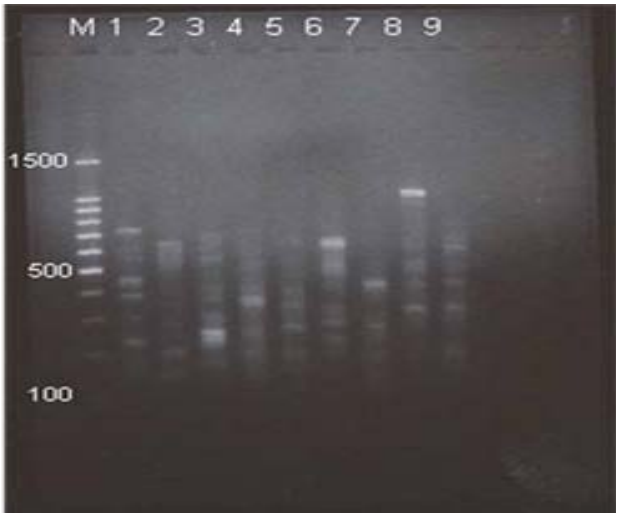


Fig. 4: Agarose gel electrophoresis of PCR-amplified DNA of astigmatid mite, *Sancassania* sp. (RAPD-PCR profiles using 9 primers)



mutations making it suitable for genotoxicity and carcinogenesis studies. Several studies have tested the reproducibility of the RAPD method in other organisms (Baruffi *et al*., 1995; Sebastiani *et al*., 2001; Bertin *et al*., 2007). An important aspect of using the RAPD-PCR method and other DNA-related identification methods is that adult specimens, which are usually required in traditional taxonomy (and which were not available for this study), are not essential for identification. In addition the genus *Sancassania* is morphologically conserved, but has high intraspecific variability and a large number of species (Klimov *et al*., 2004). Although RAPD-PCR is a very useful tool to genetically describe a species that cannot be recognized based on the currently available morphological identification keys, we recognize that DNA identification of a species is not a replacement for traditional methods based on morphology. In this research we did not suggest a name to the species found in UAE based on its DNA profile, as a comparison should be done with other known *Sancassania* species from neighboring countries, but we have provided information that could be useful when coupled with a complete morphological description when the adult stage become available.

RAPD reactions often produce a pattern of bright intense bands together with fainter bands or faintly smeared regions in the gel. Therefore the molecular size of the bright

intense bands and the total number provide a banding pattern profile specific to the UAE *Sancassania* mite, which could be compared with other mite profiles using the RAPD-PCR technique. Mites with the same profiles generated by the same set of primers should belong to the same species of phoretic mite. More work is warranted on *Sancassania* species composition in UAE ecosystem in part, because *Sancassania* DNA samples from other countries were not available to us to conduct comparisons.

CONCLUSION

Sancassania sp. was found as a phoretic mite on the fruit-stalk borer, *O. agamemnon* in UAE. The RAPD-PCR method, which is a reliable and sensitive assay, was employed to provide a DNA banding pattern of the *Sancassania* sp. which could be used to compare it with other mite profiles when they are available.

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