

The invasive coconut mite *Aceria guerreronis* (Acari: Eriophyidae): origin and invasion sources inferred from mitochondrial (16S) and nuclear (ITS) sequences

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Abstract

Over the past 30 years the coconut mite *Aceria guerreronis* Keifer has emerged as one of the most important pests of coconut and has recently spread to most coconut production areas worldwide. The mite has not been recorded in the Indo-Pacific region, the area of origin of coconut, suggesting that it has infested coconut only recently. To investigate the geographical origin, ancestral host associations, and colonization history of the mite, DNA sequence data from two mitochondrial and one nuclear region were obtained from samples of 29 populations from the Americas, Africa and the Indo-ocean region. Mitochondrial DNA 16S ribosomal sequences were most diverse in Brazil, which contained six of a total of seven haplotypes. A single haplotype was shared by non-American mites. Patterns of nuclear ribosomal internal transcribed spacer (ITS) variation were similar, again with the highest nucleotide diversity found in Brazil. These results suggest an American origin of the mite and lend evidence to a previous hypothesis that the original host of the mite is a non-coconut palm. In contrast to the diversity in the Americas, all samples from Africa and Asia were identical or very similar, consistent with the hypothesis that the mite invaded these regions recently from a common source. Although the invasion routes of this mite are still only partially reconstructed, the study rules out coconut as the ancestral host of *A. guerreronis*, thus prompting a reassessment of efforts using quarantine and biological control to check the spread of the pest.

Keywords: bioinvasion, phylogeography, Acari, molecular marker, coconut mite, biological control

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Introduction

Increase in trade, tourism, transport and travel over the past century has dramatically enhanced the spread of organisms (e.g. Wittenberg & Cock, 2001). As a result, biological invasions by non-indigenous species constitute a leading threat to natural ecosystems and biodiversity (Pimentel, 2002). Bioinvasions involving exotic pests are also an undesirable element of the globalization of agriculture. Accordingly, substantial efforts in eradication or control of invasive agricultural pests have resulted in high economic costs (Pimentel, 2000). Effective control strategies generally require knowledge of the pest in their introduced and native ranges (Roderick & Navajas, 2003) as is illustrated by the invasive spread of the Mediterranean fruit fly, *Ceratitidis capitata* (Wiedemann) (Diptera: Tephritidae). For this species, a series of studies have used molecular data to document the spread of this insect in its home range in sub-Saharan Africa (White & Elson-Harris, 1992) to reach a nearly global distribution in less than 200 years (Fimiani, 1989). Besides describing the invasion pathways of the species, knowledge of the genetic structure of fruit fly populations has helped to design control strategies including quarantine and sterile insect release (Bohonak *et al.*, 2001; Bonizzoni *et al.*, 2001; Gasperi *et al.*, 2002; Baliraine *et al.*, 2003).

Coconut, *Cocos nucifera* L. (Arecaceae), is one of the most valuable crops of the humid tropics and is considered to be among the most important crops in the world (Vietmeyer, 1986). Several hypotheses concerning the origin of this palm have been proposed but there is strong molecular evidence suggesting an origin from extreme South-East Asia to the Pacific Islands of Papua New Guinea (Persley, 1992; Lebrun *et al.*, 1998). In tropical areas, coconut is used in many aspects of everyday life and thus is often referred to as the 'tree of life'. The major coconut producing areas are in Asia, including the Philippines, Indonesia, India and Sri Lanka, and in the Pacific Islands of Papua New Guinea, but it is also an important crop in coastal areas in the Americas and in Africa (Persley, 1992).

Coconut suffers serious outbreaks of a destructive mite, *Aceria guerreronis* Keifer (Acari: Eriophyidae), in a large part of the production area. This mite has spread and established rapidly in the main coconut production areas worldwide and is now considered a key pest of this crop. Populations of the mite develop on the meristematic zone of the fruits, which is covered by the perianth. Feeding of the mites in this zone apparently causes physical damage so that as newly formed tissues expand, the surface becomes necrotic and suberized. Uneven growth results in distortion and stunting of the coconut, leading to reductions in copra yield. *Aceria guerreronis* infestations cause extensive premature dropping of coconuts (Moore & Howard, 1996). In addition to damaged fruits, *A. guerreronis* can kill coconut seedlings by feeding on growing tips (Aquino & Arruda, 1967). Reductions in copra yield have been variable from 15–40% (Hernández Roque, 1977; Julia & Mariau, 1979; Muthiah & Bhaskaran, 2000; Nair & Koshy, 2000; Seguni, 2002). Losses due to extensive premature dropping of fruits have been reported from 60% in Colombia (Zuluaga & Sánchez, 1971); 70% in Venezuela (Doreste, 1968), and 10–100% (average 21%) in Tanzania (Seguni, 2002).

Strategies for the control of coconut mite involving both biological control and quarantine require knowledge of the ancestral localities and plant hosts of the mite. However,

the origin of the coconut mite is unknown. The species was described by Keifer (1965) from specimens from Guerrero, Mexico. However, some collection records indicate that it was already present in other regions in South America. Almost simultaneously with its original description, the mite was reported in Africa and in 1966 in the Gulf of Guinea Islands, in 1967 Benin, and in the 1980s in Tanzania in the east of the continent. The most recent records of the coconut mite in new areas are from India and Sri Lanka, where the species was unknown until the end of the 1990s (table 1). Surprisingly, the mite has never been reported in the presumed region of origin of coconut, namely, the extreme South-East of Asia to Papua New Guinea (see above).

Aceria guerreronis is a serious threat in coconut growing regions and like many invasive agricultural pests displays dramatic population growth, leading to serious outbreaks resulting in high costs for control (Pimm, 1996; Pimentel, 2000). Acaricides must be applied frequently to control this mite. However, in most production areas, coconut is traditionally grown by small farmers who cannot afford continuous use of insecticides/acaricides (Moore & Howard, 1996; Muthiah & Bhaskaran, 2000; Ramaraju *et al.*, 2002). As an alternative, classical biological control has been considered as a promising strategy to check populations of *A. guerreronis* (Morales & Zacarias, 2002). Critical to the success of finding effective agents for biological control is determination of the historical range of the mite. The pattern of population spread is also economically important: the coconut mite still represents a menace to other countries in Asia, where the pest has not yet been detected and understanding its spread may help to determine its potential for future invasions as well as guide quarantine measures to intercept the pest dissemination.

One valuable approach to the study of sources and introduction routes of invasive arthropods involves the use of molecular markers (e.g. Villablanca *et al.*, 1998; Davies *et al.*, 1999; Bonizzoni *et al.*, 2001; Birungi & Munstermann, 2002; Mun *et al.*, 2003; Solignac *et al.*, 2005). Colonizing populations of invasive species are usually founded by only a few individuals (Elton, 1958), causing random genetic drift which itself often leads to founder effects (Lande & Barrowclough, 1987; Tsutsui *et al.*, 2000). The reduction of genetic variability is a common feature of invasive species and introductions in general (e.g. Lande & Barrowclough, 1987; Roderick & Navajas, 2003; Solignac *et al.*, 2005). In some cases, however, genetic variability of invasive populations may be higher than predicted by genetic drift, such as when the invasion phenomenon leads to the presence of different fixed haplotypes in diverse geographical regions (Gasparich *et al.*, 1997) or when multiple invasions stem from different regions with fixed haplotypes (Stepien *et al.*, 2002; Kolbe *et al.*, 2004).

In the present study, mitochondrial and nuclear sequence-level variation was used as well as previously published morphological data to infer the historical population structure of *A. guerreronis*. The study includes samples from the geographic regions where the mite is currently reported, including several of the main coconut production regions in the Americas, Africa, and the Indian Ocean region, as well as mites from the queen palm *Syagrus romanzoffiana* (Cham.) Glassm (Arecaceae). The results suggest that the mite originates from the Americas and not from the ancestral region of coconut in South East Asia. Thus colonization of coconut by this mite is a recent event,

Table 1. World records of *Aceria guerreronis* and presence of plant symptoms.

World region	Country	Year	Record	References
The Americas	Colombia	1948	Cn fr symp	Bain, 1948
		1971	Cn fr	Zuluaga & Sánchez, 1971
	Brazil	before 1965	Cn fr symp	Robbs & Peracchi, 1965
		1965	Cn fr	Robbs & Peracchi, 1965
		1967	Cn fr	Aquino & Arruda, 1967
		1989	Lw bd	Flechtmann, 1989
	Mexico	1965	Cn fr	Keifer, 1965
	Venezuela	1965	Cn fr symp	Doreste, 1968
		1967	Cn fr	Doreste, 1968
	Cuba	before 1975	Cn fr	Estrada & Gonzalez, 1975
	Puerto Rico	1977	Cn fr	Howard <i>et al.</i> , 1990
	Trinidad	after 1976	Cn fr	Griffith, 1984
	St Lucia	1980	Cn fr	Moore <i>et al.</i> , 1989
	Dominican Republic	1984	Cn fr	Moore, 1986
		1984	Cn fr	Howard <i>et al.</i> , 1990
	USA	1997	Sr bd	Ansaloni & Perring, 2002
		before 1985	Cn fr	Moore, 1986
Grenada & St Vincent	before 1985	Cn fr	Schliesske, 1988	
Africa	São Tomé & Príncipe	1966	Cn fr	Cabral & Carmona, 1969
	Benin	1967	Cn fr	Mariau, 1969
	Ivory Coast	1975	Cn fr	Julia & Mariau, 1979
	Togo	before 1977	Cn fr	Julia & Mariau, 1979
	Nigeria	before 1977	Cn fr	Julia & Mariau, 1979
	Cameroon	before 1977	Cn fr	Julia & Mariau, 1979
	The Gambia	before 1996	Cn fr	Moore & Howard, 1996
	Tanzania	1980	Cn fr	Seguni, 2002
Indo-ocean region	Sri Lanka	1997	Cn fr	Fernando <i>et al.</i> , 2002
		2003	Bf fr	G.J. de Moraes, personal observation
	India	1998	Cn fr	Sathiamma <i>et al.</i> , 1998
		2002	Bf fr	Ramaraju & Rabindra, 2002

Cn fr symp, symptoms on *Cocos nucifera* fruits; Cn fr, presence on *C. nucifera* fruits; Cn bd, presence on *C. nucifera* buds; Lw bd, presence on *Lytocaryum weddellianum* buds; Sr bd, presence on *Syagrus romanzoffiana* buds; Bf fr, presence on *Borassus flabellifer* fruits.

perhaps facilitated by modern transportation of coconut or propagation material of some other host palm.

Materials and methods

Biological material

Samples of *A. guerreronis* were collected on coconut, *C. nucifera*, in 28 localities in nine countries, spanning the worldwide distribution of the mite (table 2 and fig. 1). Samples were also taken from another host palm tree, *S. romanzoffiana*, from California, USA (sample USA1). In each locality, at least 100 individual mites were collected. From each locality, approximately half of the individuals were preserved in 70% ethanol to confirm species identification by morphological analysis and the remaining individuals were preserved in 100% ethanol for molecular analysis. Samples of *Aceria ficus* (Cotte) (Acari: Eriophyidae) from *Ficus carica* L. (Moraceae) collected in Montpellier, France, were sequenced for the ribosomal internal transcribed spacers (ITS) to be used as out-groups in the phylogenetic analysis; these sequences were not used again in this study because the nucleotide divergence was too high to ensure reliable alignments.

DNA analysis – extraction, amplification and sequencing

Genomic DNA was extracted using the DNeasy tissue Kit (Qiagen, USA), following the protocol for animal cultured

cells. Manufacturer's instructions were modified for DNA extraction of small mites, as described below. Preliminary extraction tests of single mites gave inappropriate DNA yields. The mite is extremely small (about 240 µm long) and the specimens analysed here arrived from several continents preserved in ethanol, which results in only very small amounts of DNA being available for extraction. Thus, all subsequent extractions were done with approximately 25 pooled adult mites (previous DNA-based work on eriophyiids used pooled mites for DNA extraction of ethanol preserved mites (Fenton *et al.*, 1996, 2000)). After removing all the ethanol, 90 µl of phosphate-buffered saline (PBS) buffer was added to the tube and the mites were crushed with a plastic pestle. In the next step, 10 µl of proteinase K 20 mg ml⁻¹ (40 mAU mg⁻¹ protein) was added followed by 100 µl buffer AL (provided in the Qiagen kit) and 5 µl of RNA as carrier. The following steps of the main Qiagen protocol were applied, except that all volumes were reduced by half. DNA was recovered adding 50 µl of ultrapure water on the membrane.

A 404bp fragment of the 16S and a 494bp of the mitochondrial COI and about 1000bp of the nuclear region spanning the ITS1-5.8S-ITS2 rDNA were polymerase chain reaction (PCR) amplified using primers indicated in table 3. The primers defined in the 18S and 28S regions corresponded, respectively, to nucleotides 1939–1962 and 3328–337 of the *Drosophila* sequence (Genbank accession number M21017). Polymerase chain reactions were carried out in 25 µl reaction volumes containing 2.5 µl of 10× buffer,

Table 2. Sampling localities of *Aceria guerreronis* collected on *Cocos nucifera* (29 samples) and *Syagrus romanzoffiana* (1 sample).

Continent	Country	Locality	DNA region			Code	nb. clones ITS	ITS haplotypes	GenBank accessions ITS	16S haplotypes	GenBank accessions 16S	
			ITS	16S	COI							
Africa	Benin	Ipinkle	x	x	x	Ben1	1	21	DQ060595	7	DQ063563	
		Ouidah	x	x	x	Ben2	2	22, 23	DQ060596–97	7	DQ063564	
	Tanzania	Micuranga	x	x	x	Tan	2	24, 25	DQ060598–99	7	DQ063562	
America	Brazil	Ilha Bela, São Paulo	x	x	x	Br1	3	18, 19, 20	DQ060592–94	1	DQ063548	
		Lagarto, Sergipe	x			Br2	2	26	DQ060600–01	nd	nd	
		Aracaju, Sergipe		x		Br3	nd	nd	nd	5	DQ063556	
		Quissamã, Rio de Janeiro	x	x		Br4	2	16, 17	DQ060590–91	4	DQ063553	
		Janaúba, Minas Gerais	x	x		Br5	3	14, 15	DQ060587–89	2	DQ063549	
		Recife, Pernambuco	x	x		Br6	2	12, 13	DQ060585–86	3	DQ063550	
		Petrolina, Pernambuco		x		Br7	nd	nd	nd	6	DQ063555	
		Maceió, Alagoas	x	x		Br8	2	10, 11	DQ060583–84	5	DQ063554	
		São Felix, Bahia	x	x	x	Br9	1	39	DQ060624	3	DQ063551	
		Ilhéus, Bahia		x	x	Br10	nd	nd	nd	3	DQ063552	
	Cuba	Habana		x		Cub	nd	nd	nd	4	DQ063559	
	USA	San Diego, California*		x	x		USA1	3	1, 2	DQ060578–80	4	DQ063557
			Fort Lauderdale, Florida	x	x		USA2	2	6, 7	DQ060573–74	4	DQ063558
Mexico	Guerrero	x	x	x	Mex	3	3, 4, 5	DQ060575–77	4	DQ063560		
Venezuela	Zulia	x	x		Ven	2	8, 9	DQ060581–82	4	DQ063561		
Asia	India	Kerala, Kayankulam	x	x	x	Ind1	3	28, 37	DQ060619–21	7	DQ063575	
		Kerala, Kasaragod	x	x	x	Ind2	2	38	DQ060622–23	7	DQ063565	
	Sri Lanka	Kalpitiya	x	x	x	SrL1	3	28, 34, 35	DQ060614–16	7	DQ063569	
		Madurankuliya		x	x	SrL2	nd	nd	nd	7	DQ063570	
		Wanathawilluwa	x	x	x	SrL3	3	31, 32, 33	DQ060611–13	7	DQ063568	
		Yakwila	x	x	x	SrL4	3	28, 29	DQ060605–07	7	DQ063573	
		Haldanduwana	x	x		SrL5	3	27	DQ060602–04	7	DQ063571	
		Unagala Vehera	x	x	x	SrL6	2	36	DQ060617–18	7	DQ063567	
		Madampe	x	x	x	SrL7	3	28, 30	DQ060608–10	7	DQ063566	
Laksha Uyana		x	x	SrL8	nd	nd	nd	7	DQ063572			
Hingurakgod		x		SrL9	nd	nd	nd	7	DQ063574			

The sequenced regions, the number of clones sequenced per ITS region amplified by PCR (nb. clones), and the observed ITS2 and mt 16S haplotypes found are indicated for each sample. nd, no data.

*Sample collected on *Syagrus romanzoffiana* buds.

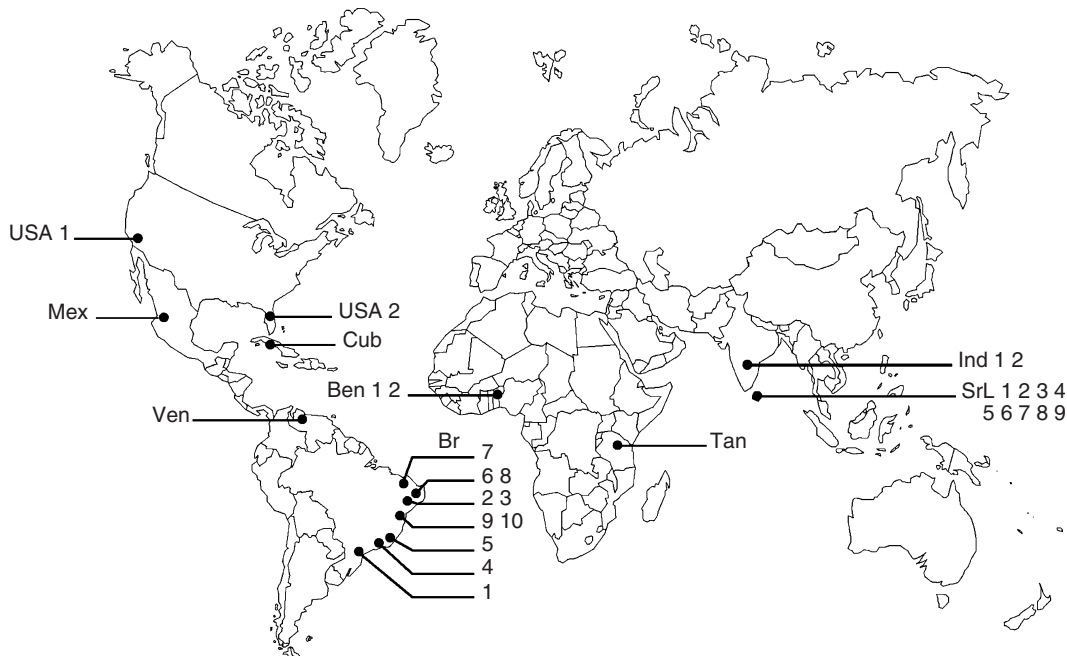


Fig. 1. Collection sites of *Aceria guerreronis*. See table 2 for locality descriptions.

Table 3. DNA amplified fragments, polymerase chain reaction (PCR) and sequencing primers and annealing temperatures used to study variation in *Aceria guerreronis* samples.

Fragment	Length (bp)	Location	Reference	PCR primers	Annealing temperature (°C)
rDNA ITS	987	3' of 18S	Ben Ali <i>et al.</i> , 2000	5' AGA GGA AGT AAA AGT CGT AAC AAG 3'	50
		5' of 28S	Ben Ali <i>et al.</i> , 2000	5' ATA TGC TTA AAT TCA GGG GG 3'	
		5.8S-InternS**	This study	5' GAT CAC TCG AAT TAC CAA TCG 3'	
		5.8S-InternSRev**	This study	5' CGA TTG GTA ATT CGA GTG ATC 3'	
mtDNA 16S	404	LR-J 12887	Simon <i>et al.</i> , 1994	5' CCG GTC TGA ACT CAG ATC ACG T 3'	53
		LR-N 13398		5' CGC CTG TTT AAC AAA AAC AT 3'	
mtDNA COI	494	C1-J 1751	Simon <i>et al.</i> , 1994	5' GGA TCA CCT GAT ATA GCA TTC CC 3'	42 and 60*
		C1-N 2191		5' CCC GGT AAA ATT AAA ATA TAA ACT TC 3'	

*Two successive PCR were performed to amplify this fragment. Annealing temperatures correspond respectively to the first and second PCR reactions (see text for more details).

**Internal primers used for sequencing.

Table 4. Molecular diversity indices estimated from nuclear ITS and mitochondrial 16S sequences obtained for samples of *Aceria guerreronis* collected from three continents.

Geographical origin	No. haplotypes (N)	No. alleles (A)	No. segregating (S)	% Nucleotide diversity (π)	Gene diversity	Main pairwise differences
ITS						
America (except Brazil)	10	9	24	0.54	0.98 ± 0.05	5.11 ± 2.71
Brazil	15	13	63	1.90	0.99 ± 0.03	18.05 ± 8.49
Africa	5	5	16	0.70	1.00 ± 0.13	6.60 ± 3.75
India & Sri Lanka	22	12	31	0.49	0.99 ± 0.02	4.59 ± 2.34
16S						
America	14	6	22	2.22	0.85 ± 0.07	8.93 ± 4.38
Africa, India & Sri Lanka	14	1	0	0	0	0

1 U Taq polymerase (Qiagen), 0.25 mM of each dNTP, 0.5 μ M of each oligonucleotide primer, 5 μ l of Q solution (Qiagen), 2.5 mM of MgCl₂ and 2 μ l of template DNA. Samples were denatured at 94°C for 4 min and then PCR was carried out for 35 cycles of 30 s denaturation at 94°C, 30 s annealing (see table 3 for annealing temperatures) and 1 min extension at 72°C. In the case of the ITS, 8 separate PCR reactions per DNA template were performed and the obtained products pooled and purified by cutting the target band from agarose gels. DNA was then purified using the QIAquick extraction kit (Qiagen). PCR reactions were pooled to obtain higher DNA concentration of the target band before DNA purification. The recovered fragments were ligated into the pGEM-T Easy vector system (Promega, USA). The ligated fragments were transfected into XL1-Blue *Escherichia coli* strain. Two to three positive clones per successful transformation were sequenced. In addition to the PCR primers defined in the 18S and the 28S, two internal ones were used to sequence the complete ITS region: InternS 5' GAT CAC TCG AAT TAC CAA TCG 3' and InternSRev 5' CGA TTG GTA ATT CGA GTG ATC 3'.

The mitochondrial 16S fragment was amplified in seven independent PCR reactions for each DNA template, subsequently pooled, the products purified by cutting the targeted band from agarose gels and the appropriate band recovered. DNA was purified as for the ITS fragments and directly sequenced. For the amplification of the cytochrome oxidase I (COI) fragment, two subsequent PCR reactions were performed. PCR products obtained in the first reaction were separated on a 1.5% agarose gel and the appropriate band

cut and the amplified DNA eluted from the gel by leaving it in a microcentrifuge tube covered with ultrapure water (about 50 μ l) for 2–3 days (Salomone *et al.*, 2002). Two μ l of the recovered DNA was then used as template for a second PCR. In the second PCR, the annealing temperature was 60°C. The product was directly sequenced. In all cases, DNA was sequenced using the BigDye Terminator method (Perkin Elmer, USA) in an ABI PRISM 377 automated DNA sequencer (Applied Biosystems Inc., USA). Both PCR primers were used to fully sequence the ITS and COI regions.

Each distinct ITS and 16S sequence obtained has been deposited in the Genbank database under accession numbers indicated in table 2.

Data analysis

Sequences were aligned using CLUSTAL W (Thompson *et al.*, 1994). For ITS, genetic variation was assessed within four major geographical areas: Brazil, America (excluding Brazil), Africa and India plus Sri Lanka. Because a single mitochondrial haplotype was detected in all the non-American samples, nucleotide variation for 16S was examined within only two groups, American and non-American samples (table 4). Standard sequence diversity indices (see Nei, 1987) were computed, including: A (number of alleles = variable haplotypes), S (number of segregating sites = variable nucleotide positions), gene diversity, and π (nucleotide diversity), using the ARLEQUIN 2.0 software: <http://anthro.unige.ch/arlequin> (Schneider *et al.*, 2000).

Intraspecific gene genealogies were inferred using the median-joining network approach (Bandelt *et al.*, 1999) implemented in NETWORK, version 2.0, software, available at <http://www.fluxus.engineering.com/sharenet.htm>. In this approach all the minimum spanning trees (MSTs) are first combined within a single network (MSN), then, using the parsimony criterion, inferred intermediate haplotypes are added to the network in order to reduce overall tree length. The method tries to resolve likely parallel events, but also retains character conflicts (in the form of reticulations) when ambiguity remains. Major genetic clusters, as well as possible areas of ambiguity, can therefore be identified.

The historical demography of the species was examined using mismatch distributions, which represent the frequency distribution of pairwise differences among all haplotypes in a sample (Rogers & Harpending, 1992). Mismatch distributions were established for samples from the Americas and their fit of each sample to a Poisson distribution was assessed by Monte Carlo simulations of 1000 random samples using ARLEQUIN 2.0. Tajima's *D* statistics were also computed with ARLEQUIN.

Results

Sequence data

From the 29 *A. guerreronis* samples examined, a total of 96 sequences were obtained for three genomic regions: 16 for the COI, 28 for the 16S and 52 for the ITS (table 2). Not every DNA template was sequenced for the three regions because of problems in obtaining PCR amplification, probably resulting from the degraded state of some specimens. The alignment of sequences contained 987 positions for the nuclear ITS region, including 111 variable sites of which 52 were parsimony informative. Twelve single-base gaps scattered along the sequence were introduced to improve alignments. The mitochondrial fragments included 404 aligned positions for the 16S (24 variable sites of which 22 were parsimony informative) and 494 for the COI (203 variable sites of which 41 were parsimony informative) fragments. The different clones sequenced from each amplified ITS region produced one to three different sequences, depending on the sample. The ITS boundaries were determined using the flanking conserved sequences of the 18S, 5.8S and 28S rRNA genes of arthropods deposited in GenBank database, mainly acarine species of the Eriophyidae (Fenton *et al.*, 2000), Ixodidae (Wesson & Collins, 1992) and Phytoseiidae (Navajas *et al.*, 1999) families.

Genetic variation

The observed *A. guerreronis* haplotypes are presented in table 2 for ITS and 16S. Diversity indices are summarized in table 4. The number of observed haplotypes was not uniformly distributed over the geographical range sampled. Of the seven different mitochondrial 16S haplotypes, six were present in the Americas (16S haplotypes 1 to 6 in table 2). All the other 14 samples shared the same single haplotype (haplotype 7). The highest diversity in 16S was found in Brazil, where the six American haplotypes were represented. The haplotypic diversity in the Americas represents a nucleotide diversity of 2.22% with a mean number of differences between haplotypes of 8.93. For mitochondrial COI, 16 sequences were obtained (four from the Americas, three

from Africa, two from India and seven from Sri Lanka). Because the COI data set was reduced to calculate diversity indices, analyses were conducted only for gene genealogies inference (see below).

Thirty-nine different ITS sequences were found in the 52 sequences obtained in this study. Among the 22 American ITS sequences, 13 were found in Brazil (ITS haplotypes 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 26 and 39: table 2), and nine more in the other American countries studied (ITS haplotypes 1 to 9). The remaining sequences belonged to samples collected in Africa (five sequences belonging to ITS haplotypes 1 and 2) and India and Sri Lanka (12 sequences, haplotypes 27 to 37). As found for 16S, the highest diversity of ITS was detected in Brazil, with 1.90% nucleotide diversity compared to 0.54% in the other American samples, 0.70% in Africa and 0.49% in the Indo-ocean region (table 4). In most cases, two or three of the different clones studied per sample had either identical or very similar sequences. For seven origins (Br1, Br6, USA1, USA2, SrL5, SrL7 and Ind1) differences between the several clones scored for a given sample were greater, but the different clones of a given sample always belonged to the same main group of samples, either America, or Africa plus Asia (see the network analysis below).

In the case of mitochondrial data where PCR products were directly sequenced, the results accounted for the variation between the consensus sequence obtained from pooled individuals for each population. Rare variants were not detected. In the case of nuclear ITS, for which several clones of PCR products were sequenced, the results accounted for sequence variation within mites of each locality. However, this variation may have resulted from both interindividual and intraindividual polymorphism. The network analysis depicts this phylogeographic structure among the mtDNA haplotypes. In fig. 2, the relationships

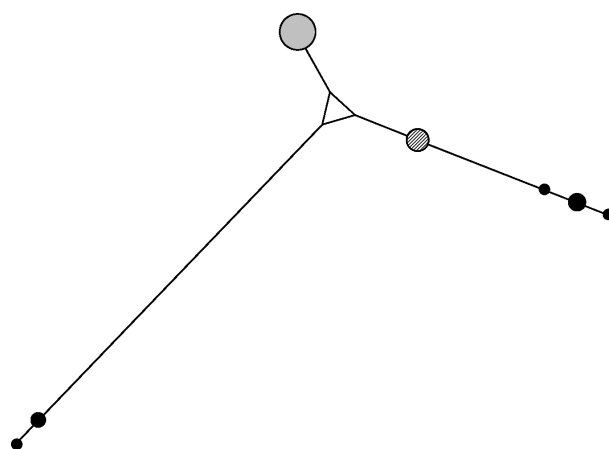


Fig. 2. Median-joining network depicting the phylogenetic relationships among all mtDNA haplotypes (circles) based on 16S sequences obtained for *Aceria guerreronis*. The size of each circle is proportional to the corresponding haplotype frequency. Branches between two haplotypes are proportional to the amount of mutational steps. The Brazilian samples are indicated by black circles ● and the other American samples by a striped circle ◐. A single haplotype found in all the non-American samples (Africa, India and Sri-Lanka) is indicated by a grey circle ○.

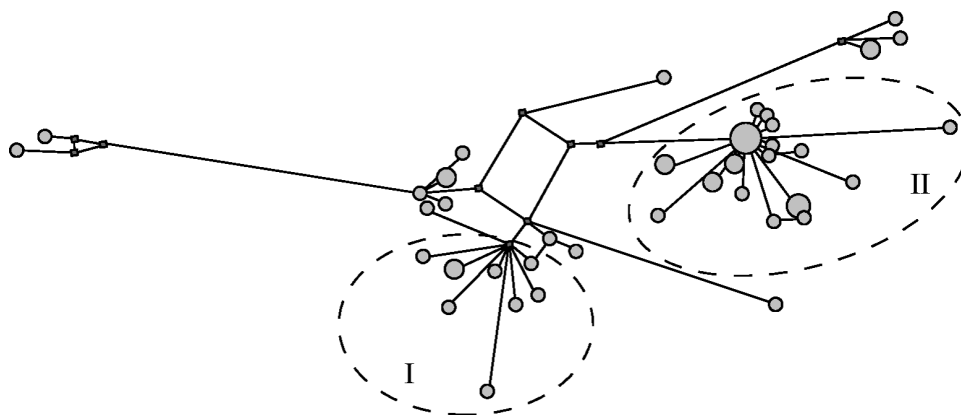


Fig. 3. Median-joining network depicting the phylogenetic relationships among all ITS sequences haplotypes (filled circles) obtained for *Aceria guerreronis*. The size of each circle is proportional to the corresponding haplotype frequency. Branches between two haplotypes are proportional to the amount of mutational steps. The Brazilian samples are dispersed in the different branches, whereas the other American samples are gathered in a cluster (I) and another cluster (II) groups all the non-American samples from Africa, India and Sri Lanka.

among all the coconut mite haplotypes based on 16S sequences include three branches, one of which is specific to non-American samples. These sequences from India, Sri Lanka and Africa were not polymorphic and are separated by at least five mutations from all the other sequences. Among the American samples, the higher diversity of the Brazilian samples is represented in fig. 2, with two branches including sequences from Brazil and only one containing those of the rest of the American samples.

Although not all samples could be sequenced for both mitochondrial and nuclear ribosomal regions, the general topology of network based on ITS was congruent with that based on 16S sequences. In fig. 3, two groups of sequences can be recognized which are identified by their star-like shape, and are associated with: (i) the American samples (USA, Venezuela, Mexico and Cuba: cluster I) and (ii) all the non-American samples from Africa and the Indo-ocean region (cluster II). As for the 16S, the Brazilian samples were very diverse with samples scattered in the different branches of the network (fig. 3). Also congruent with the ITS and 16S trees, the COI sequences (not shown) unambiguously grouped separately the African and Indo-ocean samples on one side and the American samples (three Brazilian and one Mexican) on the other.

The evolutionary relationships between groups I, II and the Brazilian samples cannot be identified. The robustness of these three groups of ITS sequences, however, was confirmed by different tree or network reconstruction algorithms (maximum likelihood, maximum parsimony, neighbour-joining and the reduced median network). These results are not reported, since all the trees they generate are included in the networks presented in figs 2 and 3.

Historical demography

Theoretical studies have demonstrated that historical demography, and in particular the pattern of population growth, has an effect on the pattern of genetic polymorphism seen within populations. In the case of populations subject to recent demographic expansions, likely subsequent to a bottleneck, mismatch distributions emerge as unimodal,

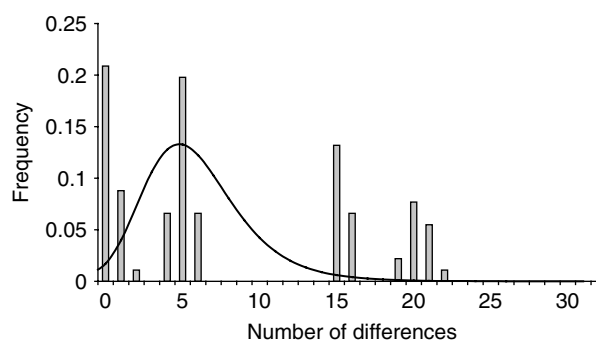


Fig. 4. Mismatch distributions established for mitochondrial 16S sequences of *Aceria guerreronis* samples. The bar represents the observed frequency of the pairwise differences among haplotypes, while the line shows the expected curve predicted for populations that have undergone a demographic expansion in the past. Corresponding results and significance are reported in table 6.

approximately fitting a Poisson (Rogers & Harpending, 1992), whereas distribution appears uneven in populations undergoing stable demographic equilibrium (Harpending, 1994). The observed mismatch distributions established here based on 16S sequences of *A. guerreronis* samples are presented in fig. 4 and table 5. The distribution does not fit well the expected Poisson model predicted for populations that have undergone a demographic expansion in the past ($\tau=20.7$, 95% confidence interval=11.2–33.1). Also, the parameters of a sudden expansion model do not indicate that an expansion occurred in *A. guerreronis* in the past. Tajima's *D* statistics was positive, though not significant. One concern about using mismatch distribution analysis for a group of unconnected populations, as found in this study, is that the assumption of random mating is likely violated. However, Rogers (1995) showed that the theory behind this analysis is robust and should approximately hold true even when populations are completely isolated.

Table 5. Mismatch distribution results based on mitochondrial 16S sequences from pooled samples of *Aceria guerreronis* collected in The Americas.

Parameters	American samples
S	22
τ	20.7 (11.2 \pm 33.1)
θ_0	0.02 (0 \pm 12.9)
θ_1	8.78 (1.95 \pm 1689)
Goodness of fit test SSD	0.069
<i>P</i>	0.289
Raggedness index	0.145
Tajima's D	0.745
<i>P</i>	0.834

The parameters of the model of sudden expansion (Rogers & Harpending, 1992) are presented as well as goodness of fit test to the model. SSD = sum of squared deviations. Tajima's (1989) *D*-test value and statistical significance are also given. S, number of polymorphic sites; θ_0 , pre-expansion; θ_1 , post-expansion; and τ , time in number of generations elapsed since the sudden expansion episode.

Discussion

Nuclear ITS and mitochondrial 16S sequences revealed a substantial genetic diversity of *A. guerreronis* collected from the three different continents. The amount of variation was not homogeneously distributed across the species range: all variability estimates showed that the Brazilian samples were strikingly more polymorphic than samples from the rest of America together, or from Africa, India and Sri Lanka.

When the results based on 16S and ITS sequences were compared with morphological data obtained from mites sampled in the same localities and often on the same palm tree as those analysed in the present study (Navia, 2004; Navajas & Navia, 2005), the same main insights emerged. The highest morphological variability was detected among American samples, mainly in those from Brazil. In addition, African, Indian and Sri Lankan samples were superimposed in a canonical analysis, indicating that not only were they morphologically similar, but they were also distinct from the American samples. In the case of inconspicuous organisms whose taxonomy relies on a few morphological characters, such as eriophyid mites, the use of molecular tools to estimate genetic variation might detect the presence of different genotypes which cluster in reciprocally monophyletic groups. This has been taken as evidence of the presence of cryptic species (Caterino *et al.*, 2000). Although the hypothesis of a complex of several cryptic species of *A. guerreronis*, at least based on mitochondrial sequences, cannot be ruled out, biological characters (host plant, behaviour and plant damage) do not vary for the different samples studied (including USA1 mites collected on *S. romanzoffiana*, which cluster with other samples collected on *C. nucifera*) and tend to corroborate the presence of a single species. Additional study of the widely accepted biological species concept, which defines a species as the group of individuals which form viable and fertile hybrids, will be useful to clarify this issue. The techniques for laboratory culturing and rearing of *A. guerreronis* have only recently been developed (Haq, 2001; Ansaloni & Perring, 2002), which should facilitate crossing experiments.

Molecular and morphological results together with the historical records of the presence of the mite in its

distribution range help to trace the recent expansion history of this pest. Reports of the presence of the mite have always been linked to the observation of damage on coconut. Despite the practically simultaneous report of the mite in the Americas and Africa (Moore & Howard, 1996), both ITS and 16S sequences suggest an American origin of the species. The most plausible origin of the pest is South America, where molecular diversity is highest. This contrasts with the great homogeneity of samples outside America. In this case, it appears that a reduction in genetic diversity has not been detrimental to the invasive ability of the mite. That genetic variation is not limiting despite clear genetic bottlenecks is not uncommon for invasive species (see for example, the invasive Argentine ant, *Linepithema humile* (Mayr) (Hymenoptera: Formicidae) (Tsutsui *et al.*, 2000). An extreme case of reduction of variability has been recently reported for a bee parasite, the mite *Varroa destructor* (Anderson & Trueman) (Acari: Varroidae)), whose host shift from the Asian bee to the Occidental bee has led to a dramatic reduction of both nuclear and mitochondrial variability but has not prevented worldwide invasion of the new host in less than 50 years (Solignac *et al.*, 2005).

With the hypothesis of an American origin of the mite with recent expansion to the rest of the world, it remains unclear why none of the American haplotypes identified in this study was present in Africa, India or Sri Lanka. One explanation could be that because genetic variation of the mite in America is high, the entire diversity of the species on this continent was only partially sampled. The high homogeneity of both mitochondrial and nuclear sequences between individuals originating in the Indo-ocean region and Africa suggests in turn a common origin of the mites infesting these regions. The appearance of the mite within a few months in different localities of India and Sri Lanka, together with the paucity of sequence variability, suggests the introduction of the pest from a very limited number of colonizers that rapidly spread in the Indo-ocean region. While samples collected from only three different African localities were examined, they most probably represent the variability in this region given the large geographic coverage (Benin and Tanzania) spanning different parts of the discontinuous range of the species on this continent (coconut is cultivated in East and West African coastal areas only). Although the colonization routes of the introduction of *A. guerreronis* to Africa and Asia remain unclear, the records of the damage to coconut tend to suggest a longer presence of the mite in Africa than in Asia. Additional surveys of the presence of mites from the two regions and the analysis of their genetic variation will be necessary to clarify this issue.

The coconut mite is now found in all the major coconut growing regions except in the area of origin of the coconut, making it unlikely that the mite's original host is coconut. An alternative hypothesis to an American origin of *A. guerreronis* found in the Indo-ocean region is that the mite was already present in the Indo-ocean area prior to its recent discovery as a pest. The mite could have been associated with a native palm tree or even the coconut, where it lived at low density. The mite is inconspicuous and it lives in hidden places on the host plants (e.g. on buds, under bracts), so that it is virtually undetectable without apparent symptoms. There are records of the presence of coconut from 2000–3000 years ago on the Sri Lanka coast and in southern India but only in the past 500 years in West Africa and the Americas

(Purseglove, 1975; Harries, 1978). Anthropogenic climatic changes or modification in cropping habits could have been favourable to coconut mite outbreaks, allowing it to reach pest status. Introduced species may stay at a fairly low population size for years and then explode at some later date (Mooney & Cleland, 2001), which can be related to environmental changes after establishment (Crooks & Soule, 1999). Intensive surveys of mites associated with coconut conducted in the Philippines (Briones & Sill, 1963), Malaysia (Kang, 1981) and India (Sathiamma, 1981; Mohanasundaram, 1984), have reported eriophyid mites associated with coconut, but *A. guerreronis* was never found. While an old and undetected presence of *A. guerreronis* in Asia cannot be formally ruled out, the absence (for 16S) or very low (for ITS) genetic variability found among samples from Sri Lanka and India, indicates that the species has not been present in the area for very long and that the invasion results from the arrival of a few colonizers.

An additional issue to resolve the hypothesis of an American origin of *A. guerreronis* is the original host plant of the mite. Eriophyid mites are typically extremely specialized on their host plant (Lindquist *et al.*, 1996) and it is commonly assumed that the mite and its host have co-evolved. In the case of *A. guerreronis*, it has, however, been suggested that the mite recently adopted coconut as a new host (Moore & Howard, 1996; Moraes & Zacarias, 2002). It is conceivable that *A. guerreronis* was present on an unknown palm and switched to coconut when this crop cultivation became more extensive in the Americas or Africa (Moore & Howard, 1996). Several facts support the hypothesis that the original host of *A. guerreronis* is not coconut: (i) the mite has never been reported from the southern tip of South-East Asia to the Pacific Islands, the recognized region of origin of coconut (Persley, 1992; Lebrun *et al.*, 1998); (ii) the mite seriously damages coconut, can cause extensive premature dropping and can kill coconut seedlings (Aquino & Arruda, 1967; Doreste, 1968; Moore & Howard, 1996); and (iii) the mite seems to adapt rapidly to new host plants (Oldfield, 1996). Many eriophyid mites co-exist with their host plant in such a way that they do not cause serious damage, perhaps as a result of co-evolution. By contrast, mite infestations that seriously harm a host plant tend to indicate a recent plant association. For example, the tomato mite *Aculops lycopersici* (Masse) (Acari: Eriophyidae), which feeds on several Solanaceae, usually lives at very low densities, with the exception of tomato on which this eriophyid mite causes serious harm. It has been suggested that the original host plant of *A. lycopersici* was a wild species of Solanaceae and its association with tomato is recent (Oldfield, 1996). The severe damage caused by *A. guerreronis* to coconut (Aquino & Arruda, 1967; Moore & Howard, 1996) is consistent with a relatively recent association between coconut and the pest.

Aceria guerreronis has been reported on three other non-coconut palm hosts. Two of them are palms of South American origin used as ornamentals: *Lytocaryum weddellianum* (H. Wendl.) (Arecaceae) in Brazil (Flechtmann, 1989), and on *S. romanzoffiana*, in southern California, USA (Ansaloni & Perring, 2002). In both cases *A. guerreronis* was found only in nurseries, and never in natural areas (Flechtmann, 1998; Santana & Flechtmann, 1998; Gondim Jr. *et al.*, 2000; Navia & Flechtmann, 2002). In the Indo-ocean region, *A. guerreronis* was reported on *Borassus flabellifer* L. (Arecaceae), in India and Sri Lanka, in the area of natural

occurrence of this palm (Ramaraju & Rabindra, 2002; G.J. de Moraes, personal observation). In the case of queen palm in California, the origin of *A. guerreronis* infestation remains unclear. However, the data presented here, including mitochondrial and nuclear sequences, all suggest that this sample is closely related to several other American samples collected on coconut. Thus, *A. guerreronis* mites infesting queen palm do not represent a host-associated biotype different from coconut mite.

Because of the extreme genetic variation of the coconut mite in southern America compared to other regions, it seems likely that the mite switched from another host species to the coconut in southern America, not in Africa. Because this transfer apparently occurred extremely recently, (i.e. in historical times) the high degree of polymorphism seen in the Brazilian populations could not have arisen on coconut, thus suggesting multiple transfer to the coconut, instead of a single accidental transfer. On the other hand, the absence of polymorphism between African and Indo-Pacific samples is compatible with a unique infestation of these regions, presumably from America.

Unexplained by the data presented here is why the mite has become invasive only in the last 50 years. Curiously, tests based on mismatch distribution of haplotypes divergences do not suggest a rapid range expansion of the species. One alternative hypothesis is an explosive growth of the mite on a palm different from coconut and our sampling from *C. nucifera* would represent only part of the total variability of *A. guerreronis* populations that are in fact on other hosts. For example, samples of *Caryedon serratus* Olivier (Coleoptera: Bruchidae), a major pest on groundnut *Arachis hypogaea* L. (Fabaceae) collected from Senegal, showed that this plant only accounted for some of the genetic variability of the species, and that other genotypes occurred on several other native hosts found in the region (Sembene, 1998).

The spread of the mite in the Americas and from there to other continents is not unreasonable. Winds have been considered as the main agent of mite spreading in relatively short distances (Griffith, 1984; Moore & Howard, 1996; Haq *et al.*, 2002). Mite colonies can be transported together with coconuts, which have been disseminated and exchanged in ancient times by ocean currents, including transoceanic migration from continent to continent or from mainland to islands or vice versa (Haq *et al.*, 2002) or by long human sea voyages and today through international trade, tourism and germplasm exchange (Baudoin *et al.*, 1998).

The suggestion that the coconut mite is not native in localities where coconut is native has profound implications for strategies to control this mite. It is expected that effective natural enemies of a pest will be found in the place of origin of the pest, where the pest and its enemies have been in contact for the longest time (Van Driesche & Bellows, 1996; Roderick & Navajas, 2003). A recent project to illustrate this point is the classical biological control of an invasive phytophagous mite, the cassava green mite *Mononychellus tanajoa* (Bondar) (Acari: Tetranychidae) in Africa, with the introduction of phytoseiid mites from South America as predators (Bellotti *et al.*, 1999). In this case, South America is the original region of both the pest and predator. When prospecting for control agents of this coconut mite, America, and especially South America, should be defined as priority areas, and not areas originally home to coconut.

The coconut mite is still a threat to the other Asian and Pacific countries where it has not yet been reported. Because it is widely accepted that the present main pathways of introduction of the coconut mite to distant areas involve transport or trade of propagation host plant material, movement of any propagation tissue palm trees, especially from America, represents a quarantine risk.

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