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SISTEMÁTICA DE ÁCAROS PLANOS DO GÊNERO *BREVIPALPUS* DONNADIEU (TROMBIDIFORMES, TENUIPALPIDAE) - ESPÉCIES ASSOCIADAS A PLANTAS CULTIVADAS E NATIVAS EM AÇORES, PORTUGAL

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JORGE LAERSON DOS SANTOS ALVES

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Dissertação apresentada à Universidade de Brasília, como parte das exigências para obtenção do título de Mestre em Zoologia

Área de concentração: Sistemática

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CAPÍTULO 4

Autor: Alves, Jorge Laerson dos Santos

Título: Sistemática de ácaros planos do gênero *Brevipalpus* donnadieu (trombidiformes, Tenuipalpidae) - espécies associadas a plantas cultivadas e nativas em Açores, Portugal.

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Keywords: phytophagous mites, Macaronesia, integrative taxonomy, molecular systematics, Prostigmata.

Resumo: O gênero *Brevipalpus* Donnadieu (Prostigmata: Tenuipalpidae) constitui um importante grupo de ácaros fitófagos que estão distribuídos em todas as regiões biogeográficas. Este gênero inclui 291 espécies, sendo algumas delas consideradas pragas em agroecossistemas. Danos diretos, causados pela alimentação, e indiretos, pela transmissão de vírus de plantas (VTBs – vírus transmitidos por *Brevipalpus*) são reportados em diversas culturas agrícolas no Brasil e no mundo, além de plantas ornamentais e florestais. A sistemática dos ácaros *Brevipalpus* tem representado um desafio para os acarologistas. Além de muitas sinonímias ao longo da história do grupo, a ocorrência de espécies crípticas tem sido confirmada nas duas últimas décadas. Os ácaros da espécie *B. phoenicis* (Geijskes) eram considerados os de maior importância econômica e um dos mais estudados, entretanto, após ser revisado algumas espécies foram ressucistadas, como *B. yothersi*, e espécies crípticas foram descritas, de forma que toda informação taxonômica e bioecológica associada precisa ser revisada com algumas outras espécies. A identificação acurada destes ácaros é fundamental para a prevenção da introdução de espécies não presentes no País, dando suporte aos procedimentos de interceptação e contribuindo para o fortalecimento dos serviços de defesa fitossanitária. O conhecimento da fauna de regiões escassamente estudadas fora do Brasil, como no Arquipélago de Açores, objeto do presente estudo, pode ajudar na identificação de espécimes interceptados. Para tanto, estudos morfológicos com a utilização de diferentes técnicas de microscopia, integrados com dados moleculares obtidos de regiões distintas do genoma, têm possibilitado um imenso avanço na taxonomia do grupo. O presente trabalho teve como objetivo geral contribuir para o avanço da sistemática de ácaros *Brevipalpus*. Essa dissertação foi organizada em três capítulos estruturados como artigos científicos, precedidos por um capítulo inicial comum (capítulo 1) contendo a introdução e contexto do trabalho, os objetivos e as referências bibliográficas. O segundo capítulo apresenta os resultados de um levantamento da fauna de *Brevipalpus* no Arquipélago de Açores, nas ilhas do Pico, Faial e Flores, realizado em 2015. Ácaros *Brevipalpus* foram procurados em 55 amostras de 32 espécies botânicas, de 25 famílias e encontrados em 15 amostras de 12 espécies de plantas, em 11 famílias. Uma abordagem integrativa baseada em dados morfológicos e moleculares foi aplicada à identificação taxonômica. Quatro espécies foram encontradas - *B. azores* Beard & Ochoa, *B. papayensis* Baker, *B. obovatus* Donnadieu e uma espécie identificada como nova para a ciência pertencente ao grupo de espécies *B. portalis*. Também foram registradas novas plantas hospedeiras para *B. azores* e *B. papayensis*. No capítulo 3 a nova espécie do Arquipélago de Açores foi descrita com base nos caracteres morfológicos e informações obtidas das sequências de DNA nuclear e mitocondrial. A taxonomia do grupo de espécies *B. cuneatus*, bem como a posição filogenética da nova espécie foram temas abordados e discutidos neste capítulo. No quarto capítulo o grupo de espécies de *B. obovatus* é redefinido com base em uma abordagem integrativa usando seqüências de DNA mitocondrial e nuclear, bem como características morfológicas detalhadas, obtidas por microscopia eletrônica e óptica e novos dados filogeneticamente informativos foram apresentados neste trabalho. Além disso, dados morfológicos e distâncias genéticas são explorados para a distinção de espécies próximas- *B. azores*, *B. feresi* Beard & Ochoa e *B. papayensis*. A integração de dados morfológicos e moleculares sugerem a ocorrência de espécies crípticas no táxon *B. papayensis*.

Abstract: The genus *Brevipalpus* Donnadieu (Prostigmata: Tenuipalpidae) is an important group of phytophagous mites that are distributed around all biogeographical regions. This genus includes 291 species of which some are considered as pests in agroecosystems. Direct damage caused by feeding, and indirect due to the transmission of plant virus (BTVs - *Brevipalpus* transmitted virus) are reported in several agricultural crops in Brazil and around the world, as well as on ornamental and forest plants. The systematics of *Brevipalpus* mites has been a challenge for acarologists. In addition to many synonyms throughout the history of the group, the occurrence of cryptic species has been confirmed along the last two decades. The *B. phoenicis* (Geijskes) species mites were considered the most economical and one of the most studied, however, after being revised some synonyms were ressurrected and cryptic species were described, in a way that all associated taxonomic and bioecological information has to be revised. Accurate identification is essential for preventing the introduction of mites not present in the country, supporting the interception procedures and contributing to improving plant protection services. The knowledge of the fauna from poorly explored regions outside Brazil, as in the Azores Archipelago, object of the present study, can be helpful in the identification of intercepted specimens. For this purpose, morphological studies using different microscopy techniques integrated with molecular data obtained from distinct regions of the genome have allowed a substantial progress in the taxonomy of the group. The present work had as general objective to contribute to the systematics of *Brevipalpus* mites. This dissertation was organized into three chapters structured as scientific articles, preceded by a common initial chapter (Chapter 1) containing the introduction and context of the paper, objectives, and bibliographic references. The Chapter 2 presents result of a fauna survey carried out in the Azores Archipelago, islands of Pico, Faial and Flores, conducted in 2015, in the. *Brevipalpus* mites were searched for in 55 samples of 32 botanic species, of 25 families and were found in 15 samples of 12 plant species in 11 families. An integrative approach based on morphological and molecular data was applied to the taxonomic identification. Four species were found- *B. azores* Beard & Ochoa, *B. papayensis* Baker, *B. obovatus* Donnadieu and a species identified as new to the science belonging to the *B. portalis* species group. New host plants for *B. azores* and *B. papayensis* were also registered. In Chapter 3 the new species collected in the Azores Archipelago was described based on morphological traits and on nuclear and mitochondrial DNA sequences. The taxonomy of the *B. cuneatus* species group as well as the phylogenetic position of the new species were discussed. In the Chapter 4 the *B. obovatus* species group is redefined based on the integrative approach using mitochondrial and nuclear DNA sequences as well as detailed morphological features obtained by electron and optical microscopy and phylogenetically informative traits were pointed out in this work. In addition, morphological data and genetic distances between closely related species- *B. azores*, *B. feresi* Beard & Ochoa and *B. papayensis*-were explored to distinguishing them. The integration of morphological and molecular data suggested the occurrence of cryptic species in *B. papayensis*.

CAPÍTULO 1

1 INTRODUÇÃO

1.1 REVISÃO BIBLIOGRÁFICA

1.1.1 A subclasse Acari

A subclasse Acari, da classe Arachnida, compreende pequenos artrópodes que habitam os mais diversos ambientes em todos os continentes, comumente conhecidos como ácaros e carrapatos. Segundo maior grupo do filo Arthropoda, os ácaros são os representantes de maior diversidade da antiga linhagem Chelicerata (Walter & Proctor, 1999; Krantz, 2009). O grupo surgiu muito provavelmente no Paleozoico, conforme indicam fósseis antigos de oribatídeos, uma subordem diversificada e abundante de ácaros de solo (Norton 1994, Behan-Pelletier 1999; Norton, 2009) datados de períodos entre 380 e 400 milhões de anos atrás (Walter & Proctor, 1999).

A subclasse Acari é composta por duas superordens: Parasitiformes e Acariformes. A primeira é constituída por ácaros que possuem de um a quatro pares de estigmas posteriores à coxa II e coxas móveis, subdividindo-se nas ordens Holothyrida, Ixodida, Mesostigmata e Opiliocarida. Por outro lado, a superodem Acariformes se subdivide em duas ordens, Trombidiformes e Sarcoptiformes, cujos ácaros não possuem estigmas visíveis posteriores à coxa II e tem as coxas fundidas à região ventral do corpo (Lindquist *et al*., 2009).

Os ácaros apresentam uma grande diversidade. Não se conhece o número exato de espécies, no entanto, estima-se que seja entre 500.000 a 1.000.000 (Brusca & Brusca, 2007; Krantz, 2009). Constitui o maior grupo de aracnídeos, com aproximadamente 55 mil espécies descritas (Walter & Proctor, 1999; Krantz, 2009), certamente devido à sua adaptabilidade nos diferentes habitats e variabilidade de hábitos alimentares- parasitas, predadores, fitófagos, fungívorus, detritívoros, necrófagos, etc. (Krantz, 2009). Outros fatores importantes para o sucesso destes pequenos animais são a sua biologia, que inclui espécies partenogenéticas e de reprodução sexuada, simbiose com outros organismos, além da sua capacidade de dispersão que pode ocorrer por caminhamento, forésia, ou até mesmo por ação dos ventos (Walter & Proctor, 1999 e Moraes & Flechtmann, 2008).

Os acarinos se diferenciam dos outros aracnídeos pela ausência de segmentação do corpo, ausência de subdivisão do corpo em tagmas separados, presença de um gnatossoma e, em sua maioria, apenas três pares de pernas no primeiro estágio móvel de desenvolvimento ontogenético (Moraes & Flechtmann, 2008). Contudo, os ácaros possuem algumas características morfológicas semelhantes às do seu grupo irmão Ricinulei, que juntos formam o grupo Acaromorpha Dubinin, 1957, próximos principalmente por duas sinapomorfias, o gnatossoma e a fusão medial das coxas palpais (Shultz, 1990). Outras características também unem os acaromorfos, Acari e Ricinulei possuem desenvolvimento pós-embrionário único apresentando, em geral, uma larva hexápode e até três estágios ninfais octópodes; gânglios segmentares pós-clericais unificados no gânglio subesofágico; faringe pós-cerebral ausente; e ausência de músculo dilatador faríngeo ventral (esternal). No entanto, as últimas quatro sinapomorfias podem não ser análogas, ou seja, resultado de evolução convergente (Wheeler & Hayashi, 1998; Shultz, 2007). O corpo dos ácaros é dividido em duas regiões principais: gnatossoma, que inclui os palpos e as peças bucais, e o idiossoma, que corresponde ao propodossoma e opistossoma (fig. 1). Como apêndices apresentam em geral quatro pares de pernas, palpo e quelícera, esta última tendo sofrido diversas modificações ao longo de sua história evolutiva, adquirindo formatos diferentes a depender do hábito alimentar (Moraes & Flechtmann, 2008).

Muitos ácaros são comumente reportados como organismos de importância médica, veterinária e agrícola. Devido à sua grande diversidade, estes grupos são estudados por diferentes subáreas da acarologia. Os carrapatos (ectoparasitas hematófagos), grupo bastante conhecido do público em geral, que pertencem a ordem Ixodida são os principais ácaros relacionados aos problemas de saúde médico-vetetinária, mencionados no meio científico em Inglês como “ticks” (Corrêa, 1971; Moraes & Flechtmann, 2008). Os ácaros também são considerados vilões quanto à saúde humana. Alguns podem viver na pele do homem, a exemplo dos ácaros do gênero *Demodex* (ordem Prostigmata), causadores do popularmente denominado “cravo” na pele dos seres humanos (Urquhart, 1996). A ordem Astigmata é composta por outro grupo de ácaros associados a problemas de saúde humana, sendo o principal causador de enfermidades alérgicas como asma, rinites e dermatites atópica, que inclui principalmente os gêneros *Blomia* e *Dermatophagoides*. Além destes, também existem grupos de ácaros de importância agrícola, representrados principalmente por algumas famílias, entre eles

estão Phytoseiidae, Ascidae, Bdellidae, Cheyletidae, Tydeidae e Cunaxidae, predadores da subordem Mesostigmata Prostigmata (Hoy, 2011). Um grande número de espécies fitófagas e consideradas pragas agrícolas são encontradas nas famílias Eriophyidae, Tarsonemidae, Tenuilpalpidae, Tetranychidae e Tuckerellidae (Krantz & Walter 2009; Hoy 2011).

1.1.2 A família de ácaros fitófagos Tenuipalpidae

Os ácaros da família Tenuipalpidae Berlese (Acari: Trombidiformes: Tetranychoidea), conhecidos como ácaros planos ou "false spider mites", são pequenos artrópodes cujo tamanho pode variar de 0,25 a 0,4 mm. Apresentam grande variabilidade na forma do corpo e também de cor, comumente encontrados na cor avermelhada, todavia, podem apresentar as cores amarelo, laranja, verde e marrom (Beard *et al*., 2013). Todos os tenuipalpídeos são exclusivamente fitófagos, alimentando-se em folhas, caule, fruto, flor, etc. (Childers *et al*., 2003c; Childers & Rodrigues, 2011). Nas folhas, podem ser encontrados tanto na face adaxial quanto abaxial. São conhecidas mais de 1.100 espécies válidas, pertencentes a 38 gêneros, distribuídos em todas as regiões zoogeográficas, das quais a maioria foram descritas na América do Norte (Mesa *et al*., 2009). Os gêneros mais numerosos são *Tenuipalpus* Donnadieu, *Brevipalpus* Donnadieu, *Aegyptobia* Sayed e *Cenopalpus* Pritchard and Baker, que juntos representam mais de 80% do total de espécies conhecidas na família.

A família Tenuipalpidae é considerada uma das mais recentes na história evolutiva da superfamília Tetranychoidea Baker & Pritchard (Walter & Proctor, 1999), a qual incluí ainda outras quatro famílias- Tuckerellidae Baker & Pritchard, Tetranychidae Donnadieu, Linotetranidae Baker & Pritchard e Allochaetophoridae Reck (Walter *et al*., 2009). Evidências morfológicas, comportamentais e a plasticidade no grupo corroboram esta hipótese. Contudo, a supressão do "processo unha-dedão", considerado uma sinapomorfia, é uma forte indicação da posição filogenética relativamente derivada dos tenuipalpídeos (Kranz, 2009). Quirós-González (1985), através de análise filogenética considerou Tenuipalpidae como um grupo monofilético.

A maioria dos gêneros de Tenuipalpidae possuem 4 pares de pernas nos estágios de protoninfa, deutoninfa e adultos, com exceção das espécies dos gêneros *Larvacarus*

Baker & Pritchard e *Raoiellana* Baker & Pritchard, as quais apresentam apenas três pares em todos os estágios de desenvolvimento (Mesa *et al*., 2009). Em geral o palpo é pequeno, com dois a cinco segmentos (sem "processo unha-dedão") e comumente as fêmeas exibem um sulco sejugal nítido, enquanto que os machos apresentam opistossoma afilado, bem como um edeago em forma de estilete alongado, com pouca variação entre as espécies (Mesa *et al*., 2009).

O desenvolvimento dos tenuipalpídeos é caracterizado pelos estágios de ovo, larva, protoninfa, deutoninfa e adultos, machos e fêmeas com dimorfismo sexual, embora os machos sejam relativamente raros em várias espécies conhecidas (Moraes & Flechtmann, 2008). Em muitas espécies da família a reprodução ocorre por partenogênese (telítoca) com fêmeas produzindo fêmeas haplóides (Childers *et al*., 2003a). Um estudo realizado por Weeks *et al*. (2001) demonstrou que uma bactéria endossimbionte, *Cardinium* (Groot & Breeuwer, 2006), foi responsável pela feminização de indivíduos geneticamente machos em *Brevipalpus yothersi* Baker (previamente identificado como *Brevipalpus phoenicis* Geijskes). Espécies diplóides que resultam de reprodução sexuada também são encontradas, a exemplo de *Raoiella indica* Hirst e *Brevipalpus russulus* Boisduval (Pijnacker *et al*., 1980).

1.1.3 O Gênero *Brevipalpus* Donnadieu

O gênero *Brevipalpus* Donnadieu, 1875 apresenta-se como um dos mais importantes da família Tenuipalpidae, figurando juntamente com *Tenuipalpus*, como os gêneros com maior número de espécies descritas (Mesa *et al*., 2009; Childers *et al*., 2003a). Atualmente, contém 291 espécies descritas distribuídas por todas as regiões biogeográficas do mundo, cerca de 133 na região Neotropical, 70 na região Neártica, 49 na região Oriental, 26 no Paleártico Ocidental, 9 no Paleártico Oriental e apenas 4 na região Afrotropical (Mesa *et al*., 2009; Beard *et al*., 2013; Navia *et al*., 2013; Beard *et al*., 2015).

Espécies deste gênero geralmente possuem coloração laranja, vermelho, amarelo ou preto-avermelhado; marcações escuras dorsalmente são bastante comuns, além de apresentarem ovos avermelhados (Childers *et al*., 2003a; Beard *et al*., 2012). É importante

destacar que este padrão de coloração aliado à movimentação lenta destes organismos facilita seu reconhecimento durante as inspeções (Childers *et al*., 2003a).

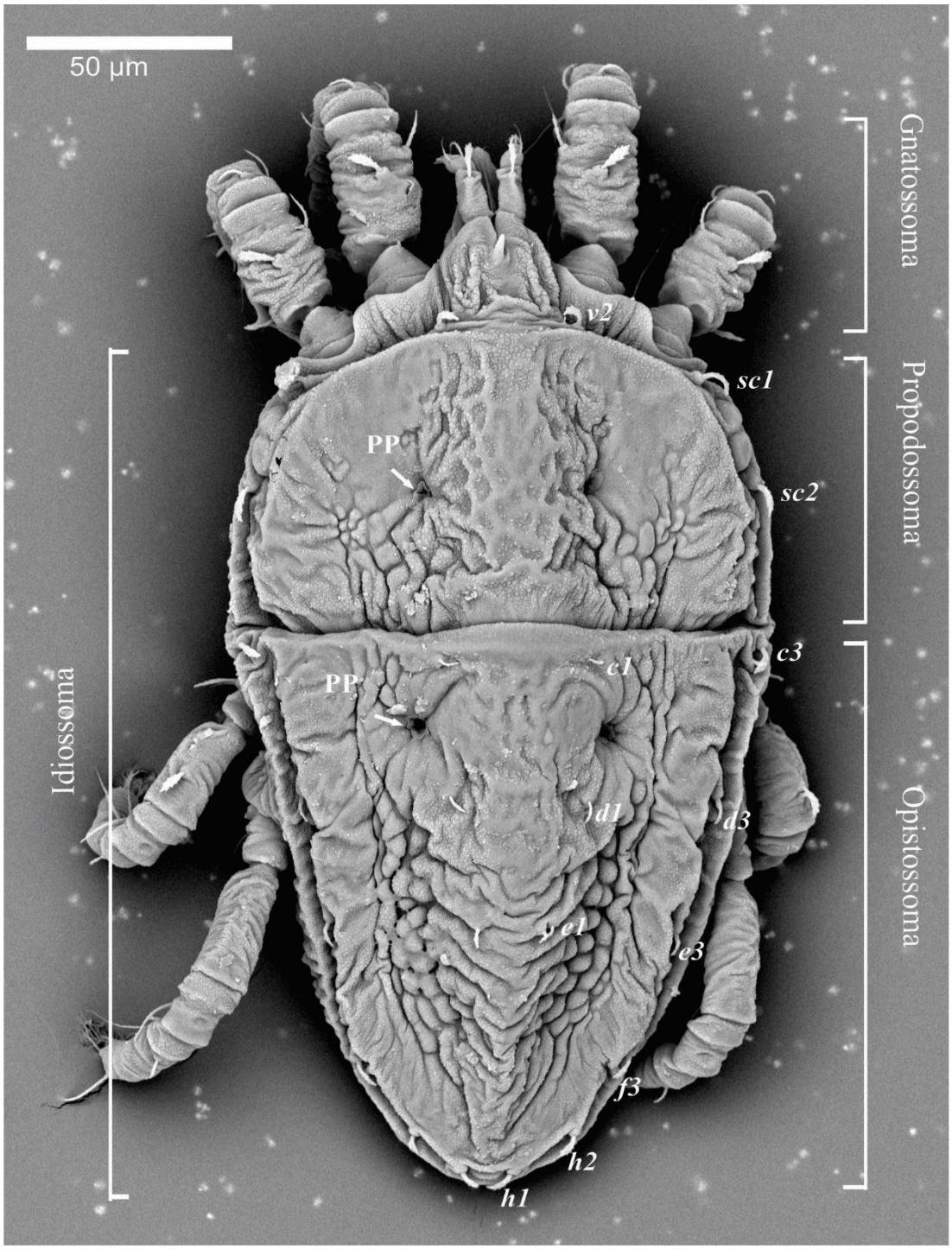

Figura 1. Aspecto geral dorsal do corpo de um ácaro (adaptado de Moraes & Flechtmann) do gênero *Brevipalp*us, *B. azores* Beard & Ochoa - poro opistossomal: PO; poro propodossomal: PP; setas: *v2, sc1, sc2 c1, c3, d1, d3, e1, e3, f3, h1* e *h2*.

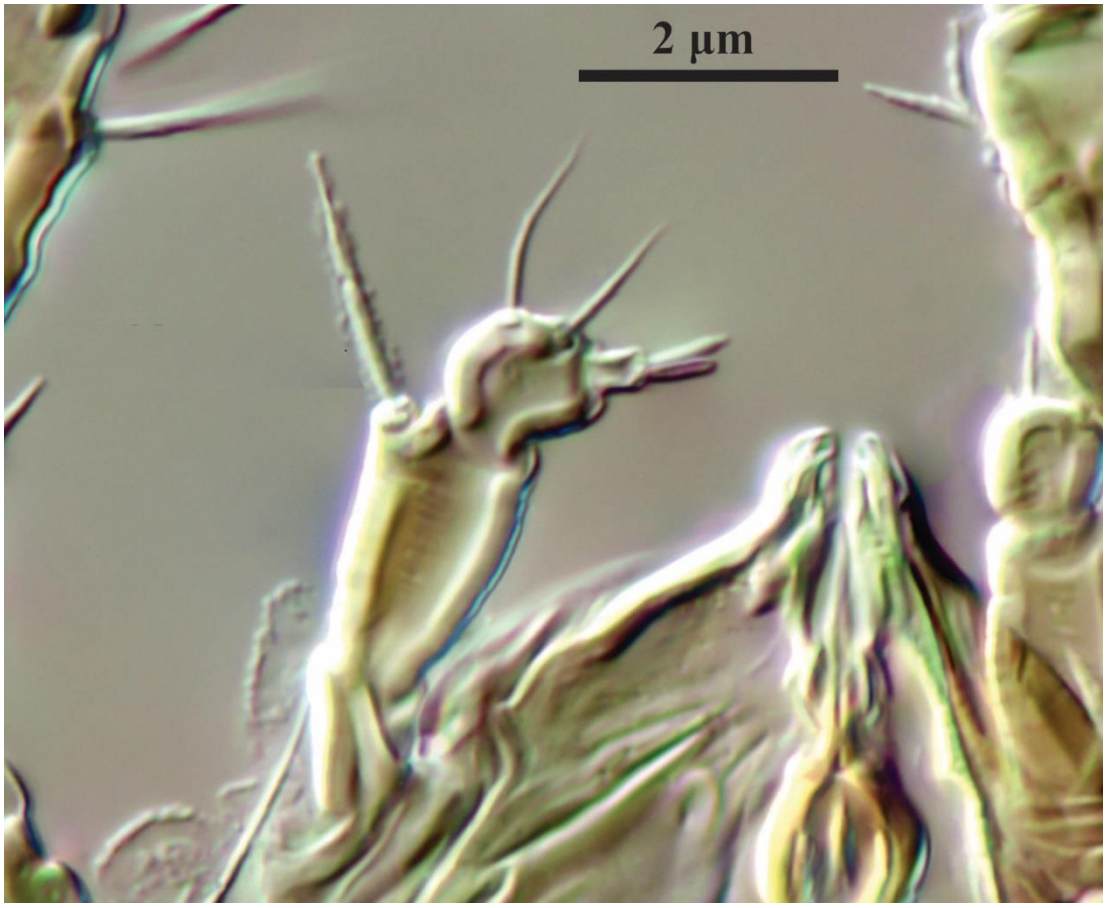

Figura 2. Palpo de fêmea de ácaro do gênero *Brevipalpus*, com quatro segmentos.

Os ácaros do gênero *Brevipalpus* podem ser diferenciados dos outros tenuipalpídeos pelos seguinte caracteres: placas genitais e ventrais separadas, a anterior elíptica, mais larga do que longa; e a segunda retangular, geralmente com uma cutícula estriada formando uma moldura ao redor da placa; palpo com 4 segmentos (Fig. 2); margem lateral do opistossoma com 6 ou 7 pares de setas (*f2* presente ou ausente); *sc1*, *c1* e *e1* não são extremamente longas e lanceoladas, conforme ilustrado na figura 1 (de acordo com Mesa *et al*., 2009). O propodossoma e o opistossoma são separados por um sulco característico em muitos tenuipalpídeos, o sulco sejugal. A face dorsal pode possuir até 12 pares de setas, variando no opistossoma de 9 a 10 pares, conforme indicado na figura 1. As setas mais centrais no opistossoma são rotuladas como "1" e as cerdas mais

laterais são classificadas como “3” (Beard *et al*., 2012). Apêndices são muito importantes na taxonomia do grupo. Presença dos poros propodossomal e opistossomal são comuns em várias espécies de *Brevipalpus*. Além de setas importantes na região ventral, é notória a presença de três estruturas que se destacam- as placas ventral, genital e anal- todas importantes na distinção entre as espécies devido à variação na ornamentação. No ácaro macho o opistossoma é subdividido, exibindo então, dois escudos opistossomais; comumente apresenta dois solenídios no tarso I e, às vezes, com um solenídio nos tarsos III e IV. Diferentemente das fêmeas, os machos não possuem placa genital, e apresentam um edeago (Beard *et al*., 2012).

O ciclo de vida destes ácaros consiste em quatro estágios ativos: larva, protoninfa, deutoninfa e adultos, com um estágio de desenvolvimento quiescente (crisálida), entre os estágios; nesses estágios quiescentes os ácaros permanecem sésseis, mas fisiologicamente ativos (Childers *et al*., 2003a). A quantidade de ovos que uma fêmea é capaz de produzir em período de oviposição pode variar de acordo com a espécie; por exemplo, em *B. californicus* Banks pode ser de um ovo por dia, enquanto que de *B. phoenicis* pode chegar a dois ovos por dia (Manglitz & Cory, 1953; Kennedy *et al*., 1996). Em média, uma fêmea adulta oviposita por cerca de 30 dias (Jeppson *et al*., 1975; Childers *et al*., 2003a).

1.1.4 Ácaros *Brevipalpus* – importância econômica e quarentenária

Nas últimas décadas, os estudos com ácaros do gênero *Brevipalpus* têm sido intensificados, sobretudo devido à sua crescente importância agrícola. A alimentação dos ácaros pode causar sintomas severos em folhas, galhos verdes, flores e até mesmo nos frutos (Childers *et al.*, 2010). Em um estudo realizado por Childers e colaboradores (2003b) 928 espécies de plantas em 513 gêneros dentro de 139 famílias foram relatadas como hospedeiros de três espécies de *Brevipalpus*- *B. californicus*, *B. obovatus* Donnadieu e *B. phoenicis*- no entanto, esta última devido a identificações errôneas, provavelmente se refira a *B. yothersi* (ver Beard *et al*., 2015). A lista de hospedeiros inclui monocotiledôneas e dicotiledôneas, com diversas espécies de palntas de importância agrícola, ervas medicinais, ornamentais, florestais e lenhosas.

Os danos causados por estes ácaros podem ser diretos e indiretos, ou seja, causados pela própria alimentação ou pela transmissão de fitovírus. Ao se alimentarem esses ácaros matam células epidérmicas e injetam saliva tóxica em tecidos de frutas,

folhas, caule e brotos de plantas hospedeiras, causando progressivamente áreas ressecadas, necróticas, com subsequente queda de folhas e frutos. Os sintomas são severos quando as populações são numerosas (Childers, 1994; Childers *et al*., 2003a). Ebeling e Pence (1949) observaram que *B. lewisi* McGregor, na California, Estados Unidos, causou a corrosão significativa e fissuras na casca em frutas de romã, com índice de dano entre 50 a 90%.

Algumas espécies de *Brevipalpus* tem a capacidade de transmitir vírus aos seus hospedeiros, os "Vírus Transmitidos por *Brevipalpus* (VTBs)", que são conhecidos desde a metade do século XX, e já foram encontrados em mais de 37 espécies de plantas ornamentais, e em importantes culturas agrícolas (Kitajima *et al.,* 2010). Em 1969, no Japão, Doi e colaboradores relataram a morfologia e citopatologia do primeiro vírus relacionado a *Brevipalpus.* Até meados do ano 2000, poucos VTBs eram conhecidos. Os VTBs infectam várias plantas de importância econômica (Kitajima *et al.,* 2010) e entre os mais importantes estão: *Citrus leprosis virus C* - CiLV-C (Childers *et al*., 2003c), *Coffee ringspot dichorhavirus* - CoRSV (Chagas et al., 2003), *Orchid fleck dichorhavirus* - OFV (Kondo et al., 2003), e *Passion fruit green spot virus*- PFGSV (Kitajima et al., 2003b). Estes vírus se apresentam em dois tipos, o nuclear (N-VTB) e o citoplasmático (C-VTB), cada um apresentando características diferenciadas de transmissão, sintomas, hospedeiros, etc (Kitajima *et al*., 2003). A primeira doença economicamente importante identificada no Brasil cujo agente etiológico é transmitido por *Brevipalpus* foi a leprose dos Citros (CL) (Kitajima *et al*., 2010). No País, esta doença causa significativas perdas econômicas, sendo considerada a doença viral mais importante da citricultura brasileira (Rodrigues, 2000). Anualmente, o controle do ácaro custa aos produtores brasileiros cerca de US $ 80 milhões (Bastianel *et al*., 2010).

Os ácaros *Brevipalpus* constituem um grupo de pragas de importância quarentenária, ou seja, praga de importância econômica potencial para uma área em perigo e ainda não presente, ou presente, mas não amplamente distribuída e controlada oficialmente (EPPO, 2019). Estes organismos exóticos podem ser introduzidos nas regiões para as quais possuem status de "praga quarentenária". Estas pragas geralmente são transportadas de um local para outro direta ou indiretamente pelo homem, através do trânsito de materiais vegetais. Podem ser agrupadas em duas categorias: PQA- pragas de importância econômica potencial para uma área em perigo, que não esteja presente em território nacional e; PQP- pragas de importância econômica potencial para uma área em

perigo, não amplamente distribuída e que se encontra sob controle oficial (MAPA, 2019). As pragas de expressão quarentenária podem se tornar Espécies Invasoras Exóticas (EIEs). A importância das mesmas é tão notória que medidas de impedimento à introdução das EIEs e seu controle ou erradicação são tratados no artigo 8 da "Convenção sobre Diversidade Biológica" (CDB, 2005).

Nos últimos anos, pesquisadores têm alertado sobre o risco da entrada de determinadas pragas quarentenárias que representam risco para o Brasil e para outros países (Mendonça *et* al., 2005; Migeon *et al*., 2009; Navia *et al*., 2010; Navajas *et al*., 2013). Algumas espécies expandiram sua distribuição geográfica e emergiram como uma grande praga agrícola invasiva em regiões onde foram introduzidas. Um exemplo é o caso de *Tetranychus evansi* Baker and Pritchard, nativo do Brasil, cuja distribuição foi sendo expandida para África e Europa (Tsagkarakou *et al*., 2007; Navajas *et al*., 2013). *Raoiella indica* é um outro exemplo de um ácaro fitófago invasivo. Originalmente da Índia, sua distribuição ampliou para outros continentes (Pritchard & Baker 1958; Gerson et al., 1983), até ser relatado por Flechtmann & Etienne (2004) ocorrendo nas Américas. No Brasil, foi registrado pela primeira no Estado de Roraima, onde provavelmente ocorreu sua introdução, na fronteira entre Brasil e Venezuela (Navia *et al*., 2010). Atualmente, *R. indica* apresenta ampla distribuição no Brasil, conhecido como ácaro vermelho das palmeiras, e é capaz de ocasionar sérios danos principalmente nas espécies das famílias Arecaceae, Heliconiaceae, Musaceae, Pandanaceae, Strelitziaceae e Zinberaceae (Mendonça *et* al., 2005). O ácaro hindustânico dos citros, *Schizotetranychus hindustanicus* Hirst, se encontra na lista de pragas quarentenárias presentes e constitui uma ameaça à citricultura no Brasil. Quanto à lista de pragas quarentenárias ausentes no Brasil, o número de espécies de ácaros é bem maior, incluindo duas do gênero *Brevipalpus*- *Brevipalpus chilensis* Baker e *Brevipalpus lewisi* McGregor (MAPA, 2017). Segundo o Ministério da Agricultura, Pecuária e Abastecimento (MAPA) e a Embrapa, *B. chilensi*s está entre as dez pragas quarentenárias mais importantes para o Brasil.

Vias mediadas pelo homem foram as principais fontes de invasões de pragas agrícolas no Brasil (Lopes da Silva *et al*., 2014). As interceptações de ácaros fitófagos tem sido frequente, sobretudo em locais onde se realiza uma inspeção acarológica detalhada de produtos vegetais (Navia *et al.,* 2006). A identificação acurada dos espécimes interceptados é fundamental para a prevenção da introdução de ácaros não presentes no País, o que só é possível com a colaboração de especialistas e a disponibilidade de coleções de referências, essenciais para darem suporte aos

procedimentos de interceptação (Navia *et al.*, 2006). A identificação e depósito de espécies que não ocorrem no Brasil, em coleções de referência, podem ajudar na identificação de espécimes interceptados, contribuindo para o fortalecimento do serviço de defesa fitossanitária.

1.1.5 Sistemática do gênero *Brevipalpus*

A sistemática do gênero *Brevipalpus* sempre foi um grande desafio para os acarologistas. Problemas relacionados à taxonomia de *Brevipalpus* têm sido reportados desde o século passado, o que é evidenciado pelo elevado número de sinonímias, criando um cenário cada vez mais confuso quanto à taxonomia do gênero (Pritchard & Baker 1952; De Leon, 1960; Meyer, 1979; Mitrofanov & Strunkova 1979; Baker & Tuttle, 1987). Castagnoli (1974) demonstrou a importância da vesícula da espermateca para a taxonomia do grupo, caráter que foi por muito tempo negligenciado ou esquecido, entretanto, ressurgiu como uma estrutura importante de discriminação taxonômica para *Brevipalpus* (Navia *et al*., 2013; Beard *et al*. 2015). Após mais de dez anos, Baker & Tuttle (1987) publicaram um trabalho repleto de caracteres morfológicos, e apresentaram a definição de grupos que é aceita na atualidade. Beard e colaboradores (2012) levantaram a questão de que os táxons *B. californicus* e *B. phoenicis* representam complexos de espécies crípticas. Tal hipótese foi corroborada pelo trabalho de taxonomia integrativa de Navia *et al*. (2013), o qual incluiu dados moleculares, e por Beard *et al*. (2015) explanando sobre o complexo de espécies de *B. phoenicis*, que inclui outras sete espécies, entre redescrições e novas descrições. Enquanto isso, novos caracteres morfológicos têm sido apontados, a exemplo da estrutura das microplacas presente na cutícula, cujo padrão diverge entre as espécies (Welbourn *et al*., 2003; Beard *et al*. 2015; Hao *et al*., 2018; Tassi, 2018).

A sistemática tem se beneficiado do desenvolvimento contínuo de ferramentas moleculares e da aplicação de técnicas de microscopia, as quais tem permitido aclarar a posição taxonômica de táxons e revelar a ocorrência de espécies crípticas (Navajas *et al*., 1999; Grismer *et al*., 2013; Zhang *et al*., 2014). Neste sentido, abordagens inovadoras, como a taxonomia integrativa são essenciais para a acurada identificação das espécies e vêm impulsionando e integrando o conhecimento taxonômico a outros campos das ciências biológicas (Bickford *et al*., 2007; Schlick-Steiner *et al*., 2010; Navajas & Navia,

2010; Navia *et al*., 2013). A taxonomia integrativa se beneficia de ferramentas distintas e complementares, como, a morfologia convencional, a morfometria, o diagnóstico molecular, fatores ecológicos e comportamentais, como provedoras de fonte de evidências confiáveis para a separação de espécies. Essa integração de informações, tem dado consistência às decisões quanto à delimitação de táxons e ajuda a revelar as relações evolutivas precisas entre os mesmos (Samadi & Barberousse, 2006; Bickford *et al*., 2007; Samadi & Barberousse, 2009; Schlick-Steiner *et al*., 2010; Navajas & Navia, 2010; Navia *et al*., 2013; Navia *et al*., 2014).

A taxonomia integrativa tem revelado uma diversidade inesperada em muitos grupos de animais, incluindo diferentes grupos de ácaros, a exemplo dos Eriophyidae Nalepa, Phytoseiidae Berlese, Tetranychidae e Tenuipalpidae (Navajas *et al*., 2000; Navajas & Navia, 2010; Skoracka & Dabert, 2010; Skoracka *et al*., 2012; Navia *et al*., 2013; Navia *et al*., 2014; Lewandowski *et al*., 2014; Chetverikov *et al*., 2015). No tocante ao gênero *Brevipalpus*, tal abordagem também tem sido utilizada para aclarar diversidade críptica. À medida que os estudos se intensifiquem, e sejam obtidos e disponibilizadas mais dados (ex. sequências de DNA) em plataformas públicas, espera-se ter avanço significativo na sistemática do gênero, incluindo a filogenia.

Atualmente, as espécies do gênero *Brevipalpus* estão classificadas em seis grupos, de acordo com Baker & Tuttle (1987). Esta classificação é baseada exclusivamente em caracteres morfológicos, como número de setas no opistossoma, número de solenídios no tarso II e número de setas do quarto segmento do palpo. Os grupos de espécies são: *B. californicus, B. cuneatus, B. frankeniae B. obovatus, B. phoenicis,* e *B. portalis*. Essa classificação dos ácaros *Brevipalpus* é artificial, isto é, não tem sido baseada nas relações evolutivas entre espécies. Entretanto, é desejável que essa classificação passe a ser baseada nas relações filogenéticas entre os táxons. O conhecimento das relações evolutivas no contexto de grupos intimamente aparentados, como de espécies de plantas hospedeiras ou animais, é fundamental para prever suas características bioecológicas, subsidiando a definição de estratégias de prevenção e controle eficientes (Yates *et al*., 2003).

2 OBJETIVOS GERAIS

Contribuir para o conhecimento da fauna de ácaros fitófagos do gênero *Brevipalpus* e avançar em sua sistemática, através de uma abordagem integrativa, com a utilização de informações morfológicas e moleculares.

2.1 OBJETIVOS ESPECÍFICOS

- Realizar identificação taxonômica de espécies do gênero *Brevipalpus* coletadas em plantas nativas e cultivadas no arquipélago de Açores, Portugal;
- Elaborar descrição de uma nova espécie do gênero *Brevipalpus* coletada em uma planta endêmica em Açores;
- Subsidiar uma revisão da composição dos grupos de espécies do gênero *Brevipalpus*, com foco no grupo *B. obovatus*, através de análises de filogenia molecular.

3 REFERÊNCIAS

Baker EW, Tuttle DM (1987) The False Spider Mites of Mexico (Tenuipalpidae: Acari). U.S. Department of Agriculture, Technical Bulletin, 1706: 237p.

Barbosa & Paranhos (2018) disponível em http://www.agencia.cnptia.embrapa.br/Agencia22/AG01/arvore/A01_116_241120051152 25.html acessado em set 2018.

Bastianel M, Novelli V, Kubo K, Kitajima EW, Bassanezi R, Machado MA, Freitas-Astúa J (2010) Citrus leprosis: centennial of an unusual mite virus pathosystem. Plant Disease, Saint Paul, 94 (1): 284-292

Beard JJ, Ochoa R, Braswell WE, Bauchan GR (2015*) Brevipalpus phoenicis* (Geijskes) species complex (Acari: Tenuipalpidae)- a closer look. Zootaxa 3944 (1): 001-067.

Beard, JJ, Ochoa R, Redford A J, Trice MD, 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. 1º Ed. http://idtools.org/id/mites/flatmites/.

Beard JJ, Ochoa R, Bauchan GR, Trice M, Redford A, Walters T, Mitter C (2013) Flat mites of the world. Disponível em: http://idtools.org/id/mites/flatmites/ acessado em dez 2018.

Behan-Pelletier VM, (1999) Oribatid mite biodiversity in agroecosystems: role of bioindication. Agriculture, Ecosystems and Environment, 74: 411–423.

Bickford D, Lohman DJ, Sodhi NS, Ng PKL, Meier R, Winker K, Ingram KK, Das I (2007). Cryptic species as a window on diversity and conservation. Trends in Ecology & Evolution, 22: 148–155.

Brusca RC, Brusca GJ, (2007) Invertebrados. Segunda edição. Editora Guanabara-Koogan, Rio de Janeiro. 968p.

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

CDB, A Convenção sobre Diversidade Biológica: entendendo e Influenciando o processo (2005) Um guia para entender e participar efetivamente da oitava reunião da conferencia das partes da convenção sobre diversidade Biológica.

Chagas CM, Kitajima EW, Rodrigues JCV (2003) *Coffee ringspot virus* vectored by *Brevipalpus phoenicis* (Acari: Tenuipalpidae) in coffee. Experimental and Applied Acarology, 30: 203–213.

Chetverikov PE, Cvrkovic T, Makunin A, Sukhareva S Vidovic B, Petanovic R (2015) Basal divergence of Eriophyoidea (Acariformes, Eupodina) inferred from combined partial COI and 28S gene sequences and CLSM genital anatomy. Experimental and Applied Acarology, 67: 219–245.

Childers CC, Rodrigues JCV (2011) An overview of *Brevipalpus* mites (Acari: Tenuipalpidae) and the plant viruses they transmit. Zoosymposia 6: 180–192

Childers CC, French JV, Rodrigues JCV (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 CC, Rodrigues JCV, Welbourn WC (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.

Childers CC, Rodrigues JCV, Derrick KS, Achor DS, French JV, Welbourn WC, Ochoa R, Kitajima EW (2013c) Citrus leprosis and its status in Florida and Texas: past and present. Experimental and Applied Acarology, 30 (1): 181-202,

Childers CC, Kitajima EW, Welbourn WK, Rivera C, Ochoa R (2001) *Brevipalpus* mites on citrus and their status as vectors of citrus leprosis. Manejo Integrado de Plagas (Costa Rica), 60: 66-70.

Childers CC (1994) Feeding injury to 'Robinson' tangerine leaves by *Brevipalpus* mites (Acari: Tenuipalpidae) in Florida and evaluation of chemical control on citrus. Florida Entomologist, 77: 265–271.

Corrêa, O. Doenças parasitarias dos animais domésticos. Porto Alegre: Livraria Sulina, 1971. 285 p.

De Leon D (1960) The genus *Brevipalpus* in Mexico, Part I (Acarina: Tenuipalpidae). Florida Entomologist, 43(4):175–187.

Doi Y, Arai K, Yora K (1969) Ultrastructure of bacilliform virus found in *Euonymus japonicum* with mosaic and *Cymbidium* with ringspot. Annals of the Phytopathological Society of Japan, 35: 388p.

Ebeling W & Pence RJ (1949) New pomegranate mite. California Agriculture, 6: 11–14.

Elmer HS & Jeppson, LR (1957) Biology and control of the citrus flat mite. Journal of Economic Entomology, 50(5): 566–570.

EPPO (European and Mediterranean Plant Protection Organization) (2002) disponível em: //www.eppo.org.// acessado em jan 2019.

Flechtmann, CHW & Etienne J (2004) The red palm mite, *Roiella indica* Hirst, a threat to palms in the Americas (Acari: Prostigmata: Tenuipalpidae). Systematic and Applied Acarology, 9: 109-110.

Gerson U, Venezian A, Blumberg D (1983) Phytophagous mites on date in Israel. Fruits, 38(2): 133–135.

Ghai, S & Shenhmar, M (1984) A review of the world fauna of Tenuipalpidae (Acarina: Tetranychoidae). Oriental Insects, 18: 99–172.

Grismer LL, Wood Jr P L, Anuar S, Muin MA, Quah ESH, Mcguire JA, Thai, PH (2013) Integrative taxonomy uncovers high levels of cryptic species diversity in *Hemiphyllodactylus* Bleeker, 1860 (Squamata: Gekkonidae) and the description of a new species from Peninsular Malaysia. Zoological Journal of the Linnean Society, 169: 849–880. http://doi:10.1111/zoj.12064

Groot TVM, Breeuwer JAJ (2006) *Cardinium* symbionts induce haploid thelytoky in most clones of three closely related *Brevipalpus* species. Experimental and Applied Acarology, 39: 257-271.

Hao DJ, Pfammatter P, Liu Q, Fan BQ, Yan Wang, Tian Zi Gu (2016) Morphological and genetic characteristics of *Brevipalpus lewisi* (Acari: Tenuipalpidae) and comparison with other three *Brevipalpus* species, International Journal of Acarology, 42:1, 34-40. http://doi:10.1080/01647954.2015.1114022

Hoy MA (2011) Agricultural Acarology: Introduction to Integrated Mite Management. CRC Press 114p.

Jeppson LR, Baker EW, Keifer HH (1975) Mites Injurious to Economic Plants. University of California Press, Berkeley.

Kennedy JS, Impe VG, Dance TH, Lebrun PH (1996) Demecology of the false spider mite, *Brevipalpus phoenicis* (Geijskes) (Acari: Tenuipalpidae). Journal of Applied Entomology, 120(8): 493-499.

Kitajima EW, Chagas CM, Rodrigues JC (2003) *Brevipalpus*-transmitted plant virus and virus-like diseases: cytopathology and some recent cases. Experimental and Applied Acarology, 30 (1/3), 135–160.

Kitajima EW, Rodrigues JCV, Freitas-Astua J (2010) An annotated list of ornamentals naturally found infected by *Brevipalpus* mite-transmitted viruses. Sci. agric. (Piracicaba, Braz.) 67 (3): 348-371.

Kondo H, Maeda T, Tamada T (2003) Orchid fleck virus: *Brevipalpus californicus* mite transmission, biological properties and genome structure. Experimental and Applied Acarology, 30 (1): 215-223.

Krantz GW, Walter DE (2009) A Manual of Acarology. Texas Tech University Press, 3ª Edição 807p.

Lewandowski M, Skoracka A, Szydło W, Kozak M, Druciarek T, Griffiths DA (2014) Genetic and morphological diversity of *Trisetacus* species (Eriophyoidea: Phytoptidae) associated with coniferous trees in Poland: phylogeny, barcoding, host and habitat specialization. Experimental and Applied Acarology, 63, 497–520. http://doi:10.1007/s10493-014-9805-z

Lindquist EE, Krantz, GW, and Walter, DE (2009). Classification, pp. 97-103 In Krantz GW and Walter DE. [eds.], A Manual of Acarology. 3 ed. Texas Tech Univ. Press, Lubbock, Texas, USA.

Lopes MS, Martinello MS, Stancioli AR, Alves G, Sugayama R (2014) The role of natural and human-mediated pathways for invasive agricultural pests: A historical analysis of cases from Brazil. Agricultural Sciences, 5: 634-646.

Manglitz GR &Cory EN (1953) Biology and control of *Brevipalpus australis*. Journal of Economic Entomology, 46:116-119.

MAPA (2017). Ministério da Agricultura, Pecuária e Abastecimento: Lista de pragas quarentenárias ausentes e presentes. Portal Eletrônico. Brasília: disponível em http://www.agricultura.gov.br/. Acessado em Nov 2018

MAPA (2019). Ministério da Agricultura, Pecuária e Abastecimento: Instrução Normativa nº 45, de 22 de agosto de 2018. Portal Eletrônico. Brasília: disponível em http://www.agricultura.gov.br acessado em dez 2018.

Mendonça RS, Navia D, Cabrera RI, Oliveira MRV (2005) O ácaro do arroz, *Steneotarsonemus spinki* (Acari: Prostigmata: Tarsonemidae) uma praga quarentenária aproximando-se das fronteiras do Brasil. In: CONGRESSO BRASILEIRO DE ARROZ IRRIGADO, 4.; Reunião Da Cultura Do Arroz Irrigado, 26., Santa Maria, RS p. 112-114.

Mesa NC, Ochoa R, Welbourn WC, Evans AG, Gilberto JM (2009) A catalog of the Tenuipalpidae (Acari) of the World with a key to genera. Zootaxa 2098: 1-185.

Meyer MKP (1979) The Tenuipalpidae (Acari) of Africa with keys to the world fauna. Entomology Memoir, Department of Agriculture Republic South Africa, Pretoria, 50: 1–133.

Migeon A, Ferragut F, Escudero-Colomar LA, Fiaboe M, Knapp M, Moraes GJ, Ueckermann E, Navajas M (2009) Modelling the potential distribution of the invasive tomato red spider mite, *Tetranychus evansi* (Acari: Tetranychidae) Experimental and Applied Acarology 48 (3): 199–212.

Mitrofanov VI, Strunkova ZI (1979) A key to false spider mites. Operdelitl`Kleshchei-ploskotelok, *USSR*, 148, 1–148.

Moraes GJ, Flechtmann CHW (2008) Manual de Acarologia – Acarologia Básica e Ácaros de Plantas Cultivadas no Brasil. Holos Editora, Ribeirão Preto. 308p.

Navajas M, Navia D (2010) DNA-based methods for eriophyoid mite studies: review, critical aspects, prospects and challenges. Experimental and Applied Acarology, 51: 257–271.

Navajas M, Lagnel J, Fauvel G, de Moraes G (1999) Sequence variation of ribosomal internal transcribed spacers (ITS) in commercially important Phytoseiidae mites. Experimental and Applied Acarology, 23: 851-859.

Navajas M, Tsagkarakou A, Lagnel J, Perrot-Minnot MJ (2000) Genetic differentiation in *Tetranychus urticae* (Acari: Tetranychidae): polymorphism, host races or sibling species? Experimental and Applied Acarology, 24: 365–376.

Navajas M, Moraes GJ, Auger P, Migeon A (2013) Review of the invasion of *Tetranychus evansi*: biology, colonization pathways, potential expansion and prospects for biological control. Experimental and Applied Acarology, 59: 43–65.

Navia D, Mendonca RS, Ferragut F, Miranda LC, Trincado RC, Michaux J, Navajas M (2013) Cryptic diversity in *Brevipalpus* mite (Tenuipalpidae). Zoologica Scripta. 42(4): 406-426.

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

Navia D, Marsaro JR. AL, Silva FRDA, Gondim JR. MGC, Moraes GJDE (2010) First report of the red palm mite, *Raoiella indica* Hirst (Acari: Tenuipalpidae), in Brazil. Neotropical. Entomology, 40(3): 409-411.

Navia D, Domingos CA, Mendonça R S, Ferragut F, Rodrigues MAN, Gondim Jr, MGC (2014) Reproductive compatibility and genetic and morphometric variability among populations of the predatory mite, *Amblyseius largoensis* (Acari: Phytoseiidae), from Indian Ocean Islands and the Americas. Biological Control, 72: 17–29. https://doi.org/10.1016/j.biocontrol.2014.01.011

Norton RA (1994) Evolutionary aspects of oribatid mite life histories and consequences for the origin of the Astigmata. In: Houck, M. (Ed.), Mites. Ecological and evolutionary analyses of life-history patterns. Chapman and Hall Publishing, New York, 99–135.

Norton RA, Behan-Pelletier VM (2009) Suborder Oribatida. In: Krantz, G.W. & Walter, D.E., (Eds.), A Manual of Acarology. 3rd Edition. Texas Tech University Press, Lubbock, Texas, pp. 430–564.

Oliveira ICS (2014) Sistemática molecular para ácaros planos do gênero *Brevipalpus* Donnadieu (Acari: Tenuipalpidae) – utilização de marcadores moleculares

mitocondriais e nucleares. 2014. 120p. Dissertação (Mestrado em Zoologia) – Instituto de Ciências Biológicas, Universidade de Brasília, Brasília.

Pijnacker LP, Ferwerda MA, Bolland HR, Helle W (1980) Haploid female parthenogenesis inthe false spider mite *Brevipalpus obovatus* (Acari: Tenuipalpidae). Genetica 51: 211–214.

Pritchard AE, Baker EW (1952) The false spider mites of California (Acarina: Phytoptipalpidae). University of California Publications in Entomology, 9 (1): 1-94.

Pritchard AE, Baker EW (1958) The false spider mites (Acarina: Tenuipalpidae). Publications in Entomology, Berkeley, 14 (3): 175-274.

Rodrigues JCV (2000) Relações patógeno-vetor-planta no sistema leprose dos citros. PhD Dissertation. Centro de Energia Nuclear na Agricultura, da Univ. São Paulo, Piracicaba.

Samadi S, Barberousse A (2006) The tree, the network, and the species. Biological Journal of the Linnean Society, 89: 509–521. https://doi.org/10.1111/j.1095-8312.2006.00689.x

Samadi S, Barberousse A (2009) Species: towards new, well-grounded practices. Biological Journal of the Linnean Society, 97: 217–222. https://doi.org/10.1111/j.1095-8312.2009.01191.x

Schlick-Steiner BC, Steiner F M, Seifert B, Stauffer C, Christian E, Crozier RH (2010) Integrative Taxonomy: a multisource approach to exploring biodiversity. Annual Review of Entomology, 55, 421–438.

Shultz JW (1990) Evolutionary morphology and phylogeny of Arachnida. Cladistics 6: 1-38.

Shultz JW (2007) A phylogenetic analysis of the arachnid orders based on morphological characters. Zoological Journal of the Linnean Society, 150: 221–65.

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(3): 263–272

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

Tassi AD (2018) Diversidade morfológica e génetica de diferentes espécies de *Brevipalpus* (Acari: Tenuipalpidae) e suas competências como vetores de vírus. Escola superior de agricultura Luiz de Queiroz.

Tsagkarakou A, Cros-Arteil S, Navajas M (2007) First record of the invasive mite *Tetranychus evansi* in Greece. Phytoparasitica 35(5): 519-522.

Urquhart, GM (1996) Veterinary Parasitology, 2nd Edition. Blackwell Publishing, 307p.

Walter DE, Proctor HC (1999) Mites: Ecology evolution and behaviour. Wallingford, Oxon, UK: CAB lnternational. p322.

Weeks AR, Marec F, Breeuwer JAJ (2001) A mite species that consists entirely of haploid females. Science, 292: 2479–2482.

Welbourn WC, Ochoa R, Kane EC, Erbe EF (2003) Morphological observations on *Brevipalpus phoenicis* (Acari: Tenuipalpidae) including comparisons with *B. californicus* and *B. obovatus*. Experimental and Applied Acarology, 30(1/3): 107–133.

Wheeler WC, Hayashi CY (1998) The phylogeny of the extant chelicerate orders. Cladistics. 14:173–92.

Yates TL, Salazar-Bravo J, Dragoo JW (2004) The importance of knowing the tree of life. In: Cracraft, J. & Donoghue, M.J. (Eds.), Assembling the Tree of Life. Oxford University Press. New York, pp. 07–17.

Zhang F, Yu D, Luo Y, Ho SYW, Wang B, Zhu C (2014) Cryptic diversity, diversification and vicariance in two species complexes of *Tomocerus* (Collembola, Tomoceridae) from China. Zoologica Scripta, 43 (4): 393–404.

CAPÍTULO 2

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Flat mites of the genus *Brevipalpus* Donnadieu (Trombidiformes, Prostigmata, Tenuipalpidae) from the Azores archipelago- new records and host plants

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Abstract:

In spite of the agricultural importance of *Brevipalpus* Donnadieu mites (Tenuipalpidae) the fauna is still scarcely known in several zoogeographical regions including the Azores archipelago in the Macaronesia. A mite survey was conducted in the islands of Pico, Faial and Flores in 2015, being collected 55 samples of wild or cultivated plants. *Brevipalpus* mites where found in 15 samples from 12 plant species, belonging to 11 families. An integrative approach based on morphological and molecular data was applied to the taxonomic identification. Three target DNA fragments were PCR-amplified and sequenced per single mite: one mitochondrial- *COI* gene; and two nuclear DNA fragment- the subunit D1-D3 region in 28S rDNA gene and ITS2 region. Four species were found- *B. azores* Beard & Ochoa, 2015; *B. papayensis* Baker, 1949; *B. obovatus* Donnadieu, 1875; and a species identified as new for science belonging to *B. portalis* species group. The current survey showed a number of new host plants for *B. azores* and *B. papayensis*.

Such reports constitute valuable contribution taking into account that the ongoing taxonomic revision of the *Brevipalpus* genus implies the need for checking and associating biological information to the known revisited or new described taxa.

Keywords: Macaronesia, false spider mite, phytophagous mite, Portugal, integrative taxonomy, DNA-based identification

Introduction

Mites in the family Tenuipalpidae, commonly known as flat mites or false spider mites, are exclusively phytophagous and can damage plants by feeding directly on the epidermal cells of stems, leaves and fruits, or by vectoring plant viruses. The genus *Brevipalpus* Donnadieu is one of the most important of this family with 291 described species distributed throughout the biogeographic regions of the world mainly in tropical and subtropical areas (Mesa et al. 2009; Navia et al. 2013; Beard et al. 2015). Some species of *Brevipalpus* can transmit virus to their host plants, which are commonly known as "*Brevipalpus* Transmitted Virus (BTVs)". In addition to agricultural crops (e.g. citrus, coffee and passion fruit), more than 37 ornamental plants have been reported as infected by BTVs (Childers et al. 2003; Kitajima et al. 2010). Despite the importance of this group of mites knowledge on the fauna in several areas around the world still scarce, including the Azores islands.

The Azorean archipelago is composed by nine volcanic islands located in the middle of the Atlantic Ocean, about 1600 km from southwestern cost of Europe and 3900 km from the east coast of North America, and it belongs to the Macaronesian biogeographical province. These islands present predominantly a wet and mild climate, with small fluctuations of temperature (annual average is 17.5°C), high precipitation rate and air humidity (Rego et al. 2015). The Azores is characterized by a high level of endemism. Originally, the islands including coastal regions were covered with dense forests of low height, but nowadays are dominated by pastures, fields and exotic forests, with remnants of natural vegetation (Schäfer 2002). Geographic location linked to historical aspects made the archipelago become a strategic point in the trade route since the sixteenth century allowing the occurrence of both endemic and introduced American, European and African species (Amaral 1987).

In the last decades surveys of flora and fauna have been conducted in the Azores islands. The arthropods have the largest number of species, 2.278 (Borges et al. 2010a), of which approximately 181 species are mites (around 8%), 27 of them are endemic (Borges et al. 2010b). In addition, 22 species of predatory mites in the Phytoseiidae family were reported by Ferragut and Navia (2017), including five new taxa. In the Suborder Prostigmata, 19 species have been registered in the archipelago, of which none is endemic (Borges *et al*., 2010b; Rego et al. 2015). The islands with the greatest biodiversity are Flores, Faial, Pico and São Jorge (Borges et al. 2010b). *Brevipalpus* fauna in the Azores is poorly known. Carmona (1981) reported for the first time the occurrence of the genus in the archipelago, with identification of two species, *Brevipalpus phoenicis* Geijskes on *Citrus sinensis* and *Brevipalpus obovatus* Donnadieu on *Camellia sinensis* and *C. sinensis*. Costa-Comelles et al. (1994) and Soares et al. (1992) also reported occurrence of *B. phoenicis* on different host plants of three islands. In other reports of *Brevipalpus* mites the specific identification is not presented (Soares et al. 1991). Recently, a new species was described from plant material originated from the archipelago- *Brevipalpus azores* Beard & Ochoa (Beard et al. 2015).

Taxonomy of *Brevipalpus* mites has represented a challenge for acarologists for decades. Due to their morphological similarity most important species have been consistently confused and misidentified (Welbourn et al. 2003). Nowadays, a taxonomic revision of the *Brevipalpus* genus is ongoing. Taxonomic confusions on the most important species belonging to the *Brevipalpus phoenicis* species complex were enlightened by Beard et al. (2015) through a meticulous morphological study showing the need for revising previous identifications. Taxonomic studies integrating morphological and molecular data has supported an accurate identification of *Brevipalpus* species (Navia et al. 2013).

In this study results of a survey of *Brevipalpus* mites on cultivated, invasive and wild plants in three islands of the Azores archipelago- Pico, Faial and Flores- are presented. To the accurate identification of species, we employed an integrative approach in which morphological and molecular data were analyzed. New records of occurrence and host plants are presented. Remarks on the identification of previously reported species are presented.

Materials and Methods

Mite collections

Brevipalpus mites were searched in three islands of the Azores archipelago- Pico, Faial and Flores- in October 2015, by D. Navia and F. Ferragut. Samples of plant material (leaves, stems and/or fruits) were taken from wild, invasive and cultivated vegetation at different sites of the islands representing diverse topographical and botanical characteristics. A total of 55 samples, from 19 localities, accounting for 32 plant species belonging to 25 families were collected to acarological inspection (Supplementary material 1).

Morphological identification

Mite specimens were collected directly from plant material under a dissecting microscope (40x) and transferred to vials with 70% alcohol and absolute ethyl alcohol (for DNA extraction). Specimens were mounted and clarified on glass slides using Heinze-PVA medium. Morphological study was conducted under microscope optical with DIC (Nikon Eclipse Ni-U) and a scanning electron microscope (JEOL JSM-IT300LV). Increases ranging from 330x to 18,000x were used to capture the images in SEM. The specimens were visualized in ventral and dorsal position.

Studied specimens were deposited in the mite collection at the Laboratory of Acarology, Instituto Agroforestal Mediterráneo, Universitat Politècnica de València, Valencia, Spain and in the Plant Mite Collection, at Embrapa Genetic Resources and Biotechnology, Brasilia, DF, Brazil.

DNA extraction, amplification and sequencing

Samples stored in 100% ethyl alcohol were submitted to molecular analysis. Total genomic DNA was extracted from a single adult female using the DNeasy Tissue kit (Qiagen Germantown, MD, USA), according to the DNA extraction protocol 'Purification of Total DNA from Animal Blood or Cells' (SpinColumn Protocol). The manufacturer's instructions were modified for DNA extraction from tiny mites, as described by Dowling et al. (2010) and Mendonça et al. (2011). It is noteworthy that the specimens were not macerated allowing them to be recovered from the extraction column and mounted on slides in Heinze PVA medium. These slides were deposited as voucher

specimens in the Plant Mite Collection, at Embrapa Genetic Resources and Biotechnology, Brasília, Brazil.

Three target DNA fragments were PCR-amplified and sequenced per single mite: one mitochondrial- the cytochrome c oxidase subunit I (*COI*) gene; and two nuclear DNA fragment - the subunit D1-D3 region in 28S rDNA gene and ITS2 region. The reactions and PCR primers are described in Table 1 and 2. PCR products (5 μL) along with 0.5 μL of loading buffer were resolved by 1% (w/v) agarose gel electrophoresis prepared in 0.5X TBE buffer and visualized on GelRed staining (Biotium, Inc, Hayward, Canada). Gel images were acquired with Gel DocTM System (Bio Rad). The amplified fragments (*COI*, ITS2 and D1-D3) containing visible and single bands were directly sequenced on both strands using an ABI 3730XL Applied Biosystems automated sequencer (at Macrogen (Seoul, Korea). No additional primers were used for sequencing.

Table 1. PCR primers used in this study.

Marker acronym	Marker name	Fragment length (bp)	Primer Name	Primer sequence	Author
COI mtDNA	Cytochrome Oxidase Subunit 1	400	DNF	5'-3' TAC AGC TCC TAT AGA TAA AAC	Navajas et al., 1996
			DNR	3'-5' TGA TTT TTT GGT CAC CCA GAA G	
ITS 2 rDNA	Internal Transcribed Spacer 2	650	Bp4 rw	5'-3' AGTGCGAATTGCAGGACACA	in this study
			28Sc	3'-5' ATA TCG TTA AAT TCA GCG GG	Navajas et al., 1998
		700	Bp3 Deg-F	5'-3' TCG ATG AVG AAC GYA GCA RGY T	in this study
			28Sc	3'-5' ATA TCG TTA AAT TCA GCG GG	Navajas et al., 1998
D1-D3 28S rRNA	subunit D1-D3 of the gene 28S	1000	D23F	5'-3' GAG AGT TCA AGA GTA CGT G	Park and Foighil, 2000
			D6R	3'-5' CCA GCT ATC CTG AGG GAA ACT TCG	

Molecular identification

Molecular sequence-based identifications were performed from the GenBank blast platform (https://blast.ncbi.nlm.nih.gov/Blast.cgi) at the genus level (species level with morphological identification). The software Staden Package v.1.6.0 (Staden, Beal & Bonfield, 1998) was used for checking, editing and assembling the raw data into sequence contigs. The sequences were aligned using software such as the ClustalW multiple alignment procedure in BIOEDIT 7.0.4 (Hall, 1999) and Muscle (Muscle: multiple sequence alignment comparison by log-expectation program (Edgar 2004).

Table 2. Protocols of the PCR reactions used in this study.

PCR conditions					
Initial denaturation	94°C	4'	95°C	3'	95°C
No. cycles	35		35		35
Denaturation	92°C	1'	95°C	45"	95°C
Annealing	48°C	1' 30"	55°C	40"	50°C
Elongation	72°C	1' 30"	72°C	1' 15"	72°C
Final Elongation	72°C	10'	72°C	10'	72°C
Reaction mix composition (µl)					
H2O	15.77		4.35		3.575
10x buffer Qiagen	2,5		2.5		2.5
MgCl2 (25mM)	1.5		1.5		1.5
Oligonucleotide (10mM) (each)	0,75		0.175		0.25
dDNTP (10mM) Qiagen	1,2		0.5		0.125
Trehalose 10% Sigma	0		12.5		12.5
Bovine Serum Albumn (10mg/ml)	0,4		0.1		0.1
Taq polymerase Qiagen (5u)	0.13		0.2		0.2
DNA template	2		3		4

Results

Brevipalpus mites were found on 15 samples, including 12 plant species from wild, invasive and cultivated dicotyledon plants, belonging to 11 botanic families, on seven localities, which represents 27.3% of the collected samples (Table 3). Four species were identified based on both morphological traits and DNA sequences of mitochondrial and nuclear markers- *B. azores* (Fig. 1a); *B. obovatus* (Fig. 1b); *B. papayensis* (Fig. 1c); and a species identified as new for science belonging to the *B. portalis* species group (Figs. 1d) according to Baker & Tuttle (1987). DNA sequences of studied specimens were deposited in GenBank and accession numbers can be found in Table 3.

The most abundant and widespread *Brevipalpus* species were *B. papayensis* and *B. obovatus*, which togheter account for more than 72% of the total number of specimens collected and were found on 21.8% of the total samples examined. *Brevipalpus azores* was the third species in importance, representing about 21% of mites collected and found on 5.5% of the samples, while the new species belonging to the *portalis* species group was more scarce, representing only 7% of the *Brevipalpus* collected and inhabiting only one sample.

Brevipalpus papayensis and *B. obovatus* were found on six host plants, while *B. azores* was collected on four host plants. Infestation of the same sample by two or three

Brevipalpus species was observed on citrus, grapevine and sweet pittosporum (*Pittosporum undulatum* Vent.). Grapevine hosted the three *Brevipalpus* species- *B. azores*, *B. obovatus* and *B. papayensis*; citrus hosted *B. azores* and *B. papayensis*; and pittosporum hosted *B. azores* and *B. obovatus*. The new taxon, *B.* n. sp. *portalis* species group, was collected only on the endemic ivy *Hedera azorica* Carrière (Araliaceae).

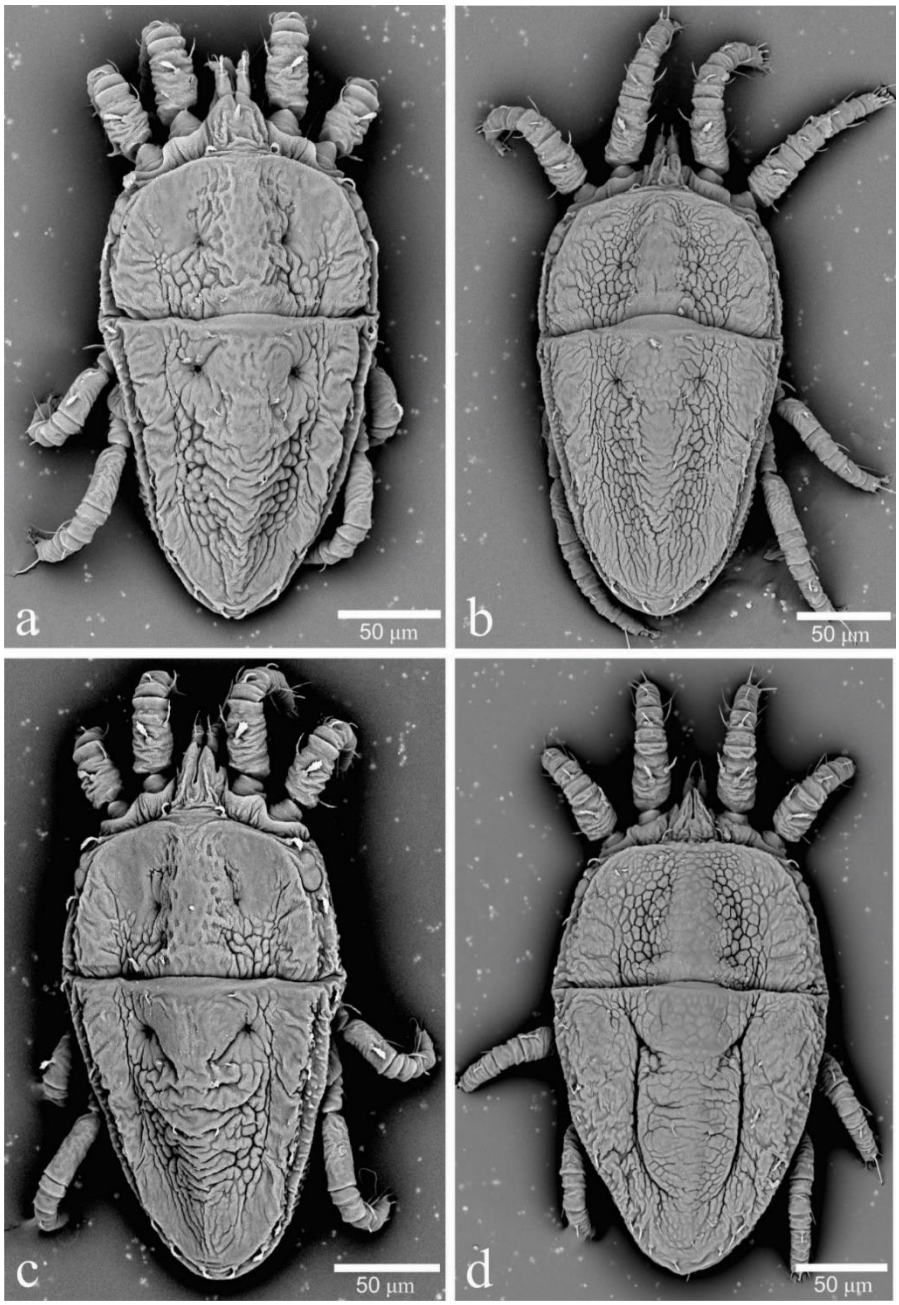

Figura 1. Dorsal view: (a) *B. azores*; (b) *B. obovatus*; (c) *B. papayensis*; (d) *B.* sp.

Table 3. Samples from where *Brevipalpus* mites were collected in this study in Azores archipelago- collection data, host plants, taxonomic identification of mites Brevipalpus and GenBank deposit accession number.

						GenBank Accession No.	
Host plant	**Host family**	**Locality**	**Coordinates**	**Date**	***Brevipalpus* spp.**	*COI*	ITS2
Citrus sinensis	Rutaceae	Faial, Praia de Almaxarife	38°33´36"N, 28°37´33"W	07/X/2015	*B. azores*		
					B. papayensis		MK508977
Eriobotrya japonica	Rosaceae	Pico, Madalena	38°25´24"N, 28°23´21"W	04/X/2015	*B. papayensis*		
Eleagnus umbellata	Eleagnaceae	Pico, Madalena	38°25´24"N, 28°23´21"W	04/X/2015	*B. papayensis*		
Hedera azorica	Araliaceae	Flores, Ponta da Fajâ	39°28´20"N, 31°15´22"W	08/X/2015	*B.* sp. nov.	MK499461 MK499462 MK499463 MK499464	
Ipomoea indica	Convolvulaceae	Pico, Madalena	38°25´24"N, 28°23´21"W	04/X/2015	*B. papayensis*		MK508966 MK508967 MK508968 MK508969
Ipomoea indica	Convolvulaceae	Faial, Horta	38°33´03"N, 28°38´21"W	07/X/2015	*B. obovatus*		

Melissa officinalis	Lamiaceae	Pico, Silveira	38°24´48"N, 28°17´02"W	06/X/2015	*B. obovatus*	MK499455 MK499456 MK499457	
Mentha sp.	Lamiaceae	Pico, Silveira	38°24´48"N, 28°17´02"W	05/X/2015	*B. obovatus*		
Phytolacca americana	Phytolaccaceae	Pico, Madalena	38°25´24"N, 28°23´21"W	04/X/2015	*B. obovatus*		
Pittosporum undulatum	Pittosporaceae	Faial, Praia de Almaxarife	38°33´36"N, 28°37´33"W	07/X/2015	*B. azores* *B. obovatus*		
Rhododendron indicum	Ericaceae	Faial, Horta	38°33´03"N, 28°38´21"W	07/X/2015	*B. azores*		MK508976 MK508978 MK508979
Tecoma capensis	Bignoniaceae	Pico, Madalena	38°25´24"N, 28°23´21"W	04/X/2015	*B. papayensis*		MK508970 MK508971 MK508972 MK508981
Vitis vinifera	Vitaceae	Pico, Madalena	38°25´24"N, 28°23´21"W	04/X/2015	*B. papayensis*		
Vitis vinifera	Vitaceae	Faial, Lonbega	38°32´03"N, 28°44´18"W	08/X/2015	*B. obovatus*	MK499459	
					B. papayensis		MK508973
					B. azores	MK499460	MK508974 MK508975
Vitis vinifera	Vitaceae	Flores, Lajedo	39°23´50"N, 31°14´40"W	11/X/2015	*B. obovatus*	MK499465 MK499469	MK508980

Discussion

Although the relatively low number of *Brevipalpus* species found during surveys in the Azores island, in wich just one non-reported species- *B. papayensis*- and one new species were found, our results included new host plants reports for the collected species and allowed elaborating interesting remarks on the taxonomic identification of *Brevipalpus* mites from the Azores and on the phytosanitary risk associated with their occurrence.

Host plants – new records and remarks on associations

The current survey showed a number of new host plants for *B. azores* and *B. papayensis*. Such reports constitute valuable contribution taking into account that the ongoing taxonomic revision of the *Brevipalpus* genus implies the need for checking and associating biological information to all the revisited or new described taxa.

Brevipalpus azores was for the first time collected on *Pittosporum undulatum* (Pittosporaceae), and *Rhododendron indicum* (L.) Sweet (Ericaceae). Main hosts previously reported for *B. azores* were citros (*Citrus* spp.), and it had also been reported on *Datura stramonium* (Solanaceae); *Camelia sinensis* (Theaceae); *Chinchona* sp. (Rubiaceae); *Hedera* sp. (Araliaceae); and *Musa* spp. (Musaceae), *Vitis* sp. (Vitaceae) (Beard et al. 2015).

New host plants pointed out for *B. papayensis* as result of this survey are *Eriobotrya japonica* (Rosaceae), *Eleagnus umbellata* (Eleagnaceae), *Ipomoea indica* (Convolvulaceae), *Tecomaria capensis* (Bignoniaceae) and *Vitis vinifera* (Vitaceae). Host plants previously confirmed for *B. papayensis* were *Camellia sinensis* (Theaceae), *Carica papaya* (Caricaceae), *Citrus* sp. (Rutaceae), *Coffea arabica* (Rubiaceae), *Polygonum lapathifolium* (Polygonaceae), and *Lenwebbia* sp. (Myrtaceae) (Beard *et al*. 2015).

The new host reports for *B. azores* and *B. papayensis* broaden its host range in plant species and even on botanical families; it includes common ornamental and agricultural plants. Among them stand out grapevine as a new host for *B. papayensis*, a valuable crop.

The new species, tentatively classified as belonging to the *B. portalis* species group, was collected only on *Hedera azorica*, which constitute a new host plant for *Brevipalpus* mites. Some *Brevipalpus* species have been reported on ivies (*Hedera* spp.) (Mesa et al. 2009), however this is the first one associated with an endemic Azorian

species. All host plants in which *B. obovatus* was found in this study had already been listed by Livshitz, Mitrofanov & Vasilieva (1972), Ehara (1966), and Beard et al. (2012).

Although a considerable number of wild/invasive/cultivated plants has been sampled during this survey the known *Brevipalpus* species- *B. azores*, *B. obovatus*, and *B. papayensis*- were collected just on cultivated or invasive ones; *P. undulatum*, *Ipomoea indica* and *T. capensis* have been considered as noxious weed or invasive plants in Azores (Silva et al. 2008; CABI 2018; GISD 2015). The only wild/native plant in which *Brevipalpus* mites were found was the ivy *H. azorica*, an endemic species, which was associated with the new taxon. It suggests that these species have been introduced in the Azores through propagation plant material and maybe have not yet adapted to wild plants in the archipelago.

Previous identifications of Brevipalpus mites in the Azores islands

Differently from Carmona (1981), Soares et al. (1992) and Costa-Comelles et al. (1994) *B. phoenicis* was not found during the surveys we conducted in the Azores archipelago. According to Costa-Comelles et al. (1994) this species was found in Faial, Pico and San Miguel on medal, orange, mint and *Hydranger*; Soares et al. (1992) and Schander et al. (1995) on citrus in São Jorge and São Miguel islands, respectively; and to Carmona (1981) on orange. These reports suggest that *B. phoenicis* should be a common species in the archipelago. On the other hand in this study one of the most common *Brevipalpus* species was *B. papayensis*, which was found on orange, loquat, blue morning glory (*Ipomoeae indica* (Burm.) Merr.), Cape honeysuckle (*Tecomaria capensis* (Thunb.) Lindl., and grapevine in Pico and Faial islands. *Brevipalpus papayensis* is a former synonym of *B. phoenicis* (Geijskes, 1939). This species was resurrected by Beard et al. (2015) during a revision on the *B. phoenicis* species complex. Furthermore other species occurring in Azores, *B. azores*, also has been previously misidentified as *B. phoenicis* until be described by Beard et al. (2015). Then it is possible that *B. phoenicis* specimens collected by Carmona (1981), Soares et al. 1992 ; and Costa-Comelles et al. (1994) indeed should be *B. papayensis* or even *B. azores* instead of *B. phoenicis* sensu stricto as informed in publications. Taking into account that *Brevipalpus* taxonomy is under revision and recent changes make necessary revisiting previous identifications it would be relevant to clarifying *B. phoenicis* report in Azores. For this purpose would be necessary proceed a detailed morphological study of voucher specimens collected during

previous surveys in Azores taking into account the taxonomic revision of the *B. phoenicis* species complex presented by Beard et al. (2015).

Brevipalpus yothersi Baker, 1949, the most common *Brevipalpus* species around the world and the proper taxonomic designation of several populations previously misidentified as *B. phoenicis* (Welbourn et al. 2003; Beard et al. 2014), was not collected during this survey. It is possible that this species is also present in Azores and that colletion efforts have not been enough to find it. By other side is possible that this species is actually not present in the archipelago similarly to observations by Jacobucci et al. (2018) on Western Mediterranean area and some Macaronesian citrus crop.

Phytosanitary risks associated with Brevipalpus mites in Azores

One of the main damage caused by *Brevipalpus* mites is the transmission of BTV's. Actually few *Brevipalpus* species have been confirmed as vectors of BTVs (Childers et al. 2003). The main vector was considered to be *B. phoenicis* (Childers et al. 2003) and due to the taxonomic revision of *B. phoenicis* species complex by Beard et al. (2015) this biological performance should be checked for each taxon in the *B. phoenicis* species complex.

Currently the main BTV vector is *B. yothersi*, which can transmit among others *Citrus leprosis virus* (Roy et al. 2015). However recent transmission studies revealed that *B. papayensis*, one of the most common species in this survey, can also transmit *Citrus leprosis virus*-Cytoplasmic and *Coffee ringspot virus* (Nunes et al. 2018) consituting a BTV vector.

Furthermore *B. obovatus*, the other more common *Brevipalpus* species found in this study, has been confirmed to be vector of BTVs- e.g. *Cestrum ringspot virus* - CeRSV (Guidotti et al. 2006); and *Solanum violaelifolium ringspot virus* – SvRSV (Ferreira et al. 2007).

Until now BTVs occurrence have not yet been reported for Azores islands, however the presence of these vectors warns to the phytosanitary risk of these ethiological agents which can seriously affect agricultural crops and similarly serious lead to imposition of phytosanitary barriers for the plant material exchange. For example *Citrus leprosis virus* is regulated as quarantine pests to several regions, including North America and Europe (EFSA et al. 2017).

General remarks

This study just provides a restrict information on the *Brevipalpus* fauna in Azores, which identifications resulted from a multi tool approach in which a detailed morphological study was integrated to molecular data. Further efforts should be conducted to increase the knowledge on the *Brevipalpus* mite fauna in the Azores archipelago. Surveys should include a higher number of plant species– endemic, invasive, cultivated-, and of microclimates. Other islands of the archipelago of Azores should be surveyed. It would be interesting also carrying out samplings along the year in a way to cover information along distinct seasons, which can influence population dynamic of species.

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References

Amaral JBM. 1987. Development of the Azores: Problems and Prospects. Athens Center of Ekistics. Ekistics, 54, (323/324): 143-146

Baker EW, Tuttle DM. 1987. The False Spider Mites of Mexico (Tenuipalpidae: Acari). U.S. Department of Agriculture, Technical Bulletin. 1706: 237p.

Beard JJ, Ochoa R, Bauchan GR, Braswell WE. 2014. *Brevipalpus phoenicis* (Geijskes) species complex-resurrection of E.W. Baker's species (Acari: Tenuipalpidae). In: International Congress of Acarology, 14, 2014, Kyoto, Japan. Abstracts, Kyoto: 2014. p. 60.

Beard JJ, Ochoa R, Bauchan GR, Trice M, Redford A, Walters T, Mitter C. 2013. Flat mites of the world. Available from: http://idtools.org/id/mites/flatmites/ (Accessed on Dez 2018).

Beard JJ, Ochoa R, Braswell WE, Bauchan GR. 2015. *Brevipalpus phoenicis* (Geijskes) species complex (Acari: Tenuipalpidae) - a closer look. Zootaxa 3944 (1): 001-067.

Borges PAV, Bried J, Costa A, Cunha R, Gabriell R, Gonçalves V, Martins AF, Melo I, Parente M, Raposeiro P, Rodrigues P, Santos RS, Silva L, Vieira P, Vieira V,

Mendonça E, Boieiro M. 2010a. Descrição da biodiversidade terrestre e marinha dos Açores [dissertation]. Açores (PT): Universidade do Açores.

Borges PAV, Cunha R, Gabriel R, Martins AF, Silva L, Vieira V, Dinis F, Lourenço P, Pinto N. 2010b. Descrição da biodiversidade terrestre dos Açores [dissertation]. Açores (PT): Universidade do Açores.

CABI, 2018. *Fallopia japonica*. In: Invasive Species Compendium. Wallingford, UK: CAB International. Available from: www.cabi.org/isc (Accessed on Nov 2018).

Carmona MM. 1981. Dois ácaros fitófagos dos Açores. Agronomia Lusitana. 41(2): 139-147.

Childers CC, French JV, Rodrigues JCV. 2003. *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.

Costa-Comelles J, Soares AO, Vercher R, Schanderl H, Ferragut F. 1994. A contribution to data on mite fauna in the Azores Islands. Relatorios e Comunicações do Departamento de Biología. Universidade dos Açores, Portugal, 22: 40–44.

Dowling APG, Bauchan GR, Ochoa R, Beard JJ. 2010. Scanning Electron Microscopy vouchers and genomic data from an individual specimen: Maximizing the utility of delicate and rare specimens. Acarologia 50(4): 479–485.

Ehara S. 1966. The tetranychoid mites of Okinawa Island (Acarina: Prostigmata). Journal of the Faculty of Science Hokkaido University, Series VI Zoology, 16(1): 1–22.

Ferraguti F, Navia D. 2017. Phytoseiid Mites (Acari: Mesostigmata) of the Azores Islands. Systematic & Applied Acarology 22(10): 1585–1621. http://doi.org/10.11158/saa.22.10.41585

Ferreira PTO, Locali-Fabris EC, Freitas-Astúa J, Antonioli-Luizon R, Gomes RT, Machado MA, Kitajima EW. 2007. Caracterização de um vírus baciliforme isolado de *Solanum violaefolium* transmitido pelos ácaros *Brevipalpus phoenicis* e *B. obovatus* (Acari: Tenuipalpidae). Summa Phytopathologica, 33 (3): 264-269.

Geijskes DC. 1939. Beitäge zur kenntnis der europäischen spinnmilben (Acari, Tetranychidae), mit bensonderer berücktigung der Niederländischen arten. Mededeelingen van de Landbouwboogeschool, Wageningen. 42: 1–68

Global Invasive Species Database (GISD). 2019. Species profile: *Tecoma capensis*. Downloaded from http://www.iucngisd.org/gisd/species.php?sc=1591on 10-02-2019.

Guidotti AR, Rodrigues V, Kitajima EW, Novelli VM, Machado MA, Freitas-Astúa J. 2006. Eficiência na transmissão do *Cestrum ringspot virus* por ácaros *Brevipalpus obovatus* após diferentes períodos de tempo. Anais do I Simpósio Brasileiro de Acarologia p.250.

Guidotti AR, Rodrigues V, Machado MA, Freitas-Astúa J. 2006. Transmissão do *Cestrum ringspot virus* por diferentes fases ativas do ácaro *Brevipalpus obovatus*. Summa Phytopathologica 32: 75 (239).

Jacobucci MA, López-Olmos S, Ferragut F. 2018. Are there *Brevipalpus* mites (Acari, Tenuipalpidae) transmitting *Citrus* Leprosis Virus on Western Mediterranean
and Macaronesian citrus crops? Integrated Control in Citrus Fruit Crops IOBC-WPRS Bulletin Vol. 132, 2018 pp. 32-37.

Kitajima EW, Chagas CM, Rodrigues JCV. 2003. *Brevipalpus* –transmitted plant virus-like diseases: cypathology and some recente cases. Experimental and Applied Acarology, 30: 135-160.

Kitajima EW, Rodrigues J CV, Freitas-Astua J. 2010. An annotated list of ornamentals naturally found infected by *Brevipalpus* mite-transmitted viruses. Sci. agric. (Piracicaba, Braz.), 67 (3): 348-371.

Livshitz IZ, Mitrofanov VI, Vasilieva EA. 1972. On the morphology and biology of *Brevipalpus obovatus* Donnadieu, 1875. Works of the Nikitsk Botanical Garden 61: 65–75.

EFSA. 2017. Panel on Plant Health (PLH). Pest categorisation of *Citrus leprosis* viruses. EFSA Journal, 15(12):5110 https://doi.org/10.2903/j.efsa.2017.5110.

Mendonça RS, Navia D, 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 NC, Ochoa R, Welbourn WC, Evans AG, Gilberto JM. 2009. A catalog of the Tenuipalpidae (Acari) of the World with a key to genera. Zootaxa 2098: 1-185.

Navia D, Mendonca RS, Ferragut F, Miranda LC, Trincado RC, Michaux J, Navajas M. 2013. Cryptic diversity in *Brevipalpus* mites (Tenuipalpidae). Zoologica Scripta. 42(4): 406-426.

Nunes MA, de Carvalho Mineiro JL, Rogerio LA, Ferreira LM, Tassi A, Novelli VM, Kitajima EW, Freitas-Astúa J. 2018. First Report of *Brevipalpus papayensis* as vector of Coffee ringspot virus and Citrus leprosis virus C. *Disease Notes* Plant Disease, 102 (5). https://doi.org/10.1094/PDIS-07-17-1000-PDN

Rego C, Boieiro M, Vieira V, Borges PAV. 2015. The biodiversity of terrestrial arthropods in Azores. Revista IDE@ - SEA, 5B: 1–24.

Roy A, Hartung J, Schneider W, Shao J, León G, Melzer M, Beard J, Otero-Colina G, Bauchan GR, Ochoa R. 2015. Role bending: complex relationships between viruses, hosts and vectors related to citrus leprosis, an emerging disease. Phytophatology 105: 1013-1025.

Schäfer HW. 2002. Chorology and Diversity of the Azorean Flora [dissertation]. Regensburg (Baviera): Vorklinische Medizin Der Universität Regensburg.

Schander JCH, Soares AO, Almeida JMP.1995. Identificação de pragas dos citrinos em pomares da Ilha de S. Miguel (Açores). Açoreana 8 (1) : 89-93.

Silva L, Corvelo R, Moura M, Guerra AS, Fernandes FM. 2008. *Ipomoea indica* (Burm. Fil.) Merr. In: Silva L, Land EO, Luengo JLR (eds) Flora e fauna terrestre invasora na Macaronésia. Top 100 nos Açores, Madeira e Canárias. Arena, Ponta Delgada. 318- 321.

Soares AO, Schanderl JH, Almeida JMP.1992. Algumas Pragas nos pomares de citrinos na da ilha do Pico (Açores). Relatórios e Comunicações do Departamento de Biologia, 92: 49-52.

Soares AO, Schanderl JH, Almeida JMP.1993. Algumas pragas de citrinos da ilha de S. Jorge (Açores). Relatórios e Comunicações do Departamento de Biologia, 21: 21-27.

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

Supplementary material 1. Total list of samples collected in this work in the Azores Archipelago.

Sample	Genus Plant	Esp Plant	Family	Location	Island	Date	*Brevipalpus*
AZ 1	*Vitis*	*vinifera*	Vitaceae	Santa Margarida	Pico	4.10.2015	
AZ 2	*Vitis*	*vinifera*	Vitaceae	Candelaria	Pico	4.10.2015	
AZ 3	*Ipomoea*	*indica*	Convolvulaceae	Candelaria	Pico	4.10.2015	X
AZ 4	*Phytolacca*	*americana*	Phytolaccaceae	Candelaria	Pico	4.10.2015	X
AZ 5	*Malus*	*domestica*	Rosaceae	Candelaria	Pico	4.10.2015	
AZ 6	*Tecoma*	*capensis*	Bignoniaceae	Candelaria	Pico	4.10.2015	X
AZ 7	*Eriobotrya*	*japonica*	Rosaceae	Candelaria	Pico	4.10.2015	X
AZ 8	*Eleagnus*	*umbellata*	Eleagnaceae	Candelaria	Pico	4.10.2015	X
AZ 9	*Vitis*	*vinifera*	Vitaceae	Candelaria	Pico	4.10.2015	X
AZ 10	*Mentha*	*suaveolens*	Labiatae	Silveira	Pico	5.10.2015	X
AZ 11	*Brugmansia*	*candida*	Solanaceae	Silveira	Pico	5.10.2015	
AZ 12	*Viburnum*	*treleasei*	Caprifoliaceae	Silveira	Pico	5.10.2015	
AZ 13	*Frangula*	*azorica*	Rhamnaceae	Lajes	Pico	5.10.2015	
AZ 14	*Cryptomeria*	*japonica*	Taxodiaceae	Silveira	Pico	5.10.2015	
AZ 15	*Calluna*	*vulgaris*	Ericaceae	Silveira	Pico	5.10.2015	
AZ 16	*Erica*	*azorica*	Ericaceae	Silveira	Pico	5.10.2015	
AZ 17	*Juniperus*	*brevifolia*	Cupressaceae	Silveira	Pico	5.10.2015	
AZ 18	*Juniperus*	*brevifolia*	Cupressaceae	Lagoa do Capitão. São Roque	Pico	5.10.2015	
AZ 19	*Hedychium*	*gardnerarum*	Zingiberaceae	Silveira	Pico	5.10.2015	
AZ 20	*Rubus*	*ulmifolius*	Rosaceae	Silveira	Pico	6.10.2015	
AZ 21	*Melissa*	*officinalis*	Labiatae	Silveira	Pico	6.10.2015	X
AZ 22	*Lycium*	*europaeum*	Solanaceae	Silveira	Pico	6.10.2015	

AZ 23	*Rubus*	*ulmifolius*	Rosaceae	São Roque	Pico	6.10.2015	
AZ 24	*Laurus*	*azorica*	Lauraceae	São Roque	Pico	6.10.2015	
AZ 25	*Vitis*	*vinifera*	Vitaceae	Silveira	Pico	6.10.2015	
AZ 26	*Ipomoea*	*indica*	Convolvulaceae	Horta	Faial	7.10.2015	X
AZ 27	*Pittosporum*	*undulatum*	Pittosporaceae	Horta	Faial	7.10.2015	
AZ 28	*Citrus*	*sinensis*	Rutaceae	Praia de Almoxarife	Faial	7.10.2015	X X
AZ 29	*Ulmus*	*procera*	Ulmaceae	Horta. Jardín Botánico	Faial	7.10.2015	
AZ 30	*Acer*	*pseudoplatanus*	Aceraceae	Horta. Jardín Botánico	Faial	7.10.2015	
AZ 31	*Camellia*	*japonica*	Theaceae	Praia de Almoxarife	Faial	7.10.2015	
AZ 32	*Pittosporum*	*undulatum*	Pittosporaceae	Praia de Almoxarife	Faial	7.10.2015	X X
AZ 33	*Pseudosasa*	*japonica*	Gramineae	Horta. Jardín Botánico	Faial	7.10.2015	
AZ 34	*Rhododendron*	*indicum*	Ericaceae	Horta. Jardín Botánico	Faial	7.10.2015	X
AZ 35	*Hydrangea*	*macrophylla*	Hydrangeaceae	Castelo Branco	Faial	8.10.2015	
AZ 36	*Malus*	*domestica*	Rosaceae	Castelo Branco	Faial	8.10.2015	
AZ 37	*Vitis*	*vinifera*	Vitaceae	Lonbega	Faial	8.10.2015	X
AZ 38	*Erica*	*azorica*	Ericaceae	Ribeira Grande	Flores	8.10.2015	
AZ 39	*Solanum*	*nigrum*	Solanaceae	Ponta da Faja	Flores	9.10.2015	
AZ 40	*Rubus*	*hochstetterorum*	Rosaceae	Ponta da Faja	Flores	9.10.2015	
AZ 41	*Lavatera*	*sp.*	Malvaceae	Ponta da Faja	Flores	9.10.2015	
AZ 42	*Hedera*	*azorica*	Araliaceae	Ponta da Faja	Flores	9.10.2015	X
AZ 43	*Erica*	*azorica*	Ericaceae	Mirador da Casinha. Fajãzinha	Flores	9.10.2015	
AZ 44	*Festuca*	*jubata*	Poaceae	Mirador da Casinha. Fajãzinha	Flores	9.10.2015	
AZ 45	*Juniperus*	*brevifolia*	Cupressaceae	Ribeira Grande	Flores	10.10.2015	
AZ 46	*Rubus*	*hochstetterorum*	Rosaceae	Ribeira Grande	Flores	10.10.2015	

AZ 47	*Erica*	*azorica*	Ericaceae	Ponta de Albarnaz	Flores	10.10.2015	
AZ 48	*Laurus*	*azorica*	Lauraceae	Ponta Delgada	Flores	10.10.2015	
AZ 49	*Festuca*	*petraea*	Gramineae	Ponta de Albarnaz	Flores	10.10.2015	
AZ 50	*Frangula*	*azorica*	Rhamnaceae	Ponta Delgada	Flores	10.10.2015	
AZ 51	*Rubus*	*hochstetterorum*	Rosaceae	Lajedo	Flores	11.10.2015	
AZ 52	*Vitis*	*vinifera*	Vitaceae	Aldeia da Quada	Flores	11.10.2015	X
AZ 53	*Festuca*	*petraea*	Gramineae	Fajã do Lopo Vaz. Lajes	Flores	11.10.2015	
AZ 54	*Solanum*	*mauritianum*	Solanaceae	Fajã do Lopo Vaz. Lajes	Flores	11.10.2015	
AZ 55	*Cryptomeria*	*japonica*	Taxodiaceae	Fajã do Lopo Vaz. Lajes	Flores	11.10.2015	

X = *Brevipalpus* found.

CAPÍTULO 3

Note: manuscript to be submitted to the ***Systematic and Applied Acarology*** *(2017 Impact Factor = 1.696)*

A new species of *Brevipalpus* (Acari: Tenuipalpidae) from the Azores islands, with remarks on the *B. cuneatus* species group

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Abstract *Brevipalpus* is the largest genus of the Tenuilpalpidae family, with about 291 species, some of which are considered of economic importance. Although the agricultural importance of *Brevipalpus* mites the fauna is scarcely known in several regions around the world, specially on wild plants. In this work a new species collected on an endemic ivy of the Azores Archipelago, *Hedera azorica* Carrière (Araliaceae), is described based on an integrative approach using morphological traits of adults, obtained through electron and optical microscopy, and in mitochondrial and nuclear DNA sequences. Phylogenetic analyses were carried out in a way to determining its phylogenetic placement. The new species is tentatively classified as belonging to the *B. portalis* species group. Morphological similarity between the new species and *B. cuneatus* Canestrini and Fanzago lead to point out some inconsistences in the current *B. cuneatus* species group classification as well as on species group concepts herein discussed.

Keywords: flat mites, false spider mite, Macaronesia, taxonomy, ivy, *Hedera azorica*, *Brevipalpus cuneatus*.

Introduction

The genus *Brevipalpus* Donnadieu is one of the most important and well-known of the Tenuipalpidae family (Tetranychoidea) and currently is the second most numerous with approximately 300 valid species occurring on all continents (Beard *et al*. 2012; Childers *et al*. 2003a; Mesa *et al*. 2009 and Navia *et al*. 2013). All species of this genus are phytophagous and can infest cultivated (ornamental, fruit and forest) and wild plants. More than 1000 host plants from 130 families have been registered for species of economic importance (Childers *et al*. 2003b and Kitajima *et al*. 2010). *Brevipalpus* mites can reach pest status especially for their role as vectors of plant viruses which result in million-dollar losses in the agricultural sector, as well as direct damages, since high populations can considerably damage fruits, leaves, shoots and stems (Childers *et al*. 2003c; Kondo *et al*. 2003; Bastianel *et al*. 2010; Kitajima *et al*. 2010 and Childers & Rodrigues, 2011). Despite the importance of this genus, many regions of the world have not yet been explored, especially natural environments, since surveys of these mites have been concentrated on cultivated plants.

The Azorean archipelago is composed by volcanic islands located in the middle of the Atlantic Ocean about 1600 km from continental Portugal and 3900 km from the east coast of North America. Azorean islands have a humid and mild climate. The biogeographic province of Macaronesia, where the archipelago is located, is characterized by a high level of endemism (Dias *et al*., 2005). Human activities such as agriculture, colonization and the introduction of exotic plants have greatly modified the local phytophysiognomy, whose original forests are being lost and native vegetation which harbours endemic species is restricted to small areas (Schäfer, 2002).

The *Brevipalpus* mite fauna is still little known in the Azores archipelago; only three species have been reported- *B. phoenicis* Geijskes; *B. obovatus* Donnadieu; and *B. azores* Beard & Ochoa (Borges, 2012; Borges *et al*., 2005; Beard *et al*., 2015).

During the surveys of plant mites in the Azores archipelago, a new *Brevipalpus* species was identified from *Hedera azorica* Carrièrre. (Araliaceae). This plant is an endemic ivy to Azores. Few records of *Brevipalpus* mites associated with plants of the genus *Hedera* are known. Only *B. obovatus* has been reported from *Hedera helix* Linnaeus, *Hedera colchica* K. Koch and *Hedera* sp. (Pritchard & Baker, 1952; Livshitz, *et al*., 1972; Ochoa & Salas, 1989).

Taxonomy of *Brevipalpus* mites has represented a challenge for acarologists for decades. Species have been consistently confused and misidentified due to their morphological similarity (Welbourn *et al*., 2003). Poor descriptions and illustrations as well as lack of critical study of their morphology have rendered *Brevipalpus* mites taxonomy a puzzle (see McGregor 1949; Beard *et al*. 2015). Through meticulous morphological studies new taxonomic traits have been pointed out and molecular studies have uncovered cryptic species (Navia *et al*., 2013; Beard *et al*., 2015). Therefore, an integrative approach, using several microscopy techniques and molecular data, is of extreme importance for the progress in the systematics of this genus.

According to Baker & Tuttle (1987) *Brevipalpus* genus is subdivided into six species groups- *B. californicus, B. cuneatus, B. portalis, B. phoenicis, B. obovatus* and *B. frankeniae*. This subdivision is exclusively based on morphological characters- number of dorsolateral setae on the opisthosoma, number of solenidia on the tarsus II of the female; and number of setae on the distal segment of palp. Knowledge on the evolutionary relationships among species in this genus is scarce and among species groups is lacking (Rodrigues *et al*., 2003; Navia *et al*., 2013).

In this article we present the description of the new Azorean species from the ivy *H. azorica* based on an integrative approach using morphological traits of females and male, obtained through electron and optical microscopy, and in mitochondrial and nuclear DNA sequences. Based on Baker & Tuttle (1987) this new species was classified as belonging to the *portalis* group, even though being very similar to the species *B. cuneatus* Canestrini and Fanzago, in the *cuneatus* group. This discrepancy highlighted some inconsistencies related to the current *B. cuneatus* species group as well as on the *B. portalis* and *B. cuneatus* species group concepts, which are herein discussed.

Material and methods

Mite collections

The mites were collected in October 2015 in the island of Flores, Azores archipelago, from *H. azorica* (Araliaceae) (Table 1). Specimens were collected by direct inspection using a dissecting microscope and transferred to bottles with 70% and 100% ethyl alcohol for morphological and molecular identification. Specimens preserved in 70% alcohol were slide-mounted in Heinze-PVA medium and dried in an oven at 50° C until clarification.

Morphological identification

The slide-mounted mites were examined at 40x and 100x magnification using differential interference contrast (DIC) in a compound microscope Nikon Eclipse Ni-U. Illustrations were performed on a graphic tablet using Concepts software (https://concepts.app). Images were captured with a digital camera Nikon DXM 1200 and modified in LightRoom CC and Photoshop CC (© Adobe Systems Inc.) and CorelDRAW X7 software (Corel Corporation). The measurements are presented from the holotype in micrometers (μm), followed by the range between the holotype and paratypes in parenthesis (both sides measured), and average in brackets. The distances between the setae were obtained from the distance from the inner edge of one base to the other (minimum distance between two base bases). Chaetotaxy follows that of Lindquist (1985).

Table 1. Samples used in this study for molecular phylogenetic analysis- collection data and GenBank accession number of nucleotide sequences.

						accession number in Genbank		
Species	**Host plant, family**	**Country, state/region, city**	**Latitude/longitude**	**collection date**	**Collector**	***COI* mtDNA**	**28S rRNA (D1-D3)**	**Reference**
B. californicus	*Pittosporum tobira* ,Pittosporaceae	Spain, Valencia, Valencia	+39° 30' 3.10", -0° 22' 15.66"	01.X.2011	Ferragut F. and Navia D.	MH204770	MK293656	
	Citrus sinensis , Rutaceae)	Spain, Cordoba, Palma del Rio	+37° 41' 58.72", -5° 16' 51.00"	X.2011	Ferragut F	MH204784	MK293708	Oliveira, 2014
B. chilensis	*Ligustrum sinensis* , Oleaceae	Chile, Santiago, Curacavi	-33° 28 '9.80", -70° 43' 26.14"	28.II.2013	Trincado R.	MH204699 and MH204701		
	Magnolia grandiflora , Magnoliaceae	Chile, Cachapoal, S. F. de Mostazal	-33° 54' 58.38", -70° 40' 31.22"	18.II.2013	Trincado R.		MK293671	Oliveira, 2014
	Magnolia grandiflora , Magnoliaceae	Chile, Cachapoal, S. F. de Mostazal	-33° 54' 58.38", -70° 40' 31.22"	08.V.2014	Kitajima E. W.		MK293716	
B. aff. *cuneatus* sp. 1	*Rosmarinus officinalis* , Lamiaceae	Spain, Valencia, Valencia	+39° 28' 11.67", -0° 22' 34.64"	12.X.2013	Navia D. and Ferragut F.	MH204728 MH204724	MK293625 MK293702	Oliveira, 2014
B. aff. *cuneatus* sp. 2	*Erica* sp., Ericaceae	Spain, Castelló, Castellon	+39° 55' 8.18", -0° 23 37.22"	09.VII.2013	Navia D. and Ferragut F.	MH204713 MH204714		Oliveira, 2014
B. incognitus	*Phoenix* sp., Arecaceae	Brazil, São Paulo, Piracicaba	-22º 43' 31", -47º 38' 57"	31.V.2013	Kitajima E. W.	MH204723	MK293687	
	Cocos nucifera , Arecaceae	Brazil, Minas Gerais, Janaúba	-15° 50' 18.60", -43° 24' 48.11"	IX.2012	Alves R. B. N.	MH204759	MK293640	Oliveira, 2014
B. obovatus	*Coniza bonariensis* , Asteraceae *Helichrysum stoechas* , Asteraceae	Spain, Valencia, Valencia	+39° 22' 57.00", -0° 19' 57.00"	13.X.2013	Navia D. and Ferragut F.	MH204740 MH204749	MK293694 MK293701	Oliveira, 2014
?	*Olea europea* , Oleaceae	Spain, Valencia, Valencia	+39° 28' 50.74", -0° 22 '3.78" '	16.X.2013	Navia D. and Ferragut F.	MH204767 MH204768	MK293661 MK293662	Oliveira, 2014
B . sulcatus sp. nov.	*Hedera azorica* , Araliaceae	Portugal, Açores, Flores	39°28´20"N, 31°15´22"W	09.X.2015	Navia D. and Ferragut F.			This study
B . tuberellus	*Lecythis lurida* , Lecythidaceae	Brazil, Bahia, Ilhéus	14° 47' 49"S, -39° 10' 23"W	14.III.2018	Souza K. S.			This study
B . yothersi	*Ipomoea batatas* , Convolvulaceae	Brazil, Alagoas, Arapiraca	-09° 45' 09"N, -36° 39' 40"W		Navia, D. and Silva E. S.	MH204706 MH204711	MK293672 MK293678	Oliveira, 2014

Some of the mites kept in 100% ethyl alcohol were submitted to scanning electron microscopy (SEM). The specimens were dried at the critical point (Baltec EM CPD 300 - Lichtenstein), and then fixed in 15 x 30 mm copper plates using ultra-soft round carbon adhesive guides. All samples were bathed with another by a metallizer (Baltec SCD 050 - Lichtenstein). The scanning electron microscope used to capture the images was a Japanese-made JEOL JSM-IT300LV at the Laboratory of Electronic Microscopy NAP, ESALQ-USP, in Piracicaba, São Paulo, Brazil. The acceleration voltage of 5 kV was used to visualize the samples with increases ranging from 330x to 18,000x. The specimens were visualized in ventral and dorsal position, and for some individuals, it was possible to obtain the complete body imaging, examining both, the dorsal and ventral surface, on the same specimen.

DNA extraction, amplification and sequencing

Some of the mites kept in 100% ethyl alcohol were submitted to molecular analysis. Total genomic DNA was extracted from a single adult female using the DNeasy Tissue kit (Qiagen Germantown, MD, USA), according to the DNA extraction protocol 'Purification of Total DNA from Animal Blood or Cells' (SpinColumn Protocol). The manufacturer's instructions were modified for DNA extraction from tiny mites, as described by Dowling *et al*. (2010) and Mendonça *et al*. (2011). It is noteworthy that the specimens were not macerated since they were recovered from the column and mounted on slides in Heinze PVA medium. These slides were deposited as voucher specimens in the Plant Mite Collection, at Embrapa Genetic Resources and Biotechnology, Brasilia, DF, Brazil.

Table 2. PCR and sequencing primer sets used to obtain mitochondrial *COI* and nuclear ribosomal D1-D3 subunit (28S) sequences of *Brevipalpus* mite specimens.

Marker acronym	Marker name	Fragment length (bp)	Primer Name	Primer sequence	Author
COI mtDNA	Cytochrome Oxidase Subunit 1	~400	DNF DNR	5'-3' TAC AGC TCC TAT AGA TAA AAC 3'-5' TGA TTT TTT GGT CAC CCA GAA G	Navajas et al., 1996
D1-D3 28S rRNA	subunit D1-D3 of the gene 28S	~1000	D23-F D6R	5'-3' GAG AGT TCA AGA GTA CGT G 3'-5' CCA GCT ATC CTG AGG GAA ACT TCG	Park and Foighil, 2000

Table 3. Protocol of the PCR reactions used in this study.

	COI		28S	
PCR conditions				
Initial denaturation	94°C	4'	95°C	3'
No. cycles	35		35	
Denaturation	92°C	1'	95°C	45"
Annealing	48°C	1' 30"	50°C	1' 30"
Elongation	72°C	1' 30"	72°C	1' 10"
Final Elongation	72°C	10'	72°C	10'
Reaction mix composition (µl)				
H2O	15.77		3.575	
10x buffer Qiagen	2,5		2.5	
MgCl2 (25mM)	1.5		1.5	
Oligonucleotide (10mM)(each)	0,75		0.25	
dDNTP (10mM) Qiagen	1,2		0.125	
Trehalose 10% Sigma	0		12.5	
Bovine Serum Albumn (10mg/ml)	0,4		0.1	
Taq polymerase Qiagen (5u)	0.13		0.2	
DNA template	2		4	

Two target DNA fragments were PCR-amplified and sequenced per single mite: one mitochondrial - the cytochrome c oxidase subunit I (*COI*) gene; and one nuclear DNA fragment - the subunit D1-D3 region in 28S rDNA gene. PCR primers are described in Table 2. The amplification reaction for *COI* was performed in 25-µL total volumes containing 2.5 µL of a 10 × buffer supplied by the manufacturer, 1.5 µL MgCl2 (25 mM), 1.2 µL dNTP (0.25 mM of each base), 0.75 µL of each primer (10 µM), 0.4 µL of bovine serum albumin solution (BSA) (10 mg mL−1 Biolabs), 0.13-µL U µL−1 (5 units) of Taq polymerase (Qiagen), 15.77 µL of sterile water and 2 µL of DNA template. A PCR for the subunit D1-D3 (28S) was also carried out in 25-µL volume containing 2.5 µL of a 10 × buffer supplied by the manufacturer, 1.5 µL MgCl2 (25 mM), 0.125 µL dNTP (0.25 mM of each base), 0.25 µL of each primer (10 µM), 0.1 µL of bovine serum albumin solution (BSA) (10 mg mL−1 Biolabs), 0.2-µL U µL−1 (5 units) of Taq polymerase (Qiagen), 12.5 µL trehalose (Sigma-Aldrich Brazil), 3.575 µL of sterile water and 4 µL of DNA template. To amplify the *COI* fragment, the thermocycler profile included initial denaturation at 94 °C for 4 min, followed by 35 cycles of 1 min denaturation at 92 °C, 1 min 30 s annealing at 48 °C, 1 min 30 s final extension at 72 °C, and a final step of 10

min at 72 °C. For D1-D3 subunit, samples were denatured at 95 °C for 3 min, followed by 35 cycles of 45 s denaturation at 95 °C, 1 min and 30 s annealing at 50 °C, 1 min and 10 s extension at 72 °C, and a final step of 10 min at 72 °C. PCR products (5 µL) along with 0.5 µL of loading buffer were resolved by 1 % (w/v) agarose gel electrophoresis prepared in 0.5X TBE buffer and visualized on GelRed staining (Biotium, Inc, Hayward, Canada). Gel images were acquired with Gel Doc™ System (Bio Rad). The amplified fragments (*COI* and D1-D3) containing visible and single bands were directly sequenced on both strands using an ABI 3730XL Applied Biosystems automated sequencer (at Macrogen (Seoul, Korea). No additional primers were used for sequencing.

Phylogenetic analyses

The software Staden Package v.1.6.0 (Staden, Beal & Bonfield, 1998) was used for checking, editing and assembling the raw data into sequence contigs. Consensus sequences were also checked against *Brevipalpus COI* sequences retrieved from GenBank (Navia *et al*., 2013; Sanchez-Velazquez *et al*., 2015; Salinas-Vargas *et al*., 2016) and used for editing a neighbor joining tree in order to verify their reliability. To initially identify candidate protein coding regions in DNA *COI* sequences searching start and stop codons, an open reading frame was determined using a graphical analysis tool (ORF Finder) available at http://www.ncbi.nlm.nih.gov/projects/gorf/. The sequences were aligned using software such as the ClustalW multiple alignment procedure in BIOEDIT 7.0.4 (Hall, 1999) and Muscle (Muscle: multiple sequence alignment comparison by log-expectation program (Edgar 2004).

The jModeltest version 2.1 (Darriba *et al*., 2012) based on the likelihood for 88 different models was used to estimate the best-fit models of nucleotide substitution using the Akaike information criterion corrected (AICc) and the Bayesian information criterion (BIC). For the *COI* dataset, the TPm1uf + G model (Kimura three-parameter, K-3P) (Kimura 1981) was selected according to AICc and to BIC. A discrete gamma distribution shape parameter (G) of 0.2910 was used to model evolutionary rate differences among sites. The ML models were tested in PhyML v. 3.0 (Guindon & Gascuel, 2003; Guindon *et al.* 2010), NJ in MEGA v. X (Kumar *et al*., 2018), and Bayesian inference (BI) was tested in MrBayes v.3.2.6 (Ronquist *et al*., 2012). The same softwares and procedures were used to test NJ, ML and BI phylogenies in the subsequent analyses of the subunit D1-D3 sequences. For the subunit D1-D3 of the 28S rDNA dataset, the GTR (Tavaré

1986) was selected according to AICc (G = 0.4050) and according to BIC, the TPM3+G model (Kimura, 1981) (G = 0.4010) was selected. The ML analyses were performed using the online version of the PhyML3.0 algorithm (Guindon *et al.* 2010). The robustness of the trees was assessed with a bootstrap analysis that involved 1000 bootstrap replicates for all analyses.

For the combined analysis, sequences of the two studied fragments (*COI* and D1-D3) were individually organized using MEGA v. X software (Kumar et al 2018). Alignments of the two fragments was carried out separately by the Muscle program (Edgar, 2004) implemented in the MEGA v. X. The files were then concatenated in a single matrix containing 18 taxa for each fragment and totalizing 1371 base pairs, using Mesquite v. 3.0.4 (a modular system for evolutionary analysis) (Maddison & Maddison, 1996). The combined analysis using Bayesian Inference (BI) was performed in MrBayes ver.3.2 (Ronquist et al. 2012). The number of categories used to approximate the gamma distribution was set at four, and four Markov chains were run for 10,000,000 generations; the final average standard deviation of split frequencies was less than 0.01, and the stabilization of model parameters (burn-in = 0.25) occurred at approximately 250 generations. *Raoiella indica* was used as outgroup in the analysis, *i.e.*, sequences accession number MH174682 and JF928437 for *COI* and D1-D3 alignments respectively.

The phylogenetic trees based on the output file (newick format) created by PhyML 3.0 algorithm and MrBayes program was edited using FigTree v.1.4.3 (http://tree.bio.ed.ac.uk/software/figtree/). The final settings to better understand the data was performed in CorelDRAW X7 software.

Inter- and intraspecific genetic distance

Analyses of the pairwise genetic distances overall and between nucleotide sequences (*COI* and D1-D3), as well as the among- and within-distance of *Brevipalpus* species and their related groups were performed using MEGA v. X (Kumar *et al.*, 2018). The most appropriate evolutionary model for estimation of inter and intra-species and group genetic variation was also selected using MEGA v. X. The Tamura 3-parameter model (T3P) (Tamura, 1992) was applied to the *COI* dataset, and the Kimura 2-Parameters (K2P) model (Kimura, 1980) was applied to the D1-D3 datasets. Standard error estimates were obtained by a bootstrap procedure (1000 replicates). Aiming to check the phylogenetic position and the species group classification for the *B.* sp. nov., three different scenarios

were considered to estimate the genetic distance values (COI and D1-D3), such as: 1) testing the hypothesis of the new species belonging to "*B. cuneatus* species group" based on the morphological similarity between the new species and *B. cuneatus* species; 2) testing the current "species group" classification based on the morphological traits as described by Baker & Tuttle (1987) – *B. phoenicis* species group (*B. yothersi* Baker + *B. incognitus* Ferragut & Navia); *B. californicus* species group (just *B. californicus* Banks); *B. obovatus* species group (*B. obovatus* Donnadieu + *B. chilensis* Baker); *B. portalis* species group, herein represented by *B.* sp. nov. and; *B. cuneatus* species group (*B. oleae* Baker *B.* aff. *cuneatus* sp1, *B.* aff. *cuneatus* sp2, and *B. tuberellus* De Leon); 3) testing the scenario revealed by the phylogeny obtained through the Bayesian combined analysis (Fig. 8), as well as through the phylogenies performed singly for *COI* and D1-D3 sequences (Supplementary Material - Figures 1S and 2S), where the species in the *cuneatus* and *portalis* species groups could be each one selected to constitute likely one group.

Sequence retrieval and dataset

All the new sequences herein obtained have been deposited in GenBank (Table 1 ou 2). Available *COI* and D1-D3 sequences (Navia *et al*., 2013; Oliveira, 2014) of the *Brevipalpus* species belonging to four species groups according to Baker and Tuttle (1987) were retrieved from GenBank and included in the analyzes: *B. yothersi* and *B. incognitus* in the *phoencis* group; *B. californicus s.s.* in the *californicus* group; *B. chilensis* Baker and *B. obovatus* in the *obovatus* group; *B. oleae*, *B.* aff. *cuneatus* sp.1, *B.* aff. *cuneatus* sp.2, and *B. tuberellus* in the *cuneatus* group (Table 1). The Tenuipalpidae mite *Raoiella indica* Hirst was used as external group in the analyses, *i.e.*, sequences accession number MH174682 and JF928437 (Oliveira, 2014, Dowling et al. 2012) for *COI* and D1-D3 fragments respectively. All dataset will be available upon request.

Results

Taxonomy

Family **Tenuipalpidae** Berlese, 1913

Genus ***Brevipalpus*** Donnadieu, 1875

***Brevipalpus* sp. nov. Alves, Ferragut & Navia** (Figures 1–7)

Diagnosis (female). Most morphological traits fitting with *B. portalis* species group (sensu Baker & Tuttle, 1987)- dorsal opisthosomal setae *f2* present; tarsus II with one solenidion; palp 4-segmented and with two distal setae. In addition to the traits above mentioned: central area of prodorsum elevated, with verrucose elements only distinct under SEM (this area appears almost smooth under DIC microscopy); sublateral area with large and polygonal cells; lateral margin wrinkled. Dorsal opisthosoma longitudinally elevated, with a remarkable deep furrow. Pores absent in both dorsal propodosoma and opisthosoma. Central opisthosomal setae (*c1*, *d1*, *e1*) setiform, very thin, and lateral setae (*c3*, *d3*, *e3*) more robust and slightly serrated. Setae *f3* in normal position, inserted in line with setae *f2* and *h2*. Ventral cuticle covered with strong pebble-like reticulation. Ventral plate well defined and with coalescent rounded warts forming distinct transverse bands. Genital plate with cells often fused to form transverse bands, more evident on the posterior part of the shield. Palp femurgenu with slightly barbed setiform dorsal seta; tarsus very small, with 2 distal setae.

Description.

FEMALE (holotype and nine female paratypes) (Figs. 1-6).

Dorsum **(**Figs. 1-2a**).** Body size measurements: distance between setae *v2-h1* 261 (243-278) [257]; *c3-c3* 189 (183-210) [194]; *c1-h1* 156 (147-166) [158]; *v2* to dorsal disjugal furrow 98 (88-99) [93]. Anterior margin of prodorsum forked, forming two central and acute projections and two lateral and rounded expansions. Length of central projections 38 (32-40) [35]; of lateral expansions 14 (14-16) [15]. No visible pores on prodorsum and opisthosoma. Prodorsum centrally elevated; elevation covered by verrucose elements not visible with DIC or Phase-contrast techniques (under compound microscopy the central area of propodosoma is almost smooth) (Figs. 1 and 2a). Sublateral area with reticulation forming polygonal and well defined cells, which laterally become less defined or weak wrinkled and anteriorly are smaller and more isolated. Lateral margins wrinkled, with irregular folds of different length, some of them reaching the shield margin. Central opisthosoma elevated; anterior part of elevation between setae *c1–d1* a semicircular lobe; posterior part elongate, tongue-shaped. Anterior and posterior part separated from the rest of the opisthosoma by a deep and wide furrow (Figs. 1 and 2a). Cuticle between setae *c1–d1* verrucose, similar in appearance to that of central prodorsum

(not observed on the slide-mounted holotype); cuticle between setae *d1–e1* with irregular wrinkles, mostly oriented transversally. Sublateral opisthosoma wrinkled, with irregular folds; some elongate cells appear posterolaterally to the dorsocentral elevation.

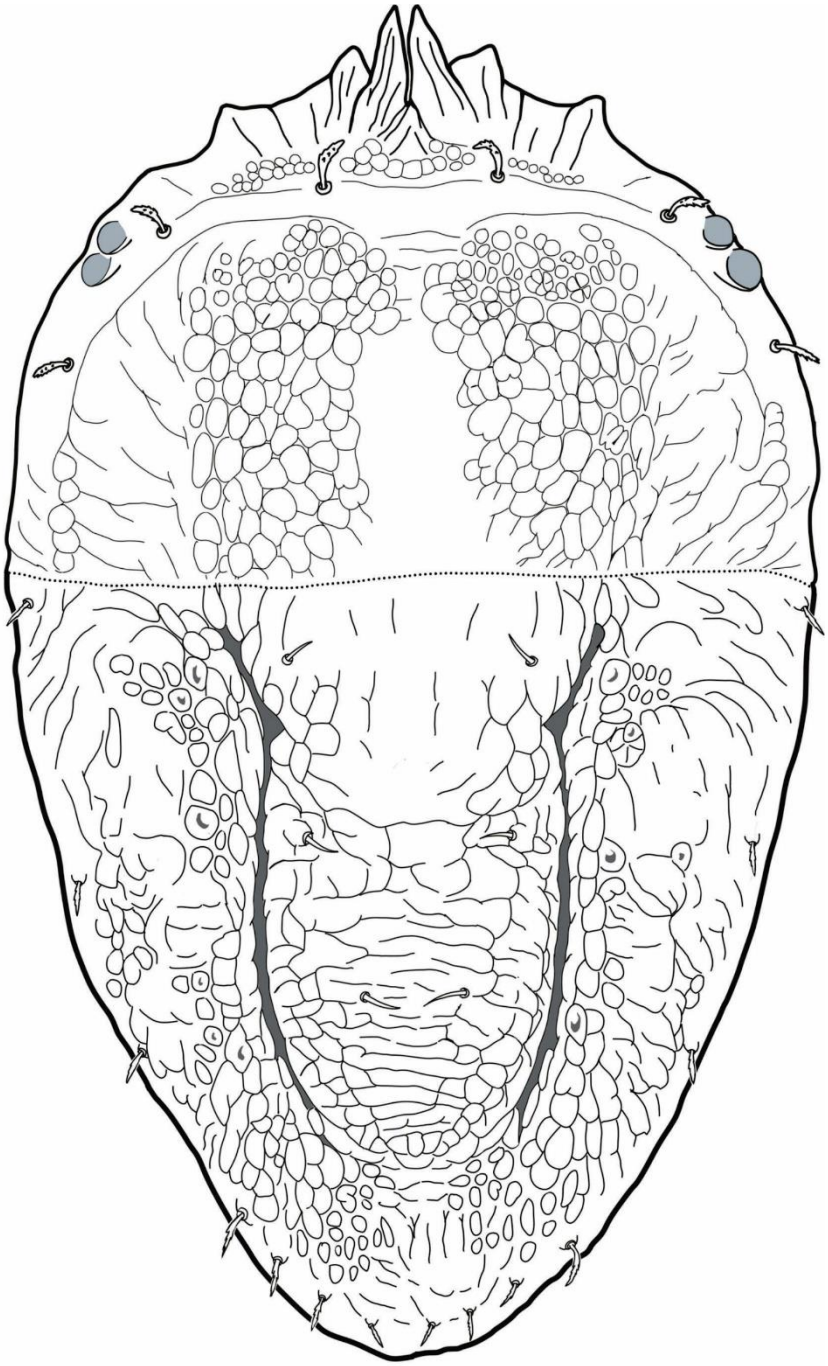


Figure 1. *Brevipalpus* sp. nov. (Female): dorsum.

Dorsal setae *v2*, *sc1* and *sc2* lanceolate; *c1*, *d1* and *e1* sublanceolate or acuminate; *c3*, *d3*, *e3*, *h1* and *h2* lanceolate; all dorsal setae strongly serrate and clearly pedunculated except for *c1*, *d1* and *e1*, which are very slightly barbed. Setal measurements: *v2* 12 (10-12) [11]; *sc1* 13 (10-13) [12]; *sc2* 11 (10-12) [11]; *c1* 8 (8-10) [8]; *c3* 11 (8- 11) [10]; *d1* 7 (6-9) [7]; *d3* 10 (9-12) [10]; *e1* 7 (6-9) [8]; *e3* 10 (8-10) [9]; *f2* 11 (9-11) [10]; *f3* 8 (7-10) [8]; *h1* 7 (5-9) [6]; *h2* 6 (5-8) [6]. Distance between setae: *v2-v2* 44 (36-48) [42]; *sc*1-*sc*1 120 (114-128) [119]; *sc*2-*sc*2 168 (157-180) [167]; *c1-c1* 64 (55-67) [61]; *d1-d1* 52 (46-53) [50]; *d3-d3* 171 (160-180) [169]; *e1-e1* 19 (19-30) [23]; *e3-e3* 134 (129-144) [134]; *f2-f2* 78 (77-88) [82]; *f3-f3* 55 (54-66) [59]; *h1-h1* 13 (13-16) [15]; *h2-h2* 37 (34-44) [37].

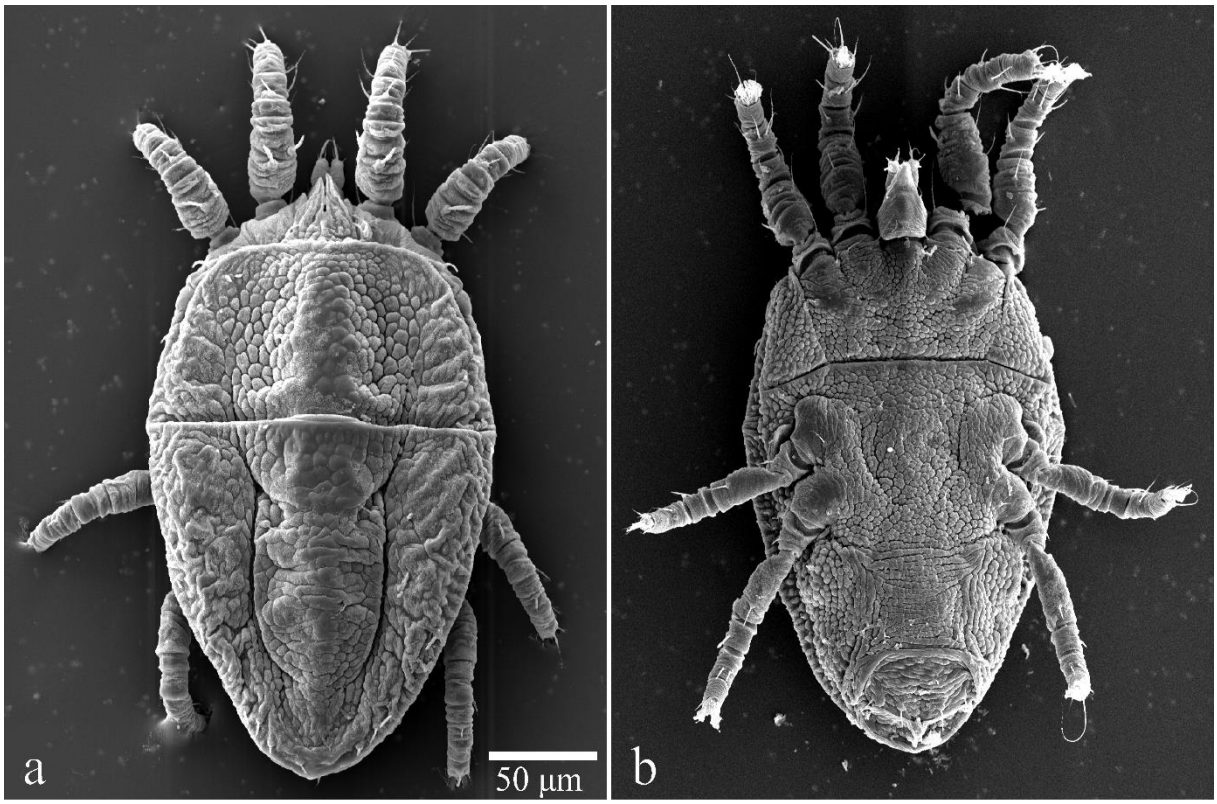
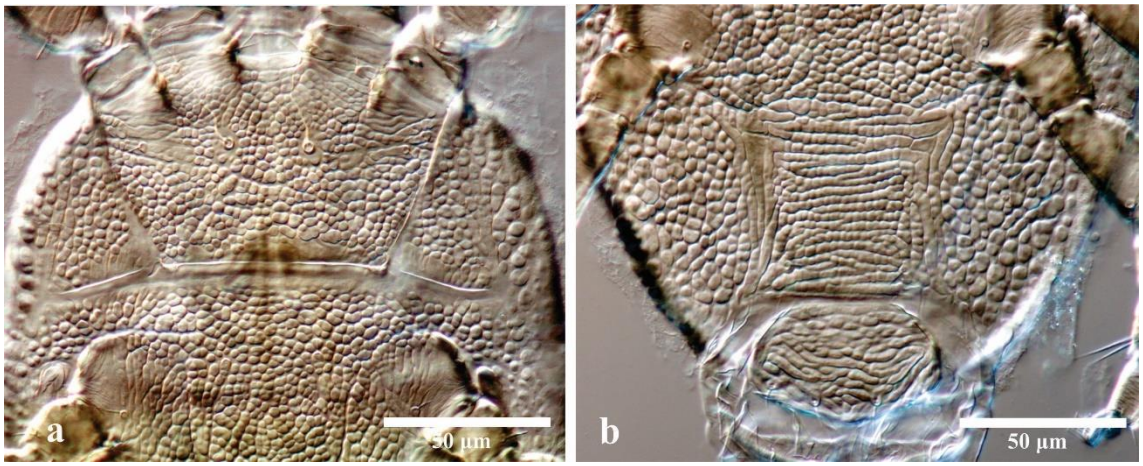


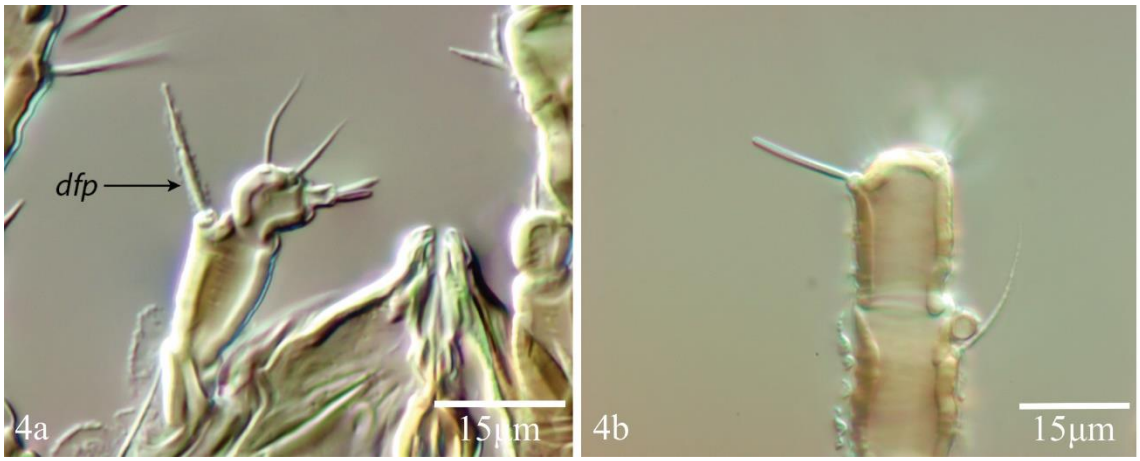
Figure 2 2a-2b. *Brevipalpus* sp. nov. (Female): dorsum (a) and venter (b).

Venter (Fig. 2b-3b). Ventral region entirely verrucose, covered by warts of different size and shape (Fig. 2b, 3a and 3b). Cuticle between setae *3a* and ventral plate with rounded, pebble-like or rosette-like warts. Coalescing warts form elongated cells

around coxae III and IV. Ventral plate subquadrate, clearly separated from the surrounding ornamentation on the venter; 56 (49-66) [57] long, 47 (44-55) [50] wide (Fig. 3b); warts fusioned forming transverse bands; laterals with elongate, longitudinal bands. Genital plate 40 (34-40) [37] long, 59 (56-63) [59] wide, with large and medium rounded weak cells transversely aligned on the anterior part and cells often fused to form transverse folds in the posterior part. Setal measurements: *1a* 109 (100-109) [103]; *3a* 11 (5-11) [8]; *4a* 8 (8-11) [9]; *ag* 7 (6-8) [7]; *g1* 12 (11-13) [12]; *g2* 9 (8-11) [9]; *ps1* 8 (8-9) [8]; *ps2* 9 (7-10) [9].



Figures 3 3a-3b. *Brevipalpus* sp. nov. (Female): ventral plate (a) and genital plate (b).



Figures 4 4a-4b. *Brevipalpus* sp. nov. (Female): palp (a) and solenidia (b). dfp = detail seta femurogenu of the palp.

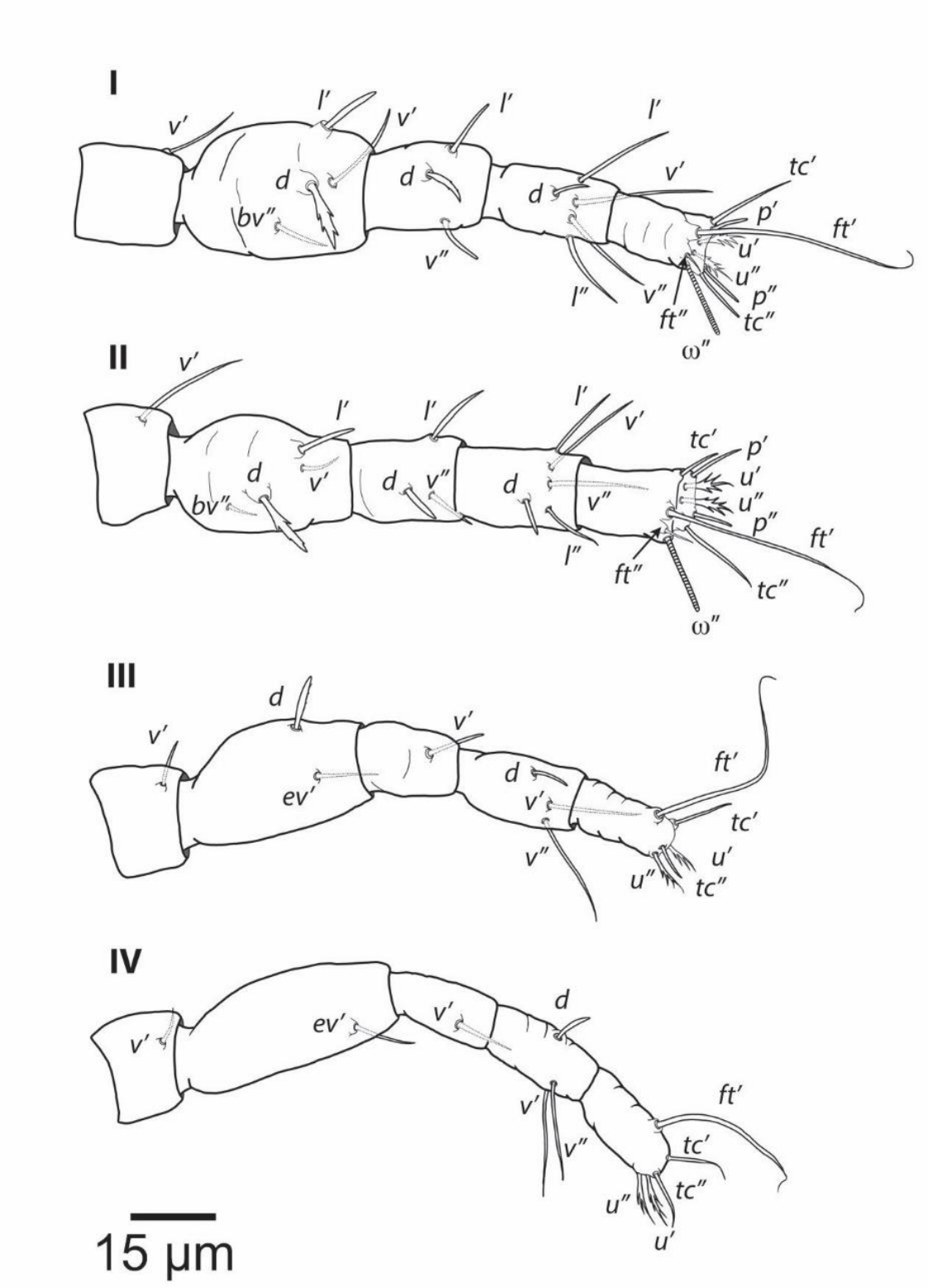


Figure 5. *Brevipalpus* sp. nov. (Female): legs I–IV, respectively.

Gnathosoma (Fig. 4a). Rostrum arriving to the distal part of femur I. Palps four-segmented. Setal formula 0-1-2-2 (1 ω solenidion + 1 *ul'* eupathidium). Segment IV very small. Palp femorogenu seta acuminate (Fig. 4a), 15 (12-15) [14] long.

Legs **(**Fig. 4b-5). Setal formula for legs I-IV (coxae to tarsi): 2-1-1-1, 1-1-1-1, 4-4-2-1, 3-3-3-3, 5-5-3-3, 9 (8+1ω")-9 (8+1ω")-5-5. Solenidia of tarsi I and II long (Fig. 4b) (antiaxial), straight, 16 (15-17) [16] and 15 (15-17) [16], respectively. Dorsal seta of femur I lanceolate, dentate, 12 (11-13) [12] long.

Dorsal microplates (Fig. 6). Separate individual rounded irregular plates, with a series of randomly distributed parallel structures of different sizes.

*Spermatheca***:** Insemination duct relatively short, distal part narrowing gradually, without forming any dilatation or bulb. Seminal receptacle not developed in the examined females.

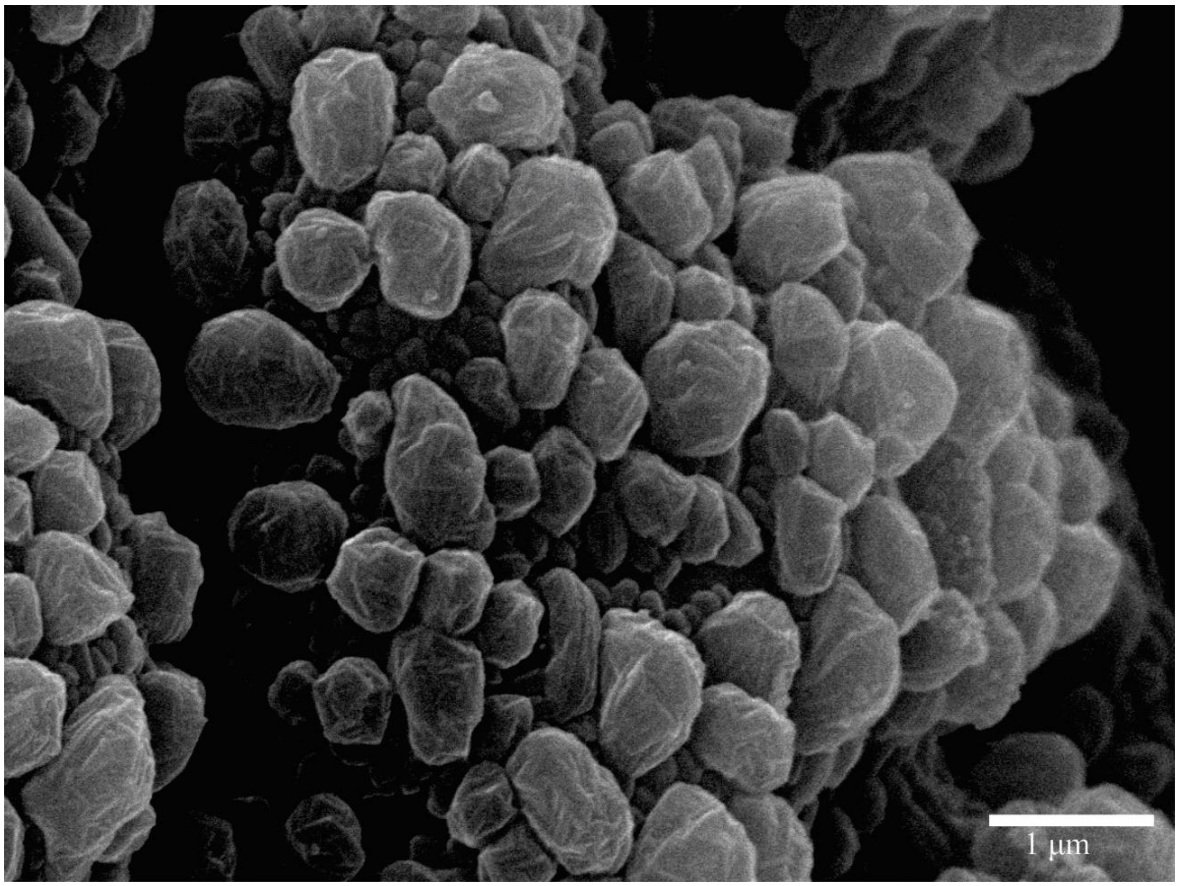


Figure 6. *Brevipalpus* sp. nov. (Female): microplates.

MALE (1 measured) (Fig. 7)

Dorsum. (Fig. 7) Body measurements: distance between setae *v2-h1* 231; *c3-c3* 145; *c1-h1* 144; *v2* to dorsal disjugal furrow 83. Idiosoma with a narrow waist between setal row *d* and *e*, separating the mesonotal and pygidial shields. Propodosoma broad, widest at level of setae *c3*. Rostral shield with forked median and acute projection 25 long and two adjacent, lateral and rounded projections, 16 long. Central area of prodorsum smooth; sublateral area reticulate, forming elongate cells more rounded and smaller on the anterior part; laterals scarcely striated. Dorsocentral area of opisthosoma mostly smooth, with a few weak striae; sublateral area with cells, more evident on the anterior part, disappearing just anterior to level of setae *f3*; laterals almost smooth.

Dorsal setae lanceolate and serrate, except *c1*, *d1* and *e1*, slenderer and smooth. Setae *v2*, *sc1* and *sc2* inserted on prodorsum; setae *c1*, *c3*, *d1* and *d3* on mesonotal shield; setae *e1*, *e3*, *f2*, *f3*, *h2* and *h1* on pygidial shield. Setal measurements: *v2* 7, *sc1* 10, *sc2* 9, *c1* 5, *c3* 8, *d1* 5, *d3* 8, *e1* 5, *e3* 9, *f2* 9, *f3* 6, *h1* 5, *h2* 6. Distance between setae: *v2-v2* 38; *sc*1-*sc*1 102; *sc*2-*sc*2 137; *c1-c1* 53; *d1-d1* 40; *d3-d3* 116; *e1-e1* 26; *e3-e3* 101; *f2-f2* 70; *f3-f3* 41; *h1-h1* 6; *h2-h2* 18.

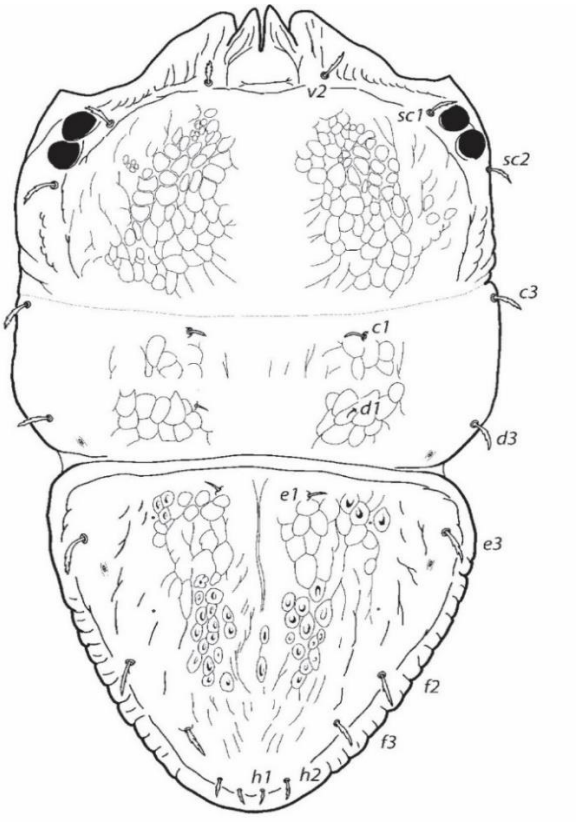


Figure 7. *Brevipalpus* sp. nov. (Male): dorsum

Four pairs of pore-like structures present on dorsal surface. Two pairs of simple punctiform pores placed between setae *e1-e3* and anteromesad to *f2*; two pairs of lirifissures, posterolaterad to *d1* and posteromesad to *d3*, the former near the furrow between mesonotal and pygidial shields.

Venter. Ventral ornamentation similar to female, formed by pebble and rosette-like warts. Setae *3a* and *4a* short, acuminate and smooth, 8 and 6 long, respectively. Anterior and anterolateral cuticle on ventral pygidial shield covered by warts of different size and shape; central area with weak, transverse ornamentation, which disappears on the posterior body part. Setae *ag* slender, 8 long. Setae *ps3* 30 long.

Gnathosoma. Extending until middle of femur I. Palp four-segmented; setal formula similar to female. Dorsal seta on palp femorogenu modified in a subconical spur, 5 long. Distal solenidion ω, 5 long.

Legs. Setation similar to female, except for the addition of a second solenidium ω' on tarsus II. Setal formula for legs I-IV (coxae to tarsi): 2-1-1-1, 1-1-1-1, 4-4-2-1, 3-3-3-3, 5-5-3-3, 9 (8+1ω")-10 (8+2 solenidia ω', ω")-5-5. Solenidium on tarsus I 17 long; on tarsus II, ω' 10, ω" 17. Dorsal seta on femur I lanceolate, serrate, 12 long.

Aedeagus. Formed by an sclerotised and straight tube surrounded by a membrane. Sclerotized part 61 long.

*Type Material***.** Holotype female, nine female paratypes and one male paratype collected on *Hedera azorica* Carrière (Araliaceae). Ponta do Fajã, island of Flores, Azores archipelago, Portugal; 39°28´20"N, 31°15´22"W, 68 m above sea level; 9. X.2015. Voucher specimens deposited in the Laboratory of Acarology, Instituto Agroforestal Mediterráneo, Universitat Politècnica de València, Spain and at the Plant Mite collection at Embrapa Genetic Resources and Biotechnology, Brasilia, DF, Brazil. Holotype and some paratypes were deposited at Museo Nacional de Ciencias Naturales (MNCN), Madrid, Spain.

Molecular diagnosis

We amplified and sequenced a 364 bp fragment of the *cytochrome c oxidase subunit I* (*COI*) gene (mtDNA) region used by Navia *et al*. (2013), Breewuer *et al*. (2006), Rodrigues *et al*. (2004) and Navajas *et al*. (1996) for four paratypes (accession numbers MK499461, MK499462, MK499463 and MK499464), and 1000 bp fragment of the subunit D1-D3 at 28S gene (rRNA) (Dowling *et al.* 2012) for five females paratypes (in

submission). No intraspecific variability in *B.* sp. nov. was detected, i.e., a single haplotype was identified for both fragments (*COI* and D1-D3). Comparison of the sequences from *B.* sp nov. and *B. tuberellus* herein obtained showed that the aver genetic divergence was 10.1% and 14.04% for *COI* and D1-D3 respectively. This mean genetic divergence was even higher than the mean divergence between *B. phoenicis* group and *B. obovatus* group (9.3% and 7.08% - *COI*/D1-D3) and between *B. californicus* group and *B. obovatus* group (8.7% and 5.94 – *COI*/D1-D3), supporting by molecular information the species status of *B.* sp. nov. and the hyphotesis that this new species could be selected as a suitable specimen to join *B. portalis* group.

Etymology: only in paper publication.

Biological observations. Mites are whitish or slightly reddish in color with a characteristic dark spot on the body. They were found on the leaves associated to the phytoseiid mite *Amblyseius largoensis* Muma.

Remarks. Females of *B.* sp. nov. were partially covered by a waxy substance that remains on body and setae after clarification and montage, and also after preparation for observation under scanning microscope. The morphology of some leg setae was difficult to establish due to the presence of this substance, which hamper the nature, smooth or barbed, of these structures.

Most characteristics of the new species fit to the *B. portalis* species group (Baker & Tuttle 1987), however it differs from all species in this group by the dorsal central setae (*c1*, *d1*, *e1*), which are slightly different in shape and size to the dorsal lateral setae (*c3*, *d3*, *e3*). In addition, it differs from *B. portalis* Canestrini & Fanzago, 1876 because it has only one seta on the trochanter of leg III (two setae in *B. portalis*).

The new species is similar to a species not belonging to the *portalis* group- *B. cuneatus* (Canestrini & Fanzago, 1876), placed in the *cuneatus* species group by sharing the general dorsal and ventral ornamentation; the presence of one long solenidion in tarsus II; one seta in trochanter III; very short (minute) ventral setae *3a* and *4a*. However, the new species differs from *B. cuneatus* by: the presence of a deep furrow in the dorsal opisthosoma (absent in *B. cuneatus* which present just a suture) and the dorsolateral seta *f3* in normal position (shifted to the side in *B. cuneatus*).

Molecular phylogeny

The final *COI* dataset consisted of 25 aligned sequences of 364 bps including four sequences of *B.* sp. nov. and three of *B. tuberellus* obtained in this study, 17 sequences of *Brevipalpus* species available in the GenBank and one of *R. indica* (MH174682) as an outgroup. The estimated frequencies of the nucleotide bases were A = 26.6 %, C = 10.3 %, G = 13.4 % and T = 49.7 %; A+T = 76.3 % and G+C = 23,7 %. The translation of the nucleotide *CO*I sequences resulted in 124 amino acid sequences, with 82 variable amino acid positions and 67 parsymony informative sites. Putative conserved domains (33) were detected that matched all sequences in the data set, producing good alignments with the *Brevipalpus* sequences retrieved from GenBank (Navia *et al.* 2013; Sanchez-Velazquez et al. 2015; Salinas-Vargas *et al*. 2016). No stop codons or frame shift mutations were observed in the alignment. No insertions or deletions were found.

The nuclear sequence data of the D1-D3 region (28S rDNA) comprised 21 aligned sequences of 1000 bps, such as five from *B.* n. sp. and one from *B. tuberellus* obtained in this study, 14 *Brevipalpus* D1-D3 sequences retrieved from GenBank and the outgroup, *R. indica* (JF928437). The estimated frequencies of the nucleotide bases were A = 26.2 %, C = 20.1 %, G = 25.4 % and T = 28.3 %; A+T = 54.5 % and G+C = 45.50 %. In the alignment, 408 sites were variable and 295 sites were parsimony informative.

The topologies of the phylogenetic trees inferred by maximum likelihood (ML) optimality criterion for the *COI* and D1-D3 (Supplementary Material – Figures 1S and 2S) subunit as well as for the combined analysis (COI + D1-D3) by Bayesian inferences (Figure 8) were similar in general and revealed two main well-supported clades comprising: I) *Brevipalpus* species in the *phoenicis*, *californicus*; and *obovatus* species groups; II) species currently classified or fitting in the *cuneatus* species group and the new species. However internal topologies of each of these two main clades were somewhat different in the subclades composition and relationships when comparing COI and D1-D3 phylogenetic trees.

Main differences concerning Clade I were: i) in the *COI* phylogeny *B. yothersi* is isolated in a subclade and not grouped with *B. incognitus* (the morphological closely species currently placed in the "*phoenicis* species group") while in the D1-D3 these species compose just one subclade. The Bayesian combined analysis showed three subclades corresponding to *B. phoenicis*, *B. californicus* and *B. obovatus* species groups, in accordance with the current group species classification by Baker & Tuttle (1987).

Concerning Clade II COI and D1-D3 phylogenies were quite similar in showing four subclades: I) the new species *B.* sp. nov.; II) *B. oleae*; III) *B. tuberellus*; and IV) *B.* near *cuneatus*. However, relationship between these subclades differed between COI and D1-D3 phylogenies as well as subclades support. For COI subclades I (*B.* sp. nov.) was closely related to subclade II (*B. oleae*) while for D1-D3 subclades I (*B.* sp. nov.) was closely related to subclade III (*B. tuberellus*). Subclades in D1-D3 phylogeny were well-supported however it was not observed for COI phylogeny. The Bayesian combined analysis showed four subclades being subclades I (*B.* sp. nov.) closely related to subclade III (*B. tuberellus*) similarly to showed in D1-D3 phylogeny.

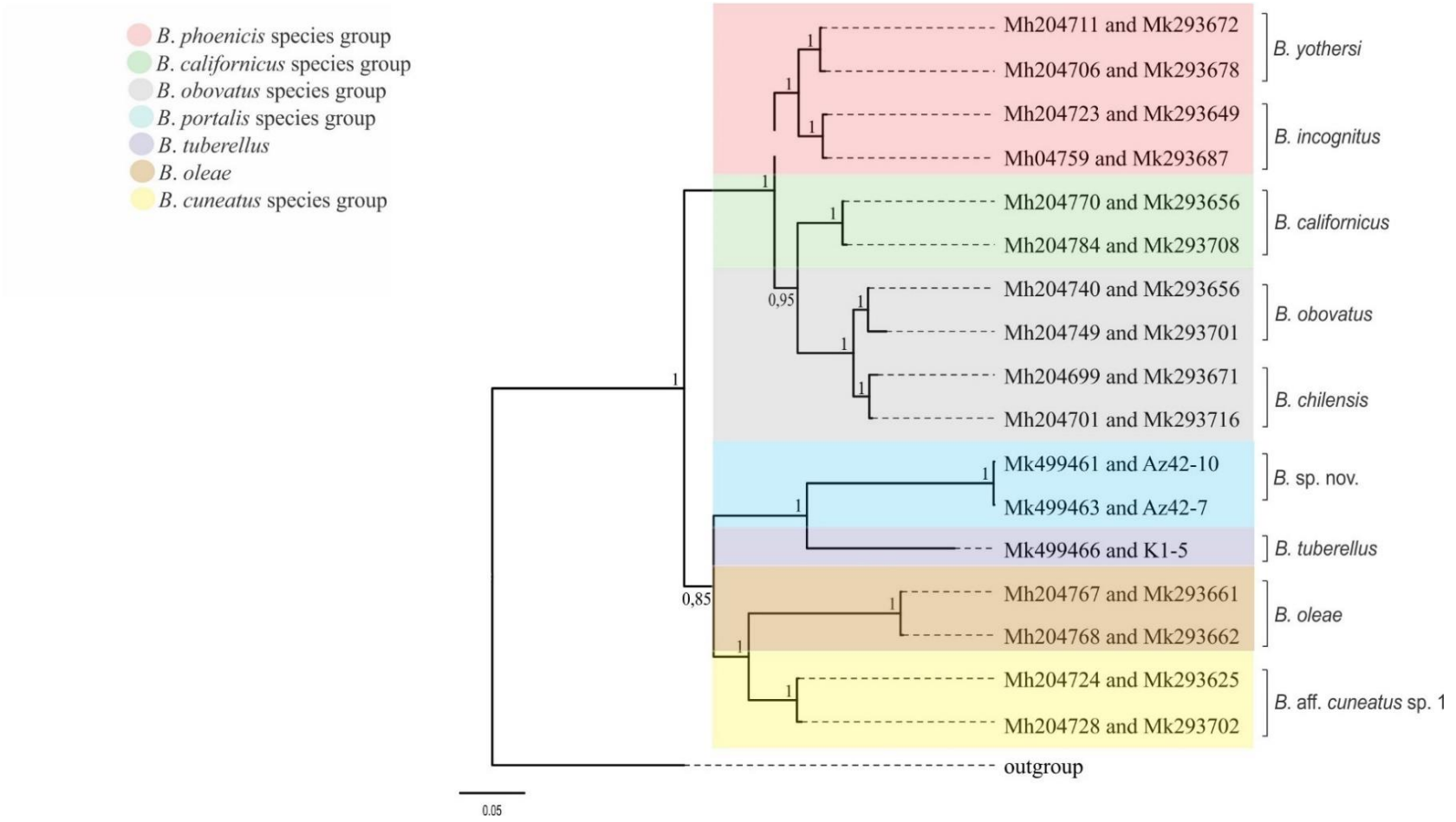


Figure 8. Combined Bayesian inference (BI) analysis tree for *Brevipalpus* species calculated from the concatenated cytochrome c oxidase subunit I sequences (COI) and 28S r-RNA subunit D1-D3 sequences. Statistical supports indicate Bayesian posterior probabilities values. Only statistical supports greater than 0.5 are indicated above branches. Putative *Brevipalpus* species groups are highlighted in colored squares. *Raiolla indica* (MH174682) was used as outgroup.

The *COI* average mean divergence over all the sequence pairs (including the outgroup taxa) was 13.0 % (SE = 2.0) and ranged from 0.0% to 25.99 %. The average mean divergence over the *Brevipalpus* sequences was 12.0 % (SE = 2.0) and ranged from 0.0 % to 19.91 %. For the subunit D1-D3 the average mean divergence over all sequence pairs (including the outgroup taxa) was 13 % (SE = 2.0 %) and ranged from 0.00 % to 26.3 %. The average mean divergence over the *Brevipalpus* spp. sequences was 12.0% (SE = 2 %) and ranged from 0.00 % to 21.17%.

Pairwise comparison of the distances between- and within-*Brevipalpus* species and -*Brevipalpus* species groups for the two fragments *COI* and D1-D3 considering the three calculated scenarios are presented in Tables 3 and 4, respectively.

In scenario 1 (*B.* sp. nov. sequences are included in the *B. cuneatus* species group), intragroup variability is 9.8%, for COI, and 11.1% for D1-D3. These values are considerably higher than those observed for other groups. For the other studied groups, the highest intragroup variabilities were observed for *B. phoenicis* species group for *COI* (6.2%) and for *B. obovatus* species group for D1-D3 (1.4%). Intragroup variability for the hypotethized *B. cuneatus* species groups was even higher than the highest intergroup distances for D1-D3 (maximum of 7.1% between *B. obovatus* species group and *B. phoenicis* species group). For COI the intragroup variability for the hypothetized *B. cuneatus* species group (9.8%) was higher than intergroup variability between *B. obovatus* species group and *B. phoenicis* species group (9.3%) and *B. californicus* species group (8.7%) and a little lower to the variability between *B. californicus* species group and *B. phoenicis* species group (10.4%).

In scenario 2. (*B.* sp.nov. sequences representing *B. portalis* species group) intergroup variability between *B. portalis* species group and *B. cuneatus* species group (12.4% for COI and 16% for D1-D3) is considerably higher than those observed between other species groups in Clade 1 (e.g. in Clade 1 for the COI the highest intragroup variability was 10.4% between *B. californicus* and *B. phoenicis* species groups; for the D1-D3 highest intragroup variability between *B. obovatus* species group and *B. phoenicis* species group (7.1%)). Distances between *B. portalis* species group (*B.* sp. nov.) and all other species group (12.4-16.8% for COI; 16-20.7% for D1-D3) are the higher observed between *Brevipalpus* species groups and come closer to distances with the outgroup *R. indica* (e.g. 17.7% between the outgroup and *B. obovatus* species group for COI; 22.3% between the outgroup and *B. californicus* speceis group). Intragroup variability for *B. cuneatus* species group was 8.7% for COI and 9.9% for D1-D3. These intragoup

variabilities are higher than those observed for all species groups in the Clade 1 for both fragments (COI- 6.2% for *B. phoenicis* species group; D1-D3 1.4% for *B. obovatus* species group) and are even higher or comparable to the intergroup variabilties observed in Clade 1 (e.g. 7.1% between *B. obovatus* species group and *B. phoenicis* species group for D1-D3; 8.7% between *B. obovatus* species group and *B. californicus* species group for COI).

In scenario 3 (hypothesis based on the topology of the Bayesian phylogenetic tree, each subclade as a probable "species group") intergroup distances between supposed "species group" in Clade 2 for COI (10.1-13.4%) were higher or similar than intergroup distances between "species groups" in Clade 1 (8.7-10.4%); for D1-D3 intergroup distances in Clade 2 (8.5%-16.8%) were also higher or considerably higher than in Clade 1 (4.7-7.1%).

Table 4. Mean genetic Tamura 3-parameter distances (%) between *COI* sequences of *Brevipalpus* species groups (below the diagonal) with the standard estimates error (%) (above diagonal) for the three predict scenarios. The shade blocks indicate the intraspecific mean distance. *Raoiella indica* was also included to represent the external group.

Scenario 1

	Group	1	2	3	4	5
1	*B. phoenicis* species group	6,2%	1,5%	1,4%	1,5%	2,4%
2	*B. californicus* species group	10,4%	0,0%	1,6%	1,9%	2,9%
3	*B. obovatus* species group	9,3%	8,7%	2,0%	1,7%	2,3%
4	*B. cuneatus* species group	13,2%	15,6%	14,3%	9,8%	2,5%
5	outgroup	20,3%	22,5%	17,7%	21,9%	n/c

Scenario 2

	Group	1	2	3	4	5	6
1	*B. phoenicis* species group	6,2%	1,5%	1,3%	1,8%	1,5%	2,5%
2	*B. californicus* species group	10,4%	0,0%	1,6%	2,3%	1,9%	2,9%
3	*B. obovatus* species group	9,3%	8,7%	2,0%	2,0%	1,8%	2,4%
4	*B. portalis* species group	13,9%	16,8%	14,5%	0,0%	1,6%	3,0%
5	*B. cuneatus* species group	12,9%	15,1%	14,2%	12,4%	8,7%	2,5%
6	outgroup	20,3%	22,5%	17,7%	23,2%	21,3%	n/c

Scenario 3

	Group	1	2	3	4	5	6	7	8
1	*B. phoenicis* species group	6,2%	1,6%	1,4%	1,9%	1,7%	2,2%	1,8%	2,5%
2	*B. californicus* species group	10,4%	0,0%	1,6%	2,3%	2,2%	2,6%	2,2%	2,9%
3	*B. obovatus* species group	9,3%	8,7%	2,0%	2,1%	2,1%	2,5%	2,0%	2,3%
4	*B. portalis* species group	13,9%	16,8%	14,5%	0,0%	1,8%	2,0%	2,1%	3,0%
5	*B. tuberellus*	11,3%	13,9%	13,1%	10,1%	0,0%	2,0%	1,7%	2,8%
6	*B. oleae*	15,7%	18,3%	17,3%	11,9%	11,9%	0,0%	2,0%	2,8%
7	*B. cuneatus* species group	12,6%	14,3%	13,5%	14,4%	10,1%	13,4%	2,5%	2,7%
8	outgroup	20,3%	22,5%	17,7%	23,2%	21,4%	20,4%	21,6%	n/c

Legenda. Scenario 1: Testing the hypothesis of the new species belongs to "*B. cuneatus* species group". Scenario 2: Testing the current "species group" classification based on the morphological traits as described by Baker & Tuttle (1987). Scenario 3: Testing the hipothesis revealed by the Bayesian combined analysis

(Fig. 8), as well as through the ML phylogenies performed singly for *COI* and D1-D3 sequences (Figure S1 and S2).

Table 5. Mean genetic Kimura 2-parameter distances (%) between D1-D3 (28S) sequences of *Brevipalpus* species groups (below the diagonal) with the standard estimates error (%) (above diagonal) for the three predict scenarios. The shade blocks indicate the intraspecific mean distance. *Raoiella indica* was also included to represent the external group.

Scenario 1

	Group	1	2	3	4	5
1	*B. phoenicis* species group	0,6%	0,8%	1,0%	1,4%	2,1%
2	*B .californicus* species group	4,7%	0,6%	0,9%	1,3%	2,0%
3	*B. obovatus* species group	7,1%	5,9%	1,4%	1,5%	2,3%
4	*B. cuneatus* species group	15,7%	16,0%	17,7%	11,1%	1,8%
5	outgroup	23,6%	22,3%	25,7%	23,2%	n/c

Scenario 2

	Group	1	2	3	4	5	6
1	*B. phoenicis* species group	0,6%	0,8%	1,0%	1,7%	1,4%	2,1%
2	*B. californicus* species group	4,7%	0,6%	0,9%	1,7%	1,3%	2,0%
3	*B. obovatus* species group	7,1%	5,9%	1,4%	1,9%	1,3%	2,3%
4	*B. portalis* species group	16,9%	17,7%	20,7%	0,0%	1,5%	2,1%
5	*B. cuneatus* species group	14,5%	14,4%	14,7%	16,0%	9,9%	1,8%
6	outgroup	23,6%	22,3%	25,7%	23,8%	22,6%	n/c

Scenario 3

	Group	1	2	3	4	5	6	7	8
1	*B. phoenicis* species group	0,6%	0,8%	1,0%	1,6%	1,8%	1,6%	1,4%	2,1%
2	*B. californicus* species group	4,7%	0,6%	0,9%	1,7%	1,8%	1,6%	1,4%	2,0%
3	*B.* obovatus species group	7,1%	5,9%	1,4%	1,9%	1,8%	1,6%	1,3%	2,2%
4	*B. portalis* species group	16,9%	17,7%	20,7%	0,0%	1,6%	1,7%	1,7%	2,0%
5	*B. tuberellus*	18,5%	18,6%	19,0%	14,0%	0,0%	1,7%	1,6%	2,2%
6	*B. oleae*	14,4%	14,5%	16,1%	16,2%	16,8%	0,0%	1,1%	2,0%
7	*B. cuneatus* species group	12,6%	12,3%	11,2%	16,8%	15,4%	8,5%	0,5%	2,0%
8	outgroup	23,6%	22,3%	25,7%	23,8%	26,0%	21,9%	21,5%	n/c

Legenda. Scenario 1: Testing the hypothesis of the new species belongs to "*B. cuneatus* species group". Scenario 2: Testing the current "species group" classification based on the morphological traits as described by Baker & Tuttle (1987). Scenario 3: Testing the hipothesis revealed by the Bayesian combined analysis (Fig. 8), as well as through the ML phylogenies performed singly for *COI* and D1-D3 sequences (Figure S1 and S2).

Discussion

Describing the new Brevipalpus species

The description of the new species has been supported by an integrative approach using a combination of morphological traits visualized through DIC microscopy and scanning electron microscopy, and mitochondrial and nuclear DNA sequences. The results obtained have demonstrated that relevant taxonomic characters of the integument ornamentation may differ depending on the optical technique used. DIC observation

revealed that the central area of the female prodorsum and the opisthosomal cuticle between setae *c1–d1* was smooth. However, images obtained with scanning electron microscopy showed an elevation on the central prodorsum and anterior part of the opisthosoma, covered by distinct verrucose and domed elements. Current mounting techniques imply the flattening of the specimens and, as a result, some details of the cuticular ornamentation may disappear or become difficult to discern. This observation emphasizes the importance to examine the external morphology of *Brevipalpus* with SEM, revealing the outline of the dorsal surface (e.g. flat, elevated, corrugated) and the true and detailed ornamentation of integumentary structures. The description of the new species has been performed using the morphological characters in the female holotype observed by DIC microscopy. In addition, we included those features which are evident on the females observed in SEM but not on slide-mounted specimens.

Brevipalpus sp. nov. species group

Based on morphological traits authors considered that the new species could be classified as belonging to the *B. portalis* species group according to Baker & Tuttle (1987), although one-character set in the *B. portalis* species group concept- dorsal central setae (*c1*, *d1*, *e1*) similar in shape to dorsal lateral setae (*c3*, *d3*, *e3*)- do not fit perfectly for the new species. In *B.* sp. nov. central setae are slightly different in shape to the dorsal lateral ones, a trait defined to the *B. cuneatus* species group. Furthermore, the remarkable general similarity between the new species and *B. cuneatus* highlighted the need for complementary phylogenetic analysis to confirm the taxonomic placement of the new taxon.

Phylogeny as well as inter and intragroup genetic distances supported the hypothesis that *B.* sp. nov. does not belong to the *B. cuneatus* group. Phylogenetic trees based on both the mitochondrial COI and the nuclear D1-D3 markers as well as the Bayesian combined analysis showed that *B.* sp. nov. comprises a subclade which is not closely related with other species in Clade 2, which are currently classified as belonging to the *B. cuneatus* species group. Intragroup variability for *B. cuneatus* species group in Scenario 1, in which *B.* sp. nov. was included in this group, was comparable (for *COI*) or even higher (for D1-D3) than the intergroup variability observed for the *Brevipalpus* studied species revealing that not closely related taxa were assembled. In Scenario 2. (*B.* sp. nov. sequences representing *B. portalis* species group) intergroup variability between *B. portalis* species group (i.e. *B.* sp. nov.) and *B. cuneatus* species group was comparable

or higher than other intergroup variabilities, supporting that *B.* sp. nov. should not be classified as belonging to *B. cuneatus* species group.

These results allow us to outline some remarks on the phylogenetic value of *Brevipalpus* mites morphological traits. According to Baker & Tuttle (1987), one of the main morphological differences between *cuneatus* and *portalis* species groups is the presence/absence of one seta in the fourth segment of the palp (3 in *cuneatus* species group and 2 in *portalis* species group). The distant evolutionary relationship between *B.* sp. nov. and *B. cuneatus* species group suggest this trait is phylogenetically informative. Following the same reasoning related to shape/size differentiation between dorsal central and lateral opisthosomal setae this trait could be considered as less informative, since in *B.* sp. nov. central setae are slightly different in shape to the dorsal lateral ones, more similar with the *cuneatus* species group. However, this trait seems not to reflect phylogenetic proximity between these taxa. It reinforces that the gain or loss of a seta (especially in the palp supposed to have sensorial functions) is a more robust phylogenetic trait than the size and shape of dorsal setae.

Brevipalpus sp. nov. is herein placed in the *B. portalis* group because we consider the absence of a seta in the fourth segment of the palp, an evolutionarily more robust character than the size and shape of specific setae, since this can vary more easily, as the selective pressure for the appearance or loss of a seta tends to be greater than the change in its shape.

Phylogenetically the new species formed a distinct subclade, not grouping with *B. phoenicis, B. obovatus, B. californicus* and *B. cuneatus* species groups. Unfortunately, information on molecular systematics of *Brevipalpus* mites still scarce and sequences of species classified as belonging to the *B. portalis* and *B. frankeniae* species group were no available in public databases and was not possible to obtain biological material of species in these groups. Therefore, it was no possible to check phylogenetic proximity between the new taxon herein described and species in the *B. portalis* species group. Since most of traits of *B.* sp. nov. fit in the concept of *B. portalis* species group we assumed that this new species belongs to *B. portalis* species group and is the first representative of this species group to have available molecular markers sequences that can be useful in systematics studies. Surely further studies and enrichment of databases with DNA sequences of other taxa will clarify uncertainties and support a phylogenetically-based taxonomic classification of this important genera of phytophagous mites.

Should Brevipalpus cuneatus be in the B. portalis species group?

Due to the remarkable similarity between *B.* sp. nov. and *B. cuneatus* morphological traits of this last species were detailed checked along this study making possible highlight an interesting inconsistency related to the classification of the species *B. cuneatus* in the *B. cuneatus* species group. According to Baker & Tuttle (1987) species in the *B. cuneatus* species group present three setae on the distal palp fragment, however *B. cuneatus* present just two setae on this fragment (Beard *et al*., 2012) similarly to *B.* sp. nov. Due to this trait *B. cuneatus* should be classified as belonging to the *B. portalis* species group. The other trait distinguishing *B. cuneatus* and *B. portalis* is the shape of dorso central setae *c1*, *d1*, *e1* & the dorso lateral *c3*, *d3*, *e3* – similar shape in *B. portalis* species group & different shape in *B. cuneatus* species group. However, this trait presents a degree of subjectivity, since this differentiation can be discrete as that observed in *B.* sp. nov.

Other remarkable similarity between *B.* sp. nov. and *B. cuneatus* is related to the leg chaetotaxy, which for now is not included in the species group concept. Both species have just one seta on trochanter of the leg III (Table 6). This trait differs them from all other *Brevipalpus* species included in the *portalis* and *cuneatus* species groups.

Table 6. Morphological characters used to distinguishing the *Brevipalpus* studied species.

Character	*B. portalis*	*B* . sp. nov.	*B. cuneatus*	*B* . aff. *cuneatus* sp. 1	*B. tuberellus*	*B. oleae*
Gnathosoma						
1. Palp	extending only to femur I	extending only to femur I	extending only to femur I	extending only to femur I	extending only to femur I	extending beyond femur I
2. Palp tarsus	2 distal setae not reduced	2 distal setae very small	2 distal setae very small	3 distal setae not reduced	3 distal setae very small	3 distal setae not reduced
Leg						
3. Omega on tarsus II	normal	long	long	long	normal	normal
4. Trochanter III	2 setae	1 setae	2 setae	2 setae	2 setae	2 setae
Dorsum						
5. Opisthosomal setal pair	body margin	body margin	inserted off the body margir	body margin	body margin	body margin
6. Dorsal central setae						
Venter						
7. Setae 4a	long	very short	very short	long	long	long
8. reticulation	ventral cuticle central mostly smooth; with strong reticulation until seta 4a	entire with strong pebble-like reticulation	entire with strong pebble-like reticulation	entire with strong pebble-like reticulation	ventral cuticle central mostly smooth	ventral cuticle central mostly smooth
9. Ventral plate	with small to medium rounded cells, often fused to form weak bands.	not well defined; warts fusioned forming transverse bands	not well defined; warts fusioned forming transverse bands	not well defined; warts fusioned forming transverse bands		with small to medium rounded cells, often fused to form weak bands.
Spermatheca	not visible	not visible	not visible	visible (Fig. 9b)	visible (Fig. 9c)	visible (Fig. 9d)

In most species of the *B. portalis* species group it is not possible to observe the formation of the vesicle of the spermatheca, except in *B. selas*. The great majority presents only the duct, and two others do not observe any structure referring to spermatheca, *B. aeoloides* and *B. combreti*. The non-visualization of the spermathecae of the *Portalis* group may have two possible explanations, the structure of the spermatheca in these species being less sclerotized and therefore difficult to preserve, or the group is very basal and lost the spermatheca vesicle throughout its evolutionary history.

The results obtained in the present work support that *B.* sp. nov. should not be classified as *B. cuneatus* species group. It would be also possible that B. cuneatus and *B.* sp. nov. belong to another phylogenetic group distinct from *B. portalis* and *B. cuneatus* species group. It will be imperative to obtain DNA sequences of *B. cuneatus* specimens and include it in the phylogenetic analyses to test this hypothesis.

Remarks on Brevipalpus cuneatus species group

Results of phylogeny and intra and intergroup distances between the studied species whose are classified as belonging to *B. cuneatus* species group- *B. tuberellus*, *B. oleae*, *B.* aff. *cuneatus* sp.1, *B.* aff. *cuneatus* sp.2- showed that these taxa are not closely related, except for *B.* aff. *cuneatus* sp.1, *B.* aff. *cuneatus* sp.2 (hereafter referred as *B.* aff. *cuneatus* spp.). Although forming a monophyletic group (similar that was observed for *B. phoenicis*, *B. obovatus* and *B. californicus* groups in Clade 1) (Fig. 8) subclades (branches) are not clustering species as expected. When these taxa were grouped for nucleotide distance analyses (Tables 4 and 5, scenario 2) comprising the "*B. cuneatus* species group" the intragroup variability was considerably higher if compared with that between another studied species group (remarkable for the D1-D3 fragment). When these taxa were analyzed separately (Tables 4 and 5, scenario 3) intergroup distances were within the value range observed between other species groups. Therefore, taking into account the limited information on the molecular systematics of *Brevipalpus* mites our results reveal that the current morphological concept of *B. cuneatus* species group is not supported by phylogeny and that the studied species can constitute at least three species group.

Another important observation about the *B. cuneatus* species group is that different vesicle structures of the spermatheca were observed among the species. At

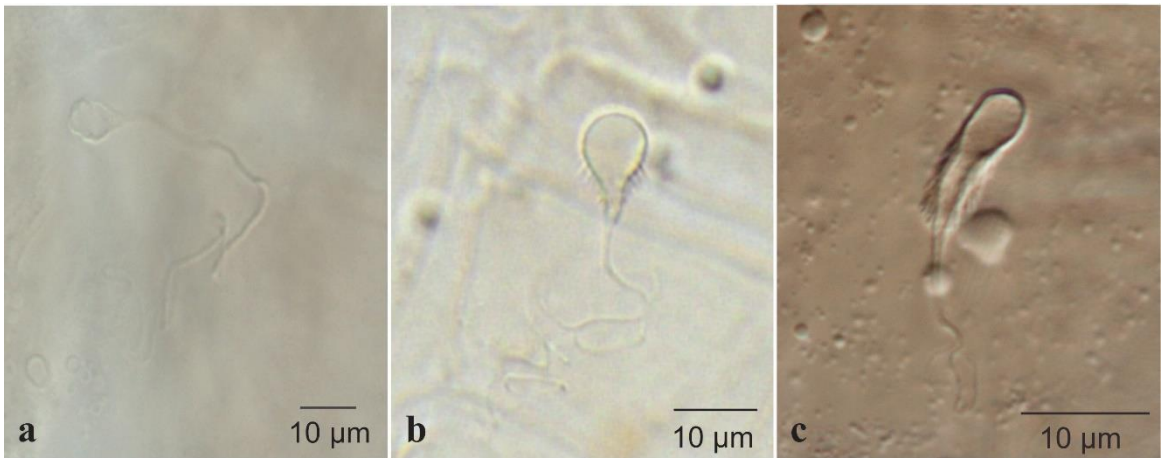


Figure 9. Different structural forms of spermatheca in three distinct species of *Brevipalpus*: *B. tuberellus* (a), *B. oleae* (b) and *B.* aff. *cuneatus* sp.1

least three groups are observed sharing the same type of spermatheca- group I (Fig. 9b): *B. oleae*, *B. olearius* Sayed and *B. oleasteri* Hatzinikolis & Panau; group II (Fig.9a): *B. tuberellus*, *B. chamaedorea* Baker, Tuttle & Abbatiello, *B. cromroy* Evans, *B. formosus* De Leon, *B. floridanus* De Leon and *B. hernandiae* De Leon and; group III (Fig.9c): *B.* aff. *cuneatus* sp. 1 and *B.* aff. *cuneatus* sp. 2. Since spermatheca is a basal character in *Brevipalpus* it can be an evolutionary signal that assists in the phylogenetic reconstruction of the group.

Currently *B. cuneatus* is the most numerous species group of *Brevipalpus* mites comprising more than 126 species (Evans, 1990; Mesa *et al*., 2009), that represents more than 40% of the total number of species in the genus. It is possible that species grouped in this species group based on the current morphological classification by Baker & Tuttle (1987) consist of multiple phylogenetic groups in addition to the taxa herein studied that revealed to be phylogenetically distinct groups. Efforts should be directed to including the described species classified as belonging to this species group in the molecular phylogeny studies supporting a taxonomic revision of the group and pointed out morphological traits phylogenetically informative. In order to clarify the relationship of the species of the *cuneatus* group, a robust revision is fundamental, and essential for new studies that focus on the species complex of the *B. cuneatus* group.

References

Baker EW, Tuttle DM (1987) The False Spider Mites of Mexico (Tenuipalpidae: Acari). U.S. Department of Agriculture, Technical Bulletin, 1706: 237.

Bastianel M, Freitas-Astúa J, Kitajima EW, Machado MA (2006) The citrus leprosis pathosystem. Summa Phytopathologica, 32:211–220. http://dx.doi.org/10.1590/S0100-54052006000300001

Beard JJ, Ochoa R, Bauchan GR, Trice MD, Redford AJ, Walters TW, Mitter C (2012) Flat Mites of the World. Available from: http://idtools.org/id/mites/flatmites/ [Accessed on June 2018].

Beard JJ, Ochoa R, Braswell WE, Bauchan GR (2015) *Brevipalpus phoenicis* (Geijskes) species complex (Acari: Tenuipalpidae)- A closer look. Zootaxa, 3944:1–67. http://dx.doi.org/10.11646/zootaxa.3944.1.1

Borges PAV, Cunha R, Gabriel R, Martins AF, Vieira V (2005) A list of terrestrial fauna (Mollusca and Arthropoda) and flora (Bryophyta, Pteridophyta and Spermatophyta) from the Azores. Direcçao Regional do Ambiente e Universidade dos Açores, Horta, Angra do Heroismo e Ponta Delgada, 318.

Borges PAV (2012) Base de dados da biodiversidade dos Açores. Available from: http://www.azoresbioportal.angra.uac.pt/pesquisa.php?sstr=8&lang=pt/ [Accessed on June 2018].

Childers CC, Rodrigues JCV (2011) An overview of *Brevipalpus* mites (Acari: Tenuipalpidae) and the plant viruses they transmit. *Zoosymposia*, 6:180–192.

Childers CC, Rodrigues JCV, Welbourn WC (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. https://doi.org/10.1023/B:APPA.0000006544.10072.01

Childers CC, Rodrigues JCV, Derrick KS, et al. (2003c) Citrus leprosis and its status in Florida and Texas: Past and present. *Experimental and Applied Acarology*, 30:181–202. https://doi.org/10.1023/B:APPA.0000006548.01625.72

Darriba D, Taboada GL, Doallo R, Posada D (2012) "jModelTest 2: more models, new heuristics and parallel computing". Nature Methods 9, 772.

Dowling APG, Bauchan GR, Ochoa R, Beard JJ (2010) Scanning electron microscopy vouchers and genomic data from an individual specimen: maximizing the utility of delicate and rares. Acarologia 50(4): 479–485.

Dowling APG, Ochoa R, Beard JJ, Welbourn WC, Ueckermann EA (2012) Phylogenetic investigation of the genus *Raoiella* (Prostigmata: Tenuipalpidae): diversity, distribution and world invasions. Experimental and Applied Acarology, 57(3-4): 257-69.

Edgar RC (2004) MUSCLE: a multiple sequence alignment method with reduced time and space complexity. BMC Bioinformatics20045:113.

Evans GA, Cromroy HL, Ochoa R, (1993) The false spider mites of Honduras (Tenuipalpidae: ACARI). Florida Entomology, 76,126–155.

Guidon S, Dufayard JF, Lefort V, Anisimova M, Hordijk W, Gascuel O (2010) New Algorithms and Methods to Estimate Maximum-Likelihood Phylogenies: Assessing the Performance of PhyML 3.0. Systematic Biology, 59(3): 307-321.

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

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

Kimura M, (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. Journal of Molecular Evolution, 16(2): 111–120.

Kitajima EW, Rodrigues JCV, Freitas-Astua J (2010) An annotated list of ornamentals naturally found infected by *Brevipalpus* mite-transmitted viruses. Sci. agric. (Piracicaba, Braz.), 67:348–371. http://dx.doi.org/10.1590/S0103-90162010000300014

Kondo H, Maeda T, Tamada T (2003) Orchid fleck virus: *Brevipalpus californicus* mite transmission, biological properties and genome structure. Experimental and Applied Acarology, 30:215–223.

Kumar S, Stecher G, Li M, Knyaz C, and Tamura K (2018) MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. Molecular Biology and Evolution 35:1547-1549.

Lindquist EE, Sabelis MW, Bruin J (1996) Eriophyoid Mites: Their Biology, Natural Enemies and Control. World Crop Pests, Vol. 6. Elsevier Science Publishers, Amsterdam. 790.

Livshitz IZ, Mitrofanov VI, Vasilieva EA. (1972) On the morphology and biology of *Brevipalpus obovatus* Donnadieu, 1875. Works of the Nikitsk Botanical Garden, 61:65–75.

Maddison WP (1996) *Pelegrina Franganillo* and other jumping spiders formerly placed in the genus *Metaphidippus* (Araneae: Salticidae). Bulletin of the Museum of Comparative Zoology 154: 215-368.

McGregor EA (1949) Nearctic mites of the family Pseudoleptidae. Memoir of the Southern California Academy of Sciences, 3(2): 1–45.

Mendonca RS, Navia D, Diniz, IR, 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 and Applied Acarology, 55(1): 1-23.

Mesa NC, Ochoa R, Welbourn WC, Evans AG, Gilberto JM (2009) A catalog of the Tenuipalpidae (Acari) of the World with a key to genera. Zootaxa 2098: 1-185.Navia D, Mendonça RS, Ferragut F, *et al*. (2013) Cryptic diversity in *Brevipalpus* mites (Tenuipalpidae). Zoologica Scripta, 42: 406–426. https://doi.org/10.1111/zsc.12013Ochoa R, Salas LA, (1989) The genus *Brevipalpus* in Costa Rica (Acari: Tenuipalpidae). International Journal of Acarology, 15:(1) 21-30. http://dx.doi.org/10.1080/01647958908683819

Oliveira ICS (2014) Sistemática molecular para ácaros planos do gênero *Brevipalpus* Donnadieu (Acari: Tenuipalpidae) – utilização de marcadores moleculares mitocondriais e) nucleares. 120p. Dissertação (Mestrado em Zoologia) – Instituto de Ciências Biológicas, Universidade de Brasília, Brasília.

Pritchard AE, Baker EW (1952) The false spider mites of California (Acarina: Phytoptipalpidae). University of California Publications in Entomology, 9(1):1-94.

Rodrigues JCV (2000) Relações patógeno-vetor-planta no sistema leprose dos citros. PhD Dissertation. Centro de Energia Nuclear na Agricultura, da Univ. São Paulo, Piracicaba.

Ronquist F, Teslenko M, Van der Mark P, Ayres D L, Darling A, Höhna S, Larget, B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic biology, 61(3), 539-42.

Salinas-Vargas D, Santillán-Galicia MT, Guzmán-Franco AW, Hernández-López A, Ortegaarenas LD, Mora-Aguilera G (2016) Analysis of the genetic variation in *Brevipalpus yothersi* (Acari: Tenuipalpidae) populations from four species of citrus host plants. PLoS One, San Francisco, v. 11, n. 10. Available from:http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0164552. [Accessed on June 2018].

Sánchez-Velázquez EJ, Santillán-Galicia MT, Novelli VM, Nunes MA, Mora-Aguilera G, Valdez-Carrasco JM, Otero-Colina G, Freitas-Astua J (2015) Diversity and genetic variation among *Brevipalpus* populations from Brazil and Mexico. PloS One, San Francisco, v.10, n.7, 2015. Available from: http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0133861. [Accessed on Octo 2018].

Schäfer HW. 2002. Chorology and Diversity of the Azorean Flora [dissertation]. Regensburg (Baviera): Vorklinische Medizin Der Universität Regensburg.

Staden R, Beal KF, Bonfield JK (1998) The Staden Package, 1998. Computer methods in molecularb. In: Misener, S. & Krawetz, S. A. (Eds). Bioinformatics Methods and Protocols, 132. Totowa: Humana Press Inc., 115-130.

Tamura K. (1992). Estimation of the number of nucleotide substitutions when there are strong transition-transversion and G+C content biases. Molecular Biology and Evolution, 9(4): 678–687.

Tavaré S (1986) Some probabilistic and statistical problems in the analysis of DNA sequences. Lectures on Mathematics in the Life Sciences (American Mathematical Society), 17: 57–86.

Welbourn WC, Ochoa R, Kane, Erbe EC, EF (2003) Morphological observations on *Brevipalpus phoenicis* (Acari: Tenuipalpidae) including comparisons with *B. californicus* and *B. obovatus*. Experimental and Applied Acarology, 30(1/3): 107–133.

CAPÍTULO 4

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Brevipalpus obovatus Donnadieu species group (Trombidiformes: Tenuipalpidae)- redefinition and cryptic diversity based on molecular phylogeny

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Abstract *Brevipalpus* Donnadieu constitute an important genus of plant feeding mites. Some species in this genus are agricultural pests. Taxonomy of this group of mites has represented a challenge for acarologists for decades. Currently a taxonomic revision of the genus is ongoing. Despite a major progress in the identification of economically important *Brevipalpus* species previously confused or misidentified, knowledge on the evolutionary relationships among species in this genus as well as among species groups is lacking. One of the main goals of taxonomic classification is to improving predictive power on different aspects of a particular taxon based on known traits of closely related taxa. *Brevipalpus* genus is currently subdivided into six species groups however subdivision is artificial, exclusively based on morphological characters. In this study the *B. obovatus* species group is redefined based on an integrative approach including molecular phylogeny and detailed morphological observations. Herein, we use *COI*, ITS and D1-D3 rRNA data of 10 *Brevipalpus* species (186 sequences). In addition, detailed study from morphological data and genetic distances showed the differences between *B. azores* Beard & Ochoa, *B. feresi* Beard & Ochoa and *B. papayensis* Baker species. Relationship between taxa in this species complex is quite different among phylogenies showing that evolutionary relationship is not well-resolved suggesting recent radiation. Cryptic diversity is revealed between populations previously morphologically identified as *B. papayensis* Baker.

Keywords: flat mites, false spider mite, taxonomy, *B. papayensis*.

Introduction

Brevipalpus Donnadieu is one of the most numerous genus among flat mites of the Tenuipalpidae family, with about 300 species described (Mesa *et al*. 2009; Navia *et al*. 2013; Beard *et al*. 2015). This genus is cosmopolitan, except for Antarctica, being present in all zoogeographic regions of the world, although they occur mainly in tropical and subtropical climates (Mesa *et al*. 2009). *Brevipalpus* constitute an important group of plant feeding mites. Some species in this genus are agricultural pests causing damage to food crops, forest or ornamental plants (Mesa et al. 2009; Childers & Rodrigues 2011). However main impact of these mites is actually due to their action as plant virus vector. Almost 40 plant species have been reported as being naturally infected by *Brevipalpus* transmitted virus (BTVs) (Kitajima *et al*. 2010).

Unambiguous identification of *Brevipalpus* species is needed to understand the role of each species in the transmission of plant virus, to guide the adoption of quarantine measures and to support the development of pest management (Beard *et al*. 2015). Taxonomy of this group of mites has represented a challenge for acarologists for decades. Due to their morphological similarity *Brevipalpus* species of greatest economic importance have been consistently confused and misidentified (Welbourn *et al*. 2003). Currently a taxonomic revision of the genus is ongoing. Main taxonomic confusions on the most important species were enlightened by Beard et al. (2015) through a meticulous study using traditional and refined microscope techniques, resulted in species redescription, synonyms resurrection, and descriptions of new species. New morphological taxonomic characters, as well as traits indicated as informative, but that have not been appropriately considered in the last decades have been taken into account (Beard et al., 2015). An integrative approach, including molecular data, has been applied allowing uncover cryptic species (Navia et al., 2013).

Despite a major progress in the identification of economically important *Brevipalpus* species previously confused or misidentified, knowledge on the evolutionary relationships among species in this genus as well as among species groups is lacking. One of the main goals of taxonomic classification is to improving predictive power on different aspects of a particular taxon based on known traits of closely related taxa. Therefore, taxonomy is especially useful if reflects evolutionary relationship among organisms, which has been challenging for biologists working with the most diverse groups of organisms (Hinchliff *et al*. 2015). Furthermore, phylogenetic information is

essential for the study of nearly all aspects of adaptation and made easier advance in the management of applied problems.

Brevipalpus genus is currently subdivided into six species groups- *B. californicus*, *B. cuneatus*, *B. portalis*, *B. phoenicis*, *B. obovatus* and *B. frankeniae* according to Baker & Tuttle (1987). This subdivision is exclusively based on morphological characters- setae on the opisthosoma, number of solenidia on the tarsus II of the female; and number of setae on the distal segment of palp. However preliminary phylogenetic studies strongly suggest that this artificial classification do not correspond to the phylogenetic groups (Navia et al., 2013).

In this study the *B. obovatus* species group is redefined based on an integrative approach using molecular phylogeny, since mitochondrial and nuclear DNA sequences, as well as detailed morphological traits, obtained through electron and optical microscopy. The phylogenetic value of morphological characters is further evaluated and characters that should be considered to defining *B. obovatus* species group are suggested. Cryptic diversity on the *Brevipalpus papayensis* Baker taxon was uncovered.

Material and Methods

Sample collection

Plant material samples (leaves, stems and/or fruits) were collected in 2015 in three islands of the Azores archipelago- Pico, Faial and Flores (Table 1). Specimens were collected by direct inspection using a dissecting microscope and transferred to bottles with 70% and 100% ethyl alcohol for morphological and molecular identification. Specimens preserved on 70% ethyl alcohol were mounted on slides in Heinz and Hoyer medium to light microscope observations while those preserved on 100% ethyl alcohol were used to molecular characterization or to Scanning Eletron Microscopy studies.

Morphological identification

The slide-mounted mites were examined at 400x and 1000x magnification using differential interference contrast (DIC) in a compound microscope Nikon Eclipse Ni-U. Editing of the images was performed in CorelDRAW X7 software (Corel Corporation). Identifications were based on a generic key using Lucid3 software (Beard et al. 2012) available in http://idtools.org/id/mites/flatmites/key.php?key=brevipalpus_key, and on

the original descriptions of the species. Lucid3 is software for creating and using interactive identification. Chaetotaxy follows that of Lindquist (1985).

Some of the mites preserved in 100% ethyl alcohol were studied under Scanning Electron Microscopy (SEM). The specimens were dried at the critical point (Baltec EM CPD 300 - Lichtenstein), and then fixed in 15 x 30 mm copper plates using ultra-soft round carbon adhesive guides. All samples were bathed with another by a metallizer (Baltec SCD 050 - Lichtenstein). The scanning electron microscope used to capture the images was a Japanese-made JEOL JSM-IT300LV. The acceleration voltage of 5 kV was used to visualize the samples with increases ranging from 330x to 18,000x. The specimens were visualized in ventral and dorsal position, and in some individuals, it was possible to obtain the complete body imaging, examining both, the dorsal and ventral surface, on the same specimen.

Table 1. Collection records of *Brevipalpus* mites sequenced in this study and respective GenBank accession numbers.

Host plant		Collection site		Date	*Brevipalpus* species	GenBank Accession No.		
Scientific name	**Family**	**Locality**	**Coordinates**			**COI**	**ITS2**	**D1-D3**
Citrus sinensis	Rutaceae	Faial, Praia de Almaxarife	38°33´36"N, 28°37´33"W	07/X/2015	*B. azores*			
					B. papayensis		MK508977	
Eriobotrya japonica	Rosaceae	Pico, Madalena	38°25´24"N, 28°23´21"W	04/X/2015	*B. papayensis*			
Eleagnus umbellata	Eleagnaceae	Pico, Madalena	38°25´24"N, 28°23´21"W	04/X/2015	*B. papayensis*			
Ipomoea indica	Convolvulaceae	Pico, Madalena	38°25´24"N, 28°23´21"W	04/X/2015	*B. papayensis*		MK508966 MK508967 MK508968 MK508969	
Ipomoea indica	Convolvulaceae	Faial, Horta	38°33´03"N, 28°38´21"W	07/X/2015	*B. obovatus*			
Melissa officinalis	Lamiaceae	Pico, Silveira	38°24´48"N, 28°17´02"W	06/X/2015	*B. obovatus*	MK499455 MK499456 MK499457		
Mentha sp.	Lamiaceae	Pico, Silveira	38°24´48"N, 28°17´02"W	05/X/2015	*B. obovatus*			
Phytolacca americana	Phytolaccaceae	Pico, Madalena	38°25´24"N, 28°23´21"W	04/X/2015	*B. obovatus*			
Pittosporum undulatum	Pittosporaceae	Faial, Praia de Almaxarife	38°33´36"N, 28°37´33"W	07/X/2015	*B. azores*			

Host plant		Collection site		Date	*Brevipalpus* species	GenBank Accession No.		
Scientific name	Family	Locality	Coordinates			COI	ITS2	D1-D3
					B. obovatus			
Rhododendron indicum	Ericaceae	Faial, Horta	38°33´03"N, 28°38´21"W	07/X/2015	*B. azores*		MK508976 MK508978 MK508979	
Tecoma capensis	Bignoniaceae	Pico, Madalena	38°25´24"N, 28°23´21"W	04/X/2015	*B. papayensis*		MK508970 MK508971 MK508972 MK508981	
Vitis vinifera	Vitaceae	Pico, Madalena	38°25´24"N, 28°23´21"W	04/X/2015	*B. papayensis*			
Vitis vinifera	Vitaceae	Faial, Lonbega	38°32´03"N, 28°44´18"W	08/X/2015	*B. obovatus*	MK499459		
					B. papayensis		MK508973	
					B. azores	MK499460	MK508974 MK508975	
Vitis vinifera	Vitaceae	Flores, Lajedo	39°23´50"N, 31°14´40"W	11/X/2015	*B. obovatus*	MK499465 MK499469	MK508980	

Morphological identification

The slide-mounted mites were examined at 400x and 1000x magnification using differential interference contrast (DIC) in a compound microscope Nikon Eclipse Ni-U. Editing of the images were performed in CorelDRAW X7 software (Corel Corporation). Identifications were based on a generic key using Lucid3 software (Beard et al. 2012) available in http://idtools.org/id/mites/flatmites/key.php?key=brevipalpus_keyand original descriptions of the species. Lucid3 is software for creating and using ineractive identification. Chaetotaxy follows that of Lindquist (1985).

Some of the mites kept in 100% ethyl alcohol were submitted to scanning electron microscopy (SEM). The specimens were dried at the critical point (Baltec EM CPD 300 - Lichtenstein), and then fixed in 15 x 30 mm copper plates using ultra-soft round carbon adhesive guides. All samples were bathed with another by a metallizer (Baltec SCD 050 - Lichtenstein). The scanning electron microscope used to capture the images was a Japanese-made JEOL JSM-IT300LV. The acceleration voltage of 5 kV was used to visualize the samples with increases ranging from 330x to 18,000x. The specimens were visualized in ventral and dorsal position, and in some individuals, it was possible to obtain the complete body imaging, examining both, the dorsal and ventral surface, on the same specimen.

DNA extraction, amplification and sequencing

Genomic DNA was extracted from single *Brevipalpus* adult female using the DNeasy Tissue kit (Qiagen Germantown, MD, USA), according to the DNA extraction protocol 'Purification of Total DNA from Animal Blood or Cells' (SpinColumn Protocol). Mites were individualized in 1.5 ml microcentrifuge tubes containing 90 µL ATL buffer and 10 µL of proteinase K provided in the Qiagen before extracting the DNA. Mites were not crushed. In order to get maximal recovered of DNA, two incubation periods were performed initially, *i.e*., at 56 °C and 70 °C for about 20 hours and then 10 minutes, respectively. The next manufacturer's instructions were modified for DNA extraction from tiny mites, as described by Dowling *et al*. (2010) and Mendonça et al. (2011). DNA was isolated from 1 to 6 specimens from each sample. Before pipetting the mixture into a DNeasy mini spin column (Qiagen ki) the specimens was recovered from the wall of the microcentrifuge tubes and mounted on slides in Heinze PVA medium. These slides were deposited as voucher specimens in the Plant Mite Collection at Embrapa Genetic

Resources and Biotechnology, Brasília, Brazil. The final DNA solution was preserved at -20 °.

Three target DNA fragments were PCR-amplified and sequenced per single mite: one mitochondrial- the cytochrome c oxidase subunit I (*COI*) gene; and two nuclear DNA fragments- the Internal Transcribed Space 2 (ITS2) region and the subunit D1-D3 in 28S rDNA gene. PCR primers are described in Table 2.

Table 2. Characteristics of the four molecular markers considered (length of the amplified fragment, primer's names and sequences) and bibliographic references.

Marker acronym	Marker name	Fragment length (bp)	Primer Name	Primer sequence 5'-3'	Reference
COI mtDNA	Cytochrome Oxidase Subunit 1 (COI)	400	DNF	TAC AGC TCC TAT AGA TAA AAC	Navajas et al., 1996
			DNR	TGA TTT TTT GGT CAC CCA GAA G	
ITS 2 rDNA	Internal Transcribed Spacer 2 (ITS2)	650	Bp4 rw	AGTGCGAATTGCAGGACACA	in this study
			28Sc	ATA TCG TTA AAT TCA GCG GG	Navajas et al., 1998
		700	Bp3 Deg-F	TCG ATG AVG AAC GYA GCA RGY T	in this study
			28Sc	ATA TCG TTA AAT TCA GCG GG	Navajas et al., 1998
D1-D3 28S rRNA	subunit D1-D3 of the gene 28S	1000	D23F	GAG AGT TCA AGA GTA CGT G	Park & Foighil, 2000
			D6R	CCA GCT ATC CTG AGG GAA ACT TCG	

The reaction mixture for *COI* amplifications was performed in 25-µL total volumes containing 2.5 µL of a 10 × buffer supplied by the manufacturer, 1.5 µL $MgCl_2$ (25 mM), 1.0 µL dNTP (0.25 mM of each base); 0.75 µL of each primer (10 µM); 0.4 µL of bovine serum albumin solution (BSA) (10 mg mL−1 Biolabs), 0.13-µL U µL−1 (five units) of Taq polymerase (Qiagen); 15.77 µL of sterile water and 2 µL of DNA template.

PCR of the ITS2 region was conducted in 25-µL reaction volume containing 2.5 µL of a 10 × buffer (Qiagen), 1.5 µL $MgCl_2$ (25 mM), 0.175 µL of each primer (10 µM), 0.5 µL dNTP (0.25 mM of each base), 0.1 µL of bovine serum albumin solution (BSA) (10 mg mL−1 Biolabs), 0.2-µL U µL−1 (five units) of Taq polymerase (Qiagen), 12.5 µL of trehalose (Sigma-Aldrich Brazil), 4.35 µL of sterile water and 3 µL of DNA template. Two new ITS2 forward primers (Bp3-Deg-F and Bp4-rw) were used in combination with the reverse 28SC (Navajas et al., 1998) (Table 2). These primers were designed by Navia et al. (personal communication, February 2019) (Navia et al. in press).

A PCR for the subunit D1-D3 (28S) was also carried out in 25-µL volume containing 2.5 µL of a 10 × buffer supplied by the manufacturer, 1.5 µL $MgCl_2$ (25 mM),

0.125 µL dNTP (0.25 mM of each base), 0.25 µL of each primer (10 µM), 0.1 µL of bovine serum albumin solution (BSA) (10 mg mL−1 Biolabs), 0.2-µL U µL−1 (5 units) of Taq polymerase (Qiagen), 12.5 µL trehalose (Sigma-Aldrich Brazil), 3.575 µL of sterile water and 4 µL of DNA template.

The amplification thermal conditions were presented in Table 3. PCR products (5 µL) along with 0.5 µL of loading buffer were resolved by 1 % (w/v) agarose gel electrophoresis prepared in 0.5X TBE buffer and for 30 min at 100 volts. Gel images were visualized on GelRed staining (Biotium, Inc, Hayward, Canada) and acquired with Gel Doc™ System (Bio Rad). The amplified fragments (*COI*, ITS2 and D1-D3) containing visible and single bands were directly sequenced on both strands using an ABI 3730XL Applied Biosystems automated sequencer (at Macrogen (Seoul, Korea). No additional primers were used for sequencing.

Table 3. Polymerase chain reaction (PCR) thermal cycling conditions for the three DNA fragments considered. For the ITS2 region two different forward primers are reported.

PCR conditions	*COI*		ITS2		D1-D3	
Initial denaturation	94°C	4'	95°C	3'	95°C	3'
No. cycles	35		35		35	
Denaturation	92°C	1'	95°C	45"	95°C	45"
Annealing	48°C	1' 30"	55°C	40"	50°C	1' 30"
Elongation	72°C	1' 30"	72°C	1' 15"	72°C	1' 10"
Final Elongation	72°C	10'	72°C	10'	72°C	10'

Sequence retrieval and datasets

All the new sequences herein obtained have been deposited in GenBank (Table 1). Available *COI*, ITS 2 and D1-D3 sequences (Navia et al. 2013; 2018, Oliveira, 2014; Sanchez-Velazquez et al. 2015) of the *Brevipalpus* species belonging to three species groups according to Baker and Tuttle (1987) were retrieved from GenBank and included in the analyzes: *B. yothersi* Baker and *B. incognitus* Ferragut & Navia and B. *phoenicis* sensu stricto (Geijskes) in the *B. phoencis* group; *B. californicus* sensu stricto Banks in the *californicus* group; *B. chilensis* Baker and *B. obovatus* Donnadieu, *B. papayensis* Baker, *B.* azores Beard & Ochoa, *B. ferraguti* Ochoa & Beard and *B. feresi* Beard & Ochoa in the *B. obovatus* group; (Supplementary Material 1; 2 and 3). The Tenuipalpidae

mite *Raoiella indica* Hirst was used as external group in the analyses, *i.e.*, sequences accession numbers MH174682, JF928437 and MH093581 for *COI*, ITS2 and D1-D3 alignments respectively (Oliveira, 2014, Dowling et al. 2012) for *COI* and D1-D3 fragments respectively (Table 1). All dataset will be available upon request.

Phylogenetic analyses

The software Staden Package v.1.6.0 (Staden, Beal & Bonfield, 1998) was used for checking, editing and assembling the raw data into sequence contigs. Consensus sequences were also checked against *Brevipalpus COI* sequences retrieved from GenBank (Navia et al. 2013; Sanchez-Velazquez et al. 2015; Salinas-Vargas et al. 2016) and used for editing a neighbor joining tree in order to verify their reliability. To initially identify candidate protein coding regions in DNA *COI* sequences searching start and stop codons, an open reading frame was determined using a graphical analysis tool (ORF Finder) available at http://www.ncbi.nlm.nih.gov/projects/gorf/. The sequences were aligned using software such as the ClustalW multiple alignment procedure in BIOEDIT 7.0.4 (Hall, 1999) and Muscle (Muscle: multiple sequence alignment comparison by log-expectation program (Edgar 2004).

The distributions and frequencies of the shared haplotypes (COI sequences) and sequences variants (ITS 2 and D1-D3 sequences) were identified using DnaSP v.6 software (Rozas et al. 2017).

The jModeltest version 2.1 (Darriba et al. 2012) based on the likelihood for 88 different models was used to estimate the best-fit models of nucleotide substitution using the Akaike information criterion corrected (AICc) and the Bayesian information criterion (BIC). For the *COI* dataset, the HKY + G model (Hasegawa et al 1985) was selected according to AICc and to BIC with the following parameters used to model evolutionary rate differences among sites: a discrete gamma distribution shape parameter (G) of 0.2910 and Ti/Tv (Trasition/Transversion) of 1.0733. The ML models were tested in PhyML v. 3.0 (Guindon & Gascuel, 2003; Guindon *et al.* 2010), NJ in MEGA v. X (Kumar et al. 2018), and Bayesian inference (BI) was tested in MrBayes v.3.2.6 (Ronquist et al.2012). The same softwares and procedures were used to test NJ, ML and BI phylogenies in the subsequent analyses of the ITS 2 region and subunit D1-D3 sequences. For the ITS 2 dataset, the GTR+G (Tavaré, 1986) nucleotide substitution model was selected according to AICc and HKY + G just as BIC with the gamma distribution shape parameter of 1.071

and 1.055 respectively. For the subunit D1-D3 of the 28S rDNA dataset, the TIM3+G (Posada and Crandall, 1998) was selected according to AICc (G = 0.3740) and according to BIC, the TrN+G model (Tamura and Nei 1993) (G = 0.3730) was selected. The ML analyses were performed using the online version of the PhyML3.0 algorithm (Guindon et al. 2010). The robustness of the trees was assessed with a bootstrap analysis that involved 1000 bootstrap replicates for all analyses.

For the combined analysis, sequences of the three studied fragments (*COI*, ITS2 and D1-D3) were individually organized using MEGA v. X software (Kumar et al. 2018). Alignments of the three fragments was carried out separately by the Muscle program (Edgar 2004) implemented in the MEGA v. X. The files were then concatenated in a single matrix containing 24 taxa for each fragment and totalizing 2015 base pairs, using Mesquite v. 3.0.4 (a modular system for evolutionary analysis) (Maddison & Maddison 1996). The combined analysis using Bayesian Inference (BI) was performed in MrBayes ver.3.2 (Ronquist et al. 2012). The number of categories used to approximate the gamma distribution was set at four, and four Markov chains were run for 10,000,000 generations; the final average standard deviation of split frequencies was less than 0.01, and the stabilization of model parameters (burn-in = 0.25) occurred at approximately 250 generations. *Raoiella indica* was used as outgroup in the analysis, *i.e.*, sequences accession numbers MH174682, JF928437 and MH093581 for *COI*, ITS2 and D1-D3 alignments respectively. The phylogenetic trees based on the output file (newick format) created by PhyML 3.0 algorithm and MrBayes program was edited using FigTree v.1.4.3 (http://tree.bio.ed.ac.uk/software/figtree/). The final settings to better understand the data was performed in CorelDRAW X7 software.

Inter- and intraspecific genetic distances

Analyses of the pairwise genetic distances overall and between nucleotide sequences (*COI*, ITS 2 and D1-D3), as well as the among- and within distance of *Brevipalpus* species were performed using MEGA v. X (Kumar et al 2018). The most appropriate evolutionary model for estimation of inter and intra-species and group genetic variation was also selected using MEGA v. X. The Tamura Nei model (Tamura et al 1993) was applied to the *COI* dataset, and the Tamura 3-parameter model (T3P) (Tamura, 1992) and Kimura 2-Parameters (K2P) model (Kimura, 1980) was applied to the ITS 2 and D1-D3 datasets respectively. Standard error estimates were obtained by a bootstrap procedure (1000

replicates). In addition to calculating genetic distances between morphological-identified taxa, for the COI, ITS and D1-D3 fragments, distances evolving branches that phylogeny showed to potentially consist in cryptic taxa particularly among *B. papayensis* populations were also calculated. Thus, to better understand the phylogenetic relationships between studied species three different scenarios were considered to estimate the genetic distance values. The first considering the known valid species singly, i.e., *B. papayensis*, *B. phoenicis*, *B. azores*, *B. feresi*, *B. ferraguti*, *B. incognitus and B. yothersi* as members of *B. phoenicis* group; *B. obovatus* and *B. chilensis* in *B. obovatus* group and *B. californicus s.s*. in *B. californicus* group (Baker and Tutle, 1987; Beard et al. 2015; Navia et al. 2013). The second simulating *B. azores* and *B. feresi* as synonym of *B. papayensis* joining all as a single valid taxon. It was checked because during the morphological examination it was observed tiny differences inside the populations herein identified as *B. papayensis*. Some were morphologically closer to the species *B. azores* and *B. feresi* recently described by Beard et al. (2015), while others cleared showed differences. So, there was a suspicion that *B. papayensis* name could keep cryptic species inside. Finally, the latter scenario, also based on this suspitioous, checking the structure revealed by the COI and ITS 2 phylogenies (Figures 1 and 2) as well as the Bayesian combined analysis (Fig. 4), where the *B. papayensis* populations grouped in different clades scattered in the middle of clades of valid species as *B. feresi* or *B. azores* and could be each one selected to constitute likely one lineage, *i.e*., *B.* aff. papayensis sp.1 and *B.* aff. *papayensis* sp.2.

Results and Discussion

Molecular Phylogeny

Three main *Brevipalpus* mites clades were observed in the topology of COI, ITS, and D1-D3 phylogenetic trees, as well as of the Bayesian combined analysis (Figures 1, 2 and 3). Composition of these clades in all phylogenies was similar, although relationship between them showed to be somewhat different according to the studied molecular marker. **Clade I**- *B. californicus*; **Clade II**- *B. azores*, *B. chilensis*, *B. feresi*, *B. ferraguti*, *B. obovatus*, *B. papayensis*, and *B. phoenicis*; **Clade III**- *B. yothersi* and *B. incognitus*.

Main differences in the topology of the obtained phylogenetic trees were observed in the: i) relationship among main clades (phylogenetic groups); and ii) in the composition and relationships among subclades of the Clade II. In both COI and D1-D3 phylogenetic

trees the closest related Clades were I and III while in ITS and in the combined Bayesian analysis were Clades I and II. In COI and ITS Clade II was composed of two subclades- *B. chilensis*, *B. ferraguti* and *B. obovatus*; and *B. azores*, *B. feresi*, *B. papayensis*, and *B. phoenicis*. In D1-D3 although Clade II has also been composed of two subclades, they were: *B. chilensis* and *B. obovatus*; and *B. azores*, *B. feresi*, *B. ferraguti*, *B. papayensis*, and *B. phoenicis*. In COI and ITS phylogenies populations morphologically identified as *B. papayensis* were not monophyletic, being distributed in three branches. Unfortunnately same populations were not included in D1-D3 phylogeny since sequences for all of them were not available.

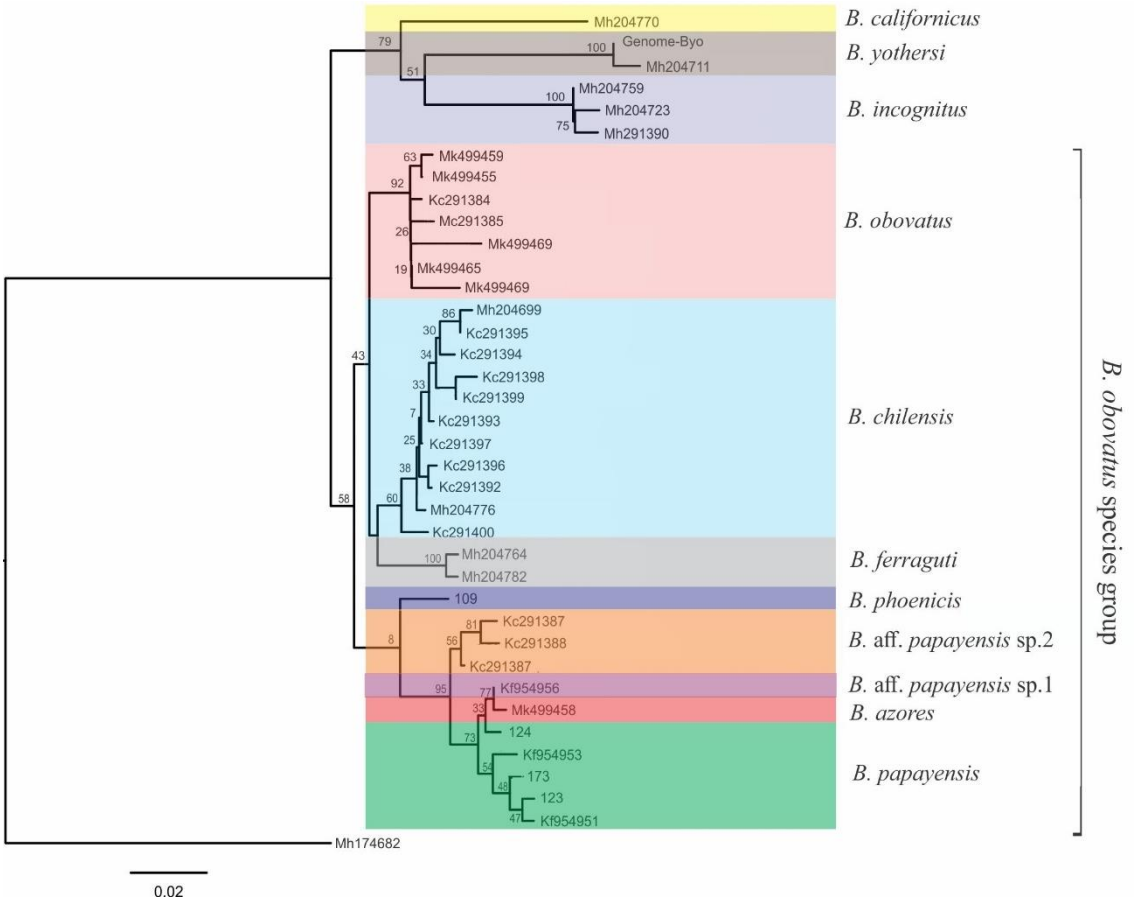

Figure 1. Molecular phylogenetic analysis inferred from *COI* sequences of *Brevipalpus* species obtained in this study and retrieved from GenBank. Maximum Likelihood (ML) method was used based on the HKY + G model (Hasegawa et al 1985) model considering a discrete gamma distribution = 0.2910 and trasition/transversion = 1.0733 to model evolutionary rate differences among sites. Information on sample codes and GenBank accession number are given in Table 2. Statistical support indicates bootstraps values greater than 50%. MH174682 = outgroup (*Raoiella*).

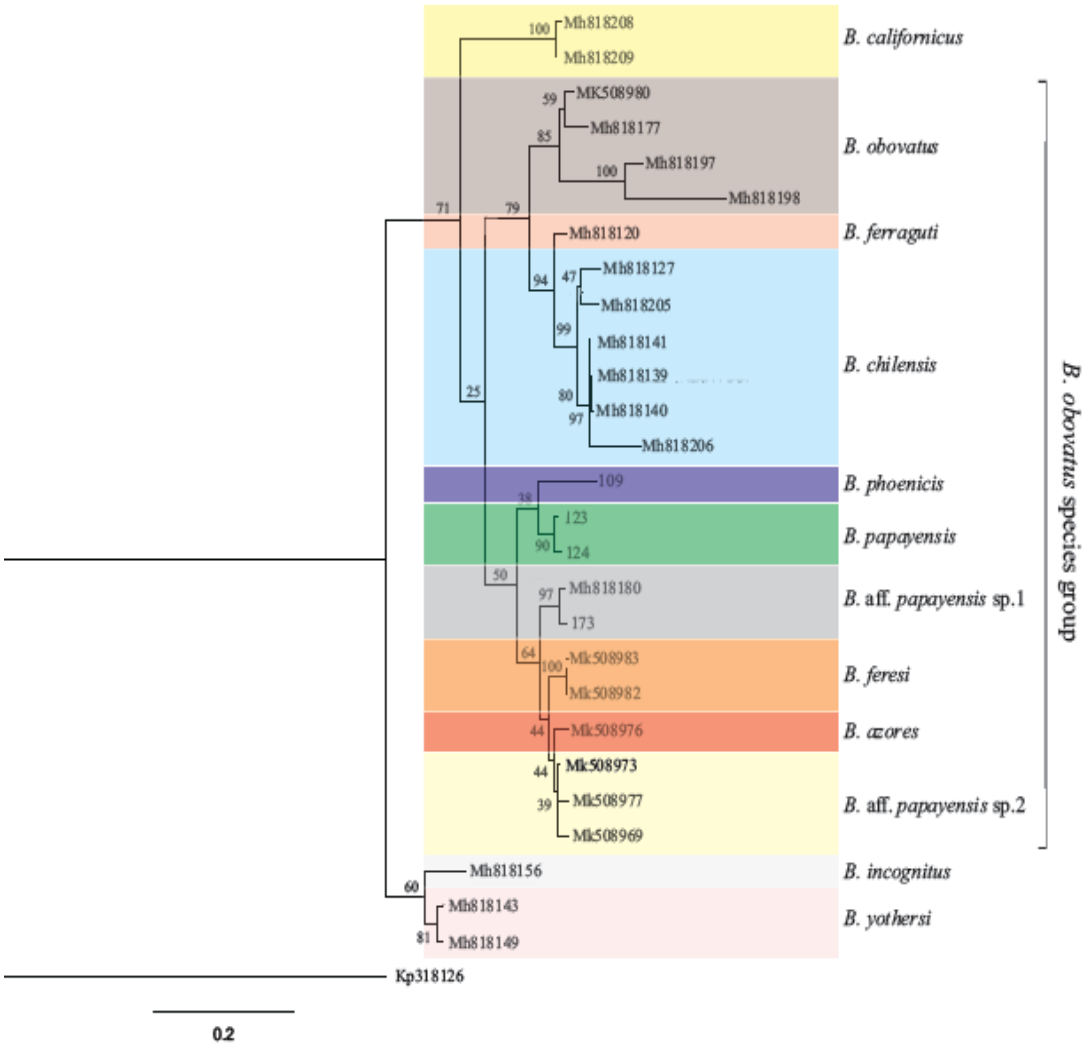

Figure 2. Molecular phylogenetic analysis inferred from ITS 2 region rRNA sequences Brevipalpus species obtained in this study and retrieved from GenBank. Maximum Likelihood method was used based on the GTR+G (General Time Reversible) model (Tavaré, 1986) considering a discrete gamma distribution = 1.071 to model evolutionary rate differences among sites. Information on sample codes and GenBank accession number are given in Table 2. Statistical support indicates bootstraps values greater than 50%. KP318126 = outgroup (*Raoiella*).

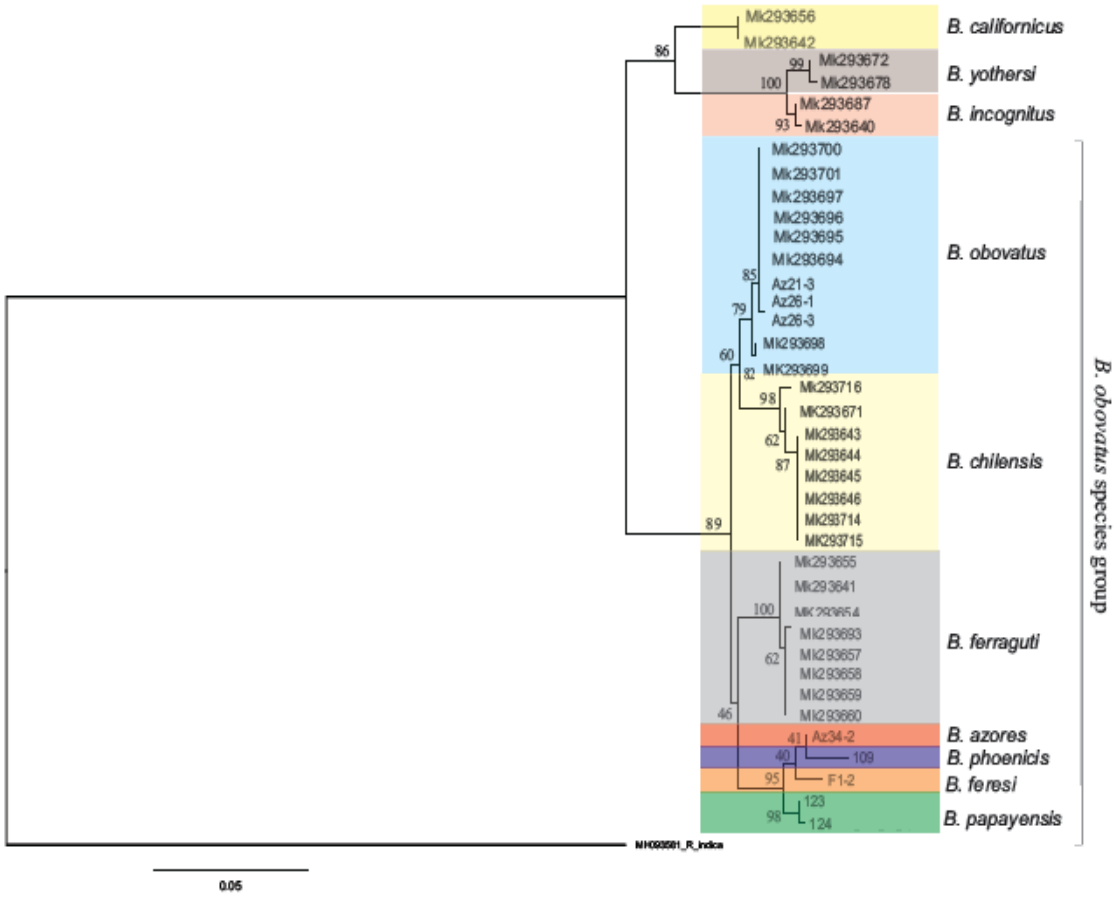

Figure 3. Molecular phylogenetic analysis inferred from 28S r-RNA subunit D1-D3 sequences Brevipalpus species obtained in this study and retrieved from GenBank. Maximum Likelihood method was used based on the TrN+G model (Tamura and Nei 1993) considering a discrete gamma distribution = 0.3730 to model evolutionary rate differences among sites. Information on sample codes and GenBank accession number are given in Table 2. Statistical support indicates bootstraps values greater than 50%. *R. indica* = outgroup.

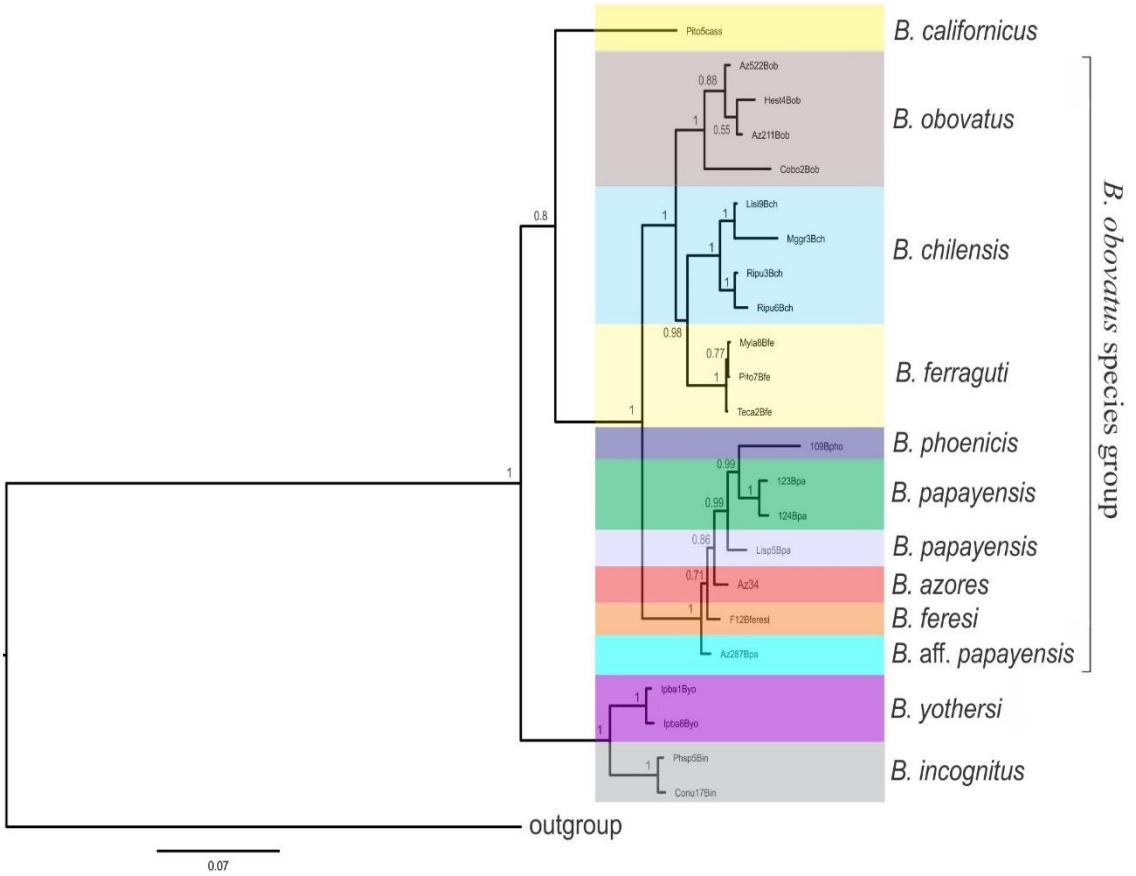

Figure 4. Combined Bayesian inference (BI) analysis tree for *Brevipalpus* species calculated from the concatenated *cytochrome c oxidase subunit I* sequences (*COI*), ITS2 region rRNA and 28S r-RNA subunit D1-D3 sequences. Statistical supports indicate Bayesian posterior probabilities values. Only statistical supports greater than 0.5 are indicated above branches. Putative *Brevipalpus* species groups are highlighted in colored squares.

The *Brevipalpus* species followed of the assigned haplotype number (mtDNA) and sequence variants (rDNA), the number of times it was found in the dataset (Frequency) and the respective GenBank accessions numbers or voucher codes are presented in Table 5 for COI, ITS2 and D1-D3 fragments.

Table 5. Data on Brevipalpus species with the morphological identification (Taxon ID), haplotype number/mtDNA or sequence variants/rDNA (Nr.), haplotype frequency/sequence variants (Freq.) and GenBank accession number for COI, ITS 2 and D1-D3 fragments.

	COI mtDNA - Haplotypes			ITS 2 rDNA - Sequence variants			D1-D3 (28sS) rRNA - Sequence variants		
Taxon ID	**Nr.**	**Freq.**	**Accession nr./voucher Code**	**Nr.**	**Freq.**	**Accession nr./voucher Code**	**Nr.**	**Freq.**	**Accession nr./voucher Code**
B. papayensis	8	1	Genome-Bpa	8	2	MK508973 MK508972	8	1	123-Coar_CAM_SP
	9	1	123-Coar_CAM_SP_Bpa	9	7	MK508969 MK508966 MK508967 MK508968 MK508970 MK508971 MK508981	9	1	124-Coar_CAM_SP
	10	1	124-Coar_CAM_SP_Bpa	10	1	MK508977			
	11	1	173-Brsp_COR_CR_Bpa	11	4	MH818180 MH818181 MH818182 MH818183			
	12	5	KF954956 KC291389 KF954950 KF954952 KF954957	12	1	123-Coar-CAM_SP			
	13	1	KF954951	13	1	124-Cosp_CAM_SP			
	14	1	KF954953	14	1	173-Brsp-COR_CR			
	15	1	KC291387						
	16	1	KC291388						
B. feresi				15	1	MK508982	7	1	AZ34_2_Baz
				16	1	MK508983			
B. azores	17	2	MK499458 MK499460	7	5	MK508976 MK508978 MK508979 MK508974 MK508975	6	1	AZ34_2
B. phoenicis	7	2	109-Cisp_IBI_SP DQ789586	6	1	109-Cisp-IBI-SP_Bpho	11	1	109-Cisp_IBI_SP_Bpho
B. ferraguti	19	4	MH204726 MH204762 MH204763 MH204780	17	13	MH818118 MH818119 MH818120 MH818121 MH818122 MH818123 MH818124 MH818125 MH818126 MH818128 MH818129 MH818191 MH818130	12	3	MK293654 MK293655 MK293641
	20	8	MH204764 MH204765 MH204766 MH204760 MH204772 MH204761 MH204774 MH204775				13	4	MK293657 MK293658 MK293659 MK293660
	21	2	MH204782 MH204781						
	18	1	MK499459	18	1	MK508980	10	2	Az26 Az26_3

	COI mtDNA - Haplotypes			ITS 2 rDNA - Sequence variants			D1-D3 (28sS) rRNA - Sequence variants		
Taxon ID	**Nr.**	**Freq.**	**Accession nr./voucher Code**	**Nr.**	**Freq.**	**Accession nr./voucher Code**	**Nr.**	**Freq.**	**Accession nr./voucher Code**
B. obovatus	22	1	KC291383	19	3	MH818177 MH818178 MH818179	15	7	Az21_3_Bob MK293694 MK293695 MK293696 MK293697 MK293700 MK293701
	23	9	MH204749 MH204740 MH204750 MH204742 AY320028 MH204704 MH204703 MH204705 Az52_2	20	3	MH818195 MH818196 MH818197	16	2	MK293698 MK293699
B. obovatus	24	1	KC291384	21	4	MH818198 MH818199 MH818200 MH818201			
	25	1	KC291385						
	26	1	KC291386						
	27	6	MH204741 MH204743 MH204744 MK499455 MK499456 MK499457						
	28	1	MK499469						
B. chilensis	29	1	MH204699	22	2	MH818141 MH818142	17	6	MK293643 MK293644 MK293645 MK293646 MK293714 MK293715
	30	1	KC291401	23	1	MH818127	18	1	MK293671
	31	4	MH204702 MH204700 MH204701 KC191391	24	1	MH818139	19	1	MK293716
	32	1	KC291397	25	1	MH818140			
	33	1	KC291393	26	1	MH818206			
	34	1	KC291394	27	1	MH818205			
	35	1	KC291395						
	36	1	KC291396						
	37	4	MH204776 MH204778 MH204779						
	38	1	KC291392						
	39	1	KC291398						
	40	1	KC291399						
	41	1	KC291400						
B. californicus	6	2	MH204770 MH204771	4	1	MH818208	5	2	MK293656 MK293642
				5	1	MH818209			
B. yothersi	1	2	Genome-Byo MH204706	1	1	MH818143	1	1	MK293672
	2	1	MH204711	2	1	MH818149	2	1	MK293678
B. incognitus	3	1	MH204723				3	1	MK293687

	COI mtDNA - Haplotypes			ITS 2 rDNA - Sequence variants			D1-D3 (28sS) rRNA - Sequence variants		
Taxon ID	**Nr.**	**Freq.**	**Accession nr./voucher Code**	**Nr.**	**Freq.**	**Accession nr./voucher Code**	**Nr.**	**Freq.**	**Accession nr./voucher Code**
	4	1	KC291390	3	2	MH818117 MH818156	4	1	MK293640
	5	1	MH204759						

Genetic distances between *Brevipalpus* with a look at closely related species in the *B. obovatus* group

Genetic distances based on COI marker

The *COI* average mean divergence over all the sequence pairs (including the outgroup taxa) was 5.36 % (SE = 0.63) and ranged from 0.0% to 25.32 %. The average mean divergence over the *Brevipalpus* sequences was 5.02 % (SE = 0.59) and ranged from 0.0% to 11.22%.

The *COI* marker showed low interspecific divergence among the species *B. papayensis* and *B. azores.* The obtained value, 1.51 %, is almost half of the observed for the interspecific divergence among *B. chilensis* and *B. obovatus* (3.18 %) known to be very closely species in the *B. obovatus* group (Table 6). The *COI* intraspecific values (2.02 % and 0.00%) found for *B. papayensis* and *B. azores* (Table 6) were compatible with COI values found in studies of Tetranychidae, Eriophyidae and Phytoseiidae mites (Navajas et al. 1998; 2003; Mendonça et al. 2010; Skoracka et al. 2012; Santos et al. 2016; Tixier et l. 2017). No *COI* sequences from *B. feresi* were obtained. Considering de scenario 2 (Table 7), including *B. azores* together with the *B. papayensis* clade, the intraspecific distance kept almost unchanged and the interspecifc value found for the closest species, *B. phoenicis*, was 3.93%. This value is nearby those observed between *B. chilensis* and *B. obovatus* (3.18%); *B chilensis* and *B. ferraguti* (3.65 %) and, *B. obovatus* and *B. ferraguti* (3.76 %). Considering *B. papayensis* as apart populations, as predicted for scenario 3, the interspecific values for the distances between *B.* aff. *papayensis* sp.1, *B.* aff. *papayensis* sp.2 and *B. azores* were lower than 2.00% as well as the intraspecific were lowers than 1.00% (Table values not showed). The results indicate that the morphological characteristics used to define the recently described species (*B. azores*) have not yet been reflected in the mitochondrial genome of *Breviapalpus* spp. This *COI* marker does not genetically distinguished *B. azores* and *B. papayensis*, neither the putatives lineages inside B. *papayenses* taxon (*B.* aff. *papayensis* sp.1 and *B.* aff. *papayensis* sp.2).

Table 6. Estimates of evolutionary divergence over sequence between and within groups- *COI* gene.

		1	2	3	4	5	6	7	8	9	10
1	*B. papayensis*	2,02%	0,41%	0,97%	1,26%	1,06%	1,24%	1,79%	1,73%	1,84%	2,75%
2	*B. azores*	1,51%	0,00%	1,10%	1,40%	1,25%	1,39%	1,89%	1,85%	1,93%	2,80%
3	*B. phoenicis*	3,95%	3,81%	0,00%	1,14%	1,10%	1,10%	1,87%	1,88%	2,04%	2,84%
4	*B. ferraguti*	6,47%	6,69%	5,02%	0,24%	1,00%	1,01%	1,92%	1,88%	2,04%	2,73%
5	*B. obovatus*	4,95%	5,41%	4,42%	3,76%	0,66%	0,88%	1,67%	1,69%	1,96%	2,84%
6	*B. chilensis*	6,28%	6,68%	4,48%	3,65%	3,18%	1,07%	1,75%	1,74%	2,01%	2,72%
7	*B. californicus*	10,82%	11,01%	10,62%	10,29%	8,82%	9,59%	0,00%	1,81%	1,79%	3,27%
8	*B. incognitus*	10,51%	10,68%	11,01%	10,98%	8,88%	9,83%	10,44%	0,82%	1,76%	3,11%
9	*B. yothersi*	10,72%	10,53%	11,20%	11,22%	10,51%	11,17%	10,15%	9,52%	0,41%	2,93%
10	outgroup	20,83%	20,57%	20,95%	19,63%	20,45%	19,73%	25,32%	24,09%	22,11%	n/c

Caption: the gray color indicates the intra-specific distance of each species.

Table 7. Estimates of evolutionary divergence over sequence between and within groups- *COI* gene (Considering *B. azores* as a population of *B. papayensis*).

		1	2	3	4	5	6	7	8	9
1	*B. papayensis*	1,88%	0,97%	1,32%	1,06%	1,24%	1,85%	1,75%	1,78%	2,73%
2	*B. phoenicis*	3,93%	0,00%	1,20%	1,08%	1,06%	1,87%	1,90%	1,96%	2,82%
3	*B. ferraguti*	6,50%	5,02%	0,24%	1,01%	1,02%	1,92%	1,91%	1,94%	2,69%
4	*B. obovatus*	5,01%	4,42%	3,76%	0,66%	0,84%	1,69%	1,69%	1,85%	2,76%
5	*B. chilensis*	6,33%	4,48%	3,65%	3,18%	1,07%	1,77%	1,78%	1,93%	2,70%
6	*B. californicus*	10,84%	10,62%	10,29%	8,82%	9,59%	0,00%	1,88%	1,79%	3,18%
7	*B. incognitus*	10,53%	11,01%	10,98%	8,88%	9,83%	10,44%	0,82%	1,76%	2,92%
8	*B. yothersi*	10,70%	11,20%	11,22%	10,51%	11,17%	10,15%	9,52%	0,41%	2,79%
9	outgroup	20,79%	20,95%	19,63%	20,45%	19,73%	25,32%	24,09%	22,11%	n/c

Caption: the gray color indicates the intra-specific distance of each species.

Genetic distances based on ITS2 marker

For the ITS 2 region the average mean divergence over all sequence pairs (including the outgroup taxa) was 12.00 % (SE = 0.01) and ranged from 0.00 % to 79.61 %. The average mean divergence over the *Brevipalpus* spp. sequences was 9.00 % (SE = 0.01) and ranged from 0.00 % to 24.23 %. The results from sequences of the ITS2 region were similar to that observed for sequences of the molecular marker *COI*, that is, the inter-specific distances among *B. papayensis* and *B. feresi* (2.58%); *B. papayensis* and *B. azores* (2.39%) or even among *B. feresi and B. azores* (2.08 %) are still lower than those observed for *B. chilensis* and *B. obovatus* (5.47 %) or between *B. yothersi* and *B. incognitus (*6.09 %), which are closely related species.

Another important fact in this analysis that can be observed in Tables 8 and 9 is that the interspecific distance of *B. papayensis* to *B. feresi* and *B. azores* is lower than intraspecific distance within *B. obovatus* and within *B. chilensis*. When compared *B. azores* and *B. feresi* the genetic distance is still lower, *i.e.*, 2x lower than the intraspecific distance in *B. obovatus* and *B. chilensis* taxa.

Table 8. Estimates of evolutionary divergence over sequence between and within groups- ITS2 region.

		1	2	3	4	5	6	7	8	9	10	11
1	*B. papayensis*	1,77%	1,05%	1,06%	2,19%	2,55%	2,73%	2,63%	3,93%	2,80%	3,14%	13,30%
2	*B. feresi*	2,58%	0,59%	1,04%	2,65%	2,48%	2,82%	2,39%	3,88%	2,72%	2,92%	13,32%
3	*B. azores*	2,39%	2,08%	0,00%	2,78%	2,39%	2,55%	2,64%	3,40%	2,87%	2,93%	13,01%
4	*B. phoenicis*	9,00%	9,04%	10,04%	n/c	2,32%	2,52%	2,12%	4,09%	2,69%	3,00%	13,77%
5	*B. ferraguti*	8,94%	9,07%	8,76%	10,73%	0,00%	2,46%	1,78%	3,13%	3,63%	4,02%	12,53%
6	*B. obovatus*	13,17%	13,42%	13,11%	13,24%	8,36%	4,02%	2,41%	4,07%	4,21%	4,31%	11,88%
7	*B. chilensis*	12,27%	11,92%	12,52%	13,17%	5,47%	11,95%	4,81%	3,42%	3,14%	3,45%	11,24%
8	*B. californicus* ss	19,09%	19,89%	17,95%	21,23%	15,32%	19,10%	19,56%	0,59%	4,43%	4,56%	12,74%
9	*B. incognitus*	20,40%	18,22%	18,56%	16,39%	17,79%	22,17%	19,95%	23,76%	0,00%	1,75%	14,57%
10	*B. yothersi*	19,42%	17,30%	17,63%	15,38%	17,63%	21,25%	20,41%	24,23%	6,09%	1,78%	13,31%
11	outgroup	79,28%	79,61%	77,84%	78,24%	71,71%	72,64%	75,53%	72,54%	77,70%	74,99%	n/c

Caption: the gray color indicates the intra-specific distance of each species.

Table 9. Estimates of evolutionary divergence over sequence between and within groups- ITS2 region (Considering *B. azores* as a population of *B. papayensis*).

		1	2	3	4	5	6	7	8	9
1	*B. papayensis*	2,00%	2,13%	2,57%	2,99%	2,58%	3,63%	4,17%	4,06%	11,24%
2	*B. phoenicis*	9,22%	n/c	2,98%	2,93%	2,90%	3,48%	3,67%	3,49%	11,66%
3	*B. ferraguti*	8,91%	10,73%	0,00%	1,97%	1,31%	3,32%	3,75%	3,89%	11,15%
4	*B. obovatus*	13,18%	13,24%	8,36%	4,02%	2,04%	3,54%	3,77%	3,37%	10,40%
5	*B. chilensis*	12,30%	13,17%	5,47%	11,95%	4,81%	3,66%	3,91%	4,03%	11,47%
6	*B. californicus* ss	18,92%	21,23%	15,32%	19,10%	19,56%	0,59%	4,02%	4,53%	10,01%
7	*B. incognitus*	19,83%	16,39%	17,79%	22,17%	19,95%	23,76%	0,00%	1,80%	11,57%
8	*B. yothersi*	18,87%	15,38%	17,63%	21,25%	20,41%	24,23%	6,09%	1,78%	12,07%
9	outgroup	79,01%	78,24%	71,71%	72,64%	75,53%	72,54%	77,70%	74,99%	n/c

Caption: the gray color indicates the intra-specific distance of each species.

According to scenario 2 (Table 9), *B. papayensis* + *B. feresi* + *B. azores* as a single taxon, the calculated intraspecific distance value of 2.00% was lower than those observed for *B. obovatus* (4.02 %) and *B. chilensis* (4.81%). So, or these three species are closely related or may also suggest that *B. obovatus* and *B. chilensis* keep inside diferent lineages. Also thinking about the scenario 3, about *B. papayensis* lineages, the interspecific values for the distances between *B. papayensis*, *B.* aff. *papayensis* sp.1, *B.* aff. *papayensis* sp.2,

B. feresi and *B. azores* were from 1.72 % to 3.91 % as well as the intraspecific were lower than 1.5% (Table values not showed).

The ITS2 sequence divergence between some species of the *Tetranychus* group ranged from 1.0 (*T. turkestani* and *T. puearicola*) to 2.2 % (*T. urticae* and *T. truncatus*), and intraspecifically from 0,1 (*T. urticae*) to 0.3 % (*T. turkestani*). The ITS2 sequence divergence for closely related species in the *Tetranychus* genus usually present low values, as 1.4% (*T. urticae* and *T. turkestani*) (Navajas et al. 1992), 1.3% (*T. urticae* and *T. kanzawai*) and 1.5% (*T. mcdanieli* McGregor, 1931 and *T. pacificus* McGregor, 1919) (Navajas et al. 1998). Ben-David et al. (2007) used ITS2 sequences as a barcoding region and effectively discriminated 16 species (9 genera) of Tetranychidae from Israel. The authors established a 2% threshold for species diagnosis and 14 among 16 species recognized by morphological criteria were accurately identified.

Considering the lowest distance value found equal to 2.08% (*B. azores* and B. *feresi*) (Table 8) as the limit to separate species based on ITS 2 sequences for *Brevipalpus* species, then *B. feresi* and *B. azores* could be confirmed as valid names as well as could indicate the presence of cryptic species in *B. papayensis* (*B.* aff. *papayensis* sp.1 *and B.* aff. *papayensis* sp.2), *B. obovatus* and *B. chilensis*.

Genetic distances based on 28S marker

The subunit D1-D3 average mean divergence over all sequence pairs (including the outgroup taxa) was 5.00% % (SE = 0.0 %) and ranged from 0.00 % to 29.25 %. The average mean divergence over the *Brevipalpus* spp. sequences was 4.00 % (SE = 0.00 %) and ranged from 0.00 % to 8.99%.

The genetic distances of the 28S nuclear region were the ones that presented the best resolutions in comparison with the other markers analyzed in this study. The data clearly indicate the distinction between the three closely related species- *B. azores*, *B. feresi* and *B. papayensis*.

When we consider *B. azores* and *B. feresi* belonging to *B. papayensis*, their intraspecific distance was superior to the interspecific distance between *B. yothersi* and *B. incognitus*> In addition, it is also much higher to all the intraspecific distance obtained in this study (for region 28S), even when compared to *B. obovatus*, whose dataset contains sequences of populations from different host/regions around the world. The genetic distance between *B. papayensis* and *B. feresi* is close to the divergence between *B.*

obovatus and *B. chilensis*. Furthermore, *B. azores*, *B. feresi* and *B. papayensis* present greater distances than those found between *B. incognitus* and *B. yothersi*, indicating the segregation of these species for this molecular marker.

The D1-D3 subunit (28S) (rRNA) has recently been used as molecular marker for phylogenetic studies of phytophagous mites (Dowling et al. 2012). So, comparative distance values for this region are scarce in the literature. However, the D2 subunit (28S) (rRNA) is widely used for Eriophyidae mites (Skirakaca et al. 2009, Skoracka et al. 2012) and distance values around 2.0% discriminate close species such as *Aceria eximia* Sukhareva and *Aceria tosichella* Keifer (Skoracka et al., 2012).

The D1-D3 genetic distance between *B. chilensis* and *B. obovatus* was 1.87 %; between *B. yothersi* and *B. incognitus* = 1.10 %; and between *B. phoenicis* and *B. azores* =1.10 %. Thus, it is possible to infer about the validity of the species *B. feresi*, *B. azores* and *B. papayensis* since distances between them were similar or larger than 1.10%.

Table 10. Estimates of evolutionary divergence over sequence between and within groups- 28S gene.

		1	2	3	4	5	6	7	8	9	10	11
1	*B. papayensis*	0,18%	0,51%	0,45%	0,55%	0,79%	0,69%	0,81%	1,26%	1,28%	1,29%	2,60%
2	*B. feresi*	1,57%	n/c	0,48%	0,59%	0,84%	0,72%	0,80%	1,24%	1,30%	1,27%	2,66%
3	*B. azores*	1,38%	1,29%	n/c	0,44%	0,77%	0,68%	0,78%	1,21%	1,22%	1,23%	2,62%
4	*B. phoenicis*	1,75%	2,04%	1,10%	n/c	0,83%	0,77%	0,81%	1,35%	1,30%	1,28%	2,73%
5	*B. ferraguti*	3,42%	3,70%	3,12%	3,65%	0,14%	0,60%	0,69%	1,18%	1,24%	1,26%	2,48%
6	*B. obovatus*	2,92%	3,13%	2,82%	3,51%	2,21%	0,18%	0,53%	1,18%	1,28%	1,30%	2,47%
7	*B. chilensis*	3,52%	3,42%	3,42%	3,80%	2,96%	1,83%	0,29%	1,20%	1,30%	1,31%	2,53%
8	*B. californicus* ss	8,02%	7,51%	7,30%	8,54%	7,23%	7,14%	7,44%	0,00%	0,99%	1,02%	2,35%
9	*B. incognitus*	8,55%	8,67%	7,83%	8,68%	8,36%	8,48%	8,79%	5,00%	0,18%	0,42%	2,39%
10	*B. yothersi*	8,75%	8,45%	8,03%	8,67%	8,56%	8,68%	8,99%	5,19%	1,10%	0,18%	2,47%
11	outgroup	28,60%	29,00%	28,42%	29,25%	26,41%	26,98%	27,73%	25,06%	26,05%	26,50%	n/c

Caption: the gray shadow indicates the intra-specific distance of each species.

Table 11. Estimates of evolutionary divergence over sequence between and within groups- 28S gene (Considering *B. azores* as a population of *B. papayensis*).

		1	2	3	4	5	6	7	8	9
1	*B. papayensis*	1,23%	0,45%	0,74%	0,65%	0,74%	1,20%	1,25%	1,25%	2,63%
2	*B. phoenicis*	1,66%	n/c	0,83%	0,78%	0,81%	1,34%	1,32%	1,30%	2,77%
3	*B. ferraguti*	3,41%	3,65%	0,14%	0,61%	0,70%	1,17%	1,25%	1,26%	2,52%
4	*B. obovatus*	2,94%	3,51%	2,21%	0,18%	0,54%	1,20%	1,30%	1,31%	2,52%
5	*B. chilensis*	3,47%	3,80%	2,96%	1,83%	0,29%	1,20%	1,31%	1,32%	2,57%
6	*B. californicus* ss	7,71%	8,54%	7,23%	7,14%	7,44%	0,00%	0,96%	0,99%	2,29%
7	*B. incognitus*	8,40%	8,68%	8,36%	8,48%	8,79%	5,00%	0,18%	0,41%	2,45%
8	*B. yothersi*	8,50%	8,67%	8,56%	8,68%	8,99%	5,19%	1,10%	0,18%	2,50%
9	outgroup	28,65%	29,25%	26,41%	26,98%	27,73%	25,06%	26,05%	26,50%	n/c

Caption: the gray shadow indicates the intra-specific distance of each species.

Brevipalpus species groups- morphological-based classification & phylogenetic groups

Results showed that the current *Brevipalpus* species group classification by Baker & Tutle (1987) do not correspond to the phylogenetic groups (Clades I, II, III) observed in this study. Based exclusively on morphological traits, according to Baker & Tutle (1987) and Beard et al. (2015), five studied species- *B. azores*, *B. feresi*, *B. ferraguti*, *B. papayensis*, and *B. phoenicis*- belong to the *B. phoenicis* species group or "species complex" respectively together with *B. yothersi*. However, phylogenetic analysis showed that *B. yothersi* is not closely related with these five species, just with *B. incognitus*, an uncovered cryptic species also classified as belonging to *B. phoenicis* species group (Navia et al. 2013). These five species were clustered with *B. chilensis* and *B. obovatus*. From these results we assumed that the "*B. obovatus* phylogenetic group" is composed by *B. azores*, *B. chilensis*, *B. feresi*, *B. ferraguti*, *B. obovatus*, *B. papayensis* and *B. phoenicis.* Other species included in this study would represent the "*B. yothersi* phylogenetic group" (*B. yothersi* and *B. incognitus*) and "*B. californicus* phylogenetic group" (*B. californicus*).

Genetic distances between taxa in Clade II (*B. azores*, *B. chilensis*, *B. feresi*, *B. ferraguti*, *B. obovatus*, *B. papayensis* and *B. phoenicis*) and between these and those in Clade III (*B. incognitus* and *B. yothersi*) also strongly supported the close evolutionary relationship inside these phylogenetic groups and distant among them (Tables 6-12).

We would like to highlight that due to the new placement of the taxon *B. phoenicis* in the *B. obovatus* phylogenetic group it would not be appropriate retain its specific designation (*phoenicis*) as the name of the "phylogenetic group". Therefore we suggest to name it "*B. yothersi* phylogenetic group", the specific designation of the former species to be described.

Actually, a disagreement between the composition of the morphological-based *Brevipalpus* classification by Baker & Tuttle (1987) and the groups based on phylogenetic analyses herein observed is not surprisingly and a previous study has already highlighted it (Navia et al. 2013). Baker & Tuttle (1987) classification was basically artificial since the value of morphological traits as phylogenetically informative characters have never been evaluated, neither by morphology or molecular traits.

The *B. obovatus* species group is currently one of the less numerous in the *Brevipalpus* genus, being composed by 16 species (Baker & Tuttle, 1987). In a way to

evaluating if these species are also evolutionarily closely related it would be important obtaining biological material for further morphological and molecular studies.

B. obovatus phylogenetic group- morphological concept

The *B. obovatus* species group (sensu Baker & Tuttle 1987) has been defined by the following morphological traits- dorsal opisthosomal seta *f2* absent; tarsus II with 1 solenidion; dorsal central setae (*c1*, *d1*, *e1*) different shape to dorsal lateral setae (*c3*, *d3*, *e3*); palp 4-segmented with 3 distal setae (Baker & Tuttle, 1987). Some of these characteres have showed not to be phylogenetically informative since not all the species clustered in this study in the *B. obovatus* phylogenetic group share the traits listed above (Table 12). For instance, number of solenidion on tarsus II and differentiation between dorso central and dorso lateral opisthosomal setae have been used to distinguishing species groups however it is a variable trait between species in the phylogenetic cluster showing that should no more be used for defining *Brevipalpus* groups. Morphological traits which are shared between species that constitute the *B. obovatus* species group are: opisthosomal seta *f2* absent; if developed spermathecal vesicle rounded, covered in finger-like projections or in tuberosities. These traits should be used to defining the *B. obovatus* phylogenetic group.

Table 12. Main morphological traits of species composing the *B. obovatus* phylogenetic group.

Species/ Traits	opisthosomal seta *f2*	No. solenidion on tarsus II	Differentiation between dorso central and dorso lateral opisthosomal setae	General shape of spermatheca vesicle	Shape/extension of palp femur seta	Microplates	Ventral plate
B. azores	absent	2	no	sclerotized spherical vesicle with a crown of minute proejctions	barbed, broadly setiform dorsal seta	Separate individual, rounded to irregularly rounded plates, with irregular multidirectional ridges on dorsal surface; no series of parallel ridges present	with small to medium circular cells, some cells fused to form transversely elongate cells
B. chilensis	absent	1	yes	vesicle is generally quite visible, and is often located near coxae IV	seta thin, tapered, barbed.		with small to medium circular cells, some cells fused to form transversely elongate cells
B. feresi	absent	2	no	small rounded vesicle with a weakly formed crown of short projections	barbed, broad flat dorsal seta	Separate individual, rounded to irregularly rounded plates, with multiple short irregular ridges on dorsal surface aligned in apparently random directions; no series of parallel ridges present	with small to medium circular cells, some cells fused to form transversely elongate cells
B. ferraguti	absent	2	no	A long narrow duct, ending in an elongate membranous bulb	barbed, broad flat dorsal seta	Sublateral: anterior cuticle reticulate with regular cells becoming longitudinally elonagate towards posterior	cuticle weakly verrucose or weakly reticulate with rounded to transversely elongate cells; central cuticle with some fused "warts" forming a few weak transverse bands

B. obovatus	absent	1	yes	rounded, covered in short finger-like projections, around the entire perimeter	seta varies from a broad flat seta with few barbs, to a thin, tapered, weakly barbed seta	Separate individual plates, rounded to oblong, with multiple series of distinct parallel crests on the dorsal surface not aligned in multiple directions	with small rounded cells, often central cells fusing to form weak transverse bands
B. papayensis	absent	2	no	sclerotized spherical vesicle with a crown of minute proejctions	barbed, broadly setiform dorsal seta	Separate individual, rounded to irregularly rounded plates, with irregular multidirectional ridges on dorsal surface; no series of parallel ridges present	cuticle with bands of mixed orientation; lateral and posterior bands tend to be transverse, central bands tend to be oblique
B. phoenicis	absent	2	no	A moderately thick convoluted duct terminates distally in a membranous bulb	barbed, broad flat dorsal seta	Separate individual, rounded to irregularly rounded plates, with irregular multidirectional ridges on dorsal surface; no series of parallel ridges present	cuticle weakly verrucose; "warts" are fused to form transverse bands; no separately formed "warts" centrally; often with some separately formed "warts" laterally

Cryptic diversity in the *B. obovatus* phylogenetic group

COI and ITS phylogenies as well as genetic distances suggested that populations morphologically identified as *B. papayensis* constituted at least three phylogenetic lineages instead of only one. Although detailed morphological observations have been conducted in this study, including SEM and DIC optical microscopy, no remarkable differences were observed between populations that constitute the three lineages. Fine variations observed in ornamentation could be attributed to intraspecific variability. Taking into account the cryptic diversity revealed by the molecular phylogeny in this study, would be important conducting further morphological studies including a higher number of specimens in an attempt to finding morphological traits that would allow to differentiate the phylogenetic lineages. It is important to note that speciation is not always followed by changes in external morphological traits. It is also possible that tools currently employed for the morphological studies are not yet enough refined to visualizing such differences.

Aiming to defining what of the *B. papayensis* genetic lineages correspond to that described by Baker in 1949, from papaya (*Carica papaya*) from Hawaii would be necessary to collecting biological material from the type locality and obtaining DNA sequences of the analyzed molecular markers to comparison with populations herein studied. Other lineages should be formally described as new taxa based on molecular traits as well as on morphological ones if differences are detected.

The B. phoenicis species complex in the B. obovatus phylogenetic species group

Brevipalpus species in Clade II- *B. obovatus* phylogenetic species group- subclade including *B. azores*, *B. feresi*, *B. papayensis*, *B. papayensis* sp.1, sp.2, and *B. phoenicis*, are morphologically and genetically closely related composing a species complex. The earliest species described in this species complex is *B. phoenicis* by Geijskes in 1939. Due to it this species complex is herein named as *B. phoenicis* species complex based on morphological and phylogenetic data.

Relationship between taxa in this species complex is quite different among phylogenies showing that evolutionary relationship is not well-resolved. This unsolved phylogeny associated with morphological similarity between species suggest that the group is in a recent radiation process.

References

Baker EW, Tuttle DM (1987) The False Spider Mites of Mexico (Tenuipalpidae: Acari). U.S.Department of Agriculture, Technical Bulletin, 1706: 237.

Baker EW (1949) The genus *Brevipalpus* (Acarina: Pseudoleptidae). The American Midland Naturalist, 42(2): 350–402.

Beard JJ, Ochoa R, Braswell WE, Bauchan GR (2015*) Brevipalpus phoenicis* (Geijskes) species complex (Acari:Tenuipalpidae)- a closer look . Zootaxa 3944 (1):001-067.

Ben-David, T, Melamed, S, Gerson, U. & Morin, S. (2007) ITS2 sequences as barcodes for identifying and analyzing spider mites (Acari: Tetranychidae). Experimental and Applied Acarology, 41,169-181.

Childers CC, Rodrigues JCV (2011) An overview of *Brevipalpus* mites (Acari: Tenuipalpidae) and the plant viruses they transmit. *Zoosymposia* 6: 180–192

Dabert J, Ehrnsberger R, and Dabert M (2008) *Glaucalges tytonis* sp. n. (Analgoidea, Xolalgidae) from the barn owl *Tyto alba* (Strigiformes, Tytonidae): compiling morphology with DNA barcode data for taxon descriptions in mites (Acari). Zootaxa, 1719: 41–52.

Dabert M, Witalinski W, Kazmierski A, Olszanowski Z, and Dabert J. (2010) Molecular phylogeny of acariform mites (Acari, Arachnida): strong conflict between phylogenetic signal and long-branch attraction artifacts. Molecular Phylogenetics and Evolution, 56: 222–241.

Darriba D, Taboada GL, Doallo R, Posada D (2012) jModelTest 2: more models, new heuristics and parallel computing. Nature methods, 9(8): 772.

Dowling APG, Bauchan GR, Ochoa R, Beard JJ (2010) Scanning electron microscopy vouchers and genomic data from an individual specimen: maximizing the utility of delicate and rare. Acarologia, 50(4): 479–485

Edgar RC (2004) MUSCLE: a multiple sequence alignment method with reduced time and space complexity. BMC Bioinformatics, 20045:113

Geijskes DC (1939) Beitäge zur kenntnis der europäischen spinnmilben (Acari, Tetranychidae), mit bensonderer berücktigung der Niederländischen arten. Mededeelingen van de Landbouwboogeschool. Wageningen, 42: 1–68

Groot, TVM. (2006) The effect of symbiont induced haploid thelytoky on the evolution of *Brevipalpus* mites. PhD dissertation, Institute of Biodiversity and Ecosystem Dynamics. University of Amsterdam, Amsterdam, the Netherlands, 154p.

Guidon S, Dufayard JF, Lefort V, Anisimova M, Hordijk W, Gascuel O (2010) New algorithms and methods to estimate maximum-likelihood phylogenies: Assessing the Performance of PhyML 3.0. Systematic Biology, 59(3): 307-321.

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

Hasegawa M, Kishino K , Yano T (1985) Dating the human-ape splitting by a molecular clock of mitochondrial DNA. Journal of Molecular Evolution, 22: 160 –174

Hinchliff CE, Smith SA, Allman JF, Burleigh JG, Chaudhary R, Coghill LM *et al*. (2015) Synthesis of phylogeny and taxonomy into a comprehensive tree of life. Proceedings of the National Academy of Sciences, 112(41): 12764–12769.

Kimura M, (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. Journal of Molecular Evolution, 16(2), 111–120.

Kitajima EW, Rodrigues J CV, Freitas-Astua J (2010) An annotated list of ornamentals naturally found infected by *Brevipalpus* mite-transmitted viruses. Sci. agric. (Piracicaba, Braz), 67 (3): 348-371.

Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms, Molecular Biology and Evolution, 35 (6), 1547–1549. https://doi.org/10.1093/molbev/msy096

Maddison WP (1996) *Pelegrina Franganillo* and other jumping spiders formerly placed in the genus *Metaphidippus* (Araneae: Salticidae). Bulletin of the Museum of Comparative Zoology 154: 215-368.

Mendonca RS, Navia D, Diniz, IR, 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 and Applied Acarology, 55(1): 1-23.

Mesa NC, Ochoa R, Welbourn WC, Evans AG, Gilberto JM. (2009) A catalog of the Tenuipalpidae (Acari) of the World with a key to genera. Zootaxa 2098: 1-185

Oliveira ICS (2014) Sistemática molecular para ácaros planos do gênero *Brevipalpus* Donnadieu (Acari:Tenuipalpidae) – utilização de marcadores moleculares mitocondriais e nucleares. 120p. Dissertação (Mestrado em Zoologia) – Instituto de Ciências Biológicas, Universidade de Brasília, Brasília.

Navajas, M., Cotton, D., Kreiter. S. & Gutierrez, J. (1992) Molecular approach in spider mites (Acari: Tetranychidae): preliminary data on ribosomal DNA sequences. Experimental and Applied Acarology, 15, 211-218.

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.

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

Rodrigues JCV, Gallo-Meagher M, Ochoa R, Childers CC and Adams BJ (2004) Mitochondrial DNA and RAPD polymorphisms in the haploid mite *Brevipalpus phoenicis* (Acari: Tenuipalpidae). Experimental and Applied Acarology 34: 275–290.

Rodríguez, F., J. F. Oliver, A. MarÍn, and J. R. Medina (1990) The general stochastic model of nucleotide substitution. Journal of Theoretical Biology, 142: 485 –501

Ronquist F, Teslenko M, Van der Mark P, Ayres D L, Darling A , Höhna S, Larget, B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic biology, 61(3), 539-42.

Rozas J, Ferrer-Mata A, Sánchez JC, DelBarrio Guirao-Rico S, Librado P, Ramos-Onsins SE, Sánchez-Gracia A (2017) DnaSP 6: DNA sequence polymorphism analysis of large data sets. Molecular Biology and Evolution, 34(12): 3299-3302.

Salinas-Vargas D, Santillán-Galicia MT, Guzmán-Franco AW, Hernández-López A, Ortegaarenas LD, Mora-Aguilera G (2016) Analysis of the genetic variation in *Brevipalpus yothersi* (Acari: Tenuipalpidae) populations from four species of citrus host plants. PLoS One, 11(10).

Sánchez-Velázquez EJ, Santillán-Galicia MT, Novelli VM, Nunes MA, Mora-Aguilera G, Valdez-Carrasco JM, Otero-Colina G, Freitas-Astua J (2015) Diversity and genetic variation among *Brevipalpus* populations from Brazil and Mexico. PloS One, 10 (7).

Santos VV, Tixier M-S (2016) Which molecular markers for assessing which taxonomic level? The case study of the mite family Phytoseiidae (Acari: Mesostigmata). Cladistics 33:251–267.

SkoracckaA. , Lechosław K, Mendonça RS, Mirosława DabertD, Wiktoria SzydłoA, Knihinicki D, Truol G and Navia D (2012) Cryptic species within the wheat curl mite *Aceria tosichella* (Keifer) (Acari : Eriophyoidea), revealed by mitochondrial, nuclear and morphometric data. Invertebrate Systematics, 26: 417–433.

Staden R, Beal KF, Bonfield JK (1998) The Staden Package, (1998) Computer Methods in Molecular Biology. In: Misener, S. & Krawetz, S. A. (Eds). Bioinformatics Methods and Protocols, 132. Totowa: Humana Press Inc., 115-130.

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 (3): 512–526.

Tamura K. (1992). Estimation of the number of nucleotide substitutions when there are strong transition-transversion and G+C content biases. Molecular Biology and Evolution, 9(4): 678–687.

Tavaré S (1986) Some probabilistic and statistical problems in the analysis of DNA sequences. Lectures on Mathematics in the Life Sciences (American Mathematical Society), 17: 57–86.

Tixier M-S, dos Santos VV, Douin M, Duso C, Kreiter S (2017) Great molecular variation within the species *Phytoseius finitimus* (Acari: Phytoseiidae): implications for diagnosis decision within the mite family Phytoseiidae. Acarologia, 57:493–515.

Welbourn WC, Ochoa R, Kane EC and Erbe EF (2003) Morphological observations on *Brevipalpus phoenicis* (Acari: Tenuipalpidae) including comparisons with *B. californicus* and *B. obovatus*. Experimental and Applied Acarology, 30(1/3): 107–133.

Supplementary Material 1. List of *Brevipalpus* species *COI* mtDNA sequences retrieved fromGenbank to construct the dataset. Information on taxon name, accession numbers, sample localities, host plant, a and the original bibliographic references are given.

Taxon ID DNF-DNR	Accession nr	Country	Locality	Host
Brevipalpus yothersi	MH204711	Brazil	BR, Alagoas, Arapiraca	*Ipomoea batatas*
Brevipalpus yothersi	MH204706	Brazil	BR, Alagoas, Arapiraca	*Ipomoea batatas*
Brevipalpus yothersi	* Genome			
Brevipalpus incognitus	MH204723	Brazil	BR, Sao Paulo, Piracicaba	*Phoenix* sp.
Brevipalpus incognitus	MH204759	Brazil	BR, Minas Gerais, Janauba	*Cocos nucifera*
Brevipalpus incognitus	ǂ KC291390			
Brevipalpus californicus s.s	MH204770	Spain	SP, Valencia	*Pittosporum tobira*
Brevipalpus californicus s.s	MH204771	Spain	SP, Valencia	*Pittosporum tobira*
B. papayensis	† KF954956			
B. papayensis	ǂ KC291389			
B. papayensis	† KF954950			
B. papayensis	† KF954952			
B. papayensis	† KF954957			
B. papayensis	† KF954951			
B. papayensis	† KF954953			
B. papayensis	KC291387			
B. papayensis	KC291388			
Brevipalpus ferraguti	MH204726	Spain	SP, Valencia	*Tecomaria capensis*
Brevipalpus ferraguti	MH204760	Spain	SP, Valencia	*Myoporum laetum*
Brevipalpus ferraguti	MH204761	Spain	SP, Valencia	*Myoporum laetum*
Brevipalpus ferraguti	MH204762	Spain	SP, Valencia	*Myoporum laetum*
Brevipalpus ferraguti	MH204763	Spain	SP, Valencia	*Myoporum laetum*
Brevipalpus ferraguti	MH204764	Spain	SP, Valencia	*Myoporum laetum*
Brevipalpus ferraguti	MH204765	Spain	SP, Valencia	*Myoporum laetum*
Brevipalpus ferraguti	MH204766	Spain	SP, Valencia	*Myoporum laetum*
Brevipalpus ferraguti	MH204774	Spain	SP, Valencia	*Pittosporum tobira*
Brevipalpus ferraguti	MH204772	Spain	SP, Valencia	*Pittosporum tobira*
Brevipalpus ferraguti	MH204775	Spain	SP, Valencia	*Pittosporum tobira*
Brevipalpus ferraguti	MH204780	Spain	SP, Valencia	*Tecomaria capensis*
Brevipalpus ferraguti	MH204781	Spain	SP, Valencia	*Tecomaria capensis*
Brevipalpus ferraguti	MH204782	Spain	SP, Valencia	*Tecomaria capensis*
Brevipalpus obovatus	MH204703	Brazil	BR, Sao Paulo, Piracicaba	*Solanum violaefolium*
Brevipalpus obovatus	MH204704	Brazil	BR, Sao Paulo, Piracicaba	*Solanum violaefolium*
Brevipalpus obovatus	MH204705	Brazil	BR, Sao Paulo, Piracicaba	*Solanum violaefolium*
Brevipalpus obovatus	MH204740	Spain	SP, Valencia	*Coniza bonariensis*
Brevipalpus obovatus	MH204741	Spain	SP, Valencia	*Coniza bonariensis*
Brevipalpus obovatus	MH204742	Spain	SP, Valencia	*Coniza bonariensis*
Brevipalpus obovatus	MH204749	Spain	SP, Valencia	*Helichrysum stoechas*
Brevipalpus obovatus	MH204750	Spain	SP, Valencia	*Helichrysum stoechas*
Brevipalpus obovatus	ǂ KC291384			
Brevipalpus obovatus	ǂ KC291385			
Brevipalpus obovatus	ǂ KC291386			
Brevipalpus obovatus	ǂ KC291383			
Brevipalpus chilensis	MH204699	Chile	CH, Curacavi, Santiago	*Ligustrum sinensis*
Brevipalpus chilensis	MH204700	Chile	CH, Curacavi, Santiago	*Ligustrum sinensis*

Brevipalpus chilensis	MH204701	Chile	CH, Curacavi, Santiago	*Ligustrum sinensis*
Brevipalpus chilensis	MH204702	Chile	CH, Curacavi, Santiago	*Ligustrum sinensis*
Brevipalpus chilensis	MH204776	Chile	CH, Parque Fray Jorge, Limari	*Ribes punctatum*
Brevipalpus chilensis	MH204778	Chile	CH, Parque Fray Jorge, Limari	*Ribes punctatum*
Brevipalpus chilensis	MH204779	Chile	CH, Parque Fray Jorge, Limari	*Ribes punctatum*
Brevipalpus chilensis	ǂ KC291401			
Brevipalpus chilensis	ǂ KC191391			
Brevipalpus chilensis	ǂ KC291397			
Brevipalpus chilensis	ǂ KC291393			
Brevipalpus chilensis	ǂ KC291394			
Brevipalpus chilensis	ǂ KC291395			
Brevipalpus chilensis	ǂ KC291396			
Brevipalpus chilensis	ǂ KC291392			
Brevipalpus chilensis	ǂ KC291398			
Brevipalpus chilensis	ǂ KC291399			
Brevipalpus chilensis	ǂ KC291400			

ǂNavia *et al*. (2013); Oliveira (2014); †Sanchez-Velazquez *et al*. (2015); *****Navia *et al*. (2018)

Supplementary Material 2. List of *Brevipalpus* species ITS 2 sequences retrieved from Genbank to construct the dataset. Information on taxon name, accession numbers, sample localities, host plant, and the original bibliographic references are given.

Taxon ID - ITS2	Accession nr.	Country	Note	Host
Brevipalpus yothersi	MH818143	Brazil	BR, Alagoas, Arapiraca	*Ipomoea batatas*
Brevipalpus yothersi	MH818149	Brazil	BR, Alagoas, Arapiraca	*Ipomoea batatas*
Brevipalpus incognitus	MH818117	Brazil	BR, Minas Gerais, Janauba	*Cocos nucifera*
Brevipalpus incognitus	MH818156	Brazil	BR, Sao Paulo, Piracicaba	*Phoenix sp.*
Brevipalpus californicus s.s.	MH818208	Spain	SP, Elche-Elx	*Citrus limon*
Brevipalpus californicus s.s.	MH818209	Spain	SP, Elche-Elx	*Citrus limon*
Brevipalpus papayensis	MH818180	Brazil	BR, Brasilia	*Ligustrum* sp.
Brevipalpus papayensis	MH818181	Brazil	BR, Brasilia	*Ligustrum* sp.
Brevipalpus papayensis	MH818182	Brazil	BR, Brasilia	*Ligustrum* sp.
Brevipalpus papayensis	MH818183	Brazil	BR, Brasilia	*Ligustrum* sp.
Brevipalpus ferraguti	MH818118	Spain	SP, Valencia	*Myoporum laetum*
Brevipalpus ferraguti	MH818119	Spain	SP, Valencia	*Myoporum laetum*
Brevipalpus ferraguti	MH818120	Spain	SP, Valencia	*Myoporum laetum*
Brevipalpus ferraguti	MH818121	Spain	SP, Valencia	*Myoporum laetum*
Brevipalpus ferraguti	MH818122	Spain	SP, Valencia	*Myoporum laetum*
Brevipalpus ferraguti	MH818123	Spain	SP, Valencia	*Pittosporum tobira*
Brevipalpus ferraguti	MH818124	Spain	SP, Valencia	*Pittosporum tobira*
Brevipalpus ferraguti	MH818125	Spain	SP, Valencia	*Pittosporum tobira*
Brevipalpus ferraguti	MH818126	Spain	SP, Valencia	*Pittosporum tobira*
Brevipalpus ferraguti	MH818128	Spain	SP, Valencia	*Tecomaria capensis*
Brevipalpus ferraguti	MH818129	Spain	SP, Valencia	*Tecomaria capensis*
Brevipalpus ferraguti	MH818130	Spain	SP, Valencia	*Tecomaria capensis*
Brevipalpus ferraguti	MH818191	Spain	SP, Valencia	*Tecomaria capensis*
Brevipalpus *obovatus*	MH818177	Brazil	BR, Brasilia	*Ligustrum* sp.
Brevipalpus obovatus	MH818178	Brazil	BR, Brasilia	*Ligustrum* sp.
Brevipalpus obovatus	MH818179	Brazil	BR, Brasilia	*Ligustrum* sp.
Brevipalpus obovatus	MH818196	Spain	SP, Valencia	*Helichrysum stoechas*
Brevipalpus obovatus	MH818197	Spain	SP, Valencia	*Helichrysum stoechas*
Brevipalpus obovatus	MH818198	Spain	SP, Valencia	*Coniza bonariensis*
Brevipalpus obovatus	MH818199	Spain	SP, Valencia	*Coniza bonariensis*
Brevipalpus obovatus	MH818200	Spain	SP, Valencia	*Coniza bonariensis*
Brevipalpus obovatus	MH818201	Spain	SP, Valencia	*Coniza bonariensis*
Brevipalpus obovatus	MH818202	Brazil	BR, Pernambuco, Recife	*Hibiscus rosa-sinensis*
Brevipalpus chilensis	MH818127	Chile	CH, Parque Fray Jorge, Limari	*Ribes punctatum*
Brevipalpus chilensis	MH818139	Chile	CH, Curacavi, Santiago	*Ligustrum sinensis*
Brevipalpus chilensis	MH818140	Chile	CH, Curacavi, Santiago	*Ligustrum sinensis*
Brevipalpus chilensis	MH818141	Chile	CH, Curacavi, Santiago	*Ligustrum sinensis*
Brevipalpus chilensis	MH818142	Chile	CH, Curacavi, Santiago	*Ligustrum sinensis*
Brevipalpus chilensis	MH818205	Chile	CH, Parque Fray Jorge, Limari	*Ribes punctatum*
Brevipalpus chilensis	MH818206	Chile	CH, Cachapoal, San Francisco de Mostazal	*Magnolia grandiflora*

Oliveira (2014)

Supplementary Material 3. List of *Brevipalpus* species D1-D3 (28s) rRNA sequences retrieved from Genbank to construct the dataset. Information on taxon name, accession numbers, sample localities, host plant and the original bibliographic references are given.

Taxon ID - D1-D3	**Accession nr**	**Country**	**Locality**	***Host***
Brevipalpus yothersi	MK293672	Brazil	BR, Alagoas, Arapiraca	*Ipomoea batatas*
Brevipalpus yothersi	MK293678	Brazil	BR, Alagoas, Arapiraca	*Ipomoea batatas*
Brevipalpus incognitus	MK293687	Brazil	BR, Sao Paulo, Piracicaba	*Phoenix sp.*
Brevipalpus incognitus	MK293640	Brazil	BR, Minas Gerais, Janauba	*Cocos nucifera*
Brevipalpus californicus s.s	MK293642	Spain	SP, Valencia	*Pittosporum tobira*
Brevipalpus californicus s.s	MK293656	Spain	SP, Valencia	*Pittosporum tobira*
Brevipalpus ferraguti	MK293654	Spain	SP, Valencia	*Myoporum laetum*
Brevipalpus ferraguti	MK293655	Spain	SP, Valencia	*Myoporum laetum*
Brevipalpus ferraguti	MK293657	Spain	SP, Valencia	*Pittosporum tobira*
Brevipalpus ferraguti	MK293658	Spain	SP, Valencia	*Pittosporum tobira*
Brevipalpus ferraguti	MK293659	Spain	SP, Valencia	*Tecomaria capensis*
Brevipalpus ferraguti	MK293660	Spain	SP, Valencia	*Tecomaria capensis*
Brevipalpus ferraguti	MK293693	Spain	SP, Valencia	*Tecomaria capensis*
Brevipalpus obovatus	MK293694	Spain	SP, Valencia	*Coniza bonariensis*
Brevipalpus obovatus	MK293695	Spain	SP, Valencia	*Coniza bonariensis*
Brevipalpus obovatus	MK293696	Spain	SP, Valencia	*Coniza bonariensis*
Brevipalpus obovatus	MK293697	Spain	SP, Valencia	*Coniza bonariensis*
Brevipalpus obovatus	MK293698	Brazil	BR, Sao Paulo, Piracicaba	*Solanum violaefolium*
Brevipalpus obovatus	MK293699	Brazil	BR, Sao Paulo, Piracicaba	*Solanum violaefolium*
Brevipalpus obovatus	MK293700	Spain	SP, Valencia	*Helichrysum stoechas*
Brevipalpus obovatus	MK293701	Spain	SP, Valencia	*Helichrysum stoechas*
Brevipalpus chilensis	MK293671	Chile	CH, Cachapoal, San Francisco de Mostazal	*Magnolia grandiflora*
Brevipalpus chilensis	MK293643	Chile	CH, Parque Fray Jorge, Limari	*Ribes punctatum*
Brevipalpus chilensis	MK293644	Chile	CH, Parque Fray Jorge, Limari	*Ribes punctatum*
Brevipalpus chilensis	MK293645	Chile	CH, Parque Fray Jorge, Limari	*Ribes punctatum*
Brevipalpus chilensis	MK293646	Chile	CH, Parque Fray Jorge, Limari	*Ribes punctatum*
Brevipalpus chilensis	MK293714	Chile	CH, Parque Fray Jorge, Limari	*Ribes punctatum*
Brevipalpus chilensis	MK293715	Chile	CH, Parque Fray Jorge, Limari	*Ribes punctatum*
Brevipalpus chilensis	MK293716	Chile	CH, Cachapoal, San Francisco de Mostazal	*Magnolia grandiflora*

Oliveira (2014)