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Original article

Characterization of mirid assemblages (Heteroptera, Miridae) in mango orchards in Reunion Island and implementation of identification and recognition tools

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Summary

Introduction – **Mango (*Mangifera indica* L.) is the second most important tropical fruit produced worldwide. In Reunion Island, serious losses are the result of damage caused by *Orthops palus* (Taylor, 1947) (Heteroptera, Miridae). This bug sucks the sap of mango inflorescences, causing them to dry out. However, little is known about this pest and the other mirid species present in mango orchards. The aim of the present study was to characterize the mirid species assemblages in mango orchards in Reunion Island and to design tools for the taxonomic identification and for the recognition of these species in the field.** *Materials and methods* – **Surveys were conducted in 14 mango orchards (var. José and Cogshall) between 2012 and 2014. Observations were made in the canopy and in ground cover. The individuals collected were identified to species level.** *Results and discussion* – **Thirteen species were inventoried of the 1695 individuals collected and identified. *Orthops palus* was the dominant species on mango (more than 80% of the individuals collected) and the only species present in significant numbers on inflorescences. In addition, three mirid identification and recognition tools were implemented: a taxonomic identification key to species level; Cytochrome c oxidase subunit I (COI) sequences of 12 of 13 catalogued species, published on GenBank and available for molecular identification of species; a flyer for mirid recognition in the field designed for farmers.** *Conclusion* – **These results and tools help assess mirid diversity in mango orchards and to develop agroecological management of *O. palus* populations.**

Keywords
Reunion Island, mango, *Mangifera indica*, plant bug, *Orthops palus*, identification key

Résumé

Caractérisation de la communauté des mirides (Heteroptera, Miridae) dans les vergers de manguiers de l'île de La Réunion et mise en place des outils d'identification et de reconnaissance.

Introduction – **La mangue (*Mangifera indica* L.) est la deuxième production fruitière tropicale mondiale. A La Réunion, d'importantes pertes de récolte sont provoquées par une punaise *Orthops palus* (Taylor, 1947) (Heteroptera, Miridae) , qui pique et dessèche les inflorescences des manguiers. Néanmoins, peu de connaissances sont disponibles sur ce ravageur et sur les autres espèces de mirides présentes dans les vergers de manguiers. La présente étude vise à caractériser la communauté des mirides présentes dans les vergers de manguiers à La Réunion et de proposer des outils d'aide à l'identification taxonomique et la reconnaissance sur le terrain de ces espèces.** *Matériel et méthodes* – **Les collectes ont été effectuées dans 14 vergers de manguiers (var. José et Cogshall) de 2012 à 2014. Les observations ont été réalisées dans les arbres et dans la couverture végétale du sol. Les mirides collectées ont été identifiées au niveau de l'espèce.** *Résultats et discussion* – **Sur les 1695 individus collectés et identifiés, treize espèces de mirides ont été recensées. *Orthops palus* est l'espèce la plus importante sur les arbres (plus de 80% des individus observés) et la seule espèce de mirides présente en nombre significatif sur les inflorescences de manguier. De plus, trois outils d'identification et de reconnaissance des mirides des vergers de manguiers ont été mis au point: une clé taxonomique pour l'identification jusqu'à l'espèce; les séquences de la sous-unité I de l'oxydase du Cytochrome c (COI) pour 12 des 13 espèces inventoriées, publiées sur GenBank et disponibles pour une identification moléculaire; une fiche**

Significance of this study

What is already known on this subject?

- In Reunion Island, each year mango inflorescences suffer severe damage attributed to plant bugs, particularly *Orthops palus*. However, this species is not clearly identified in field and its relative importance in mirid species assemblages in mango orchards is unknown.

What are the new findings?

- Mirid assemblages in mango orchards of Reunion Island is composed of 13 species which is now molecularly and morphologically identified.

What is the expected impact on horticulture?

- This work promotes to develop agroecological management of mirid populations in mango orchards to obtain a healthy and environmentally-friendly agriculture.

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de reconnaissance des mirides sur le terrain pour les agriculteurs. *Conclusion* **– Ces résultats et ces outils permettent de mieux connaître la diversité des mirides présentes en vergers de manguiers et d'envisager la mise au point de méthodes de gestion agro-écologique des populations d'*O. palus*.**

Mots-clés
La Réunion, mangue, *Mangifera indica*, punaise, *Orthops palus*, clé d'identification

Introduction

Mirids (Hemiptera: Heteroptera: Cimicomorpha), or plant bugs, are one of the most diverse and species-rich families of insects, with approximately 11,000 species described to date (Cassis and Schuh, 2012). They are present in all major biogeographic regions of the world (Schuh, 1995, 2008) and abundant in a variety of habitats (Ehanno, 1958). The majority of species in this family are phytophagous and many of them have an economic impact on crops; some species are zoophagous and zoophytophagous (Sweetman, 1958). These characteristics are found in the tropics (Atiama *et al*., 2016).

Mango (*Mangifera indica* L.) is the second most important tropical fruit produced worldwide (FAO STAT, 2016) and it is the fifth most important in Reunion Island (385 cultivated hectares divided between about 150 farms) (Agreste DAAF, 2014). Mango inflorescences suffer severe damage each year and used to be attributed to a plant bug originally identified as *Taylorilygus palus* (Agreste DAAF, 2014), described for the first time from Uganda (Taylor, 1947) in the genus *Lygus*. Now considered as the most important mango pest in Reunion Island, this species has been recognized as *Orthops palus* (Atiama *et al*., 2016). This pest is controlled by chemical treatments which give unsatisfactory results (Deguine *et al*., 2014). Apart from this pest, there are other species of mirids in mango orchards in Reunion. Mirid species have been poorly studied on Reunion Island and in the Malagasy region. Indeed, only 17 species have been reported in Reunion's agroecosystem (Kirkaldy, 1902; Vayssières *et al*., 2001) and the Miridae assemblage in mango orchards has never been studied in this island or indeed the world. Therefore, this study will aid in characterizing the mirid fauna in Reunion Island.

The identification of mirid species is not easy (Wheeler, 2001). Colors are not good criteria to differentiate species and genitalia dissection, which is complex, is often needed (Wheeler, 2001; Schuh and Slater, 1995). Identification tools use identification keys (Eyles, 1999) or molecular techniques involving Cytochrome c oxidase subunit I (COI) sequences for adults and nymphs and aid in the identification of cryptic species (Raupach *et al*., 2014; Jung *et al*., 2011; Park *et al*., 2011). These techniques are not available for mirid species in Reunion. In the fields, farmers and agricultural technicians often have difficulty recognizing mirids species and have asked for tools to help them.

This study aims to improve knowledge on mango orchard mirids, with the aim of developing agroecological management of their populations. The first aim of the study was to inventory and characterize mirid assemblages in mango orchards (in the mango canopy itself, on the ground and in border vegetation) and to ascertain if *O. palus* is the only mirid species to be a pest of mango inflorescences during the flowering season. The second aim of the study was to develop and implement tools to identify and recognize mirid species: a taxonomic identification key to species level; reference COI sequences; mirid recognition flyers to be used in the field.

Materials and methods

Ecological context and sampling sites

Reunion Island (55°39'E, 21°00'S) is a sub-tropical island situated in the western Indian Ocean (Figure 1). This island of 2,512 km^2 is located 1,200 km off the African continent and 800 km from Madagascar. Generally, the island has a tropical climate with a hot and humid season from November to April (sea level temperature: 22.6 to 30.0 °C) and a cooler, drier season from June to September (17.7 to 25.8 °C) (Raunet, 1991). However, Reunion Island is characterized by a diversity of microclimates due to a wide range of elevations with high mountains (highest point: 3,070 m), and a windward east coast and leeward west coast. These winds induce contrasted rainfalls between the east (annual rainfall: 2,000 to 5,000 mm) and west coasts (500 to 1,500 mm) (Raunet, 1991). Today, commercial mango orchards cover more than 300 ha and have expanded rapidly over the last thirty years (50 ha in 1970). West and southwest coasts are the most suitable area for mango cultivation (Vincenot and Normand, 2009). The two mango varieties commercially produced (*Mangifera indica* L. var. José and Cogshall) flower from July to October.

Surveys were conducted in 14 insecticide-free mango orchards in the production areas (Figure 1; Table 1). These orchards are part of the "Biophyto" network, a project starting in 2011 which aims to produce insecticide-free mango in Reunion Island (Deguine *et al*., 2015).

Mirid collection

In order to inventory the mirid species present during the year in the orchards, sampling was carried out every two weeks for a period of three years in each of the 14 orchards. Three different habitats were sampled: inflorescences in the canopy; the ground cover and shrubs bordering the orchard. Sampling was done via suction (modified leaf blower, STIHL BG56, Stihl, Dieburg, Germany). Three suctions of 30 sec duration each were conducted on each sample in three habitats.

In order to characterize the relative importance of the mirid species in the trees during the flowering season, collections were carried out once during the flowering season in August. They were conducted in the 14 orchards over a period of three years. Collections were performed between 7:00 am and noon in order to collect as many mirids as possible. Sampling was carried out using the same technique as for inventories. We sucked mirids from mango inflorescences at the four cardinal points of each tree during a total of 10 sec. The number of trees sampled per orchard was a function of the size of the plot (4 to 10 trees per orchard). Two criteria for analyzing the relative importance of species were considered: (i) the proportion of each species collected; and (ii) the proportion of each species per tree infested by at least one individual. This last criterion is to consider the fact that the distribution of mirid populations in orchards is not always homogeneous and may be aggregative, as seen in other species of mirid (Babin *et al*., 2010).

Identification and recognition tools

To create an identification key for mirid species present in orchards, taxonomic criteria are morphological (body shape, presence and shape of the spots, color of antenna

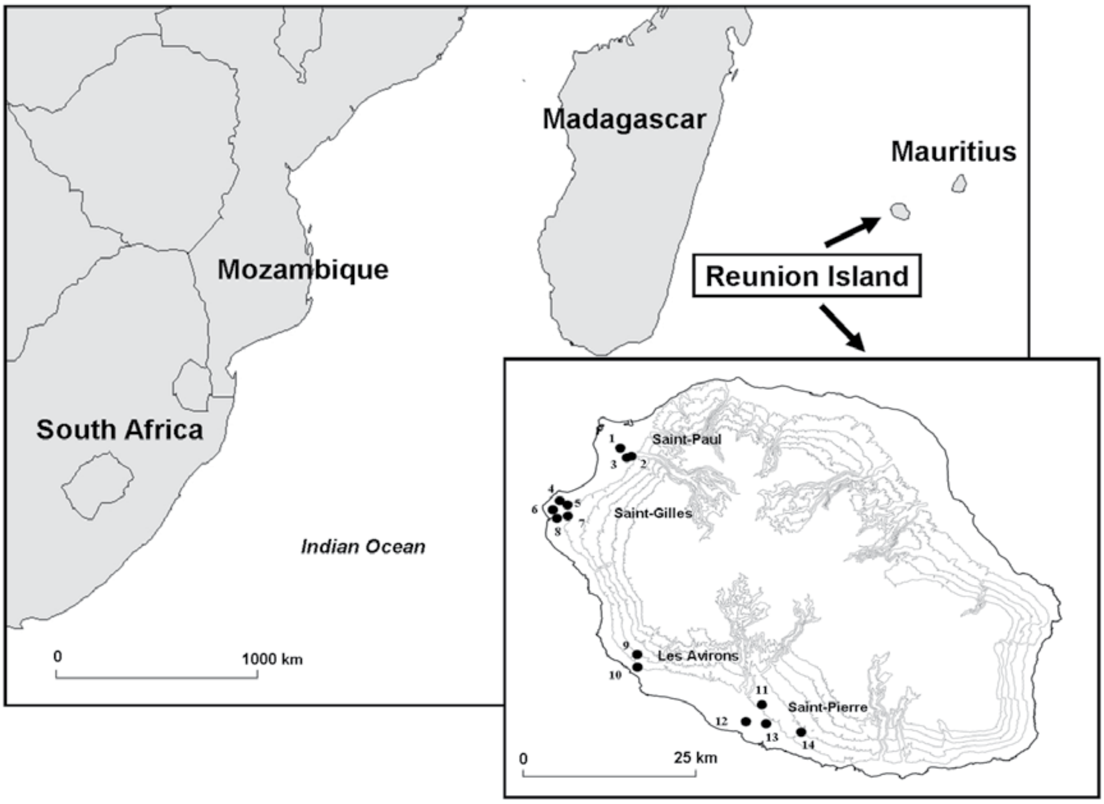

Figure 1. Maps of South West Indian Ocean and Reunion Island (with the location of the 14 mango orchards surveyed in 2012, 2013 and 2014). Each number corresponds to a mango orchard.

segments) which are widely used for the identification of mirids in general. The anatomical terminology used is that of Schuh and Slater (1995). One or two individuals of each species were deposited in the CIRAD-UMR PVBMT collection (CIRAD, Saint-Pierre, Reunion Island). Each individual was identified by a collection number. Voucher specimens, conserved in ethanol, are available upon request. Photographs were realized with a macroscope Nikon AZ100. Drawings (Figure 4a-d) have been made by one of the authors (TR) with the software Inkscape 0.91 based on pictures.

Table 1. Geographical characterization of the 14 mango orchards.

Orchard number	Localities	GPS coordinates	
		Longitude (E)	Latitude (N)
1	Saint-Paul	55°18’36”375	-20°58’02”815
2	Saint-Paul	55°19’13”128	-20°58’28”441
3	Saint-Paul	55°19’25”532	-20°58’29”458
4	Saint-Gilles	55°14’05”043	-21°01’47”555
5	Saint-Gilles	55°14’15”000	-21°02’00”591
6	Saint-Gilles	55°13’36”937	-21°02’25”297
7	Saint-Gilles	55°14’24”282	-21°02’16”205
8	Saint-Gilles	55°13’57”385	-21°02’41”121
9	Les Avirons	55°19’51”592	-21°14’21”951
10	Etang-Salé	55°19’54”463	-21°14’57”777
11	Saint-Pierre	55°29’09”743	-21°18’19”181
12	Saint-Pierre	55°27’39”446	-21°19’14”609
13	Saint-Pierre	55°29’17”950	-21°19’21”582
14	Saint-Pierre	55°31’44”451	-21°20’00”985

To obtain the COI sequences, genomic DNA was extracted with the DNeasy Tissue Kit (QIAGEN, Hilden, Germany) from one to three individuals per species. Miridae were not crushed before extraction to enable intact vouchers to be kept for collection and morphological examination. The COI sequence of each species was obtained with a COI primer cocktail for Hemiptera containing: LCO1490puc -t1 (TGTAAAACGACGGCCAGTTTTCAACWAATCATAAAGATATTGG) (Cruaud *et al.*, 2010), LCO1490Hem1-t1(TGTAAAACGACGGCCAGTTTTCAACTAAYCATAARGATATYGG) (Germain *et al.*, 2013), HCO2198puc-t1 (CAGGAAACAGCTATGACTAAACTTCWGGRTGWCCAAARAATCA) (Cruaud *et al.*, 2010), HCO2198Hem1-t1 (CAGGAAACAGCTATGACTAAACYTCDGGATGBCCAAARAATCA) (Germain *et al.*, 2013) and HCO2198Hem2-t1 (CAGGAAACAGCTATGACTAAACYTCAGGATGACCAAAAAAYCA) (Germain *et al.*, 2013) (the PCR program used was 3 min at 95 °C, 5 × [30 sec at 95 °C, 40 sec at 45 °C, 1 min at 72°C], 35 × [30 sec at 95 °C, 40 sec at 51 °C, 1 min at 72 °C], 10 min at 72 °C). Cycle sequencing reactions were performed using the primers M13F (TGTAAAACGACGGCCAGT) and M13R (CAGGAAACAG -CTATGAC) in separate reactions. These primers were used to amplify a 658 bp. region of Cytochrome c oxidase subunit I. *Orthops palus* individuals were also tested with other primers (LepF2_t1 and LepR1) (Park *et al.*, 2011) (PCR program: 5 min/94 °C; 35 cycles [30 s/94 °C, 1 min 30 s/45 °C, 1 min/72 °C]; 7 min/72 °C) which amplify the same sequence. PCR products were sent to Macrogen© for standard sequencing. COI sequences were analyzed by MEGA 6 (Tamura *et al.*, 2013) and the resulting sequences were deposited in GenBank. The BLAST tool from NCBI was used to look for similarities between our sequence

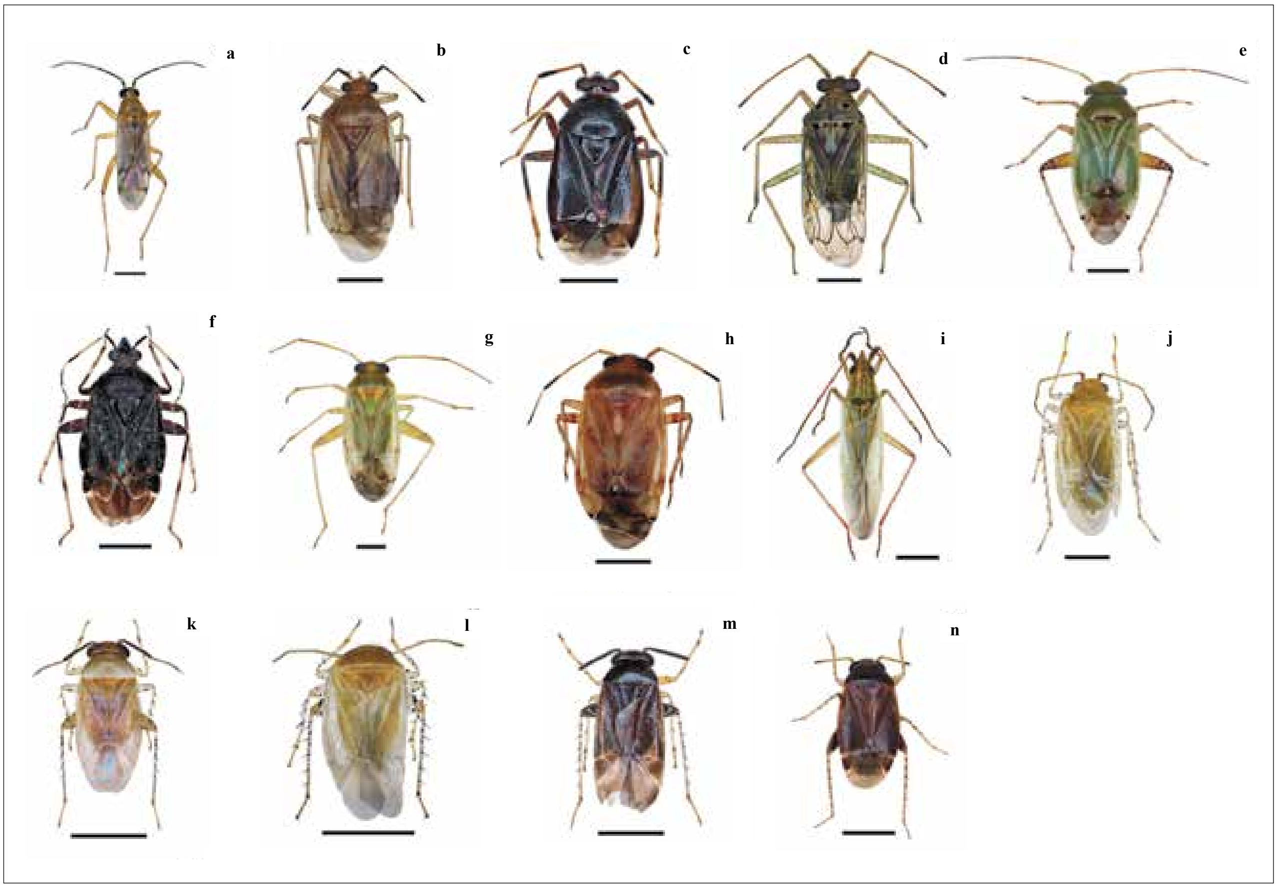

FIGURE 2. Miridae species collected in mango orchards in Reunion Island (2012–2014), dorsal view. Scale bar = 1 mm. (a) *Nesidiocoris volucer*; (b) *Deraeocoris indianus*; (c) *Deraeocoris* cf. *howanus*; (d) *Corizidolon notaticolle*; (e) *Orthops palus*; (f) *Proboscidocoris* sp.; (g) *Taylorilygus apicalis*; (h) *Taylorilygus* cf. *entadae*; (i) *Trigonotylus tenuis*; (j) *Moissonia importunitas*; (k) *Campylomma* cf. *plantarum* ♂; (l) *Campylomma* cf. *plantarum* ♀; (m) *Campylomma leucochila*; (n) *Campylomma* spA.

dataset and sequences already published in GenBank.

An illustrated recognition guide with pictures of adults of each species was created using the inventoried species and taxonomic key. It contains comments and aims to be a familiarization and field reconnaissance tool for farmers.

Results and discussion

An original study

Numerous studies have focused on larger groups of insects like Hemiptera (Tara *et al.*, 2014) or inventory insects and spiders on mango (Chin *et al.*, 2007). Some studies focused on mirids in different crops [fruit crops in Canada (Kelton, 1982)] and some studies on mango and mirids did not inventory all mirids present in mango orchards. For example, one study focused on mirids associated with a Lepidoptera, *Orthaga exvinacea,* which is a pest of mango (Rafeeq and Ranjini, 2013). Another study focused on a mirid species that is a pest of mango (Peng and Christian, 2008). But, to our knowledge, our study is the first to tackle the whole mirid complex in mango orchards.

Thirteen mirid species were inventoried in mango orchards

During the year, a total of 598 mirids bugs were collected. Thirteen species [*Corizidolon notaticolle* Reuter, 1907; *Trig-*

TABLE 2. Miridae species composition in the mango canopy on La Reunion island. Sampling was carried out with a suction device on mango inflorescences over a period of 3 years (2012–2014) (*n* = 350 trees sampled, SD: Standard deviation).

Species	Number of collected specimens per species	Proportion of species in the samples (in %)	Average proportion (in %) of species on trees infested by at least one mirid (SD)
Campylomma leucochila	103	9.39	17.26 (2.63)
Campylomma cf. *plantarum*	63	5.74	7.56 (1.82)
Corizidolon notaticolle	8	0.73	2.95 (1.27)
Deraeocoris cf. *howanus*	5	0.46	1.25 (0.6)
Orthops palus	913	83.23	69.88 (3.30)
Taylorilygus apicalis	1	0.09	0.23 (0.23)
Trigonotylus tenuis	4	0.36	0.28 (0.21)

onotylus tenuis Reuter, 1893; *Nesidiocoris volucer* Kirkaldy, 1902; *Proboscidocoris* sp., *Deraeocoris indianus* Carvalho, 1957; *Deraeocoris* cf. *howanus* Poppius, 1912; *Campylomma leucochila* (Reuter, 1905); *Campylomma* spA., *Campylomma* cf. *plantarum* Lindberg, 1958; *Taylorilygus* cf. *entadae* (Taylor, 1947)*; Orthops palus, Taylorilygus apicalis* (Fieber, 1861)*; Moissonia importunitas* (Distant, 1910)] were inventoried and eight genera were identified (Figure 2). Three species appear to be new to Reunion Island (*Campylomma* cf. *plantarum*, *Deraeocoris* cf. *howanus*, *Taylorilygus* cf. *entadae*). Several species were encountered frequently, while others were found only once or twice, with only a few specimens each time. Several mirid species known to inhabit the island were not encountered during this survey: *Creontiades pallidus* (Rambur, 1839); *Deraeocoris ostentans* (Stål, 1855); *Eurystylus bellevoyei* (Reuter, 1879); *Moissonia nigropunctata* (Poppius, 1910); *Nabidomiris clypealis* Poppius, 1914; *Nesidiocoris tenuis* (Reuter, 1895); *Proboscidocoris punctaticollis* Reuter, 1905 (Vayssières *et al*., 2001).

Orthops palus was the only significant mirid on mango inflorescence during the flowering season

A total of 350 trees were sampled and 1,097 mirids were collected. Seven of the 13 species were observed on mango inflorescences during the flowering season (Table 2). Only three species were present in significant numbers, the other four representing only 1.6% of the collected mirids. *Orthops palus* represents by far the most abundant species with more than 83% of total mirids collected and 70% of mirids captured on infested trees. It makes sense to hypothesize that *O. palus* is the only mirid pest of mango inflorescences of significant importance in Reunion. Two other species were found on mango inflorescences: *C. leucochila,* (9%) and *C.* cf. *plantarum* (6%). *C. leucochila* is often described as a zoophagous mirid (Cadou, 1994; Malipatil, 1992). It cannot therefore be considered as a mango pest. However, even though its population levels remain low (less than 10% of collections), we cannot rule out the possibility that *C.* cf. *plantarum* causes damage to mango inflorescences for two reasons: on one hand, this species morphologically resembles *O. palus* and thus may have been the subject of confusion in the past in Reunion; secondly, it is known to be phytophagous (Lindberg, 1958; Odhiambo, 1960). Furthermore, other phytophagous species should be monitored carefully such as *Moissonia importunitas* which was only found in the ground cover, but it is an important pest of *Crotalaria* species in India (Banerjee and Kakoti, 1969), Thailand (Yasunaga, 2010) and recently in Reunion Island with a presence in several localities (Ratnadass *et al*., 2017).

1 Species with two round black spots and a distinct color pattern on the pronotum (Figure 2.d)........ ***Corizidolon notaticolle***
Species without the two spots and peculiar color pattern on the pronotum........ **2**
2 Species with a longitudinal white line on the pronotum and scutellum (Figure 2.i)........ ***Trigonotylus tenuis***
Species without the white line on the pronotum and scutellum........ **3**
3 Species with a blackish spot at the apex of both cuneus and cuneal fracture (Figure 2.a and Figure 4.a)........ ***Nesidiocoris volucer***
Species with a blackish spot only at the apex of the cuneus or without a blackish spot in either place (Figure 2.e; 2.f, 4.b)........ **4**
4 Species with whitish hairs scattered in patches (Figure 2.f)........ ***Proboscidocoris* sp.**
Species with different pilosity........ **5**
5 Yellow species with a longitudinal broad black line along the claval commissure (Figure 2.b)........ ***Deraeocoris indianus***
Species without a black line along the claval commissure........ **6**
6 Brownish yellow to black species, glabrous, in which the apex of the 2nd antennal segment is broadened (Figure 2.c)........ ***Deraeocoris* cf. *howanus***
Species without the apex of the 2nd antennal segment broadened........ **7**
7 Dark colored species, reddish black or black (Figure 2.m; 2.n)........ **8**
Light colored species, yellow, brownish yellow or light green (Figure 2.e; 2.g; 2.j; 2.k; 2.l)........ **9**
8 Black species; whole cuneus dark; the first and second antennal segments black (Figure 2.m)........ ***Campylomma leucochila***
Reddish black species; a dark cuneus with a whitish base; first and second antennal ..segments dark yellow and the last third of the 2nd segment black (Figure 2.n)........ ***Campylomma* spA.**
9 Second antennal segment black (Figure 2.k)........ ***Campylomma* cf. *plantarum* (♂)**
Second antennal segment mostly yellow (Figure 2.e; 2.g, 2.h, 2.j)........ **10**
10 Apical fourth of the 2nd antennal segment black (Figure 2.h)........ ***Taylorilygus* cf *entadae***
Apical fourth of the 2nd antennal segment yellow........ **11**
11 Femur III with red to brown stripes on the apical third (Figure 2.e; 2.g)........ **12**
Femur III with black spots at least on the apical third (Figure 4.c; 4.d)........ **13**
12 Species with at most a dark spot at the apex of the corium (Figure 2.e)........ ***Orthops palus***
Species with darken areas on the clavus, the corium and the embolium (Figure 2.g; 4.g)........ ***Taylorilygus apicalis***
13 Femur III swollen, three times longer than broad; characteristic pattern of black spots (Figure 4.c)........ ***Campylomma* cf. *plantarum* (♀)**
Femur III thinner, more than four times longer than broad; characteristic pattern of black spots (Figure 4.d)........ ***Moissonia importunitas***

Figure 3. Taxonomic key of mirid species of mango orchards of Reunion Island.

A taxonomic key was implemented for the identification of mirids

A key was developed to aid in the taxonomic identification of 13 species of mirid found in mango orchards in Reunion (Figures 3 and 4). Identification to species level was not possible for all the morphospecies in the Malagasy region due to a lack of knowledge of Miridae taxonomy (Legros *et al*., 2016). The effectiveness of the proposed taxonomic key was tested in laboratory conditions by 15 technicians, engineers and researchers with different levels of entomological

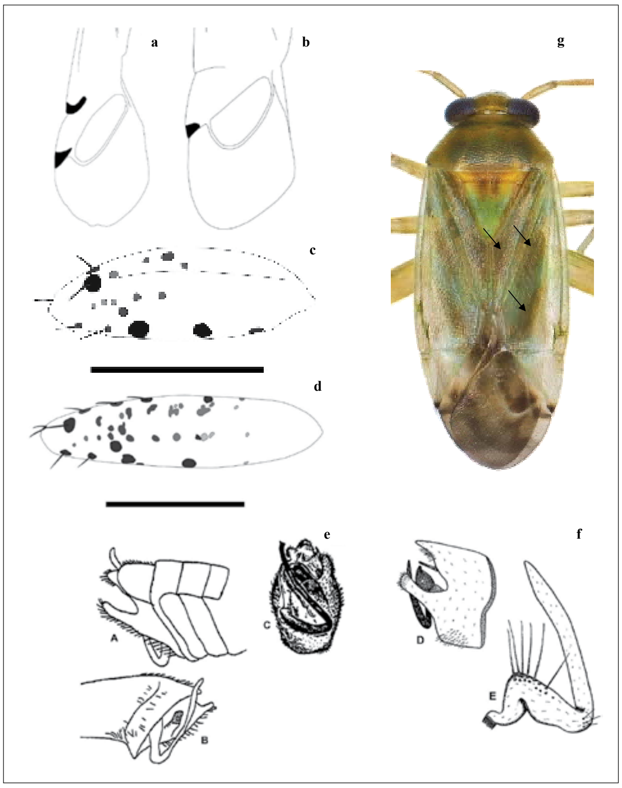

Figure 4. Anatomical characteristics used to identify mirid species of mango orchard of Reunion Island. Scale bar = 0.5 mm. Apex of left hemelytra: *Nesidiocoris volucer* (a); *Orthops palus* (b); Right femur III: *Campylomma* cf. *plantarum* (c); *Moissonia importunitas* (d); *Nesidiocoris volucer* apex of the abdomen (♂) (e) seen from the right (A), from the left (B) and from below (C) (after Lindberg, 1958); *Nesidiocoris tenuis* ♂ pygophore (f) (D) and left clasper (E) (after Carvalho, 1956); (g) Zoom on dorsal view of *Taylorilygus apicalis,* arrows show typical dark areas. The drawings (a), (b), (c) and (d) are original production from R. Thibault.

Table 3. Collection and GenBank information for Miridae species collected in mango orchards in Reunion Island. Collection numbers of the specimen sequenced (reference collection of CIRAD-UMR PVBMT), GenBank accession numbers of partial COI with first primers and second primers (in bold, first published sequences for the species).

Species	Location of sample	Collection number	Sequence size (bp)	GenBank accession number
Deraeocoris cf *howanus* (Poppius, 1912)	Etang-Salé; Maniron/Lambert	MATI00001_0101	612	KT201348
	Saint-Gilles	MATI00002_0101	658	KT201349
Campylomma leucochila (Reuter, 1905)	Saint-Paul	MATI00004_0101	612	KT201382
	Saint-Paul; Trois Roches	MATI00005_0101	487	KT201381
Deraeocoris indianus (Carvalho, 1957)	Le Tampon	MATI00014_0101	658	KT201351
Proboscidocoris sp. (Reuter, 1882)	Saint-Pierre	MATI00016_0101	637	KT201352
Taylorilygus apicalis (Fieber, 1861)	Saint-Pierre; Ligne Paradis	MATI00018_0101	658	KT201353
	Saint-Pierre; Ligne Paradis	MATI00020_0101	661	KT201354
Moissonia importunitas (Distant, 1910)	Saint-Gilles	MATI00022_0101	658	KT201355
	Saint-Pierre	MATI00023_0101	451	KT201356
Campylomma cf *plantarum* (Lindberg, 1958)	Saint-Gilles	MATI00024_0101	608	KT201357
Campylomma spA.	Etang-Salé	MATI00028_0101	582	KT201358
Trigonotylus tenuis (Reuter, 1893)	Saint-Gilles	MATI00034_0101	658	KT201359
	Saint-Gilles	MATI00036_0101	658	KT201361
Nesidiocoris volucer (Kirkaldy, 1902)	Saint-Pierre; Ligne Paradis*	MATI00035_0101	658	KT201360
	Saint-Pierre; Ligne Paradis*	MATI00012_0101	655	KT201350
Orthops palus (Taylor, 1947)	Avirons; Ravine Ruisseau	MATI00039_0101	588	KT201362
	Saint-Philippe	MATI00040_0101	655	KT201363
	Saint-Philippe	MATI00040_0102	655	KT201364
	Saint-Gilles	MATI00041_0101	661	KT201365
	Saint-Gilles	MATI00041_0102	655	KT201366
Corizidolon notaticolle (Reuter, 1907)	Saint-Pierre	MATI00042_0101	587	KT201367
	Saint-Pierre	MATI00042_0102	655	KT201368
	Saint-Pierre	MATI00042_0103	636	KT201369
Taylorilygus cf. *entadae* (Taylor, 1947)	Saint-Pierre	MATI00033_0101	-	-

(-) means that no sequence was obtained.

* DNA extracted from samples from mango orchards was of poor quality, samples of *Nesidiocoris volucer* coming from tomato (*Solanum lycopersicum* L.) were used.

knowledge who used the key to identify and level rank 10 specimens. The difficulties encountered and the questionnaire filled in by each participant helped us improve and validate the key. Even if *Nesidiocoris tenuis* (Reuter, 1895) was not collected in mango orchards, on the contrary of *N. volucer*, it may be present and may act as an auxiliary insect. *N. tenuis* is not included in the taxonomic key but both species can be distinguished by the shape of their left clasper (Figures 4eA-C and 4fD-E).

COI sequences were published for molecular identification of mirids

COI sequences of mirid species (451 bp to 661 bp) were obtained for 12 of the 13 described species (with the exception of *Taylorilygus* cf. *entadae* – failed sequencing) (Table 3). These are the first published COI sequences for 11 of the 13 species described (with the exceptions of *Taylorilygus* cf. *entadae* (failed sequencing) and *Taylorilygus apicalis* (COI sequence already available). All the sequences were submitted to GenBank (accession number KT201348 to KT201382) and they now can be used for molecular identification (Table 3). Sequences of *Orthops palus* obtained with LepF2t1 and LepR1 were also submitted to GenBank (accession number KT201370 to KT201380). COI sequences are very useful in the case of doubt on any identification, for nymph identification (easier to sample) and for customs to prevent pest species from entering a country.

A field card was proposed to farmers for mirid recognition

An illustrated field card was also produced to help farmers recognize adults of the different species (Figure 5) as the same way that Muller *et al.* (2003) produced a species recognition guide for the *Lygus* genus in crops in California. The card is A4-sized, double-sided, laminated and illustrated with pictures of the relative size of each species. The front of the card shows three species commonly encountered on mango inflorescences: *O. palus*, *C. leucochila* and *C.* cf. *plantarum*. The back shows the 13 mirid species found in orchards accompanied with comments on their densities and locations where information is available. Hundreds of copies were printed and distributed to producers and technicians in the mango industry by the Reunion Chamber of Agriculture (organization in charge of training and transfer to farmers). The field cards were particularly popular with users and were also sent to producers of other crops (*e.g.*, citrus fruits, in which some mirids species can be observed). This card helps producers understand the biotic community in orchards and better take into consideration certain beneficial species.

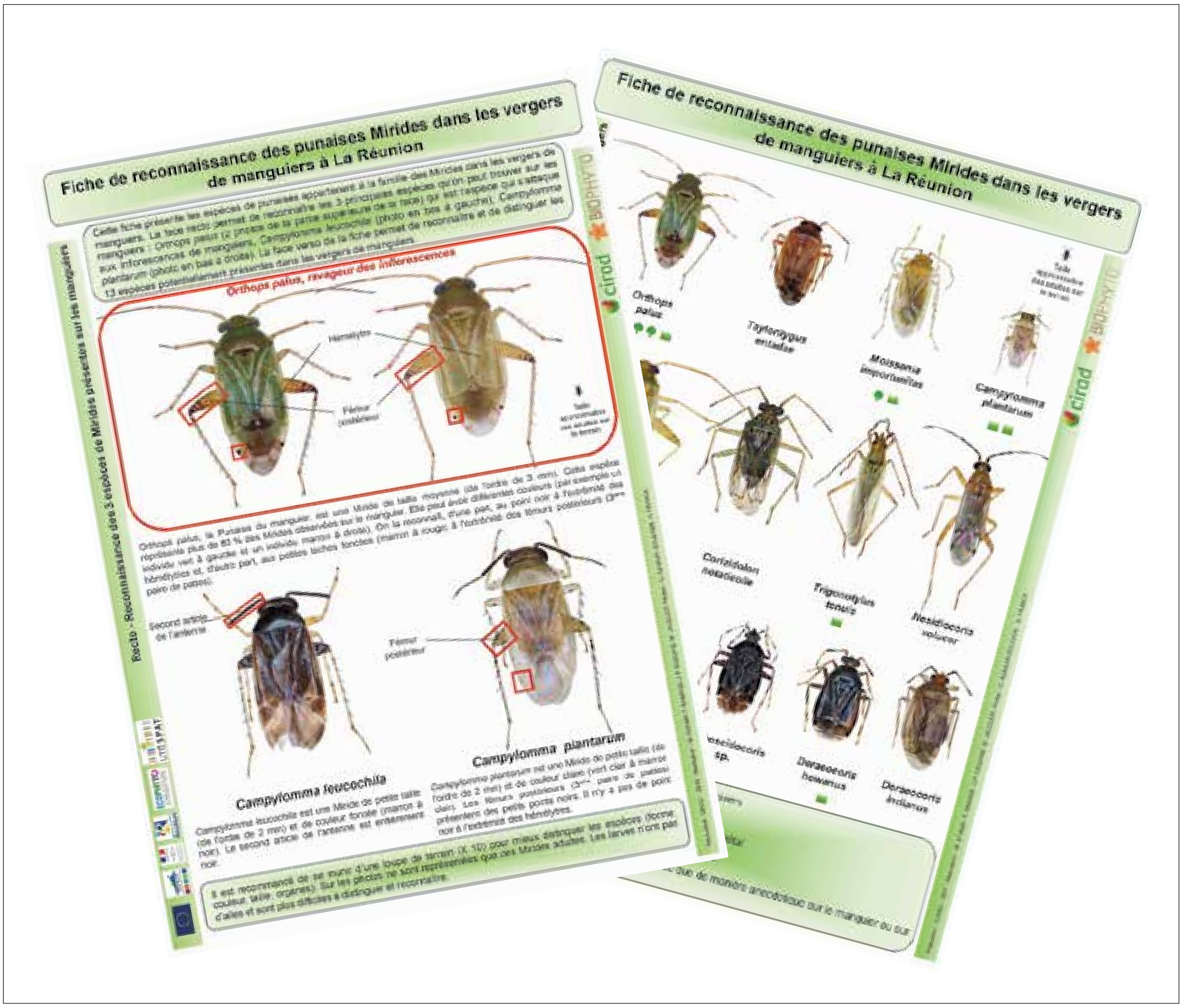

Figure 5. Field cards of mirid recognition in mango orchards. The front of the card shows three species commonly encountered on mango inflorescences: *O. palus*, *C. leucochila* and *C. plantarum*. The back shows the 13 mirid species found in orchards accompanied with comments on their densities and locations where information is available.

Applications and perspectives

Given the results of this study, on an applied level, protection efforts should concentrate on *O. palus*, the species primarily present on the mango inflorescences during flowering. Today there are intervention thresholds used when to decide whether or not to use chemical control, but this solution is not entirely satisfactory and should no longer be prioritized in agricultural policies. An agro-ecological approach is now a priority for the management of mango orchards and several strategies are currently under discussion. Three strategies have great potential: (i) use of trap plants in the framework of the push-pull technique (Bensen and Temple, 2008; Swezey et al., 2007, 2013); (ii) the use of entomopathogenic fungi such as *Beauveria bassiana* (McGuire *et al*., 2006; Portilla *et al*., 2014); (iii) the use of bio-insecticide spot treatments on trees whose population of *O. palus* is above a defined threshold.

Further studies will focus on determining the status of mirids present in mango orchards: diet (phytophagy, zoophagy, zoophytophagy) and functional role (pest, beneficial). In addition, it is essential in the future to study the status of *C.* cf. *plantarum* in Reunion mango orchards, especially as a number of *Campylomma* species are pests of fruit crops in some countries, like *C. verbasci* for apple (Reding *et al*., 2001; Kain and Agnello, 2013) and more recently *C. austrina* in mango orchards in the Northern Territories in Australia (Peng and Christian, 2008). Moreover, interspecific interactions (competition, predation) between the most abundant species on flowers (*O. palus* and *C. leucochila*) should be studied. Finally, understanding the movements of the different species between the canopy and ground vegetation represents a further area of study.

Conclusion

Thirteen morphospecies of mirids were inventoried in mango orchards in Reunion Island and two tools (a taxonomic key and COI sequences) are now available for species identification. Furthermore, the field card we developed will help farmers to better recognize mirids directly in the field. In the mirid assemblages in mango orchards, *Orthops palus* was by far the most common species and is the species responsible for most damage to mango inflorescences. Protection will need to focus on *O. palus* and include both biological and crop management.

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