Journal of Insect Science (*2016*) 16(1): 24; 1–5
doi: 10.1093/jisesa/iew007
Research article

Isolation and Characterization of 11 Polymorphic Microsatellite Markers Developed for *Orthops palus* (Heteroptera: Miridae)

M. Atiama,[1] H. Delatte, and J.-P. Deguine

CIRAD-BIOS, UMR PVBMT, 7 chemin de l'IRAT, 97410 Saint-Pierre, La Réunion, France (morguen.atiama@cirad.fr; helene.delatte@cirad.fr; jean-philippe.deguine@cirad.fr) and [1]Corresponding author, e-mail: morguen.atiama@cirad.fr

Subject Editor: Johanne Brunet

Received 20 July 2015; Accepted 17 January 2016

Abstract

Miridae (Hemiptera: Heteroptera: Cimicomorpha), or plant bugs, are one of the most diverse and species-rich families of insects. Most of them are phytophagous, but some are insect predators and used for biocontrol. Among this family, the mango bug, *Orthops palus* (Taylor 1947), is one of the most important pest of mango in Reunion Island. We developed 11 polymorphic microsatellite loci to study the population genetics of this pest species. The microsatellite markers were characterized by genotyping 78 field-collected insects sampled at different localities in Reunion Island. The number of alleles per locus ranged from 1 to 13 and heterozygosity levels ranged between 0.40 and 0.94. Several loci were not at Hardy–Weinberg equilibrium for the tested populations. These markers are the first to be developed for a species of the genus *Orthops.*

Key words: agricultural pest, mirid, population genetics, Reunion Island

Miridae (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). Plant bugs are among the most common insects, the majority are phytophagous, and many are destructive pests of crops including alfalfa, apple, cocoa, cotton, sorghum, and tea (Wheeler 2001); but a large number are predacious (Sweetman 1958) and have been used successfully in biological control programs (Wheeler 2000). Certain omnivorous plant bugs are considered both harmful pests and beneficial natural enemies of pests on the same crop, depending on the prevailing environmental conditions (Wheeler 2001). An interesting example is *Orthops palus* Taylor (Taylor 1947), better known as "The mango bug" (Heteroptera: Miridae: Mirinae), which is one of the most important pests on mango [*Mangifera indica* (L.)]. This bug was first described in Uganda in 1947 (Taylor 1947) and first reported on Reunion Island in 1974 (Etienne and Roura 1974). Although it has only been formally identified in these two countries, its presence is strongly suspected in East Africa, particularly in neighboring countries of Uganda and in the Mascarene Islands.

Mango is the main tropical fruit produced worldwide and plays an important economic role in East Africa. In Uganda, it is the most widely distributed fruit (**National Agricultural Advisory Services** [NAADS] 2013) and in Kenya, it is the third fruit in terms of area and total production (**Food and Agriculture Organization of the United Nations** [FAO] 2009). Mango losses result from damage caused by adults and nymphs of the mango bug picking and sucking mango inflorescences, and in Reunion Island, losses due to the bug have been reported to reach 100% some years (Insa et al. 2002). *O. palus* is a potential threat to mango production and to other crops due to its polyphagy (eating several plant families). However, little is known about the ecology of this pest. Knowledge of its population genetic structure would benefit the development of adequate management strategies.

The generally high level of polymorphism and high heterozygosity and the fact they are mostly neutral markers make microsatellites particularly suitable for studies of population genetic structure, gene flow and dispersal (Estoup et al. 1995, Jarne and Lagoda 1996, Balloux and Lugon-Moulin 2002, Hoy 2003). Microsatellite markers are also powerful tools to study insect evolutionary ecology linked to genetic variation at the population level and to enable the identification of biotypes. Microsatellite markers can also be used to assess the importance of wild host plants as reservoirs of the pest and to better understand the relationship between pest populations on wild plants and crops (Agata et al. 2011). Microsatellite markers have been used to study gene flow among populations of another Miridae species (*Lygus lineolaris*: Perera et al. 2007, 2015; *Lygus hesperus:* Shrestha et al. 2007) but not in the mango bug. The aim of this work was to isolate and characterize new microsatellite markers to examine the genetic diversity and population structure of some *O. palus* populations.

Materials and Methods

Specimen Collection

Collections of adults of *O. palus* were realized in three steps. First, for microsatellite development, 24 adult bugs were sampled at 17 different sites in Reunion Island (one or two individuals per site). Second, for selecting the primers and testing for polymorphism, five adult bugs from five different sites were collected. Third, for characterizing the genetic diversity and genetic structure, 78 adult bugs were collected in four different populations of *O. palus* (different plant species at each site) from Reunion Island. All collections were realized by suction using a D-Vac on flowers of different plants species but especially mango, Brazilian pepper (*Schinus terebinthifolius* Raddi), and jujube (*Ziziphus* sp.). Adult bugs were isolated. Taxonomic identification in Miridae family is difficult and morphological taxonomic identification was performed by a taxonomic expert in Miridae, Dr. Armand Matocq from the French Natural History Museum in Paris. In addition, to further verify their classification, the cytochrome oxidase subunit I was sequenced on some of the insects previously identified using taxonomy. Obtained sequences were submitted to GenBank (accession numbers KT201362 to KT201366). Reference specimens were also photographed and conserved in the CIRAD-3P La Réunion collection (vouchers MATI00039 to MATI00041).

DNA Extraction

DNA was extracted from adults preserved in ethanol. Bugs were placed individually in a 2 ml Eppendorf tube with a glass bead containing 50 µl of buffer 1 (100 mM NaCl, 200 mM sucrose, 100 mM Tris–HCl (pH 9.1), 50 mM EDTA, and proteinase K at a final concentration of 100 µg/ml). The tubes were placed in a Mixer Mills (Retsch® Technology, Haan, Germany) at 30 Hz for 1 min. After a brief centrifugation, 50 µl of buffer 2 was added (buffer 1 with 0.5% SDS) to each tube. The homogenate was incubated at 65°C for 2 h. A buffer 3 (12.5 µl) (8 M potassium acetate) was then added at a final concentration of 1 M and the mixture was centrifuged 15 min at 15,000 × *g*. The supernatant was collected and mixed with 2.5 volume of absolute ethanol, left at room temperature for 5 min, and centrifuged again for 15 min at 15,000 × *g*. The resulting precipitate was dried and suspended in 60 µl of TE (10 mM Tris–HCl, pH 8, 1 mM EDTA).

Microsatellite Development

Two pools of 12 individuals of *O. palus* sampled at 17 sampling localities were made and sent to GenoScreen® (Genoscreen, Lille, France). Microsatellite-enriched libraries were developed using 454 GS-FLX Titanium pyrosequencing (Malausa et al. 2011) by GenoScreen. The enriched library was constructed by optimization of classical biotin-enrichment methods (Kijas et al. 1994). Standard adapters were used Adap-F: GTTTAAGGCCTAGCTAGCAGA ATC and Adap-R: GATTCTGCTAGCTAGGCCTT (Malausa et al. 2011). Probes were designed to enrich total DNA with these motifs: TG, TC, AAC, AGG, ACAT, ACG, AAG, ACTC.

One-eighth of 454 run provided 336,396 sequences of average fragment length of 223 bp. Prior to obtaining the results, a first filter was applied to discard short fragments (<40 bp) and low intensity fragments which removed 21% of the sequences. The software QDD was then run on the 336,936 sequences to identify microsatellite motifs and obtain primers flanking the microsatellite region (Meglécz et al. 2010). In total, 5,743 sequences were obtained with microsatellite motifs.

Primers were designed for 356 loci (Supp Table 1 [online only]). We selected 41 primers of various lengths (Supp. Table 1 [online only] and tested them on five individuals from five different populations. The polymerase chain reaction (PCR) cycling conditions consisted of denaturation for 5 min at 95°C, 35 denaturation cycles for 30 s at 95°C, 20 s at 55°C, and 45 s elongation at 72°C, and a final elongation for 10 min at 72°C.

Eleven markers were selected due to their consistent amplification and high level of polymorphism. These 11 markers were fluorescently labeled (forward primer; Applied Biosystems) (Table 1) and multiplexed in two different PCR mixes using the same PCR program (Table 1). Multiplex PCR were performed in 15 µl reaction volumes containing 5 ng of DNA, 7.5 µl of 2 × Type-it Multiplex PCR Master Mix (Qiagen), and 0.1 µM of each primer.

Levels of variation of the 11 microsatellite loci were evaluated among the 78 individuals collected from four populations of *O. palus* from Reunion Island: Avirons ($n = 7$), St Gilles 2 ($n = 24$), Saline Bellevue ($n = 19$), and Sainte-Rose ($n = 28$) (sampling described in Materials and Methods).

One microliter of PCR products (1/20 dilution) was combined with 10.9 µl deionized formamide and 0.1 µl of dye-labeled size standard (Life Technologies Gene-Scan AB500LIZ) and fragment analysis was performed on an ABI Prism 3130xl Genetic Analyzer (Applied Biosystems).

Microsatellite Scoring and Population Genetic Analysis

Alleles were scored using GeneMapper v4.0 software (Applied Biosystems). Genepop v4.3 was used to calculate the number of alleles, the frequency of null alleles per locus according to Dempster et al. (1977), and to test for Hardy–Weinberg equilibrium and linkage disequilibrium (Rousset 2008). Observed and expected heterozygosities were estimated by Genetix v4.05.2 (Belkhir et al. 1996–2004). Population genetic structure was investigated by calculating pairwise F_{ST} values (Weir and Cockerham 1984) and the significance level was tested by 1,000 permutations of multilocus individual genotypes as implemented by Arlequin v3.5.1.3 (Excoffier et al. 2007).

Results

Primer sequences, repeat motifs, GenBank accession numbers, and fluorescent dye are listed in Table 1. The table summarizing all potential primers is available in Supp. Table 1 (online only). The 11 loci had between 5 and 19 alleles per locus among all 78 samples (Table 1). The most polymorphic loci were CIROP11 (Na = 19), CIROP10 (Na = 17), CIROP32 (Na = 17) (Table 1), and CIROP21, CIROP14, and CIROP25 were the least polymorphic loci with five and nine alleles per locus, respectively (Table 1). Expected heterozygosities ranged from 0.40 to 0.94 and observed heterozygosities from 0.048 to 0.833 (Table 2). Two loci (CIROP10, CIROP24) showed significant deviation from Hardy–Weinberg equilibrium ($P < 0.01$) for the four populations tested but eight out of the eleven loci showed HWE for at least one of the four populations (Table 2). No significant linkage disequilibrium was detected among the 55 pairwise comparisons ($P > 0.05$). Null alleles occurred at some loci, with a frequency ranging from 0.00 (CIROP18; Avirons) to 0.68 (CIROP21; Avirons) (Table 2).

Genetic differentiation (pairwise F_{ST}) ranged from −0.02 to 0.06 (Saline Bellevue and Sainte-Rose, respectively). Three F_{ST} values were statistically different from zero indicating significant levels of genetic differentiation among these three pairs of populations (Table 3).

Discussion

This is the first time that 454 pyrosequencing (next-generation sequencing) has been used to develop microsatellite markers for Miridae and

Table 1. Characteristics of the 11 microsatellite markers isolated from *O. palus*

Primer name		Primer sequence (5′ to 3′)	Repeat motif	Number of alleles	Allelic range	Fluorescent dye	PCR multiplex set	GenBank accession numbers
CIROP38	F	CACCAAGTGCTACATGGCAA	$(CA)_{15}$	11	91–137	NED	1	KR827557
	R	CACCTTCAAGACAACCCGTC						
CIROP14	F	TCCAGATGATCCTGTGAAACC	$(AG)_9$	9	279–299	NED	1	KR827558
	R	AAGACGAATTTATCTTGGGAGTG						
CIROP18	F	CCGAGTTTGCCAAAGTTTTC	$(TC)_8$	9	265–293	6-FAM	1	KR827559
	R	TAAACGAGATTCCGCGAGTT						
CIROP23	F	TTCATTTGCTGAGGAATTACAAGA	$(CT)_9$	11	107–139	6-FAM	1	KR827560
	R	CGTAAATAAGCAAGCTCTTAGACTGA						
CIROP11	F	ACCCATCAAACCAACTCTGC	$(CA)_{14}$	19	183–271	VIC	1	KR827561
	R	AAAACAACCGCTTGAAGAGC						
CIROP30	F	TTCATCATCGGGAAGAGGTC	$(AC)_{10}$	13	112–168	VIC	1	KR827562
	R	CTTTATATTTGTCGTTTATTCGAAAG						
CIROP25	F	TACTCCGTTGTATCACTACCCG	$(TC)_9$	11	124–158	PET	1	KR827563
	R	ATACAAGACTACCCGACGCC						
CIROP10	F	ACTTCACAGTGACTTCAATAAGCAA	$(AG)_{12}$	17	189–243	NED	2	KR827564
	R	CCCGCAGTACTAATTGTGAATTT						
CIROP21	F	AATGCAGATTCGCCATTTTC	$(AG)_8$	5	170–194	6-FAM	2	KR827565
	R	TCGGTTCCCTAGCCATGTAG						
CIROP32	F	TTTTCTTGAGTTGGCACCCT	$(AG)_{11}$	17	122–166	VIC	2	KR827566
	R	AATTTGCATCTTTCAAGCAATTA						
CIROP24	F	ACCACATTGTCTGTTCAATGTACC	$(TC)_9$	15	134–170	PET	2	KR827567
	R	CCTAAACTTCAATTTTCAACAAGATG						

Annealing temperature was 55°C for all primers.

Table 2. Number of alleles (Na), observed (Ho) and expected (He) heterozygosity, Fis estimates and test for Hardy–Weinberg equilibrium (*$P < 0.01$), and null allele frequency in the four populations studied

Locus	Avirons ($n = 7$)—mango					Saint-Gilles 2 ($n = 24$)—jujube					Saline Bellevue ($n = 19$)—jujube					Sainte-Rose ($n = 28$)—Brazilian pepper				
	Na	Ho	He	Fis	Null allele	Na	Ho	He	Fis	Null allele	Na	Ho	He	Fis	Null allele	Na	Ho	He	Fis	Null allele
CIROP10	7	0.33	0.91	0.66*	0.28	13	0.50	0.89	0.44*	0.23	10	0.27	0.91	0.72*	0.33	12	0.56	0.88	0.38*	0.15
CIROP21	3	0.29	0.58	0.53	0.68	2	0.05	0.40	0.88*	0.49	3	0.68	0.63	0.07	0.39	3	0.08	0.56	0.87*	0.64
CIROP24	7	0.57	0.88	0.37	0.12	10	0.35	0.89	0.61*	0.28	11	0.37	0.89	0.59*	0.29	12	0.46	0.88	0.48*	0.21
CIROP32	8	0.67	0.94	0.31	0.11	10	0.70	0.85	0.18	0.06	10	0.83	0.86	0.03	0.07	12	0.72	0.85	0.15	0.06
CIROP11	6	0.40	0.84	0.56*	0.35	11	0.13	0.91	0.86*	0.40	10	0.38	0.88	0.58*	0.26	13	0.52	0.89	0.42*	0.19
CIROP14	5	0.57	0.76	0.26	0.08	9	0.46	0.71	0.37*	0.18	5	0.39	0.79	0.52*	0.21	6	0.54	0.76	0.29*	0.12
CIROP18	5	0.67	0.58	−0.18	0.00	7	0.57	0.76	0.26*	0.09	7	0.53	0.67	0.21	0.08	6	0.56	0.67	0.18*	0.09
CIROP23	6	0.43	0.79	0.48	0.15	7	0.71	0.71	−0.00*	0.00	8	0.61	0.74	0.18	0.10	8	0.61	0.60	−0.01	0.00
CIROP25	4	0.50	0.74	0.35	0.08	5	0.44	0.68	0.36*	0.14	6	0.37	0.69	0.47*	0.19	9	0.61	0.73	0.17	0.08
CIROP30	4	0.17	0.56	0.72	0.22	8	0.21	0.86	0.76*	0.38	9	0.21	0.84	0.75*	0.37	11	0.46	0.87	0.48*	0.22
CIROP38	5	0.40	0.82	0.54	0.21	7	0.14	0.54	0.74*	0.32	1	–	–	–	–	8	0.10	0.73	0.87*	0.36

Table 3. Pairwise FST values of four populations of *O. palus* of Reunion Island

	Avirons—mango	Saint-Gilles 2—jujube	Saline Bellevue—jujube	Sainte-Rose—Brazilian pepper
Avirons—mango				
Saint-Gilles 2—jujube	0.06*			
Saline Bellevue—jujube	0.01	0.03*		
Sainte-Rose—Brazilian pepper	0.03*	0.01	−0.02	

Mango, jujube, and Brazilian pepper represent the plant on which the *O. palus* individuals were collected.
*Probability that the FST value is statistically different from zero at $\alpha = 0.05$.

the first study on microsatellite markers for a species of the genus *Orthops*. We designed and characterize 11 polymorphic primers which will permit further studies of the genetic variability and population genetic structure of *O. palus* within its geographic distribution range.

The significant excess of homozygotes observed in several populations for 6 of the 11 loci (CIROP10, CIROP21 CIROP24, CIROP11, CIROP30, and CIROP38) might result from a high incidence of null alleles (>20%). Null alleles often result from a

polymorphism in one or both of the forward and backward primers which prevent allele amplification (Callen et al. 1993, Paetkau and Strobeck 1995, Dakin and Avise 2004, Pompanon et al. 2005, Chapuis and Estoup 2007). Null alleles are common in insects (Liu et al. 2006) and have already been observed in several mirid species (Liu et al. 2006, Kobayashi et al. 2011, Babin et al. 2012). We obtained a high level of null alleles despite the fact that we used a QDD analysis to find the primers. A recent publication by Meglécz et al. (2014) underlined that PCR or genotype failure could result from several phenomena including the incomplete filter of reads that might include short stretches of cryptically simple sequence. These regions contain low complexity and/or semirepetitive motives and are often present in flanking regions of microsatellites. Similarly, the distance between the primer and the target microsatellite was also important, and amplification improved for primers that were further from the microsatellites (Meglécz et al. 2014).

Thirty-five species are recognized in the genus *Orthops*, including some closely related species that are widespread pests such as *Orthops campestris* or *Orthops kalmi* (Heshula and Hill 2012). Cross-species transferability of polymorphic markers such as microsatellites within and between genera in arthropods often yields returns (>60% transfer success between species within a genus) (Barbará et al. 2007). However, the rapid evolution of these markers can render many of them useless even in closely related species due to the absence of the repeat or low repeat number/low polymorphism or changes in the nucleotide composition in flanking regions (Deitz et al. 2012). Nevertheless, several of the microsatellite markers developed here have repeat motifs over 10 dinucleotides and are polymorphic (in average 12.45 alleles per locus), so their transferability should be tested on other species within the genus *Orthops* and on species of closely related genera (e.g., *Lygus*).

Supplementary Data

Supplementary data are available at *Journal of Insect Science* online.

Acknowledgments

We are extremely grateful to their colleague A. Matocq for his species identification. We thank C. Simiand, M. L. Moutoussamy, B. Béziat, and T. Schmitt for technical assistance. We would like to thank the anonymous reviewers and particularly the editor Johanne Brunet for improving the manuscript. This work was supported by the French *Région Réunion*, Centre de coopération International en Recherche Agronomique pour le Développement, and the BIOPHYTO is not a acronyme, is the full name of a project. This study was funded by the Regional Council of Réunion, the General Council of Réunion, the French Government, the European Union (ERDF, EAFRD), the Trust Account for Agricultural and Rural Development (CASDAR-1138 CASDAR 2011) and the CIRAD.

References Cited

Agata, K., S. Alasaad, V.M.F. Almeida-Val, J. A. Alvarez-Dios, F. Barbisan, J. S. Beadell, J. F. Beltrán, M. Benítez, G. Bino, C. Bleay, et al. 2011. Permanent genetic resources added to Molecular Ecology Resources Database 1 December 2010–31 January 2011. Mol. Ecol. Resour. 11: 586–589.

Babin, R., C. Fenouillet, T. Legavre, L. Blondin, C. Calatayud, A.-M. Risterucci, and M.-P. Chapuis. 2012. Isolation and characterization of twelve polymorphic microsatellite loci for the cocoa mirid bug *Sahlbergella singularis*. Int. J. Mol. Sci. 13: 4412–4417.

Balloux, F., and N. Lugon-Moulin. 2002. The estimation of population differentiation with microsatellite markers. Mol. Ecol. 11: 155–165.

Barbará, T., C. Palma-Silva, G. M. Paggi, F. Bered, M. F. Fay, and C. Lexer. 2007. Cross-species transfer of nuclear microsatellite markers: potential and limitations. Mol. Ecol. 16: 3759–3767.

Belkhir, K., P., Borsa, L., Chikhi, N., Raufaste and F., Bonhomme 1996–2004. GENETIX 4.05, logiciel sous Windows TM pour la génétique des populations. Laboratoire Génome, Populations, Interactions, CNRS UMR 5000, Université de Montpellier II, Montpellier, France.

Callen, D. F., A. D. Thompson, Y. Shen, H. A. Phillips, R. I. Richards, J. C. Mulley, and G. R. Sutherland. 1993. Incidence and origin of "null" alleles in the (AC)n microsatellite markers. Am. J. Hum. Genet. 52: 922–927.

Cassis, G., and R. T., Schuh 2012. Systematics, biodiversity, biogeography, and host associations of the miridae (Insecta: Hemiptera: Heteroptera: Cimicomorpha). Annu. Rev. Entomol. 57: 377–404.

Chapuis, M.-P., and A. Estoup. 2007. Microsatellite null alleles and estimation of population differentiation. Mol. Biol. Evol. 24: 621–631.

Dakin, E. E., and J. C. Avise. 2004. Microsatellite null alleles in parentage analysis. Heredity 93: 504–509.

Deitz, K. C., V. P. Reddy, M. R. Reddy, N. Satyanarayanah, M. W. Lindsey, H. J. Overgaard, M. Jawara, A. Caccone, and M. A. Slotman. 2012. Limited usefulness of microsatellite markers from the malaria vector *Anopheles gambiae* when applied to the closely related species *Anopheles melas*. J. Hered. 103: 585–593.

Dempster, A. P., N. M. Laird, and D. B. Rubin. 1977. Maximum likelihood from incomplete data via the EM algorithm. J. R. Stat. Soc. Ser. B Methodol. 39: 1–38.

Estoup, A., L. Garnery, M. Solignac, and J. M. Cornuet. 1995. Microsatellite Variation in Honey Bee (*Apis Mellifera* L.) Populations: Hierarchical Genetic Structure and Test of the Infinite Allele and Stepwise Mutation Models. Genetics. 140: 679–695.

Etienne, J., and A. Roura. 1974. Note sur l'entomofaune des manguiers à la Réunion, pp.89–92. *In* Rapport Annuel de l"IRAT. Saint-Denis, La Réunion. Institut de recherches agronomiques tropicales.

Excoffier, L., G. Laval, and S. Schneider. 2007. Arlequin (version 3.0): an integrated software package for population genetics data analysis. Evol. Bioinform. Online 1: 47–50.

(FAO) Food and Agriculture Organization of the United Nations. 2009. Value chain analysis: a case study of mangoes in Kenya. FAO, Kenya.

Heshula, L.U.P., and M. P., Hill 2012. Effect of previous feeding by *Falconia intermedia* (Hemiptera: Miridae) on subsequent feeding activity on the invasive shrub *Lantana camara* (Verbenaceae). Biocontrol Sci. Technol. 22: 671–684.

Hoy, M. A. 2003. Insect molecular genetics: an introduction to principles and applications. Academic Press, San Diego, USA.

Insa, G., G. Rossolin, and D. Vincenot. 2002. La Mangue - Dossier Technico-Economique, 2nd ed. Chambre d'agriculture de La Réunion, Saint-Pierre, La Réunion.

Jarne P. and J. L. Lagoda. 1996. Microsatellites, from molecules to populations and back. Trends Ecol. Evol. 11: 424–429.

Kijas, J. M., J. C. Fowler, C. A. Garbett, and M. R., Thomas 1994. Enrichment of microsatellites from the citrus genome using biotinylated oligonucleotide sequences bound to streptavidin-coated magnetic particles. BioTechniques 16: 656–660.

Kobayashi, T., T. Sakurai, M. Sakakibara, and T. Watanabe. 2011. Multiple origins of outbreak populations of a native insect pest in an agro-ecosystem. Bull. Entomol. Res. 101: 313–324.

Liu, Y.-D., Z.-C. Yang, and K.-M. Wu. 2006. Eight polymorphic microsatellite markers developed in the green leaf bug, *Lygus lucorum* Meyer-Dür (Hemiptera: Miridae): PRIMER NOTE. Mol. Ecol. Notes 7: 29–31.

Malausa, T., A. Gilles, E. Meglécz, H. Blanquart, S. Duthoy, C. Costedoat, V. Dubut, N. Pech, P. Castagnone-Sereno, C. Délye, et al. 2011. High-throughput microsatellite isolation through 454 GS-FLX titanium pyrosequencing of enriched DNA libraries. Mol. Ecol. Resour. 11: 638–644.

Meglécz, E., C. Costedoat, V. Dubut, A. Gilles, T. Malausa, N. Pech, and J.-F. Martin. 2010. QDD: a user-friendly program to select microsatellite markers and design primers from large sequencing projects. Bioinformatics 26: 403–404.

Meglécz, E., N. Pech, A. Gilles, V. Dubut, P. Hingamp, A. Trilles, R. Grenier, and J.-F. Martin. 2014. QDD version 3.1: a user-friendly computer program for microsatellite selection and primer design revisited: experimental validation of variables determining genotyping success rate. Mol. Ecol. Resour. 14: 1302–1313.

(NAADS) National Agricultural Advisory Services. 2013. User guide on mango production. NAADS, Kampala, Uganda.

Paetkau, D., and C. Strobeck. 1995. The molecular basis and evolutionary history of a microsatellite null allele in bears. Mol. Ecol. 4: 519–520.

Perera, O. P., G. L. Snodgrass, B. E. Scheffler, J. Gore, and C. A. Abel. 2007. Characterization of eight polymorphic microsatellite markers in the tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois). Mol. Ecol. Notes. 7: 987–989.

Perera, O. P., J. Gore, G. L. Snodgrass, R. E. Jackson, K. C. Allen, C. A. Abel, and R. G. Luttrell. 2015. Temporal and spatial genetic variability among tarnished plant bug (Hemiptera: Miridae) populations in a small geographic area. Ann. Entomol. Soc. Am. 108: 181–192.

Pompanon, F., A. Bonin, E. Bellemain, and P. Taberlet. 2005. Genotyping errors: causes, consequences and solutions. Nat. Rev. Genet. 6: 847–846.

Rousset, F., 2008. genepop'007: a complete re-implementation of the genepop software for Windows and Linux. Mol. Ecol. Resour. 8: 103–106.

Shrestha, R. B., M. N. Parajulee, O. P. Perera, B. E. Scheffler, and L. D. Densmore. 2007. Characterization of microsatellite loci in the western tarnished plant bug, *Lygus hesperus* Knight (Hemiptera: Miridae). Mol. Ecol. Notes. 7: 1342–1344.

Sweetman, H. L. 1958. The principles of biological control; interrelation of hosts and pests and utilization in regulation of animal and plant populations. The biological control of insects. W. C. Brown Co., Dubuque, IA.

Taylor, T.H.C. 1947. Some East African species of *Lygus*, with notes on their host plants. Bull. Entomol. Res. 38: 233–258.

Weir, B. S., and C. C. Cockerham. 1984. Estimating F-statistics for the analysis of population structure. Evolution 38: 1358–1370.

Wheeler, A. G. 2000. Predacious plant bugs (Miridae), pp. 657–693. *In* C. W. Schaefer and A. R. Panizzi (eds.), Heteroptera of economic importance. CRC Press LLC, Boca Raton, FL.

Wheeler, A. G. 2001. Biology of the plant bugs (Hemiptera: Miridae): pests, predators, opportunists. Cornell University Press, Ithaca, NY.