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RESEARCH ARTICLE

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Diversity of insect pests of common bean and pigeon pea in the Republic of Congo revealed by DNA barcoding

Virginie Roy[1], Joseph Mpika[2], Gael J. Kergoat[3], Gladrich Feldane Tsoungould Mboussy[2] and Attibayeba[2]

[1]*Universite Paris Est Creteil, Sorbonne Universite, Universite Paris Cite, CNRS, IRD, INRAE, Institut d'Ecologie et des Sciences de l'Environnement, Creteil, France*
[2]*Université Marien Ngouabi, Brazzaville, Faculté des Sciences et Technique, République du Congo*
[3]*CBGP, INRAE, IRD, CIRAD, Institut Agro, Universite Montpellier, Montpellier, France*

In Central Africa, the development of leguminous crops is accompanied by a proliferation of pests, such as seed-beetles (Coleoptera: Chrysomelidae: Bruchinae). Integrated biological control against insect pests requires a preliminary phase of early detection and monitoring of potential invasive species, which is often limited by the availability of diagnostic morphological characteristics. DNA barcoding represents a powerful molecular tool for identifying specimens, and the mitochondrial sequences produced can provide information concerning the origins of introduced species. In this study, we characterised the diversity of insect pests present in farmer storage sites and plots of common bean and pigeon pea, by using DNA barcoding of specimens sampled in the five main agricultural regions of the Republic of Congo. The cosmopolitan seed-beetle species *Acanthoscelides obtectus* (Say, 1831) (Coleoptera: Chrysomelidae: Bruchinae) was recognised as the major pest sampled on common bean. The sub-Saharan species *Specularius erythraeus* (Pic, 1908) (Coleoptera: Chrysomelidae: Bruchinae) was the main species found in pigeon pea plots, sometimes co-occurring with the cosmopolitan species *Callosobruchus maculatus* (Fabricius, 1775) (Coleoptera: Chrysomelidae: Bruchinae). A fourth bruchine, *Zabrotes subfasciatus* (Boheman, 1833), a weevil species of the genus *Apion* Herbst, 1797 (Coleoptera: Brentidae) and two moth species were also identified: a species of the genus *Mussidia* Ragonot, 1888 (Pyralidae) and the cosmopolitan pest of stored food, *Cadra cautella* (Walker, 1863) (Pyralidae). These results differ from species lists compiled in the 1980s, thus providing updated knowledge concerning the pest species present in this region and fundamental information for choosing appropriate methods of control.

CORRESPONDENCE
Virginie Roy

EMAIL
roy@u-pec.fr

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INTRODUCTION

Insect pests are major constraints to agricultural production in Africa. Most of the two dozen main pests recognised on this continent are cosmopolitan species, which were introduced through international trade and other human activities (Abate et al. 2000) Introduced species are responsible for loss or reduced efficiency of agricultural production and may affect food security (Diagne et al. 2021; Pimentel et al. 2008). The annual economic impact of invasive pests on the African continent was estimated at US$65.58 billion (Eschen et al. 2021) and sub-Saharan countries are among the most vulnerable to the impact of invasion by insect pests of crop plants, because they can be strongly dependent on agriculture (Diagne et al. 2021; Paini et al. 2016).

In Central Africa, the main leguminous crops are the common bean (*Phaseolus vulgaris* L., 1753), the lima bean (*Phaseolus lunatus* L., 1753), the pigeon pea (*Cajanus cajan* L., 1753) and the groundnut (*Arachis hypogaea* L., 1753) (Huignard et al. 2011). The common bean is listed as a major staple for food security; it is a very popular crop that provides the population with an important source of protein and calories (Wortmann 1998). The pigeon pea, which contains about 20–22% proteins and significant amounts of essential amino acids, is also popular because it is drought tolerant and needs minimum inputs (Saxena et al. 2002). However, the development of leguminous crops is accompanied by a proliferation of pests, such as seed-beetles (Coleoptera: Chrysomelidae: Bruchinae), that are major harmful insects for these crops throughout the tropics (Southgate 1979; Taylor 1981).

Little recent data is available concerning the diversity and distribution of insect pest species in the Republic of Congo. In the 1980s, Delobel (1984) published a provisional list of Coleopteran pests of stored products, revealing that 33 species were present in storage places of various cereals and leguminous crops throughout the country. Delobel and Epouna-Mouinga (1984a) provided a preliminary list of the Coleopteran and Lepidopteran species encountered in storage places of the Bouenza region. Later, the book by Delobel and Tran (1993) reviewed the data concerning the Coleopteran pest species of food stocks in tropical areas. These documents constitute reference data for the investigation of present species distribution, and keys to genera and illustrations represent useful tools to help identifying species.

Indeed, the correct identification of pest species and the knowledge of their distribution are crucial first steps for choosing appropriate methods of control. Integrative biological control (i.e. the management of pest populations by combining different approaches such as cultivation techniques, crop resistance and biological antagonists) requires accurate identification to improve the effectiveness of treatment, to reduce the costs and to preserve the biodiversity of ecosystems surrounding crops (Andersen and Wagner 2016). The identification of insect pest species is often

problematic, particularly in tropical countries (Balakrishnan 2005). In many cases, identification to species level requires the study of morphological traits that are particularly difficult to observe and interpret, such as male genitalia (Ribeiro-Costa and Almeida 2016). Distinguishing between closely related species can also be difficult due to intraspecific phenotypic diversity and the overlap of diagnostic traits. Finally, there is a clear decline in the number of taxonomists specialised in tropical invertebrate animals (Gaston and Williams 1993; Wheeler et al. 2004).

DNA barcoding was developed in the early 2000s with the objective of identifying unknown specimens with reference to a molecular database (Hebert et al. 2003). DNA barcoding usually targets a 658 bp fragment of the mitochondrial cytochrome *c* oxidase I subunit gene (COI) and allows the studied organisms to be affiliated with described species that are referenced in the database. It is an accessible method, as sequencing can be carried out by international platforms that receive and process samples at relatively low costs and very quick turnaround times. DNA barcoding is deemed to be quite effective if the gap between intra and interspecific diversity is well marked. Some barcoding studies focusing on a restricted geographical area and dedicated to the identification of crop pests and introduced species have provided convincing results due to the restricted number of related species and the low intraspecific diversity (Jinbo et al. 2011; Meier et al. 2008). For cosmopolitan, well-studied species, which are highly represented in public DNA sequence repositories, it can be a powerful tool for identifying specimens (Goldstein and DeSalle 2011). Furthermore, phylogeographical analysis of the COI gene can provide valuable information about the origin of introduced species and how they dispersed through human activities, by identifying multiple introductory events, privileged exchanges between geographic areas or genetic bottlenecks (Kébé et al. 2017).

In the Republic of Congo, a preliminary study assessed the post-harvest diversity of the bruchines present on common bean in two production regions of the country (Dibangou et al. 2021). The authors identified two cosmopolitan seed-beetle pests, *Callosobruchus maculatus* and *Acanthoscelides obtectus* (Chrysomelidae: Bruchinae) based on the mitochondrial 12S rRNA gene. In line with this work, the aim of the present study was to identify the pest species infesting farmer storage sites and plots of the common bean and the pigeon pea in the five main agricultural regions of the Republic of Congo, using DNA barcoding of the standard COI gene fragment. Potential sources of introduction for the species were also investigated using phylogenetical analyses including published COI sequences from various geographic origins.

MATERIAL and METHODS

Sampling sites

Insects were collected as adults or juveniles in the five main regions that produce common bean and pigeon pea: the prefectures of Bouenza, Lekoumou, Niari, Plateaux and Pool, between April and December 2019 (Supplementary Table S1). Samples were obtained based on two procedures: 1) direct survey in farmer storage places and 2) experimental storage of seeds, collected from farmer plots, until insect emergence. For the survey procedure, infected seeds containing live insects were collected directly from containers, bags or plastic sheets on which seeds are left to dry, in each locality visited. Infected seeds were recognised by the circular exit opercula cut by the larvae on the surface of the seed before pupation. For the experimental storage procedure, selected farmer plots were monitored from sowing to harvest. In the field and at harvest, seeds were collected and placed in plastic pots. The pots were stored in the laboratory until the emergence of insects. Each sampling site/plot was geo-referenced. All insects collected/emerging from a plot/site were grouped together in the same vial and were preserved in 70–95% ethanol.

DNA extraction

Before extraction, insects were sorted into morpho-groups based on their external morphology (i.e. oval body shape, elytra patterned and not fully covering the abdomen for seed-beetles, elongate rostrum and geniculate antennae for weevils and narrow forewings and dull coloration for pyralid moths). Putative species were proposed without any attempts to assign individuals to known species at this stage. One to six individuals per putative species and per plot were chosen for individual DNA extraction. Each individual was rinsed in 95% ethanol to prevent cross-contamination before DNA extraction. Total DNA was isolated from head, thorax and legs, following the DNeasy® Blood & Tissue Kit (Qiagen, France) protocol with a final elution in 50 µl of buffer.

PCR amplification and sequencing

The standard DNA barcoding region (Hebert et al. 2003) targeted for PCR amplification consists of a 658 bp fragment at the 5' end of the COI gene. PCRs were performed in a total volume of 20 µl containing 1 × Colorless GoTaq® Buffer Master Mix (Promega, France), 0.2 µM of each forward (LCO1490-GGTCAACAAATCATAAAGATATTGG) and reverse (HCO2198-TAAACTTCAGGGTGACCAAAAAATCA) primer (Folmer et al. 1994), 0.5 mM of dNTPs, 0.5 U of GoTaq® G2 DNA polymerase (Promega, France), 2 µl of template DNA and PCR-grade water (q.s.). The following thermocycling conditions were used: an initial denaturation step at 94 °C for 3 min followed by 40 cycles at 94 °C for 30 sec, an annealing step at 50 °C for 45 sec and an extension step at 72 °C for 1 min, and a final extension step at 72 °C for 10 min. PCR products were sent to Eurofins Genomics for Sanger unidirectional sequencing with primer LCO1490.

Molecular analyses

The sequences generated in this study were submitted to GenBank and accession numbers are provided in Supplementary Table S1 For one species, i. e. *A. obtectus* (see Results), visual inspection of the chromatograms with Chromas 2.6.6 (Technelysium, Australia) showed multiple haplotypes from individual specimens. We observed that sites with secondary peaks were strongly associated with third codon positions and synonymous transitions, a pattern indicating heteroplasmy rather than the co-amplification of nuclear pseudogenes (Magnacca and Brown 2010). At this step, the dominant haplotype for each individual was recorded and peaks of equal heights were coded as missing data.

Molecular taxonomic identification was initiated using the Identification System (IDS) on the Barcode of Life Data System (BOLD) (Ratnasingham and Hebert 2007) and the Basic Local Alignment Search Tool (BLAST) for searches on GenBank (NCBI). For IDS, the *Species Level Barcode Records* option was chosen first, and if it failed in returning a species-level identification, the *All Barcode Records on BOLD* option was used.

To refine specific identification or to investigate potential sources of introduction of the main pest species, haplotypes were obtained using DnaSP v6.12.03 (Rozas et al. 2017) and integrated in datasets comprising sequences from the literature. Reference sequences from various geographic origins and/or for phylogenetically closest species were retrieved from GenBank as of December 2022. Alignments were produced using Muscle program in Seaview (Gouy et al. 2010). SMS (Lefort et al. 2017) was used to select the best model of nucleotide substitution with an AIC criterion. Phylogenetic trees were reconstructed using

Maximum likelihood with PhyML (Guindon et al. 2010) with 100 bootstrap replicates, and Bayesian Inference with MrBayes (Ronquist et al. 2012) running 5 million generations, sampling every 1000 generations with a burn-in of 25%.

When heteroplasmy was suspected, phylogenetic analyses were run following two steps: firstly, considering only the dominant haplotype for each individual and secondly, using the IUPAC code and treating all heteroplasmic sites as ambiguities. In order to derive information from heterozygous sites, phasing was also performed on IUPAC-coded sequences. Haplotypes were inferred using the PHASE program implemented in DnaSP, based on alignments containing available unphased sequences. A median-joining haplotype network was constructed using PopART v1.7 (Leigh and Bryant 2015).

RESULTS

DNA barcoding

Seven putative species were recorded in the total dataset, hereafter referred to as seed-beetle putative species 1, 2, 3 and 4, weevil putative species 1, and moth putative species 1 and 2. COI sequences were obtained for 90 samples from seven subprefectures and 22 localities, representing 22 insects collected from common bean storage places, 22 which emerged from seeds collected in common bean plots, four insects collected from pigeon pea storage places and 42 which emerged from seeds collected in pigeon pea plots (Supplementary Table S1).

Seed-beetles

Thirty-six sequences were obtained from specimens recognised as seed-beetle putative species 1. They were either unambiguously affiliated to *A. obtectus* with BOLD IDS (i.e. 21 samples retrieved a "solid match", with 99.76–100% identity) or retrieved close sequences from *A. obtectus* (i.e. three samples obtained a "nearest match" with 97.8–98.35% identity, and 12 samples obtained a "no match" with 92.47–96.44% identity). A "solid match" in BOLD is a robust identification unless there is a very closely allied congeneric species that has not yet been analysed. BLAST analyses reported 91.86–100% identity with *A. obtectus*.

Thirty-six sequences were obtained from specimens proposed as seed-beetle putative species 2. They generated no match with BOLD IDS, with 86.13–86.92% identity with various bruchine species sequences depending on the sample tested. BLAST analyses reported 85.28–86.35% identity with *Bruchus pisorum* (L., 1758), *Bruchidius uberatus* (Fåhraeus, 1839) or *Bruchidius marginalis* (Fabricius, 1777) depending on the sample tested. In the absence of close sequences in public nucleotide databases, the sequences from Congo were compared to reference sequences from an unpublished molecular dataset assembled by GK. The sequences from Congo and the sequence of *Specularius erythraeus* (Chrysomelidae: Bruchinae) from Kenya (isolate GKER.00679) showed 95.3–98.72% identity.

Five sequences were obtained from specimens recognised as seed-beetle putative species 3. They matched sequences from *C. maculatus* in BOLD IDS, obtaining a "nearest match" with 98.09–98.5% identity. BLAST analyses reported 97.48–98.53% identity with *C. maculatus* sequences.

One sequence was obtained from the specimen recognised as seed-beetle putative species 4. It obtained a "no match" with 90.68% identity to *Z. subfasciatus* (Coleoptera: Bruchinae) with BOLD IDS. BLAST analyses reported 92.08% identity with a *Z. subfasciatus* sequence. The sequence from Congo and a sequence of *Z. subfasciatus* from the GK DNA sequence dataset (isolate GKER.culture) showed 96% identity.

Weevils

Four sequences were obtained from the specimens identified as the only weevil putative species. They matched a sequence of *Apion* sp. from Kenya with BOLD IDS, obtaining a "nearest match" with 97.01–97.18% identity. BLAST analyses did not provide any information concerning the identification since they reported low identity (< 88%) with sequences from various weevil species.

Moths

Six sequences were obtained from specimens classified as moth putative species 1. They retrieved sequences from an unidentified species of *Mussidia* (Lepidoptera: Pyralidae) with BOLD IDS, obtaining a "solid match" with 99.84–100% identity. BLAST analyses reported 93.81–93.96% identity with *Mussidia* sp.

Finally, two sequences were obtained from specimens classified as moth putative species 2. They matched sequences from *Cadra cautella* (Lepidoptera: Pyralidae) with BOLD IDS, obtaining a "solid match" with 100% identity. BLAST analyses reported 99.85% identity with *C. cautella*.

Phylogenetic and network reconstructions

Acanthoscelides obtectus

Because mixed sequences were observed for *A. obtectus*, a first set of ML and BI trees was constructed considering only the dominant haplotype for each individual. The corresponding COI alignment comprised 49 sequences of 616 bp: 36 sequences from Congo, 12 GenBank sequences from China, Egypt, Hungary, Finland, Germany and Serbia, and one GenBank sequence of *Acanthoscelides oblongoguttatus* (Fåhraeus, 1839) from Mexico as outgroup. The best model selected using AIC criterion was a GTR+G+I (K = 105, Lik = –1771.38, AIC = 3752.76). Sequences for the 21 individuals with a "solid match" with BOLD IDS clustered in a clade including all *A. obtectus* sequences available on Genbank in both the ML and BI trees (Figure 1). Sequences for the 15 remaining individuals, with a "nearest match" or "no match" were found paraphyletic to this clade. No geographical clustering was observed since sequences from the Republic of Congo and from different localities around the world were found dispersed throughout the phylogenetic trees.

In a second step, only sequences with no or very low noise level were retained (i.e. 29/36 sequences) to assign polymorphisms, since in this case heteroplasmy is recognisable even when the non-dominant peak is low. As the height of secondary peaks can vary widely, manual editing of chromatograms is known to be subjective (Magnacca and Brown 2010). Considering this limitation, 24/29 individuals were considered as heteroplasmic, and polymorphic sites in heteroplasmic individuals accounted for about 8% of the total sequence. The corresponding COI alignment with IUPAC codes comprised 42 sequences of 616 bp, including the 29 retained sequences from Congo. The best model selected using AIC criterion was GTR+G+I (K = 91, Lik = –1518.18, 3218.36). Results obtained for this dataset were quite similar to those obtained with the dominant haplotypes, except for PS18-1 sequence that was included in the major clade in the ML and BI trees (Supplementary Figure S1). The phasing of haplotypes resulted in 43 haplotypes and the Median-joining haplotype network showed two clearly separated groups with a few intermediate haplotypes (Figure 2). Intra-cluster diversity was 0.02 for both clusters and inter-cluster diversity was 0.08.

Specularius erythraeus

The Congolese dataset was composed of 36 *S. erythraeus* COI sequences representing 11 haplotypes. The COI alignment comprised 58 sequences of 453 bp (11 haplotype sequences from Congo, one *S. erythraeus* sequence from Kenya from GK unpublished dataset i.e. the sole COI sequence available for this species, 10 GenBank sequences from various *Bruchus* species, 35 GenBank sequences from various *Bruchidius* species and one GenBank sequence from *Pachymerus cardo* (Fåhraeus,

Figure 1. Maximum likelihood tree based on COI sequences of *Acanthoscelides obtectus* collected on common bean (storage sites and field plots) in Congo and from GenBank. Only the dominant haplotype was considered for each individual. The tree is rooted with *Acanthoscelides oblongoguttatus* (GenBank AB49967). Bootstrap values >70 and Bayesian posterior probabilities >0.95 from Bayesian Inference tree are indicated at nodes. Samples from storage sites are indicated in grey boxes and samples from field plots, in black boxes. Solid matches (>99%) to *A. obtectus* according to BOLD IDs are annotated with an asterisk.

1839) used as an outgroup). The best model selected using AIC criterion was GTR+G+I (K = 123, Lik = −6652.27, AIC = 13550.54). Congolese haplotypes were grouped in a highly supported clade including *S. erythraeus* from Kenya (ML BT = 100% and BPP = 1.00) (Figure 3).

Callosobruchus maculatus

The five *C. maculatus* sequences of the dataset from Congo were represented by three haplotypes. The alignment was composed of 85 sequences of 488 bp (three sequences from Congo, 69 sequences of *C. maculatus* from countries worldwide available on GenBank, 13 sequences from other *Callosobruchus* species from GenBank, and one sequence of *A. obtectus* from GenBank). The best substitution model selected using AIC criterion was GTR+G+I (K = 177, Lik = −3764.99, AIC = 7883.99). The three *C. maculatus* haplotypes from Congo clustered in a well-supported clade (ML BT = 95% and BPP = 0.98) (Figure 4). Again, no phylogeographic pattern was observed in ML and BI trees for *C. maculatus* at the global scale, some clades including sequences from different countries and continents (e.g. the Thailand, Sri Lanka, Brazil, Taiwan, Myanmar, Vietnam clade, supported by ML BT = 96% and BPP = 1.00).

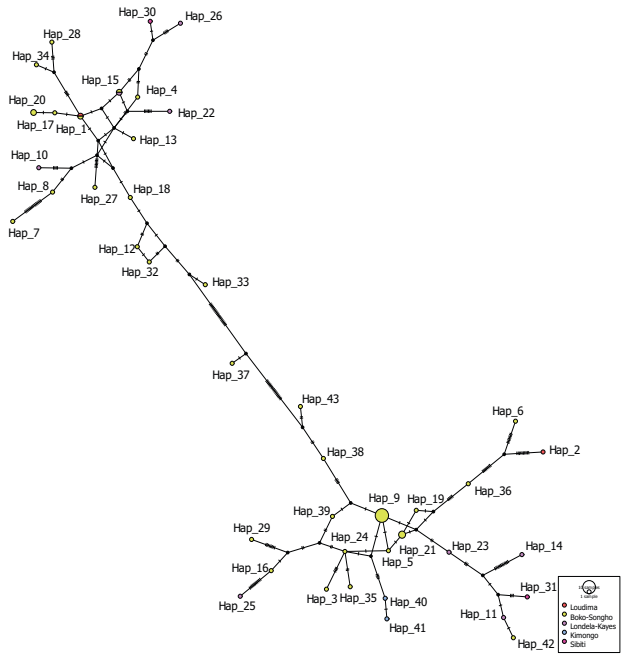

Figure 2. Median-joining network of COI haplotypes of *Acanthoscelides obtectus* from Congo after phasing. Colours indicate sampling sites and bars indicate the number of mutations between two haplotypes.

Figure 3. Maximum likelihood tree based on COI sequences of *Specularius erythraeus* found in pigeon pea field plots (haplotypes 1 to 11) in the Republic of Congo, various *Bruchidius* and *Bruchus* species from GenBank, and *Pachymerus cardo* (GenBank AY390668) as outgroup. Bootstrap values >70 and Bayesian posterior probabilities >0.95 from Bayesian Inference tree are indicated at nodes

Figure 4. Maximum likelihood tree of COI sequences of *Callosobruchus maculatus* from the Republic of Congo found at pigeon pea field plots (haplotypes 1 to 3), various *Callosobruchus* species from GenBank, and *Acanthoscelides obtectus* (GenBank KX825864) as outgroup. Bootstrap values >70 and Bayesian posterior probabilities >0.95 from Bayesian Inference tree are indicated at nodes

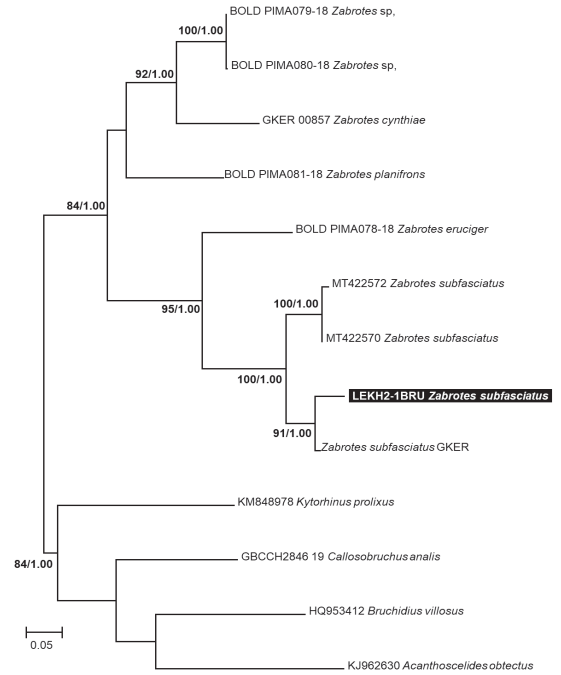

Figure 5. Maximum likelihood tree based on COI sequences of *Zabrotes subfasciatus* obtained from a common bean field plot in the Republic of Congo and from various bruchine species from BOLD and GenBank. Bootstrap values >70 and Bayesian posterior probabilities >0.95 from Bayesian Inference tree are indicated at nodes

Zabrotes subfasciatus

The COI alignment was composed of 13 sequences of 654 bp (one *Z. subfasciatus* sequence from Congo, two *Z. subfasciatus* sequences from GenBank, one *Z. subfasciatus* from the GK unpublished dataset, five sequences from various *Zabrotes* species from BOLD and the GK unpublished dataset, and four GenBank sequences from other bruchine genera). The best model selected using AIC criterion was GTR + R (K = 40, Lik = –3321.55, AIC= 6723.10). The sequence from Congo was included in a clade comprising all *Z. subfasciatus* sequences (ML BT = 100% and BPP = 1.00) (Figure 5).

Distribution of sequenced specimens

Specimens of *A. obtectus* were identified in all common bean storage sites and field plots in Bouenza, Lekoumou, Niari and Pool prefectures. In only one Boko Songho site (Bouenza), the moth *C. cautella* was found co-occurring with *A. obtectus*. The moth *Mussidia* sp. and the seed-beetle *Z. subfasciatus* were observed in the prefecture of Plateaux (Supplementary Table S1 and Figure 6), from storage places and field plots.

Specimens of *S. erythraeus* were identified from all pigeon pea plots in Bouenza, Lekoumou and Niari. Other species were more rarely observed on pigeon pea, such as *C. maculatus* and *Mussidia* sp. which were found co-occuring with *S. erythraeus* in Loudima (Bouenza) and the weevil species that was observed in pigeon pea storage places in Niari (Supplementary Table S1 and Figure 6).

Figure 6. Map of the sites for which insect samples were barcoded: common bean in small black circles and pigeon pea in small grey circles. The presence of bruchines is indicated with a square (Ao: *Acanthoscelides obtectus,* Se: *Specularius erythraeus,* Cm: *Callosobruchus maculatus,* Zs: *Zabrotes subfasciatus),* the presence of moths, by a large circle (Cc: *Cadra cautella*, M: *Mussidia* sp.) and the presence of weevils, with a triangle (W: undetermined weevil)

DISCUSSION

DNA barcoding

In this study, we used a DNA barcoding approach to identify insects collected from farmer storage sites and emerging from seeds collected in plots of common bean and pigeon pea in the five main agricultural regions of the Republic of Congo. Molecular analyses supported the presence of at least seven pest species (four seed-beetles, one weevil and two moths). Five of them could be affiliated to a described species and molecular data were produced for all of them, supplying nucleotide databases with standard COI barcodes for further research and comparison. Two putative species (weevil putative species and moth putative species 1) could not be affiliated to known species and deserve to be studied using additional genes and detailed morphological observations. These results show that the DNA barcoding approach has some limitations for regions where morphological and molecular data are scarce, and for poorly-known taxonomic groups. These limitations have been widely discussed in the literature (Meyer and Paulay 2005; Virgilio et al. 2012) and they highlight the importance of generating associated morphological and molecular data for pest species in these regions.

Because COI sequences produced in this study are the first for these species in the Republic of Congo, we advocate that DNA barcoding be implemented routinely to identify insect pest species in this region, to expand knowledge of pest diversity and genetic databases, and as a preliminary step to future biological control programmes. Furthermore, DNA barcoding allowed identification of moth caterpillars in the region of Plateaux, thus linking juvenile specimens to adult morphotypes. Identification of juvenile stages of pest species is very important for pest control, but only well-trained taxonomists can identify larvae to species level using morphological characteristics (Doskocil et al. 2008).

Our study also showed multiple mitochondrial haplotypes within an individual, in a large majority of samples of *A. obtectus*. Heteroplasmy may arise through paternal leakage, i.e. when the paternal mitochondria are not eliminated during fertilisation of the egg. Many cases of heteroplasmy were reported in insects, including various Coleoptera e.g. bark weevils (Boyce et al. 1989), the firefly *Inflata indica* (Sriboonlert and Wonnapinij, 2019) and the leaf beetle *Gonioctena intermedia* (Kastally and Mardulyn, 2017), but this is the first time it is evocated for *A. obtectus* to our knowledge. Heteroplasmy was cited as a problem for barcoding because heteroplasmic species were significantly less likely to be identified by standard methods. Magnacca and Brown (2010) made different recommendations to mitigate this difficulty. They advocate including multiple sequences from each species to cover as much genetic diversity as possible and using extractions from different tissue types to account for possible haplotype segregation. Another explanation for the mixed pattern of sequences in *A. obtectus* is the mixing of sequences from different genetic lineages, as many individuals from the same plot were conserved in the same vial. This last hypothesis seems unlikely since individuals were thoroughly rinsed in 95% ethanol before extraction, and because *A. obtectus* is the sole species concerned by this problem. Additional sampling with individual storage in ethanol could definitely rule out this possibility.

Pests of common bean

Acanthoscelides obtectus was the major pest species found in storage places and field plots of common bean in this study. This species is specialised on wild or cultivated species of the genus *Phaseolus*. Egg-laying or oviposition occurs in bean crops when the pods are ripe and *A. obtectus* causes significant losses during storage (Abate and Ampofo 1996). The species was also reported on new crop host plants such as *C. cajan*, *Cicer arietinum* L., 1753, *Lens culinaris* Medik., 1787, *Pisum sativum* L., 1753, *Vicia faba* L., 1753, *Vigna unguiculata* (L.) Walp., 1843, and *Vigna subterranea* (L.) Verdc., 1981 (Johnson 1991; Delobel and Tran 1993). In the Republic of Congo, *A. obtectus* was already mentioned by Delobel and Epouna-Mouinga (1984a) in common bean stocks in the region of Bouenza. However, it was much less common than *C. maculatus* at that time (Delobel 1984; Delobel and Epouna-Mouinga 1984a). Our results are rather in line with those of Dibangou et al. (2021), who found *A. obtectus* at ten sites sampled in Niari and Bouenza and *C. maculatus* at only two sites in Bouenza. Conversely, *A. obtectus* was not identified in Plateaux in our study, while it was the most abundant species in this region in the 1980s (Delobel and Epouna-Mouinga 1984b). Although additional sampling is necessary in the region of Plateaux, these results suggest that the distribution of *A. obtectus* could have changed substantially.

Molecular analyses also revealed that the two groups of haplotypes observed for *A. obtectus* were present in several subprefectures (Boko Songho, Loudima, Kimongo, Londela Kayes and Sibiti), indicating that they are both widely dispersed across the country. These two groups were separated by a mean K2P distance of 8%, a value that is outside the intraspecific range for 95% of insects tested by Virgilio et al. (2010) and these groups could represent genetically distinct species. The major clade in the phylogenetic analyses, comprising published sequences from various regions of the world, probably corresponds to *A. obtectus sensu stricto,* while other sequences could belong to differentiated lineage(s) or cryptic species with potential hybrids.

Acanthoscelides obtectus is native to Meso-America and followed the introduction of bean crops in Asia, Europe and Africa (Oliveira et al. 2013). Several phylogeographic studies have been carried out on *A. obtectus*, showing that the African populations of this species originated from a colonization event from South America around 500 yBP, possibly followed by minor secondary colonisation events (Alvarez et al. 2005; Oliveira et al. 2013). *Acanthoscelides obtectus* from the Republic of Congo did not show any phylogeographic structure: they were dispersed among Asian, European and other African sequences, suggesting recurrent genetic exchanges between infested continents. The number of COI sequences available for *A. obtectus* that overlap with the standard DNA barcode was, however, limited and our results must be confirmed with other markers (i.e. other mitochondrial and nuclear data), to infer precise genetic relationships with other African lineages.

Other pest species were occasionally found on the common bean. The moth *C. cautella* was observed in storage sites in the Bouenza region. To the best of our knowledge, it is the first time that the species is reported on common bean in the Republic of Congo. The moth, called "tropical warehouse moth", "almond moth", "cocoa moth" or "fig moth", is a cosmopolitan pest of stored food such as maize or wheat (Freeman 1948; Burges and Haskins 1965). It also feeds on dried fruits, beans, nuts, bananas, groundnuts, dried cocoa, and coconut. Infestation usually occurs after the harvest (Khan and Noor 2015). The larva spoils the food by its feeding activity and the production of large quantities of silk.

Another moth in the genus *Mussidia* was observed in storage sites and obtained from field plots in the region of Plateaux. A *Mussidia* species was already mentioned by Delobel and Epouna-Mouinga (1984a) as the sole insect infesting maize in Mouyondzi (Bouenza). The species was observed only at this site and, according to the authors, it seemed unable to reproduce on harvested/stored maize.

Finally, *Z. subfasciatus* was obtained from a field plot in the region of Plateaux. In the Republic of Congo, the species was already observed on common bean in the region of Mouyondzi (Bouenza) (Delobel 1984). It was mentioned as rare and of little economic importance.

Pests of pigeon pea

Specularius erythraeus was the most numerous species recovered from pigeon pea plots. It was identified in Bouenza, Lekoumou and Niari regions. This species has a sub-Saharan distribution. It is known to infest pigeon pea in the field, with egg-laying occurring on the pods and seeds, and it was also recorded on *V. unguiculata*. Interestingly, Delobel (1984) described this species, which was previously observed on pigeon pea in the region of Mouyondzi (Bouenza), as an uncommon pest in the Republic of Congo. Infestation by *S. erythraeus* has never been detected during storage in previous studies (Southgate and McFarlane 1976; Delobel and Tran 1993) nor in this study. *Specularius erythraeus* was sometimes found co-occurring with *C. maculatus* during this study, a result consistent with those of Southgate and McFarlane (1976), who described *Specularius* species occurring in association with *Bruchidius* and *Callosobruchus* species on *Cajanus*, *Phaseolus* and *Vigna* crops in East Africa.

Callosobruchus maculatus was found only in a few pigeon pea plots in Loudima (Bouenza). The species is pantropical and known to have many host plants, especially in the genus *Vigna,* but also *C. cajan*, *C. arietinum*, *Glycine max* (L.) Merr., 1917, *Lablab purpureus* (L.) Sweet, 1826, *L. culinaris*, *P. sativum* and *V. faba* (Delobel and Tran 1993). The origin of *C. maculatus* is Afrotropical, in association with its main host plant, *V. unguiculata* (Tuda et al. 2014). Oviposition takes place on maturing pods, on dry pods or directly on seeds (Viaud 1983). Females lay eggs on crops in the field as well as in storage sites. The species was already mentioned by Delobel and Epouna-Mouinga (1984a) from a single pigeon pea sample in Nsanga (Bouenza) and it was recently found at two localities in the Bouenza region (Dibangou et al. 2021).

We observed that the three Congolese haplotypes of *C. maculatus* were grouped in the phylogenetic trees and were not shared with other African localities, a result consistent with those of recent studies that focused on the genetic structure of *C. maculatus*. Indeed, in Africa a marked genetic differentiation was described between subregional populations due to geographic isolation (Tuda et al. 2014). Kébé et al. (2016, 2017) also showed genetic differentiation between geographic regions and limited gene flow between African populations. These results were explained by the authors as a combination of biogeographic processes, isolation by distance and human-mediated events such as insecticide or host plant selection.

CONCLUSION

Using a DNA barcoding approach, we identified five of the seven putative pest species found on common bean and pigeon pea in storage sites and field plots, in the five main agricultural regions of the Republic of Congo: *A. obtectus*, *C. maculatus, S. erythraeus, Z. subfasciatus* and *C. cautella*. These results allow updating the species reports obtained in the 1980s and signalling for the first time *A. obtectus* as the main pest of common bean in storage sites and plots, and *S. erythraeus* as the main pigeon pea pest in field plots. Further integrated biological control could be implemented according to these results.

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