Zoologica Scripta

Cryptic diversity in *Brevipalpus* mites (Tenuipalpidae)

Denise Navia, Renata S. Mendonça, Francisco Ferragut, Letícia C. Miranda, Roberto C. Trincado, Johan Michaux & Maria Navajas

Submitted: 25 December 2012
Accepted: 25 March 2013
doi:10.1111/zsc.12013

Navia, D., Mendonça, R. S., Ferragut, F., Miranda, L. C., Trincado, R. C., Michaux, J., Navajas, M. (2013). Cryptic diversity in *Brevipalpus* mites (Tenuipalpidae). —*Zoologica Scripta*, *00*, 000–000.

Defining the taxonomic identity of organisms is a prerequisite for their study, and in the case of economically important species, misidentification may lead to the application of inappropriate prevention and control strategies. Flat mites of the *Brevipalpus* genus include several crop pests and the systematics of these mites represents a challenge for acarologists. Many of the most economically important *Brevipalpus* species have repeatedly been inaccurately identified. Such problematic classification has been attributed to the likely occurrence of cryptic species in the genus. In this study, we used an integrative approach that combined molecular analyses, including sequence-based species delimitation, with detailed morphological identification using traits that have recently showed to be taxonomically informative. Sequences of mitochondrial cytochrome c oxidase subunit I (COI) were obtained from individuals collected from host plants belonging to 14 genera and 13 families across 29 locations in the Americas (Brazil, Chile, USA). The phylogenetic analyses included previously published *Brevipalpus* sequences from GenBank, and the final data set was classified into 44 haplotypes. Six putative species were recognised by COI-based species delimitation analysis, and morphological evidence supported each of these species. The integrative approach revealed the occurrence of cryptic species in the *Brevipalpus* genus and contributed to the clarification of previously noted incongruences. The results presented here allow for the evaluation of taxonomic characteristics in a phylogenetic context and indicate new characters for the differentiation of *Brevipalpus* species. In addition, *Brevipalpus incognitus* n. sp. Ferragut & Navia, a cryptic species detected in this study, is described based on morphological and molecular traits. Implications of the advances in *Brevipalpus* systematics presented herein with respect to pest management are briefly discussed.

Corresponding author: *Denise Navia, Embrapa Genetic Resources and Biotechnology Cx. Postal 02372, 70.770-917 Brasilia, Brazil. E-mail: denise.navia@embrapa.br*
Renata S. Mendonça, CNPq- Embrapa Genetic Resources and Biotechnology/University of Brasília, Cx. Postal 02372, 70.770-900, Brasilia, Brazil. E-mail: mendonca.rsm@gmail.com
Francisco Ferragut, Instituto Agroforestal Mediterráneo, Universidad Politécnica de Valencia, Camino de Vera, s/n, 46022, Valencia, Spain. E-mail: fjferrag@eaf.upv.es
Letícia C. Miranda, Embrapa Genetic Resources and Biotechnology/University of Brasília, Cx. Postal 02372, 70.770-917, Brasilia, Brazil. E-mail: leticiacalvoso@gmail.com
Roberto C. Trincado, Servicio Agrícola y Ganadero, Cx. Postal 71, Curacaví, Santiago de Chile, Chile. E-mail: rtrincado@artropodos.cl
Johan Michaux, INRA, UMR CBGP (INRA/IRD/Cirad/Montpellier SupAgro), Campus International de Baillarguet, CS 30016, 34988, Montferrier-sur-Lez Cedex, France. E-mail: michaux@supagro.inra.fr
Maria Navajas, INRA, UMR CBGP (INRA/IRD/Cirad/Montpellier SupAgro), Campus International de Baillarguet, CS 30016, 34988, Montferrier-sur-Lez Cedex, France. E-mail: navajas@supagro.inra.fr

Introduction

The erroneous identification of crop pests may lead to the application of inappropriate prevention and control strategies (Paterson 1991; Clarke & Walter 1995; Armstrong & Ball 2005; Bickford *et al.* 2007). Integrative taxonomy seeks concordant changes in more than one feature of an organism

together with corroboration from independent data (e.g., molecules, morphology) as reliable evidence for separating species (Bickford *et al.* 2007). Employing a multisource approach that takes advantage of complementary disciplines is necessary to build consistent alpha-taxonomies (Schlick-Steiner *et al.* 2010).

A difficulty often encountered in many groups is that speciation is not accompanied by morphological differentiation between taxa (Bickford *et al.* 2007) even though the entities might be genetically isolated. As a consequence, reliable species within such species complexes are indistinguishable on the basis of morphological criteria alone. These cryptic species might differ in physiological, behavioural and ecological traits (Pfenninger & Schwenk 2007; Calcagno *et al.* 2010; Henry & Wells 2010). With the application of molecular techniques during the last two decades, cryptic diversity has been detected in almost all taxonomic groups (e.g. Hebert *et al.* 2004; Bickford *et al.* 2007), and these species are distributed among all major metazoan taxa and biogeographical regions (Pfenninger & Schwenk 2007). Subsequent to the detection of cryptic species using DNA data, their separate status is usually confirmed with morphological or ecological data (Bickford *et al.* 2007).

Species delimitation, the process by which species boundaries are determined, has emerged as a major topic in modern systematics (e.g. Sites & Marshall 2003), and several methods have been developed and compared (Wiens 2007). Among them, the DNA-based species delimitation approach proposed by Pons *et al.* (2006) has proven to be useful for identifying meaningful entities among microorganisms (e.g. Jousselin *et al.* 2009), vertebrates (e.g. Pagès *et al.* 2010) and invertebrates (e.g. Ahrens *et al.* 2007; Fontaneto *et al.* 2008), including mites (Tixier *et al.* 2011), whose current taxonomy is incomplete or uncertain.

Plant mites (Prostigmata) can have a great economic impact on agriculture and forestry (Hoy 2011). The family Tenuipalpidae, commonly known as flat mites or false spider mites, includes several crop pests that damage plants by feeding directly on the epidermal cells of the stems, leaves and fruits, or by vectoring plant viruses. Most tenuipalpid mites that cause economic damage to cultivated plants belong to the genus *Brevipalpus* Donnadieu, which comprises approximately 280 valid species (Mesa *et al.* 2009). Three species are regarded as having significant economic importance: *Brevipalpus phoenicis* (Geijskes), *B. obovatus* Donnadieu and *B. californicus* (Banks) (Childers *et al.* 2003a). These species are highly polyphagous and infest more than 900 different plant species throughout the world (Childers *et al.* 2003b). Each of these three species has been implicated as a vector of at least one plant virus (Childers & Derrick 2003). *Citrus leprosis virus* (CiLV), *Passion fruit green spot virus* and *Coffee ringspot virus* (CoRSV) are *Brevipalpus*-transmitted viruses (BTVs) that affect food crops (Chagas *et al.* 2003; Kitajima *et al.* 2003; Rodrigues *et al.* 2003). An increasing number of BTVs are in the process of being identified, and almost 40 plant species have been reported as being naturally infected by BTVs (Kitajima *et al.* 2010). In addition, two related species, *B. chilensis* Baker and *B. lewisi* McGregor, although not reported as plant virus vectors, can cause direct damage to their host plant and reach pest status, particularly on fruit crops (Childers *et al.* 2003a); these *Brevipalpus* species are quarantine pests, regulated in the international exchange or trade of fresh fruits and propagation material of their host plants (Navia *et al.* 2006).

Unambiguous identification of *Brevipalpus* species is needed to understand the role of each species in the transmission of plant viruses, to guide the adoption of quarantine measures and to support the development of control strategies. Due to their morphological similarity, the *Brevipalpus* species of greatest economic importance have been consistently confused and misidentified for more than 50 years (Welbourn *et al.* 2003). For many years, authors have noted intraspecific variations in *B. phoenicis*, *B. californicus* and *B. obovatus*, resulting in numerous synonymous species (Mesa *et al.* 2009). These variations have raised concerns about the presence of cryptic species (Baker & Tuttle 1987; Welbourn *et al.* 2003; Rodrigues *et al.* 2004). Through a detailed morphological study, Beard *et al.* (2012) differentiated two morphological types of *B. phoenicis* and three morphological types of *B. californicus* and considered that a finer taxonomic investigation of these *Brevipalpus* species was required.

Mitochondrial cytochrome oxidase subunit I mutates at a faster rate than most nuclear DNA (Lynch *et al.* 2006), making it an appropriate marker for phylogenetic exploration at low taxonomic levels (e.g. between closely related species or infraspecific categories) (Hebert *et al.* 2003). Sequences of the mitochondrial cytochrome c oxidase subunit I (COI) gene have been extremely useful as molecular markers in systematic studies of different groups of organisms, including phytophagous mites at both the genus and species levels (e.g. Ros & Breeuwer 2007; Skoracka & Dabert 2010; Skoracka *et al.* 2012). Rodrigues *et al.* (2004) and Groot & Breeuwer (2006) have employed DNA-based methods to evaluate genetic variability and to make inferences about *Brevipalpus* taxonomy using sequences of a fragment of COI. Groot & Breeuwer (2006) studied the phylogenetic relationships among haplotypes from Brazil and the Netherlands, including representatives of populations that had been morphologically identified as *B. phoenicis*, *B. obovatus* and *B. californicus*. According to these authors, the taxonomic inferences that could be made based on the COI sequences were incongruent with the morphological based identifications.

The need to clarify *Brevipalpus* systematics has been repeatedly noted in the literature (Welbourn *et al.* 2003; Rodrigues *et al.* 2004; Groot & Breeuwer 2006; Kitajima *et al.* 2010). In this study, we use an integrative approach based on detailed morphological observations and DNA-based information and utilise recently developed phylogenetic methods to investigate species boundaries of *Brevipalpus* species. To this end, mtDNA COI sequences were obtained from individuals collected from several species and origins of *Brevipalpus* in the Americas. The phylogenetic analyses included *Brevipalpus* sequences available on GenBank. The integrative taxonomic approach applied here aimed to investigate the occurrence of cryptic species, clarify systematic inconsistencies previously reported in the literature and explore the relative importance of morphological and genetic traits for the systematics of *Brevipalpus* mites. In addition, the description of a new *Brevipalpus* taxon detected as a cryptic species in this study is presented.

Material and methods

Mite collections

Brevipalpus specimens were collected between 2006 and 2009 across 29 locations in the Americas, including eight states and Federal District of Brazil: Acre, Amapá, Bahia, Minas Gerais, Paraná, Pernambuco, São Paulo and Sergipe; four regions in Chile: the Federal District, VI, VII and the Metropolitan Region; and Florida in the USA. Mites were collected from host plants belonging to 14 genera and 13 families. The collection data are presented in Table 1. The leaves and stems of the host plants were collected in the field and transported to the laboratory for further inspection. Mites were collected through direct inspection using a stereomicroscope (40×). Mites obtained from one specific host plant and locality were regarded as a single population for analysis, hereafter referred to as a 'sample'. When possible, 50 specimens were collected for each sample for both morphological and molecular analyses. Mites were preserved in either absolute ethyl alcohol for molecular analysis or 70% ethyl alcohol for morphological identification.

DNA extraction, amplification and sequencing

DNA was isolated from 1 to 6 specimens from each sample. Genomic DNA was extracted from single adult females using the DNeasy Tissue Kit (Qiagen, Germantown, MD, USA) following the protocol for animal cultured cells. All of the ethanol used to preserve the mites was removed before extraction. Phosphate-buffered saline (PBS – 90 μL) buffer was added to a 1.5-mL microcentrifuge tube, and mites were crushed with a plastic pestle. All other steps in the Qiagen protocol were modified for DNA extraction from small mites as described by Mendonça *et al.* (2011).

A fragment of the COI gene was amplified by PCR with the primers DNF 5′ TAC AGC TCC TAT AGA TAA AAC 3′ and DNR 5′ TGA TTT TTT GGT CAC CCA GAA G 3′ (Navajas *et al.* 1998; Rodrigues *et al.* 2004). The amplification reactions were performed in 25 μL volumes containing 2.5 μL of 10× buffer supplied by the manufacturer, 0.2 μL (5 units) of *Taq* polymerase (Qiagen), 2.5 μL dNTP (0.25 mM of each base), 1.25 μL of each oligonucleotide primer (10 mM), 2.5 μL of $MgCl_2$ (25 mM), 5.8 μL of H_2O and 4 μL of the template DNA. The samples were denatured at 94 °C for 4 min, and PCR was conducted for 30 cycles of 1 min denaturation at 92 °C, 1 min annealing at 50 °C and 1.5 min extension at 72 °C, with a final elongation of 10 min after the completion of all cycles. PCR products (5 μL) were visualised on a 1% agarose gel saturated with ethidium bromide in 0.59 TBE buffer (45 mM Tris base, 45 mM boric acid and 1 mM EDTA, pH 8.0). PCRs were pooled to obtain a higher DNA concentration of the target band before DNA purification. DNA was then purified with a QIAquick PCR Purification kit (Qiagen). The amplified fragments were directly sequenced in both strands with an ABI PRISM 377 automated DNA sequencer (Applied Biosystems Inc., Foster City, CA, USA). No additional primers were used for sequencing.

Sequence data set and phylogenetic analysis

A data set of a total of 154 COI sequences (358 bp) was constructed that comprised the 102 sequences generated in this study as well as 52 *Brevipalpus* spp. sequences available in GenBank and published by Rodrigues *et al.* (2004) and Groot & Breeuwer (2006). Although other COI *Brevipalpus* sequences are available from GenBank, these were not used in the data set because they had not been published in peer-reviewed journals and no information was available on the identification procedures for those sequences. A *Cenopalpus pulcher* (Canestrini & Fanzago) COI sequence from GenBank was chosen as the outgroup for the molecular analyses. COI sequences were aligned using the CLUSTALW multiple alignment procedure (Thompson *et al.* 1994) implemented in BIOEDIT software version 7.0.4 (Hall 1999). No manual adjustments to the CLUSTAL alignment were performed. The alignment of COI sequences was confirmed by translating the aligned DNA into amino acids using GENEDOC v. 2.7 (Nicholas & Nicholas 1997). To initially identify candidate protein coding regions in DNA sequences, an open reading frame was determined using a graphical analysis tool (ORF FINDER) available at http://www.ncbi.nlm.nih.gov/projects/gorf/. The Blast tree view tool in the NCBI WEB BLAST was used, and genetic distances were calculated using Kimura's methods for protein sequences to build a phylogenetic tree using neighbor-joining methods. This graphic was helpful for recognising

Table 1 Characteristics of the samples used in this study and respective lineage in ML phylogeny, putative species in Pons analysis, haplotype, morphological identification and GenBank accession number (haplotype accession numbers are underlined).

Lineage ML phylogeny	Cluster pons analysis	HAP	Host plant species, family	Country, state/region, city	Latitude/longitude	Collection data	GenBank accession	Previous ID	Final ID	Reference
1	B1	1	*Ligustrum* sp., Oleaceae	Brazil, DF, Brasília	15.77S 47.87W	12.I.2006	KC291366	*B. phoenicis*	*B. phoenicis* type 2	in this study
1	B1	1	*Coffea arabica*, Rubiaceae	Brazil, MG, Araguari	18.63S 48.18W	05.VIII.2007	KC344702	*B. phoenicis*	*B. phoenicis* type 2	in this study
1	B1	1	*Vitis vinifera*, Vitaceae	Brazil, MG, Janaúba	15.80S 43.31W	03.IX.2008	KC344703	*B. phoenicis*	*B. phoenicis* type 2	in this study
1	B1	1	*Vitis vinifera*, Vitaceae	Brazil, MG, Janaúba	15.80S 43.31W	03.IX.2009	KC344704	*B. phoenicis*	*B. phoenicis* type 2	in this study
1	B1	1	*Rhododendron* sp., Ericaceae	Brazil, MG			DQ789584	*B. phoenicis*	*B. phoenicis* type 2	Groot & Breeuwer (2006)
1	B1	1	*Carica papaya*, Caricaceae	Brazil, MG			DQ789585	*B. phoenicis*	*B. phoenicis* type 2	Groot & Breeuwer (2006)
1	B1	2	*Ligustrum* sp., Oleaceae	Brazil, DF, Brasília	15.77S 47.87W	12.I.2006	KC291367	*B. phoenicis*	*B. phoenicis* type 2	in this study
1	B1	3	*Malpighia glabra*, Malpighiaceae	Brazil, PE, Recife	08.08S 34.90W	30.III.2007	KC291368	*B. phoenicis*	*B. phoenicis* type 2	in this study
1	B1	3	*Coffea arabica*, Rubiaceae	Brazil, SP, Piracicaba	22.65S 47.63W	20.IV.2007	KC344704	*B. phoenicis*	*B. phoenicis* type 2	in this study
1	B1	3	*Malpighia glabra*, Malpighiaceae	Brazil, RR, Bonfim	03.37N 59.83W	07.IV.2008	KC344705	*B. phoenicis*	*B. phoenicis* type 2	in this study
1	B1	3	*Citrus sinensis*, Rutaceae	Brazil, SP, Conchas			AY320019	*B. phoenicis*	*B. phoenicis* type 2	Rodrigues *et al.* (2004)
1	B1	3	*Citrus sinensis*, Rutaceae	Brazil, SP, Monte Azul			AY320020	*B. phoenicis*	*B. phoenicis* type 2	Rodrigues *et al.* (2004)
1	B1	3	*Citrus sinensis*, Rutaceae	Brazil, SP, Bebedouro			AY320021	*B. phoenicis*	*B. phoenicis* type 2	Rodrigues *et al.* (2004)
1	B1	3	*Citrus sinensis*, Rutaceae	Brazil, RJ, Teresópolis			AY320022	*B. phoenicis*	*B. phoenicis* type 2	Rodrigues *et al.* (2004)
1	B1	3	*Citrus sinensis*, Rutaceae	Brazil, SP, Araraquara			AY320023	*B. phoenicis*	*B. phoenicis* type 2	Rodrigues *et al.* (2004)
1	B1	3	*Coffea* sp., Rubiaceae	Brazil, MG, Patrocínio			AY320024	*B. phoenicis*	*B. phoenicis* type 2	Rodrigues *et al.* (2004)
1	B1	3	*Citrus reshni*, Rutaceae	Brazil, SP, Cordeirópolis			AY320027	*B. phoenicis*	*B. phoenicis* type 2	Rodrigues *et al.* (2004)
1	B1	3	*Citrus* sp., Rutaceae	Brazil, SP			DQ789576	*B. phoenicis*	*B. phoenicis* type 2	Groot & Breeuwer (2006)
1	B1	3	*Citrus* sp., Rutaceae	Brazil, SP			DQ789577	*B. phoenicis*	*B. phoenicis* type 2	Groot & Breeuwer (2006)
1	B1	3	*Monodora crispata*, Annonaceae	The Netherlands			DQ789578	*B. phoenicis*	*B. phoenicis* type 2	Groot & Breeuwer (2006)
1	B1	3	*Terminalia ivorensis*, Combretaceae	The Netherlands			DQ789579	*B. phoenicis*	*B. phoenicis* type 2	Groot & Breeuwer (2006)
1	B1	5	*Citrus sp.*, Rutaceae	Brazil, PE, Recife	08.08S 34.90W	30.III.2007	KC291370	*B. phoenicis*	*B. phoenicis* type 2	in this study
1	B1	6	*Hibiscus* sp., Malvaceae	Brazil, SP, Piracicaba	22.65S 47.63W	20.IV.2007	KC291371	*B. phoenicis*	*B. phoenicis* type 2	in this study
1	B1	6	*Citrus* sp., Rutaceae	Brazil, BA, Cruz das Almas	12.67S 39.10W	01.VIII.2007	KC344706	*B. phoenicis*	*B. phoenicis* type 2	in this study
1	B1	6	*Citrus* sp., Rutaceae	Brazil, BA, Cruz das Almas	12.67S 39.10W	13.VI.2008	KC344707	*B. phoenicis*	*B. phoenicis* type 2	in this study
1	B1	6	*Citrus* sp., Rutaceae	Brazil, BA, Muritiba	12.65S 39.15W	14.VI.2009	KC344708	*B. phoenicis*	*B. phoenicis* type 2	in this study
1	B1	6	*Citrus* sp., Rutaceae	Brazil, BA, Gov. Mangabeira	12.60S 39.02W	15.VI.2010	KC344709	*B. phoenicis*	*B. phoenicis* type 2	in this study
1	B1	6	*Citrus* sp., Rutaceae	Brazil, BA, Maragogipe	12.77S 38.92W	16.VI.2011	KC344710	*B. phoenicis*	*B. phoenicis* type 2	in this study
1	B1	6	*Citrus sinensis*, Rutaceae	USA, FL			KC344711	*B. phoenicis*	*B. phoenicis* type 2	in this study
1	B1	6	*Citrus sinensis*, Rutaceae	USA, FL, Lake Alfred			AY320007	*B. phoenicis*	*B. phoenicis* type 2	Rodrigues *et al.* (2004)
1	B1	6	*Citrus sinensis*, Rutaceae	USA, FL, Plant City			AY320008	*B. phoenicis*	*B. phoenicis* type 2	Rodrigues *et al.* (2004)
1	B1	6	*Citrus sinensis*, Rutaceae	USA, FL, Montverde			AY320009	*B. phoenicis*	*B. phoenicis* type 2	Rodrigues *et al.* (2004)
1	B1	6	*Citrus sinensis*, Rutaceae	USA, FL, Bowling Green			AY320010	*B. phoenicis*	*B. phoenicis* type 2	Rodrigues *et al.* (2004)
1	B1	6	*Citrus sinensis*, Rutaceae	USA, FL, Lake Alfred			AY320011	*B. phoenicis*	*B. phoenicis* type 2	Rodrigues *et al.* (2004)
1	B1	6	*Citrus sinensis*, Rutaceae	USA, FL, Oak Hill			AY320012	*B. phoenicis*	*B. phoenicis* type 2	Rodrigues *et al.* (2004)
1	B1	6	*Citrus sinensis*, Rutaceae	USA, FL, Merritt Island			AY320013	*B. phoenicis*	*B. phoenicis* type 2	Rodrigues *et al.* (2004)
1	B1	6	*Rhododendron* sp., Ericaceae	USA, FL, Lake Alfred			AY320015	*B. phoenicis*	*B. phoenicis* type 2	Rodrigues *et al.* (2004)
1	B1	6	*Citrus sinensis*, Rutaceae	USA, FL, Plant City			AY320016	*B. phoenicis*	*B. phoenicis* type 2	Rodrigues *et al.* (2004)
1	B1	6	*Citrus sinensis*, Rutaceae	USA, FL, Eustis Lake			AY320017	*B. phoenicis*	*B. phoenicis* type 2	Rodrigues *et al.* (2004)
1	B1	6	*Ligustrum* sp., Oleaceae	USA, FL, Lake Alfred			AY320018	*B. phoenicis*	*B. phoenicis* type 2	Rodrigues *et al.* (2004)

Table 1 Continued

Lineage ML phylogeny	Cluster pons analysis	HAP	Host plant species, family	Country, state/region, city	Latitude/longitude	Collection data	GenBank accession	Previous ID	Final ID	Reference
1	B1	6	*Citrus maxima*, Rutaceae	USA, Texas, Welasco			AY320025	*B. phoenicis*	*B. phoenicis* type 2	Rodrigues *et al.* (2004)
1	B1	6	*Citrus paradisi*, Rutaceae	USA, Texas, Donna			AY320026	*B. phoenicis*	*B. phoenicis* type 2	Rodrigues *et al.* (2004)
1	B1	7	*Coffea arabica*, Rubiaceae	Brazil, MG, Araguari	18.63S 48.18W	05.VIII.2007	KC291372	*B. phoenicis*	*B. phoenicis* type 2	in this study
1	B1	8	*Malpighia glabra*, Malpighiaceae	Brazil, RR, Bonfim	03.37N 59.83W	07.IV.2008	KC291373	*B. phoenicis*	*B. phoenicis* type 2	in this study
1	B1	9	*Citrus* sp., Rutaceae	Brazil, BA, Gov. Mangabeira	12.60S 39.02W	15.VI.2010	KC291374	*B. phoenicis*	*B. phoenicis* type 2	in this study
1	B1	10	*Hibiscus sp.*, Malvaceae	Brazil, AC, Rio Branco	09.97S 67.82W	26.VII.2008	KC291375	*B. phoenicis*	*B. phoenicis* type 2	in this study
1	B1	10	*Hibiscus rosa-sinensis*, Malvaceae	Brazil, MG			DQ789580	*B. phoenicis*	*B. phoenicis* type 2	Groot & Breeuwer (2006)
1	B1	10	*Hibiscus rosa-sinensis*, Malvaceae	Brazil, MG			DQ789581	*B. phoenicis*	*B. phoenicis* type 2	Groot & Breeuwer (2006)
1	B1	11	*Citrus* sp., Rutaceae	Brazil, MG, Janaúba	15.80S 43.31W	03.IX.2008	KC291376	*B. phoenicis*	*B. phoenicis* type 2	in this study
1	B1	12	*Hibiscus* sp., Malvaceae	Brazil, MG, Janaúba	15.80S 43.31W	03.IX.2009	KC291377	*B. phoenicis*	*B. phoenicis* type 2	in this study
1	B1	13	*Spondias purpurea*,Anacardiaceae	Brazil, AM, Ferreira Gomes	00.83N 51.16W	12.XI.2008	KC291378	*B. phoenicis*	*B. phoenicis* type 2	in this study
1	B1	14	*Citrus* sp., Rutaceae	Brazil, AM, Porto Grande	00.66N 51.44W	12.XI.2008	KC291379	*B. phoenicis*	*B. phoenicis* type 2	in this study
1	B1	15	*Malvaviscus arboreus*, Malvaceae	Brazil, AM, Ferreira Gomes	00.83N 51.16W	12.XI.2008	KC291380	*B. phoenicis*	*B. phoenicis* type 2	in this study
1	B1	16	*Malvaviscus arboreus*, Malvaceae	Brazil, AM, Ferreira Gomes	00.83N 51.16W	12.XI.2008	KC291381	*B. phoenicis*	*B. phoenicis* type 2	in this study
1	B1	36	*Hiiscus* sp., Malvaceae	USA, FL, Pembroke			AY320014	*B. phoenicis*	*B. phoenicis* type 2	Rodrigues et al. (2004)
1	B1	39	*Citrus sinensis*, Rutaceae	USA, FL, Lake Alfred	28.09N 81.72W	01.VII.2005	KC291382	*B. phoenicis*	*B. phoenicis* type 2	in this study
1	B1	41	*Citrus* sp., Rutaceae	Brazil, MG			DQ789582	*B. phoenicis*	*B. phoenicis* type 2	Groot & Breeuwer (2006)
1	B1	41	*Citrus* sp., Rutaceae	Brazil, MG			DQ789583	*B. phoenicis*	*B. phoenicis* type 2	Groot & Breeuwer (2006)
2	B2	4	*Hibiscus* sp., Malvaceae	Brazil, PE, Recife	08.08S 34.90W	30.III.2007	KC291369	*B. phoenicis*	*B.* p*hoenicis* ?	in this study
3	B3	24	*Cocos nucifera*, Arecaceae	Brazil, MG, Janaúba	15.80S 43.31W	10.V.2007	KC291390	*B. phoenicis*	*B.* n. sp	in this study
4	B4	40	Unknown	USA, FL, Lake Alfred	28.09N 81.72W	01.VII.2005	KC291402	*B. californicus*	*B. californicus*	in this study
4	B4	44	*Rhododendron* sp., Ericacea	Brazil, MG			DQ789591	*B. californicus*	*B. californicus*	Groot & Breeuwer (2006)
4	B4	44	*Rhododendron sp.*, Ericacea	Brazil, MG			DQ789592	*B. californicus*	*B. californicus*	Groot & Breeuwer (2006)
4	B4	44	*Euphorbia xanthii*, Euphorbiaceae	The Netherlands			DQ789593	*B. californicus*	*B. californicus*	Groot & Breeuwer (2006)
4	B4	45	*Thevetia peruviana*, Apocynaceae	The Netherlands			DQ789594	*B. californicus*	*B. californicus*	Groot & Breeuwer (2006)
5	B5	17	*Ligustrum sp.*, Oleaceae	Brazil, DF, Brasília	15.77S 47.87W	12.I.2006	KC291387	*B. phoenicis*	*B. phoenicis* type 1	in this study
5	B5	17	*Ligustrum japonicum*, Oleaceae	Brazil, PR, Colombo	25.32S 49.17W	23.IV.2008	KC344713	*B. phoenicis*	*B. phoenicis* type 1	in this study
5	B5	22	*Alnus subcordata*, Betulaceae	Brazil, PR, Colombo	25.32S 49.17W	23.IV.2008	KC291388	*B. phoenicis*	*B. phoenicis* type 1	in this study
5	B5	23	*Alnus subcordata*, Betulaceae	Brazil, PR, Colombo	25.32S 49.17W	23.IV.2008	KC291389	*B. phoenicis*	*B. phoenicis* type 1	in this study
5	B5	23	*Strongylodon macrobotrys*, Fabacea	The Netherlands			DQ789587	*B. phoenicis*	*B. phoenicis* type 1	RC Trincado
5	B5	23	*Beaumontia grandiflora*, Apocynaceae	The Netherlands			DQ789588	*B. phoenicis*	*B. phoenicis* type 1	Groot & Breeuwer (2006)
5	B5	42	*Malpighia glabra*, Malpighiaceae	Brazil, MG			DQ789586	*B. phoenicis*	*B. phoenicis* type 1	Groot & Breeuwer (2006)
6	B6	18	*Ocimum basilicum*, Lamiaceae	Brazil, SE, São Cristovão	11.00S 37.20W	30.III.2006	KC291383	*B. obovatus*	*B. obovatus*	in this study
6	B6	19	*Ocimum basilicum*, Lamiaceae	Brazil, DF, Gama	15.77S 47.87W	10.VII.2006	KC291384	*B. obovatus*	*B. obovatus*	in this study
6	B6	20	*Ocimum basilicum*, Lamiaceae	Brazil, DF, Gama	15.77S 47.87W	10.VII.2006	KC291385	*B. obovatus*	*B. obovatus*	in this study
6	B6	21	*Cestrum nocturnum*, Solanaceae	Brazil, SP, Cordeirópolis	22.47S 47.45W	01.VII.2007	KC291386	*B. obovatus*	*B. obovatus*	in this study
6	B6	37	Unknown	USA, FL, Lake Alfred	28.09N 81.72W		KC344713	*B. obovatus*	*B. obovatus*	in this study
6	B6	37	*Camellia sinensis*, Theaceae	USA, SC, Charleston			AY320028	*B. obovatus*	*B. obovatus*	Rodrigues *et al.* (2004)
6	B6	37	*Zingiber* sp., Zingiberaceae	Brazil, MG			DQ789589	*B. obovatus*	*B. obovatus*	Groot & Breeuwer (2006)
6	B6	43	*Hibiscus rosa-sinensis*, Malvaceae	Brazil, MG			DQ789590	*B. obovatus*	*B. obovatus*	Groot & Breeuwer (2006)
7	B7	25	*Ligustrum sinense*, Oleaceae	Chile, RM, Santa Rosa	35.55S 71.73W	23.VII.2004	KC191391	*B. chilensis*	*B. chilensis*	in this study

Table 1 Continued

Lineage ML phylogeny	Cluster pons analysis	HAP	Host plant species, family	Country, state/region, city	Latitude/longitude	Collection data	GenBank accession	Previous ID	Final ID	Reference
7	B7	25	*Crateagus* sp., Rosaceae	Chile, RM, Quinta Normal	33.47S 70.65W	23.VII.2004	KC344714	*B. chilensis*	*B. chilensis*	in this study
7	B7	25	*Ligustrum sinense*, Oleaceae	Chile, RM,, La Pintana	33.58S 70.63W	23.VII.2004	KC344715	*B. chilensis*	*B. chilensis*	in this study
7	B7	25	*Ligustrum sinense*, Oleaceae	Chile, RM, La Platina	34.45S 70.98W	23.VII.2004	KC344716	*B. chilensis*	*B. chilensis*	in this study
7	B7	25	*Ligustrum sinense*, Oleaceae	Chile, RM, La Platina	34.45S 70.98W	23.VII.2004	KC344717	*B. chilensis*	*B. chilensis*	in this study
7	B7	25	*Ligustrum sinense*, Oleaceae	Chile, RM, La Platina	34.45S 70.98W	23.VII.2004	KC344718	*B. chilensis*	*B. chilensis*	in this study
7	B7	25	*Ligustrum sinense*, Oleaceae	Chile, RM, La Platina	34.45S 70.98W	23.VII.2004	KC344719	*B. chilensis*	*B. chilensis*	in this study
7	B7	25	*Ligustrum sinense*, Oleaceae	Chile, RM, La Platina	34.45S 70.98W	23.VII.2004	KC344720	*B. chilensis*	*B. chilensis*	in this study
7	B7	25	*Cestrum parqui*, Solanaceae	Chile, RM, Lampa	33.28S 70.88W	27.I.2005	KC344721	*B. chilensis*	*B. chilensis*	in this study
7	B7	25	*Crataegus* sp., Rosaceae	Chile, RM, La Pintana	33.58S 70.63W	24.II.2005	KC344722	*B. chilensis*	*B. chilensis*	in this study
7	B7	25	*Ligustrum sinense*, Oleaceae	Chile, VI, Pichilemu	34.38S 72.02W	24.II.2005	KC344723	*B. chilensis*	*B. chilensis*	in this study
7	B7	25	*Ligustrum sinense*, Oleaceae	Chile, RM, Curacaví	33.70S 71.22W	12.VI.2006	KC344724	*B. chilensis*	*B. chilensis*	in this study
7	B7	25	*Ligustrum sinense*, Oleaceae	Chile, RM, Santiago	33.45S 70.67W	07.V.2008	KC344725	*B. chilensis*	*B. chilensis*	in this study
7	B7	26	*Juglans regia*, Juglandaceae	Chile, RM, La Platina	34.45S 70.98W	23.VII.2004	KC291392	*B. chilensis*	*B. chilensis*	in this study
7	B7	26	*Juglans regia*, Juglandaceae	Chile, RM, La Platina	34.45S 70.98W	23.VII.2004	KC344726	*B. chilensis*	*B. chilensis*	in this study
7	B7	26	*Crataegus* sp., Rosaceae	Chile, RM, La Pintana	33.58S 70.63W	24.II.2005	KC344727	*B. chilensis*	*B. chilensis*	in this study
7	B7	27	*Ligustrum sinense*, Oleaceae	Chile, RM, La Platina	34.45S 70.98W	23.VII.2004	KC291393	*B. chilensis*	*B. chilensis*	in this study
7	B7	28	*Citrus sinensis*, Rutaceae	Chile, RM, Lampa	33.28S 70.88W	27.I.2005	KC291394	*B. chilensis*	*B. chilensis*	in this study
7	B7	29	*Cestrum parqui*, Solanaceae	Chile, RM, Lampa	33.28S 70.88W	27.I.2006	KC291395	*B. chilensis*	*B. chilensis*	in this study
7	B7	30	*Crataegus* sp., Rosaceae	Chile, RM, La Pintana	33.58S 70.63W	24.II.2005	KC291396	*B. chilensis*	*B. chilensis*	in this study
7	B7	31	*Ligustrum sinense*, Oleaceae	Chile, RM, La Obra	33.58S 70.45W	07.III.2005	KC291397	*B. chilensis*	*B. chilensis*	in this study
7	B7	31	*Ligustrum sinense*, Oleaceae	Chile, VI, Placilla	34.63S 71.12W	26.IV.2008	KC344728	*B. chilensis*	*B. chilensis*	in this study
7	B7	32	*Vitis vinifera*, Vitaceae	Chile, VII, Molina	34.63S 71.35W	08.III.2005	KC291398	*B. chilensis*	*B. chilensis*	in this study
7	B7	33	*Ligustrum sinense*, Oleaceae	Chile, VI, Placilla	34.63S 71.12W	26.IV.2008	KC291399	*B. chilensis*	*B. chilensis*	in this study
7	B7	34	*Ligustrum sinense*, Oleaceae	Chile, RM, Lo Aguirre	33.37S 70.75W	06.V.2008	KC291400	*B. chilensis*	*B. chilensis*	in this study
7	B7	35	*Ligustrum sinense*, Oleaceae	Chile, RM, La Pintana	33.58S 70.63W	08.V.2008	KC291401	*B. chilensis*	*B. chilensis*	in this study
outgroup		38	*Malus pumila*, Rosaceae	USA, OR, Corvalis			AY320029	*C. pulcher*	*C. pulcher*	Rodrigues *et al.* (2004)

Abbreviations for sample collection state or regions – Brazil, AC, Acre; AM, Amapá; BA, Bahia; DF, Distrito Federal; MG, Minas Gerais; PE, Pernambuco; PR, Paraná; RJ, Rio de Janeiro; RR, Roraima; SE, Sergipe; SP, Sao Paulo; Chile; RM, Región Metropolitana; VI, VI Región; VII, VII Región; USA; FL, Florida; OR, Oregon; SC, South Carolina; TX, Texas.

[a]Sequences representing the haplotypes are underlined.

[b]*B. obovatus* by molecular methods (COI fragment).

the presence of aberrant or unusual sequences. Nucleotide composition (calculated as the base frequencies for each sequence as well as an overall average) and substitution patterns and rates were estimated under the Tamura–Nei (1993) model. The homogeneity of the substitution patterns between sequences was tested with the Disparity Index (I_D). Overall and pairwise distances of nucleotide sequences as well as distances within and among putative species were calculated using Kimura's 2-parameter (K2P) model (Kimura 1980) with codon positions including 1st+2nd+3rd and with the pairwise deletion of gaps. The number of amino acid substitutions per site based on averaging overall sequence pairs was calculated using the JTT matrix-based model (Jones *et al.* 1992). Standard error estimates were obtained with a bootstrap procedure (1000 replicates). All of the above analyses were conducted in MEGA5 (Tamura *et al.* 2011). Intra- and interspecific measures of DNA sequence variation and the ratio of synonymous to non-synonymous substitutions calculated using Tajima's test were assessed in DNA SP software version 5.0 (Librado & Rozas 2009.

The likelihood scores for 56 models of DNA substitution for COI sequences were calculated using PAUP*4.0 beta 5 software (Phylogenetic Analysis Using Parsimony) (Swofford 2003). MODELTEST version 3.8 (Posada & Crandall 1998) was used to estimate the best substitution model using the hierarchical likelihood ratio test (hLRT), approximated Akaike information criterion corrected (AICc) and Bayesian information criterion. The MODELTEST server (Posada 2006) was used to execute the MODELTEST computation.

The MODELTEST analysis K81uf + G model (= Kimura three-parameter, K-3P) (Kimura 1981) was selected by hLRTs as the best-fit model of DNA evolution for phylogenetic analysis of the COI sequence data set with the following maximum likelihood (ML) parameters: the proportion of invariable sites was 0.0, and the gamma distribution shape parameter was 0.3254. The ML tree was built using the K80 (Kimura 1980) model in PHY ML version 3 (Guindon and Gascuel 2003), and the topology was compared between the trees obtained using ML and neighbor-joining (NJ) performed with the K-2P parameter model using MEGA 5 and Bayesian inference (BI) with MrBAYES version 3.12 (Ronquist & Huelsenbeck 2003) on Phylogeny.fr: robust phylogenetic analysis for the non-specialist (Dereeper *et al.* 2008, Web Server Issue:W465-9, available at: http://www.phylogeny.fr/). The robustness of the trees was assessed with a bootstrap analysis that involved 1000 bootstrap replicates for all analyses, and the approximate likelihood ratio test (aLRT) function within PHYML (Anisimova & Gascuel 2006) was used to test the accuracy of each branch using the log-likelihood test. Phylogenies generated from COI data set were statistically tested using Shimodaira's Approximately Unbiased (AU test), Kishino–Hasegawa (KH test) and Shimodaira–Hasegawa (SH test) tests, as implemented in CONSEL program package (Shimodaira & Hasegawa 2001). The sitewise log-likelihood scores of each trees topology were estimated using PAUP v4.0b10 (Swofford 2003) and used as input file for the program to calculate *P*-values. A *P*-value cut-off of 0.05 was considered statistically significant to reject the hypothesis that the trees were significantly different. Pairwise nucleotide distances were calculated using Kimura's two-parameter correction and the software program MEGA 5 (Kimura 1980; Tamura *et al.* 2007) for the COI sequences included in the data set.

The variation in the sequence of the COI gene fragment of mtDNA in the haplotype alignment was examined to determine polymorphic sites that could be used to differentiate *Brevipalpus* putative species. Only nucleotide sequence variations that were both present in all haplotypes that comprised a putative species and exclusive to that putative species were considered diagnostic sites.

All sequences have been deposited in GenBank under the accession numbers indicated in Table 1. The alignments are available upon request.

Species delimitation: DNA sequence-based delimitation of species method

We used the DNA-based approach proposed by Pons *et al.* (2006). Using a likelihood framework, this procedure detects the transition in the rate of lineage branching of a tree from interspecific long branches to intraspecific short, budding branches and identifies clusters of specimens corresponding to putative species. Two models are implemented to account for the branching process of the entire tree. Under the null model, the whole sample derives from a single population obeying a coalescent process. The alternative model, the general mixed Yule coalescent (GMYC) model, combines equations that separately describe branching within populations (coalescent process) and branching between species (a Yule model including speciation and extinction rates). Under the GMYC model, a threshold (T) is optimised such that nodes before the threshold are considered species diversification events and branches crossing the threshold define clusters following a coalescent process. A standard likelihood ratio test (LRT) is used to assess whether the alternative model provides a better fit than the null model. If the GMYC model is favoured over the null model, the T parameter of the maximum likelihood solution allows the number of species to be estimated. This test was achieved using the R code provided by T. G. Barraclough. This latest version outputs the estimates of the number of species, the

threshold time and the 95% confidence limits of those estimates (i.e. solutions with 2-log-likelihood units of the maximum).

Because a prerequisite of the method is an ultrametric tree, we used the MESQUITE 2.75 program (Maddison & Maddison 2011) to convert our optimal phylogram tree (estimated from the PhyML analysis) into a rooted additive tree with terminal nodes equally distant from the root.

Morphological study and description of new species

For the purpose of morphological study, the collected specimens were mounted directly on microscopic preparations in Hoyer's medium, and discriminative characteristics were examined by phase- and differential interference contrast microscopy using 40× and 100× objectives (Nikon Eclipse 80i, Nikon, Tokyo, Japan). From each sample, 25 specimens (females and/or males), when available, were mounted in the dorso-ventral position for morphological identification. When haplotypes consisted exclusively of retrieved GenBank sequences (HAP 36, 41, 42, 43, 44, 45), it was not possible to examine representatives (not available). In these cases, specimens of haplotypes in the same genetic cluster were examined, and information on the primary morphological characters of those specimens was extrapolated to the closest non-examined haplotypes.

Traditionally, the morphological identification of *Brevipalpus* mites has been based on a reduced number of traits. The number of solenidia (*omega*) on tarsus II, the presence of the opisthosomal setal pair *f2*, as well as general dorsal cuticular patterns have been considered the most important characteristics for identifying the *Brevipalpus* species of greatest economic importance. Apart from these usual characteristics used in *Brevipalpus* taxonomy, some other traits have recently been shown to be taxonomically informative and deserve more attention, including the shape of the spermatheca (insemination apparatus of females), type of palp genual seta, detailed dorsal and ventral cuticular pattern, the shape of the propodosomal and opisthosomal setae, and complete leg chaetotaxy (coxa, trochanter, femur, genu, tibia and tarsus from legs I to IV) (see Seeman & Beard 2011; Beard *et al.* 2012). In this study, morphological identification of specimens of each sample was conducted in two steps: the first step consisted of an identification based on the usual characteristics employed for *Brevipalpus* taxonomy; the second step consisted of a detailed morphological identification taking into account taxonomically informative characters.

The standard system of notation based on that of Grandjean (1939), first applied to the Tenuipalpidae by Quirós-González (1986) and also followed in Welbourn *et al.* (2003) and Mesa *et al.* (2009), was followed in this paper. Leg chaetotaxy is derived from Lindquist (1985).

For the sample collected from hibiscus from Pernambuco, Brazil (HAP 04), the total number of males in the mounted slides was registered as well as the number of specimens with one or two solenidia (ω) on tarsus II.

Specimens belonging to a sample identified as a new taxon were measured and drawn with a Nikon Eclipse 80i microscope equipped with a camera lucida. The measurements correspond to the holotype, and the range in parentheses corresponds to the paratypes. All measurements were made under 100× objectives and are presented in micrometres (μm). Body size was measured as the distance between setae *v2–h1* (length) and between setae *c3–c3* (width). Leg and palp setal numbers are written as the total number of setae followed by the number of solenidia in parentheses.

Micrographs were obtained with a digital imaging system consisting of the above-mentioned microscope connected to a digital camera (Nikon DS-Ri1-U3, 12.6 megapixels, Nikon, Tokyo, Japan), which was in turn connected to a computer. HELICON FOCUS 5.2 software was used to create a whole focused image of some morphological structures from several partially focused images.

All the studied material was deposited in the mite collection at Embrapa Recursos Genéticos e Biotecnologia, Embrapa, Brasilia, Brazil. Paratypes of the new *Brevipalpus* species were also deposited in the mite collection at Instituto Agroforestal Mediterráneo, Universidad Politécnica de Valencia, Valencia, Spain.

Results

Sequence analyses, phylogenetic reconstructions and species delimitation

The final data set was classified into 44 haplotypes and one outgroup (Table 1). No insertions or deletions were needed for the alignments, with the exception of two insertions in the *C. pulcher* sequence. In the alignment, 58 (16.11%) sites were parsimony informative and 88 (24.44%) sites were variable. Among the variable sites, 75 (21.61%) were in the third codon position, 12 (3.46%) in the first codon position and eight (2.31%) in the second codon position. The COI sequences exhibited a bias in nucleotide composition. The average base frequencies were T = 49.7%, A = 27.2%, C = 10.5% and G = 12.6%. Base frequencies were homogeneous across taxa ($I_D = 0$ and $P = 0$ for each sequence pair). In the whole data set, the transition/transversion bias equalled 1.28, and for the *Brevipalpus* sequences, the transition/transversion bias was 1.39.

The translation of the nucleotide sequences resulted in 115 amino acid sequences, with 17 (17.78%) variable amino acid positions. No stop codons were revealed in translation. Putative conserved domains were detected that matched all sequences in the data set, producing good alignments with the *Brevipalpus* sequences retrieved from GenBank.

The general topologies of the phylogenetic trees inferred by NJ, ML and BI based on the nucleotide COI data set were similar and consistently demonstrated the same structure of *Brevipalpus* samples and supported the position of the outgroup. All investigated tree topologies were in the confidence set with *P*-values ranging from 4e−004 to 0.028, indicating all of them representing a good explanation of the observed data (Shimodaira & Hasegawa 2001). Only the ML tree is presented (AU test, *P*-value = 2e−004; KH test, *P*-value = 4e−004; and SH tests, *P*-value = 4e−004) (Fig. 1).

All *Brevipalpus* samples formed a monophyletic group. The *Brevipalpus* haplotypes clustered into seven lineages (five clades and two isolated branches) (Fig. 1). The divergence among these lineages averaged 8.53% (SE = 0.015) and ranged from 3.35% to 10.86%. All clades were homogenous, with a mean intra-clade sequence divergence of <3% (mean 1.96%, SE 0.51%, range 1.27% to 2.95%). Pairwise nucleotide comparisons of the COI distances within and between *Brevipalpus* lineages and between lineages and the outgroup are presented in Table S1. The ML phylogeny demonstrated that most lineages were well supported, with bootstrap values (Bp) ranging from 86% to 98%; however, the basal branch supporting lineages 3, 4, 5, 6 and 7 exhibited a low bootstrap value (58%) (Fig. 1). Information on samples that composed each lineage and haplotype is provided in Table 1. HAP 24, collected from coconut, and HAP 04, from hibiscus, both from Brazil, composed isolated lineages 5 and 7, respectively.

To perform the Pons analyses and eliminate polytomies in the tree used by this method, some haplotypes were removed from the data set, which then comprised 27 *Brevipalpus* haplotypes plus the outgroup. The seventeen haplotypes eliminated were HAP 01, 03, 06, 08, 10, 12, 14, 15, 39 and 41 from lineage 1; HAP 19 and 20 from lineage 6; HAP 25, 26, 29, 31 and 32 from lineage 7.

To fit the position of the transition in the rate of lineage branching, the method of Pons *et al.* (2006) was applied to the time-calibrated tree. The GMYC model was preferred over the null model of uniform branching rates ($\log L = 93.54$, compared with null model $\log L = 89.91$; $2\Delta L = 7.25$, chi-squared test, d.f. = 0.06, $P < 0.0001$). The model estimated the transition in the branching pattern −0.0996 (i.e. T of the ML solution), with the time separating the ingroup root from the present arbitrarily designated as 1.

The ultrametric *Brevipalpus* tree and the clusters of specimens recognised as putative species by the Pons method are presented in Fig. 2. The estimated number of species ranged from 6 to 7 *Brevipalpus* species plus the outgroup. Pons analysis clearly detected six putative species, and one branch (HAP 04) felled into the uncertainty zone.

Drawing a parallel between the ML phylogenetic (Fig. 1) and Pons ultrametric trees (Fig. 2), we observed that each lineage of the ML phylogeny was recognised as a putative species of the Pons analysis, with the exception of lineage 2 (HAP 04), which branched in the uncertainty zone in the Pons ultrametric tree. Information on the recognised putative *Brevipalpus* species and their corresponding lineages and haplotypes is presented in Table 1.

Integrating morphological identification and DNA-based delimitation of species

The morphology-based taxonomic identification of *Brevipalpus* samples presented by Rodrigues *et al.* (2004) and Groot & Breeuwer (2006) for samples whose DNA sequences were retrieved from GenBank, combined with the first step of identification of the samples collected in this study, indicated that four species were present in the samples: *Brevipalpus phoenicis*, *B. californicus*, *B. obovatus* and *B. chilensis* (Table 1). However, the second step of identification supported the detection of six taxa as putative species according to the Pons analysis. A summary of morphological traits of taxa identified in this study, which characterise the different lineages/putative species, is found in Table S2.

B1 specimens, firstly identified as *B. phoenicis*, fit with *B. phoenicis* type 2 as characterised by Beard *et al.* (2012) (Table S2). This species was the most common among the studied samples. It was detected in 56 samples from 22 localities in eight Brazilian states and the Federal District and 10 localities in two US states (Florida and Texas). Most samples of *B. phoenicis* type 2 were collected from *Citrus* (32 samples) and *Hibiscus* (six samples). Other hosts included coffee, fruit trees and ornamental plants (Table 1).

Specimens of B2, the only putative species that fell in the uncertainty zone of the ultrametric tree, are morphologically close to B1 putative species (*B. phoenicis* type 2), exhibiting similar spermatheca shape, palp genual seta and absence of opisthosomal seta *f2* (Table S2). However, some differences in the dorsal and ventral reticulation patterns, and in length and shape of propodosomal seta *v2* were observed (Table S2). Other notable differences are that most B2 female specimens (15 of 16 studied) showed only one solenidium (*omega*) on tarsus II (always two in B1 samples) and that males were present in the sample (five males among 21 studied specimens). *B. phoenicis* populations are usually composed exclusively of females that reproduce by thelytokous parthenogenesis (Weeks *et al.* 2001). In this study, no males were found in the 27 studied B1 populations (*B. phoenicis* type 2).

The specimens representing putative species B3 were firstly identified as *B. phoenicis*. During the morphological

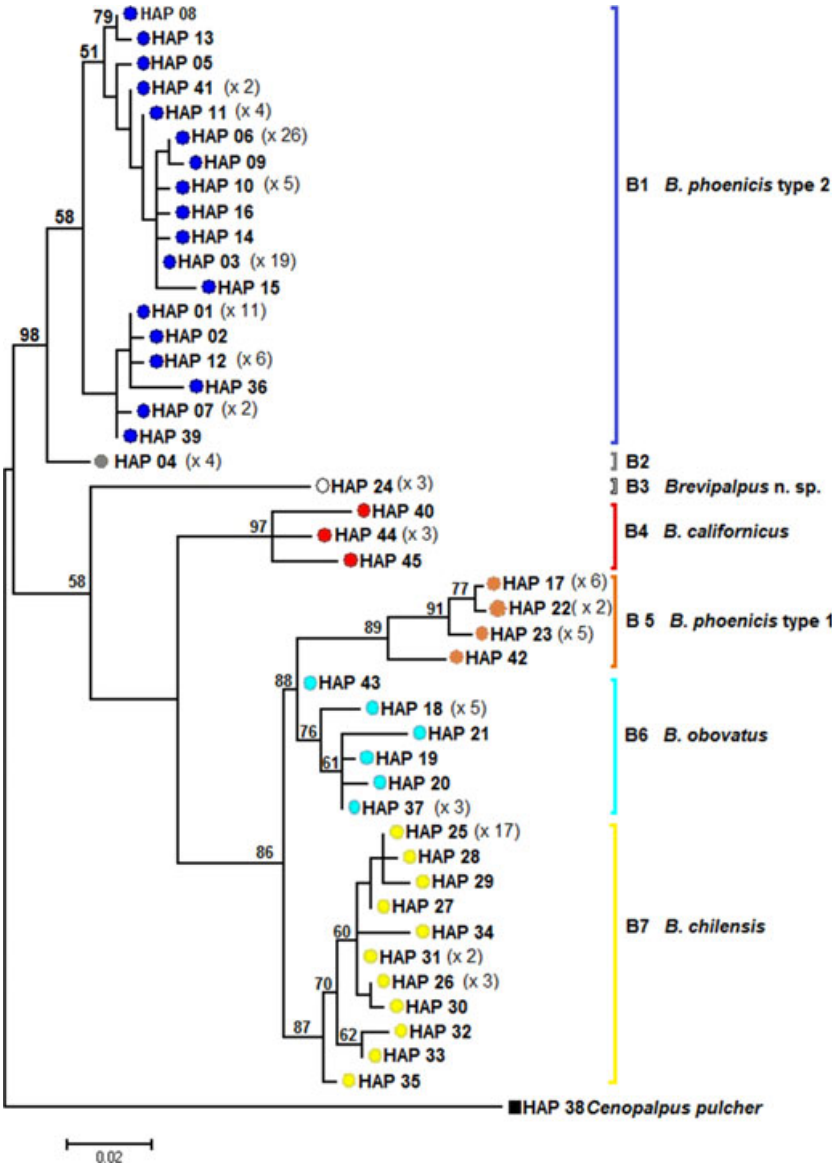

Fig. 1 ML phylogeny tree (K-3P substitution model) inferred from 358 bp COI sequences of *Brevipalpus* mites retrieved from GenBank and obtained in this study. Information on samples composing haplotypes (HAP) and the associated Genbank accessions are given in Table 1. The number of times that a haplotype was found in the data set is indicated between parentheses. ML bootstrap values are above branches. Putative species (Pons *et al.* 2006 analysis) and lineages name identification is indicated on the right.

study considering the recently assigned taxonomic characteristics, some clear differences were observed between B3 and B1 (*B. phoenicis* type 2) specimens, particularly in the shape of the dorsal opisthosomal setae and the dorsal and ventral reticulation patterns (see Table S2).

The specimens representing putative species B4 were identified as *B. californicus* during both the first and second step of morphological identification. In addition to the established morphological characteristics used to identify *B. californicus* (presence of two solenidia (*omega*) on tarsus II and of opisthosomal setal pair *f2*), the specimens of this group have a unique spermatheca vesicle (see Table S2). The specimens collected in this study are similar to those characterised by Beard *et al.* (2012) as *B. californicus* type 2.

The specimens composing putative species B5 were identified by Groot & Breeuwer (2006) and by the authors during the first step of identification as *B. phoenicis*. However, during the second step of identification, it was observed that the shape of the spermatheca of group B5 is not similar to that of other *B. phoenicis* specimens (e.g. B1 in this study), but is closer to that of *B. obovatus* and *B. chilensis* (Table S2). B5 specimens can also be clearly distinguished from other studied *Brevipalpus* species by their dorsal and ventral reticulation patterns. Beard *et al.* (2012) characterised *Brevipalpus* specimens similar to B5 as *B. phoenicis* type 1.

Specimens representing putative species B6 and B7 were identified as *B. obovatus* and *B. chilensis*, respectively, during

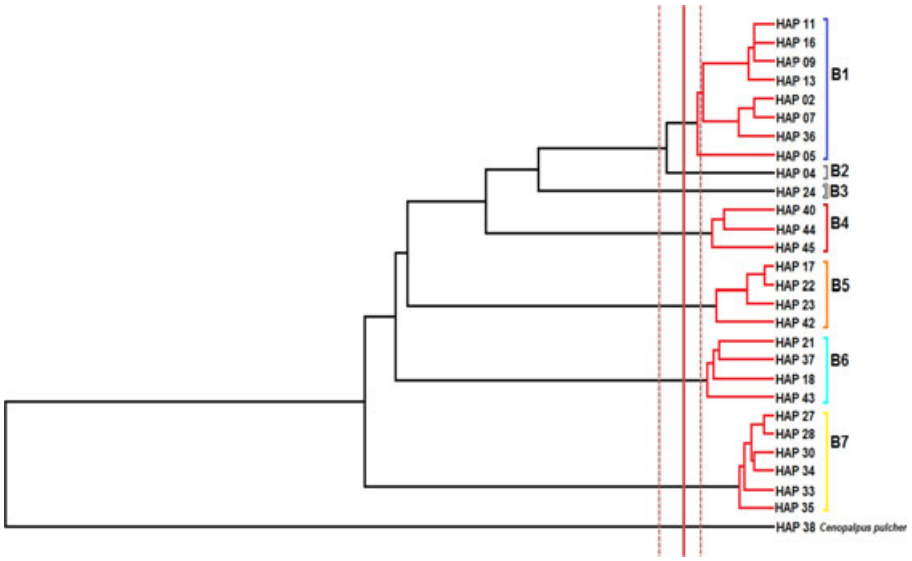

Fig. 2 *Brevipalpus* ultrametric tree and clusters of specimens recognised as putative species by the method of Pons *et al.* (2006). Genetic clusters recognised as a putative species are highlighted in red and separated by longer black branches. The solid vertical red bar indicates the threshold (Test Pons: 93.54 likelihood) which identify seven valid clusters plus the outgroup. The vertical hatched red bars indicate the incertitude zone which spans between six and eight clusters plus the outgroup.

both the first and second step of morphological identification.

Nucleotide divergences and diagnostic sites among Brevipalpus *putative species*

The pairwise nucleotide distances for the set of putative species studied here are presented in Table S1. The divergence in the COI gene fragment among *Brevipalpus* putative species ranged from 3.37% to 10.6%. The lowest interspecific genetic divergence was between B6 (*B. obovatus*) and B7 (*B. chilensis*) (3.37%), and the two highest were between B3 (*Brevipalpus* sp.) and B5 (*B. phoenicis* type 1) (10.6%) and between B1 (*Brevipalpus phoenicis* type 2) and B7 (*B. chilensis*) (10.5%). The distances between the representatives of the genus *Brevipalpus* studied here and the outgroup, *C. pulcher*, ranged from 14.5% to 19%. The highest intraspecific nucleotide divergence was observed for *B. californicus* (2.97%).

Aiming to further investigate the taxonomic status of B2 putative species, as it branched in the uncertainty zone in the Pons analysis (Fig. 2), the pairwise nucleotide distances were evaluated for two cases: 1) B2 as a different taxon from B1 (*B. phoenicis* type 2); and 2) B2 as synonymous with B1 (*B. phoenicis* type 2), its closest putative species. In case 1, when B2 was considered a different taxon, the B1 intraspecific distance was 1.58; in case 2, when B2 was placed together with B1, the intraspecific distance for this combined taxon was slightly higher, 1.68%. This value is lower than the intraspecific distance observed for B4 (*B. californicus*) (2.97%), the putative species with the highest intraspecific divergence. In case 1, the distance between B1 and B2 was 2.6%, lower than the distance observed between *B. chilensis* and *B. obovatus* (3.37%), the closest recognised taxa in this study.

A total of 51 polymorphic sites that could be used to differentiate at least two *Brevipalpus* putative species were found along the 358 bp COI alignment (Fig. S1). Two diagnostic sites (alignment positions 23 and 170) differentiated B1 (*B. phoenicis* type 2) and B2 (*Brevipalpus* sp.). The highest number of diagnostic positions (18) was observed for B3, including 11 polymorphic sites exclusive for this putative species (alignment positions 65, 71, 155, 182, 191, 230, 239, 263, 277, 280 and 335). Three diagnostic positions (alignment positions 98, 200 and 302) were observed between the closest putative species, B6 (*B. obovatus*) and B7 (*B. chilensis*).

Discussion

The systematics of the genus *Brevipalpus* represents a challenge for agricultural acarologists. Morphological studies have been conducted to characterise *Brevipalpus* species (Welbourn *et al.* 2003; Beard *et al.* 2012), and important taxonomic characteristics have been rediscovered and employed (Beard *et al.* 2012). The combination of a molecular approach together with a detailed morphological identification of *Brevipalpus* samples from this study revealed the occurrence of cryptic species in the group and contributed to the clarification of the previously noted incongruencies. Morphology was evaluated in a phylogenetic

context, and new traits were pointed out to differentiate *Brevipalpus* species.

Cryptic species in Brevipalpus *mites revealed through an integrative approach*

The Pons analysis used in this study indicated that there were six putative *Brevipalpus* species, whereas preliminary morphological identification detected only four species. Two (B3 and B5) of the six putative species were cryptic and have previously been misidentified as *B. phoenicis*. A detailed morphological study, taking into account the results of the delimitation of species, allowed us to detect taxonomic traits that characterise the cryptic taxa.

Although specimens of the putative species B3 are close to B1 (*B. phoenicis* type 2), morphological evidences observed during the detailed morphological identification supported it as a different taxon. Comparison of B3 with all other species of *B. phoenicis* group showed it represents a new taxon. The description of this new *Brevipalpus* species is presented below and it includes morphological description and molecular polymorphisms that allow the differentiation of this new taxon from the others included in this study (Fig. S1).

Putative species B5 consisted of four haplotypes (HAP 17, 22, 23 and 42) including samples collected in this study as well as by Groot & Breeuwer (2006). Those authors noted a clear conflict between the morphological identifications and the molecular phylogeny of specimens representatives of HAP 23 and HAP 42 (Table 1), which were morphologically identified as *B. phoenicis* but clustered with *B. obovatus*. Although a morphological study of the Groot & Breeuwer (2006) specimens from which the sequences in HAP 23 and 42 were obtained was not possible, we examined specimens from the same HAP 23 that were collected in this study as well as specimens of HAP 22 and HAP 17. The B5 specimens exhibited the traditional morphological traits that were usually accepted to characterise *B. phoenicis*. However, some morphological differences, mainly the shape of the spermatheca vesicle, and the group's phylogenetic position support its proximity with *B. obovatus* and *B. chilensis*, which explains the inconsistencies observed by Groot & Breeuwer (2006). The B5 specimens corresponded to *B. phoenicis* type 1 presented by Beard *et al.* (2012), and taking phylogeny into account, this taxon should be included in the *B. obovatus* group instead of remaining in the *B. phoenicis* group. According to R. Ochoa & J. Beard (personal communication), *B. phoenicis* type 1 was erroneously synonymised with *B. phoenicis* in the past, and a publication renaming and redescribing this taxon is in preparation.

B2, the only putative species that fell in the uncertainty zone of the Pons analysis ultrametric tree, is comprised by HAP 04, collected from *Hibiscus* in Pernambuco, Northeast Brazil. Pairwise nucleotide distances between B2 and B1 (*B. phoenicis* type 2) did not support the hypothesis that B2 is a putative species but rather reflects the variability of B1 (*B. phoenicis* type 2). Although specimens of B2 are closely related to B1 (*B. phoenicis* type 2), some clear morphological and biological (presence of males) differences were observed, as mentioned above. Two possible explanations for this are that B2 represents 1) a *B. phoenicis* type 2 lineage in the process of speciation; or 2) a hybrid between *B. phoenicis* type 2 and a species with one solenidia on tarsus II, for example *B. obovatus*. No clear conclusions can be drawn on the status of B2 as a new taxon based on the available data. In this study, we considered B2 as '*B.* aff *phoenicis* type 2'. Future studies to clarify B2 status should include data of other genomic regions, as for example the nuclear intergenic spacer region (ITS).

Two aspects of evolution, in particular, are responsible for the difficulties in delimiting species: variability within populations and the existence of incipient species that are in the process of genetic reconstruction and the acquisition of isolating mechanisms (Mayr & Ashlock 1991). When speciation proceeds with a complete and thorough splitting of a lineage, species are clearly demarcated and easy to recognise and describe. However, other phenomena can obscure or confuse this major pattern, particularly for asexual organisms (Winston 1999). Eukaryotic organisms can receive transferred genetic material from other organisms via the incorporation of symbionts, lateral transfer and hybridisation (Winston 1999). Hybridisation has been investigated or reported for species and races of different mites (e.g. Hau-Hong 1988; Vala *et al.* 2000; Badek & Dabert 2006), and *Wolbachia* symbionts are involved in hybrid breakdown in tetranychid mites (Vala *et al.* 2000). A peculiar reproduction method is reported for *Brevipalpus* mites; some species, such as *B. phoenicis*, reproduce by thelytokous parthenogenesis, a type of parthenogenesis in which females are produced from unfertilised eggs. In the genus *Brevipalpus*, this parthenogenesis is induced by a bacterial symbiont of the genus *Cardinium*, which is responsible for the feminisation (Weeks *et al.* 2001; Kitajima *et al.* 2007). It is possible that *Cardinium* symbionts could be involved in hybridisation among *Brevipalpus* mites, as has been reported for whitefly biotypes (see Thierry *et al.* 2011), and that B2 could be a result of this process, presenting variable biological and phenotypic characteristics that could be related to its parental origin. This hypothesis requires further investigation.

Beard *et al.* (2012) reported the occurrence of three types of *B. californicus*. Among the *B. californicus* samples evaluated in this study, just one putative species was recognised by both molecular and morphological analyses, corre-

sponding to *B. californicus* type 2 (see Beard *et al.* 2012), despite the relatively high level of intraspecific nucleotide divergence (2.97%), the highest observed in this study. Only three *B. californicus* samples were included in this study. Taxonomic status of the *B. californicus* types identified by Beard *et al.* (2012) based on morphology, whether they consist of cryptic species or simply intraspecific variability, needs to be further investigated by combining molecular data.

Brevipalpus *phylogeny and the value of morphological traits*

The integrative analysis combining two DNA-based phylogenetic approaches and a morphological study of some *Brevipalpus* species enabled an evaluation of the phylogenetic value of available taxonomic characteristics and identified those with the most power to discriminate among species in this genus. Pons analysis results were helpful to interpretating low supported nodes in ML phylogeny, which warranted that distinction among haplotypes was not clear.

The genus *Brevipalpus* has been divided into groups based on the number of dorsal opisthosomal setae, number of setae on the palpal tarsus and number of solenidia (*omega*) on the tarsus of leg II (Baker *et al.* 1975). The present study reinforce that a revision of the concept of groups in the *Brevipalpus* genus is necessary because the morphological traits currently used to define groups do not fit with its phylogeny. For example, we observed variation in the number of solenidia (*omega*) on the tarsus of leg II in monophyletic groups and even within one haplotype. The monophyletic group composed of lineages 5, 6 and 7 clustered *B. obovatus* and *B. chilensis* (both with one solenidia (*omega*) on tarsus II) together with *B. phoenicis* type 1 (with two solenidia (*omega*) on tarsus II). Similarly, the monophyletic group composed of lineages 1 and 2 that represents *B. phoenicis* type 2 and B2 is characterised by either one or two solenidia (*omega*) on tarsus II, as there is variation among specimens in B2. Intraspecific variation in the number of solenidia on the tarsus of leg II has long been reported (e.g. De Leon 1967). Another interesting observation by Kitajima *et al.* (2010) was the asymmetry in the number of solenidia in tarsi II in some *B. phoenicis* and *B. obovatus* in South America. All evidence suggests that the number of solenidia (*omega*) on tarsus II is neither a phylogenetically informative trait nor a reliable taxonomic characteristic for *Brevipalpus* mites.

The spermatheca has been widely used in the systematics of several groups of plant mites, for example predatory mites of the family Phytoseiidae (Chant & McMurtry 2007), and for phytophagous spider mites (see Vacante 1983, 1984). Surprisingly, the spermatheca has not been currently used for the identification of Tenuipalpidae mites. Castagnoli (1974) was the first to report the presence of spermatheca in the Tenuipalpidae and described this organ for eight species including two *Brevipalpus* species: *B. californicus* and *B. olivicola* Pegazzano & Castagnoli. Later, Baker & Tuttle (1987) also depicted the spermatheca of some *Brevipalpus* Mexican species. In a recent work by Beard *et al.* (2012), an identification key and taxonomic information for eleven *Brevipalpus* species were provided, including the presence and shape of the spermatheca vesicle. The present study emphasises the importance of the spermatheca, a rediscovered characteristic, for *Brevipalpus* taxonomy and phylogeny and shows that it represents an interesting taxonomic trait at the group or species level. Similar spermatheca shapes were observed in *B. obovatus*, *B. chilensis* and *B. phoenicis* type 1, close taxa that comprise a monophyletic group; in addition, the few differences in spermatheca shape that were observed among these species (e.g. the distribution of projections) could help to differentiate them (see Fig. 3, Table S2). The spermatheca of *B. californicus*, a species that is not closely associated with any other in the studied group, exhibited a unique shape that was easily differentiated from those of other species. Unfortunately, information on spermatheca shape is only available for a limited number of *Brevipalpus* species (see Castagnoli 1974; Baker & Tuttle 1987; Beard *et al.* 2012), as it is not a characteristic traditionally included in species descriptions. Detailed information on the spermatheca should be included in future taxonomic studies involving *Brevipalpus* mites.

Reticulation patterns have not been considered reliable for identification of the most important *Brevipalpus* species because a number of factors can influence their appearance. The amount of reticulation on the propodosoma and opisthosoma can vary with age and diet (Ochoa 1985; Evans *et al.* 1993). Mounting techniques can also affect how the ornamentation of the propodosoma and opisthosoma appears under light microscopy (Welbourn *et al.* 2003). In this study, the observation of reticulation patterns on the propodosoma, opisthosoma and ventral plates was essential to recognise the cryptic species identified by the Pons analysis as putative species (see Table S2). In addition, details of the propodosoma, opisthosoma and ventral plate reticulation patterns were used to characterise species and infraspecific categories within *Brevipalpus* by Beard *et al.* (2012). To benefit from the taxonomic information provided by cuticle reticulation patterns in *Brevipalpus* mites and minimise the factors that influence their appearance, mounting procedures should be standardised as much as possible, and morphological studies should consider a large number of specimens.

Leg chaetotaxy has long been considered paramount to understanding the Tetranychoidea (Lindquist 1985), the superfamily which includes Tetranychidae and Tenuipalpi-

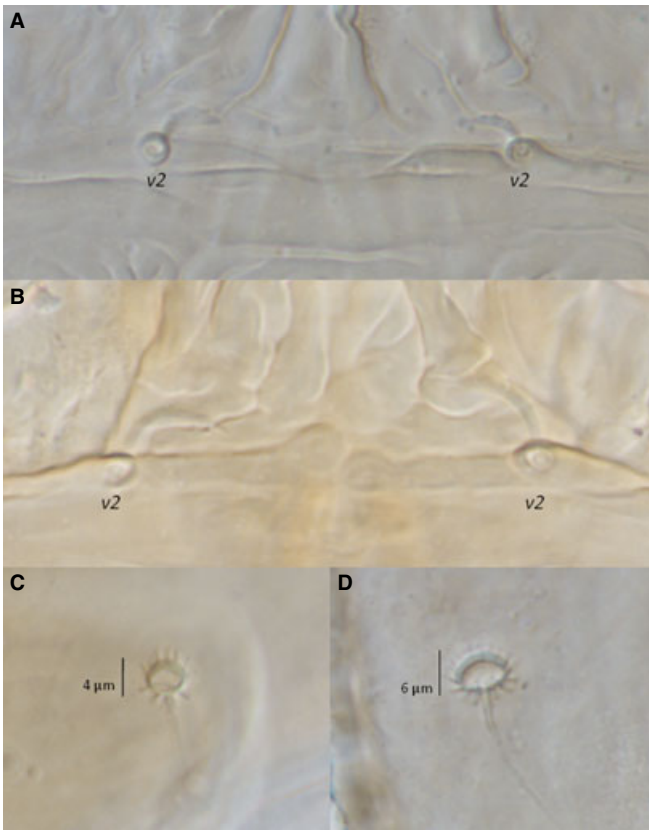

Fig. 3 Morphological differences between *Brevipalpus obovatus* Donnadieu and *B. chilensis* Baker. A and C. *B. obovatus*. —A. Prodorsal setae *v2*. —C. Spermatheca vesicle. —B and D. *B. chilensis*. —B. Prodorsal setae *v2*. —D. Spermatheca vesicle.

dae. However, these data are rarely presented for Tenuipalpidae. Seeman & Beard (2011) considered leg chaetotaxy an informative addition to descriptions in Tenuipalpidae, particularly for phylogenetic analyses. With respect to *Aegyptobia*, these authors suggested that such information could help subdivide the genus. For *Brevipalpus* mites, Welbourn *et al.* (2003) presented leg chaetotaxy for three species: *B. phoenicis*, *B. obovatus* and *B. californicus*. Those authors observed that the leg chaetotaxy was identical for these species, with the exception of the number of solenidia (ω) on tarsus II. The same was observed for the *Brevipalpus* species studied herein. However, in this study, we observed the femoral setae *d* on leg I showed clear differences in length between close taxa. Although it has not been considered a valuable characteristic for *Brevipalpus* systematics, leg chaetotaxy should still be considered in taxonomic studies, and in addition to the arrangement, information on length and shape of some setae should be provided.

The accurate identification of *B. chilensis* has been extremely important in the international trade of fresh fruits exported from Chile as it represents a quarantine pest for other South American countries, the USA and Europe, where it has often been intercepted (Navia *et al.* 2006). *Brevipalpus obovatus*, which is widely distributed around the world, is the closest species to *B. chilensis*. The trait currently used to distinguish these species is the reticulation pattern in the central area of the dorsal propodosoma (smooth in *B. obovatus* and uniform in *B. chilensis*) (Baker 1949; González 1958). Defining molecular polymorphisms that differentiate these species is very important because it would permit the identification of any intercepted developmental stage; in this study is showed that three COI polymorphic sites (see Results) can be used to differentiate *B. chilensis* and *B. obovatus* (Fig. S1). Furthermore, the availability of additional morphological characteristics to support identifications would also be very useful. In addition to differences recently noted by Beard *et al.* (2012), in this study the shape and length of the propodosomal seta *v2* is defined as a new trait to distinguish between *B. chilensis* and *B. obovatus*. This seta is shorter (length approximately 1/5 the distance between bases) and lanceolate/spatulated in *B. obovatus* vs. longer (length approximately 1/3 the distance between bases) and setiform serrate in *B. chilensis* (see Table S2, Fig. 3A–B). Associated with these characteristics,

differences in the size and in the number, distribution and length of the projections around the spermatheca vesicle were observed (see Table S2, Fig. 3C–D).

Associated with these characteristics, differences in the size and in projections around the spermatheca vesicle were observed (see Table S2, Fig. 3C–D). Vesicle spermatheca is smaller in *B. obovatus* (4 μm in diameter) than in *B. chilensis* (6 μm in diameter). In *B. obovatus*, spermatheca vesicle projections are less numerous and are concentrated, while in *B. chilensis*, they are more numerous and are distributed around the whole surface.

Advances in Brevipalpus *systematics and implications for pest management*

The recognition of cryptic species within *Brevipalpus* could have implications for prevention and pest management. Each *Brevipalpus* species is expected to have specific biological and ecological traits, for example range of host plants, associated natural enemies, population dynamics, plant genetic resistance. Control information previously obtained on *B. phoenicis*, which actually consist in a group of cryptic species, has to be readdressed. Virus vector activity was confirmed for only three *Brevipalpus* species: *B. californicus*, *B. obovatus* and *B. phoenicis* (Childers *et al.* 2003a). Control of *Brevipalpus* mites represents a key factor in the management of BTVs. Information on virus-mite relationships should include the progress made in *Brevipalpus* systematics. This study lays the foundations to better investigate the ability of different *Brevipalpus* species to vector BTVs and whether they are as widespread as previously believed. In this study, B1 (*B. phoenicis* type 2) was the dominant species associated with citrus and coffee in the Americas, and some samples were collected from BTV-affected areas. B1 most likely represents the main vector of CiLV and CoRSV. However, the efficacy of virus transmission by the 'hidden' *B. phoenicis* species recognised in this paper is unknown. Some questions arise immediately. For instance, symptoms of *Ligustrum ringspot virus* (LigRSV), a BTV associated with *B. phoenicis*, were observed on ligustrum plants from which a *B. phoenicis* type 1 was collected in Brazil. Is *B. phoenicis* type 1 the only vector of LigRSV, or can both type 1 and type 2 transmit this virus?

This study represents a first step in a long-term project aimed at a deep revision of *Brevipalpus* taxonomy based on phylogenetic and ecological data. Morphological methods are ill-suited for recognising cryptic species, and when morphology can succeed in delimiting species, other approaches can assist significantly and provide phylogenetic information on the group (Schlick-Steiner *et al.* 2010). We are confident that future investigations employing an integrative approach, combining molecular data and detailed morphological study, will help to clarify the systematics of this important and confusing group.

Taxonomy

Genus Brevipalpus *Donnadieu, 1875*

Brevipalpus incognitus *Ferragut & Navia n. sp. (Figs 4–6 and S2)*.

Holotype. f, BRAZIL, Minas Gerais, Janaúba, 15°49′49″ S 43°16′8″W, on *Cocos nucifera* L. (Arecaceae), 10 May 2007, coll. D. Navia, deposited at reference collection of Laboratory of Acarology, Embrapa Recursos Genéticos e Biotecnologia, Brasília, DF, Brazil.

Paratypes. Same locality as for holotype. 10 May 2007, D. Navia, and 3 September 2008, coll. D. Navia and L. C. Miranda. Paratypes (34♀ + 3 deutonymphs) were deposited at reference collection of Laboratory of Acarology, Embrapa Recursos Genéticos e Biotecnologia, Brasília, DF, Brazil; other paratypes (11♀ + 1 deutonymph) are deposited at Laboratory of Acarology, Instituto Agroforestal Mediterráneo, Universidad Politécnica de Valencia, Valencia, Spain.

COI sequence: GenBank accession KC291390.

Etymology. The Latin name *incognitus* means unknown, not examined, and refers to its previous cryptic status, which made it unnoticed among other related taxa.

Diagnosis (female). Dorsal surface with 12 pairs of serrated or barbed, lanceolate or foliate (leaflike) pedunculate setae. Crateriform pores present on propodosoma and opisthosoma. Dorsal propodosoma entirely reticulate, with areolae in the median area. Submedian and lateral areas covered by irregular polygonal cells with rounded angles. Dorsal opisthosoma between *c1-c1* and *d1-d1* smooth to wrinkled; V-shaped folds between and posterior to *e1-e1*. Ventral cuticle between legs III and IV mostly smooth and punctate. Reticulation pattern of ventral and genital plates with elongated and transversely aligned cells. Tarsus I with one solenidum, tarsus II with two solenidia. Seta *v2* leaflike, approximately as long as 1/2 the distance between the bases. Palp femur seta acuminate or slightly sublanceolate and barbed. Dorsal seta on femur I broadly leaflike and as long as the width of the segment. Dorsal seta on femur II foliate and shorter than the width of the segment.

Description. *Female (holotype)* (range of 10 paratypes in parentheses. Figs 4–5).

Dorsum (Figs 4A and S2). Body size measurements: distance between setae *v2-h1* 210 (209-222); *c3-c3* 140 (138-148); *c1-h1* 132 (129-139); *v2* to dorsal disjugal furrow 73 (73-80); other measurements: *v2-v2* 32 (32-40); *sc1-sc1* 86

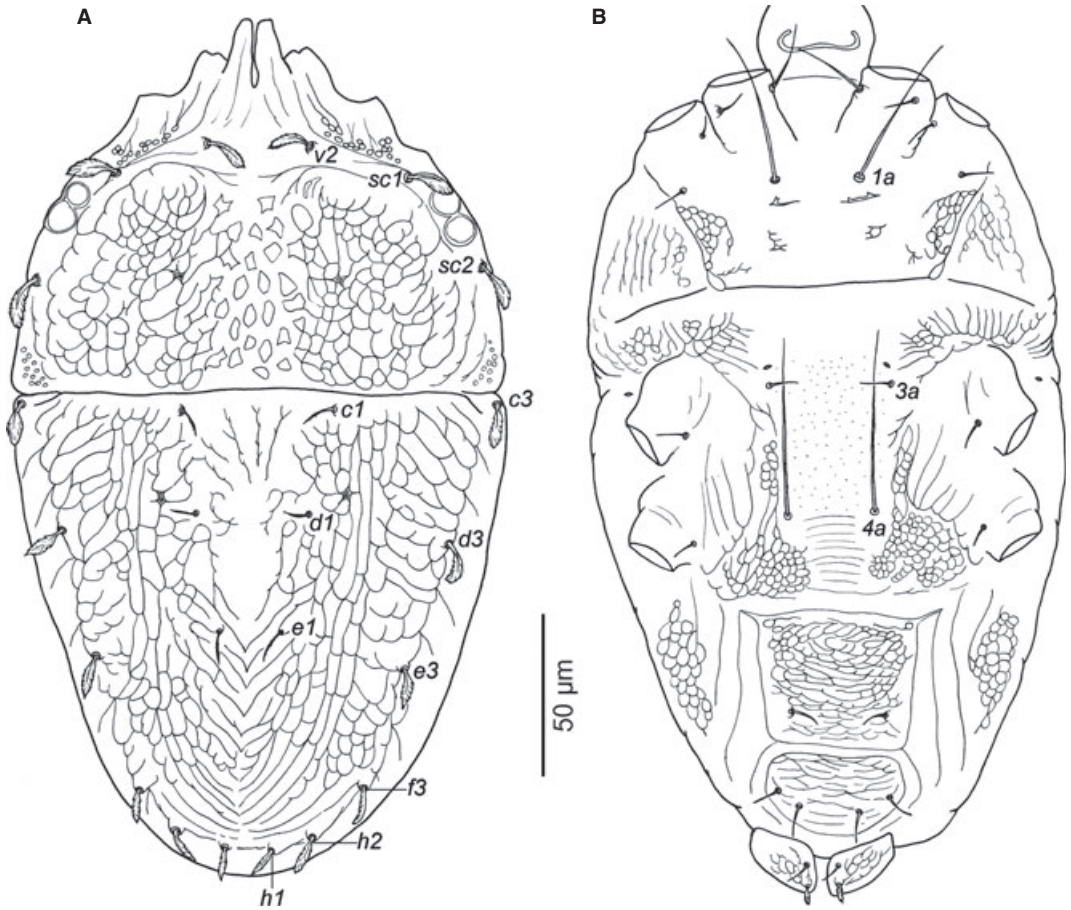

Fig. 4 —A–B. *Brevipalpus incognitus* Ferragut & Navia sp. n. female. —A. Dorsal view. —B. Ventral view.

(86-97); *sc2-sc2* 132 (128-136); *c1-c1* 45 (44-50); *d1-d1* 34 (27-35); *d3-d3* 114 (110-122); *e1-e1* 18 (15-18); *e3-e3* 93 (90-100); *f3-f3* 68 (68-73); *h1-h1*14 (14-18); *h2-h2* 40 (40-51). Rostrum extending to middle of femur I. Anterior projection of rostral shield not extending beyond the base of femur I. Rostral shield and lateral projections sometimes with a distinct basal area of small rounded cells. Rostral shield 38 (34-42). Pores visible on prodorsum and opisthosoma. Propodosoma entirely reticulated; median area areolate; submedian and lateral areas filled with irregular cells, some of them longitudinal, elongated or fused; anterolateral area with some open cells. Opisthosomal area between *c1-c1* and *d1-d1* wrinkled and between *d1-d1* and *e1-e1* mostly smooth; V-shaped folds between and posterior to *e1-e1*; sublateral area with irregular cells, some of them elongated and fused, forming longitudinal chains; reticulated or wrinkled area between longitudinal cells and dorsolateral setae; lateral area mostly smooth. Dorsal setae *v2*, *sc1*and *sc2* leaflike; *c1*, *d1* and *e1* sublanceolate; *c3*, *d3*, *e3*, *h1* and *h2* lanceolate to leaflike; all dorsal setae strongly serrate and clearly pedunculated except for *c1*, *d1* and *e1*, which are slightly barbed. Setal measurements: *v2* 16 (13-17); *sc1* 15 (13-17); *sc2* 17 (14-18); *c1* 9 (8-12); *c3* 14 (13-17); *d1* 9 (7-9); *d3* 13 (12-15); *e1*10 (7-11); *e3* 14 (12-16); *f3* 12 (11-14); *h1* 9 (9-12); *h2* 10 (10-12).

Venter (Fig. 4B) of propodosoma mostly smooth, except for regular or elongated fused cells on the areas surrounding coxa II. Anterior area of metapodosoma smooth, punctate between the insertions of legs III and IV. Four poroids transversely aligned at level of leg III. Cuticle wrinkled anterior to coxa III and ornamented with small rounded cells posterior to coxa IV. Lateral area posterior to leg IV with rounded cells grouped like a cluster. Ventral plate 41 (36-43) long, 48 (44-50) wide, reticulation pattern with medium-sized cells, mostly elongated and transversely aligned, rounded elements on anterior corners. Open, elongate cells on posterior part of the shield. Genital plate 28 (27-31) long, 45 (43-50) wide, reticulation pattern comprised mostly of transversal bands with some open cells near anterior margin. Setal measurements: *1a* 76 (76-88); *3a* 14 (12-17); *4a* 90 (87-93); *ag* 12 (12-15); *g1* 14 (12-16); *g2* 13 (12-14); *ps1* 10 (8-12); *ps2* 10 (10-12).

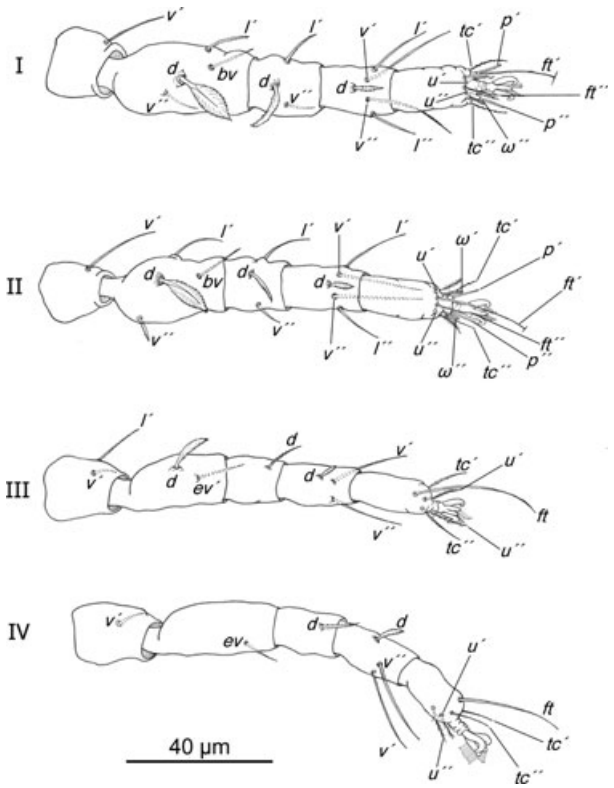

Fig. 5 *Brevipalpus incognitus* Ferragut & Navia sp. n. female. —Leg chaetotaxy (right legs). Legs I to III adaxial aspect; leg IV lateral view.

Palps Four-segmented. Setal formula 0-1-2-3 (1s+2e). Palp femur seta acuminate or slightly sublanceolate, barbed, 11 (10-13).

Legs (Fig. 5) Setal formula for legs I-IV (coxae to tarsi), 2-1-4-3-5-8(+1ω), 2-1-4-3-5-8(+2ω), 1-2-2-1-3-5, 1-1-1-1-3-5, respectively. Leg chaetotaxy as follows (trochanter to tarsus): trochanters I, II, IV *v'*; trochanter III *l'*, *v'*; femora I, II *d*, *l'*, bv, *v''*, femur III *d*, *ev'*, femur IV *ev'*; genua I, II *d*, *l'*, *v''*, genua III, IV *d*; tibiae I, II *d*, *l'*, *l''*, *v'*, *v''*, tibiae III, IV *d*, *v'*, *v''*; tarsus I *ft'*, *ft''*, ω'', *tc'*, *tc''*, *p'*, *p''*, *u'*, *u''*, tarsus II *ft'*, *ft''*, ω', ω'', *tc'*, *tc''*, *p'*, *p''*, *u'*, *u''*, tarsi III, IV *ft*, *tc'*, *tc''*, *u'*, *u''*. Dorsal seta *d* on femora I and II broadly leaflike and pedunculate. Dorsal seta on femur I 15 (15-17) as long as width of segment: dorsal seta on femur II 14 (13-15) shorter than width of segment.

Spermatheca (Fig. S2B) A long thin duct ending in an oval vesicle, 4 (4-6) long, with a long distal stipe-like projection, 12 (10-14). Stipe-like projection divided distally into two or three tips.

Male. Unknown.

Deutonymph (n = 4, Fig. 6)

Dorsum (Fig. 6A). Body size measurements: distance between setae *v2-h1* 198-221; *c3-c3* 118-132; *c1-h1* 125-138; *v2* to dorsal disjugal furrow 73-86; other measurements: *v2-v2* 29-35; *sc1-sc1* 82-86; *sc2-sc2* 116-124; *c1-c1* 30-36; *d1-d1* 26-35; *d3-d3* 94-102; *e1-e1* 7-10; *e3-e3* 78-82; *f3-f3* 60-64; *h1-h1*14-18; *h2-h2* 38-42. Rostral shield 26-28, mostly smooth with short longitudinal striae basally. Propodosoma and opisthosoma mostly smooth. Dorsal setae *v2*, *sc1*and *sc2* leaflike pedunculate; *c1*, *d1* minute, lanceolate and barbed, *e1* short, palmate serrate; *c3*, *d3*, *e3*, *h1* and *h2* leaflike pedunculate serrate. Setal measurements: *v2* 14-18; *sc1* 16-18; *sc2* 19-22; *c1* 5-6; *c3* 17-18; *d1* 5-6; *d3* 18-20; *e15*-6; *e3* 16-18; *f3* 17-20; *h1* 14-18; *h2* 17-18. One of the deutonymphs showed longer and foliate setae *c1*, *d1* and *e1* (*c1* 16, *d1* 11, *e1* 17).

Venter thoroughly striate. Cuticle between setae *1a* with longitudinal striae forming a basket posterior to *1a*. Other propodosomal areas with transverse striae. Opisthosoma with central area transversally striate. Setal measurements: *1a* 42-48; *3a* 10-14; *4a* 43-48; *ag* 5-8; *g1* 4-7; *ps1* 3-6; *ps2* 5-7.

Palps four-segmented. Setal formula 0-1-1-3 (1s+2e). Palp femur seta acuminate or slightly sublanceolate, barbed, 9-10.

Legs (Fig. 6B). Setal formula for legs I-IV (trochanter to tarsus), 2-1-3-3-5-8(+1ω), 2,-0-3-3-5-8(+1ω), 1-1-2-1-3-5, 1-0-1-1-3-5, respectively.

Protonymph and *larva*. Unknown.

Remarks. The new species belongs to the *B. phoenicis* species group (Baker & Tuttle 1987) and can be distinguished from other species in the group by the dorsal and ventral ornamentation and by the shape and/or relative length of the dorsal setae, palp femur seta and dorsal setae on femur I. *Brevipalpus incognitus* is similar to *B. phoenicis* type 2 (*sensu* Beard *et al.* 2012) in the presence of areolae on the median area of prodorsum, the dorsocentral opisthosomal ornamentation and the shape of the spermatheca. However, *B. incognitus* has a strongly reticulate prodorsum with cells almost uniform in size and shape, rather than smaller and narrower cells in anterior propodosoma in *B. phoenicis* type 2; the ventral and genital shields bears elongated cells, rather than broad and rounded cells in *B. phoenicis* type 2, and the dorsal seta on femur I is longer, being as long as the width of the segment (Fig. 5B), whereas in *B. phoenicis* type 2, it is shorter, approximately 60% of the width of the segment. In addition, females of *B. incognitus* are larger than those *B. phoenicis* type 2, and dorsolateral setae on the propodosoma and opisthosoma are longer, foliate and pedunculated in *B. incognitus*, instead of shorter, more lanceolated and much less pedunculated in *B. phoenicis* type 2. Molecular polymorphisms. 22 diagnostic sites along COI sequences distinguished the new species and *B. phoenicis* type 2 (alignment positions 2, 9, 65, 71, 80,

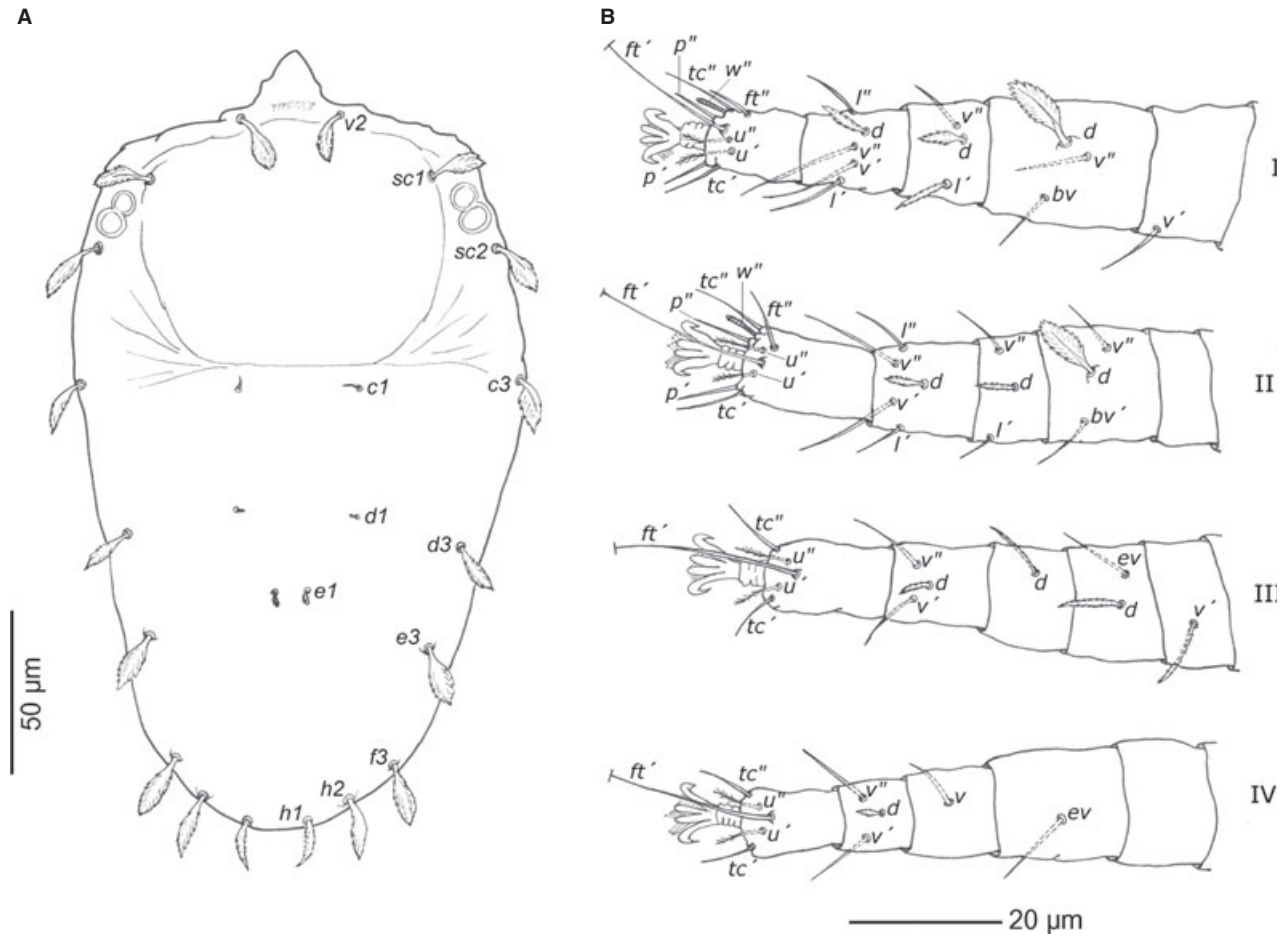

Fig. 6 —A–F. *Brevipalpus incognitus* Ferragut & Navia sp. n. deutonymph. —A. Dorsal aspect. —B. Leg chaetotaxy (right legs, adaxial aspect).

116, 125, 155, 179, 182, 191, 200, 224, 230, 239, 263, 277, 280, 284, 288, 326, 335) (Fig. S1).

Brevipalpus incognitus differs from *B. phoenicis* type 1 in the dorsal ornamentation and the shape of the spermatheca. Other related species such as *B. araucanus* González, *B. hondurani* Evans, *B. rugulosus* Chaudhri, Akbar & Rasool and *B. tarus* González lack areolae longitudinally aligned on the median area of the prodorsum.

DNA barcode. We amplified and sequenced a 358-bp fragment of the cytochrome oxidase subunit I (COI) gene in the DNA barcode region (a standardised genomic fragment of 650 bp) chosen by the Consortium for the Barcode of Life, http://barcoding.si.edu, for three females paratypes (haplotype acc. KC291390), as well as for specimens of *B. phoenicis* type 2. (haplotypes acc. KC291366-KC291368; KC291370-KC291382). No intraspecific variability in *B. incognitus* n. sp. was detected. Intraspecific K2P divergence in *B. phoenicis* type 2 was 1.58%. Comparison of the sequences from *B. incognitus* n. sp. and *B. phoenicis* type 2 showed that the average genetic divergence was 9.4% (8.6–10.9%). This mean genetic divergence was higher than the divergence between *B. phoenicis* type 2 and *B. californicus*, a species belonging to a different *Brevipalpus* species group (see Table S2).

Acknowledgements

We sincerely thank Dalva L. de Queiroz Santana, Embrapa Florestas, Colombo, Paraná; Aloyseia Noronha, Eduardo Chumbinho de Andrade, Juliana Freitas-Astúa and Francisco F. L. Barbosa, Embrapa Mandioca e Fruticultura, Cruz das Almas, Bahia; Alberto Luiz Marsaro Jr, Embrapa Roraima, Boa Vista, Roraima; Ricardo Adaime da Silva, Embrapa Amapá, Macapá, Amapá; Patricia Maria Drumond, Embrapa Acre, Rio Branco, Acre; Manoel Guedes C. Gondim Jr and Aleuny C. Reis, Universidade Federal Rural de Pernambuco, Recife, Pernambuco; José. C. M. Poderoso, Universidade Federal de Sergipe, São Cristóvão, Sergipe, all from Brazil;

and José Carlos V. Rodrigues, University of Puerto Rico, San Juan, Puerto Rico, for their help with sampling; Tatiane G. C. M. Galasso, Brazil, for help with extensive laboratory work. We also thank Maries Pages and Fabien Condamine, INRA, UMR CBGP, France, for their help concerning the Pons analysis. This study was funded by Brazil – National Council for Scientific and Technological Development (CNPq) (call CNPq/MAPA/SDA Nº 064/2008, grant 578353/2008-3), Embrapa Macroprograma 3 and Fundação de Apoio a Pesquisa do Distrito Federal (FAP-DF). Authors DN and RSM are also grateful to CNPq for the research and pos-doc fellowships, respectively.

References

Ahrens, D., Monaghan, M. T. & Vogler, A. P. (2007). DNA-based taxonomy for associating adults and larvae in multi-species assemblages of chafers (Coleoptera: Scarabaeidae). *Molecular Phylogenetics and Evolution*, *44*, 436–449.

Anisimova, M. & Gascuel, O. (2006). Approximate likelihood-ratio test for branches: a fast, accurate, and powerful alternative. *Systematic Biology*, *55*, 539–552.

Armstrong, K. F. & Ball, S. L. (2005). DNA barcodes for biosecurity: invasive species identification. *Philosophical Transactions of the Royal Society B*, *360*, 1813–1823.

Badek, A. & Dabert, J. (2006). The possible hybrid origin of the feather mite *Avenzoaria canuti* (Astigmata: Analgoidea) from the red knot *Calidris canutus* (Aves: Charadriiformes) – a morphological approach. *Biological Letters*, *43*, 119–130.

Baker, E. W. (1949). The genus *Brevipalpus* (Acarina: Pseudoleptidae). *The American Midland Naturalist*, *42*, 350–402.

Baker, E. W. & Tuttle, D. M. (1987). The false spider mites of Mexico (Tenuipalpidae: Acari). United States Department of Agriculture, Agricultural Research Service, Technical Bulletin, 1706, 1–236.

Baker, E. W., Tuttle, D. M. & Abbatiello, M. J. (1975). The false spider mites of northwestern and north central Mexico (Acarina: Tenuipalpidae). *Smithsonian Contributions to Zoology*, *194*, 1–22.

Beard, J. J., Ochoa, R., Redford, A. J., Trice, M. D., Walters, T. W. & Mitter, C. (2012). Flat mites of the World – Part I *Raoiella* and *Brevipalpus*. Identification Technology Program, CPHST, PPQ, APHIS, USDA; Fort Collins, CO, USA. Available via http://idtools.org/id/mites/flatmites/.

Bickford, D., Lohman, D. J., Sodhi, N. S., Ng, P. K. L., Meier, R., Winker, K., Ingram, K. K. & Das, I. (2007). Cryptic species as a window on diversity and conservation. *Trends in Ecology & Evolution*, *22*, 148–155.

Calcagno, V., Bonhomme, V., Thomas, Y., Singer, M. C. & Bourguet, D. (2010). Divergence in behaviour between the European corn borer, *Ostrinia nubilalis*, and its sibling species *Ostrinia scapulalis*: adaptation to human harvesting? *Proceedings of the Royal Society B: Biological Sciences*, *277*, 2703–2709.

Castagnoli, M. (1974). La spermateca in alcune specie di Brevipalpini (Acarina, Tenuipalpidae). *Redia*, *55*, 85–88.

Chagas, C. M., Kitajima, E. W. & Rodrigues, J. C. V. (2003). Coffee ringspot virus vectored by *Brevipalpus phoenicis* (Acari: Tenuipalpidae) in coffee. *Experimental and Applied Acarology*, *30*, 203–213.

Chant, D. A. & McMurtry, J. A. (2007). *Illustrated Keys and Diagnoses for the Genera and Subgenera of the Phytoseiidae of the World (Acari: Mesostigmata)*. West Bloomfield: Indira Publishing House.

Childers, C. C. & Derrick, K. S. (2003). *Brevipalpus* mites of unassigned rhabdovirus in various crops. *Experimental and Applied Acarology*, *30*, 1–3.

Childers, C. C., French, J. V. & Rodrigues, J. C. V. (2003a). *Brevipalpus californicus*, *B. obovatus*, *B. phoenicis*, and *B. lewisi* (Acari: Tenuipalpidae): a review of their biology, feeding injury and economic importance. *Experimental and Applied Acarology*, *30*, 5–28.

Childers, C. C., Rodrigues, J. C. V. & Welbourn, W. C. (2003b). Host plants of *Brevipalpus californicus*, *B. obovatus*, and *B. phoenicis* (Acari: Tenuipalpidae) and their potential involvement in the spread of viral diseases vectored by these mites. *Experimental and Applied Acarology*, *30*, 29–105.

Clarke, A. R. & Walter, G. H. (1995). "Strains" and the classical biological control of insect pests. *Canadian Journal of Zoology*, *73*, 1777–1790.

De Leon, D. (1967). Six new false spider mites from southern Florida (Acarina: Tenuipalpidae). *Florida Entomologist*, *39*, 55–60.

Dereeper, A., Guignon, V., Blanc, G., Audic, S., Buffet, S., Chevenet, F., Dufayard, J. F., Guindon, S., Lefort, V., Lescot, M., Claverie, J. M. & Gascuel, O. (2008). Phylogeny.fr: robust phylogenetic analysis for the non-specialist. *Nucleic Acids Research*, *36* (suppl 2), W465–W469.

Evans, G. A., Cromroy, H. L. & Ochoa, R. (1993). The Tenuipalpidae of Honduras (Tenuipalpidae: Acari). *Florida Entomologist*, *76*, 126–155.

Fontaneto, D., Boschetti, C. & Ricci, C. (2008). Cryptic diversification in ancient sexual: evidence from the bdelloid rotifer *Philodina flaviceps*. *Journal of Evolutionary Biology*, *21*, 580–587.

González, R. H. (1958). *Biología y control de la falsa arañita de la vid*, *Brevipalpus chilensis Baker (Acarina: Phytoptipalpidae)*. Boletín Técnico nº 1. Maipú: Estación Experimental Agronómica–Universidad de Chile.

Grandjean, F. (1939). Les segments post-larvaires de l'hystérosoma chez les Oribates (Acariens). *Bulletin de la Société Zoologique de France*, *64*, 273–284.

Groot, T. V. M. & Breeuwer, J. A. J. (2006). *Cardinium* symbionts induce haploid thelytoky in most clones of three closely related *Brevipalpus* species. *Experimental and Applied Acarology*, *39*, 257–271.

Guindon, S. & Gascuel, O. (2003). A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Systematic Biology*, *52*, 696–704.

Hall, T. A. (1999). BIOEDIT: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series*, *41*, 95–98.

Hau-Hong, L. (1988). Studies on hybridization of Trombiculidae mites. *Acta Entomologica Sinica*, *3*, 268–272.

Hebert, P. D. N., Ratnasingham, S. & de Waard, J. R. (2003). Barcoding animal life: cytochrome c oxidase subunit I divergences among closely related species. *Proceedings of the Royal Society of London B Biological Sciences*, *270*, S96–S99.

Hebert, P. D. N., Penton, E. H., Burns, J. M., Janzen, D. H. & Hallwachs, W. (2004). Ten species in one: DNA barcoding

reveals cryptic species in the neotropical skipper butterfly *Astraptes fulgerator*. *Proceedings of the National Academy of Sciences of the USA*, *101*, 14812–14817.

Henry, C. S. & Wells, M. M. (2010). Acoustic niche partitioning in two cryptic sibling species of *Chrysoperla* green lacewings that must duet before mating. *Animal Behaviour*, *80*, 991–1003.

Hoy, M. A. (2011). *Agricultural Acarology: Introduction to Integrated Pest Management*. Boca Raton: CRC Press.

Jones, D. T., Taylor, W. R. & Thornton, J. M. (1992). The rapid generation of mutation data matrices from protein sequences. *Computer Applications in the Biosciences*, *8*, 275–282.

Jousselin, E., Desdevises, Y. & Coeur d'acier, A. (2009). Fine-scale cospeciation between *Brachycaudus* and *Buchnera aphidicola*: bacterial genome helps define species and evolutionary relationships in aphids. *Proceedings of the Royal Society B: Biological Science*, *276*, 187–196.

Kimura, M. (1980). A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution*, *16*, 111–120.

Kimura, M. (1981). Estimation of evolutionary distances between homologous nucleotide sequences. *Proceedings of the National Academy of Sciences*, *78*, 454–458.

Kitajima, E. W., Chagas, C. M. & Rodrigues, J. C. V. (2003). *Brevipalpus*-transmitted plant virus and virus-like diseases: cytopathology and some recent cases. *Experimental and Applied Acarology*, *30*, 135–160.

Kitajima, E. W., Groot, T. V. M., Novelli, V. M., Freitas-Astúa, J., Alberti, G. & de Moraes, G. J. (2007). In situ observation of the *Cardinium* symbionts of *Brevipalpus* (Acari: Tenuipalpidae) by electron microscopy. *Experimental and Applied Acarology*, *42*, 263–271.

Kitajima, E. W., Rodrigues, J. C. V. & Freitas-Astúa, J. (2010). An annotated list of ornamentals naturally found infected by *Brevipalpus* mite-transmitted viruses. *Scientia Agricola*, *67*, 348–371.

Librado, P. & Rozas, J. (2009). DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics*, *25*, 1451–1452.

Lindquist, E. E. (1985). External anatomy. In W. Helle & M. W. Sabelis (Eds) *Spider Mites: Their Biology, Natural Enemies and Control*. World Crop Pests, Vol. *1A* (pp. 3–28). Amsterdam: Elsevier.

Lynch, M., Koskella, B. & Schaack, S. (2006). Mutation pressure and the evolution of organelle genomic architecture. *Science*, *311*, 1727–1730.

Maddison, W. P. & Maddison, D. R. (2011). Mesquite: a modular system for evolutionary analysis. Version 2.75. http://mesquiteproject.org

Mayr, E. & Ashlock, P. D. (1991). *Principles of Systematic Zoology*, 2nd edn. Singapore: McGraw-Hill.

Mendonça, R. S., Navia, D., Diniz, I. R., Auger, P. & Navajas, M. (2011). A critical review on some closely related species of *Tetranychus* sensu stricto (Acari: Tetranychidae) in the public DNA sequences databases. *Experimental & Applied Acarology*, *55*, 1–23.

Mesa, N. C., Ochoa, R., Welbourn, W. C., Evans, G. A. & Moraes, G. J. (2009). A catalog of the Tenuipalpidae (Acari) of the world with a key to genera. *Zootaxa*, *2098*, 1–185.

Navajas, M., Lagnel, J., Gutierrez, J. & Boursot, P. (1998). Species-wide homogeneity of nuclear ribosomal ITS2 sequences in the spider mite *Tetranychus urticae* contrasts with extensive mitochondrial COI polymorphism. *Heredity*, *80*, 742–752.

Navia, D., Moraes, G. J. & Flechtmann, C. H. W. (2006). Phytophagous mites as invasive alien species: quarantine procedures. In: J. B. Morales-Malacara, V. Behan-Pelletier, E. Ueckermann, T. M. Pérez, E. Estrada, C. Gispert & M. Badii (Eds). *Proceedings of the XI International Congress of Acarology, Acarology XI*. (pp. 87–96). Mexico DF: UNAM/ Sociedad Latinoamericana de Acarología.

Nicholas, K. B. & Nicholas, H. B., Jr (1997). *GeneDoc: A Tool for Editing and Annotating Multiple Sequence Alignments. Ver. 2.7.000*. Pittsburgh, PA: Pittsburgh Supercomputing Center's National Resource for Biomedical Supercomputing.

Ochoa, R. C. (1985). Reconocimento preliminar de los ácaros fitoparásitos del género *Brevipalpus* (Acari: Tenuipalpidae) en Costa Rica. PhD Thesis, Facultad Agronomia, Universidad de Costa Rica, 124 pp.

Pagès, M., Chavall, Y., Herbreteau, V., Waengsothorn, S., Cosson, J. F., Hugot, J. P., Morand, S. & Michaux, J. (2010). Revisiting the taxonomy of the Rattini tribe: a phylogeny-based delimitation of species boundaries. *BMC Evolutionary Biology*, *10*, 184.

Paterson, H. E. H. (1991). The recognition of cryptic species among economically important insects. In: M. P. Zalucki (Ed.) *Heliothis: Research Methods and Prospects* (pp. 1–10). Berlin: Springer-Verlag.

Pfenninger, M. & Schwenk, K. (2007). Cryptic animal species are homogeneously distributed among taxa and biogeographical regions. *BMC Evolutionary Biology*, *7*, 121.

Pons, J., Barraclough, T. G., Gómez-Zurita, J., Cardoso, A., Duran, D. P., Hazell, S., Kamoun, S., Sumlin, W. D. & Vogler, P. (2006). Sequence-based species delimitation for DNA taxonomy of undescribed insects. *Systematics Biology*, *55*, 595–609.

Posada, D. (2006). ModelTest Server: a web-based tool for the statistical selection of models of nucleotide substitution online. *Nucleic Acids Research*, *34*, Web Server issue, W701–W703.

Posada, D. & Crandall, K. A. (1998). Modeltest: testing the model of DNA substitution. *Bioinformatics*, *14*, 817–818.

Quirós-González, M. J. (1986). *A Systematic and Phylogenetic Analysis of the World Genera of Tenuipalpidae Berlese (Acari: Tetranychoidea)*. PhD Thesis, Maryland: University of Maryland.

Rodrigues, J. C. V., Kitajima, E. W., Childers, C. C. & Chagas, C. M. (2003). Citrus leprosis virus vectored by *Brevipalpus phoenicis* (Acari: Tenuipalpidae) on citrus in Brazil. *Experimental and Applied Acarology*, *30*, 161–179.

Rodrigues, J. C. V., Childers, C. C., Gallo-Meagher, M., Ochoa, R. & Adams, B. J. (2004). Mitochondrial DNA and RAPD polymorphisms in the haploid mite *Brevipalpus phoenicis* (Acari: Tenuipalpidae). *Experimental and Applied Acarology*, *34*, 274–290.

Ronquist, F. & Huelsenbeck, J. P. (2003). MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, *19*, 1572–1574.

Ros, V. I. D. & Breeuwer, J. A. J. (2007). Spider mite (Acari: Tetranychidae) mitochondrial COI phylogeny reviewed: host plant relationships, phylogeography, reproductive parasites and barcoding. *Experimental and Applied Acarology*, *42*, 239–262.

Schlick-Steiner, B. C., Steiner, F. M., Seifert, B., Stauffer, C., Christian, E. & Crozier, R. H. (2010). Integrative Taxonomy: a

Multisource Approach to Exploring Biodiversity. *Annual Review of Entomology*, *55*, 421–438.

Seeman, O. D. & Beard, J. J. (2011). A new species of *Aegyptobia* (Acari: Tenuipalpidae) from Myrtaceae in Australia. *Systematic and Applied Acarology*, *16*, 73–89.

Shimodaira, H. & Hasegawa, M. (2001). CONSEL: for assessing the confidence of phylogenetic tree selection. *Bioinformatics*, *17*, 1246–1247.

Sites, J. W., Jr & Marshall, J. C. (2003). Delimiting species: a renaissance issue in systematic biology. *Trends in Ecology and Evolution*, *18*, 462–470.

Skoracka, A. & Dabert, M. (2010). The cereal rust mite *Abacarus hystrix* (Acari: Eriophyoidea) is a complex of species: evidence from mitochondrial and nuclear DNA sequences. *Bulletin of Entomological Research*, *100*, 263–272.

Skoracka, A., Kuczynski, L., Mendonça, R. S., Dabert, M., Szydlo, W., Knihinicki, D., Truol, G. & Navia, D. (2012) Cryptic speciation of the wheat curl mite *Aceria tosichella* (Keifer) (Acari, Eriophyoidea) revealed by mitochondrial, nuclear and morphometric data. *Invertebrate Systematics*, *26*, 417–433.

Swofford, D. L. (2003). *PAUP* Phylogenetic Analysis Using Parsimony (*and other methods) Ver. 4*. Sunderland, MA: Sinauer Associates.

Tamura, K. & Nei, M. (1993). Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution*, *10*, 512–526.

Tamura, K., Dudley, J., Nei, M. & Kumar, S. (2007). MEGA4: molecular evolutionary genetics analysis (MEGA) software. Version 4.0. *Molecular Biology and Evolution*, *24*, 1596–1599.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. & Kumar, S. (2011). MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution*, *28*, 2731–2739.

Thierry, M., Becker, N., Hajri, A., Reynaud, B., Lett, J. M. & Delatte, H. (2011). Symbiont diversity and non-random hybridization among indigenous (Ms) and invasive (B) biotypes of *Bemisia tabaci*. *Molecular Ecology*, *20*, 2172–2187.

Thompson, J. D., Higgins, D. G. & Gibson, T. J. (1994). ClustalW — improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research*, *22*, 4673–4680.

Tixier, M. S., Mireille, O. & Kreiter, S. (2011). An integrative morphological and molecular diagnostics for *Typhlodromus pyri* (Acari: Phytoseiidae). *Zoologica Scripta*, *41*, 68–78.

Vacante, V. (1983). Prima raccolta di acari Tetranichidi in Sicilia. *Phytophaga*, *1*, 41–132.

Vacante, V. (1984). Note sul Briobiini italiani (Acari: Tetranychidae) I. *Frustula Entomologica*, *7–8*, 559–573.

Vala, F., Breeuwer, J. A. J. & Sabelis, M. W. (2000). *Wolbachia*-induced "hybrid breakdown" in the two spotted spider mite *Tetranychus urticae* Koch. *Proceedings Royal Society London B*, *267*, 1931–1937.

Weeks, A. R., Marec, F. & Breeuwer, J. A. J. (2001). A mite species that consists entirely of haploid females. *Science*, *292*, 2479–2482.

Welbourn, W. C., Ochoa, R., Kane, E. C. & Erbe, E. F. (2003). Morphological observations on *Brevipalpus phoenicis* (Acari: Tenuipalpidae) including comparisons with *B. californicus* and *B. obovatus*. *Experimental and Applied Acarology*, *30*, 107–133.

Wiens, J. J. (2007). Species delimitation: new approaches for discovering diversity. *Systematic Biology*, *56*, 875–878.

Winston, J. (1999). *Describing Species. Practical Taxonomic Procedure for Biologists*. New York: Columbia University Press.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig. S1. Sequence variation of the mitochondrial COI alignment of *Brevipalpus* haplotypes.

Fig. S2. A-B. *Brevipalpus incognitus* Ferragut & Navia sp. n. female. – A. Prodorsum . – B. Spermatheca vesicle.

Table S1. Pairwise distance among COI sequences (358 bp) of six *Brevipalpus* putative species recognised by Pons analysis plus B2 (a putative species in the uncertainty zone of the ultrametric tree not completely recognised by Pons analysis), calculated using Kimura's two-parameter correction.

Table S2. Morphological traits of *Brevipalpus* putative species (females) recognised by Pons analysis (including B2, a putative species in the uncertainty zone of the ultrametric tree), present identification (*Brevipalpus* type identification according to Beard *et al.* (2012)) and previous identification (see Material and methods).