IDENTIFICATION OF THREE ARMYWORM SPECIES (LEPIDOPTERA: NOCTUIDAE) USING DNA BARCODES AND RESTRICTION ENZYME DIGESTION

By

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Abstract

SIX SPECIES of sugarcane armyworms (*Mythimna* spp.) are known to occur in Mauritius, namely *M. pseudoloreyi*, *M. loreyi*, *M. insulicola*, *M. phaea*, *M. tincta* and *M. pyrausta*. Due to the close resemblance of the adult moths, morphological distinction among the different species is difficult and may lead to misidentification. One new approach for their characterisation involves the use of DNA barcodes to analyse the sequence diversity within a short standardised segment of their genome. Polymorphism in the 5' end of the mitochondrial *cytochrome oxidase* I (COI) gene is extensively used as a DNA barcoding system in Lepidopterans. In 2008, adult moths of *M. insulicola*, *M. phaea* and *M. pseudoloreyi* were reared from field-collected larvae. Two nucleic acid extraction methods were evaluated, both yielding high quality DNA for molecular studies. DNA extractions were performed from different body parts including abdomen, leg and wing. A ~700 bp PCR fragment amplified from the three species, using primers HCO2198/LCO1490, was digested using restriction enzymes. A combination of four enzymes *Rsa*1, *Taq*I, *Pvu*II and *Sac*I successfully allowed distinction of the three species of armyworms tested. The PCR products from the three species were cloned and sequenced. A 658 bp fragment from each of *M. insulicola*, *M. phaea* and *M. pseudoloreyi* was submitted to Genbank and respectively recorded as *Leucania insulicola*, GQ353294; *Leucania phaea*, GQ353295; and *Leucania loreyi*, GQ353296. Using the Barcode of Life identification engine (BOLD-ID), high sequence identities were obtained with *Leucania* species (Noctuidae: Hadeninae)– 99.54% for *L. phaea* and *Leucania* sp. (from Kenya), 100% for *L. loreyi* and *Leucania* sp. and 99.85% for *L. insulicola* and *L. striata* (from Madagascar). The *L. phaea* sequence diverged by 7.3% and 10.6% from those of *L. loreyi* and *L. insulicola* respectively. A sequence divergence value of 8.5% was observed between *L. loreyi* and *L. insulicola*. DNA barcoding and sequencing could provide useful information for classification and characterisation of armyworms.

Introduction

In Mauritius, infestations of sugarcane fields by armyworms belonging to the genus *Mythimna,* (Lepidoptera: Noctuidae) were initially reported in 1959 (MSIRI, 1960). In subsequent years, these pests did not pose a major threat to sugarcane and were under control.

With the introduction of mechanised sugarcane harvesting and the adoption of trash blanketing practices, severe outbreaks were observed after 1992. Attacks by armyworms cause retardation in shoot development and this is more pronounced when associated with other stress factors including drought conditions.

During severe outbreaks, total defoliation of affected fields may occur. To-date six species of sugarcane armyworms are known to occur in Mauritius, namely *M. pseudoloreyi*, *M. loreyi*, *M. insulicola*, *M. phaea*, *M. tincta*, and *M. pyrausta* (Ganeshan, 2007).

Control of armyworms is achieved through the use of insecticides. Chemical control can have negative impacts on the cane ecosystem in Mauritius, where a biological control strategy has always been adopted for the management of sugarcane pests. Six species of parasitoids have been identified from *Mythimna* spp. in Mauritius (Ganeshan, 2001) and the entomopathogen *Metarhizium anisopliae*, has also been observed on larvae and pupae (Beehary-Panray and Rajabalee, 1998). However, the application of biological control agents is reliant on accurate identification of the pest species involved and for *Mythimna* spp. this has been a major difficulty. Morphological distinction is problematic due to the close resemblance of the adult moths and this may lead to misidentification of species.

Molecular biology tools, particularly the use of 'DNA barcodes', can complement traditional morphologically based taxonomy to determine the identity of insect pest species (Hebert *et al*., 2003). This method relies on the sequence diversity in the *cytochrome-c oxidase* I (COI) gene of the mitochondrial DNA. Application of DNA barcodes in taxonomy is gaining momentum and there are currently various efforts worldwide in this area namely: Consortium for the Barcode of Life, All Lepidoptera Barcode of Life Initiative, Fish Barcode of Life Initiative, Canadian Centre for DNA Barcoding, Canadian Barcode of Life Network and All Birds Barcode of Life Initiative. These form part of the International Barcode of Life Initiative, which aims to have a global barcode system for all the species present on earth (Savolainen *et al*., 2005). The Barcode of Life Data Systems (BOLD) has thus been set-up (http://www.barcodinglife.org) and can be considered as an online barcoding information system for collection, management and analysis of DNA barcodes.

For Lepidoptera, which constitute the second most diverse insect order, with more than 180 000 known species and many more unknown ones, DNA barcodes are expected to identify more than 95% of species. In Costa Rica, in a study comprising of 4260 specimens of which 521 were *Lepidoptera* species, Hajibabaei *et al*. (2006) unambiguously identified 97.9% of the test species. Using DNA barcodes, it was shown that the sequence of *Diatraea saccharalis* shared 99% homologies with members of the Crambidae family (Bravo *et al*., 2008). Previously, there was confusion regarding classification of these moth borers in either the Pyralidae or Crambidae families. Similarly, it is expected that barcodes will provide more insight into the classification of the six *Mythimna* spp. in Mauritius. The genus *Leucania* has been reviewed and several species belonging to this genus were assigned to the *Mythimna* genus (Holloway *et al.,* 1987). However, there is still some confusion on the taxonomic nomenclature of this genus, which needs revision.

The main objective of this project is to investigate the usefulness of DNA barcodes for distinguishing the six *Mythimna* spp. present in Mauritius.

Material and methods

Armyworm collection

Larvae were collected from sugarcane fields in 2008 and were reared to adult in the laboratory. Adult moths were identified using morphological characters (Ganeshan, 2007). The adult specimens were preserved in 90% ethanol until processed. Only three species were encountered: *M. pseudoloreyi*, *M. insulicola* and *M. phaea*. DNA was extracted from the three species (Table 1).

DNA extraction

An appropriate DNA extraction technique is a critical step for successful application of a DNA barcoding system for insects. It is important to have good quality DNA for amplification of the mitochondrial DNA fragment by the polymerase chain reaction (PCR). Two DNA extraction protocols were evaluated. Before homogenising, the specimens were removed from the alcohol and allowed to dry.

In the first method, the protocol described by Zhou *et al*. (2000) was followed for extraction of DNA from single moths**.** After removing the wings and legs, the moth was homogenised using a sterilised mortar and pestle (with 1 mL of extraction buffer – 10 mM Tris HCl (pH 7.5), 60 mM NaCl and 10 mM EDTA). 600 µL of the homogenate were transferred to a 2 mL microcentrifuge tube and an

equal volume of post-grinding buffer (200 mM Tris HCl pH 9.0, 30 mM EDTA and 2% SDS) were added. 140 µg of proteinase K was added and the tubes were incubated in a water bath at 50°C overnight. Sodium acetate (pH 4.8) was added to a final concentration of 0.3 M. The contents of the tubes were transferred to a 15 mL falcon tube and an equal volume of phenol was added and centrifuged at 8000 rpm. The aqueous phase was recovered and extracted with an equal volume of chloroform: isoamyl alcohol (24:1). To the supernatant, two volumes of 95% ethanol were added and DNA precipitated at –20°C for 2 h. The DNA was recovered by centrifugation and after air-drying, was resuspended in 100 µL of sterile distilled water.

In the second method, a modified CTAB method was adopted. Adult moths devoid of wings and legs were homogenised in 1 mL CTAB buffer (2% CTAB, 1.4 M NaCl, 20 mM EDTA pH 8.0, 100 mM Tris-HCl pH 8.0, 0.1% mercaptoethanol and 200 µg proteinase K) using a mortar and pestle. 1 mL of the homogenate was transferred to a centrifuge tube and incubated for 45 min at 60°C. An equal volume of phenol: chloroform: isoamyl alcohol (25:24:1) was added and centrifuged for 10 min at 14 000 rpm. A second extraction step was performed on the supernatant using an equal volume of chloroform: isoamyl alcohol (24:1). DNA was precipitated with 1/10 volume of sodium acetate (3.0 M) and an equal volume of 95% ethanol (–20°C for 2 h). The DNA was pelleted by centrifugation and following air-drying, was resuspended in 500 µL of sterile distilled water.

Improvements to the two methods included an optional RNA digestion step. This was performed after the chloroform:isoamyl alcohol step; the supernatant was treated with 100 µg of RNase A and incubated at 37°C for 3 h. Attempts were also made to extract DNA from detached wings and legs of specimens (Table 1).

Table 1—Different extraction methods performed.

Specimen number	Sample number	*Mythimna* species	Extraction method followed	Insect part used
1	AR 001	*M. pseudoloreyi*	Zhou *et al*. (2000)	Abdomen
2	AR 002			
3	AR 003			
4	AR 004			
5	AR 005		Modified CTAB	
6	AR 006			
7	AR 007			
8	AR 008			
9	AR 009	*M. insulicola*	Modified CTAB	Abdomen
	AR 010			Legs and wings
10	AR 011*			Abdomen
	AR 012			Legs and wings
11	AR 013	*M. insulicola*	Zhou *et al*. (2000)	Abdomen
12	AR 014	*M. pseudoloreyi*		
13	AR 015	*M. insulicola*		
14	AR 016	*M. pseudoloreyi*		
15	AR 017*	*M. phaea*	Modified CTAB	Abdomen
16	AR 018*			
15	AR 019			Legs and wings
16	AR 020			

* optional RNase digestion performed

Amplification of DNA barcodes

Primer pair LCO1490/HCO2198 (Folmer *et al.*, 1994) amplifies a ~700 bp fragment of the mitochondrial COI gene. PCR was performed in a total volume of 50 µL with 0.2 mM dNTPs, 1X PCR buffer, 0.3 µM of forward primer (LCO1490, 5'-GGTCAACAAATCATAAAGATATTGG and reverse primer HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA) and 1 U of *Taq* polymerase (Roche diagnostics, USA) and 1.5 µL of template DNA. The mixtures were set in a thermal cycler using the following thermal profile: denaturation at 94°C for 10 min followed by 35 cycles of 94°C for 30 s,

50°C for 30 s and 72°C for 1 min, and a final elongation step at 72°C for 10 min. Amplified fragments were separated by 1% agarose gel electrophoresis and visualised under UV light.

Restriction fragment length polymorphism (RFLP)

The ~700 bp PCR fragments amplified using HCO1490/HCO2198 were digested with enzymes *Rsa*I, *Pvu*II, *Taq*I and *Sac*I as per manufacturer's instructions (Roche Diagnostics). Digestion products were separated by 1% agarose gel electrophoresis and visualised under UV light.

Cloning and sequencing

One ~700 bp fragment amplified from each of the three species (*M. pseudoloreyi, M. insulicola and M. phaea*) was purified from agarose gel using the QIAquick Gel Extraction Kit (Qiagen) and cloned using the pGEM T Easy Vector system (Promega). Subsequently, the inserts were sequenced using an ABI 310 Genetic Analyser using the Big dye 3.1 Sequencing Kit (Applied Biosystems, USA) using universal primers.

Sequence analysis

COI DNA sequences from *M. pseudoloreyi*, *M. insulicola* and *M. phaea* were compared with other sequences from Genbank. The sequences were also used for species identification using the BOLD-IDS (barcode identification engine http://www.barcodinglife.org/). In order to establish the phylogenetic relationship of the three *Mythimna* spp., the primer sequences were trimmed and sequences were aligned with other sequences retrieved from BOLD and Genbank (Table 2) using Clustal X. Mega3 was used to construct a neighbour-joining phylogenetic tree based on Kimura 2 parameter (K2P) genetic distances and 1000 replicated bootstrap values.

Table 2—Sequences included in phylogenetic analysis of *Mythimna* sp.

Species	Accession number
M. insulicola (*Leucania insulicola*[1])	GQ353294
M. phaea (*Leucania phaea*[1])	GQ353295
M. pseudoloreyi (*Leucania loreyi*[1])	GQ353296
Noctua atlantica	AY600452
Schinia pulchripennis	EU768965
Spodoptera frugiperda	EU768963
Spodoptera ornithogalli	EU768964
Helicoverpa zea	EU768942
Helicoverpa punctigera	EU768941
Helicoverpa pallida	EU768940
Helicoverpa hawaiiensis	EU768939
Helicoverpa gelotopoeon	EU768938
Helicoverpa assulta	EU768937
Helicoverpa armigera	EU768936
Busseola fusca	DQ337200
Busseola phaia	DQ337196
Schinia felicitata	EU768955
Erythroecia suavis	EU768900
Schinia oleagina	EU768966
Schinia miniana	EU768961
Leucania inermis	AF549772
Plutella xylostella	EF380092
*Mythimna albipuncta**	GWOR4022-09|BC ZSM Lep 21306
*Mythimna conigera**	GWOR4020-09|BC ZSM Lep 21304
*Mythimna ferrago**	GWOR3909-09|BC ZSM Lep 21193
*Mythimna impura**	GWOR3906-09|BC ZSM Lep 21190
*Mythimna l-album**	GWOR4026-09|BC ZSM Lep 21310
*Mythimna unipuncta**	GWOR3947-09|BC ZSM Lep 21231
*Mythimna vitellina**	GWOR3879-09|BC ZSM Lep 21163
Leucania striata[2]	LTOL390-09
***Leucania* sp**[2]	PMANL304-09
Leucania stenographa[2]	ANICB181-06

* Sequences obtained from Barcode of Life database; [1] Name as accepted by Genbank (this study)
[2] Non-Public sequences in BOLD (Permission sought from authors)

Results and discussion

Amplification of DNA barcodes

The ~700 bp fragment was successfully amplified with all 20 DNA extracts, AR001-AR020 (Figure 1).

Extracts obtained using the Zhou *et al*. (2000) method (AR001-004 and AR013-016) as well as the remaining ones (CTAB method) were suitable for the generation of DNA barcodes from *Mythimna* spp.

The optional RNase step was performed for only three extracts (AR011, AR017 and AR018 in Lanes 11, 17 and 19 respectively) and there was not much difference in product amplification.

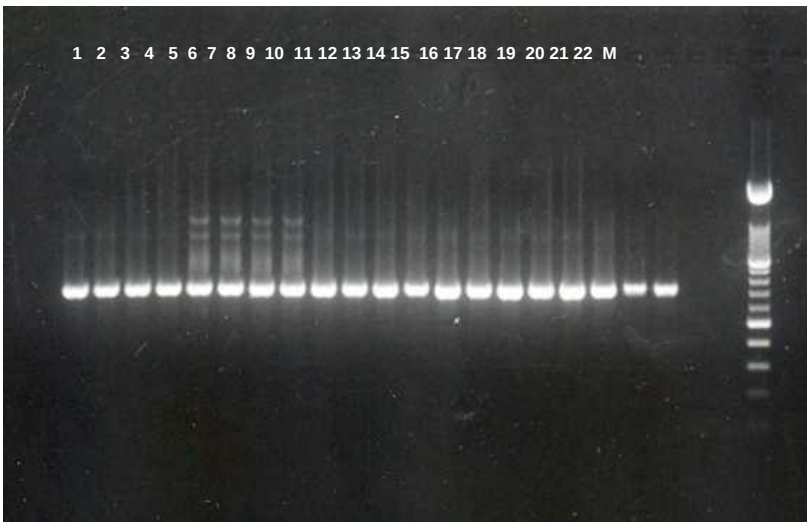


Fig. 1—Amplification of the ~700 bp mitochondrial COI DNA fragment from extracts AR001-AR020 (Lanes 1–20), Lanes 21–22 are water controls and M is 100 bp marker XIV (Roche).

Restriction fragment length polymorphism (RFLP)

The ~700 bp products amplified with primers HCO1490/LCO2198 were digested using restriction enzymes *Rsa*I, *Taq*I, *Sac*I and *Pvu*II (Figure 2). A combination of restriction patterns with at least two enzymes allowed discrimination between the three *Mythimna* spp. tested. *Rsa*I and *Taq*I could differentiate *M. pseudoloreyi* and *M. insulicola* from *M. phaea*.

The *Sac*I recognition site, which is only present in the *M. pseudololreyi* fragment, was used to separate *M. pseudoloreyi* from *M. insulicola*. Enzyme *Pvu*II also confirmed the slight difference existing between *M. pseudoloreyi* and *M. insulicola* (Figure 2), but the digested product was very close to 700 bp.

The PCR-RFLP described above is a simple technique for differentiating the three armyworm species *M. pseudoloreyi, M. insulicola* and *M. phaea*. In 2009, additional specimens including *M. loreyi*, *M. tincta*, and *M. pyrausta* will be collected and the method further validated.

Sequence analysis

Sequences obtained from cloned HCO1490/LCO2198 PCR products of *M. insulicola*, *M. phaea* and *M. pseudoloreyi* were edited to remove the primer region and deposited in Genbank. The curators have accepted the entries as *Leucania insulicola*, *L. phaea* and *L. loreyi* respectively (GQ353294-6). These names will be used for further discussion in the current paper in order to avoid confusion. It is expected that, with further analysis of barcode data from the three other *Mythimna* species in Mauritius (*M. loreyi*, *M. tincta* and *M. pyrausta*), the issue of classification will be clearer.

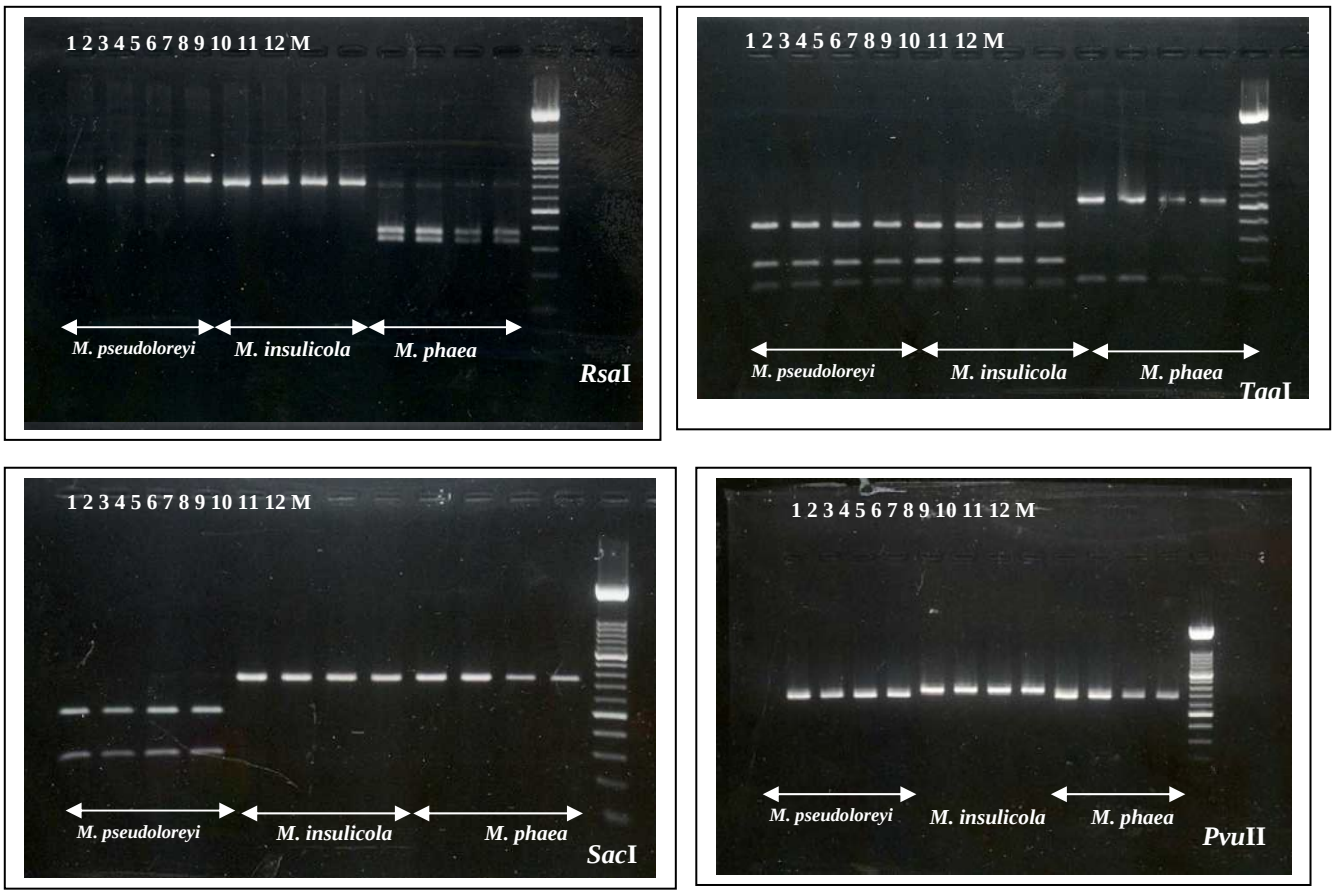


Fig. 2—Restriction digestion of HCO1490/LCO2198 PCR products using enzymes *Rsa*I, *Taq*I, *Sac*I and *Pvu*II. Lanes 1–12 consist of the three *Mythimna* spp. as specified, M is 100 bp ladder.

Analysis in the Barcode of Life Identification Engine (BOLD-ID) was performed using the full barcodes and very high (99%) homologies were obtained with specimens belonging to the *Leucania* genus (Noctuidae; Hadeninae) (Table 3). However, these sequences are not currently publicly available and requests were made to the authors for access. Interestingly, the closest matches were either regional specimens, e.g. *L. striata* (LTOL390-09) was collected in Madagascar while the unclassified *Leucania* sp. (PMANL304-09) was collected in Kenya, or pests of sugarcane: specimen *L. stenographa* (ANICB181-06) originates from Australia where this species (=*L. loreyimima*) is known to infest sugarcane plantations (Edwards, 1992).

Table 3—Sequence similarities of *Leucania* (*Mythimna*) from this study with those from related species.

Species	Homologies
L. insulicola	99.85 % with *Leucania striata* (LTOL390-09)- from Madagascar
L. phaea	99.54% with unidentified *Leucania* sp. (PMANL304-09)- from Kenya
L. loreyi	100 % with unidentified *Leucania* sp. (PMAN310-09)- 98.1 % with *Leucania stenographa* (ANICB181-06)- from Australia

The sequences from this study were compared with those from other *Mythimna* and *Leucania* species publicly available from BOLD. The COI sequence of *L. insulicola* (GQ353294) diverged by 10.6% from *L. phaea* while there is a divergence of 7.3% from *L. loreyi*. For *L.*

insulicola and *L. loreyi*, there is a sequence divergence of 8.5% (Table 4). With such high divergence at nucleotide level, it is very easy to distinguish between the three species being studied. The average overall mean divergence for the 15 species considered in Table 4 is 8.5% (±0.7% SE). It should be noted that only *Mythimna* and *Leucania* sp. have been included in Table 4.

Table 4—Average pairwise Kimura 2 parameter distances (below diagonal) and standard errors (above) between *L. phaea*, *L. insulicola* and *L. loreyi* and selected sequences.
1. *Leucania inermis*, 2. *Mythimna l-album*, 3. ***Leucania phaea****, 4. *Mythimna impura*, 5. *Mythimna conigera*, 6. *Mythimna vitellina*, 7. ***Leucania insulicola****, 8.*Mythimna albipuncta*
9. *Mythimna ferrago*, 10. ***Leucania loreyi****, 11. *Mythimna unipuncta*, 12. *Leucania* sp-Kenya, 13. *Leucania stenographa*, 14. *Leucania striata* and 15. *Leucania* sp-PMAN310-09.
(*this study).

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1		0.011	0.012	0.012	0.013	0.012	0.013	0.015	0.013	0.012	0.013	0.012	0.012	0.013	0.012
2	0.066		0.011	0.010	0.013	0.011	0.013	0.014	0.012	0.012	0.013	0.011	0.013	0.013	0.012
3	0.081	0.075		0.011	0.013	0.014	0.014	0.014	0.013	0.012	0.013	0.003	0.012	0.014	0.012
4	0.077	0.060	0.071		0.013	0.012	0.012	0.013	0.011	0.01	0.011	0.011	0.01	0.012	0.01
5	0.099	0.097	0.103	0.085		0.013	0.014	0.015	0.015	0.013	0.014	0.013	0.012	0.014	0.013
6	0.081	0.073	0.104	0.081	0.09		0.013	0.015	0.013	0.012	0.014	0.013	0.012	0.013	0.012
7	0.097	0.098	0.106	0.085	0.113	0.093		0.015	0.013	0.013	0.015	0.014	0.013	0.000	0.013
8	0.107	0.099	0.114	0.091	0.119	0.117	0.112		0.012	0.013	0.013	0.014	0.013	0.015	0.013
9	0.087	0.083	0.092	0.074	0.105	0.095	0.091	0.078		0.012	0.012	0.012	0.012	0.013	0.012
10	0.079	0.093	0.073	0.062	0.089	0.087	0.085	0.087	0.073		0.012	0.012	0.006	0.013	0.000
11	0.092	0.100	0.098	0.073	0.111	0.104	0.120	0.096	0.091	0.079		0.012	0.011	0.015	0.012
12	0.076	0.070	0.005	0.070	0.097	0.098	0.104	0.108	0.087	0.072	0.092		0.012	0.014	0.012
13	0.081	0.093	0.079	0.060	0.083	0.089	0.095	0.087	0.077	0.019	0.077	0.073		0.013	0.006
14	0.097	0.098	0.106	0.085	0.113	0.093	0.000	0.112	0.091	0.085	0.12	0.104	0.095		0.013
15	0.079	0.093	0.073	0.062	0.089	0.087	0.085	0.087	0.073	0.079	0.079	0.072	0.019	0.085	

Using sequences of the mitochondrial COI gene of moths, Hebert *et al*. (2003) showed that there is an average sequence divergence of 0.25% for conspecific individuals while for congeneric species, an average of 6.5% was observed. For the sugarcane stem borer, *Busseola* sp., interspecific divergence of > 4.5% has been reported (Assefa *et al*., 2007). However, caution should be made using COI sequence divergence information for species delimitation (Whinnett *et al*., 2005). It is also important to consider morphological characteristics for taxonomy purposes.

A phylogenetic tree was constructed using the three *Leucania* (*Mythimna*) spp. from this study and selected related sequences from Genbank and BOLD (Figure 3). These species clustered within the *Mythimna*/*Leucania* group.

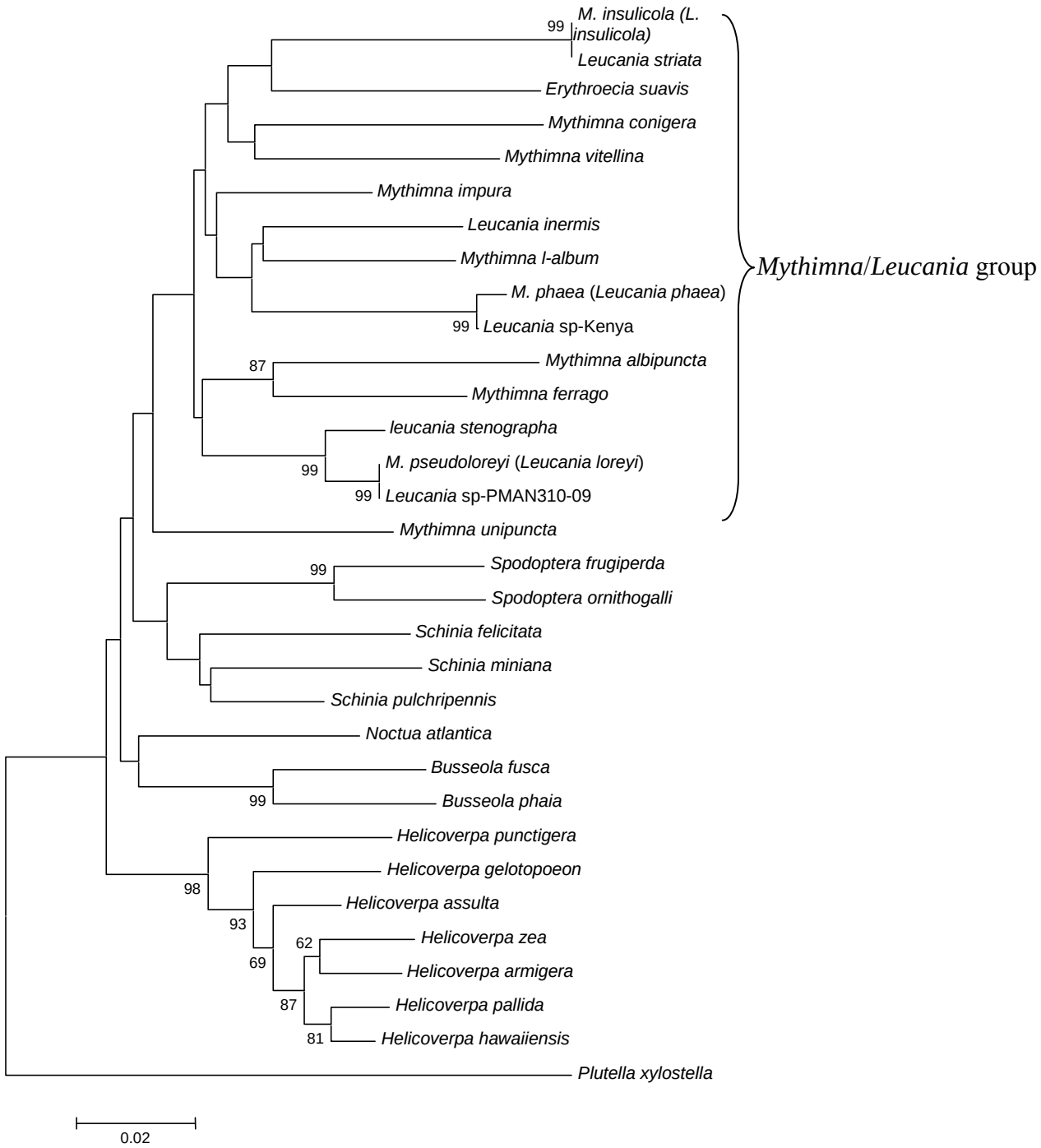
Conclusions

A simple and quick method for distinguishing among *L. insulicola*, *L. phaea* and *L. pseudoloreyi*, based on PCR-RFLP of part of the mitochondrial COI gene is described in this study.

At nucleotide level, the three species shared high homologies with moths of *Leucania* spp., which occur either in neighbouring countries e.g Madagascar or as pests of sugarcane in Australia.

These sequences have currently been deposited in Genbank as *L. insulicola*, *L. phaea* and *L. loreyi.* The high percentage divergence among the three sequences indicates the occurrence of three

distinct species. Divergence of 7.3% and 10.6% was observed between *L. phaea* sequence and those of *L. pseudoloreyi* and *M. insulicola* respectively. The latter two species had 8.5% sequence divergence. DNA barcoding is effective and very convenient for distinguishing closely related and morphologically similar species.



* This study

Fig. 3—Neighbour-Joining phylogenetic tree based on Kimura-2 parameter genetic distances. *Plutella xylostella* is an out group. Only bootstrap values (1000 replicates) greater than 50% are shown.

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L'UTILISATION DE BARCODES ADN ET DES ENZYMES DE RESTRICTION POUR IDENTIFIER TROIS ESPÈCES DE CHENILLES LÉGIONNAIRES (LÉPIDOPTERA: NOCTUIDAE)

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MOTS-CLÉS: *Mythimna* spp., Chenilles Légionnaires, Barcodes ADN, Identification Des Espèces.

Résumé

SIX ESPÈCES de chenilles légionnaires de la canne à sucre (*Mythimna* spp.) sont connues à Maurice, notamment *M. pseudoloreyi*, *M. loreyi*, *M. insulicola*, *M. phaea*, *M. tincta* et *M. pyrausta*. En raison de la ressemblance morphologique des adultes et de la difficulté de distinguer les papillons, des erreurs d'identification sont existantes. Une nouvelle approche implique l'utilisation des barcodes moléculaires. Le polymorphisme, sur une courte séquence d'un fragment d'ADN conservé du gène mitochondrial codant pour la première sous-unité de la cytochrome oxydase (COI), est couramment utilisé comme barcodes pour identifier des Lépidoptères. En 2008, les larves collectées aux champs provenant de trois espèces *M. insulicola*, *M. phaea* et *M. pseudoloreyi* ont été élevées en laboratoire. Deux méthodes d'extraction d'ADN ont été testées sur les pattes, les ailes et l'abdomen des adultes. Les deux méthodes ont donné des résultats satisfaisants. Des fragments de ~ 700 pb, amplifiés des trois espèces par la technique PCR avec les amorces HCO2198/LCO1490, ont été coupés à l'aide des enzymes de restriction. La distinction des trois espèces de chenilles légionnaires a été possible avec une combinaison de quatre enzymes notamment *Rsa*I, *Taq*I, *Pvu*II et *Sac*l. Les produits PCR amplifiés de *M. insulicola*, *M. phaea* et *M. pseudoloreyi* ont été clonés et séquencés et un fragment de 658 pb pour chaque espèce a été enregistré dans Genbank comme suit: *Leucania insulicola* (numéro d'accession GQ353294), *L. phaea* (GQ353295) et *L. loreyi* (Q353296). L'analyse de ces barcodes avec le moteur de recherche de BOLD-ID (base de donnés 'Barcode of Life Datasets') indique une forte identité de séquence avec les membres du genre *Leucania* (Noctuidae: Hadeninae); 99.54% entre *L. phaea* et *Leucania* spp. (de Kenya), 100% entre *L. loreyi* et *Leucania* spp. et 99.85% entre *L. insulicola* et *L. striata* (de Madagascar). *L. phaea* présentait un pourcentage de variation de 7.3% et de 10.6% par rapport à *L. loreyi* et *L. insulicola* respectivement. D'autre part, une divergence de 8.5% au niveau de la séquence a été observée entre *L. loreyi* et *L. insulicola*. Les informations obtenues avec les barcodes ADN seront très utiles pour déterminer les espèces de chenilles légionnaires.

IDENTIFICACIÓN DE TRES ESPECIES DE GUSANOS COGOLLEROS (LEPIDOPTERA: NOCTUIDAE) USANDO CÓDIGOS DE ADN Y DIGESTIÓN POR ENZIMO DE RESTRICCIÓN

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PALABRAS CLAVE: *Mythimna* spp., Gusano Cogollero, Códigos de Barras del ADN, Identificación de Especies.

Resumen

SEIS ESPECIES de gusanos cogolleros de la caña de azúcar (*Mythimna* spp.) existen en Mauricio, en específico, *M. pseudoloreyi*, *M. loreyi*, *M. insulicola*, *M. phaea*, *M. tincta* y *M. pyrausta*. Debido al gran parecido de las mariposas adultas, la identificación morfológica entre las distintas especies se dificulta. Un nuevo enfoque para su caracterización involucra el uso de códigos de barra del ADN para analizar la diversidad de secuencia dentro de un pequeño segmento estándar de su genomio. El polimorfismo en el extremo 5' del gen mitocondrial *citocromo oxidasa* I (COI, por sus siglas en Inglés) es usado ampliamente como un sistema de código de barras de ADN en los Lepidópteros. En 2008, mariposas adultas de *M. insulicola*, *M. phaea y M. pseudoloreyi* fueron criados a partir de larvas colectadas en el campo. Dos métodos de extracción de ácidos nucléicos se evaluaron, ambos rindiendo ADN de alta calidad para estudios moleculares. Extracciones de ADN se efectuaron a partir de distintas porciones corpóreas, que incluyeron abdomen, pata y ala. Un fragmento de ~700 pares de bases (pb) de cada una de las tres especies se amplificó por medio de la reacción en cadena de la polimerasa (PCR, por sus siglas en Inglés) usando los iniciadores (*primers*) HCO2198/LCO1490, luego de lo cual se digirió usando enzimos de restricción. Una combinación de cuatro enzimos *Rsa*1, *Taq*I, *Pvu*II y *Sac*I permitió distinguir exitosamente a las tres especies de gusanos cogolleros evaluados. Los productos de PCR de las tres especies fueron clonados y secuenciados. Un fragmento de 658 pb de cada una de las especies *M. insulicola*, *M. phaea* y *M. pseudoloreyi* fueron enviadas a la base de datos 'Genbank' y registrados, respectivamente, como *Leucania insulicola*, GQ353294; *Leucania phaea*, GQ353295; y *Leucania loreyi*, GQ353296. Usando el mecanismo de identificación de Código de Barras de la Vida (en Inglés, *Barcode of Life identification engine,* BOLD-ID), se identificaron (con gran probabilidad) secuencias con las especies de *Leucania* (Noctuidae: Hadeninae)- 99.5% para *L. phaea* y *Leucania* sp. (de Kenia), 100% para *L. loreyi* y *Leucania* sp., y 99.8% para *L. insulicola* y *L. striata* (de Madagascar). La secuencia de *L. phaea* fue diferente en un 7.3% y 10.6% de aquellas de *L. loreyi* y *L. insulicola*, respectivamente. Un valor de divergencia de 8.5% se observó entre *L. loreyi* y *L. insulicola*. La secuencia de *L. phaea* fue divergente en un 7.3% y 10.6% de aquellas de *L. loreyi* y *L. insulicola*, respectivamente. El código de barras de ADN y la secuenciación pueden proveer de información útil para la clasificación y caracterización de gusanos cogolleros.