



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 Moraes^d, Marie-Stéphane Tixier^e, Manoel G.C. Gondim Jr.^b

^a Embrapa Recursos Genéticos e Biotecnologia, Cx. Postal 02372, 70.770-900 Brasília, 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

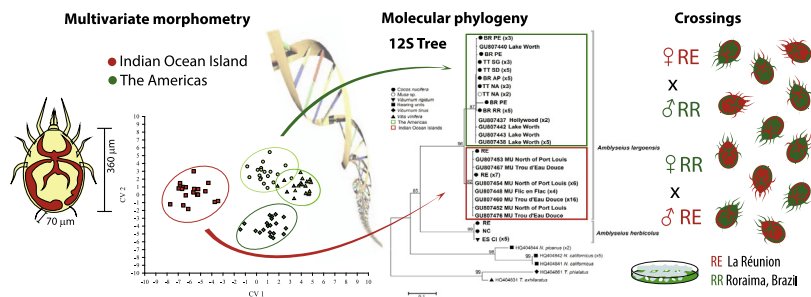
^d Embrapa Roraima, Laboratório de Entomologia, 69301-970 Boa Vista, Brazil

^e Montpellier SupAgro, UMR CBGP, INRA/IRD/CIRAD, Campus International de Baillarguet, Montpellier 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 length.
- *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



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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 mitochondrial 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).

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.

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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 Hoyer's 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

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