Biological Control 72 (2014) 17–29

Contents lists available at ScienceDirect

Biological Control

journal homepage: www.elsevier.com/locate/ybcon

Reproductive compatibility and genetic and morphometric variability among populations of the predatory mite, *Amblyseius largoensis* (Acari: Phytoseiidae), from Indian Ocean Islands and the Americas

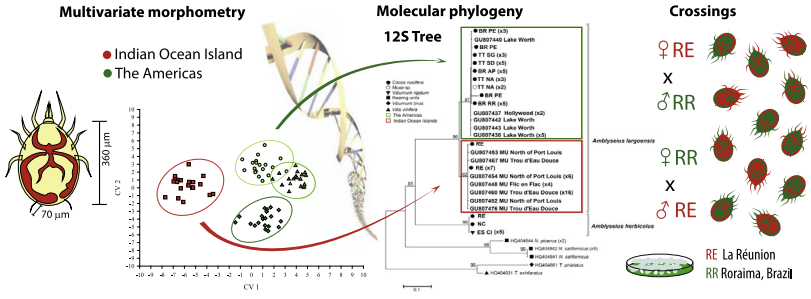
Denise Navia [a,*], Cleiton A. Domingos [b], Renata S. Mendonça [a], Francisco Ferragut [c], Maria Angélica N. Rodrigues [a], Elisângela G.F. de Morais [d], Marie-Stéphane Tixier [e], Manoel G.C. Gondim Jr. [b]

[a] *Embrapa Recursos Genéticos e Biotecnologia, Cx. Postal 02372, 70.770-900 Brasilia, DF, Brazil*
[b] *Universidade Federal Rural de Pernambuco, Departamento de Agronomia, 52171-900 Recife, Brazil*
[c] *Instituto Agroforestal Mediterraneo, Universidad Politécnica de Valencia, Valencia, Spain*
[d] *Embrapa Roraima, Laboratório de Entomologia, 69301-970 Boa Vista, Brazil*
[e] *Montpellier SupAgro, UMR CBGP, INRA/IRD/CIRAD, Campus International de Baillarguet, Montferrier sur Lez cedex, France*

HIGHLIGHTS

- *A. largoensis* from the Americas and La Réunion Islands are morphometrically distinct.
- Most differences among *A. largoensis* studied populations are on the setae lenght.
- *A. largoensis* from the Americas and Indian Ocean Islands consist a taxonomic unity.
- *A. largoensis* from the Americas and Indian Ocean Islands consist two genetic groups.
- Genetic and morphological differences among *A. largoensis* populations can be markers.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 21 June 2013
Accepted 24 January 2014
Available online 3 February 2014

Keywords:
Red palm mite *Raoiella indica*
Classical biological control
Phytoseiid mite
Multivariate morphometry
Molecular systematics
Biosystematics

ABSTRACT

The red palm mite (RPM), *Raoiella indica* Hirst (Acari: Tenuipalpidae), is an invasive phytophagous mite that was recently introduced into The Americas. The predatory mite *Amblyseius largoensis* Muma (Acari: Phytoseiidae) has been the only natural enemy consistently found in association with RPM. This study aimed to determine if *A. largoensis* populations from the Indian Ocean Islands (La Réunion and Mauritius) and the Americas (Brazil, Trinidad and Tobago and the USA) consist a taxonomic unit or a group of cryptic species. First, the morphological variability among the *A. largoensis* populations from these areas was evaluated through morphometric analyses of 36 morphological traits. Then, their genetic variability and phylogenetic relationships were assessed based on two target DNA fragments: the nuclear Internal Transcribed Spacer and the mithochondrial 12S rRNA. Finally, reproductive compatibility of the populations from La Réunion and Roraima, Brazil was evaluated. Morphometric differences between the *A. largoensis* specimens from La Réunion Island and the Americas were observed, most of them on the length of the setae. Molecular analysis indicated that the *A. largoensis* populations from the Indian Ocean Islands and the Americas belong to the same taxonomic entity, although to two well defined genetic groups. Crossings involving the *A. largoensis* populations from La Réunion Island and Roraima, Brazil revealed

* Corresponding author. Fax: +55 61 34484624.
E-mail address: denise.navia@embrapa.br (D. Navia).

http://dx.doi.org/10.1016/j.biocontrol.2014.01.011
1049-9644/

complete reproductive compatibility between these populations. Information on the morphometric and genetic variability among studied *A. largoensis* populations can be further exploited in future studies to follow colonization of Indian Ocean Islands populations in the Americas, in the case of field releases.

1. Introduction

The red palm mite (RPM), *Raoiella indica* Hirst (Acari: Tenuipalpidae), is an invasive phytophagous mite that was recently introduced into the Caribbean (Flechtmann and Etienne, 2004). In only a few years, it was disseminated to North (FDACS, 2007) and South America (Vazquez et al., 2008) and was first reported in Brazil in 2009, in the northern region, state of Roraima (Navia et al., 2011). Red palm mite populations can reach high densities and cause significant damage to their host plants. These populations tremendously expanded their host range in the invaded areas of the Americas, and more than 90 monocot species have already been listed as host plants by Carrillo et al. (2012a), most of which are palm trees of the family Arecaceae. The coconut tree (*Cocos nucifera* L.) is the main host plant for the RPM in the Americas, and significant losses due to high infestations have been reported in the affected areas, e.g., in Trinidad and Tobago and in Venezuela. In Brazil, extensive coconut production areas are expected to be affected by the pest, in addition to other major palm trees and banana trees. Coconuts are widely cultivated along the Brazilian coast and in the irrigated areas of the northeastern region, making this country the fifth largest coconut producer in the world (FAOSTAT, 2011). Furthermore, in addition to its economic value, this crop has great social importance.

Although the RPM has not yet affected these production areas, great attention should be paid to its impact in Brazil, and management strategies must be defined (Navia et al., 2014). Several constraints on the widespread use of chemicals to control the pest exist. Coconut, its main host, is produced mainly by small growers, who cannot afford the continuous use of acaricides. In addition, coconut plants can grow too tall to allow easy chemical application. Thus, alternative methods of controlling this pest should be explored, and biological control is a promising strategy (Carrillo et al., 2012c; Moraes et al., 2012). Efforts have been undertaken to identify and evaluate potential biological control agents of *R. indica* that can be used in Neotropical regions (Hoy, 2012; Taylor et al., 2012).

In the search for natural enemies of RPM in the areas of the eastern hemisphere where it occurs, Moraes et al. (2012) considered La Réunion, an Indian Ocean Island, to be an interesting prospection site because this mite has been found there in low-density populations that do not cause major crop damage. While conducting surveys in the lowlands of this Island in February 2011, the authors determined that the predatory mite *Amblyseius largoensis* Muma (Acari: Phytoseiidae), widely distributed in tropical and subtropical areas around the world, was the only natural enemy of *R. indica* that is consistently found in association with it. This phytoseiid mite has also been found in other areas where RPM is present, e.g., in Asia and Africa (Bowman and Hoy, 2012; Gallego et al., 2003; Taylor et al., 2012; Zannou et al., 2010), as well as in the recently invaded areas of the Americas (Carrillo et al., 2011, 2012b; Peña et al., 2009; Roda et al., 2008), including Roraima State in Brazil (Gondim Jr. et al., 2012). Because geographically limited populations of a predatory mite can exhibit variable abilities to control a target pest, an *A. largoensis* population from La Réunion Island was officially introduced into Brazil for comparison with a Brazilian population from Roraima under laboratory conditions. Domingos et al. (2012) evaluated the development, reproduction and predation of these two populations and found no significant differences between them in relation to the duration of various immature stages or their total viability. However, the oviposition period, prey consumption and net reproductive rate values of La Réunion population were significantly higher than those of the Roraima, Brazil population.

An accurate identification of biological control agents is the first step for a biological control program because imprecise identification may lead to unsuccessful control (Moraes, 1987). Strains or cryptic species have been found among widely distributed phytoseiid mites (e.g. Famah-Sourassou et al., 2010, 2012; Noronha and Moraes, 2004; Tixier et al., 2010). The question of whether *A. largoensis* is truly a single species or a group of cryptic species has been posed (Bowman and Hoy, 2012; Carrillo et al., 2012c). Genetic variability between the *A. largoensis* populations from the Indian Ocean Island, Mauritius, and southern Florida, USA, was evaluated by Bowman and Hoy (2012); however, these authors did not reach a conclusion about the species status of the studied populations, stating that additional investigation was needed. Because such information is crucial for the biological control of the RPM in Brazil and other Neotropical areas, this study aimed to determine if *A. largoensis* populations from Indian Ocean Islands (La Réunion and Mauritius) and the Americas (Brazil, Trinidad and Tobago and the USA) are taxonomically identical. First, the morphological variability among the *A. largoensis* populations from these areas was evaluated through morphometric analyses. Then, their genetic variability and phylogenetic relationships were assessed, based on two target DNA fragments: the nuclear ribosomal region Internal Transcribed Spacer (ITS) and the mitochondrial 12S rRNA marker. Finally, to investigate the reproductive compatibility of the populations from La Réunion and Roraima, Brazil, crossings and backcrossings were conducted. The results obtained represent an essential step in the RPM biological control project in Brazil and provide preliminary data for tracking the colonization of the *A. largoensis* population imported into Brazil from La Réunion that can be used if field releases are implemented.

2. Materials and methods

2.1. Morphometric analyses

Morphometric analyses for four *A. largoensis* populations – two from Brazil, from Roraima and Pernambuco States; one from Trinidad and Tobago; and one from La Réunion Island (Table 1) – were conducted. Approximately 30 specimens of each population were slide-mounted in Hoyeŕs medium, and the best 20 females in dorsoventral position were selected for examination. For each female, the 36 morphological trait parameters (see list of characteristics in Table 2) that are commonly used for phytoseiid mite identification (e.g., Chant and McMurtry, 1994, 2005, 2007) were measured. The measurements were obtained by phase- and differential-contrast microscopy (Nikon Eclipse 80i, Nikon, Tokyo, Japan) at 400× magnification, using an ocular micrometer. All of the measurements are given in micrometers. The setal nomenclature follows that of Lindquist and Evans (1965), as applied to the phytoseiids by Rowell et al. (1978) and Chant and Yoshida-Shaul (1991).

An analysis of variance (PROC ANOVA) followed by Student–Newman–Keuls multiple range comparison tests ($\alpha = 0.05$) was performed to test the significance of the differences between the populations in each of the 36 characteristic morphological traits. Three multivariate statistical analyses were also performed on

Table 1
Characteristics of the populations of *Amblyseius largoensi*, *A. herbicolus*, ingroups and outgroup species studied and their accession numbers in Genbank database for 12S rRNA and ITS rRNA.

Mite taxon	Voucher code	Code	Country, Territory/ County/State	Locality	Date	Host plant		Variant/Haplotype		Genbank accessions	
						scientific name	Family	ITS	12S	ITS	12S
A. largoensis	Al 1	BR PE	Brazil, Pernambuco	Recife	24.vi.2011	*Cocos nucifera*	Arecaceae	V1	H1, H2, H3	**KF219618**-KF219624	KF234094-KF234095 **KF234098**
	Al 3	BR RR	Brazil, Roraima	Boa Vista	25.iv.2011	*Cocos nucifera*	Arecaceae	V1	H2	**KF219625**-KF219630	KF234099-KF234103 **KF234101**
	Al 8	BR AP	Brazil, Amapá	Macapá	29.iv.2011	*Cocos nucifera*	Arecaceae	V1	H2	**KF219645**-KF219648	KF234117-KF234121 **KF234118**
	Al 4	TT SG	Trinidad and Tobago, Trinidad, Saint George	Arima	12.v.2011	*Cocos nucifera*	Arecaceae	V1	H2	**KF21963**1-KF219633	**KF234104**-KF234106
	Al 5	TT NA	Trinidad and Tobago, Trinidad, Nariva	Saint Margaret	10.v.2011	*Cocos nucifera*	Arecaceae	V1	H2	**KF219634**-KF219636	**KF234107**-KF234109
	Al 6	TT NA	Trinidad and Tobago, Trinidad, Nariva	Saint Margaret	10.v.2011	*Musa* sp.	Musaceae	V1	H2	**KF219637**-KF219638	**KF234110**-KF234111
	Al 7	TT SD	Trinidad and Tobago, Trinidad, Saint David	Cumana Bay, Toco	09.v.2011	*Cocos nucifera*	Arecaceae	V1	H2	**KF219639**-KF219644	**KF234112**-KF234116
	Al 2 RE	RE	Reunion		10.iii.2011	*Cocos nucifera*	Arecaceae	V2	H7	**KF219649**-KF219655	**KF234122**-KF234128
	Al 3 RE	RE	Reunion	St Joseph	10.v.2011	*Cocos nucifera*	Arecaceae	n/a	H8	n/a	**KF234129**
	[a]	FL	USA, Florida	Hollywood	[a]	[a]	[a]	–	H2	–	GU807437, GU807446 [a]
	[a]	FL	USA, Florida	Lake Worth	[a]	[a]	[a]	–	H2	–	GU807438, GU807439 [a] GU807441,GU807444, GU807445 [a]
	[a]	FL	USA, Florida	Lake Worth	[a]	[a]	[a]	–	H4	–	GU807440 [a]
	[a]	FL	USA, Florida	Lake Worth	[a]	[a]	[a]	–	H5	–	GU807442 [a]
	[a]	FL	USA, Florida	Lake Worth	[a]	[a]	[a]	–	H6	–	GU807443 [a]
	[a]	MU	Mauritius	Flic em Flac	[a]	[a]	[a]	–	H7	–	GU807448-GU807451 [a]
	[a]	MU	Mauritius	North of Port Louis	[a]	[a]	[a]	–	H7	–	GU807454-GU807459 [a]
	[a]	MU	Mauritius	Trou d'Eau Douce	[a]	[a]	[a]	–	H7	–	GU807460-GU807464 [a] GU807468-GU807475 [a] GU807477-GU807479 [a]
	[a]	MU	Mauritius	North of Port Louis	[a]	[a]	[a]	–	H9	–	GU807452 [a]
	[a]	MU	Mauritius	North of Port Louis	[a]	[a]	[a]	–	H10	–	GU807453 [a]
	[a]	MU	Mauritius	Trou d'Eau Douce	[a]	[a]	[a]	–	H11	–	GU807467 [a]
	[a]	MU	Mauritius	Trou d'Eau Douce	[a]	[a]	[a]	–	H12	–	GU807476 [a]
A. herbicolus	Ah ES	ES CI	Spain, Canary Islands	La Palma, Breña Alta	12.ii.2011	*Viburnum rigidum*	Caprifoliaceae	V3	H13	**KF219656**-KF219660	**KF234130**-KF234134
	Ah RE	RE	France, Reunion	–	17.v.2011	*Cocos nucifera*	Arecaceae	n/a	H13	n/a	KF234135
	Ah NC	NC	France, New Caledonia	–	17.vi.2011	*Cocos nucifera*	Arecaceae	n/a	H13	n/a	KF234136
N. californicus	[b]		Rearing units		[b]	[b]	[b]	V4	H14, H15	HQ404802-HQ404807 [b]	HQ404836-HQ404839 [b] HQ404841-HQ404842 [b]
N. picanus	[b]		Rearing units		[b]	*Citrus* sp.	Rutaceae	V5	H16	HQ404809-HQ404810 [b]	HQ404844-HQ404845 [b]
T. phialatus	[b]	FR	France		[b]	*Viburnum tinus*	Caprifoliaceae	V6	H18	HQ404829 [b]	HQ404861 [b]
T. exhilaratus	[b]	FR	France		[b]	*Vitis vinifera*	Vitaceae	V7	H17	HQ404830 [b]	HQ404831 [b]

Where none is specified, sequences were deposited in Genbank by the present authors. Sequences representing the haplotypes in the phylogeny are in bold.
[a] Bowman and Hoy (2012).
[b] Tsolakis et al. (2012).

Table 2
Mean, standard error and range of 36 morphometric characters measured on females specimens identified as *Amblyseius largoensis* from four geographic origins. (ANOVA, SNK $\alpha = 0.05$).

Morphological characters	La Réunion Island		Recife, Brazil		Roraima, Brazil		Trinidad & Tobago	
	Mean ± SE	Min–Max	Mean ± SE	Min–Max	Mean ± SE	Min–Max	Mean ± SE	Min–Max
Length of dorsal shield	371.1 ± 3.50 **b**	337.5–397.5	362.0 ± 3.61 **b**	320.0–377.5	368.8 ± 3.60 **b**	312.5–387.5	383.0 ± 3.73 **a**	340.0–407.5
Width of dorsal shield	222.5 ± 2.70 **b**	200.0–240.0	225.6 ± 2.08 **b**	212.5–242.5	229.25 ± 1.72 **b**	212.5–242.5	236.3 ± 0.94 **a**	225.0–242.5
Distance St1-St1	71.1 ± 0.53 **a**	67.5–75.0	67.4 ± 0.53 **b**	62.5–70.0	68.5 ± 0.52 **b**	65.0–72.5	68.3 ± 0.63 **b**	62.5–72.5
Distance St2-St2	72.8 ± 0.54 **a**	70.0–77.5	68.8 ± 0.43 **c**	67.5–72.5	70.6 ± 0.44 **b**	67.5–72.5	71.7 ± 0.57 **ab**	67.5–75.0
Distance St3-St3	76.5 ± 0.70 **a**	72.5–85.0	72.4 ± 0.50 **c**	70.0–75.0	74.4 ± 0.75 **b**	67.5–80.0	76.7 ± 0.51 **a**	72.5–80.0
Distance St1-St3	67.4 ± 0.42 **a**	62.5–70.0	67.6 ± 0.38 **a**	65.0–70.0	67.1 ± 0.49 **a**	62.5–70.0	68.0 ± 0.53 **a**	65.0–72.5
Distance St2-St3	30.9 ± 0.38 **b**	30.0–35.0	33.5 ± 0.42 **a**	30.0–37.5	32.1 ± 0.33 **b**	30.0–35.0	32.1 ± 0.44 **b**	30.0–35.0
Distance St4-St4	81.9 ± 0.96 **a**	75.0–87.5	76.2 ± 0.56 **b**	70.0–80.0	81.1 ± 1.41 **a**	72.5–92.5	83.6 ± 0.84 **a**	80.0–90.0
Distance St5-St5	75.1 ± 0.59 **a**	70.0–80.0	70.6 ± 0.57 **b**	67.5–75.0	72.3 ± 0.68 **b**	67.5–77.5	70.5 ± 0.70 **b**	65.0–75.0
Length of ventrianal shield	118.5 ± 1.70 **a**	105.0–130.0	116.4 ± 1.26 **ab**	107.5–125.0	113.0 ± 1.48 **a**	100.0–122.5	120.7 ± 1.50 **a**	112.5–135.0
Width of ventrianal shield (level of ZV2)	47.5 ± 1.01 **a**	40.0–55.0	50.1 ± 0.70 **a**	45.0–55.0	49.3 ± 0.81 **a**	42.5–57.5	47.5 ± 0.81 **a**	40.0–52.5
Width of ventrianal shield (level of anus)	72.9 ± 1.27 **ab**	62.5–82.5	70.6 ± 0.68 **b**	65.0–75.0	75.5 ± 0.53 **a**	72.5–80.0	74.1 ± 0.80 **a**	70.0–80.0
Seta j1	38.9 ± 0.64 **a**	35.0–45.0	36.4 ± 0.61 **b**	32.5–40.0	34.6 ± 0.58 **c**	30.0–40.0	37.6 ± 0.30 **ab**	35.0–40.0
Seta j3	53.9 ± 0.46 **a**	50.0–57.5	53.4 ± 0.52 **a**	50.0–57.5	53.8 ± 0.38 **a**	50.0–57.5	54.7 ± 0.57 **a**	50.0–60.0
Seta j4	6.1 ± 0.29 **b**	5.0–7.5	8.1 ± 0.25 **a**	7.5–10.0	8.0 ± 0.23 **a**	7.5–10.0	6.6 ± 0.28 **b**	5.0–7.5
Seta j5	5.3 ± 0.13 **a**	5.0–7.5	5.75 ± 0.26 **a**	5.0–7.5	5.90 ± 0.27 **a**	5.0–7.5	5.1 ± 0.13 **a**	5.0–7.5
Seta j6	7.0 ± 0.23 **b**	5.0–7.5	9.1 ± 0.38 **a**	7.5–12.5	7.6 ± 0.13 **b**	7.5–10.0	7.8 ± 0.30 **b**	5.0–10.0
Seta J2	9.3 ± 0.26 **c**	7.5–10.0	11.8 ± 0.32 **a**	7.5–12.5	9.5 ± 0.40 **c**	7.5–12.5	10.7 ± 0.30 **b**	10.0–12.5
Seta J5	9.0 ± 0.33 **b**	7.5–12.5	11.0 ± 0.28 **a**	10.0–12.5	9.1 ± 0.27 **b**	7.5–10.0	10.5 ± 0.24 **a**	10.0–12.5
Seta z2	9.9 ± 0.29 **c**	7.5–12.5	12.0 ± 0.23 **a**	10.0–12.5	10.6 ± 0.25 **b**	10.0–12.5	10.9 ± 0.28 **b**	10.0–12.5
Seta z4	8.6 ± 0.29 **b**	7.5–10.0	10.8 ± 0.27 **a**	10.0–12.5	9.0 ± 0.28 **b**	7.5–10.0	9.3 ± 0.30 **b**	7.5–10.0
Seta z5	5.4 ± 0.20 **c**	5.0–7.5	8.5 ± 0.28 **a**	7.5–10.0	7.1 ± 0.20 **b**	5.0–7.5	6.6 ± 0.30 **b**	5.0–7.5
Seta Z1	11.3 ± 0.29 **b**	10.0–12.5	13.4 ± 0.13 **a**	10.0–12.5	10.8 ± 0.26 **b**	10.0–12.5	12.2 ± 0.18 **a**	10.0–12.5
Seta Z4	106.4 ± 0.7 **a**	102.5–112.5	97.8 ± 0.72 **b**	92.5–102.5	94.1 ± 1.25 **c**	87.5–102.5	100.1 ± 0.82 **b**	90.0–105.0
Seta Z5	285.5 ± 2.34 **b**	270.0–307.5	290.8 ± 2.02 **b**	277.5–307.5	283.4 ± 2.90 **b**	262.5–320.0	301.3 ± 1.46 **a**	292.5–315.0
Seta s4	106.6 ± 1.08 **a**	100.0–117.5	96.8 ± 0.83 **c**	90.0–105.0	97.5 ± 0.85 **c**	90.0–102.5	100.70 ± 1.01 **b**	92.5–107.5
Seta S2	12.5 ± 0.18 **c**	10.0–15.0	15.0 ± 0.41 **a**	12.5–17.5	11.90 ± 0.25 **c**	10.0–12.5	13.42 ± 0.28 **b**	12.5–15.0
Seta S4	11.5 ± 0.28 **b**	10.0–12.5	14.1 ± 0.33 **a**	12.5–17.5	11.13 ± 0.29 **b**	10.0–12.5	13.6 ± 0.30 **a**	12.5–15.0
Seta S5	10.0 ± 0.31 **c**	7.5–12.5	13.1 ± 0.36 **a**	10.0–15.0	11.4 ± 0.29 **b**	10.0–12.5	11.6 ± 0.30 **b**	10.0–12.5
Seta r3	12.5 ± 0.18 **b**	10.0–15.0	14.1 ± 0.33 **a**	12.5–17.5	12.3 ± 0.40 **b**	10.0–15.0	13.4 ± 0.28 **a**	12.5–15.0
Seta R1	11.38 ± 0.29 **bc**	10.0–12.5	13.1 ± 0.25 **a**	12.5–15.0	10.6 ± 0.25 **c**	10.0–12.5	12.1 ± 0.35 **b**	10.0–15.0
Seta JV5	78.0 ± 0.88 **b**	70.0–87.5	69.8 ± 0.91 **a**	62.5–77.5	63.1 ± 0.80 **c**	57.5–67.5	72.2 ± 0.90 **d**	65.0–77.5
Calyx of spermatheca	32.1 ± 0.50 **a**	27.5–35.0	32.8 ± 0.68 **a**	30.0–37.5	33.6 ± 0.60 **a**	30.0–37.5	30.53 ± 0.45 **b**	27.5–35.0
Macroseta genu IV	132.3 ± 0.91 **b**	125.0–137.5	133.0 ± 1.08 **b**	125.0–142.5	131.4 ± 1.40 **b**	125.0–142.5	137.5 ± 0.83 **a**	132.5–145.0
Macroseta tibia IV	103.3 ± 1.00 **a**	97.5–112.5	100.8 ± 1.00 **a**	95.0–110.0	100.3 ± 0.92 **a**	90.0–107.5	100.5 ± 0.85 **a**	95.0–110.0
Macroseta tarso IV	72.1 ± 0.84 **a**	67.5–77.5	68.4 ± 0.60 **b**	65.0–72.5	63.3 ± 0.63 **c**	57.5–67.5	67.1 ± 0.64 **b**	62.5–72.5

the 36 quantitative variables. First, a principal component analysis (PCA) was applied to reveal any discontinuities in the morphological variation among the specimens originating from the different geographic areas. Second, a canonical discrimination analysis (CDA) was performed to determine the patterns of morphological variation and to identify the morphological characteristics that contributed the most to the morphological differentiation between populations. Third, a discriminant function analysis was conducted to evaluate if the individuals had been correctly assigned to their original groups. All of the analyses were performed using SAS version 9.2 software (SAS, 2005).

The specimens that were measured for the morphometric analyses were deposited as voucher specimens in the Mite Collection of Embrapa Genetic Resources and Biotechnology in Brasília, Brazil.

2.2. Molecular analyses

To assess their genetic variability and intra-interspecific variation, and to know the phylogenetic relationships among the studied *A. largoensis* populations, two target DNA fragments were PCR-amplified and sequenced: the nuclear ribosomal gene section including the ITS1-5.8S-ITS2 (ITS) regions and the 12S rRNA mitochondrial marker. The former region has been widely used in taxonomy for mite species identification and phylogenetic studies (e.g., Gotoh et al., 1998; Hillis and Dixon, 1991; Navajas and Fenton, 2000; Navajas et al., 1999) and has been utilized to assess the phylogenetic relationships between related taxa of phytoseiid mites (e.g., Kanouh et al., 2010; Okassa et al., 2011; Tixier et al., 2006, 2011a,b, 2012). The latter fragment was successfully used for clarifying mites synonymies and phytoseiid species discrimination (Jeyaprakash and Hoy, 2002; Kanouh et al., 2010; Murrel et al., 2001; Okassa et al., 2009, 2010, 2011; Tixier et al., 2010, 2011a,b, 2012). These markers were chosen for this study because they are independent (nuclear ITS and mitochondrial 12S) complementary markers that exhibit different evolutionary rates.

The DNA sequences from nine *A. largoensis* populations from three countries- Brazil (Amapá, Roraima and Pernambuco States), France (La Réunion Island), and Trinidad and Tobago (Arima, Saint Margaret and Toco)-, collected from palm trees and banana plants, were obtained in this study (Table 1). The specimens from Roraima, Brazil and La Réunion were collected from laboratory-reared populations (see details in Sections 2 and 2.3), whereas those from the other populations were directly collected from the field. For the analysis using the 12S rRNA marker, *A. largoensis* sequences from another Indian Ocean Island, Mauritius, and from Florida, USA, as presented in Bowman and Hoy (2012) were retrieved from GenBank (GU807437-GU807479, except for GU807447, GU807465 and GU807466, which do not cluster with the *A. largoensis* sequences) and included in the dataset.

In conducting phylogenetic analyses of closely related taxa, selecting informative ingroups and outgroups is extremely important, and it is desirable to include at least one closely related spe-

cies as a control for determining the genetic distances within the genus, as well as among the species in the subfamily and other subfamilies. *A. largoensis* belongs to a group of nine closely related species that are referred to collectively as the *A. largoensis* group in the subfamily Amblyseiinae (McMurtry and Moraes, 1984). They are distinguished mostly by differences in the shape of their spermathecae. Except for *A. largoensis*, *A. herbicolus* (Chant) and *A. eharai* Amitai and Swirski, the other species in the group have been reported only in the Australasian biogeographic region (Moraes et al., 2004). Three *A. herbicolus* populations from the Canary Islands, New Caledonia and La Réunion that were directly collected in the field were sequenced and included in the analysis as ingroup data (Table 1). In addition, the DNA sequences of both ITS and 12S rRNA of *Neoseiulus californicus* (McGregor) and *N. picanus* (Ragusa) from the subfamily Amblyseiinae, and of *Typhlodromus phialatus* Athias-Henriot and *T. exhilaratus* Ragusa, from the subfamily Typhlodrominae (in Tsolakis et al., 2012), were retrieved from GenBank and included in the analyses as outgroup data (Table 1).

Ten to 50 females per population were collected. At least ten of these specimens were preserved in 100% ethanol for DNA extraction. Ten to fifteen specimens from the populations that were not enough to be used for the morphometric analysis were slide-mounted in Hoyer's medium and morphologically identified by microscopic examination (see details in Sections 2 and 2.1). These specimens were also deposited as voucher specimens in the Mite Collection of Embrapa Genetic Resources and Biotechnology in Brasilia, Brazil.

2.2.1. DNA extraction and amplification

The total DNA was extracted from single adult females using the DNeasy tissue kit (Qiagen, Brazil) according to the DNA extraction protocol (Purification of Total DNA from Animal Blood or Cells; Spin-Column Protocol). The mite specimens were preserved in 100% ethanol and not crushed. The manufacturer's instructions were modified for DNA extraction from small mites, as described by Kanouh et al. (2010) and Mendonça et al. (2011).

ITS primers that were previously designed for tetranychid and phytoseiid mites were used. They were 5′-AGAGGAAGTAAAAGTCGTAACAAG-3′ (Ben-Ali et al., 2000; Navajas et al., 1999) and 5′-ATATGCTTAAATTCAGCGGG-3′ (Mendonça et al., 2011; Navajas et al., 1998). The 12S rRNA fragment was amplified using the primers 5′-TACTATGTTACGACTTAT-3′ and 5′-AAACTAGGATTAGATACCC-3′ (Jeyaprakash and Hoy, 2002).

The amplification reactions were performed in a 25 μl volumes containing 2.5 μl of 10× buffer supplied by manufacture, 1.0 μl $MgCl_2$ (25 mM), 0.5 μl DNTP (0.25 mM of each base), 0.175 μl of each oligonucleotide primer (10 μm), 0.125 μl uni μl^{-1} (5 units) of *Taq* polymerase (Qiagen), 18.525 μl of sterile water and 2 μl of DNA template for ITS. PCR for 12S fragment was performed as describes above except that 0.4 μl bovine serum albumin solution-BSA (10 mg mL^{-1} Biolabs) and was 0.25 μl uni μl^{-1} (5 units) of *Taq* polymerase (Qiagen) was added to the reaction, and the water volume decreased at 18 μl. To amplify the ITS fragment the thermocycler profile included initial denaturation at 94 °C for 2 min followed by 35 cycles of 15 seg denaturation at 94 °C, 45 s anneling at 50 °C and 1 min final extention at 72 °C. For 12S fragment, samples were denatured at 95 °C for 1 min and then PCR was carried out for 40 cycles of 30 seg denaturation at 94 °C, 30 seg annealing at 40 °C and 1 min extension at 72 °C. After amplification, 5 mL of the PCR reaction was analysed by electrophoresis on a 1% agarose gel and visualized by GelRed staining. Both strands of the amplified fragments (ITS and 12S) were directly sequenced using an ABI 3730 automated DNA sequencer (Applied Biosystems Inc., Lille, France). No additional primers were used for sequencing.

2.2.2. Sequences and phylogenetic analyses

The Staden Package version 1.6.0 (Staden et al., 1998) was used for editing and assembling the raw data into sequence contigs. The sequences were aligned using the CLUSTAL W multiple alignment procedure (Thompson et al., 1994), implemented with BIOEDIT software, version 7.0.4 (Hall, 1999). No manual adjustments to the CLUSTAL alignment were performed. Shared haplotypes (12S sequences) and sequence variants (ITS sequences) were identified using DnaSP version 5 software (Librado and Rozas, 2009). The overall and pairwise distances between nucleotide sequences, as well as the within- and among-clade distances, were calculated using Kimura's 2-parameter (K2P) model (Kimura, 1980). The standard error estimates were obtained using a bootstrap procedure (1000 replicates). All of the above analyses of the ITS and 12S sequences were conducted using MEGA version 5 software (Tamura et al., 2011).

Phylogenetic analysis using both the ITS and 12S DNA fragment data were conducted using the maximum likelihood (ML) optimality criterion. The best-fit models of nucleotide substitution were selected using the jModeltest version 2.1.1 program (Darriba et al., 2012) based on the likelihood scores for 88 different models, and both the Akaike information criterion (AIC) and the Akaike information criterion corrected (AICc), as well as the Bayesian information criterion (BIC), were calculated. The ML analyses were performed using the online version of the PhyML3.0 algorithm (Guindon et al., 2010). The analyses were set to optimize the branch lengths and to search the tree topologies using the nearest neighbor interchange algorithm. Phylogenies were edited using MEGA version 5 software (Tamura et al., 2011) based on the output file (newick format) created by PhyML 3.0 algorithm (Guindon et al., 2010). A representative sequence of each population was included in the alignment to produce the ML tree. If different variants/haplotypes were detected within a population, they were also included (see Table 1). The robustness of the trees was assessed by bootstrap analysis, with 1000 bootstrap replicates for all of the analyses, and the approximate likelihood ratio test (aLRT) function within PhyML (Anisimova and Gascuel, 2006) was used to test the accuracy of each branch using the log-likelihood test. Similar supports were obtained, and only the bootstrap values are shown. Phylogenetic analyses using the distances obtained by the neighbor-joining (NJ) algorithm were performed with the K2P parameter, maximum parsimony (MP) and Bayesian methods and yielded similar topologies (not shown).The NJ and MP analyses were conducted using MEGA version 5 software, and the Bayesian inference (BI) analysis using MrBayes version 3,12 (Ronquist and Huelsenbeck, 2003) was conducted using Phylogeny.fr: robust phylogenetic analysis for the non-specialist (Dereeper et al., 2008).

All of the new sequences in the dataset have been deposited in GenBank. The alignments are available upon request. The number of specimens of each population that were analyzed is shown in Table 1, along with their GenBank accession numbers.

2.3. Crossing experiments

2.3.1. Populations and rearing techniques

Specimens of *A. largoensis* from La Réunion Island were obtained from a colony that was introduced into Brazil for experimental purposes in February 2011 (Brazilian Ministry of Agriculture permit 21016.000668/2010-38). The imported population consisted of approximately 190 mites of all instars collected from coconut palm trees and an unidentified palm species in different parts of the Island (21°06′S, 55°36′E) (Moraes et al., 2012). Approximately 100 specimens of this imported colony were used to establish a colony at Laboratory of Entomology, Embrapa Roraima, Boa Vista, Roraima, Brazil. This colony was established on banana (*Musa*

sp.) leaf discs 14 cm in diameter with the abaxial surfaces facing up. The leaf discs were surrounded with hydrophilic cotton and placed on 1-cm thick polyethylene foam discs of a similar diameter, which were set in 16-cm diameter Petri dishes. To prevent the mites from escaping and to maintain the turgidity of the leaf discs, the foam mat was maintained permanently wet by daily addition of distilled water. Each day, the colony was provided with a fresh supply of prey consisting of immature stages and adults of *R. indica* that were collected from coconut palms using a small brush. The Roraima, Brazil *A. largoensis* specimens were obtained from 'Green Dwarf' coconut leaves at the Embrapa Roraima experiment station (57°03.4′N; 60°42′19.7″W) and they were reared and fed as described above for La Réunion. Both colonies were maintained in a room under controlled environmental conditions at 27 °C, with 60 ± 10% RH and a 12-h photophase. Care was taken to prevent mutual contamination.

2.3.2. Crossings and backcrossings

The crossing and backcrossing experiments were conducted at the Laboratório de Entomologia, Embrapa Roraima, Boa Vista, Roraima, Brazil. The experimental units were established on pieces of banana leaves (11 × 11 cm) placed with the abaxial surface up on a piece of foam mat covered with filter paper that was placed in Petri dishes (16 cm in diameter). Each piece of banana leaf was divided into nine experimental units (3 × 3 cm each) by strips of cotton wool. The experiments were conducted under the same conditions under which the *A. largoensis* rearing was maintained.

Each experiment used a cohort of eggs of uniform ages, obtained by placing a gravid female in each of the 50 experimental units for each of the two populations. After a period of 12 h, the females had oviposited, and excess eggs were removed, leaving a single egg per unit. The predators were always fed a surplus amount of prey throughout the experiment. When the mite developing in a unit reached adulthood and was found to be female, a male was transferred to the unit from one of the stock colonies; dead males were replaced by new males from the same source until the death of the female. Additionally, ten virgin females of each population were kept in isolation to ascertain that mating is necessary for oviposition to occur. Each couple or isolated female was observed daily to determine egg production, egg hatching and female longevity. The eggs laid in the first ten days of the oviposition period were isolated to determine the sex ratio. For the heterogametic crosses, female deutonymphs taken from the stock colony of each population were isolated in experimental units; upon reaching adulthood, a male taken from the colony of the other population was introduced into the respective unit (♀ La Réunion vs ♂ Roraima; ♂ La Réunion vs ♀ Roraima). The offspring (males and females) of each cross were backcrossed with both populations to assess the fertility of the hybrids. The evaluations conducted were similar to those described for the homogametic crosses.

A single factor ANOVA and a Tukey-adjusted *t*-test ($\alpha = 0.05$) were used to analyze the effect of the crossing combination on fecundity, using SAS version 9.2 software (SAS, 2005).

3. Results

3.1. Morphometric analyses

The mean values, the standard errors and the range of the values for the 36 morphometric characteristics of the *A. largoensis* females from the populations analyzed are shown in Table 2. The multivariate morphometric analyses (PCA and CDA) revealed morphological variations among the studied populations.

Approximately 38.1% of the total morphological variability was explained by two principal components (PC 1: 20.6%; PC2: 17.5%). Partial overlap of the characteristics of distinct populations in the space of the two PCs was observed (Fig. 1), implying that there are no morphometric discontinuities among them. However, some tendencies were observed. The specimens from La Réunion were plotted mainly along the positive section of the PC1 axis, whereas the specimens from Pernambuco, Brazil were plotted mainly along the negative section of the same axis; thus, the overlap between these populations was extremely small, indicating large morphometric differences between them. In contrast, the populations from Roraima, Brazil and Trinidad and Tobago overlapped broadly in the central area of the ordination graphic, indicating their morphometric similarity.

The first two canonical variables (CV1 and CV2) jointly explained 83.7% of the total variance (CV1: 56.1%; CV2: 27.6%) (Fig. 2). The first canonical variable completely separated the La Réunion specimens from all of the American specimens, with the former plotted along the CV1 negative axis and the latter mostly plotted along the positive CV1 axis. All of the Roraima, Brazil specimens were plotted at the extreme edge of the CV2 negative axis, completely separate from other American specimens. The specimens from Trinidad and Tobago and Pernambuco, Brazil are the most morphometrically similar. An analysis of the loading structure (Table 3) indicated that the setal lengths are the main characteristics that distinguish the studied populations. A joining analysis of the loadings (Table 3) and morphometric characteristics (ANOVA) (Table 2) indicated that the La Réunion specimens had longer setae j1, Z4, s4, and JV5; longer macroseta on tarsus IV; a greater distance between the setae St5 and shorter seta z5. The Roraima, Brazil specimens differed from all of the other specimens by having shorter setae S2 and Z4, and they differed from the Pernambuco, Brazil and Trinidad and Tobago specimens by the greater distance between their setae St5.

The predicted classification of the specimens based on the discriminant analysis showed that overall, 93.75% of the specimens were classified in the population of origin; 90% were correctly classified in the Pernambuco, Brazil population, and 95% were correctly classified in the Roraima, Brazil, La Réunion and Trinidad and Tobago populations. Two misclassifications occurred in the Brazilian population from Pernambuco (2 specimens). Only one specimen each from La Réunion Island, Roraima, Brazil, and Trinidad and Tobago populations was misclassified.

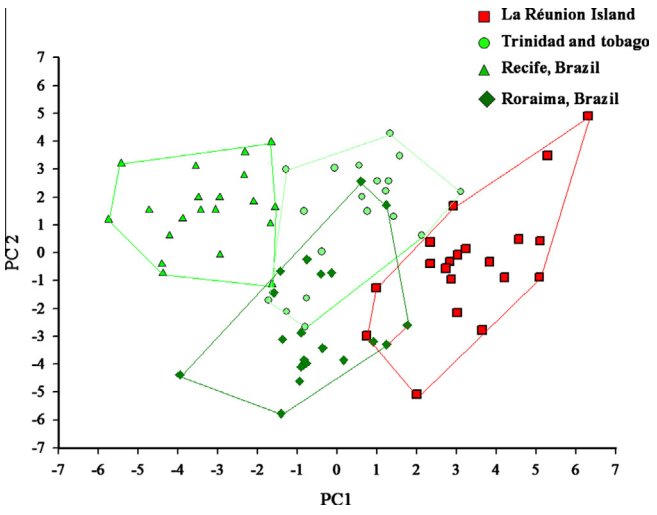


Fig. 1. Principal component analysis of 36 morphometric characters measured on females of *A. largoensis* populations from La Réunion Island and the Americas. Individuals plotted against their values for the first two principal components.

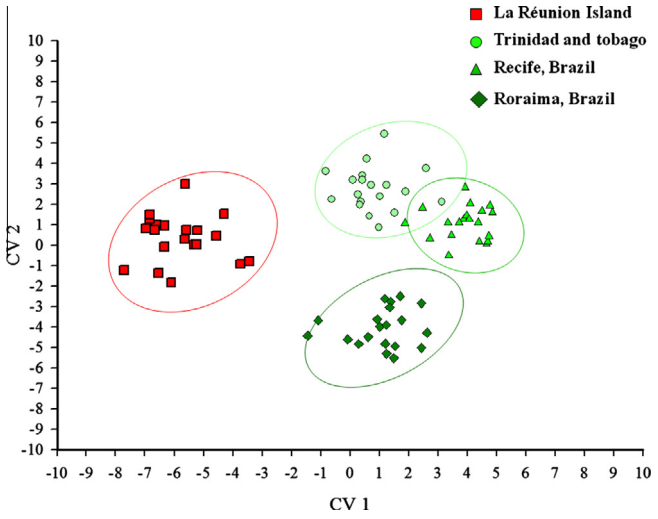


Fig. 2. Canonical discriminant analysis of 36 morphometric characters measured on females of *A. largoensis* populations from from La Réunion Island and the Americas. Individuals plotted against their values for the first two canonical variables.

3.2. Molecular analyses

Partial DNA sequences of ITS region (620 bp) and the 12S mitochondrial rRNA (400 bp) gene of the *A. largoensis* and *A. herbicolus* populations were obtained. The sequences of the ITS region and the 12S rRNA of 41 and 36 *A. largoensis* specimens, and five and seven *A. herbicolus* specimens, respectively, were sequenced (Table 1).

3.2.1. ITS rDNA

The resulting ITS dataset consisted of 43 sequences from the genus *Amblyseius*, including 38 from *A. largoensis* (17 from Brazil; 14 from Trinidad and Tobago; and 7 from La Réunion Island) and 5 from *A. herbicolus* (Canary Islands, Spain). In addition, as ingroup and outgroups in the dataset were included eigth sequences from the genus *Neoseiulus* (*N. californicus* HQ404802-HQ404807; *N. picanus* HQ404809-HQ404810) and two from the genus *Typhlodromus* (*T. phialatus* HQ404829; *T. exhilaratus* HQ404830) (Tsolakis et al., 2012). Sequences were collapsed into seven different sequence variants/genotypes.

TrN + G model (Tamura and Nei, 1993) was chosen for both the maximum likelihood (ML) and Bayesian inference (BI) analyses of the ITS dataset, with the gamma distribution shape parameter of 0.7830. The topologies of the major branches of both trees were similar, and therefore, for the clarity of presentation, only the ML tree is shown (Fig. 3). The estimated base frequencies were A = 0.2647, C = 0.2039, G = 0.2281, and T = 0.3033. The phylogenetic tree indicated a strong correspondence between the clades and the previously assigned taxa. The *A. largoensis* populations from the Americas clustered together with the populations from the Indian Ocean Islands, La Réunion, in one clade, which is fully supported by its booststrap score (100%), although the populations from the different continents were separate within this clade. The *A. herbicolus* genotype remained apart from the *A. largoensis* clade. Both clades (*A. largoensis* and *A. herbicolus*) formed a monophyletic cluster in a high bootstrap-supported branch (100%).

Table 3
Canonical discriminant analysis for females of four *A. largoensis* populations from La Réunion Island and the Americas. First ten morphological characters (eigenvectors) with higher loadings (eigenvalues) for two canonical axis (CV1 and CV2), in decrescent order of absolute values.

Morphological characters	CV1 loadings	Morphological characters	CV2 loadings
Seta Z4	−0.9144	Seta S2	0.9517
Seta z5	0.7632	Length of dorsal shield	0.7479
Seta s4	−0.5961	Seta Z4	−0.6778
Seta S2	0.5556	Seta J5	0.6759
Seta S4	0.5535	Calyx spermatheca	−0.6679
Length of dorsal shield	0.5475	Distance St5-St5	−0.6545
Seta JV5	−0.4905	Distance St2-St2	−0.6478
Seta j1	−0.4715	Distance St3-St3	0.6387
Macroseta tarsus IV	−0.4569	Seta z2	0.6246
Distance St5-St5	−0.4290	Seta z4	−0.5814

A pairwise comparison of ITS-based distances between the *A. largoensis* populations, the ingroup, and the outgroup species is presented in the Table 4. The average overall mean divergence of the ITS sequence pairs (including the outgroup taxa) was 6.68% (SE = 0.5), ranging from 0% to 40.2%. The average mean divergence within the *A. largoensis* and *A. herbicolus* populations was 0.05% and 0.0%, respectively.

Two genotypes were identified within the 38 ITS sequences of *A. largoensis*, differing by only a T (n = 34) or C (n = 7) at nucleotide position 123 (Fig. S1), which represented the populations from the Americas (V1) and La Réunion Island (V2), respectively. The *A. largoensis* ITS sequences differed from those of *A. herbicolus* by 12 nucleotide positions (47, 87, 111, 112, 120, 124, 130, 133, 135, 141, 162, 166) (Fig. S1), and the average mean divergence between these species was 5.89% (SE = 0.25) (Table 4A).

3.2.2. 12S mitochondrial rRNA fragment

The final 12S mitochondrial rRNA dataset consisted of 83 sequences from the genus *Amblyseius*, including 76 from *A. largoensis* (30 from Mauritius; 15 from Brazil; 13 from Trinidad and Tobago; 10 from Florida (USA); and 8 from La Réunion Island) and 7 from *A. herbicolus* (5 from the Canary Islands, Spain; 1 from La Réunion; and 1 from New Caledonia). In addition, as ingroup and outgroups in the dataset were included eight sequences from *Neoseiulus* (*N. californicus* HQ404836-HQ404839 and HQ40484HQ404842; *N. picanus* HQ404844-HQ404845) and two from the genus *Typhlodromus* (*T. phialatus HQ404861*; *T. exhilaratus HQ404831*). All the 12S rRna sequences represented 18 haplotypes with a haplotype diversity (Hd) of 0.7497. Six haplotypes were identified within the 38 sequences of *A. largoensis* from the Americas, and six haplotypes were identified within the 38 sequences from the Indian Ocean Islands. A single haplotype of *A. herbicolus* was found in the three different geographic regions that were sampled: the Canary Islands, New Caledonia and La Réunion.

The best-fit model for nucleotide substitution obtained from both the maximum likelihood (ML) and Bayesian inference (BI – not shown) analyses was the HKY + G model, in which the Ti/Tv ratio was 0.9801, the kappa value was 3.2034, and the gamma distribution shape parameter (G) was 0.5630. The estimated base frequencies were A = 0.3932, C = 0.0835, G = 0.1156, and T = 0.4078. Consistent with the ITS results, the maximum likelihood analysis based on the 12S sequences/haplotypes (Fig. 4) revealed a similarly well-resolved species phylogeny. The clade containing the *A. largoensis* haplotypes indicated the presence of two groups, which clustered the American haplotypes apart from the Indian Ocean Islands haplotypes, with bootstrap values of 87% and 82%, respectively. Moreover, a clear separation between the specimens of *A. largoensis* and those of *A. herbicolus* was observed.

The average mean divergence for all of the 12S sequence pairs (including the ingroup and outgroup taxa) was 11.6% (SE = 1.3%), ranging from 0% to 42.3%. The average mean divergence among all of the 12S rRNA *Amblyseius* sequences was 7.3% (SE = 1.1%), and those within the *A. largoensis* and *A. herbicolus* sequences were 3.79% and 0.0%, respectively (Table 4B). The estimated values for the average divergence between the sequence pairs within each

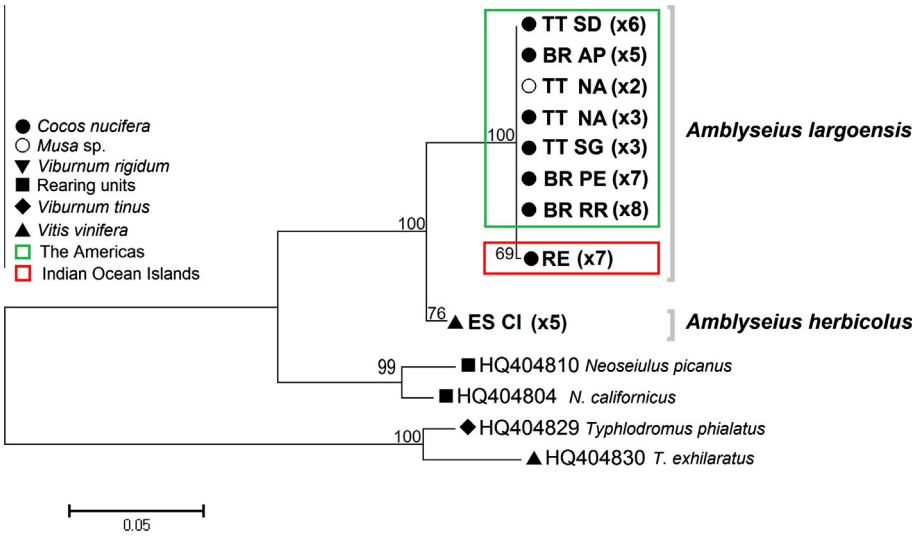


Fig. 3. ITS tree. Maximum likelihood (ML) tree performed with TrN + G model of the ribosomal region ITS of *Amblyseius largoensis* mites, ingroup and outgroups with sequences obtained in this study. Concordant trees were obtained by maximum likelihood (ML) and Bayesian inference (BI) analyses which produced the same topology in defining groups. Statistical supports indicate maximum parsimony bootstraps. The number of times that a haplotype was found in the dataset is indicated between parentheses. Codes of sequences/variants specified in Table 1.

group (the Americas and the Indian Ocean Islands) were 0.22% and 0.12%, respectively, and the divergence between these two groups was 5.68%. Considering the present 12S sequences dataset, the mean distance between *A. largoensis* and *A. herbicolus* was 22.98%.

The *A. largoensis* haplotypes from the Americas (H1–H6) and the Indian Ocean Islands (H7–H12) (Table 1) differed in 24 nucleotide positions in the 12S alignment (Fig. S2). Seventeen of the observed polymorphisms are the result of transitions, and seven are the result of transversions. The *A. largoensis* and *A. herbicolus* 12S sequences differed at 80 nucleotide positions.

3.3. Crossings

The results of the crosses and backcrosses between members of the *A. largoensis* populations from La Réunion Island and Roraima, Brazil are presented in Table 5. Unmated females from the La Réunion and Roraima population did not oviposit when a male was not present in the experimental unit, whereas 100% of the females of all crosses and backcrosses oviposited. The average number of eggs produced from homogametic crosses was 1.8 and 2.2 eggs/female/day for Roraima and La Réunion populations, respectively. The offspring viability for both of these populations was higher than 97%. For heterogametic crosses between ♀ La Réunion vs ♂ Roraima and between ♂ La Réunion vs ♀ Roraima, the fecundity rate was1.9 and 1.7 eggs/female/day, respectively, and the sex ratio was 0.6 and 0.5, respectively. All of the offspring from both of the heterogametic crosses were viable during the first ten days of the oviposition period. For the backcrosses, the fecundity rate ranged from 2 to 1.3 eggs/female/day, and the egg viability ranged from 89% to 100%, while the sex ratio ranged from 0.5 to 0.7, being significantly different among some of the crosses.

4. Discussion

A fundamental requirement for the classical biological control of the RPM in the invaded areas of the Americas is accurate taxonomic identification of its natural enemies in the eastern hemisphere. In this study, morphometric, molecular and biological data were integrated to determine if populations of the phytoseiid mites from Indian Ocean Islands and the Americas that are morphologically identified as *A. largoensis* actually belong to the same taxonomic entity. In addition, molecular polymorphisms and morphometric differences between these populations were determined and constitute basic information for further studies aiming to follow colonization of Indian Ocean Islands populations in the Americas, in the case of field releases. Finally, the observed morphometric and molecular variability among the *A. largoensis* populations that were studied is discussed in the context of phytoseiid mite systematics.

Morphometric differences between the *A. largoensis* specimens from La Réunion Island and the Americas were observed; most of them pertaining to the length of the setae (see Table 2). Species discrimination within the family Phytoseiidae is largely based on differences in the setal lengths (e.g., Chant and McMurtry, 2007), and sometimes, such continuous characteristics are the only features available to distinguish between close species. However, intraspecific variability in these characteristics has been widely observed among phytoseiid mites (e.g., Tixier et al., 2008), hampering specific identification in this group. Tixier (2012) presented a statistical approach to assess, for a character in a lot of specimens, if differences between means referred to intra- or interspecific variability. This general approach was based on the lengths of the setae of phytoseiid mites and highlighted that the minimal difference between the mean values of two specimen lots belonging to two species should be of 10.58 μm (for setae <65 μm) and 33.99 μm (for setae >65 μm). According to these criteria, the differences observed between the *A. largoensis* populations from La Réunion and each of the American localities reflects intraspecific variability.

The results of the molecular analysis agreed with those of the morphometric analysis, indicating that the *A. largoensis* populations from La Réunion Island and the Americas (Roraima and Pernambuco States in Brazil and Trinidad and Tobago) belong to the same taxonomic entity. In addition, the results indicated that the other American populations (Amapá in Brazil and Florida in the USA), as well as those from another Indian Ocean Islands (Mauritius) also belong to the same species. Although belonging to the same taxon, the *A. largoensis* from the Indian Ocean Islands and the Americas remained away from one another in the phylogeny, using both of the genetic markers (ITS and 12S). This is highlighted by the 12S genetic marker. Similar results were obtained by Bowman and Hoy (2012) in a study of specimens from South Florida and Mauritius that was based on the 12S fragment.

Table 4
Pairwise distance calculated among ITS (**A**) and 12S (**B**) sequences of *Amblyseius* species including the ingroup and outgroup taxa using Kimura's two-parameter correction. *Amblyseius largoensis* populations from the Americas and the Indian Ocean Islands were considered as composing a single taxon (**1**) or different taxa (**2**).

A(1)

		1	2	3	4	5	6
1	*A. largoensis*	0.05%					
2	*A. herbicolus*	5.89%	0.00%				
3	*N. picanus*	21.43%	18.29%	0.05%			
4	*N. californicus*	21.08%	16.90%	4.17%	0.00%		
5	*T. phialatus*	35.38%	31.43%	31.80%	27.73%	n/c	
6	*T. exhilaratus*	34.96%	32.75%	33.61%	30.16%	4.75%	n/c

A(2)

		1	2	3	4	5	6	7
1	*A. largoensis* (The Americas)	0.00%						
2	*A. largoensis* (Indian Ocean Islands)	0.16%	0.00%					
3	*A. herbicolus*	5.89%	5.89%	0.00%				
4	*N. picanus*	21.43%	21.43%	18.29%	0.48%			
5	*N. californicus*	21.08%	21.08%	16.90%	4.17%	0.00%		
6	*T. phialatus*	35.38%	35.38%	31.43%	31.80%	27.73%	n/c	
7	*T. exhilaratus*	34.96%	34.96%	32.75%	33.61%	30.16%	4.75%	n/c

B(1)

		1	2	3	4	5	6
1	*A. largoensis*	3.00%					
2	*A. herbicolus*	22.98%	0.00%				
3	*N. picanus*	33.65%	36.40%	0.10%			
4	*N. californicus*	30.22%	34.22%	12.06%	0.00%		
5	*T. phialatus*	30.89%	37.03%	41.48%	37.00%	n/c	
6	*T. exhilaratus*	34.68%	39.22%	42.26%	41.52%	13.54%	n/c

B(2)

		1	2	3	4	5	6	7
1	*A. largoensis* (The Americas)	0.22%						
2	*A. largoensis* (Indian Ocean Islands)	5.68%	0.12%					
3	*A. herbicolus*	22.09%	23.82%	0.00%				
4	*N. picanus*	28.89%	31.49%	34.22%	0.15%			
5	*N. californicus*	32.89%	34.37%	36.40%	12.06%	0.00%		
6	*T. phialatus*	33.59%	35.71%	39.22%	41.52%	42.26%	n/c	
7	*T. exhilaratus*	29.54%	32.16%	37.03%	37.00%	41.48%	13.54%	n/c

Shade blocks = intraspecific values; white blocks = interspecific values.

Phylogenetic analysis based on the ITS and 12S fragments showed that the populations from the Indian Ocean Islands and the Americas comprise a monophyletic group. The genetic distances that were obtained in this study using both the 12S and ITS sequences fit within the intra-specific distances of other phytoseiid species that have been reported in the literature.

The pairwise nucleotide distance between the ITS sequences of the *A. largoensis* populations from Indian Ocean Islands and the Americas (0.16%) is comparable to and even lower than the intraspecific distances determined for phytoseiid species in the genera *Typhlodromus* (*T. pyri*, *T. exhilaratus*, *T. phialatus* and *T. athiasae* Porath and Swirski) (Tixier et al., 2006, 2012), *Neoseiulella* (Kanouh et al., 2010), and *Neoseiulus* (Okassa et al., 2011; Tixier et al., 2011a) confirming these *A. largoensis* populations belong to the same species. The interspecific distance between the closely related species, *A. largoensis* and *A. herbicolus*, based on their ITS sequences that were obtained in this study, was 5.89%, and that for the above mentioned *Typhlodromus* species ranged from 4.4% to 5.1% (Tixier et al., 2012), similar to that obtained by several authors (Kanouh et al., 2010; Okassa et al., 2011; Navajas et al., 1999; Tixier et al., 2006) for other phytoseiid congeneric species (3–7.2%). These interspecific distance values are significantly higher than the distance value between the Indian Ocean Islands and American populations that was obtained in this study.

Similarly, the 12SrRNA-based pairwise distance between the Indian Ocean Islands and American populations of *A. largoensis* (5.68%) is comparable to or even lower than the intraspecific distances observed for other phytoseiid species (see Okassa et al., 2009, 2010; Tixier et al., 2010, 2012); and much lower than interspecific distances in different genera, e.g., between *A. largoensis* and *A. herbicolus* (22.98%) observed in this study; between *Typhlodromus* species (25.5–26.7%) observed by Tixier et al. (2012b); between *Euseius* species (14–22%) reported by Okassa et al. (2009); and between *N. californicus* and *Neoseiulus fallacis* (Garman) (9%) reported by Jeyaprakash and Hoy (2002) or between *N. californicus* and *N. idaeus* (12.5%) by Okassa et al. (2011).

The lack of oviposition by unmated females has been observed for the phytoseiid mites studied to date (e.g., see Croft, 1970; Moraes and McMurtry, 1981; Noronha et al., 2003). In this study, unmated females from both La Réunion and Roraima, Brazil populations did not oviposit during their entire life cycle, which demonstrated that crossing is a prerequisite for oviposition. The number of oviposited eggs, the egg viability and the sex ratio determined for the heterogametic crosses and the backcrosses involving the *A. largoensis* populations from La Réunion Island and Roraima, Brazil revealed complete reproductive compatibility between these populations, without evidence of pre- or post-zygotic isolation mechanisms. No differences in the sex ratio among the offspring of crosses or backcrosses were observed. The fecundity rate was not significantly different between the homogametic and heterogametic crosses (see Table 5); however, this value was somewhat lower for all of the offspring of backcrosses in which the parental strains were from Roraima, Brazil. The lower oviposition rates of the offspring of heterogametic crosses and backcrosses

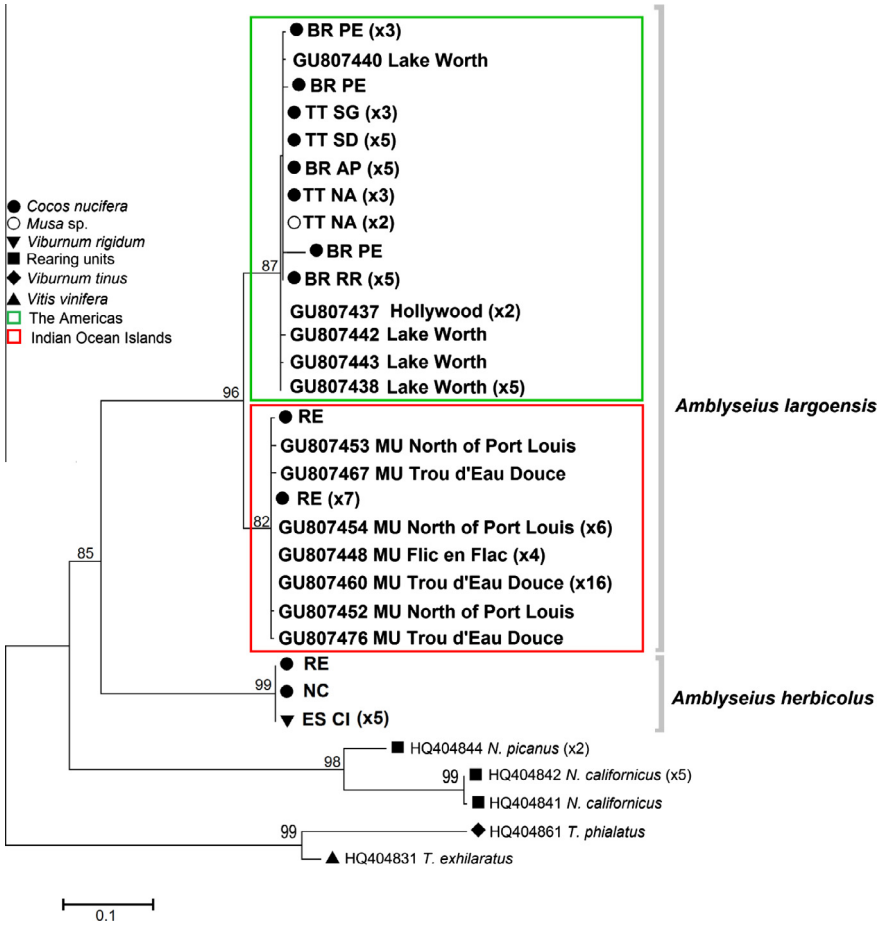


Fig. 4. 12S tree. Maximum likelihood (ML) tree performed with HKY + G model of the fragment 12S (mtDNA) of *Amblyseius largoensis* mites, ingroup and outgroups with 12S sequence retrieved from Genbank and obtained in this study. Concordant trees were obtained by maximum likelihood (ML) and Bayesian inference (BI) analyses which produced the same topology in defining groups. Statistical supports indicate maximum parsimony bootstraps. The number of times that a haplotype was found in the dataset is indicated between parentheses. Codes of sequences/variants specified in Table 1.

Table 5
Fecundity, egg viability and sex ratio obtained for crosses and backcrosses between populations of the predatory mite *A. largoensis* from La Réunion Island (RI) and Roraima, Brazil (RO). Oviposition was observed during the 10 first days. Experiments were conducted at 27 °C, 60 ± 10% RH and 12 h photophase.

♀ X ♂		Number of couples	Ovipositing (%) ♀	Egg viability (%)	Number of eggs	Eggs/♀/day (±SE)	Sex ratio (±SE)
RI	–	10	0	–	–	–	–
RO	–	10	0	–	–	–	–
RI	RI	10	100	97.6	216	2.17 ± 0.21 a	0.61 ± 0.01 abc
RI	RO	10	100	100	188	1.90 ± 0.04 abc	0.60 ± 0.05 abc
RI	$F1_{(BR\ vs\ RI)}$	10	100	98.57	140	1.98 ± 0.25 abc	0.61 ± 0.04 abc
RO	RO	10	100	100	167	1.78 ± 0.20 abc	0.75 ± 0.01 a
$F1_{(RO\ vs\ RI)}$	RI	10	100	100	120	1.71 ± 0.04 abc	0.55 ± 0.05 bc
RO	RI	10	100	100	162	1.67 ± 0.02 abc	0.47 ± 0.05 c
$F1_{(RI\ vs\ RO)}$	RI	10	100	88.97	127	1.67 ± 0.07 abc	0.56 ± 0.05 bc
RI	$F1_{(RI\ vs\ RO)}$	10	100	98.65	148	1.61 ± 0.05 abc	0.71 ± 0.03 ab
$F1_{(RI\ vs\ RO)}$	RO	10	100	98.26	115	1.54 ± 0.11 bc	0.60 ± 0.04 abc
RO	$F1_{(RI\ vs\ RO)}$	10	100	98.05	103	1.41 ± 0.07 bc	0.63 ± 0.06 abc
$F1_{(RO\ vs\ RI)}$	RO	10	100	100	110	1.37 ± 0.04 c	0.49 ± 0.02 c
RO	$F1_{(RO\ vs\ RI)}$	10	100	100	107	1.34 ± 0.04 c	0.67 ± 0.03 ab

are most likely a function of the lower innate reproductive capacity of the Roraima population (see Domingos et al., 2012), not evidence of partial incompatibility. These results indicate that the two populations belong to the same taxon, according to the biological species concept (Mayr and Ashlock, 1991).

In summary, all of the strategies adopted in this study—morphological, biological and molecular—indicated that the American and the Indian Ocean Islands populations identified as *A. largoensis* belong to the same taxonomic entity, despite some observed morphological and genetic differences.

Bowman and Hoy (2012) defined population-specific primers for identifying specimens of *A. largoensis* from Mauritius in the event that they were released in south Florida. Considering the 12S alignment obtained in this study, the similarity of the sequences from the Mauritius and La Réunion populations and the similarity among the sequences from the American populations,

such population-specific primers are expected to distinguish also between La Réunion and Roraima, Brazil specimens. Concerning the ITS polymorphism observed among variants/genotypes V1 (the Americas) and V2 (La Réunion) (Table 1), the C-T transition (alignment position 123, see Fig. S1) can also be used as a trait to differentiate these geographic populations. The morphometric differences between the *A. largoensis* populations that were studied made it possible to match specimens with their source populations and could be used as "morphological markers" for taxonomy at the intraspecific level. However, in the case of field releases of Indian Ocean Islands populations in the Americas and considering there is no reproductive incompatibility between them, both genetic or morphometric traits could be useful to distinguish specimens of those populations until they start to interbreed. Further evaluations of these diagnostic traits in the progeny would be necessary to determine whether they will be identifiable in the resulting mixed population. In addition, microsatellite markers have been used for mite population studies (see Bailly et al., 2004; Uesugi et al., 2009) and could be tested for traceability of the colonization processes of introduced *A. largoensis*.

Differences between populations of a predatory mite in response to a specific prey could be associated to genetic variability and/or to learning. Carrillo et al. (2012b) detected variations in the response to *R. indica* among *A. largoensis* populations from Florida previously exposed to different diets and previous feeding experience. Domingos et al. (2012) also observed differences in biological parameters between *A. largoensis* populations preying on *R. indica*, but in this case authors studied populations from different geographic areas. Authors observed that oviposition period, prey consumption and net reproductive rate were significantly higher for La Réunion population than for Roraima, Brazil population. The genetic differences observed in the present paper between the Indian Ocean Islands and American populations of *A. largoensis* can be related to the biological differences observed by Domingos et al. (2012), although they are not the genetic characteristics that determined those biological differences. Differences in the biological performance of populations of the same phytoseiid species have also been reported. A strain of *Phytoseiulus longipes* Evans from South Africa was described as ineffective in controlling the tomato red spider mite *Tetranychus evansi* Baker and Pritchard, which is a major pest of solanaceous crops (Moraes and McMurtry, 1985). However, tests conducted with specimens of this predatory mite that were collected in southern Brazil and northern Argentina indicated that *T. evansi* was a suitable food for them (Ferrero et al., 2007; Furtado et al., 2007); this biological difference was also associated with variability in the 12S rDNA fragment (Tixier et al., 2010). Furthermore, intraspecific biological differences were observed in populations of phytoseiid mites introduced to Africa to control the cassava green mite, *Mononychellus tanajoa* (Bondar). Brazilian populations of *Typhlodromalus aripo* De Leon, *Neoseiulus idaeus* Denmark and Muma and *Amblydromalus manihoti* (Moraes) were successfully established in Africa after repeated unsuccessful attempts involving populations of the same species from Colombia (Yaninek et al., 1993). It is possible that the differences in the performance of these phytoseiid mite populations are also related with their genetic differences. These findings reinforce the importance of integrating population genetic characterization into the efficacy evaluations of phytoseiid mites for use as biological control agents.

The predatory mite *A. largoensis* is an important natural enemy of several other phytophagous mite species that attack not only palms but many other plant hosts (see Galvão et al., 2007; Rodríguez and Ramos, 2000). Since populations from the Indian Ocean Islands and the Americas constitute a different strain would be important evaluate possible ecological consequences of introducing an exotic strain on the native population of predators, e.g. direct effect on rare or native species, competition with native species, or effect of hybridization with native species (Sato et al., 2012).

Acknowledgments

We thank the Agricultural Research Centre for International Development (CIRAD), in special Dr. Serge Quilici, for the logistical support in obtaining the population from La Réunion. We are grateful to Serge Kreiter, Gilberto J. de Moraes, Tatiane Marie Martins Gomes de Castro, Ricardo Adaime da Silva, and Farsan Hosein for their assistance in the sample collection. To José Wagner Melo and Daniela Duarte Rezende for the support with SAS statistical analyses. To Bruno de Paiva Rocha and Adilson Werneck for graphical abstract edition. Finally we are grateful to Gilberto J. de Moraes for valuable manuscript review. This research was partially funded by the National Council for Scientific and Technological Development (CNPq), Brazil, call CNPq/SDA/MAPA Call 064/2008. Authors D.N., R.S.M. and M.G.C.G. Jr. also thanks CNPq for research and post-doc fellowships.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.biocontrol.2014.01.011.

References

Anisimova, M., Gascuel, O., 2006. Approximate likelihood ratio test for branchs: a fast, accurate and powerful alternative. Syst. Biol. 55, 539–552.

Bailly, X., Migeon, A., Navajas, M., 2004. Analysis of microsatellite variation in the spider mite pest *Tetranychus turkestani* (Acari: Tetranychidae) reveals population genetic structure and raises questions about related ecological factors. Biol. J. Linn. Soc. 82, 69–78.

Ben-Ali, Z., Boursot, P., Said, K., Lagnel, J., Chatti, N., Navajas, M., 2000. Comparison of ribosomal ITS regions among *Androctonus* spp. *scorpions* (Scorpionida: Buthidae) from Tunisia. J. Med. Entomol. 37, 787–790.

Bowman, H.M., Hoy, M.A., 2012. Molecular discrimination of phytoseiids associated with the red palm mite *Raoiella indica* (Acari: Tenuipalpidae) from Mauritius and South Florida. Exp. Appl. Acarol. 57, 395–407.

Carrillo, D., Navia, D., Ferragut, F., Peña, J., 2011. First report of *Raoiella indica* Hirst (Acari: Tenuipalpidae) in Colombia. Fla. Entomol. 94, 370–371.

Carrillo, D., Amalin, D., Hosein, F., Roda, A., Duncan, R., Peña, J.E., 2012a. Host plant range of *Raoiella indica* Hirst (Acari: Tenuipalpidae) in areas of invasion of the new world. Exp. Appl. Acarol. 57, 271–289.

Carrillo, D., de Coss, M.E., Hoy, M.A., Peña, J.E., 2012b. Variability in response of four populations of *Amblyseius largoensis* (Acari: Phytoseiidae) to *Raoiella indica* (Acari: Tenuipalpidae) and *Tetranychus gloveri* (Acari: Tetranychidae) eggs and larvae. Biol. Control 60, 39–45.

Carrillo, D., Frank, J.H., Rodrigues, J.C.V., Peña, J., 2012c. A review of the natural enemies of the red palm mite, *Raoiella indica* (Acari: Tenuipalpidae). Exp. Appl. Acarol. 57, 347–360.

Chant, D.A., McMurtry, J.A., 1994. A review of the subfamilies Phytoseiinae and Typholodrominae (Acari: Phytoseiidae). Int. J. Acarol. 20, 223–310.

Chant, D.A., McMurtry, J.A., 2005. Review of the subfamily Amblyseiinae Muma (Acari: Phytoseiidae) Part IV. The tribe Euseiini N. tribe, subtribes Typhlodromalina, N. subtribe, Euseiina N. subtribe and Ricoseiina N. subtribe. Int. J. Acarol. 31, 187–222.

Chant, D.A., McMurtry, J.A., 2007. Illustrated keys and diagnoses for the genera and subgenera of the Phytoseiidae of the world (Acari: Mesostigmata). Indira Publishing House, West Bloomfield, USA.

Chant, D.A., Yoshida-Shaul, E., 1991. Adult ventral setal patterns in the family Phytoseiidae (Acari: Gamasina). Int. J. Acarol. 17, 187–199.

Croft, B.A., 1970. Comparative studies on four strains of *Typhlodromus occidentalis* (Acarina: Phytoseiidae). I. Hybridization and reproductive isolation studies. Ann. Entomol. Soc. Am. 63, 1558–1563.

Darriba, D., Taboada, G.L., Doallo, R., Posada, D., 2012. jModelTest 2: more models, new heuristics and parallel computing. Nat. Methods 9, 772.

Dereeper, A., Guignon, V., Blanc, G., Audic, S., Buffet, S., Chevenet, F., Dufayard, J.-F., Guindon, S., Lefort, V., Lescot, M., Claverie, J.-M., Gascuel, O., 2008. Phylogeny.fr: robust phylogenetic analysis for the non-specialist. Nucleic Acids Res. 36, W465–W469, doi: 10.1093/nar/gkn180.

Domingos, C.A., Oliveira, L.O., Morais, E.G.F., Navia, D., Moraes, G.J., Gondim Jr., M.G.C., 2012. Comparison of two populations of the pantropical predator *Amblyseius largoensis* (Acari: Phytoseiidae) for biological control of *Raoiella indica* (Acari: Tenuipalpidae). Exp. Appl. Acarol. 60, 83–93.

Famah-Sourassou, N., Hanna, R., Zannou, I., Breeuwer, J.A., Moraes, G.J., Sabelis, M.W., 2012. Morphological, molecular and cross-breeding analysis of geographic populations of coconut-mite associated predatory mites identified as *Neoseiulus baraki*: evidence for cryptic species? Exp. Appl. Acarol. 57, 15–36.

Famah-Sourassou, N., Hanna, R., Zannou, I., Moraes, G.J., Negloh, K., Sabelis, M.W., 2010. Morphological variation and reproductive incompatibility of three coconut-mite-associated populations of predatory mites identified as *Neoseiulus paspalivorus* (Acari: Phytoseiidae). Exp. Appl. Acarol. 53, 323–338.

FAOSTAT, 2011. World Production. Available from: <http://faostat.fao.org> (Accessed 19.03.2012).

FDACS, 2007. Red palm mite infestation identified in palm gardens. In: Florida Department of Agriculture and Consumer Services. Available from DIALOG. <http://www.doacs.state.fl.us/press/2007/12052007_2.html> (Accessed 29.09.2012).

Ferrero, M., Moraes, G.J., Kreiter, S., Tixier, M.-S., Knapp, M., 2007. Life tables of the predatory mite *Phytoseiulus longipes* feeding on *Tetranychus evansi* at four temperatures (Acari: Phytoseiidae, Tetranychidae). Exp. Appl. Acarol. 41, 45–53.

Flechtmann, C.H.W., Etienne, J., 2004. The red palm mite, *Raoiella indica* Hirst, a threat to palms in the Americas (Acari: Prostigmata: Tenuipalpidae). Syst. Appl. Acarol. 9, 109–110.

Furtado, I.P., Moraes, G.J., Kreiter, S., Tixier, M.S., Knapp, M., 2007. Potential of a Brazilian population of the predatory mite *Phytoseiulus longipes* as a biological control agent of *Tetranychus evansi* (Acari: Phytoseiidae, Tetranychidae). Biol. Control 42, 139–147.

Gallego, C.E., Aterrado, E.D., Batomalaque, C.G., 2003. Biology of the false spider mite, *Rarosiella cocosae* Rimando, infesting coconut palms in Camiguin, northern Mindanao (Philippines). Philipp. Entomol. 17, 187.

Galvão, A.S., Gondim Jr., M.G.C., Moraes, G.J., Oliveira, J.V., 2007. Biologia de *Amblyseius largoensis* (Muma) (Acari: Phytoseiidae), um potencial predador de *Aceria guerreronis* Keifer (Acari: Eriophyidae) em coqueiro. Neotrop. Entomol. 36, 465–470.

Gondim Jr., M.G.C., Castro, T.M.M.G., Marsaro Jr., A.L., Navia, D., Melo, J.W.S., Demite, P.R., Moraes, G.J., 2012. Can the red palm mite threaten the Amazon vegetation? Syst. Biodivers. 10, 527–535.

Gotoh, T., Gutierrez, J., Navajas, M., 1998. Molecular comparison of the sibling species *Tetranychus puercicola* Ehara and Gotoh and *T. urticae* Koch (Acari: Tetranychidae). Entomol. Sci. 1, 55–57.

Guindon, S., Dufayard, J.F., 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. Syst. Biol. 59, 307–321.

Hall, T.A., 1999. BIOEDIT: a user-friendly biological sequence alignment editor and analysis program for windows 95/98/NT. Nucleic Acids Symp. Ser. 41, 95–98.

Hillis, D.M., Dixon, M.T., 1991. Ribosomal DNA: molecular evolution and phylogenetic inference. Q. Rev. Biol. 66, 411–453.

Hoy, M.A., 2012. Overview of a classical biological control project directed against the red palm mite in Florida. Exp. Appl. Acarol. 57, 381–393.

Jeyaprakash, A., Hoy, M.A., 2002. Mitochondrial 12S rRNA sequences used to design a molecular ladder assay to identify six commercially available phytoseiids (Acari: Phytoseiidae). Biol. Control 25, 136–142.

Kanouh, M., Tixier, M.-S., Guichou, S., Kreiter, S., 2010. Two synonymy cases within the genus *Neoseiulella* (Acari: Phytoseiidae): is the molecular evidence so evident? Biol. J. Linn. Soc. 101, 323–344.

Kimura, M., 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide-sequences. J. Mol. Evol. 16, 111–120.

Librado, P., Rozas, J., 2009. DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25, 1451–1452.

Lindquist, E.E., Evans, G.O., 1965. Taxonomic concept in the Ascidae, with a modified setal nomenclature for the idiosoma of the Gamasina (Acari: Mesostigmata). Mem. Entomol. Soc. Can. 47, 1–64.

Mayr, E., Ashlock, P.D., 1991. Principles of Systematic Zoology, second ed. McGraw-Hill, New York.

McMurtry, J.A., Moraes, G.J., 1984. Some phytoseiid mites from the South Pacific, with descriptions of new species and a definition of the *Amblyseius largoensis* species group. Int. J. Acarol. 10, 27–37.

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

Moraes, G.J., McMurtry, J.A., 1981. Biology of *Amblyseius citrifolius* (Denmark and Muma) (Acarina – Phytoseiidae). Hilgardia 49, 1–29.

Moraes, G.J., McMurtry, J.A., 1985. Comparison of *Tetranychus evansi* and *T. urticae* (Acari: Tetranychidae) as prey for eight species of phytoseiid mites. Entomophaga 30, 393–397.

Moraes, G.J., Castro, T.M.M.G., Kreiter, S., Quilici, S., Gondim Jr., M.G.C., Sá, L.A.N., 2012. Search for natural enemies of *Raoiella indica* Hirst in Reunion Island (Indian Ocean). Acarologia 52, 129–134.

Moraes, G.J., 1987. Importance of taxonomy in biological control. Insect Sci. Appl. 8, 841–844.

Moraes, G.J., McMurtry, J.A., Denmark, H.A., Campos, C.B., 2004. A revised catalog of the mite family Phytoseiidae. Zootaxa 434, 1–494.

Murrel, A., Campbell, N.J.H., Barker, S.C., 2001. A total-evidence phylogeny of ticks provides insights into the evolution of life cycles and biogeography. Mol. Phylogenet. Evol. 21, 244–258.

Navajas, M., Fenton, B., 2000. The application of molecular markers in the study of diversity in Acarology: a review. Exp. Appl. Acarol. 24, 751–774.

Navajas, M., Lagnel, J., Fauvel, G., Moraes, G.J., 1999. Sequence variation of ribosomal Internal Transcribed Spacers (ITS) in commercially important Phytoseiidae mites. Exp. Appl. Acarol. 23, 851–859.

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

Navia, D., Marsaro Jr., A.L., Silva, F.R., Gondim Jr., M.G.C., Moraes, G.J., 2011. First report of the Red Palm Mite, *Raoiella indica* Hirst (Acari: Tenuipalpidae), in Brazil. Neotrop. Entomol. 40, 409–411.

Navia, D., Morais, E.G.F., Mendonça, R.S., Gondim Jr., M.G.C., 2014. Ácaro-vermelho-das-palmeiras, *Raoiella indica* Hirst (Prostigmata: Tenuipalpidae). In: Vilela, E., Zucchi, R.A. (Eds.), Pragas introduzidas no Brasil-insetos e ácaros. FEALQ, Piracicaba, in press.

Noronha, A.C.S., Moraes, G.J., 2004. Reproductive incompatibility between mite populations previously identified as *Euseius concordis* (Acari: Phytoseiidae). Exp. Appl. Acarol. 32, 271–279.

Noronha, A.C.S., Mota, A., Moraes, G.J., Coutinho, L.L., 2003. Caracterização molecular de populações de *Euseius citrifolius* Denmark and Muma *Euseius concordis* Chant (Acari: Phytoseiidea) utilizando o sequenciamento das regiões ITS1 e ITS2. Neotrop. Entomol. 32, 591–596.

Okassa, M., Tixier, M.-S., Cheval, B., Kreiter, S., 2009. Molecular and morphological evidence for new species status within the genus *Euseius* (Acari: Phytoseiidae). Can. J. Zool. 87, 689–698.

Okassa, M., Tixier, M.-S., Kreiter, S., 2010. Morphological and molecular diagnostics of *Phytoseiulus persimilis* and *Phytoseiulus macropilis* (Acari: Phytoseiidae). Exp. Appl. Acarol. 52, 291–303.

Okassa, M., Kreiter, S., Guichou, S., Tixier, M.-S., 2011. Molecular and morphological boundaries of the predator *Neoseiulus californicus* McGregor (Acari: Phytoseiidae). Biol. J. Linn. Soc. 104, 393–406.

Peña, J.E., Rodrigues, J.C.V., Roda, A., Carrillo, D., Osborne, L.S., 2009. Predator-prey dynamics and strategies for control of the red palm mite (*Raoiella indica*) (Acari: Tenuipalpidae) in areas of invasion in the Neotropics. Proceedings of the 2nd meeting of IOBC/WPRS, Integrated control of plant feeding mites. Florence, Italy, 9–12 March 2009, pp. 69–79.

Roda, A., Dowling, A., Welbourn, C., Peña, J.E., Rodrigues, J.C.V., Hoy, M.A., Ochoa, R., Duncan, R.A., De Chi, W., 2008. Red palm mite situation in the Caribbean and Florida. Proc. Caribbean Food Crops Soc. 44, 80–87.

Rodríguez, H., Ramos, M., 2000. Evaluation of rearing methods for *Amblyseius largoensis* (Muma) (Acari: Phytoseiidae) on *Polyphagotarsonemus latus* (Banks) (Acari: Tarsonemidae). Rev. Prot. Veg. 15, 105–108.

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

Rowell, H.J., Chant, D.A., Hansel, R.I.C., 1978. The discrimination of setal homologies and setal patterns on thedorsal shield in the family Phytoseiidae (Acarina: Mesostigmata). Can. Entomol. 110, 859–876.

SAS, 2005. SAS Institute, version 9.2 Inc., Cary.

Sato, Y., Mochizuki, M., Mochizuki, A., 2012. Introduction of non-native predatory mites for pest control and its risk assessment in Japan. Jpn. Agric. Res. Quart. 46, 129–137.

Staden, R., Beal, K.F., Bonfield, J.K., 1998. The Staden package. In: Misener, S., Krawetz, S.A. (Eds.), Computer Methods in Molecular Biology, 132. The Humana Press Inc., Totowa, NJ, USA, pp. 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. Mol. Biol. Evol. 10, 512–526.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., Kumar, S., 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance and maximum parsimony methods. Mol. Biol. Evol. 28, 2731–2739.

Taylor, B., Rahman, P.M., Murphy, S.T., Sudheendrakumar, V.V., 2012. Within-season dynamics of red palm mite (*Raoiella indica*) and phytoseiid predators on two host palm species in south-west India. Exp. Appl. Acarol. 57, 331–345.

Thompson, J.D., Higgins, D.G., Gibson, T.J., 1994. Clustal-W Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. 22, 4673–4680.

Tixier, M.-S., 2012. Statistical approaches for morphological continuous characters: a conceptual model applied to Phytoseiidae (Acari: Mesostigmata). Zool. Scr. 42, 327–334.

Tixier, M.-S., Ferrero, M., Okassa, M., Guichou, S., Kreiter, S., 2010. On the specific identity of specimens of *Phytoseiulus longipes* Evans (Mesostigmata: Phytoseiidae) showing different feeding behaviours: morphological and molecular analyses. Bull. Entomol. Res. 17, 1–11.

Tixier, M.-S., Hernandes-Akashi, F., Guichou, S., Kreiter, S., 2011a. The puzzle of DNA sequences of Phytoseiidae (Acari: Mesostigmata) in the public Genbank database. Invertebr. Syst. 25, 389–406.

Tixier, M.-S., Kreiter, S., Moraes, G.J., 2008. Biogeographic distribution of the mites of the family Phytoseiidae (Acari: Mesostigmata). Biol. J. Linn. Soc. 93, 845–856.

Tixier, M.-S., Kreiter, S., Ragusa, S., Cheval, B., 2006. The status of two criptic species: *Typhlodromus exhilaratus* Ragusa and *Typhlodromus phialatus* Athias-Henriot (Acari: Phytoseiidae): consequences for taxonomy. Zool. Scr. 37, 115–122.

Tixier, M.-S., Okassa, M., Kreiter, S., 2012. An integrative morphological and molecular diagnostics for *Typhlodromus pyri* (Acari: Phytoseiidae). Zool. Scr. 41, 68–78.

Tixier, M.-S., Tsolakis, H., Ragusa, S., Poinso, A., Ferrero, M., Okassa, M., Kreiter, S., 2011b. An integrative taxonomical approach demonstrates the synonymy between *Cydnodromus idaeus* and *C. picanus* (Acari: Phytoseiidae). Invertebr. Syst. 25, 273–281.

Tsolakis, H., Tixier, M.-S., Kreiter, S., Ragusa, S., 2012. The concept of genus within family Phytoseiidae (Acari: Parasitiformes): historical review and phylogenetic analyses of the genus Neoseiulus Hughes. Zool. J. Linn. Soc.-Lond. 165, 253–273.

Uesugi, R., Kunimoto, Y., Osakabe, M., 2009. The fine-scale genetic structure of the two-spotted spider mite in a commercial greenhouse. Exp. Appl. Acarol. 47, 99–109.

Vazquez, C., Quirós, G.M., Aponte, O., Sandoval, D.M.F., 2008. First report of *Raoiella indica* Hirst (Acari: Tenuipalpidae) in South America. Neotrop. Entomol. 37, 739–740.

Yaninek, J.S., Onzo, A., Ojo, J.B., 1993. Continent-wide releases of Neotropical phytoseiids against the exotic cassava green mite in Africa. Exp. Appl. Acarol. 17, 145–160.

Zannou, I.D., Negloh, K., Hanna, R., Houadakpode, S., Sabelis, M.W., 2010. Mite diversity in coconut habitat inWest and East Africa. In: XIII International Congress of Acarology, Recife, Brazil, Abstract book, p. 295.